

**Bioaccumulation and bioaccessibility of arsenic and mercury in the common carp
(*Cyripnus carpio*) of the Hex river catchment in rustenburg, south africa**

By

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DECLARATION

I, the undersigned, hereby confirm that the work contained in this research dissertation is my own original work for a Degree of Masters of Science in Agriculture in Animal Health working under the supervision of Professor Upenyu Marume and Doctor Mathew Nyirenda. This dissertation has not been submitted to any University. Materials and evident information from any other sources have been fully recognized.

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GENERAL ABSTRACT

Mining is one of the anthropogenic activities that increase heavy metal contamination in the aquatic environment. Arsenic (As) and Mercury (Hg) are well-known cancer causing agents and they are a major concern all over the world. Their effects may be on the freshwater rivers, fish or marine biota and consumers' health. Since this is in a liquid ecological condition, As and Hg might be converted into organic species that are able to bioaccumulate through the trophic food chain to get to the highest level of heavy metal bioaccumulation in fish that will end up causing health risk to the consumers. This study investigated bioaccumulation and accessibility of As and Hg concentrations on the Common carp (*Cyprinus caprio*) in the Hex River of Rustenburg, North West, South Africa and associated health risks to consumers. Bioaccumulation levels of As and Hg Common carp were determined on raw and cooked (Frying and Boiling) fish muscle using microwave acid digestion. While the assessment of consumer health risk due to As and Hg was investigated using the static *in-vitro* digestion model, by quantifying the bioaccessibility levels of the elements, As and Hg, to consumers this could be able to estimate their health risk. Both methods were analysed using inductive coupled plasma mass spectrometry (ICPMS). According to the results, Bioaccumulation levels of As and Hg on the fish were very low on raw and cooked fish, except for mercury levels in the fried fish muscles were above the acceptable limits recommended by South African Department of Health (SA-DOH). These suggest that Hg levels in the fried fish can cause bad effect to consumers. While Bioaccessibility estimates suggest that cooking processes can elevate the concentrations of As and Hg bioaccessibility by solubilisation and volatilisation effects

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ABBREVIATIONS

As	Arsenic
ATSDR	Agency for Toxic Substances and Disease Registry
BW	Body Weight
CLT	Central limit theorem
CR	Carcinogenic risk
CRM	Certified reference material
CSF	Cancer slope factor
DMA	Dimethylarsinic
dw	Dry weight
DWAF	Department of Water Affairs and Forestry
EC	European commission
EDI	Estimated daily intake
EFSA	European Food Safety Authority
FAO	Food and Agricultural Organization
GIT	Gastrointestinal tract
Hg	Mercury
iAs	inorganic Arsenic
ICP-MS	Inductively Coupled Plasma – Mass Spectroscopy
JECFA	Joint FAO/WHO Expert Committee on Food Additive
MAL	Maximum Allowable limits
MMA	Monomethylarsonic
ppm	parts per million

PTWI	Provisional Tolerable Weekly Intake
THQ	Target Hazard Quotient
WHO	World Health Organization
ww	Wet weight
µg/g	microgram per gram
USEPA	United States Environmental Protection Agency

CHAPTER 1

1. INTRODUCTION

1.1 Background

Heavy metals are huge ecological contaminants and their poisonous characteristics are an issue of critical importance for environmental, evolutionary, ecological, food and water footprints (Jaishankar *et al.*, 2014; Nagajyoti *et al.*, 2010). Heavy metals get into the aquatic environment by natural contamination and through human actions. Different causes of heavy metals contamination include soil erosion, natural weathering of the earth's crust, mining, industrial effluents, urban runoff, sewage discharge, bug or infestation control agents used on crops, and numerous others (Morais *et al.*, 2012). All these events and processes result in the build-up of heavy metals in soils, aquatic habitats, water sources for livestock and humans and the human food chain. In this way, nutrition, particularly consumption of marine and aquatic protein sources, become the primary route of exposure to these heavy metals by the consumers (Kim & Lee, 2010).

Fish constitute an essential supply of animal protein for most of the human population globally. In the next coming ten years, it is anticipated that protein production from undomesticated fisheries and aquaculture will surpass production of poultry, beef and pork (Commission, 2011). In 2010, about 16.7% of the global animal protein consumed was supplied by fish and the global fish food supply had increased by 3.2% from 1961 to 2012 on an annual basis (Moffitt & Cajas-Cano, 2014). Due to a rapid growing population globally, wild fish is also expected to play an important role in food security serving as source of nourishment particularly to the resource poor rural communities (Moffitt & Cajas-Cano, 2014), through the supply of cheap organic protein,

important nutrients and generation of income (Thilsted *et al.*, 1997; Roos *et al.*, 2007; Welcome *et al.*, 2010; Youn *et al.*, 2014).

1.2 Problem statement

In spite of the potential of dietary fish as a source of protein, particularly in mining and agricultural production areas, the heavy metals that might be contained in the fish have stimulated anxiety among the regular fish eating consumers (Domingo, 2007, Dórea, 2008, Martorell *et al.*, 2011). The common heavy metal contaminants that may be present in soil, water sources and fish include arsenic, cadmium, lead and mercury (Agusa *et al.*, 2005, Mohamad *et al.*, 2012). Of all these metallic contaminants, arsenic is the most hazardous heavy metal which is known to cause cancer and genotoxic conditions where people drinking contaminated water may end up having chromosomal abnormalities (Chou *et al.*, 2001; Chen *et al.*, 2005). Mercury has also been observed to induce extreme neurological effects such as brain damage, muscle tremors, cognitive loss, etc. in humans (Liu *et al.*, 2008; Díez *et al.*, 2009). Their impacts can be particularly extreme on unborn children, since these metals can be transmitted from the maternal to her child (Sanfeliu *et al.*, 2003).

Each heavy metal has its own values of concentration for health hazard assessment, such as maximum allowable limits (MAL) and provisional tolerable weekly intake (PTWI) for heavy metals in different products to prevent people from their health effects (Tressou *et al.*, 2004). According to the South African Department of Health, arsenic concentration standard for fish and processed fish are set at 3.0 mg/kg (SA-DOH, 2004) and inorganic arsenic in seafood as a provisional tolerable weekly intake (PTWI) of 15 µg/kg body weight(bw)/week (Falcó *et al.*,

2006). Estimated daily intake for arsenic in fish products is 0.13 µg/kg bw/day (Meeting & Organization, 2000).

In many resource poor communities of South Africa, particularly in mining towns, fish forms an important source of nutrients. For example, Rustenburg is a mining town of the North West province of South Africa which is home to two platinum mines and a refinery that processes around 70% of the world's platinum. These mining activities predispose the surface water sources, including the Hex River catchment, to heavy metal contamination (McMurry & Fay, 2004; Mendie, 2005). The Hex River catchment habitats contain fish that is consumed by the majority of poor in the area. Currently, there is no information on the effects of heavy metal contamination on fish meat wholesomeness before it is consumed by the people in the area. Most studies have focused mainly on water and fish microbiology (Bacteria, viruses, fungi) rather than on bioaccumulation and bioaccessibility of heavy metals in fish. It is therefore critical to evaluate the extent of heavy metal build up in fish in the areas and their potential effects on human health.

1.3 Justification

In the absence of information on bioaccumulation in fish, there is a growing risk of the heavy metals reaching toxic levels in the water source, the fish and subsequently in humans. Empirical information from the study will therefore be critical in formulating Veterinary Public Health (VPH) procedures that will include heavy metal testing during meat wholesomeness examination and strategies that improve the efficiency of mining waste management and minimise the flow of heavy metals into the water sources.

1.4 Objectives

The major objective of the study was to determine the bioaccumulation of arsenic and mercury in Common carp (*Cyripnus carpio*) of Rustenburg's Hex River catchment and the associated health risks to consumers.

1.4.1 The specific objectives were:

- To determine the levels of arsenic (As) in raw and cooked Common carp from the Rustenburg's Hex River catchment,
- To determine the levels of mercury (Hg) in raw and cooked Common carp from the Rustenburg's Hex River catchment,

To conduct an exposure assessment and risk characterization among consumers of Common carp from the Rustenburg's Hex River catchment.

1.5 Hypothesis

- The degree of bioaccumulation of As and Hg in Common carp muscles in raw treatment is an indication of Hex River exposure to heavy metals contamination.
- The degree of bioaccumulation and accessibility of As and Hg in raw and cooked treatments of Common carp from Hex River is an indication of whether the fish is safe or not for consumers.

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CHAPTER 2

2. LITERATURE REVIEW

2.1 Introduction

The earth's most essential metals and minerals are produced from Africa for example platinum group metals (Services, 2008). There are two large platinum mines in South Africa, Rustenburg North West Province, the Anglo platinum mine and the Lonmin platinum mine are the largest producers of platinum around the globe and contribute to the economic growth of Rustenburg (Mahlati, 2012). But these good benefits always come at a great price to the ecological surroundings such as soil contamination produced by dangerous wastes, heavy metals polluted water overflow into the rivers and air pollution from the mine dumping site (Mahlati, 2012). Metal contamination takes place on account of remediation and dumping of sludge constituting metals from various companies such as processing, smelting and mining companies (Wang *et al.*, 2007; Perelló *et al.*, 2008; Wang *et al.*, 2010). South Africa is one of the very significant mining nations around the globe, therefore mining contamination is a very critical aspect to the country as the mines may cause negative effects to the water quality, largely because South Africa is a water scarce country (Ochieng *et al.*, 2010). Water has been considered as a basic need for humans' survival from the beginning of life (Stock, 2012). It was suspected that there are some mining activities that take place at Hex River resulting in introduction of effluents from underground and surface seepage channels, which run and gather into the Bospoort Dam (Du Preez *et al.*, 2006). Hex River receives treated effluents from the Rustenburg town, including effluents from mines. The effluents undergo oxidation process at the Bospoort Dam prior to being deposited to Vaalkop Dam and this water is more contaminated than that of Hartbeespoort Dam (Motshegoa, 2014). Pollution of the river water is way to introduce Heavy metals to aquatic

animals and accumulate especially in fish at the top of the trophic food web (Jarapala *et al.*, 2014). Monitoring heavy metal contamination in river systems, bioaccumulation in fish and bioaccessibility by consumers helps to assess the quality of aquatic ecosystem (Adams & Onorato, 2005).

2.2 Bioaccumulation of heavy metals in fish

A process whereby toxic substances are absorbed straight from the water via gills or via ingestion of tainted feed is called bioaccumulation (Titilayo & Olufemi, 2014). Marine species gather high levels of metallic contaminants, fish being the most exposed in terms of natural and chemical substance accumulation. The accumulation process of these contaminants relies on the fish's fat content, feeding behaviour and age (Jorgenson *et al.*, 2001; Eneji *et al.*, 2011; Hien *et al.*, 2012) also the types of pollutants, location of sample collection, trophic level and the fish species all are critical (Asuquo *et al.*, 2004). Bio-accumulation of pollutants in fish tissues occurs when the fish gets them in their bodies by ingestion of tainted feed or through gills from the water they live in (Abdel-Khalek *et al.*, 2016). Fish can accumulate heavy metals in any of five ways mainly through skin, gills, food or food particles and water; after ingestion. These heavy metals will travel through the bloodstream to different organs in the body such as liver (Jabeen & Chaudhry, 2010). Liver is considered a vital organ in mitigating metal toxicity as it serves as the organ for biotransformation, accumulation and excretion of contaminants (Shinn *et al.*, 2009). Fish that habituate polluted environment gather high amount of heavy metals to pose possible health hazards to those who eat such fish and the risk level is determined by different factors like concentration of elements, exposure time and ecological factors like change in temperature, salinity and pH (Copat *et al.*, 2012). Fish that are at the bottom of food chain are vulnerable to

comparatively lower pollution while those at the top of food chain are highly exposed to accumulate more heavy metals (Terra *et al.*, 2008). Since fish survive in the aquatic environment, they are prone to gathering metals from the organisms they eat and water they live in, especially fish that feed at the bottom may consume heavy metal contaminated sediment (Martin & Griswold, 2009). The ability of fish to adapt to environmental changes qualifies it to be the best indicator of environmental contamination. as well as its ability to gather too much quantity of heavy metal in their bodies (Batvari *et al.*, 2008).

