International Toxicology
Symposium in Africa

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7th International Toxicology Symposium in Africa

7th International Toxicology Symposium in Africa

Jointly hosted by University of Johannesburg and North-West University in South Africa, and Hokkaido University in Japan.

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Prof Victor Wepener (North-West University)
Prof Johan van Vuren (University of Johannesburg)

THE ISTA7 2015 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 the following scientists:

- Prof Mayumi Ishizuka (Japan) – Guest Editor
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- Dr Shouta Nakayama (Japan)
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The Editor and Assistant editor evaluated and forwarded the feedback from the reviewers to the submitting authors. A total of 82 papers were submitted for review. Twenty six papers were rejected and 56 were accepted of which 12 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

This is the seventh annual International Toxicology Symposium in Africa and it has become one of the anticipated events on the ecotoxicological sciences calendar in Africa. It is with great pleasure that the co-hosts of this symposium, Hokkaido University and North-West University, welcome you to a day of stimulating debate on environmental issues that are relevant to the African continent. This year we have participants from thirteen African countries and as it has become synonymous with the International Toxicology Symposia in Africa, there is a very good balance between student and senior scientist participation. The Symposium therefore provides an ideal opportunity for both junior and senior scientists to showcase their latest research and enter into debate.

This year is also the first time that the presentations will be published as peer-reviewed conference proceedings. A total of 82 proceedings were received and following a review process 56 manuscripts were selected as poster and oral presentations. These presentations promise to provide an exciting overview of environmental issues that the continent is currently facing. The presentations address issues from human to ecological health posed by the traditional “culprit” chemicals, i.e. metals, PAHs and DDT to new emerging contaminants such as perfluorooctanesulfonic acid and nanomaterials. The influences of these chemicals are studied across the full range of biological organization from molecular effects to community responses. As organizing committee we would like to invite you to participate in active discussion on scientific issues raised, meet with old friends and make new friends.

Prof Victor Wepener

ISTA7 – Organising Committee

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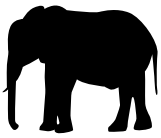

Keynote Address
Special Lecture
Oral Session

KA-1

Interspecies differences in xenobiotic metabolism

Mayumi Ishizuka

Hokkaido University, JAPAN

1. Introduction

The biotransformation of xenobiotics, including toxicological substances is divided into phase I and II reactions. In phase I reactions, the main enzymes are the cytochromes P450 (CYP) that perform hydroxylation and hence act as monooxygenases, dioxygenases, and hydrolases. Phase II enzymes also play an important role in the biotransformation of xenobiotic metabolites to more water-soluble forms. Xenobiotic-metabolizing ability is an important factor in determining sensitivity to foreign chemical compounds because the metabolites are readily excreted into urine.

In our previous study, we found clear inter / intra species differences of xenobiotic metabolizing ability in vivo, in vitro and in silico analyses, which reflect the difference in P450 and UDP-glucuronosyltransferase (UGT) in phase II reactions. Here, we would like to introduce inter and intra species differences in xenobiotic metabolism of bird and mammalian species.

2. CYP in bird species

We aimed to clarify and classify all of the existing isoforms of CYP1-3 in avian species using available genome assemblies for chicken, zebra finch, and turkey. Furthermore, we performed qRT-PCR assay to identify dominant CYP genes in chicken liver. Our results suggested that avian xenobiotic-metabolizing CYP genes have undergone unique evolution such as CYP2C and CYP3A genes, which have undergone avian-specific gene duplications. qRT-PCR experiments showed that CYP2C45 was the most highly expressed isoform in chicken liver, while CYP2C23b was the most highly induced gene by phenobarbital. Considering together with the result of further enzymatic characterization, CYP2C45 may have a dominant role in chicken xenobiotic metabolism due to the constitutive high expression levels, while CYP2C23a and CYP2C23b can be greatly induced by chicken xenobiotic receptor (CXR) activators. These findings will provide not only novel insights into avian xenobiotic metabolism, but also a basis for the further characterization of each CYP gene.

3. Phase II reaction in mammalian species

Several xenobiotics (environmental pollutants and drugs) and endogenous hormones were used to determine the interspecies differences in phase II conjugation reaction.

Firstly, we collected urine from 16 mammalian species and analyzed environmentally exposed Pyrene (PY) metabolites. Interspecies differences in urinary PY metabolites, especially in the concentration and composition of phase II conjugated metabolites were apparent. Glucuronide conjugates are dominant metabolites in the urine of many species, including deer, cattle, pigs, horses, and humans. However, they could not be detected in ferret urine even though the gene for ferret UDP-glucuronosyltransferase (UGT) 1A6 is not a pseudogene. Sulfate conjugates were detected mainly in the urine of cats, ferrets and rabbits. Interestingly, sulfate conjugates were detected in pig urine. Although pigs are known animal to have limited aryl sulfotransferase (SULT) activity, this study demonstrated pig liver was active in 1-hydroxypyrene sulfation. In Pinnipeds, several species showed drastically low ability of UGT conjugation toward pyrene. In the results of sequencing analyses of UGT, Steller sea lion and Northern fur seal possessed 2 bps insertion in UGT1A6 exon 1 with associated stop codons. According to synteny analysis, some species of Pinnipeds (e.g, Pacific walrus, Ferret) might not have U*GT1A7-1A10* homologues.

4. Summary

We found clear interspecies differences of substrate metabolism ability in *in vivo*, *in vitro* and *in silico* analyses, which reflect the difference in phase I and phase II reactions. These differences should causes the chemical sensitivity differences in each species.

Is Indoor Residual Spraying broken and what should be fixed?

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Indoor residual spraying (IRS) has been and is still a very successful method to control malaria. We are concerned that not enough research attention is given to improving IRS and that most funding goes towards modern but seemingly still ineffectual methods. We believe that there is ample scope for improving IRS, while reducing insecticide exposure

Keywords: Indoor residual spray, IRS, Botha de Meillon, policy

1. Introduction

The effectiveness of DDT and other insecticides when properly used as indoor residual spray (IRS) to prevent the transmission of malaria is not in question [1] (Fig 1). We can safely assume that millions of lives have been saved in the more than 80 years since it was first used [1]. The high body burden of DDT of those protected by IRS, as well as the human health consequences of those protected by all IRS insecticides are of great concern [1,2]. However, many promises of 'silver bullets' (using anything but IRS) to beat malaria over the last couple of decades have come and gone on a road littered with good intentions, vast investments, and less impressive results when confronted with realities, especially in Africa.

Yet the one proven method, IRS, hardly gets recognition. IRS interrupts transmission where most infections occur - the home. It is also at home where those most likely to be infected by malaria – babies, children and pregnant mothers – are found. The negative part of the IRS approach though, remains the inevitable co-exposure of the very same susceptible groups and mosquitoes to IRS insecticides. Protection by IRS comes at a cost, creating a paradox – protection against malaria that kills hundreds of thousands of people per year with something that may be or is harmful [1,3].

2. Where did IRS start and why is it so effective?

IRS as a method has remained almost unchanged since Botha de Meillon pioneered it in South Africa and published in 1936 [4] (Figs 2 & 3). Initial IRS chemicals were pyrethrum, BHC, and DDT, often used with kerosene. DDT is now applied as a water wettable powder. Currently, pyrethroids, organophosphates, and a carbamate are recommended by the WHO for IRS. IRS initially was not very successful everywhere, but in most areas of the world it reduced infections, although the mode of action (lethality, irritancy, repellency, or combinations thereof) was not always clear. Indiscriminate use and use in agriculture may have led to resistance in many areas. Combining basic biological knowledge about reproductive behaviour of the female vector mosquito with residual toxic chemicals within and close to residential areas where most infections occur, is effective at preventing transmission, but bad at preventing chemical exposure and uptake of the chemicals by residents - posing a paradox.

3. Who little attention on improving IRS?

Policy formulation, negotiating fora, and the development of research priorities via consensus (many or all burdened with other agendas) seem not to be good platforms to deal with such seemingly intractable paradoxes. IRS using chemicals seems out of vogue and often relegated in favour of the promises of 'high-tech' or new methods. Many of these methods are promoted from a developed country perspective, but with little or no attention to improve on a method (IRS) that has worked so well. DDT, one of the most effective and dependable IRS chemicals, is facing many pressures for premature elimination, creating 'official' resistance in countries that recognise IRS as the best practical way of protecting its populations.

4. Why maintain and improve IRS?

Maintaining a proven top-down IRS strategy supported by an effective hospital and clinic system requires a minor inconvenience but no other behavioural changes by the inhabitants [5], ecological engineering, biological interventions, environmental modifications, or vaccines. The mostly non-intrusive IRS allows inhabitants and communities the freedom for social interactions and economic betterment unhindered by the inconvenience required by some currently promoted forms malaria prevention that requires active community participation. For the foreseeable future, IRS will remain a mainstay of malaria prevention, will most likely have a role in malaria elimination in any endemic area, and/or will remain the fall-back

method in case of failure of alternatives.

5. Recommendations

We believe that a vast scope of options to improve IRS remain to be explored that will significantly reduce human exposure to chemicals while maintaining effective prevention of transmission.

Options for further exploration include:

- Study the behaviour of humans, IRS chemicals and mosquitoes following the Total Homestead Environment Approach (THEA) (Fig 4).
- Investigating how to apply insecticides more selectively, depending on (and further looking at) mosquito behaviour
- Exploring the seemingly many opportunities available regarding mosquito irritability and repellency while preventing resistance
- Investing in better formulations for IRS, including removal of compounds from formulations that are not required for control, but that may harm human health [1]
- Investigating and developing new chemicals
- Based on Thea (fig 4), develop an Integrated Host and Environmental Protection Approach

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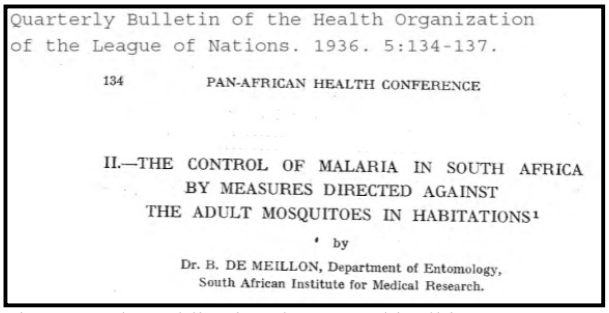
Figure 1: Malaria control by indoor residual spraying.

Figure 2: Botha de Meillon collecting mosquito larvae



Quarterly Bulletin of the Health Organization of the League of Nations. 1936. 5:134-137.

134 PAN-AFRICAN HEALTH CONFERENCE

II.—THE CONTROL OF MALARIA IN SOUTH AFRICA BY MEASURES DIRECTED AGAINST THE ADULT MOSQUITOES IN HABITATIONS[1]

by

Dr. B. DE MEILLON, Department of Entomology, South African Institute for Medical Research.

Figure 3: The publication that started it all in 1936.

Figure 4: An illustrated concept of the Total Homestead Environment Approach (THEA).

O-1

Vector-control pesticide DDT detected in free-ranging chickens from South Africa

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Dichloro-diphenyl-trichloroethane (DDT) has been administered in indoor residual spraying as part of the KwaZulu-Natal Province's malaria control program for several years. Toxicity of this pesticide has been demonstrated in many avian and mammalian species. For this study, meat was sampled from free-ranging chickens (n=25) reared for consumption within the province, and analysed for the presence of DDT congeners. Total concentration of DDTs ranged from 0.01 to 71.1 ng/g ww, with a mean of 15.9±19.8 ng/g ww (9,169±17,401 ng/g lw). In order to assess potential risk to people from consumption of the chicken meat, a risk analysis was performed. Based on questionnaires, average daily consumption was estimated at 27.2 g/person. Although the estimated daily intake of DDTs was below the acceptable daily intake, cancer risk estimates and hazard ratios of DDTs exceeded stated thresholds, indicating a potential concern, with a lifetime cancer risk greater than 1 in 10^6. DDT use is likely to continue until alternative malaria control methods are developed and successfully implemented, and environmental persistence means this potential risk will remain for many years.

Keywords: DDT, vector control, risk assessment, chickens

1. Introduction

The World Health Organization estimated 198 million cases of malaria occurred worldwide in 2013, with 584,000 deaths (WHO 2014). Of these, 90% were in Africa, predominantly children under five years old. The vector of the malaria-causing *Plasmodium* parasite, the *Anopheles* mosquito, is often controlled using pesticides such as dichloro-diphenyl-trichloroethane (DDT). Toxicity in wildlife resulted in banning of DDT in many countries, and its use is now strictly regulated. Although initially thought to be safe for humans, some reports suggest exposure may result in neurotoxic, carcinogenic, immunotoxic and reproductive effects (Van den Berg 2009). South Africa has an integrated malaria control program in three provinces, including KwaZulu-Natal (KZN). Intervention focusses on vector control using insecticides in bed nets (insecticide-treated nets, ITN) and indoor residual spraying (IRS), along with timely case diagnosis and use of medication.

Although parts of KZN have significant industry and economic development, the area studied has a subsistence economy and low education rate. Many inhabitants maintain small gardens and free-range chickens as sources of nutrition. This study was undertaken to assess the levels of DDT congeners present in such chickens in order to make an assessment of the potential risk to human health from consumption of the chicken meat.

2. Materials and Methods

2.1 Study area, sampling and analysis

The study area is located in the Jozini and uMhlabuyalingana Local Municipalities in KZN Province. Sampling was conducted in October 2014 just prior to annual IRS application with DDT. Procedures were conducted in accordance with the guidelines of Hokkaido University Institutional Animal Care and Use Committee. Free-ranging chickens (n=25) were purchased and muscle samples collected. Samples were processed and analysed following the method of Yohannes (2013). Briefly, DDTs (*o,p'*-DDT, *p,p'*-DDT, *o,p'*-DDE, *p,p'*-DDE, *o,p'*-DDD and *p,p'*-DDD) were extracted from 5g samples using Soxhtherm apparatus (S306AK Automatic Extractor, Gerhardt, Germany), applied to florisil for clean-up, and analysed by gas chromatography with electron capture detector (Shimadzu GC-2014, Kyoto, Japan with ENV-8 MS capillary column, 30 m x 0.25 mm i.d., 0.25 µm film thickness).

2.2. Human health risk assessment

Questionnaires were conducted to estimate daily average consumption of chicken. Estimated dietary intake (*EDI*) of DDTs was then calculated:

$$EDI = \frac{C \times DR}{BW}$$

where *C* is the measured concentration of DDTs (ng/g ww) at the 50th and 95th percentiles, *DR* is average daily consumption rate (g/d/person) and

BW is body weight (kg), which was set at 60kg (WHO 2010). To further assess public health risk from consumption of chicken meat, cancer risk estimates and hazard ratios (*HR*s) were assessed based on United States Environmental Protection Agency (USEPA) guidelines (USEPA 2005). Cancer risk estimates were calculated using the USEPA cancer slope factor (*CSF*):

$$\text{Cancer risk estimate} = EDI \times CSF$$

According to public screening criteria for carcinogens set at a carcinogenic risk threshold of 10^{-6}, cancer risk estimates below this level are considered acceptable, those above 10^{-4} unacceptable, and those between the two values are an area of concern. *HR* for cancer risks was assessed by comparing *EDI* with *BMC*:

$$HR = \frac{EDI}{BMC}$$

Benchmark concentration (*BMC*) is derived from *CSF*. This represents an exposure concentration at which lifetime cancer risk is one in a million. A *HR* value greater than one indicates potential risk to human health.

3. Results and Discussion

DDTs were present in muscle samples from all chickens sampled. The mean sum of DDTs detected was 15.9±19.8 ng/g ww (9,169.9±17,401 ng/g lw). The main congener was *p,p'*-DDE (Figure 1). The presence of *p,p'*-DDT indicates recent exposure to the pesticide, although hut records showed the most recent IRS application was nine months prior to sampling.

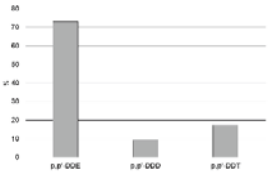

Figure 1: Relative abundance of individual DDT components detected in free-ranging chicken meat.

Although the national *DR* of chicken is 99.1 g/d (South African Poultry Association 2012), it was estimated from questionnaires to be 27.2 g/d in the study area. *EDI* from consumption of the chicken meat was lower than the acceptable daily intake (ADI) for DDTs (10,000 ng/kg *BW*/d), indicating no risk to human health. However, the cancer risk estimate increased from 1.5 (at the 50th percentile exposure level) to 9.8 (95th percentile) x 10^{-3}, suggesting an unacceptable risk with a chance between 2 and 10 in 1,000 of developing cancer due to DDTs present in the meat. *HR*s based on the 50th and 95th percentile exposure levels were greater than one, suggesting a potential risk to human health (Figure 2). It should be noted that these analyses were conducted on uncooked samples. Previous studies have shown a reduction in concentration of DDTs by approximately 25% after cooking, reducing further if water used for boiling is discarded (Morgan *et al* 1972).

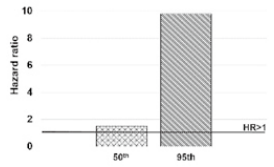

Figure 2: Cancer risk (50th and 95th percentiles) from consumption of free-ranging chicken meat.

IRS results in DDT coating of the homestead including wall/floor surfaces, which are sources of exposure to chickens. DDTs accumulating within poultry are in turn a potential source for people through consumption. Further analyses of homestead samples are needed to clarify the most significant routes of exposure.

Other methods of controlling malarial vectors—including novel insecticides with lower health risks—are being developed. Meanwhile, storage and administration of chemicals such as DDT should be performed carefully to minimise exposure and toxic effects to both humans, particularly with regard to pregnant women and children, and animals.

4. Acknowledgments

This study was supported in part by the Leading Program at Hokkaido University, and in part by the Japan Society for the Promotion of Science, awarded to Lesa Thompson (JSPS KAKENHI Grant Number 16J02013). Support was also received from Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan awarded to Mayumi Ishizuka (No. 24405004 and 24248056) and Yoshinori Ikenaka (No. 26304043, 15H0282505 and 15K1221305), and the foundation of JSPS Core-to-Core Program (AA Science Platforms) and Bilateral Program.

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O-2

Lead pollution in the children in Kabwe mining area, Republic of Zambia- Source identification by stable isotope analysis

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Childhood lead poisoning is a serious public health concern worldwide. Blood lead levels (BLLs) exceeding 5 µg/dL are considered elevated. In Kabwe, the capital of Zambia's Central Province, extensive Pb contamination of township soils in the vicinity of a Pb-Zn mine and posing serious health risk to children has been reported. We investigated BLLs in children under the age of 7 years in townships around the mine; where blood samples were collected and analysed using an ICP-MS. Almost all of the sampled children had BLLs exceeding 10 µg/dL. Children in these areas could be at serious risk of Pb toxicity as 18% of the sampled children in Chowa, 57% (Kasanda) and 25% (Makululu) had BLLs exceeding 65 µg/dL. Eight children had BLLs exceeding 150 µg/dL with the maximum being 427.8 µg/dL. We recommend that medical intervention be commenced in the children with BLL exceeding 45 µg/dL. The results of stable Pb isotope ratios suggested that the main source of Pb is soil around the mine.

Keywords: Lead, Kabwe, Zambia, stable isotope

1. Introduction

In Africa, major sources of childhood Pb poisoning include Pb mining and smelting.

Childhood lead (Pb) poisoning is a serious public health concern worldwide. Young children under the age of 7 years are particularly vulnerable to Pb poisoning because of behavioral factors, such as frequent hand-to-mouth activities and biological factors including greater gastrointestinal absorption compared to adults and developing neurological systems (Calabrese et al. 1997; Manton et al. 2000; Bellinger 2004). Lead exposure among children is associated with developmental abnormalities including impaired cognitive function, reduced intelligence, impaired hearing and reduced stature.

In Kabwe, the capital of Zambia's Central Province, extensive contamination of Pb in soils, wild rats as well as offal of cattle and chicken in townships in the vicinity of a lead-zinc mine has been reported and poses a serious health risk to children in these townships (Nakayama et al. 2010; Yabe et al. 2011; Yabe et al. 2013). The concentrations of Pb (9-51188 mg/kg) in Kabwe soil (n=101) were much higher than benchmark values. Pb levels in tissues of Kabwe cattle were higher than those in other Zambian towns. Moreover, mean concentrations of Pb exceeded maximum levels for human consumption in some organs including muscle in free-range chickens, in contrast to low levels in broiler chickens. Therefore, this study investigated blood lead levels (BLLs) in children in townships around the Pb-Zn mine in Kabwe and to identify children with BLLs that require medical intervention so as to mitigate the toxic effects of Pb. The current study also conducted analysis of stable Pb isotope ratios (Pb-IRs) to identify possible sources of Pb since Pb isotopic analysis has proven to be a very efficient tool for tracing the sources of local and global Pb pollution (Gulson et al., 1981, Dolgopolova et al., 2006, Iglesias et al., 2010).

2. Materials and Methods

The study was approved by the University of Zambia Research Ethics Committee and the Ministry of Health. Zambia. After informed and written consent was obtained from the parents or guardians, blood samples up to 3 mL (17 samples at Chowa, 100 samples at Kasanda and 129 samples at Makululu) were collected by qualified laboratory technicians from the children at clinics in the study areas. For each child, data on the age, sex and residential area were recorded. The blood samples were promptly transferred and stored at -20 °C at the laboratory of the Kabwe District Health Offices. The samples were transported to the Graduate School of Veterinary Medicine, Hokkaido University, Japan and analyzed for Pb concentrations by ICP-MS (7700 series, Agilent technologies, Tokyo, Japan) after extraction with Microwave digestion system (Speedwave MWS-2; Berghof, Germany). The analysis of Pb-IRs was performed by the method of Nakata et al. (2015) with slight modification. For precise analysis, the extract solutions were diluted such that the total

Pb concentration in the solutions became less than 20 µg/L. In order to correct for mass bias and dead time effects, standard reference material (SRM) 981 (National Institute of Standards and Technology (NIST), Gaithersburg, MD, USA) was measured every 10 samples. During the analytical procedure, the following isotopes were measured: 204Pb, 206Pb, 207Pb, and 208Pb. However, only the 208Pb/206Pb and 207Pb/206Pb ratios are discussed in this study, as they show the most significant differences between the contaminated and natural background materials. Moreover, these ratios have been the most commonly interpreted ratios in previous research (Monna et al., 1998). The ratios of the samples were corrected every 10 samples using the average value of each isotope ratio obtained by measurement of SRM 981. The standard error for the 208Pb/206Pb and 207Pb/206Pb measurements was < 1.0 % of RSD (relative standard deviation). All the statistical analyses were carried out using JMP 11 (SAS Institute, Cary, NC, USA) in order to evaluate significant differences in the data.

3. Results and Discussion

Almost all of the sampled children in the current study had indications of Pb poisoning, with BLLs exceeding 10 µg/dL. Children in these areas could be at serious risk of Pb toxicity as 18% of the sampled children in Chowa, 57% (Kasanda) and 25% (Makululu) had BLLs exceeding 65 µg/dL. Eight children had BLLs exceeding 150 µg/dL with the maximum being 427.8 µg/dL. When children were grouped according to age, younger children between the ages of 0 – 3 years accumulated higher BLLs than their older counterparts (4 – 7 years). Significant negative correlation between age and BLLs supported this finding. This study demonstrated that childhood Pb poisoning in Kabwe is among the highest in the world. Although clinical cases and deaths due to Pb poisoning among children in Kabwe are rare, these findings indicate that more studies are needed to establish the health effects of Pb poisoning in children exposed to Pb pollution in townships around the Pb-Zn mine in Kabwe. The results of Pb-IRs suggested that the main source of Pb is soil in and around the mine. In conclusion, further study and appropriate countermeasure are urgent to reduce the Pb levels in children.

4. Acknowledgements

This work 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 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). We also acknowledge the financial support by The Mitsui & Co., Ltd. Environment Fund. We are grateful to Mr. Takahiro Ichise (Laboratory of Toxicology, Graduate School of Veterinary Medicine, Hokkaido University) for technical support.

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O-3

Relationship between urinary bisphenol A and male reproductive function markers in rural (Djutitsa) and urban (Yaoundé) populations in Cameroon

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This study was designed to investigate the effects of bisphenol A (BPA; a widely used synthetic xenoestrogen) on male reproductive function among farmers (agropesticides users, who showed reduced testosterone levels) in Djutitsa (rural area, west Cameroon) and townsmen in Yaoundé (urban area, Centre region, Cameroon). To this end, urinary samples from these populations were screened for BPA, and correlation between BPA exposure and markers of male reproductive function assessed. BPA was detected in 92.6% of the urine samples; the average concentration being 3.86±3.49 µg/g creatinine. When grouped according to residence area, the urinary BPA levels did not show significant difference between rural and urban populations. However, positive correlation ($P<0.05$) was found between urinary BPA levels and sex-hormone-binding globulin (SHBG) and testosterone (free and bioavailable) levels in the rural population only. These findings indicated exposure of the urban and rural populations to BPA. Considering exposure to agropesticides and decreased testosterone levels which were previously reported among the rural population, the present results suggest a positive interaction between agropesticides and BPA, leading to detrimental effects on reproductive function in the farmers.

Keywords: Bisphenol A (BPA), male human, reproductive toxicity, endocrine disruption

1. Introduction

Bisphenol A [2,2-bis(4-hydroxyphenyl)propane] (BPA) is an environmental contaminant, resulting from epoxy resin linings in food and beverage cans, degradation of industrial plastic-related wastes, etc. BPA is one of the highest volume chemicals produced worldwide, with over 100 tons released into the atmosphere by yearly production (see Manfo *et al*., 2014, for review). The latter chemical is thus widely spread in environmental matrices, such as water, soil, and foodstuffs. It gets into human system mainly through food ingestion (Manfo *et al*., 2014; Zhang *et al*., 2011). BPA is rapidly metabolized after absorption, and completely excreted in urine after 24h, mainly as BPA glucuronide (BPA-G, a glucuronide conjugate that constitutes about 79% of urinary BPA) (Harthé *et al*., 2012). BPA have been reported as endocrine disruptor, which alters the physiologic function of endogenous hormones. Up to dates, existing reports on BPA exposure are mainly focused on western countries and Asia. BPA exposure in African populations is still unclear, even though its exposure sources are wide spread in the continent.

The present work therefore aimed at determining exposure to BPA among Cameroonian urban (Yaoundé, Centre Region) and rural (Djutitsa, West Region) populations, and assessing its effect on male reproductive function.

2. Materials and Methods

Urinary samples were obtained from small scale male farmers resident in Djutitsa, and townsmen in Yaoundé. The urinary levels of BPA-G was quantified (Harthé *et al*., 2012), and used for determination of BPA. BPA levels were then corrected using urine creatinine levels. Furthermore, urinary BPA concentration for each individual was associated with his respective serum levels of reproductive function markers (sex-hormone-binding (SHBG), gonadotropins, steroid and thyroid hormones that were previously reported (Manfo *et al*., 2012)). Spearman's correlation coefficients were then determined for each hormone/marker using

SPSS 17.0.

3. Results and Discussion

BPA was detected in 92.6% of the urine samples; the average and highest concentrations being 3.86±3.49 µg/g creatinine and 17.6 µg/g creatinine, respectively. When grouped according to the area of residence the urinary BPA levels did not show significant difference between rural and urban populations (3.89±2.77 µg/g creatinine vs. 4.31±4.71 µg/g creatinine, P>0.05).

Among the markers of reproductive function investigated, only SHBG and testosterone (free and bioavailable testosterone) were significantly correlated (P<0.05) with urinary BPA levels in the rural population (Figure 1). SHBG concentrations were positively correlated to BPA levels, while testosterone levels decreased with increasing concentrations of BPA in the latter population. Conversely, the markers of reproductive function from men living in the urban area didn't correlate with BPA exposure (data not shown).

It was previously reported that rural residents were exposed to agropesticides, and showed decreased testosterone, as well as high frequency of reproduction difficulties (Manfo *et al*., 2012). Testosterone is well known as the main androgen that stimulates spermatogenesis, and elevated SHBG levels were associated with decreased sex drive in men (Ahn *et al*., 2002). Testosterone present in human blood is strongly bound to SHBG, or loosely bound to albumin or free; and the sum of free and albumin-bound testosterone (bioavailable testosterone) (Sá *et al*., 2014) represents the fraction of testosterone that readily enters cells. This fraction better reflects the bioactivity of testosterone than does the serum total testosterone.

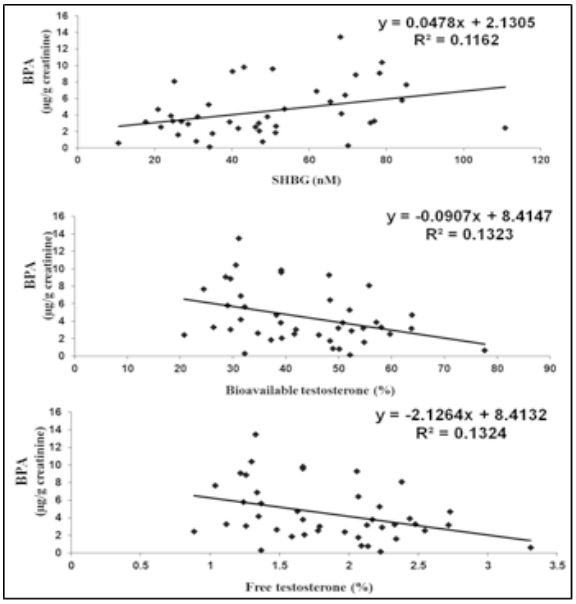

Figure 1: Correlation between urinary BPA and markers of male reproductive function (testosterone and SHBG) in rural residents (agropesticides users)

Both urban and rural populations had similar BPA levels, while rural residents were the only participants exposed to agropesticides (Manfo *et al*., 2012). Therefore, the observed correlations between BPA and hormones/SHBG suggest a positive interaction between agropesticides and BPA leading to detrimental effects on reproductive function in the male farmers. There is thus a need to strengthen regulatory measures for BPA, particularly among agropesticides users.

4. Acknowledgments

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SL-2

Metallothionein as a biomarker for chicken exposure to heavy metals

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Chickens are exposed during their life to a vast array of xenobiotics such as heavy metals, pesticides and antibiotics. This study aimed firstly to investigate the heavy metal bioaccumulation levels in the liver, kidneys and muscles of free-range chicken in Tarkwa, Ghana, compared with the controlled-reared ones. Furthermore, establishing a molecular biomarker for heavy metal exposure in chickens was our second target from this study. Examined chickens had high residual levels of cadmium (Cd), lead (Pb), and accumulated copper (Cu) and arsenic (As) in the kidneys and liver. Metallothionein 4 (MT4) mRNA was significantly induced in the free-range chickens especially in the liver and kidneys. Scatter plots between MT4 and heavy metal load in the different tissues showed positive and significant correlations. Thus, MT4 is an ideal biomarker for chicken exposure to heavy metals especially Cd, Cu, Pb and As.

Keywords: Chicken, heavy metals, metallothionein, biomarker

1. Introduction

Chicken (*Gallus gallus*) is considered as a major source for animal derived protein. Free-range chicken is reared for their meat or egg production. This rearing system gives rise to exposure of chicken to different environmental pollutants such as heavy metals, polycyclic aromatic hydrocarbons and pesticides. Chicken, especially which lives in highly polluted areas may be exposed to heavy metals through various sources such as air, food and water.

Heavy metals such as lead (Pb), cadmium (Cd) and arsenic (As) are toxic at even minute concentrations. Since some of them may accumulate in the food chain. Heavy metals constitute a major threat to human, animals and different avian species due to their significant toxicological implications. Heavy metals can interfere with the immune defence system increasing susceptibility for disease and affecting the growth parameters for different poultry species including chicken. Thus introducing a diagnostic biological marker for chicken exposure to heavy metals is of significant importance for the field of poultry industry.

Metallothionein (MT) is a cysteine-rich, metal-binding protein that has a significant role in the metabolism and detoxification of heavy metals such as cadmium and mercury (Darwish et al. 2014). Thus, the induction of MT gene expression is considered as a valuable marker for heavy-metal exposure (particularly with respect to cadmium, mercury, and zinc). However, the relationship between Pb, As and Cu exposure and MT gene expression has been less well defined especially at avian species.

Thus, this study was undertaken to screen the residual levels of heavy metals such as Pb, Cd, Cu and As in the different tissues (liver, kidneys and muscles) of free-range chicken reared in Tarkwa, Ghana. Moreover, Metallothionein 4 (MT4) gene expression was investigated in these tissues in comparison with the liver of broiler chicken reared in laboratory using the real-time PCR (qPCR) method. Correlation analysis between the heavy metal levels and MT4 expression in the examined tissues was performed.

2. Materials and Methods

All procedures used in this study were according to the guidelines of the Hokkaido University Institutional Animal Care and Use Committee.

2.1 Sample collection

Ten free-range chickens were collected alive from townships and households within 1km of the two major gold mines in Tarkwa, Ghana. Chickens were disected in Ghana and samples including RNA later from liver, kidneys and thigh muscles were imported to laboratory of Toxicology, Graduate schoold of Veterinary Medicine, Hokkaido University, Japan after getting the required permissions. Liver samples collected from broiler chicken reared at laboratory of Toxicology were used as control samples for this study.

2.2 Heavy metal extraction and measurements

Approximately 0.5 g of individual samples were

dried in an oven at 40°C and digested in a closed microwave extraction system, Speed Wave MWS-2 microwave digestion (Berghof, Germany). Acid digestion used solution consisted of 5 mL of (65%) nitric acid, and 1 mL (30%) hydrogen peroxide, H_2O_2 (Kanto Chemical Corp., Tokyo, Japan). An Inductively Coupled Plasma-Mass Spectrometer (ICP-MS; 7700 series, Agilent technologies, Tokyo, Japan) was used for quantification.

2.3 mRNA expression of MT4

RNAs were extracted and cDNAs were synthesized from examined tissues (control liver, liver, kidney and muscle) using our previously used method (Darwish et al., 2014). Quantitative Real-Time PCR method was used to investigate the comparative mRNA expression of MT4 using SYBR green reagents and β-actin as a house-keeping gene according to Darwish et al. (2014). The sense primer for chicken MT4 was 5'-GCAACAACTGTGCCAAGGGC-3', and the anti-sense was 5'-TTTCGTGGTCCCTGTCACCC-3', while the sense primer for chicken β-actin was 5'-GAGAAATTGTGCGTGACATCA-3' and the anti-sense was 5'- CCTGAACCTCTCATTGCCA-3' (Gene bank accessions for chicken MT4 and β-actin are NM_205275.1 and NM_205518.1 respectively). Comparative CT method was used and all measurements were run in duplicates.

2.4 Statistical analysis

Statistical significances and correlations were evaluated using Tukey-Kramer HSD difference test and Spearman's test respectively using (JMP) (SAS Institute, Cary, NC, USA). $P<0.05$ was considered to be significant.

3. Results and Discussion

The increased occurrence of heavy metals and metalloid contamination in the world is associated with increased anthropogenic activities such as mining, waste disposals, fertilizers, high industries and pesticides application.

Tarkwa city, Ghana is very famous with gold mining activities and thus high load of heavy metals in the environment (Borty-Sam et al., 2015)

The chronic exposure of living species including chicken to heavy metals in the polluted environments resulted in lower immunity and high susceptibility of infectious diseases. Measuring the heavy metal residual concentrations in different tissues of free-range chicken in Tarkwa, Ghana revealed high levels of both Pb and Cd than the recommended levels by WHO. However, Cu and As levels were within the acceptable levels (Table 1). Establishing a diagnostic molecular biomarker for chronic exposure of chicken to heavy metals is a matter of significant importance. Interestingly, measuring mRNA expression level of MT4 in the different tissues examined showed a significant induction of MT4 in the liver and kidneys of free-range chickens compared with the control. This high induction positively and significantly correlated (P values ranged from <0.0001 to 0.03) with the accumulation levels of the different metals examined particularly, Cd and Cu (Fig. 1).

Table 1: Mean ± SE of heavy metal residues (mg/kg wet weight) & MT mRNA relative expression in chicken different tissues

	Control	Liver	Kidney	Muscle
Pb	0.01 ± 0.003^{C}	0.13 ± 0.03^{B}	1.08 ± 0.42^{A}	0.01 ± 0.003^{C}
Cd	0.003 ± 0.001^{B}	0.22 ± 0.05^{B}	3.00 ± 1.05^{A}	0.003 ± 0.001^{B}
Cu	1.07 ± 0.11^{C}	3.67 ± 0.17^{B}	11.74 ± 0.51^{A}	1.07 ± 0.11^{C}
As	0.01 ± 0.001^{B}	0.08 ± 0.02^{B}	0.59 ± 0.15^{A}	0.008 ± 0.001^{B}
MT4	1.00 ± 0.23^{B}	12.66 ± 3.88^{A}	10.79 ± 4.16^{A}	0.44 ± 0.12^{B}

A,B, C significantly different within the same row; ($p< 0.05$).

Moreover, MT4 was positively and significantly correlated with both Pb and As levels in the kidney (Fig. 1). Induction of MT4 is a detoxification trial from chicken to adapt to pollution in the surrounding environment. In conclusion, this study shows that MT4 is an ideal biomarker for chicken exposure to heavy metals such as Cd, Cu, Pb and As.

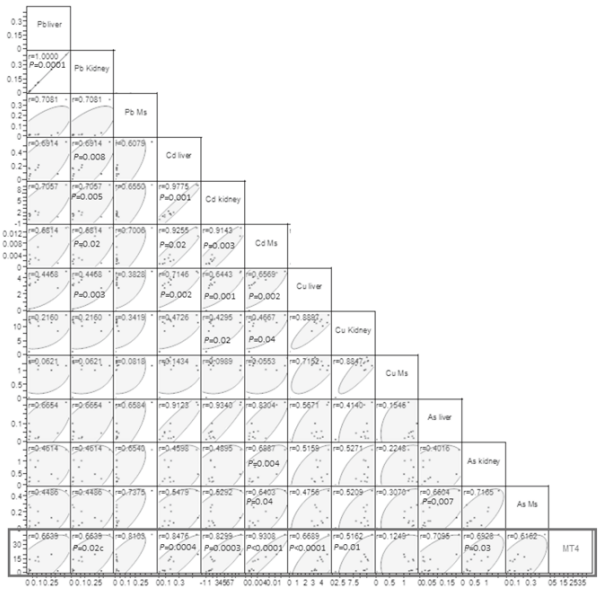

Figure 1: Correlation analyses between Pb, Cd, Cu, As & MT4 mRNA expression in liver, kidneys and muscles (Ms) of chicken

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O-4

Distribution, sources and ecological risk of polycyclic aromatic hydrocarbons (PAHs) in sediments of the Orange-Senqu River Basin, Southern Africa.

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Sediment samples in the catchment of the largest river basin in southern Africa were analysed for the 16 priority PAHs. The ΣPAH ranged from 0.006–0.87 µg/g dm. The highest sum of carcinogenic PAHs (ΣCPAH) was 0.37 µg/g dm and the greatest toxic equivalency (TEQ) was 0.015 µg/g dm. Approximately 48% of the sites had petrogenic PAHs. PAH levels exceeded 10 of the 12 Canadian threshold effects guidelines for protection of benthic organisms. The acenaphthene guideline was exceeded at 33% of the sites. There is potential risk to fish within the basin.

Keywords: PAHs, sediment, South Africa, Orange-Senqu catchment, guidelines

1. Introduction

The Orange-Senqu River Basin is the second largest river basin in southern Africa draining 1 000 000 km^2. It is shared by Botswana, Lesotho, Namibia and South Africa. Its water supports the economic centre of especially South Africa—most activities here are potentially producers of PAHs.

The 16 priority PAH congeners (US EPA, 2008) are toxic, carcinogenic and mutagenic. Their widespread occurrence, high volume releases and slow degradation allow them to remain in the environment at high concentrations. Pyrogenic PAHs are created by the incomplete combustion of organic material. Petrogenic PAHs have their origin in crude oils and refined petroleum products. Sediment becomes a sink for these pollutants (Chen and Chen 2011). When sediment dwelling organisms ingest PAHs they enter the food chain and may have negative effects on the biota as well as bioaccumulate within higher trophic levels.

2. Materials and Methods

Composite samples from 61 sites (Figure 1) were air dried, ground, and sieved. PAH congeners naphthalene [Nap] acenaphthylene [Acey], acenaphthene [Acea], fluorene [Fl], phenanthrene [Phe], anthracene [Ant], fluoranthene [Fla], pyrene [Pyr], benzo(a)anthrancene [BaA], chrysene [Chr], benzo(b+k)fluoranthene [BbkF], benzo(a)pyrene [BaP], indeno(1,2,3-cd)pyrene [InP], benzo (ghi)perylene [BgP] and dibenzo(ah+ac)anthracene [DBA] were analysed by an accredit German laboratory (method ISO 18287; ISO 2006).

PAH diagnostic ratios were used to distinguish between pyrogenic and petrogenic sources (Pies *et al*. 2008) and between biomass vs petroleum combustion (Yunker *et al*. 2002).

The toxicity of the PAHs was gauged in terms of the guideline values described by MacDonald *et al.* (2000) which are sediment quality guidelines (SQG) established on the compound's toxicity to sediment-dwelling organisms. "Toxicity" includes multiple mechanisms of toxic action and was determined for the following congeners: Nap, Fl, Phe, Ant, Fla, Pyr, BaA, Chr, BaP and DBA, here referred to as "guideline PAHs" (GPAHs).

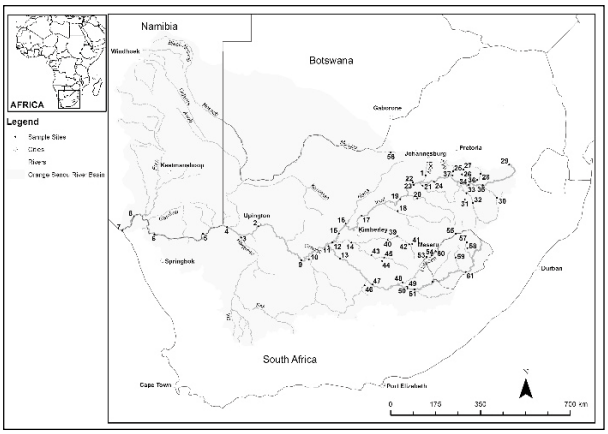

Figure 1: Distribution of the sites in the catchment.

The sediment's potential to activate specific toxicity through the AhR was also calculated. Villeneuve *et al.* (2002) devised toxic equivalent values (TEFs) for these PAH congeners—BaA, BaP, BbkF, Chr, DBA, InP—that actively bind to the AhR using the H4IIE-*luc* bioassay. These were used to calculate a toxic equivalent quotient (TEQ). The TEQs was compared to the Canadian interim sediment quality guideline (ISQG) of 0.85 ng/kg dm for dioxin-like compounds (CCME 2002). This value was based on TEFs for fish (Van den Berg *et al*. 2006).

3. Results and Discussion

The ranges of the various PAH categories are summarised in Table 1 as well as the guidelines to which they were compared.

The highest ΣPAH concentration (0.867 µg/g) was at site 22, located in a gold mining area, followed by sites 60 and 54. Sites 60 and 54 are downstream from the industrial region of the capital of Lesotho. The greatest ΣLPAH and ΣHPAH concentrations

were measured at sites 36 and 22 respectively and the highest ΣCPAH levels were at site 53. Sites of concern contains were 53, 54 and 60.

Table 1: Ranges of PAHs (µg/g dm) and TEQ (ng/g).

	Range	Mean
$\Sigma PAH_{16}s$	0.006 – 0.87	0.11
ΣLPAHs	0.004 – 0.22	0.04
ΣHPAHs	0.003 – 0.69	0.07
ΣCPAHs	0.002 – 0.37	0.04
ΣGPAHs	0.003 – 0.61	0.08
TEQ	0.00004 – 0.015	0.0015
Sediment quality guidelines		
Total GPAHs	Threshold Effects Concentration (TEC)	1 610
	Probable Effects Concentration (PEC)	22 800
ISQG	0.00000085 TEQ µg/g dm	

ΣLPAHs: low molecular mass PAHs (2 & 3 ringed); ΣHPAHs: high molecular mass PAHs (4–6 ringed); ΣCPAHs: carcinogenic PAHs; ΣGPAHs: guideline PAHs

48% of the sites had petrogenic PAHs. The nature of the pyrogenic sources was mostly from biomass combustion, as only 3% of the sites had petrogenic PAHs (Figure 2).

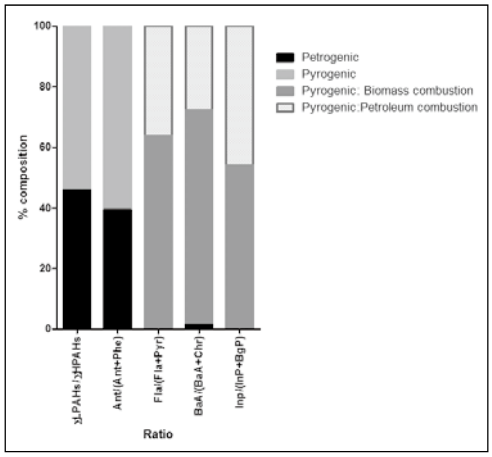

Figure 2: PAH congener ratios: pyrogenic vs petrogenic

When comparing the data of this study to the SQGs of MacDonald *et al*. (2000) and to the ISQG (CCME 2008), contrasting conclusions were drawn. All PAH levels were below the threshold effects concentration (TEC) of MacDonald *et al*. (2000) indicating that the PAHs in the sediments are probably non-toxic to benthic organisms. In contrast, the PAH levels exceeded 10 of the 12 TECs of the CCME (2008) ISQGs. Site 54 was the site with the most guideline failures, not meeting 8 of the PAH guidelines (Acea, Acey, Fla, Pyr, BaA, Chr, BaP and DBA). Other congener guideline exceedance was between 2 and 3% for Nap, Acea, Acey, Flu, Fla, Pyr, BaA, Chr, BaP and DBA. The more sensitive Canadian guidelines (CCME 2008) thus showed potential risk, especially from Acea, indicating that site 54 has potentially the most risk to aquatic life. Potential toxic risk for fish within the catchment, cf. TEQ values, were very high (Table 1).

4. Acknowledgments

This study was supported by the United Nations Development Programme—Global Environment Facility, Orange-Senqu River Strategic Action Programme.

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O-5

Evaluation of acute toxicity, micronuclei abnormalities and oxidative stress of effluents from a paint manufacturing industry in Midwestern Nigeria

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Toxicity testing of soluble fractions of industrial wastes with a battery of bioassays is extremely important since chemical analysis alone may not adequately provide integrated information on the effects of the pollutants on aquatic life. In this study, the acute toxicity of a paint effluent was investigated on *Clarias gariepinus* juveniles using a renewable static bioassay. At the end of each trial, the fish were dissected and their livers isolated for antioxidant enzymes activity determination. Toxicosis symptoms were observed in the fish juveniles exposed to various concentrations of the wastewater for 96 hrs under laboratory conditions. The LC_{50} value obtained was 28.1 ml/L with lower and upper limits being 3.59 ml/L and 20.8 ml/L respectively. The genotoxic effects of fish exposed in the effluents for 28 days using the micronucleus test on peripheral blood erythrocytes showed that the wastewater induced significant ($p<0.05$) concentration-dependent increase in micronuclei, binucleated and immature erythrocytes. Activities of superoxide dismutase increased significantly ($p<0.001$) while catalase and glutathione peroxidase activities decreased with increase in effluent concentration. The data shows that the tested effluent was capable of causing cyto-genotoxic effects in exposed individuals.

Keywords: Effluent, *Clarias gariepinus*, toxicity, Micronucleus, Antioxidant enzymes

1. Introduction

Effluents from paint industries are among the problematic environmental issues faced by the Nigerian Chemical Manufacturing Sector. Industrial wastes inappropriately treated releases toxic compounds that can contaminate water bodies. Evaluation of toxicity of these effluents should not be by chemical analysis alone but by bioassays (Tigini *et al*. 2011).

Fish are extremely valuable in toxicity monitoring as they appear to possess the same biochemical pathways with mammalian species to deal with the effects of toxic agents. Genotoxic effects of chemicals using micronucleated erythrocytes of fish have been used for cytogenetic studies, so also is the micronucleus (MN) test. Variations in the antioxidant enzymes of fish also serve as useful biomarkers for the detection of pollutants in the aquatic ecosystem. Catalase and the peroxidases are the major enzymes involved in H_2O_2 detoxification.

The need to establish the nature of treated wastes generated by paint industries in Nigeria necessitated this case study. It was undertaken using a battery of bioassays to ascertain the level of compliance of a paint factory in Midwestern Nigeria with the regulatory bodies. The results obtained would provide a more thorough evaluation of hazards associated with industrial paint effluents discharged into the environment.

2. Materials and Methods

2.1 Sample collection and analysis

The paint effluent used for this study was obtained from a paint manufacturing industry in Agbor (6°16'0"N, 6°9'0"E) in Delta State of Nigeria. The samples were collected at the point of discharge in Orogodo River with washed plastic containers in ice chest and stored in the refrigerator at 4°C until needed.

2.2 Acute Toxicity and Micronucleus Assay

Juveniles of *C. gariepinus* used (17±1.0 cm long, 12.4±0.5 g mean weight) were purchased from a fish farm in Benin City (6°15′N, 5°25′E). The fishes were prepared for acute toxicity test and micronucleus (MN) following previous methods (Olorunfemi *et al*., 2015). The frequencies of micronuclei and other nuclei lesions were scored along with MN as biomarkers of cyto-genotoxicity.

Results obtained for the confidence limits of the LC_{50} were subjected to regression statistical analysis with Duncan's multiple range test in one way ANOVA, using Windows SPSS version 16.0 to compare paint effluent concentrations and control.

2.3 Enzyme assays:

The fish used in the 96 hr acute toxicity test were

dissected and their livers removed and kept at -80°C for the enzyme assays. Organs were homogenized in phosphate buffer (0.2 M, pH 6.5) with a ratio of 1:4, respectively and the homogenates centrifuged at 10,000 rpm for 15 minutes at 4°C to produce a clear supernatant for the enzyme assays. SOD activity was determined according to methods of Misra and Fridovich, (1989). Catalase was determined by the method of Cohen *et al*., (1970) while peroxidase activity was determined according to Civello *et al*. (1995).

