8th International Toxicology Symposium in Africa

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2016

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International Toxicology Symposium in Africa

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JAPAN SOCIETY FOR THE PROMOTION OF SCIENCE
日本学術振興会

Outline for Chemical Hazard Commission for Africa (CHCA)

In recent years, resource development has rapidly progressed in various African countries by advanced and emerging nations. But at the same time, this has led to the creation of environment pollution at an unprecedented pace. Regarding this accelerated output of environmental pollution in African countries, there is very little data, and current conditions are not well understood. The fact that toxicological surveillance of the ecosystem, wildlife, and humans has not been carried out is particularly problematic, and is one reason for the delay in the development of countermeasures. According to our preliminary survey, environment pollution has already deteriorated to the level that threatens the food safety of several nations, and chemical hazard due to environmental pollution is an urgent issue faced by many nations. Over the past six years, an international symposium has been held under the title, “International Toxicology Symposium in Africa,” which has led to the formation of a network for the survey and study of environmental pollution in Africa. Through this symposium, toxicology researchers from various countries have been able to exchange opinions, and at the last symposium, researchers and students from more than 10 nations participated. This symposium has served as a bottom-up engine for toxicology in research institutes in Africa. In addition, data, as the black box of environmental research, has been collected through the joint surveillance by African nations, and foundational data of environmental toxicology has been established. A consortium to continue these activities in 2014 was constructed, but strong opinions regarding the activities of this consortium were voiced, especially for the continuation of the research network and contributions toward further human resource development. There is a strong demand from African countries for capacity building, and support for young researchers and student training has been requested by Japan. Also, development of human resources with international management capability and leadership from the Japan side, which has been pointed out by Europe and the U.S. as a weak point of Japan, is expected to improve by responding to actual conditions regarding chemical hazard in Africa. Consequently, the purpose of this project is to establish the Chemical Hazard Commission for Africa (CHCA) which seeks to resolve chemical hazard issues by focusing on the human resource development of young researchers and graduate students, in addition to the implementation of surveillance research.

Coordinator in Japan
Prof. Mayumi Ishizuka
Prof. Yoshinori Ikenaka

Organizing Committee

Egypt side
Prof. Alaa Eldin Morshdy
Prof. Eageh Sobhy Darwish
Food Control Department, Faculty of Veterinary Medicine, Zagazig University, Egypt

Japan side
Prof. Mayumi Ishizuka
Prof. Yoshinori Ikenaka
Graduate School of Veterinary Medicine, Environmental Veterinary Sciences, Laboratory of Toxicology, Hokkaido University, Japan

THE ITSA 2016 REVIEW PROCESS

The International Symposium on Toxicology in Africa annual meeting provides the opportunity for scientists to publish their work in the conference proceedings. All papers in this Proceedings Book were subjected to a double blind review process to ensure scientific quality and credibility. Each paper was sent to two reviewers who are regarded as specialists in the field of Environmental Toxicology. The review panel consisted of following scientists.

- Prof. Paul Fawou Moundipa (Cameroon)
- Prof. Yoshinori Ikenaka (Japan)
- Prof. Wageh Sobhy Darwish (Egypt)
- Dr. Yared Beyene (Ethiopia)
- Dr. Osei Akoto (Ghana)
- Prof. Ezemonye Lawrence Ikechukwu (Nigeria)
- Prof. Johan van Vuren Johan (SA)
- Prof. Victor Wepener (SA)
- Prof. Nico Smit (SA)
- Dr. Yabe John (Zambia)
- Dr. Shouta M.M. Nakayama (Japan)
- Prof. Mayumi Ishizuka (Japan)
- Dr. Aksorn Saengtienchai (Japan)

The Editor in Chief and Associate Editor evaluated and forwarded the feedback from the reviewers to the submitting authors. A total of 98 papers were submitted for review. Forty papers were rejected and 58 papers were accepted of which 7 required major revision and the remainder minor revisions. The editorial team ensured that all required corrections were made prior to the final acceptance of the paper for inclusion in the Symposium Proceedings. Reviewers were instructed not to discuss any aspects with the authors of the papers until after the conference presentations.

WELCOME ADDRESS

Environmental Pollution is a magnified problem in different African countries, resulting in sever biological consequences and adverse health effects on animals and human. Over the past seven years, with sincere efforts from our Japanese partners (Professor Mayumi Ishizuka, Professor Yoshinori Ikenaka and all stuff members of Graduate School of Veterinary Medicine, Hokkaido University) and great funding from Japanese Society for Promotion of Science (JSPS), we succeeded to held International Toxicology symposium in Africa and establish our consortium to discuss hot environmental issues in Africa. As a result of this symposium, many young African researchers had good chances to improve their scientific knowledge and skills by attending our annual conference.

Fortunately, this year the 8th International Toxicology Symposium will be held in Egypt and co-hosted by Zagazig University, Egypt and Hokkaido University, Japan. We take this chance to acknowledge JSPS for funding and to welcome all invitees from Japan and different African countries. We hope to enjoy your stay in Egypt and have fruitful discussions to find good solutions and recommendations to minimize the risks and hazards of Environmental pollution. Welcome to Egypt.

Professor Dr. Alaa Eldin Morshdy,
Professor Dr. Wageh Sobhy Darwish,
Food Control Department
Faculty of Veterinary Medicine,
Zagazig University, Egypt

Professor Dr. Alaa Eldin Morshdy

Professor Dr. Wageh Sobhy Darwish

TIME TABLE

— Welcome reception (29th Aug) —

18:00-20:00 Restaurant at Hotel

— Opening (30th Aug) —

Moderator **Prof. Wageh S. Darwish and Prof. Yoshinori Ikenaka**

8:00-8:10 **Opening Remarks**

Prof. Mayumi Ishizuka

Session : Keynote address

KA-1 8:10-8:35 **Prof. Dr. Ebied Saleh**

President of Damanhur University and Progessor of Food Safety

Chair person Prof. Ezemonye Lawrence

Session A: Food Poisoning and food contamination 1; Heavy Metals

Chair person Prof. Morshdy Alaa Eldin and Prof. Wageh S. Darwish

O-1 8:35-8:50 **ABD-EL AAL Salah Fathy**

Toxic metals screening in concentrated milks and assessment of transfer from packaging materials

O-2 8:50-9:05 **BAYOUMI Mohamed Abdelhakim**

Camel milk metal's status, a mini survey on Libyan camel milk

O-3 9:05-9:20 **Abd El-Kader Mahdy**

Chemical quality of water supplies to poultry farms in Egypt

9:20-9:40 **Coffee break**

Session B: Environmental Pollution and Public health

Chair person Dr. Shouta Nakayama and Prof. Hayder Abdelgader

SL-1 9:40-10:00 **YABE John**

Kabwe childhood lead poisoning: past, present and future

O-4 10:00-10:15 **TOYOMAKI Haruya**

Lead exposure on human samples in African countries: a mini review

O-5 10:15-10:30 **DARKO Godfred**

Estimated health risks associated with consumption toxic metals in fish raised from sewage-fed aquaculture

O-6 10:30-10:45 **OGBOMIDA Emmanuel Temiotan**

Risk Assessment of Heavy Metals via Consumption of Muscle and Offal of Free-Range Animals from Benin City

O-7 10:45-11:00 **MOUNDIPA Paul**

Study of Oxidative stress and Epigenetic modifications in High Blood Pressure Individual Leaving in an Environment Containing Mycotoxins and Bisphenol A

11:00-12:20 **Poster Discussion Time**

11:00-11:40 Core time for odd numbers

11:40-12:20 Core time for even numbers

12:20-13:40 **Lunch**

Session C: Situation of environmental pollution in Africa

Chair person Dr. Hazuki Mizukawa and Dr. Darko Godfred

O-8 13:40-13:55 **ABDELGADER Hayder**

Pesticide Use in Sudan: Historical Background and Future Prospective

O-9 13:55-14:10 **EZEMONYE Lawrence**

Heavy Metals Concentrations in Sediment and Benthic Fauna (Chrysichthys auratus and Tympanotonus fuscatus) of Benin River: Application of Risk Assessment Indices.

O-10 14:10-14:25 **Greenfield Richard**

Heavy metals in Mopane worms from Kruger National Park

O-11 14:25-14:40 **PIETERS Rialet**

Polycyclic aromatic hydrocarbon toxicity assessment of urban sediments, South Africa

O-12 14:40-14:55 **WOLMARANS Corrie**

The Possible Association between Selected Sediment Characteristics and the occurrence of heavy metals in a highly Pristine River in South Africa

14:55-15:15 **Coffee break**

Session D: Food poisoning and food contamination 2; Other chemicals

Chair person Dr. Osei Akoto and Prof. Rialet Pieters

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Levels of Pesticide Residues in Fruits and Vegetables from Markets in Dar es Salaam, Tanzania

O-14 15:30-15:45 **MOHAMED Mohamed Elsayed**

Distribution of the gene encoding thermostable direct hemolysin in Vibrio paraehemolyticus isolates of marine fish in Alexandria, Egypt

O-15 15:45-16:00 **ATANDA Olusegun**

Dietary exposure to mycotoxins and risk assessment for adult consumers of locally processed rice from Nigeria

O-16 16:00-16:15 **OGBEIDE Ozekeke**

Risk Characterization of Pesticide Residues in Tympanotonus fuscatus (periwinkle) obtained from selected markets in Benin City, Nigeria.

Session E: Diagnosis and treatment

Chair person Prof. Paul Moundipa and Prof. Yoshinori Ikenaka

O-17 16:15-16:30 **SAKAI Ryosei**

Basic sciences for safety of glutamate and MSG: intestinal metabolism of dietary glutamate in rats

O-18 16:30-16:45 **OSEI Akoto**

Effects of Per-household treatments on chlorpyrifos residues in Lettuce (Lactuce sativa)

O-19 16:45-17:00 **OLORUNFEMI Daniel Ikudayisi**

Application of random amplified polymorphic DNA (RAPD) to detect the genotoxic effects of cassava effluents

O-20 17:00-17:15 **ISHII Chihiro**

Creating a model of renal damage in chicken and glycomic approach to identify novel biomarkers for kidney injury in birds

— Closing Remarks —

17:15-17:30 **Prof. Alaa Morshdy & Dr. Wageh S. Darwish**

— Symposium Photo/Banquet —

17:30-18:00 / 18:30-20:30

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P-4 Metal concentrations in selected macroinvertebrate families, water and sediment of a pristine river system in the North-West Province of South Africa.
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P-5 Susceptibility of the African bollworm, Helicoverpa armigera to two commonly used insecticides in Sudan
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P-9 Mycological evaluation of retailed edible offal
Hafez Abd El Salam (Egypt)

P-10 Heavy metal residues in marketed fish in Manzala city, Egypt
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P-12 Freshness parameters of marketed sausage
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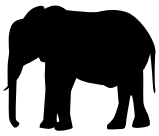

Keynote Address
Special Lecture
Oral Session

KA-1

Food-borne intoxication

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Food poisoning is a common, yet distressing and sometimes life-threatening problem for millions of people throughout the world. People infected with food-borne pathogens or intoxicated with their performed toxins may be symptom-free or may have symptoms ranging from mild intestinal discomfort to severe dehydration and bloody diarrhea. Depending on the type of infection, people can even die as a result of food poisoning.

Food borne-intoxication includes mainly two types of food poisoning botulism and Staph. aureus intoxication.

The bacterium *Clostridium botulinum* is responsible for causing the rare but serious illness botulism. Foodborne botulism is caused by eating foods that contain the botulism toxin. Botulism can be deadly and are considered medical emergencies. Symptoms of botulism include blurred vision, double vision, droopy eyelids, slowed or slurred speech, difficulty swallowing, dry mouth and muscle weakness. If these symptoms are untreated, they may lead to paralysis of the arms, legs, trunk, and respiratory muscles. Symptoms of foodborne botulism usually develop 18 to 36 hours after consuming contaminated food, but symptoms can occur as early as six hours or as late as a week to 10 days.

Staph food poisoning is a type of food poisoning caused by infection with the *Staphylococcus aureus* (*S. aureus*) bacterium. The bacteria multiply in foods and produce toxins especially if food is kept at room temperature. The toxins may be present in dangerous amounts in foods that have no signs of spoilage, such as a bad smell. Symptoms of staph food poisoning include nausea, vomiting, retching, stomach cramping, and diarrhea. In more severe cases, dehydration, headache, muscle cramping, and changes in blood pressure and pulse rate may occur. Symptoms typically come on quickly. The condition is typically over in 2 days. But it is not unusual for complete recovery to take 3 days and sometimes longer in severe cases. In this lecture we will give a full account on food-borne intoxication and other cases of food-borne infection.

O-1

Toxic metals screening in concentrated milks and assessment of transfer from packaging materials

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180 different local and exported concentrated milk samples (60 each of sweetened condensed milk, evaporated milk and dried milk) were collected from different markets in Mansoura city, Dakahlia Governorate, Egypt and classified into three groups: each group include 20 cans of each product. All samples stored at room temperature (17.5-31.5◦ C) for 210 days (from January to July 2015). All groups were analyzed by Atomic Absorption Spectrophotometer (the first group at zero day, the second at 60 days and the third at 210 days) to determine the level of toxic heavy metals (Pb, Cd, Al and Sn) for the estimation of the level of contamination as well as the ability of transfer from packaging materials. The statistical analysis of the data indicated that 100% of the examined samples contained Pb, Cd, Al and Sn. When comparing the obtained results, there were significant change ($p<0.05$) in the level of Pb, Cd, Al and Sn in all examined concentrated milk samples from 0, 60 and 210 days. The public health hazards of these metals were discussed.

Keywords: Lead, cadmium, aluminium, tin, storage, concentrated milks.

1. Introduction

Milk and its products may contain varying amounts of different toxic contaminants (Ataro et al., 2008). The levels of toxic metals are an important component of safety and quality of milk and dairy products. Metals are widely released in the environment, and have two major origins: human activities and geological background (Loska et al., 2004), where they are present in soil through fertilizers or following atmospheric deposition and from natural weathering of rocks.

The risk associated with the exposure to toxic metals in food products had aroused widespread concern in human health. Dizziness, nausea, vomiting, diarrhea, sleeping disorders, loss of appetite and reduced conception rate are the symptoms of heavy metal toxicity. Also, it connected to Alzeihemer's, Parkinson's, autism, lupus, amyotrophic lateral sclerosis, cardiovascular disease, depressed growth, impaired fertility, nervous and immune system disorders, increased spontaneous abortions, and elevated death rate among infants (Jack, 2005).

So, the present study was undertaken to determine the level of contamination of concentrated milks with some toxic heavy metals (Pb, Cd, Al and Sn) as well as to assessment their migration during 210 days of storage.

2. Materials and Methods

2.1 **Collection of samples**: This were done following Ortega and Garcia (1992).

2.2 **Preparation of samples**: according to AOAS (2000).

2.3 **Analysis of the prepared samples**: Following the method described by Tsoumbaris and Papadop (1994) and Dabeka and Mckenzie (1992).

2.4 **Statistical analysis**: All the data analyzed using SPSS/PCT (Foster, 2001). One way ANOVA were performed to evaluate differences.

3. Results and Discussion

The statistical analysis indicated that 100% of the examined concentrated milk samples contained lead, cadmium, aluminium and tin and there were considerable changes in the levels of all elements but the change occurred may be significant or non-significant.

The analyzed data revealed that the mean values of Pb, Cd, Al and Sn in sweetened condensed milk samples during storage period (0, 60 and 210 days) were (0.55, 0.115, 0.60, 1.40); (0.66, 0.25, 1.65, 1.40) and (0.77, 0.24, 2.30, 1.52) ppm, respectively.

While, in case of evaporated milk samples were (0.41, 0.210, 1.35, 1.55); (0.56, 0.28, 1.35, 1.76) and (0.81, 0.28, 1.85, 1.66) ppm, respectively and in dried milk samples were (0.48, 0.225, 1.15, 1.58); (0.57, 0.33, 1.65, 1.50) and (0.80, 0.34, 2.50, 1.76) ppm, respectively.

From the previous results, there were different significant changes in the level of examined toxic metals between storage periods. It seem that storage has its effect on migration of toxic metals between packaging materials and included food matrices.

Table (1): Heavy metals concentration in examined concentrated milks samples during 210 days storage period (N=20)

Concentrated Milks	Zero day				60 days				210 days			
	Cd	Sn	Al	Pb	Cd	Sn	Al	Pb	Cd	Sn	Al	Pb
Sweetened	0.115^{b}	1.40^{b}	0.60^{b}	0.55^{a}	0.25^{c}	1.40^{c}	1.65^{a}	0.66^{a}	0.24^{c}	1.52^{c}	2.30^{b}	0.77^{a}
Evaporated	0.210^{a}	1.55^{a}	1.35^{a}	0.41^{c}	0.28^{b}	1.76^{a}	1.35^{b}	0.56^{b}	0.28^{b}	1.66^{b}	1.85^{c}	0.81^{a}
Dried	0.225^{a}	1.58^{a}	1.15^{a}	0.48^{b}	0.33^{a}	1.50^{b}	1.65^{a}	0.57^{b}	0.34^{a}	1.76^{a}	2.50^{a}	0.80^{a}

*Means with different superscript (a, b and c) in each raw are significantly differed at level ($P<0.05$).

It is evident that the toxic metal contents of concentrated milks are variable because of factors such as characteristics of the manufacturing procedures and possible contamination from the equipment during the process, packaging, and storage. So, it is necessary to control the manufacturing process at each step, in order to determine the source and levels of contamination and to ensure the desired product quality (Ayar et al., 2009).

The obtained results can be attributed to the fact that the migration of metals from the surface of the can to the products is caused not only by the length of storage, but also by the consistency of milk product, in this products. Also, may be due to the nature of tinned can manufacture (which consists of 98.8% of Tin, small amounts of lead beside soldering of seams with lead-containing paste, copper, cadmium, aluminium and some other metals) capable of participating in electrochemical transformation (Arvanitoyannis and Bosnea, 2004).

From this conducted study we concluded that the chemical composition of concentrated milk depends on the mode of packing and quality of the packing materials and on the conditions and time of storage.

Also, it is preferable that consumption of milk products should be as early as possible from the date of manufacture to reduce the migration of heavy metals from the containers to the products during storage period.

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O-2

Camel milk metal's status, a mini survey on Libyan camel milk

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Interest in camel milk consumption or use in manufacturing of dairy products has recently increased. Although, a little information is known about camel milk metal (essential or toxic) status. Therefore, this study was conducted to determine some essential and toxic metals in Libyan's camel milk. Samples were freshly collected from some regions near Misurata, Libya. Analysis was done using microwave plasma atomic emission spectroscopy. Obtained results revealed a mean level of 0.03, 0.04, 0.01, 0.23, 0.19 and 0.06 for Lead, Cadmium, Chromium, Iron, Zinc and Copper, respectively. None of the toxic metals has exceeded the international recommended permissible limit. Moreover, camel milk was found to be a good source of iron, especially for children and iron-deficiency anemia patients.

Keywords: Camel Milk, Toxic metals, Essential metals, Food chemical safety.

1. Introduction

Recently there is an increasing interest in camel milk as a potential traditional therapeutic and a wholesome nutritional element. Several studies proved many health associated benefits of camel milk, like its hypoglycaemic effect (Agrawal et al., 2007; antihypertensive effect (Tagliazucchi et al., 2016); anticarcinogenic effect (El Miniawy et al., 2014); antiviral (Redwan & Tabll, 2007) and many more. Camel milk characterized by excellent nutritional values over milks of other dairy animal, for instance, camel milk contains high amounts of lactoferrin, lysozymes, vitamin C, immunoglobulins and α-lactalbumin (Kappeler et al., 2004), with a very minute amount of short chain- and higher amount of long chain-fatty acids in comparison to cow and goat milk.

Unfortunately, as far as or knowledge, there is very little information about the metal content of camel milk. In regards to human food, metals have three main types; essential (copper, zinc, iron, Calcium, chromium, etc) and toxic (lead, arsenic and cadmium). Although essential metals are required to ensure normal body mechanisms, they may turned toxic if they exceeded certain limits. Metals play vital roles in many body metabolic (anabolic and catabolic) reactions. In contrary, accumulation of metals in body leads to sever adverse detrimental effects. Carcinogenicity, mutagenicity, and teratogenicity are associated with heavy metal accumulation, in addition to kidney, cardiovascular and nervous disorders.

The main aim of this study is to explore metal contents in Libyan camel milk and to determine its concordance with standard permissible limits.

2. Materials and Methods

2.1 Camel milk samples

Ten raw camel milk samples from two regions (figure 1); Alkhorjah and Saso valleys' regions, near Misurata, Libya were collected from hand milked camels (*Camelus dromedarius*). Samples collected during spring season of 2014. Samples were kept cooled until analysis.

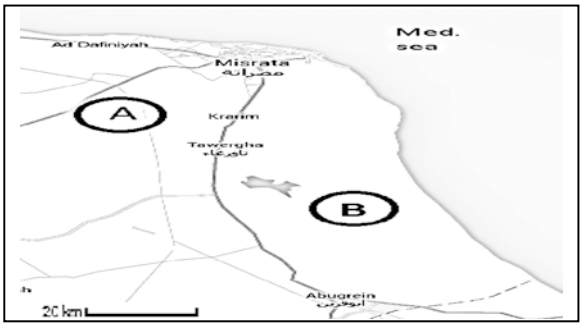

Figure 1: Sampling regions. A; Saso valley and B; Alkhorjah valley.

2.2 Metals analysis

1 mL of each sample was initially oven-dried at 100°C and then transferred into Pyrex glass digestion tubes contained a mixture of 3 mL of concentrated nitric acid and 1 mL of Hydrogen peroxide was added. Heated digestion was completed and colourless solution was collected. To which, 2 mL of concentrated nitric acid was added and then diluted to 10 mL with deionised water to be used for analysis. Blank and 5 standard solutions (0.05, 0.1, 0.3, 0.5 and 0.7 ppm) were regularly used to calibrate the instrument. Microwave plasma atomic emission spectrometer (Agilent Technologies, Japan) was used. Three replicates were used and the average were calculated.

2.3 Permissible limits compliance

Permissible metals' levels in milk were compared against European commission regulation standards (EC, 2001; 2006).

3. Results and Discussion

Mean of metals concentrations and their ranges in analyzed camel milk samples are reported in table 1.

Table 1: Statistical analytical results of analyzed metals.

Metal	Mean ± SD (range) ppm	No. of positive samples (≥LOD)
Lead	0.03 ± 0.01 (0.02-0.07)	10
Cadmium	0.04 ± 0.01 (0.03-0.04)	4
Chromium	0.01 ± 0.0 (0.01-0.01)	10
Iron	0.23 ± 0.13 (0.11-0.52)	10
Zinc	0.19 ± 0.07 (0.06-0.29)	10
Copper	0.06 ± 0.01 (0.03-0.09)	10

Among these stated results, a notable iron level draws attention. In contrary to cow milk which is very deficient in iron, camel milk is rich in this element and this may explain the high percent of lactoferrin it has. Zinc and copper levels were seemed to be in their regular values. Metals reach animal mainly through both water and feed. Thus, camel feed would certainly have a great impact on milk metals containment. In Libya, most camels' owners depend predominantly on rural grazing rather than in-house feeding. Thus, levels of reported metals certainly reflect environmental and natural levels in soil.

Regarding toxic metals, Cadmium was found to have the highest mean (0.04 ppm) followed by lead (0.03). Only 4 samples were found to have cadmium levels over the LOD, while all analysed samples have lead residues over the LOD. In comparison to standard value, European Commission regulations declared a maximum permissible level of 0.2 ppm in raw milk for lead, 0.05 ppm for cadmium, while no such level was clearly determined for chromium. Obtained results revealed that neither of the analysed samples have exceeded these permissible limits for lead and cadmium. This definitely reflects the superior environmental condition at these regions where camels are reared. In addition, it was also clear that there is no significance difference between results of samples of the two selected regions.

Unfortunately, only one literature could be found regarding metals status in camel milk in Kazakhstan (Konuspayeva et al., 2011). Our result agreed with their results in regards to lead, cadmium and copper. However, they reported higher zinc levels. This might be attributed to types of feed (natural or artificial).

Based on obtained results of this first exploratory study of metals concentrations in Libyan camel milk, it can be concluded that all analysed samples had complied with international standards regarding toxic metals. In addition, camel milk seemed to be an admirable nutritious source of iron and may be very helpful for toddlers, children and iron-deficiency anaemia patients. Further nationwide studies should be followed to assess overall camel milk qualities.

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O-3

Chemical quality of water supplies to poultry farms in Egypt

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The water quality in poultry farms may affect broilers and layers production performance which indicated by decreased food conversion ratios, depressed egg production and low egg quality. The aim of this study is to determine the chemical quality (Ammonia, Nitrate, Nitrite, Chloride, Sulphates, total hardness, lead and cadmium residues) of water supplies introduced to different broiler and layer farms in Sharkia governorate, Egypt. The recorded results indicated that the water of drinkers in poultry farms had significantly higher contents of ammonia, nitrates, nitrites, total hardness, sulphates, lead and cadmium. The obtained concentrations were compared with the maximum permissible limits established by WHO for the guidelines of drinking water. The water in the drinkers of broiler farms had higher concentrations of the examined chemical parameters compared with the water in layer farms. The recorded results in this study indicate unsatisfactory chemical water quality parameters in the poultry farms in Egypt, which should be improved to keep high productivity of broilers and layers.

Keywords: Water quality, Chemicals, Poultry farms

1. Introduction

Poultry farms may use water from municipal sources (potable for humans), wells, ponds and springs. Because of its nature of potential hydrogen bonding, water is an excellent solvent for both organic and inorganic substances and for this reason; water is an ideal medium for the proliferation and distribution of harmful components such as chemical elements and microorganisms. The water quality may affect layer production performance which indicated by depressed egg production and egg quality. Moreover, inferior or poor water quality may depress water consumption (Koelkebeek et al., 1999). On the other hand, in broiler farms, water quality has determined effects on broiler performance, which is negatively correlated with body weight as well as immune resistance (Grizzle et al., 1997).

Heavy metals may be found primarily in ground water or leached from geologic formations; while in surface water may be run-off from land application, dumped from domestic and industrial sewage, atmospheric deposition of mining practices, smelter operations, improper handling of mining tailings, or as a result of corrosion of distribution system materials (Calderon, 2000). In broilers and layers, there was a linear correlation between increasing lead concentrations in drinking water and some detrimental effects such as decrease in egg production, weight gain, and increase of embryonic mortality (Vadela et al., 1997).

Thus, the aim of this study is to determine the chemical quality (Ammonia, Nitrate, Nitrite, Chloride, Sulphates, Total hardness, lead and cadmium residues) of water supplies introduced to different broiler farms in Sharkia governorate, Egypt.

2. Materials and Methods

Collection of samples

A total of 100 water samples were collected from 24 broiler farms and 27 layer farms located at different districts at Sharkia Province during the period of August 2008 to April 2009. These samples were classified as 49 samples main water supplies, and 51 water samples from bell-shaped drinkers.

pH measurement:

The pH value was determined by using digital pH meter model Accumet 395, USA.

Ammonia measurement:

Ammonia was determined by using the direct Nesslerization method recommended by APHA, 1998.

Nitrites measurement:

Nitrites content were measured in the examined water samples using the Diazotization method recommended by APHA, 1998.

Nitrates measurement:

Nitrates in the examined water samples were measured using the Brucine method according to APHA, 1998.

Chlorides measurement:

Chlorides in water samples were measured using Argentometric method recommended by APHA, 1998.

Sulphates measurement:

Sulphates in water samples were measured using the gravimetric method with ignition of residues recommended by APHA, 1998.

Total hardness:

Total hardness in water samples was measured using EDTA trimetric method recommended by APHA, 1998.

Heavy metals measurements:

Lead and cadmium were quantitatively measured using the atomic absorption spectrophotometer.

Statistical analysis:

Statistical analysis was done using Tukey's Kramer HSD test.

3. Results and Discussion

The chemical parameters for water samples collected from main water supplies and water of drinkers in both broiler and layer farms were examined. The obtained results were recorded in tables 1, 2 and 3. It is clear from the obtained results that only 11.8 % of the water of drinkers had pH values higher than the recommended levels.

Concerning ammonia, ammonia concentrations in water of drinkers were significantly higher than the main water supply, additionally; broiler farms had higher levels compared with drinkers of layer farms.

Water of drinkers had significantly higher concentrations of nitrate and nitrites compared with the main water supply. The recorded concentrations were higher than the MPL. Likely, total sulphates and total hardness were significantly higher in water of drinkers compared with the main water supply, despite no significant difference between broiler and layer farms.

The mean values of Cd were higher in water of drinkers compared with the main water supply, especially in broiler farms.

The concentrations of Pb were higher than MPL in 98 and 96.1% of the examined samples of main water supply and water of drinkers, respectively.

The chemical parameters of the examined water samples in this study indicate unsatisfactory quality parameters, which have significant effects on the poultry performances.

Table 1: Chemical contents of water samples collected from main water supplies and drinkers in all examined poultry farms

	Main water supply (n=49)	Water of drinkers (n=51)
Ammonia	0.14 ± 0.02	0.65 ± 0.07*
Nitrite	0.03 ± 0.005	0.12 ± 0.01*
Nitrate	5.09 ± 0.52	24.50 ± 2.14*
Chloride	113.35 ± 8.22	193.1 ± 10.22
Sulphates	98.1 ± 14.36	200.58 ± 31.88*
Total hardness	112.86 ± 6.27	420.11 ± 31.39*
Lead	0.27 ± 0.037	0.34 ± 0.04
Cadmium	0.0004 ± 0.0001	0.005 ± 0.004*
pH	7.32 ± 0.04	7.16 ± 0.14

Means carrying star mark in the same raw are significantly different at p<0.05.

Table 2: Percentage (%) of samples exceeding maximum permissible limits among main water supply and water of drinkers

	MPL (mg/L)	Main water supply (n=49)	Water of drinkers (n=51)
Ammonia	0.2	16.3	72.5
Nitrite	0.2	0	17.6
Nitrate	25	0	47
Chloride	250	0	17.6
Sulphates	250	14.3	31.4
Total hardness	180	4	100
Lead	0.02	98	96.1
Cadmium	0.01	0	2
pH	6-8	0	11.8

Values were compared with MPL recommended by WHO, 2006.

Table 3: Chemical contents of water samples collected from drinkers in relation to type of production

	Broiler farms	Layer farms
Ammonia	0.85 ± 0.11*	0.46 ± 0.08
Nitrite	0.125 ± 0.02	0.109 ± 0.02
Nitrate	25.98 ± 3.02	23.18 ± 3.05
Chloride	204.12 ± 15.68	183.31 ± 13.36
Sulphates	204.24 ± 52.14	197.33 ± 39.41
Total hardness	444.81 ± 49.53	398.15 ± 40.14
Lead	0.425 ± 0.07*	0.26 ± 0.04
Cadmium	0.009 ± 0.008*	0.001 ± 0.0002
pH	7.23 ± 0.22	7.09 ± 0.18

Means carrying star mark in the same raw are significantly different at p<0.05.

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SL-1

Kabwe childhood lead poisoning: past, present and future

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Childhood lead (Pb) poisoning is a serious public health concern worldwide. Young children are particularly vulnerable to Pb exposure and eventual poisoning. Blood lead levels (BLLs) > 10 µg/dL in children are considered elevated and at higher BLLs > 60 µg/dl, clinical symptoms of Pb toxicity become visible. BLLs exceeding 100 µg/dL can cause encephalopathy, convulsions, coma and death. It has been recommended that intensive medical management and chelation therapy be initiated at levels ≥ 45 µg/dL. In Africa, major sources of childhood Pb poisoning include Pb mining and smelting. In Kabwe, the capital of Zambia's Central Province, extensive Pb contamination of township soils in the vicinity of a Pb-Zn mine has been reported and poses a serious health risk to children in these townships.

Keywords: Childhood, Lead, Poisoning, Kabwe, Zambia

Introduction

Childhood lead (Pb) poisoning is a serious public health concern worldwide. Exposure to Pb affects multiple organ systems resulting in numerous morphological, biochemical and physiological changes that include hematological disorders, nervous system disturbances and impairment of liver and kidney functions (Needleman 2004). Children are more vulnerable to Pb poisoning compared with adults as the central nervous system is most sensitive to Pb toxicity during developmental stages.

In the last decade, BLLs in children have reduced significantly in a number of developed countries following the phasing out of leaded gasoline. However, childhood Pb toxicity continues to be a major public health problem in most developing countries. In Africa, major sources of childhood Pb poisoning include Pb mining and smelting, paint and battery recycling (Yabe et al., 2010). The recent Pb poisoning disaster in Nigeria, where more than 400 children died, was attributed to gold ore–mining and processing, especially that metals were processed in their dwellings

Kabwe Mining Town - Zambia

Study site

Kabwe town, the provincial capital of Zambia's Central Province, is located at about 28°26′E and 14°27′S. Kabwe has a long history of Pb-Zn mining. The mine operated almost continuously from 1902 to 1994 without addressing the potential risks of metal pollution. Dense fumes rich in Pb and other metals were emitted from smelters and they polluted the environment in the surrounding communities extensively. Despite closure of the mine, scavenging of metal scraps from the abandoned tailings and wastes stored on the mine has continued to serve as a source of metal pollution, especially dusts emanating from the mine dumps.

Fig. 1: Scavenging for scrap metals

Past activities by our research team

Soil: Median concentrations of Pb (mg/kg) in soils from Kasanda (3,008), Makandanyama (1,613), Chowa (1,233), Mutwe Wansofu (1,148), Makululu (870) and Luangwa (507) townships exceeded the 150 mg/kg maximum limit recommended by FAO/ISRIC (2004).

Cattle: The maximum metal concentration, expressed in mg/kg and dry weight, in the liver was 1.8. Concentrations of Pb in Kabwe cattle were higher than levels in other Zambian towns. The study highlighted the dangers of offal consumption of cattle from Kabwe.

Chickens: Concentrations of Pb in tissues of 17 free-range and 32 commercial broiler chickens were determined. Mean concentrations of Pb exceeded maximum levels for human consumption in edible organs of village chickens.

Humans: Blood Pb (B-Pb, µg/dL) levels were determined in children aged 0-7 years. Mean blood

Pb levels in children from Chowa (39.0), Kasanda (82.2) and Makululu (57.1) townships exceeded the WHO guideline level of 10 µg/dL for public health protection.

- Reference values in blood
  - 10 µg/dL – level of concern
  - 45 µg/dL – initiate treatment e.g. chelating therapy
  - 65 µg/dL – toxicity levels and clinical signs
  - ≥ 150 µg/dL – encephalopathy, death

Current and future activities - SATREPS Research project

Title: Visualization of impact of chronic / latent chemical hazard and Geo-Ecological Remediation in Zambia
Project duration – (2016-2020)

Given our earlier investigation in human (Yabe et al., 2015), we plan to expand our survey in the current project. Since there is a need to investigate a broader area of Kabwe, we intend to collect samples from many townships in addition to three townships (Kasanda, Makululu, Chowa).

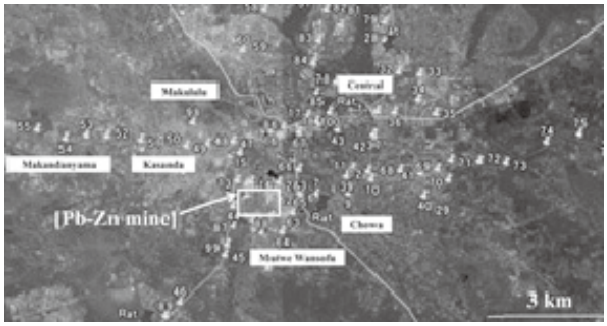

Fig. 2. Sampling sites around the mine

In addition to blood, urine and fecal samples, breast milk and hair samples also will be collected. Stable Pb isotope ratios (Pb-IRs) will also be analyzed to identify and distinguish the possible Pb pollution sources. Biomarkers will be analysed in order to determine the effect of metal exposure on human health. Furthermore, intelligence quotient (IQ) of children will be determined to evaluate the effects of Pb exposure.

The new project will create and develop an education and research base for chemical hazards from an international perspective. Project output will include the following:

1) Monitoring and risk assessment in Kabwe mining area (and geo-ecological surveillance of broad areas of Zambia); 2) Establishment of an economic assessment system for human / animal health and ecosystem and visualization; 3) Development of geo-ecological database and remediation technology; lastly, 4) Capacity building.

Remediation technology

Based on "visualized" data and health risk visualization, new remediation methods will be established.

1. Chemo-remediation: Several soil conditioners will be tested to investigate their influences on the mobility of Pb in soils.
2. Bio-remediation: Soil microbes that can immobilize Pb will be investigated.
3. Phyto-remediation: Mechanisms of Pb uptake by plants will be studied to select the best crops that should be introduced in contaminated sites.

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O-4

Lead exposure on human samples in African countries: a mini review

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Understanding lead exposure on human in the world is an important issue. To understand the recent situation of lead exposure on human in African countries, the information of human samples was collected and reviewed. Articles published after 2009 which estimated lead levels of human samples in African countries were collected mainly from Pubmed using advanced search. Papers studied about lead exposure on human samples in 16 African countries were found. Samples were mainly whole blood samples and some study analysed umbilical cord blood, serum, semen, urine, hair, nail, bone and maternal milk samples. All samples were analysed by atomic absorption spectrometry, inductivity couples plasma mass spectrometry or LeadCare® series. Most studies estimated only lead levels and not mentioned well about adverse effects by lead exposure. In the future, the other remaining African countries are necessary to be studied about lead exposure on human, and the background and the effects should be understood well.

Keywords: Lead exposure, Human, Africa, Review

1. Introduction

Lead is one of the earliest metals used by human and still used in manufacturing industries nowadays. Lead toxicity is also known from Ancient Roman era and cause neurological symptoms and death in the worst case. In 2003, a new observation was revealed that blood lead levels (BLLs), even those below 10 μg per decilitre, are inversely associated with children's IQ scores by Canfield et al. and a reference level of blood lead concentrations as reduced by 5 μg/dL decilitre. The link between lead exposure and violence crime is also well known. Children should be protected from lead exposure for their health and the future.

In developing countries, lead mining, smelting and recycling battery under not well managed situation are the most important problems of lead exposure.

In Africa, two recent large incidents of lead poisoning in Dakar, Senegal (Haefliger et al., 2009) and Zamfara, Nigeria (Dooyema et al., 2012) made an impact to the world. Those incidents might be a tip of iceberg. Kabwe, Zambia where is a mining area is listed as one of the World's Worst Polluted Places by Pure Earth. Moreover, traditional cosmetics and medicines in some African countries have been revealed that it contains high heavy metals levels and important source of lead exposure. Lead levels of environmental samples have been studied and the situation of environmental lead exposure is being revealed gradually. However, the information about lead exposure on human in African countries is limited. Understanding lead exposure on human in whole African countries is an important issue.

To understand the recent situation of lead exposure on human in African countries, the information of human samples was collected and reviewed.

2. Materials and Methods

Articles published after 2009 which estimated lead levels of human samples in African countries were collected mainly from Pubmed using advanced search. Some papers were removed because of not sufficient quality.

3. Results and Discussion

Papers studied about lead exposure on human samples in 16 African countries were found. Samples were mainly whole blood samples and some study analysed umbilical cord blood, semen, urine, hair, nail, bone and maternal milk samples.

All samples were analysed by atomic absorption spectrometry, inductivity couples plasma mass spectrometry or LeadCare® series.

Average lead levels (LLs) of human samples in African countries were summarised below.

BLLs of children in village A (n = 44) and village B (n = 42) Zamfara, Nigeria, 2010 were 153.3 μg/dL and 107.5 μg/dL respectively (Dooyema et al., 2012).

BLLs and semen LLs of men with primary infertility (n = 29) in Egypt, 2010 were 20.08 μg/dL and 11.40 μg/dL respectively (Awadalla et al., 2011).

Umbilical cord blood LLs of newborns (n = 150) in Tanzania were 4.1μg/dL (Azayo et al., 2009).

Urine LLs of mining community men (n = 57) in Ghana, 2009 were 1.34 μg/L (Basu et al., 2011).

Hair and finger nail LLs of children in Kisumu (n = 10), Kendu Bay (n = 13), Karungu (n = 13) and Port Victoria (n = 13), Kenya, were 12.4 μg/g dw, 6.9 μg/g dw, 5.4 μg/g dw and 2.9 μg/g dw, 34.4 μg/g dw and 20.6 μg/g dw, 9.6 μg/g dw and 4.6 μg/g dw, respectively (Oyoo-Okoth et al., 2010 and personal communication).

Cortical femora bone LLs of black (n =72) and white (n =29) male who died between 1943 and 2012 in South Africa were 3.92 μg/g and 10.04μg/g, respectively (Hess et al., 2013).

Maternal milk LLs of mothers drinking surface water (n = 30) and groundwater (n = 22) in Egypt were 2.5 μg/dL and 3.5 μg/dL, respectively (Mandour et al., 2013).

Primary teeth LLs of children in Addis Ababa (n =46), village A (n = 49) and K (n = 51) Ethiopia between 1998 and 2000 were 1.27 μg/g, 0.59 μg/g and 0.39 μg/g, respectively (Tvinnereim et al., 2011).

Most studies estimated only lead levels and not mentioned well about adverse effects by lead exposure. Some study showed high lead levels, however the source of lead exposures have not been identified clearly. It is difficult to say that the results of these studies show whether normal or topical levels in these countries, because of the limited information. In the future, the other remaining African countries are necessary to be studied about lead exposure on human, and the background and the effects should be understood well.

4. Acknowledgments

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O-5

Estimated health risks associated with consumption toxic metals in fish raised from sewage-fed aquaculture

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We determined the health risk hazard due to consumption of toxic metals in fish raised from a sewage fed aquaculture. Toxic metals were analyzed in fish, sediment and water samples from sewage fed aquaculture with atomic absorption spectrophotometer. All the fish samples had some amount of toxic metals in them. The maximum concentrations of Pb, Cd, Cu, Cr and Hg in the water samples were 28.7, 18.2, 246.0, 310.0 and 150.0 µg/kg respectively which were all below the US EPA limits. The maximum concentration of Pb, Cu, Cr and Hg in all sediment samples are 27.4 µg/kg, 323.0 µg/kg, 240.0 µg/kg and 150.0 µg/kg respectively. The maximum concentration of Pb, Cd, Cu, Cr and Hg in the fish samples were 48.6, 18.9, 434.0, 300 and 320 µg/kg respectively which were below the EU maximum residual limit. The estimated daily intake calculated ranged from 1.0x10-6 to 6.0x10-5 mg/kg/day. Mercury showed the highest health risk index (HRI), whiles Pb had the lowest. There was no HRI >1, indicating that humans being who consume fish from the pond would not be exposed to health hazards due to toxic metals in the fish.

Keywords: heavy metals, wastewater, sewage, sediment, health risk index

1. Introduction

Agricultural reuse of untreated, partially treated and diluted wastewater is a common reality in and around three out of four cities in low-income countries (Drechsel et al. 2008). The economic benefits derived from reuse of wastewater include its reliable flow, nutrient recovery, contributions to urban food supply and safeguarding of livelihoods (Drechsel et al. 2010). However, health risks to farmers and consumers, such as worm infections, diarrhoea and disorders of the nervous system due to toxic metals are major possible drawbacks, limiting formal recognition and support of wastewater reuse. In several instances, raw wastewater is used in irrigation and for fish farming.

The use of waste stabilization pond for commercial fish farming has been adopted in Ghana to justify the occupancy of the ponds on scarce urban land. In Kumasi, the second largest city in Ghana, all sewage treatment ponds are waste stabilization ponds. They include three community-scale waste stabilization ponds (in Asafo, Ahensan and Chirapatre suburbs) and a municipal-scale faecal sludge stabilization pond system (in Dompoase). The maturation ponds of the two community-scale waste stabilization ponds at Ahensan and Chirapatre have been converted to sewage-fed aquaculture. The treated wastewater in these maturation ponds would inevitably contain contaminants such as toxic metals. If the treated wastewater is used for aquaculture, then it is obviously necessary to monitor the concentrations of toxic compounds accumulated in the fish produced by the aquaculture. This is crucial to avert possible health effect to consumers.

The objectives of this study were therefore: to determine the concentrations of toxic metals in muscle of Clarias gariepinus (African Sharptooth catfish); to estimate the daily intake and target hazard quotient of the toxic metals through consumption of this fish in Kumasi and; to determine the concentrations of toxic metals in sediment and water of all the waste stabilization ponds.

2. Materials and Methods

2.1 Site Description

The two community-satellite waste stabilization ponds studied are located in Kumasi, Ghana, a metropolis with a population of about 2 million inhabitants. The waste stabilization ponds (WSP) located at Ahensan in Kumasi was designed to handle sewage from the suburb. Currently about 200 houses, with an estimated population of 1500 have been connected to the facility (Murray and Yeboah-Agyepong 2012). The Ahensan waste stabilization ponds comprises of an anaerobic pond, one facultative pond and two maturation ponds. The maturation ponds are used for aquaculture. The effluents from the WSP, are discharged into the Wiwi River through a nearby stream which is used for vegetable irrigation downstream. Chirapatre waste stabilization pond comprises of an anaerobic pond, two facultative pond and two maturation ponds. Main pollution sources are soaps and detergents, oil and greases, human excreta and solid wastes that are washed down the drains. Chirapatre waste stabilization ponds are situated at Chirapatre Township in Kumasi with the sewer lines connected to 300 household with an estimated population of 1800 people. The maturation ponds

are used for aquaculture.

2.2 Sampling and Sample Preparation

In January 2014, 61 samples of fish, water and sediment were collected in one sampling campaign from Chiraprate WSP and Ahensan WSP in Kumasi, Ghana. Permission was obtained from the Kumasi Metropolitan Assembly - Waste Management Division to catch fish from the WSP Composite water samples (200 mL each, pooling 5 aliquots collected from the same site with 1-h intervals) were collected from influents of each WSP pond. The effluent of the ponds was also sampled.

The fish samples were caught from the maturation pond of each waste stabilization pond using dragnet. The samples were then kept in an iced chest containing iced blocks and transported to the laboratory where they were kept in a deep freezer at -4°C prior to analysis. The frozen fish sample was thawed and portions of muscle tissue cut out, homogenized prior to acid digestion.

2.3 Digestion of samples

A portion (0.5 g) of homogenized fish or sediment samples was placed in 50 mL digestion tube and 1 mL H_2O, 2 mL HCl, 5 mL of 1:1 HNO_3:$HClO_4$ were added. Samples were then heated in a digestion block on a hot plate at 200 ° C until a clear solution was obtained. The digests were left to cool, filtered into 50 mL volumetric flask and diluted to 50 mL mark with distilled water. Aliquot of 1 L of water sample was digested and concentrated with 5 mL concentrated nitric acid. The water was boiled until the digest volume about 25 mL solution was obtained. The digest was left to cool. It was then filtered into 50 mL volumetric flask and diluted to 50 mL mark with distilled water.

2.4 Heavy metals determination

Concentrations of Pb, Cd, Cr and Cu were determined using Perkin Elmer Spectra AA 220 flame atomic absorption spectrophotometer (Perkin Elmer Ltd, USA). An automatic mercury analyzer model HG-5000 (Sanso Seisakusho Co., Ltd, Japan), operating at a wavelength of 253.7 nm was used to determine Hg concentration in samples (Voegborlo and Akagi 2007).

3. Results and Discussion

Recoveries of the spiked elements ranged from 95-99%. The detection limits ranged from 0.001 to 0.006 µg/kg. The regression coefficients ranged from 97 to 99%. The precision expressed as percent coefficient of variation ranged from 3 to 5%.

3.1 Concentrations of toxic metal in water

The concentration of Pb, Cd, Cu, Cr, and Hg in water from Ahensan ranged from 21.3 – 26.1, 14.6 – 15.6, 232.0 – 246.0, 120.0 – 310.0, and 1.1 – 2.0 µg/L respectively. The concentration of Pb, Cd, Cu, Cr, and Hg in water from Chirapatre ranged from 26.9 – 28.7, 14.9 – 18.2, 216.0 – 222.0, 180.0 – 250.0, and 0.9 – 1.4 µg/L respectively. The maximum concentration of Pb, Cd, Cu, Cr, and Hg in the water samples are 28.7, 18.2, 246.0 310.0 and 2.0 µg/L respectively which were all below the US EPA thresholds for short term reuse of wastewater of 10000.0, 50.0, 5000.0 and 1000.0 µg/L respectively(US EPA 2012). However, Cd and Cr concentrations in all water samples analysed were above the US EPA thresholds for long term reuse of wastewater. Therefore, Cd and Cr could bio-concentrate in fish due to long term use of sewage-fed aquaculture in Kumasi, Ghana. The concentrations of toxic metals could be due to natural sources, surface run-off and wastewater from estate houses connected to the waste stabilization ponds.

The mean concentration of Cu, Pb and Cd were determined to be 10970.0, 93330.0 and 2230 respectively (Maiti and Banerjee 2012). These high concentration is because, the wetlands receive city sewage, storm water run-off and effluents from thousands of industries in India.

In the Ahensan waste stabilization ponds, Pb, Cd, Cu and Hg concentrations did not change significantly in the various ponds ($p > 0.05$). However, the concentration of Cr varied significantly in the various pond ($p<0.0001$). Mwakaboko and colleagues, also determined the mean concentration of Cr in influent of waste stabilization ponds at Mikocheni to be 3.7 µg/L and in effluent to be <1 µg/L (detection limit). The variation of Cr concentration in various ponds could be due to aquachemistry of chromium. Settling of precipitated chromium (III) hydroxide and other insoluble chromium compound could account for this variation (JØrgensen and Fath 2013). In the Chirapatre waste stabilization ponds, Pb, Cd Cu and Hg concentrations did not also change significantly in the various ponds ($p > 0.05$). However, the concentration of Cr also varied significantly ($p=003$) in the various ponds. The concentration of Cr found in the facultative pond was 180 µg/L, the level had increased in the first maturation pond (250 µg/L) and dropped in the second maturation pond (190 µg/L). The non-parametric t-test performed showed statistical differences in concentrations of Pb ($p=0.016$) and Cu ($p=0.020$) from Ahensan and Chirapatre WSP. However, Cd, Cr, and Hg concentrations in water were not statistically different in the 2 WSP studied.

3.2 Concentrations of toxic metal in sediment

The concentration of Pb, Cd, Cu, Cr, and Hg in sediment from Ahensan ranged from 26.2-27.1, 14.7–18.1, 299.0–323.0, 190.0–220.0, and 90.0–140.0 µg/kg respectively. The concentration of Pb, Cd, Cu, Cr, and Hg in sediment from Chirapatre ranged from 24.7–27.4, 12.5–14.2, 241.0–253.0, 190.0–240.0, and 100.0–150.0 µg/kg respectively. The maximum concentration of Pb, Cu, Cr and Hg in the sediment samples is 27.4, 323.0, 240.0 and

150.0 µg/kg respectively. These were below the pollution limits of 40000, 25000, 25000 and 1100 µg/kg respectively (US EPA 1997) and WHO maximum tolerable soil concentrations of 84000, 7000 and 4000 for Pb, Hg and Cd respectively (WHO 2006).

Mwakaboko and colleagues, determined the mean concentrations of Pb, Cu, and Cr in sediment of waste stabilization ponds at University of Dar es Salaam main campus to be 601500.0, 276000.0 and 740000.0 µg/kg respectively (Mwakaboko et al. 2014). Cu, Pb and Cd in sediment samples from waste stabilization ponds used in sewage-fed aquaculture, at East Kolkata Wetlands, West Bengal, India. The mean concentrations reported of Cu, Pb and Cd were determined to be 41000.0, 32000.0 and 3480 µg/kg respectively (Maiti and Banerjee 2012). These high concentrations were higher than the ones reported in this study. In the Ahensan sampling site, Pb, Cu and Hg concentrations in the various ponds increased slightly from pond to pond. The Cd concentration was slightly higher in the facultative pond than was in the anaerobic pond, the level decreased in the first maturation pond and remained the same in the second maturation pond. The Cr concentration (µg/kg) in the first maturation pond increased slightly from 200.0 to 220.0 before dropping to 190.0 in the second maturation pond. The concentration of Pb, Cu, and Cd concentrations in the sediment samples from Chirapatre did not change significantly ($p>0.05$). However, the concentration of Cr was higher than Hg in the anaerobic pond, and showed a drop in the second facultative pond.

3.3 Concentrations of toxic metal in fish

Fish is a good indicator of heavy metal contamination in aquatic systems because they occupy different trophic levels (Burger et al. 2002). Heavy metal intake by fish in a polluted aquatic environment vary depending on factors such as ecological requirements, metabolisms, salinity, and water pollution level. Fish accumulates metals directly from water and through the food it feeds on. The consumption of contaminated fish could causes acute and chronic effects to humans. All the fish samples had some amount of the metals in them. The concentration of Pb, Cd, Cu, Cr, and Hg in fish samples from Ahensan ranged from 36.7–48.6, 12.0–18.9, 291.0–434.0, 120.0–300.0, and 217.0–320.0 µg/kg respectively. The concentration of Pb, Cd, Cu, Cr, and Hg in fish samples from Chirapatre ranged from 23.5– 36.1, 10.8 – 18.4, 212.0 – 432.0, 10.0 – 180.0, and 203.0 – 278.0 µg/kg respectively. The maximum concentration of Pb, Cd, Cu, Cr and Hg in the fish samples were 48.6, 18.9, 434.0, 300.0 and 320.0 µg/kg respectively. These were below the EU maximum tolerable limits of 300.0, 50.0, 20000.0, 1000.0 and 1000.0 µg/kg respectively (EU 2006) The WHO provisional tolerable weekly intake for man are 50 and 8.3 µg/kg body-weight for Pb, and Cd (JECFA 1972) and are below the maximum Pb and Cd toxic metals measured in this study being 0.8, and 0.3 µg/kg body-weight respectively. Lead and Cd are classified among the most toxic heavy metals which have no known biochemical benefits to animals and humans but could pose serious systemic health problems in humans (Akoto et al. 2014). Cu, Pb and Cd in four fish species; Cirrhinus mrigala, Oreochromis nilitica, Catla catla and Labeo rohita reared in the sewage fed ponds at East Kolkata Wetlands, West Bengal, India. The maximum concentrations reported of Cu, Pb and Cd were 5580.0, 52160.0 and 3680.0 µg/kg respectively (Maiti and Banerjee 2012) were higher than the ones reported in this study. Lead, Cr, and Hg showed a strong positive correlation with length and weight of fish. These correlations were significant with p- values <0.05. On the contrary, Cd revealed a weak negative correlation with length and weight of fish, which was statistically significant ($p<0.05$).

3.4 Toxicity indices in fish

There was no HRI value >1, indicating that humans being who consume fish from the pond would not be exposed to health hazards due to toxic metals in the fish. Mercury showed the highest HRI value of 0.106, whiles Pb gave the lowest HRI (4 x 10-5). The estimated daily intake (EDI) calculated ranged from 1.0x10-6 to 6.0x10-5 mg/kg/day.

4. Conclusion

The present study investigated the occurrence of 5 toxic metals in fish raised in sewage-fed aquaculture along with the occurrence of these metals in sediment and water in the WSP where the fish are raised in Kumasi, Ghana. It has been shown that, all the fish we took had some amount of toxic metals in them but their quantities were lower than those that could harm the human body. This shows that those who consume the fish reared in the pond systems we investigated are not exposed to health hazards as far as the metals in the fish is concerned. In addition, the levels of the 5 toxic metals were higher in sediment than in water and fish. It is worrying that the Cd and Cr concentrations in all water samples were above the US EPA thresholds for long term reuse of wastewater. This indicate that there is an urgent need to identify diffuse sources of exposure and more appropriate use of conventional methods of sewage treatment.

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O-6

Risk Assessment of Heavy Metals via Consumption of Muscle and Offal of Free-Range Animals from Benin City

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The use of free range animals for monitoring environmental health offers opportunities to detect exposure and assess the toxicological effects of pollutants in terrestrial ecosystems. Potential human health risk of dietary intake of metals and metalloid via consumption of offal and muscle of free range chicken, cattle and goats by the urban population in Benin City was evaluated. Muscle, gizzard, liver and kidney samples were analyzed for Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Cd, and Pb concentrations using inductively coupled plasma mass spectrometer (ICP-MS) while Hg was determined using Hg analyzer. Mean concentrations of metals (mg/kg ww) varied significantly depending upon the tissues and animal species. Human health risk estimations for children and adults showed estimated daily intake (EDI) values of tissues below oral reference dose (RfD) threshold for non-essential metals Cd, As, Pb and Hg thus strongly indicating no possible health risk via consumption of animal based food. Calculated Hazard quotient (HQ) was less than 1 for all the metals anlysed for both adult and children. Hazard Index (HI) for additive effect of metals were higher in chicken liver and gizzard for children and chicken liver for adults. Principal component analysis (PCA) and correlation analysis showed a clear species difference in metal accumulation between chickens and the ruminants. This study provides valuable evidence of anthropogenic impacts necessary to initiate national and international policies for control of heavy metal and metalloid content in food items.

Keywords: Heavy metals, Metalloid, Offal, Muscles, Hazard Quotient (HQ), Hazard Index (HI), Principal component analysis (PCA)

1. Introduction

Human exposure to heavy metals through food, air, and water has increased exponentially in the last century, as a result of different anthropogenic activities such as industrialization, mining, wastewater irrigation, sludge application, used of agrochemicals, and vehicular emission (Cherfi et al. 2015; Li et al. 2014; Khan et al., 2015a). Thus, the heavy metal pollution has become of great concern because of food safety issues, potential health risks, and its detrimental effects on soil ecosystems (Li et al. 2014). Therefore, monitoring of heavy metal contents in foods including meat and meat products are becoming a desideratum. Indeed, meat and meat products in human diet are important components across the world both in terms of quantities consumed and nutritional value (Khan et al. 2015b). They are excellent sources of proteins, some essential fats, soluble vitamins and minerals. It also provide fat including saturated fatty acids (SFA), unsaturated fatty acids (USFA), cholesterol, triacylglycerol and phospholipids. In Nigeria heavy metals pollution has become a serious problem due to non-compliance and enforcement of existing environmental laws and regulations, inadequate monitoring capabilities, weak institutional structures and poor legal framework. As a result large scale metal poisoning leading to several deaths in some part of Nigeria due to artisanal/illegal gold mining activities have been recorded. Regular surveys and monitoring programs of heavy metal contents in animal based food have been carried out for decades in many countries (Bortey-Sam et al., 2015; Ihedioha and Okoye, 2013). However human health risk via consumption of meat and meat products from free range animals in Benin City, Edo State is still very scarce. This study was therefore carried out to determine the levels and distribution pattern of metals in organs of animal species and also assess the possible human health risk associated with consumption of edible parts of free-range cattle, goats and chicken in Benin City.

2. Materials and Methods

2.1 Study area

The study was conducted in Benin City, Edo State around markets, abattoirs and dumpsite areas. Benin City is located 6.3176°N, 5.6145°E and is the capital and largest City of Edo State in Southern Nigeria. The city is a commercial centre strategically positioned as the gateway to the northern, eastern and western states of Nigeria. Notable economic activities include breweries, wood carving, traditional brass and bronze casting, wood and timber processing, printing and publishing. Major markets within the city are Oba, New Benin, Oliha, Uselu, Agbado and Edaiken markets.

2.2 Sample Collection

Fresh samples of kidney, liver and muscle of free-range goats (*Capra hircus*) and cattle (*Bos Taurus*) were collected from the abattoirs while kidney, liver, gizzard and muscle of free-range chickens (*Gallus gallus domesticus*) were collected from live adult chickens after exsanguination and dissection in the laboratory. Samples were kept frozen in labelled plastic bags in the National Centre for Energy and Environment laboratory, University of Benin and was later transported to the Laboratory of Toxicology, Graduate School of Veterinary Medicine, Hokkaido University, Japan and stored in -3^{0}C until analysis.