A review by Bashir *et al.* (2012) on the bioaccumulation of metals by fish showed that the muscle, liver and gills are the critical organs with high levels of heavy metal concentrations. This is attributed to the fact that the level of metals in the gills symbolize the level of metals in the water the fish resides in, while the level of metals in the fish's liver symbolizes the level of metals accumulated in the fish and metal found in the muscles symbolizes the fraction that is possibly available to customers (Bashir *et al.*, 2012). deghani Firouzabadi *et al.* (2012) also showed that the liver, as the most functional metabolic organ of the body is bound to contain the highest concentrations of heavy metals followed by the muscles and the gills. Heavy metals build-up in the marine system has effects on human and the biological system (Dural *et al.*, 2007). The effect of elevating levels of such heavy metals in the ecosystem is additionally upgraded by their low degradability, bringing about bioaccumulation which is transmitted through the progressive food web (Ciesielski *et al.*, 2010). Ecosystem is mainly contaminated by heavy metals because they are poisonous towards the environment and organisms, but heavy metal absorption rate from contaminated rivers, dams, lakes, etc. varies due to the biological desire, digestion and pollution rate in foodstuff and water residue, in addition other ecological

factors, for example, temperature, saltiness and interfacing agent do play a role (Rauf *et al.*, 2009).

In South Africa, one of the popular fish breeds found and consumed is the Common carp. It is among the popular river fish of the teleost breeds (FAO, 2014). The fish is a significant breed in the *Cyprinidae* family, which has spread all over the Eurasian nations and was distributed in America and Africa more than 2000 years ago (FAO, 2007). Even though it is not a South African native breed, it is presently found almost everywhere around the Southern parts of Africa (Winker, 2010; Wakida-Kusunoki & Amador-del Ángel, 2011) . Meanwhile its arrival in South Africa has made it to be the most significant breed for angling and aquaculture business (Skelton, 2001). Common carp is also economically recognized as an experimental organisms in the fields of ecology (Kulhanek *et al.*, 2011), developmental biology (Cheng *et al.*, 2010), evolution (Zhang *et al.*, 2008), immunology (Kongchum *et al.*, 2010) and environmental toxicology (Bervoets *et al.*, 2009; Kroupova *et al.*, 2010).

Common carp like rivers with gentle underground sediment and smooth moving water since it is one of the fish that spends most of its time swimming down the river, to coming to the surface in search for food and can as well survive in severe and critical situations (Motshegoa, 2014). In contrast to other aquatic organisms, for example shrimp and salmon, Common carp are considered to be the most eco-friendly fish ever due to the fact that they are omnivorous feeding on vegetation and small organisms in the river (Xu *et al.*, 2014). Their mating period is during spring and summer time, they lay eggs on shallow plants, egg hatching starts 4-8 day after mating and larvae mature very fast after hatching (Skelton, 2001; Holtan, 2011). *Cyprinus*

carpio (Common carp) has the ability to survive and acclimatize in acid and heavy metal contaminated ecological rivers, lakes, ponds and dams (Vinodhini & Narayanan, 2009).

2.3 Bioaccessibility of heavy metals to consumers

Bioaccessibility can be utilized in the establishment of policy and human health risk evaluations (Koki *et al.*, 2015). Oral bioavailability and bioaccessibility are models basically essential for quantifying the danger related to oral exposure to environmental pollutants (Oomen *et al.*, 2003). Bio-accessibility entails the fraction of a pollutant that is leached from soil into solvent by digestive enzymes (Oomen *et al.*, 2003). It symbolizes the greatest quantity of pollutant that is accessible for intestinal assimilation (Hernout *et al.*, 2015). Usually, just a fraction of these bioaccessible pollutants can be picked up by the intestinal epithelium (Hernout *et al.*, 2015). Inorganic pollutants are afterwards carried to the liver by means of the portal vein for biotransformation (Meca *et al.*, 2012). The fraction of parent element that arrives at the systemic circulation is the bio-available fraction. It was established that bioaccessibility is one of the main factors restricting the bioavailable fraction, but it is an essential tool to quantify for risk evaluation aims (Van de Wiele *et al.*, 2007; Wragg *et al.*, 2011). Solving the bioaccessibility gaps requires a high quality of artificial *in-vitro* digestion model, where consumption of substance polluted by heavy metals undergoes artificial physiological environment of the gastrointestinal tract (GIT), such as high pH levels and proteolytic enzymes (Oomen *et al.*, 2002; Van de Wiele *et al.*, 2007). It is vital to take into consideration that while food is passing through the GIT during digestion, most nutrients are assimilated at the intestinal level (Alverdy & Chang, 2008).

Food ingested through the mouth might possess different uptake rates in the intestine, the inner layer of the small intestine epithelia O-glycans are essential for the food substance bioaccessible fraction and hence the level of each chemical absorbed can be predicted since mucus plays an important role in chemical binding (Sergent *et al.*, 2008). This information is of high significance for hazard evaluation of pollutants in foodstuff among buyers, especially kids, as it is outstanding that in infants and little kids the inner layer epithelium of the digestive system is not yet fully developed and is substantially less glycosylated compared to the adults (Blažević, 2014). Along these lines, children are more defenceless against the potential lethal impacts of contaminated substance in foodstuff (Lidsky *et al.*, 2007). A few substances that are absorbed into epithelial cells can finely tune the articulation or potentially act as transport proteins associated with the uptake procedure, and may likewise clearly influence the bioaccessibility of different chemicals, being agents in charge of possibly harming contacts (Sergent *et al.*, 2008). A few techniques of intestinal uptake are portrayed in other authors' works, for example, static *in-vitro* digestion techniques that utilise simulated layers or *in silico* modelling, and *in vivo* techniques that depend on organism, separated tissue parts and cell prototypes (Van Breemen & Li, 2005). All methods possess strengths and weaknesses. *In vivo* intestinal uptake includes differing modes of action as passive dissemination (Para-cellular and trans-cellular), transporter interceded or restricted transportation and vesicular transportation (liquid stage endocytosis, receptor intervened endocytosis and transcytosis) (Hidalgo, 2001).

2.3.1 Different techniques used to determine bioaccessibility.

Basic techniques with simulated layers can anticipate just transcellular passive dissemination (Balimane *et al.*, 2000). Cell techniques incorporate all above expressed modes of action to

investigate penetrability, as appeared for Caco-2 monolayer on small pores layer that is the most generally utilized cell technique for such examinations (Markowska *et al.*, 2001). Assessing the effects of food-borne heavy metal in humans from the metal bioavailability can be done either by *in vivo* or *in-vitro* techniques (Dyck *et al.*, 1996). Human *in vivo* method give best results than the other methods such as *in-vitro* test and animal *in-vivo* experiments where animals such as rodents, dogs, rabbit, primates and pigs are used to conduct human risk experiments on medical and ecosystem fields, because of their close resembles of nutritional needs, mineral breakdown, bone development, bodily processes and digestive tube to humans (Juhasz *et al.*, 2008). The animals most used in the *in vivo* experiments are primates, but their use has been restricted due to financial constrains (Basta & Juhasz, 2014). Little pigs are the best choice for proper functioning prototype for gastrointestinal absorption of a pollutant, because they have a gall bladder that secretes bile into the duodenum and ileum during digestion and coprophagia is unwanted to retain nutritional level (Rees *et al.*, 2009). Pigs might be taught to consume polluted food because of simple movement of pollutant within their gastrointestinal tract, therefore collecting blood samples from pigs is simpler than collecting from other animals, hence there is a potential of conducting cross-over experiments in order to decrease variables and quantity of animals needed (Cheli *et al.*, 2015). Though, experimenting with animals is costly, and tough to conduct, there also are ethical limitations, and it gives restricted information in every test (Hansen & Spears, 2009). As a result various *in-vitro* techniques were being utilised as substitutes for the evaluation of heavy metal bioavailability (Intawongse & Dean, 2006). There are important factors to be considered for conducting *in-vitro* techniques to be identical to human digestion process such as the pH, chemical contents, temperature and the shaking of components (Moreda-Pineiro *et al.*, 2011). There are many ways to conduct *in-vitro* digestion for assessment:

- (1) the extreme absorbable concentration of the element of concern in the simulated gastrointestinal fluid after filtration or centrifugation (bioaccessible fraction).
- (2) the absorbable portion of the element (bioaccessible fraction) made present after digesting with human gastrointestinal microbiota (Simulator of the Human Intestinal Microbial Ecosystem, SHIME) (Van de Wiele *et al.*, 2007; Sivieri *et al.*, 2011),
- (3) the dialyzable fraction of the element, which may be dialyzed within a semi-permeable membrane with an identified pore size (dialysate or bioavailable fraction) at equilibrium (Miller and Schricker, 1982) or non-equilibrium (Wolters *et al.*, 1993; Shen *et al.*, 1994; Shiowatana *et al.*, 2006) conditions; and,
- (4) the portion of the element capable of being reserved or moved within a compact or small pores supports (bioavailable fraction) in which human Caco-2 cells grown are combined (intestinal epithelial model) (Ekmekcioglu, 2002).

These techniques produce good estimations to *in vivo* conditions and provide the benefits like easy to conduct, fast, simple procedures, cheap, high accuracy and excellent results (Cardoso *et al.*, 2015). Saliva is being used in other *in-vitro* digestion protocols (Woolnough *et al.*, 2010). The surface area is increased when big portions of samples are mechanically broken down into little portions in the mouth, while this takes place the sample is mixed with saliva (Woolnough *et al.*, 2010). It is well acknowledged that this food softening substance, saliva, is mucus which constitutes of salivary amylase (an enzyme which starts the breaking down process of starch) and buffers which retain the pH lubricating substance (Afonso *et al.*, 2015). Since this whole procedure takes little duration under approximately neutral pH (6.5) in the mouth, elements of concern cannot be expected at this level of digestion from food (Intawongse & Dean, 2006). Hence it is generally implicated that saliva poses merely a minor influence on the level of

liberation of chemicals (Oomen *et al.*, 2002). Imitations of stomach and intestine are the most preferred stages of *in-vitro* digestion techniques and these techniques are mainly utilised for assessment of concentration of metals on food and soil samples, oral bioaccessibility (Intawongse & Dean, 2006).

2.3.2 Advantages and disadvantages of using Static *in vitro* digestion technique

Research has been carried out on arsenic bioaccessibility utilizing *in-vitro* digestive methods that are cheap and consistent with the purpose to imitate the human digestive tract (physiochemical and enzymatic) processes (Laird *et al.*, 2007; Van de Wiele *et al.*, 2010). A traditional device for assessing oral bioavailability is oral bioaccessibility, which is characterized as the pollutant portion that solubilises from its matrix throughout gastrointestinal digestion and gets presented for intestinal absorption (Etcheverry *et al.*, 2012). Bioaccessibility is utilized as a marker of highest oral bioavailability (Versantvoort *et al.*, 2005) and is thus an essential device to be utilized in danger evaluation. Fish species and co-utilization of food consisting of phytochemicals and dietary fibre simultaneously might affect Hg bioaccessibility (Cabañero *et al.*, 2007; Shim *et al.*, 2009). Reports on the bioaccessibility of Hg in predatory fish from Spain is rare and have only been done in uncooked fish (Cabañero *et al.*, 2004; Cabañero *et al.*, 2007; Torres-Escribano *et al.*, 2010). Just because the levels of heavy metals in fish muscles are below the maximum limits does not give the guarantee that the fish is safe for consumption. There are a lot of unknown associations of toxicological evaluation of pollutants, opposing impacts of microflora, various pollutants, nutrients and so forth that might be in the GIT that are overlooked in static *in-vitro* digestion and may enhance heavy metal bioavailability (Marques *et al.*, 2011). Besides the limitations related with the uncertainty and population variability, this approach does

not consider the behaviour of the contaminants once they enter the body, and more specifically, their fate through the gastrointestinal system (Mutagen & Mutagen, 2009).