3. Results and Discussion

Unlike the control groups of *C. gariepinus* juveniles, abnormalities were observed in fish exposed to different concentrations of the effluent. The LC_{50} of the test organisms derived at 95 hours was 28.1 ml/L with lower and upper limits being 3.59 ml/L and 20.8 ml/L respectively. The computed regression equation was Y= -5 + 4* (R = 1.37, Y = probit kill) (Fig. 1).

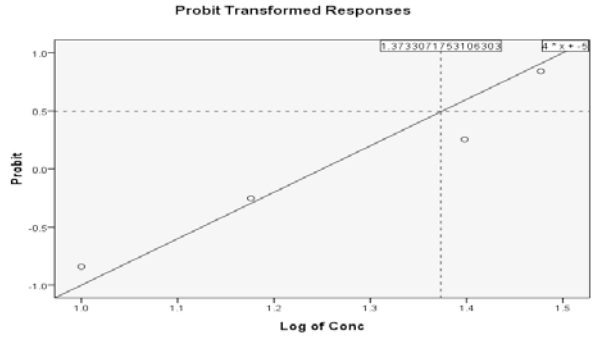

Fig. 1: Mean probit mortality and log conc. of *C. gariepinus* juveniles in paint effluent for 96 hours

The respiratory abnormalities observed suggest that mortality of the exposed fish is due to impaired metabolism and nervous disorder. The MN genotoxicity showed that compared with the control, the frequency of micronucleus induction in mature and immature erythrocytes of *C. gariepinus* increased significantly ($p<0.05$) with increase in effluent concentration (Data not shown). Our results are in agreement with Kligerman (1982) who stated that MN and nuclear abnormalities in fish are useful indicators of cyto-genotoxic effects of contaminants in aquatic organisms.

Paint effluent treatment of the fish resulted in significant concentration-dependent increase in SOD activities and concomitant decrease in CAT and GSH-Px activities (Table 1). These findings are consistent with earlier reports with heavy metal treatment in catfish (Batool, 2014) which shows that variations in the antioxidant enzymes can also serve as useful biomarkers for the detection of pollutants in the aquatic ecosystem

Table 1**:** Changes in anti-oxidative enzyme activities cultivated in paint effluents concentrations

Conc. of Paint Effluent (ml/l)	Mean ± SD of Enzyme Activity (µ/mg protein)		
	SOD	CAT	GSH-Px
Control	70.10 ±1.00	410.58±18.6	0.85±0.05
5	80.30±0.50	482.40±9.40	0.58±0.00
10	86.42±0.58	276.50±4.31	1.00±0.05
20	93.16±0.58	250.60±4.60	0.45±0.00
30	95.60±1.00	146.80±3.60	0.15±0.00

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O-6

Histopathologic assessment of co-joint Cd and Pb exposure in rats

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Lead (Pb) and cadmium (Cd) are the two most abundant toxic metals in the environment. Although numerous research studies on Pb and Cd toxicity focused on single metal exposure, it has been recognized that humans are exposed to a combination of metals in the environment. The current study investigated co-joint Cd and Pb toxicity in rats by intravenous injections (i.v.) of single or combined Cd and Pb to evaluate the effects of co-joint exposure of these metals. Sixteen Sprague Dawley rats were randomly assigned to four groups as follows: control (0.5 ml saline), Cd (0.5mg/kg), Pb (2.5 mg/kg) and Cd/Pb (0.5mg/kg and 2.5 mg/kg of Cd and Pb, respectively). A total of 30 injections were administered to each rat via the lateral tail vein for 2 months. At the end of the exposure, the rats were sacrificed and samples were collected for histopathologic and metal analysis. In the liver, mean concentrations of Cd between the Cd (0.45 mg/kg) and Cd/Pb (0.40 mg/kg) groups were not different ($p > 0.05$). Similarly, mean concentrations of Pb between the Pb (0.01 mg/kg) and Cd/Pb (0.01 mg/kg) groups were not different ($p > 0.05$). Histopathologic lesions including renal tubular degeneration/necrosis and hepatocellular apoptosis were similar between Cd and Cd/Pb groups as well as between Pb and Cd/Pb groups. These preliminary findings indicate that co-joint Cd and Pb exposure in rats did not induce greater than additive (synergism and potentiation) nor less than additive (antagonism and inhibition) effects.

Keywords: Cadmium, Lead, Co-joint toxicity, Intravenous exposure, Histopathology

1. Introduction

Cadmium (Cd) and lead (Pb) are toxic metals that co-exist ubiquitously in the environment. Exposure to Pb causes hematological disorders, nervous system disturbances and impairment of liver and kidney functions. Cases of Pb poisoning have been reported in children in regions with long history of mining (Yabe et al., 2015). Mortalities due to Pb poisoning have been widely reported.

Similarly, Cd toxicity results in a wide range of biochemical and physiological dysfunctions in humans. Toxicity in multiple organs such as kidneys, liver, lungs, brain and bones has been reported. Chronic Cd poisoning can result in nephrotoxicity, osteoporosis, cardiovascular diseases, renal failure and neurodegenerative conditions (Umemura 2000). One of the most severe forms of chronic Cd toxicity in humans is *itai-itai* disease (meaning ‘‘Ouch-ouch disease’’ in English), a syndrome that mainly occurs in post-menopausal women and is characterized by osteoporosis with osteomalacia, renal tubular disorder, and renal anemia.

Although numerous research studies on Pb and Cd toxicity focused on single metal exposure, more work need to be focused on the simultaneous effects of these metals as it has been recognized that humans are exposed to a combination of metals in the environment. Earlier studies indicate that interactions between or among metals occur and are characterized by alterations in both tissue metal concentrations and toxicity. Three categories of joint toxicity that have been identified include greater than additive (synergism and potentiation); additive (no interaction); and less than additive (antagonism and inhibition). Thereore, the current study investigated co-joint Cd and Pb toxicity in rats by intravenous injections (i.v.) of single or combined Cd and Pb to evaluate the effects of co-joint exposure of these metals. Ovariectomized female rats were used since characteristic effects of itai-itai disease could only be reproduced in ovariectomized female rats by i.v. injections (Umemura 2000).

2. Materials and methods

2.1 Experimental Animals and Treatment

Sixteen Sprague Dawley rats (Crj: CD (SD) IGS, Charles River Japan, Kanagawa, Japan) were obtained at seven weeks of age. At week

8, ovariectomy was done under anesthesia with xylazine (10 mg/kg i.p.) and ketamine (100 mg/kg i.p.). At ovariectomy, the body weights ranged from 236 to 295 g. Three weeks later (10 weeks old, weighing 307 – 406 g), the rats were randomly assigned to four groups and allowed 1 week of acclimatization. Administration of Pb and Cd began at eleven weeks of age with weights ranging from 307 to 398 g. The rats were assigned to the following groups as follows: (I) saline injection (Saline, Japan); (II) CdCl2 injection (CdCl2, Japan); (III) PbCl2 injection (PbCl2, Japan) and CdCl2 + PbCl2 injection. Four rats were randomly selected and assigned to each of the four groups in plastic cages.

A total of 30 injections were administered to each rat via the lateral tail vein for 2 months. In the control group, 0.5 ml of saline was injected. The rats in the Cd group were injected with 0.5 ml CdCl2 dissolved in saline (0.5mg/kg) as reported in previous studies (Umemura 2000). The rats in the Pb group were injected with PbCl2 dissolved in saline water (2.5 mg/kg). The rats in the Cd + Pb groups were injected with 0.5 ml consisting of a mixture of CdCl2 (0.5mg/kg) and 2.5 mg/kg of PbCl2 dissolved in saline water. Since literature for Pb injection studies in scarce, a Cd:Pb ratio of 1:5 used in per os administration studies in rats was adopted (Grosicki and Kowalski 2002). At the end of the exposure, the rats were sacrificed and samples including liver, kidney, spleen, lung and brain were collected for histopathologic and metal analysis.

3. Results and Discussion

There were no differences in weight gains between rats exposed to single metals and metal mixtures. In the liver, mean concentrations of Cd between the Cd (0.45 mg/kg) and Cd/Pb (0.40 mg/kg) groups were not different ($p > 0.05$). Similarly, mean concentrations of Pb between the Pb (0.01 mg/kg) and Cd/Pb (0.01 mg/kg) groups were not different ($p > 0.05$). Findings in the kidneys were also not different.

Histopathologic lesions including renal tubular degeneration/necrosis and hepatocellular apoptosis were similar between Cd and Cd/Pb groups as well as between Pb and Cd/Pb groups.

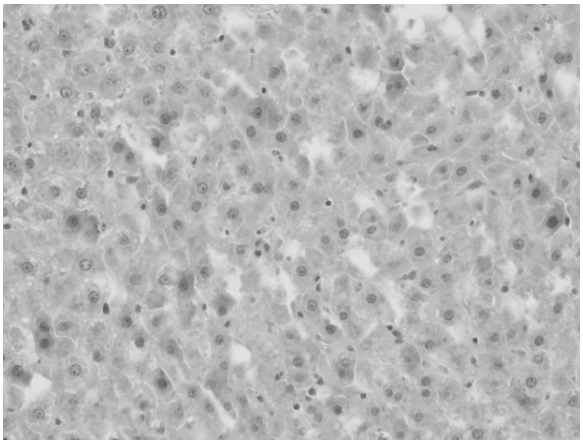

Figure 1: Liver - Co-joint Cd and Pb group showing single cell necrosis (apoptosis), degeneration, increased eosinophilia, etc. H&E stain.

These preliminary findings indicate that co-joint Cd and Pb exposure in rats did not induce greater than additive (synergism and potentiation) nor less than additive (antagonism and inhibition) effects. However, haematological and biochemical analyses as well as biological response need to be conducted to fully determine the interaction of these metals.

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SL-3

Application of a sediment quality triad to evaluate the risk posed by metals to a freshwater wetland ecosystem

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The sediment quality triad (SQT) has been applied successfully to evaluate the risk that contaminants in marine sediments pose to the marine ecosystem. A modified SQT based on a weight of evidence (WoE) approach was applied to sediments from a known metal contaminated wetland in the Taaibosch Spruit. Five sites were sampled during a wet and dry season and the standard three lines of evidence (sediment chemistry, sediment toxicity and benthic macroinvertebrate diversity). Results for sites adjacent to an industrial complex indicated that there was a strong relationship between benthic invertebrate community structures and sediment contamination levels. This was further supported by acute toxicity recorded in the sediments from these sites. Bioaccumulation data supported the increased metal exposure and toxicity at these sites. Thus the WoE indicated that sediments from the two sites adjacent to the industrial complex posed a high risk to the aquatic ecosystem but decreased further downstream.

Keywords: toxicity testing, macroinvertebrate assemblages, weight of evidence

1. Introduction

The sediment quality triad (SQT) has successfully been used to assess the effect of chemical contamination on sediment quality. The SQT assesses the chemical concentrations, benthic macroinvertebrate assemblages and toxicity of the sediments under laboratory conditions to evaluate the effect of the various contaminants in an ecosystem (Chapman *et al*. 2013). Shaddock and Wepener (2015) used the SQT to demonstrate how the different components (i.e. lines of evidence – LoE) of the triad can be integrated using a weight of evidence (WoE) approach.

De Klerk *et al*. (2013) found that the sediments of the Taaibosch Spruit wetland had amongst other metals, very high Hg concentrations. The high metal concentrations were attributed to point source releases from a large industrial complex adjacent to the wetland. The aim of this study was therefore to determine whether the SQT, using the WoE approach, is a suitable tool to assess the overall risk of the contaminated sediments to the Taaibosch Spruit wetland.

2. Methods

Five sites were selected along the length of the wetland. Sampling was undertaken during the summer rainy season and the winter non-rain season. Water, sediment and macroinvertebrate community sampling was undertaken at each site during the two surveys. The SQT assessment with different LoE was undertaken as follows:

Chemistry: Metal concentrations were measured in filtered water (dissolved fraction) and sediment samples (total extraction) using an X-7 Thermo Elemental ICP-MS. Other sediment parameters determined were total organic carbon (TOC) and sediment grain size. For bioaccumulation the metal concentrations were measured in the surviving chironomids that were used in the toxicity assessment.

Sediment toxicity: Sediment samples were subjected to two standard OECD sediment toxicity bioassays, i.e. the Daphnid and Chironimid mortality tests.

Benthic macroinvertebrate assemblages: The standard sampling protocols used for the gravel/ sand/mud and stones-in-current biotopes of the SASS5 macroinvertebrate technique were applied at all the sites. Samples were preserved using bufferd formalin and a vital dye. Samples were identified and enumerated in the laboratory. The community structures from each site were assessed using univariate (Shannon-Weiner diversity index) and multivariate (redundancy analyses) statistical analyses.

Interpreting LoEs: an ordinal ranking scheme was developed to categorize the results obtained for the different chemistry, toxicity and macroinvertebrate assemblage LoEs into three different risk categories (Table 1).

3. Results

The different risk ranks for each of the LoEs considered during this study are presented in Table 2. Site 5 was the proposed reference site for this study. The results obtained for Site 5 in summer indicated that this site had a negligible potential for adverse effects on the aquatic environment as a result of sediment contamination. The remaining

sites and as well as Site 5 in winter indicated a potential for the sediment to pose a high risk to the aquatic environment. With the exception of Site 3 in summer, which had a risk for adverse effects, the remaining sites had either the same or a lower risk in summer when compared to the winter overall WoE.

Table 1. Ordinal ranking scheme to assign risk categories to each of the lines of evidence (LoE) for the different components of the sediment quality triad.

RISK	● - High risk	◍ - Moderate risk	○ - No risk
CHEMISTRY			
Water Metal Concentrations	> Acute Effect Value	> Chronic Effect Value < Acute Effect Value	< Chronic Effect Value
Total Metal concentrations	Concentrations> upper bound PEL	Concentration >lower bound ISQG < PEL	Concentrations< lower bound ISQG
Bioavailable Concentration	> Acute Effect Value	> Chronic Effect Value < Acute Effect Value	< Chronic Effect Value
Bioaccumulation	10 – fold Δ control	2 – fold Δ control	No Δ control
BENTHIC DIVERSITY			
Benthic diversity	Marked decrease (>30%) from reference Shannon-Weiner diversity index value	Slight decrease (10% - 30%) from reference Shannon-Weiner diversity index value	<10% decrease from reference Shannon-Weiner diversity index value
Benthic community structure		"Fine Site" – generally characterized by lower species diversity and a substrate composed mainly of fine sand, silt and clay	"Course Site"- generally characterized by a higher species diversity and a substrate composed mainly of gravel, course and medium sand
TOXICITY			
Chironomid Toxicity Test	Δ of ≥50% of the endpoint relative to control	Δ of ≥20% of the endpoint relative to control	Δ of <20% (or no Δ) of the endpoint to negative control
Daphnia Toxicity Test	Δ of ≥30% of the endpoint relative to control	Δ of ≥10% of the endpoint relative to control	Δ of <10% (or no Δ) of the endpoint to negative control

The sites around the industrial complex (Sites 1 and 2) indicated a degree of sediment contamination, with the effects being compounded in the non-rainy winter season due to reduced dilution capacities and increased deposition. These sites therefore have the potential to release sediment bound contaminants into the water column to levels that would pose a high risk to the aquatic ecosystem.

The higher TOC content of these sites would have contributed to the expressed toxicity and increased availability of metals once these samples were aerated in the toxicity exposures. Therefore it is not possible to assume that low species diversity indicates an impaired system. Generally, species richness did correlate with contamination levels and particularly with mercury levels at Site 1. It is however, not possible to rule out habitat alteration as a factor driving benthic community structure, but changes in the species diversity due to contaminants could be secondary to habit changes.

4. Discussion and conclusion

The WoE risk categories were determined assuming that those sites with fine sediments, high TOC, and low species diversities were impaired. However, there were sites in the study area which exhibited toxicity responses and elevated contaminants on analysis, which were grouped with coarse sediments which had low TOC and increased species diversities. There are however large margins of uncertainty when trying to extrapolate the effects of laboratory based toxicity tests to field conditions.

Table 2. Lines of Evidence (LoE) used to classify the overall risk of sediments to the Taaiboschspruit wetland. For the water and sediment chemistry, the following metals are represented by the circles: Water (Al, Cd, Cr, Cu, Mn, Pb, Zn) – Total Metal (Cd, Cr, Cu, Hg, Pb, Zn) – Bioavailable Metal (Al, Cd, Cr, Cu, Mn, Pb, Zn) – Bioaccumulation (Al, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Zn).

Site	Season	Chemistry: Water-Total-Bioavailable-Bioaccumulation	Benthic fauna: Diversity – Community structure	Toxicity: Chironomid-Daphnia	Overall WoE: Risk ranking
1	Summer	○○○●○●○○●○○●○●○●●●●●●●○●○○○○●○○○○	○○○	●○○	○
	Winter	○○○●○●○○●○○●○●○●●●●●●●○○○○○○●○○○○	●○○	●○●	●
2	Summer	○○○●○●○○●●○●○○●○●●●●○○○○○●○○○○	○○○	○○○	○
	Winter	●○○●○●●○●○○●○●○●●●●●●●○○○○○○●○○○○	●○○	○○○	○
3	Summer	○○○●○○○●○○○○○○○●○●●●●●○○○○○○○○○○	●○○	●○○	●
	Winter	○○○●○●●○●○○○○○○●○●●●●●○○○○○○○○○○	○○○	●○○	○
4	Summer	○○●●○○○●○○○○○○○●○●●●●●○○○○○○●○○○	○○○	●○○	○
	Winter	○○○●○●○○●○○○○○○●○●●●●●○○○○○○○○○○	○○○	●○○	○
5	Summer	○○○●○●●○●○○○○○○●○●●●●●●○○○○○○○○○	○○○	○○○	○
	Winter	○○○●○●○○●○○○○○○●○●●●●●○○○○○○●○○○	○○○	○○○	○

It is apparent from this study site that when using of the WOE approach to assess the sediment quality in an area, it is necessary to include the benthic community structure data. It is also imperative that these data are correctly interpreted so that the data can be used appropriately. The use of benthic communities prevents the results from being misinterpreted, and effects being estimated as the current ecological state of the site. For this reason the benthic community LoE provides the most ecologically relevant information for interpreting the SQT. Thus the overall risk was greatest at Sites 1 and 2 and dissipated downstream to Site 5 before the Taaibosch Spruit flows into the Vaal River.

5. Acknowledgements

The authors wish to thank Spectrum (University of Johannesburg) for the metal analyses.

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O-7

Health assessment and biomarker responses of *Clarias gariepinus* from impoundments in an urban area, South Africa

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The Klip River that flows through the largest urban area in South Africa, Soweto/ Lenasia, is subjected to various forms of pollution and anthropogenic influences. A health assessment on the sharptooth catfish, *Clarias gariepinus*, from four water bodies within this aquatic system was conducted during the peak low flow season of 2013 and 2014. The fish health assessment index (HAI) and hepato-somatic index (HSI) were calculated and biomarkers of exposure response included acetylcholinesterase activity (AChE) and cytochrome P450 demethylating activity (CYP) were determined. Fish from Orlando had the highest HSI scores. The fish AChE activity was inhibited for both the 2013 and 2014 seasons, and the CYP was up-regulated relative to the control. The fish from all sites were in poorer health compared to the control group, with fish from Nancefield having the highest HAI score for both seasons. The poor health and biomarker responses in fish from the Klip River system, indicate potential risk to the health of humans and other wildlife.

Keywords: Health assessment index, hepato-somatic index, acetylcholinesterase, cytochrome P450, *Clarias gariepinus*, ecotoxicology

1. Introduction

The Klip River drains the Witwatersrand area in Gauteng, the most populated province in South Africa. It flows through the densely populated urban areas of Soweto and Lenasia. The Klip River is considered one of South Africa's most polluted rivers (McCarthy et al., 2006) and subjected to various forms of anthropogenic stressors. The introduction of stressors into an aquatic system may lead to changes within the different levels of organization in organisms—from molecular to population level. The possible effect of these stressors was assessed on *Clarias gariepinus*, a hardy fish species that is abundant in South Africa (Skelton, 2001). The fact that they can survive in harsh conditions makes them an ideal indicator of health in the polluted Klip River system. Over the years various health- and organo-somatic indices (Adams *et al*., 1993) and biochemical response assays (Van der Oost *et al*., 2003) have been used to express the overall health and condition of fish. Using these methods it makes it possible to assess the aquatic ecosystem on different levels of organisation.

2. Materials and Methods

2.1 Fish sampling

Clarias gariepinus were sampled during the peak low flow season of 2013 and 2014 from four impoundments in Soweto/Lenasia (Figure 1). Fish were collected using gill nets (118- & 150 mm) and kept in aerated containers until field analysis commenced.

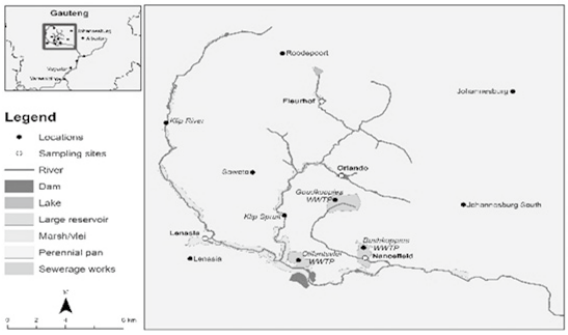

Figure 1: Fish sampling sites in Soweto/Lenasia

2.2 Health assessment and biomarker response assays

The health of the fish was analysed using a modified version of Adams *et al.*'s (1993) necropsy based health assessment index (HAI). Fish were weighed, measured and macroscopically examined for external abnormalities before euthanasia. Following dissection, the internal organ were macroscopically assessed for internal abnormalities. The liver masses were recorded in order to calculate the hepato-somatic indices (HSI).

Acetylcholinesterase activity (AChE) was measured according to the protocol of Ellman (1961). The demethylating activity of cytochrome P450 (CYP) was determined with a kit from Arbor Assays (K011-F1). The HAI and biomarker response assays were also completed on a control group of fish kept at the NWU under standard aquarium conditions. Gaussian distribution was determined using the D'Agostino and Pearson omnibus normality test. The non-parametric results were tested for significant differences using the

Kruskall-Wallis test coupled with Dunn's multiple comparison post hoc test.

3. Results and Discussion

The HAI, HSI and biomarker response (AChE and CYP) results are shown in Figure 2.

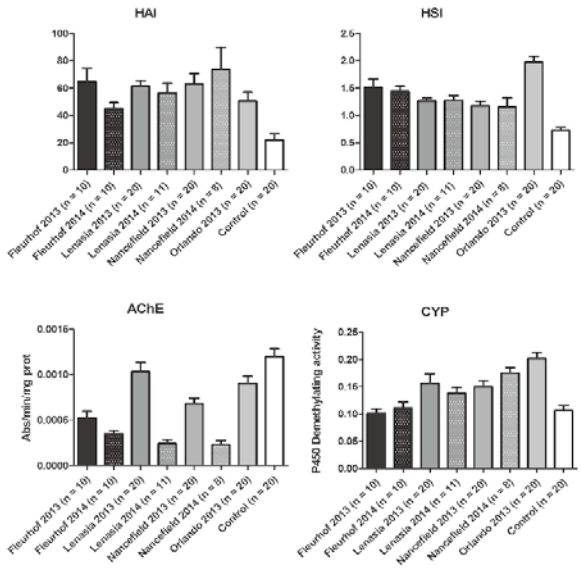

Figure 2: Health assessment and biomarker response results of *Clarias gariepinus* from Soweto/Lenasia

Nancefield 2014 had the highest HAI score (73.8±29.8) (90% liver abnormalities). Fleurhof 2014 had the lowest HAI and was the only site not significantly higher than the control ($p < 0.0001$). The health of all the fish sampled in the study area was poorer than the control—as indicated by their higher mean HAI score—as well as lower than *C. gariepinus* health from the Okavango Delta panhandle, a pristine system (Van Dyk *et al*., 2009). Compared to Pongolopoort Dam's HAI—exposed to organohalogens (McHugh *et al*., 2013)—only Fleurhof 2014 and the control were in better health.

The HSI of the study ranged from 1.17–1.98, which is higher than the HSI from the Okavango floodplain (0.5%±0.1) (Van Dyk *et al*., 2009) as well as Pongolapoort Dam, which was 0.93%±0.005 (McHugh *et al*., 2013) and is consistent with the liver abnormalities seen during the HAI necropsy. The site with the greatest HSI was Orlando (1.98%±0.44) and similar to the HAI, Fleurhof 2014 was the least affected.

AChE was inhibited—lower than the control—as well as seasonal decrease in activity. The greatest significant inhibition was seen at Nancefield 2014 and Lenasia 2014 ($p < 0.0001$). Lenasia 2013 had the smallest inhibition. The up-regulation of the CYP enzymes, relative to the control, shows that the organisms were actively bio-transforming xenobiotics. Orlando had the highest CYP activity, followed by Nancefield 2014 ($p < 0.0001$). The Fleurhof fish (2013 & 2014) had low CYP activity.

These biomarker responses indicated the potential presence of xenobiotics in the system and supported the effects seen for the HAI and HSI.

According to the results, the worst affected sites were Orlando and Nancefield. Orlando is situated in central Soweto and is thus the most susceptible site to anthropogenic stressors in the study area. Secondly Nancefield was the final sampling site downstream, possibly receiving the cumulative load of stressors from upstream. Fleurhof—the most upstream site—was the least affected, even though it is situated in an urban environment.

The fact that the fish in the urban areas are in poor health and that xenobiotics may be present indicates that the health of humans and other wildlife in the area, utilising this system, may be at risk.

4. Acknowledgments

This study was supported by the Water Research Commission of South Africa (WRC, K2/2242).

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O-8

Assessment of water quality and documentation of macro- and micro-invertebrates in dams and rivers of Qwaqwa, South Africa

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This study was aimed at assessing water quality status and documentation of macro- and micro-invertebrate organisms in freshwaters of Qwaqwa area of Maluti-A-Phofung municipality in South Africa. Water samples were collected seasonally from 5 rivers (Metsimatsho, Namahadi, Khoptjwane, Kollatshwene and Elands) and 2 dams (Metsimatsho and Fikapatso). The mean electrical conductivity (EC) levels were the highest in Kollatshwene, Elands and Khoptjwane rivers and Fikapatso dam. The TDS levels were high in rivers ranging from 21 mg/l to 297 mg/l than that of dams with 13 to 60 mg/l dams. The most prevalent taxa was Baetidae 34.22%; followed by Corixidae (12.08%) with the least being Ceratopogonidae (6.04%). Kollatshwene, Elands and Khoptjwane rivers had the highest percentage occurrence of tolerant families, 71.4%; 61.9% & 60.0% respectively. The coliform bacteria were also identified from Qwaqwa waters.

Keywords: macro-invertebrates; micro-invertebrates; water quality of dams and rivers.

1. Introduction

South Africa's freshwater resources include rivers, man-made lakes, dams, wetlands and aquifers (Oberholster & Ashton, 2008). Water resources in this country are scarce and extremely limited in global terms (DWAF, 2009) due to country's climatic variations from desert to semi-desert in the west to sub-humid along the coastal area. Consumption of water containing pathogens or toxic chemicals may result in poor hygiene that poses a serious risk to human and animal health. Many water quality studies conducted in South Africa (Oberholster & Ashton, 2008; DWAF, 2009) indicated that poor maintenance of wastewater and sewage treatment infrastructure contributes to the pollution of water resources upon which rural communities depend for all their domestic and other purposes. There is no information on the water quality of Qwaqwa dams and rivers, as a result the current study was conducted to document such information.as it is of importance to both animal and human health.

2. Materials and Methods

2.1 Sample Collection and water quality assessment

Water samples were collected seasonally from 5 rivers (Metsimatsho; Namahadi; Khoptjwane; Kollatshwene and Elands) and 2 dams (Metsimatsho and Fikapatso). *In situ* measurements of temperature; pH; salinity; total dissolved solids (TDS) and electrical conductivity (EC) were taken using HANNA HI 9828 multiparameter instrument, (HANNA Inc., Romania).

2.2 Macro- and Micro-invertebrate Detection

Water was collected in 1 liter bottles from rivers and dams and then dispensed in petri dishes in the lab. Macro-invertebrates were observed and recorded by used of dissecting micrscope. Bacterial cultures were prepared on agar paltes in the lab for obervation on gram-neative and gram positive bacteria.

2.3. Ethics approval

No ethics approval was needed as the study was conducted in rivers and dams and only invertebrates (insects) were collected.

3. Results and Discussion

Water quality status through the *in situ* assessment of the three rivers, Khoptjwane, Kollatshwene and Elands advocates seasonal decline in water quality. This is due to constant increase of total dissolved solids, salinity and electrical conductivity values which subjects water bodies to eutrophication particularly if concentration of phosphates and nitrates increase (Oberholster & Ashton, 2008). The results indicate high solubility of elements or salts in water particularly in spring resulting in high concentrations of salts. This could be due to low river flow and erratic rainfall distributions during autumn and spring. Electrical conductivity (EC) also increased with increasing salt concentration especially when temperature increased in spring. The EC levels in rivers were elevated by concentration of the water TDS including nitrates and ammonium compounds. These were the

Table 1: Analysis of nutrient levels detected from the sampled rivers and dams.

	National standard ranges	MAP Internal standards	Observed measurements (mg/l)						
			Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7
NH_3	<6	<4	0.40	0.05	0.20	0.50	0.65	*	*
NO_3	<15	<9	1.40	1.50	0.10	0.50	1.80	*	*
PO_4	<10	<6	0.57	0.20	0.23	0.10	0.83	*	*
TSS	<25	<15	30	9	8	3	40	4	14
Cl	Optional	0.15 – 0.25	**	**	**	**	**	**	**
COD	≤75	≤50	30	29	09	17	11	40	15
pH	5.5 – 9.5	6.5 – 8.7	7.4	7.7	7.9	7.8	8.2	8.1	6.9

Site1 = Metsimatsho River; site 2 = Namahadi River; Site 3 = Khoptjwane river; Site 4 = Kollatshwene River; Site 5 = Elands River; Site 6 = Fikapatso Dam & Site7 = Metsimatsho Dam.

rivers with much of faecal contamination due to the dense township settlements in their proximity whose dumping areas are either close to the rivers or directly into rivers. Salinity levels of rivers were higher than that of dams because rivers receive the effluent discharge from municipality sewage system. This makes waters from these rivers not suitable for human consumption than the potable water provided by dams. Nutrient levels of NO_3^-, PO_4^- and NH_4^+ observed from the sampled waters (Table 1) were at minimal levels that comply with municipality water standards. In contrast, Metsimatsho and Elands rivers fail the municipality water standards with high TSS levels (30 mg/l and 40 mg/l, respectively) which could have been influenced by high faecal contamination.

Elands river constituted three (3) macro-invertebrate families of highly sensitive SASS status and these includes Oligochaeta, Culicidae and Syrphidae, all with the SASS value “1”; whereas

Kollatshwene river has Oligochaeta and Culicidae. According to the sensitivity scale by Dickens & Graham, (2001), Culicidae, Oligochaeta and Syrphidae are bioindicators of deteriorated water quality. It can be concluded that water quality status at sampled rivers and dams may be of different pollution levels since Qwaqwa rivers comprises wastewater with municipality sewer discharge effluent, whereas dams provide potable water for the Qwaqwa communities.The results based on microinvertebrate occurrence indicate a poor water quality status among all the sampled rivers.

4. Competing interests

Authors have no conflict of interest.

5. Acknowledgements

The study was made possible by the NRF-NSTF TW Kambule 2013-2014 Award (GUN92546) and NRF Incentive grant for rated researchers (GUN94187) both made available to OMMT. We thank Mr Tello Mphuthi (MAP Water) for the analysis of water samples.

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O-9

The presence of heavy metals, regarded as toxic to aquatic biota, in the Mooi River catchment area, North West Province South Africa.

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The heavy metal concentration in water samples, collected during a low and high flow period from six different sites in both the Wonderfontein Spruit/ Mooi River and Loop Spruit/ Mooi River areas, was determined by means of ICP-MS. Only Zn, Cu and Al exceed the TWQR values. It was found that the nearby gold mine activities were not significantly involved regarding heavy metal pollution of the Moor River catchment area.

1. Introduction

Heavy metals in running water mainly originate from soils in the drainage area and are generally the result of weathering and anthropogenic activities. The presence of gold mine activities in the catchment area investigated during this study, may act as a source for toxic heavy metals for the two main tributaries of Mooi River, namely the Wonderfontein Spruit which meanders through a dolomite rich area and the Loopspruit with a soil surface basin.

The aim of this study was to establish to what extent the gold mine activities may contribute to the dissolved fraction of heavy metals in these two rivers and if the concentrations exceed the water quality ranges relevant to South Africa.

2. Materials and Methods

2.1 Study Area

Six sites each in the Wonderfontein/Mooi River and in in the Loop Spruit/Mooi River areas were respectively selected for the sampling water during the low and high flow season (Fig.1) Site1 in the Wonderfontein Spruit is near Carletonville, a gold mining district. Site 2 is at the Bovenste Eye and is regarded as a pristine habitat possibly due to the fact that the Gatsrand geological ridge prevents it from getting contaminated by mine activities. Site 3 in the Mooi River at Muiskraal, is joined by the Wonderfontein Spruit. Site 4 is adjacent to the upper part of Boskop Dam while Site 5 is situated in the Wonderfontein Spruit at Turffontein, also characterized by a number of eyes. Site 6 is at the Eye of the Gerrit Minnebron, another pristine site and which acts as an important water source for the Town of Potchefstroom. Its water joins the Mooi River at Site 4. With regard to the Loopspruit Site 1 is situated near Fochville and was characterized by a number of eyes. Site 2 is more or less 200m downstream from Site1 and is joined by a stream draining a gold mine area. Site 3 is in small stream feeding into the Loop Spruit and next to a scrapyard while Site 4 is at Ensel Spruit next to the Potchefstroom-Vereeniging Road. Site 5 is below Klipdrif Dam and Site 6 in the Mooi River at the South Bridge in Potchefstroom.

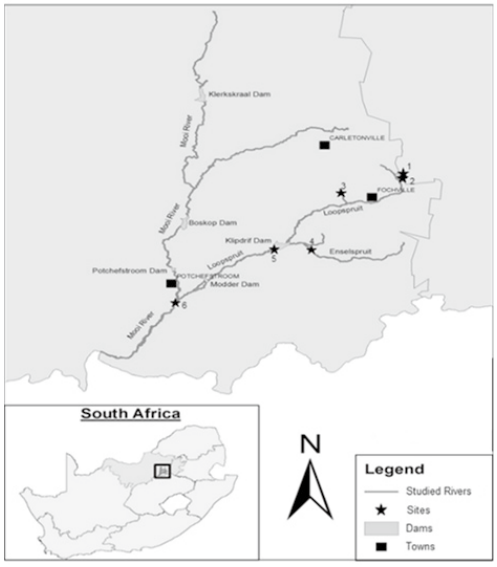

Fig 1: The Wonderfontein Spruit/ Mooi River survey area.

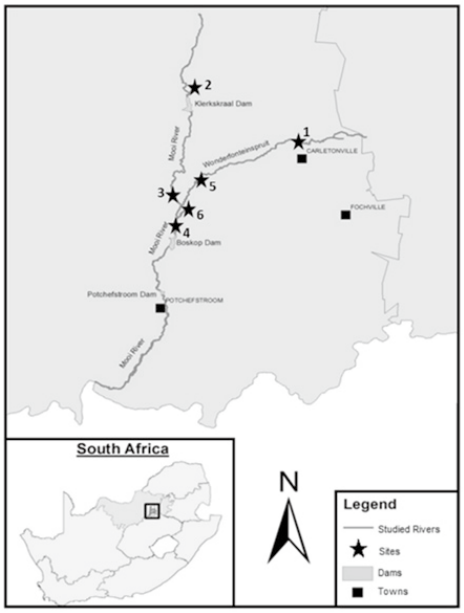

Fig 2: The Loop Spruit/ Mooi River survey area.

2.2 Heavy metal analysis and abiotic factors

Water samples were collected at each of the sites during a low and high flow season. The samples were filtered through Whatman filter paper (0.45 µm) and the filtrate was used for heavy metal analysis in a ICP-MS Concentrations of the following toxic metals were determined: Al, Cr, Mn, Cu, Zn, As, Se, Cd and Pb. Temperature, conductivity, turbidity, flow rate and pH were also measured at each of the sites.

3. Results and Discussion

The results obtained for the Wonderfonten Spruit and Loop Spruit are respectively presented in Tables 1 and 2. From Table 1 it is evident that only Al, Cu and Zn exceeded the TWQR in both the surveyed areas at all the sites. The high concentration of Al at Site 1 (Table 1) is probably due to its abundance in minerals and clay while it is also inert in the aquatic environment. Aluminium and Zn is however also present in contaminated groundwater coming from nearby mining activities. The significant decrease in the concentrations of Al, Zn and Cu from Site 2 downstream may be ascribed to the dolomite basin characterized by a neutral to alkaline pH leading to a decrease in the solubility of heavy metals, as well as to a dilution effect by the water from Bovenste Eye .The relatively high concentration of As and Cu at Site 1 is probably related to the gold mine activities nearby. With regard to Cr and Mn it was found that these two metals were present in higher concentrations in the high flow than in the low flow period. It must be kept in mind that Cr is naturally present in water and rocks while Mn is a dominant metal in dolomite structures Natural weathering which may be enhanced during the high flow season may be partially responsible for the higher concentrations found for these two metals.

With regard to the Loopspruit (Table 2) it is evident that as in the case of the Wonderfontein Spruit only Al, Cu and Zn exceeded the TWQR. The extremely high concentration of Al at Site 1 during the low flow period is possibly due to natural weathering and anthropogenic activities in the Town of Fochville. Although there was a significant decrease in this concentration downstream this phenomenon continues for the entire surveyed area. The entering of gold mine effluent at Site 2 may be responsible for the high concentration of Zn at this site while the relatively high concentration of Cr at Site 3 during the high flow period may be caused by painting activities at the mentioned scrapyard, as well as natural weathering processes. The higher concentration of Cu at this site may also be the product of activities performed at this industry. To conclude it is obvious that the nearby gold mine activities did not contribute significantly in increasing the concentration of more heavy metals to values exceeding TWGR in the Mooi River catchment area.

Table 1: Heavy metal concentrations present at the Wonderfontein Spruit/ Mooi River sites

	Site U1		Site U2		Site U3		Site U4		Site U5		Site U6		TWQR
	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low	
Al	0	61.35	0	23.18	0	27.64	0	14.36	0	19.50	0	29.47	10
Cr	7.20	1.59	9.02	4.19	5.92	0.86	7.09	2.39	7.16	2.59	10.17	4.49	12
Mn	8.59	1.35	3.20	0.82	4.53	1.10	5.21	0.69	5.59	0.90	4.81	1.07	180
Cu	2.60	5.24	0.30	2.02	0.90	2.28	0.53	2.39	0.17	2.13	0.15	2.12	0.8
Zn	13.05	64.53	10.26	50.67	12.18	39.15	11.57	37.18	13.76	47.72	14.02	38.95	2
As	10.05	12.98	0.15	0.04	0.78	0.06	0.35	0.07	0.20	0.02	0.17	**ND**	10
Se	2.26	0.64	0.12	**ND**	0.39	**ND**	0.65	**ND**	0.89	**ND**	1.41	**ND**	2
Cd	0.01	**ND**	0	**ND**	0	**ND**	0	**ND**	0	**ND**	0	**ND**	0.25
Pb	0.20	0.17	0.01	0.11	0.15	0.10	0.09	0.04	0.27	0.08	0.06	0.11	0.5
ph	7.13	7.53	6.77	6.75	7.48	7.8	7.26	7.44	7.06	7.18	7.05	7.12	
EC	1109	1001	435	457	491	456	677	697	758	798	781	785	
Temperature	20.7	13.9	19.9	19.2	22.3	10.2	17.1	11.3	21	19.8	21	19.7	
Turbidity	72	69	100	100	82	98	93	99	90	99	100	100	
Flow Rate	0.2	0.1	0.1	0.1	0.9	0.8	1.2	1.1	0.1	0.1	0.1	0.1	

Table 2: Heavy metal concentrations present at the Loop Spruit/Mooi River sites

	Site E1		Site E2		Site E3		Site E4		Site E5		Site E6		TWQR
	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low	
Al	0	295.90	0	35.58	0	23.7	0	27.18	0	54.31	0	37.87	10
Cr	5.68	1.71	5.50	1.08	9.15	2.86	5.82	0.93	7.48	1.89	6.34	1.24	12
Mn	2.31	0.25	0.77	3.53	2.54	1.21	3.70	1.92	4.90	6.36	3.77	3.54	180
Cu	1.12	2.26	0	3.33	2.52	6.40	1.69	4.23	1.31	3.94	0.52	2.95	0.8
Zn	15.65	18.70	6.12	60.14	3.11	49.57	9.30	59.35	8.30	54.65	10.92	40.96	2
As	0.41	0.10	0.26	0.10	1.14	0.62	1.13	0.36	1.38	0.78	0.43	0.27	10
Se	0.17	ND	0.16	ND	4.07	1.59	1.09	ND	2.07	0.26	0.91	ND	2
Cd	0	ND	0	ND	0	ND	0.001	ND	0.001	ND	0	ND	0.25
Pb	0.28	0.37	0	0.38	0.03	0.15	0	0.25	0.04	0.27	0.10	0.13	0.5
ph	6.11	7.2	6.43	6.66	6.46	6.82	7.65	8.34	6.43	7.43	8.04	7.73	
EC	121	65	853	901	1499	1207	535	480	790	781	641	695	
Temperature	16.4	9.2	18.7	12.3	16.3	11.7	16.1	10.7	16	7.3	19.3	12.3	
Turbidity	64	63	96	98	85	94	20	23	75	78	76	80	
Flow Rate	0.3	0.2	1.4	1.2	0.3	0.2	0.2	0.1	0.3	0.3	0.4	0.3	

4. Acknowledgments

We want to thank the Unit for Environmental Sciences and Management, North-West University, Potchefstroom for financial support and infrastructure.

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O-10

Chromium, copper, nickel and zinc accumulation within selected fish species from a Ramsar site in Southern Africa

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Barberspan was one of the first Ramsar sites that were declared in South Africa in 1975. It receives water from the Harts River which is impacted by urban, industrial and agricultural effluent. During April 2014, *Clarias gariepinus* Burchell 1822 and *Labeo capensis* A. Smith 1841 were collected to determine the concentrations of chromium, copper, nickel and zinc. Fish muscle tissue were collected, digested with an Mars 5 microwave digester and analysed on an ICP-MS. Results indicated that chromium, copper and nickel concentrations were higher in *Clarias gariepinus* while zinc concentrations were similar. No reference metal concentrations were available for comparison for the catchment, and sediment concentrations were below international guidelines. Thus, metal concentrations in *Labeo capensis* and *Clarias gariepinus* are mostly from natural origins.

Keywords: *Barberspan*, *Clarias gariepinus*, *Labeo capensis*,

1. Introduction

Barberspan Bird Sanctuary is one of the first two Ramsar sites that were declared in South Africa in 1975. It was artificially created in 1918 by flooding a temporary endorheic depression and it receives water from the Harts River that is subjected to industrial, urban and agricultural effluent. Anecdotal evidence has suggested that metal pollution is a potential problem in Barberspan due to these effluents that flow into the system.

Thus, this study was initiated to determine the metal concentrations present within selected fish species from the Barberspan Ramsar site.

2. Materials and Methods

The selected fish species, *Clarias gariepinus* and *Labeo capensis* were collected during April 2014 using a set of gill nets (mesh size 70mm, 90mm and 110mm) and small fyke nets (hoop size of 60cm). Fish were sacrificed and a subsample of muscle tissue from each fish was dissected out. Muscle tissue was frozen until analysis at the North-West University laboratory.

Samples were thawed and weighed to determine wet mass. Each sample was then dried in a drying oven for 96 hours at 60°C, allowed to cool, and then reweighed to determine the dry mass and the water content of the tissue (Wepener *et al.*, 2012).

A known mass of each dried sample was digested with 7mℓ nitric acid (HNO_3) in a Mars 5 Microwave digester at 200°C for 20min. The samples were then cooled, diluted to 50mℓ and the concentrations of selected metals were determined on an Inductively Coupled Plasma – Mass Spectrophotometer (ICP-MS; Agilent 7500 CE).

Certified Reference Materials (NCS DC – 73310) were analysed concurrently as quality control of the digestion and ICP-MS analysis.

3. Results and Discussion

Bioaccumulation results for *C. gariepinus* and *L. capensis* (Figure 1) indicate that *C. gariepinus* generally has higher concentrations of chromium, copper, nickel and zinc. Statistically, using one way Analysis of Variance (ANOVA), none of the concentrations between *C. gariepinus* and *L. capensis* were significant ($p > 0.05$).

A study by Enslin (1966) on the feeding ecology of fish from Barberspan found *L. capensis* fed mostly on detritus and plant material on the muddy bottom substrates and *C. gariepinus* had detritus, decaying fish material and zooplankton comprising the largest percentage of its stomach contents. Thus, it could be expected that *C. gariepinus* have higher metal concentrations due it omnivorous diet.

The Enslin (1966) study also found that *Labeo capensis* had similar feeding preferences to *L. umbratus* in Barberspan. Therefore, metal concentrations from this study were compared to a study on *L. umbratus* from the Olifants River (Coetzee *et al.*, 2002) that is known for mining pollution. However, the blue lines in Figure 1 indicated that those concentrations were much higher for chromium and nickel than found in this study. The zinc concentration from the Olifants River corresponded to the standard error of the Barberspan results.

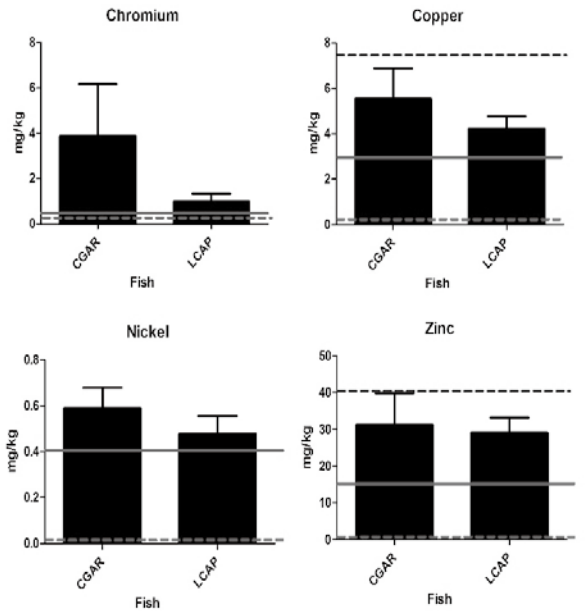

Figure 1: Chromium, copper, nickel and zinc concentrations in muscle tissue of *Clarias gariepinus* (CGAR) and *Labeo capensis* (LCAP) from Barberspan Nature Reserve in April 2014. Solid red line – LCAP data from Wepener *et al*. (2012); Dashed red line – CGAR data from Pheiffer *et al.* (2014); Blue line – *Labeo umbratus* data from Coetzee *et al.* (2002).

Pheiffer *et al*. (2014) looked at metal concentrations in the Vaal River that are subjected to urban, industrial and agricultural land uses. Pheiffer *et al*. (2014) analysed various metals in *C. gariepinus* from a downstream site at the confluence of the Vaal River with the Harts River. These results are depicted by the dashed red line in Figure 1 and are noticeably lower than all the concentrations obtained in the current study. A study on the Vaal River around Vereeniging and Sasolburg studied the relative risk of pollutants to the aquatic environment. The metal concentrations from this study on *L. capensis* (solid red line in Figure 1) are generally within the standard error of the Barberspan results with the exception of zinc that was higher in Barberspan.

Chromium, copper, nickel and zinc are all essential trace metals and nutrients necessary for a variety of biochemical functions. These metals can also come from natural weathering processes and in high concentrations could lead to toxic effects. No baseline bioaccumulation data is available for metal concentrations within biota of the Harts River catchment. Sediment concentrations of these metals indicated lower concentrations than those measured in the Vaal River and international guidelines. As Barberspan is an endorheic system, increased metal bioaccumulation could occur due to the concentration of metals in the system over time.

It is recommended that additional bioaccumulation and ecotoxicological studies are carried out within the Harts River system upstream of Barberspan to determine if metal effects are present due to anthropogenic sources or natural processes.

Baberspan has Ramsar status due to the multitude of bird species that visit the reserve and as such is under the protection of numerous national and international legislations. Therefore, it is essential that the system be continuously monitored to ensure improved management in the future as the pressure on the water resources of the Harts River is not going to decrease.

4. Acknowledgments

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O-11

Level of mercury in fish from the Ethiopian Rift Valley Lakes: its implications in dietary exposure

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Environmental contaminants in fish pose a potential human health hazard. The level of mercury (Hg) has been investigated in three fish species, i.e., *Labeobarbus intermedius, Oreochromis niloticus and Clarias gariepinus,* from Lake Koka and Lake Ziway, Ethiopia. The concentrations of Hg found in *C. gariepinus* and *O. niloticus* from Lake Koka and Lake Ziway were in general lower than the International Marketing Limit (IML) (0.5 μg g^{-1}) and World Health Organization (WHO) guidelines (0.2 μg g^{-1}) for consumption and this finding is, in general, in agreement with most studies conducted in other African lakes. However, of the total fish samples for each species, 67 % of *L. intermedius* from Lake Koka and 27 % of *L. intermedius* from Lake Ziway showed Hg concentrations that exceeded WHO guidelines (0.2 μg g^{-1}) for consumption. Species variation in total Hg (THg) accumulation is attributed to trophic position, and therefore, consumption of fish from a high trophic level may represent a possible health hazard. Consumption of *L. intermedius* from both lakes may pose a special health risk to children and pregnant women.