2.3 Sample Preparation and Metal Extraction

Individual animal liver, kidney, gizzard and muscle samples of about 0.5g wet weight were digested in concentrated nitric acid 65% (Kanto Chemical Corp., Tokyo, Japan) and 1ml of (30%) hydrogen peroxide, H_2O_2 (Kanto Chemical Corp., Tokyo, Japan) spectrometry grade using a closed microwave digestion system (Speed wave MWS-2; Berghof, Germany) according to the method of Nakayama et al. (2011). Within each digestion series, appropriate blanks with ultra-pure water were also subjected to the same procedure to account for background contamination levels. After cooling, solutions were transferred to a standard volume with ultra-pure water.

2.4 Metal Analysis

Determination of metals was done using an Inductive Coupled Plasma-Mass Spectrometer (ICP-MS) model 7700 series, Agilent technologies, Tokyo, Japan). Total mercury (Hg) was measured by thermal decomposition, gold amalgamation and atomic absorption spectrophotometry (Mercury Analyzer, MA-3000; Nippon Instruments Corporation, Tokyo, Japan), after preparation of calibration standards.

3. Results and Discussion

3.1 Concentration of Metals and Metalloid in Offal and Muscle

The results showed that chicken liver accumulated the highest levels of Fe, Mn and Hg, chicken kidney (Pb, Cd and As) and chicken gizzard (Ni and Cr) while cattle liver accumulated Co, Cu and Zn. This result conform to Demirezen *et al*. (2006) who reported that the primary site for heavy metals accumulation is the liver and kidney. This is because these organs are important in metabolism and excretion of xenobiotics hence help to detoxify the system thereby leading to accumulation. The chicken gizzard also is an organ found in the digestive tract which help disintegrate absorbed food particles as a result leads to high accumulation of metals. Neisheim *et al.* (1979) reported that free range local chickens usually accumulate higher levels of pollutants in their body system as they have no oral discrimination in their feeding habit. This could the reason why free-range chickens accumulated more metals than the cattle and goat. The findings of this study conform with Mukesh *et al*. (2008) that food chain has becomes the main gateway for persistent heavy metals to higher organisms. Animals at the top of food chain may generally accumulate a large amount of metals and metalloids in their tissue, according to age, size and feeding habits. Principal component analysis (PCA) of metals and metalloid distribution in offal and muscle of animal based food samples showed a clear separation between chicken grouped on one side, and the ruminants, cattle and goat clustered on another side in both offal and muscle fig 1.

3.2 Human Health Risk Assessment

EDI values of metals and metalloid in free range chicken, cattle and goat offal and tissue for human non-essential metals Cd, As, Pb and Hg were far below RfD values recommended by the international regulatory bodies (USEPA, 2009) thus strongly indicating no possible human health risk via consumption of these food based animals. HQ value in this study was less than 1 which means the exposed population is unlikely to experience obvious adverse effects (USEPA, 2000) from the intake of individual metals through offal and muscle consumption fig 2 HI for residents ingesting these metals by consuming tissues and offal of free range chicken cattle and goat were greater than 1 (>1) for children. This suggests that there is overtly adverse health effects from their consumption of chicken liver and gizzard fig 3. This suggests that combined effects of all the metals pose a greater health risk than any lone metal (Cao *et al*., 2010).

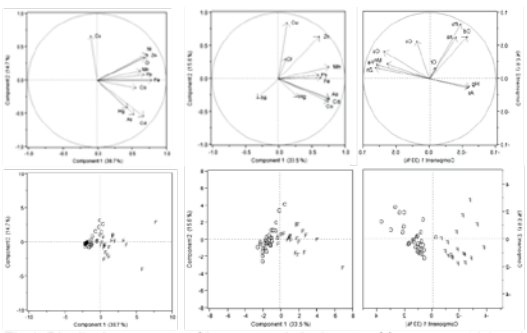
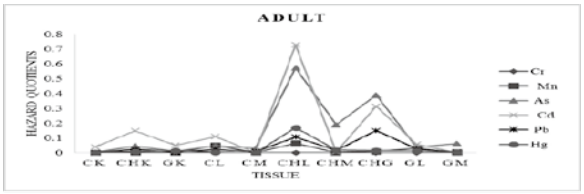


Fig 1: Distribution patterns of heavy metals in tissues of free range chicken (*Galius gallus domesticus*), Cattle (*Bos taurus*) and Goat (*Capra hircus*) characterized by PCA (C: Cattle; F: Chicken; G: Goat).



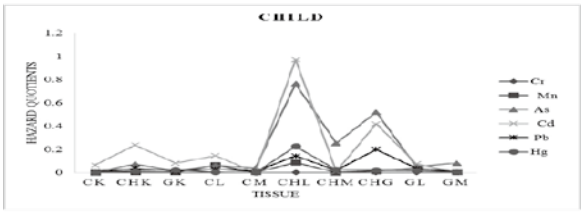


Figure 2: Hazard quotient (HQ) of heavy metals (Cr, Mn, As, Cd, Pb and Hg) in children and adults via consumption of cattle liver CL, chicken liver CHL, goat liver GL, cattle kidney CK, chicken kidney CHK, goat kidney GK, cattle muscle CM, chicken muscle CHM, goat muscle GM, chicken gizzard CHG

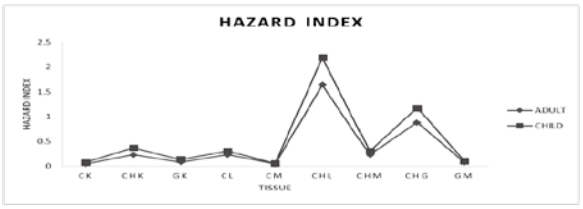


Fig 3: Hazard Index (HI) for children and adults through the consumption of offal and muscle

4. Acknowledgments

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O-7

Study of oxidative stress and epigenetic modifications in high blood pressure individuals leaving in an environment containing bisphenol A and Mycotoxins

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The study of mycotoxin contamination in Cameroon started during the years 1980, with some reports on the contamination of food staple with aflatoxins, this was followed by reports on aflatoxin detection in breast milk and blood sample respectively in kwashiorkor children and in cancer patients (Domngang et al 1989, Tchouanguep et al. 1994, Tchana et al, 2010). Recently (Abial et al 2013, 2014) multiple mycotoxin were found in common food samples and in urinary sample of HIV/AIDS patients. In addition to these studies, high level of BisphenolA (BPA) and heavy metal were found to be consumed by Yaounde inhabitants (Unpublished results). At the same time metabolic diseases as Diabetes mellitus, Hypertension, Cancer has been found to increase within Cameroonian leaving in urban area.

1. Objectives.

The objective of this study was to evaluate the influence of medication on oxidative stress and epigenetic modifications namely DNA methylation in new and old hypertensive patient living in an environment containing food contaminants.

2. Material and Methods

To achieve this, a study for which ethical clearance was obtained was designed using high blood pressure (HBP) patients. The urine samples were collected from 91 participants selected on the basis of criteria for inclusion and non-inclusion divided into 38 former HBP patients, hypertensive participants 30 newly recruited HBP patients and 23 control participants. An epidemiological survey was conducted in all participants. The following analyzes were performed in the urine samples from each participant using referenced methods: creatinine, total protein, carbonyl protein, and electrophoretic analysis of urinary proteins. For the analysis of oxidative stress (Morrow et al, 1990, Kadiiska et al 2005), ELISA kit for assay of 15-F_{2t}-Isoprostane was used (Oxford Biomedical Research). Epigenetic modifcations was assessed by measuring DNA methylation (Epigentek). In addition, Mycotoxins, heavy metals and BPA were also analyzed.

3. Results and discussion

The study population were mainly public servants, housewives, people with individual activities (traders, pastor, writer and unemployed), and people on retirement and farmers represented respectively 53.83%, 17.58%, 12.08%, 9. 89% and 6.59% of the study population. Within this population, 16.48% had normal weight, 36.26% were overweight and 43.25% obese.

The urinary protein content ranged from 0.08 to 5.04 mg / 24 hours in former HBP patients, 0.09 to 1.75 mg / 24 hours in newly recruited HBP patients,

Table : Urinary 15-isoprostane (ng/mg of de creatinine) in patient and control groups

Groups	Sexe	number	Min	Max	Moy±SD
fHBP	M	17	$7.88.10^{-4}$	$5.70.10^{-3}$	$2.31.10^{-3}$±$1.14.10^{-3}$
	F	21	$8.26.10^{-4}$	$8.05.10^{-3}$	$2.91.10^{-3}$±$1.63.10^{-3}$
nHBP	M	8	$1.2.10^{-3}$		$2.88.10^{-3}$±$1.22.10^{-3}$
	F	20	$8.78.10^{-3}$	1,18	$3.94.10^{-3}$±$2.52.10^{-3}$
Control	M	12	$9.70.10^{-4}$	$6.44.10^{-3}$	$3.45.10^{-3}$±$1.75.10^{-3}$
	F	8	$2.06.10^{-6}$	$1.37.10^{-3}$	$6.99.10^{-3}$±$3.32.10^{-3}$

fHBP : Former hypertensive patients, nHBP newly hypertensive patients. Values are mean ± SD in each group. M, male; F, female

and 0, 15 to 0.51 mg / 24 hours in controls. Most of the urine samples of former HBP patients had protein bands on electrophoresis analysis whereas, less protein bands were found in urine sample of the control and nwely recruited patients. The evaluation of oxidative stress using isoprostane showed that 15 - Isoprostane was detected in 95.60 % of the samples in the study population. Concentrations of 15 - Isoprostane varied from 0,6ng /ml to 4,99ng / ml. With a respective average amount in former HBP patients, new hypertensive patients and controls of 3.33 ± 2.22ng/ml, 4.14 ± 3.31ng/ml and 3.54 ± 2.33ng/ml.

Oxidized proteins were detected in 100 % of the samples in the study population. These results are an indication of less oxidative stress in former HBP patients compared to newly recruited HBP and control. Levels of mycotoxins, BPA and heavy metal in patients are discussed to find out the influence of theses contaminants on oxidative stress and epigenetic modification.

Keywords: mycotoxins, bisphenol A, protein carbonyl, isoprostane, DNA methylation.

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O-8

Pesticide Use in Sudan: Historical Background and Future Prospective

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In the Sudan Gezira, cotton spraying started as early as season 1945/46 when only 1% of the cotton area was sprayed once. By 1978/79 the problem caused by the cotton insect pests, particularly the cotton whitefly (*Bemisia tabaci*) flared up. The number of sprays per season went up, reaching 9.25 sprays in season 1978/79, which might be attributed partly to the diversification and intensification of cropping systems. The number of sprays declined after that and reached 5.11 sprays in season 1982/83. This might be due to the use of more potent chemicals or to the drought condition prevailing during the early 1980's through which low whitefly and ABW (*Helicoverpa armigera*) infestation occurred. In recent years the economic importance of the African bollworms increased and most sprays are directed towards the control of this pests. Sudan's over reliance on pesticides has caused severe problems, including resurgence of secondary pest, pesticide resistance, and greatly increased costs of production, human and environmental pollution. As a result, Sudan has to deal with escalating costs and decreasing effectiveness of pesticides. A study attributed the striking increase in pesticide cost to the increase in taxes against pesticides. The possibilities to reduce the cost of pesticides to render cotton a profitable production and hence it's competitiveness in a liberalized economy are discussed.

Keywords: Pesticide sprays, Cotton, insect pests, Sudan

1. Introduction

The use of insecticides to combat insect cotton pest is essential for the economic cotton production. However many problems are associating with the use of insecticides which include environmental contamination, resurgence of secondary pest and the development of insecticide resistance by some noxious insect pests. Sudan has a long history of pesticide dependence and pesticide-related problems. Sudan began large-scale cotton production under British colonialism in the 1920s and started applying pesticides to its cotton plantations in the 1940s. In Sudan Gezira, cotton spraying started as early as the season 1945/46 when only 1% of the cotton area was sprayed once. In season 1946/47 around 8,348 feddan were sprayed with DDT to control cotton pests, maily the cotton jassid E. lybica (Abdelgader 1987).

Pest Management: Current Situation

Chemical Control is the main tool used in Sudan to control insects pest, particularly on cotton plantations. The use of chemical pesticides to control pests has many advantages. These include their quick action, spraying of large areas in short time and the easiness of finding the suitable formulation to combat specific pest situation.

2. Methods

This paper tried to investigate process of Pesticides Registration in Sudan as well as the number of different pesticides registered in Sudan at various periods.

The study also looked at the relationship between Cotton Yield and insecticide Spraying during different periods

3. Results and Discussion

Introduction and use of pesticides in Sudan is governed by law. Documentation on the Procedures and Regulation Governing Research on Agricultural Pesticides for Registration in Sudan was published in 2000 (Zorgani et al 2000). This documentation showed the requirement for the introduction of a pesticide, testing of a pesticide, seed dressing materials, evaluation of efficacy, release of pesticides, testing fees and some general rules.

Following the regulations governing pesticides use in Sudan, about 619 pesticides are registered in Sudan as follows

* 455 pesticides (73.5%) on the Agricultural Sector (Agriculture, Irrigation and Sugar).
* 156 pesticides (25.2%) on Public Health Sector.
* 8 pesticides (1.3%) for Veterinary use

The Actual need of Pesticides for the period (1997/98-2002/03) is shown in a report from the ministry of Agriculture and can be seen in Table (1). Accordingly, the number of sprays decreased from more than 9 sprays to about 4 sprays. However the Percentage of the cost of pesticides increased from 20% (with 9 sprays) to 35% (with 4 sprays).

The study found out that this was mainly due to the increase in taxes.

Table1. Actual need of Pesticides for the period (1997/98-2002/03)

Season	Quantity Imported (Tons)	Value in US$
1997/98	3581	42.219.999
1998 /99	1827	19.296.567
1999 /2000	2202	19.394.564
2000 /2001	1438	15.239.457
2001 /2002	2774	27.208.251
2002 /2003	1621	15.308.462

Cotton Yield and insecticide Spraying

Relationship between number of sprays and yield in Gezira and Rahad scheme (during the period 1977/78- 1997/98) is presented in Figures 1 and 2. A smillar trend can be observed in both situation. An inverse linear relationship ($R^2 > 0.7$) between the number of sprays and years indicating a steady decrease in numbers of sprays with years. On the other hand the yield remained almost at the same level all through ($R^2 < 0.03$).

This indicates that the pest control program during this period was successful in reducing the amount of pesticides needed for the control of pests without reducing the productivity of cotton. This might be attributed to the implementation of new effective strategies for pest management (e.g. selective spraying, IPM…etc). This indicate that these efforts should continue to implement new approaches for pest management aiming at further reduction of the cost of control measures and reducing risks to the environment.

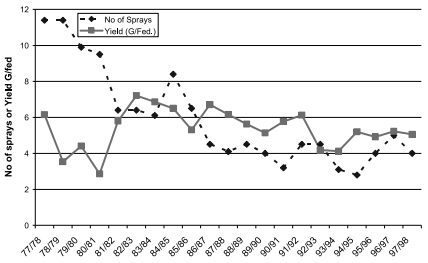

Figure 1 (a). Spraying and Yield in Rahad Scheme (1977-1998)

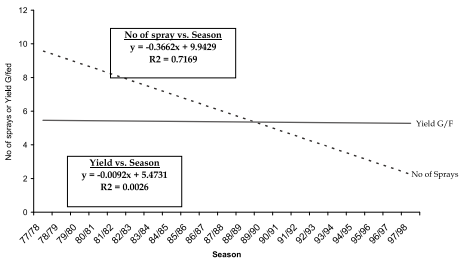

Figure 1(b). Relationship between Seasons and No of Sprays and/or Yield in Rahad Scheme (1977-1998)

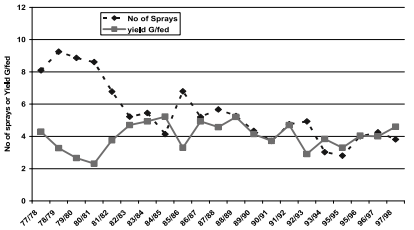

Figure 2 (a). Spraying and Yield in Gezira Scheme (1977-1998)

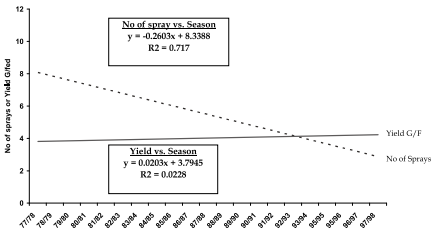

Figure 2(b). Relationship between Seasons and No of Sprays and/or Yield in Gezira Scheme (1977-1998)

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O-9

Heavy Metals Concentrations in Sediment and Benthic Fauna (*Chrysichthys auratus* and *Tympanotonus fuscatus*) of Benin River: Application of Risk Assessment Indices.

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The concentrations of heavy metals Cadmium (Cd), Cobalt (Co), Copper (Cu), Iron (Fe), Lead (Pb), Manganese (Mn), Nickel (Ni) and Zinc (Zn), in sediments and benthic fauna (fish: *Chrysichthys auratus* and periwinkle: *Tympanotonus fuscatus*) from Benin River were assessed with the aim of estimating the attendant ecological and human health risk. Samples were prepared and digested using standard methods while heavy metal content in each matrix was analyzed using an atomic absorption spectrophotometer (AAS). Ecological and human health risk indices were employed to assess the degree of contamination of the sediments, anthropogenic influence on the sediment quality, sensitivity of the biota to the toxic heavy metals and non-cancer effects to humans. Results showed the presence of target metals, with benthic fauna having the highest concentration of heavy metals. Enrichment Factor and Pollution load index showed that heavy metals in sediment were from anthropogenic sources while the geoaccumulation index (I_{geo}) showed extreme pollution of sediment samples with Cd and Fe, and Pb. Transfer factor confirmed biomagnification and trophic level enrichment of metals was more in *T. fuscatus* than *C. auratus*. The human health risk assessment indicated adverse health risk for non-carcinogenic effect (Hazard Index (HI) values > 1).

Keywords: Heavy metal, *health risk, pollution index, transfer factor*

1. Introduction

The recent rapid rate of development, industrialization and urbanization around the bank of Benin River has resulted in the release of a substantial volume of untreated or inadequately treated wastewaters. This has provided the potential for deterioration of the water quality, leading to significant negative impacts on the aquatic ecosystems (Lin et al. 2010). Heavy metals from these sources pose serious threats to the environment and human health which is of major concern because of their toxicity, persistence, and bioaccumulation (Bonanno and Lo Giudice 2010). Heavy metals have the tendency to accumulate in various organs of the aquatic organisms, especially fish and shellfish, which in turn accumulate in human through consumption resulting in serious health hazards (Puel et al., 1987).

This study was aimed at determining the concentration of heavy metals (Cd, Cr, Cu, Fe, Mn, Pb, and Zn) in sediments, *C. auratus* and *T. fuscatus* from Benin River. Furthermore, the potential trophic level enrichment and biomagnification along the fish - periwinkle food chain was also established while ecological and human health risk were also estimated.

2. Materials and Methods

2.1 Description of the study sites

Three sampling sites along Benin River were selected for this study. Each station was associated with agricultural and industrial activities. Station 1 is a mangrove forest and runs through Ebialegbe rural community (5°, 58.264´ N and 5°, 29.433´E), Therefore prone to considerable domestic waste water. Station 2 (5°, 59.830´ N and 5°, 27.859´E) and 3 (5°, 59.938´ N and 5°, 27.019´E) runs through industrial and oil processing installations (Ebenco Global Link Limited, the Optima Energy Resources Limited and Total Nigeria PLC). The samples (sediment, *C. auratus* and *T. fuscatus*) were collected monthly from December, 2014 to May, 2015.

2.2 Sample preparation and analysis

Samples were prepared based on the method described by AOAC (1990). The sediment samples were oven dried, homogenized, and sieved using a 2mm sieve. Edible portions of biota samples were also dried and homogenised.0.5 g of the sediment and biota samples were digested using 10:1:4 mixture of nitric acid (HNO_3), sulphuric acid (H_2SO_4) and perchloric acid ($HClO_4$) until digestion was complete. The solution was cooled at room temperature, diluted, and adjusted to 25 mL with deionized water. Analysis of heavy metal concentrations was carried out using an atomic absorption spectrophotometer (Model 210 VGP, Buck Scientific). The absorption wavelengths for each metals were as follows 283.3nm (Pb), 228.8nm (Cd), 357.9nm (Cr), 279.5nm (Mn), 248.3nm (Fe), 232nm (Ni), 324.8nm (Cu), 213nm (Zn) and 240nm (Co).

2.3 Ecological Risk Assessment of Heavy:

The following indices were employed in assessing the degree of contamination of the sediments, anthropogenic influence on the sediment quality and describing the sensitivity of the biota to the toxic heavy metals.

1. Enrichment factor (EF): this was used to determine the source of heavy metal pollution.

$$EF = \frac{\left(C_x/_{Fe}\right)_{sample}}{\left(C_x/_{Fe}\right)_{background}}$$

2. Pollution Load Index (PLI): this was also used to determine the source and level of heavy metal pollution in sediment samples. PLI represents the number of times by which the metal content in the sediment exceeds the background concentration and gives a summative indication of the overall level of heavy metal toxicity in a particular sample.

$$PLI = (CF_1 \times CF_2 \times CF_3 \times CF_n)^{1/n}$$

Where $CF = \frac{Metal\ concentration\ in\ sediment}{Background\ value\ of\ metal}$

3. Contamination Degree (CD): this refers to the sum of all contamination factors. It is used to assess the contamination level of contaminants in sediment.

$CD = \sum_{i=1}^{n} CF$

4. Geo-accumulation Index (I_{geo}): this is used to determine the extent of metal accumulation in sediments.

$$I_{geo} = \log_2\left[\frac{C_n}{1.5B_n}\right],$$

5. The Potential Ecological Risk Index (RI): this is used to quantify the potential ecological hazard of heavy metals in contaminated sediments to biota.

$E_r^i = T_r^i \times CF$

The sum of the individual potential risks (E_r^i) is the potential ecological risk index (RI) for the water body. It is represented as: $RI = \sum_{i=1}^{n} T_r^i \times CF$

6. Sediment to benthos transfer assessment: Sediment to benthic fauna metal transfer was computed as transfer factor (TF). TF values indicate the level of bio-magnification that has occurred in *C. auratus* and *T. fuscatus* respectively.

$$TF = \frac{C_{fauna}}{C_{sediment}}$$

2.4 Human Health Risk Assessment

Human health risk assessment for heavy metals will be carried out using the target hazard quotient.

Target hazard quotient (THQ): This was used to estimate the potential risk of heavy metals to humans through dietary exposures to contaminated *C. auratus* and *T. fuscatus.* THQ above 1 indicate non-cancer risk while THQ below low indicates no risk.

$$\text{THQ} = \frac{Efr\ X\ EDtot\ X\ FIR\ X\ C}{RfDo\ X\ BW\ X\ ATn}\ X\ 10^{-3}$$

Where EFr is exposure frequency (days/year); ED_{tot} is the exposure duration, average lifetime); FIR is the food ingestion rate (g/day); C is the heavy metal concentration in biota (ppm); RfDo is the oral reference dose (mg/kg/day). BW is the average body weight (70 kg) and AT_n is the averaging exposure time for non-carcinogens (365 days/year × number of exposure years assuming 52 years).

3. Results and Discussion

The mean heavy metal concentrations (ppm) in sediment, *C. auratus* and *T. fuscatus* obtained from Benin River is presented in Fig 1. Fe was the dominant metal in all samples and matrices. However, higher concentrations of Pb, Zn Mn and Cu were observed in biota (*C. auratus* and *T. fuscatus*) compared to sediment samples. From the results there is a clear evidence of bioaccumulation from contaminated sediments sediment. Related studies have shown that mangrove sediments act as a trap for chemical contaminants because such sediments contain high percentage of silt and clay that cause an increase in the metals adsorption (Vallejuelo et al., 2010). Concentrations of Pb and Cd in *C. auratus* and *T. fuscatus* collected from Benin River were higher than the permissible limits set by EU and WHO (0.3 and 0.05 mg/kg respectively).

3.1 Ecological risk assessment

Low enrichment factor (EF) values and pollution Load index (PLI) (0.8) showed that the sources of heavy metals in sediment samples were a mixture of anthropogenic and natural sources. However, estimated contamination factor (CF) for Cd, Fe and Pb in sediment samples indicated severe anthropogenic inputs of these metals. Correspondingly, very high contamination degrees (CD>24) were observed in sediment indicating serious anthropogenic pollution. Estimated I_{geo}, showed extreme pollution of sediment samples with Cd and Pb and Fe. The individual potential risks (E_r^i) of heavy metals showed that there the possibility of adverse effect to benthic fauna exposed to contaminated sediments. The potential ecological risk (RI) for heavy metals in sediment samples showed values lower than the recommended threshold (150), indicating low ecological risk to benthic fauna. Estimated transfer factor values for heavy metals in *C. auratus* and *T. fuscatus* showed biomagnification of some toxic metals (Fig 2). A transfer factor ≥ 1 indicates that the metal is biomagnified. Biomagnification could lead to high toxicity of these metals in organisms, even when the exposure level is low.

3.2 Human Health Risk Assessment

Estimated hazard quotient (HQ) for the benthic fauna (*C. auratus* and *T. fuscatus*) (Fig 3), showed that there could be potential non-cancer risk to consumers. HI values for both *C. auratus* (17.5) and *T. fuscatus* (27.5) were greater than 1 indicating adverse health risk for non-carcinogenic effect.

The observations from this study were consistent with the enrichment of contaminant along the trophic level. Concentrations of heavy metals increased with an increase in the source of contaminants while bioaccumulation was a function of the presence of contaminants in the matrices. Heavy metal concentrations followed the order of sediment > *T. fuscatus* > *C. auratus*.. This calls for more efforts by relevant stake holders to monitor the discharge of waste water into aquatic systems.

4. Acknowledgement

This project was funded by the University of Benin tertiary education trust fund (TETFUND) as research grant for the year 2014.

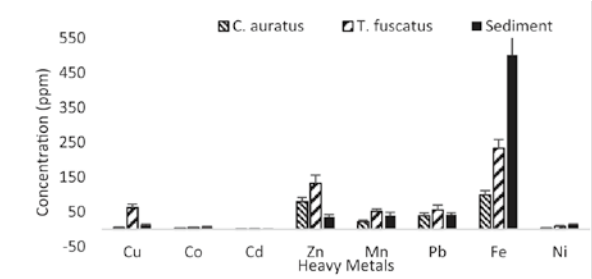

Fig 1: Mean Concentration of Heavy metals in sediment, *C. auratus and T. fuscatus* obtained from Benin River

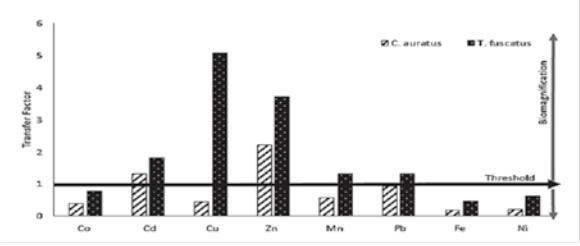

Fig 2: Transfer Factor for Heavy Metals in *C. auratus and T. fuscatus*

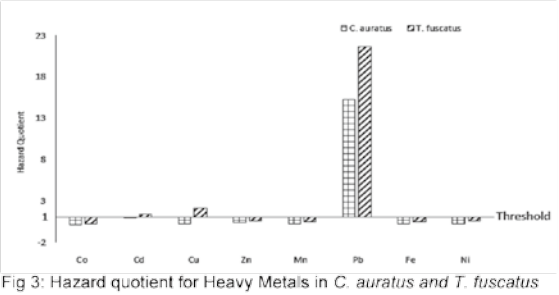

Fig 3: Hazard quotient for Heavy Metals in *C. auratus and T. fuscatus*

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O-10

Heavy metals in Mopane worms from Kruger National Park

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This study was undertaken to assess metal concentrations in mopane worms in the Kruger National Park. Mopane worms, gut content, leaves and particulate matter on the surface of leaves was analysed using ICP-MS analysis to determine if worms were contaminated and possible sources of contamination. The results indicated that the metal concentrations found in the worms are from dust particles ingested while foraging rather than from the leaves themselves. The results also indicate that metal concentration further away from the mining town of Phalaborwa are lower. Sustainable harvesting of Mopane worms should take place further away from mining activities as they pose a smaller potential health risk to rural consumers.

Keywords: ICP-MS, metal contamination, Mopane Worms, Kruger National Park.

1. Introduction

Between 2010 and 2012 FAO estimated that approximately 870 million people were undernourished. This is about 12.5% of the global population (Obopile and Seeletso, 2013). One of the ways to alleviate this global problems is by eating insects. Insects form part of the staple diets of many people around the world with some insects being seen as delicacies. *Imbrasia belina* commonly known as the mopane worm is an important source of food amongst many southern African cultures (Ditlhogo, 1996). Mopane worms are the larval stage of emperor moths from the order *Lepidoptera* that feed on the mopane tree (*Colophospermum mopane)* (Ditlhogo, 1996). The mopane worm is estimated to generate $3.3 million during a good season providing employment for approximately 10 000 people (Ghazoul, 2006). Mopane worms are highly nutritious providing a high protein content of approximately 64% which is higher than that of meat and fish (Obopile and Seeletso, 2013). The Kruger National Park (KNP), introduced mopane worm harvesting projects in the northern region of the park between Punda Maria and Phalaborwa areas in December 2010 (Strauss, 2011). The harvesting projects are aimed at uplifting the community, while monitoring the ecological, social and economic impacts of harvesting the worms (Strauss, 2011). Anthropogenic activities such as mining, agriculture and the use of vehicles have many negative impacts on the environment. Greenfield et al., (2014) published findings that Mn levels were above threshold values for the safe consumption of mopane worms in the Phalaborwa region of the KNP. The worms however in this paper were not gutted, making conclusions as to the safety of these worms for consumption difficult. The aims of this study were thus to determine the safety of consuming mopane worms harvested and prepared in a traditional manner from the Kruger National Park.

2. Materials and Methods

Four sampling sites (PWB, SW, N, WD) were chosen by KNP management for sustainable harvesting. Twenty worms and 20 leaves were collected from each site. The worms were gutted according to traditional methods, and dried in brown bags until analysis could take place.

In the laboratory the leaves were washed three times with distilled water and the solution was filtered through pre-weighed nitrocellulose filter paper (0.45um pore size). Samples (worms, filter paper, leaves and guts) were dried at 60°C for 48 h (Wepener et al., 2011) to attain constant mass.

The filter papers were digested using HCl/HNO_3 and the worms, guts and leaves were digested using H_2O_2/HNO_3 in a MARS 6 CEM microwave digester. Metal concentrations were determined using ICP-MS. Quality control of metal measurements in samples were verified with process blanks and certified reference materials (Wepener et al., 2011).

Results were analysed using SPSS v18. The data underwent student T tests for significance ($p \leq 0.05$) between variables at each site. Levene's test for homogeneity of variance was used to determine data distribution. A one way analysis of variance (ANOVA) was run to determine significant differences between the sites. The data further underwent a discriminant function analysis (DFA) and the first two functions were plotted on a Bi plot.

3. Results and Discussion

The CRM values fell within the 85-120% range indicating acceptable recovery from sample preparation.

The DFA biplot of the worm, gut content and leaf data represents 61% of the variance in the data on the first two axes. The triangle indicates that little variance in the leaf data. The squares shows that the metal concentration for both the N and WD sites in the guts and worms were similar. The dashed

squares show that the WD and PWB site had similar concentrations for the worms and guts and the same applies for the PWB worms and guts.

High concentrations of metals on filter papers compared to the leaves for all sites suggests that Al, Ni, Pb, Se and Zn are atmospheric in nature.

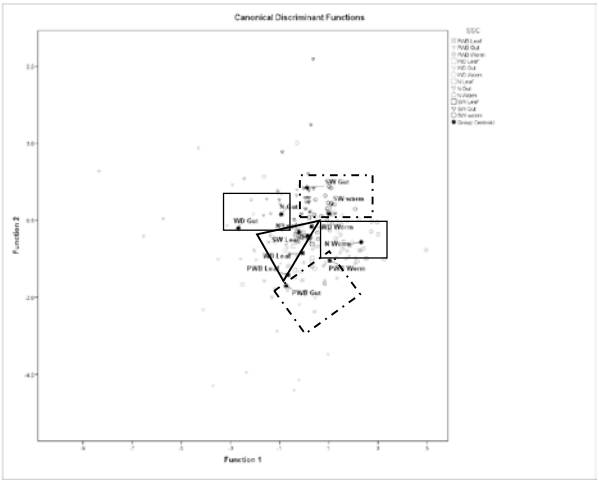

Figure 1: Discriminant Function Plot indicating combined variance between the Worms, Gut content and Leaves from the different sites.

Results indicate that chromium comes from the leaves which suggests it is naturally occurring. Cr concentrations from WD were highest on the filter paper suggesting it sourced from the atmosphere.

The low As concentrations in the leaves compared to the filter papers suggests that the As is atmospheric in nature.

Copper concentrations in the gut content except from PWB indicate that gutting the mopane worms thoroughly may reduce the risk of contamination from copper. The high Cu concentration in worms from PWB correlates with the study by (Grobler and Swan 1999) whereby Cu poisoning was labelled as the reason for chronic Cu poisoning of Impala around the PWB site.

The high Fe concentration on the filter paper compared to the leaves for the PWB site suggests an atmospheric source. The worms from the other sites respectively accumulate Fe from the leaves.

High Mn concentrations obtained for all sites may pose a potential human health risk. The PWB worms once again rank highest with a concentration of about 48 mg/g with the lowest rank held by the N worms with 8mg/g. The leaf concentrations for all sites except PWB are higher than the filter paper concentrations. Worms from the PWB site most probably obtain their high concentrations from the dust particles that settle on the leaves.

In the PWB site the Se concentration in worms is about 2mg/g and approximately 0.6mg/g for the WD and N site worms. The worms in the PWB site pose a potential risk to human health.

The high metal concentrations in the worms is not from the leaves but dust particles which settle on the leaves as reflected for Al, Ni and Zn. The WD and N worms are exposed to similar conditions. This is supported by the worm metal concentration for Cu, Pb and Se. this holds true for both the PWB and SW sites. Indication is that the worms accumulate the metals from the gut content. This is supported by the Cu concentrations in the PWB site and the Mn and Ni concentrations in the SW site as depicted in Figure 1.

4. Acknowledgments

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O-11

Polycyclic aromatic hydrocarbon toxicity assessment of urban sediments, South Africa

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The largest urban area in Gauteng South Africa, Soweto, is transversed by the Klip River. Sediment samples were collected in this area and analysed for the 16 priority PAHs. The ΣPAH ranged from 421–5 370 ng/g dm, and the carcinogenic PAHs (ΣCPAH) ranged from 157–1 015 ng/g dm. The toxicity of these sediments were assessed using toxic equivalencies (TEQ) and gauging these against international guidelines. The greatest TEQ measured was 525 ng/kg dm, far greater than the guidelines for protection of aquatic health.

Keywords: Sediments, Polycyclic aromatic hydrocarbons, Toxicity assessment, Toxic equivalence, Fish potency factors

1. Introduction

The Klip River and its tributaries drain the largest urban area in South Africa's most populated province, Gauteng. As the Klip River is subjected to various forms of anthropogenic stressors, including polycyclic aromatic hydrocarbons (PAHs), it is considered one of South Africa's most polluted rivers (McCarthy et al., 2006). The 16 priority PAHs are of specific concern as all are toxic and some are known carcinogens referred to as CPAHs (USEPA, 2008). PAHs introduced into an aquatic system bind to sediment and can be introduced into the food web through benthic fauna. This in turn can lead to negative effects within the food web. The negative effects of PAHs on biota from the Klip River catchment has been reported (Pheiffer et al., 2015). The toxicity of the PAHs were compared to that of 2,3,7,8-TCDD toxicity in fish using fish potency factors (FPFs) as determined by Barron et al. (2004). The FPFs these authors determined express the ability of the PAHs to induce CYP1A or bind to the aryl hydrocarbon receptor (AhR).

2. Materials and Methods

2.1 Sediment sampling and chemical analysis

Composite sediment samples were collected during the peak low flow season of 2014 from nine sites in Soweto (Figure 1). Samples were air dried and homogenised prior to the extraction for the 16 priority PAHs through accelerated solvent extraction (USEPA, 1998). The PAH fraction was separated from the raw extract by size exclusion chromatography (USEPA, 1994). The final extract was purified using Florisil/silica solid phase extraction (SPE) cartridges (USEPA, 1996, 2007). The target analytes were analysed by the National Metrology Institute of South Africa (NMISA) using gas chromatography coupled to a time-of-flight mass spectrometry (GC-TOFMS). Quantification was done using isotope dilution mass spectrometry.

The sum of all PAHs were calculated as well as the total CPAH (chrysene, benzo(b+k)fluoranthene, benzo(a)pyrene, indeno[1,2,3-c,d]pyrene, and di-benz(a,h)anthracene). The ΣPAHs was compared to the total PAH guidelines of MacDonald et al. (2000) for benthic organisms with the lower threshold effects concentration (TEC) of 1 160 ng/g dm) and the higher probable effects concentration (PEC) of 22 800 ng/g dm.

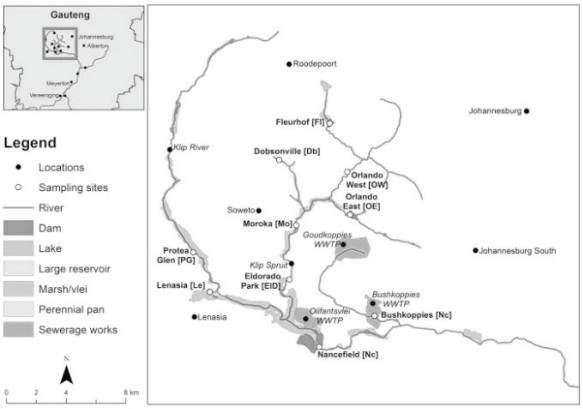

Figure 1: Sediment sampling sites in the Klip River, Soweto

2.2 Toxicity assessment

Individual PAH toxicity was compared to that of TCDD in fish systems specifically by multiplying the sediment concentrations of a PAH to its FPF (Barron et al., 2004) and adding them to create a toxic equivalency (TEQ) per site:

$$TEQ=\sum(C_i \times FPF_i)$$

The calculated TEQs were compared to the TEQ based Canadian sediment quality guidelines for dioxin-like compounds where the lower interim sediment quality guideline (ISQG) is 0.85 ngTEQ/kg and the higher probable effects level (PEL) of 21.5 ngTEQ/kg (CCME, 2002).

3. Results and Discussion

The PAH levels ranged from 421.3 ng/g to 5 369 ng/g. These levels were comparable to sediment levels previously measured from the same area: Nieuwoudt et al., (2011) reported 580 ng/g and Quinn et al., (2009) calculated a mean of

641 ng/g (maximum 2 799 ng/g). In a different catchment mainly impacted through agriculture, Nieuwoudt et al., (2011) reported 120 ng/g and Pieters et al., (2015) a mean of 110 ng/g (maximum 870 ng/g).

Table 1: Sum of PAHs and CPAHs (ng/g dm) quantified in the sediments of Soweto

Sites	ΣPAHs	ΣCPAHs
Protea Glen	757.8	223.4
Lenasia	2 089.7	445.0
Fleurhof	902.8	214.9
Moroka	5 369.5	1 014.5
Eldorado Park	2 412.9	563.1
Orlando West	946.7	222.8
Orlando East	421.3	156.6
Nancefield	676.3	211.9
Dobsonville	435.5	188.2

Grey indicates exceedance of TEC (1 160 ng/g) (MacDonald et al., 2001).

The two highest concentrations of ΣPAHs and ΣCPAHs were found at Moroka and Eldorado Park (Table 1) both within the Klip Spruit tributary downstream from each other (Figure 1). Lenasia also exceeded the TEC of MacDonald et al., (2000), indicating an increased toxicity toward benthic organisms with increasing PAH concentrations.

In terms of toxicity towards fish, all the sediments of Soweto exceeded the PEL of the Canadian guideline (Figure 2), by 4 to 21 times. These fish TEQs indicate that in spite of the apparent "safe" PAHs levels in the sediment (as shown in Table 1), there is increased probability of adverse biological effects within the fish population.

The CPAHs contribute more towards the TEQ that the ΣPAHs (Spearman correlation r = 0.95; p = 0.0004).

In conclusion, this particular urban area is influenced by PAHs in its aquatic environment. Applying guidelines to instrumentally determined levels benthic organisms have low risk to PAH toxicity. However, calculating toxic equivalence predicted a very high probability of dioxin-like toxic effect in fish.

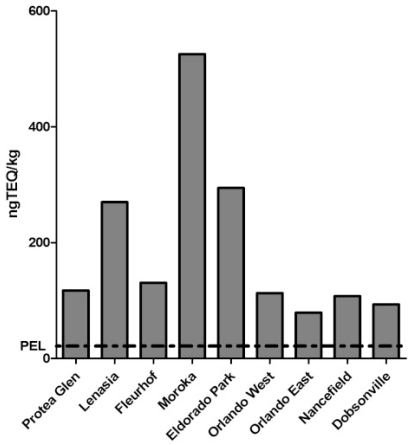

Figure 2: Toxic equivalencies of the sediments from Soweto compared to sediment quality guidelines

4. Acknowledgments

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O-12

The Possible Association between Selected Sediment Characteristics and the occurrence of heavy metals in a highly Pristine River in South Africa

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This study was undertaken to investigate the possible association between selected sediment characteristics including particle size, secondary lithology, and anthropogenic activities with heavy metals in the sediment of the Marico River. Sediment particle fractionation was done to determine the percentage fine sediment (<2mm) and larger particles (>2mm) at different sites. Heavy metal analyses were done on both these fractions while secondary minerals were identified in the sediment<2mm. Higher metal concentrations associated with sediment, <2mm while a possible association between secondary minerals and heavy metal was established.

Keywords: sediment heavy metals, secondary lithology.

1. Introduction

It is well known that sedimentation of rivers can lead to stream pollution and habitat degradation while fine sediment, in excess, can have negative effect on the survival macroinvertebrates. Although studies have been done to emphasize the toxic effects of metals of anthropogenic origin, less is known about the effects caused by heavy metals which enter natural water sources via processes which include weathering of secondary minerals and atmospheric deposition. (Tchounwou, Yedjou, and Sutton. 2012), Interfacial processes such as mineral dissolution, mineral precipitations and the sorption and desorption of chemical species are responsible for the release and/or sequestration of heavy metals that may eventually become pollutants in soils and ground (Brown, Foster and Ostergren, 1999) while various mineral surfaces also act as sorption media for metals of anthropogenic origin including quartz, koalinite, calcite, hematite and montmorillonite

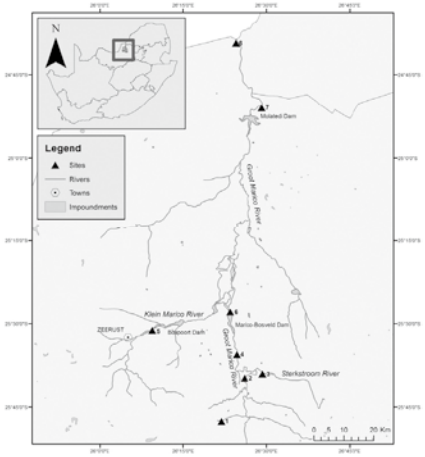
The Marico River originates at the Marico Eye in the North-West Province. Except for urban development near Zeerust limited anthropogenic activities are practised in the catchment. The main lithology surrounding the study area includes quartzite, ferruginous shale, hornfels, ferruginous quartzite, andesite, basaltic lawa, agglomerate, tuff, shale, rhyolite and dacite.

The aim of this study was to establish the possible association between selected sediment characteristics including particle size and secondary lithology with heavy metals in the sediment of the Marico River

2. Material and Methods

Sediment samples were collected in triplicate at each of the sites from the upper 7 cm of the basin. The size distribution of each sample was determined by the dry sieving method. The sediment fractions of each sample were divided between fractions, <2mm and those >2mm.

Figure 1 Location of sampling sites (triangles) within the Marico River catchment, North-West Province, South Africa



Analyses of heavy metals regarded to be toxic to aquatic macroinvertebrates present in the total sediment, as well as in clay fractions < 50µm were done by making use of a ICP-MS.

Mineral identification was done by characterization of the crystalline materials present in the sample. Sample preparation was done using a back loading technique. Samples were scanned using X-rays generated by a Cu X-Ray tube which generates a unique diffractogram for each sample.

3. Results and Discussion

The origin of the heavy metals analysed (Table 1) are most probably, because of the limited anthropogenic activities in the catchment of this river, due to natural weathering of the surrounding primary lithology which results in the deposition of secondary minerals in the sediment. Clay was only present at Sites 1, 3, 5, 7 and 8. Heavy metal concentrations, (ppm per 1g) were for the majority of metals measured, higher in the fractions > 50µm at Sites 1 and 8 while only the concentrations of Al, Cu and Pb were higher in fractions <50µm at

site 1 This was the case for Zn at Site 8. For Sites 3, 5 and 7, higher concentrations of nearly all the heavy metals were measured in the clay except for Cu at Site 3, and Cr and Cu at Site 5. In the case of Site 7 it is evident that the concentration in the clay fractions was for nearly all the metals measured, considerable higher than those in the sediment >2mm.It is further clear that Fe, Al and Mn were present in the high concentrations at all the sites and are according to the literature some of those elements that are of those most abundant in the environment (Chapman, 1998, Rosseland, Eldhuset and Staurnes, 1990**)** The phenomenon that these metals are also adsorbed by, amongst others, sediment particles, organic material,(Lin and Chen, 1998) sulfide ions (Fu and Wang, 2011) not toxic to macroinvertebrates while As and Cd were present in relatively small concentrations.

From Table 2 it is obvious that quartz was dominant at all the sites, representing a maximum of 99.8% of all the minerals at Site 8 to a minimum of 66,5% at Site 7. Muscovite was present at six of the eight sites namely at sites 2-7. Kaolinite was the third most abundant and was present at all the sites except Sites 4, 6 and 8 hematite and pyrophyllite were only present at Site 1. Montmorillonite and chlinochore were only found at Site 4, goethite at Site 6, chrysolite at Sites 7 and 8 and magnetite and calcite also only at Site 7. Muscovite was, although not detected at Sites 1 and 8, the most abundant at the remaining sites and varied from 23% (Site4) to 8% at Site 7.

Taking the heavy metals identified in the sediment samples into account it is clear that these metals are also present in number of the identified minerals including, Fe in hematite, goethite, chrysolite and magnetite Al in kaolinite, pyrophyllite, muscovite and montmorillinite, Na, Ca, and Mg in montmorillinite and Mg, Al. From these results it is however, in most of the cases, not possible to distinguish between metals forming an integral part of the mineral crystals and those adsorbed by the mineral itself. It is however possible that all the metals can be released into the water by means of amongst others, weathering, erosion and acid rain deposition and can thus reach concentrations that may be toxic to macroinvertebrates.

Conclusion From this study it seems clear that there was a strong association between high concentrations of heavy metals and sediment particles < 2mm. It is further evident the secondary minerals identified mainly consist of those metals nor regarded as toxic to aquatic organisms. This was also confirmed by the elemental pomposition of the clay collected at the various sites.

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5. Acknowledgements

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Table.1 The percentage clay and the heavy metal concentrations in the total sediment as well as clay samples

	mg/kg	Al	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Cd	Pb	Ti	Particle sizes %
H1	Total	37298.8	506.88	20360.12	133201.2	106.92	257.49	214.19	343.38	45.65	3.57	145.43	392.524	25.28
	Clay	60208.87	296.67	9416.83	101337.71	36.26	119.92	1893.17	230.08	35.91	2.92	183.25	345.6042	74.72
H3	Total	23707.2	578.56	2871	92963.2	64.13	131.75	180.17	92.44	44.25	2.62	75.76	180.576	7.53
	Clay	27064.4	625.24	3121.36	113480.4	75.75	151.36	163.02	104.24	48.61	2.68	101.49	211.948	92.43
H5	Total	31636	534.82	3544.64	109894.4	63.25	126.9	189.51	148.28	44.77	2.78	99.8	572.088	19.83
	Clay	46068	443.17	6750.92	119323.6	65.46	153.21	179.78	206.1	46.88	2.92	363.62	723.8	80.17
H7	Total	16361.23	317.43	1831.51	48231.63	38.19	85.77	93.37	83.02	23.21	2.31	27.12	111.23	4.76
	Clay	42631.6	402.34	1343.85	77792	51.9	178.64	171.25	136.84	26.45	2.76	173.04	224.18	95.33
H8	Total	43256.4	459.36	626.47	73568	60.71	194.88	208.69	116.65	14.73	1.94	73.96	191.9588	1.4
	Clay	36330.8	399.74	601.96	63113.6	48.32	172.7	180.36	141.33	13.29	1.87	45.24	167.816	98.59

Table 2 The minerals and its metal composition identified at each of the sites expressed as a percentage of all the minerals at a specific site.

Sample Identification	H1	H2	H3	H4	H5	H6	H7	H8	
Mineral Identification	Percentage Values (%)								Metal composition
Quartz	66.5	73.4	85.5	69.8	80.3	79.9	85.2	99.8	Si
Hematite	2.2								Fe, Mn
Kaolinite	21.1	1.8	3.7		4.7		2.8		Si, Al, Fe, Mg, Ca K,
Pyrophyllite	10.2								Si, Ti, Al, Fe,
Muscovite		24.7	10.8	23.3	15	18.3	8		Si, Al, Mg, Na, K
Montmorillonite				0.7					Si, Al, Fe, Mg, Ca, Na, K
Clinochlore				6.2					Si, Al, Fe, Cr,
Goethite						1.7			Si, Fe
Chrysotile							0.1	0.2	Si, Mg,
Magnetite							1		Fe, Mn, Mg, Zn, Ni, Al, Cr
Calcite							2.9		Fe, Mg, Ca

O-13

Levels of Pesticide Residues in Fruits and Vegetables from Markets in Dar es Salaam, Tanzania

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The aim of this study was to assess the levels of contamination by pesticide residues in fruits and vegetables from markets in Dar es Salaam city. Fresh fruits and vegetables from selected major local markets were analysed for eighteen (18) organochlorine, organophosphorus and pyrethroid pesticide residues. Pesticide residues were detected in 81.7% of all the samples. The detected pesticide residues were *p,p'*-DDT, *p,p'*-DDD, *o,p'* DDD, *p,p'*-DDE, α-HCH, α-endosulfan, β-endosulfan, chlorpyrifos and cypermethrin. Their highest concentrations varied from 0.002 mg/kg to 3.81 mg/kg. The concentrations of some of the contaminants in the samples were above the maximum residue limits (MRLs). The findings indicated risks and concerns for public health.

Keywords: Pesticide, Contamination, Fruits, Vegetables, Tanzania

1. Introduction

Pesticides are widely used in fruits and vegetables to control pests and diseases during farming, transportation and storage. Pesticides are known to be the most important tool for the production of adequate food supply for an increasing world population and for the control of vector-borne diseases (EPA, 2005). However, pesticides have some toxicological and environmental consequences, which include toxic residues in food substances and adverse effects on non-target organisms. The gross and improper use of synthetic pesticides is a matter of much concern. Pesticides have been associated with a wide variety of human health hazards, ranging from acute impacts such as headache, vomiting and diarrhoea to chronic impacts like cancer, reproductive harm, and endocrine disruption. Many people die from pesticides poisoning and other people suffer from various health effects (WHO, 2010).

Fruits and vegetables are among the most frequently consumed food types in Tanzania. As fruits and vegetables are eaten either fresh or semi-processed and due to improper agricultural practices, it could be expected that they contain high pesticide residue levels. Dar es Salaam is a big city hosting major transportation and commercial networks, markets and industrial activities. Among the largest and busiest markets in Tanzania are found in Dar es Salaam. These markets are well known for their massive sales of fruits and vegetables that come from different areas of the country where pesticides are widely and commonly used. Thus, assessment of pesticides in samples from these markets could reflect the contamination status in the area and other areas.

2. Materials and Methods

2.1 Sampling

Samples were collected from Buguruni, Mwananyamala, Ilala and Kariakoo markets in Dar es Salaam city. The selected vegetables and fruits collected were tomatoes (*Lycopersicon esculenta*), cabbage (*Brassica oleracea var. capitata*), spinach (*Spinacia oleracea*), watermelon (*Citrullus lanatus*) and onions (*Allium cepa*). Sampling was conducted by applying standard guidelines (Åkerblom, 1995) with some modifications. Sixty samples were collected. The samples (200-500 g each) were separately wrapped in aluminium foil and placed into polythene bags. The samples were kept deep frozen until extraction was performed. Sample extraction was conducted within 24 hours after sampling.

2.2 Sample Homogenization, Extraction and Clean-up of Extracts

Each sample was homogenized by using a clean motor and pestle. The homogenized sample (20 g) was extracted with acetone (20 mL) and a mixture of dichloromethane: cyclohexane (1:1, 20 mL) by sonication in ultrasonic bath for 30 min. The mixture was filtered through glass wool containing sodium sulfate for drying. The extract was concentrated in a rotary evaporator operated at 40 ºC. Clean-up was conducted using activated florisil topped up with sodium sulfate in a chromatographic column; eluted with cyclohexane: acetone (9:1, 20 mL) and concentrated to 2 mL (Åkerblom, 1995).

2.3 Analysis and Quality Assurance

Instrumental analyses of all the samples were conducted at Chemistry Department, University of Dar es Salaam using a gas chromatograph coupled to a mass spectrometer (GC-MS) equipped with a capillary column (Rtx-5MS) and an autosampler.

Analytical quality assurance included analysis of matrix blanks, recovery tests, determination of detection limits and calibration. All sample types were analysed concurrently with matrix blanks. A mixture of pesticide standards was spiked into blank samples. The spiked samples were prepared/ processed and analysed just like the ordinary samples. No significant peaks were found in the blanks. The mean percentage recoveries (n = 10) for the analytes ranged from 71.2% to 110% and were within an acceptable range. Method detection limits for the compounds analysed were established based on a 3:1 signal to noise ratio. Every signal below this limit was treated as not detectable. The detection limits ranged 0.0001 – 0.0007 mg/kg for most compounds and 0.0017 – 0.0036 mg/kg for endosulfans.

3. Results and Discussion

Nine pesticides and metabolites were detected in the samples. Their detection frequencies varied from 8.33% to 91.7% in the species and the overall detection was 81.7%. *p,p'*-DDT was detected in 8.33% of cabbage samples, *p,p'*-DDE was detected in 8.33% of watermelon samples and α-HCH was detected in 16.7% of the watermelon samples. Other compounds were detected as summarised below in cabbage, spinach, onion, tomato and watermelon samples, respectively:
p,p'-DDD: 83.3%, 75%, 50%, 91.7% and 66.7%;
o,p'-DDD: 8.33%, 16.7%, 0%, 0% and 16.7%;
α-Endosulfan and β-Endosulfan: 33.3%, 33.3% 16.7%, 50% and 16.7%;
Chlorpyrifos: 33.3%, 41.7%, 25%, 41.7% and 50%;
Cypermethrin: 25%, 33.3%, 16.7%, 33.3% and 33.3%. The most frequently detected compounds were *p,p'*-DDD and chlorpyrifos.

The compounds aldrin, dieldrin, fenitrothion, pirimiphos methyl and most of the HCH isomers (β-HCH, γ-HCH and δ-HCH) were not detected in all samples. This suggested that they were not used for the fruits and vegetables.

The highest concentrations of the pesticides and metabolites (mg/kg) varied as follows:
p,p'-DDT: up to 0.004 in cabbage;
p,p'-DDD: 0.01 (onions) to 0.64 (spinach);
o,p'-DDD: up to 0.01 (spinach);
p,p'-DDE: up to 0.002 in watermelon;
α-Endosulfan: 0.22 (onions) to 0.59 (cabbage);
β-Endosulfan: 0.07 (onions) to 0.21 (cabbage);
Chlorpyrifos: 2.12 (onions) to 3.81 (watermelon);
Cypermethrin: 0.014 (onions) to 0.05 (watermelon) and α-HCH: up to 0.004 in watermelon.

The DDT residues were mostly represented by DDD suggesting that the degradation of DDT was dominated by anaerobic pathway. The concentrations of α-endosulfan were higher than β-endosulfan in all samples. This represents the technical formulation, which suggests recent inputs (ATSDR, 2013). The concentrations of chlorpyrifos were higher than other pesticide residues. This represents the current use of the pesticide as it is allowed in horticultural production in Tanzania.

Generally there were no significant variations of all pesticide residues among fruits and vegetables (One way ANOVA, F (4, 55) = 0.3454–1.170, p = 0.3341–0.8461), indicating that the same pesticides were applied on them. Therefore, the findings suggested similar contamination patterns for all pesticides and metabolites among the fruits and vegetables investigated. The results from statistical analyses also showed no significant variations of the pesticide residues among all the sampling sites indicating that the fruits and vegetables from all the markets had similar sources.

The concentrations of the compounds detected were generally greater than those found in studies in other countries. The concentrations of contaminants were above the MRLs in 8.33–50% of the samples. These findings suggest possible health risks to the consumers. The levels of contamination found could be linked to improper farmer practices i.e., the farmers may not be following good agricultural practices. It might be partly due to their ignorance about judicious use of pesticides.

The levels of contamination have been indicated to be generally high, suggesting the need for remedial or control measures.

4. Acknowledgments

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O-14

Distribution of *tdh* gene encoding thermostable direct hemolysin in *Vibrio paraehemolyticus* isolates of marine fish in Alexandria, Egypt

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Marine fish is a food substrate for zoonotic *Vibrio parahaemolyticus* that cause food poisoning and diarrhea in humans. A total of 170 marine fish samples including Gilthead sea bream and European seabass (85, each) were collected then cultured on Thiosulphate Citrate bile salts sucrose agar media. The isolation percentages of *V. parahaemolyticus* were 22.3% and 14.1 % in Gilthead sea bream and European seabass, respectively. Regarding the molecular detection of *tdh* gene among *V. parahaemolyticus* isolates, only 5 out of 31 isolates (16.1%) were positive for *tdh* gene. Also, *tdh* gene was detected in 3 out of 19 *V. parahaemolyticus* isolates (15.8%) from Sea bream and in 2 out of 12 isolates (16.7%) from Seabass. This study confirmed that the PCR- targeted the detection of *tdh* virulent gene is a more reliable technique in distinguishing the pathogenic V. parahaemolyticus of marine fish that poses a public health hazard.

Keywords: *Vibrio parahaemolyticus*, Sea bream, Seabass, Thermostable direct hemolysin gene.

1. Introduction

Vibrio parahaemolyticus is a human pathogenic Gram-negative halophilic bacterium, a natural inhabitant of the marine environment and could be found in crabs, shrimps, marine fish, oysters, mussels and other seafoods. *Vibrio parahaemolyticus* is an important enteric pathogen that induces gastroenteritis and traveler'diarrhoea in humans. This microrganism was first discovered in Japan in 1950 with a food poisoning case associated with the consumption of raw or undercooked seafood and food recontaminated with the bacterium after cooking.

The pathogenicity of this microorganism in humans is correlated with the production of thermostable direct hemolysin (TDH) and TDH-related hemolysin (TRH). Many studies have reported that virulent strains of *V. parahaemolyticus* possess either the gene *tdh* or *trh*, or both (Lee and Pan, 1993). In Egypt, Little literature is available on the occurrence and molecular detection of *V. parahaemolyticus* positive isolates for *tdh* gene in shellfish (Abd-Elghany and Sallam, 2013). Thereby, the objectives of this study to investigate the distribution of *tdh* gene in *V. parahaemolyticus* isolated from two species of marine fish particularly *Gilthead* sea bream (Sparus aurata) and *European seabass (Dicentrarchus labrax)* in Alexandria, Egypt.

2. Materials and Methods

2.1 Sampling, isolation and identification

One hundred and seventy marine fish samples including Gilthead sea bream (Sparus aurata) and European seabass (Dicentrarchus labrax) (85, each) were collected from Borg-El Arab city at Alexandria, Egypt. Five grams of individual fish flesh were homogenized in 45 ml of 3% NaCl containing 1% alkaline peptone water (APW, pH: 8.6),then incubated at at 37°C for 18 hr. The fish homogenate were inoculated on Thiosulphate Citrate bile salts sucrose agar media (TCBS, Hi Media, India) followed by incubation at 37°C for 18 hrs (Colakoglu et al., 2006). The colonies were grown at various salt concentrations by transferring colonies into tubes containing peptone water and 0%, 3%, 6% and 10% NaCl, then followed by incubation of tubes at 37°C for 24 hrs. The bacterial colonies growing on TCBS plates were streaked onto Trypticase Soya agar slants (TSA; Oxoid, UK) supplemented with 2% NaCL, and followed by incubation at 37°C for 24 hrs for further biochemical identifications.