Although the effect of gastrointestinal juices on toxic elements has been scarcely investigated, preliminary studies indicate that only a fraction of the ingested chemicals, known as bioaccessible fraction, could finally be absorbed (Maulvault *et al.*, 2011; Calatayud *et al.*, 2012). The remaining fraction may be embedded in the un-absorbable fraction and excreted or changed its chemical form through a speciation process (Versantvoort *et al.*, 2005). To fill this gap, in recent years, *in-vitro* gastrointestinal models with a varied complexity level, have been successfully developed and validated, offering a reproducible and economic approach (Berti *et al.*, 2015; Maulvault *et al.*, 2011).

2.4 Effects of cooking methods on heavy metal bioaccessibility

Fish is normally preferred to be consumed when cooked using different cooking methods (Ouédraogo & Amyot, 2011). The reason for fish to be consumed with different kinds of cooking methods including baking, boiling, frying or grilling is to enhance its flavour and taste for more enjoyable consumption (Sartal *et al.*, 2012). Alipour *et al.* (2010) stated that cooking can alter the physical and chemical properties of the fish such as loss of mass, changes in water accumulation ability, texture because of protein denaturation, loss of fluids and taste enhancement. Cooking methods can modify the heavy metal components of the marine foodstuff and this might increase the bioaccessibility of heavy metal to consumers (Cardoso *et al.*, 2010; Liang *et al.*, 2011; Cardoso *et al.*, 2012; Hu *et al.*, 2016). This is because the high heat that is used during the cooking process is able to convert the nature of heavy metals, such as As and Hg, by releasing the bound heavy metal (Ünlüsayın *et al.*, 2001; Schmidt *et al.*, 2015).

Nevertheless, according to Devesa *et al.* (2001) differences in the total heavy metal levels in marine foodstuff after being cooked might occur due to the following two reasons: (a) the reduction in weight caused by the drip of fluids, volatiles and to some extent the gross sample contents such as fats, carbohydrates and proteins, (b) the reduction in the sum of arsenic due to volatilization or solubilization (Devesa *et al.*, 2008).

As much as it is of paramount importance to prepare and cook food in the family, the levels of As in foodstuff, level of As in sauce used to cook (either water or oil) and the level of skill used to prepare are the important aspects in the assessment of As in the cooked foodstuff (Bundschuh *et al.*, 2012). Frying and boiling treatments can sometimes be the reason for elevation or decline of As concentration in certain seafood, when conducting human health risk assessment (Visciano *et al.*, 2013). If a product is boiled with water that contains elevated concentrations of As, during cooking process the As concentration will increase in that product (Cubadda *et al.*, 2015), while boiled with water that contains less concentration of As, the concentration of As will decline in the product (Cubadda *et al.*, 2003). This is the reason why boiling treatment is conducted with ultrapure water, because there will be no any heavy metal impact from the water to the assessed foodstuff (Omar *et al.*, 2014). Study on impacts of culinary treatments of Arsenic in a boiled, grilled, roasted, stewed, microwaved and steamed fish, obtained that the level of total arsenic was elevated from 2466 ng g⁻¹ wet weight of raw to 3048 ng g⁻¹ wet weight after the fish was cooked (Devesa *et al.*, 2001).

Kalogeropoulos *et al.* (2012) observed that mercury concentration levels were elevated on wet weight in fish specimens due to water loss and lipids loss during culinary treatments. While on

fried and roasted fish specimens, on dry weight, the mercury level was found to have increased due to these culinary treatments (Schmidt *et al.*, 2015). He and Wang (2011) and Shim *et al.* (2009) reported that mercury bioaccessibility increased in boiled and fried foodstuff due to the effects of culinary treatment on the heavy metal concentration and protein nature. Generally, most conducted research on effects of culinary treatments on wet fish samples have shown an elevation of mercury concentrations because of the loss of moisture and lipids during the process (Morgan *et al.*, 1997; Burger *et al.*, 2003; Perelló *et al.*, 2008; He & Wang, 2011).

2.5 Heavy metal poisoning impacts on fish consumers' health

Human operations discharge the heavy metals that may bioaccumulate in fish and cause bad impacts to consumer health (Ginsberg & Toal, 2009). Due to this, fish is regarded as the main factor of heavy metal exposure to consumers (Squadrone *et al.*, 2013; Milanov *et al.*, 2016). Since fish and fish products are part of human diet, they have been regulated for heavy metal maximum limits and there are test in place that can be used to determine the heavy metal content of the fish and fish products (Fernández *et al.*, 2015). Diet and environment are the sources of these heavy metals and are needed in micro amounts for the normal functioning of the body, because when present in large amounts they may lead to health hazards or poisonous effects in the body. Heavy metals such as arsenic, selenium and mercury are very lethal, because of this they are considered to be of environmental significance (Cai *et al.*, 2012).

2.5.1. General impacts of heavy metal to consumers

Heavy metals may change their chemical form in water to become more toxic becoming hazardous to human and wildlife wellbeing as they end up in the food-chain (Hamilton, 2004;

Munthe *et al.*, 2007; Scheuhammer *et al.*, 2007; Khokiattiwong *et al.*, 2009). The ability of metals to poison relies on the biological functions of the metal, the type of organism vulnerable to the metal and the type of metal itself (Lenntech, 2012). There are serious consequences that heavy metals may pose on marine flora and fauna that will affect the food chain and subsequently pose a serious threat to the health of those who eat fish (Lokhande *et al.*, 2011). The symptoms of heavy metal poisoning include decreased body energy level, decreased or impaired central nervous and mental role, as well as, severe damage of important organs like liver and kidneys and abnormalities in blood cells (Mohod & Dhote, 2013). Prolonged contact with heavy metals will cause muscular, neurological and physical damage that result in muscular dystrophy (continuous muscle weakness), multiple sclerosis (neurological illness that target the spinal cord and brain), Alzheimer's illness (sickness that cause malfunctioning of the brain) and Parkinson's disease (deteriorating illness of the brain), additionally lead is among the always available heavy metal in potable water, if its present at a high level than the recommended dose, it acts as an enzyme blocker and thus metabolic toxicity (Mebrahtu & Zerabruk, 2011). Cancer can be as a result of repeated prolonged contact with some metals and their elements (Järup, 2003).

Specific effects of consumption of As include heart infections, dermal abrasions, GIT conditions and some severe health issues, ultimately resulting to fatalities (Organization, 2010; Zhang *et al.*, 2017). The marine foodstuff may also contain trace of monomethylarsonic (MMA) and dimethylarsinic (DMA). MMA affects the gastrointestinal tube secondly thyroid, renal organ and lately reproductive system, while DMA cause severe impacts on thyroid, urinary bladder,

renal organ and deadly changes were reported on *in vivo* oral experiments (Food & Administration, 2016).

Specifically, Mercury toxicity is known to cause a number of conditions to the wellbeing of children and adults such as heart, lung, and nervous system conditions as well as, in addition, alter the capacity of the renal system, hepatic system, thyroid hormone and suppresses immune system (Counter & Buchanan, 2004; Holmes *et al.*, 2009; Park & Zheng, 2012). Conditions associated with mercury toxicity to the renal system include death of the tubular tissues, inflammation of the kidney, kidney illness, and kidney tumors (Tchounwou *et al.*, 2003; Li *et al.*, 2010; Park & Zheng, 2012). Mercury can last up to one or two months in the human body (Dart, 2004), while it is said that it can last up to two decades in the human brain (Rice *et al.*, 2014).

Mercury (Hg) as a constant and poisonous ecological contaminant and as an element can experience various changes in the ecosystem, ionic Hg may be changed into the worse harmful species, such as methylmercury (MeHg), by both environmental and organism pathways (He *et al.*, 2007). Methylmercury has been demonstrated in various examinations to possess the ability to cross the membrane of the placenta and *in utero* which result in the negative impacts on the foetus development , such as impaired nervous system, studying disorder, memory loss and low immune system (Zahir *et al.*, 2005; Holmes *et al.*, 2009; Park & Zheng, 2012). This type of mercury that causes severe side effects in many people is found in fish and fish products (Björkman *et al.*, 2007).

2.6. Mechanism of Arsenic poisoning in the human body

Uptake of arsenic when its particles are inhaled is much dependent on its solubility and the size of the particles, which are discharged into the lungs (Saha *et al.*, 1999). Arsenic uptake in the stomach and intestines occurs when it is consumed through the mouth (solvent arsenic elements are effortlessly assimilated from the stomach and intestines) (Saha *et al.*, 1999). The physiological half-span of consumed As is around 10 h, and 50– 80% is discharged from the body in around 3 days while the methylated arsenic has a half-existence of 30 h (Casaret & Klaassen, 2001; Gosselin *et al.*, 2009). Consumed As can pass via the placenta and lead to heavy metal contamination binding to red blood cells from mother to child through the cord that takes blood from the mother to the foetus (Patrick, 2003). Erythrocytes carry the Arsenic to every part of the body, then produce arsenates and methylated products as by products to arsenite after it is assimilated in the stomach and intestines. Biomethylation is a process where the As (+3) species go through enzymic methylation mainly in the liver to produce MMA and DMA; this procedure may occur many times again and again to bring about dimethylated arsenic metabolites. Most arsenic is quickly discharged from the body through urine; a blend of As (+3), As (+5), MMA, and DMA; DMA is normally the main content of the urine in human consuming food, drinking water and inhaling air contaminated with arsenic (Casaret & Klaassen, 2001; Abernathy *et al.*, 2003; ATSDR, 2003). Nevertheless MMA(III) is not released, but it accumulates in the cell like an intermediate product and is discovered to be more poisonous when compared to other arsenic species, potentially responsible for starting cancerous growths (Singh *et al.*, 2007). Arsenic has a preference for sulfhydryl-rich keratin in the skin and tends to accumulate in the skin, however it can likewise be stored in bones, teeth, hair and nails particularly when susceptibility is constantly occurring (Casaret & Klaassen, 2001; Gosselin *et al.*, 2009). The main biomarkers generally

utilized for speciation proof and evaluation of arsenic susceptibility are urine, whole blood, hair and nails (Kakkar & Jaffery, 2005).

2.7 Mechanism of mercury poisoning in human body

In 1997 the American Association of Poison Control Centers reported about 3,596 acute cases of heavy metal toxicity and mercury was responsible for all those cases. Mercury is well recognized to be a dangerous residual metal. Methylmercury is an element that poisons the nervous system which results in mitochondrial impairment, fat peroxidation, microtubule damage and buildup of toxin particles in nerves (Patrick, 2002). The quantity of mercury released to the environment per year is estimated to be close to 2,200 metric tons (Ferrara *et al.*, 2000). The reports from both the National Academy of Science and Environmental Protection Agency have agreed that there is 8 to 10% chances that a child born to an American woman can have neurological disorder due to mercury poisoning (Haley, 2005). Alteration of behavior and severe neurological disorder are symptoms that show that animals have been vulnerable to mercury toxicity, for example rabbits show symptoms such as severe cellular deterioration, brain cell death and ambiguous pathological alterations when they are subjected to 1 to 13 weeks of 28.8 mg/m³ gaseous form of mercury (Ashe *et al.*, 1953). Mercury may cause damage to any organ, disorders of kidneys, muscles and nerves it also interferes with intracellular regulation and distraction of membrane strength. The brain remains the main target and mercury freely attaches to accessible thiols (Patrick, 2002). Mercury plays a major part in destroying the tertiary and quaternary protein structures. It also binds to the selenohydryl and sulfhydryl groups that will react with methyl mercury and obstruct the cellular structure. Vaporous mercury can cause asthma, short-term

respiratory difficulties and bronchitis. Mercury causes the annihilation of the endoplasmic reticulum; the function of natural killer cells; and vanishing of ribosomes by interfering with the stages of transcription and translation; however the energy of a cell is also affected, resulting in free radical shape. The heavy metal chelation is a mercury sulfhydryl bond and is distributed to nearby sulfhydryl containing ligands; it also donates free sulfhydryl groups to encourage metal movement inside the ligands (Bernhoft, 2012).