Keywords: Fish species, Rift Valley Lakes, Hg, Fish consumption

1. Introduction

Mercury in fish is of global concern, and a widely studied topic, because of its established relevance to human health risks. Generally, organic mercury forms are more toxic to human than the inorganic forms (Boening, 2000). It is also documented that virtually all 50–98% of the total Hg (THg) in the edible tissue of fish is in the form of MeHg (e.g.,Carrasco et al. 2011). The key factor dictating total concentration of Hg in the biota (e.g. fish) is the MeHg concentration in water (Morel *et al*., 1998), and the important sources are precipitation, runoff from wetlands and in-lake methylation Rudd, 1995; Downs *et al*., 1998). Hg can accumulate to elevated levels in the biota even in remote areas which are devoid of local industrial sources; due to long-range atmospheric transport followed by deposition (Fitzgerald *et al*., 1998). Local factors such as the presence of hot springs around lakes is also thought to account for high Hg concentrations in the waters of Ethiopian Rift Valley soda lakes (Zinabu Gebremariam and Pearce, 2003). It is also important to take into account biological factors such as the food web structure and age of the fish, to explain the accumulation of Hg in the biota. The objective of the present study was therefore to determine the level of THg and the influence of size, diet and trophic position in key fish species: Big barb (*Labeobarbus intermedius*), African sharp tooth (*Clarias gariepinus*) and Nile tilapia (*Oreochromis niloticus*) from Lake Koka and Lake Ziway in order to assess the potential human health risks related to consumption of fish by the local population.

2. Materials and Methods

Stomach content analysis was carried out at the Department of Applied Biology, University of Hawassa, Ethiopia whereas; the stable isotope and THg analyses were done at the Environmental Chemistry Section, Department of Plant and Environmental Sciences, Norwegian University of Life Sciences (UMB), Norway. Stable isotopes of nitrogen (15N and 14N) and carbon (13C and 12C) were determined by a Continuous Flow-Infrared Mass Spectrometer (CF-IRMS).

The wet muscle tissues (200 mg) were first weighed, and then digested; using an Anton Paar microwave oven and THg concentrations were analyzed using the Perkin-Elmer model FIMS 400 flow injection Hg system. The equipment was calibrated by plotting calibration curves using the measurement values of four different synthetic standards. The curves were linear, and calibration was rechecked after every five samples. The concentrations of synthetic standards varied depending on the concentrations of Hg in the samples. The accuracy of the method was controlled against DORM-2 (piked dogfish *Squalus acanthias* L.), certified reference material, National Research Council of Canada, Ottawa. The concentrations of Hg found in the fish species were compared against the International Marketing Limit (IML) (0.5 μg g^{-1}) and World Health Organization (WHO) guidelines (0.2 μg g^{-1}) for consumption.

3. Results and Discussion

The concentrations of Hg found in *C. gariepinus* and *O. niloticus* from Lake Koka and Lake Ziway were in general lower than the International Marketing Limit (IML) (0.5 µg g^{-1}) and World Health Organization (WHO) guidelines (0.2 µg g^{-1}) for consumption (Fig 1). However, of the total fish samples for each species, 67 % of *L. intermedius* from Lake Koka and 27 % of *L. intermedius* from Lake Ziway showed Hg concentrations that exceeded WHO guidelines (0.2 µg g^{-1}) for consumption (WHO, 2007). The concentrations of THg found in *L. intermedius* were higher than those found in the other fish species in our study, as well as in most other studies carried out in other African lakes, although fish size, sample size etc. varied in these studies, and may make direct comparisons difficult.

For all fish species and individuals pooled, a significant positive relationship was found between log transformed THg and $\delta^{15}N$ both in Lake Koka ($P < 0.05$, $R^2 = 0.35$) and Lake Ziway ($P < 0.05$, $R^2 = 0.26$) (Fig 1). The present study demonstrates the accumulation of heavy metals (e.g, Hg) in ERVLs, and species variation in THg accumulation is attributed to size, diet and trophic position of the fish; and therefore, a person with a daily intake of old fish from a high trophic level such as *L. intermedius* may be exposed to possible health hazards. Children and pregnant women of the local community, especially the local subsistence fishermen and their families are the most vulnerable population sub-group.

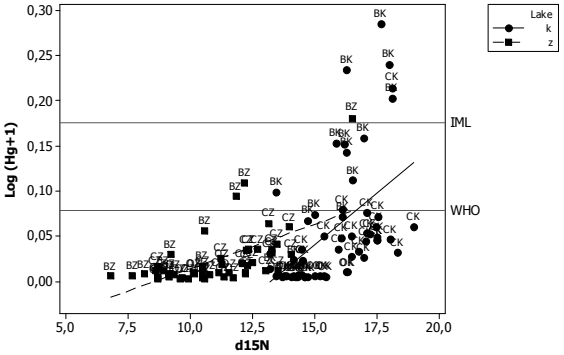

Figure 1: The relationship between log transformed THg (mg.kg-1 ww) and $\delta^{15}N$ signatures and comparison of the level of THg in fish sampled from Lake Koka and Lake Ziway with WHO guideline values set for safe fish consumption (WHO; 0.2 µg g^{-1} ww) and International Marketing Limit (IML; 0.5 µg µg g^{-1} ww) (BK: *Labeobarbus intermedius* in Koka, OK: *Oreochromis niloticus* in Koka, CK: *Clarias gariepinus* in Koka, BZ: *Labeobarbus intermedius* in Ziway, OZ: *Oreochromis niloticus* in Ziway, and CZ: *Clarias gariepinus* in Ziway).

4. Acknowledgments

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O-12

Assessment and monitoring of drins from a premier conservation area

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The assessment and monitoring of persistent pesticides such as aldrin, endrin and dieldrin in aquatic ecosystems are important in order to determine their environmental fate and risks posed to aquatic biota. *Hydrocynus vittatus* were collected from sections of the Olifants, Letaba and Luvuvhu Rivers within the Kruger National Park, and analysed for the aforementioned OCPs. Aldrin was only detected from *H. vittatus* from the Luvuvhu River, endrin was detected from all three rivers and dieldrin was not detected in any of the tigerfish although it was found in the sediments. Concentrations during low flow periods exceeded European Union regulations and concentrations of aldrin and endrin are the highest ever measured from South African freshwater systems and pose potential risks to aquatic biota.

Keywords: aldrin, endrin, dieldrin, *Hydrocynus vittatus*, Kruger National Park

1. Introduction

South Africa has the top gross domestic product and one of the top four pesticide importers in Africa, including organochlorine pesticides (OCPs) which are used extensively throughout the world. Contaminants such as OCPs are known to be ubiquitous anthro-pogenic pollutants in aquatic ecosystems and known to bioaccumulate in the fatty tissues of organisms and threaten biological compo-nents of the receiving environment. As such monitoring is essential for the assessment of potential impacts (Yohannes et al. 2013). Endrin, aldrin and dieldrin are highly toxic pesticides once used worldwide as a replacement for DDT and concentrations are rarely assessed and never assessed within the Kruger National Park (KNP). Tigerfish (*Hydrocynus vittatus*) were chosen as representative organism for the study as they are important freshwater fish and top predators. The aims of the study were to determine concentrations of drins in *H. vittatus*, and compare accumulation on a temporal scale.

2. Materials and Methods

Tigerfish were sampled during the period September 2009 to June 2011 at sampling sites along the Olifants (OR), Letaba (LeR) and Luvuvhu Rivers (LR) within the KNP. After capture, fish were sacrificed, and axial muscle removed and frozen. Analytical and quality control procedures to measure aldrin, dieldrin and endrin concentrations were done according to Yohannes et al. (2013). A one way ANOVA was performed differences between groups were determined using Tukey-Kramer post hoc analyses ($p < 0.05$). Principal component analysis was also applied to the data to relate accumulated levels with environmental concentrations.

3. Results and Discussion

Aldrin was only detected in *H. vittatus* from the LR (Figure 1A). Aldrin is readily changed into dieldrin in both the environment and biota, this however, does not seem to be the case, as no dieldrin was found in *H. vittatus*, although there were measurable levels of dieldrin in sediments from the LR (data not shown here). Either aldrin is not metabolised to dieldrin, or dieldrin is regulated and excreted by *H. vittatus*. The dieldrin in sediments is likely due to fresh inputs of aldrin that have been transformed. Endrin was detected during the majority of the surveys and individuals sampled (Figure 1B). Endrin is easily biode-graded and the high concentrations in *H. vittatus* are testament to its wide scale application in the various catchments. During the LF2010 surveys a number of individuals from each of the rivers and a single individual sampled from LR in 2010 exceeded the maximum residue levels in edible fat for endrin (50 $ng.g^{-1}$) and aldrin (200 $ng.g^{-1}$) as set by the European Union (EC 2005) (refer to Figure 1). The measured concentrations of endrin and aldrin during the LF2010 survey of the LR are the highest levels recorded in fish from South African freshwater systems (see review by Ansara-Ross et al. 2012).

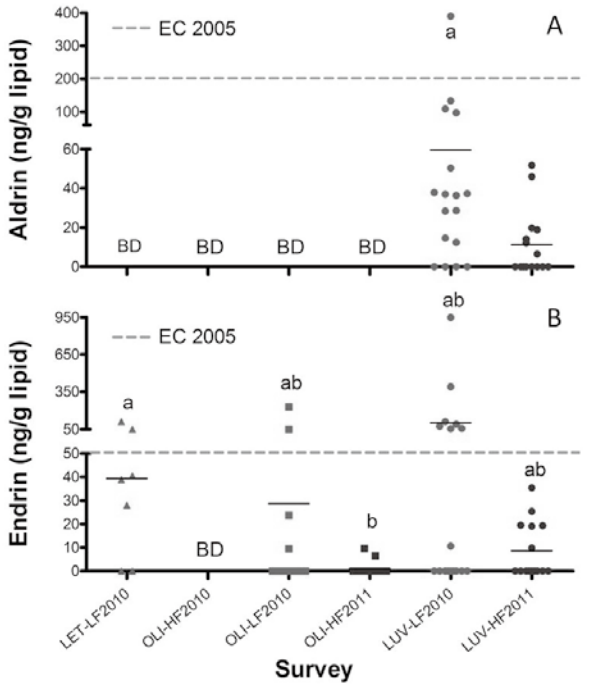

Figure 1: Concentrations of aldrin (A) and endrin (B) in *Hydrocynus vittatus* muscle during the various surveys.

The biplot in Figure 2 explains 99.8% of the variation of the bioaccumulation and sediment data for OCPs. With the more contaminated Luvuvhu River separating from the other two rivers along the first axis explaining 74.9% of the variation, with temporal differences occur-ring along the second axis with LF surveys associated with higher bioaccumulation and HF surveys with increased sediment contam-ination, explaining a further 24.9%. The data clearly demonstrated that the LR is more impacted by these OCPs than the OR and LeR and pose possible risks to the aquatic biota.

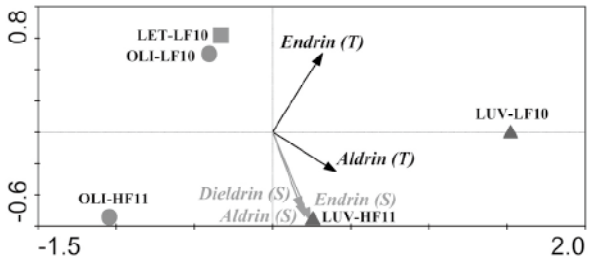

Figure 2: PCA biplot of aldrin, dieldrin and endrin concentrations in abiotic and biotic compartments of the Olifants, Letaba and Luvuvhu Rivers.

4. Acknowledgments

Financial support for this project was pro-vided by the Water Research Commission (WRC) of South Africa (Project K5-1922, NJ Smit, PI) and the National Research Foun-dation (NRF) of South Africa (Grant No. 86632). Opinions expressed here are those of the authors and not of the WRC or NRF.

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O-13

HCH's in two fish species from a large floodplain pan within a subtropical conservation area.

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The use of fish as biological indicators of aquatic system health is a well-documented practice. This study was conducted in order to establish whether specific organochlorine pesticides (OCP) are present within the Nyamiti Pan, situated in the Phongolo floodplain of KwaZulu-Natal, South Africa. Hexachlorocyclohexanes (HCH's) were one group of OCP's found within the water body, and levels of three isomers, namely α-HCH, β-HCH and γ-HCH, were determined. The results indicate seasonally dependant trends in α-HCH and γ-HCH. The percentage composition of each isomer shows a varied spraying regime, with possible combination spraying of both technical grade Lindane and HCH in the area. Levels of HCHs are lower in *Hydrocynus vittatus* than in *Synodontis zambezensis* and may be attributed to dietary and habitat usage differences as well as complex differences in bioaccumulation patterns.

Keywords: Organochlorine pesticides, *Hydrocynus vittatus*, *Synodontis zambezensis.* South Africa, Ndumo, Nyamiti Pan

1. Introduction

Demand for produce of higher quality and quantity has led to increased use of pesticides in agriculture. Pesticide exposure, poses a significant threat to biota. This threat is linked directly to the chemical makeup of pesticides that gives them the potential to bioaccumulate and become persistent within ecosystems. Extensive use of organochlorine pesticides (OCP) in the past has led to their dispersal throughout the globe (Ansara-Ross *et al*., 2012). Their bioaccumulation potential is not only hazardous to biota, like fish, but also to the human populations that depend on fish as a source of food.

Lindane (γ-Hexachlorocyclohexane; γ-HCH) has been used in the past as both an agricultural insecticide and a pharmaceutical treatment for insect infestations in humans, like lice and scabies. The distribution and trade of this OCP is banned under the Rotterdam Convention and its use in the agricultural sector is banned under the Stockholm Convention on persistent organic pollutants. This chemical is however still used in the treatment of lice and scabies under specific exemption.

Although the γ isomer is the main isomer used today, there is concern that it may break down into other isomers (α-HCH and β-HCH) which may prove to be more persistent within the environment and have greater toxicity. Technical grade HCH consists of five isomers: α-HCH (60-70%), β-HCH (5-12%), γ-HCH (10-15%), δ-HCH (6-10%) ε-HCH (3-14%) (Kutz *et al*., 1991). All forms of HCH show greater ability to dissolve in water which is uncommon in OCP's and this ability means that its bioavailability within aquatic systems is greater. All HCH isomers exist as either gasses in the atmosphere or dissolved in water, and only a small percentage bind to particles (Walker *et al*., 2014).

This study was conducted to establish whether OCP's are present within two fish species *Hydrocynus vittatus* and *Synodontis zambezensis*, and at what levels they appear. This information will confirm whether they are suitable species to use as indicators of exposure. The use of these chemicals within the surrounding catchments will be better understood through data collected in this study.

2. Materials and Methods

Ethical clearance was approved for all sampling techniques used and appropriate permits were issued for the species sampled as well as the study area. Fish were sampled from the Nyamiti Pan during November 2012, April 2013 and September 2013. The fish were sacrificed by severing the spinal cord and 10 g of muscle tissue was removed, wrapped in tin foil and frozen in Ziploc bags until analysis.

Preparation of the samples, standards used and analysis of samples were all done according to Yohannes *et al*. (2013). Analysis of OCP's was carried out with a gas-chromatography (GC) equipped with 63Ni electron capture detector (GC-ECD: Shimadzu GC-2014, Kyoto, Japan).

3. Results and Discussion

The results show that HCH is present within both fish species and that it is present in the isomers

α-HCH, β-HCH and γ-HCH. The data represented in Figure 1A shows a steady increase of α-HCH concentrations from November 2012 to September 2013 within both fish species. This corresponds with the changes in season within the area, with September being the dry season. This means that rainfall is decreased and concentrations in the Nyamiti Pan increase due to the lack of dilution from incoming fresh water. Spraying in the area may have been conducted in preparation for the rainy season and more HCH may have entered the system through spray drift. The higher concentrations in *S. zambezensis* may be linked to feeding habits and dietary pathways. They may be exposed to higher concentrations because of their varied dietary pathways.

Figure 1B shows that concentrations of γ-HCH showed little concentration variation in both species from November 2012 to September 2013, and this may mean that the presence of this isomer in the system is a result of scheduled spraying throughout the year in the area.

Figure 1C presents the percentage composition of each isomer within the total HCH concentration. From November 2012 to about mid-April 2013, the largest percentage present is that of γ-HCH, which may mean that during this period technical grade Lindane (99.9% γ-HCH) was used in this area. In a study by Yohannes *et al*. (2013), γ-HCH was also the highest isomer present, at 60%, from Lake Awassa, and recent spraying was the reason for this large percentage. The percentages of γ-HCH in the present study were higher than those reported by Yohannes *et al*. (2013). The shift in percentage composition from the end of April 2013 to September 2013 shows that the largest percentage is now α-HCH, which may indicate that during these periods, technical grade HCH (60-70% α-HCH) may have been applied to crops or used in some way in this area. In the same study by Yohannes *et al*. (2013), α-HCH was present in lower concentrations than γ-HCH, but they too were the most predominant isomers found within the system studied. This variation in spraying regime may mean that HCH's are being used for various things within the same small area. These uses may include treatment of crop seeds like corn, and wheat in preparation for the planting season. Another common use is the dipping of cattle and sheep, for treatment of lice, scabies and ticks.

The ban on both technical grade Lindane and technical grade HCH means that these chemicals should not be used for agriculture in this area, but the isomers of both are present within the aquatic ecosystem which means that they are being used in some way in this area. Chemical waste may be entering the system through improper disposal of chemicals once treatment of livestock or crop seeds had been completed.

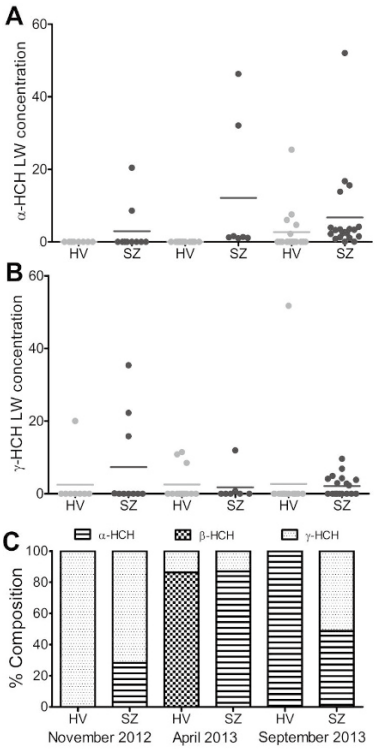

Figure 1: Scattered dot plots of α-HCH (1A) and γ-HCH (1B) concentrations (ng/g) and the percentage composition (1C) of each isomer in *Hydrocynus vittatus* (HV) and *Synodontis zambezensis* (SZ).

4. Acknowledgments

Thanks go to the University of Johannesburg, North West University and Hokkaido University for support through grant number UID 92424 of the Japan/SA Bi-lateral Programme. The WRC, project number WRC-K5-2185 and the NRF grant number, SFH1208209210 for funding.

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O-14

Assessment of the food web structure of *Xenopus muelleri* from the lower Phongolo River floodplain using stable isotope analysis

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Stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) were measured in 11 different food web components inside and outside Ndumo Nature Reserve in order to determine the effect of direct anthropogenic activity on the food web structure of *X. muelleri* from the Lower Phongolo River floodplain. The analysis showed clearer distinctions between trophic groups inside the reserve with slight carbon source shifts and significant nitrogen enrichment observed outside the reserve. This was attributed to possible external nitrogen input from small scale agricultural activities surrounding the outside sites. It was concluded that definite food web structure shifts had occurred outside the reserve, but not to such an extent that the position of *X. muelleri* within the food web is affected.

Keywords: nutrient enrichment, trophic position, amphibian ecology, apex predator

1. Introduction

The combination of $\delta^{13}C$ & $\delta^{15}N$ ratios can determine the trophic levels and food web structure of organisms. The dynamics of these ratios as atoms are transferred through the food web (Fry, 1991). Stable Isotope analysis (SIA) is widely used to study ecosystem functioning (Fry, 1991) as it provides integrated data on the dietary dynamics of target organisms (Davis *et al.*, 2012). The aim of this study was to determine whether the food web structure pertaining to *Xenopus muelleri* (Müller's clawed frog) outside Ndumo Nature Reserve (NNR) is affected by direct anthropogenic activity in the area, with the food web structure inside NNR serving as a control site.

2. Materials & Method

Sample collection occurred in February 2014 at six sites, three within NNR and three outside. Samples representing 11 different food web components relating to *X. muelleri* were collected (Table 1). Frogs were euthanized through means of double pithing (Amitrano & Tortora, 2012) after which muscle tissue was dissected out. All collected samples were stored at -20 ˚C until analysis.

After collection samples were dried (50 °C) and ground into a fine powder. Lipids were removed through the addition of chloroform:methanol (2:1 v/v) solution. The sample was centrifuged (1,500 G, two minutes), the supernatant carefully discarded and the pellet dried.

Analysis was done using an IsoPrime100-vario MICRO cube (Jasco). After determining individual optimal analysis mass (0.8 – 2.0 mg), samples from each component were processed and analysed to determine the $\delta^{13}C$ and $\delta^{15}N$ SIAs. Nitrogen SIA data were used to calculate Tropic Positions (TP) using the following equation: $TP = ([\delta^{15}N_{comp} - \delta^{15}N_{ref}] / 2.8) + 1$, with $\delta^{15}N_{comp}$ referring to the $\delta^{15}N$ of the specific food web component and $\delta^{15}N_{ref}$ referring to the basal source (sediment). The constant value (2.8) is the mean N enrichment difference between trophic levels (Jepsen & Winemiller, 2002).

Table 1: Food web components with corresponding sampling methods

Food web component	Short Label	Sampling method
Sediment	Sed	Scooping
Leaf litter (L)	L	Scooping
Biofilm	Bio	Toothbrush
Oligochaeta	O	Sweep net
Baetidae	Ba	Sweep net
Mollusca	M	Sweep net
Atyidae	At	Sweep net
Ghomphidae	G	Sweep net
Small fish	F	Sweep net
Tadpoles (*X. muelleri*)	*X. m.* T	Sweep net
Adult Frogs (*X. muelleri*)	*X. m.*	Baited traps & night frogging

3. Results & Discussion

The SIA results from inside the reserve showed clear distinctions between the primary sources and invertebrates with *X. muelleri* clearly depicted as the apex predator of the represented food web (Figure 1a). The outside sites had a less clear distinction between the different trophic groups (Figure 1b). For inside sites biofilm and leaf litter were displayed as the primary food sources for all invertebrates, except molluscs correlated to sediment as carbon source. *Xenopus muelleri* tadpoles had similar nitrogen enrichment to Atyidae, but the tadpoles' carbon sources seemed to be more closely correlated to leaf litter whilst Atyidae correlated more closely to biofilm as primary carbon source.

When compared to the outside sites significant differences in the food web structure were observed. The sediment carbon source shifted even further away from the rest of the food web and all primary sources had higher nitrogen enrichment. There was no longer clear distinction between trophic groups and carbon levels of higher trophic position organisms did not correlate to the same primary sources indicating dietary shifts, especially in the case of molluscs. The tadpoles of *X. muelleri* still correlated to leaf litter as primary source, but nitrogen enrichment was higher within the reserve, most likely due to differences in Gosner stage of development (Gosner, 1960). Small fish collected showed fairly similar N and C levels to invertebrates in both food webs. *Xenopus muelleri* was still depicted as the apex predator, but the distinction between it and other food web components was smaller outside the reserve.

Trophic positions revealed higher TP values for primary sources outside the reserve (Table 2). Molluscs, Baetidae and *X. muelleri* adults had lower TP values outside while all other components showed higher values outside. For both inside and outside, *X. muelleri* was approximately four trophic positions above the basal source (sediment in both cases).

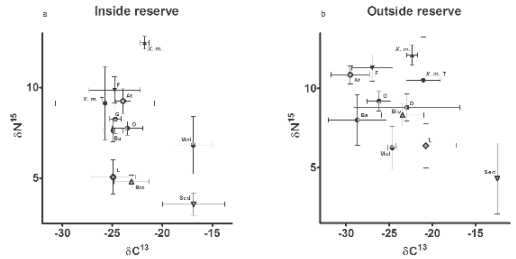

Figure 1: Biplot of δ^{15}N and δ^{13}C isotope ratios for all food web components analysed from inside (a) and outside (b) NNR. Data plots (mean ± SEM) are a composition of all sites sampled per location.

The main reason for a shift in food web structure as observed with the outside sites is most likely due to eutrophication of the water body causing an increase in the nitrogen enrichment of biofilm as primary food source and causing a chain reaction at higher trophic levels (Graening & Brown, 2003). This is supported by the TP value difference observed for biofilm between inside and outside the reserve (Table 2). However even with the shift in food web structure the TP of *X. muelleri* is fairly similar between within and without indicating that no major dietary change has occurred or that the shift occurred recently prior to sampling and has not yet affected the apex predator to the same extent as other food web components.

Table 2: The mean Trophic Positions (TP) of all food web components, according to trophic groups

Food web component	Mean Trophic Position	
	Inside	Outside
Amphibians		
Xenopus muelleri	4.2	3.8
Xenopus muelleri tadpoles	3.0	3.2
Fish		
small Fish (*Barbus sp.* inside, *Tilapia sp.* outside)	3.3	3.5
Invertebrates		
Atyidae	3.0	3.3
Gomphidae	2.7	2.7
Oligochaeta	2.5	2.6
Baetidae	2.5	2.3
Molluscs	2.2	1.7
Primary sources		
Leaf litter	1.5	1.7
Biofilm	1.4	2.4
Sediment	1	1

4. Acknowledgements

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SL-4

Assessment of chemical additives and heavy metals in selected canned foods in Nigeria: Levels and human health implication

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Levels of food additives (Nitrite and Nitrate, Sodium Chloride) and heavy metals (Pb, Cd, As, Fe, Mn and Zn) in canned foods frequently consumed in Nigeria, were assessed to estimate human health risks associated with consumption. Chemical additives were assessed using a Perkin-Elmer spectrophotometer while Heavy metal levels were determined using Atomic Absorption Spectrophotometer (AAS). Human Health risk was estimated using standard indices (Estimated daily intake (EDI), Target Hazard Quotient (THQ), Hazard Index (HI) and Dietary Exposure (DE)). Results showed varying concentrations of additives and heavy metals in all the canned food categories. Nitrate, Fe and Cd in all the canned food categories exceeded recommended limit set by EU. Health risk estimations showed EDI values for Cd in all the canned food categories above the tolerable daily intake, while DE for Fe in canned sweet corn, Fe, Zn and Pb in canned beans/peas had values above recommended limits. THQ values for all the canned foods were above 1 in the canned beans/peas while HI was above 1 in the canned fish category. The study revealed the potential for Cd toxicity and risk of non-carcinogenic health effects from canned beans/peas consumption. Constant monitoring of canned foods is therefore imperative.

Keywords: Heavy metals, Chemical additives, Canned foods, Health implication.

1. Introduction

Canned foods are considered as safe foods. They are easy to prepare, have long shelf life and are quite economical. However, different levels of contamination has been reported in these canned foods (Voegborlo *et al*., 1999).

Chemical hazards in foods include food additives, environmental contaminants such as mercury, dioxins and residues of heavy metals, pesticides and veterinary drugs. These chemical may pose long-term adverse effects on public health (FAO/ WHO, 2006). Consequently, information about levels of food additives in canned foods as it relates to dietary intake is imperative to avert health risks associated with consumption.

Canned foods are frequently consumed in Nigeria, and there is limited information on the levels of heavy metals and chemical additives in these canned foods marketed in Nigeria. More worrisome is the lack of information on health risks associated with consumption.

The objectives of the study was therefore to assess the levels of food additives (Nitrite and Nitrate, Sodium Chloride) and heavy metals (Pb, Cd, As, Fe, Mn and Zn) in canned foods frequently consumed in Nigeria, to estimate human health risks associated with consumption.

2. Materials and Methods

2.1 Sample collection

A total of hundred samples of canned foods made up of different brands and manufacturers were purchased from local supermarkets in Benin City, Nigeria. They were grouped into four categories: fish, meat, beans/peas and sweet corn.

2.2 Sample Analysis

The samples were homogenized, digested and analysed according to standard procedure. The pH of the samples was determined using a HANNA pH meter, chloride was determined using Titrimetric analysis, chemical additives were assessed using a Perkin-Elmer spectrophotometer while Heavy metal levels were determined using Atomic Absorption Spectrophotometer (AAS)(Radojevic, and Bashkin, 1999).

2.3 Health risk assessment:

Health risk indices were reported as Estimated daily intake (EDI), Target Hazard Quotient (THQ), Hazard Index (HI) and Dietary Exposure (DE). Estimated Daily Intake was calculated as the product of the heavy metal concentration in the exposure medium (mg/kg),the Ingestion rate (kg/ day), the exposure frequency (365 days/year) and the exposure duration (54 years, equivalent to the average lifespan) divided by the product of the body weight (kg) and time period over which the dose is averaged (365 days/year × number of exposure years, assumed to be 54 years in this study). Hazard Quotient was calculated as the product of the Estimated daily intake and the Reference dose.

Dietary exposure was calculated by multiplying consumption data with data on the concentration of chemicals in food (JECFA, 2005). (USEPA, 2007).

3. Results and Discussion

3.1 Concentrations of Food Additives and Heavy Metals in Canned Foods

The mean concentration of the pH, Na, Cl, Nitrate and Nitrite, heavy metals (Fe, Zn, Cd, Pb, Mn and As across the canned food categories are presented in Figures 1 to 2. Mean pH values ranged from 5.02±0.02 to 6.16±0.07 for the canned food and were all within standard limits. For the chemical additives, mean values for nitrates ranged from 54.22±9.52 to 93.92±3.73 mg/kg and the values were above Food Standards Australia New Zealand (FSANZ) bench marks. Nitrites mean values ranged from 21.1±3.69 to 36.54±1.45mg/kg across the canned food categories and were below FSANZ bench marks. The values of Na and Cl ranged from 332.6±45.08 to 497.58±42.35 mg/kg and 85.16±3.38 to 111.96±9.53mg/kg respectively. Heavy metals concentrations across the canned food categories ranged from 130.68±1.74 to 303.08±11.57 mg/kg (Fe), 1.44±0.34 to 6.3±0.35 mg/kg (Mn), 15.72±3.49 to 91.88±3.95 mg/kg (Zn), 0 to 0.23±0.04 mg/kg (Cd), and 0 - 0.17±0.08 mg/kg (Pb). Cd was above EU standard in all canned groups while Pb exceeded same standard in canned meat.

3.2 Health risk estimations

Health risk estimations showed EDI values for the additives ranging from 0.033 to 0.953 mg/kg bw/day and 0.013 to 0.371 mg/kg bw/day, for nitrates and nitrites respectively. EDI for heavy metals were within Joint FAO/WHO Expert Committee on Food Additives Provisional Tolerable Daily Intake (PTDI) except for the EDI of nitrites in canned beef and beans/peas and Cd in all the canned food categories which were above the PTDI limits. Human health risk assessment showed that the DE for the chemical additives and heavy metals were within recommended daily requirement except DE of Fe in canned sweet corn, Fe, Zn and Pb in canned beans/peas which had values above recommended limits. THQ values for all the canned foods were <1 except in the canned beans/peas which had values >1. HI was <1 in canned fish but >1 in the other canned food categories. THQ and HI >1 indicates potential health risk. Results from this study revealed potential Cd toxicity and risk of non-carcinogenic toxic effect from canned beans/peas consumption; it is therefore imperative for constant monitoring of canned foods for possible contamination.

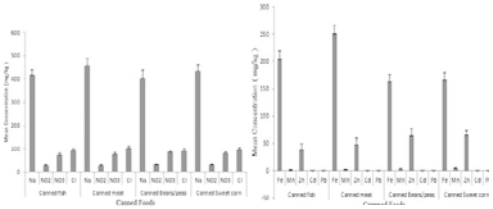

Figure 1: Mean concentration (mg/kg of chemical additives across canned food categories

Figure 2: Mean concentration (mg/kg) of heavy metals across canned food categories

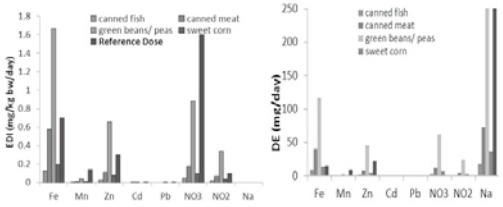

Figure 3: Estimated Daily Intake (mg/kg bw/day) for Heavy Metals and chemical additives in canned fish, canned meat, green beans and sweet corn

Figure 4: Dietary Exposure (mg/day) for Heavy Metals and chemical additives in canned fish, canned meat, green beans and sweet corn.

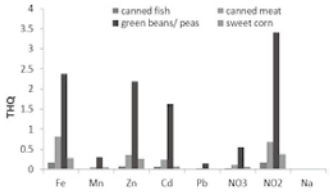

Figure 5: Target Hazard Quotient for Heavy Metals and chemical additives in canned fish, canned meat, green beans and sweet corn

4. References

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O-15

Antibiotic and heavy metal residues in camel meat

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Camel meat is a very popular in many parts of the world especially in Arabian countries like Egypt and Saudi Arabia. Camel meat is characterized by its low intramuscular fat and high protein contents. Chemical residues in meat have become a problem of magnitude worldwide. Among these residues, antibiotics and heavy metals of a particular importance due to their significant adverse effects on consumers. This study was undertaken to estimate the antibiotic and heavy metal residues in the camel meat in comparison with cattle and buffalo meat. Interestingly, among 150 camel carcasses screened for antibiotic residues, none was recorded as positive. Unlikely, 9.2% and 5% of examined cattle and buffalo carcasses were positive, respectively. Toxic metal residues (lead and cadmium) exceeded the permissible limits in liver, and kidneys of the three animal species examined. Public health importance of the residues was discussed.

Keywords: Camel, meat, antibiotics, heavy metals, public health

1. Introduction

Camels are one of the most fundamental pillars of the national economy and food security for many countries in the world, because they occupy a very important role in providing human food, especially meat. The camel meat is distinguished from other animals by their low levels of intramuscular fats, so camel meat is considered as much healthier and recommended for cardiovascular diseases and atherosclerosis patients.

Antibiotics are used in food producing animals not only to treat disease but also to maintain health and promote growth. However, lack of proper application and handling can lead to occurrence of residues in food of animal origin particularly meat, milk and egg. Farm animals treated with antibiotics and their edible products could lead to residues in food animal origin, with potential adverse effects on human health (Darwish et al., 2013).

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

Thus, this study was undertaken to screen the incidence of antibiotic residues in the meat of camel in comparison with that of cattle and buffalo. Moreover, the residual levels of heavy metals such as lead (Pb), cadmium (Cd), copper (Cu) and aluminium (Al) were measured in the muscle, liver and kidneys of these meat producing animals. Public health importance of such residues was also discussed.

2. Materials and Methods

2.1 Sample collection

Antibiotic residues were screened in the liver, kidneys and muscle of 150, 120 and 80 camel, cattle and buffalo carcasses respectively. Heavy metal residues were measured in the liver, kidneys and muscles of 20 each of camel, cattle and buffalo. All samples were collected from butcher shops in Sharkia governorate, Egypt and transferred in a cooled ice-box to food control department, Faculty of Vet. Medicine, Zagazig University for chemical analysis.

2.2 Screening of antibiotic residues

The screening of the collected samples for presence of antibiotic residues was done using the four-plate method (Aerts et al., 1995) using nutrient agar plates seeded with *Bacillus subtilis* (ATCC-6633) at pH 6 and pH 8. Quantitative estimation of oxytetracycline residues in the positive samples using High performance liquid chromatography (HPLC) was adopted according to our previously used method (Morshdy et al., 2013).

2.3 Extraction and heavy metal 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 perchloric acid were added to the tissue sample. The tubes were allowed to stand overnight at room temperature. Then tubes were heated for three hours at water bath adjusted at 70^{o} C to ensure complete digestion of samples. The tube were cooled at room temperature and then diluted with 5 ml deionized water, capped with plastic film and thoroughly mixed. The digested solution was filtered through whatt-man filter paper. 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 metal contents. The metals were measured using Atomic

Absorption Spectrophotometer (AAS) (Perkin-Elmer 2380).

2.4 Statistical analysis

Statistical significances were evaluated using Tukey-Kramer HSD difference test (JMP) (SAS Institute, Cary, NC, USA). $P<0.05$ was considered to be significant.

3. Results and Discussion

Chemical residues in food constitute a major problem worldwide. The first objective of this study was to screen antibiotic residues in camel meat compared with that of the cattle and buffalo. Antibiotics are widely used in veterinary field on large scales for prophylaxis and treatment of different diseases. Additionally, they may be used as growth promoters and feed additives. Subsequently drug residues may persist in foods derived from animals, which may pose an adverse health effect for the consumer. In this study, screening of antibiotic residues in camel, cattle and buffalo carcasses revealed 0, 9.2 (11 carcasses) and 5 % (4 carcasses) respectively as shown in figure 1. Quantitative estimation for antibiotic residues using HPLC revealed that the major antibiotic found was oxytetracycline. The recorded concentrations in the positive samples exceeded the maximum permissible limits (200, 600 and 1200 µg/kg in muscle, liver and kidney respectively). These results agree with Darwish et al. (2013), who reported that the major antibiotic used in African countries is oxytetracycline. Interestingly, there was no residues for antibiotics in the camel carcasses examined. This phenomenon may be attributed to either rapid metabolism for recently administered antibiotics in the camel or the high immunity profile in camel compared with cattle and buffalo. Thus, further investigations are still needed in order to explain this interesting phenomenon. Antibiotic residues may cause allergic reactions, imbalance of intestinal microflora and antibiotic resistance.

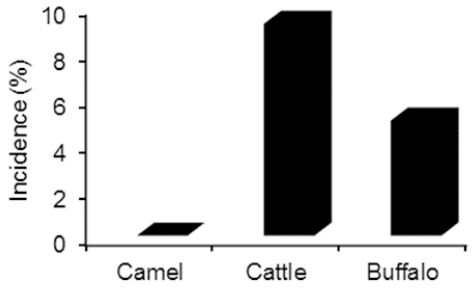

Figure 1: Incidence (%) of antibiotic residues in camel, cattle and buffalo carcasses marketed at Sharkia, Egypt

Thus, efficient cooking of meat and strict legislations to observe antibiotic withdrawal times should be followed. Moreover, strict precautions should be taken to avoid using antibiotics as animal feed additives.

The second part of this study was concerned with measurement of heavy metals (Pb, Cd, Cu and Al) in the liver, kidney and muscle of examined camel, cattle and buffalo carcasses. The results obtained were shown in table 1.

Table 1: Heavy metal residues (ppm wet weight) in the camel, cattle and buffalo carcasses marketed at Sharkia, Egypt

		Camel	Cattle	Buffalo
Pb	Liver	$2.01\pm0.51^{a,B}$	$3.51\pm0.68^{a,A}$	$2.18\pm0.21^{a,B}$
	Kidney	$1.76\pm0.59^{a,A}$	$1.87\pm0.38^{b,A}$	$1.47\pm0.61^{a,A}$
	Muscle	$1.11\pm0.40^{b,A}$	$1.43\pm0.22^{b,A}$	$1.17\pm0.20^{b,A}$
Cd	Liver	$0.10\pm0.02^{a,B}$	$0.34\pm0.11^{a,A}$	$0.18\pm0.05^{a,B}$
	Kidney	$0.10\pm0.01^{a,B}$	$0.45\pm0.09^{a,A}$	$0.11\pm0.01^{b,B}$
	Muscle	$0.06\pm0.01^{b,A}$	$0.08\pm0.01^{b,A}$	$0.08\pm0.02^{b,A}$
Cu	Liver	$1.92\pm0.61^{a,C}$	$6.93\pm0.11^{a,A}$	$3.89\pm0.64^{a,B}$
	Kidney	$0.54\pm0.07^{b,C}$	$3.43\pm0.15^{b,A}$	$2.01\pm0.11^{b,B}$
	Muscle	$0.58\pm0.06^{b,C}$	$3.14\pm0.12^{b,A}$	$1.78\pm0.09^{b,B}$
Al	Liver	$3.35\pm1.11^{a,B}$	$6.35\pm1.53^{a,A}$	$5.47\pm2.67^{a,A}$
	Kidney	$3.17\pm0.95^{a,C}$	$6.45\pm2.76^{a,A}$	$4.67\pm1.66^{a,B}$
	Muscle	$2.77\pm0.60^{a,B}$	$3.99\pm0.57^{b,A}$	$2.81\pm1.66^{b,B}$

A,B, C significantly different for same metal among different animals; a,b, c significantly different for same metal in same animal species among different tissues ($p< 0.05$).

Pb residual concentrations exceeded the permissible limits (0.2 ppm) in all animal species examined. Cadmium exceeded the permissible limits (0.1 ppm) in cattle and buffalo livers and kidneys. Copper and Aluminium were within the permissible limits decided by Egyptian authorities (15 and 12 ppm for Cu and Al respectively). Liver had the highest heavy metal load followed by kidney and finally muscle as clear in table 1 for all examined metals.

It is clear from the results that camel meat had the lowest residual concentrations of the heavy metals examined. This interesting finding indicates a low contamination level of camel meat compared with other marketed meat, or rapid detoxification systems in the camel body compared with other animals examined. Thus, we highly recommend consumption of camel meat compared with other meat distributed in the Egyptian market concerning antibiotic and heavy metal residues.

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O-16

Improving seed treatment methods: A key factor to reduce the risk of chemical insecticides to the environment

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Drift of pesticides generated from drilling seed dressed with pesticides can have a great role in environmental contamination. Bee, this small little insect is considered as one of the reasons for the possibility of human development on earth. However these important species are endangered through the use of pesticides. The current study investigated the amount of drift generated from seeds of two varieties of cotton using two formulation of the neonicotinoid insecticide imadocloprid through measuring the fine dust particles from various treatments using the Heubach methods. The aim was to improve the seed treatment methodology to reduce the drift generated from seeds by drilling and hence saving of bees and other pollinators as well as reducing the risk of people handling the treated seeds during the sowing activities and people located in the vicinity of the sowing site. The Heubach vaules were higher in case of WS formulations. The results of the study indicated in General that the Flowable concentrate formulation for seed treatment is better than the Water dispersible powder formulation in reducing the drift generated from pesticide treated seeds and can play important role in improving seed dressing technology to save various pollinators.

Keyword: Seed treatment, Insecticides, neonicotinoid, cotton varities

1. Introduction

In late April and early May of 2008, numerous cases of increased bee mortalities were recorded in the Upper Rhine Valley (SW Germany). Typically, the affected bees showed symptoms of acute intoxication, in most cases these effects were seen in adult bees only. Approximately 11,500 bee hives were affected. The investigation of the incident was started by regional and Federal authorities immediately after the first records of conspicuous mortality. From the beginning, there were indications which linked the increased Hazards of pesticides to bees – 10th International Symposium of the ICP-Bee Protection Group Julius-Kühn-Archiv 423, 2009 133 mortalities with the drilling of corn, which took place simultaneously in the affected region. In dead honeybees and samples of vegetation adjacent to drilled corn fields, residues of clothianidin were detected.

Clothianidin is a neonicotinoid insecticide contained in the seed-dressing product Poncho Pro® (Clothianidin FS 600, 1.25 mg a.s./kernel), which is applied as a seed-dressing product to corn seeds and was used in the Upper Rhine Valley for control of the western corn rootworm (*Diabrotica virgifera*), an economically

devastating pest in corn. Some farmers in the affected area reported unusually high amounts of dust in the bags of treated corn seeds and the emission of red dust during the drilling of these seeds. These reports provided indications that dust from abraded particles of

the seed-dressing, which contained the intrinsically bee-toxic clothianidin, was released during the drilling process with the outlet air of neumatic drilling machines and deposited on flowering, bee-attractive crops and weeds in adjacent vegetation strips and fields where bees were exposed during foraging.

2. Materials and Methods

In this study the Assessment of free floating dust and abrasion particles of treated seeds as a parameter of the quality of treated seeds using HEUBACH TEST was carried. Cotton seeds were either dressed with Water dispersible powder for slurry (WS) formulation or Flowable concentrate (FS) of imadocloprid. Treated seeds were mechanically stressed inside a rotating drum. A vacuum pump creates an air flow through the rotating drum, the connected glass cylinder and the attached filter unit. By the air flow, abraded dust particles are transported out of the rotating drum through the glass cylinder and subsequently through the filter unit. Coarse non-floating particles are separated and collected in the glass cylinder while floating dust particles are deposited onto a filter. The amount of floating dust collected on the filter is determined gravimetrically.

3. Results and Discussion

The results of the experiment is shown in Table 1. The results indicated clearly a high reduction of drift of tested pesticides (imadocloprid + tebuconazole) measured through the Heubach Apparatus for the

Flowable Concentrate for seed treatment relative to water dispersible powder for slurry treatment. The reduction in drift for FS formulation over WS formulation amounted to 64% and 90% for the medium sta[le cotton (hamid) and the long staple cotton (Barakat), respectively.

Table (1). Drift measured in Heubach Apparatus for different cotton seed varieties treated with various seed dressing

Cotton Variety	Chemical seed dressing	g drift/kg seed	Drift Reduction Percentage for FS formulation
Hamid (Medium Staple cotton)	***Imadocloprid + Tebuconazole (WS)***	1.23	64
	Imadocloprid + Tebuconazole (FS)	0.44	
	Untreated Control	0.22	
Barakat (Long staple cotton)	***Imadocloprid + Tebuconazole (WS)***	1.19	90
	Imadocloprid + Tebuconazole (FS)	0.12	
	Untreated Control	0.38	

O-17

Carcinogenic and non-carcinogenic risk of organochlorine pesticide residues in processed cereal-based complementary foods for infants and young children in Ghana

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Fourteen organochlorine pesticides (OCPs) residues were analyzed in 10 brands of processed cereal-based complementary foods with the aim of assessing the health risk to infants and young children. The QuEChERS method was used for extraction and clean-up of pesticide residues. Subsequent detection and a quantification were done using GC with ECD and PFPD. Levels of *p,p'*-DDE, dieldrin, β-endosulfan, β-HCH, and γ-HCH detected in the processed cereal-based complementary food were higher than their respective MRL. The mean estimated daily intakes of OCPs in infants were significantly higher than that of young children. Exposure levels of heptachlor and dieldrin were higher than their respective ADI's. Their HIs recorded were greater than 1 indicating the possibility of adverse health effect on consumers. Hazard Ratio for carcinogenic risk posed by β-HCH, dieldrin, heptachlor, γ-HCH and γ-chlordane were greater than 1. This result raises concerns of possible carcinogenicity for infants and young children.

Keywords: Hazard Ratio, Carcinogenicity, Dietary Intake, Organochlorine, Pesticides

1. Introduction

Despite enormous restrictions on the use of OCPs due to their toxic and persistent nature, there is recent evidence of OCP residues in food samples. (Akoto *et al.,* 2013). Children, when exposed to contaminated food are at risk higher than adults due to their higher basal metabolic rate and energy requirements.

Despite the higher risk posed by pesticides to children, studies on dietary exposure are limited in Ghana. There is therefore the need for dietary exposure studies on pesticide residues for children considering their unique vulnerabilities. The objective of this study is to assess the risk posed to infants and young children by OCP residues following the consumption of processed cereal-based complementary foods sold in Ghana.

2. Materials and Methods

Five samples of different batches comprising ten brands of processed cereal-based complementary foods, five (5) locally produced and 5 imported produced were sampled for this study. Brands designated as baby food A to E were locally produced whiles F and J were imported.

The QuEChERS method developed by Anastassiades *et al*., (2005) was used for extraction and clean-up. Separation and quantification of pesticides were done using Varian CP-3800 GC with equipped with an ECD.

Risk assessment

EDI for detected was calculated using the equation below.

$$\mathrm{EDI} = \frac{\mathrm{CxCR}}{\mathrm{B_W}} - - - - - - - - - (1)$$

Risk characterization

For non-carcinogenic risk, the ratio of EDI to Acceptable Daily Intake (ADI) was used to obtain the Hazard Index (HI).

For carcinogenic effects, the hazard ratios (HRs) were calculated using the equation below.

$$\mathrm{HR} = \frac{\mathrm{EDI}}{\mathrm{CBC}} - - - - - - - - - (2)$$

Where CBC is the Cancer Benchmark Concentration which was calculated using the formula by Dougherty *et al., (*2000).

3. Results and Discussion

Fourteen (14) different types of OCP residues comprising (β-HCH, γ-HCH, δ-HCH, heptachlor, aldrin, γ-chlordane, *p,p*'-DDE, *p,p*'-DDT, dieldrin, endrin, α-endosulfan, β-endosulfan, endosulfan sulfate and methoxychlor) were analysed. Residues of 8 OCPs were detected in 9 brands of the samples. The mean concentration ranged from 0.002 ± 0.001 $mgkg^{-1}$ in baby food F to 0.022 ± 0.007 $mgkg^{-1}$ in baby food E.

γ–HCH, detected in 5 different brands was the most frequent encountered pesticide residue. Brands E, F and G recorded the highest number of different pesticide. Each recorded the presence of 4 different pesticides residues. All the 5 selected locally based processed food recorded the presence

of at least 3 different types of OCP residue even though the use of OCPs is banned in Ghana. On the other hand dieldrin and *p,p'*-DDE which are metabolites of aldrin and *p,p'*-DDT were the most frequent residues detected in the foreign based processed foods.

γ-HCH in sample D and E recorded high EDI than their ADI for infants consequently, their HI >1. Again the EDIs for heptachlor in baby food E for both infants and young children were greater than their corresponding ADI resulting in an HI of 1.43 for infants and 1.13 for young children. The HI for dieldrin calculated for young children in sample G was 1.13. Again the EDI of dieldrin in samples H were higher than their ADIs for both infants and young children with HRs of 2.86 and 1.13 respectively. Dieldrin in processed food H recorded the highest HI of 2.26 for infants whiles heptachlor in food sample E recorded the highest HI for young children.

Carcinogenic risk

Hazard ratio obtained for β-HCH in samples A, C, D and I could pose potential carcinogenic risk to both infants and young children since the HRs were far greater than 1. The HR for γ-Chlordane in sample E was 2.58 and 1.62 for infants and young children respectively. γ-HCH recorded HRs of 4.42 for infants and 2.79 for young children in sample B. The HR for γ-HCH in baby food F was however less than 1 for both infants and young children. Heptachlor also recorded hazard ratios of 15.3 for infant and 9.61 for young children in baby food E. Baby food F recorded HR of 38.3 and 4.7 for infants and young children respectively. Dieldrin recorded the highest HR of 109 in sample H for infant. The HR values obtained for dieldrin in baby food F, G and H were all greater than 1. These HR values indicate that the cancer benchmark concentrations exceeded the EDI for the respective OCP in the food samples, thus raising serious concerns of possible carcinogenicity. *p,p'-DDE* detected in process food F recorded hazard ratio of less than 1, indicating that it was unlikely for infants and young children to experience carcinogenic effect from dietary exposure.