2.2 Molecular detection of pathogenic *V. parahaemolyticus* carrying *tdh* gene

The template DNA of *V. Parahaemolyticus* isolates was extracted using QIA amp DNA Mini Kit (QIAGEN, Germany). The sequences of primers ; TDH-L, ′GTA AAG GTC TCT GAC TTT TGG AC 3′ and TDH-R, 5′TGG AAT AGA ACC TTC ATC TTC ACC 3′ (Bio Basic Inc. ,Canada) were used to amplify *tdh* gene in *V. Parahaemolyticus* (Bej et al. ,1999). The program of PCR reaction was optimized at 95°C for 15 min as initial denaturation, and then followed by 30 cycles of 94°C for 30 sec as denaturation, 60°C for 30 sec as annealing and 72°C for 45 sec as extension. The final extension was followed at 72°C for 10 min. The amplicon was electrophorezed in 1.5% agarose gel containing

0.5 X TBE at 70 volts for 60 min., and visualized under ultraviolet light. A positive control of *V. parahaemolyticus* isolate was kindly supported by Department of Zoonoses, Faculty of Veterinary Medicine, Zagazig University.

3. Results and Discussion

Vibrio parahaemolyticus is a zoonotic pathogen that occurs naturally in marine and estuarine environment and has been implicated in seafood poisoning in many countries including Japan, USA, India and Taiwan. Based on the cultural and biochemical properities, the overall incidence rate of *V. parahaemolyticus* in marine fish was 18.2% (31 out of 170). Moreover, *V. parahaemolyticus* was isolated with percentages of 22.3% (19/85) and 14.1 % (12/85) in Gilthead sea bream and European seabass , respectively. All isolates of *V. parahaemolyticus* are not considered pathogenic, but the pathogenic strains are characterised by production of TDH (thermostable toxins) and/ or TDH related hemolysin (TRH) encoded by *tdh* and *trh* virulent genes, respectively. Out of 31 isolates of *V. parahaemolyticus* from marine fish, only 5(16.1%) were positive for *tdh* virulent gene using PCR (Table 1). The positive *V. parahaemolyticus* isolate (tdh^+) was confirmed by presence of 269 bp DNA fragment (Figure 1). In this study, *tdh* gene was detected in 3 out of 19 *V. parahaemolyticus* isolates (15.8%) from Sea bream and in 2 out of 12 isolates (16.7%) from Seabass.

Table 1. Distribution of *tdh* gene among pathogenic *V. parahaemolyticus* isolates from marine fish

Marine fish	Number of isolates (%)		
	Total isolates	tdh^+ (%)	tdh^- (%)
Sea bream	19	3 (15.8)	16(84.2)
Seabass	12	2(16.7)	10(83.3)
Total	31	5(16.1)	26(83.8)

Previous studied recorded lower incidence rates for *tdh* gene than that found in this study. Out of 27 *V. parahaemolyticus* isolates from shellfish, Egypt; three(11%) were positive for *tdh* and *trh* genes (Abd-Elghany and Sallam, 2013). In another study, out of 20 sea fish, PCR gave 4 (11.4%) positive result for *tdh* gene (Nelpati and Krishaiah, 2010). Also, lower positivity of *tdh gene* among *V. parahaemolyticus* (11.1%) was cited in sea fish (Raghunath et al., 2008). Otherwise, higher percentage (20%) for *tdh* gene in the confirmed *V. parahaemolyticus* islotes of marine fish was recorded (Baffone et al., 2000).

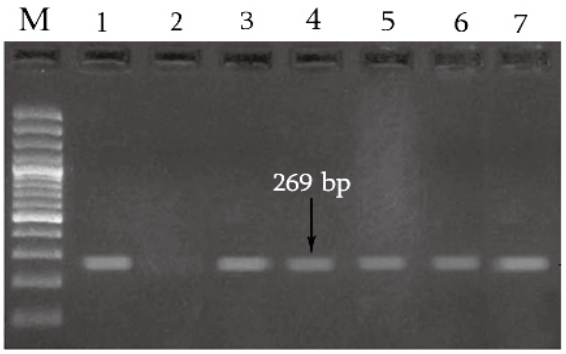

Figure 1:. Molecular detection of *tdh* virulent gene in confirmed *V. parahaemolyticus* isolates of marine fish.
M: 100 bp DNA ladder; Lane 1(positive control); lane 2 (negative control); lanes 3, 4&5(positive *tdh* in isolates from Sea bream); lanes 6 &7 (positive *tdh* in isolates from Seabass).

In conclusion, the PCR- targeted the detection of *tdh* gene is more accurate and reliable technique in identifying the pathogenic *V. parahaemolyticus* of marine fish that poses a a zoonotic risk and seafood borne illness in Alexandria, Egypt.

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O-15

Dietary exposure to mycotoxins by adult consumers of locally processed rice from Nigeria

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Locally processed rice collected from five agro-ecological zones (AEZ) of Nigeria were analysed for some important mycotoxins by a liquid chromatography tandem mass spectrometric method. The data obtained was subsequently used to determine the probable dietary intake (PDI) of the mycotoxins. The range of mycotoxin contamination was between 0.27 ng/g for sterigmatocystin and 464 ng/g for zearalenone. The PDIs of the mycotoxins varied significantly ($p<0.05$) across the zones and the the mean national PDIs for total fumonisin , ochratoxin A, deoxynivalenol, zearalenone, sterigmatocystin, beauvericin, nivalenol and moniliformin was estimated to be 19.13, 1.50, 5.97, 157.36, 24.85, 15.19, 20.81 and 39.77 ng/kg bw/day respectively. The study showed that daily intake of mycotoxins especially aflatoxins and zearalenone from the locally processed rice may predispose consumers to harmful health effect of mycotoxins.

Keywords: Rice, Agro-ecological zone, Aflatoxins, Zearalenone, Probable daily intake

1. Introduction

Rice is a very important staple food in the diet of Nigerians. The Food and Agriculture Organization of the United Nations estimated that approximately 25% of the cereals produced in the world are contaminated by mycotoxins, the toxic secondary metabolites of fungi.

One of the main routes of human exposure to contaminants is the diet and mycotoxins have been ranked as the most important chronic dietary risk fact. There is dearth of information on mycotoxin exposure through rice consumption in Nigeria despite the fact that it is a staple food.

In this study we determined the levels of some important mycotoxins in locally processed rice samples from Nigeria and calculated the rate of dietary exposure to mycotoxins by adult consumers

2. Materials and methods

2.1 Collection and preparation of samples

Milled rice samples were collected from five out of the seven AEZs (Sudan Savannah (SS) = 7, Northern Guinea Savannah (NGS) = 4, Southern Guinea Savannah (SGS) = 4, Derived Savannah (DS) = 11 and Humid Forest (HF) = 12) of Nigeria. The details of sample collection, geographical location, temperature and rainfall patterns of the zones are as reported by Abdus-Salaam et al. (2015). Samples were not collected from the Sahel and Mid- Altitude zones of the country due to the security challenges of these zones. Samples were collected from different processing centres, where rice are traditionally soaked, parboiled, dried and milled within the AEZs and milled rice samples collected from available commercial processors. The subsamples were further pooled together to form a composite sample per centre.

2.2 LC-MS/MS quantification

Samples (5g) were extracted with (acetonitrile/ water/acetic acid 79:20:1, v/v/v) mixture, diluted with the same volume of dilution solvent and the extracts injected. The LC-MS/MS screening of target microbial metabolites was performed with a QTrap 5500 LC-MS/MS System (Applied Biosystems, Foster City, CA, USA) equipped with TurboIonSpray electrospray ionisation (ESI) source and a 1290 Series HPLC System (Agilent, Waldbronn, Germany). Chromatographic separation was performed at 25°C on a Gemini® C18-column, 150 × 4.6 mm i.d., 5 µm particle size, equipped with a C18 4 × 3 mm i.d. security guard cartridge.

2.3 Exposure assessment (PDI)

The exposure assessment of adult rice consumers in Nigeria to the mycotoxins was determined by the method of Liu and Wu (2010). Briefly, the mycotoxin exposure was estimated by multiplying the mycotoxin concentraion of the rice grains in the AEZs by the consumption rate of rice in Nigeria (87.67g/person/day, as estimated by USAID, 2009) and the product divided by the average weight of an adult Nigerian (60kg).

3. Results and Discussion

Table 1 shows the mean and maximum

concentration of the mycotoxins as well as the probable daily intake (PDI) in ng/kg bw/day. Both the mean and maximum PDI of the mycotoxins differed significantly ($p<0.05$) across the zones. The national dietary exposure to AFs was 20.78 ng/kg bw/day. Table 1 also showed that adult consumers at the NGS zone had the highest exposure to aflatoxins while those in the SS zone had the highest exposure to total fumonisin. In contrast, the HF zone had the lowest exposure to total fumonisin and BEA.

The national PDI of total fumonisin (95.65 ng/kg bw/day) was much lower than the provisional maximum tolerable daily intake (PMTDI) of 2 µg/kg bw/day proposed by the JECFA (2001).

The mean national dietary exposure to OTA (2.99 ng/kgbw/day) was below the TDI of 17 ng/kg bw/day specified by EFSA (2006a).

The national PDI of DON (11.93 ng/kg bw/day) reported in this work was below the TDI limit of 1 µg/kg bw/day set by SCF (1999). The national dietary exposure to ZEA was 786.80 ng/kg bw/day, which was much higher than the 25 µg /kg bw/day specified by EFSA, (2006a).

Similarly, the national dietary exposure to STER and BEA was 124.24 and 75.94 ng/kgbw/day respectively, which was lower than hypothetical value of 0.1 µgkgbw/day suggested by Rodriguez-Carrasco et al. (2013).

The national dietary exposure to NIV (83.22 ng/kg bw/day) was below the recommended 1.2 µg/kgbw/day recommended by EFSA (2013a).

The study showed that intake of low, daily doses of mycotoxins especially aflatoxins from the locally processed rice may predispose consumers to harmful health effect of mycotoxins, most especially chronic aflatoxicosis

Table 1: Exposure assessment (ng/kg bw/day) of rice by Nigerian adult consumers

AEZ	$^{1}AF_T$ ^{2}(ng/g)	^{3}PDI AF_T	^{4}Total FB (ng/g)	PDI FB_T	OTA (ng/g)	PDI OTA	DON (ng/g)	PDI DON	ZEA (ng/g)	PDI ZEA	STERY (ng/g)	PDI STERY	BEA (ng/g)	PDI BEA	NIV (ng/g)	PDI NIV	MON (ng/g)	PDI MON
SS	-	^{7}-	23.37	34.14^{e}	-	-	-	-	6.26^{b}	9.14^{b}	0.34^{b}	0.52^{b}	3.96^{c}	5.78^{c}	-	-	9.78^{a}	14.31^{a}
NGS	-	-	60.71^{e}	88.73^{e}	-	-	-	-	8.88^{b}	12.97^{b}	0.36^{a}	0.52^{a}	14.27^{c}	20.86^{c}	-	-	41.55^{c}	60.71^{c}
	37.04^{e}	^{5}10.66^{d}	8.61^{b}	12.57^{b}	0.59^{b}	0.86^{a}	5.76^{c}	8.42^{a}	464.17^{e}	678.25^{e}	41.98^{e}	61.36^{e}	34.72^{e}	50.73^{e}	36.32^{e}	53.07^{d}	43.11^{d}	62.97^{d}
	20.17^{e}	^{6}29.48^{d}	20.32^{b}	29.67^{b}	0.59^{b}	0.86^{a}	5.76^{c}	8.42^{a}	927.85^{c}	1355.74^{e}	124.96^{e}	182.58^{e}	131.01^{e}	191.42^{e}	42.17^{e}	61.64^{d}	110.27^{d}	161.15^{d}
SGS	2.87^{d}	4.17^{c}	16.46^{d}	24.05^{d}	-	-	-	-	4.11^{a}	5.98^{a}	0.27^{a}	0.37^{a}	2.63^{b}	3.84^{b}	2.72^{b}	3.96^{a}	21.23^{c}	31.01^{c}
	6.12^{d}	8.94^{c}	35.58^{c}	51.97^{c}	-	-	-	-	8.53^{a}	12.48^{a}	0.65^{b}	0.95^{b}	5.35^{b}	7.81^{b}	2.72^{b}	3.96^{a}	26.04^{a}	38.04^{a}
DS	1.97^{b}	2.88^{a}	11.93^{c}	17.43^{c}	-	-	2.40^{b}	3.51^{b}	56.11^{d}	81.96^{d}	39.51^{d}	57.73^{d}	8.78^{d}	12.85^{d}	11.78^{d}	17.24^{c}	51.68^{e}	75.51^{e}
	3.71^{c}	5.42^{b}	55.08^{d}	80.48^{d}	-	-	2.40^{b}	3.51^{b}	231.74^{d}	338.61^{d}	77.87^{d}	113.81^{d}	73.22^{d}	106.96^{d}	26.82^{d}	39.16^{c}	143.03^{e}	208.97^{e}
HF	2.12^{c}	3.07^{b}	5.11^{a}	7.46^{a}	1.46c	*2.13^{b}	-	-	7.85^{c}	11.47^{c}	2.93^{c}	4.26^{c}	1.41^{a}	2.74^{a}	6.13^{c}	8.95^{b}	10.27^{b}	15.03^{b}
	3.67^{b}	5.35^{a}	13.03^{a}	19.03^{a}	1.46^{c}	*2.13^{b}	-	-	62.96^{c}	91.97^{c}	10.96^{c}	16.07^{c}	3.53^{a}	5.15^{a}	9.44^{c}	13.77^{b}	36.08^{b}	52.74^{b}
NATIONAL	14.00	20.78	65.48	95.65	2.05	2.99	8.16	11.93	538.50	786.80	85.03	124.24	51.50	75.94	56.95	83.22	136.07	198.83
	33.67	49.19	184.72	269.88	2.05	2.99	8.16	11.93	1239.96	1811.77	214.80	313.93	227.38	332.20	81.15	118.53	356.97	521.61

Values with different superscript along the same columm are significantly different at $p<0.05$. [1]Sum of aflatoxin B_1, B_2, and G1.. [2]Concentration of mycotoxins (ng/g). [3]Probable daily intake of mycotoxin. [4]Sum of fumonisin B_1, B_2 and B_3. [5]Mean mycotoxin concentration [6]Maximum mycotoxin concentration. [7]less than the limit of detection . *One sample only with detectable level of mycotoxins

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O-16

Risk Characterization of Pesticide Residues in *Tympanotonus fuscatus* (periwinkle) obtained from selected markets in Benin City, Edo State, Nigeria.

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A study was carried out to access the residual levels of pesticides in *Tympanotonus fuscatus* with the aim of estimating the carcinogenic and non-carcinogenic effects that could arise through consumption. Samples were collected from major markets in Benin City Edo state, Nigeria. Extraction and subsequent clean-up of pesticides was done while subsequent detection and quantification was done using a Gas Chromatograph equipped with an Electron capture detector (ECD). Cancer and non-cancer risk was estimated using the estimated daily intake (EDI) and the cancer bench mark concentration (CBC) respectively. 16 pesticides residues ranging from organochlorines, organophosphate, triazines and carbamates were detected at varying concentrations in *Tympanotonus fuscatus* obtained in Benin City. On a market comparison, *Tympanotonus fuscatus* obtained from Oba market had the highest total pesticide (2.5 µg/kg ww) although there was no significant difference ($p>0.05$) in the concentration of pesticide residues between the markets. The organochlorine pesticide; Alpha HCH (0.3 µg/kg ww) was the most dominant pesticide in all samples. Non-carcinogenic estimates showed that the consumption of *Tympanotonus fuscatus* contaminated with pesticides residues will not pose any non-carcinogenic effect as HQ and HI values were below the threshold of 1. However, the hazard ratios (HRs) based on cancer risk were greater than 1.0 for Heptachlor epoxide, Aldrin and Dieldrin, indicating carcinogenic effects to consumers through *Tympanotonus fuscatus* consumption.

Keywords: Non-cancer risk, cancer risk, daily intake, *Tympanotonus fuscatus*.

1. INTRODUCTION:

Pesticide contamination of major food stuffs in Nigeria and the world at large has been on the increase in an alarming rate. This might be attributed to an increase in the use of pesticides during agricultural activities in an attempt to boost productivity and achieve food security (Ntow *et al.,* 2007). Reports have shown that these pesticides are used without proper monitoring or adherence to good agricultural practices (GAP). In Nigeria, there is also the unwanted proliferation of banned and toxic pesticides in local markets available to farmers due to the down turn in the economy, poor awareness of existing enforcement agencies, bottle necks in the enforcement of the regulations; illegal trade and porous borders (PAN, 2007). This situation has led to the high distribution of pesticides in the environment and since most organic pesticides are highly resistant to degradation, their presence in food is very difficult to avoid (Rychen et al., 2014). This is worrisome because food stuffs are the primary route of human exposure to POPs from numerous chemical classes (Vogt et al., 2012). Despite the fact that several studies have determined the presence of pesticides in food stuffs, the risk of cancer and non-cancer effects of such exposure remains an unexplored area in most studies in Nigeria. This assessment is necessary because of the tendency of pesticides to accumulate and persist in body tissues, leading to acute or chronic health effects (Pardío et al., 2012). *Tympanotonus fuscatus* (periwinkles) are marine gastropod molluscs known as edible sea snails (Fasakin 2015). They are rich in protein, hence widely consumed in Nigeria especially states in the Niger Delta region including Edo State. However, its ability to bioaccumulate toxic substances from its habitat makes consuming them a source of concern. This study is therefore aimed at determining the concentration of pesticide residues in *Tympanotonus fuscatus* obtained from selected markets with the attendant health risk that could arise from continuous consumption.

2. METHODOLOGY

Samples of *Tympanotonus fuscatus* were obtained from four major markets (Uselu, Oba, New Benin and Oliha markets). Samples were extracted and cleaned up and analysed for pesticides. Corresponding results were obtained using a Hewlett-Packard (hp) 5890 Series II equipped with 63Ni Electron Capture Detector (ECD) of activity 15 mCi with an auto sampler (USEPA, 2007).

Risk Characterization.

***Non-Carcinogenic risk*:** Hazard quotient (HQ) was

estimated by using the ratio of estimated daily intake (EDI) to acceptable daily intake (ADI). A ratio less than 1.0, can be concluded that there is essentially no probability of adverse effect. However, if the ratio exceeds 1.0, then there is potential for adverse effects (US EPA, 2000). Estimated daily intake was obtained using the formula

$$\mathbf{HQ} = \frac{\mathbf{EDI}}{\mathbf{ADI}}$$

Where $\mathbf{EDI} = \frac{\mathbf{C \times CR}}{\mathbf{BW}}$ (Dougherty et al., 2000)

Where C is the concentration of pesticide µg/kg), CR is the consumption rate of periwinkles (kg d^{-1}) and BW is body weight (Kg).

***Carcinogenic risk*:** the hazard ratios for estimating carcinogenic effects (HRs) were calculated using the equation below

$$\mathbf{HQ} = \frac{\mathbf{EDI}}{\mathbf{CBC}}$$ (Dougherty et al., 2000)

Where CBC is the cancer bench mark concentration and calculated using he formula

$$\mathbf{CBC} = \frac{(\frac{\mathbf{RL}}{\mathbf{OSF}}) \times \mathbf{BW}}{\mathbf{CR}}$$ (Dougherty et al., 2000)

Where RL is the risk level ($1x10^{-6}$), OSF is the oral slope factor (kg d^{-1}).

3. RESULTS AND DISCUSSION

Analysis of *Tympanotonus fuscatus* shows varying concentration of pesticides comprising of alpha HCH, gamma HCH, Heptachlor, Heptachlor epoxide, Endosulfan I, Endosulfan II, Endosulfan aldehyde, Endosulfan sulfate, Aldrin, Dieldrin, Endrin, DDT, Diazinon, Phosphomethylglycine, Carbofuran, and Atrazine. Samples obtained from Oba Market had the highest concentration of pesticide residues. While alpha HCH (0.32 µg/kg) was the most dominant pesticide, making up 14% of the total composition of pesticide residues. The presence of pesticides in periwinkles indicates the bioaccumulation of pesticides from the natural habitats of these organisms.

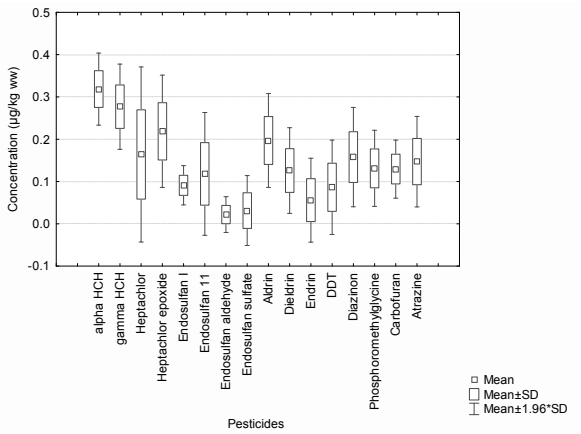

Fig 1: Mean concentration of pesticides residues in *Tympanotonus fuscatus*

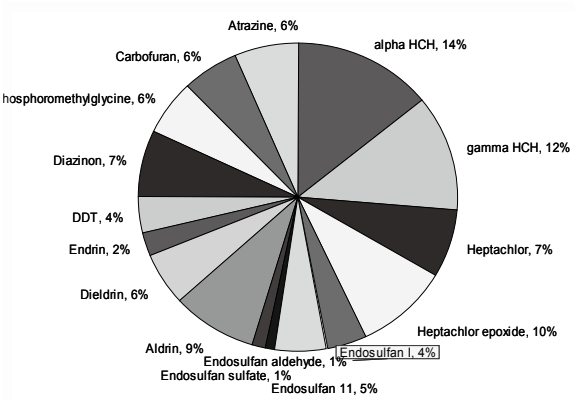

Fig 2: percentage distribution of pesticides residues in *Tympanotonus fuscatus*

Risk Characterization

Food consumption has been identified as an important route of human exposure to pesticides (Pardío et al., 2012). In this study, non-carcinogenic risk estimations showed that estimated dietary intake (EDI) was lower than the acceptable daily intake while HR for each pesticide was below the threshold value (1). The presence of pesticides in *Tympanotonus fuscatus* demonstrates that there are no potential for adverse non-carcinogenic effects. However, carcinogenic risk assessment showed that HR estimated for Aldrin (2.5) and Dieldrin (1.5) where above the threshold value (Fig 3). This is further confirmed by the hazard index (6.2) which is above the threshold of 1. These HR values indicate that the cancer benchmark concentrations exceeded the EDI for the pesticides in *Tympanotonus fuscatus,* thus raising serious concerns of possible carcinogenicity.

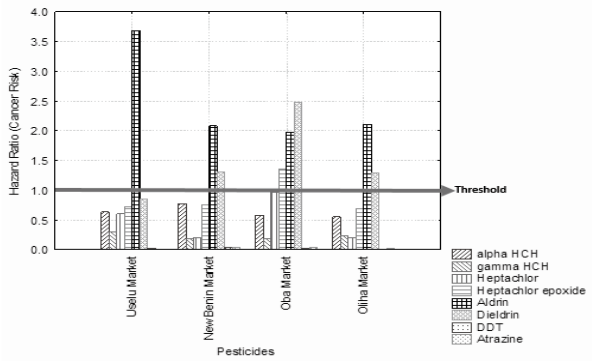

Fig 3: Risk Characterization of pesticides residues in *Tympanotonus fuscatus*

Conclusion:

This study has shown the levels of contamination of *Tympanotonus fuscatus* obtained from major markets in Edo State with pesticides. Although concentrations where minute, health risk estimations showed that there is a great potential for carcinogenic effects through the consumption of contaminated *Tympanotonus fuscatus.*

This findings suggest that there is a need to

monitor the distribution of pesticides in various environmental samples in other to ensure public health safety. It's also imperative that extension services be extended to rural farmers, to ensure proper usage of pesticides during food production.

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O-17

Basic sciences for safety of glutamate and MSG: intestinal metabolism of dietary glutamate in rats

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Glutamate is one of major excitatory neurotransmitters and exposure of excessive glutamate or its salts such as MSG cause neural cell death via its excitotoxic activity. However, glutamate intake from general foods never causes neural cell death despite the fact that glutamate is the most abundant amino acid in foods. Since almost all dietary glutamate is metabolized in the first pass by the gut, glutamate intake does not change plasma concentrations of glutamate. This is why glutamate intake does not affect internal glutamatergic neurons. The present study investigated intestinal metabolism of dietary glutamate using stable isotope techniques in rats. Fischer strain male rats were given fixed amounts of amino acid defined diet containing [U-^{13}C] or [^{15}N] glutamate hourly. Amino acid concentrations and their isotopic enrichments in portal and arterial blood were measured to calculate their net releases into portal blood. The results indicated that: 1) half of dietary glutamate carbon was metabolized into CO_2 in the gut, but only 8% was released into the portal vein. 2) glutamate nitrogen was incorporated into various amino acids such as alanine (75%) and citrulline (8%) in the gut, but only 4% was released into the blood stream. 3) plasma glutamate concentrations did not increase after feeding despite glutamate is major dietary amino acid in this study. These results indicated intensive intestinal metabolism of dietary glutamate and suggested crucial roles of the gut in the maintenance of glutamate homeostasis in the circulation. Since similar results were reported also in piglets and human, such active intestinal metabolism of glutamate would be common features in all mammals. Metabolic features of dietary glutamate would be one of important basis for safety of glutamate and its salts such as MSG existing in the foods regardless of their origin from the nature or added ones.

Keywords: monosodium glutamate, food additive, gut, nutrient, blood brain barrier

1. Introduction

Glutamate is one of major neurotransmitters and exposure of excessive glutamate or its salts such as monosodium glutamate (MSG) causes neural cell death via its excitotoxic activity. However, glutamate intake from general foods never causes neural cell death despite the fact that glutamate is the most abundant amino acid in foods. This is because of the strict regulation on glutamate homeostasis in the body. Our fundamental interest is how glutamate homeostasis is maintained despite the presence of quantitative variation of dietary input of glutamate. Therefore we studied the fate of dietary glutamate using stable isotope techniques. Rats were fed diets containing [U-^{13}C]glutamate or [^{15}N]glutamate, and the intestinal metabolism of dietary glutamate-carbon (C) and –nitrogen (N) was estimated. Further, we estimated incorporation of plasma glutamate into brain across the blood brain barrier (BBB) to understand how neurons are protected from possible elevation of blood glutamate.

2. Materials and Methods

2.1 Animals and Experimental Procedure

Male 6 week-old Fischer (F344) rats (Charles River Japan, Atsugi, Japan) were fed a purified diet based on AIN-93G containing crystalline amino acids (values in g/kg; arginine: 4.3, histidine: 2.8, isoleucine: 6.2, leucine: 10.7, lysine hydrochloride: 11.5, methionine: 6.5, cysteine: 3.3, phenylalanine: 6.8, tyrosine: 3.4, threonine: 6.2, tryptophan: 2.0, valine: 7.4, alanine: 4.0, aspartate: 4.0, glycine: 6.0, proline: 4.0, serine: 4.0, asparagine monohydrate: 4.6, glutamate: 20.0 and glutamine: 20.0) following the recommendations of the National Research Council. The rats were adapted to these conditions for 2 weeks and were then fed hourly with 1.0 g (approximately 80% of ad libitum intake) of an experimental diet in which half of or all of the natural glutamate was replaced with [U-^{13}C]glutamate or [^{15}N]glutamate after a 15 h fasting period. The rats were anesthetized by ether after 0, 0.5, 1, 1.5, 3, 6 or 9 h of feeding, and blood was simultaneously collected from the portal vein and the descending aorta. Plasma was separated and stored at –80°C until analysis.

2.2 Analysis

Concentrations of ammonia, urea and amino acids were measured using automated amino acid analyser (Hitachi). The isotopic enrichments of ammonia, urea, acidic and neutral amino acids in the plasma were measured using GCMS (Agilent Technologies). The isotopic enrichments of plasma arginine, ornithine and citrulline were determined using LC-MS/MS (Agilent Technologies). $^{13}CO_2$

enrichment was measured using isotope ratio mass (Thermo Fischer Scientific).

2.3 Estimations

Releases of individual ^{13}C and ^{15}N-metabolites from the gut were estimated from their arterial-portal concentration differences and portal blood flow. Brain uptakes of individual amino acids were estimated from their plasma concentrations and kinetic constants for the transporters in the BBB reported by Smith Q.

3. Results and Discussion

Although hourly ingestion of experimental diet increased concentrations of circulating essential amino acids such as methionine, phenylalanine, leucine, isoleucine and valine, no increase was seen in glutamate concentrations (Figure 1). Tracer study using [U-^{13}C]glutamate indicated that most of dietary glutamate-C was metabolized to CO_2 and other metabolites such as alanine, lactate and proline and that only minor part was released into portal vein (Figure 2) as was reported in piglets and preterm infants. Study using [^{15}N]glutamate indicated that virtually all the dietary glutamate-N was utilized for the synthesis of other amino acids such as alanine and citrulline (Figure 2). These results that intensive metabolism of glutamate in the gut would contribute to the maintenance of postprandial glutamate homeostasis in the circulation. Further the results indicated contribution of dietary glutamate to energy production and supply of amino-N for the synthesis of other amino acid synthesis in the gut.

Estimation of cerebral uptakes of amino acids indicated virtually no blood glutamate enters into the brain across the BBB, while significant amount of neutral and basic amino acids enters into the brain (Figure 3). Thus limited transport of amino acids across the BBB also would contribute to the maintenance of glutamate homeostasis in the brain.

These metabolic features of glutamate in the gut and brain would be the basis for safety of glutamate and its salts such as MSG existing in the foods regardless of their origin from the nature or added ones.

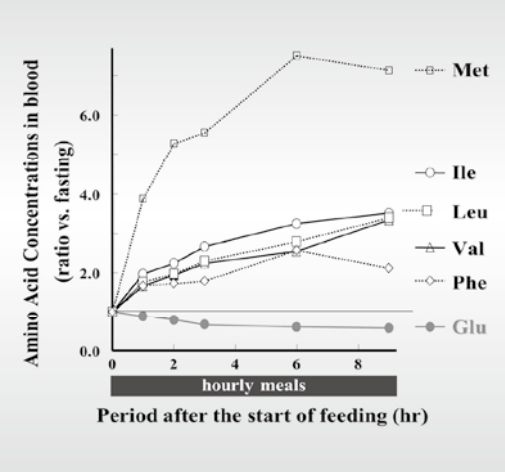

Figure 1: Amino acid concentrations in arterial plasma before and during hourly feeding

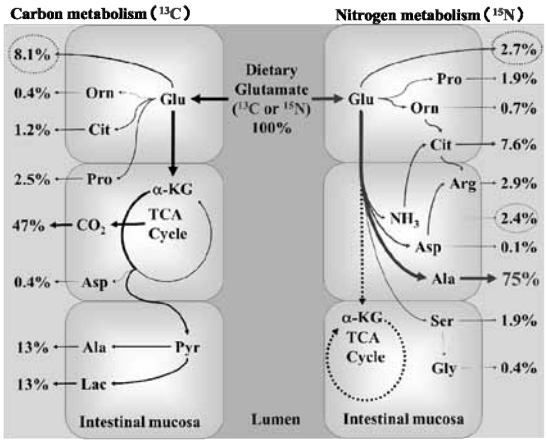

Figure 2: Fate of dietary glutamate-C& N in the gut

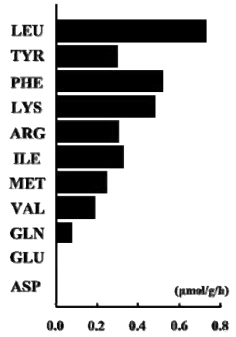

Figure 3: Estimation of cerebral uptakes of amino acids across the BBB

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O-18

Effects of Per-household treatments on chlorpyrifos residues in Lettuce (Lactuce sativa)

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The study was organized to evaluate the extent of accumulation of chlorpyrifos on *Lactuca sativa* during cultivation and also examine the effect some pre-household treatment procedure on residue levels. Concentrations of Chlorpyrifos applied at different stages of growth of lettuce were examined using GC equipped with LCD. at different time intervals of 1 h 24 h and 7 days after pesticide application. The results showed that residue levels detected at 1 h and 24 h after application were all above the MRL of 0.05 $mgkg^{-1}$ and can pose health risk to consumers while that recorded 7 days after application were far below the MRL. Accumulation of chlorpyrifos on crop during cultivation was not observed since no significant differences were observed 7 days after application at all the different stages. There is therefore the need for farmers to allowed 7 days' re-entry intervals before harvesting. All the pre-household treatment procedures caused significant reduction in residues levels. Treatment T_3 was most effective in removing residues. Hence to reduce the risk associated with intake of chlorpyrifos through lettuce, T_3 procedure should be followed before consumption.

Keywords: Chlorpyrifos, Consumers, Health risk, Removal, Treatments

1. Introduction

Consumption of diets high in fresh vegetables such as lettuce by Ghanaians is growing because vegetables are known to contain high amount of vitamins, antioxidants and dietary fiber which is linked to lower incidence of cardiovascular disease and obesity (Slavin and Lloyd, 2012). However, pests and diseases militate against the successful cultivation of the crop. As a result, pesticides are use (Gerken et al.,2010). In Ghana, pesticide residues including that of chlorpyrifos have been found at concentrations above the acceptable limits in lettuce (Amoah *et al*., 2006), that have not been subjected to any household treatment procedures. Studies have shown that pre-household treatments help to reduce pesticide residues (Kim et al., 2015). The effects of pre-household treatments on residue levels are important in evaluating the risk associated with ingestion of pesticides contaminated vegetable. But information on household treatment procedure on chlorpyrifos residue in lettuce is scarce. The objectives of the study were to measure chlorpyrifos residue levels in lettuce and examine the effect of pre-household treatment procedures on the residues.

2. Materials and Methods

Fresh lecture samples were collected at different time intervals of 1 h, 24 h and 7 days after pesticide application at three different stages of growth. Samples were then cut into small pieces, and sub-samples subjected to three (3) pre-household treatment procedures, thus: washing under tap water (T_1), dipping 2% salt solution (T_2) and dipping in 1% detergent solution ($T_{3)}$. Control samples were analysed without any pre-household (T_0). FAO/ WHO procedure for extraction and clean-up of pesticide residues was followed with little modification. Residues of chlorpyrifos levels were analyzed using GC equipped with ECD.

3. Results and Discussion

Mean chlorpyrifos residues detected after the various household treatment and that of the control at all the three (3) different stages of growth are presented in Table 1. The mean concentrations of chlorpyrifos in the control samples, at 1 h and 24 h after application at all the stages of growth were above the MRL of 0.05$mgkg^{-1}$. These levels reduced significantly to values far below the MRL, 7 days after application. The mean concentrations of chlorpyrifos in samples from T_1 at 1 h and 24 h after application for the 3 stages of growth were lower than the MRL value. But their differences with T_0 were not significant ($P > 0.05$). It was noted that washing with tap water reduced chlorpyrifos levels to 4 - 16%. This may be due to the types of adjuvants added during formulation, insolubility nature of chlorpyrifos in water and nature of the surface of the lettuce leaves. It is lined with thing layer of cuticle which makes the binding of the compound to the surface stronger.

Relatively lower levels of chlorpyrifos were detected in the samples that were taken at 1 h after application and treated with T_2 when compared with the control. For treatment T_2, percentage reduction was found to be in the range of 35-43% in the samples that were collected 1 h and 24 h after application at all the 3 growth stages. Levels of chlorpyrifos residues in all the samples were far below MRL set for chlorpyrifos on lettuce after

treatment T_2 and T_3. Treatment T_3 reduced the residues by a mean percentage of 54.4%. This show that, detergent is more effective in reducing chlorpyrifos levels on lettuce than washing with tap water and with salt solution. According to Hui *et al*., 2010, hydrolysis of chlorpyrifos occurs readily at pH > 7. Detergent which was used in T_3 caused an increase in the pH of solution. This resulted in destabilizing chlorpyrifos residue levels on the lettuce resulting in its higher removal.

Chlorpyrifos had direct contact with foliage and remained on the surface after application. This was evident from the results since high residue level were observed 1 h after application. Gradual but continuous deterioration of the chlorpyrifos residues on the lettuce were observed as a function of time. Mean percentage reduction of 38.1% and 96.3% were observed 24 hours and 7 days respectively after application on the control samples. The decrease may be due volatilization and/or photodegradation. Chlorpyrifos adsorbed on leaf surfaces are usually lost through volatilization especially in hot climates (Roberts and Hutson, 1999). Obviously, a higher temperature tends to favour volatilization and photodegradation of pesticides from plants, because the vapour pressure of the pesticide compound is temperature-dependent and additionally the adsorption to the leaf surface decreases with increasing temperature.

Stage of growth at which pesticide is applied and frequency of application during growth are important factor affect residue levels and dissipation rate on a crop. In this work relatively higher concentration of residues were observed on the stage 1 samples than stage 2 and stage 3 samples that were collected at 1 hour after application. This may be due to accumulation of applied pesticide on the foliage which have small surface area. But at the second and third stages the leaves sizes were larger and therefore the applied pesticides spread to cover the whole surface thereby reducing the residue levels. The rete of pesticide degradation was fast with the second and the third stages of the growth than the first stage. This is because larger foliage sizes at the second and the third stages provided large surface areas for volatalisation and photodegradation. Mean percentage reduction of 38.1% and 96.3% were observed 24 hours and 7 days respectively after application on the control samples.

4. CONCLUSION

Residue levels of chlorpyrifos were above the MRL, 1 h and 24 hours after pesticide application in the control samples but reduced significantly after 7 days. Therefore, it is important to allow at least a 1-week (7 days) withholding period. Effects of pre-household treatment on chlorpyrifos residue in samples, indicated that residues levels were reduced significantly by washing with salt solution and 1% detergent solution. Tap water washing did not show any significant effect on the chlorpyrifos levels. Hence to reduce the risk associated with intake of chlorpyrifos through lettuce, mild detergent washing should be carried out. This must be followed by thorough rinsing with tap water otherwise the detergent may be consumed together with the vegetables.

Table 1: Chlorpyrifos residue levels (mg kg^{-1}) in treated and control lettuce samples at different stages of growth

	Stage 1			Stage 2			Stage 3		
Treatments	1 hr	24 hr	7 days	1 hr	24 hr	7 days	1 hr	24 hr	7 days
Control (T_0)	0.059	0.052	0.006	0.055	0.053	0.007	0.055	0.034	0.002
T_1	0.049	0.048	0.006	0.052	0.046	0.005	0.053	0.03	0.002
T_2	0.039	0.023	0.004	0.041	0.035	0.004	0.043	0.025	0.002
T_3	0.018	0.01	0.003	0.028	0.027	0.004	0.027	0.019	0.001

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O-19

Application of random amplified polymorphic DNA (RAPD) to detect genotoxic effects by cassava effluents

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The random amplified polymorphic DNA (RAPD) assay was used to assess the level of DNA polymorphism in onion roots exposed to three types of processed cassava effluents at 0.001, 0.01 and 0.1% (v/v) concentrations. Compared to the control, the DNA obtained caused great changes in the RAPD patterns. These were discernible with appearances/disappearances of bands in the treated plants. A total of 89 RAPD bands were obtained using five oligonucleotide primers; 44 (49.4%) of these showed polymorphism. Onion bulbs exposed to *garri* effluent produced 37 RAPD bands; 19 (51.35%) of which were polymorphic; plants treated with *akpu* effluent produced 31 RAPD bands and 12 (38.71%) of these were polymorphic while those treated with *lafun* effluent showed 21 RAPD bands; 13 (61.90%) of which were polymorphic. The loss and gain of bands decreased with increase of effluent concentration. The genetic distances shown on the dendrogram revealed that genotoxicity of the effluents were concentration-dependent and they caused different types of mutations. The data obtained from the RAPD screening implied that the cassava processing effluents should be properly treated to minimize and possibly eliminate the risks associated with its discharge into the environment as the wastewaters are capable of inducing genotoxic effects.

Keywords: Processed cassava effluents, random amplified polymorphic DNA (RAPD), toxicity, DNA damage

1. Introduction

Although Nigeria is the world leader in cassava (*Manihot esculenta* Crantz) production, majority of cassava produced in the country is used for human food as *gari*, *lafun* and *fufu* (IITA, 2010). During the processing, large amounts of the effluents generated are discharged into the public sewers and nearby shallow wells, thereby polluting the soil, ground water, streams or rivers due to high solid content in the effluents (Afuye and Mogaji, 2015) and oil and grease from the lubricated parts of the grinding machines (Olukanni, et al., 2013).

Among the DNA based techniques in molecular biology for DNA analysis in eco-genotoxicology, the Random amplified polymorphic DNA (RAPD) technique has the potential to detect a wide range DNA damage as well as mutation in plant seedlings (Liu *et al.*, 2007).

Cassava effluents have been implicated in the cytotoxicity and induction of chromosome aberration in onion (*Allium cepa* L.) root tips in previous studies (Olorunfemi et al., 2011). This study was undertaken to evaluate the genotoxic effects of three types of processed cassava effluents (*gari*, *lafun* and *fufu*) on onion roots exposed to the effluents using the RAPD technique.

2. Materials and methods

2.1 Plant material and sample collection

Average-sized onion bulbs were purchased in Benin City, Nigeria and sun-dried for two weeks. The dried roots at the base of the onion bulbs were carefully shaved off with a sharp razor blade to expose the fresh meristematic tissues.

Fresh effluents of processed *garri* and *akpu* were obtained from small-scale cassava processing mills in Uselu community (6°15′N, 5°25′E) in Edo State while the *lafun* effluent was obtained from Oba-Akoko ($7^0$31'N, $5^0$78'E) in Ondo State. The effluents were scooped from different positions in the mills into washed plastic containers and used for the RAPD assay.

2.2 DNA extraction and RAPD finger printing

Root tips of (2.5-3.5 cm long) obtained from the suspension of onion bulbs on the effluent samples were collected, ground in liquid nitrogen, and the total genomic DNA extracted by a CTAB method with minor modifications (Qari, 2010; Olorunfemi et al. 2014). The DNA purity was determined by measuring the optical density using the nanodrop spectrophotometer at 260 nm/280 nm ratios. The quality of DNA was determined using gel electrophoresis and observing under a UV illuminator.

The conditions of DNA amplification followed the procedure of Williams *et al.* (1990) with some modifications (Qari, 2010). RAPD was performed using primers OPA2 (TGCCGACTG), OPA6 (GGTCCCTGAC), OPA9 (GGGTAACGCC), OPA10 (TGTCTGGATG) and OPA11 (CAAATCGCCGT) (Operon technologies Inc., Alameda, California, USA) for each amplification.

PCR amplifications of microsatellite loci were performed on a thermocycler (A & E Laboratories, UK Model Cyl-005-1). For comparison, DNA molecular size marker (1 kb) was used for each agarose gel.

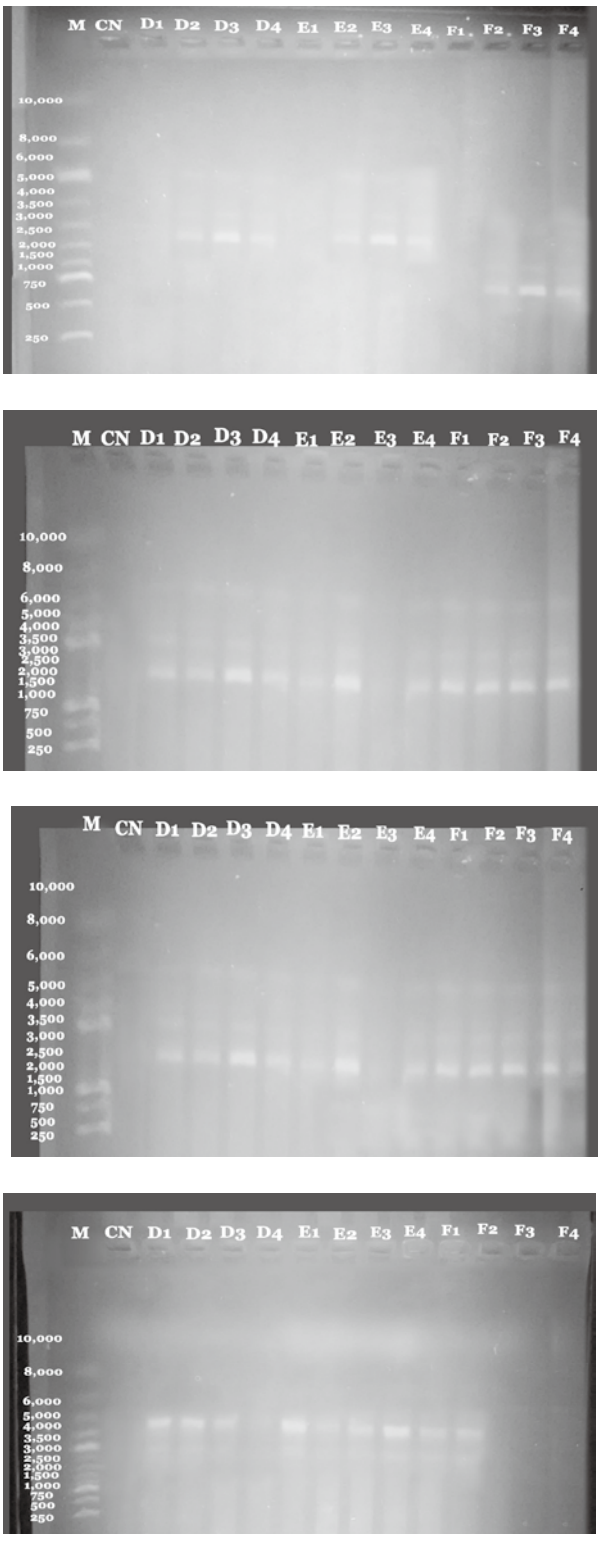
The percentage genomic template stability was calculated as 100 − (100 **a**/**n**), where **a** was RAPD polymorphic profiles detected in each treated sample while **n** was the total number of bands in the control. Polymorphisms observed in RAPD profiles include disappearance of a normal band and appearance of a new band in comparison with control RAPD profiles (Williams *et al*., 1990).

3. Results and discussion

The range of DNA purity extracted from the onion root tips in the test and control samples was 1.76 - 1.89. The RAPD profile obtained with the five oligonucleotide primers used produced bands between 500 and 6000 bp in length. Four of these primers amplified, the most amplified being OPA2; OPA6 did not (Plates 1-4). In all, 89 bands were scored, 44 (49.4%) were polymorphic. Onion bulbs exposed to *garri* effluent produced 37 RAPD bands, 19 (51.35%) of which were polymorphic; plants treated with *akpu* effluent produced 31 bands and 12 (38.71%) of these were polymorphic while those treated with *lafun* effluent showed a total of 21 RAPD bands, 13 (61.90%) of which were polymorphic. The loss and gain of bands decreased with increase of effluent concentration.

The genetic distances shown on the dendrograms revealed that genotoxicity of the effluents were concentration-dependent and that the three types of effluents caused different types of mutations (Fig. 1).

DNA-RAPD fingerprinting has been used by several workers as a biomarker assay to detect DNA damage and mutational events in cells of *Allium cepa* (Olorunfemi et al. 2014). In this study, DNA damage/polymorphism was evident in RAPD profiles via appearance or disappearance of bands compared with the control.



Plates1-4: RAPD profiles of genomic DNA from *A. cepa* root cells suspended in cassava effluents at different concentrations using primers OPA-2, OPA-9, OPA-10 and OPA-11 respectively.

Disappearing bands are likely due to changes in oligonucleotide priming sites, originated from rearrangements and less likely from point mutations and DNA damage in the primer binding sites (Liu et al. 2007).

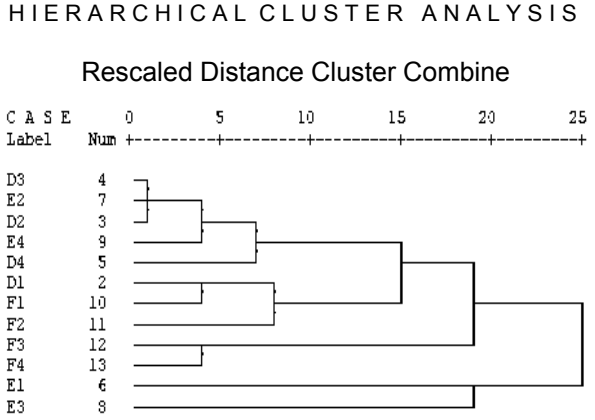


Figure 1: UPGMA dendrogram showing clustering of control and treated samples of *A. cepa*. D1: *Garri* (Control), D2: *Garri* (0.001), D3: *Garri* (0.01), D4: *Garri* (0.1), E1: *Akpu* (Control), E2: *Akpu* (0.001), E3: *Akpu* (0.01), E4: *Akpu* (0.1), F1: *Lafun* (Control), F2: *Lafun* (0.001), F3: *Lafun* (0.01), F4: *Lafun* (0.1).

The cluster analysis method is considered as one of the most effective methods in numerical analysis regarding band scoring and analysis of RAPD fingerprinting. In this study, cluster analysis was done to estimate the level of DNA polymorphism between the control plant and those treated with the cassava effluents. The observed wider generic distances between control and untreated effluents are indicative of genotoxic effects of heavy metals and other substances present in wastewaters (Olorunfemi et al. 2011).

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O-20

Creating a model of renal damage in chicken and glycomic approach to identify novel biomarkers for kidney injury in birds

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Kidney injury in avian species is caused by various factors, such as diclofenac (NSAIDs) and heavy metals. However, evaluation of levels of renal damage is difficult in avian species and identification of a novel renal biomarker is needed. Glycomics has been at the forefront of biological and medical sciences and glycans are used as biomarkers of carcinoma in human. In this study, chicken model of renal damage was created by the administration of diclofenac sodium. From the results of histopathological examination, kidney-damaged chickens were selected for analysis of plasma *N*-glycan using the glycoblotting method. In our chickens, 38 *N*-glycans were detected and levels of 16 glycans were changed between pre- and post-administration in kidney-damaged chickens. Kidney injury was associated with increased levels of both sialylated and non-fucosylated glycans. Some of these glycans increased in patients of renal carcinoma. Therefore, these 16 glycans have the possibility to be a novel kidney biomarker in avian species.

Keywords: Avian species, Renal biomarker, Glycan

1. Introduction

In avian species, kidney injury is caused by various factors. Diclofenac, one of the non-steroidal anti-inflammatory drugs (NSAIDs), has caused serious damage to kidney of vultures in the Indian subcontinent (Oaks et al. 2004). In addition, heavy metals, such as lead (Pb) and mercury (Hg) cause renal damage in birds. Birds have a unique kidney structure and kidney is exposed to various materials which are carried by the abundant blood flow.

Evaluation of levels of kidney dysfunction is difficult in avian species. Although uric acid (UA) levels in blood are typically used as an indicator, altered level of UA can only be detected when 70% or more kidney function is lost. In mammals, blood urea nitrogen (BUN), creatinine concentrations is generally used for monitoring renal function. However, in avian species, both BUN and creatinine concentrations has limited diagnostic value because their levels are normally low. Therefore, identification of the novel kidney biomarker is needed to monitor the kidney function of birds.

Recently, Glycomics has been at the forefront of biological and medical sciences, and gave insight into the biological significance of plasma *N*-glycome in human health and disease. Therefore, there is a possibility that glycans of avian species would also become a kidney biomarker.

2. Materials and Methods

Male Rhode Island Red chickens (*Gallus gallus domesticus*) (n=11, 10 weeks) were randomly assigned to three groups and injected with either 20% Dimethyl sulfoxide (DMSO) or diclofenac sodium which was diluted by 20% DMSO in the pectoral muscle once daily in morning for four consecutive days. The following groups were considered: control group (n=2, ID: Cont.-1, 2, 3): injected 20% DMSO; low dose group of diclofenac sodium (1.5 mg/kg body weight) (n=4, ID: Low-1, 2, 3, 4); high dose group of diclofenac sodium (2.0 mg/kg body weight) (n=4, ID: High-1, 2, 3, 4).

Blood was collected every morning (24, 48, 72 and 96 h after first exposure) and UA, AST, ALP, ALT, TPP, BUN, creatinine, LDH, CPK, TCHO, iP, Ca, Na, and K levels were performed by Cobas Ready® or DRI-CHEM 7000V. After euthanasia, six different tissues were dissected, including kidney, liver, lung, heart, spleen, and muscle. The excised tissues were stored in 10% neutral buffered formalin for histopathological analysis. Paraffin-embedded kidney sections were stained with periodic acid-

Schiff and the other tissue sections were stained with haematoxylin and eosin.

For the glycomic analysis, four chickens which had renal dysfunction and two control chickens were selected. Plasma specimens were pretreated for the release of *N*-glycans and directed to glycoblotting for the enrichment and quantification of *N*-glycans prior to the MALDI-TOF/MS analysis.

3. Results and Discussion

In this study, elevation of UA levels was confirmed in chicken Low-1, High-1, High-2 and High-4, indicating severe renal damage. Another indicator of renal lesion; iP, Na, and K levels changed in Low-1 and High-1. Levels of iP and Na in High-2 altered slightly. Histopathological analysis showed that High-1 and Low-1 had severe renal damage, such as infiltration of heterophils to tubulointerstitium and proximal tubular lumens, dropping cells in proximal tubular lumens, and dilation of proximal and distal tubular lumens (Fig. 1a and b). High-2 chicken also showed severe damage and High-4 showed mild degenerative lesions (Fig. 1c, 1d). The other chickens of injection groups had milder degenerative lesions than High-4.

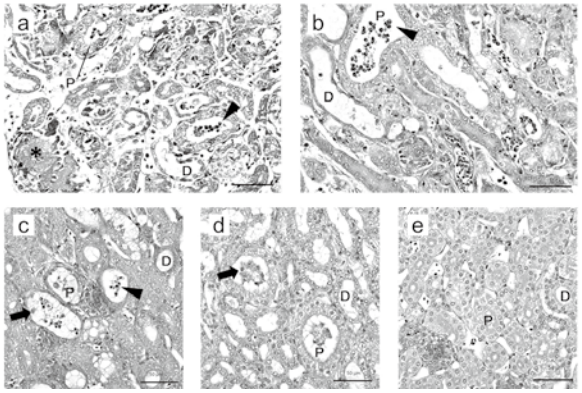

Figure 1. Histopathological features of kidneys in diclofenac-treated or control chickens.

From the plasma of chickens, 38 *N*-glycans were detected. There were 16 glycans which expression increased or decreased between pre- and post-administration in diclofenac injection group. High damaged kidney was associated with increased levels of both sialylated and non-fucosylated glycans. Although this tendency was not applicable to five glycans, the other 33 glycans correspond with this rule. Especially, No. 17 and 27 glycans showed high expression levels and the levels of these two glycans occupied approximately 50-70% of total glycans levels. The glycan of No. 27 has a full length of non-fucosylated glycans and No. 17 has a defect of one sialic acid from full length in synthesis pathway of glycans.

In human, increase of three *N*-glycans (No. 8, 26 and 29) had significant intergroup differences between patients with renal cell carcinoma and healthy volunteers (Hatakeyama et al. 2014). Moreover, three *N*-glycans (No. 12, 17 and 29) showed higher levels in patients of clear cell renal cell carcinoma although the changing of No. 17 levels were different depend on the structure (Gbormittah et al. 2014). From our study, we could identify the presumptive composition of each glycan but not accurate structure. However, original levels of No. 17 and 27 were higher than other glycans and there was correlation between No. 17 and renal damage score (ρ = 0.93, p = 0.008). Therefore, these glycans have the high potentiality to become the renal biomarker in birds. 16 glycans which levels increased or decreased in chickens are needed to be analyzed in various avian species for clinical use.

4. Acknowledgments

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Poster Session

P-1

Monitoring of organic contaminants in the Ethiopian Rift Valley Lakes using Semi Permeable Membrane Devices

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Semi permeable membrane devices (SPMDs) were deployed for one month (September to October, 2009) at 2 sites in Lakes Hawassa, Ziway and Koka, Ethiopia, and subsequently extracted for analysis of organochlorine pesticides (OCPs) and polychlorinated biphenyles (PCBs). The predominant OCPs in SPMDs deployed in these Lakes were DDT which comprises more than 66% of the total OCPs detected and followed by endosulfan 23%. The mean concentrations of OCPs, in general, were higher in the SPMDs deployed in Lake Ziway than in Lakes Hawassa and Koka, which reflects continuing discharges and in-place of OCPs from the local farm and glass houses. However, the concentrations of PCBs were higher in the SPMDs deployed in Lake Hawassa than Lakes Ziway and Koka, which reflects continuing, discharges of PCB from nearby city given that Lake Hawassa is an urban lake. At sites from the Lake, the concentration of OCPs and PCBs is in, general, decrease with distance from site of discharge i.e. from point and non-point sources. The result indicates that SPMDs are useful in situ monitoring devices for determining spatial trends in the contamination of the aquatic environment for the possible management and conservation action, but several factors can modify rates of uptake into these devices.

Keywords: SPMD, Rift Valley Lakes, OCPs, PCBs

1. Introduction

Lakes Hawassa, Ziway and Koka are part of the ERVLs which have a significant environmental, economic and cultural importance to the region. Due to the intensive agricultural and deforestation activities in the catchments of these lakes and the pressure from the expansion of human settlement and urbanization, there is a risk of chemical pollution from fertilizers, pesticides and industrial wastes. Run off and erosion from the surrounding catchment could release organic chemicals sequestrated in the soil to the lake may enter the food chain, and reaching out to the fish. Semi-permeable membrane devices (SPMDs) are a tool for passive, in situ monitoring of concentrations of organic contaminants in the aquatic environment. This device mimics the processes by which aquatic organisms bioconcentrate hydrophobic pollutants in the aquatic systems (Huckins et al., 2003). Therefore, the objective of the present study is monitoring these lakes using SPMDs to take the possible management and conservation action.

2. Materials and Methods

The SPMDs were deployed at the lakes in September to October, 2009 in cylindrical cage. Two cages, each containing one SPMD, were deployed at each of the 2 sampling sites of the lakes. As soon as SPMDs are recovered from the environment, they were taken out of the cages on-site and immediately placed and sealed in the original can and placed on ice in a cooler for shipping. SPMDs were stored in the sealed cans (shipping containers) in a freezer at -20^{o}C until analysis. To permit the recovery of residues, the SPMD were cleared up and analyzed following the method Huckins et al. (2003) at the laboratory of Norwegian Institute for Agricultural and Environmental Research (Bioforsk): Chemistry and Pesticide Section, Norway

3. Results and Discussion

The predominant OCPs in SPMDs deployed in the ERVLs were the DDT and followed by endosulfan (Figure 1). Reports from other African Lakes, for instance in Lake Malawi (Kidd et al. 2000) and in previous studies in the same Ethiopian Rift Valley Lakes: Koka, Ziway and Hawassa (Ermias Deribe et al. 2011; 2014) also indicated much higher levels of DDT in fish compared to other OCPs. Such high concentrations of DDTs may be attributed to the intensive and continuous use of DDT in vector control in the study areas and the use of endosulfan largely on horticulture farms. Moreover, endosulfan is a chlorinated insecticide that is chemically similar to DDT that may be used as alternatives to DDT in the study areas.

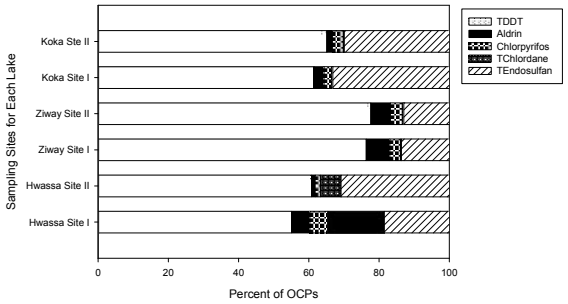

Figure 1. The percentage of OCPs in the SPMDs deployed in each sampling sites of the lakes.