2.8 Summary

Mining towns are likely to experience contamination of their aquatic environment such as rivers, dams, lakes etc. due to effluents from the mines. Mine effluents contains heavy metals pollutants such as arsenic and mercury which might be harmful to fish and other aquatic organisms. Since fish are the inhabitants of this aquatic environments that get polluted and are at the top of the trophic food web, they tend to bioaccumulate these heavy metals in their bodies, therefore this makes the fish an excellent choice as a biomarker or bioindicator. Wild fish is readily available and is a supplier of high quality organic proteins. Due to this benefit fish happen to be a favourite part of human daily diet and is mostly preferred consumed cooked rather than when raw, because cooking methods (boiling and frying) are known to improve the flavour and taste of food. However, cooking methods might have a negative or positive impact on heavy metal concentrations in the fish, either increase or decrease the bioaccessibility of As and Hg to consumers. This research has therefore provided the possible impact of mines on fish bioaccumulation of heavy metal and the negative impacts of As and Hg bioaccessibility to consumers.

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CHAPTER 3

**BIOACCUMULATION AND ACCESSIBILITY OF ARSENIC AND MERCURY IN
COOKED COMMON CARP (*CYRIPNUS CARPIO*) FROM THE HEX RIVER
CATCHMENT IN RUSTENBURG**

Abstract

Mercury and Arsenic are well-known cancer causing heavy metals in humans. There has been serious concerns raised all over the world regarding pollution of freshwater rivers and other water bodies that has resulted in detrimental effects on fish or marine biota, subsequently increasing risks of human exposure. This study investigated the bioaccumulation and bioaccessibility of arsenic and mercury in Common carp *Cyripnus carpio* in the Hex River of Rustenburg, North West province, South Africa and the possible associated effects to consumers. Freshwater Common carp samples were collected from Hex River (Bospoort Dam) and analysed for arsenic and mercury by inductive coupled plasma mass spectrometry (ICPMS). Raw samples were also heat treated and digested to determine the bio accessible fraction. The mean concentration levels of the heavy metals in muscle tissue of Common carp from the referenced site of collection for Arsenic were 0.10 ppm in raw fish, 0.98 ppm from boiled fish, and 0.13 for fried fish while for Mercury there was 0.01 ppm from raw fish, 0.13 ppm in boiled fish and 741.89 from fried fish. For the static *in-vitro* digestion the findings for As were 0.93 ppm (raw fish), 4.24 ppm (boiled) and 1.39 ppm (fried fish) while for Mercury were 0.01 ppm (raw fish), 0.27 ppm for (boiled fish) and 0.22 ppm (fried fish). Boiled fish and raw fish showed an increase from acid digestion to static *in-vitro* digestion, while the fried fish showed a decrease in arsenic and mercury concentrations ($p < 0.001$). The reason for the increase on arsenic and mercury was due to the effect of heat (high temperature denatures protein) and enzymes (pepsin on protein). This research shows that temperature and enzymes (pepsin) have major impact in the increase

and decrease of arsenic and mercury concentration in different cooking methods. Moreover the high concentrations of heavy metals in fish muscles of Common carp have detrimental effects on people.

3.1 Introduction

Heavy metal bioaccumulation in fish has been of great concern around the globe. This is because fish provide organic nutrients with high nutritional value to the human diet (Elnabris *et al.*, 2013). Heavy metals enter the aquatic habitats e.g. rivers, lakes, dams etc. through contamination due to human activities such as mining (Sary and Mohammadi, 2012). Elevated levels of heavy metals in rivers will have a negative impact on the lives of the aquatic organisms at the top of the trophic level (Kamaruzzaman *et al.*, 2011). As predators in the trophic food web, fish are exposed to heavy metals and may be the end reservoir of the bioaccumulated heavy metals in a food web, consequently posing health risk concerns to the consumers (Martin-Rios *et al.*, 2018). Nevertheless, not all the amount of heavy metals that have bioaccumulated in fish will be accessible to people who consume the fish, only the ones that have accumulated in edible parts will be absorbed during fish consumption (Amiard *et al.* 2008; Gao and Wang 2014; He *et al.* 2016). The heavy metal fraction that is absorbed into human body is the bioaccessible fraction and the process of taking it up is bioaccessibility (Ng., 2015). The rates of heavy metal bioaccumulation are very different and they rely on different factors including the type of organism and its position on the food web (Ivanović *et al.* 2016; Milošković *et al.* 2016; Marshall *et al.*, 2016), the length of exposure, weight and the maturity stage (Aich *et al.* 2012; Zubcov *et al.* 2012); the examined muscles, the gender and the amount of fat (Jankovská *et al.* 2014; Mieirol *et al.* 2014), the circumstance (Noël *et al.* 2013), the term and the climatic weather (Ben Salem *et*

*al.*2014; Mieiro *et al.*2014; Wang *et al.*2016) in addition to all these variables, the place and the ecological location (Aydin-Onen *et al.*2015; Salamat *et al.*2015; Milošković *et al.*2016).

In the Northwest Province of South Africa, the Hex River is known to be contaminated by the effluents that are disposed from the nearby platinum mines. These contaminations affect the water quality, fish health and consequently pose health risks to fish consumers. Recently, a study was conducted by Motshegoa (2014) on the bioaccumulation of organic and inorganic pollutants and human health risk assessment. Although this study was conducted using raw fish muscles only, no study has been conducted on the effects of cooking procedure on the bioaccessibility of the heavy metals in fish. Therefore this study was conducted to determine the bioaccumulation and bioaccessibility of As and Hg in raw, boiled and fried Common carp muscles from the Hex river.

3.2 Materials and methods

3.2.1 Study site

The study was conducted on the water sources around the platinum mining areas in Rustenburg in the North West Province of South Africa. Platinum mining has been practised in the Province for the past 75 years. Owing to the recent boom in platinum mining, which has been stimulated by the recent high global demand for the platinum group elements, several new surface and underground operations have been started up during the last decade (Fearnley, 2005, Wiseman and Zereini, 2010). Rustenburg is located at 25°40'0" S latitude and 27°15'0" E Longitude, about

1150 meters above sea level. This study was carried out with samples from the Hex River, Bospoort Dam, and Vaal kop dam.

3.2.2 Fish and water sample collection

Samples were collected from a 1 km stretch at the point where the mine's treated waste water drains into the Hex River. This river is considered as a spoilt catchment because of more than 80 years of mining activities and other changes within the catchment (Swanepoel *et al.*, 2017). Fifty four Common carp of about the same size of 30 ± 5 cm in length were purchased twice from local fisherman at the research site. Fish consumers prefer Common carp of 30 cm to 60 cm in length size (Hyman *et al.*, 2017) The Common carp samples were purchased using the Central Limit Theorem (CLT) sampling procedure. This approach is the most suitable method for generating food composition database (Greenfield and Southgate, 2003). Purchased fish samples were immediately kept in ice, within polystyrene boxes, and transported to the laboratory to retain freshness. Upon arrival at the laboratory, the fish were individually measured for their total body weight and length.

3.2.3 Reagents for determination of heavy metals using the inductively coupled plasma-mass spectrometry (ICP-MS).

All chemicals that used were of analytical grade quality. Ultrapure water was obtained from a Millipore water system (Millipore), ultrapure Nitric acid (HNO_3 , Merck) was used to digest the samples. Stock standard solutions of Arsenic and Mercury containing $10\mu\text{g/mL}$ in 2% HNO_3 were procured from Sigma Aldrich, USA, and prepared as per the procedure (Uluozlu *et al.*, 2007). The certified reference materials (CRM) were purchased from National Institute of

Standard Technology (NIST-8436) and used for the standardization and validation of the method.

3.2.4 Reagents for static in-vitro digestion

The following reagents were utilised to determine *in-vitro* digestion: NaCl (Fluka), NaOH (Fluka), KCl (Sigma Aldrich), 1 UK α -amylase (Sigma-aldrich A1031, EC232-565-6, pH 6.9), 250 gm pepsin (Sigma-aldrich P6887, EC 232- 629-3, pH 1.5), pancreatin lipase (Sigma-aldrich type II, EC 232-619-9, pH 7.5), bile extract (Sigma-aldrich B8631, EC232-369-0, pH 7.5), HCl (Merk) and Distilled Water (NWU-AH Laboratory).

3.2.5. Sample preparation and tissue sampling

Common carp samples were stored in ice cooler box and transported to the laboratory. The Common carp were then processed (decapitated, gutted, washed, and filleted). Special care was taken to prevent metal contamination of the samples. All laboratory glassware were soaked in 2 M HNO₃ for 48 h and rinsed several times with distilled water and deionised water prior to use so as to sterilize them. Fish that were small in size were pulverized and the separated skin and the edible portions' weights were recorded. Aliquots of edible tissue were taken for analysis for heavy metals and stored in a freezer at -20 °C till further analysis. Samples of muscles were dissected from each fish on polypropylene dissection boards using stainless steel dissecting equipment. Samples were placed in 25 ml labelled CONSOL glass bottles, and dried prior further analysis.

3.2.6 Heavy metal analysis in fish

3.2.6.1 Microwave digestion.

For the digestion assisted by microwave radiation, the method published by Batista *et al* (2012) was performed with some modifications. In the present study, 0.5g of fish sample were weighed and transferred to the PTFE flask specific for the microwave oven used (ETHOSONE from Milestone, Sorisole, Bergamo, Italy). About 6 mL of HNO₃ and 1 mL of HCl acid were added to the sample in the flask after an overnight pre-digestion step. The following program was run: 1000W at 100 °C (5min. ramp, 5min. holding) and 1000W at 130 °C (3min. ramp, 3min. holding). Before the digestion, the samples were spiked to reach a final concentration of 30.0, 10.0, and 1.0 µg L⁻¹ of arsenic and mercury, respectively, in the final sample solution (volume topped up to 100mL by adding deionised water).

3.2.6.2 Inductively Coupled Plasma Mass Spectroscopy (ICPMS).

The levels of Arsenic and Mercury in fresh water Common carp were determined using ICPMS (Perkin Elmer Elan 9000-USA). For better operating conditions the ICP-MS was adjusted to nebulizer gas flow 0.91 L/min, radio frequency (RF) 1200 W, lens voltage 1.6 V, cool gas 13.0 L/min, and auxiliary gas 0.70 L/min (Noël *et al*, 2005). CRM samples were procured from National Institute of Standard Technology (NIST) and used for method standardization and validation. Standard graphs were drawn. Recovery study was also done.

3.2.7 Determination of digestible fraction

In vitro digestions were done on raw and cooked fish to determine the digestible fraction following methods by Cabañero *et al.* (2004) and Goñi *et al.*, (2006). The fish was divided into

five treatment groups, with six fish per group. The first group comprised the raw. The other two groups were fried and boiled. The skin of the fish was not removed after cooking. Raw and cooked samples (muscle) were then homogenized in a stainless-steel meat mincer and blender and 50 g test portions was stored at -20 °C and each group was analysed in the same way. A static *in-vitro* digestion model based on the three main gastrointestinal compartments (mouth, stomach and small intestine) were applied here (Maulvault *et al.*, 2011). Figure 3.1 shows the digestion process while Table 3.1 shows the enzymes that were used for the digestion process. Briefly, 0.5 g of cooked sample (in triplicate) was mixed with 2 mL of artificial saliva for 16 hours. Then, 3 mL of gastric juice was added and stirred for 2 hours, then after 4.5 mL of duodenal juice were added then stirred for 6 hours, prior to adding 2.5 mL of bile juice, being the final extract digested for 6 hours. The whole process was carried out in a rotating (60 rpm) incubator (Mettler W1314, Germany) at 37 °C. By the end of the whole process, the digestion was slowed down by lowering the temperature to 4 °C, and the bio-accessible fraction (i.e. supernatant) was separated from the non-digested fraction (i.e. pellet) by centrifuging at 10,000 *g* for 10 min at 4 °C (Sigma 3k30, Postfach, Germany). Raw and cooked fish samples were analysed for arsenic and mercury and the metals contents were expressed as g/g wet wt. of fresh Common carp muscle tissues.

3.2.8 Quantification of total As in raw and digested fish

Each sample (~0.5 g) was treated with 5 mL of 65% Suprapur HNO₃ (Merck, Darmstadt, Germany) in hermetic Teflon vessels. Samples were kept for 8 h at room temperature, being subsequently heated to 80 °C for 8 h. After cooling, the solutions were filtered and made up to 25 mL with ultrapure water. The concentrations of As was determined by inductively coupled

plasma-mass spectrometry (ICP-MS, Perkin–Elmer Elan 6000). The accuracy of the instrumental method and analytical procedure were checked by assessing the levels of As in duplicate per sample, as well as by using reference materials for trace elements (TORT-2 Lobster hepatopancreas, NRC, Canada). Rhodium was used as internal standard. Replicate measurements were performed. Uncertainty sources were identified and quantified according to the ISO 17025:2005 norm.