4. Conclusion

This study has shown the level of contamination of ten different brands of processed cereal-based complementary food obtained from the Ghanaian marker with OCP residues. γ–HCH which recorded the highest concentration was the most frequently encountered pesticide residue. Health risk assessment for the detected pesticide residues indicated that the EDI of heptachlor; and dieldrin were higher than their respective ADI's. Non-carcinogenic hazard indices for these pesticides were greater than 1, signifying that food containing these pesticides could pose adverse health risk to infants and young children.

Cancer hazard risks calculated for for γ-HCH, β-HCH, heptachlor, γ-chlordane and dieldrin were greater than 1 with dieldrin recording the highest HR of 109. Hence there is the possibility for carcinogenicity among consumers.

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Poster Session

P-1

Heavy metal residues in fish and shell fish marketed in Zagazig city, Egypt

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This study was undertaken to investigate the concentration of arsenic, cadmium and lead in the flesh of different fish and shell fish that consumed by Egyptian consumers in Zagazig city, Egypt and comparing these residues with the established legislation. The highest arsenic and cadmium residues were detected in *Neptunus pelagicus* while the lowest were in *Tilapia nilotica*. The highest lead residue was detected in *Sardinella aurita* while the lowest was in *Mugil cephalus*.

Keywords: Lead, Cadmium, Arsenic, Fish.

1. Introduction

Fish is considered a very valuable food, rich in proteins, minerals and vitamins. Moreover, beneficial effects of fish consumption such as the reduction of human cardiovascular diseases and different disorders have also been attributed to the presence of polyunsaturated fatty acids, particularly omega-3 and omega-6 fatty acids. Moreover, the intake of fish is beneficial to children's growth and development and against some diseases such as rheumatoid arthritis, psychiatric disorders and lung disease.

The activities of industrial production generate waste of various kinds which, discharged into the environment, are likely to adversely affect the ecosystem. They have mostly led to a rapid increase of various pollutants. In recent decades, much attention has been paid to the study of essential and toxic trace element content in foodstuffs, as a result of a growing concern about the health benefits and risks of food consumption. Therefore, exposure to the different heavy metals through fish consumption as an important food is obvious. Investigations regarding the existence of heavy metals in fish have increased in latter decade world wide.

There is a group of heavy metals which has no any function in the body like arsenic, cadmium, lead and mercury. Other metals such as, copper and zinc have some essential roles for many enzymatic functions; however, their intake of more than the safe recommended levels may produce toxic effects.The main goal of this study was to record the residual levels of toxic metals and trace elements in fish and shell fish marketed at Zagazig city, Egypt.

2. Materials and Methods

2.1 Materials

A total of 72 representative fish and shell fish samples salted *Sardinella aurita, salted Mugil cephalus, salted and smoked Clupea harengus, Tilapia nilotica, Neptunus pelagicus and Sepia savignngi (12 of each)* collected from Zagazig fish market. The collected fish individuals were wrapped separately. The samples were frozen at -20 ^{o}C in deep freezer unit. The frozen samples were transported to Japan for analysis.

Metal analysis; nitric acid and hydrogen peroxide were purchased from Kanto Chemical, Japan. All glass vessels were soaked in 1:1 nitric acid for 12h then rinsed with de-ionized water for several times.

2.2 Methods

After removal of fines and scales, one gram of fish flesh (back muscle) collected in identified boat then put it in hot air oven at 45 $^{\circ}$C for 48 hours tell drying. Samples were then taken and digested promptly. The fish samples were incinerated wetly in a microwave system under pressure with nitric acid. Metal contents were determined by inductively coupled plasma-mass spectrometry (ICP-MS, Agilent 7500 Series ICP-MS.

3. Results and Discussion

3.1 Arsenic

The results of this study indicated that arsenic (As) residues (ppm) in examined species arranged in descending manner as following: *Neptunus pelagicus* > *Sepia savignngi* > *Clupea harengus* > *Sardinella aurita* > *Mugil cephalus* > *Tilapia nilotica.* Significant differences in arsenic concentration ($p < 0.01$) were found between examined species indicating that co-accumulation differs from species to another depending on As naturally present in the fish's food chain. The highest concentrations of As residues were detected in *Neptunus pelagicus* samples and lowest in *Tilapia nilotica*. This may attribute to the ability of Marine organisms to accumulate arsenic and convert it into organo arsenicals **(Neff, 2002)**. Occurrence of As in various chemical forms in marine water, more than twenty different chemical forms, have been identified and characterized before (**Julshamn et al. 2001)**.

There is no Europe-wide regulation of As in food. In the UK, the As in Food Regulations (SI 1959 no.

831) as amended lay down a general limit of 1 ppm for total As in food. However, this does not apply to fish and edible seaweed, which mainly contain two forms of arsenic that are not considered to be a significant risk to health.

3.2 Cadmium

Cadmium (Cd) bio-accumulates in all levels of aquatic and terrestrial food chains. It accumulates largely in the liver and kidneys of vertebrates and not in muscle tissue. Intestinal absorption of Cd is low and biomagnifications through the food chain may not be significant **(Sprague 1986).**

There was considerable variation of Cd levels among the examined species. Cd residual concentrations exhibited the following decreasing order: *Neptunus pelagicus* > *Sardinella aurita* > *Sepia savignngi* > *Clupea harengus* > *Mugil cephalus* > *Tilapia nilotica.* With a mean value of 0.46 ± 0.29, 0.35 ± 0.19, 0.053 ± 0.035, 0.003 ± 0.001, and 0.001± 0.0005 and 0.0002 ± 0.0001 (mg/kg wet weight), respectively. Considering the existing legislated regulations on permissible limits of cadmium 0.05 mg/kg wet weight, according to Regulation **No. 78/2005 of EU**. We observe that *Neptunus pelagicus, Sardinella aurita and Sepia savignngi* samples exceed this limits, while *Clupea harengus* > *Mugil cephalus* > *Tilapia nilotica* samples located within the permissible limit.

There were significance between the examined fish species ($p < 0.01$) thus, may attribute to crustacean (*Neptunus pelagicus*) and cephalopod (*Sepia savignngi*) have in common the ability to concentrate cadmium at extremely high levels more than fish (**Bustamante et al. 1998, 2000**). *Sardinella aurita* fish species differed significantly with other fish species may be due to the effect of processing and preservation of *Sardinella aurita* in metal cans. The salt added act as harsher corrosive to cans so the producer usually use steel plated with cadmium in cans material. This plating is indispensable especially resistant against salts.

The lowest arsenic and cadmium residues among samples were detected in *Tilapia nilotica* this attributed to the lower trophic level in food chain and fast growth rate under natural and farming condition.

3.3 Lead

Lead (Pb) concentration ranged as follows: *Sardinella aurita* > *Sepia savignngi* > *Tilapia nilotica* > *Neptunus pelagicus* > *Clupea harengus* > *Mugil cephalus* with mean ± SE values of 0.025 ± 0.013, 0.023 ± 0.01, 0.0067 ± 0.0073, 0.0054 ± 0.0055, 0.0053 ± 0.0042 and 0.003 ± 0.001 mg.kg-1, respectively. All examined samples located within the legislated regulations on permissible limits of lead, **according to Regulation No. 78/2005 of EU** estimated to be 0.2 mg.kg-1 of wet weight. The examined samples were significantly different ($p < 0.01$) depending on the habitat of fish on water, pelagic species (*Sardinella aurita* and *Sepia savignngi*) that present in shallow water in which Pb dissolved more than deep water could accumulate Pb more than deep water inhabitant fish, this agrees with **Castro-González and Méndez-Armenta, (2008)**. who reported that Pb amounts in deep ocean waters are about 0.01–0.02µg/L, but in surface ocean waters, it is about 0.3µg/L.

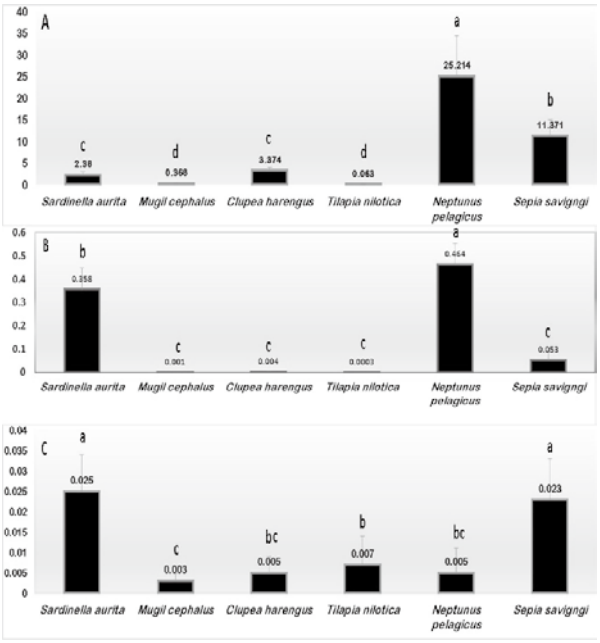

Figure 1: A- Arsenic residues in fish species expressed in $mg.kg^{-1}$. B- Cadmium residues in fish species expressed in $mg.kg^{-1}$. C- Lead residues in fish species expressed in $mg.kg^{-1}$. Means carrying different letter are significantly different ($P < 0.01$).

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P-2

The effect of a sulphuric acid spill on metal concentrations in the Nyl River.

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Toxic metal contamination in aquatic ecosystems has been a major focus in recent environmental studies. The Nyl River Floodplain located downstream of where the spill took place, is the largest wetland classified as a floodplain in South Africa. The aim of the study was to determine the effect of the spill of 28000litres of sulphuric acid into the Nyl River. Concentrations of Al, Fe, Co and Zn were analysed by means of ICP-MS. The levels of conductivity and pH were also taken *in situ.* The pH levels downstream of the spill were decreased to approximately 6. The conductivity was increased after the acid spill which could be due to the inadequacy of the Sewage Treatment Works. All metals showed an increase downstream from the acid spill with Al and Zn levels above target water quality ranges for all sites. Overall the sulphuric acid spill caused changes in the chemical composition of the water downstream, with the sites in close proximity to the acid spill showing increased conductivity, decreased pH and markedly higher levels of Fe and Zn.

Keywords: Metals, acid spill, floodplain, Nylsvley.

1. Introduction

In recent years there has been major focus in the scientific community on toxic metal contamination in aquatic ecosystems. Water pollution is constantly on the rise, with aquatic ecosystems consistently receiving an influx of polluted water from anthropogenic activities (Dahms et al. 2014). The Nyl River Floodplain is the largest ephemeral floodplain wetland in South Africa with an abundance of threatened and endangered biota (Vlok et al. 2006). These factors contributed to its current Ramsar wetland status. The Nyl floodplain is a tributary of the Limpopo River which is located in the Limpopo Province of South Africa (Greenfield et al. 2007). The wetland covers an area of approximately 16000Ha when fully inundated which contributes to its environmental significance. This system was directly affected by the spill of sulphuric acid from an overturned chemical transport truck on the 16th of May 2015 (SABC, 2015). The truck was carrying 28000 litres of sulphuric acid which spilled directly into the Nyl River in the town of Modimolle, Limpopo. It is well documented that toxic metal contamination is a serious threat to many aquatic ecosystems. It is also well-known that pH can influence the concentration and form of many toxic metals in aquatic systems (Munk & Faure, 2004). The aim of this study was to determine the influence of the sulphuric acid spill on the metal concentrations in the Nyl River.

2. Materials and Methods

Seven sites were selected along the upper part of the Nyl River based on their relation to possible pollution sources. Three sites (KNO, DPD and GC) were situated before the spill, one site at the STW directly below the spill site and three sites further downstream from the spill site (JAS, NYL and MDD). Water was sampled during the wet and dry seasons of 2014 as well as four days after the acid spill took place in the dry season of 2015. Readings were taken along the Nyl River from the origin of the Klein Nyl River (Modimolle) to the Moorddrift Dam (Mokopane). Water quality parameters were measured *in situ* and included pH and conductivity (µS/cm). Water samples were also taken for metal analysis. Water samples were filtered using 0.45µm pore size filter paper and acidified to 5% using 65% Suprapur Nitric Acid. Total metal make-up was analysed in the water using an Inductively Coupled Plasma Mass Spectrometer (ICP-MS). Two internal standards, Lu and Rh were added to ensure quality control throughout the analysis.

3. Results and Discussion

Figure1 indicates the levels of metals before and after the acid spill. The spill took place downstream of Donkerpoort Dam (DPD) and upstream of the Sewage Treatment Works (STW). It is evident that the conductivity of the water showed an increasing trend over the seasons and were notably increased after the acid spill, this could also be attributed to the STW not functioning during the time of sampling. The pH of the sites in close proximity to the location of the spill showed a decrease in pH regardless of the Catchment Management Agency adding lime to the water (SABC, 2015).

The metal levels of the site directly downstream of the spill have increased. This could be due to a mobilization of metals from sediment due to the decreased pH (Munk & Faure, 2004). This trend doesn't seem to follow through the system for Co. Co ranged from 0 – 9.0999µg/l. The levels of Fe which ranged from 11.44 - 5007µg/l and Zn

which ranged from 0 – 229.7µg/l, show decreasing concentrations of metals as one moves further downstream from the location of the spill. This can be attributed to dilution. The levels of Al seem to be the most affected downstream of the spill as levels remained higher than previous sampling trips for both wet and dry seasons with a range of 21.142 - 402.976µg/l. Though Al levels were affected by the acid spill, they are still lower than those found a decade ago for all sites (Vlok et al. 2006). Fe concentrations were found to be lower than those found a decade ago except for that of STW after the acid spill. Zinc levels are lower for all sites and seasons except for Nylsvley (NYL) which had similar levels to those 10 years ago and STW which had levels up to 16 times higher than a decade ago. The levels of Co acquired in this study are similar to those found in a surface water assessment from 2011, however the levels of Co increased notably after the acid spill (AED, 2012).

Al and Zn levels were all above the South African Target Water Quality Range (TWQR) for Aquatic Ecosystems (DWAF, 1996).

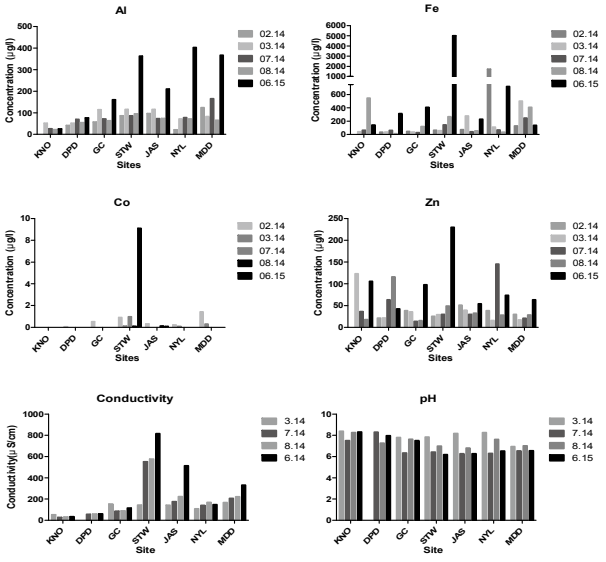

Figure 1: Levels of affected metals, conductivity and pH of water samples pre and post acid spill, in the Nyl River.

4. Conclusion

Although metal concentrations are above the TWQR for aquatic ecosystems, they are generally lower than the metal concentrations recorded in 2001/2002. Levels of Fe and Zn were notably higher for sites in close proximity to the spill. Though continued monitoring should take place, the system should recover in due time.

5. Acknowledgments

We would like to acknowledge the National Research Foundation of South Africa as well as the University of Johannesburg and Mrs Eve Kroukamp from the Spectrum Facility for technical assistance. We would also like to thank Mr. R. Musa, Mr N.J Baker, Mr. G.R.J Van Rensburg and Ms. K.S Dyamond for fieldwork assistance.

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P-3

Chemical speciation and distribution of heavy metals in surface soil from abandoned waste disposal sites in Kumasi, Ghana.

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The study seeks to assess heavy metal pollution concentration, spacial distribution and chemical form of metals in surface soils at Aboabo and Santasi abandoned landfills in the Kumasi Metropolis, Ghana. 30 soil samples were collected from both abandoned landfill sites. A control sample was also taken from KNUST Botanic Gardens. Concentration of metals were analyzed using AAS after digestion. BCR sequential extraction method was used for the chemical speciation. The mean concentrations of the analyzed metals ranged from 34.41±99.92 mg/kg to 13947.67±3320.51 mg/kg in Aboabo soil and 12.23±31.84 mg/kg to11771.54±3640.75 mg/kg in Santasi soil. The speciation result revealed that, most of the analyzed metals are predominantly associated with the residual fraction. Analyses of the physicochemical parameters revealed that, mean pH of soils from both study sites were alkaline and the soils have a sandy loam texture. Correlation analysis of metal concentrations in the study areas indicated that, only Pb/Cu correlated at the Aboabo abandoned landfill, whereas Cu/Zn, Fe/Pb and Cr/Co showed strong positive correlation in Santasi abandoned landfill. PCA classified metals in Aboabo landfill into two main components; PC-1 (containing Al, Fe, Co, Zn and Cr) and PC-2 contains Cu and Pb). However PCA for the metals in Santasi abandoned landfill revealed three main component; PC-1 (Fe, Co, Cr, Cu, Co and Al), PC-2 (Zn) and PC-3 (Pb). Spatial distribution maps of the analyzed metals in the study areas revealed that Co, Zn and Fe whereas Al and Pb are similarly distributed in the northern part of the site. In the Santasi abandoned landfill site however, Co, Cr and Cu exhibited similar spatial patterns in the south-western region of the site whiles Al and Fe also showed similar spatial trends in the north-eastern, central and western portions. The study generally revealed high heavy metal pollution at the two abandoned landfills.

Keywords: Abandoned landfill, spacial distribution, Heavy metals, Pollution.

1. INTRODUCTION

Environmental pollution by heavy metals is a universal problem, because metals are none-destructive and can impose toxic effects on living organisms when the permissible concentrations are exceeded in the environment (Oluyemi et al., 2008). In most populated cities however, several waste from different sources end up in landfill, and due to their heterogeneity and complexity, these waste eventually release a variety of contaminants including heavy metals into landfill soil (Sukop et al., 1979).

The Kumasi city is characterized with high rate of commercial and industrial activities, due its high human population (KMA, 2010). The exponential increase in population and boost for commercial activities persistently creates a huge volume of waste on daily basis with landfilling as the main means of containment (Owusu-Sekyere et al., 2013). Meanwhile, most abandoned landfill sites in the city are currently used for crop cultivation, rearing livestock and as sites for unauthorized settlements. Existence of biologically labile heavy metals beyond acceptable threshold in abandoned landfills within the city may therefore impose heath risk to humans and other lives within the city by accumulating in plants cultivated in the sites, animals fed on plants in the sites or leachate of metals into underground water.

The study seeks to assess levels of Fe, Pb, Cr, Cu, Co, Zn and Al pollution in surface soil from Aboabo and Santasi abandoned landfill sites in the Kumasi, by measuring the total concentration, spacial distribution, as well as the chemical form in which these metals exist in these soils.

2. Materials and Methods

30 composite top-soil samples were collected with stainless steel spatula from the two study areas. A control sample was also collected from the KNUST Botanical Garden. Samples for total metal concentrations were digested with aqua regia solution while metal speciation was carried out using the BCR extraction method. Heavy metal concentrations from the digested samples were analyzed using AAS. pH and EC were measured from a 1 : 2.5 (w/v) soil : water suspension. Organic matter content was determined by ashing the

samples at 500 0C for 5hrs. The soil texture was determined by the Hydrometer method. IBM SPSS Statistics 20 was used to carry out correlation analysis and Principal Component (PCA) of metal concentrations. The IDW interpolation method was used to develop spatial distribution maps, with ArcGIS (version 10.2).

3. Results and Discussion

Physicochemical parameter analysis revealed that, both Aboabo and Santasi abandoned landfill soils were alkaline with pH of 7.53±0.47 and 8.05±0.21 respectively. The soil in both abandoned landfills were found to be sandy loam and organic matter contents of the soils were 8.67±7.19% for Aboabo and 19.23±4.80% for Santasi sites. Electrical conductivities recorded for the two study areas were 3118.05±3254.80 µS cm-1 and 261.49±66.93 µS cm-1 for Aboabo and Santasi respectively.

The mean concentrations analyzed metals ranged from 34.41±99.92 mg/kg of Cu to 13947.67±3320.51 mg/kg of Al in Aboabo soil, whereas the average metal concentration in Santasi soil fell within 12.23±31.84 mg/kg of Cu to 11771.54±3450 mg/kg of Al range. However, the mean concentrations of all the analyzed metals in the control environment (KNUST Botanical garden), ranged from 1.29 mg/kg of Co to 1250 mg/kg of Fe.

Pearson's correlation analysis of metal concentrations in both landfills indicated that, only Pb/Cu ($r = 0.530$, $P < 0.05$) correlated at the Aboabo abandoned landfill, whereas in the Santasi abandoned land fill, Cu/Zn ($r = 0.507$, $P < 0.01$), Fe/Pb ($r = 0.626$, $P < 0.05$) and Cr/Co ($r = 0.966$, $P < 0.01$**)** showed strong correlation. Principal component analysis classified metals in Aboabo landfill into three main components; PC-1 (contains Al, Co and Zn with variance of 27.262%), PC-2 (contains Cu and Pb with variance of 25.529%) and PC-3 (contains Cr with variance of 16.349%). PCA for metals in the Santasi site also revealed three main component; PC-1 (Co and Cr), PC-2 (Zn, Cu and Al) and PC-3 (Fe and Pb) with total variance of 76.746%.

The spatial distribution maps developed for the total concentrations of the analyzed metals in Aboabo abandoned landfill revealed that Co, Zn and Fe show similar distribution in the eastern part whiles Al and Pb are similarly distributed in the northern part of the site. In the Santasi abandoned landfill site however, Co, Cr and Cu exhibited similar spatial patterns their concentration hotspots occurred in the south-western region of the site whereas Al and Fe showed similar spatial trends in the north-eastern, central and western portions of the site. The speciation result revealed that, all the analyzed metals except Cr, are predominantly associated with the residual forms (residual fractions of the studied metals ranged from obtained in Aboabo was 57.11% to 91% whereas the range of residual fractions in Santasi was 39.82% to 95.88%). However, Cr in Aboabo soil was mainly found in the reducible fraction (33.73%).

The entire study revealed high degree of contamination by Fe, Pb, Cr, Cu, Zn, Co and Al at both abandoned landfills in the Kumasi Metropolis, which may be a cause of concern for their surrounding environment and organisms. Hence, remediation of the sites may be necessary to safeguard the environment and organisms from the possible effects of these heavy metals.

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P-4

Zinc and cadmium concentrations in the House Sparrow (*Passer domesticus*), Thohoyandou, Limpopo, South Africa

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An increase in environmental pollution, specifically heavy metal contamination, has resulted in widespread environmental degradation. Heavy metals can have adverse effects on both humans and other living organisms, thus a terrestrial bio-monitoring species in South Africa is needed. The House Sparrow (*Passer domesticus*), an abundant, sedentary bird, was chosen as the bio-monitoring species for this study. Sites relating to a bigger project focussing on DDT were chosen and included: Muledane, Tshakhuma, Makonde, Magondi, Lefule and Makula. Plume feather, flight feather and muscle tissue were dissected from the specimens and analysed for Zn and Cd using ICP-OES. Plume feathers exhibited the highest Zn and Cd concentrations compared to that of the flight feathers and muscle tissue across all sites. Tshakhuma, a more industrialised region of Thohoyandou, showed the highest concentrations of Zn. This was attributed to increased numbers of cars on the roads, thus increased fossil fuel emissions. Informal housing settlements could also be a cause of heightened Zn levels, as Zn sheets are often utilised as a building material. Magondi and Makula represented the highest Cd concentrations, which was accredited to the disturbance of sediment as a result of sewage infrastructure construction.

Keywords: Flight feathers, plume feathers, muscle tissue, *Passer domesticus*, Thohoyandou, ICP-OES

1. Introduction

Africa, once thought safe from environmental pollution, is now threatened as a result of exponential population growth, urbanisation and industrial development. Metals are persistent contaminants with the capacity to bio-magnify in varying intensities within food webs. Metal pollution is, therefore, a growing concern due to the adverse effects it has on both humans and other organisms (Swaileh & Sansur 2006). A bio-indicator species for the monitoring of metal contamination in terrestrial environments across South Africa is thus needed. The House Sparrow (*Passer* domesticus) is a sedentary bird (non-migratory) that lives very close to humans (Swaileh & Sansur 2006). It is abundant, can be found throughout South Africa and it tends to predominantly inhabit small villages, farms, houses and even industrial facilities (Swaileh & Sansur 2006). The House Sparrow is endemic to much of Europe and Asia, however, recently, its distribution has become essentially global due to human intervention (Swaileh & Sansur 2006). For the aforementioned reason, the House Sparrow has been chosen as the test organism for this study. Moreover, there are many advantages in the use of feathers as a bio-monitoring tissue, the most significant being the relatively non-invasive way in which they can be sampled. During molt, birds readily sequestrate endogenously accumulated metals in the feathers, thus, metal concentrations in the feathers tend to be higher than that of muscle tissue (Ansara-Ross et al. 2013).

Trace metals are an essential part of life, however, in high concentrations, they negatively affect organisms in different ways. In birds, metals adversely affect behaviour, reproduction and immunological defences (Burger 1993). The aim of this study was to determine the most suitable avian body tissue for use in bio-monitoring of Zinc (Zn) and Cadmium (Cd) pollution in Thohoyandou, South Africa.

2. Materials and Methods

The House Sparrows were sampled in November 2014 from various sites within and around the Thohoyandou region. Sites were chosen as part of a bigger project looking at the effects of DDT on House Sparrows. These sites included: Muledane (MUL), Tshakhuma (TSH), Makonde (MDE), Magondi (MAG), Lefule (LUF) and Makula (MLA).

Birds were collected using mist nets, ethically sacrificed according to the Society for the Prevention of Cruelty to Animals (SPCA) standards and frozen at -20°C until dissection. Plume feathers (P), flight feathers (F) and breast muscle (M) tissues were excised. Before drying, plume and flight feathers were washed with MilliQ water and all samples were then dried at 60°C for 96 hours. The dried flight feathers were stripped of their vanes as only the rachises were analysed.

0.5g of dried tissue were digested (10ml 65% Suprapur Nitric Acid and 3ml 30% Suprapur

Hydrogen Peroxide – Merck) using a CEM Mars 6 Microwave Digester. The digested samples were then analysed using the Spectro Arcos ICP-OES. Certified Reference Material (CRM) (Dogfish Liver Tissue) was analysed and recoveries for Zn and Cd were within the 80 to 100% range. A Discriminant Function Analysis was performed to relate Zn and Cd levels to specific tissues and sites.

3. Results and Discussion

Cd concentration for plume feathers ranged from 0.01 - 53.65 mg/kg, flight feathers from 0.00 - 65.49 mg/kg and muscle tissue from 0.00 - 21.30 mg/kg. Zn concentrations ranged from 176.01 - 584.62 mg/kg in plume feathers, 80.6 - 273.06 mg/kg in flight feathers and 26.93 - 71.38 mg/kg in muscle tissue. Cd and Zn concentrations were higher in the plume feathers from all sampled sites (Figure 1). Zn is an essential component of normal feather growth and formation and was thus expected to be higher in the feathers. Cd in contrast, is not an essential metal, does not have a biological function and in high concentrations can have detrimental effects on organisms (Malik & Zeb 2009). To account for increased endogenous metals, birds actively sequestrate endogenously accumulated metals into their feathers during molt (Ansara-Ross et al. 2013). This accounts for higher Zn and Cd concentrations in feathers than in muscle tissue. Plume feathers are molted and replaced more frequently than flight feathers and thus sequester more metals.

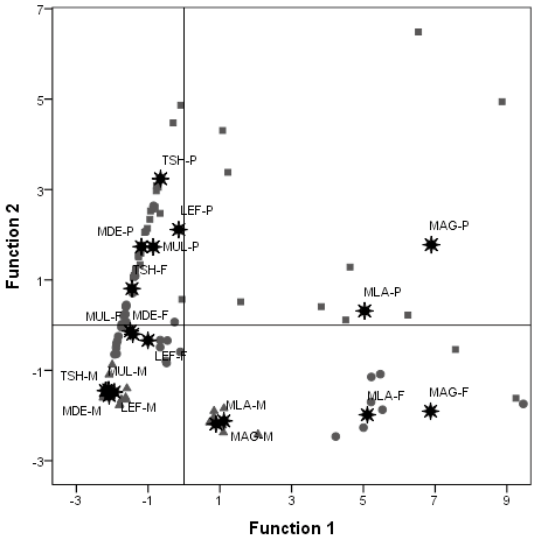

Figure 1: DFA plot showing Zn and Cd levels in plume feathers (square), flight feathers (circle) and muscle tissue (triangle) of the House Sparrows between sampling sites. Group centroids are represented by stars.

In all tissues, the highest concentrations of Zn occurred at TSH (Figure 1). This is attributed to increased industrialisation, urbanisation and a greater population density. With a larger network of tarred roads, travelling becomes easier and more frequent. Zn released from motor vehicle emissions, and increased human activity, has caused increased levels of Zn in this area.

Sites MAG and MLA showed higher Cd concentrations compared to that of the other sample sites (Figure 1). The plume feathers showed the highest Cd concentrations between the two sites. For the duration of sampling in MAG, construction work was underway to upgrade sewage systems in the area. This construction work involves digging up large portions of ground using heavy machinery, and may be the cause of the increased Cd levels in this area. Causes for the increased Cd levels in MLA, however, are not certain and further research is needed.

In conclusion, feathers, specifically plume feathers, are a good tissue for monitoring metal contamination and can be used to show local levels of Zn and Cd pollution.

4. Acknowledgments

The authors would like to thank Mrs E. Kroukamp at Spectrum for assistance with ICP analysis.

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P-5

Used Lead Acid vehicle battery challenges in Zimbabwe: A case study of the City of Chinhoyi

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This paper discusses significant cases of human and environmental exposure to some of the hazardous materials contained in spent Lead Acid vehicle batteries in Zimbabwe. Key exposure cases are emanating from improper disposal and unregulated reconditioning activities observed in the city of Chinhoyi. These have been noted to be fuelled by general lack of public awareness of safe disposal options, lack of stricter product specific regulation on end of life management on this kind of waste and general prevalence of poverty and unemployment cases in the country. This has seen Zimbabwe and the majority of other third world countries lagging behind with regards to interventions on used lead acid battery waste threats to human health and environmental pollution, a gap that needs urgent attention.

Keywords: Used Vehicle Batteries, Lead Poisoning, Recycling

1. Introduction

Used Lead Acid batteries are classified as a hazardous waste under the Basel convention. Despite its well-documented health impacts and efforts to curb its use, lead (Pb) remains a pervasive global neuro-toxin capable of causing serious and in some cases irreversible neurological damage (van der Kuijp et al. 2013). In 2014, the global consumption of refined lead was 11.55 million tonnes (Sucden Financial, 2015), the bulk of which found applications in the production of lead acid batteries. According to the Blacksmith institute, 2014, used lead acid batteries appear in the top ten of the toxic twenty, the top four least addressed problems and the top eight pollution problems most affecting children. Many countries already have a strong set of regulations dealing with chemicals, hazardous waste and toxic pollution, but enforcement varies, and the issues compete with other national priorities for funding and resources (GAHP, 2013). In the case of used lead acid batteries, recycling has become so popular with more success stories coming from the industrialised and high income countries. Developing countries on the other hand are lagging in the establishment of product oriented legislation. Due to economic and social circumstances, minimal concern has been devoted to recovery operations (Harraz, 2011).

2. Materials and Methods

A survey was conducted in the city of Chinhoyi mainly focusing on how spent vehicle batteries were being dealt with after their useful life on a vehicle. Information was gathered by observations at specific site of interest in the C.B.D, High density residential areas and main public transport terminus. A sample representative of the general public in particular those that were vehicle user or owners was also interviewed as a way of finding out the level of public awareness with regards to proper end of life management and disposal of spent vehicle batteries.

3. Results and Discussion

The results of the survey revealed a number of challenges that were prominent in the city with regards to spent vehicle batteries. These included issues to do institutional capacity challenges of the local Authorities, unregulated reconditioning practices and general lack of public awareness.

3.1 Institutional capacity challenges

The economic challenges currently being faced in the country have really hit hard on the city of Chinhoyi municipality's institutional capacity to deliver on most of its obligations that includes refuse collection. An observation which makes this worse is that the rate of residential expansion in Chinhoyi is overwhelming the local authority's capacity to deliver some of the basic services, especially in the high density areas. The institution does not have enough refuse collection trucks to adequately service all these residential areas. The resulting crisis forces local residence to dump waste at unregulated points within the residential areas, a typical site is shown in figure 1. These sites were observed at an average of 2 – 3 points per square kilometre around most of the high density suburbs. Of interest in the one captured in figure 1, was the presence of dumped dead vehicle batteries among the solids waste heap. It was also observed that the waste at these dump sites will eventually be

incinerated, another dangerous practice given the fact that almost anything and everything is dumped there.

Figure 1: An unregulated dump site in a high density suburb in Chinhoyi.

3.2 Unregulated lead acid battery reconditoning

Unregulated lead acid battery reconditioning was observed to be notably popular in the survey conducted. A total of 12 backyard reconditioners were encountered during the survey. Battery reconditioning is proving to be a prominent line of business generally because of the low levels of disposable income of most individual who prefer to repair their dead batteries compared purchasing a brand new unit they consider to be expensive. The biggest challenge observed with these backyard enterprises is that, the industry has a lot of malpractices that violet environmental and human health. There are serious irregularities with regards to storage, handling and discarding of material that does not find use in their operations. A typical backyard recondition spot in the city is pictured in figure 2.

Figure 2: a backyard reconditioning facility in the CBD of the Chinhoyi.

3.3 General lack of public awareness

From a sample of vehicle operators and owners that were interviewed at Chinhoyi University, the results summarized in figure 3 can portray a picture of the state of public awareness on safe disposal of spent vehicle batteries in the city of Chinhoyi. The respondents in the interviews conducted were simply asked what they do with their vehicle batteries after they reach the end of their functional life on the vehicle.

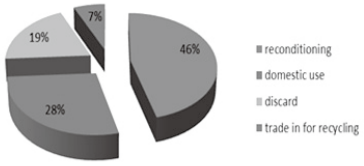

Figure 3: Popular end of life options being used by a sample of the typical of the population of vehicle owners and users in the city of Chinhoyi.

From the results presented above, it should be noted that only trading in spent batteries for recycling at regulated retail outlet, is the safe pathway for dealing spent batteries.

4. conclusions

From this paper, it can be agreed that there is need for National institutions e.g. Local Authorities and the Environmental Management Authority through the necessary legislature, statutory instruments and regulations to implement interventions that promote safe pathways of safe recovery and recycling of used lead acid batteries. If proper guidelines, diligent enforcement of regulations and education are pursued, individuals, communities and even small scale enterprises can be persuaded to adjust accordingly which will convert into behavioural change that will bring about "Smart Cities".

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P-6

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

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This study was undertaken to determine the metal composition in the Groot Marico catchment. Eight sites were selected in the Marico River, Klein Marico River and Sterkstroom, where water and sediment samples were collected for metal analysis. Selected physico-chemical parameters were measured *in situ*. With the exception of the pH at Site H3, all the physico-chemical parameters recorded, were within the DWAF Target Water Quality Ranges and only a few of the metal concentrations (P, Cu and Pb) exceeded the guideline values for freshwater systems. From the results, it appears that the water of the tributaries was not impacted to such an extent that it had any substantial effect on the water of the Groot Marico River**.** The elevated levels of metals were possibly due to natural geological weathering and thus poses little to no threat for the ecosystems in this study area.

Keywords: Metal concentration, water, sediment, river

1. Introduction

In South Africa, nearly 71% of the main rivers are considered as either endangered or critically endangered, mainly due to anthropogenic activities such as mining and industrialisation, contaminating these waterbodies. It is thus of great importance to conserve the few remaining so-called pristine rivers such as the Marico River, a tributary of the Limpopo River and one of the main rivers in the North-West Province.

No information is currently available regarding the metal composition of this river system. This study aims to contribute in closing the knowledge gap regarding the metal concentrations in the catchment, as well as the possible influence of tributaries by sharing the data obtained from two seasonal surveys.

2. Materials 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).

Sites were selected based on the importance and possible impacts of the tributaries on the Marico River. 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.

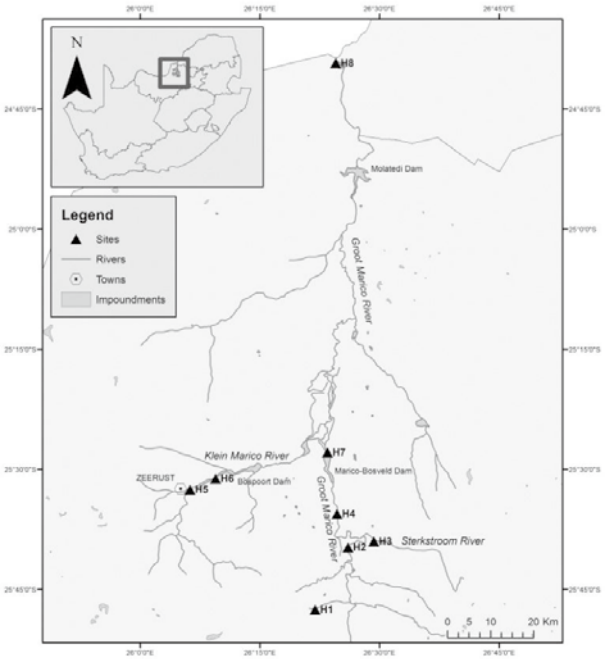

Figure 1: Sampling sites (triangles) within the Marico River catchment, North-West, South Africa.

Two surveys were conducted, one during a high-flow period (April 2013) and one during a low-flow period (June 2013). Water and sediment samples were collected separately in polyethylene bottles, from all the sites. Physico-chemical parameters including water temperature (Temp), electrical conductivity (EC) and pH were measured *in situ* at all the sites during each survey with portable digital instruments. Water samples were filtered and the filtrate was used directly for a baseline 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.

The values were, where possible, compared to existing target water quality guidelines (TWQG) as established for South Africa (Holmes, 1996) and only the metals that exceeded these values 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). 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.

When comparing and evaluating these metal concentrations to the water quality guidelines, only three elements exceeded the guideline values. These include phosphorous (P), copper (Cu) and lead (Pb). As for the sediments, no guideline values have yet been established for South Africa.

The levels of P and Cu exceeded the TWQR at the majority of the sites during the high-flow season, possibly due to runoff surface water. In contrast to this, the increased levels of Pb at all the sites during the low-flow season may indicate a possible concentration effect. Copper, a common environmental metal, was the highest at Site H1, the Marico Eye, and Site H7 in the Marico River. The main sources of Pb in the aquatic environment are generally regarded as originating from anthropogenic impacts however, as in this study; it can also be released into the water through the weathering of sulphide ores. High P concentrations at most sites, possibly originates from phosphorous bearing rocks and the decomposition of organic matter. At Site H5 however, the elevated P concentrations during both the flow periods might be ascribed to the sewage water discharge from the town of Zeerust.

Although the pH values and EC varied between the different sites and the two flow periods, it was, with the exception of pH at Site H3, never above or below the target water quality ranges acceptable for aquatic ecosystems (Holmes, 1996). From the results, it appears that the water of the tributaries was not impacted to such an extent that it had any substantial effect on the water of the Groot Marico River**.** The dolomitic geology of the area is further responsible for a buffer capacity, explaining the relatively constant pH values and ultimately stable metal concentrations throughout the system.

The fact that elevated levels of metals were present at the source of the Marico River (Site H1), where no known impacts are described in literature, and at many sites downstream of the Eye, indicates that these metal concentrations were most likely from natural sources such as geological weathering and not from any specific anthropogenic impacts.

4. Acknowledgments

We are indebted to the Unit for Environmental Sciences and Management, North-West University, Potchefstroom, South Africa for 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 range* values.

Site	Flow	Temp (°C)	EC (µS/cm)	pH	P	Cu	Pb
H1	High	20.7	256	7.14	76.64	6.4	0.06888
Marico Eye	Low	21.8	282	7.2	1.224	0.1703	4.213
H2	High	22.4	310	7.7	81.15	0.951	0.07446
Marico River	Low	15.8	295	7.38	1.538	0.1968	3.977
H3	High	22.3	137	6.45	78.03	0.8948	0.06687
Sterkstroom	Low	11.9	120	6.04	0.9869	0.07734	6.259
H4	High	25.3	297	6.98	83.97	0.004728	0.07462
Marico River	Low	17.2	302	8.05	0.9115	0.1773	4.277
H5	High	23.4	951	8.29	554.7	0.001177	0.06606
Klein Marico River	Low	10.5	773	7.86	742.3	0.01733	4.912
H6	High	21.5	522	7.61	85.83	1.018	0.07154
Klein Marico River	Low	11.3	560	7.9	0.8468	0.1056	3.782
H7	High	25.5	271	7.17	85.56	4.507	0.07395
Marico River	Low	12.9	265	7.61	0.9116	0.2239	4.779
H8	High	24.7	436	8.7	81.76	0.9921	0.06197
Marico River	Low	17.7	456	8.52	0.6633	0.1661	5.909
TWQR* Values				6.5 - 9	5-250	<0.3	<0.5

P-7

Heavy metal contamination in corals from Sodwana and Aliwal Shoal Marine Protected Areas, South Africa

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Coral reefs are one of the most biodiverse, yet delicate biomes on earth. No known published study had been conducted on the accumulation of heavy metals in corals from reefs along the South African coast. In July 2014, we collected nine coral species –five hard corals and four soft corals- from Sodwana and Aliwal Shoal MPA. We analysed 2 g of each coral sample for 18 heavy metals, using ICP-MS. The results show no distinct difference between corals from Aliwal Shoal and Sodwana, but clear difference between hard and soft corals. A type specific rather than site specific trend was shown.

Keywords: anthropogenic, coral reef, hard coral, soft coral

1. Introduction

Coral reefs are invaluable marine ecosystems, containing over a quarter of known marine species (Ko *et al*. 2014). They are extremely susceptible to anthropogenic damage (Peters *et al*. 1997). No known study has addressed heavy metal pollution in corals from South Africa. Traces of heavy metals can be taken from sea water and incorporated into the coral skeleton of hard corals (Ramos *et al*. 2009).

We predict that Aliwal Shoal will have higher concentrations of heavy metals in the coral tissue than corals from Sodwana, due to industrial activity on the banks of the Umkumaas River (SAPPI, 2014).

2. Materials and Methods

Corals were collected- with all the necessary permits- from Aliwal Shoal and Sodwana Reef in July 2014. Both areas are marine protected areas (MPAs). Fragments of coral colonies were removed, using either cutting pliers or a diving knife. Scleractinian (hard) corals collected were *Acropora spp., Fungia fungites, Pocillopora verrucosa, Stylophora pistillata* and *Dendrenophyllia robusta*. Alcyonarian (soft) corals were *Dendrenopthytha spp., Sarcophyton crassocaule, Eleutherobia grayi* and *Sinularia spp*.. The corals were transported in a cooler box from the dive site to land. On land, the corals were divided by species, placed in ziplock bags, and frozen at -20°C. Prior to analysis, the corals were frozen at -80°C and freeze dried.

The EPA 3050B method was used to completely digest 2 g of dried coral fragments. Inductively coupled plasma mass spectrometer (ICP-MS) was used to analyse the digested solution. Concentrations of 18 heavy metals were measured as mg/kg dry mass (dm).

3. Results and Discussion

The presence of different heavy metals in corals correlated to different coral types (Figure 1) rather than different sites (Figure 2). Our hypothesis was thus incorrect.

Soft corals had relative higher concentrations of As, Cu, Cd, Ni, Pb and Zn. *Sinularia* of both reefs had at least an order of magnitude higher concentration of Ni than any other corals– 803.5 mg/kg in Sodwana and 543 mg/kg in Aliwal. The nearest concentration was 23.9 in *Pocillopora* from Sodwana. It is known that *Sinularia* poses an effective anti-predator defence by releasing extracts and sclerites from both the base and the tips of the colony to deter predatory fish such as *Chaetedon* (Van Alstyne *et al*. 1994). There might be a correlation between Ni concentration and the predator deterrent. However, this remains to be examined.

Hard corals had higher affinity toward V, Mn, Al, Fe, Cr, Ag, U, Ti and Hg. Au, Pt and Co did not have any particular affinity to either hard or soft corals.

Metals that have been proven to have detrimental consequences on corals include Cu, Zn, Fe, Ni and Cd (Table 1). These effects include mortality, bleaching, metamorphoses and fertilization inhibition, and reduced larvae settlement (Van Dam *et al*. 2011).

Antifouling paints of boats, industrial waste or sewage outflow could be possible sources of heavy metal contamination (Ali *et al*. 2011).

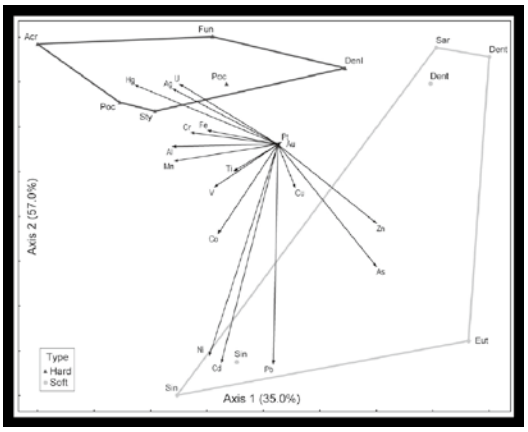

Figure 1. NMS of the associations of heavy metals to hard and soft coral types.

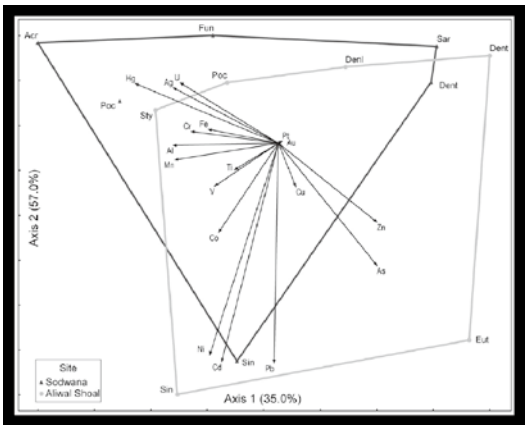

Figure 2. NMS of the associations of heavy metals to corals from Aliwal Shoal and Sodwana.

Species	Site	Ni	Cu	Zn	Cd	Hg	Fe
Acropora	Sod	18.51	0.71	65	0.05	0.28	2109
Dendrenopthytha	Sod	1.6	1.11	61	0.33	0.017	649.5
Fungia	Sod	9.94	1.02	68	0.07	0.031	1561.75
Pocillopora	Sod	23.91	0.9	65	0.001	0.022	2607.5
Sarcophyton	Sod	1.38	0.95	70	0.2	0.013	471.5
Sinularia	Sod	803.5	2.31	61	11.61	0.017	979.75
Eutherobia	Aliwal	1.49	1.77	62	0.42	0.015	458.5
Dendrenophyllia	Aliwal	1.72	0.74	73	0.17	0.014	992
Dendrenopthytha	Aliwal	1.2	1.05	66	0.25	0.013	599.5
Pocillopora	Aliwal	16.67	0.62	70	n.d	0.012	1911
Sinularia	Aliwal	543	1.19	65	10.7	0.015	1461.75
Stylophora	Aliwal	19.69	0.6	75	0.006	0.013	2253.25

Table 1: Concentrations of heavy metals (mg/kg) found in different corals from Aliwal Shoal and Sodwana

4. Acknowledgments

Thanks to Fanie and Cobus van der Schyff, Miekie van der Walt, JP Huisamen and Marinus du Preez, Scuba Xcursion and Ocean Divers.

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P-8

Experimental approaches of cytotoxicity and genotoxicity assessment of cadmium, mercury and their mixture on clarias gariepinus

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In order to assess the genotoxicity and cytotoxicity effects of cadmium, mercury and their mixture on catfish, 300 of *Clarias gariepinus* were divided into control and experimental groups. Three groups (A,B,C) were respectively exposed to cadmium (15.3 mg/L, 7.6 mg/L and 5mg/L), another set (D,E,F) was respectively exposed to mercury (0.2 mg/L, 0.1 mg/L and 0.06 mg/L) and the last experimental set (G,H,I) was exposed to combined metals. At the end of first, second and third week, five fish were randomly selected from each group and Blood for the smears was taken from the caudal vein with heparinised syringes and was immediately smeared. The study of the slides at 1000 x magnification revealed highly significant frequencies in MN and NAs reaching their peak at the end of the first week followed by a gradual decrease over the end of two and three weeks. The data obtained in the present work demonstrate that the environmental contamination with cadmium, mercury and their mixture can represent a great threat for the fish populations and also a serious problem for the aquaculture.

Keywords: *Clarias gariepinus*, blood erythrocytes, heavy metals, sub-lethal concentrations, MN, NAs

1. Introduction

A large number of pollutants in aquatic environment are responsible for multiple effects at the organisms, including human beings and ecosystem levels, affecting organ function, reproductive status, species survival, population size and ultimately biodiversity (Bickham et al., 2000; Dixon et al., 2002; Edorh et al., 2011). Among these pollutants, heavy metals known as carcinogenic and mutagenic compounds (Pruski and Dixon, 2002; Yadav and Trivedi, 2009) are the most problematic since their effect may exert damage beyond that of individual and may be active through following generations (Bolognesi and Hayashi, 2011). In fact environmental contamination with heavy metals is a potential cause of damage to genetic material (Prá *et al.*, 2006). The effects of genotoxic substances on fish genomes have been the theme of many studies, especially when seeking to establish the response of genes to environmental stimuli (Bücker *et al.*, 2006). Since the response from fish to toxicants is often similar to that found in the higher vertebrates, they can be useful in screening for chemicals potentially capable of inducing teratogenic and carcinogenic effects in humans (Al-Sabti and Metcalfe, 1995).

The aim of this research is to assess the genotoxicity and cytotoxicity effects of cadmium, mercury and their mixture on African catfish. *Clarias gariepinus* was chosen as an experimental animal on the basis of important criteria. This organism is a representative species and widely used in aquaculture in Africa. Moreover it is largely tolerant for high concentration of heavy metals. In addition, Clarias gariepinus is a hardy fish and can survive difficult conditions.