The mean concentrations of OCPs were, in general higher, in the SPMDs deployed in Lake Ziway than Lakes Hawassa and Koka, which reflects continuing discharges and in-place OCPs from the local farms and green houses in the shoreline of Lake Ziway. However, the mean concentrations of PCBs were higher in the SPMDs deployed in Lake Hawassa than Lakes Ziway and Koka, which reflects continuing discharges and in-place PCB contamination from the urban settings, the fact that Lake Hawassa is being an urban Lake.

Table 1. The concentration of OCPs in ng/SPMD or ng/ 1 ml triolein.

Lakes	OCPs and PCBs	Site I	Site II	Mean
Hawassa	TDDT	131.3	86.7	109.0
	Aldrin	12.3	1.4	6.8
	Chlorpyrifos	12.2	2.2	7.2
	TChlordane	38.4	8.4	23.4
	TEndosulfan	44.0	43.9	44.0
	TPCB	89.3	11.1	50.2
Ziway	TDDT	204.3	194.9	199.6
	Aldrin	18.0	14.0	16.0
	Chlorpyrifos	8.1	8.5	8.3
	TChlordane	0.8	1.0	0.9
	TEndosulfan	36.4	32.5	34.4
	TPCB	12.4	6.6	9.5
Koka	TDDT	172.3	162.0	167.1
	Aldrin	8.0	4.0	6.0
	Chlorpyrifos	6.2	7.7	7.0
	TChlordane	0.7	0.7	0.7
	TEndosulfan	93.0	74.3	83.7
	TPCB	4.1	3.9	4.0

Another point worth mentioning is that, the mean concentration of OCPs and PBCs in SPMDs deployed at sites in the littoral zone is, in general, higher than in SPMDs deployed at the pelagic sites (Table 1). The reason could be linked to the fact that, the marked decrease in the mean concentrations of total OCPs and PCBs with increase in distance from the point and non point sources. Our result indicates that SPMDs are useful cost effective in situ monitoring devices for determining spatial trends in the contamination of the aquatic environment in Africa where there is limited resources, but several assumptions should be taken into contamination for its application.

4. Acknowledgments

This study was financially supported by the Norwegian Program for Development, Research and Higher Education (NUFU); Project ID: NUFU PRO 2007/10115.

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P-2

Health Risk Implication of Heavy Metal Concentrations in Surface Water, Shrimps (*Macrobrachium macrobrachion*) and Fish (*Brycinus longipinnis*) From Benin River.

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The distribution of heavy metals Manganese(Mn), Iron(Fe), Copper(Cu), Cadmium(Cd), Nickel(Ni), Lead(Pb), Cobalt(Co) and Zinc(Zn) levels in surface water, fish (*Brycinus longipinnis*) and shrimp (*Macrobrachium macrobrachion*) in Benin River was studied to ascertain the concentrations and health risk implication from consumption. Results showed that mean concentrations of heavy metals ranged from 0.01mg/L – 0.42mg/L (water), 0.93mg/kg – 114.78mg/kg (shrimp), and 0.98mg/kg – 66.13mg/kg (fish). Heavy metal levels in water were generally below recommended limit set by WHO and SON except for Cd, Ni and Pb. Shrimp and fish samples were generally above limits set by FAO and USEPA. Target Hazard Quotient (THQ) estimated for heavy metals in water (dermal exposure) and shrimp were below the threshold value of 1. However, THQ for heavy metals in water (oral exposure) and consumption of fish were above threshold value of 1 indicating health risk. Total Target Hazard Quotient (TTHQ) estimated for heavy metals (water and fish) were above 1 indicating potential non-carcinogenic health risk to consumers. Continuous monitoring of heavy metals in Benin River is of necessity in order to ensure the safety of aquatic organisms and humans who rely on aquatic resources.

Keywords: Heavy metals, fish, shrimp, health risk.

1. INTRODUCTION

Heavy metal contamination of aquatic inland and coastal ecosystems of the Niger delta region have in recent times received much attention due to anthropogenic activities like discharge of untreated industrial effluents, gas flaring, oil spills and sewage discharges. Aquatic systems in Nigeria are contaminated with heavy metals from industrial and agricultural activities (Ezemonye and Enuneku, 2012). The accumulation of heavy metal in tissues of organisms can result in chronic illness and cause potential damage to the population. Human exposures to heavy metals have become a major health risk (Yabe et al, 2011). The aim of this study was to assess the health risk from heavy metal contamination concentration in surface water, fish tissue and shrimp in Benin River, Koko, Delta State, Nigeria.

2. MATERIALS AND METHODS

2.1 Study Area

Benin River lies within longitude and latitude (Latitudes 05059'43.6'' – 05059'35.7''N; Longitude 005028'06.7''- 005025'56.2''E). It is a tributary of the Benin River The climate in this area is tropical with two main seasons; the wet (April –October) and dry (November – March) seasons. Along this stretch is located bitumen blending plant belonging to Total Nig. Ltd, facilities of Optima petroleum company and watercraft maintenance workshop. The river receives copious amounts of residential and industrial wastes with partial or no pre-treatment.

2.2 Sample collection/Preparation

Water samples were collected in 1 litre acid washed polyethylene bottles at a depth of 20cm, 10% HNO_3 was added insitu and transported to the laboratory. Samples were refrigerated at 4^0C until further analysis. The fish and shrimp samples were preserved in ice and taken to the laboratory. They were kept frozen in the refrigerator pending heavy metals analysis in the laboratory. Water samples were analyzed directly without further treatment. Fish and shrimp samples were oven dried at 105^0C to constant weighed, 0.5g was measured for digestion after homogenization according to procedures of AOAC (AOAC, 1990). Heavy metal levels in all matrices were analyzed using Buck scientific atomic absorption spectrophotopmeter, Model VGP 210.

3. Result / Discussion

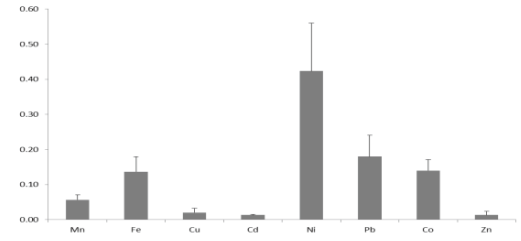

Figure 1: Mean heavy metal concentrations in surface water from Benin River.

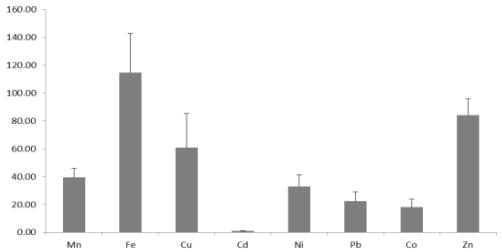

Figure 2: Mean heavy metal concentrations in *Macrobrachium macrobrachion* from Benin River.

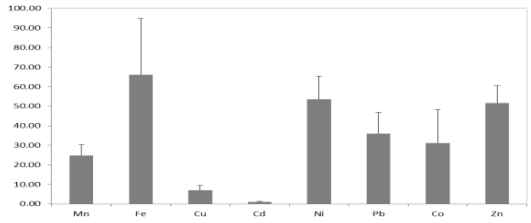

Figure 3: Mean heavy metal concentration in *Brycinus longipinnis* from Benin River.

There was significant difference in the concentration of heavy metals in water, shrimp and fish, with shrimp having the highest concentration. Nickel (Ni) was observed to be the most dominant heavy metal in water, while Iron (Fe) was most dominant in shrimp and fish. Heavy metal levels in water were generally below recommended limit set by World Health Organisation (WHO) and Standard Organisation of Nigeria (SON) except for cadmium (Cd), nickel (Ni) and lead (Pb). Shrimp and fish samples were generally above limits set by Food and Agricultural Organisation (FAO) and United States Environmental Protection Agency (USEPA). Health risk indices were reported as Target Hazard Quotient (THQ), and Total Target Hazard Quotient (TTHQ). Target Hazard Quotient (THQ) estimated for heavy metals in water (dermal exposure) and shrimp were below the threshold value of 1. However, THQ for heavy metals in water (oral exposure) and consumption of fish were above threshold value of 1 indicating health risk. Total Target Hazard Quotient (TTHQ) estimated for heavy metals in water (oral exposure) and consumption of fish were above 1 indicating potential non-carcinogenic health risk to consumers. The continuous monitoring of heavy metals in Benin River is of necessity in order to ensure the safety of aquatic organisms and humans who rely heavily on aquatic resources.

4. Acknowledgement

This project was funded by the University of Benin Tertiary Education Trust Fund (TETFUND) as research grant for the year 2014.

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P-3

Seasonal variation in metal concentrations and physico-chemical parameters of water and sediment in the Loop Spruit, North-West Province, South Africa.

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Unit for Environmental Sciences and Management, Potchefstroom Campus of the North-West University, South Africa.

This study was undertaken to investigate the seasonal variation and to determine the metal concentrations in the water and sediment of the Loop Spruit catchment. The study was conducted at six sampling sites situated in the Loop Spruit, Ensel Spruit and Kraalkop Spruit where water and sediment samples were collected and selected physico-chemical parameters were measured *in situ*. Water and sediment samples were analysed for metals and a total of 34 and 35 metals were detected, respectively. Only four elements including Cu, Zn, Se and P exceeded the Target Water Quality Guideline values set for South African freshwater. The fact that elevated levels of metal concentrations were measured during both surveys suggests that little to no seasonal variation was present. Sites where mining activity were present, were probably the most impacted, as reflected by the high metal concentrations, high EC and low pH values.

Keywords: Metal concentrations, river, water, sediment

1. Introduction

Anthropogenic activities, such as mining, urbanisation and industrialisation are the main factors that contaminate river systems all over the world. In South Africa, nearly 71% of the main river systems are considered as endangered or critically endangered due to these factors. It is thus crucial to monitor and protect river systems in order to maintain high quality freshwater for human consumption, as well as thriving ecosystems. The Loop Spruit is situated in one of the richest goldmine districts of South Africa and can possibly be impacted by mining effluent.

Little to no information regarding the metal composition in the water and sediment of this river system is available at present. This study thus aims to narrow the gap concerning the metal concentrations, as well as the possible origin and the influence of tributaries on metal concentrations in the river system.

2. Materials and Methods

2.1 Study Area

The study was conducted in the Gauteng and North-West Provinces, at six sampling sites in the Loop Spruit and selected tributaries (Ensel Spruit and Kraalkop Spruit).

Sites were selected based on the availability of water, accessibility to the river, mostly natural areas and most impacted areas. This was done in order to evaluate the possible influence of anthropogenic impacts on the river system. Site 1 is the Eye of the Loop Spruit, situated on the outskirts of the town of Fochville, with few to no impacts. Just 500 m downstream of Site 1 is Site 2, where an underground gulley spills mine effluent from Kloof goldmine into the Loop Spruit. Site 3 is situated in Kraalkop Spruit, which receives Mponeng, the world's deepest goldmines', effluent. Furthermore, this site is impacted by a scrapyard next to the sampling site. Site 4 is in the Ensel Spruit, a tributary of the Loop Spruit, which is considered as an unimpacted river. The site is located above the Klipdrift Dam, which supplies water for irrigation to farmers in the area.

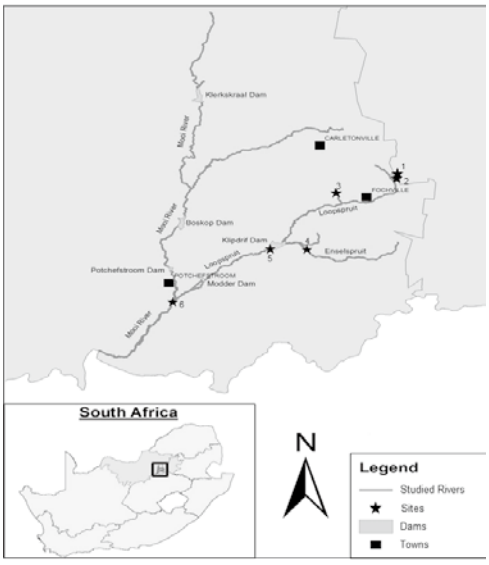

Figure 1: Sampling sites (stars) within the Loop Spruit catchment, North-West, South Africa.

Site 5 is below the Klipdrift Dam, which alters the flow of the river to an unacceptable level with highly impacted areas around the river due to farming activities. Site 6 is the last site in the Loop Spruit, just before its confluence with the Mooi River in the town of Potchefstroom.

2.2 Field Surveys

Two surveys were conducted, one during a high-flow period (April 2014) and one during a low-flow period (July 2014). Water and sediment samples were collected at each site in polyethylene bottles. Physico-chemical parameters of the water, including temperature, electrical conductivity (EC) and pH were measured *in situ* at all the sites, during each survey, with portable digital instruments.

2.3 Metal Analyses

Water samples were filtered through a 0.22 Whatmann filter paper and the filtrate was used directly for a baseline analysis in an Inductively Coupled Plasma Mass Spectrometer (ICP-MS), using standard recognised techniques. Sediment analyses were carried out after air drying the samples at 70°C for 48 hours before the EPA Method (3050B) was used to digest the samples for ICP-MS analyses.

Values were compared to existing target water quality guidelines (TWQG) as established for South Africa (Holmes, 1996). Only the metals that exceeded these values will be discussed.

3. Results and Discussion

A total number of 34 and 35 metals were detected in the water and sediment samples, respectively from all the sampling sites (data not shown). Concentrations in the sediment were significantly higher than those in the water. However, no guideline values for sediment have yet been established for South Africa.

When comparing and evaluating the metal concentrations to the water quality guidelines, four elements exceeded the guideline values. These include copper (Cu), zinc (Zn), selenium (Se) and phosphorous (P). Except for Se which exceeded the TWQG only during the high-flow, no other seasonal variation was evident. The high concentration of Se at Sites 3 and 5 may probably be ascribed to the surface runoff from the scrapyard and pesticides at the two sites, respectively. As the levels of Cu, Zn and P concentrations were higher than the TWQG at almost all the sites during both the high- and low-flow surveys, it might be attributed to natural sources like geological weathering. Elevated levels of Cu, Zn and P can, however, also be due to runoff surface water where pesticides and fertilizers are used. High P concentrations, indicating organic enrichment, can originate from the decomposition of organic material or the weathering of phosphorus bearing rocks.

The physico-chemical parameters varied considerably between the different sites, but not between the two seasons. The pH values were below the lowest TWQG during the high-flow surveys at Sites 2, 3 and 5 while the EC ranged from a low 65 µS/cm at the Loop Spruit Eye, a mostly natural area, to 1499 µS/cm at Kraalkop Spruit, a highly impacted area.

The fact that elevated levels of metal concentrations were measured during both surveys, suggests that little to no seasonal variation was present. Sites 2 and 3, where mining activity were present, were probably the most impacted, as reflected by the high metal concentrations, high EC and low pH values.

4. Acknowledgments

We are indebted to the Unit for Environmental Sciences and Management, North-West University, Potchefstroom, South Africa for the financial support and infrastructure.

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Table 1: Metal concentrations (µg/ℓ) and selected physico-chemical parameters recorded from each site, as well as the target water quality guideline values*.

Site	Flow	Temp (°C)	EC (µS/cm)	pH	Cu	Zn	Se	P
1 Loop Spruit Eye	High	15	82	6.91	1.12	15.65	0.17	17.72
	Low	9.2	65	7.20	2.29	18.70	0	47.52
2 Loop Spruit	High	18.7	853	6.43	0	6.12	0.16	15.18
	Low	12.3	901	6.66	3.33	60.14	0	55.07
3 Kraalkop Spruit	High	16.3	1499	6.46	2.52	3.11	4.07	41.34
	Low	11.7	1207	6.82	6.40	49.57	1.59	121.50
4 Ensel Spruit	High	16.1	535	7.65	1.69	9.30	1.09	40.28
	Low	10.7	480	8.34	4.23	59.35	0	58.82
5 Loop Spruit	High	16	790	6.43	1.31	8.30	2.07	77.55
	Low	7.3	781	7.43	3.94	54.65	0.26	71.01
6 Loop Spruit	High	16.4	693	7.24	0.52	10.92	0.91	14.44
	Low	12.3	695	7.73	2.95	40.96	0	84.37
TWQG*				6.5-9	<0.8	<2	<2	25-250

P-4

Metal concentrations in selected macroinvertebrate families, water and sediment of a pristine river system in the North-West Province of South Africa.

Hilde Kemp* and Corrie Wolmarans

Unit for Environmental Sciences and Management, Potchefstroom Campus of the North-West University, South Africa.

This study was undertaken to determine the metal concentrations in selected macroinvertebrate families, as well as in the water and sediment in the Groot Marico catchment. Eight sites were selected in the Marico River and tributaries where macroinvertebrates, water and sediment samples were collected for metal analyses. Seven families including Notonectidae, Coenagrionidae, Atyidae, Libellulidae, Baetidae, Caenidae and Chironomidae were analysed to determine their metal concentrations. A total of 35 metals were detected in the macroinvertebrates, water and sediment. Metal concentrations in the sediment were higher than those in the water and the slightly elevated levels of metals were probably due to natural geological weathering. Metal concentrations were the highest in specimens of Baetidae, Caenidae and Chironomidae, which is most probably due to the fact that these families live in close proximity to the sediments while the other families reside in the water, on the surface or on plants.

Keywords: Metal concentration, water, sediment, river, macroinvertebrates

1. Introduction

Aquatic macroinvertebrates can take up and accumulate both essential and non-essential trace elements in the water and sediment, and the concentrations of these metals show enormous variability across metals and invertebrate taxa. Excessive metal concentrations can have adverse effects on these organisms, however, invertebrates are able to react to, metabolize, and thus regulate, both essential and non-essential metals within their internal systems.

This study was conducted to determine the baseline metal concentrations in selected macroinvertebrate families, as well as in the water and sediment of a pristine river system.

2. Material and methods

The study was conducted in the North-West and Limpopo Provinces, at eight samplings sites in the Marico River and selected tributaries (Klein Marico River and Sterkstroom) (Figure 1).

Site H1 is the dolomitic Marico Eye, the source of the Marico River. Sites H1 and H2 are both situated in unimpacted areas. Sites H3 is in the Sterkstroom, described as moderately impacted. Sites H4 and H7 are in the Marico River upstream and downstream of the Marico Bosveld Dam while sites H5 and H6 are situated in the Klein Marico River, upstream and downstream of the Klein Maricopoort Dam, respectively. This tributary is described as being in a fair to poor state and is contaminated by effluent from the town of Zeerust. Site H8 is situated in the Marico River at 3de Poort near the Botswana border.

One survey was conducted during the low-flow period of 2013. Macroinvertebrates, water and sediment samples were collected from all the sites.

Macroinvertebrates were collected by sampling the marginal and aquatic vegetation, as well as the substratum, using a Perlon® gauze net. Samples were preserved with 70% ethanol and transported to the laboratory for identification and analyses.

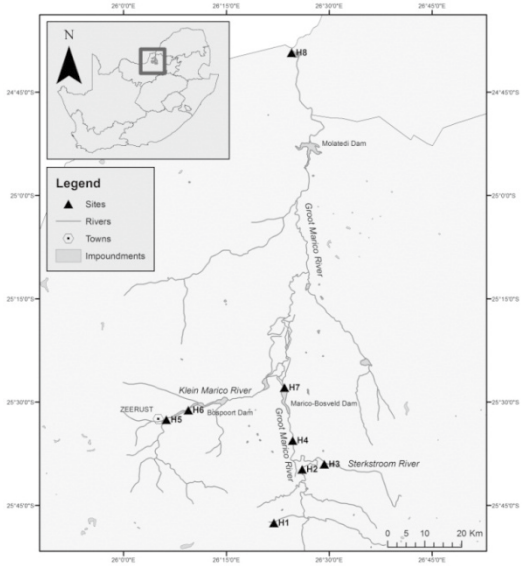

Figure 1: Sampling sites within the Marico River catchment, North-West, South Africa.

Macroinvertebrates were identified up to family level. From each sample, three specimens per family were selected, weighed and placed in a multiple cell Teflon container with 1ml 65% nitric acid per cell. Samples were then digested under pressure at 70 °C for 24 hours. Digested samples were diluted with double distilled water and analysed with an ICP-MS.

Water samples were filtered and the filtrate was used directly for analysis in an ICP-MS using standard recognized techniques. Sediment analyses were carried out after air drying the samples at 70°C for 48 hours before the EPA Method (3050B) was used to digest the samples for ICP-MS analyses. Only selected metals will be discussed.

3. Results and discussion

A total number of 35 metals were detected in both the water and sediment samples from all the sampling sites (data not shown). Only selected metals were used for statistical analyses and discussion. Metal concentrations in the sediment were higher than those in the water, however, the fact that these metals were all present at both the anthropogenically impacted and unimpacted sites, suggests that it is most probably from a geological origin (Kemp and Wolmarans, 2015).

Seven families including Notonectidae, Coenagrionidae, Atyidae, Libellulidae, Baetidae, Caenidae and Chironomidae were analysed to determine their metal concentrations. A total number of 35 metals were also detected in the different macroinvertebrate samples at the various sites. Concentrations varied considerably between the different families as well as between the different sites (data not shown). The mean metal concentrations were the highest in the families Baetidae, Caenidae and Chironomidae. This is most probably due to the fact that these families are known to live in close proximity to the sediments while the other families reside either in the water, on the surface or on plants.

According to the PCA tri-plot (Figure 2), metal concentrations in the macroinvertebrate families are strongly associated with the metal concentrations in both the water and sediment. This is in accordance with literature, where linear correlations between metal concentrations in macroinvertebrates and sediments where found (Santoro et al. 2008). Furthermore, on the PCA tri-plot, Baetidae, Caenidae and Chironomidae also associates slightly more with the metal concentrations in the sediment while Notonectidae, Coenagrionidae, Atyidae and Libellulidae associate slightly more with the metal concentrations in the water.

Concentrations of Al, Mn, Fe and Zn were constantly the highest in all of the families, as well as in the water and sediment, which is not surprising as these are the most common elements in the earths' crust.

To conclude, positive correlations were evident between the metal concentrations in the selected macroinvertebrate families and the metal concentrations in the water and sediment. As the metal concentrations in the water and sediment were most probably from geological origin, it does not, under the current circumstances, pose a serious threat to the organisms.

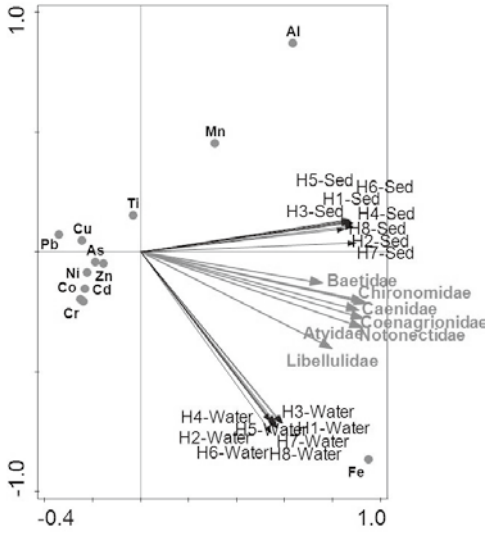

Figure 2: PCA tri-plot of the metal concentrations in the water and sediment at the various sites as well as the mean metal concentrations in the selected families. The tri-plot describes 79.48% of the total variation, with 68.17% being described on the first axis and 11.31% on the second axis

4. Acknowledments

We are indebted to the Unit for Environmental Sciences and Management, North-West University, Potchefstroom, South Africa for financial support and infrastructure.

5. References

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Kemp, M. and Wolmarans, C.T. 2015. Metal concentrations in the water and sediment of a pristine river system in the North-West Province of South Africa. 7th International Toxicology Symposium in Africa, Johannesburg, South Africa, pp. 55 – 56.

Table 1: Mean metal concentrations (ppb/mg) in macroinvertebrate families collected at the various sites.

ppb/mg	Notonectidae	Coenagrionidae	Atyidae	Libellulidae	Baetidae	Caenidae	Chironomidae
Al	137.27±114.32	215.74±82.10	184.96±85.33	234.79±149.44	855.09±604.23	865.52±499.70	892.81±945.22
Ti	3.87±2.97	6.52±3.95	7.22±2.33	5.32±2.72	15.60±7.68	21.01±25.61	20.77±28.12
Cr	1.82±1.20	3.02±1.17	2.24±0.98	3.16±2.3967	11.86±6.81	12.74±10.78	11.50±13.09
Mn	86.89±99.73	123.37±56.39	211.56±193.54	137.90±104.99	405.52±344.79	357.38±240.34	157.59±118.90
Fe	232.84±138.32	450.11±122.28	523.13±315.50	799.24±638.77	1619.59±677.16	2046.97±762.19	1479.99±1391.61
Co	0.30±0.15	0.50±0.24	0.50±0.28	0.53±0.37	2.46±1.45	1.93±0.94	1.50±1.39
Ni	1.71±1.37	2.80±1.54	1.76±1.34	3.72±4.96	7.00±5.26	6.37±3.30	5.60±4.01
Cu	25.12±12.88	23.27±18.56	17.60±7.89	23.61±29.37	103.01±142.69	59.83±65.67	105.24±181.45
Zn	197.44±193.69	153.57±183.63	67.91±87.78	385.81±748.78	298.82±406.12	226.37±244.17	134.93±155.49
As	1.13±1	1.41±1.23	1.55±2.23	2.62±4.05	0.40±0.31	0.48±0.27	0.12±0.14
Cd	0.26±0.10	0.33±0.31	0.17±0.23	0.20±0.14	0.25±0.44	0.01±0.02	0.04±0.05
Pb	18.85±17.04	29.62±22.20	23.10±30.23	15.10±14.47	96.46±143.20	65.98±74.51	48.48±61.75

P-5

Susceptibility of the African bollworm, *Helicoverpa armigera* to two commonly used insecticides in Sudan

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The African cotton bollworm (ABW), *Helicoverpa armigera* Hubner (Lepidoptera: Noctuidae) is one of the major threats to present day intensive agriculture in Sudan. There is a belief that this pest has developed resistance to some of the most commonly used insecticides (e.g. Endosulfan, Dimethoate). The expected consequence of resistance is using higher dosage rates to overcome the problem, which leads to increasing contamination to the environment. A study was carried out aiming at generating data for field-collected strains of cotton insect pest against endosulfan and dimethoate using a discriminating-dose technique and to conduct log-dose assays on 'suspect resistant' strains those do not show 100% mortality at the discriminating-dose. Fourth instars larvae of the African bollworm *Helicoverpa armigera* were exposed to residues of insecticides on cotton leaves. The percentage reduction of damage in treatment relative to the control was calculated. The results showed that the LC_{50} for dimethoate was 1075.2 ppm, which was less than half of the Field Recommended rate of this insecticide (2560 ppm). On the other hand the dose required to cause 99% (i.e. Discriminating Dose) response was relatively high (6219.5 ppm) and the ration of Field Recommended Dose/Discriminating Dose was 0.25. Results with endosulfan showed that the LC_{50} for endosulfan was 175.27 ppm, which only 0.05 the Field Recommended rate of this insecticide (3750 ppm). On the other hand the dose required to cause 99% (i.e. Discriminating Dose) response was (660.9 ppm) and the ration of Field Recommended Dose/Discriminating Dose was 5.7. This indicates that the 4th instar larvae of ABW were still highly susceptible to Endosulfan. It is highly recommended to use the Discriminating dose calculated from this study for future monitoring any tendency of an increase in the frequency of tolerant individuals to Endosulfan in the ABW population

Keywords: African bollworm, *Helicoverpa armigera*, resistance, insecticides, Sudan

1. Introduction

The African cotton bollworm (ABW), *Helicoverpa armigera* Hubner (Lepidoptera: Noctuidae) is one of the major threats to present day intensive agriculture in Sudan. There is a belief that this pest has developed resistance to some of the most commonly used insecticides (e.g. Endosulfan, Dimethoate). Over-dependence on a particular group of chemistry is one of the important reasons for rapid development of resistance. Reports from India using log dose probit bioassays indicated Significant levels of cypermethrin and fenvalerate resistance were found in all field strains, demonstrating that resistance to at least some pyrethroids is now ubiquitous in *H. armigera* populations in the Indian subcontinent; cypermethrin and fenvalerate resistance levels ranged from 5– to 6500–fold and 16– to 3200–fold respectively (Nigel, 1996)

(The present study tried to generate base-line information of two commonly used insecticides to combat insect cotton pest (i.e. endosulfan and dimethoate) on 4th larval instar of *Helicoverpa armigera*, adults of the cotton jassid and adults of the cotton flea beetle *Podagrica spp*. The aims of this study was to generate data for field-collected strains of the African bollworm to endosulfan and dimethoate using a discriminating-dose technique.

2. Materials and Methods

Insecticides tested in the present study against larvae of the ABW were prepared based on the field recommended rate (FRD) in a preliminary experiment. In subsequent experiments a dose response was carried out for each insecticide based on the results of the first experiment (Preliminary). Test insecticides were prepared as follows (Considering an application volume of 70 l/fed):

1. Dimethoate 32% EC: The FRD was calculated to be 2560 ppm (i.e. 2560 mg a.i. /l).
2. Endosulfan 50% EC: The FRD was calculated to be 5000 ppm (i.e. 5000 mg a.i./l)

Test larvae of the African bollworm were exposed to residues on cotton leaves. Leaf discs (2 cm*cm) from potted cotton plants were dipped in the appropriate concentration for 10 seconds and

allowed to dry. The leaf discs were then caged with test insect. Mortality was assessed at different periods after exposure.

3. Results and Discussion

Dose Mortality experiment with Dimethoate:

In Table (1) a significant difference was observed between the higher dose tested (FRD) and other concentrations. Doses required to produce a responses in the range from 1% to 99% were calculated. The LC_{50} for dimethoate was 1075.2 ppm, which was less than half of the Field Recommended rate of this insecticide (2560 ppm). On the other hand the dose required to cause 99% (i.e. Discriminating Dose) response was relatively high (6219.49) and the ration of FRD/DD was 0.25. This might be taken as indication of an increase in the frequency of tolerant individuals among the African bollworm population in Central Gezira (Table 2).

Dose Mortality experiment with Endosulfan:

Mortality caused to 4th ABW larvae after exposure to various doses of Endosulfan on cotton leaves are shown in Table (1). The tested larvae highly susceptible to endosulfan. as a result of exposure to 1/10 the FRD (500 ppm) caused 100 mortality. Doses required to produce a responses in the range from 1% to 99% are presented in Table 2. This table shows that the LC_{50} for Endosulfan was *175.27* ppm, which only 0.05 the Field

Recommended rate of this insecticide (3750 ppm). On the other hand the dose required to cause 99% (i.e. Discriminating Dose) response was (*660.90*) and the ration of FRD/DD was 5.7. This indicates that the 4th instar larvae of ABW were still highly susceptible to Endosulfan

4. Conclusions

A discriminating dose of dimethoate of 6220 ppm should be used to monitor the development of resistance of the 4th instar larvae of the African bollworm Helicoverpa armigera using Leaf-dipping method.

2. A discriminating dose of endosulfan of 661 ppm should be used to monitor the development of resistance of the 4th instar larvae of the African bollworm Helicoverpa armigera using Leaf-dipping method.

Table (1). Susceptibility of the 4th larval instars to Dimethoate and Endosulfan 24 hours after exposure.

Dimethoate		Endosulfan	
Dose (ppm)	Mortality %	Dose (ppm)	Mortality %
0	0.0 (0.0) a	0	0.0 (0.0) a
320	7.0 (4.2) a	62.5	6.1 (3.3) a
640	33.1 (30.6) b	125	34.1 (31.7) b
1280	43.9 (48.1) b	250	53.4 (64.2) c
2560	62.1 (92.5) c	500	90.0 (100.0) d
SE	6.5	8.9	

Figures followed by the same letter were not significantly different, Values in parentheses are actual data.

Table (2). Dosae –Mortality (Respose) of Dimethoate and Endosulfan on the $th Larval instar of Helicoverpa armigera in the laboratory

	Dimethoate			Endosulfan		
	Dose	95% Confidence Limits			95% Confidence Limits	
Response	(ppm)	Lower	Upper	DOSE	Lower	Upper
LC 50	1075	845	1390	175.27	146.18	210.7
LC90	2827	2023	5278	364.12	288.46	534.04
LC 99	6219	3726	17317	*660.9*	*466.98*	*1225.49*

5. Reference:

Nigel J. Armes, Deepak R. Jadhav and Kenneth R. DeSouza. 1996. A survey of insecticide resistance in *Helicoverpa armigera* in the Indian subcontinent. Bulletin of Entomological Research : 86 :499-514

P-6

Organochlorine pesticide residues in Nile talipia and African catfish from Sharkia Province, Egypt

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This study investigated the residual levels of organochlorine pesticide (OCPs) in highly consumed types of freshwater fish in Egypt (tilapia and catfish). A total of ten Nile tilapia (Oreochromis niloticus) and ten African catfish (Clarias gariepinus) were collected from Zagazig city in Sharkia Province, Egypt. Levels of 11 OCPs such as hexachlorocyclohexanes (HCHs), aldrin, endrin and dichlorodiphenyltrichloroethanes (DDTs) residues were investigated. Generally, contamination pattern of OCPs was in order of HCHs > other OCPs (heptachlor, heptachlor-epoxide, aldrin, γ chlordane and endrin) > DDTs.The levels of OCPs residues detected in examined fish samples in this study were below the maximum residue limit for food safety recommended by U.S.FDA and Codex Alimentarius Commission except γ-HCH and endrin in catfish. Moreover, higher levels of OCPs were detected in catfish than tilapia. The present results demonstrated the extensive and recent use of these types of pesticides in the present time in Egypt.

Keywords: Organochlorine pesticide, Talipia, Catfish, Egypt

1. Introduction

Organochlorine pesticides (OCPs) have been used for agricultural and industrial purposes for a long time and on a large scale throughout the world owing to their exceptional insecticidal and fungicidal properties. Due to their persistence, bioaccumulation, and adverse effects on wild-life and human, production and application of these compounds were banned in the early 1970s in developed countries but some developing countries are still using these chemicals because of their low cost and adaptability in industry.

In aquatic systems, OCPs are distributed in the environmental and biological compartments where they are affected by their octanol–water partition coefficients, organic matter in the sediments, and the lipid contents in biota. In comparison with terrestrial mammals, fish have been shown to have an increased ability to bioaccumulate organic pollutants due to their lower mono-oxygenase activity (detoxification enzymes) and therefore, they are useful bioindicators of pollution in aquatic habitats.

Nile tilapia and African catfish are the most popular freshwater fish species in Egypt. They play an important role in fish consumption in Egypt and around the world. OCPs were detected in freshwater fish in previous studies in Egypt (Yahia and Elsharkawy, 2014). The possible sources of this pesticide group originated from previous or illegal use.

The objective of this study was to investigate the levels of OCPs residues in the most famous and highly consumed types of freshwater fish in Egypt collected from Zagazig city in Sharkia Province; to compare the levels of pesticide residues between Nile tilapia and African catfish and to compare the obtained levels with the international maximum residue limits (MRL).

2. Materials and Methods

2.1 Sample collection

Twenty samples of Nile tilapia (Oreochromis niloticus) and African catfish (Clarias gariepinus) (10 fish samples per each spcies) were collected from local markets in Zagazig, Sharkia Province, Egypt for detection and determination of 11 OCPs (αBHC, βBHC, γBHC, heptachlor, heptachlor epoxide, aldrin, γ chlordane, pp-DDE, pp-DDD and pp-DDT and endrin). Approximately 200 g of muscles from each fish samples was obtained and kept at – 20ºC until analysis.

2.2 Analysis of OCPs residues

Extraction and clean up of the samples were carried out using methods described by McMahon and Hardin (1994) and Wainwright et al. (2001) respectively. The cleaned extracts was fractionated by passing through a column of 12 gm activated florisil and eluted with hexane (first fraction) followed by 20% dichloromethane in hexane (second fraction).

Quantitative determination of OCPs was performed using Hewlett Packard gas chromatograph (GC) equipped with Ni 63-electron capture detector (ECD). GC conditions were as follows: Db-608 capillary column (30 m length × 0.25 mm internal diameter × 0.25 µm film thickness); carrier gas: N2 at a flow rate of 3 ml/ min., and injector and detector temperatures were 280 ºC and 300 ºC, respectively.

The initial column temperature was 160 ºC for 2 min., raised at 5ºC/min. to 260 ºC then held for 10 min. The recovery rate of OCPs residues ranged from 80-92%.

3. Results and Discussion

Our results showed that the residual levels of OCPs in freshwater fish on wet weight basis (ng/g ww) were dominated by HCHs (α-, β- and γ-HCH) followed by other OCPs (heptachlor, heptachlor-epoxide, aldrin, γ chlordane and endrin) then DDTs (p,p-DDE, p, p-DDD and p, p-DDT). Total HCH in fish was in range of ND-380 ng/g ww and ND-598 ng/g ww in Nile talipia and catfish respectively. The γ-HCH isomer was dominant of HCHs in all examined fish followed by α-HCH then β-HCH (Table 1). This reflects recent exposure to HCH.

Total DDT was ranged from ND to 94 ng/g ww and 180 ng/g ww in Nile talipia and catfish respectively. DDT and its derivatives followed this general order in this study: DDE>DDD>DDT (Table 1). Our results are consistent with reported order of metabolites of DDT in fish from Iran (Hosseini et al., 2008).These results may be attributed to the high chemical stability and hydrophobicity of p,p-DDE and its long half-life and, hence, persistence in both abiotic and biotic components of the aquatic ecosystems. p,p-DDT levels were very low in all fish, most probably because of its shorter half-life (8 months) than p,p-DDD and p,p-DDE (7 years).

Regarding the residue levels of other OCPs in freshwater fish, total other OCPs concentrations ranged from ND to 355 ng/g ww in Nile talipia and 584 ng/g ww in catfish. γ Chlordane was dominant among other OCPs with the range of ND-150 ng/g ww and ND-250 ng/g ww in Nile talipia and catfish respectively(Table 1). Yahia and Elsharkawy (2014) detected some OCPs as aldrin, p,p-DDE and heptachlor in Nile talipia and African catfish in Assiut, Egypt but their values were lower than levels obtained in our study.

Interestingly, all detected OCPs levels were higher in catfish than in Nile tilapia. These results were in accordance with those recorded by Yahia and Elsharkawy (2014) in Assiut, Egypt. These findings may be attributable to the relatively high fat content of catfish meat consequently; the fat soluble environmental pollutants such as OCPs are the more probable pollutants present in catfish meat. In addition, the nature of catfish habitat which lives in cloudy water and preys on another fish, worm and insects could explain the high concentration of OCPs in catfish than in Nile tilapia. The levels of OCPs residues in all examined fish samples were below MRL adopted by United States Food and Drug Administration (U.S. FDA, 1983, 2000) and Codex Alimentarius Commission (2009) except γ-HCH and endrin in catfish (Table 1).

Table 1: Concentrations (range) of OCPs residues (ng/g ww) in examined Nile tilapia and catfish samples from Sharkia Province.

OCPs	Type of fish		MRL
	Nile tilapia	Catfish	
α-HCH	ND-120	ND-180	200(1)
β-HCH	ND-10	ND-18	200(1)
γ-HCH	ND-250	ND-400	300(2)
Total HCH	ND-380	ND-598	
Heptachlor	ND-140	ND-190	300(2)
Heptachlor-epoxide	ND-10	ND-17	300(2)
Aldrin	ND-5	ND-7	300(2)
γ Chlordane	ND-150	ND-250	300(2)
p,p-DDE	ND-60	ND-110	
p, p-DDD	ND-24	ND-50	
p, p-DDT	ND-10	ND-20	
Total DDT	ND-94	ND-180	5000(2)
Endrin	ND-50	ND-120	100(3)
Other OCPs	ND-355	ND-584	

ND; non detected. (1): U.S.F.D.A. (1983) (2):U.S.F.D.A. (2000). (3): Codex Alimentarius Commission (2009).

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P-7

Aflatoxins and fumonisins levels in selected maize- based dishes in Cameroon and Human exposure

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The aim of this research was assessed the levels of total aflatoxin (B_1, B_2, G_1 and G_2) denoted AFT and total fumonisin (B_1, B_2, B_3) denoted FUT using ELISA TEST in eleven selected types of traditional maize-based dishes to estimate the health risk associated with their consumption in Bafia (Centre Cameroon) where maize is an ingredient in most of their dishes. Human exposure was estimated using standard indice (Estimate Daily Intake or EDI). A food survey was carried out using deterministic method on 366 individuals (102 children [5-8 years], 106 adolescents [9-15 years]. and 156 adults [>15 years]). The results showed varying concentration of AFT in all maize-based dishes samples analysed and FUT in 95% of samples. Human exposure showed average, median and percentile $_{95}$ EDI values for AFT and FUT in at least of 90% above the tolerable daily intake in the three subgroup of population. This study revealed the potential for AFT and FUT toxicities and risk of carcinogenic health effect from maize-based dishes consumption. Constant monitoring and control of these food is urgent in Cameroon

Keywords: Maize-based dishes, Total aflatoxin, Total fumonisin, dietary intake, Cameroon

1. Introduction

In Sub-saharian Africa, many individuals are not only food insecure, but also are chronically exposed to high levels of mycotoxins in their diet (FAO,2007). Aflatoxin and fumonisin are mycotoxins, compromises food security in the most vulnerable groups of people in Africa.Aflatoxins may cause liver cancer, suppressed immune systems. Children are the most sensitive to the effects of aflatoxin- contaminated food. Reports have linked maize consumption with high levels offumonisins and high incidence of human oesophageal carcinoma in somme parts of South Africa and China. Some of the highest and most persistent human exposures to aflatoxin and fumonisin occur in Cameroon (Njumbe *et al.* 2014) : Maize is one of the cereals most susceptible to aflatoxinand fumonisin contamination. The objective of this work is to investigate total aflatoxin (B_1, B_2, G_1 and G_2) and total fumonisin (B_1, B_2, B_3) in eleven selected type of traditional maize based dishes from various villages in Bafia (Centre Cameroon) where maize is an ingredient in most of their dishesand estimate the human health risk

2. Material and methodology

2.1 Study site and analysis

This study was carried out in *Bafia* located in the agro-ecological humid zone of Cameroon between March and December 2012.. A total of 33 samples of maize based dishes, each weighing approximately 500 g was obtained.Maize based dishes included maize beer; dry or fresh maize cake with vegetables; dry or fresh maize cake with groundnuts; maize porridge; maize *fufu* (*couscous*); maize milk; vegetables with maize; roasted maize; boiled maize; fried maize with groundnuts. :Solid samples were dried and then reduced to powder by milling, while liquid samples were lyophilized and reduced to powder in the same manner. Total aflatoxin and total fumonisin T levels were analyzed using a competitive ELISA according to the manufacturer's instruction (RENEKABIO, No: AF012714, No: FU012714, USA).

2.2 Dietary intake

Estimates of dietary intakes of AFT and FUT contaminated maize-based dishes were obtained for 366 individuals that consisted of 108 children (4-8 years), 102 adolescents (9-14 years) and 156 adults (15 years and over) by multiplying individual

intake of maize based dishes by the average concentration of AFT and FUT in each contaminated food consumed and then summing the contributions of all the maize based dishes. The mean, median and 95th percentile denoted P_{95} exposures to AFT and FUT were estimated for each of the three study population subgroups. Survey data and analysis were analyzed using SPSS software Version 10 (2000).and ANOVA tests.

3. Results and Discussion

The results (Table 1 and table 2) for AFT and FUT showed that mean, mediane and percentile 95 estimate dairy (EDI) intake levels varied significantly from the different subgroups of study population ($P<0.05$) : The average EDI value of AFT ingested per maize-based dishes exceeds nearly 44 times the TDI proposed 1 ng/kg bw/day (Matumba, 2014) in children, 32 times in adolescents and 27 times in adults. In the three subgroups of the population, all subjects (100%) exceeded the TDI. The major vectors of this exposition are the fried maize with groundnuts and dry or fresh flat maize cake with vegetables, Analysis of the results showed that the different maize-based dishes contain AFT at levels that may chronically affect health of the populations especially in causing liver damage. These populations are more exposed than the other population in Africa which are exposed to much lower levels. Our data on AFT intake showed that children are most at risk when compared to adolescents and adults. The average EDI levels of FUT by the consumption of maize-based dishes exceeds 2 times the TDI proposed by the European standards set at 2 µg/kg bw/day. in adults, 2.5 times in adolescents and 6.5 times in children. In the three subgroups studied, 98% of children, 90% of adolescents and 89% of adults exceed the TDI. The majority of these exposures are found in maize milk which is the most consumed. The cumulative effect of the long-term FUT exposure can contribute to an increased incidence of esophageal cancer especially in children who are most at risk. Based on the results of the present studyit is imperative to develop global control strategies to reduce the health risks associated with these toxins of African population.

Table 1: Total aflatoxin estimate daily intake among the population of *Bafia (centre Cameroon)*

Sample population of Bafia	***Total Aflatoxin estimate daily intake (ng/kg bw/day)***			
	Mean	**Median**	**P_{95}**	**% of individuals that exceeded TDI* (**1 ng/kg bw/day)
Children (4-8 years) n=108	43.8±0.6[c]	43.3	53.4	100
Adolescents (9-14 years) n=102	31.9±0.3[b]	32.0	37.6	100
Adults (15 years and above) n=156	27.4±0.5[a]	25.7	41.0	100

Table 2: Total fumonisin estimate daily intake among the population of *Bafia (centre Cameroon)*

Sample population of Bafia	***Total Fumonisin estimate daily intake (µg/kg bw/day)***			
	Mean	**Median**	**P_{95}**	**% of individuals that exceeded TDI* (**2 µg/kg bw/day
Children (4-8 years) n=108	13.2±0.3[c]	13.4	16.7	98
Adolescents (9-14 years) n=102	9.0±0.1[b]	9.2	10.8	90
Adults (15 years and above) n=156	4.0±0.[a]	3.8	5.8	89

Numbers in a column with different superscript letters are significantly different ($P<0.05$). Mean body weigh children: 23.2 kg; Adolescents: 32.0 kg;
Adults: 56.4 kg. *TDI = Tolerable Daily Intake ;P_{95}=95th percentile

4. Acknowledgments

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P-8

Infant formulae and baby foods as source of Ochratoxin A, a mini survey in Egypt.

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This study was conducted to determine the occurrence of ochratoxin A in marketed infant formulae and baby foods in Egypt. 50 random samples were collected, 25 each of locally produced and imported brands, and were prepared and examined using an ochratoxin A enzyme-linked immunosorbent assay commercial kits. 60 and 48 % of examined samples were found positive for ochratoxin A with a mean level of 0.81 and 0.74 ppb in locally produced and imported samples, respectively. In addition, 8 and 3 samples had an ochratoxin A content exceeding that recommended by European commission regulation. Our findings revealed the importance of strict monitoring of all marketed infant formulae and baby foods types. Besides, these results may help to determine possible causes of mycotoxins associated children disorders.

Keywords: Infant formulae and baby foods, Mycotoxin, Ochratoxin A.

1. Introduction

Infants and toddlers are nearly considered the most vulnerable group for food contaminants' problem. Different categories of contaminants have their access to infant foods, mycotoxins constitute a prominent category among them. Many particular types of fungi are able to produce mycotoxins, mainly of *Aspergillus* and *Penicillium* genera. Hundreds types of mycotoxins were identified, and they can be classified into six main groups (CAST, 2003). Principally, Aflatoxins and ochratoxins are mainly associated with foods as a post-harvest faulty storage. Ochratoxin A is a secondary metabolite produced by *Penicillium verrusocum*, Aspergillus ochraceus, A. carbonarius, A. niger and A. terreus. Cereals, beans, juices, malt, meat, milk and dairy products were reported to have ochratoxin A.

Adverse effects of ochratoxicosis ranged from minor renal dysfunctions to sever fatal cancers. Nephrotoxicity and Balkan Endemic Nephropathy, a fatal human kidney disease, are also among potential ochratoxin associated problems. In addition, Ochratoxin is a well-documented mutagen, immunotoxin and teratogen (Rosa et al., 2004).

As infant formulae and baby foods contain numerous ingredients that Ochratoxin-producing fungi can easily grow on them, these foods are highly suspicious. Bad storage conditions, faulty handling and lack of rigorous inspection contribute in increasing chances of presence of Ochratoxin in infant formulae and baby foods. Consequently, this study was conducted to assess the presence of Ochratoxin A in some locally produced and imported infant formulae and baby foods.

2. Materials and Methods

2.1 Samples

Fifty samples of baby foods, infant formulae (25 each of locally produced and imported brands) were collected randomly from local markets, pharmacies and retail stores from cities of Cairo, Zagazig and Mansoura, Egypt through the period from August 2015 till January 2016. All samples were kept in their original packaging until analysis.

2.2 Ochratoxin A determination.

Initially extraction of samples was done using OchraStar™ immunoaffinity columns. According to the manufacturer instructions, 2.5 mL of 1 N HCl was added to 2 g of powered sample, with shaking for 2 hours and then extraction was accomplished using 4 mL dichloromethane. 2 mL of 0.13 M sodium bicarbonate, (pH: 8.1) was added to dichloromethane separated phase, then aqueous phase was collected and 1 mL of dilution buffer was added. The final solution was used for ochratoxin determination. 50 µL was added to wells of 96-well microplate which was read using microplate reader (BioTek) at 450 nm. Results were expressed as parts per billion (ppb). Ochratoxin detection limit was 0.025 ppb.

2.3 Statistical analysis.

Three readings were taken for each sample and their average was recorded. Any sample has contained ochratoxin A below the detection limit was considered as a negative result.

3. Results and Discussion

Presence of ochratoxin A in examined infant formulae and baby foods samples were presented in table 1. As shown, ochratoxin A present in 27 samples out of examined 50 samples (54%). According to brand types, 60 and 48 % of locally produced and imported infant formulae and baby foods samples had ochratoxin A contents above detection limits, respectively. Ochratoxin A levels ranged from 0.025 to 1.4 ppb in locally produced types with a mean value of 0.81 ± 0.06 ppb, and 0.025 – 0.92 in imported samples with a mean

value of 0.74 ± 0.04.

Table 1: Ochratoxin A in Infant formulae and baby foods samples

Sample type	No. of samples	Positive samples* (%)	Mean ± SD of Ochratoxin A conc.	Range
Local	25	15 (60)	0.81 ± 0.06	0.025 – 1.4
Imported	25	12 (48)	0.74 ± 0.04	0.025 – 0.92

* Samples with Ochratoxin A over detection limit (0.025 ppb).

Numerous worldwide studies have determined levels of ochratoxin A in infant formulae and baby foods, and they reported different prevalence percentages and levels of contaminations (Araguás et al., 2005; Darouj et al., 2016; Juan et al., 2014; Ozden et al., 2012; Rubert et al. 2012). Our results agreed to some extent with their findings in the prevalence percentages, while according to the levels, no clear pattern of agreement was found.

Regarding composition of examined products, they were mainly composed of modified milk powder and cereals (wheat, rice, barley, corn, and/ or oat). Ochratoxin A finds its way into milk powder through indirect transfer via contaminated animal feed and in turn into produced milk. Concerning cereals, the only documented route is via direct growth of Ochratoxin producing fungi on cereals under favourable conditions, which later will be used in manufacturing of baby foods.

The European commission regulation stated that the maximum allowed ochratoxin residues in cereal-based foods and baby foods for infants and young children is 0.5 ppb (EC, 2012). Nearly 53 and 25 % of examined locally produced and imported infant formulae and baby foods were found to exceed this limit (figure 1). This would certainly reflect the worsen increase in children cancers ratios.

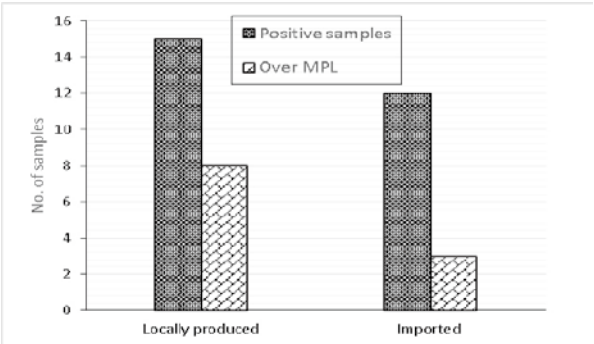

Figure 1: Samples over maximum permissible limits according to EC, 2012.

In brief conclusion, strict regulatory measures should be taken against infant formulae and baby foods safety.

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P-9

Mycological evaluation of retailed edible offal

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Contamination of carcasses and their offal with moulds is of a high significance for their quality and shelf life. Mycological contamination of meat may lead to its spoilage and produce mycotoxins with potential health hazards to human due to their carcinogenic effects, liver diseases and organ damage. Therefore, this work was planned to determine the incidence and type of moulds in the edible offal of different slaughtered animals at Zagazig city, Egypt. One hundred and fifty random samples of raw edible offal of slaughtered cattle, camel and sheep (fifty of each) were collected from different localities in different sanitation levels at Zagazig city and districts. Ten samples were collected from each animal represented by liver, lung, rumen, intestine and head muscle. Among the examined meat animal species, we found that cattle samples had significantly the highest total mould count values in all examined organs liver, lung, rumen, intestine and muscle followed by camel and sheep offal.

Keywords: Offal, mould, Aspergillus, mycotoxins

1. Introduction

Contamination of meat either chilled or frozen with different kinds of fungi either moulds or yeast is of a particular importance in meat industry. Although, many reports studied the contamination of meat with different kinds of moulds but there is little information about the contamination of offal with such moulds. Edible offal is defined as animal by products other than skeletal muscles that are eaten. Offal is contributing to enjoyment of food with unique flavors, textures, aromas and a lot of vitamins, high quality protein and energy. The incidence of meat contamination with different mould genera were investigated in different localities of the world such as Australia and Japan. The common isolated mould genera from retailed meats were Aspergillus, Penicillium, Alternaria, Cladosporium, Mucor and Rhizopus as mentioned by (Pitt and Hocking, 1997). Contamination of carcasses and their offal with moulds is of a high significance for their quality and shelf life. Serious contamination takes place from soil and water, also raw meat becomes contaminated from meat contact surfaces, equipment, utensils, handling by workers and during transportation (Van Laack, 1994). Many factors control the mould growth such as moisture, pH, oxygen, substrate and the interaction with other microbiological agents. It is well established that mould can grow over a wide range of pH from 2 to 11; over a water activity (^{a}W) value ranges from 0.620 to 0.995; over a temperature ranges from -10 to around 60 °C and over a wide range of nutrient limitations (Pitt and Hocking, 1997). Mycological contamination of meat may lead to its spoilage and produce mycotoxins with potential health hazards to human due to their carcinogenic effects, liver diseases and organ damage. Therefore, this work was planned to determine the incidence and type of moulds in the edible offal of different slaughtered animals at Zagazig city.

2. Materials and Methods

Samples

One hundred and fifty random samples of raw edible offal of slaughtered cattle, camel and sheep (fifty of each) were collected from different localities in different sanitation levels at Zagazig city and districts. The ten samples collected from each animal represented by liver, lung, rumen, intestine and head muscle. Samples were identified, packed and transferred to the laboratory in an ice box and subjected to the mycological examination.

Preparation of samples:

Twenty five grams of offal sample were aseptically excised and homogenized in 225 ml of sterile buffered peptone water 0.1% at 2500 rpm for 2 min with 225 ml of 0.1% sterile peptone water using a sterile homogenizer (type M-P3-302, mechanic, precyzina, Poland). Such homogenate represents the dilution of 10-1, and then decimal dilutions were done.

Determination of the total mould count:

The total mould counts were determined by culturing duplicate plates on each of malt extract agar media and Czapeck-Dox agar with 6% Nacl (Oxoid, Basingstoke, UK) followed by incubation at 25 ˚C for 5-7 days. During the incubation time, the plates were examined daily for the star-shape mould growth which is picked up under aseptic conditions with its surrounding cultivated medium and transferred into malt extract slope agar (Oxoid) then kept for further examination. Estimation of total mould count was obtained by counting of the cultured agar plates of acidified malt extract agar and osmophilic moulds on Czapeck-Dox agar (APHA, 2001). The identification of isolated

mould genera were carried out based on their micromorphological properties using variable mould determination keys. The isolates were subcultured on malt extract agar and Czapeck Dox agar, incubated at 25 ˚C for 5-7 days. The identification of the colonies was carried out by careful observation and measurements of the macroscopical and microscopical characteristics of the mould colonies which were recorded in the data sheet.

Statistical Analysis: was conducted using Tukey's Kramer HSD test, p value of 0.05 was considered to be significant.

3. Results and Discussion

Among the examined meat animal species, we found that cattle samples had significantly the highest total mould count values in all examined organs liver, lung, rumen, intestine and muscle followed by camel and sheep offal. Within the organs, the total mould count in intestine samples was the highest among all other offal samples in all animal species and this may be owing to the difficulty of complete removal of intestinal contents despite of sever washing. The total mould count in the rumen samples came second to intestine and that also may be reasonable due to the difficulty of complete removal of the ruminal contents and ingesta and this lead to high chance of mould contamination. The high total mould count in intestine and rumen may lead to big troubles, especially if they are used for the manufacturing of the natural sausage casings. We could succeed to isolate and identify different mould genera from these samples such as Aspergillus, Penicillium, Mucor, Rhizopus, Alternaria, Fusarium, Cladosporium and other mould genera. Their prevalence ranged from 20% to 100% in the examined offal samples, and this indicates bad hygienic measures adopted during handling or processing of these edible offal parts. The predominant mould genera was Aspergillus followed by Penicillium and this may be attributed to their ability to grow over a wide range temperatures besides they need a very low concentration of

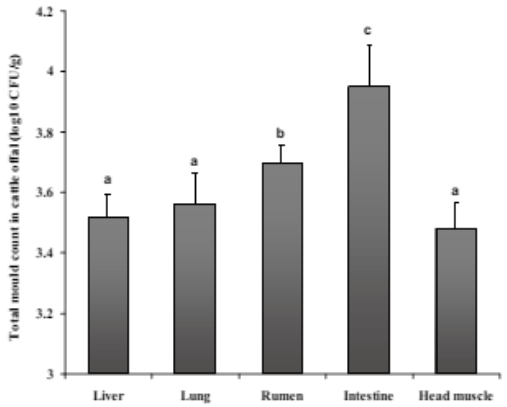

Figure 1. Total mould count in the examined raw edible offal samples of cattle.

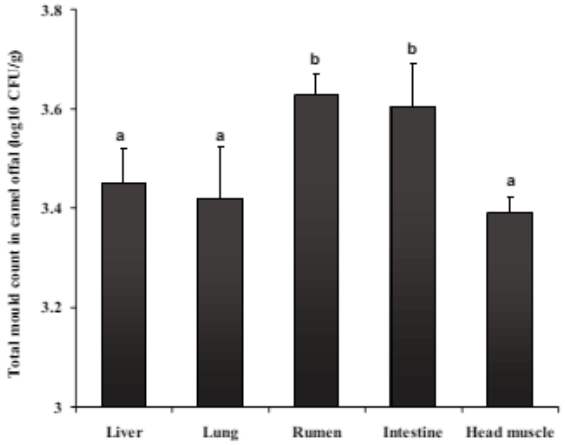

Figure 2. Total mould count in the examined raw edible offal samples of camel.

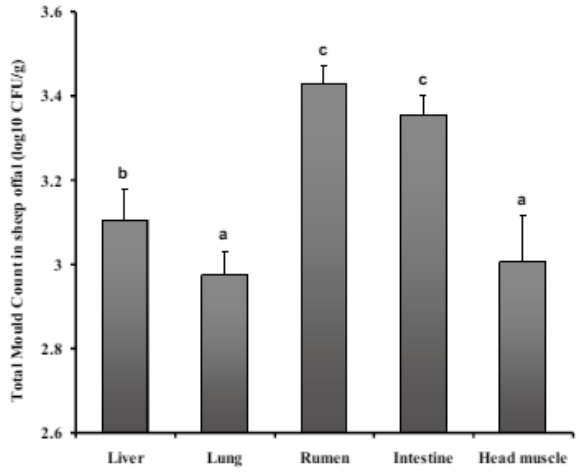

Figure 3. Total mould count in the examined raw edible offal samples of sheep.