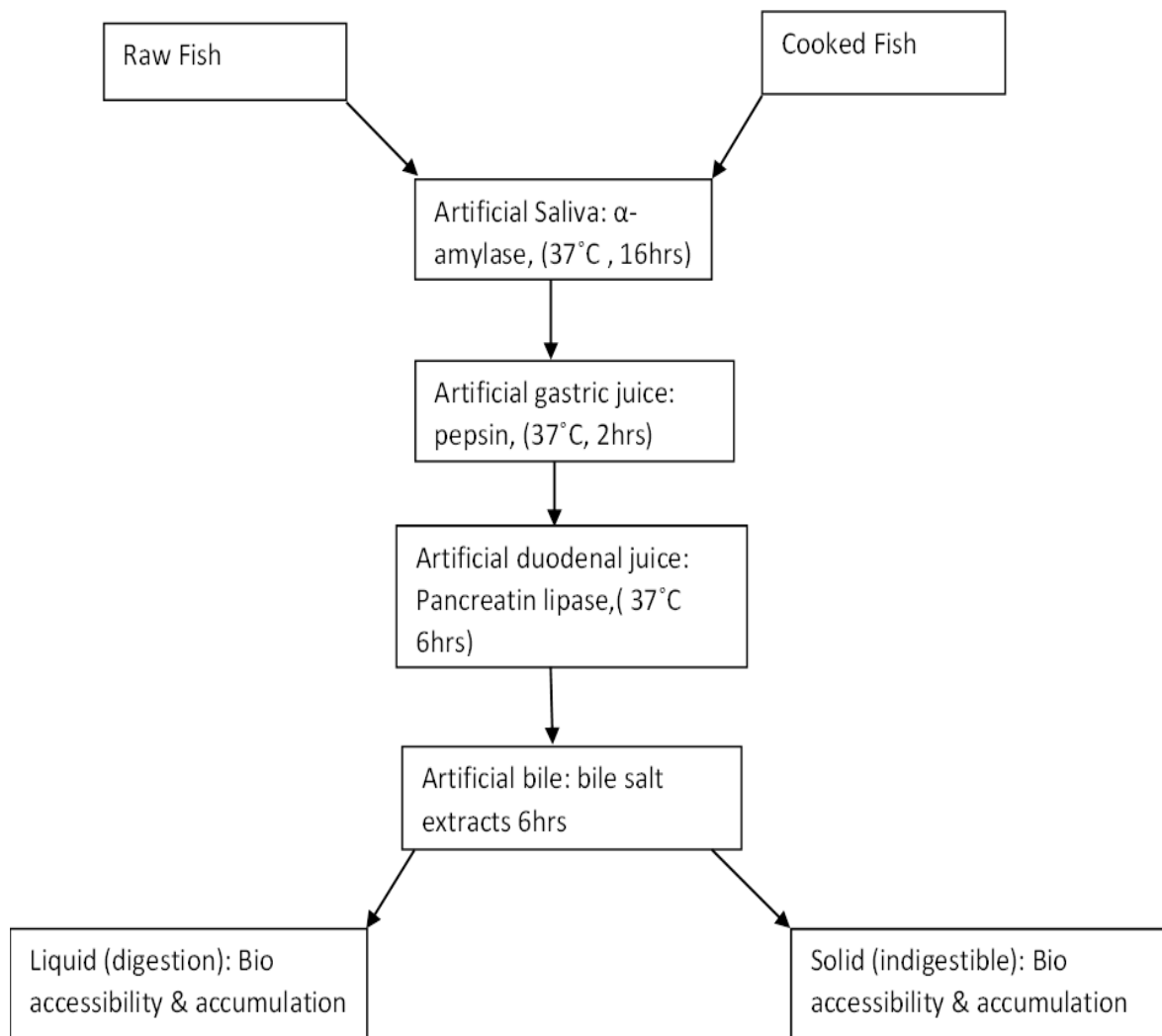


Figure 3. 1: Procedure for analysing the digestible fraction

Table 3. 1: Detailed information of the enzymes that were used for *in-vitro* digestion

Enzymes	Buffer	Fish (0.5g)	
		Concentration(M)/ml	Volume(ml)
α -amylase	Na ₂ HPO ₄	0.1M	2.0ml
Pepsin	KCl & HCl	1.0M	2.5ml
Pancreatin	Na ₂ HPO ₄	0.1M	4.5ml
Lipase	Na ₂ HPO ₄	0.1M	4.5ml
bile salt extract	Na ₂ HPO ₄	0.1M	3.0ml

3.2.9 Quantification of total Hg in raw and digested fish

Mercury concentrations in fish samples were quantified by ICP-MS, using an automatic Hg analyser (AMA 254, Leco, St. Joseph, MI, USA). Briefly, for total Hg determination, 10–20 mg of solid sample or 100–200 µL of liquid sample was placed on a sample boat of the automatic analyser. After their drying and combustion, samples undergo to amalgamation at 700 °C. The elemental mercury (Hg^0) was pre-concentrated, released and detected at a wavelength of 254 nm. Mercury concentrations were calculated from linear calibration with a 1000 mg/L Hg (II) nitrate standard solution (Merck, Darmstadt, Germany) diluted in nitric acid (0.5 mol/L, Merck) at concentrations ranging between 0.10 and 40 ng of Hg. For the quantification of organic Hg (MeHg), fresh water Common carp samples (~150 mg freeze-dried solid sample or 150 µL of liquid sample) was hydrolysed in hydrobromic acid (10 mL, 47% w/w, Merck), followed by MeHg extraction with toluene (Merck). Duplicate measurements of the analyses were performed, blank samples were also analysed in the same conditions as the samples.

3.2.10 Quality assurance

Blanks were prepared by loading the enzyme treatment in series without fish samples. The blank levels were less than LOD. To test the recovery, Heavy Metals (HMs) standards were spiked into fish in pre-analysis. The recovery was analyzed by calculating the ratio of the sum of digestible and indigestible portions to the total concentration.

3.2.11 Calculation of bioaccessibility

Bio accessibility of chemicals in this study can be defined as the fraction of the contaminants mobilized into the digestive juices from food which would be available for absorption through

the small intestine (Oomen et al 2002). The bioaccessibility (% BA) of HMs was calculated as the ratio of the concentration of HMs in liquid phase to that in the sum of liquid and solid, which could minimize the influence of loss during the digestion process.

$$\%BA = \frac{\text{BA extracted HMs}}{\text{total HMs}} \times 100\%$$

BA = Bioaccessibility

HMs = Heavy metals

% = Percentage

3.2.12 Statistical analysis

Analysis of Variance (ANOVA) was used to statistical significance of the different cooking methods on bioaccumulation and bioaccessibility of concentrations of heavy metals in the fish samples. The probability differences (**PDIFF**) option of SAS (2010) was used to perform pairwise comparisons of the least square means.

3.3. Results

3.3.1 Bioaccumulation of Arsenic and Hg concentrations in fish.

The concentrations of As and Hg in raw and cooked Common carp samples are presented in Table 3.2. From the results, cooking method had a significant ($P < 0.05$) effect on both As and Hg concentrations (Figure 3.2 and 3.3). In this study, the As heavy metal's mean concentrations for boiled fish were significantly higher compared to those of raw fish and fried fish concentration means. The concentrations were in the following order: concentration of As in boiled fish > As in fried fish > As in raw fish. Mercury heavy metal concentration for fried fish was significantly higher compared to those concentrations of raw fish and boiled fish

concentrations. The Hg concentrations were in the following order: Hg in fried fish> Hg in boiled fish> Hg in raw fish.

Table 3. 2: The As and Hg concentrations in Common carp muscles (Mean $\pm$ SE)

Parameters	Fish Muscle Samples			South African standard limits (DOH,2004)
	Raw fish	Boiled fish	Fried fish	
Weight (g)	331.93 $\pm$ 100.63	331.93 $\pm$ 100.63	331.93 $\pm$ 100.63	
Length (cm)	25.01 $\pm$ 1.43	25.01 $\pm$ 1.43	25.01 $\pm$ 1.43	
As (ppm)	0.10 $\pm$ 0.07	0.98 $\pm$ 0.07	0.13 $\pm$ 0.07	3.0 mg/kg or ppm
Hg (ppm)	0.01 $\pm$ 88.13	0.13 $\pm$ 88.13	741.89 $\pm$ 88.13	1.0 mg/kg or ppm
As: Wt index	32.53	324.63	42.82	
Hg:Wt index	2.66	43.48	246,255.63	

The Hg mean concentration for boiled fish was significantly ($P < 0.05$) higher than that of raw fish.

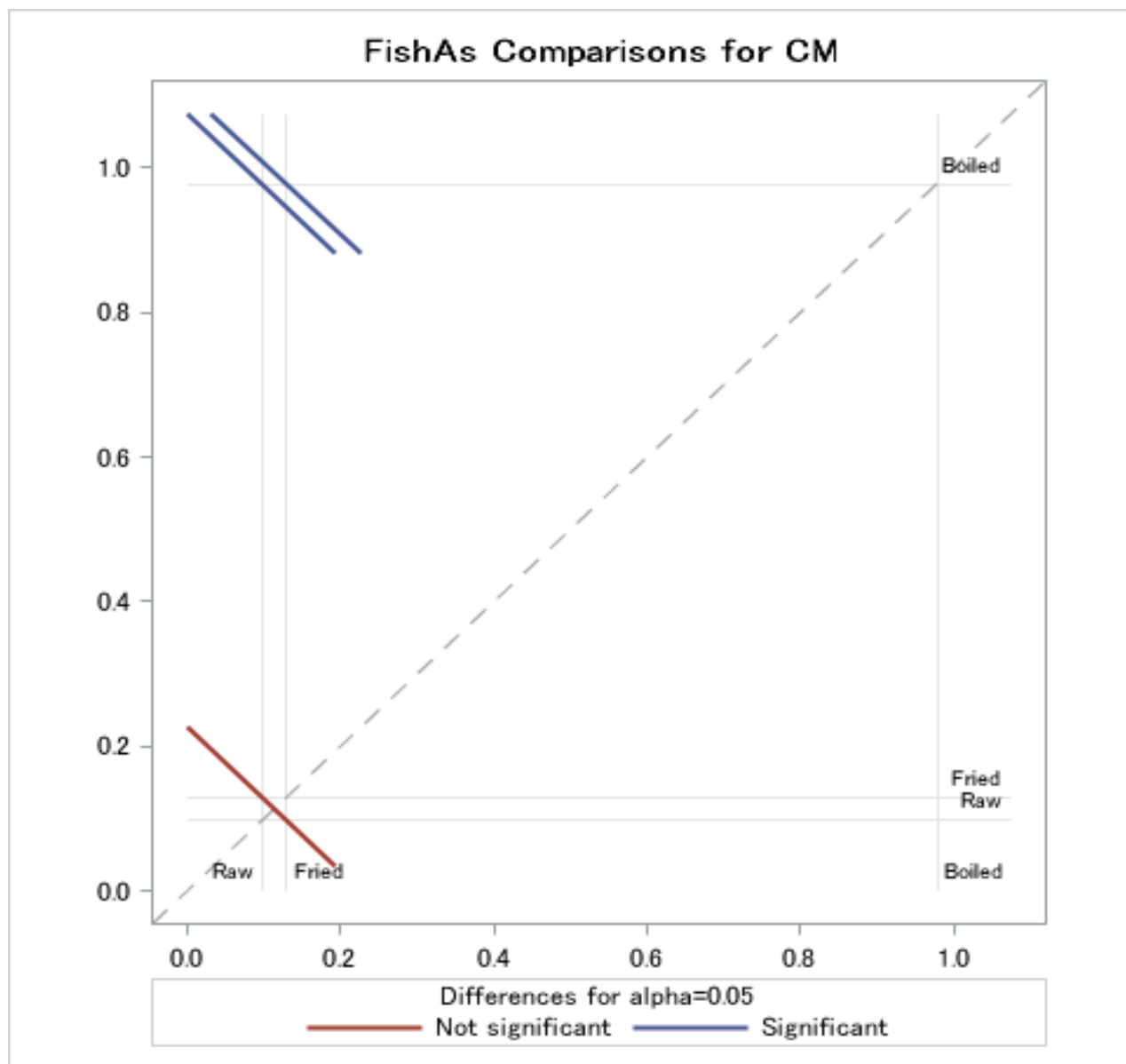


Figure 3. 2: Comparison of As concentration in raw and cooked Common carp muscles