2. Materials and methods

Three hundred Juveniles of C. gariepinus were divided into 2 control treated with dilled water and benzene and 9 experimental groups treated with Cd, Hg and Cd +Hg.

Three doses were set for each toxicant from LC50 (Guedenon et al. 2011 and 2012).

At the end of the first, second and third week five fish were randomly selected from each group and venous blood was collected and was immediately smeared for MN and NAs assessment.

3. Results and discussion

The different frequencies of micronuclei, bi-nucleated cells, blebbed and notched nuclei recorded during our investigation of genotoxicity and cytotoxicity connected to the exposure of *Clarias gariepinus* to sub-acute doses of cadmium, mercury and combined cadmium and mercury over one week, two weeks and three weeks exposure are presented in figures 1, 2 and 3.

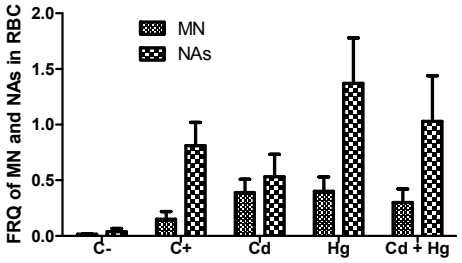

Fig 1: Frequencies of MN & NAs of C. gariepinus according to the groups

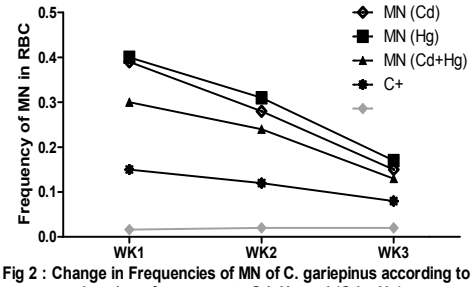

Fig 2 : Change in Frequencies of MN of C. gariepinus according to duration of exposure to Cd, Hg and (Cd + Hg)

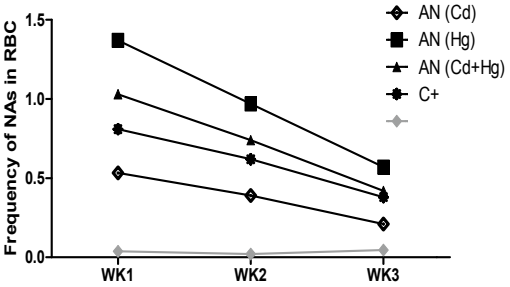

Fig 3: Change in Frequencies of NAs of C. gariepinus according to duration of exposure to Cd, Hg and (Cd + Hg)

The comparison of the frequencies of MN and NAs in blood erythrocytes over one, two and three weeks(Figures 2 and 3) revealed that the highest values of MN and NAs were recorded at the end of the first week followed by a gradual and steady decrease over the time (second and third weeks). Besides, the cytogenotoxic effects of the heavy metals (Cd, Hg and Cd+Hg) were observed to be severe at elevated concentrations. This report of dose-dependent and time-dependent increase in the induction of micronuclei and nuclear abnormalities in peripheral blood in the present investigation accords with those of Çavaş et al.(2005) and Yadav and Trived (2009).

Our study demonstrated that cadmium, mercury and combined cadmium and mercury are highly genotoxins and cytotoxin since they were the main responsible for all observed nuclear alterations. According to Da Silva Souz and Fontanetti (2006) and Ventura et al. (2008), chemicals such as heavy metals involved in nuclear alterations interfere in the DNA synthesis of exposed organisms resulting in nuclear abnormalities. It was proved that most of the toxic chemicals that produce genotoxic effects trigger the formation of reactive oxygen species (ROS) as well as electrophilic free-radical metabolites. The reactive oxygen species then interact with DNA to cause disruptive changes (Chandra and Khuda-Bukhsh, 2004). Additionally, Çelik et al. (2009) and Liu et al. (2009) demonstrated that ROS production could reduce cellular antioxidants and lower cellular defence against oxidative stress. The ensuing effects are inhibition of normal cell division, damage of erythrocyte chromosomes, interruption of DNA duplication and MN induction occurs as consequence.

Conclusion: The data obtained in the present work demonstrate that the environmental contamination with cadmium, mercury and their mixture can represent a great threat for the fish populations and also a serious problem for the aquaculture.

4. References

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P-9

The influence of acid volatile sulphides (AVS) on metal bioavailabilty from sediments of the Olifants River, South Africa

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Acid volatile sulphides (AVS) are believed to reduce the bioavailability of metals from sediments. The protective role of AVS in the metal contaminated Olifants River was studied by relating metal bioaccumulation in the Leaden mudfish, *Labeo molybdinus*, to different chemical characteristics of water and sediments from the same sites. The same level of bioaccumulation occurred irrespective of the AVS concentrations in the sediments. Other factors such as total metal concentrations and competing ions in the water column (e.g. calcium) also played a role in predicting the bioaccumulation of metals in epi-benthic feeding organisms. It was thus concluded that AVS is not a significant sediment characteristic in describing variation in sediment bioaccumulation.

Keywords: mudfish, simultaneously extracted metals, environmental modifying factors

1. Introduction

The Olifants River is often regarded as the most mineralized river in South Africa with the sediments acting as a major sink for the high metal concentrations (Wepener *et al.* 2000). External environmental factors are able to modify the chemical potential to which the organisms are subjected (Di Toro et al. 1990). As a consequence, different sediments will exhibit different degrees of toxicity for the same total quantity of chemical. Metal availability and subsequent bioaccumulation within benthic dwelling aquatic species is thus a consequence of a number of factors.

The acid volatile sulphide (AVS) concentrations present within aquatic sediments are a function of anaerobic bacterial action, exerting a strong influence on cationic metal activity and toxicity (Di Torro et al., 1990). The protective role of AVS is based on the premise that when AVS (molar based) concentrations in sediments exceed simultaneous extracted metal (SEM) concentrations (SEM–AVS < 0) the metals will be bound to sulphides rendering them non-bioavailable and therefore non-toxic.

The aim of this study was to determine the influence of AVS concentrations in sediments of the Olifants River of the bioavailability of metals by measuring the bioaccumulation of these metals in tissue of the Leaden mudfish, *Labeo molybdinus*. This is a an epibenthic dwelling fish that occurs in the Olifants River and it is exposed to both metals in sediments as well as in the water column.

2. Methods

Water, sediment and Leaden mudfish were collected from three sites along the length of the Olifants River inside the Kruger National Park, South Africa during September 2011. Samples and livers tissue, excised in the field, were frozen in acid pre-cleaned polypropylene containers.

The purge-and-trap method was used for AVS determination (De Jonge et al. 2009).

The remaining extract was used to measure the SEM fraction. The organic matter content of the sediment was determined using the Loss of Ignition (LOI) method. For this purpose approximately 1 g of dried sediment was incinerated at 600°C for 6 hours.

Concentrations of Cd, Cr, Cu, Ni, Pb and Zn in the extraction solutions were measured by means of a Thermo X-series 2 quadrupole-based ICP-MS instrument. Significance ($p<0.05$) was tested using Analysis of Variance. Spearman's correlation analyses were done to identify significant relationships between metal bioaccumulation and environmental factors while linear regression was used to describe these relationships.

3. Results

The AVS concentrations showed a high variability among the sampling sites and increased from 84.12 µmol/g at site 1 to 548.34 µmol/g at site 3. The clay content also varied between sites, ranging from 8.1 % at site 3 to 12.6 % at site 2. The organic carbon content (expressed as LOI) was low at all sites, ranging from 0.41 % at site 2 to 1.18 % at site 3. The spatial bioaccumulation results of Cd, Cu, Ni, Pb and Zn in liver tissue of *L. molybdinus* revealed a significant decrease in Cu, Ni, and Pb from site 1 to 3 (Figure 1). Zinc is the only metal that remained high at all three sites.

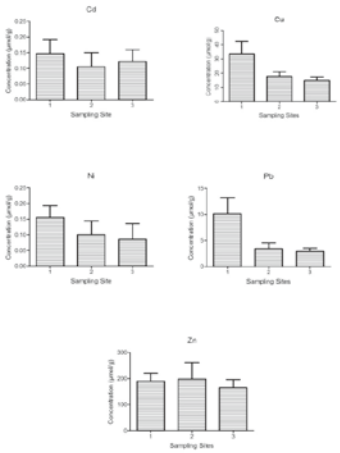

Figure 1. Mean + standard error of metal bioaccumulation in the liver of *Labeo molybdinus* (µmol/g dry weight).

Metal bioaccumulation was best correlated with total metal content in the sediment normalised for organic carbon (LOI) and suspended metal concentrations in the surface water. For Cd, Cu, Ni, Pb and Zn SEMME showed significant correlations with accumulated tissue concentrations even when [SEM-AVS]<< 0. There was a positive relationship between Cu, Ni and Zn bioaccumulation and SEMME-AVS and Total metals in sediment/ LOI, while no relationship was found for Cd and Pb. Positive correlations were present between Ni concentrations of *L. molybdinus* and SEM-AVS and Zn bioaccumulation and Total metals in sediment/LOI, AVS, SEM, SEM-AVS, SEM-AVS/ LOI and dissolved metal concentrations. The linear regression models that best described the bioaccumulation processes were for Cu and Zn with AVS-SEM and AVS respectively (Figure 2) and Ni bioaccumulation is decreased through increased water hardness.

4. Discussion and conclusion

In this study we found that the AVS levels were highly variable in the Olifants River and supports the findings of De Jonge et al. (2009) who indicated this high variability in rivers in Flanders. High levels of Cu, Pb and Zn were accumulated even when AVS concentrations largely exceeded SEM concentrations. These results are in agreement with De Jonge et al. (2009). Recent studies have indicated that the relationship between AVS and metal accumulation in aquatic invertebrates is highly dependent on many variables, including feeding behaviour and ecology (De Jonge et al., 2010) and the results from this study further support these findings.

It is therefore concluded that AVS does not necessarily play a protective role in bioaccumulation of metals from sediments.

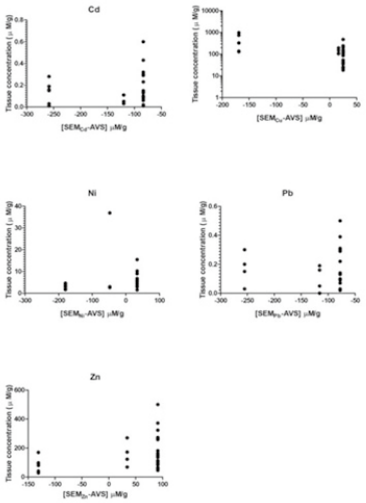

Figure 2. Relationship between metal bioaccumulation in Labeo molybdinus liver tissue (µM/g) and [SEMMe-AVS].

5. Acknowledgements

Financial support for this project was provided by the Water Research Commission (WRC Project No.K5/1922, NJ Smit PI)

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P-10

The use of bird feathers as indicators of historical metal contamination in Gauteng, South Africa

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Feathers are commonly used as non-invasive tissues for bioaccumulation studies in birds. In this study metal bioaccumulation in breast feathers collected from Weavers during 2002 and 2005 was compared to museum samples from 1899 to 1959 to assess the degree of incresed metal contamination in the highly industrialised region of Gauteng, South Africa. Levels of Co, Cu, Mn and Ni have not changed in the past centuary, while Al, Cd, Cr, Fe, Pb, Sr and Zn have increased. When compared to international studies Al, Fe and Zn levels in Weaver feathers were extremely elevated. This study demonstrated the value of access to historical samples to allow for comparison with current levels.

Keywords: Weavers, breast feathers, museum samples

1. Introduction

The use of feathers has been proposed as a non-invasive biomonitoring method for bioaccumulation of metals in birds (Burger 1993). In an extensive study on owls in South Africa, Ansara-Ross *et al*. (2013) demonstrated that there was a good correlation between the metal levels in feathers and internal organs to warrant the application of feathers as a non-invasive bioaccumulation indicator tissue for birds. Since feathers do not degrade over time, historical contamination may be determined through the use of museum samples (Burger 1993).

The aim of this study was to determine the historical metal exposure patterns in birds from the industrialised Gauteng Province, South Africa. Weavers were chosen as they fulfil most of the criteria for being used in biomonitoring (Burger 1993). For this study three weaver species were chosen, the Southern Masked Weaver (*Ploceus velatus*), Red Bishop (*Euplectes orix*) and Red-billed Quelea (*Quelea quelea*).

2. Methods

Four sites were selected in the province of Gauteng, South Africa. The sites were sampled during the autumn months of February to May in 2002 and 2005. During this time period the birds are either building nests or already breeding and they are thus expected to stay in that specific area. For the determination of historical metal levels, feather samples were collected from the Transvaal Museum in Pretoria. The date of the samples ranged from 1899 to 1959, most being from the period 1900 to 1910. All of the samples were collected from Gauteng.

The feather samples were prepared according to the methods of Dauwe *et al.* (2004). The feathers were washed and acidified with a 1:1 mixture of HNO_3 (70%) and H_2O_2 (30%). The samples were subjected to microwave digestion and the metal content of all the samples were measured using an inductively coupled plasma mass spectrophotometer (X-7 ICP-MS, Thermo Elemental).

Data were tested for normality and homogeneity of variance using Kolmogorov-Smirnoff and Levene's tests, respectively. Post-hoc multiple comparisons between sampling periods were made using the appropriate Scheffé (parametric) or Dunnette-T3 (non-parametric) test to determine which values differed significantly ($p<0.05$).

3. Results

Initial statistical analyses showed there were no significant differences in feather metal bioaccumulation between the three weaver species. For this reason the data from individuals of the three species were pooled for analyses. The metal concentrations are divided into five different time periods, i.e. 1899-1919, 1920-1939, 1940-1959, 2002 and 2005. Distinct temporal metal bioaccumulation patterns were observed. For Al (Fig. 1A), Cd (Fig. 1C), Cr (Fig. 1E) and Pb (Fig. 2D) there were no significant differences between the first three year groups.

Several metals once again displayed similar concentrations for the first three year groups, but with 2002 concentrations higher than 2005. Metals that followed this pattern were Co (Fig. 1D), Fe (Fig. 2A), Mn (Fig. 2B) and Zn (Fig. 2F). Once again the 2002 concentrations were higher than the 2005 concentrations. Although there were differences among the year groups, the difference were not significant. It was only for As (Fig. 1B) were more than a thousand times higher in the museum specimens than in the field specimens.

4. Discussion and conclusion

For most metals there is an increase from 1899

to 2005. This increase is however not significant for all metals measured during this study. The current (2002/2005) levels of Al , Fe and Zn are very high compared to levels recorded in Great Tit feathers from polluted sites in Belgium (Janssens *et al*. 2001; Dauwe *et al.* 2004) and owl feathers from South Africa (Ansara-Ross *et al*. 2013). The levels of Cr recorded in weaver feathers were also much higher than levels recorded in Europe and owl species in South Africa. Although Cd and Pb concentrations increased from historical levels to 2005 in weaver feathers these levels were still much lower than levels recorded in feathers from Great Tits from industrially polluted sites in Belgium (Janssens *et al*. 2001). Cobalt, Cu, Mn and Ni concentrations have not changed when compared to historical levels. These concentrations are also low when compared to concentrations in Great Tits (Janssens *et al*. 2001).

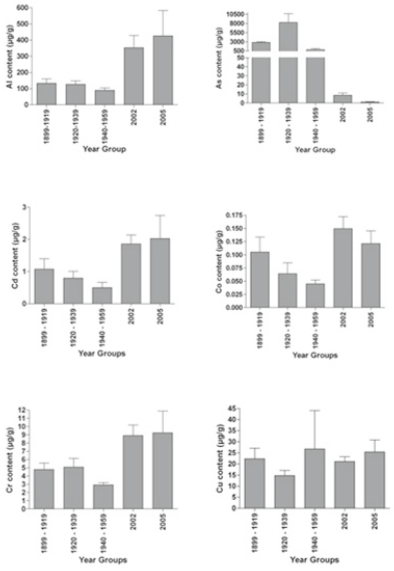

Figure 1. The concentrations (µg/g dry weight) of Al (A), As (B), Cd (C), Co (D), Cr (E) and Cu (F) in feathers of weavers for the various year groups.

The As concentrations from the museum samples were significantly higher than the current levels. This can be attributed to the use of As soap for the preservation of museum specimens. The results from this study provide a good baseline for evaluating the degree of metal contamination in terrestrial ecosystems.

5. Acknowledgements

The authors wish to thank the Transvaal Museum in Pretoria for allowing us to collect feathers from museum specimens.

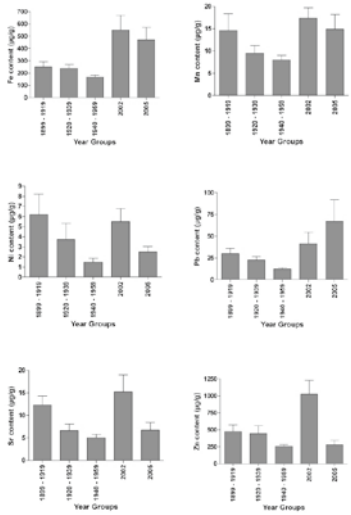

Figure 2. The concentrations (µg/g dry weight) of Fe (A), Mn (B), Ni (C), Pb (D), Sr (E) and Zn (F) in feathers of weavers for the various year groups.

6. References

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P-11

Assessment of exposure to toxic elements (As, Pb, Cd and Hg), and DDTs in four species of birds from Ethiopia

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Exposure to contaminants is a hypothesis to explain the decline in bird populations. In the present study, accumulation levels of toxic elements (As, Cd, Pb and Hg), and DDTs were investigated in liver of four bird species (*Scopus umbretta, Threskiornis aethiopicus*, *Leptoptilos crumeniferus*, and *Pelecanus onocrotalus*) from Ethiopia. Results showed liver concentrations of Cd (mean 1042 ng/g dw) and Hg (mean 749 ng/g ww) in *P. onocrotalus*, Pb (mean 91 ng/g dw) in *L. crumeniferus*, and As (mean 78 ng/g dw) in *T. aethiopicus* were found high. However, levels of these toxic elements were below their toxicological thresholds. Levels of ∑DDTs ranged from 103 to 4578 ng/g ww, and the highest concentration was observed in *L. crumeniferus*. Among the metabolites, *p,p′*−DDE was the most abundant isomer followed by *p,p′*−DDD, interpreting as contamination by old DDT. This hypothesis may not apply in Ethiopia since DDT is still in use because of malaria. In addition, *p,p′*−DDT was detected in the samples, indicating exposure to a “fresh” source of DDT. The risk characterization associated with exposure to DDE demonstrated potential risks to birds.

Keywords: Toxic element, DDT, Bird, Liver, Ethiopia

1. Introduction

Many of the bird species that we value are in decline (http://www.birdlife.org), and multiple threats have been suggested such as contaminant exposure from anthropogenic sources. Exposures to toxic elements and DDTs are of increasing concern in developing countries. Once in the environment, these pollutants can enter the food web where bioaccumulation and/or biomagnification can occur. Elements are naturally present in the environment, and distributed in biota (Pacyna and Pacyna 2001). Although the use of chlorinated pesticides has been banned, DDTs are still in use for agricultural and public health purposes in Africa. Ethiopia has implemented indoor residual spraying with DDT for malaria control for nearly few decades.

Birds are used as ecotoxicology sentinel species due to their trophic position, widespread distribution and long life span. Birds are liable to consume prey with high level of contaminants through their food. Toxic elements contamination can cause avian mortality directly, but more commonly have sub-lethal effects. Adverse effects of DDTs on birds include reduced reproductive success, eggshell thinning, and alteration of liver functions (Gisey et al. 2003). In spite of the great variety and number of birds in Africa, most studies have been carried out on species inhabiting developed countries. There is a paucity of information about the impacts of environmental pollutants in birds from Africa.

Therefore, aims of this study are to: (1) assess levels of toxic elements and DDTs in liver of four bird species in Ethiopia, and (2) compare pollutant concentrations to avian toxic effects thresholds to assess any threat to avifauna at the study site.

2. Materials and Methods

2.1 Study area and sampling

The Ethiopian Rift Valley, which encompasses seven lakes is an important area for agricultural, commercial and industrial development. The region serves as breeding and wintering ground habitat for several resident and migratory bird species. However, anthropogenic sources of DDTs and trace elements lead to unprecedented environmental contamination to the ecosystem.

23 birds belonging to 4 species (*Scopus umbretta*; *N* = 5, *Threskiornis aethiopicus*; *N* = 7, *Leptoptilos crumeniferus*; *N* = 6, and *Pelecanus onocrotalus*; *N* = 5) were captured in 2012 at the shore of Lake Ziway. All specimens were dissected, and livers were collected and stored at −20°C until analysis. Frozen samples were transported to Japan for analysis.

2.2 Toxic elements analysis

Dry liver sample (~0.5 g) was digested with 65% HNO_3 and 30% H_2O_2 in a microwave digestion system (Speed Wave MWS-2; Berghof, Germany). Levels of three toxic elements (As, Cd, and Pb) were analyzed using an inductively coupled plasma-mass spectrometer (ICP-MS; 7700 series, Agilent

technologies, Tokyo, Japan). Mercury levels were assessed by MA-3000 mercury analyzer (Nippon Instruments Corporation, Tokyo, Japan). Reference standard materials, DORM-3 and DOLT-4, were used for method validation and quality control.

2.3. DDTs analysis

Liver (2 g) was homogenized with anhydrous sodium sulfate, and extracted using a Soxtherm apparatus (S306AK Automatic Extractor, Gerhardt, Germany) with hexane:acetone (3:1, v/v) using a method described by Yohannes et al. (2014). The lipid content was determined gravimetrically, and the rest of the extract was cleaned up on a column filled with activated florisil and eluted with hexane:dichloromethane (7:3, v/v). Analysis of DDTs (*o,p′*−, and *p,p′*−DDT, DDE, and DDD) were performed using a gas chromatography equipped with electron capture detector (GC-μECD, Shimadzu, Kyoto, Japan) and an ENV-8MS capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness). Procedural and spiked blanks, and standard reference material SRM 1947 were analyzed for QC/QA process.

3. Results and Discussion

3.1 Levels of toxic elements

Levels of toxic metals showed significant difference ($p < 0.05$) among the bird species except for Pb (Figure 1). Concentrations of Cd (1042 ± 87 ng/g dw), and Hg (749 ± 302 ng/g dw) were found high in the liver of *P. onocrotalus*, aquatic bird species, indicating the accumulation ability of Cd and Hg in aquatic ecosystems. *T. aethiopicus* and *L. crumeniferus*, having a wide range of feeding habits from both mainly terrestrial and aquatic food webs, had high levels of As (78 ± 24 ng/g dw), and Pb (91 ± 85 ng/g dw), respectively.

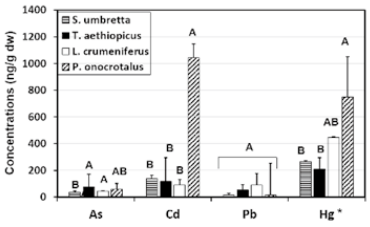

Figure 1: Toxic metal levels in liver of 4 bird species from Ethiopia. (* concentration expressed as ng/g ww)

Overall, concentrations of As, Cd, Pb and Hg were lower than their corresponding threshold levels. In this context, levels of these toxic elements could be considered as background levels for birds from Ethiopia.

3.2. Levels of DDTs

The levels of ΣDDTs ranged from 103 to 4578 ng/g ww, and exhibited high intra and inter variation among the species (Figure 2). The magnitude of pollutant bioaccumulation has been associated with feeding ecology, trophic level, ability to metabolize contaminants, and seasonal variation of food compositions (Jasper et al. 2006).

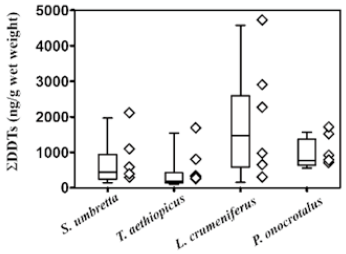

Figure 2: Levels of ∑DDTs in liver of 4 bird species from Ethiopia

p,p′−DDE was the most abundant isomer owing to its high chemical stability, and persistence in the environment and in living organisms. The abundance of this isomer compared to *p,p′*−DDT might be interpreted as the result of old DDT contamination. This hypothesis may not apply in Ethiopia, since DDT is still in use because of high incidence of malaria and the corresponding fatalities. Additionally, *p,p′*−DDT was detected in the liver, indicating exposure to a “fresh” DDT source.

Concentrations of *p,p′*−DDE in liver samples ranged from 100.8 to 4498 ng/g ww (2000 to 107000 ng/g lw) could pose a threat in terms of toxicity i.e., eggshell thinning and survival of young birds because DDT is still in use in the region.

4. Acknowledgments

This study was supported in part by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan, which was awarded to M. Ishizuka and Y. Ikenaka.

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P-12

Exposure levels of polycyclic aromatic hydrocarbons (PAHs) and heavy metals in wild rats in Kumasi, Ghana

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Wild rats were trapped from communities in Kumasi, Ghana, to determine the concentrations of polycyclic aromatic hydrocarbons (PAHs) and heavy metals. The results of the study showed that phenanthrene (0.51 ± 0.08 ng/g dw) was the most abundant individual PAH in the liver of wild rats. The ∑PAH concentrations in liver ranged from 0.05 to 1.64 ng/g dw in Bomso (a community) and city centre, respectively. Abundance of individual PAHs in the liver of wild rats decreased in the order: phenanthrene > pyrene > acenaphthene > naphthalene > fluoranthene and > anthracene. Levels of phenanthrene and pyrene detected were significantly higher than other PAHs. Naphthalene and fluoranthene were detected in 80% and 78% of the samples collected respectively. Heavy metals in the liver of wild rats in Kumasi decreased in the order Zn > Cu > As > Ni > Pb > Co > Cd > Cr.

Keywords: Wild rats, Polycyclic aromatic hydrocarbons, Heavy metals, Ghana

1. Introduction

As a developing country, the economic and population growth rates in Ghana over the past few years have seen tremendous increase. Currently, the gross domestic product and annual population growth rates of Ghana stand at 6.1% and 2.5% respectively. The growing rate of industrialization is gradually leading to contamination and deterioration of the environment, and pollution is likely to reach disturbing levels. Previous studies of PAHs in air and soils showed high concentrations in the city centre, including benzo[a]pyrene, and that fuel combustion was the dominant source of PAHs in the region (Bortey-Sam et al. 2013; 2014; 2015). Rats could be used as sentinels to measure the environmental pollution state because they are mammals that share many processes with humans and are appropriate for use to answer many research questions. They tend to pick up food and water from the ground which could be contaminated with various pollutants e.g. PAHs and heavy metals. The objective was therefore to determine the exposure levels of wild rats in Kumasi to environmental pollutants such as 16 USEPA PAHs and heavy metals.

2. Materials and Methods

2.1 Study area and sampling

Kumasi is one of the most industrialized and economically significant cities in Ghana, and has been subjected to heavy anthropogenic influences as a result of rapid economic development and urbanization. The human population has drastically increased, the number of cars has doubled during the past decade leading to greater fuel combustion rate. Wild rats (n = 46) were trapped from communities in Kumasi in May 2011. They were euthanized and liver samples collected. The samples were stored at –20 °C in KNUST, Ghana and later transported to the Laboratory of Toxicology, Hokkaido University, Japan where they were stored at –30 °C until analysis. Treatment of all animals were according to the guidelines of the Hokkaido University Institutional Animal Care and Use Committee.

2.2 PAHs analysis

For PAHs analysis, liver sample (~2 g) was homogenized and extracted using acetone:hexane mixture (1:2, v/v). The extract was reduced to ~1 mL, passed through a column packed with silica gel, and eluted with diethyl ether:hexane mixture (1:4, v/v). The eluate was collected, concentrated and made up to 100 µL using methanol. Samples were analysed using HPLC 20A series (Shimadzu) with a fluorescence detector (RF-1AXL; Shimadzu), equipped with an ODS column (ODS-120T 2.1 mm × 300 mm; Tosoh). Mobile phase A consisted of 10 mM ammonium acetate buffer (pH 5.0), and B was methanol/acetonitrile/water solution (38:57:5, v/v/v). Concentrations of 16 USEPA priority PAHs; naphthalene (Naph), acenaphthylene (Acl) acenaphthene (Acen), fluorene (Fle), phenanthrene (Phe), anthracene (Ant), fluoranthene (Fluo), pyrene (Pyr), benz[a]anthracene (BaA), chrysene (Chr), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene

(BkF), benzo[a]pyrene (BaP), indeno[1,2,3–cd] pyrene (IP), dibenz[a,h]anthracene (DBahA), and benzo[g,h,i]perylene (BghiP), were measured in each sample. Results were expressed in ng/g dry weight (dw).

2.3 Heavy metal analysis

Dried liver sample (~0.5 g) was digested with 65% HNO_3 and 30% H_2O_2 in a microwave digestion system (Speed Wave MWS-2; Berghof, Germany). Levels of heavy metals (Cr, Co, Ni, Cu, Zn, As, Cd, and Pb) were analysed using an inductively coupled plasma-mass spectrometer (ICP-MS; 7700 series, Agilent technologies, Tokyo, Japan). Standard reference materials, DORM-3 and DOLT-4, were used for method validation and quality control.

3. Results and Discussion

3.1 Levels of PAHs

Of the 16 PAHs analysed, 6 were detected in the rat livers. Ten PAHs, namely Acl, Fle, BaA, Chr, BbF, BkF, BaP, IP, DBahA, and BghiP, were not detected in any of the samples. The 6 compounds detected were considered for ∑PAHs. The ∑PAH concentrations in liver ranged from 0.05 to 1.64 ng/g dw, in Bomso (a community) and the city centre respectively. The minimum (0.05 ng/g dw) and maximum (1.64 ng/g dw) concentration of ∑PAHs were both in female rats that weighed ~42 and 300 g respectively. As shown in Figure 1, the abundance of PAHs (ng/g dw) in liver of wild rats collected decreased in the order: Phe (0.51 ± 0.08) > Pyr (0.39 ± 0.20) > Acen (0.19 ± 0.01) > Naph (0.15 ± 0.01) > Fluo (0.11 ± 0.11) and > Ant (0.07 ± 0.07). The levels of Phe and Pyr were significantly higher than other PAHs (Figure 1). Naph and Fluo were detected in 80% and 78% of the samples respectively. In surface soils in Kumasi, Pyr was the second most abundant PAH (Bortey-Sam et al. 2014), and rats could have been exposed (to Pyr and other PAHs) from the soil through dermal contact or through ingestion during feeding or drinking.

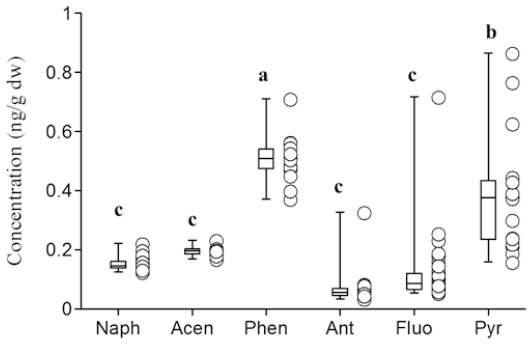

Figure 1: Distribution of PAHs in the livers of wild rats in Kumasi, Ghana (different letters (a, b, and c) indicate statistical difference, Tukey's test: $p < 0.05$)

3.2 Levels of heavy metals

The mean (± SD) concentrations of heavy metals in the livers of wild rats in Kumasi, and decreased in the order Zn > Cu > As > Ni > Pb > Co > Cd > Cr (Table 1). The results of Zn, As, Cu, Ni, from this study were higher than the study by Nakayama et al. (2013) on the accumulation and biological effects of metals in wild rats in mining areas in Kabwe, Zambia. The high concentrations of these heavy metals in liver samples of rats within the area in Kumasi could be due to exposure through combustion of fossil fuels and metal smelting.

Table 1: Heavy metal concentrations (mg/kg dw) in livers from wild rats

Rat liver	Cr	Co	Ni	Cu	Zn	As	Cd	Pb
Average	0.077	0.18	4.9	14	265	9.6	0.11	0.70
SD	0.039	0.029	4.6	2.5	56	4.4	0.034	0.24
Minimum	0.034	0.14	2.4	10	199	1.2	0.087	0.44
Maximum	0.15	0.21	15	17	342	15	0.16	1.1

4. Acknowledgments

This study was supported in part by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan, which was awarded to M. Ishizuka (No. 19671001).

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P-13

PAH Levels in smoked fish species from selected markets in Benin City, Nigeria: Potential Risks to Human Health

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The levels of sixteen (16) priority Polycyclic Aromatic Hydrocarbons (PAHs) in three locally available and commonly consumed smoked fish species: *Clarias gariepinus* (Cat fish), *Ethmalosa fimbriata* (Bonga fish) *and Tilapia zilli* (Mangala) from selected markets in Benin City, Nigeria were assessed to determine possible human health risks associated with consumption. Assessment of human health risks were estimated for two (2) age/weight categories: 1-11years/30kg (children) and 70kg (adult) using standard health risk indices. Risks were categorized for non-carcinogenic effects and assessed at the average measured exposure concentrations (MEC). Total PAHs ranged from 0-0.204 mg/kg (*C. gariepinus)*, 0-0.315 mg/kg (*T. zilli)*, and 0-0.288 mg/kg (*E. fimbriata)*. There was a considerable predominance of the 5-ring PAH (Benzo(b) floranthrene) in *C. gariepinus* and *E. fimbriata,* while Naphthalene (2-ring) was the most dominant congener in *T. zilli*. Concentrations of benzo[a]pyrene in all the smoked fish samples (except for *C. gariepinus*) exceeded the acceptable maximum residual limit (MRL) of 5 µg/kg wet weight, which calls for concern. Human health risk estimations for children and adult showed EDI values for most of the PAHs exceeding threshold values. Non-cancer health risk estimates showed hazard index (HI) exceeding 1, indicating the possibility of non-cancer health risks to consumers from consumption.

Keywords: PAHs, Smoked fish, Dietary intake, Health risk.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are important group of compounds of major environmental concern. The health effects posed by these compounds has been reported (Palm *et al.,* 2011). Dietary exposures has been implicated as the major source of exposure to PAHs (Phillips, 1999). One significant food source of PAHs is smoked fish (Palm *et al.,* 2011). Smoke not only gives the fish special taste and aroma, it also improves preservation due to its dehydrating and bactericidal properties (Palm *et al.,* 2011; Silva *et al.,* 2011). However, smoke especially wood smoke contains PAHs, many of which are carcinogenic (Phillips, 1999).

In developing countries, smoking is the most common method employed in preserving fish. In Nigeria, smoked fish products constitutes about 61% of the total 194,000 metric tons of dry fish produced (Silva *et al.,* 2011). In Benin City, smoking is the most common fish preservation technique and smoked fish products are the most available form of fish product for consumption (Silva *et al.,* 2011). This could be attributed to the fact that most of the fishing communities have limited access to electricity to preserve their fish products (Silva *et al.,* 2011). This has however increased the risk of PAHs contamination through consumption. Several studies have shown the presence of PAHs in smoked fish (Phillips, 1999; Silva *et al.,* 2011). However, despite these reported studies, limited data exists on human health risks associated with PAHs contamination through consumption.

This study was therefore carried out to determine the concentration of PAHs in three locally available and commonly consumed smoked fish species (*Clarias gariepinus* (Cat fish), *Ethmalosa fimbriata* (Bonga fish) *and Tilapia zilli* (Mangala)) from selected markets in Benin City, Nigeria, to assessed possible human health risks associated with consumption.

2. Materials and Methods

2.1 Sample collection, Extraction and Analysis

All experiments using animal specimens were performed according to the guidelines of the Committee of Animal Care and Use, University of Benin.

Fish samples, *C. gariepinus* (Cat fish) *E. fimbriata* (Bonga fish) *and T. zilli* (Mangala) were randomly collected from three markets in Benin City, Edo state: Oregbeni (6°21' 0.09" N and 5° 39' 32.67" E), New Benin (6°23' 59.96" N and 5° 36' 67.37" E) and Santana market (6°17' 44.6" N and 5° 38' 8.9" E). Samples were packaged in polythene bags and were refrigerated at 4°C until extraction. Samples were collected for a period of four months (June-September). 10 g of the homogenized fish sample was thoroughly mixed with anhydrous Na_2SO_4 to dehydrate the sample. PAH extraction and analysis was done according to the method described by Pena *et al.,* (2006).Quantification was carried out using a Hewlett Packard (HP) 5890 series II Gas chromatograph model equipped with an electron capture detector (ECD). A standard mixture of 16 priority PAHs were obtained and used for the analysis.

2.2 Human health risk estimation

Human health risk estimates for individual PAHs concentrations in the fish species were computed using estimated daily intake (EDI) (mg/kg/day),

hazard quotient (HQ) and hazard index (HI). Risks were estimated for two (2) age/weight categories: 1-11years/30kg (children) and 70kg (adult).Risks were categorized for carcinogenic and non-carcinogenic effects and assessed at the average measured exposure concentrations (MEC).

3. Results and Discussion

3.1 Concentrations and Possible Source of PAH in Smoked Fish.

A total of 16 priority PAHs were analysed in the three smoked fish species (*E. fimbriata*, *T. zilli* and *C. gariepinus*), however, benzo(k)fluoranthrene, indeno(1,2,3)pyrene, dibenzo(a,h) anthracene and benzo(g,h,i)perylene were not detected in the fish samples while Benzo(b)floranthrene and Benzo(a) pyrene were not detected in *T. zilli.* The mean PAHs concentrations (mg/kg) in *E. fimbriata*, *T. zilli* and *C. gariepinus* are presented in Figure 1. Varying concentrations of PAHs were observed in the fish species with higher concentrations of PAHs observed in *T. zilli,* concentrations were however not statistically significant ($p>0.05$, $F= 0.02$) between the species. PAHs concentrations in the fish samples showed the predominance of the 5-ring PAHs with the carcinogenic congener, benzo(b)fluoranthrene being the most dominant in *E. fimbriata* (41.5%) and *C. gariepinus* (28.5%), while Naphthalene, the 2-ring PAH was dominant (33.2%) in *T. zilli* (Figure 2). Concentrations of benzo[a]pyrene in all the smoked fish samples (except for *C. gariepinus*) exceeded the acceptable maximum residual limit (MRL) of 5 µg/kg wet weight. Assessment of PAHs sources were identified using the ratio of phenanthrane to anthracene (Phe/Ant).The ratio of Phe/Ant obtained for *E. fimbriata*, *T. zilli* and *C. gariepinus* were 0.32, 1.56 and 1.72 respectively, which suggests that the PAH contamination was predominantly of pyrolytic origin.

3.2 Human Health Risks through Fish Consumption

The estimated EDI values of acenaphthene, phenanthrene and chrysene in the three fish species for children and adult were above threshold values (Table 1). Likewise, EDI values of acenaphthylene, fluorene, benzo(b)floranthrene and benzo(a)pyrene in *C. gariepinus* and *E. fimbriata* were also above threshold values for children and adult (Table 1).

To further assess the threat to human health attributable to PAHs in smoked fish, risks were categorized for carcinogenic and non-carcinogenic effects using HQ and HI values (Figures 3, 4).The estimated human health risks showed no potential carcinogenic health effects while estimations for non-cancer health risk showed HI values in the three species of fish and HQ values for acenaphthylene, phenanthrene and chrysene above one for children and adult, while HQ values for *C. gariepinus* and *E. fimbriata* for benzo(b)floranthrene and benzo(a)pyrene were also above one for both age/weight categories, indicating potential non-carcinogenic health effects from consumption of these fish species.

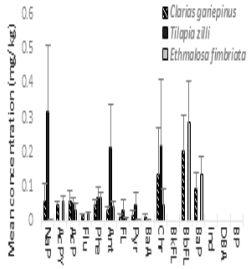

Figure1: Mean Concentrations (mg/kg) of PAHs levels in smoked fish species from selected markets in Benin City, Nigeria.

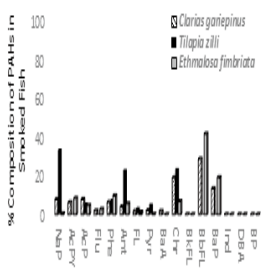

Figure 2: Percentage (%) Concentrations of PAHs levels in smoked fish species from selected markets in Benin City, Nigeria.

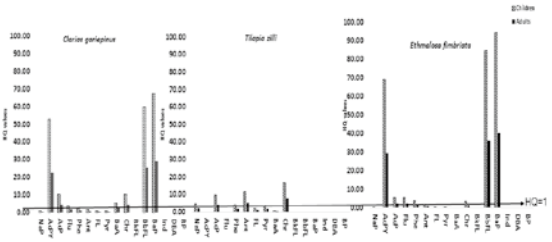

Figure 3: Hazard quotient for non-cancer risk assessment based on the average measured exposure concentrations for Children and Adult.

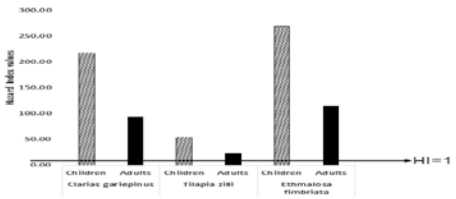

Figure 4: Hazard Index for non-cancer risk assessment based on the average measured exposure concentrations for Children and Adult.

Table 1: Estimated Daily Intake (EDI) of PAHs in Smoked Fish

			EDI					
			Clarias gariepinus		*Tilapia zilli*		*Ethmalosa fimbriata*	
PAH		ADI	1-11years/30kg	Adult/70kg	1-11years/30kg	Adult/70kg	1-11years/30kg	Adult/70kg
Naphthalene	NaP	0.02	0.0167	0.0072*	0.0946*	0.0405*	0.0009	0.0004
Acenaphthylene	AcPY	0.00025	0.0132*	0.0057*	0.0000	0.0000	0.0173*	0.0074*
Acenaphthene	AcP	0.00161	0.0166*	0.0071*	0.0155*	0.0067*	0.0097*	0.0041*
Fluorene	Flu	0.00098	0.0040*	0.0017*	0.0000	0.0000	0.0057*	0.0024*
Phenanthrene	Phe	0.00513	0.0134*	0.0058*	0.0207*	0.0089*	0.0201*	0.0086*
Anthracene	Ant	0.0058	0.0086*	0.0037	0.0645*	0.0276*	0.0117*	0.0050
Fluoranthene	FL	0.0043	0.0035	0.0015	0.0091*	0.0039	0.0024	0.0010
Pyrene	Pyr	0.00397	0.0045*	0.0019	0.0144*	0.0062*	0.0004	0.0002
Benzo(a)anthracene	BaA	0.00065	0.0036*	0.0015*	0.0004	0.0002	0.0000	0.0000
Chrysene	Chr	0.0039	0.0406*	0.0174*	0.0661*	0.0283*	0.0141*	0.0060
Benzo(k)fluoranthrene	BkFL	0.00032	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Benzo(b)floranthrene	BbFL	0.00102	0.0612*	0.0262*	0.0000	0.0000	0.0864*	0.0370*
Benzo(a)pyrene	BaP	0.00042	0.0285*	0.0122*	0.0000	0.0000	0.0396*	0.0170*
Indeno(1,2,3)pyrene	Ind	0.00055	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Dibenzo(a,h)anthracene	DBA	0.00017	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Benzo(g,h,i)perylene	BP	0.0076	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

*=EDI>ADI

4. Acknowledgments

The authors appreciate the contributions of Francis Chigozie, Emmanuel Akpeji, Jane Mkpa and Ruth Agbudumeh.

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P-14

Analysis for persistence organic pollutants (PAHs, PCBs and OCPs) in water, fish and humans from Lagos Lagoon and industrial environment

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As a result of acute toxicities even at low concentrations, several regulatory bodies including WHO have included PAHs, PCBs and OCPs among the priority pollutant list and recommends their routine monitoring in environmental samples. Our recent studies investigate parents PAHs, OCPs and PCBs in fish and water samples of Lagos lagoon, as well as in plasma samples of several humans' residence in Lagos. Extraction was by solid phase extraction method and analysis with GC-^{63}Ni–ECD and Flame ionization detectors. RSD for both recoveries and mean concentrations (MC) were lower than 6.5 % in all cases. For water samples, MC varied from ND-9.8 µg/L, while 31.5% of samples analyzed were positive for PAHs. The MC of PAHs in 28.8% of samples analyzed were sufficiently high (> 0.01µg/L) to cause acute toxicity to the exposed organisms. Also, MC of OCPs in water samples varied from ND-0.996 µg/L, while concentrations in 37.3% of samples analyzed were greater than allowable MCL limit (0.1µg/L) in water. The MC of PCB and OCP congeners in fish samples varied from 0.00–0.78 and 0.01-8.92 mg/kg wet weight respectively. MC of PCBs was higher, while those of OCPs were lower than extraneous residue limit. For the human plasma samples, MC of PCBs and OCPs congeners varied from ND-1.33 and 0.00031-0.95 ng/µl respectively. MC of PCB 28 and lindane were highest while those of PCB 180 and heptachlor epoxide were lowest. However, values in plasma samples were below the approved MCL. Results were analysed by principal component analysis, while concentration levels were compared with levels detected in other parts of the world. Also, in comparison of our result with conventional SPE sorbent, electrospun polystyrene (PS) nanofibers (130-500 nm) incorporating a potassium salt of imidazole-1-carbodithioate were evaluated as potential sorbents for quantification of OCPs. Results indicated that electrospun nanofibers as sorbent material may provide an opportunity to eliminate organic solvents especially for procedures aimed at monitoring organic pollutants in the environment.

Keywords: priority pollutants, plasma samples, analysis, mean concentrations

1. Introduction

Persistence organic pollutants (POPs) are diverse group of compounds many of which are endocrine disruptors, altering normal function of the endocrine and reproductive systems and posing serious health hazards to humans. The toxicity profile in humans is largely due to stimulation of the nervous system (El-Shahawi et al., 2010). For instance endosulfan and lindane are gamma aminobenzoic acid antagonists and inhibit Ca^{2+} influx, and also inhibit Ca and Mg adenosine triphosphatase. DDT affects potassium and voltage-dependent Na channels which could result into cell proliferation and several neurological effects such as agitation, confusion and seizures, while the cardiac effects have been attributed to sensitization of the myocardium to circulating catecholamines (Kang et al. 2008). As a result of acute toxicity, several regulatory bodies have set allowable concentration limit in biological samples. Massive quantity of domestic wastewater and industrial effluents are often discharged into the sea thereby contaminating coastal and underground waters as well as marine biota (Clarke et al. 2013). In current studies, we investigated mean concentrations (MC) of parents PAHs, OCPs and PCBs residues in fish and water samples of Lagos lagoon, as well as in plasma samples of several humans' residence in Lagos. We also compared efficiencies of C-18 and electrospun nanofibers as sorbents in SPE.

2. Materials and Methods

2.1 Standard stock solutions of analytes were all prepared in isooctane. For human, blood (5.0ml) was extracted by venipuncture, collected in heparin bottles and plasma separated by centrifugation. C-18 cartridges were conditioned [2 ml methanol + 1ml x 2 mixture of water-1 propanol (v: v, 85:15)]. The Sampli Q Alumina B cartridges were washed [2 ml n-hexane + dichloromethane, DCM (v: v 50:50)] and attached to the lower end of the C-18 cartridges and samples were then percolated through at a steady flow rate. To remove interfering compounds, cartridges were washed [2ml de-ionized water] and the sorbent bed was dried thoroughly under N_2 stream and analytes eluted with 1ml mixture of hexane-DCM (v: v, 80:20). The eluate

was concentrated under N_2 gas and reconstituted with 1.0ml of isooctane for GC analysis.

3. Results and Discussion

Addition of water-1-propanol (v:v,85:15) mixture prior to extraction serve as protein denaturant and prevent protein co-precipitation with analytes, hence, the extraction cartridge was not clogged. R^2 values varies from 0.998-0.999 for analytes, while mean recoveries (n=4) vary from 90-92 %. However, reported MC values were not corrected for recoveries. RSD for both recoveries and MC were lower than 7.0 %. For human plasma samples, MC of PCBs and OCPs varied from ND-1.33 and 0.00031-0.95 ng/µl respectively (Figure 1). MC of PCB 28 and lindane were highest while those of PCB 180 and heptachlor epoxide were lowest. However, values in plasma samples were below the European Union approved MCL. The MC of OCPs (mg/L) detected in humans in this study (0.3781-1.2473) were higher when compared with values (0.0014 – 0.0084) obtained for similar studies from Rio de Janeiro Brazil (Delgado et al., 2002), Portugal (ND -0.391) and Mexico (0.0021-0.0271) respectively (Waliszewski et al, 2004). Lower chlorinated biphenyls (PCB 28, 52 and 101) are more rapidly metabolised and eliminated and hence, may not pose significant health hazards, however, their detection in all samples in this study probably indicates that the subject participants were recently exposed to the chemicals. For water samples collected from Lagos lagoon, MC of PAHs (Table 1) varied from ND-9.8 µg/L. 31.5% of samples analyzed were positive for PAHs and MC in 28.8% of samples analyzed were sufficiently high (> 0.01µg/L) to cause acute toxicity to the exposed organisms. Also, For OCPs, the mean concentrations (MC) in water samples (Figure2) varied from ND-0.996 µg/L and concentrations in 37.3% of samples were higher than allowable MCL limit (0.1µg/L) in water. The MC of PCB and OCP congeners in fish samples varied from 0.00–0.78 and 0.01-8.92 mg/kg wet weight respectively. MC of PCBs was higher (Figure 3), while OCPs were lower (Figure 4) than extraneous residue limit.Some OCPs have been used in Nigeria for pest management. For instance, technical HCH (mixtures of 60-70% α, 5-12% β, and 10-15% γ HCH) were widely used as pesticides and commercialized as lindane or 'gammalin 20'. Certain process also converts congeners and for instance, about 20% of heptachlor and chlordane may be oxidized readily into heptachlor epoxide by bacterial action. Also, aldrin can be converted readily to dieldrin by epoxidation under environmental conditions (Hongtao et al., 2008). The optimum extraction of DDE stock on functionalized electrospun nanofibers and conventional C-18 sorbent were 94.6 % and 92% respectively.