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P-10

Heavy metal residues in marketed fish in Manzala city, Egypt

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Fish is a healthy food, which is considered as a valuable source of high quality protein, polyunsaturated fatty acids, minerals and vitamins. Heavy metals are natural trace components of the aquatic environment, but their levels have increased due to domestic, industrial, mining and agricultural activities. Heavy metals have a higher tendency to be incorporated into food chains and become accumulated in tissues and organs of fish and other aquatic organisms and this represent serious health hazards to consumers. Thus, the aim of this study is to estimate heavy metal concentrations in muscles of the four fish species (*Oreochromis niloticus, Tilapia zilli, Mugil cephalus* and *Claris lazara*) marketed in Manzala city, Egypt. The recorded results showed that *Clarias lazera* had the highest load of all examined metals followed *by Mugil cephalus, Oreochromis niloticus* and finally *Tilapia zilli*. In a trial to reduce heavy metal load in fish, it is observed that all metals were reduced with different percentages after dipping in water or lemon juice followed by grilling.

Keywords: Heavy metals, Fish, Egypt

1. Introduction

Fish is a healthy food for most of the world population particularly developing countries in contrast to meat and poultry and considered as a valuable source of high quality protein, polyunsaturated fatty acids, minerals and vitamins which are associated with health benefits and normal growth.

Heavy metals make up one of the most important group of pollutants from the point of food analysts.

Heavy metals are natural trace components of the aquatic environment, but their levels have increased due to domestic, industrial, mining and agricultural activities.

At low levels, heavy metals such as copper, cobalt, zinc, iron and manganese are essential for enzymatic activity and many biological processes, but metals such as cadmium, mercury and lead have no known essential role in living organisms, and are toxic at even low concentrations. The essential metals also become toxic at high concentration.

Heavy metals have a higher tendency to be incorporated into food chains and become accumulated in tissues and organs of fish and other aquatic organisms and this represent serious health hazards to consumers.

Since the fish meat represents a major component of human diet, the presence of heavy metals in the aquatic environment and their accumulation in fish have been well studied and documented.

The determination of metals in human foods is of significant concern due to their double-edged roles which range from the nutritional requirement of essential elements to the toxicity associated with the excessive intake of both the essential and toxic metals.

Thus, the aim of this study is to estimate heavy metal concentrations in muscles of the most commercially available fishes (*Oreochromis niloticus, Tilapia zilli, Mugil cephalus* and *Claris lazara*) marketed in Manzala city, Egypt. The measured metals include Lead (Pb), Cadmium (Cd), Arsenic (As), Cupper (Cu), Zinc (Zn), Nickel (Ni), Cobalt (Co), Iron (Fe), Manganese (Mn), Chromium (Cr) and Mercury (Hg) using Inductively Coupled Plasma- Optical Emission Spectroscopy (ICP-OES) and compare the obtained values with the permissible limits of different organizations and discuss of the public health significance of these heavy metals. Moreover, some reduction trials for heavy metal load in *Oreochromis niloticuc* were done.

2. Materials and Methods

Collection of samples:

One hundred and eighty fish samples of different species (*Oreochromis niloticus, Tilapia zilli, Mugil cephalus* and *Claris lazera*) were collected in a random way during winter of 2014 from Manzala city. Additionally, sixty random fish samples of *Oreochromis niloticus* were collected from Manzala city for subsequent processing. Each sample was wrapped in a light polyethylene bag and placed in ice after identification according to the specie and location and taken to laboratory without delay.

Preparation of collected samples:

All collected fish species were washed with tape water several times to remove slime, mud and adhering blood and then prepared using common household practices, namely eviscerating and beheading. The scales were removed with plastic knife.

Samples of *Oreochromis niloticus* collected from Manzala city were classified into two groups, one dipped in distilled water for 60 minutes and the other in lemon juice, (300 ml lemmon:700 ml distilled water) (30%) for 60 minutes.

According to common household methods, the fish samples of *Oreochromis niloticus* were cooked but after dipping. The two groups subjected to grilling (10 of each group).

Grilling: Fish samples were previously powdered with bran then grilled at 180 °C for 10 min on each side using a grilling plate made of tin.

All raw and cooked fish samples were cooled at room temperature, wrapped in a light polyethylene bag then kept in the refrigerator at about 5 ºC.

Heavy metals were measured using Inductively Coupled Plasma- Optical Emission Spectroscopy (ICP-OES)

Statistical analysis:

Statistical analysis was done using Tukey's Kramer HSD test.

3. Results and Discussion

Heavy metals represent public health concern in many countries worldwide. For instance, Pb caused many poisoning cases worldwide. This study was undertaken to investigate heavy metal residual concentrations in many fish species marketed in Manzala city, Egypt.

The recorded results in table 1 showed that *Clarias lazera* had the highest load of all examined metals followed *by Mugil cephalus, Oreochromis niloticus* and finally *Tilapia zilli* (Table 1).

The results recorded in table 2 declared the effect of grilling on the metal load in *Oreochromis niloticus* after dipping in either distilled water or lemon juice.

It is observed that all metals were reduced after dipping in water or lemon juice followed by grilling with different percentages. It notes worthy that dipping in lemon juice followed by grilling achieved much clear reduction compared with dipping in water then grilling, which may be reasonable due to the effect of citric acid found in lemon juice, which helps in the elution of the metal from the fish tissue.

Several studies have reported that the common cooking methods such as frying and grilling of fish do not effectively remove heavy metals where some studies have reported a considerable reduction of the heavy metals in food after cooking **(Erosy et al., 2006; Abdelrahman et al., 2014)**. The studies that revealed a reduction of toxic elements using processes depended on cooking conditions such as time, temperature, and cooking medium. In this study, we used the lemon juice in dipping before cooking as a natural substrate rich in citric acid, which is one of the organic acids commonly used as a chelating agent.

The results achieved clear reduction of all metal load especially after dipping in lemon juice. This, we strongly recommend using of dipping in lemon juice or in water as an easy method of reduction of the metal load in the fish before consumption.

Table 1: Heavy metal concentrations (Mean± SE) (ppm/wet weight) in the examined fish samples

	O. niloticus	*Tilapia zilli*	*Mugil cephalus*	*Claris lazera*
Pb	0.55± 0.35	0.48± 0.27	0.55± 0.376	0.63± 0.36
Cd	0.01± 0.0	0.02± 0.00	0.02± 0.002	0.02± 0.00
As	0.49± 0.22	0.38± 0.19	0.51± 0.20	0.81± 0.19
Cu	0.21± 0.15	0.23± 0.14	0.22± 0.15	0.55± 0.23
Zn	1.84± 0.46	1.85± 0.51	2.03± 0.49	2.48± 0.36
Ni	0.06± 0.03	0.03± 0.01	0.10± 0.03	0.19± 0.09
Co	0.02± 0.01	0.02± 0.01	0.02± 0.02	0.34±12
Fe	17.6± 4.99	15.6±4.4	18.4± 5.2	19.3±4.4
Mn	0.02± 0.01	0.03± 0.02	0.03± 0.02	0.04±0.1
Cr	0.01± 0.01	0.04± 0.02	0.04± 0.02	0.06±0.0
Hg	0.02± 0.02	0.02± 0.01	0.04± 0.01	0.11±0.2

Table 2: Reduction percentage of heavy metal load due to grilling after dipping in either water or lemon juice

Element	Dipping in water + Grilling	Dipping in lemon juice + Grilling
Pb	29.3%	38.2%
Cd	44.4%	50.0%
As	13.0%	23.4%
Cu	34.8%	51.0%
Zn	39.3%	40.1%
Ni	20.0%	22.8%
Co	36.6%	43.3%
Mn	39.2%	52.9%
Fe	27.6%	33.1%
Cr	52.8%	60.3%
Hg	29.5%	34.0%

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P-11

Detection Of Staphylococcus aureus Enterotoxin In Some Dairy Products

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A total of 85 samples of (35 of small scale plain yoghurt, 25 of each small scale ice cream and locally manufactured fresh white soft cheese “talaga”) were aseptically collected in their retail packages from different super markets and dairy shops in Sharkia Governorate, Egypt. The collected samples were transferred directly to the laboratory in an ice box for isolation and identification of total staphylococci and detection of *Staphylococcus aureus* and it's enterotoxins production. The obtained results revealed that, total staphylococci could be detected in 25 samples (71.43%), 25 samples (100%) and 24 samples (96%) of examined small scale plain yoghurt, small scale ice cream and locally manufactured fresh white soft cheese, respectively. *Staph. aureus* could be detected in (75%), (50%) and (62.97) of positive staphylococci yoghurt, ice cream and cheese samples, respectively. Detection of enterotoxins by using ELISA revealed that no enterotoxins were detected in isolated *Staph. aureus* strains in yoghurt samples, enterotoxin (D) could be detected with percentage of (13.33% and 5.88%) in ice cream and cheese samples, respectively and enterotoxin (A) & (A&C) detected only in cheese samples with percentage of (5.88% and 5.88%), respectively.The public health importance and hygienic significance of the isolated *Staph. aureus* and their entertoxins as well as the suggested measures for improving the quality and safety of the yoghurt was discussed.

Keywords: total staphylococci, *Staph. aureus*, entertoxins

1. Introduction

Yoghurt is one of the most popular consumed dairy products all over the world. It is obtained by lactic acid fermentation of milk by the action of a starter culture containing *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. Ice cream today is a major product of dairy industry and becomes dominant interest of large segments of the population. The ingredients of ice cream may be various combinations of milk, cream, evaporated or condensed milk, dried milk, coloring material, flavors, sweetening agents and stabilizers. Any of these may become source of various specific species of bacteria. Cheese is an excellent source of protein, fat and minerals such as calcium, iron, phosphorus, vitamins and essential amino acids and therefore it is an important food in the diet of youngsters and elderly. Microbial contamination of cheese may originate from various sources, such sources might be during cheese production, storage or from humans contamination. *Staph. aureus* is ubiquitous microorganism, its major habitats are the skin and the mucous membrane of nasal passage. Lactating animals and human handlers are the main sources for this bacterium and frequently implicated in the transmission of this pathogen.

Milk products act as a good medium for *Staph. aureus*. *Staph. aureus* in food article is an index of its contamination from personnel sharing in production, handling and processing.

Staph. aureus is a leading cause of food poisoning resulting from the consumption of contaminated food with staphylococcal enterotoxins. Enterotoxins are highly thermostable, normal cooking and pasteurization cannot totally inactivate them, so they cause food poisoning.

Staphylococcal food poisoning is characterized by a sudden onset of the symptoms. Vomiting, abdominal pain, and stomach cramps being the most common.

2. Materials and Methods

2.1 Collection of samples:

A total of 85 samples of (35 of small scale plain yoghurt, 25 of each small scale ice cream and locally manufactured fresh white soft cheese “talaga”) were aseptically collected in their retail packages from different super markets and dairy shops in Sharkia Governorate, Egypt.The collected samples were transferred directly to the laboratory.

2.2 Isolation and identification of staphvlococci:

After making of serial dilution of each samples, spread on the dry surface of Baird-Parker agar medium plates using a sterile bent glass rod. Inoculated plates were incubated at 37°C for 48 hours and Staphylococci count were calculated and recorded. Each suspected colony of staphylococci (gray to black colonies) and (black with narrow white margin and surrounded by clear halo-zone extended into the opaque medium)(*Staph.aureus*) was picked up and cultured on slope agar for further

microscopical and biochemical identification.

2.3 Detection of Staph.aureus enterotoxins by ELISA (Ewalid, 1988):

Accurately, RIDASCREEN set C (Art No.: R4101, R-Biopharm AG, Darmstadt, Germany) is an enzyme immunoassay for the determination of *Staph. aureus* enterotoxins by using their definite kits.

3. Results and Discussion

Table (1): Total staphylococcal count/ml. or g. in the examined dairy products samples.

Examined dairy products samples	No. of examined samples	Positive samples	
		No.	%
Yoghurt	35	25	71.43
Ice cream	25	25	100
White soft cheese	25	24	96

Fig. (1): Incidence of isolated *Staph. aureus* in examined dairy products samples.

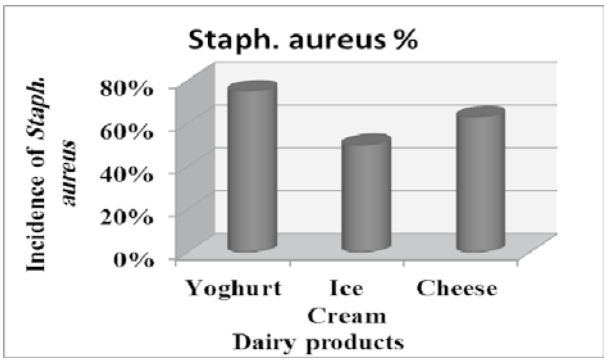
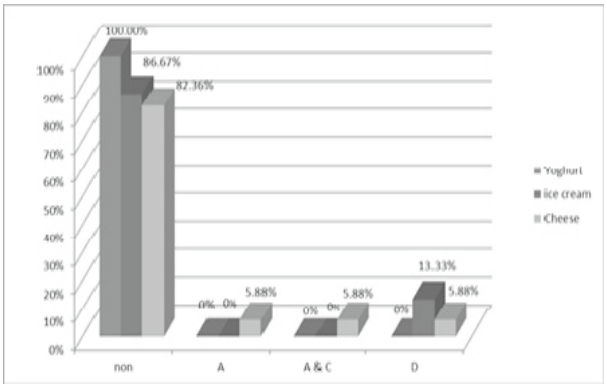


Fig.(2): Incidence of enterotoxins in isolated *Staphylococcus aureus*.



The results tabulated in Table (1) revealed that (71.43%, 100% and 96%) of examined yoghurt, ice cream and cheese samples, respectively were contaminated by staphylococci.

The results showed in fig. (1) showed that *Staph. aureus* were detected in (75%, 50% and 62.97%) of positive staphylococci yoghurt, ice cream and cheese samples, respectively.

Fig.(2) showed that no enterotoxins were detected in isolated *Staph. aureus* strains of yoghurt samples. Higher results were detected by Mohamed and Mazyed (2015) who detected enterotoxigenic, type (A) and (D) with percentage of (33.33% and 50%) respectively.

While in ice cream samples 2 (13.33%) out of 15 isolated *Staph. aureus* strains had enterotoxin D while enterotoxin (A) and (A&C) failed to be detected in all isolated *S. aureus* strains. Higher results were reported by Ertas et al. (2010). (5.88% each) of isolated *Staph. aureus* strains of cheese samples had enterotoxin A, enterotoxin A&C and enterotoxin D.

The presence of staphylococci in dairy products may originated from mouth, skin or nose of workers handling the food. Staphylococci are good indicator of personal hygiene of workers with respiratory infection. *Staph. aureus* is one of such organisms which can transmitted to human through contaminated and untreated milk and milk products.

4. Acknowledgments

I am sincerely grateful for the help provided by all members of food control department, Faculty of Veterinary Medicine, Zagazig University, Egypt.

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P-12

Freshness parameters of marketed sausage

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Two hundreds of different sausage types were examined for detection of freshness parameter. The hydrogen ion concentration (pH) and total volatile basic nitrogen (TVB-N) of all examined samples were within the normal range. The Thiobarbituric acid reactive substances (TBA) expressed by malondialdehyde (MDA) were within the permissible limit in cooked types, while exceed the limit in fresh types. The high level of TBA in fresh type sausages lead develop warm over flavor (WOF).

Keywords: TVB-N – TBA – Sausage- Hot dog- Frankfurter.

1. Introduction

Sausages are comminuted processed meat products made from red meat, poultry or a combination of these with water, binders and seasoning. They are usually stuffed into a casing and may be cured, smoked or cooked. Sausages as one of the oldest forms of meat processing in which meats go through various modification processes to acquire desirable organoleptic and keeping properties. The manufacture of sausage is a simple process of allowing meat to undergo series of controlled structural and chemical changes. These are basic to all cultures but the changes rely on varied methods of preparation and spicing to achieve desired distinctive characteristics. Even though the size and scope of operation have undergone a remarkable level of change the principles and idea behind modern day sausage manufacture in achieving products of high organoleptic value and improved shelf life remain the same. Lipid oxidation of unsaturated fatty acids is initiated by the abstraction of hydrogen in carbon adjacent to the unsaturated bound. The reaction continues by the propagation step that is characterized by decomposition of unstable peroxides and finally results in the production of stable lipid oxidation products of the termination step. Most of the toxic products are formed in the termination step. Aldehydes (malonaldehyde, hydroxynonenal, hydroxyhexenal), ketones, hydrocarbons, epoxides, alcohol, and other organic molecules are formed (**Ferrari 1999**). This work was planned out to determine the freshness parameter of different sausage types.

2. Materials and Methods

2.1 Materials

Two hundreds of (large scale fresh frozen meat sausage, small scale fresh frozen meat sausage, hotdog and frankfurter) were collected randomly from supermarkets and butchery shops at Zagazig city, Sharkia governorate, Egypt, at different levels of sanitations**.**

2.2 Methods

2.2.1. Determination of pH values according to **(Pearson 1984)** pH was determined by using of digital pH meter (Orion research model 2001).

2.2.2. Determination of total volatile basic nitrogen TVB-N according to Conway's micro diffusion technique recommended by **(FAO 1992)**.

2.2.3. Determination of thiobarbituric acid (TBA) TBA test: it was determined as malonaldehyde (end product of lipid peroxidation). The extent of oxidative rancidity was normally reported as TBA numbers or values and expressed as milligrams of malonaldehyde equivalent per kilogram samples.

3. Results and Discussion

3.1 Hydrogen ion concentration (pH)

The pH value is the important physicochemical characteristic to decide the quality and shelf life of sausage.

The Data presented in (Fig.1.A) showed that the pH of oriental small scale, oriental large scale, hot dog and frankfurter were ranged from 5.52 to 6.13, 5.36 to 5.94, 5.47 to 6.21 and 5.24 to 5.98 with mean values of 5.65 ± 1.9, 5.61 ± 1.72, 5.92 ± 1.41and 5.85 ± 1.32, respectively.

The statistical tests revealed significant differences ($P < 0.05$) between raw sausage (small scale and large scale) and cooked sausage (hot dog and frankfurter). The differences due to the effect of heat treatment during cooking which affect microorganisms which lead to sour fermentation of sausage. Additionally pH different between raw and cooked sausages may be due to the pH of meat introduced in manufacturing and the use of alkaline phosphate in cooked sausage **(Puolanne *et al.*, 2001).**

3.2 Total volatile basic nitrogen (TVB-N)

The Data presented in (Fig.1.B) showed that the TVB-N of oriental small scale, oriental large scale, hot dog and frankfurter were ranged from 7.54 to 15.36, 13.4 to 19.22, 6.36 to 9.1 and 5.96 to 10.32 with mean values of 12.1 ± 2.12, 15.2 ± 3.01, 6.3 ± 2.1 and 7.5 ± 1.39 mg/100 g., respectively. All examined sausage samples located within the

permissible limit of the **EOS. (2005)** where the level shouldn't exceed 20mg/ 100g.

Nearly similar value for TVB-N (7–18 mg VBN/100 g) in stored Chinese-style sausage has been reported **(Lin and Lin 2002)**.

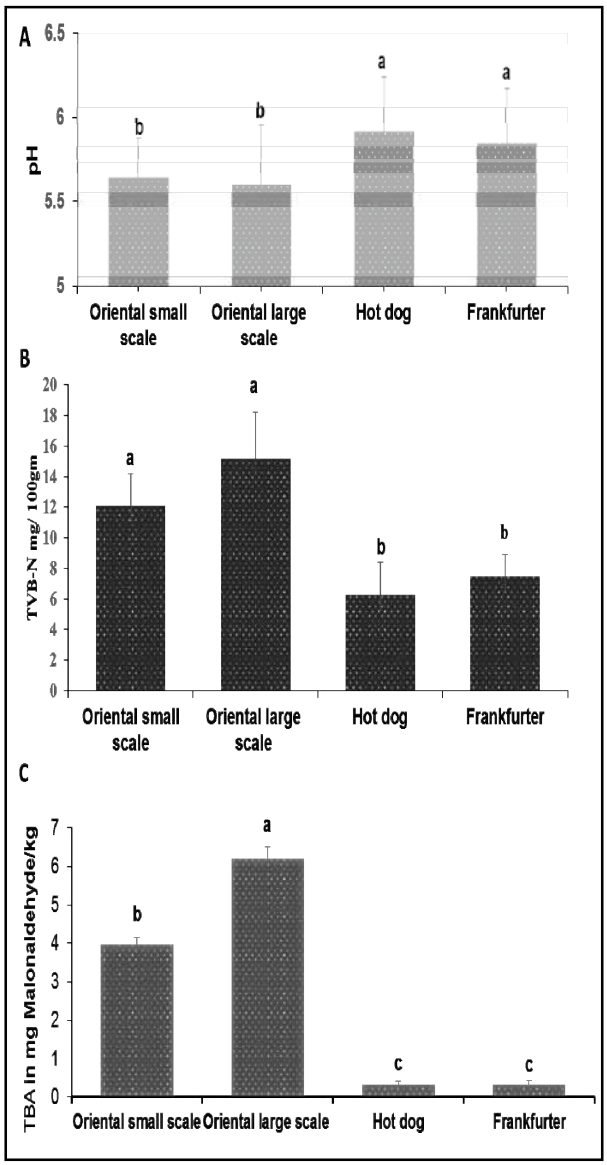

Figure 1: A- pH of different sausage samples. B- TVB-N in (mg/100g). of different sausage samples. C- TBA in (mg Malonaldehyde/Kg) of different sausage samples. Means carrying different letter are significantly different (P< 0.05).

3.3 Thiobarbituric acid (TBA)

Thiobarbituric acid (TBA) number is important relevant characteristics of meat product that indicates the degree of fat oxidation state and later on stage rancidity. The Data presented in (Fig.1.C) showed that the TBA of oriental small scale, oriental large scale, hot dog and frankfurter were ranged from 2.45 to 5.24, 2.98 to 8.14, 0.13 to 0.51 and 0.21 to 0.48 with mean values of 3.95 ± 0.22, 6.2 ± 0.31, 0.31 ± 0.14 and 0.32 ± 0.16 mg malondialdehyde / Kg, respectively.

The results of TBA in fresh sausage were higher than the permissible limit, while that for cooked types were within the permissible limit of the **EOS**. **(2005)** where the level shouldn't exceed 0.9 mg malondialdehyde / kg.

It is important to note that chronic ingestion of lipid peroxidation products such as MDA, is associated with an increased risk of many chronic diseases such as changes in blood LDL lipoproteins, resulting in the formation of atherosclerotic plaques and, subsequently, of atherosclerosis and coronary artery disease. In addition to MDA is also mutagenic and carcinogenic in vitro and in vivo **(Ferrari, 2000)**. There were a significant difference (P< 0.05) between different sausage types and this may be due to the facts obtained from the manufacturing process where fat percentages may reach up to 30% in fresh sausage. In addition to the storage of fat used during processing sausage.

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P-13

Effect of some decontaminants on heavy metals load in fish fillets

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This study was undertaken to investigate the effect of some treatments on the concentration of lead, cadmium and mercury in *Claris lazera* fillets consumed by Egyptian consumers .treatments of fish fillet with distilled water, acetic acid 5%, garlic extract 2%, garlic extract 4% reduced cadmium and lead concentrations. Garlic extract 4% led to a significant reduction of mercury contents in *Claris lazera* fillets.

Keywords: lead, cadmium and mercury

1. Introduction

Fish is the main supply of cheap and healthy protein to a large percentage of the world's population The proper human diet should satisfy the requirements for energy and nutritive components including essential polyunsaturated fatty acids, exogenous amino acids being the component of standard proteins, mineral components, fat and water-soluble vitamins.

Heavy metals such as lead, cadmium and mercury which are widely distributed in the air, agricultural land and water into the food chain (**Daoud et al., 1998)**

Heavy metals such as lead, cadmium and mercury are toxic at even minute concentrations. Since some of them may accumulate in the food chain. The risk of heavy metals contamination is of great concern for both human health and food safety.

Heat treatment of food for long period of time cannot destroy heavy metals, because they considered as non-degradable pollutants which cannot be degraded by natural or artificial means **(Mohamed, 2004)**

Thus, this study was undertaken to show the effects of some decontaminants on heavy metal load in fish fillets .Public health importance of such residues was also discussed.

2. Materials and Methods

A Total of 50 *Claris lazera fillets* were collected randomly from Zagazig markets in polyethylene bags and transferred immediately to the Central laboratory, Faculty of Veterinary Medicine, Zagazig University .Each sample was 35 g and their length with average 10cm and thickness 1cm .samples were divided into 5 groups (each of 10 fillets samples).these groups were classified as control group (non treated),second group with distilled water, third group with acetic acid 5%,fourth group with garlic extract 2% and fifth group with garlic extract 4%. Exposure time for all treatments for 30 min.

Extraction and heavy metals measurement: one gram from each sample was macerated in screw capped tube. Five millimeters of digestion mixture consists of three parts of nitric acid and two parts of per chloric acid were added to the tissues samples. The tubes were allowed to stand overnight at room temperature. The tubes were heated for three hours at water bath adjusted at 70 C to ensure complete digestion of samples. The tubes were cooled at room temperature and then diluted with 5ml deionized water; capped with plastic film and thoroughly mixed the filtrate was collected in Pyrex glass test tubes. These tubes were capped with polyethylene film and kept at room temperature until analyzed for heavy metals contents. The metals were measured using Atomic Absorption Spectrometry (AAS).

3. Results and Discussion

3.1 Cadmium

The mean values of cadmium content after dipping of *Claris lazera* fillets in distilled water , acetic acid 5%, garlic extract 2%, garlic extract 4% were 0.072 ± 0.016, 0.050 ± 0.001 ,0.030 ± 0.003, 0.040 ± 0.005, 0.016 ± 0.002 ppm wet weight respectively. There was significant reduction of cadmium content. There were no significant differences between all treatments with each other.

3.2 Lead

The mean values of Lead content after dipping *Claris lazera* fillets in distilled water, acetic acid 5%, garlic extract 2%, and garlic extract 4% were 0.610 ± 0.080, 0.540 ± 0.140, 0.650 ± 0.110, 0.530 ± 0.060 ppm respectively. There was significant reduction in lead content but there weren't significant differences between all with each other.

3.3 Mercury

The mean values of mercury content after dipping fillets of *Claris lazera* fillets in distilled water, acetic acid 5%, garlic extract 2%, garlic extract 4% is 0.480 ± 0.210, 0.420 ± 0.080, 0.440 ± 0.140, 0.340 ± 0.110, and 0.180 ± 0.060 ppm respectively .There weren't significant reduction in mercury content, except in garlic extract 4%.

In conclusion it was clear from the results that garlic extract 4% had the most powerful effect in

decontamination of lead, cadmium and mercury in *Claris lazera* fillets. Thus, we highly recommend soaking the fillets of *Claris lazera* with the extract 4% of garlic.

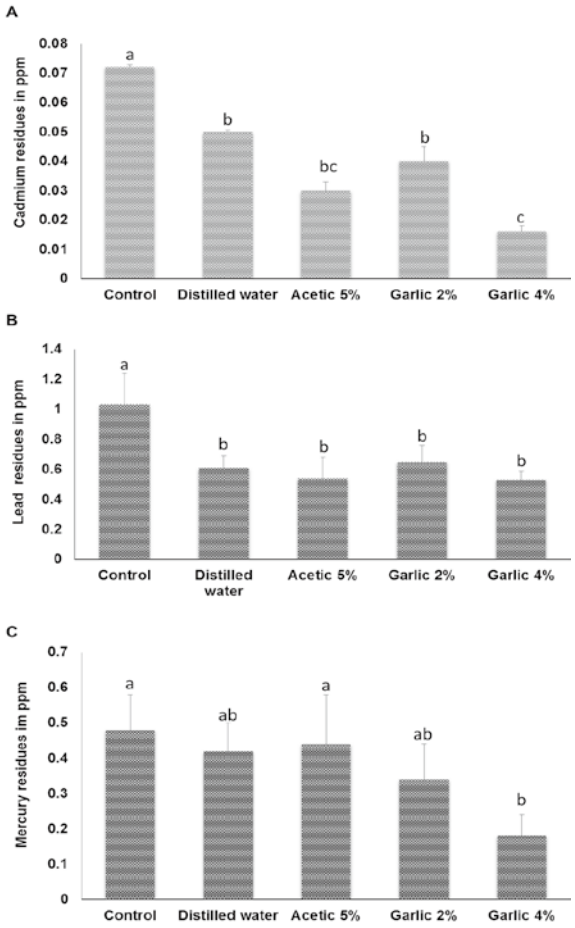

Figure 1: Effect of some decontaminant on A. cadmium residues. B. Lead residues. C. Mercury residues in *Claris lazera* fillet.

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P-14

Using of some volatile oils for improving the quality of some poultry meat products

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Food-borne illnesses in human due to bacterial pathogens and their toxins are of public health concern especially when it is correlated to the consumption of poultry meat and its products. Therefore, there is a special attention paid for the hygiene practices in meat production and storage. This study was carried out to assess 100 random samples of poultry meat products represented as (chicken fillet, chicken nuggets, chicken luncheon and chicken pane) 25 of each in Zagazig City, Sharqia province, Egypt. All samples were investigated bacteriologically for determination of Total *Staph. aureus* count, isolation and identification of *Staph. aureus,* in addition to detection of its enterotoxins. Moreover, some trials for improving the quality of the examined chicken meat products using natural biocide as volatile oils (cumin oil and marjoram oil) were conducted. The results revealed that the mean values of *Staph. aureus* count in the evaluated samples of chicken fillet, chicken nuggets, chicken luncheon and chicken pane were 4.39 ± 0.14, 4.09 ± 0.24, 4.94 ± 0.47 and 4.30 ± 0.21 cfu/g, respectively. While *Staph. aureus* was isolated from the aforementioned samples with percentages of 64%, 40%, 32% and 24%, respectively. Three different enterotoxins were identified serologically and classified into SEA, SEC and SED. While, in the experimental part of the study, cumin oil and marjoram oil had proved their effect on reducing not only *S. aureus* but also, on other microbial load that had been detected before. Their effect varied depending on the concentration (0.5%-2%-3%) and the time of exposure (0.5hour-2hours).

Keywords: Food-borne illnesses, *Staph. aureus*, poultry meat products, enterotoxins, essential oils.

1. Introduction

Even with the global awareness, food-borne diseases still show an upward trend every year resulting from bacterial contamination of the food with different pathogens. These microbes could find their ways to the food from variable sources such as improper handling, processing, packaging and storage of the products leading to reducing the shelf life of the products, rendering them of inferior quality. Moreover, these products might conistitute a public health hazard leading to serious outbreaks.

The most common potentially pathogenes causing foodborne illness are *Staph. aureus, E. coli*, Salmonella spp., *Campylobacter, Clostridium botulinum, Listeria monocytogenes* and others. *Staph. aureus* is a substantial food-borne pathogen due to the ability of enterotoxigenic strains to produce heat stable staphylococcal enterotoxins (SEs) serologically classifed into SEA, SEB, SEC, SED and SEE, which preformed in food causing staphylococcal food poisoning. Staphylococcal intoxication occurs as a result of consumption of food containing staphylococcal enterotoxins in short period ranged from 30 minutes to 8 hours leading to several symptoms include abdominal cramping, vomiting, diarrhea, nausea, and chills and the recovery witnin 1-2 days (Argudín et al., 2010).

Essential oils (EOs) are aromatic oil liquids derived by various methods from all the plant parts. They exert antiviral, antibacterial, antimycotic, antitoxigenic, antiparasitic and insecticidal properties. Also, they used not only as flavouring agents, in pharmaceuticals and perfumes but also in food preservation in order to prevent bacterial and fungal growth because EOs can act against wide variety of Gram-positive and Gram-negative pathogens(Burt, 2004). Thus, this study was performed to investigate the antimicrobial efficiency of volatile oils (cumin and marjoram oil).

2. Materials and Methods

2.1 Collection of samples

A total of 100 random samples of poultry meat products classified into 25 samples of raw poultry products (chicken fillet), 25 samples of half cooked poultry products (chicken nuggets) and 50 samples of cooked poultry products (chicken luncheon and chicken pane) (25 of each) were collected randomly from local slaughter poultry shops and different supermarkets of different sanitation levels from Zagazig city with different trade names. The samples were taken under aseptic condition in sterile polyethelene bags then transferred to the laboratory immediately where carry out a microbiological assessment of the collected

samples.

2.2 Preparation of samples:

- Enumeration and Isolation of *Staph. aureus* (APHA, 1992)
- Demonstration of *Staph. aureus* enterotoxins by ELISA (Ewalid, 1988).

3. Results and Discussion

From Fig. (1), it was displayed that *Staph. aureus* count was ranged from (3.00 to 5.00 cfu/g with a mean value 4.39±0.14 cfu/g for chicken fillet), (3.00 to 5.48 cfu/g with average 4.09±0.24 cfu/g in chicken nuggets), (3.48 to 6.48 cfu/g with a mean value 4.94±0.47 cfu/g In chicken luncheon) and (3.00 to 5.48 cfu/g with a mean amounted to 4.30±0.21 cfu/g in chicken pane). While, the results in Fig. (2) showed that positive *Staph. aureus* was occurred in chicken fillet by 16 (64%) followed by 10(40%) in chicken nuggets, then chicken luncheon by 8(32%) after that, 6(24%) in chicken pane.

Staph. aureus gain its public concern due to its ability to produce heat stable enterotoxins which causing Staphylococcal intoxication. The results from Fig. (3) illustrated the positive *Staph. aureus* isolates which have the ability to produce the enterotoxins. It was clear that, SEA was expressed as 2 (8%), 1(4%), 2(8%) in chicken fillet, chicken nuggets and chicken luncheon, respectively. While, SEC only revealed by 1 (4%) from chicken pane. Moreover, SED valued to be 1 (4%) in chicken fillet and 2(8%) in chicken nuggets. Only two isolates from chicken fillet, one produce (A&C) and the other produce (A&D).

This study included an experimental part that seeks to approve the bioactivity of some essential oils (cumin &marjoram oil). In Fig.(4) comparing with the control group, it was so obvious that both of cumin oil and marjoram oil have antistaphylococcal effect. This effect varied according to the concentration (0.5%-2%-3%) and the time of exposure (0.5 hours-2hours).

Concerning the cumin oil, cumin oil 0.5% for 0.5hour reduce the *Staph. aureus* count by 2.66% and after 2hours, the reduction amounted to 6.38%. On the other hand, when increasing the concentration to 2%, the reduction was 6.38% for 0.5 hours and 9.93% for 2hours. While, 3%cumin oil caused reduction equal to 9.22% in 0.5 hours and 13.83% in 2 hours.

However, regarding to marjoram oil, 0.5% marjoram oil has the ability to diminish *Staph. aureus* count in 4.96% after 0.5hours and 7.8% within 2hours. This reduction percentage increased when the concentration reached to 2% and recorded the reduction % as 7.8% within 0.5hours and 10.46% in 2hours. While, 3% marjoram oil decline *Staph. aureus* count in 0.5hour to be 9.22% and 13.3% in 2hours. In addition, the effect of these oils have been recorded in reducing the APC, TMC and TCC. From the aforementioned results, it was highly recommended to use cumin oils and marjoram oil to increase shelf life of poultry meat products.

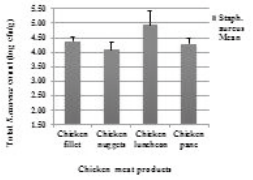

Fig. (1): Total *Staph. aureus* Count (log CFU/g) in the examined chicken meat products (n=25 of each).

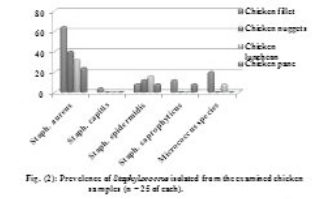

Fig. (2): Prevalence of *Staphylococcus* isolated from the examined chicken samples (n = 25 of each).

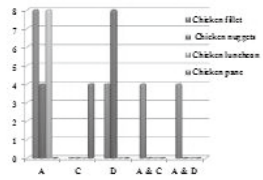

Fig. (3): Prevalence of *Staph. aureus* and its enterotoxin in the examined chicken samples (n = 25 of each).

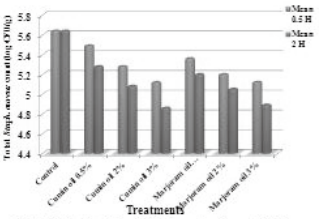

Fig. (4): Effect of the Cumin oil and Marjoram oil on the count of *Staph. aureus* in contaminated chicken meat (log_{10} CFU).

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P-15

Polycyclic aromatic hydrocarbons in grilled *Tilapia nilotica* and *Mugil cephalus*

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Polycyclic aromatic hydrocarbons (PAHs) present in the two types of fish by different percentages, but the most important compound was benzo (a) pyrene because of its public health effect and it exceeded the permissible limits in (12%) in grilled *Tilapia nilotica* fish samples and (16%) in grilled *Mugil cephalus* fish samples. PAHs effects on health appear by long exposure, so we must do our best to decrease their level in food. This can be achieved by hygienic treatment of the industrial effluents before their drainage in fresh water streams and marine water coasts, fishing areas must be away from sources of the industrial and petroleum effluents and continuous monitoring of PAHs residues in different fish types in Egyptian markets is also recommended.

Keywords: Benzo (a) pyrene, PAHs, *Mugil cephalus*, *Tilapia nilotica*.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a group of chemicals that are formed during incomplete burning of charcoal, oil, gas, wood, garbage, or other organic substances, such as tobacco and charbroiled meat or fish. The ubiquitous nature of PAHs makes them present as trace contaminants in air, water and soil. Although air and drinking water may be responsible for some human exposure, the highest PAHs intake is typically associated with their occurrence in diet. However, during industrial smoking, heating and drying processes, combustion products come into direct contact with food and PAHs contamination can occur **(Chen and Chen, 2005).** Moreover, PAHs are lipophilic compounds and commonly found in petroleum fuels, tar and various edible oil, the common examples of these compounds were naphthalene, anthracene, phenanthrene, benzo (a) pyrene (BaP), benzo (b) fluoranthene, fluoranthene

Food exposure to carcinogenic PAHs can be said to generally emanate either from their contamination during processing such as from roasting, smoking and charcoal grilling **(Chen and Chen, 2005)** or from the external environment due to anthropogenic activities.

Concerning the public health importance, some PAHs comprise the largest group of chemical compounds known to be cancer causing, producing tumors in epithelial tissues in practically all animal species tested. The present study was designed to throw light on the occurrence of PAHs residues in grilled fish species (*Tilapia nilotica* &*Magil cephalus*) marketed in Sharkia Governorate and to study the public health effect of these residues.

2. Materials and Methods

2.1 Materials

Fifty samples of grilled *Tilapia nilotica* and *Mugil cephalus (*25 for each) were collected randomly from different markets in different localities in Sharkia Governorate for detection of 11 polycyclic aromatic hydrocarbons (PAHs) compound residues: naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, chrysene, benz (b)fluoranthene, benzo (a) pyrene and dibenzo(a,h) anthracene. The soft parts of fish were removed and the muscle tissue sample was taken from the dorsal muscle in aluminum foil and kept frozen at (-18ºc) until analysis.

2.2 Methods

The analysis of PAHs residues was conducted according to the method described by (Ahmed et al., 1998). Extraction procedures and clean up were performed in Animal Health Research Institute, Zagazig Provincial Lab. Estimation of PAHs levels by Gas Chromatography was conducted in Pesticide Residue Dept. Central Pesticide Lab., Agriculture Research Center, Giza.

3. Results and Discussion

3.1 Incidence of PAHs

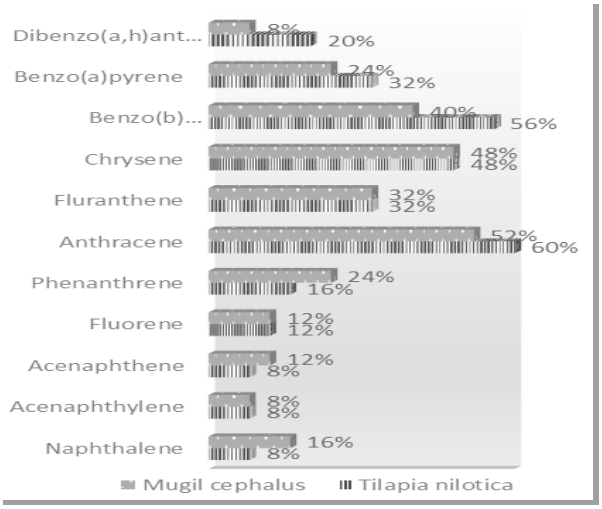

Figure 1: Incidence PAHs residues in examined grilled *Tilapia nilotica &Mugil cephalus* fish samples.

The illustrated data in Fig. (1) showed the comparison between grilled *Tilapia nilotica* and *Mugil cephalus* samples according to PAHs incidence. It was revealed that the incidence of anthracene, benzo (b) fluranthene, benzo (a) pyrene, and dibenzo (a,h)anthracene residues which were detected in grilled *Tilapia nilotica*

fish samples are higher than those detected in grilled *Mugil cephalus* fish samples. On the other hand, the incidence of presence of naphthalene, acenaphthene, and phenanthrene residues were detected in grilled *Tilapia nilotica* fish samples are lower than those detected in grilled *Mugil cephalus* fish samples. Meanwhile, acenaphthylene, fluorine, fluoranthene and chrysene residues were detected at the same percentage in both grilled *Tilapia nilotica* and *Mugil cephalus* fish samples. The difference between the percentages of PAHs in the two species may be attributed to the different pollution sources. Thus, PAHs residues in grilled fish are the products of both water pollution and grilling process.

Generally the exact mechanism of formation of PAHs in grilled or smoked foods is not precisely known. it is generally due to the pyrolysis of organic matter such as fat, protein and carbohydrates at temperature above 200 °C, PAHs formation is favored at temperature range of 500- 900 °C . The greatest concentrations of PAHs have been shown to arise from pyrolysis of fat **(Bartle, 1991).**

3.2 Concentration of PAHs

Results given in table (1) showed that the mean values of total PAHs in grilled *Tilapia nilotica* and *Mugil cephalus* were 3034 and 3561µg/kg, respectively. Our results were higher than those of **Nasr *et al.* (2010)**, which estimated that total PAHs residues were found in levels ranged between 466 to 2019 µg/kg in fresh water fish. This result means that grilling lead to increase of PAHs residues by the yield of direct contact of lipids dripping at intense heat directly over the flame. This condition can generate volatile PAHs that in turn be adhered to the surface of the food as the smoke rises. **Essumang *et al.* (2014)** recorded that the mean total PAHs in the smoked sardines from the various communities ranged from 510.59 µg/kg to 1461.79 µg/kg for all seasons with a mean value of 716.84 µg/kg in smoked cured fish.

Because of the serious public health significance of benzo (a) pyrene, the European union determined only a permissible limit of 2µg/kg in fish among all the PAHs compounds (Commission Regulation (EC) No 188/2006).

Benzo (a) pyrene residues exceeded the permissible limit in 3 samples (12%) in grilled *Tilapia nilotica* fish samples and 4 samples (16%) in grilled *Mugil cephalus* fish samples. The relatively high level of benzo (a) pyrene in the fresh water *Tilapia nilotica* fish (32%) than those in the marine water *Mugil cephalus* (24%) may be explained by the difference of the source of PAHs pollution. Although the increase in number of samples exceeded the permissible limit in grilled *Mugil cephalus* than those grilled Tilapia nilotica mean that there is a positive correlation between the fish fat% and the total PAHs level.

Concerning the public health point of view, PAHs can be harmful to the human health under some circumstances. Several PAHs, including benzo (a) pyrene, benzo (b) fluoranthene, chrysene, di benzo (a,h) anthracene cause tumors in the human **(ATSDR, 2011).** So the Commission Regulation (EC) No 1881/ 2006 amended by Commission Regulation (EC) No 835/2011 has set maximum levels for 4 PAHs in the meat of smoked fish of 30 µg/kg with the exception of fishery products 12 µg/kg.

The health risk associated with the high concentration of benzo (a) pyrene, benzo (a) anthracene and chrysene. The PAHs were proven to be animal carcinogens and in human they are suspected of causing cancer, so special attention must be given to intake of grilled fish since high amounts of PAHs can be taken in single meal.

Table (1) PAHs in grilled fish samples (µg/kg).

PAHS	**Tilapia nilotica** Mean ± SE	**Mugil cephalus** Mean ± SE
naphthalene	475 ± 23	405 ± 203
acenaphthylene	235 ± 105	705 ± 205
acenaphthene	18 ± 3	212 ± 104
fluorene	180 ± 90	38 ± 8
phenanthrene	76 ± 30.2	52 ± 26
anthracene	167 ± 52	483 ± 103
fluranthene	481 ± 241	189 ± 90
chrysene	947 ± 389	1061 ± 505
Benzo(b) fluranthene	430 ± 211	188 ± 97
Benzo(a)pyrene	21 ±10	220 ± 113
Dibenzo(a,h)anthracene	4 ± 2	8 ± 3
Total	3034	3561

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P-16

Detection of non-O157 shiga toxin producing *E.coli* and heavy metal residues in some ready-to-eat meat products marketed in Egypt

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The demand of ready to eat meat products has been increased in the Egyptian food markets and contamination of these meat products with pathogenic microorganisms and presence of heavy metal residues rendering them unacceptable for human consumption since they may be consumed without further cooking. So, the objectives of this study were firstly to detect of non-O157 Shiga toxin producing *E.coli* strains and secondly to determine the residual levels of lead, cadmium and aluminum in ready to eat meat products (kebab, grilled kofta, hawawshi and fried liver) which considered as the most popular meat products in Egypt. The isolated non-O157 STEC strains were O111, O26, O103, O113, O119, O55, O91 and O127. Despite, aluminum concentrations didn't exceed the permissible limits, lead and cadmium were present in higher concentrations than the permissible limits in most of the examined samples.

Keywords: E.coli, meat products, lead, Shiga toxin, cadmium

1. Introduction

In Egypt, the demands of ready to eat meat products have been increased because they represent quick easily prepared meat meals with delicious taste, containing high nutritive values beside solving the problem of the shortage of fresh meat of high price, but the risk of contamination of such kind of food is considered a great problem since they already heat treated and consumed without further cooking. So, they may be good substrates for pathogenic microorganisms such as Shiga toxin producing *E.coli* (STEC) that constitute health hazards to consumers and have been incriminated in several outbreaks of food poisoning (Ghoneim *et al*., 2014).

Although, *E.coli* O157 is the most recognized STEC serotype that causing severe complications, some of non-O157 STEC strains have been implicated in several outbreaks which appear as severe as that caused by O157. So, the awareness of non-O157 as a food safety concern has been growing which can be due to the tendency of these strains to become pathogenic and causing serious impacts on human health that ranging from bloody diarrhoea and haemorrhagic colitis to life-threatening haemolytic uremic syndrome ending with renal failure (Mathusa *et al*., 2010).

Not only has the attention about the presence of non-O157 STEC increased, but the risk of the presence of toxic heavy metals such as lead, cadmium and aluminum in meat also became a great concern for both food safety and human health because it is considered as one of the most serious problems in the world and this is due to their toxic and cumulative nature and the multiplicity of their contamination sources as they are widely distributed in the air, soil and even in water. Heavy metals can't be destroyed or breakdown through heat treatment of food even for long period of time. Thus, there is a great risk associated with the consumption of meat products even their presence in small amounts. Since meat is a significant part of human feed, thus, levels of heavy metals should be measured in meat intended for human consumption, especially if they were prepared from already contaminated raw meat.

Therefore, this study was undertaken to detect non-O157 STEC strains in 60 ready to eat meat products represented by (15 each of kebab, grilled kofta, hawawshi and fried liver), beside measurement of lead, cadmium and aluminium residual levels in these samples.

2. Materials and Methods

Preparation of samples

25 grams of each sample was added to 225 ml of buffered peptone water and thoroughly homogenized. Then 1 ml of initial dilution was transferred to sterile tube containing 9ml of sterile buffered peptone water and mixed well to obtain the next dilution from which further decimal serial dilutions will be prepared.

Isolation and identification of non-O157 STEC:

The most probable number (MPN) technique was used. 1ml from each dilution was used to inoculate three series of three test tubes containing Macconkey broth with Durham's tube. (The tubes were incubated at 44 °C for 24–48 h). The production of (acid yellow color) and gas (appear in Durham tube) from lactose indicate *E. coli* positive. A loopfull of the positive tubes was cultured on Eosin Methylene Blue (EMB) agar and incubated at 37 °C for 24 h. Colonies appear greenish, metallic with dark purple center were transferred to nutrient agar slants and incubated at 37 °C for 24 h and then stored at 4 °C for further identification.

Identification of isolates was carried out based on staining and biochemical tests (catalase, oxidase, indol, methyl red, Voges Proskauer test, citrate utilization, nitrate reduction, urease, H2S production, gelatin liquefaction and Eijkman test).

Serodiagnosis of *E. coli*

The confirmed *E. coli* isolates were serologically identified by using rapid diagnostic *E. coli* antisera sets (DENKA SEIKEN Co., Japan) for diagnosis of the enteropathogenic types.

Extraction and heavy metal measurement

One gram from each sample was macerated in screw capped tube. 5 ml of digestion mixture consists of 3 parts of nitric acid and 2 parts of Perchloric acid were added to the tissue sample. The tubes were allowed to stand overnight at room temperature. Then tubes were heated for 4 hrs in water bath starting from 60 °C till reach 110 °C ensure complete digestion of the samples. The tubes were then left to cool at room temperature and diluted with 5 ml deionized water, capped with plastic film and thoroughly mixed. The digested solutions were filtered through Whattman filter paper. The filtrates were collected in Pyrex glass test tubes capped with polyethylene film and kept at room temperature until analyzed for their lead, cadmium and aluminum concentrations by using Flame Atomic Absorption Spectrophotometer (VARIAN, Australia, model AA240 FS).

3. Results and Discussion

STEC is recognized worldwide as one of the most foodborne pathogens that responsible for sporadic cases to serious outbreaks and its pathogenicity is comprised from the production of shiga toxin with/ without eaeA. So, the majority of human diseases are associated with strains of STEC that produce either Stx1 and/or Stx2. Recently, it is obvious that O157 isn't the only strain able to produce Stx, but also there are many non-O157 strains producing Stx and eaeA as recorded in table 1. Which declared the isolated non-O157 from the examined samples and the detection of such strains is considered a big problem because some of them such as O111 and O103 were previously isolated in most cases of non-O157 HUS outbreaks (Brooks *et al*., 2005) and also, other strains have been associated with human infections. Almost all the examined meat products were contaminated with non-O157 STEC which mustn't be detected in such kind of food that previously heat treated. So, their detection provided an estimate of poor personal hygiene and poor sanitation during preparation and handling which considered as a case of public health concerns. In addition, the increasing of the environmental pollution which considered as a main cause of the presence of high concentrations of heavy metals such as lead, cadmium and aluminium. It was declared from figure 1 that the highest concentrations of Pb, Cd and Al were recorded in the examined fried liver samples and it may be due to that heavy metals are mainly stored in liver that is responsible for detoxification of xenobiotics. Although, aluminium concentrations in all examined meat products didn't exceed the permissible limits, most of the examined meat products had high concentrations of lead and cadmium that exceeding the permissible limits decided by Egyptian authorities. Even its presence in lower concentrations is considered objectionable as it is well known that Pb and Cd are toxic cumulative metals that causing injury to health through progressive and irreversible accumulation in human bodies as a result of repeated ingestion of small repeated amounts of food contaminated with these elements for long periods that leading to serious illnesses.

It is obvious from these findings that ready to eat meat products marketed in Egypt constitutes a potential hazard to human health, especially RTE fried liver due to the presence of non-O157 STEC strains and high concentrations of lead and cadmium. So, Measures to control the quality of the raw material, environmental and hygienic conditions during preparation and handling should be taken.

Examined samples	+ve samples (%)	The identified serotypes	E.coli biotype	Stx1	Stx2	eaeA
Kebab	8 (53.3%)	O26	EHEC	+	-	+
		O127	ETEC	+	-	-
		O103	EHEC	+	+	+
Grilled kofta	4 (26.7%)	O55	EPEC	-	+	+
		O119	EPEC	+	+	-
Hawawshi	3 (20%)	O91	EPEC	+	-	-
Fried liver	4 (26.7%)	O113	EPEC	-	+	-
		O111	EHEC	+	+	+

Table 1: Detection of non-O157 STEC in examined meat products.

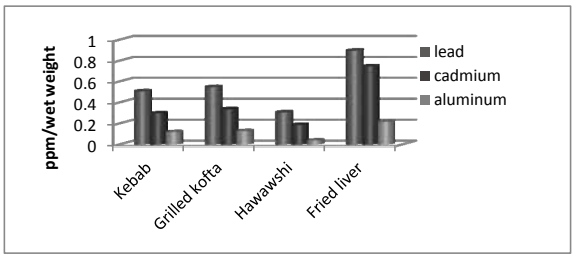

Figure 1: Mean concentrations of lead, cadmium and aluminium residues (ppm/wet weight) in the examined samples of ready-to-eat meat products.

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P-17

Effect of some processing on biogenic amines in beef burger

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This study was undertaken to investigate the effect of different treatments (chilling, freezing and addition of thyme oil) on the formation of histamine or delayed the accumulation of it in burger samples. In addition, we investigated the prevalence of biogenic amines producing microorganisms in the examined samples. Production of histamine is an indicator of spoilage of meat, so we investigated the total Enterobacteriacae count, Proteolytic count and Psycrophilic count in the burger samples before and after treatment. The results revealed that chilling, freezing or chilling and freezing with thyme significantly reduced the histamine levels. Similarly, microorganisms load was reduced after different treatments.

Keywords: biogenic amines, thyme oil, histamine, burger.

1. Introduction

Awareness concerning the importance of food safety, and related impacts on human health has grown significantly in recent years due to potential toxicological and physiological effects of food ingredients on individuals. Much emphasis has been given to biogenic amines (BAs), as toxic substances usually present in fermented food and used as indicators for food subjected to poor hygienic conditions during food processing. During food spoilage, microorganism can produce high concentration of biogenic amines by decarboxylating the free amino acids. Free amino acids either may be liberated through proteolysis of food.

The control of biogenic amines formation mainly focused on the controlling the growth of biogenic amines forming bacteria. Temperature is the major factor for controlling the biogenic amines formation in food. Freezing is one of the most common methods used to prolong the shelf life of food products. The freezing process slows down the rate of many physico-chemical and microbiological changes. Thyme essential oil is well known for its high phenolic compounds content including carvacrol, thymol, p-cymene and γ-terpinene with strong antioxidant and antimicrobial activities against a wide range of spoilage and pathogenic microorganisms **(Roby et al., 2013).** Spices (including thyme), in general, are well known for their antimicrobial ability mainly due to their content of phenolic compounds. The possible mechanisms for antimicrobial effect of phenolic compounds include altering microbial cell permeability; interfering with membrane function including electron transport, nutrient uptake, protein and nucleic acid synthesis, and enzyme activity; interacting with membrane proteins causing deformation in structure and functionality; and substituting alkyls into phenol nucleus **(Zhang *et al*., 2010)**. This study was planned to throw a light on the effect of different processing on biogenic amines as chilling, freezing and thyme oil as natural plant extract which has antimicrobial activity. Also its effect on related microbial load of Enterobacteriacae, Proteolytic counts and Psychrophilic counts which has decarboxylase activity was examined.

2. Materials and methods:

Sample collection: A total of 20 Random samples of fresh beef burger (1000 grams of each) were collected from different local butcher shops of different sanitation levels in Zagazig city, Sharqia province, Egypt. Each sample was divided into four portions. First portion was used as a control without any treatment (TEO, freezing or mix) which stored at 4 ºC, the second portion was mixed with TEO (1.5%) then stored at 4 ºC. Third portion was stored at -18º C. Fourth portion was mixed with TEO (1.5%) then stored at -18º C. Each portion was packed in sterile plastic bag and stored according to each treatment and subjected to the following examination:

Determination of biogenic amines in beef burger by using ELISA technique: The basis of the test resides in the antigen-antibody interaction. The microtiter wells are coated with histamine. Anti histamine antibodies and standards, respectively sample solutions, are added. The free and the immobilised histamines compete for the antibody binding sites. After washing, secondary antibodies labelled with peroxidase are added. These bind to the antibody histamine complexes. Any unbound enzyme conjugate is then removed by the washing step. Enzyme substrate (urea peroxide) and chromogen (tetramethylbenzidine) are added to the wells and incubated. Bound enzyme conjugate converts the colorless chromogen into a blue product. The addition of the stop reagent causes the colour change from blue to yellow. The measurement is done photometrically at 450 nm. The resulting absorbance values are inversely

proportional to the histamine concentration in the sample.

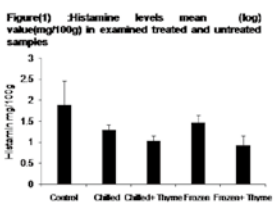
Bacteriological examination: 1-Total Enterobacteriacae count was enumerated on Violet red bile glucose agar and incubated at 37°C for 24 hours. 2- Total Proteolytic count was enumerated on Skim Milk Agar medium containing 10% Skim Milk. 3- Psychrophilic count was enumerated on standard plate count agar medium and incubated at 7ºC for 10 days.

3. Results and Discussion

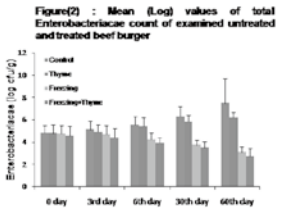
Histamine concentration was measured in meat burger sample as a control and the concentration ranged from 10.69 mg/kg to 18.83 mg/kg with a mean value of 18.83 mg /kg. From the results shown in figure (1) it is clear that chilling and thyme treatment for meat burger samples could reduce histamine concentration from 1.88 ± 0.57 mg/100g to 1.03 ± 0.11 mg/100g with a reduction percent of 45 % while chilling only recorded a concentration of 1.29 ± 0.10 mg/100g with a reduction percentage of 31%. From the results shown in figure (1), it is clear that freezing and thyme treatment for meat burger samples could reduce histamine concentration from 1.88 ± 0.57 mg/100g to 0.92 ± 0.23 mg/100g with a reduction percentage of 50% while freezing only recorded a concentration of 1.466 ± 0.18 mg/100g with a reduction percentage of 22%.

Production of histamine is an indicator of spoilage of meat, so we investigated the total Enterobacteriacae count, Proteolytic count and Psychrophilic count in the burger samples before and after treatment. From the results shown in figure (2.3.4), it is clear that chilling and Thyme treatment for meat burger samples at 6th day could reduce the total Enterobacteriacae count from 5.53 ± 0.76 (log cfu/g) to 5.41± 0.74 (log cfu/g), the total Proteolytic count from 5.77 ± 0.95 (log cfu/g) to 5.22 ± 0.81 (log cfu/g) and the total Psychrophilic count from 6.86± 0.76 (log cfu/g) to 6.33 ± 0.51 (log cfu/ g). It is sclear that freezing and Thyme treatment for meat burger samples at 60th day could reduce the total Enterobacteriacae count, total Proteolytic count and the total Psychrophilic count.

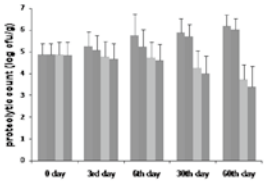
Thus, it is highly recommended to use thyme oil beside freezing or chilling to reduce histamine formation and its producing bacteria.