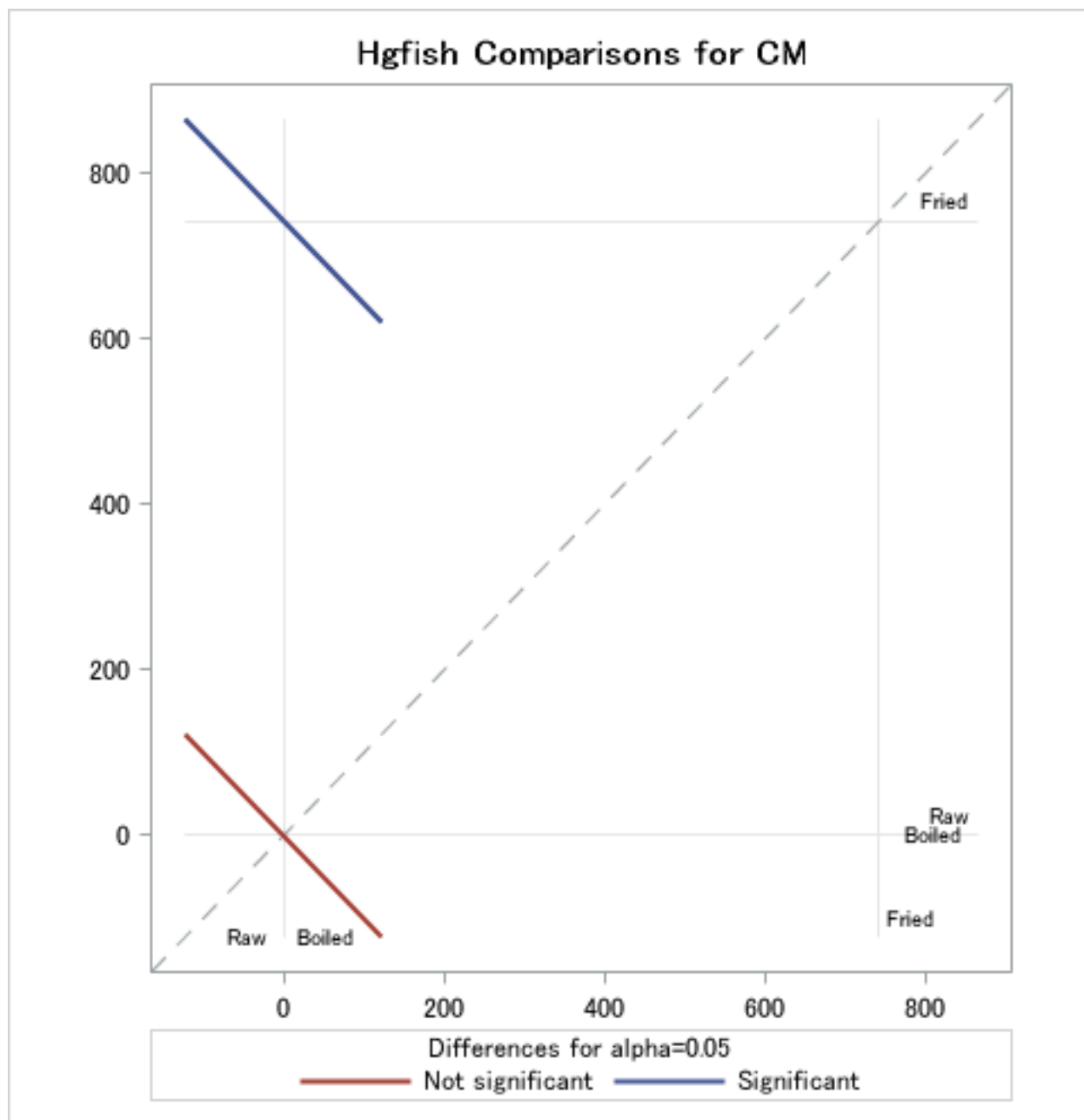


Figure 3. 3: Comparison of Hg concentrations in raw and cooked Common carp muscles

3.3.2 Bio-accessibility of As and Hg fish samples.

Table 3.3 shows the solubilisation of As and Hg after the static *in-vitro* digestion of Common carp found in Rustenburg dam. From the results, the cooking method had a significant effect ($P < 0.05$) on bio accessibility. The bio-accessible fractions of As concentrations were significantly higher in the fried fish (4.240) followed by the boiled fish (Figure 3.4 and 3.5).

Table 3. 3: The bioaccessible fraction of As and Hg of fish muscle tissues on static in-vitro digestion (Mean $\pm$ SE)

Parameters	Raw fish	Boiled fish	Fried fish	Standard limits
Enzyme digested	0.93 $\pm$ 0.24	4.24 $\pm$ 0.24	1.39 $\pm$ 0.24	3.0 mg/kg or
As				ppm
Enzyme digested	0.01 $\pm$ 0.06	0.27 $\pm$ 0.06	0.22 $\pm$ 0.06	1.0 mg/kg or
Hg				ppm

The raw fish had the lowest concentrations of As. The concentrations of As was well below the recommended limits by SA-DOH, except for boiled fish. The digestible Hg concentration for boiled fish obtained in the study was significantly higher ($P < 0.05$) than those of fried and raw fish. However, the Hg bioaccessible fractions for the raw and both cooking methods (boiled and fried) were below recommended levels of the DOH South African standard limit for Hg concentration in fish and fish after static *in-vitro* digestion.

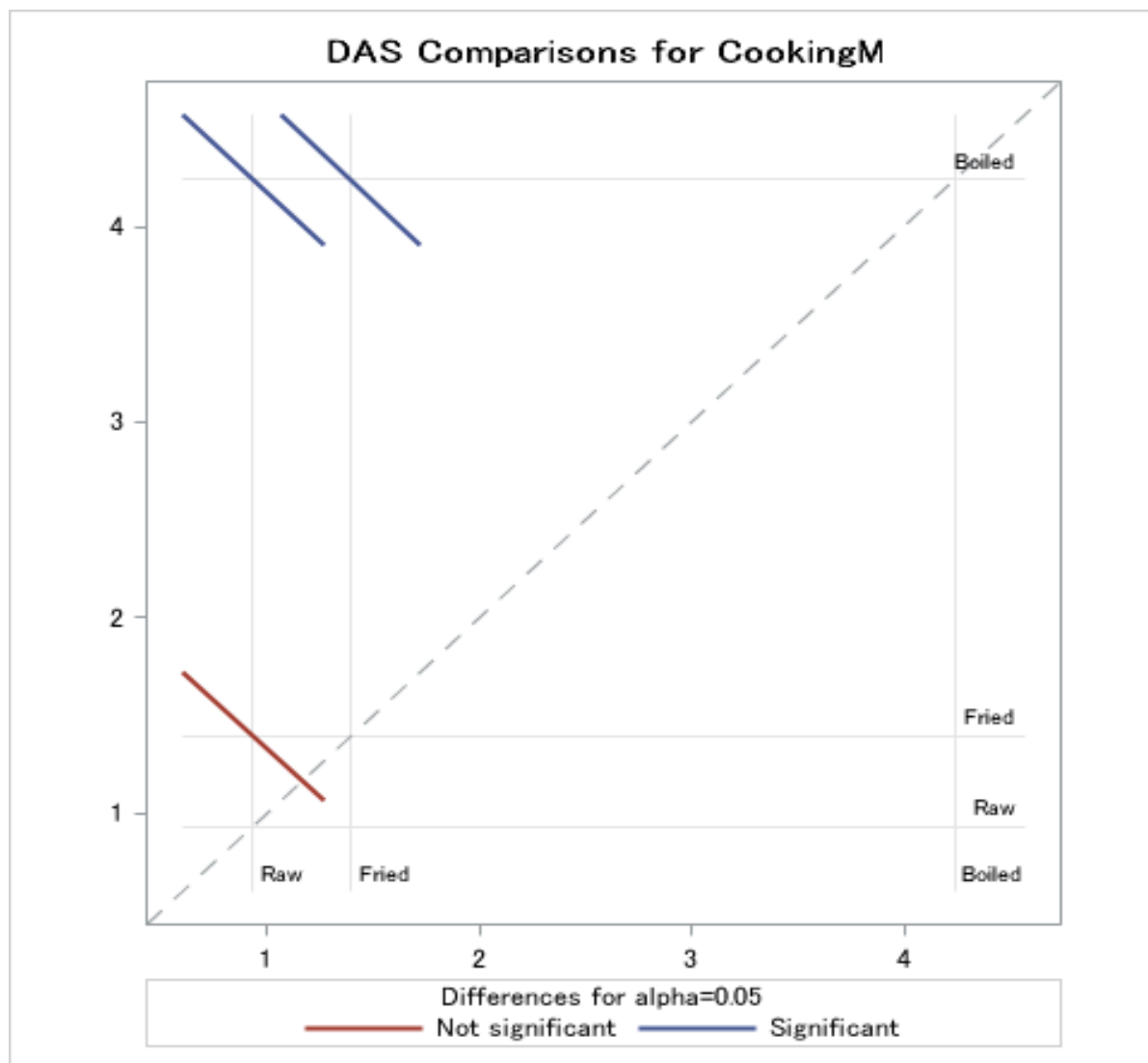


Figure 3. 4: Comparison of bio-accessible fractions of As in raw and cooked fish muscles after static *in-vitro* digestion

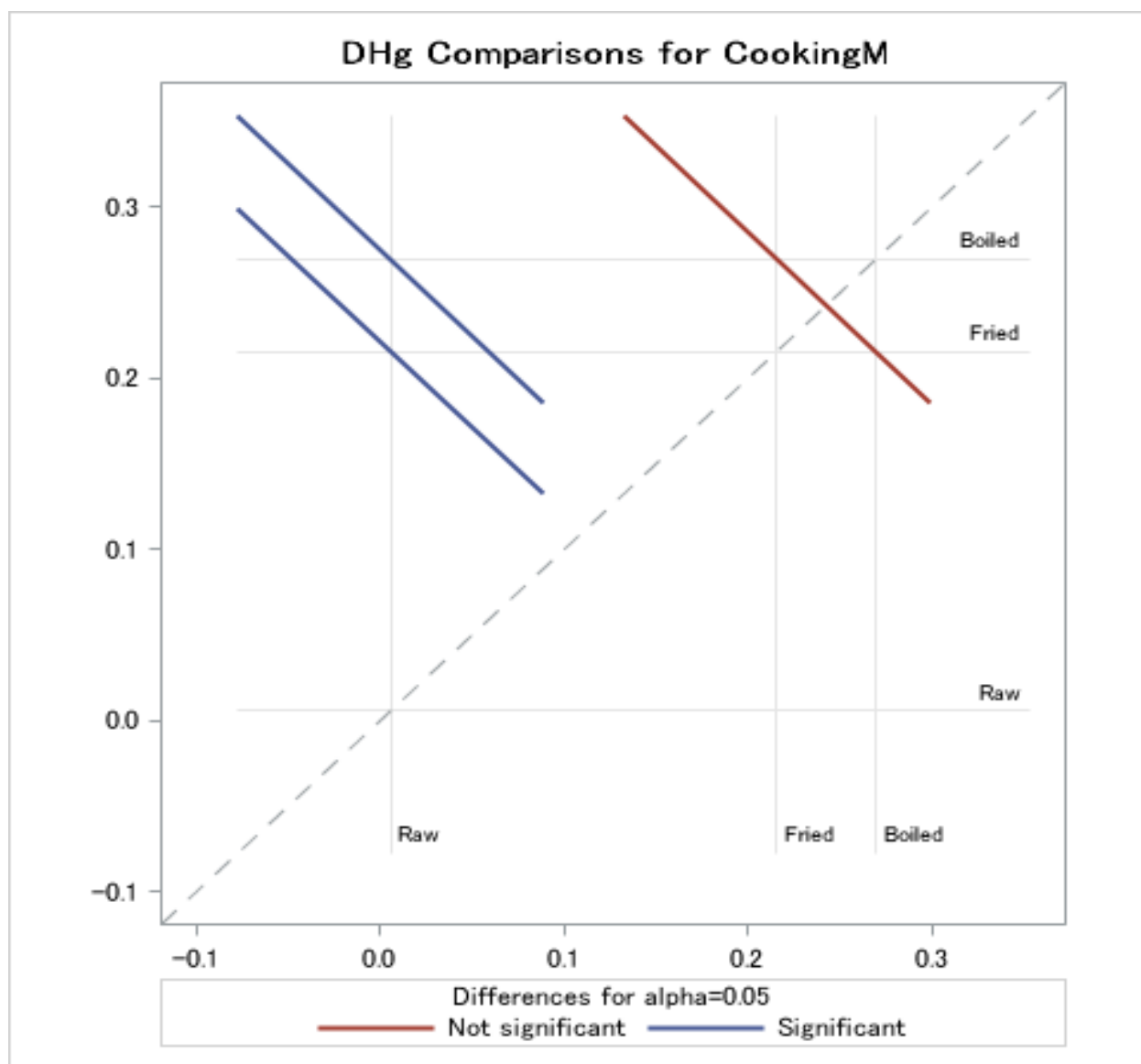


Figure 3. 5: Comparison of bio- accessible fractions of Hg in raw and cooked fish muscles after static *in-vitro* digestion