The Principal component analysis (PCA) illustrates that PCBs and OCPs were present in similar range in all the samples investigated. For example, all the variables gave a positive loading to PCA-1 suggesting similar origin of contamination. As a result, these two analyses confirmed that the contaminants in the investigated samples have been contacted through the same pathway either food or air. Notably, variables KT1, KT2, DK1, DL1, MB2 and AJ2 have negative values in PCA-2 factors of both score plots and were strongly correlated among each other both in PCBs and OCPs. These were also observed and confirmed in the correlation (*p* value 0.05) results for the PCBs and OCPs variables.

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Figure 1: mean concentrations of PCBs and OCPs (mg/L) in plasma samples

Figure 2 : OCPs distribution in water samples

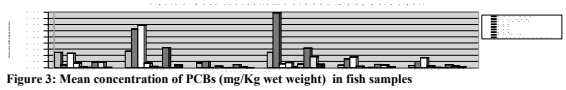

Figure 3: Mean concentration of PCBs (mg/Kg wet weight) in fish samples

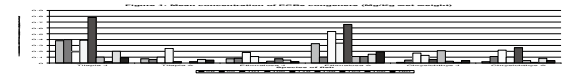

Figure 4: Mean concentration of OCPs (mg/Kg wet weight) in fish samples

Table 1: Mean concentration (µg/L) of PAHs detected in water samples

Priority PAHs pollutants	NSW	NBW	BSW	BBW	FSW	FBW	ASW	ABW	ISW	IBW	OSW	OBW	Mean conc. PAHs (µg/L)
acenaphthylene	N	N	N	N	0.3	N	N	N	0.2	N	N	N	0.25
Fluorene	5.0	N	N	N	N	N	N	N	N	N	N	N	0.42
Phenanthrene	9.8	4.7	6.0	6.3	6.1	6.4	5.3	4.7	5.5	7.9	6.6	5.6	5.73
anthracene	0.2	N	N	N	N	N	N	N	N	0.1	N	N	0.02
Fluoranthene	2.9	2.2	2.3	2.3	2.3	2.4	1.9	2.0	2.1	2.7	2.6	2.7	2.18
Pyrene	2.3	1.8	1.8	1.8	1.8	1.9	1.5	1.6	1.7	2.1	2.1	1.8	1.88
Chrysene	N	N	N	N	N	N	0.1	N	N	N	N	N	0.01
benzo[a] anthracene	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.20
benzo[a] pyrene	0.3	0.4	0.4	0.4	0.4	0.3	0.3	0.4	0.3	0.3	0.3	0.4	0.35
Benzo [g,h,i] perylene	N	N	N	0.6	N	N	N	N	N	N	N	0.6	0.11

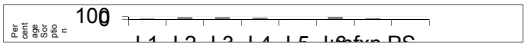

Figure 5: Pre-concentration of DDE with polystyrene electrospun nanofibers incorporating different functional ligands.

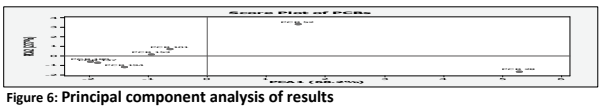

Figure 6: Principal component analysis of results

P-15

The effect of DDT and its metabolites on the structure of the shells of the eggs of the House Sparrow, *Passer domesticus*: A morphometric study

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DDT is a toxic and persistent insecticide that withstands natural breakdown processes. Together with its metabolites, DDE and DDD, it negatively affects the reproductive cycle and the development of eggs in birds. This study examined the effect of DDT and its metabolites on the eggshell thickness and pore numbers of the House Sparrow, *Passer domesticus*. The eggs were collected at five sites in the Thohoyandou area of the Limpopo Province of South Africa. The sites comprised of; two non-sprayed sites, one site sprayed five years ago and two recently sprayed ones. Analysis of the shells was done on micrographs taken with a scanning electron microscope using ImageJ. The preliminary results show that there were no statistically significant differences between the number of pores of the different categories of sites. There were significant differences between the thickness of the shells of eggs from the site sprayed five years ago and the recently sprayed ones. The thinning of the shells of the eggs exposed to DDT corresponds with observations reported in other birds by various investigators.

Keywords: DDT, House Sparrow, egg, shell morphometry, pores, malaria

1. Introduction

The pollution of the environment through spraying of Dichlorodiphenyltrichloroethane (DDT) in South Africa has become a serious problem. In the manner of the so-called 'two edged sword', DDT is the most effective insecticide in the fight against malaria and yet a very toxic environmental pollutant (Bouwman *et al.* 2011). DDT is persistent and resists natural breakdown processes of contaminants in the body (Wells & Leonard 2006). It accumulates in fat tissue and is passed from mother to foetus (Wells & Leonard 2006). The insecticide as well as its metabolites, DDE and DDD, has various harmful effects on humans and animals. It affects the reproductive success of birds by altering sperm count, egg formation, hatchling success, and eggshell thickness (Wells & Leonard 2006). The decrease in shell thickness is due to inhibition of Ca2þ-ATPase in the eggshell gland (Miriero & Iamiceli 2008). Data are lacking on the impact of DDT on the structure of the House Sparrow, *Passer domesticus*, eggs. The House Sparrow is a good bioindicator of the impact of DDT on birds due to its close association with humans and is one of the most abundant species in the sampling area.

2. Materials and Methods

Sampling took place in Thohoyandou area of the Limpopo Province of South Africa in November 2014, where malaria is controlled by indoor spraying with DDT (Bouwman *et al.* 2011). The collection of the eggs was done after obtaining the necessary ethical clearance from the University of Johannesburgs' Animal Ethics Committee and permits from the Department of Environmental Affairs of the Limpopo Province. Eggs were collected at five sites which were divided into three categories; eggs from two sites where DDT was recently sprayed (within 6-7 months); eggs from one site which was sprayed 5 years ago; and eggs from two non-sprayed sites. Thirty nine eggs were analysed.

2.1 Shell thickness

Three pieces of ~7mm^2 were taken from the sharp, the blunt and the equatorial regions of the egg. The shells were coated with gold for 2 minutes at 40mA on an Emscope SC500 corning OVF chamber gold coater and examined on a Tescan Nanospace Scanning Electron Microscope (SEM). The stage of the SEM was appropriately tilted to allow images of the shell wall to be viewed and photographed perpendicular to the outer surfaces. Three points were marked and a micrograph was taken at a magnification of 500X. These micrographs were imported into ImageJ (Open source program) and orthogonal measurements of the shell thickness made.

2.2 Pore numbers

Two pieces of shell (~7 mm^2) were taken from the equatorial regions of the opposite sides of the egg shell. The pieces were coated with gold. On the SEM the exact middle of the pieces was determined and the pores were counted at a magnification of 4100 X. A quadratic lattice grid with an area of 10 µm^2 was superimposed over the image. The number of pores which perforated the shell was determined. Perforating pores were identified by the neck of the pore that penetrated the shell. To

ensure that a correct distinction was made between the pores in the count and that only the perforating pores were analysed, the stage was tilted and the magnification was increased on the pores where the investigator was uncertain. Statistical analysis of the normality of the data was performed using the Shapiro-Wilk test and differences between various measures were determined with the Mann-Whitney non-parametric test.

3. Results and Discussion

There were nonsignificant differences in the pore numbers recorded on the eggshells collected from the three site categories (Figure 1).

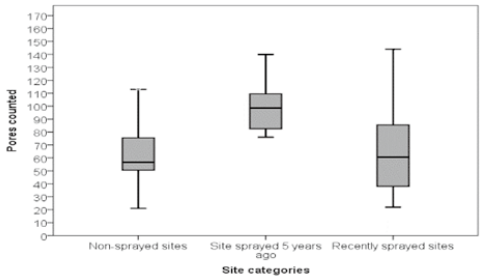

Figure 1: The number of pores counted on the eggshells for each of the site categories based on DDT spraying.

For all three site categories, small pores (<1.5µm) predominated (Figure 2).

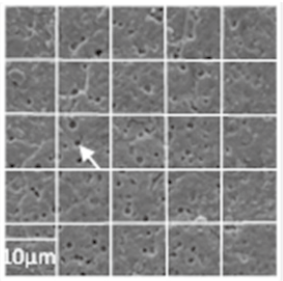

Figure 2: A Scanning electron micrograph at 4100 X magnification with a superimposed quadratic lattice grid showing the variations in the pore sizes. Arrow, small pore (area <1.5µm)

Regarding shell thicknesses, there was a significant difference between the eggshells from non-sprayed sites and recently sprayed ones (P=0.001) (Figure 3) and between shells from eggs collected from the site sprayed five years ago and the recently sprayed ones (P=0.008). The difference between the shell thicknesses of eggs from the five year ago sprayed site and the non-sprayed sites was not significant (P=0.322). From these preliminary results it can be speculated that exposure to DDT and its metabolites do not affect the pores numbers but does affects the shell thickness. The determined thinning of the eggshells corresponds with the observations made by Bouwman *et al*. 2013 in Cattle Egret eggshells, where a difference of 33% was observed between the eggs collected from DDT exposed compared to non-sprayed sites. These preliminary results suggest that DDT may affect the reproductive success of the House Sparrow in particular and birds in general.

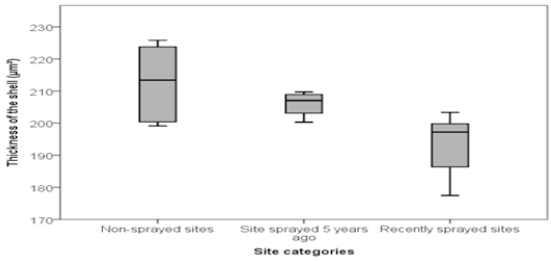

Figure 3: The shell thicknesses of the eggs collected at the three categories of site.

4. Acknowledgments

This study was funded by the National Research Foundation of South Africa (NRF). Opinions expressed and conclusions arrived at are those of the authors, and are not necessarily to be attributed to the NRF.

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P-16

DDT concentrationss in *Xenopus sp*. fat and the Grey Heron eggs in the Limpopo Province of South Africa.

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The negative endocrine disruptive effects of DDT on non-target species have been well documented. The regulated use and application of DDT to control the insect vector of malaria is still allowed in certain parts of South Africa. Concentrations of DDT was analysed in Grey Heron eggs and *Xenopus sp*. of frogs naturally occurring in and around the DDT-sprayed area. DDT concentrations in Grey Heron eggs were the highest reported concentrations for wild bird eggs this century and were at concentrations considered to be detrimental to other bird species of the same family or sharing the same feeding regime. We also identified that *Xenopus sp*. could serve as a vector for DDT to higher trophic levels. Our findings also suggest possible illegal use of DDT outside of designated areas or at least that regulated use of DDT is still inadequate to prevent the spread of DDT into ecosystems outside and upstream of the application sites.

Keywords: DDT, Grey Heron, *Xenopus sp*., Frogs, Birds, Limpopo Province

1. Introduction

DDT is well known for its ability to persist in the environment and have negative endocrine disruptive effects on non-target species. The use of DDT has been banned in most parts of the world with certain countries being exempt from this ban to facilitate the control of the insect vector of malaria through the controlled use of DDT. South Africa is one of these countries with parts of the Limpopo Province subject to indoor residual spraying (IRS) of DDT for malaria control.

The movement of DDT through the ecosystem is strongly associated with the water cycle. Additionally, DDT also accumulates in the higher trophic levels of the food web. We therefore focused our sampling effort firstly on predatory aquatic birds and birds that have a strong association with the aquatic system, and secondly, on the two obligatory aquatic clawed frog species present in the area.

The aim of our study was to compare DDT concentrations in biota from an area where IRS occurs with adjacent areas where DDT is not used. This will give an indication of the ecological threat posed by the use of DDT. For the purpose of this paper, the focus will mainly be on the results obtained for the Grey Heron (*Ardea cinerea*), an aquatic feeding bird that includes amphibians in its diet (Hockey et al. 2005).

2. Materials and Methods

2.1 Sampling

The study area was in the Vhembe district of Limpopo Province, South Africa. In general, IRS is employed from the city of Thohoyandou eastwards to the border of the Kruger National Park (Bouwman et al. 2013). We obtained ethical clearance and permits to sample bird eggs and frogs. For bird eggs, we scouted all accessible areas along the Luvuvhu River for breeding colonies and nests; this included two dams (Albisini dam in the non-DDT sprayed area and Nandoni dam straddling the sprayed and non-sprayed areas). Using satellite imagery and local knowledge we also identified water bodies within the Luvuvhu River valley that were not directly connected to the Luvuvhu River system. Additionally, we also visually tracked returning, colony breeding birds in the late afternoon in order to identify breeding colonies that were situated away from water bodies. When breeding colonies or nests were identified, we would sample eggs from the nest and leave at least one egg for the parents to hatch. Due to safety constraints not all nests could be sampled.

Frogs were sampled by placing three or four baited funnel traps in selected water bodies and leaving them overnight. The following morning the traps were inspected and the trapped frogs were sexed and placed in individual containers to be transported to the lab where they were then anaesthetised, measured, and tissue samples taken.

2.2. Analyses and data treatment

The methods for processing and analyses of eggs as well as data analyses is described in Bouwman et al. (2013). Only 21 frogs could be analysed for DDT due to financial constraints. The fat bodies were sent for analysis to FDA Laboratories (Pty) Ltd in Pretoria, South Africa by gas chromatography – mass spectrometry. Limits of quantification (LOQ)

were 15 µg/kg wet mass (wm) for *p,p'*-DDT, 10 µg/kg wm for *p,p'*-DDE and *o,p'*-DDT, 5 µg/kg wm *p,p'*-DDD, and 1 µg/kg wm for *o,p'*-DDE. Descriptive data analyses were done using GraphPad Prism version 5.04 for Windows, GraphPad Software, San Diego California USA (www.graphpad.com).

3. Results and Discussion

There was an apparent absence of active aquatic bird breeding colonies to the east of Thohoyandou. Grey Heron eggs could only be sampled at Nandoni Dam. The Grey Heron colony located in the non-sprayed area was out of reach and could not be sampled. Analyses of the Grey Heron eggs yielded the highest ∑DDT concentrations in wild bird eggs reported this century. The mean ∑DDT in Grey Heron eggs were 13 mg/kg wm (wet mass). This is substantially higher than 3 mg/kg wm, the value calculated to be the "critical level for reproductive success" for Brown Pelicans in the USA (USDoI, 1998). Previous studies found DDE concentrations of 2.8 mg/kg wm associated with reproductive impairment of piscivore bird populations while 1 mg/kg wm has been linked to reduced heron survival (Connell et al. 2003). In this study, the mean *p,p'*-DDE values for Grey Heron eggs were at 13 mg/kg, since more than 98% of the ∑DDT consisted of p,p'-DDE.

∑DDT concentrations in *Xenopus sp*. were not in the same order of magnitude as that found in the Grey Heron eggs, with a mean ∑DDT concentration of 206 ng/g and 391 ng/g wm respectively for male and female frogs from the DDT sprayed area. Interestingly, DDT was also present in both male and female frogs from the non-sprayed area, although at lower concentrations. In general the females were larger than the males with smaller fat bodies. For both the non-sprayed and sprayed areas, females had higher ∑DDT concentrations than males. There is no published data available for DDT concentrations in *Xenopus sp* from anywhere in the world. Our data are at the lower end of DDT ranges reported for other frog species worldwide. However, none of these studies reported on DDT concentrations in frogs from areas where DDT is sprayed for malaria control. This is surprising, as the first report of DDT in frogs sounded a warning when DDT is considered for use in malaria control (Ellis et al. 1944).

The frogs were sampled in ponds not connected by rivers or streams and were long distances away from each other. Therefore, movent of frogs between the water bodies was considered improbable. This, together with the fact that DDT was found in frogs and the eggs of other bird species (Bouwman et al. 2013) outside of the DDT sprayed area could be indicative of the legacy of historical DDT use in the area. Alternatively it might indicate illegal current use of DDT outside of the designated malaria control area or even long range transportation not directly linked to runoff water.

Our findings suggest a current threat posed to piscivores birds. Additionally, our findings suggest that the *Xenopus sp.* could act as a vector for DDT to higher trophic levels.

4. Acknowledgments

The project was funded by the Water Research Commission of South Africa. The views expressed in this paper are the views of the authors and should not be ascribed to the WRC.

We like to thank Anuschka Polder, JP Huisamen, Riana Bornman, Ben van der Waal, Cecila Kwinda, Salphina Tshilamulela and Grace Bulunnga, Ahmed Abdelghani and Katharina Løken.

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P-17

Pesticide residues in water from rivers and lakes in Lake Tanganyika basin, Tanzania

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The aim of this study was to investigate the types, levels and distribution of pesticide residues in water in Lake Tanganyika basin, Tanzania. Water samples were collected from Kigoma region during April and June and analysed for pesticide residues. The pesticides and metabolites detected were *p,p*'-DDT, *o,p*'-DDT, *p,p*'-DDE and *p,p*'-DDD. The concentrations of total DDT in water were up to 200 and 221 ng/L in lakes and rivers, respectively. The ratios of (*p,p*'- DDE + *p,p*'-DDD)/*p,p*'-DDT indicate recent inputs in most samples. The study reveals that there is recent use of DDT in the region. Continued use of DDT indicates concerns and risks for public and environmental health. The area therefore needs serious environmental monitoring.

Keywords: DDT, water, lake, Tanzania

1. Introduction

The increased use of various types of pesticides in agriculture has led to serious environmental contamination arising from their use. Pesticides have been linked with many health effects and they have serious implications to the environment (WHO 1990; ATSDR, 2002). A variety of agricultural activities are carried out in Lake Tanganyika basin. Different types of pesticides are used to control crop pests, suggesting the possibility of water contamination in the rivers and lakes. Therefore, this study was undertaken to assess the level of pollution due to pesticides.

2. Materials and Methods

Water samples were collected from five sites namely Lake Tanganyika, Luiche River, Malagarasi River, Lake Nyamagoma and the streams at Kalinzi in April and June 2012. From Lake Tanganyika, water samples were collected from two sampling stations at Forodhani and Kibirizi. Samples from Malagarasi River were collected from Malagarasi Bridge in Uvinza district (upstream of the river) and Ilagala (downstream of the river). Samples from Luiche River were collected from the point located five kilometers from where the river enters the Lake. From Lake Nyamagoma samples were collected at Mtegowanoti village in Uvinza district. The lake discharges into Malagarasi River. The samples from Kalinzi were collected from a stream which runs through the coffee farms.

Water samples were collected in 1-litre glass sampling bottles with Teflon caps and preserved with NaCl (100 g each). The samples were kept in a refrigerator at 4 °C until extraction. Water samples were extracted by Liquid-Liquid Extraction method (Åkerblom, 1995) using dichloromethane (60 mL x 3) and dried with anhydrous sodium sulfate (20 g). The extracts were concentrated using a rotary evaporator at 35 °C, solvent changed to cyclohexane/acetone (9:1) and concentrated to 1 mL. Analyses were performed using GC-ECD and confirmed by GC-MS. Analytical quality assurance included analysis of matrix blanks (distilled water) and recovery tests. Blank samples (n = 6) were spiked with standards of all the compounds investigated at concentrations of 0.002 mg/L and 0.005 mg/L and extracted using the same procedures as for the samples. All the blanks did not contain any detectable levels of the analytes. The mean recoveries ranged 87.4–109.6%.

3. Results and Discussion

3.1 Pesticides and Metabolites in Water Samples

The pesticides and metabolites detected in water samples were *p,p'*-DDT, *o,p'*-DDT, *p,p'*-DDE and *p,p'*-DDD. Although other pesticides like organophosphorus pesticides, pyrethroids and carbamates are used in the study areas, no residues were found. This is because they are highly water soluble and chemically unstable, while organochlorine pesticide residues are stable and therefore highly persistent in the environment (ATSDR, 2002).

3.2 Levels and Distribution of DDT Residues in Water Samples

Table 1 shows the concentrations of DDT and the major metabolites in water samples. In all sampling sites the concentrations of *p, p'*-DDT were higher than those of *o,p'*-DDT indicating contamination by technical DDT (ATSDR, 2002). The concentrations of *p,p'*-DDD were higher than those of *p,p'*-DDE in almost all water samples except those from Ilagala. This suggests that *p,p'*-DDT was dominantly degraded to *p,p'*-DDD under anaerobic conditions. The presence of *p,p'*-DDE is due to degradation of *p,p'*-DDT under aerobic conditions. In case of

water samples from Ilagala collected in April, the concentrations of *p,p'*-DDE were higher than those of *p,p'*-DDD, which implies aerobic degradation of *p,p'*-DDT (ATSDR, 2002).

Table 1: Concentrations of DDT residues in water samples collected in April and June (ng/L)

Sampling sites (points)	SP	*p,p'*-DDE	*p,p'*-DDD	*o,p'*-DDT	*p,p'*-DDT	∑DDT
Malagarasi (2 points)	Apr	5.1	14.0	41.01	56.4	116.5
		4.4	39.6	85.22	90.1	219.3
	Jun	16.03	33.3	85.0	87.0	221.3
		7.3	20.11	47.0	83.31	158
Ilagala (2 points)	Apr	11.1	5.4	6.4	10.3	33.2
		97.22	24.0	11.5	20.6	153.3
	Jun	2.5	13.7	25.1	35.11	76.4
		3.24	12.1	28.0	35.0	78.3
Luiche (2 points)	Apr	14.13	21.0	44.3	61.2	141
		13.0	25.1	52.0	60.0	150.1
	Jun	6.43	19.0	30.63	38.0	94.1
		1.64	12.0	22.4	32.24	68.3
Forodhani (2 points)	Apr	9.0	25.0	60.3	99.0	193
	Jun	4.7	12.6	32	37.3	86.6
		1.6	8.6	13.5	34.0	57.7
Kibirizi (2 points)	Apr	5.6	9.0	25.21	34.4	74.2
		3.51	18.5	29.3	30.1	81.4
	Jun	1.4	11.23	17.1	86.1	116
		10.6	46.0	65.24	78.1	200
Kalinzi (1 point)	Apr	13.1	13.53	27.33	33.32	87.3
L. Nyamagoma (2 points)	Apr	28.2	43.3	47.5	53.0	172
		4.13	10.3	19.6	31.5	65.5

SP = Sampling period; Apr = April; Jun = June

The maximum total DDT concentrations were 221.3 and 153.3 ng/L in Malagarasi and Ilagala samples, respectively. The concentrations of DDT residues were significantly higher at Malagarasi than Ilagala (t = 2.199, p = 0.034), suggesting greater contamination upstream. Also residue concentrations depend on both volume of water and flow rate. At Ilagala station the volume and flow of water were very high compared to Malagarasi bridge station, therefore big volumes of water result in dilution of the concentrations of pesticides. Also Malagarasi bridge is near many agricultural fields where crops are grown while Ilagala is too far, therefore residue concentrations tend to be reduced due to adsorption and evaporation. In samples from Luiche River, the highest concentration of ∑DDT residues was 150 ng/L. The agricultural activities taking place along the rivers could be significant sources of contamination. Contamination might partly be facilitated by the streams which run through the fields and discharge into these rivers. There was significant difference between the mean concentrations of total DDT in Malagarasi and Luiche Rivers (t = 3.58, p = *0.0373*). This is because Malagarasi River is bigger than Luiche River, therefore it has many contamination sources like streams and other small rivers; furthermore it passes through many farms which used the pesticides.

The maximum concentrations of total DDT in samples from Lake Tanganyika at Forodhani and Kibirizi were 193 and 200 ng/L, respectively. There was no significant difference in concentrations of DDT residues between these two points from the lake (t = 0.4798, p = 0.636). Possible sources of contamination might be rivers, streams and runoffs which discharge into the lake from farms and vegetable gardens around the lake. Contamination might also come from neighbouring countries. The highest concentration of total DDT in samples from Lake Nyamagoma was 172 ng/L. Possible sources of contamination might be runoff from tobacco fields near the lake as well as from streams which discharge into the lake. Samples from the stream which runs through coffee fields at Kalinzi had a maximum concentration of total DDT of 87.3 ng/L. This concentration is lower than the concentrations obtained from rivers and lakes, maybe because the stream was small compared to lakes and rivers; it passes through few coffee fields.

The ratios of (*p,p'*-DDE + *p,p'*-DDD) to *p,p'*-DDT ranged from 0.15 to 0.80 in 86.4% water samples, suggesting that new sources of technical DDT existed in the study area. Water samples collected in April from Ilagala and Lake Nyamagoma had ratios ranging from 1.35 to 5.89, indicating input of aged DDT or significant degradation (Mahugija et al., 2015). The results indicate some marked differences in the levels of DDT residues between April (rainy) and June (dry) suggesting differences in input/ application, changes in water volumes and dilution effects. The results indicate moderate level of contamination. There are risks and concerns for public health and the environment because of the fresh application indicated and the compounds are persistent and bioaccumulative.

4. Acknowledgments

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P-18

Effect of chronic pesticides exposure in farm workers health of a Camerounian community.

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This study was undertaken to investigate the effect of agropesticides on liver and kidneys function in 40 farm workers chronically exposed to pesticides in Nde Division (West – Cameroon). A transversal comparative analysis was performed between a group farm workers and a control group of 32 workers in Mfoundi Division (Centre – Cameroon) not exposed to agro pesticides. After signing an informed consent form, each participants was interviewed and his blood was collected by vein puncture for determination of biochemical parameters such as aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) activities, direct and total bilirubin (D-Bil and T-Bil, respectively), urea and creatinine. The most used pesticides by the farm workers were insecticides, herbicides and fungicides. A significant increase ($P<0.05$) of AST, PAL, D-Bil and creatinine levels was observed in a group of farm workers exposed to agropesticides compared to controls.

Keywords: agropesticides, farm workers, liver, kidneys

1. Introduction

The use of pesticide to improve crop protection and elimination of some diseases is increasing worldwide, and particularly among rural populations of the developing world whose economy relies mostly in agriculture (McKinlay et al., 2008; Haylamichael and Dalvie, 2009)

As defined by Food and Agriculture Organization of the United Nations (FAO), a pesticide is any substance or mixture of substances destined to prevent, destroy, or control any plague, including human or animal disease vectors and unwanted animal or plant species capable of inflicting damage on the production, transformation, storage, transport, or commercialization of food and farm products. These substances are ubiquitous in our environment, with substantial amounts detected in air, water, soil and human/animal tissues and blood samples. Particular concern is raised by indoor use of pesticides in large quantities. Pesticides are not only released in the environment with the purpose of protecting agricultural production, but are also applied in substantial amounts in houses, schools, and workplaces in order to control several insects or disease vectors.

Previous studies have shown that unsafe use of pesticides is common in developing countries. Indeed, the WHO estimates 200,000 deaths each year worldwide with a high number of cases occurring in developing countries **(Jeyaratnam, 1990)**. Moreover, correlation between pesticide exposure and the occurrence of disorders in humans has been established. Indeed, exposure to these can lead to cancer (Baldi et al., 1998), endocrine disruption with impact on reproductive function (**Manfo et al., 2012**), hematological and immune disorders, neurological disorders, liver and kidney dysfunction (**Dewailly et all., 2000; Devilleirs et al., 2005**.)It was previously reported that people from west region of Cameroon are highly exposed to agropesticides which impair male reproductive function through the inhibition of testosterone synthesis; however the effect on liver and kidney was not reported.

Pesticides like many xenobiotics, when ingested by humans, are chiefly metabolized by the liver, and mainly eliminated by the kidneys.These two organs are therefore the main targets of pesticides in the body, and previous studies were able to establish the link between unsafe use of pesticides and the occurrence of disorders of liver and kidney function among farmers **(Asnal at al., 2011; El-Wakiel et al, 2012)**.

Given all the above, it appears that the damage caused by pesticides on health pose a serious public health problem. Thus assessing and highlighting the influence of pesticides on the health of farmers, we contribute to the sensitization of people exposed to a safer use of these hazardous products.

This study aimed at investigating the effect of agropesticides use on the health of farmers in Ndé Division (West – Cameroon), with particular interest to liver and kidney functions.

2. Materials and Methods

2.1 Study population

A transversal comparative study was performed from July to September 2013. The study population comprised 40 farmers in West Region (Ndé Division) who were currently using agropesticides and 32 farmers in Centre Region (Mfoundi Division) not exposed to pesticides.

Exclusion criteria for the subjects included among

others, age <18 years old, residence in the locality for <5 years, pregnant women, individuals who presented any pathology who could interfere with results interpretation, those who did not accept to participate in the study.

The study protocol was approved by the institutional Ethical Committee of Université des Montagnes, certification N° 2013/068/CIE-UdM/Pr.

2.2 Sample collection

After signing an informed consent form, each participant was interviewed and his blood (10 mL) was collected by vein puncture. Serum was thereafter prepared from the blood and used for biochemical analyses.

2.3 Determination of Biochemical and physiological parameters

Serum enzymes and other parameters were measured using a Biochemical autoanalyzer. The biological markers of cytotoxicity measured and other serum components were Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) activities, direct bilirubin (D-Bil), total bilirubin (T-Bil), urea and creatinine. The creatinine clearance was determined using the formula of the MDRD (Modification of Diet in Renal Disease).

Statistical analysis of data was done using SPSS software version 18.

3. Results and discussion

The pesticides used by the farmers were insecticides, herbicides and fungicides. The average ages of the group exposed to agropesticides and the control group were 39 years (39 ± 13) and 37 years (37 ± 14), respectively. The average exposure period to pesticides was 14 years (14 ± 6).

Table 1: Biochemical and physiological parameters of the control group and agropesticides – exposed group

Variable	Reference value	Unexposed group	Exposed group	P value
ALT	< 41 U/L	16.77 ± 4.67	19.26 ± 7.91	NS
AST	< 41 U/L	14.83 ± 5.43	23.74 ± 8.43	<0.05
D-Bil	< 0.3 mg/dL	0.15 ± 0.01	0.23 ± 0.02	<0.05
T-Bil	< 1 mg/dL	0.66 ± 0.04	1.23 ± 0.19	NS
ALP	40-129 U/L	72.25 ± 25	85.12 ± 21	<0.05
Urea	10-50mg/dL	0.68 ± 0.21	1,20 ± 0.44	NS
Creatinine	53-115 µmol/L	59.22 ± 14.11	59.86 ± 12.48	NS
Creatinine clearance	> 90ml/min	215 ± 13	160 ± 21	<0.05

As shown in table 1, a significant increase in AST, PAL, and D-Bil was observed in subjects exposed to agro-pesticides when compared to the control group. This may result from liver damage caused by direct exposure to agropesticides. Indeed, pesticides like many xenobiotics, when absorbed by humans, are mainly metabolized by the liver, and this makes the liver one of the main targets of agropesticides in the body. For example, organochlorines were shown to accumulate in the liver to induce hepatomegaly. The latter pesticides also accumulate in kidneys, leading to kidney failure. A significant decrease in creatinine clearance for subjects exposed compared to non-exposed was also observed. This decrease could signal a beginning of renal failure.

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P-19

Bioaccumulation of PBDEs in the mudfish from the Vaal River, South Africa

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This study was undertaken to investigate the levels of PBDEs in *Labeo capensis* from the Vaal River, South Africa. The samples were extracted with Soxhlet and analysed with gas chromatography coupled to a mass spectrometer (GC-MS). Concentrations ranged from <LOD–78.7 ng/g (lw), with variations in concentrations between the sample locations. The most abundant congener was BDE-47. The main source of PBDEs is probably flame retardants emanating from the large industries in the catchment. These concentrations are higher than any levels measured in freshwater fish reported from other African systems.

Keywords: Labeo capensis, spatial and temporal differences, BDE-47 congener

1. Introduction

Polybrominated diphenyl ethers (PBDEs) are synthetically produced compounds that have been produced, and used in large quantities worldwide since the 1970s as flame retardants (Cheung *et al.* 2008).

PBDEs enter the environment during manufacture, or the disposal of products containing these compounds. These compounds are ubiquitous and persistent in the environment, where they accumulate in sediments, and pose risk to biological life due to the bioaccumulation potential (Cheung *et al*. 2008). Detrimental effects caused by these compounds include endocrine disruption, neurobehavioural deficits, and possibly cancer. High concentrations of PBDEs have been found in human tissues, and it was found that these concentrations were positively correlated with fish consumption.

The Vaal River forms part of the largest river basin in South Africa and the upper reaches are situated in the industrial heartland of South Africa. As a consequence it is often regarded as one of the most contaminated river systems in the country. Studies by Wepener *et al.* (2011) found that high levels of contaminates such as PCBs, metals and PBDEs could contribute to the fish kills that often occur during the early spring.

The aim of this study was therefore to determine the current spatial levels of PBDEs in muscle tissue of the mudfish, *Labeo capensis,* and compare the values to those measured during the 2010 study of Wepener *et al*. (2011).

2. Methods

Sampling was carried out at two sites that were also used in the 2010 study, i.e. Vischgat (V), situated below the Vaal Dam, and farther downstream Vaal Barrage (B). Fish, *L. capensis,* were collected using a Smith and Root electro-shocker during September 2010 and October 2014. Land use along the river, and its tributaries, includes mining, agriculture, industry, and formal and informal settlements. The river is also used for recreational activities.

Fish muscle samples were collected in the field and wrapped in aluminium prior to analysis. Tissue was extracted using Soxhlet and spiked with internal standard (PBDE 77). After gravimetric lipid determination samples were cleaned using acid silica (44% H_2SO_4). The sample was concentrated and reconstituted in *iso*-octane (Verhaert *et al*. 2013).

Analyses was performed with an Agilent 6890-5973 gas chromatograph coupled to a mass spectrometer (GC-MS), operated in electron capture negative ionisation (ECNI) and equipped with a 30 m × 0.25 mm × 0.25 µm DB-5 capillary column. BDE-28, -47, -99, -100, -153, and -154 were analysed. Significance ($p<0.05$) of spatial and temporal differences in total PBDE log-transformed concentrations was tested using Analysis of Variance.

3. Results

A total of 9 fish (B: n=4 and V: n=5) were collected in 2010 and 8 fish (B: n=2 and V: n=6) were collected in 2014. All PBDEs analysed, were detected in the samples. The most abundant congener was BDE-47 for both sites and sample years (Figure 1.)

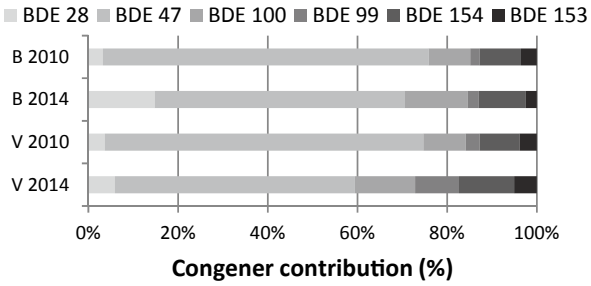

Figure 1. BDE congener contributions in muscle tissue of *Labeo capensis* from the Vaal River collected during 2010 and 2015.

Barrage had the highest concentrations when compared to Vischgat, while the 2010 concentrations were higher than the 2014 results. Concentrations at Vischgat were significantly lower ($p<0.05$) in 2014 than in 2010 with no significant temporal differences for Barrage. There were significant spatial differences in PBDE concentrations during 2014 survey, which were not found for the 2010 study.

Table 1: PBDE concentrations within muscle tissue of *L. capensis* sampled in 2010 and 2014 from Barrage (B) and Vischgat (V) (ng/g lw).

	Range	Mean ± SE
B2010	19.8 – 93.3	43.4 ± 15.2
B2014	3.0 – 38	20.5 ± 11.1
V2010	11.5 – 70.8	31.9 ± 11.3
V2014	0.4 – 11.2	2.8 ± 2.1

4. Discussion and conclusion

Concentrations were the greatest at Barrage, for both sampling years. This region has many tributaries that collect run-off from heavy industries in the Gauteng province and then flow into the Vaal River upstream of this site. The Vaal Dam, which is just upstream, of Vischgat, regulates flow of the water exiting the dam. The dam wall can also reduce the transfer of sediments, and hence the PBDEs, into lower reaches of the river. This, as well as the fewer tributary inputs explains the lower concentrations at this site.

Concentrations reduced from the 2010 to the 2014 sampling events, significantly at Vischgat. It is unlikely that inputs reduced, or that the compounds present would have degraded, due to their persistence. These fish were sampled during the spawning season, it is likely that the fish sampled in 2014 had spawned, and released their pollution loadings (Larsson *et al.* 1991).

The two most common congeners (BDE-47 and 100) are commonly used penta-BDE formulations in flame retardants (Verhaert *et al*. 2013). The dominant congener recorded in this study was BDE-47 and support the findings by Cheung *et al.* (2008) for fish muscle tissue collected from Hong Kong, tigerfish in Pongolopoort Dam (Wepener *et al.* 2011) and fish from the Congo River (Verhaert *et al*. 2013).

The total PBDE concentrations in this study are were similar or higher than concentrations measured in other South African systems (Phongolo, Wepener *et al.* 2012) and the Congo River (Verhaert *et al*. 2013). The higher degree of PBDE contamination of the Vaal River compared to the Phongolo River is demonstrated in the fact that although the tigerfish (*Hydrocynus vittatus*), is a top predator the levels were still lower than those found in *L. capensis*.

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P-20

Non-cancer risk associated with the consumption of alestes baremoze and synodontis bastiani contaminated with organochlorine pesticides from warri river, Nigeria

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An investigation into the Non cancer risk assessment of organochlorine pesticides residues in *Elestes baramoze* and *Synodontis bastiani* in the Warri River was carried out. A total of 48 samples (24 *Elestes baramoze* and 24 *Synodontis bastiani*) from Warri River were collected. Organochlorine pesticides residues were extracted from the samples. The extracts were cleaned-up and analysed using Gas Chromatography (GC) equipped with electron capture detector (GC-ECD). The health risk was calculated using the Hazard Quotient (HQ) and Hazard Index approaches. The Hazard Quotients of each of the OCPs in *E. baramoze* for child and adults were lower than unity (HQ<1) except for Heptachlor for children which was 1.5. The Hazard Quotients of each of the OCPs in *S. bastiani* for Children and Adults showed that heptachlor and Endosulfan I were higher than unity (HQ>1) indicating a potential health risk. The Hazard index (HI) for the mixture of OCPs in both *A. baremoze* and *S. bastiani* was higher than unity (HI>1) indicating potential health risk of the mixture to consumers. There is need for monitoring of Pesticides in the Warri River and Niger Delta in General.

Keywords: Non cancer Risk, Organochlorine Pesticides, Fishes

1. Introduction

Organochlorine pesticides (OCPs) are persistent organic pollutants which have caused worldwide concern as toxic environmental contaminants (Yared *et al.*, 2014). They are lipophilic, hydrophobic and are ubiquitous contaminants that have been detected far from their sources of origin because of long-range transport stemming from atmospheric exchange, water currents, animal migration and other pathways (Zhi-Yong *et* al., 20013). Human health risk assessment of chemicals refers to methods and techniques that apply to the identification and evaluation of hazards, exposure, dose-response and harm posed by chemicals, which in some cases may differ from approaches used to assess risks associated with biological and physical agents (USEPA, 1999). Assessments of risks to human health have been undertaken globally to examine the potential health risk due to exposure to toxic contaminants in various environmental media and foodstuff (USEPA 1999; Tsakiris *et al*., 2011). This study is therefore carried out to investigate the concentration of pesticides in *Elestes baramoze* and *Synodontis bastiani* from Warri River to evaluate the pollution status and the potential health risk

2. Materials and Methods

2.1 Sample collection

The study was carried out at Warri River (one of the most important coastal rivers of the Niger Delta) in Delta State. Two commonly consumed fish species (*Alestes baramoze* and *Synodontis bastiani*) in the area were collected monthly for a period of 6 months. The fishes were caught using fishing gears that are used by fishermen in the area (cast nets, gill net). The samples caught were wrapped in aluminium foil, transported in ice to the laboratory and stored at -4^{0}C followed by extraction within few days. A total of 48 samples (24 *E. baramoze* and 24 *S. bastiani*) were collected for this study.

2.2 Extraction and analysis

Edible portion of *E. baramoze* and 24 *S. bastiani* samples were extracted and cleaned-up according to the method described by Steinwandter (1992). The cleaned up extracts were analysed for pesticides (Alpha-HCB, Beta-HCB, Gamma-HCB,

Heptachlor, Heptachlor Epoxide, Aldrin, Dieldrin, Endrin and Endosulfan 1) using gas chromatograph (GC) equipped with electron capture detector (GC-ECD).

2.3 Human health risk estimation

Non-cancer risk assessment was calculated using the hazard index (HI) approach (EPA 1999, Ezemonye *et al*., 2015). i. e. HI = HQ_a+ HQ_b+ HQ_c… HQ_z. The hazard quotient (HQ) is the ratio between the estimated daily intake (EDI), and the reference dose (RfD) or Acceptable Daily intake (ADI) (USEPA 1999). The estimated daily intake (EDI) is calculated as the product of the residual concentration with fish consumption rate and dividing the product with the body weight of the individual. If the hazard quotient is more than unity (1), the chemical has a potential

health risk and if the hazard index exceeds unity (1), the mixture has a potential health risk (USEPA 1999; Zhi-Yong *et al*., 2013). It has been reported that the body weight of children is 30kg and adults is 60kg (USEPA, 1999). Consumption rate of fish in Nigeria is 9 kg per person (FAO, 2011).

3. Results and Discussion

Table 1: **Non-cancer risk associated with the consumption of *A. baremoze* from Warri River, Nigeria**

	ADI	Residual Conc.	Child		Adult	
			EDI		EDI	
Components	mg/kgday	(mg/kg)	mg/kgday	HQ	mg/kgday	HQ
Alpha-HCB	0.0005	0.0004	0.00012	0.24	0.00006	0.12
Gama-HCB	0.0125	0.0003	0.00009	0.0072	0.000045	0.0036
Beta-HCB	0.0005	0.0003	0.00009	0.18	0.000045	0.09
Heptachlor	0.0001	0.0005	0.00015	**1.5**	0.000075	0.75
Aldrin	0.0001	0.0002	0.00006	0.6	0.00003	0.3
Heptachlor epox	0.00013	0.00025	0.000075	0.576923	3.75E-05	0.288462
Endosulfan 1	0.00005	0.00015	0.000045	0.9	2.25E-05	0.45
Dieldrin	0.0001	0.0001	0.00003	0.3	0.000015	0.15
Endrin	0.0002	0.0001	0.00003	0.15	0.000015	0.075
Total				**4.454123**		**2.227062**

Table 2: **Non-cancer risk associated with the consumption of *S. bastiani* from Warri River, Nigeria**

	ADI	Residual Conc.	Child		Adult	
			EDI		EDI	
Components	mg/kgday	mg/kg	mg/kgday	HQ	mg/kgday	HQ
Alpha-HCB	0.0005	0.00135	0.000405	0.81	0.000203	0.405
Gama-HCB	0.0125	0.00035	0.000105	0.0084	5.25E-05	0.0042
Beta-HCB	0.0005	0.0009	0.00027	0.54	0.000135	0.27
Heptachlor	0.0001	0.0009	0.00027	**2.7**	0.000135	**1.35**
Aldrin	0.0001	0.0006	0.00018	**1.8**	0.00009	0.9
Heptachlor epoxide	0.00013	0.00015	0.000045	0.346154	2.25E-05	0.173077
Endosulfan 1	0.00005	0.00035	0.000105	**2.1**	5.25E-05	**1.05**
Dieldrin	0.0001	0.0001	0.00003	0.3	0.000015	0.15
Endrin	0.0002	0.0002	0.00006	0.3	0.00003	0.15
Total				**8.904554**		**4.452277**

The residual concentration, estimated daily intake (EDI) and hazard quotients of each OCPs for *A. baremoze* in both age group is shown in table 1. The results showed that the hazard quotient for each of the OCPs was lower than unity (1) for both children and adults except for Heptachlor in children that was 1.5 (table 1), indicating potential health risk. The hazard indexes for both age groups were 4.4554123 (children) and 2.227062 (adult) indicating potential health risk although individual OCPs hazard quotient was lower than unity.

The residual concentration, estimated daily intake (EDI) and hazard quotients of each OCPs for *S. bastiani* in Children and Adult is shown in table 2. The results showed that Heptachlor, Aldrin and Endosulfan I in children had HQ more than unity and heptachlor and endosulfan I for adults had HQ more than unity indicating potential health risk. The hazard indexes for both age groups were more than unity (1) which indicated potential health risk of the mixture. It has been reported that if the hazard index is more than unity (1), the mixture has exceeded the maximum acceptable level (Tsakiris *et al*., 2011). To ensure the safety of consumers, it is therefore imperative for continuous monitoring of OCPs in Warri River and its fish resources.

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P-21

Growth inhibition due to light blocking effects of gold nanoparticles (nAu) on *Pseudokirchneriella subcapitata* (algae)

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Gold nanoparticles are being developed as drug delivery vectors due to their unique properties which allow for targeted cell delivery. In an aquatic exposure study nAu and ionic gold were exposed to algae using an AlgaltoxkitF®. At 24h intervals a standard *chlorophyll-a* extraction protocol was employed using growth inhibition as an end point. Visualization of nAu on the surface of algae was done using CytoViva imaging. The nAu concentrations showed a hormetic response based on the ability of nAu, in the medium and adhered to the algal surface, to absorb and scatter light. The ionic gold exposures showed a dose dependent response due to the bioavailability and toxicity of free metal ions further exacerbated when coupled with the exposure to light.

Keywords: nAu, algae, shading effects, CytoViva

1. Introduction

The ability of nano gold (nAu) particles to undergo plasmon resonance with light is of great importance within the therapeutic fields as it can be used to resonate the electrons to destroy tissue. Gold nanoparticles have the ability to scatter and absorb visible light, a property, which is highly dependent on the size and concentrations of the particles. Since algae are reliant on light energy for growth they can be used as a model organism to gain a better understanding of nAu phototoxicity.

2. Materials and Methods

The algal (*Pseudokirchneriella subcapitata*) growth inhibition test was performed using AlgaltoxkitF® according standard protocols as set out by the OECD-TG218/219 and to eliminate particle interference the spectrometer step was replaced by a chlorophyll-a extraction method (Sartory, 1982). For the test only glassware was used as to avoid the nAu particles from adhering to the surface and samples were done in triplicate for each 24h exposure interval, therefore nine samples were prepared in total per concentration.

Concentrations ranging from 6.25 mg/l to 100 mg/l for nAu and citrate dispersant; and 1 mg/l to 10 mg/l for chloroauric acid (to differentiate between particle and metal ion toxicity) were made up in exposure media containing 1.10^6 algal cells/ml. Samples flasks were plugged with an aerating cotton wool and were kept at 23°C ±2°C with continuous illumination for 72 h.

At each 24h interval *chlorophyll-a* concentrations were determined using a Hiatachi 150-20 spectrophotometer. A volume of 49.5 ml of sample was filtered through Whatman GF/C filter paper using a vacuum pump. The chlorophyll which gathered on the filter paper was extracted using 10 ml of 95% ethanol in a water bath set to 78°C for a period of 5 minutes. Once removed the samples were left to cool down in a dark room. The samples were read at 665 nm and 750 nm where 95% ethanol was used as a blank, samples and blank were then acidified with 0.3 M HCl and read again at 665 nm and 750 nm after a period of 2 minutes. The following calculation was used to calculate the *chlorophyll-a* concentration:

***Chlorophyll-a* (µg/l) = [(A665-A750) * (A665a-A750a) x 28.66 x extract volume]/volume of sample**

Where A665 = absorbency at 665nm; A750 = absorbency at 750 nm; A665a = absorbency at 665nm after acidification; A750a = absorbency at 750nm after acidification; extract volume = 10mL 95% ethanol; volume of sample = volume of water sample filtered in litres (0.049L).

CytoViva® dark field hyperspectral imaging of algae

One millilitre of algal suspension from the control, 25 mg/l and 50 mg/l of nAu exposure concentrations were placed on a slide with a cover slip and sealed with Entellan® and then viewed using CytoViva® dark field hyperspectral imaging. CytoViva® 150 Unit integrated onto the Olympus BX43 microscope was used where HSI system 1.1 and ENVI software at 60X magnification was used to confirm the nAu particles in the medium by spectral profiles. Images were captured using the Dagexcel X16 camera and DAGE Exponent software at 60X magnification.

3. Results and Discussion

The acute algal test revealed after 48 h there was a similar increase in algal growth except for all ionic gold concentrations, which resulted in marked growth inhibition (Figure 1). From 48h it could be seen that nAu 6.25mg/l and 100mg/l had a lower

growth rate. After 72h, the lowest concentration (6.25mg/l) had the highest growth inhibition and was more affected than the serial concentrations above it and 25mg/l showed the lowest affect but still had approximately 45% less algal growth than the control. As expected the ionic gold concentrations showed a dose dependent response, where from 5mg/l there was almost no growth seen after 72h. The dispersant concentrations showed no effect on algal growth. García-Cambero *et al*. (2013) showed an EC50 of 1.91mg/l for algae exposed to chloroauric acid, which are similar to the results obtained in this study. The bioavailability of the Au^{3+} ions as well as their phototoxicity above 1mg/l provides the rationale for toxicity to aquatic organisms at low concentrations.

Particles interacting and agglomerating to one another is caused by nAu surface charge. These charges are also offering the ability of the nAu particles to form interactions with the algal cells present in the media (Figure 2). The larger the nAu agglomeration the longer the wavelength of light absorbed by the particle which leads to shading effects and will therefore have the ability to increase the toxicology of the particle on those organisms that are dependent on light for their growth.

All nAu concentrations showed a decrease in algal growth over 72h when compared to the dispersant and control exposures. As the nAu concentration increases so does the amount of surface adhesion of nAu to the algae thereby causing a reduction in the interaction of the algae with the environment, this in turn results in a lower metabolic rate and in turn the ability to for algae to reproduce (Lin *et al*., 2009). This cellular adhesion may also weaken the wall and influence vital exchanges at this site. In this study there was no evidence that the nAu particles were able to penetrate the plasma of the algae. Positively charged nAu particles adhere to the cell wall by attractive negative charges on the membrane (García-Cambero *et al*. 2013). However it has been proposed that even though nanoparticles do not enter the cell the adhesion to the surface causes the release of free metal ions thereby increasing the toxicity which when coupled with shading effects could be detrimental to algal growth.