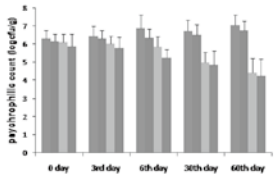
Figure(1) :Histamine levels mean (log) value(mg/100g) in examined treated and untreated samples



Figure(2) : Mean (Log) values of total Enterobacteriacae count of examined untreated and treated beef burger



Figure(3) : Mean (Log) values of total Proteolytic count of examined untreated and treated beef burger



Figure(4) : Mean (Log) values of total Psychrophilic count of examined untreated and treated beef burger

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P-18

Veterinary drug residues in chicken parts from five selected poultry Farms in the Kumasi Metropolitan, Offinso-South Municipality and Kwabre-East District of Ashanti Region- Ghana

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Samples of liver, kidney and muscles of chicken from three different Districts in Ashanti-Region of Ghana were analyzed for veterinary drug residues (including albendazole, piperazine, tiamulin, chloramphenicol, levamisole, sulphathiazine, sulphamethoxazole and oxy-tetracycline) with HPLC equipped with photodiode array detector. Homogenized samples were extracted using acetonitrile and cleanup on C-18 solid phase column. The average recoveries of the drug residues in veterinary drugs in chicken parts were in the range of 76.0-98.8%. Kentinkrono registered the lowest concentration of 343.7±35.85 µg/kg. Residues of all veterinary drugs were higher in liver and/or kidney tissue as compared to muscle tissue. Liver parts registered the highest mean concentration (1455.0±85.7 µg/kg) followed by kidney parts which registered 887.5±45.8 µg/kg mean concentration. Muscle parts registered the lowest concentration (371.2±33.6) in all the farms. The general order of decreasing total drug residue levels is liver>kidney>muscle. Levamisole present in the meat may pose risk to the consumers as their mean concentrations mostly exceeded the recommended JECFA MRLs.

Keywords: Poultry, veterinary drugs, residues

1. Introduction

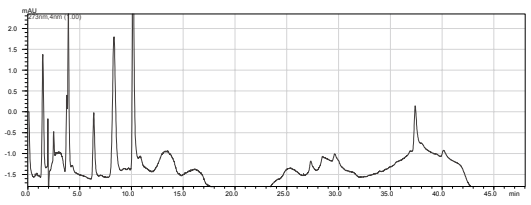
The worldwide commercial poultry industry is well-developed and is the largest supplier of animal protein in the form of meat and eggs (Bello *et al.*, 2011). Its significance is even greater in developing countries where poultry are relatively cheap and can be kept in a small area usually providing both protein and some income for a family (Ovwigho *et al*., 2009). It provides protein needed in daily food menu in terms of meat and eggs, but it is not sufficient against the need of our large population. Poultry industry forms a major portion of the agriculture sector in developing countries including Ghana. It plays a significant role in the provision of protein. The estimated protein consumption in Ghana is 53 g/day (Annan-Prah *et al*., 2012). The annual poultry production in Ghana is estimated at 14,000 metric tonnes of meat and 200 million units of eggs (Aning *et al.*, 2008). The estimated poultry products consumption in Ghana is 12 eggs and 1.2 kg meat per person per annum. The world average is 154 eggs and 9.7 kg meat per person per annum (Addo *et al.,* 2011).

2. Materials and Methods

A total of 180 birds were slaughtered and 540 samples were obtained from the liver, kidney and muscles from each bird for veterinary residue analysis from the various poultry farms in and around Kumasi. The samples were stored in refrigerator at 4^{o}C until the time of analysis. Samples were taken from 3 localities/towns from Ashanti Region. These are Mamponteng in Kwabre East District located almost in the central portion of the Ashanti region, Offinso in the Offinso South Municipal located in the extreme north-western part of the Ashanti Region. Offinso-South Municipality and Kentinkrono which are about 20 km from the centre of Kumasi

Chromatogram of the standard mix solution

HPLC with a photo diode array was chosen to allowed the separation and identification of the multi-veterinary drugs by its retention time and spectrum accordance with (Kao *et al*., 2001; Zhao *et al*., 2010). The various drugs were identified in the sample by their retention time and absorption spectrum. The multiresidue spectrum obtained from the sample was almost identical with that of standard.



Chromatogram of the standard mix Solution of all the drug residues

2.1 Extraction procedure

A 5 g each of kidney, liver and muscle samples were homogenized. The homogenates and 50 mL of acetonitrile were then added and mixed for 3 min. After filtration, the residue was mixed with another 50 mL of acetonitrile. The mixing and

filtration procedures were repeated. The combined filtrate was transferred into a separating funnel containing 30 mL of acetonitrile-saturated n-hexane and shaken for 5 min. The acetonitrile layer was collected into a concentration bottle and evaporated to dryness at 40 ^{o}C using a rotary evaporator.

3. Results and Discussion

3.1 Comparism of the body parts (liver, kidney and muscle) from all the 3 Districts visited

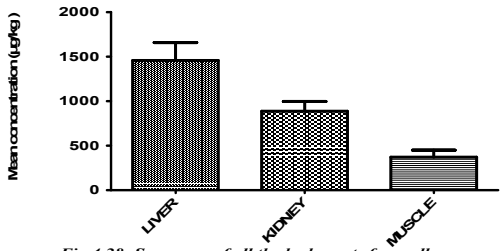


Fig 4.28: Summary of all the body parts from all the five farms

Liver parts (samples) registered the highest mean concentration (1455.0±85.7 µg/kg) followed by kidney parts which registered 887.5±45.8 µg/kg mean concentration. Muscle parts registered the lowest concentration (371.2±33.6) µg/kg in all the farms. Residues of all veterinary drugs are higher in liver and/or kidney tissue as compared to muscle tissue. This is similar to observation made by the Food Research Institute from the University of Wisconsin–Madison (Doyle, 2006). Mehdizadeh *et al*., (2010), screened chloramphenicol residue in kidney, liver and thigh muscle samples and found more than half of the samples (54.8%) showed detectable concentrations of chloramphenicol. They detected the highest concentrations of the drug residues in the kidney and liver. Another study was carried out by Pavlov *et al.,* (2008) on the presence of antimicrobial drug residues in chicken in edible tissues (breast muscles, liver and kidneys), slaughtered in two abattoirs in Bulgaria. A four-plate agar diffusion test using *Bacillus subtilis* and *Bacillus mycoides* as the test microorganisms was evaluated for the detection of antibiotic availability. From 75 samples from the first abattoir, two positive samples were found, while in the second there were no positive samples from breast muscles. The great number of samples with antimicrobial residues was found in kidneys and livers of the chicken.

3.2 Comparism of the levels of veterinary drug residues in all the 3 Districts visited

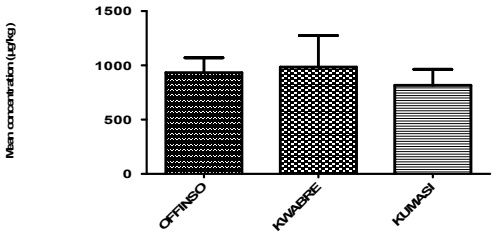


Fig 4.29: Summary of drug residues from all the three Districts

Mamponteng in the Kwabre-East District registered the highest mean concentration of 993.5±10.10 µg/kg and closely followed by Offinso-South Municipality which recorded 961.6±6.74 µg/kg. Kentinkrono in the Kumasi Metropolitan recorded the lowest mean concentration 896.3±62.6 µg/kg drug residues. The mean concentrations of the veterinary drug residue in all the 3 Districts were very close.

4. Recommendations for further development

Farmers should be made to observe the withdrawal period of the veterinary drug residue. This can be achieved by taking sample of the birds for analysis before they are allowed to be sold to the public.

Monitoring laboratories must be set up throughout Ghana to control drugs residues in eggs, meat and other food products sold to the populace.

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P-19

Carcinogenic and Genotoxicity of some PAHs in commonly consumed smoked fish (*Parachanna obscura* and *Ethmalosa fimbriata*)

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The dietary exposure of Polycyclic Aromatic Hydrocarbon (PAHs) and potential risk to human health was instigated in two different traditionally smoked species of fish (*Parachanna obscura* and *Ethmalosa frimbriata)* purchased from three markets in Benin City. Identification and quantitative analysis of PAHs components were achieved by Gas Chromatography/High Performance Liquid Chromatography. The result obtained showed that , Benzo(a)pyrene had an occurrence of 83.33% in all samples analysed. Risk assessment conducted using benzo(a)pyrene carcinogenic and mutagenic toxicity equivalent factor (TEQ & MEQ) showed slight to high risk (7.44×10^{-5} -1.95×10^{-3}) and exceeded the USEPA guideline (1.0×10^{-5}) for potential Cancer. Levels of PAHs present in smoked fish prepared using traditional method may pose elevated cancer risks if consumed at high rates over many years.

Keyword: Health Risk, *Parachanna obscura, Ethmalosa fimbriata,* PAHs**,** GC/HPLC**,**

1. INTRODUCTION

When food particularly meat, meat products and fish is smoked, roasted, barbecued, or grilled; PAHs are formed as a result of incomplete combustion or thermal decomposition of the organic materials (WHO, 2005). Pyrolysis of the fats in the meat/ fish generates PAH that become deposited on the meat/fish. PAH production by cooking over charcoal (barbecued, grilled) is a function of both the fat content of the meat/fish and the proximity of the food to the heat source (Phillips, 1999). Several analyses of charcoal roasted/grilled common food items have proven the presence of PAHs such as benzo[a]pyrene, anthracene, chrysene, benzo[a] anthracene, indeno[1,2,3-c,d]pyrene (Camargo *et al*., 2011). Most of these PAHs have been found to be carcinogenic while some are not (Pikuda and Ilelaboye, 2009). Traditional smoking techniques involve treating of pre-salted, whole or filleted fish with wood smoke in which smoke from incomplete wood burning comes into direct contact with the product, this can lead to its contamination with PAHs if the process is not adequately controlled or if very intense smoking procedures are employed (Gómez-Estaca *et al.,* 2011). Potential health hazards associated with smoked foods may be caused by carcinogenic components of wood smoke; mainly PAHs, derivatives of PAHs, such as nitro-PAH or oxygenated PAH and to a lesser extent heterocyclic amines (Stołyhwo and Sikorski, 2005Among PAHs, the benzo[a]pyrene (BaP) concentration has received particular attention due to its higher contribution to overall burden of cancer in humans, being used as a marker for the occurrence and effect of carcinogenic PAHs in food (Rey-Salgueiro *et al.,* 2009).Smoked fish may contribute significantly to the intake of PAHs if such foods form a large part of the usual diet. The primary purpose of this study, is to identify and quantify the concentration levels and distribution of PAHs in smoked fish consumed by people in Benin City, Nigeria.

2. MATERIALS AND METHODS

2.1 Sampling

Locally smoked fish (about 5 g) of two different species commonly consumed in Benin city, namely *Ethmolosa fimbriata* (bonga fish) and *Parachanna obscura* (Traditionally called Ewi), were purchased from three different market centres from local vendors in Benin city, Edo state. The quantification of PAHs was performed using an Agilent 6890 Series Gas Chromatography, HPLC system with a quaternary pump, vacuum degasser, a temperature controlled column oven and UV diode-array detector. Separation of the PAHs was performed on a monomeric type octadecyl silica column, Supelcosil LC PAH 2cm x 4.6 mm i.d containing 5µm particles at ambient temperature (25± 1˚C) at a flow rate 1.0ml/min. gradient elution using acetonitrile and water was employed (60:40 to 0:100).

2.2 Human Health Assessment

$TEQ_{Bap} = \Sigma(TEFi \times Ci)$ .....(1)TEF (Nisbet & LaGoy 1992)

$MEQ_{Bap} = \Sigma(MEFi \times Ci)$.....(2)MEF (Durant *et al*., 1996 & 1999)

Average Daily Dose of Carcinogenic (Mutagenic PAH) = TEQ (or MEQ) x IR x CF/BW.....(3)

IR= Ingestion Rate (65.5g/day), CF= Conversion Factor (0.001mg/µg), BW= Body Weight (70kg)

Hard Quotient (HQ) = Average daily dose (ADD/ RFD).....(4)

RFD (mg/kg/day) adopted from USEPA, 2004

Hazard Index (HI)= $\Sigma(HQ_1 + HQ_2 ..... HQ_n)$.....5

2.3 Statistics

Analysis of variance was performed to estimate the significance of the differences between the means of individual and total PAHs content in both species of fish. An effect was deemed statistically significant for $p \leq 0.05$

3. Result and Discussion

In total, 60 samples prepared using traditional smoking methods were chemically analyzed. Of the 16 PAHs analyzed, 10 were consistently above WHO/EU limits in both fishes. These included Dibenzo(a,h)anthracene, Benzo(a)pyrene and Indeno(1,2,3-cd)pyrene, Fluorene, Benzo(g,h,i) perylene. PAHs with high molecular weights occurred more than the low molecular weight in all samples. Individual PAH levels ranged from < 1 –93 µg kg^{-1}. Benzo (a)pyrene was the most abundant PAH found in all fish samples, this was followed by Benzo(b)fluoranthene, fluorene, Benzo(g,h,i)perylene and Benzo(k)fluoranthene . The summation of 5 congeners accounted for 75–80% of the total mass of PAHs measured across all smoked *Ethmalosa fimbriata* while 6 accounted for 87-89% of the ΣPAHs in *Parachanna obscura* . Risk values for the studied fishes prepared by traditional smoking reveals that 2 out of 200,000 adults are likely to suffer cancer in their 70 years life time. This implies that daily consumption of traditional smoked *Ethmalosa fimbriata* and *Parachanna obscura* for 70 years is likely to pose risk, because it is higher than USEPA (1993, 2009) carcinogenic limit of 1.0 x 10^{-5}. The Carcinogenic and Mutagenic equivalents recorded for both species of fish ranged (Carcinogenic: 1.96-14.2 and Mutagenic 3.91-8.47) respectively, these high levels of risk assessment lead to higher EQ_{BaP} daily dose in both fishes. Therefore the Mutagenic and carcinogenic risk involved in daily consumption of traditional smoked *Ethmalosa fimbriata* and *Parachanna obscura* for 70 years was calculated to be far >1.The result further reveals that 2 out 200,000 and 2 out of 2000 adults are likely to suffer cancer and non cancer related diseases if they exposed to oral ingestion of traditional smoked *Ethmalosa fimbriata* and *Parachanna obscura* for 70 years on a daily bases. Non carcinogenic PAHs produced hazard >1, a level that can trigger the development of non-cancer health effects through oral ingestion.

Table 1: Risk Assessment (Carcinogenic Equivalent)

Carcinogenic	*Parachanna obscura*			*Ethmalosa fimbriata*		
Equivalency	A	B	C	A	B	C
Benzo(a)anthracence	0.31	0.15	0.03	0.15	ND	ND
Benzo(b)fluoranthene	0.08	ND	0.05	0.07	ND	0.27
Benzo(k)fluoranthene	0.04	0.07	0.05	0.08	0.05	0.07
Benzo(a)pyrene	4.20	2.61	2.40	2.29	2.30	ND
Indeno(1,2,3-cd)pyrene	0.21	0.27	0.81	0.96	0.65	0.72
Dibenzo(a,h)anthracene	7.92	1.35	7.26	10.3	11.2	0.90
ΣBaP-TEQ	12.8	4.45	10.6	14.0	14.2	1.96

Table 2: Risk Assessment (Mutagenic Equivalent)

Mutagenic	*Parachanna obscura*			*Ethmalosa fimbriata*		
Equivalency	A	B	C	A	B	C
Benzo(a)anthracence	0.25	0.13	0.03	0.13	ND	ND
Benzo(b)fluoranthene	0.63	0.35	ND	0.19	ND	0.68
Benzo(k)fluoranthene	0.41	0.82	0.53	0.96	0.59	0.73
Benzo(a)pyrene	4.23	2.61	2.40	2.29	2.30	ND
Indeno(1,2,3-cd)pyrene	0.65	0.84	2.51	3.02	2.03	2.24
Dibenzo(a,h)anthracene	2.30	0.27	2.11	2.98	3.24	0.26
ΣBaP-TEQ	8.47	5.02	7.57	8.16	8.16	3.91

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P-20

Incidence of heavy metals and trace elements in chicken meat

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The present study was carried out to determine heavy metal residual concentrations in muscle of recently slaughtered local broiler chicken, muscle of frozen local broiler chicken and muscle of imported chicken consumed in Sharkia governorate, Egypt. Cadmium, copper, lead and zinc were determined after nitric acid/perchloric acid digestion using atomic absorption spectrophotometry. The order of the heavy metal residual concentrations in recently slaughtered chicken, local frozen chicken and imported frozen chicken meat was as followed Zn>Cu>Pb>Cd. The concentrations of copper, zinc were below the permissible limits while those of cadmium and lead in some samples were at levels above the permissible limits. Thus, it is highly recommended to reduce the load of these metals in order to avoid their significant health hazards.

Keywords: Cadmium, copper, lead, zinc and chicken meat.

1. Introduction

Heavy metals can induce a significant health risk to humans, particularly in elevated concentrations above the very low body requirements. In general, these metals have long biological half-lives, not biodegradable and having the potential for accumulation in different body organs leading to unwanted adverse effects.

Heavy metals such as cadmium and lead are non-essential metals, as they are toxic and can be very harmful even at low concentrations while copper and zinc are essential metals and can produce toxic effects when their intake is excessive. Toxic effects of metals have been described in animals under relatively low levels of metal exposure **(Kostial, 1986)**. Lead is a metabolic poison and a neurotoxin that binds to essential enzymes and several other cellular components and inactivates them **(Cunningham and Saigo, 1997)**.

Copper is one of the most critical trace minerals in livestock feeding because it plays a vital role in haemoglobin synthesis, connective tissue maturation especially in the cardiovascular system and in bones, proper nerve function and bone development **(SCAN, 2003)**. Copper is also an essential component of many enzyme systems such as cytochrome C oxidase, amino oxidase, polyphenoloxidase, ferroxidase and superoxide dismutase **(Gaetke and Chow, 2003)**. In addition, zinc is an essential element in our diet, but too little or too much can be harmful.

2. Materials and Methods

2.1 Samples collection

Muscle samples (n=30, 10 each of recently slaughtered, local frozen and imported chicken) were collected from carcasses of chicken from poultry shops in Sharkia Governorate and from carcasses of imported chicken.

2.2 Determination of heavy metal residues by Atomic absorption spectrophotometer (A.A.S)

The use of atomic absorption technique is the best choice because of their wide spread availability and ease of use. Quantitative determination of heavy metals was carried out by “Buck scientific 210VGP Atomic Absorption Spectrophotometer” at the Faculty of Veterinary, Zagazig University.

2.3 Analysis:-

The digest, blank, standard solutions were aspirated by atomic absorption spectrophotometer (AAS) and analyzed for heavy metals content.

Analysis of cadmium, copper, lead and zinc was conducted at Central Laboratory, Faculty of Veterinary medicine, Zagazig University. It was conducted by air / Acetylene flow (5.5/1.11/m) flame (A.A.S).

2.4 Quantitative determination: -

cadmium, copper, lead and zinc concentrations were recorded directly from the digital scale of AAS and they were calculated according to the following equation:

$$\text{Element (ppm)} = R*D/W$$

Where;

R=Reading of element concentration, ppm from digital scale of AAS.

D=Dilution of prepared sample.

W=Weight of the sample.

The concentration or the absorption values of heavy metals in blank samples were also calculated and subtracted from each analysed sample. The registered values for cadmium, copper, lead and zinc were expressed as mg/g wet weight (ppm).

3. Results and Discussion

Cadmium (Cd) mean ± SE residual concentration in breast muscle of recently slaughtered, local frozen and imported chicken was 0.035 ± 0.01078 µg/g, 0.044 ± 0.01593 µg/g and 0.029 ± 0.0096 µg/g respectively. All samples (100%) were within the permissible limits (0.1 ppm) according to **(EOSQC, 2007)**. The present results show the cadmium in different groups was within the safe value in Sharkia Governorate in comparison to maximum permissible hygienic limits for Cd in chicken meat (0.1 ppm). These results guarantee that the consumer is protected against unfavourable effects of cadmium as a result of chicken meat consumption.

Copper (Cu) mean ± SE residual concentration in breast muscle of recently slaughtered, local frozen and imported chicken was 4.439 ± 0.38146 µg/g, 1.344 ± 0.21653 µg/g and 1.708 ± 0.1489 µg/g respectively.

All samples (100%) were within the permissible limits (15 ppm) according to **(EOSQC, 2007)** and lower than the permissible limits of **(FAW/WHO, 1992)**. The present results show the copper in different groups was within the safe value in Sharkia Governorate in comparison to maximum permissible hygienic limits for Cu in chicken meat (15 ppm). Copper poisoning include nausea, vomiting, diarrhoea, hematemesis and jaundice, while chronic disease from excessive copper storage was epitomized by (Wilson's disease) which characterized by excessive copper deposition in most organs (liver, kidney, brain and eyes) **(Zenz, 1988)**.

Lead (Pb) mean ± SE residual concentration in breast muscle of recently slaughtered, local frozen and imported chicken was 0.464 ± 0.8505 µg/g, 1.24 ± 0.20039 µg/g and 1.45 ± 0.36724 µg/g respectively. The levels found in this study were slightly higher than permissible limits (0.1 ppm) according to **(EOSQC, 2007)** in local frozen and imported frozen chicken groups. Excessive amount of Pb in chicken meat could not be attributed to industrialization alone. High levels of metals in poultry products emanate mainly from contamination of feed and water sources.

Zinc (Zn) mean ± SE residual concentration in breast muscle of recently slaughtered, local frozen and imported chicken was 4.951 ± 0.68144 µg/g, 4.668 ± 0.41166 µg/g and 4.465 ± 0.36566 µg/g respectively. All samples (100%) were within the permissible limits (15 ppm) according to **(EOSQC, 2007)** and lower than the permissible limits of **(FAW/WHO, 1992)**. The present results show the zinc in different groups was within the safe value in Sharkia Governorate in comparison to maximum permissible hygienic limits for Zn in chicken meat (15 ppm). Zinc is essential nutrient required for the normal structure and function of Zn-containing enzymes, including those involved in gene expression, cell division, apoptosis and synaptic signalling **(IZNCG, 2004)**.

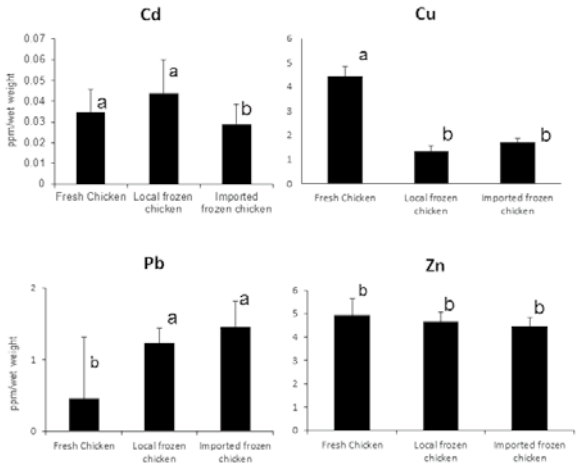

Figure 1: Cd, Cu, Pb and Zn concentrations in breast muscles of chicken.
Columns carrying different superscript letters are significantly different ($p<0.05$)

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P-21

Evaluation of environmental waters and aromatic herbs contamination by Carbamate pesticides in Bamenda and Santa (North West Region, Cameroon)

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This study aimed to determine carbamates (CRBs) concentration in water and fresh herbs samples from Santa and Bamenda (Cameroon), using solid phase extraction (SPE) and a modified QuEChERS extraction, respectively, followed by ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS). A total of 23 and 28 CRBs pesticides were screened in water samples and edible fresh aromatic herbs, respectively. Full validation of the method, showed recoveries ranged from 72 to 99% for water analysis, with relative standard deviations (RSD) lower than 17%, and limits of detection/quantification (LOD/LOQ) between 0.003 and 0.397 µg/L. In herb analysis, LOD/LOQ ranged from 0.04–3.39 µg kg^{-1}, with recoveries between 60 and 125%, and RSD lower than 15%.Results revealed the presence of propoxur (at the level 0.072µg/L) in a stream water sample from Santa, while carbemedazim-benomyl was detected in celery (23.15 ± 2.11 µg kg^{-1}) and parsley (12.84 ± 0.26 µg kg^{-1}). .This study validated the use of SPE and QuEChERS combined with UHPLC-MS/MS analysis of CRBs in water and fresh herbs, and highlighted the necessity to strengthen regulation of pesticides use and handling in developing countries, particularly in Cameroon.

Keywords: carbamates, herbs, QuEChERS, solid phase extraction, UHPLC-MS/MS, water

1. Introduction

Agriculture is the basis and key for sustainable development of African countries. However its activities use increasingly quantities of pesticides that can contaminate water sources and food stuffs.

Carbamates (CRBs) act as insecticides, herbicides, fungicides, nematocides and ascaricides, and represent a major group pesticides used in agriculture for crop protection, especially following the ban of organochlorine pesticides in the 1970s (EPA, 2015). These pesticides are commonly used for crop protection in Cameroon, where agriculture represents a source of income and nutrition for more than 70% of the population (Amin, 2001). Agropesticides are largely used to protect edible herbs from pests. Unfortunately, some residues of the pesticides may contaminate the edible herbs. A good quantity of CRBs can also leached away by rains that drain the majority of the chemicals into rivers and therefore contaminate groundwater and other sources of fresh water. The contaminated foodstuff and water therefore constitute a substantial exposure source for humans and wild animals. This study therefore aimed to use SPE or QuEChERS and UHPLC-MS/MS to evaluate the contamination of CRB pesticides in environmental waters and fresh herbs from the localities of Santa and Bamenda town in the North West Region of Cameroon (NWRC).

2. Materials and Methods

2.1 Reagents and materials

Standard CRBs were purchased from Fluka Analytical (St Louis, USA). Other chemicals used include NaCl and MgSO4 anhydrous were obtained from Panreac ITW Companies (Darmstadt, Germany),HCl, formic acid,ethylacetate (EtOAc), methanol (MeOH), acetonitrile (MeCN) obtained from VWR Prolabo (Leuven, Belgium), or Fisher Scientific (Geel, Belgium). Ultrapure water (Milli-Q Plus system, Millipore Bedford, MA, USA) was used throughout the work. Oasis® HLB Cartridges (200 mg, 3 mL) were supplied by Waters Milford, MA, USA). C_{18} was supplied by Agilent Technologies (Waldbron, Germany).

2.2 Instrumentation

The UHPLC analyses were performed in a 1290 Infinity LC using a C_{18} column (Zorbax Eclipse plus RRHD 50×2.1 mm, 1.8 µm) (Agilent Technologies, Waldbron, Germany). The mass spectrometer measurements were performed on a QqQ mass spectrometer API 3200 with electrospray ionization. The instrumental data were collected using the Analyst® Software version 1.5 with Schedule MRM™ Algorithm (ABSCIEX).

2.3 Sample collection and treatment

Five water samples (2 stream water samples and 2 tap water samples from Santa, and 1 tap water from Bamenda. samples were from mille 12 Santa) were collected from NRWC. Samples of fresh herbs collected from the same region included parsley, celery (*Apium graveolens*), leek (*Allium ampeloprasum*), welsh onion (*Allium fistulosum*) and sweet pepper (*Capsicum annuum*).

The SPE procedure was done as described by Moreno-González et al. (2011) with a few modifications. Briefly water samples were filtered (0.45 µm filter). In order to prevent the degradation of this compounds the pH was adjusted to 3.Oasis® HLB Cartridges were conditioned and 25 mL of water samples were loaded. The cartridges were washed with 3 mL of water and air-dried. The CRBs were thereafter eluted using 3 mL acetone and the eluate dried under N_2 flow at room temperature. The residue was reconstituted with 250 µL of H_2O/MeOH (80:20, v/v), filtered (0.22 µm filter) and analysed using UHPLC-MS/MS. Herb samples were chopped with a domestic blender. For the QuEChERS procedure, 1g of the grinded sample was introduced in a tube containing 10 mL of MeCN and after brief homogenization 4 g of $MgSO_4$ and 1 g of NaCl were added and the mixture was centrifuged (5000 rpm, 10 min, 25°C). 3 mL of the supernatant was transferred into a tube containing 200 mg of $MgSO_4$ and C_{18}, and the mixture was centrifuged and 2 mL of the supernatant was taken into vials and dried under N_2 flow stream. The residue was reconstituted and analyzed as described above.

2.4 UHPLC-MS/MS analysis

UHPLC separations were performed as described elsewhere (Moreno-González, et al., 2013), on a C_{18} column using a mobile phase consisting of 0.01% aqueous formic acid solution (solvent A) and MeOH with the same percentage of acid (solvent B) at a flow rate of 0.5 mL min^{-1}. The gradient profile was 0% B at the beginning; 20% B from 0.7 to 1.2 min; 50% B from 2.5 to 3 min; 95% B from 6.5 to 7.0 min; and finally in order to come back to the initial conditions, 0% B at 7.5 min, equilibrating for 2 min. The running time for each injection was 10.5 min. The temperature of the column was 25°C and the injection volume was 10 µL.

3. Results and discussion

The suitability of the method for the determination of CRBs in water samples showed good linear dynamic ranges (r^2≥0.99), limits of detection and quantification .between 003-0.397 µg/L, relative standard deviations (RSD) below 17%, and recovery ranging from 72% to 99%. For fresh herbs, the proposed method allowed recoveries between 60 and 125%, with RSD lower than 15% at 3 concentration levels (2, 8 and 40 µg kg^{-1}). The LOQs were < 3.4 µg kg^{-1}. Analyses revealed the presence of propoxur (PX) in one stream water of NWRC (Bamock centre, Santa) at the level of 0.072 ± 0.007 µg/L (figure 1A), while other water samples did not show presence of any CRB residue. Out of different herbs analyzed, parsley and celery showed detectable levels of carbamendazin-benomyl (CBZ-BY) in celery (23.2± 2.1 µg kg^{-1}) and parsley (12.8 ± 0.3µg kg^{-1}) samples from Santa/Bamenda (figure 1, B & C).

The detected level of PX is close to the maximum residue limit (MRL) in the European Commission (EC) which is 0.100µg/L (EC, 1998). The populations which use the contaminated water for multiple purposes including drinking, washing, irrigation, etc., are therefore exposed to the pesticide PX, which may induces symptoms and diseases related to PX exposure.

With respect to their action, CBZ and BY act as fungicides and fungi may be the most prevalent pests attacking crops in Santa. Preferential use of fungicide in agriculture was reported among small scale farmers in Djutitsa, West Cameroon (WHO, 1986; Manfo et al., 2012). The current levels of pesticide residues found in herbs are below the MRLs in the EC. However, the presence of such chemicals in water and food commodities represents a risk for consumers since some of these pesticides are considered as endocrine disruptors, including PX, CBZ and BY, and may thus affect cell mechanisms or signalling at the concentrations even far below the MRL (Mnif et al., 2011; Vandenberg et al., 2012).

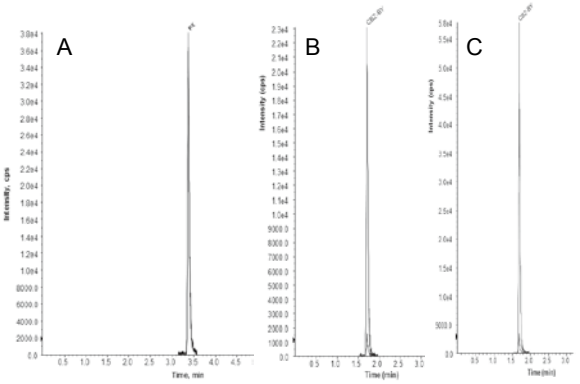

Figure 1: Chromatogram of Stream Water of Bamock Centre-Santa (SWBC) (A), parsley (B) and celery (C). The quantification and identification ions are shown in blue and red line, respectively.

The current study demonstrated that water and fresh herbs samples were contaminated with CRB pesticide residues. Although levels of detected pesticides were lower than the MRLs authorized by the EC, regulation on pesticide residues in water and aromatic herbs for human use and consumption should be strengthened, given their possible endocrine disrupting effects.

4. Acknowledgments

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P-22

Role of radiation in fish preservation

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This study investigates the effect of gamma irradiation (3-5 KGy) on biochemical and microbiological quality of fresh mullet stored at 4˚C and extending shelf life of refrigerated mullet. In this study, we are focussing on the biochemical changes which include: Total volatile basic nitrogen (TVBN), trimethylamine (TMA), thiobarbituric acid (TBA) and pH. One of chemical indicators is TVBN value which increases during storage time up to 45 mg /100 gm for non irradiated fish at 14th day, while in irradiated mullet samples, TVBN values lowered to 25.48 and 20.48 mg /100 gm for 3 and 5 KGy, respectively. In addition, TMA and TBA values of irradiated samples were lower than that of non-irradiated fish. Non-irradiated mullet can reach 7 days with refrigeration while shelf life of irradiated sample can reach up to 14 day for 3KGy irradiated mullet, and extend up to 21 day for 5 KGy irradiated mullet. Results confirm the advantage of combination of gamma irradiation with refrigeration to enhance chemical properties and extending shelf life of mullet.

Keywords: Gamma irradiation, shelf-life, biochemical changes, quality changes

1. Introduction

There is a great demand for an effective method of preservation of seafood to maintain the quality for consumer acceptance and permit shelf life extension. As fish is an extremely perishable food as compared to other food sources.

Irradiation is recognized as an effective, widely used food processing technique. The process is about exposure of food to a carefully controlled amount of energy in the form of high speed particles or rays that reduce the risk of food poisoning, control food spoilage and extend the shelf life of food without any risk to health and minimal effect on nutritional or sensory quality. This process has no effect on food taste, colour and odour and it does not leave radioactive residues (ICGF, 2002). Food irradiation in a combination with good refrigeration and handling practices might provide a mean to increase the shelf life of fish and fish products. Many studied reports that gamma irradiation kill most of microorganisms without deterioration of food.

Testing trimethylamine (TMA) or total volatile basic nitrogen (TVBN) content can be useful due to their close relation with the fish organoleptic characteristics and microbial spoilage. Maximum allowed level of TMA is accepted as 10-15 mg/100gm (Varlık et al., 1993). Maximum limit of TVB-N at 35 mg/100 g is generally acceptable (Kim et al. 2002). Malondialdhyde (MDA), a major degradation product of lipid hydroperoxides, has attracted much attention as a marker for assessing the extent of lipid peroxidation (Raharjo and Sofos, 1993).

The objective of this study was to investigate the effects of medium dose (3 and 5 KGy) of irradiation during storage (4˚C±1) on chemical properties of mullet (*Mugil Cephalus*).

2. Materials and Method

2.1 Sample preparation

Fresh mullet (n=30) were obtained from local market of Sharkia governorate, and transported to laboratory in ice box. Fish is divided into three groups (10 each), first group is control one kept at refrigerate 4˚C and the other groups were sent to the National Centre for Radiation Research and Technology for irradiation.

2.2 Irradiation and storage of samples

The fish were exposed to gamma irradiation at dose 3 and 5 KGy. Al sample were refrigerated stored at 4±1˚C.Then samples subjected to the periodical analysis.

2.3 Chemical examination

Evaluation of total volatile bases nitrogen (TVB-N) and triethylamine (TMA): TVB-N and TMA evaluated according to Malle and Poumeyro (1989).

Thiobarbituric acid was measured according to method described by Tarladgis et al., (1960).

pH value was determined according to Vyncke (1981).

3. Results and Discussion

In this study TVB-N contents of non-irradiated and 3-5 KGy irradiated mullet stored at 4˚C in refrigerator were 9.74, 10.08, and 11.76 mg/100g respectively, in the flesh of mullet at the beginning

of storage. TVB-N increase according to time of storage which become at 14th day 45.7, 25.2 and 20.48 for non irradiated samples, 3 and 5 KGy irradiated mullet respectively, while at 21th day TVBN reached 38.24 and 32.84 for 3 and 5 KGy irradiated mullet respectively. TVBN of non irradiated mullet exceeded 35 mg/100 at 14th day which is considered maximum acceptable level for fish. 3KGy irradiated fish exceeded this level at 21th day and 5kGy exceeded it at 30th day.

Table (1)
Effect of irradiation on total volatile basic nitrogen and trimethylamine during storage period:

Storage Day	TVBN			TMA		
	0KGy	3KGy	5KGy	0KGy	3KGy	5KGy
1st	9.74	10.08	11.76	3.36	3.36	3.52
7th	21.84	15.12	13.26	6.04	3.36	3.69
14th	45.7	25.2	20.48	10.72	5.04	4.54
21th	R	38.24	32.84	R	8.72	7.22
30th	R	45.96	38.74	R	10.4	9.56

Table (2)
Effect of irradiation on thiobarbituric acid and pH during storage period:

Storage Day	TBA			PH		
	0KGy	3KGy	5KGy	0KGy	3KGy	5KGy
1st	0.67	0.98	1.32	6.5	6.5	6.5
7th	1.53	1.2	1.79	6.7	6.6	6.4
14th	2.79	.975	1.5	7.3	6.8	6.7
21th	R	2.89	2.5	R	7	6.8
30th	R	3.45	3.87	R	7.2	7.2

TMA is produced by the decomposition of TMAO caused by bacterial spoilage and enzymatic activity, and it is a valuable tool in evaluation of fish quality due to rabid accumulation in muscle during refrigeration. The initial value of TMA was found to be 3.36, 3.36 and 3.52 for non-irradiated and 3-5 KGy irradiated fish respectively, indicating the freshness. During storage, TMA increases in all samples.TMA formation is lower in irradiated fish than that in non irradiated fish; but remain lower than rejection level in irradiated sample.

Ahmed et al. (1997) reported that the rate of formation of TMA-N and TVB-N were reduced in irradiated fish compared to non-irradiated samples, because of radiation sensitivity of Pseudomonas, which is the microorganism responsible for the decomposition of tri-methylamine oxide.

TBA value is variable, and did not show a consistent trend, this variation as shown in the table 2, and can be explained as a result of different phase of decomposition of peroxides. At the beginning, we found that TBA value of 5 KGy is higher due to effect of radiation on lipid, TBA contents were 0.67, 0.98 and 1.32 for non irradiated and 3-5 KGy irradiated fish, and it also increases with storage, then decreases after the 7th day due to breakdown of the malonaldehyde to tertiary degradation.

pH value of all samples was 6.5 at the beginning and increased with time due to accumulation of alkaline compounds, such as ammonia compounds and trimethylamine, which are mainly derived from microbial action.

The results of this study revealed that radiation at high dose (5KGy) enhanced lipid oxidation, while protein oxidation and microorganisms were inhibited. In conclusion, combination between irradiation and refrigeration result in significant stabilizing of chemical characteristics of mullet meat.

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HEAVY METALS RESIDUES IN SOME MARKETED CRUSTACEA

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Eighty random samples from shrimp and crab were collected from Damietta and Suez coasts (40 for each). For detection of heavy metals contamination in these samples as lead, mercury, cadmium and arsenic using atomic Absorption spectrophotometer and found that most of the samples contaminated with heavy metals that exceed the permissible limit in Egyptian Organization for Standardization quality and Control (2007) and some trails were used in order to reduce heavy metals concentrations in samples. Most of these trails had clear effect in reducing the proportion of heavy metals in samples under study.

Keywords: Heavy metals, Lead, Mercury, Cadmium, Arsenic, Crustacea, Crab, Shrimp, Dried coriander, Sodium bicarbonate, Garlic Heavy metals reduction.

1. Introduction

Crustaceans have the ability to accumulate heavy metals in their tissues, so they could be used as bio-indicators for heavy metal pollution in water, Shrimp can absorber dissolved Pb by the exoskeleton so this organism could serve as effective long-term bioindicator of lead contamination in marine water (Boisson et al. 2002), thus they represent public health hazard if consumed. Heavy metals pollution is a dangerous problem of magnitude and ecological significance because they are not biodegradable and are not easily eliminated from the ecosystem. The main source of this pollution is the industrial zones which is the most significant problem affecting food safety. Heavy metals constitute serious health hazard depending on their relative levels. Some of these metals such as lead, mercury and cadmium cause symptoms of chronic toxicity, including impaired kidney and hepatic function; Alzheimer's disease; Itai-Itai disease (or Ouch-Ouch disease which includes kidney damage and skeletal disorder); lung and testicular cancer and sorts of disorder including neurological, immunological, cardiac, motor, reproductive and even genetic.

So, it is important to investigate the levels of heavy metals in these organisms and to assess whether the concentration is within the permissible level according to Recommended Egyptian Organization for Standardization and Quality Control (EOSQC) and either it poses any hazard to the consumers or not.

2. Materials and Methods

(1) Collection of samples:

A total of (80) random samples of shrimp and crab, (40) of each of them (20) from Damietta and (20) from Suez coasts from Egypt were collected. Each group consists of (20) samples of moderate size (in crab samples sex was differentiated into 10 males and 10 females).The samples were collected in polyethylene bags, all of which were kept in ice box and transferred immediately to the Meat Hygiene laboratory, The samples were then analyzed to estimate the level of Pb, As, Hg and Cd by Atomic Absorption Spectrometry (AAS).

(2) Washing procedures:

According to (Seedy 2001) and (EL-Mowafi 1995

(3) Digestion of tissue samples:

According to (Julshaman 1983).

To determine heavy metals, per kin Elmer model (spectra-AA 10, USA) flam atomic absorption spectrometer (AAS) with computer system was employed.

(4) Determination:

Quantitative determination of heavy metals was carried out by "Buck scientific 210VGP Atomic Absorption Spectrophotometer" at the Faculty of Veterinary Medicine, Zagazig University.

(5) Reduction of the heavy metals load in the tissue of shrimp and crab of collected samples.

Experimental design contained two groups one group of shrimp and the other of crab neglecting the site of collection and sex of crab samples, each group was divided into control sample (without treatment) and treated groups were subjected to one of the following solution for specific time as mentioned:

a) Depuration in distilled water for (0.5 hour).
b) Depuration in distilled water for (2 hours).
c) Treated with sodium bicarbonate solution 5% for (2 hours).
d) Treated with sodium bicarbonate solution 2 % for (2 hours).
e) Treated with dried coriander solution 0.5 % for (2 hours).
f) Treated with dried coriander solution 2 % for (2 hours).
g) Treated with dried garlic solution 0.5% for (2 hours).
h) Treated with dried garlic solution 2 % for (2 hours).

The samples were then analyzed to estimate the level of Pb, As, Hg and Cd by Atomic Absorption Spectrophotometer.

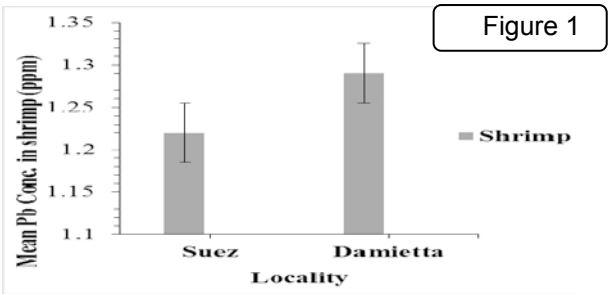
(6) Statistical analysis:

By general linear models (GLM) procedure of the Statistical Package for Social Sciences version 21.0 (SPSS for Windows 21.0, Inc., and Chicago, IL, USA).

3. Results and Discussion

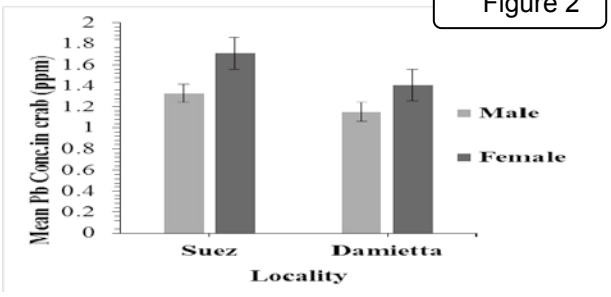
The mean values of lead concentration in examined shrimp samples from Suez and Damietta cities were with mean values of 1.22±0.24 and 1.29±0.21(ppm) wet weight ,respectively. The obtained results in examined shrimp samples were higher than what had been reported by Gawish (1998), Zaki (2004) and Abo samra (2007). , While higher results were obtained by Mohamed (1999), El abed (2001) and Sameh and Hagag (2004).as in figure (1).

Figure 1



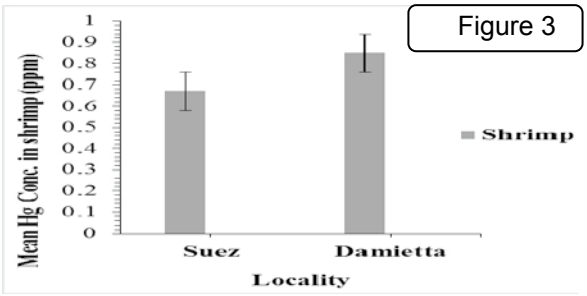
In examined male crab samples from Suez and Damietta lead residual concentrations mean values were 1.33±0.18 and 1.15±0.26 ppm wet weight, respectively, while in examined female crab samples the mean values were of 1.71±0.36 and 1.41±0.22 ppm wet weight in Suez and Damietta, respectively In crab samples higher results were obtained by Mohamed (1999), Sallam (2000) and El abed (2001). While lower results were reported by Gawish (1998) and Abo samra (2007).as figure (2).

Figure 2



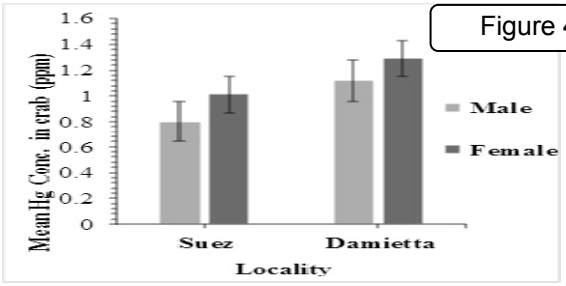
The mean values of mercury in examined shrimp samples from Suez and Damietta cities were 0.67±0.16 and 0.85±0.15 (ppm) wet weight, respectively. The obtained results in examined shrimp samples were higher than what has been reported by Gawish (1998), El abed (2001) and Abo samra (2007) as in figure (3).

Figure 3



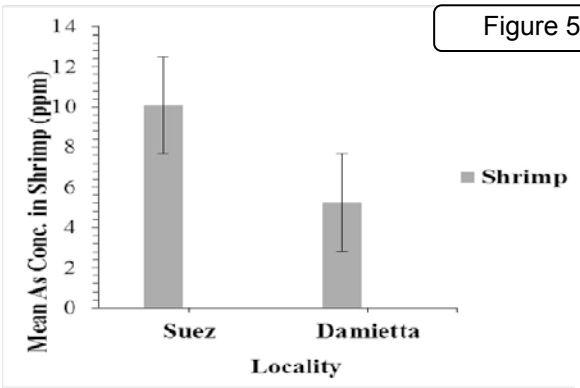
In examined male crab samples from Suez and Damietta mercury concentrations were of mean values of 0.80±0.16 and 1.12±0.23 ppm wet weight .while in examined female crab samples mean values were 1.01±0.20 and 1.29 ±0.22 ppm wet weight in Suez and Damietta, respectively. In crab samples lower results were obtained by Gawish (1998), El abed (2001) and Abo samra (2007) as in figure (4)

Figure 4



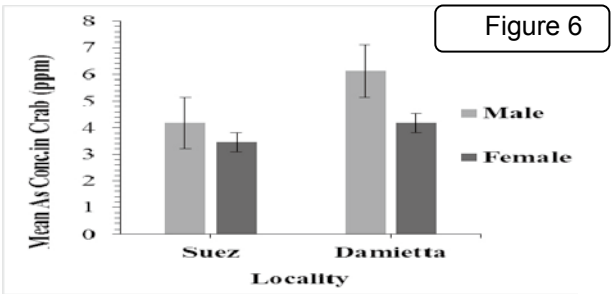
Mean values of arsenic concentration in examined shrimp samples from Suez and Damietta were 10.07±2.17 and 4.04±1.28 (ppm) wet weight, respectively as in figure (5).

Figure 5



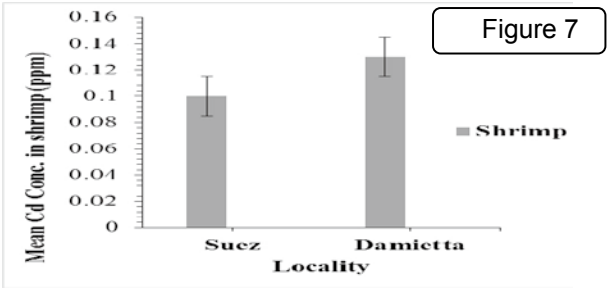
In examined male crab samples from Suez and Damietta arsenic concentrations were with mean values 4.18±1.16 and 6.13±1.94 ppm wet weight, respectively, while in examined females these values of mean were 3.46±0.94 and 4.19±1.34 ppm wet weight, respectively as in figure (6).

Figure 6



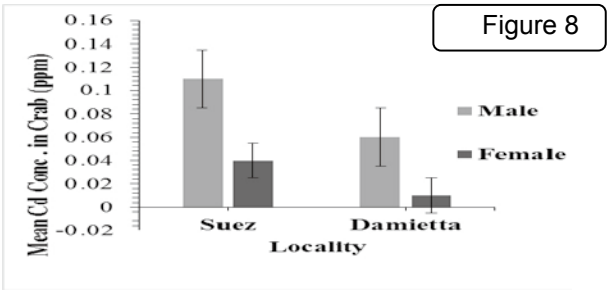
The mean values of cadmium concentration in examined shrimp samples from Suez and Damietta cities were 0.1±0.03 and 0.13±0.02 (ppm) wet weight, respectively The obtained results in examined shrimp samples were nearby what has been reported by El abed (2001) while higher results obtained by Gawish (1998) Sallam (2000), Zaki (2004) and Abo samra (2007). Lower results were reported by Mohamed (1999) as in figure (7).

Figure 7



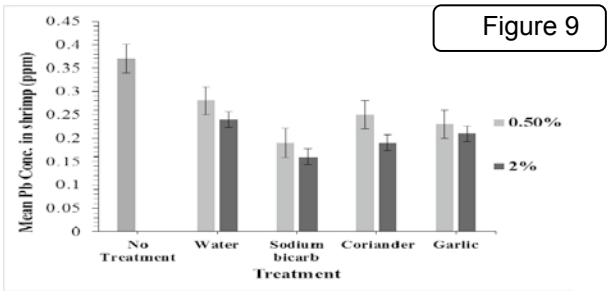
In examined male crab samples from Suez and Damietta cadmium mean values were 0.11±0.01 and 0.06±0.01 ppm wet weight, respectively, while in examined female crab samples from Suez and Damietta areas the mean values were 0.04±0.01 and 0.01±0.01 ppm wet weight, respectively. In crab samples the obtained results nearby what had been reported by Sallam (2000) and El abed (2001). While higher results were reported by Mohamed (1999), Zaki (2004) and Abo samra (2007) as in figure (8).

Figure 8



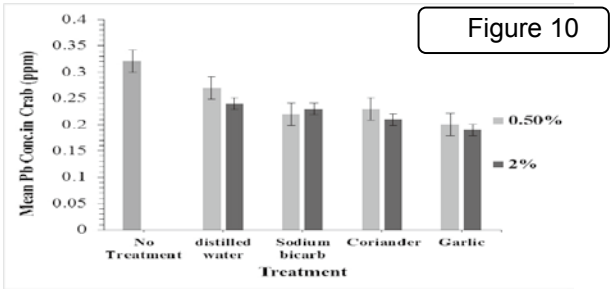
The mean values of lead in examined shrimp samples before treatment was 0.37±0.06 ppm and after treatment with distilled water for 0.5 hour, distilled water for 2 hours, sodium bicarbonate solution (0.5%) for 2 hours, sodium bicarbonate solution (2%) for 2 hours, dried coriander solution (0.5%) for 2 hours, dried coriander solution (2%) for 2 hours, dried garlic solution (0.5%) for 2 hours and dried garlic solution (2%) for 2 hours became 0.28±0.09 ,024±0.14, 0.19±0.15, 0.16±0.15, 0.25±0.17, 0.19±0.19, 0.23±0.12 and 0.21±0.12 respectively as in figure (9).

Figure 9



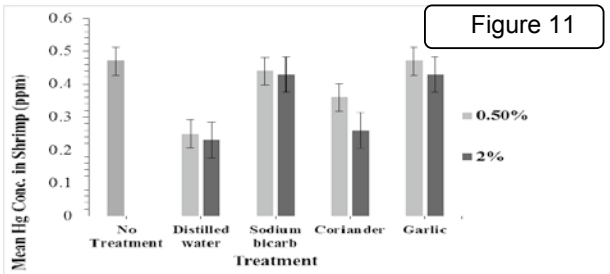
Mean values of lead in examined crab samples before treatment was 0.32±0.05 ppm. After treatment with distilled water for 0.5 hour, distilled water for 2 hours, sodium bicarbonate solution (0.5%) for 2 hours, sodium bicarbonate solution (2%) for 2 hours, dried coriander solution (0.5%) for 2 hours, dried coriander solution (2%) for 2 hours, dried garlic solution (0.5%) for 2 hours and dried garlic solution (2%) for 2 hours, these values were reduced to 0.27±0.10, 0.24±0.14, 0.22±0.09, 0.23±0.09, 0.23±0.12, 0.21±0.15, 0.20±0.02 and 0.19±0.01 respectively as in figure (10).

Figure 10



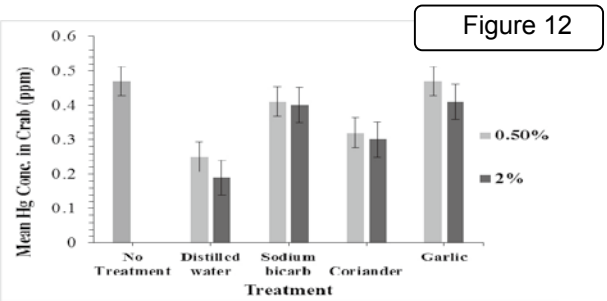
The mean values of mercury in examined shrimp samples before treatment was 0.47±0.07 ppm and after treatment with distilled water for 0.5 hour, distilled water for 2 hours, sodium bicarbonate solution (0.5%) for 2 hours, sodium bicarbonate solution (2%) for 2 hours, dried coriander solution (0.5%) for 2 hours, dried coriander solution (2%) for 2 hours, dried garlic solution for 2 hours (0.5%) and dried garlic solution (2%) for 2 hours these values were reduced to 0.25±0.07 , 0.23 ±0. 18 , 0.44±0.16 , 0.43±0.12 , 0.36±0.16 , 0.26±0.10 , 0.4 7±0.01 and 0.43±0.12 respectively as in figure (11).

Figure 11



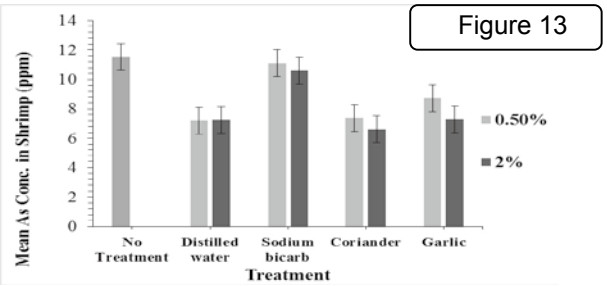
The mean value of mercury in examined crab samples before treatment was 0.47±0.06 ppm while after soaking in distilled water for 0.5 hour and 2 hours, sodium bicarbonate solution (0.5%) for 2 hours, sodium bicarbonate solution (2%) for 2 hours, dried coriander solution (0.5%) for 2 hours, dried coriander solution (2%) for 2 hours, dried garlic solution (0.5%) for 2 hours and dried garlic solution (2%) for 2 hours became 0.25±0.14, 0.19 ±0.06, 0.41±0.16, 0.40±0.07, 0.32±0.19, 0.30±0.12, 0.47±0.17 and 0.41±0.10 respectively as in figure (12).

Figure 12



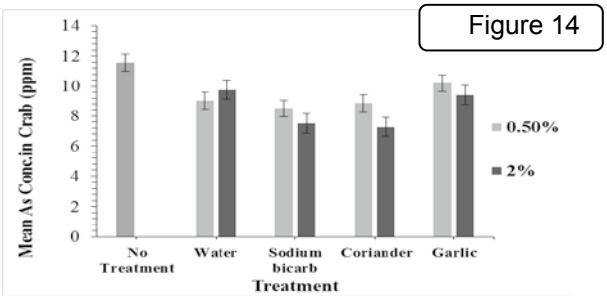
The mean value of arsenic in examined shrimp samples before treatment was 11.52±1.28 ppm and after treatment with distilled water for 0.5 hour, distilled water for 2 hours, sodium bicarbonate solution (0.5%) for 2 hours, sodium bicarbonate solution (2%) for 2 hours, dried coriander solution (0.5%) for 2 hours, dried coriander solution (2%) for 2hours, dried garlic solution (0.5%) for 2hours and dried garlic solution (2%) for 2 hours reduced to 7.20±2.22, 7.25±2.06, 11.10±1.12, 10.06±2.90, 7.39±1.97, 6.62±2.22, 8.73±0.19 and7.30±1.86 respectively as in figure (13).

Figure 13



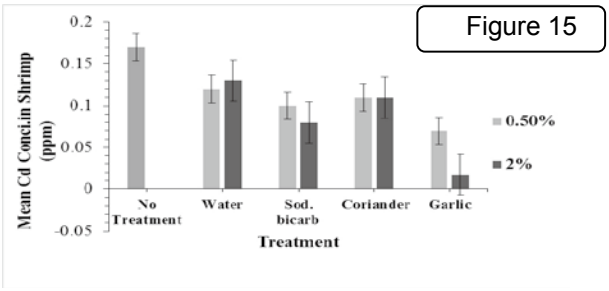
The mean value of arsenic in examined crab samples before treatment was 11.57±1.27 ppm and after treatment with distilled water for 0.5 hour, distilled water for 2 hours, sodium bicarbonate solution (0.5%) for 2 hours, sodium bicarbonate solution (2%) for 2 hours, dried coriander solution (0.5%) for 2 hours, dried coriander solution (2%) for 2 hours, dried garlic solution (0.5%) for 2 hours and dried garlic solution (2%) for 2 hours reduced to 9.02±2.81, 9.77±3.46, 8.51±1.86, 10.7±3.64, 8.85±1.45, 7.28±3.53, 10.20±2.14 and 9.42±3.16 respectively as in figure (14).

Figure 14



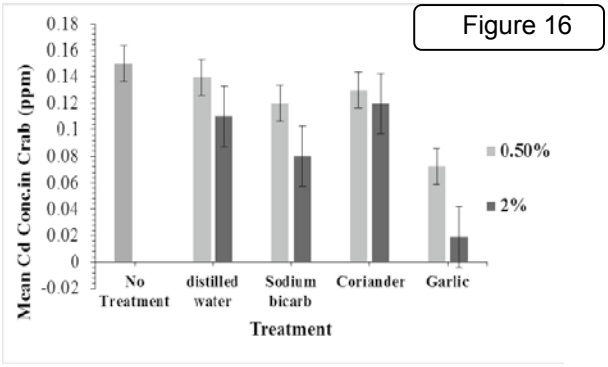
The mean values of cadmium in examined shrimp samples before treatment was 0.17±0.03 ppm and after treatment with distilled water for 0.5 hour, distilled water for 2 hours, sodium bicarbonate solution (0.5%) for 2 hours, sodium bicarbonate solution (2%) for 2 hours, dried coriander solution (0.5%) for 2 hours, dried coriander solution (2%) for 2 hours, dried garlic solution (0.5%) for 2 hours and dried garlic solution (2%) for 2 hours reduced to 0.12±0.02 , 0.13±0.03 , 0.10±0.03 , 0.08 ±0.03 , 0.11±0.02 , 0.11±0.03 , 0.07 ±0.02 and 0.017±0.002 respectively as in figure (15).

Figure 15



The mean values of cadmium in examined crab samples before treatment was 0.15±0.03 ppm and after treatment with distilled water for 0.5 hour, distilled water for 2 hours, sodium bicarbonate solution (0.5%) for 2 hours, sodium bicarbonate solution (2%) for 2 hours, dried coriander solution (0.5%) for 2 hours, dried coriander solution (2%) for 2 hours, dried garlic solution (0.5%) for 2 hours and dried garlic solution (2%) for 2 hours reduced to 0.14±0.02, 0.11±0.02, 0.12±0.02, 0.08±0.01, 0.13±0.01, 0.12±0.01, 0.072 ±0.01 and 0.019±0.01 ppm respectively as in figure (16).