3.3.3. Differences between acids digestion and static *in-vitro* digestion.

The effects of different digestion method's bioaccumulation and bioaccessibility of As and Hg using three different methods (raw, boiled and fried) are presented in Table 3.4. The concentration of As in acid digested boiled fish (0.943 ppm) was significantly less than that of static *in-vitro* digested fish samples (4.240 ppm). Similarly, the As concentrations in acid digested fried fish and raw fish samples were significantly lower than those of static *in-vitro* digested fish samples. With regards to Hg, acid digested boiled fish had lower ($P < 0.05$) concentration compared to the static *in-vitro* digested boiled fish (Table 3.4). However, the Hg concentration of acid digested fried fish samples was higher than that of static *in-vitro* digested fried fish. The Hg concentrations of acid digested raw fish and static *in-vitro* digested raw fish were similar.

Table 3. 4: Comparison between Acid and Static *in-vitro* digestion methods on As and Hg on Common carp muscles (Mean $\pm$ SE).

Parameters	Boiled fish	Fried fish	Raw fish	Standard limits
Acid digested As	0.94 $\pm$ 0.10	0.09 $\pm$ 0.10	0.10 $\pm$ 0.10	3.0 mg/kg or ppm
Static <i>in-vitro</i> digested As	4.24 $\pm$ 0.24	1.39 $\pm$ 0.24	0.93 $\pm$ 0.24	
Difference between acid and Static <i>in-vitro</i> digestion	3.42 $\pm$ 0.24	1.34 $\pm$ 0.24	0.89 $\pm$ 0.24	
Acid digested Hg	0.15 $\pm$ 79.90	302.14 $\pm$ 79.90	0.01 $\pm$ 79.90	1.0 mg/kg or ppm
Static <i>in-vitro</i> digested Hg	0.27 $\pm$ 0.06	0.22 $\pm$ 0.06	0.01 $\pm$ 0.06	
Difference between acid and Static <i>in-vitro</i> digestion	0.22 $\pm$ 79.89	302.32 $\pm$ 79.89	0.01 $\pm$ 79.89	

3.4 Discussion

3.4.1 As concentration due to different cooking methods

Bioaccumulation is a process whereby toxic substances are absorbed from the aqueous system via gills or via ingestion of tainted feed by organisms. Aquatic species gather high levels of metals, with fish being the most exposed in terms of natural and chemical substance accumulation as the accumulation process of these contaminants rely on the fish's fat content, feeding behaviour and age (Jorgenson, 2001; Eneji *et al.*, 2011; Hien *et al.*, 2012). Arsenic has been frequently classified in the health data base as the most poisonous metal due to its ability to cause severe toxic effects to consumers (ATSDR, 2015). Vu *et al* (2017) reported that As contamination in foodstuff can appear in organic and inorganic forms and these contaminations can be a result of human activities or natural occurrences. The standard limit concentration of As set by South African Department of Health (SA-DOH) is 3.0 ppm on a wet weight basis in fish and fish products. Thus the concentrations found in this study were well below the recommended limits by SA-DOH.

The concentrations of As found in the current study in raw fish, boiled fish and fried fish were lower than the threshold levels set by the South African department of health (DOH) for fish (DOH, 2004). This suggests that the fish from this river is still considered to be safe for human consumption without risk of toxicity in consumers. In the current study, an increase in the concentrations of heavy metals was observed after culinary procedures (boiling and frying), which is in agreement with observations in a study by Ackurt (1991). Similarly, Kalogeropoulos *et al.* (2012) also reported an increase in the levels of elements in fish and shellfish when culinary processes were applied on raw fish in this order: fried > grilled > raw. Furthermore, Ersoy *et al.* (2006) showed that cooking of fish resulted in increased arsenic levels. The observed increase in concentrations of heavy metals in the current study could be attributed to the release of fluids and the drip process while frying or boiling as reported elsewhere (Bendini *et al.*, 2007). For example, virgin olive oil polyphenols might attach to elemental ions available in fish, thus resulting in high element concentration in fried than in raw fish (Kalogeropoulos *et al.*, 2012) as observed in the current study. These researchers also observed that there are some metals that are bound in muscles by complex structures that do not release metals into boiling water and stay bound to the muscle while fish is being cooked (Perello *et al.*, 2008; Maulvaut *et al.*, 2012; Afonso *et al.*, 2016). This observation agrees with the results obtained in the present study showing that As in a boiled fish was higher compared to that of raw fish. Nevertheless, in some investigations it was obtained that culinary treatments have no influence in metal concentrations of fish (Gall *et al.*, 1983; Ackurt., 1991; Steiner *et al.*, 1991; Marti-Cid *et al.*, 2007).

3.4.2 Hg concentrations due to different cooking methods

The acceptable limits concentration of Hg set by South African Department of Health is 1.0 ppm on wet weight basis in fish and fish products. The present results showed that Hg concentrations in raw and boiled fish were well within the acceptable limits for human consumption, while the fried fish exceeded acceptable limits with an extremely high concentration level for Hg. The levels of Hg concentrations obtained in the present study for the raw and boiled fish were below the South African Department of Health standard limits, while the concentration for fried fish exceeded Hg standard limits (DOH, 2004), suggesting that the frying of fish can actually increase the risk of Hg toxicity on consumers. Mercury is a poisonous chemical that is found in the environment and aquatic species are capable of converting it to methylmercury that is even more poisonous, affecting mostly the brain and causing nervous conditions in human adults (Diez, 2008; Monteiro *et al.*, 2009; Crespo-Lopez *et al.*, 2011). Consumption of food with high concentration of Hg has also been observed to results in deafness, blindness, nose bleeding, and general debilitation (Renzoni *et al.*, 1998; Cano-Sancho *et al.*, 2015; Perugini *et al.*, 2016). The concentrations of Hg in acids digested Common carp were in the follow order: fried>boiled>raw which was consistent with the findings by Francisca *et al.* (2014). Some previous researcher obtained results that are in disagreement with the present study, where they found that Hg was decreased in meat after boiling (Fidanza *et al.*, 2002). Other investigations also showed that culinary time and culinary treatments had no effect on the level of Hg after the fish muscles were cooked (Germiachikh *et al.*, 2007; Morgan *et al.*, 1997; Ouedraogo and Amyot., 2011). However, Burger *et al.*, (2003), Perello *et al.*, (2008) and Costa *et al.*, (2016) demonstrated that Hg levels were elevated due to culinary methods. Perugini *et al.*, (2013) also reported high concentration of Hg in boiled samples compared to the raw white meat. They explained that the

elevated levels of Hg in boiled fish muscles were due to the degradation of enzyme astaxanthin and molecules that bind Hg by heat during boiling culinary treatment on a Norwegian lobster fish. Since Hg is well known to be more attracted to protein than lipids (Ouedraogo and Amyot, 2011), high temperature during boiling culinary treatment degrade protein, releasing the protein bound Hg and making it available to mix with inorganic Hg, hence the increase (Bacerra *et al.*, 2014). Kalogeropoulos *et al* (2012) observed that Hg elemental concentration in culinary processed fish and shellfish were greater compared to the uncooked specimens, ranking in this order: fried>grilled>raw. This is in agreement with observations of the current study.

3.4.3 Effect of different cooking methods on Bioaccessibility of As

Bio-accessibility refers to the fraction of a pollutant that is leached from the muscle into solvent by digestive juices. It symbolizes the greatest quantity of pollutant that is accessible for intestinal assimilation by static *in-vitro* digestion (Van de Wiele *et al.*, 2007; Wragg *et al.*, 2011). Kulp *et al* (2003) reported that cooking procedures are essential in determining the As bioaccessible fraction in meat. In the current study, the bio-accessible concentrations of As were in the following order boiled>fried>raw. Similar observations were made by Perello *et al* (2008) and Maulvult *et al* (2011) who reported the elevation of As level when raw fish was fried showing that culinary treatments can have an effect on the As bio accessible fraction. Nevertheless, contrary to the findings of the current study, He and Zheng (2010) observed that boiling decreases the bioaccessibility of As in little sea bass and big sea bream fish specimen due to the fact the resultant protein degradation of fish muscle tissues due to heat cause the formation of muscle fibre complexes that are less available for enzymatic digestion. Although Moreda *et al* (2012) reported that there was no relationship between the amount of fish protein and quantity of

As accessibility, some studies have shown that there are some other factors that can influence the solubility of As on the gastrointestinal tract (GIT) digestion process such as pH, digestion's duration and enzymes (Sun *et al.*, 2012, Rocha *et al.*, 2013). The low pH conditions in the gut degrade protein by weakening and breaking down the peptide bonds (Goodman, 2010). Bioaccessibility techniques that simulate the mouth, stomach and the intestines have shown that there is increased release of protein-bound As as digestion processes occurs (Sun *et al.*, 2012) in the human body. Oomen *et al* (2003) reported that low pH found in the stomach is the main factor that increases As solubility and the presence of As in the GIT stage. The pancreas and bile enzymes are responsible for the digestion of polysaccharides into monosaccharides and to degrade proteins into free amino acids and oligopeptides possessing 6-12 amino acids residues. As a result of this, As elements easily become bioaccessible for uptake through the intestines (Cabanero *et al.*, 2004; Goodman, 2010)

3.4.4 Effect of different cooking methods of Hg bioaccessibility on fish

The bio-accessible fraction of Hg in the raw and cooked fish samples were in the following order: boiled>fried>raw. The Hg quantity found from fish muscle tissues (raw, boiled and fried) in this study was lower than 1.0 ppm and cannot cause any severe pathological impacts to consumers (SA-DOH, 2004). Even if the level of contaminants are below the maximum levels of permissible limits set by the authorities, constant intake of fish can sometimes result in heavy metal build-up and contamination to people (Bosch, 2015). Therefore, people are supposed to design their diet in a way that restricts the fish intake that have elevated metal concentrations and when they eat fish continually they should choose one that has less heavy metal content (OCEANA, 2008). Since culinary treatments elevate the heavy metals bioaccessibility, people

are generally at risk due to the present heat treatment of fish (Marques *et al.*, 2011). The increase of Hg in cooked samples might be due to the alteration of fish proteins by high temperature that hinders the enzyme reaction in static *in-vitro* digestion. (Rooney, 2007). While there was an increase in bio accessible Hg fraction with culinary treatment in the current study, Cano *et al.* (2015) and Matos *et al.* (2015) reported that the cooking process decreased the bioaccessibility of the metals in marine products (methylmercury). The contrasting observations among different studies including the current study might be due to the different physiological and environmental dynamics such as the pollutants, weight, length, age and life stage of the fish used in the different studies (Nussey *et al.*, 2000; Canli and Atli, 2003; Costa *et al.*, 2009). Despite the cooking process, the portion of Hg bioaccessibility was discovered to be less in the mouth of the consumers than in the small intestine with the muscles of the black scabbard (Wang *et al.*, 2013). Fish is digested in the mouth by mastication and enzyme activity (salivary amylase) to digest starches in micro-molecule particles, while the residual proteins and fats are digested in the stomach by pepsin, in the intestine by a mixture of enzymes known as pancreatin and low pH play a role in digestion of proteins coupled with peristaltic movements of the GIT (Hur *et al.*, 2011). Therefore, the variances in bioaccessibility of Hg along the GIT are mostly correlated to the different breaking down techniques utilized, like enzymatic activities and concentration levels, kind of specimen employed, quantity of digestion solution and amount of water (Maulvault *et al.*, 2011). Liard *et al.* (2009) and Torres-Escribano *et al.* (2011) also demonstrated that the bio-accessible portion of Hg can be influenced by mechanism of transfer among the bio-accessible and non-bio-accessible portions. Most of the proteins that bind the Hg are found in marine foodstuff, hence their uptake depends on their solubility as the foodstuff moves along the GIT.