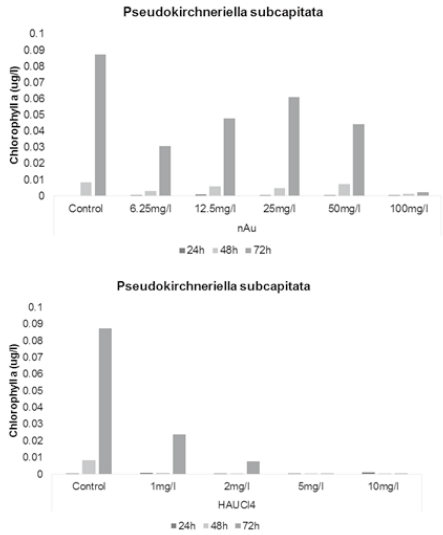

Figure 1: *Chlorophyll a* concentrations of algae exposed to a) nAu b) ionic gold

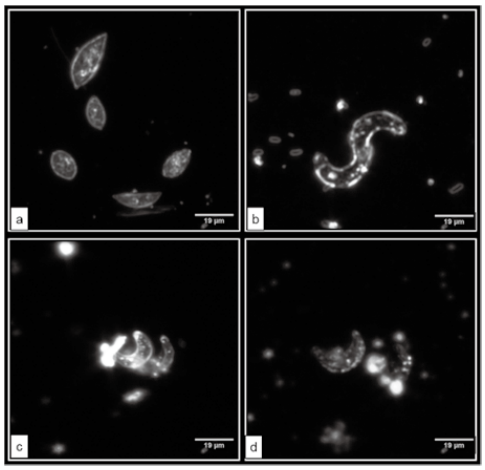

Figure 2: Algae suspensions visualized using CytoViva in a) control c-d) nAu exposure concentrations

4. Acknowledgments

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P-22

Aflatoxin and fumonisin in corn production chain in Bafia, centre cameroon: impact of processing techniques.

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In addition to aflatoxin and fumonisin being responsible for many mycotoxicoses in animals, they are also long term carcinogens in humans. The aim of this research is to determine the levels of these toxins in the variety of corn most consumed, after harvest, during drying and storage stages and also estimate the reduction levels of these toxins depending on the different culinary treatments. The total aflatoxin (AFT) and total fumonisin (FT) was carried out using quantitative ELISA in 45 composite samples of corn and corn based-dishes at different stages of production. The results in critical composites samples (samples with level of total mycotoxin more than tolerated level) have been confirm by HPLC-MS. The results show that all samples (45/45) were contaminated with AFT (range: 0.79 to 19.98 ppb) while (41/45) were contaminated with FT (range: 0.01 to 5.99 ppm). Amont the critical samples, 75% were contaminated by AFB_1 and AFB_2 whereas 100% were contaminated with FB_1 and FB_2. The culinary techniques reduce the levels of FT detected in all samples up to 94.74% mainly in corn dishes that have a screening phase and up to 60.73% (corn porridge) of the levels of AFT.

Keywords: Mycotoxin, corn and derivatives, culinary treatment, Cameroon.

1. Introduction

Food safety is a call for concern nowadays. FAO (2004) estimated that 25% annual harvests of grains worldwide are contaminated with mycotoxins. Mycotoxins are toxic compounds, ubiquitous, thermostable issuing from secondary metabolism of molds. There are more than 300 types of mycotoxins but those most commonly encountered in hot and humid countries are aflatoxins and fumonisins. It turns out that the main route of contamination by these mycotoxins is ingestion of contaminated food. Corn is the preferred substrate for mold secreting these toxins because it is rich in carbohydrates. However, corn makes up a large portion of the diet of rural people in Sub Saharan Africa and 85% of corn products are directly consumed by man. In Cameroon, corn-based dishes are the staple food of about 12 million people. The environmental conditions in Cameroon particularly favor the proliferation of toxigenic molds (Nguegwouo *et al,* 2011). The general objective of our work is to contribute to the study of mycotoxins in the traditional corn based-dishes and estimate the levels of reduction of these toxins depending on the different culinary treatments.

2. Materials and methods

This study was carried out in *Bafia*. It is located in the centre Cameroon. Five villages of Bafia (*Goufan I, Goufan II, Donenkenk I, Binya* and *Tchekané*) were randomly selected with respect to a high corn production and consumption. Forty five composites sample of corn and derivatives were collected between March and December 2012. Theses sample were collected and transported directly in coolers to the Food and Nutrition Research Center of the Ministry of Scientific Research and Innovation. In the laboratory, AFT (AFB_1, AFB_2, AFG_1 and AFG_2) and FT (FB_1, FB_2 and FB_3) levels were assessed using ELISA test according to the manufacturer's instruction (RENEKABIO, The AFT Assay; Fumonisin ELISA Assay) on all pulverized composite samples while AFB_1, AFB_2, FB_1 and FB_2 were evaluated by High-performance liquid chromatography coupled to mass spectrometry (HPLC-MS) according to Njumbe *et al.* (2011) on samples previously having high-risk of contamination following the analysis by ELISA.

3. Results and Discussion

The results (table 1) show that all the variety of corn seed cultivated in *Bafia*, including the yellow unselected grains which is the most consumed, are contaminated with aflatoxins and fumonisins. Levels of FT found in corn seed in *Bafia* (0.13 to 3.48 ppm) are lower than those found in corn from South Africa which, after some years, may be contaminated up to 140 ppm (Wagacha Muthomi,

2008).The detection and quantification of AFT and FT in composite samples of the main variety (table 2) consumed in Bafia (unselected yellow corn) at three degrees of maturity at harvest (Table 2) show that 100% of samples are contaminated with levels that vary significantly ($p < 0.05$) from fresh (80 days) to semi dry (85 days) and not significantly ($p > 0.05$) from semi dry to dry (90days).Fresh corn has a higher water content of about 60% compared to semi dry corn having a water content of about 45%.. The levels of AFT in yellow corn samples after one and two weeks of sun drying or in the barn show no significant difference ($p > 0.05$). Quantitative analyses of AFT and FT in composite samples of yellow corn after one, two and three months of storage in a barn show no significant difference ($p < 0.05$). However the average levels of FT found after two months of storage in the analyzed samples of yellow corn exceeds the recommended maximum levels of 2 ppm. The different culinary (table 3) processes affect more rates Fum.T (6.51 to 94.74%). The presence of some samples contaminated with these toxins at levels beyond prescribed limits highlights the need for regulatory actions, control and monitoring plans for AFT and FT to reduce their level in the food chain and subsequently reduce the health risks associated with their presence.

Table 1: Total aflatoxin (AFT) and Total fumonisin(FT) content in corn seed in *Bafia* sub-division

Corn varieties n= 8	**(AFT) (ppb)**	**(FT) (ppm)**
Unselected soft corn	2.02±0.83^{a*}	0.13±0.06^{a}
Unselected white corn	1.29±0.16^{a}	2.45±0.23^{b}
Unselected yellow corn	1.23±0.08^{a}	0.89±0.16^{c}
Selected red corn	1.42±0.07^{a}	3.48±0.12^{d}

Table 2: Total aflatoxin and Total fumonisin content in yellow corn at harvest, after drying and storage in *Bafia*

Treatments and samples (Yellow corn) n= 16	**Total aflatoxin (AFT) (ppb)**	**Total fumonisi (FT)(ppm)**
Harvest		
(80 days)	1.52±0.16^{b}	0.01±0.01^{b}
(85 days)	1.00±0.11^{a}	0.11±0.02^{a}
(90 days)	0.91±0.06^{a}	0.13±0.01^{a}
Drying (week)		
one week in a sun	1.36±0.19^{a}	5.17±0.02^{a}
Two weeks in a sun	1.22±0.01^{a}	0.07±0.00^{b}
One week in a barn	1.80±0.15^{a}	4.49±0.35^{a}
two weeks in a barn	1.32±0.20^{a}	0.50±0.07^{a}
Storage (month)		
one month	1.30±0.12^{a}	0.12±0.03^{b}
two months	1.59±0.10^{a}	2.01±1.13^{a}
three months	1.60±0.21^{a}	5.16±0.07^{a}

Table 3: Total Aflatoxin and Total Fumonisin contents in corn-based dishes compared to corn grains before cooking

Corn-based dishes n= 21	**% reduction AFT**	**% reduction FT**
Corn beer (*Kwata**)	+47.07%	+22.13%
Fresh or dry flat corn cake with vegetables *(kpwindim*	+15.66%	+94.74%
Fresh or dry flat corn cake with groundnuts (*Kekumba*	+ 8.5%	+36.84%
Corn porridge (*Kenouk**)	60.73%	+88.97%
Corn fufu (Couscous) (*Kepen**)	+56.88%	+93.66%
Corn milk *(Melek me Baazi**)	+59.05%	+6.51%
Corn vegetables (*Safon* or sanga**)	+13.36%	+31.25%
Roasted corn (*Baazi hangue**)	+38.71%	ND
Boiled corn *Baazi**)	+16.93%	ND
Corn fritters (*Kpwaa**)	+40.74%	+17.24%
Fried corn with groundnuts (*Baazi hangue de keezoo**)	- 91.9%	+86.21%

*Vernacular names in *Bafia*. Numbers in a column with different superscript letters are significantly different ($P<0.05$). figures are Mean ± SEM

4. Acknowledgement

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P-23

Nutrient loads on an important watercourse. Pre- and Post-Acid spill

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Exponential population growth has led to increasing amounts of polluted effluents contaminating aquatic ecosystems. An increase in nutrients from both, point and diffuse sources, leads to the eutrophication of aquatic systems, reducing ecosystem health. To ensure the sustainability of this resource, for both human and environmental use, adequate management is required. The Department of Water and Sanitation (DWS) has developed the South African Water Quality Guidelines, providing Target Water Quality Ranges (TWQR) for safe utilisation of the resource for humans and the natural environment. Water from representative sites along the Nyl River (Limpopo) was tested to determine changes in nutrient concentrations. It was noted that adjacent land use practices, impacted the system, introducing nutrients into the system when compared to the reference site. Mean NO_3, NH_3 and NH_4 concentrations exceeded the TWQR. In May 2015, an accident led to 28000L of concentrated sulphuric acid being spilt into the river system. A comparison between pre- to post-spill nutrient availability was also conducted. The mean concentrations of NO_3, NH_3, NH_4, PO_4 as well as conductivity were all higher after the spill.

Keywords: Nyl, river, pollution, water quality, eutrophication, Limpopo, South Africa.

1. Introduction

Globally, pollution in every aspect tends to degrade freshwater aquatic systems and the processes they facilitate. Recently, due to population growth, agricultural, industrial and social developments, eutrophication has been regarded as one of the most severe problems to these fragile ecosystems (Zamparas & Zacharias, 2014). Point sources of main concern include industrial, urban and rural effluents, mining sites as well as sewage treatment plants. Diffuse sources on the other hand comprise of agricultural activities, runoff and atmospheric deposition (de Villiers & Thiart, 2007).

Located in the arid Limpopo Province, South Africa, the study focuses on sites that occur before (reference sites) major settlements, through to sites located after (representative sites), which also comprises the largest floodplain of South Africa, the Ramsar accredited Nylsvley wetland.

Increased nutrient levels not only alter the natural cycles of carbon, nitrogen and phosphorous, but can pose problems associated with toxicity and decreased oxygen content, resulting in a decrease in general health of water bodies (DWAF, 1996).

An accident in May 2015, led to a major catastrophe, whereby 28000 L of concentrated sulphuric acid was spilt into the Nyl River at Modimolle. Of major concern is the fact that the watercourse leads into the largest inland floodplain in South Africa, and a Ramsar accredited wetland (Nylsvley). Disaster management teams introduced lime into the system to counteract the effect of the acid (SABC, 2015).

The aims of this study are to therefore determine the nutrient concentrations of the river course, and to determine if any impact was made by the acid spill.

2. Materials and Methods

2.1 Field procedures

Sites under study in this project were chosen prior to the commencement of the project; based on adjacent land use activities, and therefore a representation of potential anthropogenic impacts along the course of the river and wetland. Klein Nyl Oog (KNO), Donkerpoort Dam (DPD), Golf Course (GC), Sewage Treatment Works (STW), Jasper (JASP), Nylsvley (NYL) and Moordrift (MOOR) are the 7 sites respectively. *In situ* parameters of water quality, such as pH and conductivity were measured using an Extech DO700 meter. Clean 1 litre bottles were used to collect surface water for nutrient analysis. The water was then frozen to mitigate growth and process actions, up until laboratory analysis could take place..

2.2 Laboratory procedures

The frozen water samples were left to defrost to room temperature before being analysed for various concentrations of nutrients. The tests considered were ammonia, ammonium, nitrates, nitrites, phosphates along with total hardness. The procedure followed, was that which was stipulated by the Merck nutrient test kits. The concentrations were determined using a Pharo-100 spectroquant (MERCK).

2.3 Statiscal analysis

Statistical analysis was carried out using SPSS v20 and CANOCO 4.5. A PCA biplot was drawn

using CANO-DRAW in conjunction with CANOCO. Trip data of the system prior to the spill is indicated with the epithet (_1), while the data presented for post-spill indicated as (_2).

3. Results and Discussion

The PCA biplot, represented in Figure 1 explains 90.8% of variation within sites regarding the different environmental variables. 70.4% is explained along the first axis, whilst 20.4% is explained on the second axis. The groupings demonstrate the drivers with which sites are most correlated to, for instance, it can be seen that STW can be explained with high conductivity, hardness and phosphate concentrations. JASP on the other hand, can be attributed to high nitrogen content. It is also evident that the first axis separates downstream sites (STW, JASP and MOOR) on the right, from the upstream sites on the left.

The spill occurred upstream of STW, meaning that sites above should not be impacted. Therefore changes in pre- to post-acid spill comparisons only take into account the sites that include and follow STW.

The highest values in general, for pre-acid spill of NO_2 (0.25 mg/l), PO_4 (4.42 mg/l), NH_3 (3.43 mg/l), NH_4 (3.63 mg/l) $CaCO_3$ (0.495 mmol/l) and conductivity (381.1 µs/cm) occurred at STW, whilst the highest amount of NO_3 (3.3 mg/l) occurred at JASP. With regards to post-spill values, besides conductivity (817 µs/cm), $CaCO_3$ (2.08 mmol/l) and PO_4 (8.7 mg/l) occurring at highest concentrations at STW, NO_2 (0.17 mg/l), NO_3 (17.3 mg/l), NH_3 (3.65 mg/l), NH_4 (3.86 mg/l) occur highest at JASP, possibly indicating mobilisation of nutrients downstream from STW.

The pH values occurring upstream of the spill, tended to be more alkaline in comparison to pre-spill values eg. KNO_1 (7.34) whilst KNO_2 (8.32). This is expected as many land owners intervened by placing lime into the system. Whereas pH values from STW tended to decrease at all sites, which is attributed to the acid introduced.

The average natural background values for nitrogen (NO_2, NO_3, NH_3 & NH_4) is less than 0.5 mg/l, of which TWQR values do not exceed 15%. Phosphorous has natural values of less than 5000 mg/l, with the TWQR not exceeding 5% thereof (DWAF, 1996).

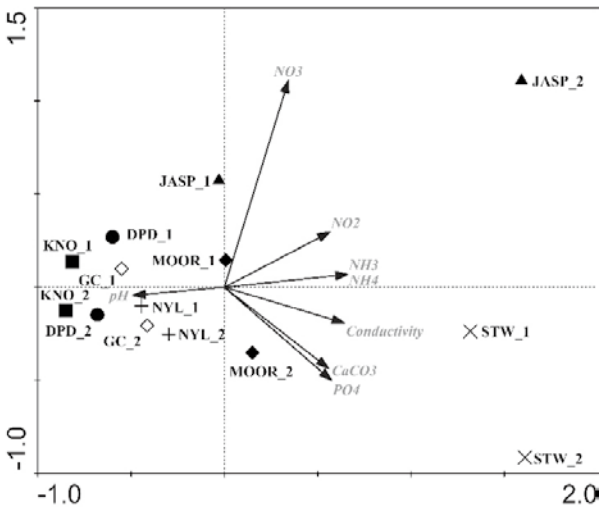

Figure 1: PCA biplot illustrating groupings of sites in relation to respective drivers. 70.4% of variation explained on axis 1, and 20.4% explained by axis 2.

4. Conclusion

It can be seen that human activities do play a role in affecting the nutrient loads introduced into the system. The increased concentrations found post-spill is attributed to mobilisation of nutrients. The buffering capacity of the water seemed to aid in controlling the impact of the acid.

5. Acknowledgments

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P-24

Estimated dietary exposure to veterinary residues in chicken and eggs

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Chicken tissues and eggs were analyzed for residues of 8 veterinary drugs including albendazole, piperazine, tiamulin, chloramphenicol, levamisole, sulphathiazole, sulphamethoxazole and oxytetracycline. Mean residue concentrations of all drugs varied by tissue and ranged from 0.004 µg/kg for tiamulin in eggs to 8.598 µg/kg for chloramphenicol in liver. Total residues of all drugs in the liver generally exceeded that of the kidney, muscles and eggs by at least a two-fold difference. The general order of decreasing total drug residue levels is liver>kidney>muscle>eggs. Chloramphenicol registered the highest exposure of 0.461 µg/kg. The exposure due to albendazole and sulphamethiazine in liver were 2- and 7-folds higher than their recommended average ADI. Exposure due to piperazine, sulphamethoxazole, levamisole and tiamulin were, however, lower than their respective recommended limits. Besides the levels of levamisole in muscle, none of the detected drugs showed levels that were higher than their respective maximum residual limits. Dietary exposure assessments of all drugs based on quantitated residue levels are within the JECFA safe regulatory exposure limits. This study informs the public about veterinary drug residues in poultry and helps address policy and regulatory changes in the use of veterinary drugs in poultry.

Keywords: Poultry, veterinary, drugs, residues

1. Introduction

Veterinary drugs are routinely used on poultry for therapy and for prophylaxis that may encompass control and prevention of diseases, assistance in relieving stress (Kao et al., 2001), rehydration of livestock, promotion of growth (Kabir et al., 2004) and stimulation of egg production. Use of veterinary drugs is so prevalent in modern animal husbandry that an estimated 80% of all food-producing animals receive a regimen of drug medication for part or most of their lives (Pavlov et al., 2008).

Consequently, incorrect use of veterinary drugs as occurs in overdose, inappropriate use times lack of adherence to optimal use instructions and non-observance of withdrawal periods prior to slaughter or to laying of eggs may leave residual levels of drugs in tissues and eggs at concentrations that may be deemed harmful to human health.

Chronic and acute human exposure to veterinary drugs through ingestion of drug residues in poultry products at concentrations above the threshold of risk are associated with the development of allergic reaction in hypersensitive individuals (Shankar et al., 2010), the alteration of human intestinal micro-flora (Shankar et al., 2010) as well as induction and generation of multi-drug resistant strains of human pathogenic bacteria (Kabir et al., 2004). The presence of veterinary drug residues in poultry above tolerance constitute a healthcare problem and represents a public health concern (Verbeke and Viaene, 2000). Safe and appropriate use of use of veterinary drugs therefore remains one of the most challenging public health issues.

2. Materials and Methods.

The homogenates and 50 mL of acetonitrile were then added and mixed. 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 separation funnel containing 30 mL of acetonitrile-saturated n-hexane and shaken. The acetonitrile layer was collected into a concentration bottle and evaporated to dryness. The above dry residue was reconstituted with 20 mL of 0.05M NaH_2PO_4 and applied onto a 1 g ENVI-18 SPE cartridge, which was pre-conditioned with 10 mL of methanol and 10 mL of 0.05M NaH_2PO_4

3. Results and Discussion

A total of 540 samples of liver, kidney and muscles, from 180 chickens and 200 eggs homogenized samples were extracted using acetonitrile and cleanup on C-18 solid phase column.

Sampled farms cover a 3 urban District areas located in the Ashanti region of Ghana follow different drug use protocols that includes more than one use frequency at different doses and at different times for either therapy or prophylaxis or for both. None of the sampled farms observed drug withdrawal period prior to slaughter and marketing of chicken products. Six of the 8 drugs including albendazole, piperazine, chloramphenicol, levamisole, sulphathiazole and sulphamethoxazole recorded their highest total concentrations in the liver. Except tiamulin that showed very low liver

residual levels, liver residues of all drugs were higher than muscles residues by at least two-fold difference and higher than egg residues by at least five-fold difference. Relatively high liver residues of the 6 drugs implicate the liver as first site of biochemical action for the drugs' metabolism. Oxytetracycline and tiamulin deviate from the pattern of quantitative distribution exhibited by the 6 other drugs. Highest residues of oxytetracycline were recorded in the kidney with a marginally lower value in the liver.

Similarly, the highest recorded values of tiamulin were found in the kidney while the liver showed a 4-fold decrease in residual levels. The highest recorded levels of tiamulin and oxytetracycline in the kidney leads to the suggestion that the kidney is the possible first site for the metabolism of both drugs.

i. Global Estimated Acute Dietary Exposure Assessments (GEADE)

The global estimated acute dietary exposure for the veterinary drugs residues studied have not yet been established. Computed GEADE of the residues was therefore compared to their ADIs, where available. All the residues had estimated values lower than their respective ADIs. Chloramphenicol recorded the highest exposure of 0.031, 0.024 and 0.012 µg/kg respectively in liver, kidney and muscles.

ii. Chronic Dietary Exposure Assessments

Chronic dietary exposure estimates cover food consumption over the long term and are usually intended to be compare with a chronic toxicity health-based guidance value such as an ADI, in a risk assessment process (FAO/WHO, 2011; FAO-JECFA, 2014). Once again, chloramphenicol residues in liver samples registered the highest level of exposure 0.461 µg/kg, The exposure due to albendazole in liver samples was twice higher than the recommended ADI of 0.05 µg/kg while sulphamethiazine recorded an exposure 7 times higher than the recommended ADI of 0.02 µg/kg. The exposure due to piperazine, sulphamethoxazole, levamisole and tiamulin were, however, lower than their respective recommended ADIs. Albendazole recorded highest residual exposure from kidney sample which was about twice higher than the recommended ADI of 0.05 µg/kg. Chloramphenicol recorded 0.079 µg/kg exposure and was closely followed by oxytetracycline which registered 0.074 µg/kg. Oxytetracycline registered the highest level of exposure in the muscle samples which was about 2 times higher than the recommended ADI. The rest of the residues were lower than the recommended ADI values.

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P-25

Catalase Activity and Malondialdehyde Content in two crustacean species from sub-tropical river sections in a leading conservation area.

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The Ndumo Game Reserve is situated in KwaZulu Natal, South Africa and is an area with uniquely high biodiversity. However like many protected areas, environmental contaminants entering from outside the conservation areas pose threats to the biota which are being protected. To establish the possible risks posed to the aquatic biota of the system, *Cherax quadricarinatus* (invasive) and *Macrobrachium. petersii* (indigenous) were selected as indicator species from the Usuthu and Pongola Rivers. Biomarker analyses were done to quantify the level of malondiadehyde (MDA) caused by reactive oxygen species oxidizing lipids, as well as if there is up or down regulation of the catalase (CAT) protective mechanism which facilitates the removal of H_2O_2. Levels of both biomarkers within each species were not significantly different between the two river systems. Impacts upstream of both systems are similar, e.g. informal agriculture, which may be a possible explanation as to why the biomarker responses within the species are similar from both sites. Comparing CAT and MDA results between species, the invasive *C. quadricarinatus*, appears to be more tolerant than the indigenous *M. petersii*. Invasive organisms are generally more tolerant to changes in environmental conditions possibly attributing to their lower MDA content and CAT activity.

Keywords: *Cherax quadricarinatus, Macrobrachium petersii,* South Africa, Ndumo Game Reserve

1. Introduction

Floodplain systems across the world are socially, economically and ecologically important. The Pongola River (PR) floodplain, located in the northern part of Kwazulu Natal, is South Africa's largest floodplain system. This area supports a vast array of biota, some of which are critically endangered. The floodplain is however under threat from anthropogenic activities within the catchment. The Ndumo Game Reserve (NGR) is a protected area which protects a very small portion of the floodplain. It is located on the border of South Africa and Mozambique and was demarcated for the protection of biodiversity. The Usuthu River (UR) forms the northern border of the reserve and part of the border between the aforementioned countries. The two rivers converge shortly downstream of the reserve. Land use practices along the Usuthu River are similar to those along the Pongola River, e.g. irrigation schemes and informal agriculture.

Selected biochemical markers were used to establish whether organisms in the rivers of the NGR are experiencing oxidative stress due to upstream human activities. Biomarker responses are well established in the field of aquatic health. They occur low down the biological system and therefore can be used as early warning signals before the stressors are able to affect populations or communities (de la Torre et al., 2007).

Reactive oxygen species (ROS) are continually produced in organisms as a result of normal metabolic processes. These ROS, such as H_2O_2, cause oxidative damage to tissues such as lipids. Catalase (CAT) is an antioxidant that has the sole function of binding and neutralizing H_2O_2 to form water and oxygen. Malondialdehyde (MDA) is a by-product of lipid peroxidation that occurs when there is an increase in ROS (Van der Oost et al., 2003). Both biomarkers can be used to ascertain information regarding oxidative stress of the organism

The invasive *Cherax quadricarinatus* (Australian red-clawed crayfish) and the indigenous *Macrobrachium petersii* (South-East coast river prawn) were chosen as indicator species. The aim of this study is to identify differences in CAT and MDA biomarker responses between the two species as well as the selected rivers.

2. Materials and Methods

Ethical clearance for the use of animals in research was granted for the project by the University of Johannesburg's ethics committee. Organisms were captured using electroshocking techniques and baited fyke nets during a low flow survey (October 2014) at selected sites in the Usuthu River and the Pongola River sections within the NGR. Organisms were sacrificed and dissected to excise muscle and hepatopancrease tissue, which were stored separately in Hendricksons

buffer and frozen in liquid nitrogen. In the lab, samples were placed in a -80°C freezer for storage. Thereafter samples were weighed out and placed in corresponding buffers for each biomarker analysis. Biomarker analyses were done following the procedures of Cohen et al. (1970) for CAT activity, and Üner et al. (2006) for MDA content. A one-way ANOVA was performed to identify differences among groups, with a Tukeys post-hoc test to identify significant differences ($p < 0.05$).

3. Results and Discussion

Figure 1 reports mean values of CAT in *C. quadricarinatus* from UR and PR, 81,3903 $\mu molH_2O_2$/min/mg protein and 70,7099 $\mu molH_2O_2$/min/mg protein respectively, are not significantly different. The same follows for CAT in *M. petersii* with UR 245,4082 nmol/mg protein and PR 272,5560 nmol/mg protein.

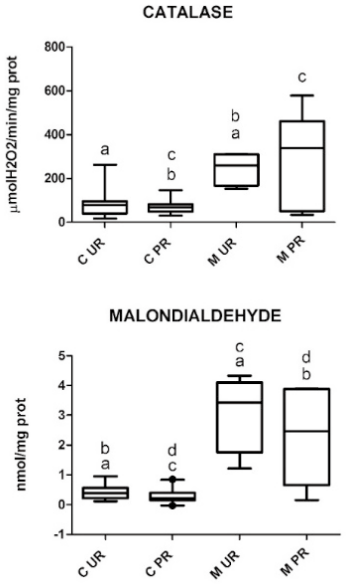

Figure 1: Biomarker responses in *C. quadricarinatus* (C) and *M. petersii* (M) from the Usuthu (UR) and Pongola (PR) Rivers. Common superscripts indicate statistically significant difference.

Mean values of MDA for *C. quadricarinatus* from UR 0,4181 and PR 0,2717 are similar, which is also observed in CAT, with mean values for *M. petersii* UR 3,0970 nmol/mg protein and PR 2,3116 nmol/mg protein. Similar land use practices upstream of both sampling sites may be the cause for the similar biomarker responses,

CAT activity and MDA show similar trends when comparing the two species. Levels of CAT activity in *M. petersii,* from PR, although not significantly different show a clear increased response compared to *C. quadricarinatus.* All other species comparisons in CAT and MDA are of statistical significance, showing a greater response in the indigenous species. These results indicate that the indigenous *M. petersii* are experiencing greater lipid peroxidation despite these individuals having elevated protective mechanisms through the function of CAT, which is attempting to neutralize the H_2O_2.

Invasive species have been found to be more tolerant of changes in the environment, compared to indigenous species that occur in the same systems. (Karatayev et al., 2009) *Cherax quadricarinatus* is an invasive species and shows a potentially higher tolerance to environmental factors causing oxidative stress in *M. petersii.*

4. Acknowledgements

Collaborators and team members from the VLIR-UOS collaboration that made this project possible. This study was made possible through funding awarded to G. Jansen van Rensburg (Grant No. 89629) from the National Research Foundation (NRF) Opinions expressed here are not necessarily those of the NRF.

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P-26

An assessment of the freshwater mollusc diversity in the Mooi River catchment area, North-West Province, South Africa

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This study was undertaken to investigate the status of the freshwater mollusc diversity in the Mooi River catchment. North-West Province, South Africa. This catchment has three major sub-catchments, the Wonderfontein Spruit, the Mooi River proper and the Loop Spruit. Large scale mining commenced in the 1930's, and water from these mines, as well as from several urban and industrial areas and informal settlements drain towards this catchment, which all contribute towards contamination of the water environment. Two surveys were conducted during a late and early low-flow period in 2014 at 21 sites selected for this study. All available biotopes at each site were sampled and pH and electrical conductivity (EC) were measured *in situ.* Molluscs were identified up to species level. A total of 15 different species were collected. The alien invasive snail, *Physa acuta,* was found for the first time in this catchment. The mollusc diversity recorded during this study, compared favourably to earlier reports from the same area. Although EC values were relatively high, indicating a possibility of organic pollution, it is concluded that ecological integrity of the Mooi River catchment has not yet been compromised to such an extent as to have a negative effect on its mollusc diversity.

Keywords: Freshwater mollusc diversity, Mooi River, North-West Province, South Africa

1. Introduction

The rate of extinctions of freshwater organisms is as high as 4% per decade, while the highest number of extinctions of any major taxonomic group is attributed to the Mollusca. Major reasons for these extinctions is largely ascribed to factors such as climate changes, habitat destruction, deforestation, urbanization, agricultural activities, dumping of acidic mine effluents in natural waters and damming of rivers.

With regard to the Mooi River catchment, large scale mining already commenced in the 1930's, and water from these mines, as well as from several urban and industrial areas and informal settlements drain towards this river, which all contribute towards contamination of the water environment (Van der Walt et al. 2002). These authors report a general increase in electrical conductivity (EC) and sulphate concentration from 1969 to 1996 in some sections of the Mooi River and according to them, the increase in formal and informal settlements contributed towards the organic pollution in this river.

In view of the concerns expressed with regard to the rate of extinction of freshwater molluscs worldwide and about the state of the ecological health of the Mooi River catchment in general, this study was undertaken to assess the effects of the anthropogenic stressors mentioned above, on the freshwater mollusc diversity of this river and its main tributaries.

1.1 The study area

The Mooi River catchment has three major sub-catchments, the Wonderfontein Spruit (north-eastern reach), the Mooi River proper (northern reach) and the Loop Spruit (eastern reach). The predominant land-uses in the north-eastern reach are farming and large scale mining, in the northern sub-catchment, crop farming, grazing and small scale diamond diggings, and in the eastern sub-catchment (Loop Spruit), crop farming, grazing, as well as gold mining. The localities of the 21 sites selected for this study are depicted in the Figure 1.

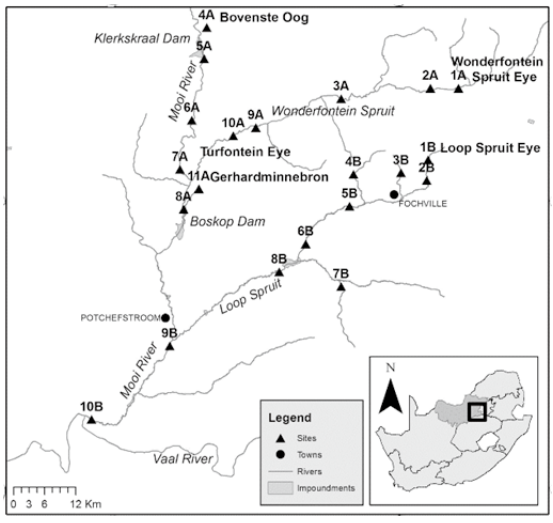

Figure 1: The study area and sites where molluscs were collected

1.2 Sampling equipment and techniques

Two surveys were done in 2014 at all 21 sites during a late and early low-flow period. All available biotopes were sampled for molluscs at each site. Sampling was done with custom made scoops. Molluscs were preserved in 70% ethanol and transported to the laboratory for identification up to species level. Coordinates and altitude were determined with a Garmin Nuvi 500- GPS at each site and pH and electrical conductivity (EC) were measured *in situ* with portable digital instruments.

2. Results and Discussion

Fifteen different species were collected during this study, of which the two most dominant species, *Bulinus tropicus* and *Burnupia mooiensis* were present at 14 and 13 sites respectively (Table 1). In contrast to this, *Bulinus africanus* were recovered from one site only and *Lymnaea truncatula* and *Gyraulus connollyi*, from two sites each. The alien invasive species, *Physa acuta* which was collected at 12 sites, was recorded for the first time from this catchment. This species was not found in an extensive survey conducted during four different seasons at 15 sites in the Mooi River (De Kock and Van Eeden 1969). Furthermore, there are also no records of this species in the database of the National Freshwater Snail Collection (NFSC) from surveys conducted between 1964 and 1980 by staff of the State Health Department and of the Snail Research Unit of The Medical Research Council at Potchefstroom. *Physa acuta* is associated with polluted water and have difficulties in establishing itself far away from human activities (Brown, 1994). The fact that it has become established at 12 sites in the Mooi River catchment is indicative of organic pollution, which is further supported by the presence of *B. tropicus* at 14 sites, a snail also known to flourish under such conditions (Table 1).

Table 1: The mollusc diversity at the selected sites and the abiotic factors measured at each site

	Mooi River upper zone and Wonderfontein Spruit											Loop Spruit and Mooi River lower zone										
Site numbers	1A	2A	3A	4A	5A	6A	7A	8A	9A	10A	11A	1B	2B	3B	4B	5B	6B	7B	8B	9B	10B
Electrical conductivity (µS/cm)	1014	1041	1004	450	434	445	466	701	1007	781	776	73	842	248	1285	865	789	519	1042	742	763
pH	7.4	7.3	7.1	6.9	7.8	7.6	7.7	7.4	8.2	7.1	7.1	6.6	6.5	6.6	6.5	6.8	6.8	7.8	7.5	7.4	7.7
Bulinus africanus				X																	
Bulinus tropicus	X	X	X		X	X			X			X	X	X	X			X	X	X	X
Burnupia mooiensis	X		X	X	X			X		X					X	X	X	X	X	X	X
Ceratophallus natalensis			X		X	X	X		X	X	X										
Ferrissia cawstoni			X			X	X	X			X	X					X	X		X	X
Gyraulus connollyi				X	X			X		X	X	X			X			X		X	X
Gyraulus costulatus					X		X														
Lymnaea columella							X		X			X		X		X					X
Lymnaea natalensis				X	X			X	X	X	X	X		X		X		X			
Lymnaea truncatula						X						X									
Physa acuta	X	X	X			X	X		X	X					X	X			X	X	X
Corbicula fluminalis africana			X		X		X										X			X	X
Pisidium costulosum							X	X				X	X	X	X			X	X	X	X
Pisidium langleyanum				X		X	X	X		X		X	X				X		X	X	
Pisidium viridarium							X	X		X		X	X	X	X	X		X	X	X	
Total number of species	3	2	6	5	7	6	9	7	5	7	4	9	4	5	6	5	4	7	6	9	8

The pH values recorded during this study ranged from 6.5 at Sites 2B and 4B to 8.2 at Site 9A (Table 1) and were generally lower than the values reported by De Kock and Van Eeden (1969) for the Mooi River as such, but were still within the tolerance ranges reported for freshwater molluscs in general (Brown 1994). The EC values were generally much higher than the values reported by De Kock and Van Eeden (1969) for the Mooi River as such. On account of the wide tolerance range of molluscs in general for this parameter, this did not seem to affect the mollusc population negatively, because the highest diversity of nine species found during this study, were recorded for Sites 1B, 7A and 9B where EC values of 73, 466 and 742 µS/cm were measured respectively.

Although the Bovenste Oog (Site 4A) is currently considered as the only site in the Mooi Rivier catchment which is still relatively pristine, the mollusc diversity recorded during the current study compared favourably to that reported from the earlier surveys mentioned above. To conclude, it seems that the ecological integrity of the Mooi River catchment was not compromised to such an extent by the anthropogenic impacts that it had a negative effect on its mollusc diversity.

3. Acknowledgments

I am indebted to the Unit for Environmental Sciences and Management of the Potchefstroom Campus, NWU, Potchefstroom 2520, for financial support and infrastructure and to dr W. Malherbe for compiling the detailed map of the study area.

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P-27

The aquatic macroinvertebrate diversity and selected abiotic factors of the Mooi River catchment area, North-West Province, South Africa

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The Mooi River catchment area has been the sole water supply of Potchefstroom since 1842. This study was undertaken to provide an indication of water quality and ecosystem health of the Mooi River catchment area regarding the macroinvertebrate diversity. One survey was conducted in the low-flow season at six preselected sites. Macroinvertebrate samples were collected at each of these sites. Selected abiotic factors (pH, electrical conductivity (EC) and temperature) were measured *in situ*. The macroinvertebrate samples were sorted, identified, counted and classified as tolerant to highly sensitive. From this study it was evident that a relatively moderate biodiversity was found. Although there are many anthropogenic impacts such as mining and agricultural activities that affect this river, the eyes that feed into this system may possibly dilute these effects and therefore sustain healthy populations of macroinvertebrates.

Keywords: Mooi River, macroinvertebrates, abiotic factors.

1. Introduction

The assessment of biota in rivers is a widely recognized method for determining the condition or health of a river. According to Wolmarans *et al*. (2014), macroinvertebrates are well known to have different sensitivities to organic pollution and habitat transformation. Different taxa exhibit different tolerances towards specific water quality variables such as pH, conductivity temperature etc. and it is thus important that these abiotic factors must be suitable for the survival and reproduction of macroinvertebrates.

Regarding the total macroinvertebrate diversity of the Mooi River catchment, a limited amount of research has been done and therefore the amount of data is insufficient to a large extent. The aim of this study was thus to provide an indication of water quality and ecosystem health in terms of the macroinvertebrate diversity.

2. Materials and Methods

One survey was conducted in the low-flow season at six preselected sites (Table 1). Macroinvertebrates were collected by making use of a standard net over a period of 15 minutes. Marginal and aquatic vegetation were sampled by pushing the net vigorously into the vegetation while the sediment was sampled by shuffling the feet whilst sweeping the net over the disturbed area. The content of the net was transferred to a plastic container and 90% ethanol was added to preserve the samples. Electrical conductivity (EC), pH, temperature, turbidity and flow-rate were measured *in situ* at all sites using portable instruments. Identification was done with a stereomicroscope,

Table 1: Site and habitat description.

Site, River, Coordinates and Altitude	Habitat Description	Landuse
Site 1 - Wonderfontein Spruit Eye S26°19'05.8" E27°23'25.6" 1476m	Stones in streambed, marginal and aquatic vegetation, algae present in water, mud and sand substratum, riffle and run biotopes, headwater zone.	Agricultural activities (cultivated land and livestock), mining. Organic enrichment present.
Site 2 - Bovenste Oog S26°11'53.3" E27°09'53.1" 1472m	Stones in streambed, sandy substratum, marginal, aquatic and overhanging vegetation, pool biotope, headwater zone.	Limited livestock practises.
Site 3 - Mooi River at Muiskraal S26°26'43.1" E27°07'06.4" 1412m	Stones in streambed, marginal, aquatic and overhanging vegetation, sandy substratum, stones in current, riffle and run biotopes present, middle water zone.	Agricultural activities (livestock).
Site 4 - Mooi River above Boskop Dam S26°30'52.3" E27°07'28.5" 1399m	Marginal and overhanging vegetation, muddy substratum, run and pool biotopes, middle water zone.	Above impoundment, no agricultural activities.
Site 5 - Turfontein Eye S26°24'33.8" E27°10'43.3" 1426m	Stones in streambed, limited marginal, aquatic and overhanging vegetation, sandy substratum, stones out of current, pool biotope, headwater zone.	Agricultural activities (livestock).
Site 6 - Gerhard Minnebron S26°28'44.8" E27°09'03.4" 1415m	Stones in streambed, marginal, aquatic and overhanging vegetation, substrate mostly sand and mud, stones out of current, pool biotope, headwater zone.	No agricultural activities.

with the aid of *Guides to the Freshwater Invertebrates of Southern Africa* (De Moore *et al* 2003). Organisms were identified up to species level, where possible, otherwise identification was done up to genus or family level and all specimens were counted and classified. The macroinvertebrates collected were sorted into three groups, based on their sensitivity to organic enrichment, namely highly tolerant (scores 1- 5), moderately sensitive (scores 6-10) and highly sensitive (scores 11-15) families, adapted from the SASS 5 scores. To further analyse the community structure within the Mooi River catchment, a combination of biological indices and

exploratory statistical analyses were conducted.

3. Results and Discussion

Although it is accepted that the Wonderfontein Spruit is highly modified due to mining activities, it is evident that Site 1 showed the highest species richness as well as the highest diversity (H') within the macroinvertebrate community. About 80% of taxa sampled here were classified as highly tolerant (Figure 1) and included the dominant taxa *Alboglossiphonia* sp. and *Tubifex* sp. This large number of organisms may possibly be attributed to the sulphates resulting from mining activities, which in turn may contribute to organic enrichment. This observation is further supported by the elevated (EC) at this site.

Site 2, the Bovenste Oog, is one of the main eyes that feed into the Mooi River and is often referred to as a pristine habitat. Site 2 had the second highest species richness, as well as a high species diversity (H'). The phenomena above may also be the result of the availability of suitable biotopes and aquatic vegetation.

Along the river banks, particularly at Site 3, numerous agricultural activities, such as livestock practises, occur and may possibly contribute to the organic enrichment. However, it was still possible for a highly sensitive taxon, Schoenobiinae, to occur here.

Site 4 showed the second highest species richness and included the dominant taxa, *Acanthiops* sp. The most moderately sensitive (40%) and highly sensitive (4%) taxa, which included the subfamily Nymphulinae, were collected here. Although the highly enriched Wonderfontein Spruit enters the Mooi River at this site, these effects may to a great extent be minimized on account of the rejuvenating effect from water of the Gerhard Minnebron.

Site 5, the Turfontein Eye, showed the lowest species richness which can probably be ascribed to the limited organic enrichment and also the lack of aquatic vegetation, as well as suitable biotopes for the macroinvertebrates. The presence of the alien invasive species, *Physa acuta*, was possibly due to its association with the limited organic enrichment. From Table 2 it is evident that Site 5 had the highest Pielou's evenness value, indicating an even distribution and thus the lack of a dominant species.

From Table 2 it is further clear that Site 6 had the lowest diversity (H') within the macroinvertebrate community, as well as the lowest Pielou's evenness, which indicated an uneven distribution. The low evenness of the Site 6 is due to the large number of *Caridina nilotica* collected, a species known for its low of sensitivity. Nymphulinae, a highly sensitive subfamily, was also collected at this site. The Gerhard Minnebron is also one of the main water sources of the Mooi River, with naturally low organic enrichment and thus limited food sources for the macroinvertebrates. The low diversity, Pielou's evenness and the occurrence of a highly sensitive subfamily, can possibly be ascribed to the limited organic enrichment at this site.

Statistical associations (CCA not shown) between turbidity, temperature and the macroinvertebrates were found.

From this study it was evident that a relatively moderate biodiversity was found. Although there are many anthropogenic impacts such as mining and agricultural activities that may affect this river system, the eyes that feed into this system may possibly dilute or nullify these effects to a great extent.

Site	1	2	3	4	5	6
Number of taxa (SR)	34	27	25	27	19	23
Sensitive taxa	6	7	9	11	4	9
H'	2.504	2.228	1.899	1.787	2.375	1.401
J'	0.710	0.676	0.590	0.542	0.807	0.447
pH	7.53	6.75	7.80	7.44	7.18	7.12
Temperature (°C)	13.9	19.2	10.2	11.3	19.8	19.7
EC (µS/cm)	1001	457	456	697	798	785
Turbidity	69	100	98	99	99	100
Flow-rate	0.1	0.1	0.8	1.1	0.1	0.1

Table 2: The species richness (SR), abiotic factors, Shannon–Wiener index (H') and Pielou's evenness (J') for each of the selected sites.

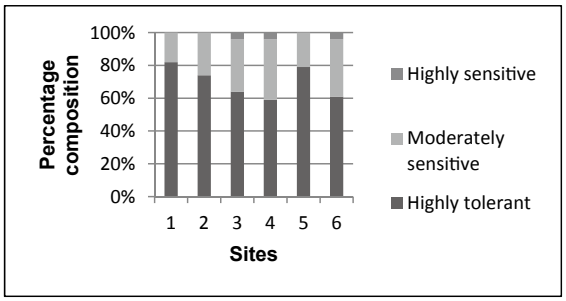

Figure 1: The percentage of the sensitivity composition of all species during the study.

4. Acknowledgments

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

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P-28

Effects of perfluorooctanesulfonic acid (PFOS) on the embryonic development of the freshwater mollusc *Bulinus tropicus*

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This study was done to determine the chronic effect of the persistent organic pollutant (POP) PFOS on the freshwater mollusc *Bulinus tropicus* at concentrations of 0, 0.001, 0.01, 0.1, 1, 10 and 100 µg/ml. The endpoints were embryonic growth and hatching. Embryonic growth was retarded at low concentrations, possibly due to endocrine disruption, and there were indications of toxicity at higher concentrations.

Keywords: Perfluorinated compounds, toxicity, persistent organic pollutants

1. Introduction

Persistent Organic Pollutants (POPs) are compounds that persist in the environment, breaks down slowly, are toxic, can travel long distances via water and air, and can bio-accumulate in the food web (Pozo *et al.*, 2006; Viljoen, 2010). Perfluorooctane sulfonic acid (PFOS) and its salts were added to Annex B of the Stockholm Convention in 2009 (Dai *et al.*, 2013; Wang *et al.*, 2010). Both direct (manufacture and use) and indirect sources for the emission of PFCs to the environment exist (Ding & Peijnenburg, 2013), and they are widely distributed in the environment (La Farre' *et al.*, 2008). PFOS is easily transported in aquatic environments and is highly water soluble compared to other POPs (Dai *et al.*, 2013).

PFOS has been found in the tissues of birds, fish and marine mammals, indicating bioaccumulation in aquatic biota (Shi *et al.*, 2011). There is a considerable ongoing effort to better understand the toxicity, potential exposure, and risks associated with PFOS in water, as it can spread through surface and ground water from contaminated sites (Funkhouser, 2014), and is found mainly in the water phase (Ding & Peijnenburg, 2013).

Molluscs are used as sentinel organisms due to their sedentary mode of life and their feeding behaviour that makes them susceptible to pollutant bioaccumulation. Molluscs have also been recommended as test organisms for endocrine disrupting chemicals (Gagnaire *et al.,* 2008). Molluscs have relatively high bioaccumulation factors due to their limited ability to metabolise exogenous organic chemicals and to eliminate pollutants through excretion (Hudson, 2011). The genus *Bulinus* can survive in stagnant waters, can tolerate a wide temperature range, and can breed and grow rapidly (Viljoen, 2010). We measured the effects of PFOS on the embryonic development of *B. tropicus*.

2. Materials and Methods

2.1 Culture conditions and exposure

Bulinus tropicus were from shallow water near Potchefstroom and have been reared for several generations. The light in the laboratory was on a 12-hour cycle and the temperature kept at 26°C (Roos, 2012). Filtered air through glass pipettes was used to constantly aerate the egg packets and the snails fed with Tetra Pro fish food. For this test, 800 ml polypropylene (PP) jars was used.

Five adult snails (*Bulinus tropicus*) were placed in each jar to lay egg packets. Each jar was monitored until there were two big egg packets laid on the same day, when the adult snails were removed and the water spiked. Six concentrations of PFOS were used (0, 0.001, 0.01, 0.1, 1, 10 and 100 µg/ml). Photos were taken every day with a Pro Scope microscope to measure embryonic development.

3. Results and Discussion

No embryos failed to develop at all concentrations. The growth of the embryos exposed to 0.001 and 0.01 ug/ml PFOS were significantly retarded at day 6 compared to the control and the concentrations higher than 1 ug/ml (ANOVA, $p<0.05$; Figure 1).

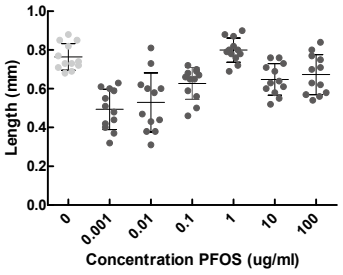

Figure 1: Length of embryos on day 6.

Embryos exposed to 0.001 ug/ml PFOs took significantly longer to hatch than exposures at 0 and 0.01 ug/ml (ANOVA, $p<0.005$). From 0.01 to 100 ug/l, there was a gradual increase in time to hatching

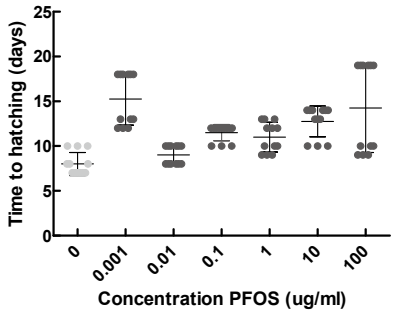

Figure 2. Age of the embryos at hatching

Embryos at the lowest exposure (0.001 ug/ml) therefore grew slower (when measured on Day 6; Figure 1) and took longer to hatch (Figure 2) than the other exposures. The slower growth but longer time to hatching resulted in significantly larger embryos when they eventually hatched (Figure 3; ANOVA, $p<0.05$) when compared with the control and 0.01 ug/ml exposures, but not the others.

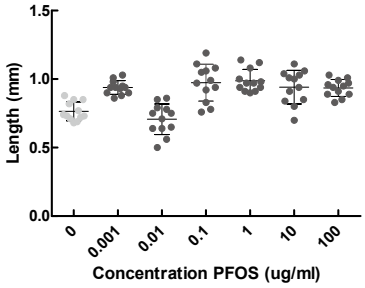

Figure 3. Length of the embryos at hatching

It seems therefore that at low concentrations there were effects different from effects at higher concentrations. At PFOS concentrations between 0 and 0.01 ug/ml, a U-shape effect on growth resulted in an inverse U-shape for time to hatch and length at hatching. U-shaped and inverted U-shaped effects are known from literature and are generally ascribed to endocrine disruptive effects (Jobling et al., 2004; Davis et al., 1992; Calabrese et al., 2001). At higher concentrations, there were indications of toxicity. [The exposures were continued with the hatchlings, all of whom died at 100 ug/ml/.] The wide range of concentrations we employed was able to capture both hermetic effects at low exposures and toxicity at higher exposures.