Figure 16



4. Acknowledgments

This study was supported and appreciated with all help in department of Food hygiene and quality control, Faculty of veterinary medicine Zagazig University, Egypt.

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Cytochrome P450 expression in the rabbit

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This study was undertaken to investigate the tissue specific expression of different Cytochrome P450 (CYP) isoforms in the rabbit. CYP1A subfamily, which includes CYP1A1 and CYP1A2, is mainly responsible for the metabolism of xenobiotics such as dioxins, polycyclic aromatic hydrocarbons and heterocyclic amines. CYP1A1 was expressed both in the liver and extrahepatically in the rabbit. CYP1A2 is expressed only in the liver. CYP2C1 and CYP2J1 are responsible for the metabolism of arachidonic acid. CYP2C1 was expressed hepatically only. CYP2J1 was expressed mainly in the colon, stomach, lungs and kidney. The effect of sex on mRNA expression of different CYP isoforms was also studied.

Keywords: Rabbit, CYPs, Xenobiotic Metabolizing Enzymes

1. Introduction

Cytochrome P450 (CYP) superfamily is divided into a number of families, which in turn are divided into subfamilies, each of which consists of one or more enzymes. These enzymes metabolize a wide range of endogenous and exogenous xenobiotic compounds, resulting in either the activation or detoxification of the xenobiotics, depending on the enzyme involved (Mureithi et al., 2012). Thus, the response of the body to physiological substrates, therapeutic drugs, carcinogens and other toxicants and pollutants can be greatly influenced by the differential expression of CYP enzymes in different tissues. In many species, the liver shows the highest expression of these enzymes, but these enzymes are also expressed in extrahepatic tissues, such as the kidney, intestine, lung and tongue (Darwish et al., 2010).

Rabbit is a very important animal species in many countries of the world for the production of meat and fur. However, this species is exposed during its lifetime to a vast array of xenobiotics. Thus, this study was undertaken to investigate the tissue-specific constitutive expression of different cytochrome P450 isoforms in the rabbits. Moreover, the effect of sex on CYP expression in rabbit was also investigated

2. Materials and Methods

2.1 Collection of samples

Ten tissue samples from each of liver, lung, heart, spleen, kidney, muscle, fat, tongue, stomach and colon (5 for each of male and female) were collected from adult white New Zealand rabbits, which received laboratory feeding.

2.2 RNA extraction

Total RNA was prepared from each tissue by the method described before (Darwish et al., 2010), using TRI reagent from Sigma. The concentration and purity of the RNA fraction was determined spectrophotometrically at 260 and 280 nm, respectively.

2.3 cDNA synthesis

cDNA was synthesized as follows: a mixture containing 5 mg total RNA and 0.5 ng oligo Dt primer was incubated in a total volume of 24 ml sterilized ultrapure water at 70ºC for 10 min. This mixture was then removed from the thermal cycler and made up to 40 ml with 4 ml of (53) RT buffer, 8 ml of 10 mM dNTP, 2 ml of DEPC (diethylpyrocarbonate) water and 2 ml of RT-ReverTra Ace. The mixture was then reincubated in the thermal cycler at 30ºC for 10 min, 42ºC for 1 h and 90ºC for 10 min to prepare the cDNA.

2.4 Quantitative real-time polymerase chain reaction

qPCR was performed to analyze the mRNA levels of rabbit CYP1A1, 1A2, 2C1, 2J1 and GAPDH, which was used as an endogenous control as it was equally expressed in all examined tissues, using the Step One Plus Real-Time PCR system (Applied Biosystems, Foster, CA, USA) and the DyNAmo HS SYBR Green qPCR kit (Finnzymes Oy, Keilaranta, Finland), according to the manufacturer's instructions. The primer sets and PCR conditions used have been described previously (Yang et al., 2003). The measurements of the specific enzymes were performed in duplicate and repeated three times. The expression of each gene was normalized to the expression of GAPDH and was calculated relative to that of controls using the comparative threshold cycle (Ct) method.

3. Results and Discussion

Studying the tissue specific expression of different CYP isoforms in the rabbit is a matter of significance in understanding the metabolic ability of the rabbit to different xenobiotics.

CYP1A subfamily (1A1 and 1A2) has been shown to be involved in the metabolism of environmental pollutant such as polycyclic aromatic amines, dioxins, aflatoxin and heterocyclic amines. Regulation of CYP1A family occurs via activation of the AhR-dependent pathway by direct binding of AhR-ligands to the receptor, AhR. The results of this study showed that CYP1A1 is expressed both in the liver and extrahepatically as clear in Fig.1A. Liver had the highest expression followed by kidney, lungs and heart. Males showed higher expression of CYP1A1 than females in livers, kidneys and lungs (Fig.1A). In regard to CYP1A2, it was expressed only hepatically. Likely, males had higher expression than females (Fig. 1B).

Tissue-specific mRNA expression of CYP2C1 and CYP2J1, which are mainly catalyst of arachidonic acid metabolism in many species were also studied. CYP2C1 was only expressed in the liver but CYP2J1 was expressed strongly in the colon, lungs, kidney and spleen (Fig. 1C& D). These results go in agreement with Kikuta et al. (1991) who recorded high expression of CYP2J1 in the gastrointestinal tract of the rabbit. There were no effect for sex difference in the expression patterns for both CYP2C1 and CYP2J1. The results achieved in this study help us to understand the tissue-specific expression of different CYPs in the rabbit.

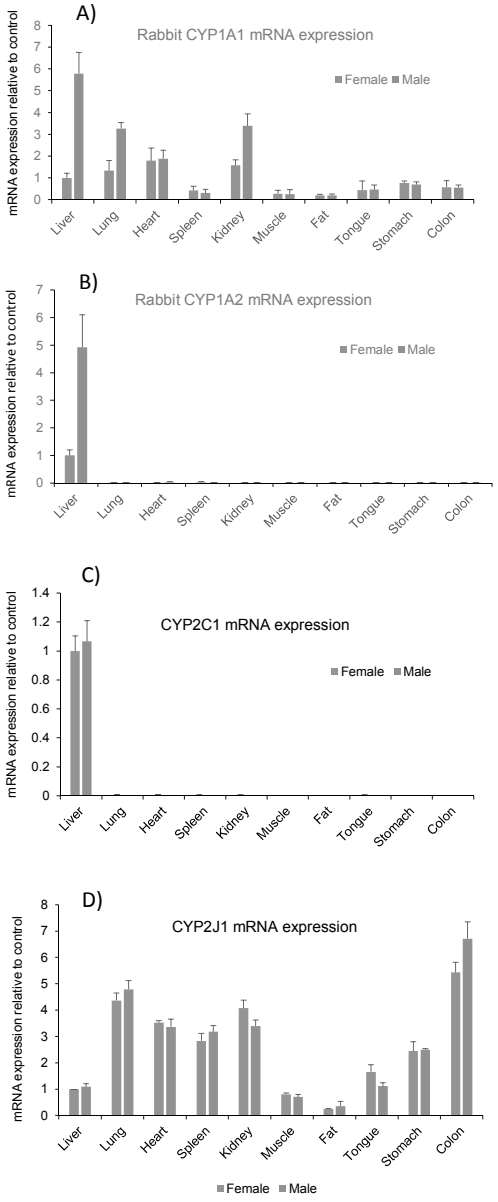

Figure 1: Cytochrome P450 (CYP) mRNA expressions in different tissues of rabbit (A) CYP1A1, (B) CYP1A2, (C) CYP2C1, (D) CYP2J1.

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P-25

Behavioural alteration following fipronil exposure with evaluation of the recovery ability of Japanese quail *(Coturnix japonica)*

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The aim of the current study was to assess the behavioural consequences of fipronil in the Japanese quail. Through15-day gavage study of fipronil exposure as well as studying the recovery of a 60-day depuration period after exposure. Our results showed that fipronil exposure had a profound negative influence on the ingestive behaviour and plumage condition of the quails. Also, such changes were reversed after an off-dose period of 30 and 45 days.
In conclusion, the fipronil causes behavioural changes, besides, the recovery study shows regeneration potential of organisms following an off dose of 30 and 45 days and confirmed that the responses of Japanese quail were in most indices reversible when exposure was terminated.

Keywords: Fipronil, quail, Phenypyrazole insecticides, behaviour

1. Introduction

Fipronil is a member of the phenylpyrazole class of insecticides. It is more effective than organophosphate, carbamate and pyrethroids insecticides against resistant pests. The mechanism behind fipronil action is interfering with the passage of chloride ions through the GABA chloride channel having an effect on the neurotransmission leading to death of insects, Fipronil is used to control ants, beetles, cockroaches, rootworms, and other insects. It is also being applied to control fleas and ticks on the domestic animals. Thus, fipronil is utilized in commercial (agricultural and veterinary purposes) and household applications (Das et al., 2006).

Several studies showed that fipronil caused endocrine interruption and adverse impacts in female rats reproduction (Ohi et al., 2004), central behavioral impacts in rats (Terçariol and Godinho, 2011). There are little data concerning the impact of fipronil in avian species. Thus, the behavioral alterations of oral dosing of fipronil for 15 days exposure and during a recovery period of 60 days in Japanese quail *(Coturnix japonica)* were assessed, on the basis of growth performance parameters and behavioral evaluations.

2. Material and methods

2.1 Tested compound and experimental design

Fipronil insecticide was used as an available commercial formulation (Fipronil 20% Sc) provided by Yong- nong Bioscience Co, Ltd. China. Sixty mature male Japanese quail (*Coturnix coturnix Japonica*) weighing (200-230 gm) were obtained from Poultry farm, Faculty of Agriculture, Zagazig University, Egypt. Fipronil was dissolved in DMSO and used for treatment groups. The birds were randomly divided into two dosage groups, group 1 (n=10) kept as a control which was orally administered a DMSO vehicle only using gastric tube. Fipronil treated birds (n=50) were received fipronil orally at a dose of $\frac{1}{5}$ LD_{50} (LD50; 11.3 mg／kg [24]) daily for 15 days representing the fipronil group. Following exposure, 10 birds were sacrificed, the other 40 birds were kept for a recovery study, which subdivided into four equal groups; R_{15}, R_{30}, R_{45} and R_{60} which considered as a recovery groups after an off-dose period of 15, 30, 45 and 60 days, respectively.

2.2 Behavioural observations

Direct observations were conducted to record different behavior for 4 hrs. /weekly in each, by focal sample technique (Shimmura et al., 2007) after identification of quails by using different colored wing bands. The observer stood inside the room 10 min before starting the direct observation to allow the quails to acclimatize. All experimental groups were observed two times/daily, directly for 3×10 minutes in the morning (8:00 am till 12:00 am) and for 3×10 minutes. The following frequencies of behavioural patterns were recorded:

- Ingestive behavior: eating or drinking.
- Locomotion behavior: quails walking or flying.
- Comfort behavior: including sham dust bathing, preening and body shaking.

After observation, the frequencies of behavior were counted in the observed quails and these numbers were used to calculate the frequencies of activities/ one hour. Evaluation of the plumage condition for all groups was scored 3 times for all quails in each experimental period. The plumage condition was assessed by using Tauson scale (Tauson et al., 1984) i.e. the scoring system assigned value of 1 to 4 for each part, where 4 was

the best condition and 1 was the worst.

2.3 Statistical analysis

Data were expressed as mean ± SE. Statistical comparisons were performed by one way-ANOVA followed by Tukey's multiple comparison post-hoc test. A value of $p<0.05$ was considered as statistically significant.

3. Results and Discussion

Quail administered fipronil orally via gastric intubation over a period of 15 days showed alterations in behavioural responses. The frequency of eating, drinking and walking was significantly decreased in fipronil administered group with non significant change in R_{60}, R_{30} and R_{30} groups, respectively whereas their frequency was reversed. On the other hands, the flying, sham dust bathing, preening and body shaking frequency was non significantly altered in all experimental groups. Concerning the pluming score, it showed highly significant decrease in fipronil which restored in R_{30} group in comparison to control.

The injurious impacts of fipronil may result from its generation of ROS that causes oxidative stress of various organs. Increased oxidative stress and lipid peroxidation are involved in the pathogenesis of pesticides-induced damage. These results may be due to behavioral endocrinology, and these results are in agreement with Jackson *et al.* (2009) demonstrating that the action of fipronil is similar to that of an endocrine disruptor.

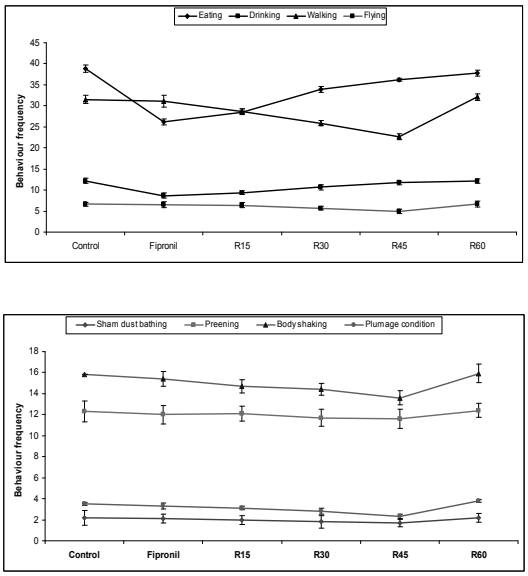

Figure 1: Effect of fipronil oral administration on Japanese quail on behavioural response. the data are represented as mean ± SE (n = 10)

4. Conclusion

This study gives further evidence that fipronil is toxic to Japanese quail and causes behavioural changes. Besides, the recovery study showed a regeneration potential of organisms following an off dose of 30 and 45 days and confirmed that the responses of Japanese quail were in most indices reversible when exposure was terminated.

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P-26

The hepatotoxic effect of Linezolid antibiotic abuse in male albino rats

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The mode of antibiotic actions on bacteria is well characterized but the mechanistic effects on mammalian tissues remain unclear. Therefore, the present study was designed to examine the effects of linezolid abuse on the antioxidant status of rat's liver. Thirty six male albino rats were segregated into random three groups of control, linezolid therapeutic treated group and double therapeutic treated rats orally for twenty eight days. There were significant increases in hepatic malondialdehyde and serum 8 hydroxyguanosine level in a dose dependent manner when compared with control rats. Meanwhile, the activities of superoxide dismutase and glutathione peroxidase with reduced glutathione exhibit a statistically significant decrease when compared with control values. The histopathological changes confirmed the aforementioned biochemical changes. We conclude that linezolid has hepatotoxic effects through induction of oxidative stress.

Keywords: Linezolid; malondialdehyde; superoxide dismutase; reduced glutathione

1. Introduction

Linezolid (LZD) is a member of the oxazolidinone group of antimicrobials that inhibits bacterial ribosomes by binding to the 23S ribosome and preventing the 30S-50S fusion. Disruption of mammalian mitochondrial protein synthesis has been implicated in three side effects of linezolid which includes hyperlactatemia and neuropathies (Garrabou et al.2007). Mitochondria represent the physiological store house of ROS in mammals. Due to their detrimental role in damaging of DNA and oxidative stress so, our study aimed to evaluate the effect of LZD antibiotic treatment on antioxidant status and histopathological changes in liver of experimental rats.

2. Material and Methods

Male Wistar rats (36 rats), 10 weeks old, weighing 180-220 g, were selected randomly. Rats were exposed to 12 h light/dark cycle with free access to food and water. The ethical guidelines of Zagazig Veterinary Medicine were followed. They were divided into 3 different groups (12 rats/ each). Control group: rats were received normal saline during the experimental period. Therapeutic treated group (LZD1): rats were given125mg/kg body weight orally LZD (manufactured by Averroes Pharma) through gastric intubation for 28 days (DeVriese et al.2006). Double therapeutic group (LZD2): rats were given 250mg/kg orally of LZD for twenty eight days. At the end of experimental period, all rats were sacrificed where blood samples were collected for serum analysis of 8 hydroxy guanosine (8-OHdG). Liver tissues were excised, weighted and homogenated using tissue homogenizer for measurement of antioxidant status. Another portion of liver samples were embedded in neutral formalin for histopathological examination. Results are expressed as means± S.E. for 12 independent rats per each group. Statistical analysis was done using SPSS and Fischer's post hoc test. The results were considered statistically significant when $p<0.05$.

3. Results and discussion

The effect of therapeutic dose and double one were epitomized in table 1. There were a significant *($P<0.05$)* increase in hepatic malondialdehyde (MDA) level and serum 8-OHdG with a significant *($P<0.05$)*decrease in the activities of superoxide dismutase (SOD), glutathione peroxidase (GPx) and reduced glutathione (GSH) content throughout the experimental period in a dose dependent manner when compared with control rats. These results came in agreement with Moraza et al (2015) who reported that the oxidative stress and hematological side effect of LZD occurs in erythrocytes manifested by alteration in SOD and CAT activities after administration of LZD for 14 days. In the present study the repeated administration of LZD resulted in H_2o_2release that can be detoxified by catalase which removes it when present at high concentration and GPx, which destroys it when present at steady state (Casado et al. 1995). Therefore, the reduction in the GPx activity may render the liver more susceptible to H_2O_2 and $OH^{\bullet}$- Induced oxidative stress. The level of GSH is a measure of cellular redox status. Hence alteration in GSH concentration may affect the overall redox status of the cell. These results matched with Wang et al (2014) who reported that LZD induces a decrease of WBC, RBC and platelet numbers in rat blood and enzymatic activities of SOD and catalase in rat serum in a dose-dependent manner. A significant increase in 8-OHdG level due to mitochondrial dysfunction which lead to steatohepatitis and high level of lipid peroxidation

act synergistically with DNA damage which represented by increased level of 8-OHdG and hepatic mitochondrial dysfunction is an alternate contributory factor to the genesis of lesions in steatohepatitis, with reduced activity of respiratory complexes I, III, and IV, and ATP synthase complex V and enhanced ROS generation. The histopathological changes were portrayed in figure A. Liver of control rats showed normal hepatocyte architecture (Fig.A1) mean while, LZD1treated rats exhibited congestion of hepatic blood vessel, sinusoids, periportal hydropic degeneration, steatosis, portal area with round cells aggregation (arrow), and biliary hyperplasia (Fig. A2-4). The liver of f LZD2 treated rats showed periportal area of coagulative necrosis, microvesicular steatosis in the hepatocytes, hypertrophied kupffer cells, macrovesicular steatosis and leukocytes infiltration (Fig. A5-8). These results provide direct evidence that LZD showed macro-and microvesicular steatosis as result of reactive oxygen species which induced oxidative stress with potentially severe clinical consequences in a dose dependent manner.

Table1. Effect of linzolide (LZD) administration for 28 days on hepatic malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GPx), reduced glutathione (GSH) and serum 8-OHdG level(Mean± SE).

	MDA nmol/g tissue	GSH mg/g tissue	SOD U/g tissue	GPx U/g tissue	8-OHdG ng/ml
Con.	50±0.6^{c}	4.7±0.1^{a}	39.9±0.2^{a}	27.2±0.3^{a}	0.66±0.1^{c}
LZD1	89±0.7^{b}	2.4±0.1^{b}	36.5±0.72^{b}	22±0.2^{b}	1.23±0.1^{b}
LZD2	107.4±1^{a}	1.7±0.1^{c}	31.5±0.5^{c}	17±0.4^{c}	1.5±0.2^{a}

^{a,b,c}The means within the same column and bearing different superscripts are significantlydifferent at $P < 0.05$.

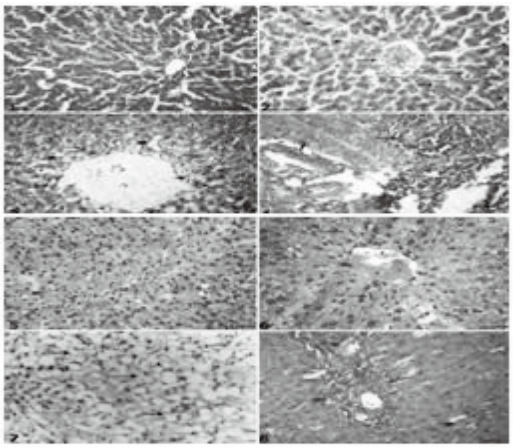

Fig. A Photomicrograph of sections in rat's liver treated with linzolide (LZD) for 28 days. Histopathological of (1) control group,(2-4) LZD therapeutic treated rats,(5-8) LZD double therapeutic treated rats.

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P-27

Pb levels and isotope ratio profiles in kidneys of Japanese wild rats

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Although Japan has been considered to have little lead (Pb) pollution in modern times, the actual pollution situation is unclear. The present study aims to investigate the Pb pollution extent and to identify the pollution source in Japan using stable Pb isotope analysis with kidney of wild rats. Wild brown (*Rattus norvegicus*) and black (*R. Rattus*) rats were captured from various sites in Japan. Mean Pb concentrations in the kidney of rats from several cities were above the threshold for histological kidney changes. However, although it was considered that composite factors are involved in Pb pollution, identification of concrete pollution source has not been accomplished due to limited differences among previously reported values of Pb isotope composition in Pb products circulated in Japan. Namely, the current study established the limit of Pb isotope analysis for source identification. Further detailed research about monitoring Pb pollution in Japan and demonstration of novel method to identify Pb source are needed.

Keywords: Wild rat, Stable Pb isotope, Source identification, Japan

1. Introduction

Pb pollution not only occurs locally but also worldwide due to its volatile character. Additionally, Pb is a proven non-essential and toxic metal for humans and animals. For humans, Pb is known to be neurotoxic, especially to children because of its ability to compete with calcium (Ca^{2+}) in nerve. Pb poisoning cases have been reported in humans worldwide. For instance, more than 160 people, mainly children under the age of five, died in the Pb poisoning disaster in Zamfara, Nigeria (Blacksmith Institute 2010).

In addition to considering the total levels and chemical/mineralogy composition of Pb, the contribution of Pb to the environment from multiple sources should be accurately determined. Among the natural abundance of four stable isotopes of Pb, i.e., ^{208}Pb, ^{207}Pb, ^{206}Pb, and ^{204}Pb, only ^{204}Pb is not radiogenic. The abundance of ^{204}Pb in the Earth's crust has remained since Earth solidified. On the other hand, ^{208}Pb, ^{207}Pb, and ^{206}Pb are radiogenic isotopes, and are the products of the radioactive decay of ^{232}Th, ^{235}U, and ^{238}U, respectively. Identifying the Pb pollution source using stable Pb isotope ratios (Pb-IRs) has been widely carried out in many fields of environmental research.

To evaluate the biological effects of Pb in detail, a model based on a living organism is needed. Inherent and external factors, such as size, sensitivity, physiological characteristics, position in the food chain, migration, abundance, and ability to propagate in captivity were widely regarded as criteria for a good sentinel. From these standpoints, wild rat is large enough as sentinels, and actually have been used as mammalian sentinels for terrestrial metal pollution. Previous report revealed that Pb-IRs in rat kidney comparatively accurately reflect those of Pb source, although biological fractionation of Pb isotopes was speculated (Liu et al. 2014). Thus, it is likely that Pb pollution source could be identified using Pb isotope analysis of rat kidneys as mentioned above. The current study aims to elucidate the extent of Pb contamination and to identify Pb source in Japan using kidney of wild rats. To the best of our knowledge, this is the first study that focuses on Pb-IRs in wild rats.

2. Materials and Methods

Sampling of wild rats

Wild rats, including brown (*Rattus norvegicus*, n = 43) and black rats (*R. rattus*, n = 98) were collected from various sampling sites in Japan from July 2004 to November 2013 using gauze cage traps with food. We treated these as same group since there was no clear difference in Pb profiles.

Sample preparations and analysis

For acid digestion, a dry thermal unit was used following methodology modified from Nakayama et al. 2011. Kidney samples (1.0 g or available amount) were dried in heat resistant glass tubes for 48 h. The dry weight was calculated, and the dried samples were then digested in 5 mL of 60 % HNO3 for 48 h in a dry thermal unit. The temperature was increased gradually to 140°C, and after complete digestion, the acid was evaporated at 160°C to

reduce the volume to 0.5 mL. The cooled samples were transferred into 15 mL plastic tubes, followed by dilution to 10 mL with 2 % HNO3. The Pb concentration was determined with a flame/flameless atomic absorption spectrophotometer (AAS, Z-2010, Hitachi High-Technologies Corporation, Tokyo, Japan) equipped with a Zeeman graphite furnace or an inductively coupled plasma–mass spectrometer (ICP-MS: 7700 series, Agilent Technologies, Tokyo, Japan). The Analyses of the Pb-IRs ($^{208}Pb/^{206}Pb$ and $^{207}Pb/^{206}Pb$) were conducted using ICP-MS, according Nakata et al. 2015.

3. Results and discussion

Rats from Aichi accumulated significantly higher concentrations of Pb in kidney compared with rats from Chiba and Hokkaido. Similarly, significantly higher level of Pb was observed in the rat kidneys from Niigata than those from Kanagawa, Chiba, and Hokkaido. Additionally, the Pb concentrations in the kidney of the rats from Ibaraki were significantly higher compared with those of the rats from Hokkaido. A high variability in the Pb level was observed in the kidney samples from every location. Although the sample size was small in some regions, a mean Pb level above the threshold of 2.50 mg/kg was found in the kidneys of the rats from Okinawa, Aichi, Niigata, Fukuoka, Ibaraki, Kyoto, Osaka, Kanagawa, and Tokyo.

Pb-IRs in rat kidneys of which Pb level was < 5.0 mg/kg converged closely at around approximately 2.080 to 2.140 ($^{208}Pb/^{206}Pb$) and 0.840 to 0.880 ($^{207}Pb/^{206}Pb$), and generally showed positive linear relationship. These values of Pb-IRs were very similar to the previous reported values in sediment and roadside dust, airborne particulate matter from several regions. Although Pb-IRs in rat kidneys of which Pb concentration was ≥ 5.0 mg/kg recorded generally similar values with that in rat kidneys of which Pb level was < 5.0 mg/kg, some individuals whose Pb concentration greater than 5.0 mg/kg from Ibaraki (2.175, 0.922), Kanagawa (2,083, 0.884 and 2.056, 0.825), and Chiba (2.162, 0.909) showed distinctive values. However, it was difficult to estimate or identify “Exact” Pb pollution source due to absence of previous report about Pb-IRs close to these values in natural or anthropogenic Pb from Japan, and of environmental samples from habitat of rat in the current study.

4. Acknowledgments

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P-28

Metabolic capacities of polychlorinated biphenyls (PCBs) in cats and dogs

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Recently, there are suspicions of the relationship between increasing feline hyperthyroidism (HT) in pet cat and exposure to organohalogen contaminants and/or those hydroxylated metabolites, in addition to the number of health problems related to the pets has been increasing with the recent pet boom. The present study determined *in vitro* and *in vivo* metabolism of polychlorinated biphenyls (PCBs) by cat and dog. *In vitro* test, after exposure to 62 PCB mixtures, 4'OH-CB18, 3'OH-CB28, 4'OH-CB79, 4OH-CB107 were found in cat liver microsomes. In the dog, 13 identified OH-PCBs and 22 unknown OH-PCBs were detected. In contradiction to cats, PB type PCBs were metabolized more easily than MC type in the dog liver. The present study supports a NIH shift with para-hydroxylation as a metabolic process than the direct insertion of oxygen in the formation of OH-PCBs. *In vivo* test, no change of the activities of the conjugation enzymes was detect, although AROD activity was increased, which indicates that the PCBs exposure does not affect the conjugation ability in cats. Cats may not metabolize lower chlorinated OH-PCBs due to lack of glucuronate conjugation ability. This study is suggesting PCBs absorption, metabolism and excretion ability of cats differ from dogs, and cats have a higher risk of toxic effects from low-chlorinated OH-PCBs. Especially, it is concern that affects to thyroid hormone homeostasis such as the depressed thyroid hormone levels of cats by PCB exposure, and thus disruptions of thyroid function.

Keywords: polychlorinated biphenyls (PCBs), Cat, Dog, Metabolic capacities

1. Introduction

Polychlorinated biphenyls (PCBs) have been widely used for industrial applications since 1960s. As a consequence, PCB levels have been increasing dramatically in the environment, wildlife and humans, because of their persistent properties and the characteristics of bioaccumulation in the food web.[1-2] The toxic effects of these compounds are already well known such as adverse effects endocrine systems and neurodevelopment.[3] It is suspected that the disturbance of thyroid hormone homeostasis is induced by not only PCBs but also their hydroxylated metabolites.[4] Hydroxylated PCBs (OH-PCBs) are the metabolites of PCBs, which are formed by oxidative metabolism by cytochrome P450 (CYP) monooxygenases enzyme system.[5]

In our previous studies, it was investigated the congener pattern of PCBs in the blood of pet dogs and cats. Higher chlorinated (6-8Cl) OH-PCBs were dominant in dog blood, in contrast, lower chlorinated (3-5Cl) OH-PCBs were dominant in cat blood.[6-7] These results suggested that metabolic capacities of PCBs between dogs and cats were totally different although of both carnivore species. It is also suspected that the thyroid hormone homeostasis is disturbed by these hydroxylated metabolites because of structural similarity with thyroid hormone. However, there are only a few studies on metabolic capacity in cats, and there are many unclear points about the toxic effects and risks of chemical exposures. In this study, we exposed PCBs as model compounds to cats *in vivo* and *in vitro*, and analysed congener pattern, hepatic enzyme activity and gene expressions to collect the foundational information about the metabolic mechanism in cats.

2. Materials and Methods

2.1 In vitro test

The methods used for in vitro experiment including the chemical analysis and molecular analysis have been reported elsewhere.[7-9] Briefly, reaction mixture contained buffer, 1μM 62 congener mixed PCBs, and liver microsomal suspension containing 200 pmol CYP enzymes were combined on ice. The metabolic assay was started by the addition of NADPH regenerating solution sequentially to all tubes and followed to proceed for a series of 180 min in a 37 °C, shaking (90 rpm) in water bath after pre-incubation for 10 min. After incubation, metabolic reaction was stopped by 1ml methanol.

2.2 In vivo test

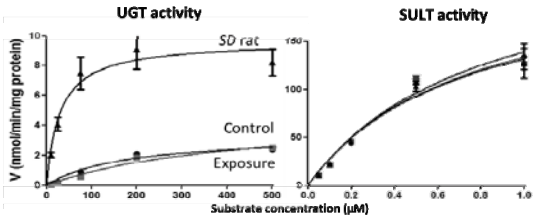
Adult male cats (*Narc: catus*) were divided into two groups (n=4 each): G1 treated with vehicle

control, and G2 with PCBs (a mixture of 12 congeners: IUPAC No. 18, 28, 70, 77, 99, 101, 118, 138, 153, 180, 187, and 202). The intraperitoneal administration (i.p.) test was performed according to the guideline approved by The Institutional Animal Care and Use Committee in Hokkaido University. After 5 days of exposure to PCBs, all cats were euthanized, and liver and blood samples were collected. The methods used for the chemical analysis and molecular analysis have been reported elsewhere. [7-9]

3. Results and Discussion

After exposure to 62 congener mixed PCB mixtures, 4'OH-CB18, 3'OH-CB28, 4'OH-CB79, 4OH-CB107, and 13 unknown hydroxylated PCBs (3-5Cl OH-PCBs) were found in cat liver microsomes by *in vitro* test (Table 1). 3'OH-CB28, 4'OH-CB79 and 4OH-CB107 metabolites were formed from MC type PCBs such as CB28, CB77, and CB118. It is suggested that CYP1A enzymes in cats were mainly intermediated these PCB metabolisms. Only few percentages (%) of PCBs were metabolized to OH-PCBs in cats (0.23-1.2 %) (Table 1). Interestingly, 1~2Cl PCBs were occurred biodegradation via CYPs, suggesting these compounds may be biotransformation to dihydroxylated metabolites and/or 1~2 chlorinated OH-PCBs. On the other hand, 13 identified OH-PCBs and 22 unknown OH-PCBs (3-7Cl OH-PCB) were found in the dog liver microsomes (Table 1). In contradiction to cats, PB type PCBs were metabolized more easily than MC type in the dog liver. Moreover, higher metabolic rates (0.77-7.9 %) than cats were found in the dogs (Table 1). The present study supports a NIH shift with para-hydroxylation as a metabolic process than the direct insertion of oxygen in the formation of OH-PCBs.

Comparing the results with a similar *in vivo* experiment conducted in dogs at the Ehime University, interspecies differences were found, and the metabolite patterns in cat blood were quite similar with *in vitro* results using liver microsomes. We also analyzed the change of hepatic enzyme activity and gene expressions of the exposed cats. No change of the activities of the glucuronosyltransferase and sulfotransferase enzymes was detect (Fig. 1), although EROD, PROD and MROD activity was increased, which indicates that the PCBs exposure does not affect the conjugation ability in cats. Also, the expression levels of the CYP1A1 and 1A2 genes were increased, suggesting they were inducted due to the exposure. This study is suggesting PCBs absorption, metabolism and excretion ability of cats differ from dogs, and cats have a higher risk of toxic effects from low-chlorinated OH-PCBs. As cats may have unique disposition kinetics for other chemicals too, there is a need for further studies about the metabolism and pharmacokinetics of cats.



	Vmax (nmol/min/mg)	Vmax/Km (μl/min/mg)	Vmax (pmol/min/mg)	Vmax/Km (μl/min/mg)
Control	3.42	18.9	251	311
Exposure	4.91	10.9	233	312
SD rat	9.61	325	226	314

Fig. 1 Enzyme kinetics for the glucuronidation (UGT) and sulfoconjugation (SULT) of hydroxylated pyrene in exposure cat, control cat and rat liver microsomes. Table shows Michaelis–Menten Constants for Glucuronidation (left) and sulfoconjugation (right).

Table 1 Formation rate (metabolites/precursors×100) (%) in the liver microsomes of cats and dog after exposed to 62 PCB mixtures.

Compounds	Cat	Dog
4'OH-CB18/CB18	0.85	4.2
3'OH-CB28/CB28	1.2	0.77
4'OH-CB79/CB77	0.23	7.6
4OH-CB97/CB99	-	3.4
4'OH-CB101/CB101	-	6.6
4OH-CB107/CB118	0.38	-
3'OH-CB118/CB118	-	3.1
4'OH-CB120/CB118	-	7.9
4OH-CB146/CB138	-	4.1
4OH-CB146/CB153	-	3.6
4OH-CB201/CB201	-	0.9
4OH-CB202/CB202	-	2.1

-: not detected

4. Acknowledgments

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P-29

Is there speceies difference of biological variation of lead (Pb) isotopic composition between livestock and poultry?

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It has been reported that stable Pb isotopic compositions are beneficial to identify lead sources. Recently, however, it has become evident that Pb isotopic composition of biological samples are different from those of the exposure source. To evaluate the utility of Pb isotopic analysis using livestock and poultry, we conducted this administration experiment. Goats and chickens were divided into control and exposure group and conducted single-dose injection of lead acetate. Tissues samples were collected from both species and Pb isotope ratios were measured. As a result, biological variations had observed in both species. However, the range of biological variation in the chickens was much smaller than that in the goats. Variation behaviour of goat was similar to that of the previous study in the rat. Conversely, variation behaviour of chicken was different from that of rat and goat and isotope ratios in chicken were less scattered. We concluded chicken could be a good indicator to identify Pb source by analysing stable Pb isotopic compositions.

Keywords: lead source; goat; chicken

1. Introduction

Lead (Pb) pollution currently constitutes one of the most serious environmental problems and has drawn worldwide attention. Sources of Pb exposure are numerous. So as to decrease the health risk of Pb to human, exact identification of the exposure sources and route is essential.

A lot of studies have indicated that Pb isotope compositions are useful for identifying Pb sources. Briefly, Pb exists in the environment as four main stable isotopes: ^{208}Pb, ^{207}Pb, ^{206}Pb, and ^{204}Pb. Only ^{204}Pb is non-radiogenic and has remained at a basically constant abundance of 1.4%. The other isotopes are the radiogenic products of the disintegration of ^{232}Th ,^{235}U, and ^{238}U, respectively. Variations in their abundance result from radioactive decay. Four stable Pb isotopes are considered to undergo slight fractionation through natural processes because of their large atomic weight and relatively small weight differences. Since the isotopic compositions of Pb is fixed and unique to the origin of the ore, analysing isotopic compositions can be clue in determining the source of Pb.

Recently, it has become evident that the Pb isotopic compositions of biological samples are sometimes different from those of the exposure source. Smith et al. reported that Pb isotopic compositions differed between paired blood and bone samples from human subjects (Smith et al. 1996). Other studies have founded that there were differences in Pb isotopic compositions among biological samples from Pb administrated rats (Wu et al. 2012, Lu et al.2014). Liu and Wu et al. suggested the hypothesis that biological fractionation of Pb isotopes occurs in live bodies. Although the mechanism is unknown, this appears to lead to wide variation of Pb isotopic compositions.

In a recent field study, our group observed a possible species difference between goat and chicken in terms of the behaviour of biological fractionation (Nakata et al. 2015). It was concluded that biological fractionation of Pb isotopes did not occur in chickens but did so in goats. Further animal experiments investigating the utility of livestock and poultry for Pb isotope analysis to determine the origin of Pb exposure are essential to reduce human health risks. In the current study, an animal experiment focusing on the biological variation of Pb isotopic compositions and on species differences in this process was conducted.

2. Materials and Methods

Goats and chickens were purchased from a local farm. After 1 week acclimation, single-dose intramuscular injections of the test substance lead acetate (Wako Pure Chemical Industries, Osaka, Japan) were administered. After 24 h, animals were dissected after exsanguination and the blood, kidney, muscle, lung, heart, bone, and brain were collected. These samples were kept frozen in the laboratory until extraction. Pb was extracted from the biological samples by acid digestion using the method of Nakata et al. 2015 with minor modifications. Briefly, approximately 0.5 g samples of kidney, muscle, lung, heart, brain, and 0.03 g of bone were dried for 48 h in an oven at 50 °C. Then, 1.0 ml samples of blood and the dried biological samples were placed in pre-washed digestion vessels, followed by acid digestion using 5 mL of nitric acid and 1 mL of hydrogen peroxide (Cica reagent, 30 %; Kanto Chemical). The digestion vessels subsequently underwent a ramped temperature program in a closed microwave extraction system (Speed Wave MWS-2 microwave digestion system; Berghof, Eningen, Germany). After cooling, the extracted solutions were transferred into 15 mL plastic tubes and diluted to a final volume of 10 mL with bi-distilled and de-ionized water (Milli-Q). Analysis of the Pb isotope ratios was performed using inductively coupled plasma-mass spectrometer (ICP-MS 7700 series; Agilent Technologies, Tokyo, Japan)., according to the method of Nakata et al. 2015 with minor modifications. To minimize mass bias and dead time effects, standard reference material (SRM) 981 (National Institute of Standards and Technology (NIST), Gaithersburg, MD, USA) was generally measured after every 12 samples. Sample ratios were corrected using the average value of each isotope ratio obtained from measurements of SRM 981, of which relative standard deviation (RSD) was ≤ 0.4% for $^{208}Pb/^{206}Pb$ and $^{207}Pb/^{206}Pb$. Each sample was measured based on 10 replicates.

3. Results and discussion

In both species, isotope ratios varied among the different tissue samples in control and exposure group. This finding could support the hypothesis of biological fractionation mentioned above.

Note that in the present study, the ranges of isotope ratios in chicken tissue samples were much smaller than those from goat tissues, regardless of group. In other words, biological variation of chicken might be slight.

When conducting stable Pb isotopic analysis in goats and chickens to investigate their use as sentinel animals to identify a pollution source, it should be kept in mind that biological fractionation may occur in both species. In goats, the ranges of isotope ratios of tissue samples were comparatively wide. Therefore, we cannot ignore the effects of biological fractionation. It may consequently be difficult to identify Pb exposure sources by analysing the isotope ratios of goat tissues.

In contrast to goat, biological fractionation of Pb might be slight in chicken. That is the choice of tissue did not have a critical effect in Pb isotope analysis of chicken tissues, as all tissues had similar isotope ratios. Hence, chickens can serve as indicators for stable Pb analysis to estimate Pb polluted source.

4. Acknowledgments

This study was supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan awarded to M. Ishizuka (No. 24405004 and 24248056) and Y. Ikenaka (No. 26304043, 15H0282505, 15K1221305), and S.M.M. Nakayama (No. 16K16197), and the foundation of JSPS Core to Core Program (AA Science Platforms) and Bilateral Joint Research Project (PG36150002 and PG36150003). We also acknowledge financial support from The Mitsui & Co., Ltd. Environment Fund and The Nihon Seimei Foundation. This research was supported by JST/JICA, SATREPS (Science and Technology Research Partnership for Sustainable Development). The analyses were technically supported by Mr. Takahiro Ichise.

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P-30

Pharmacokinetic analysis of warfarin resistant roof rats (*Rattus rattus*) in Tokyo.

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Roof rats (*Rattus rattus*) live mainly in human habitats. Heavy use of rodenticides, such as warfarin, has led to the development of drug resistance, making pest control difficult. There have been many reports regarding mutations of vitamin K epoxide reductase the target enzyme of warfarin, in resistant rats. However, it has been suggested there are other mechanisms of warfarin resistance. To confirm these possibilities, closed colonies of warfarin-susceptible roof rats (S) and resistant rats from Tokyo (R) were established, and the pharmacokinetics of warfarin in rats from both colonies were investigated. R rats had low levels of warfarin in serum and high clearance activity. These rats can rapidly metabolize warfarin by hydroxylation. The levels of accumulation in the organs were lower than those of S rats. R rats administered warfarin showed high expression levels of CYP2B, 2C, and 3A, which play roles in warfarin hydroxylation. The mechanism of warfarin resistance in roof rats from Tokyo involved not only mutation of VKOR but also high clearance ability due to high levels of CYP2B, 2C and 3A expression possibly induced by warfarin.

Keywords: warfarin, rodenticide, *Rattus rattus*, drug resistance, Pharmacokinetics, P450

1. Introduction

Wild roof rats (*Rattus rattus*) mainly live in proximity to human habitats. They are harmful to humans as they carry various zoonotic diseases. Therefore, extermination of them is necessary for public health. Rodenticides, such as warfarin, have been used since the 1950s. However, their continual use has resulted in the development of drug resistance in rodents, thus making rodent pest control difficult, especially in urbanized areas.

Warfarin inhibits blood coagulation by inhibiting the activity of vitamin K epoxide reductase (VKOR), which is the enzyme necessary for producing vitamin K-dependent clotting factors. Loss of these clotting factors leads to lethal haemorrhage.

Rost et al. (2004) [1] reported that warfarin resistance of brown rats (*Rattus norvegicus*) is due to mutation of the vitamin K epoxide reductase complex subunit 1 (vkorc1), which encodes VKOR. However, it has been suggested that vkorc1 mutation is not the only mechanism of warfarin resistance. Heiberg (2009) [2] reported warfarin-resistant rats without vkorc1 mutation. Another possible mechanism for warfarin resistance may involve enhanced metabolism of the drug by cytochrome P450 (CYP or P450). In rats, warfarin is hydroxylated to 4'-, 6-, 7-, 8-, and 10-OH warfarin, which is mainly catalyzed by CYP1A, 2B, 2C, and 3A subfamilies in rats.

For screening of warfarin resistance factors in roof rats, the pharmacokinetics of warfarin in resistant rats should be assessed.

The present study was performed to investigate the pharmacokinetics/ pharmacodynamics of warfarin in Japanese wild warfarin-resistant roof rats (Rattus rattus) using these closed colonies to determine their mechanism of warfarin resistance.

2. Materials and Methods

Warfarin-susceptible (S) and warfarin-resistant (R) roof rats having a mutation in vkorc1 (Leu76Pro) were originally caught in the wild, and maintained as closed colonies in the laboratory of Ikari Corporation (Tokyo, Japan). S-rats were from Ogasawara Islands, Japan, while R-rats were from Shinjuku, one of urbanized areas of Tokyo. All rats used in this study were male (mean age; 208 days).

Before administration, the cervical cutis was incised to visualize the jugular vein. Then, 10 mg kg^{-1} of warfarin dissolved in distilled water was administered orally (n = 4) or i.v. (n = 4). Blood were taken from the jugular vein at 5 minutes, and 1, 2, 4, 6, 10, 26, 33, and 51 hours after administration. Administration and blood collection were performed under isoflurane anesthesia. After sampling rats were euthanized and tissues were collected.

Aliquots of sample were added to centrifuge tubes with 0.1 M sodium acetate, 1 μM glucuronidated oxazepam (100 μl, as an internal standard (IS) of warfarin), 1 μM phenyl-d5-7-hydroxywarfarin (100 μl, as an IS of hydroxy warfarin), and 5000 unit β-glucronidase (100 μl).

The mixtures were incubated overnight at 37°C. After incubation, diethyl ether (5ml × 2 times) was added to the tubes and vortexed, followed by centrifugation at 3000 × g for 10 minutes. The

organic layer was obtained and evaporated under N2 gas. The residue was redissolved in MeOH (1ml). The solution was filtered with 0.2 μm Syringe Filters (GL Science, Tokyo, Japan).

Samples were analyzed by high-performance liquid chromatography - mass spectrometry (Shimadzu, Kyoto, Japan) using a C18 column (Symmetry Shield, Waters, Milford, MA). Pharmacokinetic analyse was performed with Phoenix WinNonLin (Certara, Princeton, NJ)

3. Results and Discussion

Fig.1 shows warfarin plasma concentration–time profiles after p.o. or i.v. administration. The area under the curve (AUC) of R rats of both p.o and i.v. administration was significantly lower than that of S rats, and the clearance of R rats of both administration, which was calculated by dividing AUC by the dose was significantly higher than that of S rats. In contrast, Both S and R rats had bioavailability of 70%. The concentrations of warfarin in the liver and kidney of S rats (1378.4 ± 509.6 ng mg^{-1} in the liver and 487.5 ± 300.9 ng mg^{-1} in the kidney) were significantly higher than those of R rats (61.3 ± 16.2 ng mg^{-1} in the liver and 29.3 ± 28.2 ng mg^{-1} in the kidney). Although there was no difference in BA of warfarin, R rats seemed to have the ability to metabolize warfarin rapidly.

Fig. 2 also shows the result of 4'-OH warfarin. The Cmax of i.v. administration of R rats was significantly higher than that of S rats. And the Tmax of i.v. administration of R rats was significantly earlier than that of S rats. Tmax of 4'-, 6- and 7-OH warfarin of R rats was also significantly earlier than that of S rats (data not shown). Thus, the ability of CYPs, which catalyze warfarin hydroxylation, is higher in R rats than S rats.

Immunoblotting analysis revealed that the relatively high expression levels of CYP2B, 2C and 3A in R rats after warfarin administration (data not shown). These results suggest that the high warfarin metabolic activity of R rats seems to be due to the high expression level of these CYPs.

These CYPs are induced through several nuclear receptors, i.e., constitutively active receptor (CAR) and pregnane X receptor (PXR) If warfarin also can activate these nuclear receptors in R rats, it is likely that warfarin-resistant rats from Tokyo used in these experiments would have high induction of CYPs when exposed to rodenticides.

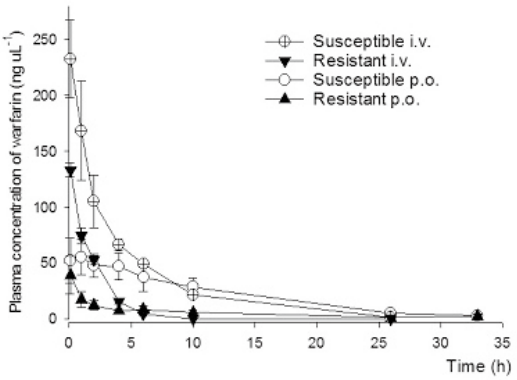

Fig.1: Time course of changes in plasma warfarin concentration after p.o. or i.v. administration.

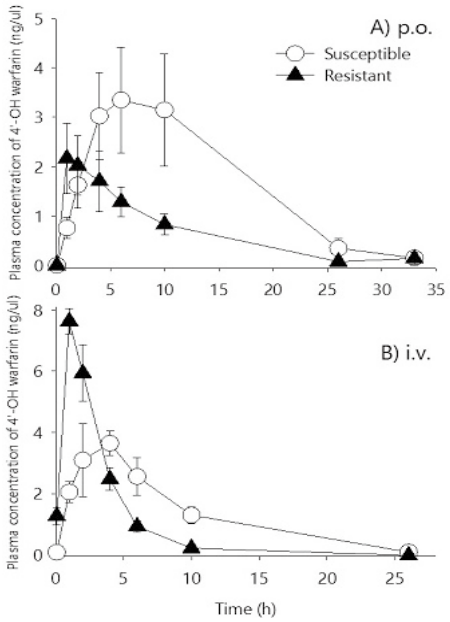

Fig.2: Time course of changes in plasma 4'-OH warfarin concentration after p.o. or i.v. administration.

4. Acknowledgments

We would like to acknowledge the financial support by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan awarded to M. Ishizuka (No. 24405004 and No. 24248056) and Y. Ikenaka (No. 26304043, 15H0282505, 15K1221305), and the foundation of JSPS Core to Core Program (AA Science Platforms) and Bilateral Joint Research Project (PG36150002 and PG36150003).

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P-31

DI-2-ETHYLHEXYL PHTHALATE (DEHP) INDUCE HISTOLOGICAL ALTERATIONS IN TESTES OF *CLARIAS GARIEPINUS*

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Di-(2-ethylhexyl) phthalate (DEHP) is among the commonly reported phthalate esters, ubiquitous in environmental matrices with the ability to sequestrate into environmental samples.

It is known to exert toxic effect on reproductive systems of organism as an estrogenic chemical. Thus this present study aimed at investigating histological changes of male *clarias gariepinus* gonads exposed to DEHP for a long term period of 112 days. Nominal concentrations were (1.0mg/$L^{-1,}$ 10.0mg/L^{-1}, 20.0mg/L^{-1}, 40.0mg/L^{-1}) under a static renewal condition in twelve glass tanks. Histological examination using light microscopy revealed severe effects on the fish testes such as complete absences of germ cells, degenerating or dead spermatids and wide spread calcification in the seminiferous tubules. In control fish, active spermatogenesis was observed with seminiferous lobules containing spermatogenic cyst and numerous spermatoids. The deleterious consequences in this study were observed to be based on dose dependents of DEHP exposure. Gonadosomatic index (GSI) was significantly decreased ($p < 0.05$) in males exposed to DEHP than unexposed fish. This study confirms that DEHP is a testicular toxicant on developing male reproductive system and its negative impact can affect fish survival recruitment and species diversity.

Keywords: DEHP, fish, spermatogenesis, gonads, estrogenic chemical

1. Introduction

There has been a growing concern about the likely impacts of exposure to chemical compounds with endocrine disrupting activity in the environment (Game *et al*. 2006). Many of these compounds such as polychlorinated biphenyl (PCB), alkylphenols, organochlorine pesticides, dioxins and phthalates elicit estrogenic responses in organisms (Olujumi *et al*. 2010). Thus a cause for concern as many phthalate esters have been found at measurable concentration in surface water, sediment and even drinking water.

Di-(2-ethylhexyl) phthalate (DEHP) is one of the most widely occurring phthalate in the environment and commonly used plasticizer for numerous building materials and consumer products (ATSDR 2002). Considering the increasing incidences of male reproductive abnormalities as a result of environmental pollution by man-made chemicals that have oestrogenic effects (Colborn *et a*l. 1993). This present study aimed at investigating histopathological changes of male *clarias gariepinus* exposed to DEHP at concentrations similar to those reported in the environment.

2. Materials and Methods

2.1 Experimental protocol

Thirteen week old juvenile African mud catfish (*Clarias gariepinus)* with mean body mass (13 ± 0.04g) were classified into 2 groups: a control positive (with ethanol) and negative (de-chlorinated tap water). Four other groups with nominal concentration (1.0mg/$L^{-1,}$ 10.0mg/L^{-1}, 20.0mg/L^{-1}, 40.0mg/L^{-1}) were used for long term exposure of 112 days (Zanotelli *et al*. 2010).

2.2 Histopathological preparations

Two male fishes of each group were sacrificed, quickly excised, weighted and their sizes were expressed as a percentage of the total body mass (gonadosomatic index, GSI). The gonads (testes) were immediately fixed in Bouins fluid. After six hours the Bouins fluid was decanted and the organs preserved in 10% phosphate buffered formalin. Fixed tissues were processed routinely for paraffin embedding technique (Drury and wallington 1973). Embedded tissues were sectioned at 5µm thickness. Standard histopathological procedures according to (Roberts 1989) were followed for investigation.

3. Results and Discussion

The GSI decreased significantly ($p < 0.05$) in fishes treated with DEHP in comparison with control fishes. The decrease was significant with increase DEHP doses. This observation supports previous work concerning oestrogen treatment in male fishes (Chang *et al.* 1995; Christiansen *et al.*1998).

The main histological examinations of the testes of exposed fishes were complete absences of germ cells especially in the highest exposure groups. Fig (1b-d) summarized the severe effects after exposure to DEHP concentrations; which generated either dead or degenerating spermatids and in some cases wide spread calcification in the seminiferous tubules.

Although, endocrine mode of action is still not well understood for phthalates (Adeogun *et al*. 2015). The abnormalities in the testes in this study may possibly be as a result of disruption process of steroidgenesis or changes of sex cells, blocking the spermatogenesis, which may in turn cause a decline on the quality of sperm cells and this could lead to sterility.

In conclusion the exposure of DEHP to *Clarias gariepinus* showed high doses have severe effects on testes growth and testicular structure. Therefore, it's key to note that DEHP is a testicular toxicant and these alterations in the reproductive organs can have a negative impact on fish survival recruitment to the next size class.

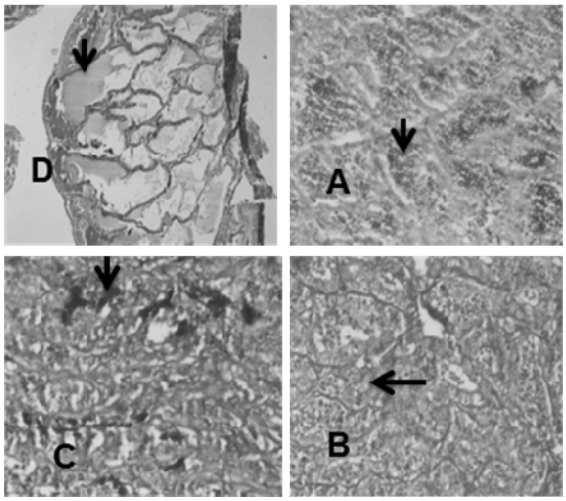

Fig 1: Light micrographs of male testes tissue in *Clarias gariepinus:* (A) normal testes from control (X400); (B) Dead spermatids (x400); (C) degenerating spermatid (x400); cyst formation& empty seminiferous tubule (x400).

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P-32

Methanol extract of *Nymphaea lotus* (Linn) ameliorated carbon tetrachloride induced hepatotoxicity

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This study was undertaken to investigate the anti-genotoxic and hepatoprotective effect of the methanol extract of *Nymphaea lotus* (NL) in mice and rat exposed to methyl methanesulfonate (MMS) and carbon tetrachloride (CCl_4), respectively. Frequency of MMS-induced micronucleated polychromatic erythrocytes was significantly ($p< 0.05$) reduced in mice exposed to MMS and NL. NL significantly ($p< 0.05$) lowered the levels of alanine aminotransaminase (ALT) and aspartase aminotransferase (AST) in rats exposed to CCl_4 relative to the control values. NL significantly ($p< 0.05$) increased CCl_4-induced decrease in hepatic reduced glutathione and also decreased the level of hepatic thiobarbituric acid reactive substances (TBARS) in CCl_4-intoxicated rats. Histopathological findings revealed cellular infiltration and fibrosis in rats that received CCl_4-only. These alterations were ameliorated in rats that received NL+CCl_4. The data suggest that NL exhibited anti-genotoxic and hepatoprotective effects via antioxidative mechanism.

Keywords: Antioxidant, Micronucleus, Hepatotoxicity, Nymphaea lotus

1. Introduction

Nymphaea lotus (L) is an aquatic herbaceous plant localized to Central and Southern Europe, Asia, the Middle East, Northern Africa, tropical mountains in Africa and West Africa especially in Nigeria. It has several folkloric uses and is used traditionally for the management of cancer and liver diseases. Its antibacterial, antidiabetic, antioxidant, and cytotoxic activities have been reported. However, to the best of our knowledge, there is no information on the ability of this plant to ameliorate genotoxic and/or hepatotoxic effect induced by established chemical toxicants.

MMS is a mono-functional alkylating agent, with the capacity to generate methylating and ethylating species that interact with macromolecules, such as DNA. CCl_4, a hepatotoxin, induces oxidative stress and massive inflammatory cell infiltration in rat's liver which mimics symptoms of various human liver dysfunctions (Lee et al. 2005).

This study was therefore designed to evaluate possible antigenotoxic and hepatoprotective effect of the methanol extract of *N. lotus* against MMS-induced genotoxicity and CCl_4-induced chronic liver injury in mice and rats, respectively.

2. Materials and Methods

2.1 Collection and extraction of plants

Whole plants of *N. lotus* were collected from Eleyele River, Ibadan, Nigeria. These were identified and authenticated, with voucher specimen (UIH-22349) deposited at the University of Ibadan Herbarium. The plant was washed, dried in shade, ground to coarse material and serially extracted in petroleum ether and methanol using simple maceration method. Each resultant mixture was filtered and the solvent was evaporated using rotary evaporator at 40°C. The methanol extract (NL) was used for this study.

2.2 Animal care and use

Young mice (6 weeks old) and male Wistar rats (8 weeks old) were obtained from the animal breeding unit of the Department of Anatomy, University of Ibadan, Nigeria. Mice for antigenotoxic study were orally exposed to NL for 10 days and a single dose of MMS ip. Rats for hepatoprotective were co-exposed to CCl4 + NL for 6 weeks. All experiments were carried out in accordance with guidelines for the care and use of laboratory animals, and the University of Ibadan animal care and use research ethics committee approved the study (UI-ACUREC/App/2015/019).

The frequencies of MN were scored (Alabi and Bakare, 2011) as biomarkers of genotoxicity and biochemical assays for hepatoprotective study

2.3 Biochemical assays

Rats were sacrificed and blood and liver were collected. Serum was used for analysis of liver marker enzymes, such as ALT and AST, using Randox diagnostic kits according to the manufacturers' protocol.

Liver was homogenized in phosphate buffer and the homogenates centrifuged at 10,000 rpm for 10 minutes at 4°C to produce a clear supernatant for assay of antioxidants and lipid peroxidation. SOD activity was determined according to methods of Misra and Fridovich, (1989). Catalase was determined by the method of Claiborne, (1985),

GSH according to Beutler et al. (1963) while lipid peroxidation (TBARS) was determined according to Rice-Evans *et al*. (1986).

2.4 Histopathological analysis

Small piece of each liver was used for Histopathologic analysis.

2.5 Statistical analysis

Data were analysed using one-way ANOVA followed by the post-hoc Duncan multiple range test for analysis of biochemical data using SPSS (20.0).

3. Results and Discussion

Co-exposure of NL and MMS led to a significant reduction in MMS-induced micronucleus (Fig 1). This implies antigenotoxic effect of NL. NL reduced the activities of markers of hepatotoxicity (ALT and AST) and lipid peroxidation (Fig 2), and increased the activities of antioxidant enzymes [(SOD, CAT, GSH and Gpx) Table 1] in rats exposed to CCl_4+NL. NL also ameliorated CCl_4-induced hepatic lesions (Fig 3) which corroborated the findings in the biochemical assays. This shows that NL ameliorated CCl_4-induced hepatic injury/toxicity. Thus NL exhibited antigenotoxic and hepatoprotective effect *in vivo*. The underlying mechanisms for this antigenotoxic and hepatoprotective effect may be via inhibition of oxidative stress.

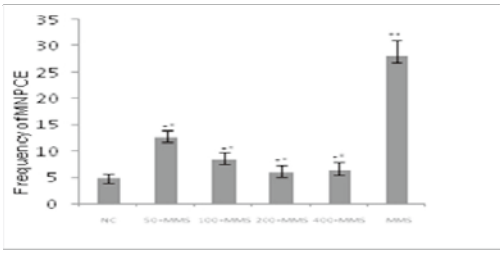

Fig 1: Frequency of MNPCE in mice exposed to NL.

Table 1: Effect of NL on hepatic antioxidant enzymes activities in rats exposed to CCl_4.

Treatment (mg/kg)	SOD	CAT	GSH
Corn oil	84.62 ± 17.95	7.15 ± 0.24	5.92 ± 0.96
CCl_4	90.60 ± 7.45	11.00 ± 0.88**	4.32 ± 0.41*
200	66.67 ± 10.88	11.67 ± 1.09**	11.95 ± 1.68**†
CCl4 + 50	88.46 ± 23.16	12.23 ± 0.91**	4.30 ± 0.74*
CCl4 + 100	67.69 ± 6.36	13.00 ± 1.31**	8.31 ± 1.18**†
CCl4 + 200	63.25 ± 12.00	8.55 ± 1.56**†	7.73 ± 0.48**†

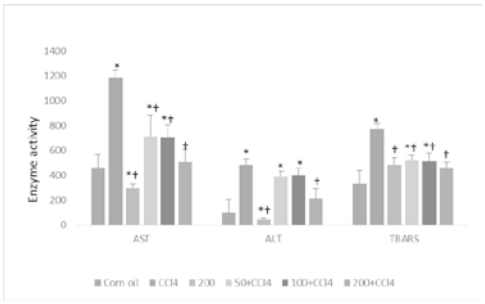

Fig 2: Effect of NL on hepatic markers of toxicity and lipid peroxidation in rats exposed to CCl_4.

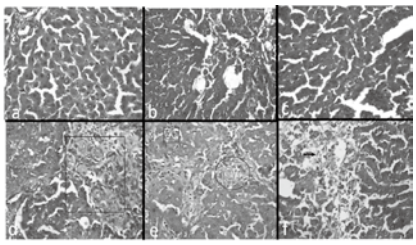

Fig 3: Effect of NL on hepatic morphology and architecture in rats exposed to CCl_4. X400

4. Acknowledgments

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P-33

Biomarker responses in *Macrobrachium petersii* (Hilgendorf, 1878) from two sub-tropical river sections

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The Ndumo Game Reserve protects a portion of the ecologically important and sensitive Phongolo River floodplain. An accredited Ramsar site, the reserve seeks to protect the floodplain and mitigate possible impacts to ecosystem functioning. However, aquatic systems are dynamic, and pollutants from upstream of the reserve enter the protected area and can have devastating effects on the aquatic fauna and flora that reside within its borders. To determine the potential impact, monitoring of water quality is essential. Passive biomonitoring techniques, with the use of *Macrobrachium petersii* as a bioindicator species, were used to determine water quality along the two main rivers that flow within the Ndumo Game Reserve namely the Phongolo and Usuthu Rivers. Biomarker analyses were used to determine any pollutant-induced biochemical alterations that may be occurring within the organisms' tissues. Multivariate statistical analysis (a discriminant function analysis) was then applied to the resulting biomarker data to determine the influence of several variables on the separation of the data sets. Limited correlations are seen between the multivariate analyses and site separation with the exception of a strong CYP response that correlates to a higher degree of oxidative damage in organisms from the UR during the LF survey.

Keywords: Phongolo River, Usuthu River, Ndumo Game Reserve, Multivariate analysis, South Africa

1. Introduction

The lower Phongolo River floodplain, in the KwaZulu-Natal province, is one of the most extensive floodplain systems in South Africa and extends into Mozambique after the confluence with the Usuthu River. This region is ecologically sensitive with high biodiversity, though it is under threat from anthropogenic activities. A portion of the floodplain is protected within the boundaries of the Ndumo Game Reserve, an internationally accredited Ramsar site (Dube et al., 2015). The reserve is located in the north of the province and borders Mozambique. Part of the border is demarcated by the Usuthu River, a transboundary river that flows from Swaziland. The Usuthu is Swaziland's largest river system and is heavily utilised mainly for agriculture, transportation of wastes as well as domestic activities (Kowalkowski et al., 2007).

The Phongolo River flows through the fertile region of the Makhathini flats. Impacts along the course of the river are mainly related to commercial and subsistence agriculture, the latter of which takes place within the floodplain along the banks of the main river course. These practises possibly have a negative impact on water quality along the two river systems. Monitoring water quality along these rivers is important so as to understand the potential impacts to these sensitive systems.

Biomarker responses were analysed in *Macrobrachium petersii* (South-East coast river prawn) tissue samples to elucidate water quality in the two rivers. Biomarker responses have been used extensively in the ecotoxicology field, however there is a limited information available on the responses in freshwater crustaceans, especially in South Africa. This study thus aims to identify spatial and temporal differences in biomarker responses in *M. petersii* from the Usuthu and Phongolo River systems.

2. Materials and Methods

Electroshocking techniques were used to capture organisms which were then sacrificed and dissected in the field. Hepatopancreas and muscle tissues were excised, stored in Hendricksons buffer and flash frozen in liquid nitrogen. Biomarkers of exposure (Acetylcholinesterase activity (AChE), Metallothioneins (MT) and Cytochrome P450 (CYP)) as well as biomarkers of effect (Reduced glutathione (GSH), Super oxide dismutase (SOD), Catalase (CAT), Malondialdehyde content (MDA), Protein carbonyls (PC) and Cellular energy allocation (CEA)) were used in the analysis. All biomarker analyses followed standard protocols used in the ecotoxicology laboratory at the University of Johannesburg. A Discriminant Function Analysis (DFA) was applied to the data to determine the influence of multiple independent variable (biomarker responses) on the discrimination of dependent variables (sample sets) from one

another.

3. Results and Discussion

A DFA of biomarkers of exposure, biomarkers of effect and cellular energy allocation and its components is illustrated in Figure 1A, 1B and 1C respectively. The structure matrix table (Table 1) indicates which of the included biomarkers was the strongest driver and on which function. Temporal and spatial distinctions can be seen across the DFAs. There are limited correlations between site/ sampling period across the plots. Samples from the UR during the LF survey were most notable in exposure and effect biomarker DFAs.

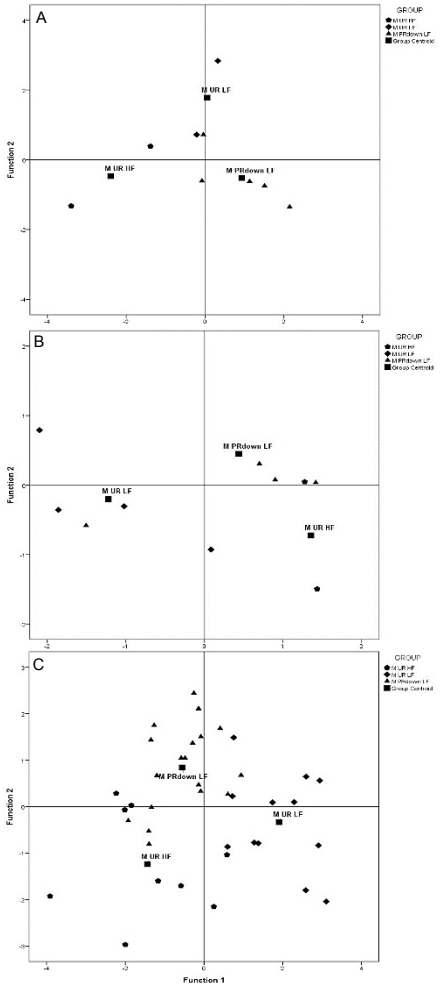

Figure 1: Canonical variates derived from a discriminant function analysis of biomarkers of exposure (A), effect (B) and cellular energy allocation and its components (C).

Organisms from this site are being discriminated for their increase in CYP activity and resulting PC content. Cytochrome P450 system is involved in metabolism of endogenous and exogenous toxicants such as PAHs and PCBs. Some of these toxicants can increase the production of reactive oxygen species that can damage macromolecules such as proteins and form PCs (Almroth et al., 2005).

Table 1: Structure matrix indicating the factor loadings from each corresponding discriminant function analysis (DFA. The variable with the greatest discriminating power towards group classification is indicated as a bold value.

Structure Matrix

		Function	
		1	**2**
Biomarkers of exposure	**MT**	**0.927**	0.225
	ACHE	0.447	0.153
	CYP	0.240	**0.837**
Biomarkers of effect	**PC**	**-0.876**	-0.091
	SOD	-0.344	0.264
	MDA	-0.078	**-0.814**
	GSH	0.471	0.510
	CAT	-0.080	-0.334
Cellular energy allocation	**Lipids**	**0.715**	-0.528
	Energy allocation	0.707	0.127
	CEA	0.663	0.077
	Protein	0.504	0.262
	Energy consumption	0.496	**0.547**
	Glucose	0.097	0.285

4. Acknowledgements

Collaborators and colleagues from the VLIR-UOS collaboration who made this project possible. Thanks to the National Research Foundation (NRF) who made this project possible through funding that was awarded to G. Jansen van Rensburg (Grant No. 89629). Opinions expressed in this study do not necessarily represent those of the NRF

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P-34

Hypoglycemic and High Dosage Effects of *Bidens pilosa* in Type-1 Diabetes Mellitus

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The study aimed to determine the anti-diabetic and high dosage effects of *Bidens pliosa* in type 1 DM (T1DM). Thirty rats were divided into six groups and subgrouped into the extract and non extract treatment groups. The extract treated group was subdivided into three groups which received 200 mg/kg, 400 mg/kg and 800 mg/kg dosage treatments respectively. The blood glucose levels were monitored using a standard glucometer for one month, and biochemical analysis of the two liver function enzymes; Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) were carried out at the Institute of Biomedical Research (IBR-KIU-WC) at the end of week IV. The study revealed that *Bidens pilosa* maintained hypoglycemia for a period of two weeks and this statuswas ly lost in subsequent weeks. T1DM rats treated with a dosage of 200 mg/kg showed a better recovery (355.25-164.5 mg/dl) of the glucose levels, followed by those that were being treated at 400 mg/kg. The mean of the AST and ALT enzymes varied with a mean ± SEM (33.72 ± 32.32 to -7.23± 12.61 IU and 22.98 ± 11.12 to 42 ± 38.2 IU, respectively) in blood in both the glibenclamide® and in the 800 mg/kg treatment groups in the study. High dosages of extract were associated (P = 0.049) with increased systemic enzyme leakage. In conclusion, tissue degeneration caused by high levels of the extract was accompanied by leakage of various enzymes (AST and ALT) into the blood, which could be a major etiological factor for the development of secondary systemic pathologies thus potentially worsening the effects of an existing T1DM prognosis in human patients. The preliminary results indicate that a dose of *Bidens pilosa* has an anti-diabetic effect for a limited initial duration before starting to cause systemic toxicological effects.

Keywords: *"Type 1 Diabetes Mellitus" "Bidens pilosa and Diabetes," "Ethno-pharmaceutical medicine in Uganda," "Sub-Saharan Africa and Diabetes"*

1. Introduction

Global prevalence of diabetes mellitus (DM) is estimated to be over 10% especially with increasing disease incidence from Sub-Saharan Africa coupled with its low case reporting (Shaw et al., 2010). The prevalence of DM in association with other metabolic diseases has been reported in Uganda (Baalwa et al., 2010). Analyses of selected enzyme activities in blood serum gives valuable diagnostic information for a number of metabolic disease conditions. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are important in the diagnosis of organ (esp. of heart and liver) damage caused by heart attack, drug toxicity, or infection due to the leakage of a variety of enzymes, including these aminotransferases, leak from the injured heart cells into the bloodstream (Ugarte 2012).

2. Materials and Methods

2.1 Plant Collection and study groups

The leaves were processed using standard protocols (Yang, W. 2014a). Experimental animals were divided into six groups each consisting of a minimum of five adult male rats (n = 5) aged 4 months. Grouping was made according to concentration of extract for treatment. These included: 200 mg, 400 mg, 800 mg extract groups as well as controls which included normal saline group, Glibenclamide® (0.5 mg/kg) group and the sixth group consisting of normal non diabetic rats for a period of 4 weeks.

2.2 Diabetes Induction

Diabetes was induced by injecting 200 mg/kg of alloxan monohydrate intraperitoneally to overnight-fasted rats (the LD_{50} was determined by an initial induction of the condition until successful results were obtained). 10% glucose solution bottles

were kept in the cages for the next 24h to prevent hypoglycemia. After 72h of injection, fasting blood glucose (FBG) levels were measured. Animals which develop more than 200 mg/dl glucose levels were used for the study.

2.3 Data Analysis

Data was expressed as means from the samples analyzed. The means of fasting blood glucose (FBG) levels for the test and control groups were compared at different times by one-way Analysis of Variance (One-Way ANOVA) using SPSS 16 software. Linear regression was carried out to determine treatment associations and a p value < 0.05 was considered statistically significant.

3. Results and Discussion

Bidens pilosa maintained hypoglycemia for a period of two weeks, and this status was lost in the subsequent period as shown in **Table 1**. The initial rise in FBG seems to offset the early anti-hyperglycemic effect of the crude extract which is in agreement with previous studies (Lans, C. A. 2006). There was no statistical significance (P = 0.637) in the hypoglycemic effects towards the end of the study due to the loss of the efficacy of the extract probably as a result of onset of systemic injury, thus cancelling the positive effects observed earlier. This would probably be due to the accumulation of reactive oxygen species due to the oxidative properties of alloxan or the extract. This was followed by an increase in the liver enzymes AST and ALT and subsequent death as described in **Table 2** and **Table 3**. High dosages of the extract were shown to be associated (P = 0.049) with increased systemic damage.

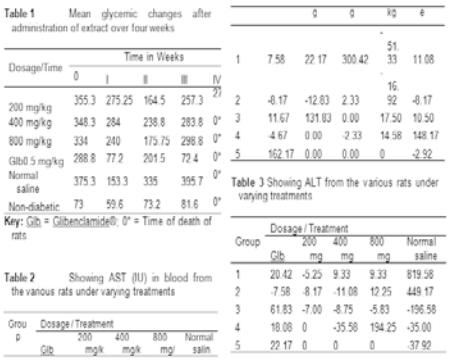

Table 1 Mean glycemic changes after administration of extract over four weeks

Dosage/Time	Time in Weeks				
	0	I	II	III	IV
200 mg/kg	355.3	275.25	164.5	257.3	27
400 mg/kg	348.3	284	238.8	283.8	0*
800 mg/kg	334	240	175.75	298.8	0*
Glib0.5 mg/kg	288.8	77.2	201.5	72.4	0*
Normal saline	375.3	153.3	335	395.7	0*
Non-diabetic	73	59.6	73.2	81.6	0*

Key: Glb = Glibenclamide®; 0* = Time of death of rats

Table 2 Showing AST (IU) in blood from the various rats under varying treatments

Group	Dosage / Treatment				
	Glb	200 mg/k	400 mg/k	800 mg/	Normal salin
1	7.58	22.17	300.42	51.33	11.08
2	-8.17	-12.83	2.33	16.92	-8.17
3	11.67	131.83	0.00	17.50	10.50
4	-4.67	0.00	-2.33	14.58	148.17
5	162.17	0.00	0.00	0	-2.92

Table 3 Showing ALT from the various rats under varying treatments

Group	Dosage / Treatment				
	Glb	200 mg	400 mg	800 mg	Normal saline
1	20.42	-5.25	9.33	9.33	819.58
2	-7.58	-8.17	-11.08	12.25	449.17
3	61.83	-7.00	-8.75	-5.83	-196.58
4	18.08	0	-35.58	194.25	-35.00
5	22.17	0	0	0	37.92

Figure 1: Showing glucose, ALT and AST levels in blood over a period of four weeks in wister rats.

4. Acknowledgments

No financial assistance was offered for this work.

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P-35

Public exposure to naturally occurring radioactivity in gold mining communities in Ghana

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Public exposure to ^{226}Ra, ^{232}Th and ^{40}K radiations in sediments, water and fish in mining communities in the Ankrobra Basin was assessed. The average activity concentrations of ^{226}Ra, 232Th and 40K in the sediment were found to be 48.6, 96.7, and 1439.7 Bqkg^{-1}, respectively. For the water samples, the average activity concentrations of ^{226}Ra, ^{232}Th and ^{40}K were 1.4, 1.2, and 24.9, BqL^{-1}, respectively. The average activity concentrations of ^{226}Ra, 232Th and 40K for fish were 2.8, 4.9, and 37.3 BqL^{-1} respectively. The average annual effective doses estimated from direct external gamma exposure from natural radioactivity concentrations in sediment, water and fish were 0.17 0.11 and 0.03 mSvy-1, respectively with a total effective dose of 0.31 mSvy^{-1} which is below the International Commission on Radiological Protection recommended level of 1 mSvy-1 for public exposure control. The average radium equivalent activity in the samples was 290.1 Bqkg^{-1}. The mean external and internal indices were 0.78 and 0.93 respectively. The average values of the radium equivalent activity, external and internal hazard indices for sediments were below the recommended acceptable limit. However continuous monitoring of the study area is imperative as about 30% of water samples used for domestic purposes was found to potentially pose exposure risks.

Keywords: Radioactivity dose, radiological protection, radium equivalent activity

1. Introduction

Environmental radioactivity results mainly from naturally-occurring primordial radionuclides, such as 40K and decay products from 238U, 235U and 232Th series. Most of the radioactivity associated with uranium in nature is due to its gaseous progeny, 222Ra, which escapes the soil matrix when disturbed (Pakade et al., 2012). Mining has been identified as one of the potential sources of exposure to naturally occurring radioactive materials. ^{226}Ra is a major contaminant in mine tailings and milling wastes because it adheres well to soil particles and sips into ground water due to its ability to form soluble sulphates, chlorides and carbonates. Measurement of natural radioactivity in the environment is, therefore, important to determine the amount of change of the background activity with time as a result of any radioanuclide release (Darko et al., 2014).

There are more than 200 mining companies in Ghana operating from small, medium to large scale. However, there is only a limited data on the levels of radioactivity in the environment and exposure to the public (Darko et al., 2010; Faanu et al., 2010). However, there is generally low level of knowledge and awareness of the radiological hazards among the populace. The levels and distribution of radiation in the environment must be known in order to safeguard the populace from the hazards.

In this work, we measured the activity concentrations of ^{226}Ra, ^{232}Th and 40K in surface water, fish (tilapia) and sediment in the mining communities in the Ankobra basin to assess the radiological risks in the environment. The results from this study will help build a databank for the Radiation Protection Board, as part of a national programme to establish data on environmental radioactivity. It will also help the regulatory authorities make informed decisions in formulating policies on environmental radioactivity in mining communities.

2. Materials and Methods

2.1 Description of the Study Area

The Ankobra Basin (latitudes 4° 52'N and 6° 27'N; longitudes 1° 42'W and 2° 33'W) is in the south-western part of Ghana. The basin covers an area of 8,403 km^2 spanning 11 districts and is well-endowed with many natural resources including gold.

2.2 Sampling

Sampling was done along the River Ankobra Basin in four towns (Dumasi, Besekyem, Mansi Aboi and Prestea). Sampling in Dumasi was done about 100 m downstream from where Apepra stream joins the Ankobra River. Five batches of samples were picked from 4 points each at Dumasi, Besekyem, Mansi Aboi and Prestea townships. In all, five batches of 20 samples each of fish surface water and water bed sediments were collected between August 2013 and March 2014.

2.3 Sample Preparation

The water samples were collected into clean

1L plastic bottles and acidified on-site with 1 M HNO_3 to preserve. The samples were not filtered prior to preparation and measurements. Sediment samples were air dried for 5 days and homogenized after removing extraneous materials from them. They were then oven-dried at 105 ^{o}C to a constant weight. They were ground into fine powder using ball mill grinder and sieved through a 2 mm mesh. The samples were transferred into a Marinelli beaker up to the recommended level and weighed to determine the mass and then sealed and stored in the laboratory for 30 days to allow for equilibration between long-lived parent radionuclides and their short-lived daughter radionuclides. The frozen fish samples were partially thawed and cleaned on plastic sheets using scalpels with steel blades and plastic forceps. Whole taxa of a designated length range were cut into small pieces with teflon-tipped scissors. The samples were freeze-dried at 20 ^{o}C for 72 h and then homogenized into fine powder in an acid-free Biospec blender (Fisher Scientific, Pittsburgh, PA).

2.4 Gamma spectrometry system

Measurements were done on a gamma spectrometry system (Saint-Gobain Crystals, USA). The gamma-spectrometry system consists of high efficiency scintillation detector which is made up sodium iodide crystals doped with thallium, NaI(TI) and is coupled to ORTEC multichannel buffers for data acquisition and processing using a MAESTRO®-32 software.

2.5 Calibration of gamma spectrometer

The energy and efficiency calibrations of the detector were performed using mixed radionuclide certified reference standard (DKD-3, QSA Global GmBH, Germany). Background counts were taken for the same period and corrections carried out where appropriate. The samples were counted for 36000 seconds using the same geometry as the standard. Energy calibration was performed by matching the energies of the principal ɣ-rays in the spectrum of the standard reference material to the channel number of the spectrometer.

2.6 Efficiency calibration

The efficiency calibration was performed by acquiring a spectrum of the standard until the count rate at the peak of total absorption can be calculated with statistical uncertainty of less than 1% at a confidence level of 95%. A plot of the efficiency of each radionuclide and its corresponding energy was made using power series function and extrapolated to determine the efficiencies at other peak energies to determine the radionuclides of interest (Darko et al., 2014) .

2.7 Calculation of activity concentrations

Specific activities (A_t) of the radionuclides were computed from their efficiencies using the analytical expression:

$$\text{Specific activity}(A_t) = \frac{N}{P\varepsilon t_c M} \qquad (2)$$

where N is the net count, At is the specific activity at any time t, P is the gamma-ray yield or gamma emission probability, ε is the efficiency, tc is the counting time, M is the mass (kg) or volume (L) of the sample (Oresengun et al., 2010).

2.8 Committed Annual Effective Dose

Committed annual effective dose (Sv/yr) from ingestion of radionuclides in water was calculated on the basis of the mean activity concentrations of the radionuclides. The average daily water consumption rate was taken to be 2 litres per day. The committed annual effective dose owing to ingestion of ^{226}Ra, ^{232}Th, ^{40}K and ^{137}Cs in water was then estimated using the relation shown in equation 3.

$$(E_T) = \sum (A_t * F_w) I_w \qquad (3)$$

Where, Iw is the water consumption rate which is 730 L/yr, At is the activity concentration of radionuclide in the water (Bq/L), and Fw is the dose conversion factor of water (Sv/Bq).

3. Results and Discussions

Specific Activity

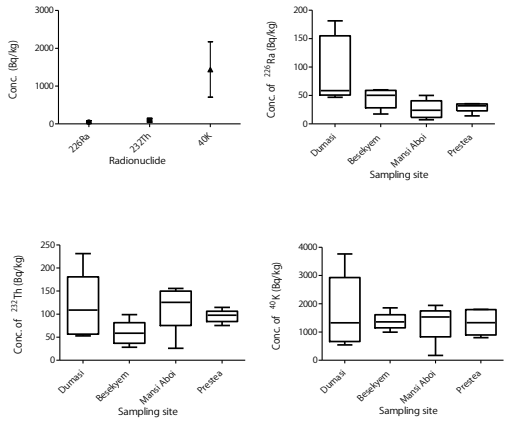

Figure 1: Activity concentrations of ^{226}Ra, ^{232}Th and ^{40}K in the sediment samples

Figure 1 shows the activity concentrations of ^{226}Ra, ^{232}Th and ^{40}K in the sediment samples. The average value of the activity concentrations of ^{226}Ra is 48.6±40.5 Bqkg^{-1}. For ^{232}Th, the average activity concentration is 96.8±48.9 Bqkg^{-1} and that of 40K is 1439.7±733.9 Bqkg^{-1}. The worldwide average activity concentration of ^{226}Ra, ^{232}Th and ^{40}K in soil samples from similar studies carried out around the world are 33, 45 and 420 Bqkg-1, respectively (UNSCEAR, 2000a, 2000b). By comparison, the activity concentrations of ^{226}Ra and ^{232}Th in this study are by about twice higher than

the world average, whilst that of 40K is about four times higher than values in normal continental soils (UNSCEAR, 2000b). The high activity of 40K could be attributed to the feldspar type of ore from the gold mines. Feldspar belongs to a group of hard crystalline minerals that consist barium.

The mean activities of ^{226}Ra, ^{232}Th and ^{40}K were 1.4±1.7, 1.2±0.8 and 24.9±22.5 BqL^{-1} respectively. The average activity concentration of ^{226}Ra and ^{232}Th in water were 40% and 20% higher than World Health Organisation (WHO) guideline of 1.0 BqL^{-1} (IAEA, 2009). This high activity may pose a risk to the members of the community who use the water for domestic purposes.

The mean specific activities of ^{226}Ra, ^{232}Th and ^{40}K were 2.8±2.2, 4.9±4.8 and 37.3±25.7 mBqkg-1 respectively. The recommended reference value of radionuclides in fish products are 100 mBq/kg and 10 mBq/kg for ^{226}Ra and ^{232}Th respectively (UNSCEAR, 2000a). Hence the activity of ^{226}Ra and ^{232}Th in the fish samples are lower than the recommended values and may not pose a risk to consumers.

3.1 Absorbed Dose and Effective Doses

The calculated absorbed dose rate in the sediments varied from 30.13 to 319.55 nGy/h with an average value of 137.56 nGy/h. The average absorbed dose rate is twice higher than the worldwide average value of 60 nGy/h (UNSCEAR, 2000a). This difference could be attributed to differences in geology and geochemical nature of the bedrocks at the sampling sites. The corresponding estimated annual effective dose was 0.17 mSvy^{-1}.

The committed annual effective doses due to ^{226}Ra, ^{232}Th and ^{40}K in water was found to be 0.11 ± 0.10 mSv. The average annual effective dose in water was within the WHO recommended guideline of 0.1 mSvy^{-1} (WHO, 2004). However, it should be noted that about 30% of water samples had committed annual effective doses exceeding the WHO guideline level of 0.1 mSvy^{-1}. The average committed annual effective doses due to ^{226}Ra, ^{232}Th and ^{40}K in fish was calculated to be 0.032 ± 0.025 mSv in a range of 0.0067–0.096 mSv which is lower than the recommended limit of 1 mSv.

3.2 Total annual effective dose

Dose limits and the total annual effective dose was estimated from all the potential exposure scenarios and compared with the recommended limits. The mean annual effective doses estimated from direct external gamma ray exposure from natural radioactivity in sediments, from drinking water, and ingestion of fish were 0.17, 0.11, and 0.03 mSv respectively. The corresponding total annual effective dose for all the exposure pathways was 0.31 mSv. The total annual effective dose of 0.31 mSv/year is also below the recommended dose limit of 1 mSv/y.

3.3 Sediment hazards assessment

In order to assess whether the sediment could be a source radiation exposure if used for building purposes, the radium equivalent activity (Raeq), external hazard (Hex) and the internal hazard (Hin) were computed. Radium equivalent activity is related to the external gamma dose from the terrestrial radionuclides and the internal dose due to radon and its decay products of ^{210}Pb and ^{210}Po. The maximum value of Raeq, in building materials must be less than 370 Bq/kg for the material to be considered safe for use. The external and internal hazard indices must also be less than unity in order to keep the radiation hazard insignificant. This implies that, the radiation exposure due to the radioactivity from these radionuclides in materials to be used for constructions must be limited to 1.5 mSv/y.

The mean radium equivalent activity was found to be 290.1±13.2 Bq/kg. The external and internal indices were 0.78±0.35 and 0.93±0.47 respectively. The mean Raeq, was within the acceptable limit of 370 Bq/kg, and the Hex and Hin were both within the acceptable limits of <1. This indicates that sediment from the study area that are used for building purposes for shelter may generally not pose a significant radiological hazard to human health, and thus the sediment is safe for use as a building material. It was, however, noted that about 25% of the sediment samples had the Raeq, Hex and Hin exceeding their recommended limit implying that these materials if used for building purposes could present radiological hazards.

4. Conclusion

This study considered public exposure to environmental radioactivities through external gamma ray exposure from natural radionuclides in sediments, internal exposure from water and consumption of fish containing 226Ra, 232Th and 40K. The average annual effective doses estimated from direct external gamma-ray exposure from natural radioactivity concentrations in sediment, water and fish were within the recommended limits.

Hazard indices including radium equivalent, external and internal hazard indices were determined in order to assess whether the sediment in the study area could be a source of public radiation exposure if used for building purposes. The external and internal hazard indices as well and the radium equivalent measured were all within their limits indicating the sediment material is radiologically safe as building material. The average absorbed dose rate in this study, however, is about two times higher than the worldwide average value. The total annual effective dose was also lower than the 1 mSv per year dose limit recommended. However, in view of the fact that some of the samples recorded higher values, it is recommended that a periodic (every 3 years) monitoring programme for environmental

radioactivity is established as the operations may alter the geochemical and radiological state of the mining communities.

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Can *Saccharomyces cerevisiae ameliorate immunotoxic and residual effects of* aflatoxin B_1 in *Oreochromis niloticus*?

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This study was undertaken to evaluate the ability of *Saccharomyces cerevisiae to ameliorate immunotoxic and residual effects of* aflatoxin B_1 in *Oreochromis niloticus*. Therefore, a total number of 120 healthy cultured monosex Nile tilapia *Oreochromis niloticus* were randomly allocated into 4 equal groups. Group 1 (Control): Fish were fed the basal diet only. Group 2 (*S. C*): Fish were fed a basal diet supplemented with 0.25% *Saccharomyces cerevisiae*. Group 3 (AFB_1): Fish were fed a diet intoxicated with 2.5 ppm aflatoxin B_1. Group 4 (AFB_1+*S. C*): Fish were fed a diet intoxicated with 2.5 ppm AFB_1 and supplemented with 0.25% *S. cerevisae*. Fish fed AFB_1 toxicated diet showed Leucopenia, lymphopenia and neutrophilia. As well as significant decrease in phagocytic % and index. Moreover, accumulation of aflatoxin residues in Nile tilapia flesh and liver which reach to 5 ppb in muscle and 15 ppb in liver. Supplementation of AFB1 intoxicated diet with 0.25% *Saccharomyces cerevisiae* succeed in mitigation of aforementioned effects of aflatoxin in Nile tilapia. It could be concluded that the *Saccharomyces cerevisiae* has the ability to counteract the adverse effects induced by AFB_1 in Nile tilapia (*Oreochromis niloticus*) and its use considered a prevention rather than a therapy of aflatoxicosis.

1. Introduction

Mycotoxins, unavoidable contaminants in foods and feed stuffs, are a major problem all over the world **(Santos et al., 2010).** Aflatoxins (AFs) are a group of structurally related mycotoxins produced as food-borne metabolites by toxigenic strains of *Aspergillus flavus, A. parasiticus* and *A. nominus* **(Verma, 2004 and Milita et al., 2010).**

Aflatoxins adversely affecting the fish industry by reducing growth rate, feed conversion, immune response and fish meat quality **(Royes and Yanong, 2008).** In the family of aflatoxins, Aflatoxin B_1 is the most prevalent and toxic for human, land animals and aquatic organisms, mostly by its strong carcinogenic, mutagenic and immunosuppressive effects **(Han et al., 2008).**

Despite good screening programs, selection of high quality raw materials and feed ingredients and good storage conditions it is very difficult to guarantee the absence of mycotoxins in aquaculture feeds **(Santos et al., 2010).** Therefore, supplementation of fish feeds with substances, which counteract the adverse effects of mycotoxins in fish, will be of great value.

Live yeast (*Saccharomyces cerevisiae*), initially used as a performance promoter in the early 1990, was found to have significant improvements against aflatoxicosis and has immunostimulant in poultry **(Stanley et al., 1993 and Yildirim and Parlat, 2003).** It able to degrade aflatoxin and other mycotoxins as T-2 toxin and zearalenone **(Freimund et al., 2003; Kusumaningtyas et al., 2006).**

Therefore, the current study was planned to assess the drastic effects of AFB_1 on immunity and its residues in Nile tilapia (*Oreochromis niloticus*) and attempting to ameliorate these effects by using *Saccharomyces cerevisiae.*

2. Materials and Methods

Aflatoxin B_1 ($C_{17}H_{12}O_6$), produced from toxigenic *Aspergillus flavus* using polished raw rice as a substrate for growth **(Shotwell et al., 1966)** with minor modifications by **Mehrim et al., (2006).** Its recommended dose for Nile tilapia is 2.5mg/kg diet by oral route of administration **(Tuan et al., 2002).**

Saccharomyces cerevisiae: Trade name, Diamond v original xp, it contains dried yeast (*Saccharomyces cerevisiae*) fermented product 100%, made in U.S.A. 0.25% *S. cerevisiae* in diet is orally administered **(Khalil et al., 2014).**

Experimental design

A total number of 120 healthy cultured monosex Nile tilapia *Oreochromis niloticus,* with average body weight (35±5 g), were obtained from Abbassa Fish hatchery, Sharkia Governorate, Egypt. were randomly allocated into 4 equal experimental groups (3 replicates per group), each replicate contains 10 fish kept in fully prepared and continuously aerated aquarium (80x40x30cm) containing dechlorinated tap water, experiment had lasted for 42 days. The experiment conducted at Pharmacology department, Faculty of Veterinary Medicine, Zagazig University, Egypt.

Group 1 (Control): Fish were fed the basal diet only. Group 2 (*S.C*): Fish were fed a basal

diet supplemented with 0.25% *Saccharomyces cerevisiae*. Group 3 (AFB_1): Fish were fed a diet intoxicated with 2.5 ppm aflatoxin B_1. Group 4 (AFB_1+*S.C*): Fish were fed a diet intoxicated with 2.5 ppm AFB_1 and supplemented with 0.25% *S. cerevisae*.

Immunological analysis

At the end of experimental period blood samples were collected from the caudal blood vessels according to the method described by **Lucky, (1977)** using a disposable tuberculin syringe. Two blood samples were collected from each group. The 1st sample was collected in clean sterilized tubes containing heparin as anticoagulant for phagocytic activity estimation according to method illustrated by **Wilkinson, (1977) and Lucy and Larry, (1982).** The 2nd blood sample was collected in clean sterilized tubes containing EDTA for determination of total and differential leucocytic count.

Analysis of aflatoxin B_1 Residues

Liver and muscle samples of five fish from each group at the end of experiment were pooled and thoroughly homogenized in a mortem. AFB_1 was extracted, filtrated and quantitatively analyzed by HPLC **(AOAC, 2005)** with a reverse phase column. The mobile phase consisted of 45% methanol, which pumped through the system at a flow rate of 1ml/min. The column temperature was set to 40°C, and analyses were detected using a fluorescence detector. Aflatoxin Standards were purchased from sigma-Aldrich (USA).

Statistical analysis

All data were analyzed using SPSS 16.0 package program with Duncan's Multiple Range Test at $P \leq 0.05$ significance value to determine statistical differences among groups

3. Results and discussion

Effects on total leukocytes and differential leucocytic count.

Table (1) illustrated that the fish group treated with *S. cerevisiae* (G2) showed a significant increase ($P \leq 0.05$) in total leukocytic and lymphocytic counts comparing with control group group. Our results are on the same direction with **Abdel-Tawwab et al., (2008)** who reported S.C. contains various immunostimulating compounds such as β-glucans, nucleic acid as well as mannan oligosaccharides that have the ability to stimulate non-specific defense mechanisms. The total leukocytes count significantly decreased ($P \leq 0.05$) in AFB_1 toxicated fish group. Leucopenia, lymphopenia and neutrophilia is the main picture of the leukogram. these toxins can damage macrophage systems **(Godish and Thad, 2001)** and hemopiotic toxicity causes the lymphocytolysis **(Sahoo et al., 2001),** The total leukocytic count was found severely decreased. Fish exposed to AFB_1 in combination with *S. cerevisiae* (G4) showed a significant increase ($P \leq 0.05$) in WBCs counts when compared with fish exposed to AFB_1 (G3). This may be attributed to the ability of *S. cerevisiae* to degrade mycotoxins and prevent their toxic effects **(Yiannikouris et al., 2006).**Our results nearly similar to **El-Boshy et al., (2008)** who stated that dietary β-glucan supplement counteracts leucopenia and lymphopenia in AFB_1 treated group.

Effects on phagocytosis.

Adding of *S. cerevisiae* to the basal diet (G2) significantly increase both phagocytic percent and phagocytic index when compared with control group. This may be due to various immunostimulating compounds, which present in S.C. **(Li and Gatlin, 2004)** beside S.C. had the ability to capture the mycotoxin molecule changing it into non toxic substance **(Yiannikouris et al., 2004).** There was a significant decrease ($P \leq 0.05$) in phagocytic % and phagocytic index as indicator for non specific immunity in AFB_1 toxicated fish. which may be attributed to immunesuppressive effect of aflatoxin. Our results were on the same direction with **Sahoo and Mukherjee, (2001)** who cleared that AFB_1 cause suppression of serum bactericidal activity, neutrophil function, macrophage phagocytic activity, humoral immune response and globulin levels in rohu (*Labeo rohita)*. Supplementation of

Table (1): Effect of aflatoxin B_1 and S. cerevisiae on leukogram of O. niloticus

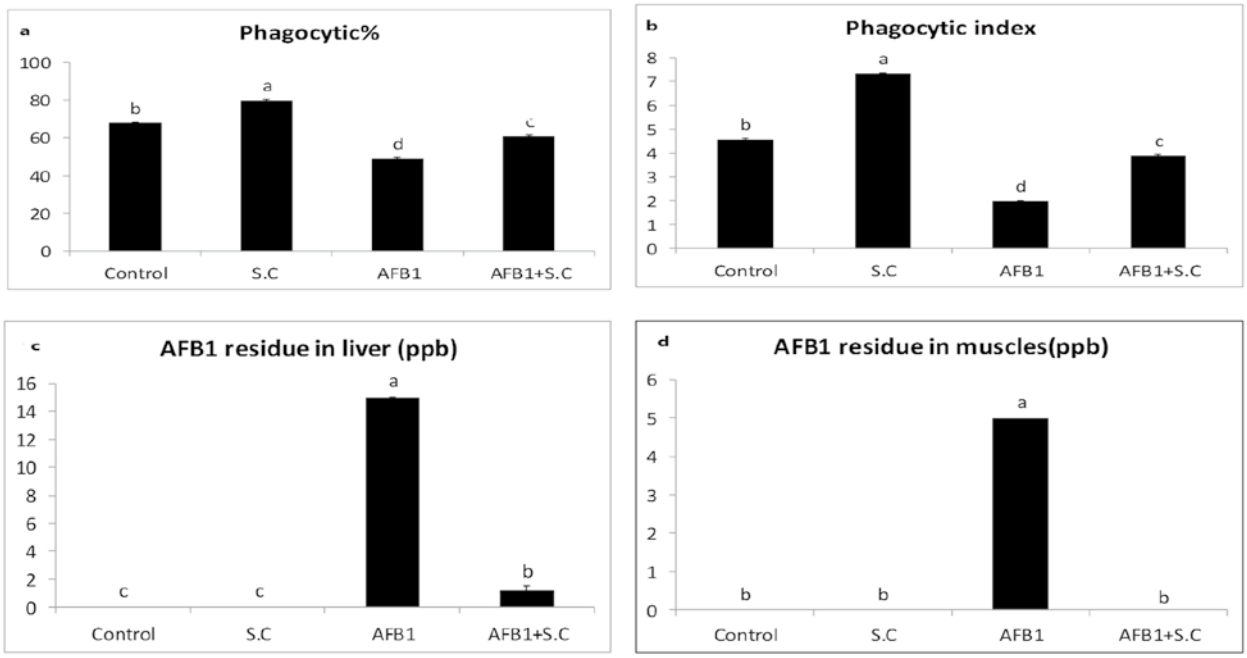
Parameter	Groups			
	G1 (Control)	G2 (S.C.)	G3 (AFB_1)	G4 (AFB1+S.C.)
WBCs (10^3/ul)	25.67± 0.02^{b}	27.25± 0.38^{a}	16.64± 0.03^{e}	22.99± 0.04^{c}
Neutrophil%	45.80± .37^{bc}	44.20± 0.20^{d}	56.60± 0.24^{a}	45.40± 0.24^{c}
Lymphocyte %	38.40±0.24^{b}	41.00± 0.37^{a}	27.60±0.24^{c}	39.60± 0.24^{ab}
Monocyte%	11.20±0.24^{ab}	11.80±0.37^{a}	8.80± 0.37^{d}	11.20± 0.20^{ab}
Eosinophil %	4.60± 0.24^{b}	3.00±0.32^{c}	7.00±0.45^{a}	3.80± 0.20^{bc}

aflatoxicated diets with *S. cerevisiae* significantly increased (P≤0.05) both phagocytic % and index compared to AFB_1 intoxicated groups fig.1 (a &b). Likewise, **Abd El-Ghany et al., (2014)** documented that S. cerevisiae has the ability to counteract immunosuppressant induced by AFB1 in Nile tilapia.

Effects on aflatoxin residues

It was clearly evident from fig 1 (b &c) that exposure to AFB_1 at a dose of 2.5 mg/kg diet for 42 day resulted in accumulation of aflatoxin residues in Nile tilapia flesh and liver which reach to 5 ppb in muscle and 15 ppb in liver. The bioaccumulation of AFB1 in the whole body of fish confirmed with similar findings of **Ayyat et al., (2013) and Hessein et al., (2014)** who concluded that residual of aflatoxin was increased significantly in fish group received AFB1 contaminated diet by 94.90% and 95.88% respectively comparing with control. Addition of *S. cerevisiae* to AFB_1 toxicated diet significantly reduced (P≤0.05) aflatoxin residues in both liver and muscle of *O. niloticus.* Our results supported by similar findings of **Shetty et al., (2007)** who recorded that S. cerevisiae was able to remove AFB1 from liquid medium.

Fig (1): Effect of aflatoxin B_1 and S. cerevisiae on phagocytic % and phagocytic index (a & b) and residues in liver and muscles (c & d)of O. niloticus



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Cadmium and Lead residues in raw cow's milk marketed in Misurata city, Libya

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Thirty random samples of raw cow's milk were collected from different markets in Misurata city, Libya to be analyzed for estimation of Cadmium and Lead concentrations and comparing these concentration with international permissible limits. The results declared that the mean concentration of Cadmium and Lead in examined raw cow's milk were 0.133 and 0.168 ppm respectively. 70% and 60% of examined samples had Cadmium and Lead residues above the international permissible limits respectively. Public health significance of Cadmium and Lead was discussed. Moreover, preventive sanitary measures required for heavy metals contamination of raw cow's milk were suggested to ensure food safety and public health.

Keywords: Lead, cadmium, cow's milk, toxic metals.

1. Introduction

Heavy metal is a term used to describe the group of toxic metals which include Lead, Cadmium, Mercury, and Arsenic; non-toxic metals as Aluminum and Tin and essential elements which include Iron, Manganese and Selenium (Beliz and Grosch, 1999).

Milk is important food for human especially for Children. However, lactating cattle may be exposed to quantities of toxic metals in the environment by air, water and ingestion of polluted feed. These animals act as a very efficient biological filter against heavy metals contamination, where it is valid when the animals are grazing near motorways and roads with heavy car traffic (Carl, 1991).

As milk is a unique essential food for rapid growth and healthy development, contamination of milk with heavy metals cause a serious risk for human health.

Thus, this study was done to estimate the concentrations of Cadmium and Lead in raw cow's milk sold in Misurata (Libya).

2. Materials and Methods

2.1 Milk samples

A total of 30 random samples of raw cow's milk (each 500 ml) were collected from different dairy shops and markets in Misurata City, Libya during the period from May to July 2015.

The samples were transferred to the laboratory to be analyzed for Cadmium and Lead residues.

2.2 Metals determination

The samples were digested according to (Tsoumbarism and Papadopoulou, 1994) and measured by Flame Atomic Absorption spectrophotometry (Hitachi 180-30, A-10) to estimate Cadmium and Lead concentrations.

2.3 Statistical analysis

Results are the average of three replicates of each sample. Results were compared with international standards of maximum permissible limit.

3. Results and Discussion

Results given in table (1) showed that the average concentrations of cadmium and lead residues in raw cow's milk samples was 0.133 and 0.168 ppm respectively. Table (2) revealed that 30% and 40% of examined raw cow's milk samples were within the permissible limit of cadmium and lead respectively according to IDF (1991) and European Commission (1997).

Table (1): Cadmium and Lead concentrations (ppm) in examined raw cow's milk samples (N =30)

Metal	Positive samples		Concentration (PPM)		
	No.	%	Min.	Max.	Mean ± SE
Cadmium	30	100	0.01	0.84	0.133 ± 0.05
Lead	30	100	0.01	0.91	0.168 ± 0.09

These results similar to some extend with Abdel-Hamid (2002). Lower results for lead concentration and higher results for cadmium concentration in raw cow's milk were reported by Enas (2005).

Table (2): Frequency distribution of Cadmium and Lead residues in examined raw cow's milk

Metal	PL. (PPM)	Within PL.		Over PL.	
		No.	%	No.	%
Cadmium	0.03*	9	30	21	70
Lead	0.05**	12	40	18	60

PL. permissible limit. * European Commission. 1997; ** IDF, 1991.

Cadmium induce chronic renal disease, cadmium poisoning may results in a case called Itai-Itai or Ouch-Ouch disease which characterized by sever

pain, soft bones and the death may occur as results of renal failure (Peter, 1993). Lead toxicity inhibits haemoglobin synthesis leading to anaemia and encephalopathia by toxic effects on nervous tissues (Gossel and Bricker, 1990).

Figure (1) showed that 21 and 18 samples have exceeded the recommended permissible limit of Cadmium and lead, respectively. This would certainly attributed to heavily environmental pollution.

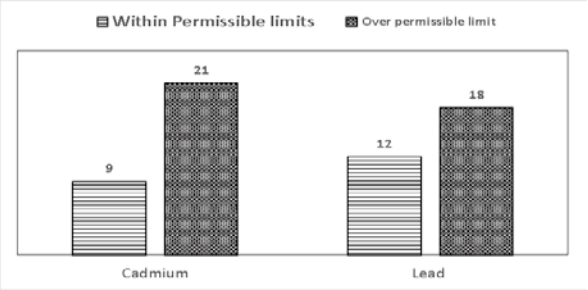

Figure (1): Distribution of samples according to permissible limits.

In a conclusion, food safety programs (HACCP) must be applied to reduce Cadmium and Lead exposure and contamination of milk and to ensure food safety and public health.

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P-38

The modulating effect of ascorbic acid against adriamycin- induced oxidative stress in male albino rat

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This work was designed to investigate the ameliorating effects of pre-and post-treatement of ascorbic acid (ASA) in the oxidative stress induced by adriamycin (ADR) in male albino rats by estimating myocardial antioxidant enzymes activity and serum malondialdehyde (MDA). Forty eight rats were randomly divided into four experimental groups treated with different treatments of ADR (15 mg / kg B.W divided in 6 injections every other day over two weeks) and ASA (10 mg / kg B.W, orally). Two, 12, 36 hrs and one week, post- drug administration animals were sacrificed. Activity of myocardial catalase (CAT) was elevated at all time points, the highest level was recorded after 36 hrs of ADR injection while those of superoxide dismutase (SOD) were decreased at 2 and 12 hrs and returned to normal after 36 hrs post injection of ADR. Myocardial reduced glutathione (GSH) level induced significantly after 12, 36 hrs and one week post injection of ADR, similarly serum MDA increased all over times in ADR treated groups, which declined after one week of ADR. Such alterations induced by ADR injection was mitigated significantly by ASA administration. In conclusion, ADR elucidated marked ruinous effects on the oxidative impacts which were alleviated by ASA.

Keywords: Adriamycin, Ascorbic acid, Oxidative stress,

1. Introduction

Adriamycin (Doxorubicin) (ADR) is a quinone-containing antitumour antibiotic and one of the most important anticancer agents. It is a valuable component of various chemotheraputic regimens used to treat breast carcinoma and small – cell lung carcinoma, Hodgkin's disease and non Hodgkin's lymphomas. It has been used in multidrug regimens which shown efficacious for treatment of canine hemangiosarcoma . However, its clinical use has been restricted by dose-limiting cardiotoxicity which lead to cardiomyopathy and heart failure. Irreversible Doxorubicin-associated myocardial failure is a well-recognized problem with this drug and unfortunately, this specific type of heart failure does not respond to the usual medical therapy as other kinds of heart failure. Myocardial failure may lead to dilated cardiomyopathy, congestive heart failure and sudden death in dogs administered 25 mg/m^2/wk for long periods (< 15 weeks). Many efforts have been made to increase the myocardial antioxidant capacity as an approach to decrease the ADR cardiotoxicity of and enhance its anticancer therapeutic efficacy. Therefore, the purpose of this study was to evaluate the protective and antioxidant effect of ascorbic acid (ASA) which is considered as the most water soluble antioxidant in extracellular fluids against ADR-induced cardiotoxicity via oxidative stress, through investigation of its effect on myocardial enzymes activities and serum MDA concentration.

2. Materials and Methods

Forty-eight adult male albino rats (150–175 g) were used in this study. The animals were assigned into four experimental groups (12 animal each) as follow, control vehicle group ASA control group: ASA was orally gavaged at a dose of 10 mg / kg B.W. orally administered (Khan and Sinha, 1999), ADR group: ADR was injected intraperitoneally at a dose of 15 mg/ kg B.W., six injections over 2 weeks (3 times/ week) (Siveski- Iliskovic et al., 1994), and ADR/ASA group: ASA was administered for 30 days (one week before, 2 weeks during and 1 week after, ADR injection). At the end of the experiment, rats were sacrificed 2, 12, 36 hours and 7 days post- drug administration, blood samples were collected for serum preparation and used for biochemical determination of MDA. Heart immediately removed, homogenized and used for determination of antioxidant enzyme activity and GSH level. The data were analyzed using an analysis of variance (one-way ANOVA) followed by Duncan's multiple range test. *P*-Values <0.05 were considered statistically significant.

3. Results and Discussion

The data of the present study revealed a highly significant increase in mean values of serum MDA concentration of ADR exposed rats which decrease by time (one week) but it still higher than control level. This increase in lipid peroxidation induced by ADR was significantly mitigated by ASA. These results may attributed to generation of free radical

(ROS) resulted in increased oxidative stress, lipid peroxidation and cell injury (Singal et al., 1995). only in tumor cells and heart but not in liver.

Concerning antioxidant enzymes activity, induction of CAT enzyme activity in rat heart following ADR treatment was remarked at 2, 12, 36 hrs and begin to recovered at one week. The change in this activity at early time points may represent an early adaptive response to counteract oxidative stress; however this adaptation is not sustained for a longer time. The SOD enzyme catalyze the conversion of superoxide radical ($O_2^{-\cdot}$) to hydrogen peroxide and molecular oxygen in ground state, thus the enzyme quenching of the ROS. CAT and GSH suppress the formation of free radicals that may result from H_2O_2 production. Therefore, the rise in SOD activity must have a parallel rise in both CAT and GSH.

Our results showed a transient decrease in SOD activity at 2, 12 hrs post last ADR injection, this transient decrease was returned to the control level after 36 hrs post last ADR administration may be due to several factors resulted from free radical produced by ADR. It may be due to decreasing the synthesis of enzyme protein level as determined by Li and Singal (2000) which explain the transient decrease in SOD may have some role in the pathogenesis of heart failure. The free radical produced by ADR inhibits DNA replication through DNA strand cleavage, resulting in a reduction in protein synthesis. The ROS are also responsible for protein oxidation resulted in the formation of protein peroxide, as well as inactivation of the detoxifying enzyme by splitting of the peptide chain. The SOD activity returned to normal level after 36 hrs post ADR administration which supported by the fact that said, at longer post treatment durations, there was no change in SOD activity.

In conclusion, the findings of the present study lead us to speculate that ASA would be beneficial, as it modulates ADR induced toxicity. Therefore, it may be used as supplements to protect against ADR toxicity.

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Table 1. Effect of oral administration of ASA (10 mg/kg B.W.) for 30 day on ADR (15 mg/kg B.W in six injections over 2 weeks) induced changes on serum MDA, myocardial SOD, CAT and GSH levels at different time of scarification (2, 12, 36 hrs and 1 week) (mean ± S.E).

Groups	MDA *(µM/L)*	CAT*(µM/g tissue)*	SOD *(µg/g tissue)*	GSH*(µM/g tissue)*
Control	1.07± 0.05 ^{f}	124.96±8.8 ^{e}	1.101±0.025 ^{a}	39.07±2.2 ^{h}
ASA	1.4 ± 0.04 ^{f}	166.91±9.60 ^{cde}	1.036±0.045 ^{abc}	41.89±2.05 ^{gh}
ADR				
ADR (2hrs)	5.8± 0.20 ^{a}	209.34±13.13 ^{abc}	0.525±0.104 ^{d}	46.927 ± 1.26 ^{efgh}
ADR (12hrs)	5.90± 0.21 ^{a}	215.93±19.00 ^{abc}	0.88±0.045^{c}	54.66 ± 0.75 ^{def}
ADR (36hrs)	5.32± 0.27 ^{ab}	231.84±11.073 ^{a}	1.042±0.081 ^{abc}	63.62 ± 2.24 ^{bc}
ADR (7 days)	3.58± 0.62 ^{cde}	213.13±7.97 ^{ab}	1.030±0.076 ^{abc}	77.86 ± 9.43 ^{a}
ADR/ASA				
ADR/ASA (2hrs)	3.93±0.59 ^{cd}	183.57±7.06 ^{abcd}	0.975±0.066 ^{abc}	40.81± 1.46 ^{gh}
ADR/ASA(12hrs)	4.53± 0.39 ^{bc}	200.38±22.11^{abcd}	0.972±0.065 ^{abc}	42.35 ± 1.15 ^{gh}
ADR/ASA (36hrs)	4.32±0.64 ^{bc}	207.58±22.6 ^{abc}	0.972±0.045 ^{abc}	48.68 ± 1.51 ^{efgh}
ADR/ASA (7days)	3.81±0.66 ^{cde}	173.99±14.8 ^{bcd}	1.072±0.067 ^{abc}	50.71± 0.55 ^{defg}

Values which have different letters are different significantly from each other (at P≤0.05).

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Histopathological evaluation of the possible protective role of royal jelly and cod liver oil against reproductive toxicity of tartrazine in adult male albino rats

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Tartrazine (T) is a synthetic dye extensively used to color food products, drugs and cosmetics despite its potential toxicological risk. Royal jelly (RJ) and cod liver oil (CLO) are natural remedy with many protective effects. This study aimed to evaluate the ameliorating effects of both RJ and CLO against toxicity effects of T on the testes. For this purpose, thirty-six male rat pups were allocated into six groups. The 1st group received distilled water (control group), the 2nd group was given 300 mg RJ/kg bw (RJ group), the 3rd group was given 0.4 ml CLO/kg bw (CLO group), the 4th was given 500 mg T/kg bw (T group). The 5th group was given T concurrently with RJ (TRJ group) and the 6th group was given T concurrently with CLO (TCLO group), at the same doses as the former groups. All treatments were given orally for 30 consecutive days. The testes were directly fixed in 4% paraformaldehyde and histologically examined. An immunohistochemical staining was applied with the anti-Sox 9 (Sertoli cell marker), anti-ssDNA antibody (apoptotic cell marker) and anti-PCNA (proliferating cell marker) to reveal the changes in testes structure. The testes in T group revealed many tubules with wide lumina where the most advanced cell lineage are spermatocyte. Furthermore, numerous ssDNA-positive cells as well as few Sox 9 and PCNA positive cells were observed in the seminiferous epithelium. Interestingly, in the TCLO and TRJ groups few ssDNA-positive cells as well as more Sox 9 and PCNA positive cells were observed in the seminiferous epithelium however not restored to that in the control, RJ and CLO groups. These results conclusively demonstrate that RJ and CLO administration provides partial protection against the ruinous effects of T on rat testes. Thus further investigations should be carried out on different products to achieve full protection.

Keywords: Tartrazine, Cod Liver Oil, Royal Jelly, Testes

1. Introduction

Recently, numerous food additives including synthetic food dyes were added to different foodstuffs to improve its colour, attractiveness, zest, texture, savour, as well as for preservation of the food. Among the widely used synthetic food dyes is Tartrazine (T). T is an orange-colored powder extensively used to color food products, known as synthetic lemon yellow. It is an artificial azo dye derived from coal tar (Elhakim and Heraud,2007). Also, T has been widely used to color human pharmaceuticals released from sources such as capsules of vitamins, antacids, cosmetics and other hair products. Furthermore, T has been used as a substitute for saffron for cooking in many developing countries (Mehedi et al., 2009).

Several investigations revealed potential toxicological risk (hepato-cellular damage and reproductive alterations) from administration of T to mice in the diet (Mehedi et al., 2009 and Gautam et al., 2010).

Previously we revealed the neurotoxic effect of oral administration of T in rat diet on the brain tissue. Moreover, we conclusively demonstrated that co-administration of RJ or CLO (as natural remedy with many protective effects) with T provided sufficient protection against the ruinous effects of T on rat pups brain tissue function and structure (Mohamed et al. 2015). On the other hand, no reports about the possible ameliorating effect of royal jelly or cod liver oil against reproductive toxicity of tartrazine in adult male albino rats.

Thus, this study was undertaken to investigate the toxically effect of T oral administration on different cells within the testes. In addition, revealing the possible protective role of royal jelly and cod liver oil against such toxicity.

2. Materials and Methods

2.1 Chemicals

Tartrazine C16H9N4O9S2Na3 (T), cod liver oil (CLO) and royal jelly (RJ) were orally administered to different experimental rat groups for one month.

2.2 Experimental animals and treatments

Thirty-six male rat pups were allocated into six groups. The 1st group received distilled water (control group), the 2nd group was given 300 mg RJ/kg bw (RJ group), the 3rd group was given 0.4 ml CLO/kg bw (CLO group), the 4th was given 500 mg T/kg bw (T group). The 5th group was given T concurrently with RJ (TRJ group) and the 6th group was given T concurrently with CLO (TCLO group), at the same doses as the former groups. All treatments were given orally for 30 consecutive days.

2.3 Tissue preparation

The testes were directly fixed in 4% paraformaldehyde and histologically examined. An immunohistochemical staining was applied with the anti-Sox 9 (Sertoli cell marker), anti-ssDNA antibody (apoptotic cell marker) and anti-PCNA (proliferating cell marker) to reveal the changes in testes structure.

3. Results and Discussion

The testes in T control, RJ and CLO groups showed normal seminiferous tubules at different spermatogenic stages and spermatozoa in their lumina. On the other hand, the testes in T group, showed many affected seminiferous tubules with wide lumina where the most advanced cell lineage are spermatocyte. However, such tubules in TRJ and TCLO groups were decreased in number (Figure 1). These results clarify the harmful effect of T on the spermatogenesis and the ameliorating protective role of both RJ and CLO against the toxic effect of T on the spermatogenesis. These results are in agreement with Gautam et al. (2010) demonstrating the reproductive toxicity following T oral administration. Also with our previous report of the protective role of both RJ and CLO against the neurotxic effect of T on rat brain (Mohamed et al., 2015). Furthermore, numerous ssDNA-positive cells as well as few Sox 9 and PCNA positive cells were observed in the seminiferous epithelium of T group. Interestingly, in the TCLO and TRJ groups few ssDNA-positive cells as well as more Sox 9 and PCNA positive cells were observed in the seminiferous epithelium however not restored to that in the control, RJ and CLO groups. The decrease in the number of apoptotic ssDNA positive cells in TRJ and TCLO groups than that in T group suggest the possible protective role of both RJ and CLO against the DNA damage that can be resulted from. The T induced dose related DNA damage in the glandular stomach, colon, and/or urinary bladder was previously reported (Himri et al., 2011).

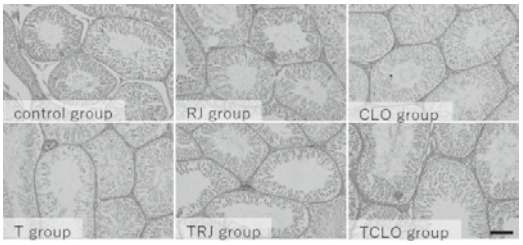

Figure 1: Testes of different experimental groups (PAS stain).

4. Acknowledgments

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