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P-29

The aquatic macroinvertebrate diversity and physical parameters in the Loop Spruit and Mooi River, North-West Province

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The Loop Spruit is subjected to various negative impacts which include industrial and mining activities, agricultural return flows, as well as treated and untreated sewage. The above mentioned pollutions sources do not only pose a threat to the Loop Spruit but can affect the Mooi, as well as the Vaal River. This study was undertaken to determine the effects of these negative impacts on the aquatic macroinvertebrate diversity in the Loop Spruit. One survey was conducted at six preselected sites during the low-flow season, where macroinvertebrates and water samples were collected at each site. The physical parameters of the water were measured *in situ*, while the macroinvertebrates were identified in the laboratory, up to genus level, where possible. The total number of taxa per site ranged from 24 to 35, which were dominated by highly tolerant taxa. To conclude, mining, urban and agricultural activities are possibly responsible for negative impacts on the health and habitat integrity of this river system.

Keywords: Pollution sources, macroinvertebrates, physical parameters

1. Introduction

The Loop Spruit originates from various springs eight kilometres north-east of Fochville and flows through the town and its informal settlements. The Loop Spruit supplies water to various impoundments before confluence with the Mooi River south of Potchefstroom. The Mooi River flows into the Vaal River 20 km further south-west of Potchefstroom.

According to the Department of Water Affairs and Forestry (DWAF) the habitat integrity of the Loop Spruit is generally in a moderately to largely modified state. This is due to major negative impacts like mining and urban activities, as well as treated and untreated sewage discharged in the upper reaches. In the middle reaches of the river, farming activities, as well as water abstraction for irrigation and the removal of riparian vegetation further reduce the habitat integrity (DWAF, 2007). Klipdrift Dam is situated in the middle reaches, which significantly alters the river flow and degrades the habitat integrity to an unacceptable level (DWAF, 2007). The habitat integrity at the lower reaches, near Potchefstroom, is extensively modified due to urban activities and treated sewage discharged into the river. All of these impacts not only pose a threat to the Loop Spruit, but can affect the Mooi-, as well as the Vaal River.

Biota assessment in rivers is an international recognised method for determining the health of rivers, with aquatic macroinvertebrates in particular, as valuable bio-indicators due to their different sensitivities to pollution and habitat alteration. According to Batty (2010), excessive pollution not only reduces the overall species diversity, but also changes community structure. Different taxa also exhibit different tolerances to specific water quality variables (Thirion, 2006), while water of suitable quality is essential to sustain healthy populations of aquatic organisms.

In this study it was decided to do a semi-quantitative survey of these organisms to provide an indication of water quality and ecosystem health at six preselected sampling sites in the Loop Spruit and its tributaries.

2. Materials and Methods

2.1 Field Survey

Table 1: Site description.

Site, River, Coordinates and Altitude	Habitat Description	Land use (State of the Rivers Report)
Site E1 Loop Spruit Eye S 26°25'49.3'' E 27°33'09.4'' 1560m	Sandy/ muddy substratum, marginal and aquatic vegetation with algae in the water, run biotope, headwater zone.	Livestock and grassland.
Site E2 Loop Spruit S 26°25'54.5'' E 27°33'08.7'' 1556m	Sandy streambed, marginal and aquatic vegetation with algae in the water, run biotope, headwater zone.	Mining effluent, livestock and grassland.
Site E3 Mponeng Mine effluent S 26°28'41.7'' E 27°25'44.4'' 1460m	Sandy substratum, overhanging tree canopy, marginal and aquatic vegetation, sand, run and pool biotopes, middle water zone.	Mining effluent, livestock and grassland.
Site E4 EnselSpruit S 26°37'08.3'' E 27°22'16.2'' 1389m	Sandy/muddy substratum, overhanging tree canopy, marginal vegetation, algae in water, pool biotope, lower water zone.	Cultivated land, livestock and grassland.
Site E5 Loop Spruit below Klipdrift Dam S 26°37'07.1'' E 27°17'49.9'' 1366m	Muddy substratum, marginal vegetation and run biotope, middle water zone.	Cultivated land, livestock and grassland, below impoundment.
Site E6 Mooi River (Suidbrug) S 26°45'08.6'' E 27°06'01.2'' 1324m	Clay substratum, overhanging tree canopy with marginal vegetation and algae in water, run and pool biotopes, lower water zone.	Urban, industrial, livestock.

The materials and methods are based on the methodology in Wolmarans *et al*. (2014). One low-flow, semi-quantitative survey was conducted at the six preselected sites, where macroinvertebrates have been collected by sampling the marginal and aquatic vegetation, as well as the substratum, using a Perlon® gauze net, for approximately 15 minutes.

Contents of the net were filtered with habitat

water and most of the debris/ course material was carefully removes by hand. Thereafter the content of the net was transferred to a plastic container with 70% ethanol. The physical parameters were measured *in situ* at each site, using portable instruments.

2.2 Identification

All macroinvertabrates were identified by using a stereomicroscope and organisms were identified up to genus level, where possible, otherwise identification was done up to family level with the aid of: *The guides to the freshwater invertebrates of Southern Africa*. Subsequently all specimens were counted and classified.

The South African Scoring System (SASS5) scores were adapted for this study to classify the taxa into three groups, namely tolerant (scores 1-5), moderately sensitive (scores 6-10) and highly sensitive (scores 11-15) taxa. Taxa collected during this study not included in the SASS5 scores, was allocated a neutral sensitivity score of six.

3. Results and Discussion

In total 79 taxa were collected during this study, where Site 1 had the lowest species richness and Site 2 the highest species richness.

Sites	Total Number of Taxa	Sensitive Taxa	Temp	EC	pH	Turbidity	Flow Rate
E1	24	7	9.2	65	7.20	63	0.2
E2	35	9	12.3	901	6.66	98	1.2
E3	26	8	11.7	1207	6.82	94	0.3
E4	33	13	10.7	480	8.34	23	0.1
E5	32	9	7.3	781	7.43	78	0.3
E6	29	7	12.3	695	7.73	80	0.3

Table 2: Number and sensitive taxa, with physical parameters at each site.

The macroinvertebrates classified into three groups showed that tolerant taxa dominated the percentage composition at each site. Site 1 and 4 were the only sites with highly sensitive taxa present and can be due to the fact that Site 1 is the eye and Site 4, is the Ensel Spruit, which is subjected to few negative impacts. These families include Perlidae and Crambidae at Sites 1 and 4 respectively.

A Canonical Correlation Analysis (CCA) was compiled (not shown) to indicate the possible association between the physical parameters and the taxa. The results revealed that only flow rate had a significant influence with a p value of 0.01. Simuliidae, Hydropsychidae and Hydroptilidae are some of the families that associated with a high flow rate.

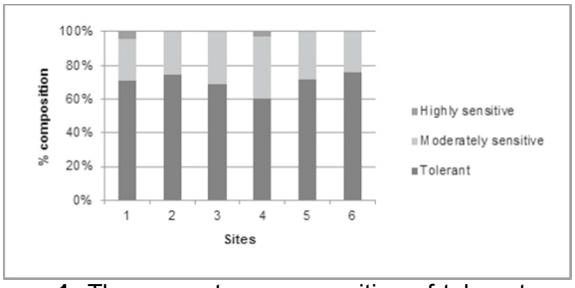

Figure 1: The percentage composition of tolerant, moderately sensitive and highly sensitive taxa at each site.

Of the 95 macroinvertebrate families found in South Africa, only 49 were recorded during this study, while only two of the 11 highly sensitive families were found. The lack of highly sensitive families can be ascribed to the high organic enrichment in the Loop Spruit catchment area and although a large number of species were collected in this study, the majority are categorised as tolerant towards organic enrichment.

To conclude, mining, urban and agricultural activities possibly had a negative impact on the health and habitat integrity of this river system.

4. Acknowledgments

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

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P-30

Antioxidant enzymes in *Oreochromis niloticus* as early warning signals in assessing pollution from Acid Mine Drainage and diffuse sources

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This study investigated the use *Oreochromis niloticus* in active biomonitoring of pollutants of two rivers of Zimbabwe. Fish samples were exposed effluent contaminated sections of the rivers for six weeks in 2011. Expression of antioxidant enzymes, catalase (CAT) and glutathione S- transferase (GST) from liver, gills, and muscle tissues of treatment and control (fish from unpolluted conditions) was measured at four and six weeks and compared. Expression of CAT and GST at both time points was significantly higher ($p<0.05$) in the liver than gills and muscles of test samples. Expression was also significantly higher at six weeks compared to four weeks. Using GST and CAT we concluded that *O. niloticus* can be used as bioindicator and GST and CAT can be successfully used as biomarkers in assessing pollution from point sources such as acid mine drainage and diffuse sources such as commercial agriculture.

Keywords: Acid mine drainage, active biomonitoring, biomarkers, fish, pollutants

1. Introduction

Acid mine drainage is the microbially mediated generation of acid from sulphide mine wastes and has been identified as the largest environmental liability facing the mining industry and the world today (Price, 2005). Problems related to mining waste may be rated second to global warming and ozone depletion in terms of ecological risk as they can result in profound and generally irreversible destruction of ecosystems (USEPA, 1987). Acid mine drainage poses a threat to native aquatic life (William and Smith, 2000) through elevated acidity and the accumulation of metals, metalloids and sulphate and increased mobility and bioavalability of heavy metals.

Biomarkers provide early warning of potential higher level effects that may not be obtainable through chemical analyses or other methods of investigation. They give information on biological effects of pollutants rather than a mere quantification of their environmental levels (Wepener *et al*, 2005). Managing diffuse sources of pollution of the Zambezi tributaries must be a strategic environmental concern for Zimbabwe. Studies done on water quality monitoring of the Yellow Jacket and Mazowe Rivers have concentrated on the chemical characterization of pollutants and only a few have looked at the effects of acid mine drainage on benthic macroinvertabrates. There is no study that has employed active biomonitoring in the two Rivers and in most of Zimbabwe's Rivers. The purpose of this study was to evaluate the usefulness of GST and CAT in the liver, gills and muscle tissue in *O. niloticus* as biomarkers.

2. Materials and Methods

Ethical approval for this study was provided by the University Of Johannesburg Faculty Of Science Ethics Committee. Water physicochemical parameters were measured in situ on deployment and sampling. *O. niloticus* were given two weeks to acclimatise before sampling at four and six weeks. Ten fish per cage were deployed in duplicate at selected sites. The fish were sacrificed on site and dissected for the liver, gills and muscle tissues. CAT activity was determined following the method of Cohen et al. (1970). For quantitative determination of CAT activity, Phosphate buffer (pH 7.0), 6mM H_2O_2, 6N H_2SO_4 and $kMnO_4$ were mixed and the rate of H_2O_2 consumption at 490nm was measured. GST Activity was determined by the method of Habig et al. (1974). GST activity was measured by following the change in absorbance at 340 nm of the substrate conjugated with reduced glutathione. The enzyme activities were measured as specific activities expressed in units of activity per mg of protein. The Bradford method (Bradford, 1976) was used to determine protein concentration in the samples.

3. Results and Discussion

The observed increased GST activity in the liver of *Oreochromis niloticus* at all sites compared to the control site shows its usefulness as a biomarker of exposure. This is in agreement with other studies (Farombi *et al.*, 2007) where GST expression in fish liver as a biomarker of exposure to xenobitics has been discussed. High GST activity at sites 4, 5 and 6 after both 4 weeks and 6 weeks could be a result of pollution of these sites by agrochemicals from the surrounding farming community and illegal gold mining activities. A combination of agrochemicals and metals are known to modulate antioxidant defence systems, which can cause oxidative stress

in aquatic organisms (Conners, 2002). The findings of this study supports the observations of Gadagbui and James (2000) that GST activity in fish, appears to be the most sensitive and most widely used catalytic probe to monitor pollution of aquatic ecosystems.

The observed higher CAT activity in the liver, followed by the gills is in agreement with Velkova *et al*., (2008) who observed that CAT activity varies greatly in tissues and is highest in the liver, kidney and lowest in connective tissue. The liver is genetically programmed to be the metabolic regulator with highly oxidative tissue and may concentrate xenobiotics. Increased CAT activity is usually observed in the face of environmental xenobiotics since it is part of the SOD-CAT system which is the first line of defence against oxidative stress. CAT activity was also shown to be elevated in mussels exposed to the insecticide cypermethrine and in freshwater snails *Lymnaea natalensis* exposed to polluted dam water (Kenan *et al*., 2010).

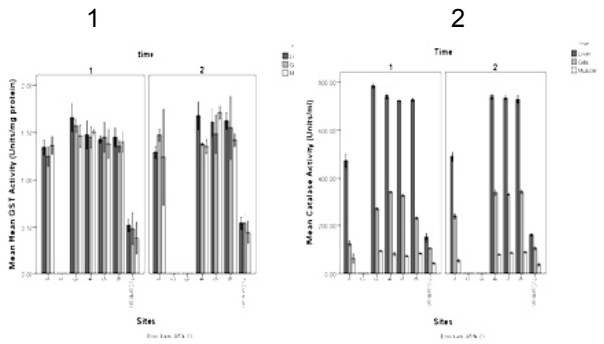

Figure 1: A comparison of GST (1) and CAT (2) expressions at four and six weeks in the different tissues.

4. Acknowledgments

This study was supported in part by the Zimbabwe Ministry of Higher and Tertiary Education, Science and Technology Development, University of Johannesburg and the Organisation for Women in Science for Developing countries (OWSD).

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P-31

Health Implications of Metals Assessed in Frequently Consumed Canned Sardines and Corned Beefs in Benin City Metropolis.

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This study investigated the levels of heavy metals (Fe, Zn, Mn, Cd, Pb and As) in canned sardines and corned beefs commonly consumed in Benin City metropolis, Nigeria. Fifty samples were randomly purchased, prepared and analysed using an AAS and the results revealed that As was not detected while other metals showed a trend of Fe > Zn > Mn > Cd > Pb in both categories. Cd exceeded European Union set standard. The health risk assessment of the investigation revealed that Estimated Daily Intake values were within Joint FAO/WHO Expert Committee on Food Additives Provisional Tolerable Daily Intake (PTDI). Similarly, the results of the Dietary Exposure (DE) were within recommended daily requirement except DE of Fe in canned corned beef, which had values above recommended limits. Target Hazard Quotient (THQ) values were <1 and Hazard Index (HI) <1 in canned sadine and >1 in canned corned beef, indicating potential health risk. It is therefore imperative for regular monitoring of canned foods sold within the metropolis and Nigeria in general.

Keywords: Heavy metals, canned fish, canned meat, Benin City, Health implication.

1. Introduction

Changing global patterns of life style, food production, international trade, technology, public expectations for health protection and many other factors have created an increasingly demanding environment in which food safety systems operate. Ensuring food safety to protect public health and promote economic development remains a significant challenge in both developing and developed countries. However, unacceptable rates of foodborne illness still remain and new hazards continue to enter the food supply (FAO/ WHO, 2006). The ingestion of food is an obvious means of exposure to metals, not only because many metals are natural components of food stuffs but also because of environmental contamination and contamination during processing. In order to ensure that the maximum permissible limits are not exceeded, routine surveillance of levels of these toxic metals in food is a necessity hence the essence of this study.

2. Materials and Methods

2.1 Sample collection and preparation

A total of fifty samples of canned sardines and corned beef of different brands were purchased, homogenized and digested using Wet digestion method. Digested samples were prepared with standard procedures and Heavy Metals content was read using AAS (Radojevic, and Bashkin, 1999).

2.2 Health risk assessment:

The average Estimated Daily Intake (EDI*)*

EDI was calculated using the following equation, US EPA (1997).

$$EDI = \frac{C \times IR \times EF \times ED}{BW \times AT}$$

Target Hazard Quotient, (USEPA 2007).

$$THQ = \frac{EDI}{RfD}$$

THQ <1, means low risk of non-carcinogenic toxic effects. THQ > 1 means potential health risks associated with overexposure. HI is the sum of more than one THQ for multiple substances HI=

$$\sum THQ_1 + THQ_2 + THQ_n \ldots\ldots\ldots\ldots$$

Dietary Exposure (DE) =∑ (FCC X FCR) (USEPA, 2007).

3. Results and Discussion

Table 1: Summary of data from the analysis of canned fish

	CANNED SARDINES							
PARAMETERS mg/kg	Product Type A mean±SE	Product Type B mean±SE	Product Type C mean±SE	Product Type D mean±SE	Product Type E mean±SE	P-Value	STANDARD	Ref.
Fe	*228.2±17.93	161.78±16.44	245.7±3.09	170.4±27.34	216.1±9.42	P<0.01	15µ/g	Tuzen & Sylak, 2007b
Mn	1.97±0.80	1.44±0.34	2.03±0.54	2.02±0.53	2.66±0.38	P>0.05		
Zn	48.14±9.45	*60.2±12.61	39.8±11.29	15.72±3.49	34.36±7.92	P<0.05		
Cd	0.18±0.05	0.084±0.02	0.06±0.06	0.13±0.08	0.13±0.08	P>0.05	0.1 µg/g	EU 2008 EU 2006
Pb	*0.17±0.08	0.09±0.04	0	0	0	P<0.05	0.30 µg/g	

Na, Fe, Mn, Zn standards are available as Recommended Maximum daily intake
P<0.05, significant, P<0.001, Highly significant, P>0.05, no significance
ND- Below detected limit

Canned sardines recorded mean Fe level which ranged from 161.78±16.44 to 245.7±3.09mg/kg. Fe was highest in canned corned beef canned sardine.

Table 2: Summary of data from the analysis of canned corned beef

CANNED CORNED BEEF								
PARAMETERS mg/kg	Product Type A mean±SE	Product Type B mean±SE	Product Type C mean±SE	Product Type D mean±SE	Product Type E mean±SE	P-Value	STANDARD	Ref
Fe	*270.28±22.99	259.24±10.23	303.08±11.57	205.28±8.13	220.72±15.32	P<0.001	15µ/g	Tuzen & Sylak, 2007b
Mn	2.46±0.43	3.27±.36	2.73±0.48	4.02±0.41	*4.64±0.47	P<0.01		
Zn	36.4±6.18	36.12±11.51	46.46±8.29	68.94±17.09	53.24±14.12	P>0.05		
Cd	0.19±0.08	0.03±0.03	0.08±0.08	0	*0.22±0.07	P<0.05	0.05 µg/g	EU 2006
Pb	0	0	0	0.08±0.08	*0.16±0.04	P<0.05	0.10mg/kg	EU 2008

Na, Fe, Mn, Zn standards are available as Recommended Maximum daily intake
P<0.05, significant, P<0.001, Highly significant, P>0.05, no significance
ND- Below detected limit

Table 3: Comparing EDI with recommended intake values from various standards

Canned food	Parameter	EDI (mg/kg bw/day) Range	Provisional Tolerable Daily Intake (mg/kg bw/day	Reference
Sardine	Fe	0.099 - 0.151	0.8	JECFA 1983
	Mn	0.001 - 0.002	0.067	FSIS 2004
	Zn	0.01 - 0.0037	1	JECFA 1982
	Cd	0- BDL	0.001	JECFA 2013
	Pb	0- BDL	0.00357	JECFA 1993
Corned beef	Fe	0.469 - 0.693	0.8	JECFA 1983
	Mn	0.006 - 0.011	0.067	FSIS 2004
	Zn	0.083 - 0.158	1	JECFA 1982
	Cd	0 - 0.001	0.001	JECFA 2013
	Pb	0- BDL	0.00357	JECFA 1993

Table 4: Comparing DE with recommended intake values from various standards

Canned food	Parameter	DE (mg/day)	Recommended Daily Requirement (mg/day)	Reference
Sardine	Fe	6.957-10.564	10-15	NCM 1995
	Mn	0.062 - 0.114	2-9	WHO 1981
	Zn	0.676 - 2.589	15-22	JEFCA 1982
	Cd	0 - 0.008	-	-
	Pb	0 - 0.007	0.075	(US FDA, 1993)
Corned beef	Fe	32.845 - 48.493	10-15	NCM 1995
	Mn	0.394 - 0.742	2-9	WHO 1981
	Zn	5.779 - 11.030	15-22	JEFCA 1982
	Cd	0 - 0.035	-	-
	Pb	0 - 0.026	0.075	(US FDA, 1993)

The Fe concentration in this study was far higher than that reported by Zarei *et al.,* (2010). Mn concentration across the canned foods sampled where quite low and there is no known standard for this metal. Zn in this study was higher than the findings of Dallatu *et al.,* (2013). Cd exceeded EU standards in all canned categories and this draws a cause of concern. This finding corroborates with that of Dallatu *et al.,* (2013) whose values were higher than WHO standards. Certain brands had Pb concentrations way above EU standards. EDI were within JECFA PTDI. Dietary Exposure (DE) was within the recommended daily requirement except for Fe in canned corned beef. The THQ values were < 1 suggesting that there is no risk of a non-carcinogenic toxic effect. HI ranged from 0.27 – 1.810 in the food categories.

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P-32

Assessment of heavy metal levels and petroleum hydrocarbons in *Pomadasys peroteti* (Cuvier, 1830) of Benin River in relation to human health.

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Assessment of heavy metals and total petroleum hydrocarbon levels in fish (*Pomadasys peroteti*) from Benin River, Southern Nigeria and human health risk through consumption was studied. Metal concentrations were analysed in fish using Atomic Absorption Spectrophotometer (PG550) while TPH concentrations were analysed using GC-MS, 6890 series model G1530 A, with flame ionization detector (FID). Metal concentrations showed the trend: liver > kidney > muscle. The highest concentrations of heavy metals occurred in the liver. This could be due to the physiological role played by this organ in the detoxification of xenobiotics. TPH showed mean values of 21.53±2.14, 8.93±0.89, and 18.90±1.88 in the liver, muscles and kidney respectively. TPH concentrations also showed the trend: liver > kidney > muscle. The total THQ (TTHQ) was 0.0938 indicating no health risk with respect to heavy metal risk. Levels of TPH in the studied fish species were higher than EU recommended benchmark of 2µg/kg wet weight for fish, thus suggesting that the consumption of *P. peroteti* is likely to cause adverse health effects to consumers with respect to petroleum hydrocarbons. Regular monitoring of Nigerian waters is necessary in order to ascertain the health risk of contaminated fauna to humans who consume them.

Keywords: Heavy metal, TPH, Fish, Risk, Nigeria.

1. Introduction

In recent decades, food safety has become a widespread public concern worldwide due to the increasing demand for food. Fish are known to be living organisms most sensitive to trace concentrations of toxicants in the aquatic habitats (Nadir, 2011. Heavy metals accumulate in the tissues of aquatic animals and may become toxic when accumulation reaches a substantially high level. Both aliphatic and polycyclic aromatic hydrocarbon fractions of dissolved petroleum are readily absorbed by finfish and shellfish because of their high lipid solubility and are bioconcentrated in them. The accumulation of aliphatic hydrocarbons and PAHs in fish can negatively affect the health and productivity of fish and pose health risk to human populations who consume them.

Benin River is located in the coastal belt of Southern Nigeria at the Western boundary of the upper Delta and the lowlands. Enuneku *et al*., 2014 reported that shrimps and crabs from Benin River were contaminated by heavy metals from anthropogenic activities of oil and gas industries around the river. This study was conducted to determine the levels of heavy metals and TPH in *P. peroteti* in Benin River. This fish species is of high commercial importance in Southern Nigeria.

2. Materials and Methods

2.1 Study area

The study area, Benin River (Koko Town) is situated in the North Central part of Delta State, Nigeria between latitudes and longitude (N05^{0}54'14.9'', E005^{0}41'50.7'' – N05^{0}59'54.9'', E005^{0}27'006'') respectively. Anthropogenic activities around this zone of the river include loading and offloading of petroleum products, oil and gas industries and watercraft maintenance workshops.

2.2 Fish Collection Sample Preparation and metal analysis.

The collection of fish samples from the river was done over a period of three months. Fish were caught with gill nets, and local. Fish samples were preserved in ice and taken to the laboratory. They were kept frozen in the refrigerator pending heavy metals analysis. Sample digestion was according to Raghuramulu *et al*. 2003. Samples were analysed for heavy metals using AAS (PG 550) after caliberation.

2.3 Gas Chromatography Analysis

Each extract transferred to 1.5ml vial was loaded into a gas chromatography system 6890 series model G1530 A, with flame ionization detector (FID), and cold on-column injection.

2.4 Target Hazard Quotient

THQ was determined according to US EPA 1989. THQ was determined by the following equation:

$$THQ = \frac{EFr \ x \ ED_{tot} \ x \ FIR \ x \ C}{RfDo \ x \ BWa \ x \ ATn} \ x \ 10^{-3}$$

Where; EFr is exposure frequency (365 days/ year); EDtot is the exposure duration 52 years, average lifetime); FIR is the food ingestion rate (9kg/day); C is the heavy metal concentration in *P. peroteti* (mg/kg); RfDo is the oral reference dose (mg/kg/day). BWa is the average adult body weight (70 kg), and ATn is the averaging exposure time for non-carcinogens (365 days/year × number of exposure years assuming 52 years).

Total THQ in this study is treated as the arithmetic sum of the individual metal THQ values.

3. Results and Discussion

Fig. 1 shows the concentration of the six heavy metals in *P. peroteti*.

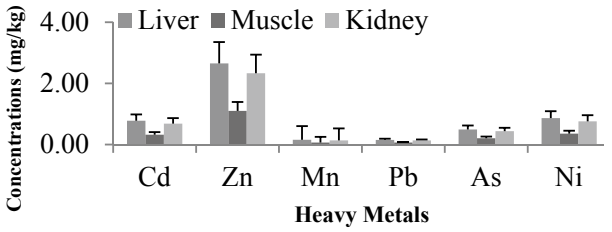

Figure 1: Heavy metal concentrations in *P. peroteti*

The highest concentration of all heavy metals occurred in the liver while the lowest concentrations occurred in the muscle. This could be due to the physiological role played by this organ in the detoxification of xenobiotics.

The oral reference doses for the heavy metals and the target hazard quotients (THQs) of studied metals through the consumption of *P peroteti* for residents are shown in table 2. The THQ values for *P. peroteti* was in the order As>Cd>Ni>Pb Zn>Mn. The total THQ (TTHQ) was 0.0938. TTHQ in the studied fish was less than 1, suggesting that the consumption of *P. peroteti* is unlikely to cause any adverse health effects to consumers.

Table 2: THQs of the studied heavy metals in *P. peroteti.*

Element	RfDo	THQ
Cd	0.001	0.0411
Pb	0.0036	0.0021
As	0.0003	0.0476
Ni	0.02	0.0023
Mn	0.14	0.0002
Zn	0.3	0.0005
	TTHQ	0.0938

Table 3 shows the mean concentration of TAH, PAH and TPH in the liver, muscle and kidney of *P. peroteti*.. The liver had the highest mean concentration of TPH (21.53mg/kg) while the muscle had the lowest (8.93mg/kg). The levels of TPH in sampled fish species were higher than EU recommended benchmark of 2µg/kg wet weight for fish. This indicates health risk to consumers with respect to petroleum hydrocarbons.

Table 3: Mean concentrations of TAH, PAH and TPH concentrations (mg/kg) in organs of *P. peroteti.*

Petroleum Hydrocarbons (mg/kg)	Liver $\overline{X}$±SD (Min- Max)	Muscle $\overline{X}$±SD (Min- Max)	Kidney $\overline{X}$±SD (Min- Max)	p-Value	EU Standard
Total Aliphatic Hydrocarbon (mg/kg)	18.12 [a] ±2.86 (16.00-22.07)	7.51 [b] ±1.18 (6.64-9.15)	15.91 [a] ±2.51 (14.05-19.38)	p<0.05	2µg/kg
Aromatic Hydrocarbon (mg/kg)	4.77 [a] ±3.77 (0.81-10.98)	1.98 [b] ±1.56 (0.34-4.55)	4.19 [a] ±3.31 (0.72-9.64)	p>0.05	2µg/kg
Total Petroleum Hydrocarbon (mg/kg)	21.53 [a] ±2.14 (18.15-24.29)	8.93 [c] ±0.89 (7.52-10.07)	18.90 [b] ±1.88 (15.93-21.33)	p<0.05	2µg/kg

p<0.05 – Significant Difference. Similar Superscript Row-wise – No Significant Difference

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P-33

Heavy metal concentrations in surface water and bioaccumulation in fish (brycinus longipinnis) and shrimp (macrobrachium macrobrachium) from Koko river, Koko, Delta state.

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The distribution of heavy metals (Mn, Fe, Cu, Cd, Ni, Pb, Co and Zn) levels in surface water, fish (*Brycinus longipinnis*) and shrimp (*Macrobrachium macrobrachium*) in Benin River was studied to ascertain the residual concentrations and compare with recommended standards. Mean heavy metal concentration across the matrices ranged from 0.010mg/l – 0.202mg/l (Water), 1.107mg/kg – 92.848mg/kg (shrimp), 1.685mg/kg – 53.896mg/kg (fish). Co had the highest concentration in water (0.202mg/l), Fe had the highest concentration in shrimp (92.848mg/kg) while Zn had the highest concentration in fish (53.896mg/kg). Heavy metals concentration in shrimp was higher than in fish. The estimated levels of Cd,Ni,Pb in water was higher than WHO maximum limits. Mn, Cu, Ni, Pb, Co and Zn in fish were above WHO limits. Mn, Ni, Pb and Co also had values above WHO limits and shrimp were higher than the maximum limit set WHO and FEPA. Regular monitoring of these and other heavy metals in the water body to ensure continuous safety of people in the area is recommended.

Keywords: Heavy metals, bioaccumulation, fish, shrimp.

1. Introduction

Heavy metal contamination of aquatic the 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 concentration of Mn, Fe, Cu, Cd, Ni, Pb, Co and Zn in surface water, fish tissue and shrimp in Benin River, Koko, Delta State, Nigeria.

2. Materials and Methods

2.1 Study Area

Koko 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

The result of heavy metal concentrations in the various matrices are shown below.

Table 1: Heavy metal Concentrations (mg/l) in surface water from Koko River.

Stations	Abialegbe	Ebenco/Optima	Total	WHO LIMITS
Heavy Metals	$\bar{x}$±SE (Min-Max)	$\bar{x}$±SE (Min-Max)	$\bar{x}$±SE (Min-Max)	
Mn	0.057±0.022 (0.027-0.121)	0.060±0.025 (0.029-0.133)	0.066±0.032 (0.026-0.162)	0.5
Fe	0.183±0.053 (0.090-0.290)	0.144±0.051 (0.025-0.255)	0.137±0.040 (0.050-0.241)	0.3
Cu	0.033±0.017 (0.007-0.081)	0.025±0.012 (0.006-0.056)	0.029±0.014 (0.002-0.054)	2.25
Cd	0.005±0.003 (0.000-0.011)	0.008±0.004 (0.000-0.019)	0.009±0.005 (0.000-0.022)	0.01
Ni	0.128±0.067 (0.047-0.327)	0.200±0.071 (0.096-0.408)	0.274±0.077 (0.152-0.491)	0.02
Pb	0.115±0.089 (0.011-0.379)	0.140±0.110 (0.014-0.468)	0.256±0.145 (0.022-0.623)	0.01
Co	0.141±0.029 (0.061-0.191)	0.190±0.056 (0.077-0.346)	0.276±0.112 (0.090-0.600)	-
Zn	0.032±0.012 (0.000-0.053)	0.017±0.006 (0.007-0.029)	0.008±0.005 (0.000-0.018)	5.0

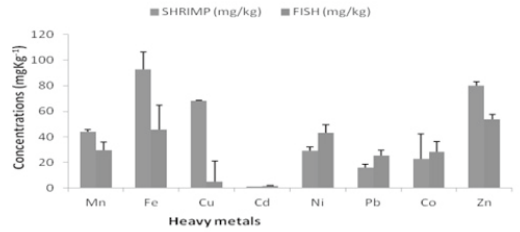

Figure 1: Bioaccumulation levels (mg/kg) in fish and shrimp.

Heavy metals have been used as indicator of pollution because of their high toxicity to human and aquatic life (Ezemonye and Kadiri, 1998). In the present study, the eight heavy metals (Mn, Fe, Cu, Cd, Ni, Pb, Co and Zn). Statistical analysis showed no significant difference in heavy metal levels across the stations (P>0.005). But there was significant difference in heavy metal levels across the different matrices. Duncan multiple range test (DMR) showed that this difference was from the water, as the values were much lower compared to heavy metal levels in shrimp and fish.

Co had the highest concentration in surface water (0.202mg/L) while Cd had the lowest concentration (0.001mg/l). The result showed that Cd, Ni, Pb had values higher than the WHO recommended benchmark (WHO, 2003).

From the heavy metals studied, Fe had the highest concentration in shrimps (92.848mg/kg). The concentration of Cd was lowest (1.107mg/kg) and below the FAO limit of 2.0mg/kg. In shrimp Mn, Cu, Ni, Pb, Co, Zn had values above the regulatory limits. This shows that the river is heavily polluted with heavy metals.

In fish, Zn had the highest concentration (53.896mg/kg) while Cd had the lowest concentration (1.685mg/kg) Mn, Ni, Pb, Co had values above the WHO regulatory limits. Periodic monitoring of these and other heavy metals in both the fish and shrimp to ensure continuous safety of people in the area is recommended.

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P-34

Human Health Risk of Pesticide Residues in Sediments through non dietary exposures.

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The distribution of α-HCH, γ-HCH, β-HCH and ∑DDT in sediment samples from major agricultural producing areas in Edo state Nigeria were determined to assess the attendant human health risk associated with non-dietary exposure. Samples obtained were extracted and analysed using Gas Chromatography (GC) equipped with Electron Capture Detector (ECD), while health risk assessment was carried out using standard models: Incremental Lifetime Cancer Risk (ILCR) and Chronic daily intake (CDI). Results showed varying concentrations of α-HCH, γ-HCH, β-HCH and ∑DDT pesticides in sediment samples with Hexachlorocyclohexane (∑HCHs) (4.6µg/g/dw) being the dominant contaminant as it was widely detected in all samples and stations. Source identification revealed that the current levels of HCHs and DDT in sediments were attributed to both historical use and fresh usage of pesticides. Estimated chronic daily intake for non-cancer risk showed values below the reference dose while cancer risk estimates using ILCR showed that there was potential cancer risk for children and adults on exposure through ingestion and dermal contact. Health risk was higher through ingestion than dermal and inhalation, with children being at higher risk.

Key words: Non Dietary, cancer risk, daily intake, sediment.

1. Introduction

It's been reported that a significant proportion of OCPs, are retained in soils and eventually sediments following application (Miglioranza et al. 2003). This implies that sediments in close proximity to areas of intense agricultural activities are reservoirs of pesticides. Despite the risk posed by pesticides, there is still a dearth of information on pesticide contamination of sediments in Nigeria. Humans are also exposed to toxic pesticides in sediments through; direct ingestion of substrate particles; dermal absorption of trace elements in particles adhered to exposed skin; and inhalation of re-suspended particles emitted from sediment through the mouth and nose (Qu *et al*. 2014). Therefore, the chronic daily intake (CDI) and the incremental lifetime cancer risk (ILCR) are employed in order to estimate the cancer and non-cancer risk that may arise from human exposure to contaminated sediments. This study aims to provide data on pesticide (α-HCH, γ-HCH, β-HCH and ∑DDT) levels in sediments from Edo State, Nigeria and also the potential human health risk that could arise from subsequent exposures.

2. Methodology

Several sampling sites in three agro ecological zones (Illushi, Owan and Ogbesse) with intense agricultural activities were selected for this study. Farming activities range from rice, cocoa, plantain and pepper production. Sediment samples were collected from each river using standard methods (Ezemonye et al. 2008). Samples were extracted and cleaned up and analysed for α-HCH, γ-HCH, β-HCH and ∑DDT. 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).

2.1 *Non carcinogenic risk*

CDI was used to estimate the non-carcinogenic risks for adults and children from non-dietary exposure to pesticides (Haung *et al*. 2014).

$$\mathrm{CDI}_{\text{ingestion}} = \frac{\mathrm{C\ (sediment)} \times \mathrm{IR\ (sediment)} \times \mathrm{CF} \times \mathrm{EF} \times \mathrm{ED}}{\mathrm{BWxAT}}$$

$$\mathrm{CDI}_{\text{inhalation}} = \frac{\mathrm{C\ (sediment)} \times (1/\mathrm{PEF}) \times \mathrm{IAR} \times \mathrm{EF} \times \mathrm{ED}}{\mathrm{BWxAT}}$$

$$\mathrm{CDI}_{\text{dermal}} = \frac{\mathrm{C\ (sediment)} \times \mathrm{SA} \times \mathrm{CF} \times \mathrm{EF} \times \mathrm{ED} \times \mathrm{ABS} \times \mathrm{AF}}{\mathrm{BWxAT}}$$

2.2 *Carcinogenic risk*

The ILCR represents the incremental probability that an individual will develop cancer during his lifetime as a result of exposure to a potential chemical carcinogen (Qu *et al*. 2014).

$$\mathbf{ILCRs}_{ingestion} = \frac{CS \times (\mathrm{CSF}\, ingestion \times 3\sqrt{(BW/70)}) \times \mathrm{IRsed} \times ED \times \mathrm{EF}}{\mathrm{BWxATxCF}}$$

$$\mathbf{ILCRs}_{inhalation} = \frac{CS \times (\mathrm{CSF}\, inhalation \times 3\sqrt{(BW/70)}) \times \mathrm{IRair} \times ED \times \mathrm{EF}}{\mathrm{BWxATxCF}}$$

$$\mathbf{ILCRs}_{dermal} = \frac{CS \times (\mathrm{CSF}\, dermal \times 3\sqrt{(BW/70)}) \times SA \times FE \times \mathrm{ABS} \times AF \times EF \times ED}{\mathrm{BWxATxCF}}$$

Where $\mathbf{C_S}$ is concentration of sediment samples, $\mathbf{I_R}$ is the ingestion rate of sediment, $\mathbf{C_F}$ is the carcinogenic slope factor, ($\mathbf{E_F}$) is the Exposure Frequency, $\mathbf{E_D}$ is Exposure Duration, $\mathbf{B_W}$ is the Body Weight, $\mathbf{F_E}$ is the Dermal Exposure Ratio, $\mathbf{A_F}$ is the Dermal Surface Factor, **ABS** is the Dermal Absorption Factor, $\mathbf{A_T}$ is the Average Life Span, $\mathbf{S_A}$ is the Surface Area and **PEF** is Particle Emission Factor. **CSF** is carcinogenic slope factor, obtained from USEPA Integrated Risk Information System (IRIS).

3. Results and Discussion

3.1 *Distribution of α-HCH, γ-HCH, β-HCH and ∑DDT*

Concentration of each isomer of HCH in sediment samples were in the following order β-HCH > α-HCH > γ-HCH. Distribution of HCHs in the various agro-ecological region, shows that the Illushi region had

higher concentrations of β-HCH while the Ogbesse region had the highest concentration of γ-HCH and the Owan region had the highest concentration of α-HCH. High concentrations of the isomers of HCH is attributed to their higher water solubility, vapour pressure, biodegradability, lower lipophilicity and particle affinity (Yang et al. 2005). Also High distribution and occurance of the unstable α-HCH in each site, reflects the recent use of technincal HCH within these areas. This is further confirmed by the ratio of α-HCH and γ –HCH, which ranged from 0-2.02 x 10^3 with mean ratio of 12.60. A ratio of this magnitude suggests that the current HCHs pollution in the area resulted from other HCH-like pesticides applications in the vicinity. The concentration of ∑DDT, in sediment samples ranged from ND-6.1 ug/kg/dw. The presence of ∑DDT in varying concentrations might be attributed to a fresh input of DDT and its slow degradation (Hu et al. 2009). Furthermore, the reintroduction of DDT as an insecticide for the control of malaria vector could be responsible for DDT contamination. Although the use of DDT in agriculture still remains prohibited, in 2010, most African countries including Nigeria authorised the use of DDT in combatting the scourge of malaria.

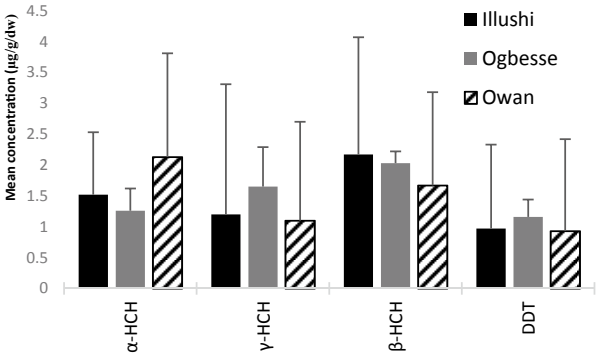

Figure 1: Concentration of OCPs in sediment samples

3.2 Health risk estimates

Chen and Liao (2006) reports an ILCR between 10^{-6} and 10^{-4} denotes potential risk, while values larger than 10^{-4} indicates potentially high health risk. An ILCR of 10^{-6} or less denotes virtual safety. It was also observed that for both groups (children and adults), the potential cancer risk was highest when exposure route was via ingestion, while exposures through inhalation had no potential cancer risk to humans. While children were at higher cancer risk compared to adults for all exposure routes (table 1). Chronic Daily Intake (CDI) estimates for non-carcinogenic risk for each pesticides were below the reference dose, indicating no potential risk (table 2). Results from this study indicate that there is a potential cancer risk from non-dietary exposures to contaminated sediments. This calls for an increased effort in regulating pesticide use and subsequent clean-up programmes.

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Table 1: Estimated Incremental Lifetime Cancer Risk (ILCRs)

	Ingestion		Dermal		Inhalation	
	Children	**Adults**	**Children**	**Adult**	**Children**	**Adults**
αHCH	3.30E-05*	2.06E-05*	5.22E-06*	1.33E-05*	1.32E-09	1.65E-09
γHCH	7.58E-06*	4.74E-06*	1.85E-06*	4.71E-06*	3.04E-10	3.8E-10
βHCH	8.13E-06*	5.08E-06*	1.86E-06*	4.74E-06*	4.66E-10	5.83E-10
∑DDT	1.11E-06*	6.93E-07	3.52E-07	8.96E-07	4.44E-11	5.56E-11

(*) Indicates potential cancer risk

Table 2: Estimated Chronic Daily Intake for OCPs

	Ingestion		Dermal		Inhalation		
	Children	**Adults**	**Children**	**Adult**	**Children**	**Adults**	**Reference Dose**
αHCH	3.1E-05	2.8E-06	1.1E-05	4.2E-06	1.3E-09	3.6E-10	8.00E-03
γHCH	2.5E-05	2.3E-06	9.2E-06	3.3E-06	1.0E-09	2.9E-10	3.00E-04
βHCH	3.8E-05	3.4E-06	1.4E-05	4.9E-06	1.5E-09	4.3E-10	8.00E-03
∑DDT	1.9E-05	1.8E-06	7.1E-06	2.6E-06	7.8E-10	2.3E-10	5.00E-04

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Levels of Benzo(a)pyrene (BaP) in Smoked and Barbecued Fish within Benin Metropolis.

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Smoked fishes such as Atlantic Mackerel (*Scomber scombrus*), Senegal Jack (*Caranx senegalus*) and barbecued fishes such as BoboCroaker **(***Psuedotolitu selongatus*), Catfish **(***Clarias gariepinus*)sold and consumed in Benin City and obtained in selected markets were screened for the presence BaP. Column chromatography, packed with anhydrous Na_2SO_4 and silica gel was used for PAH extraction with dichloromethane as the eluting solvent. The identification and concentration of BaP was carried out by liquid chromatography (HPLC) with the aid of 16 standards. Benzo(a)pyrene concentration in smoked *Caranx senegalus* ranged from 36.23 to 270.48 µg/kg. There was a significant difference ($p<0.05$) in the mean level of BaP in smoked fish samples across the sampled markets. There were no significant difference $p>0.05$ in the mean BaP for all samples of *Scomber scombrus* from the sampled markets. The difference in BaP concentration in barbecued fish among the sampled markets was statistically significant ($p>0.05$). The BaP levels of all smoked and barbecued fish samples (24) examined in this study were found to be higher than the acceptable limit (5µg/kg) specified by the European Commission and FAO/WHO limits (10µg/kg).

Key words: Benzo(a)pyrene (BaP), Smoked and Barbecued Fish, Nigeria, *Scomber scombrus, Caranx senegalus, Psuedotolitu selongatus, Clarias gariepinus*

1. Introduction

Polycyclic aromatic hydrocarbons constitute a large class of organic compounds that have the carcinogenic activity. Traces of PAHs have been detected in many foods (EFSA, 2007) including vegetable oils, fruits, sea food, grilled and roasted meat, smoked fish, tea and coffee (Simko, 2002). In particular, benzo[A]pyrene has been found in these samples at concentration levels between 0.1 and 100mg/kg and hence pose a health risk to consumers (Rey-Salgueiro*et al.,* 2008). Smoking has been used for centuries as a means for food preservation and is still used widely for this purpose. In the developing world up to 70% of the total fish catch is preserved by smoking (Ward, 1995). The process of smoking requires the penetration of food products by smoke resulting from thermal destruction of wood . Research has shown that BaP concentration of charcoal fire cooked meat samples was much higher than gas fire cooked meat (Anderson *et al.,* 2002). However, Rivera *et al.* (1996) detected BaP concentrations of 4 to 19µg/kg in charcoal grilled meat. The levels of BaP in smoked foods, including turkey, pork, chicken, beef and fish products were found to be between 0.15 and 8.93µg/kg by Gomaa *et al.* (1993). This study investigated the presence and the levels of benzo[a] pyrene in some smoked and barbecued fish in Nigeria.

2. Materials and Methods

Locally smoked fish (*Caranx senegalus* and *Scomber scombrus*) and barbecued fish **(***Psuedotolitus elongates* and *Clarias gariepinus)* (about 5 g) of different species commonly consumed in Benin city, were purchased from three different market centres from local vendors in Benin city, Edo state Nigeria and analysed using Solid phase extraction (SPE) Cartridge, Agilent 6890 series Gas chromatograph, vacuum degasser, liquid chromatography (HPLC), UV diode-array detector .

3. Results

Benzo[a]pyrene Levels in Smoked Fish(*Caranx senegalus* and *Scomber scombrus*)**.** Mean BaP levels in smoked fish are presented in (Figure 1). Benzo[a]pyrene concentration in smoked *Caranx senegalus* ranged from 36.23 to 270.48 µg/kg among the sampled markets. The least BaP concentration was recorded in smoked fish (*Caranax senegalus*) samples from Oliha Market while the highest was recorded in samples from New-Benin Market. There was a significant difference ($p<0.05$) in the mean level of BaP in smoked fish samples across the sampled markets.

Benzo[a]pyrene Levels in Barbecued Fish (*Psuedotolitus elongates* and *Clarias gariepinus*). The difference in BaP concentration in barbecued fish among the sampled markets was statistically

significant (p>0.05). The levels of BaP in barbecued fish samples ranged from 128.377 to 206.68 µg/kg, the highest value was reported in fish samples (*Psuedotolitus elongates)* from Ekewan Road (Tips Bar) while the least was reported in fish samples from S & T Barracks, Ugbowo. *Clarias gariepinus* had a mean value of 145.25 ug/kg across all stations as seen in figure 1. These levels were significantly higher as compared to the levels in the smoked fish samples as presented in figure 1. The difference between smoked and barbecued groups was significant (p<0.05).

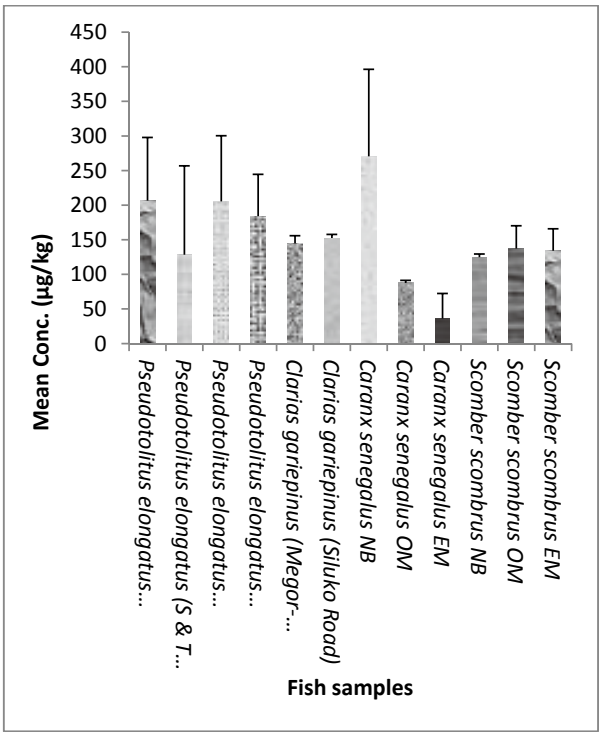

Figure 1: Determination of Benzo(a)pyrene in smoked and barbecued fish in Benin Metropolis.

4. Discussion

The variation in the levels of BaP observed among sampled smoked and barbecued fish from the selected markets in this study could be related to differences in processing (Duration of smoking), differences in the type of wood used for smoking or even differences in construction of smoking kilns. The levels of BaP in barbecued fish samples ranged from 128.377 to 206.68 µg/kg and for the smoked fish 36.23 to 270.48 µg/kg and exceeded the acceptable limit (5µg/kg) specified by the European Commission (E.C, 2005) and FAO/WHO limits (10µg/kg). Other findings were consistent with the findings from this study, where high level of BaP exceeding 100 ug/kg was determined (Guillén *et al*.,2009). Benzo[A]pyrene has been found in food samples at concentration levels between 0.1 and 100mg/kg and hence pose a health risk to consumers (Rey-Salgueiro *et al*., 2008). Variable levels of BaP were detected ranging from 7.46 to 18.79 µg/kg in smoked fish (Muyela *et al*., 2012). This study however, found much higher values. All smoked and barbecued fish samples (24) examined in this study were found to far above the acceptable limit (5µg/kg) specified by the European Commission (E.C, 2005) and FAO/WHO limits (10µg/kg).

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