3.5 Conclusion

Results from this investigation have shown that concentrations of As and Hg in Common carp muscles were significantly affected by the different cooking procedures tested. Fried fish muscles had Hg bioaccumulation levels exceeding standard limits, while cooking and enzyme digestion treatments in boiled fish muscles resulted in As bioaccessibility levels that exceeded standard limits. The concentration levels of As and Hg obtained in the study suggest that the consumption of Common carp in the Rustenburg community ought to be monitored. Therefore, it is important to impose strict measures which will monitor the measure of poisonous quality, bioaccessibility and absorption of chemical pollutants in foodstuff. Therefore, the exposure and risk characterisation of the consumption of fish from the polluted Hex River need to be assessed.

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CHAPTER 4

EXPOSURE ASSESSMENT AND RISK CHARACTERIZATION OF COMMON CARP FISH (*CYRIPNUS CARPIO*) CONSUMERS IN THE HEXRIVER CATCHMENT IN RUSTENBURG

Abstract

Fish are the basic supplier of organic proteins and have become a recently popular component of daily dietary intake to the globally rapidly growing population. This study investigated the estimate of human exposure levels to arsenic (As) and mercury (Hg) using the health risk assessment calculations such as estimated daily intake (EDI), target hazard quotient (THQ) and carcinogenic risk (CR) through the consumption of Common carp by the Rustenburg community for both children and adults. The range for consumer health risk parameters were estimated daily intake of As (0.037 to 0.119 for adults and 0.021 to 0.067 for children) and Hg (0.002 to 0.007 for adults and 0.003 to 0.010 for children) with regard to THQ of As (0.125 to 0.389 for adults and 0.070 to 0.224 for children) and Hg (0.002 to 0.008 for adults and 0.003 to 0.011 for children), while for CR of As (0.056 to 0.179 for adults and 0.032 to 0.101 for children) were compared with the acceptable values recommended by South African department of health (SA-DOH) and the United States Environmental Protection Agency (USEPA). The results for THQ and EDI showed that the intake of As and Hg through fish consumption were within the recommended limits, except for CR as both on children and adults were found to be higher than the recommended limits. These suggest that the Rustenburg community might encounter health risk from the consumption of this fish. However, these results must be validated by *in vivo* methods using live animal cells.

4.1 Introduction

Human exposure cases to heavy metals have extensively increased over the years due to rapid growth of industries, urban developments, city sewages and agricultural practises, which have all contributed to the increased pollution of the ecosystem. Consumption of polluted meat from the contaminated food web is therefore one of the root causes of introducing consumers to heavy metals (Ogbomida *et al.*, 2018). Heavy metal exposure of people through the consumption of contaminated fish can also be associated to mining activities (Khemis *et al.*, 2017). It has been observed that the mining activities such as purifying, and refining can introduce heavy metals and metalloids to the water sources in extensive amounts and these are regularly transmitted via rivers or straightforwardly released into the oceans (Akçay and Moon 2004; Altas and Buykgungor, 2007; Cevik *et al.*, 2008). At the point of heavy metals discharge into aquatic environment, natural water conditions such as pH, redox potential, and complexing specialists will determine bioaccumulation of the heavy metals in the water and ultimately in the tissues and organs of fish as they feed and breath (Sekhar *et al.*, 2004; Mendi *et al.*, 2010; Zhang *et al.*, 2015).

Heavy metals entering the aquatic system get bioaccessible to consumers via drinking of contaminated water and through consumption of fish from contaminated water bodies (Jarapala *et al.*, 2014). Being knowledgeable about the source of As and Hg pollution to the environment and the ways in which it can be introduced to the consumers is very important for the assessment of consumer health risk (Li *et al.*, 2017). Few studies have been conducted on the human health risk assessment of heavy metals on consumption of fish from contaminated water bodies in

South Africa, therefore the objectives of the study were to determine the estimated daily intake (EDI), target hazard quotient (THQ) and carcinogenic risk (CR) potential consumer health risk on the consumption of Common carp from Hex river catchment of Rustenburg.

4.2 Materials and Methods

4.2.1 Study site, sample collection and preparation and analysis methods

The description of the study area is explained in Chapter 3.2.1 – 3.7.2, whilst Section 3.8 – 3.13 explain the cooking treatments and bioaccessibility analysis methods and bioaccessibility calculation.

4.2.2 Estimated daily intake (EDI)

To estimate the daily intakes (EDI) of heavy metals by Rustenburg residents via the consumption of freshwater fish, the following equation (EPA, 2000) was used:

$$EDI = C_{fish} \times CR/BW$$

Where C_{fish} is measured metal concentration in a given species of fish; CR is the consumption rate (g/d) and BW the body weight (kg) of consumer. In the present study, BW of 60 kg was used for adults (Cheung *et al.*, 2007) and 15 kg for preschool children (USEPA, 1997), and CR was 142.2 g/d for adult (EPA, 2000) and 50 g/d for children (Leung *et al.*, 2000), respectively.

4.2.3 Non-carcinogenic risk

The target hazard quotient (THQ), which is used to express the risk of non-carcinogenic effects, is the ratio between the EDI and the oral reference dose (RfD, mg/kg bw/day)(EPA, 2000). The RfD represents an estimate of daily exposure to which the human population may be continually

exposed over a lifetime without an appreciable risk of deleterious effects. The RfDs are based on 0.3, and 0.1 (µg/kgbw/day) for inorganic As and inorganic Hg (Heshmati *et al.*, 2017), respectively. The THQ was calculated based on the following equation (EPA, 2000).

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

If the THQ value is less than 1, the exposed population should not experience any adverse health hazard. Conversely, the population may experience non-carcinogenic health risks if the THQ is equal 1, with an increasing probability (of risk) as the value increases. The method for determining THQ was provided in USEPA Region III risk-based concentration table (EPA, 2000). The THQ calculations were made based on the facts that the ingestion dose is equal to the absorbed contaminant dose, cooking has no effect on the contaminants (Means, 1989). and average body weight of an adult is 60 kg (Saha & Zaman, 2013).

4.2.4 Carcinogenic risk

Carcinogenic risk (CR) indicates an incremental probability of an individual of developing cancer over a lifetime due to exposure to a potential carcinogen. Cancer risk over a lifetime exposure to As was obtained using cancer slope factor (CSF), provided by USEPA (EPA, 2000). The equation used for estimation of the cancer risk is as follows (EPA, 2000).

$$CR = CSF \times EDI$$

Where CSF is the carcinogenic slope factor of 1.5 (mg/kg/day) set by USEPA only for inorganic As. EDI is the estimated daily intake of heavy metals. The acceptable lifetime carcinogenic risk considered by USEPA is 10^{-5} (risk of developing cancer over a human lifetime is 1 in 100,000), which is applied in this study.

4.2.5 Statistical analysis

The effect of culinary procedures on EDI, THQ and CR values was evaluated using the Proc Glm procedures of SAS (2010). Differences among the culinary procedures were determined using the PDIFF option of SAS (2010) at $\alpha = 0.05$ level of significance.

4.3 Results

4.3.1 Estimated daily intake (EDI)

The mean estimated daily intake (EDI) of As and Hg from consumption of the raw and cooked samples are presented in Table 4.1. From the results, cooking methods had a significant ($p < 0.05$) effect on EDI of As and Hg for both adults and children. In this study the boiled fish had significantly higher EDIs of heavy metals compared to the raw fish and fried fish for both adult and children respectively (Figure 4.1 and 4.2).

Table 4. 1: Estimated daily intake (EDI) of As and Hg in Common carp for adults and children in the Hex River Catchment in Rustenburg (Mean $\pm$ SE)

Parameters	Raw fish	Boiled fish	Fried fish
As (Adults)	0.037 $\pm$ 0.005	0.119 $\pm$ 0.005	0.068 $\pm$ 0.005
As (Children)	0.021 $\pm$ 0.003	0.067 $\pm$ 0.005	0.038 $\pm$ 0.005
Hg (Adults)	0.002 $\pm$ 0.001	0.007 $\pm$ 0.001	0.006 $\pm$ 0.001
Hg (Children)	0.003 $\pm$ 0.002	0.010 $\pm$ 0.002	0.009 $\pm$ 0.002

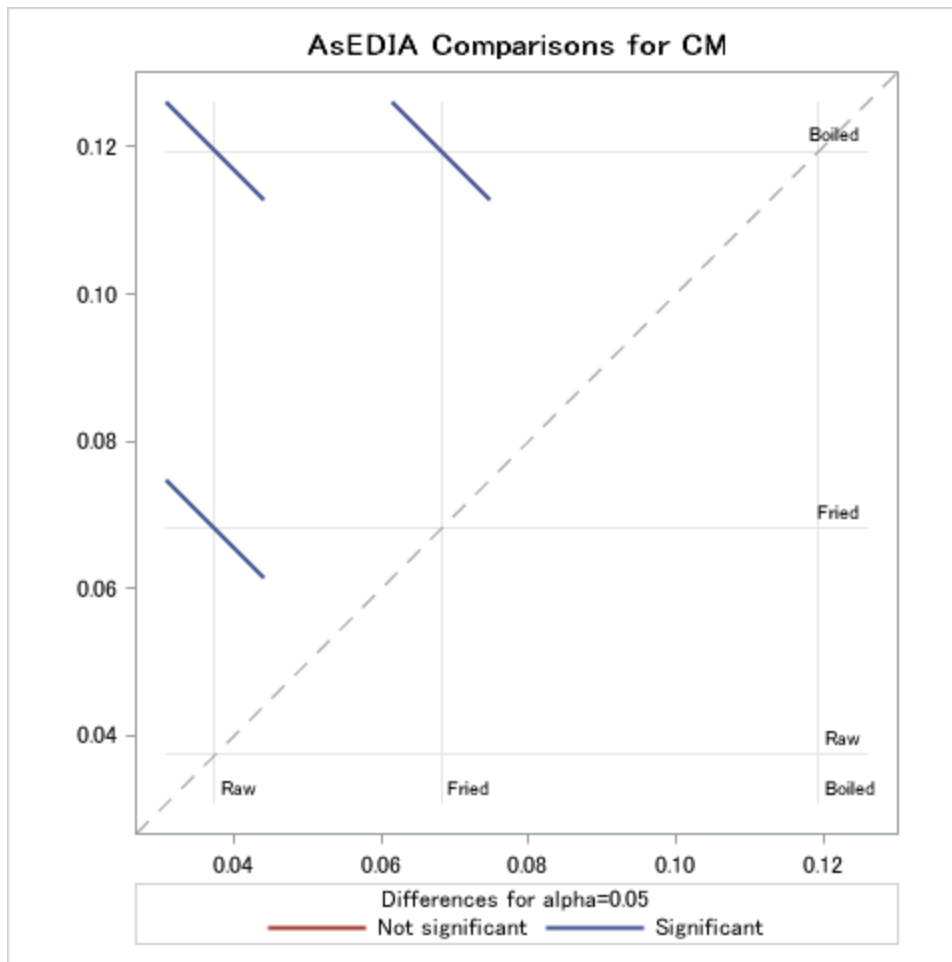


Figure 4. 1: Comparison of EDIs of the As in adult population/groups in raw and cooked Common carp muscles

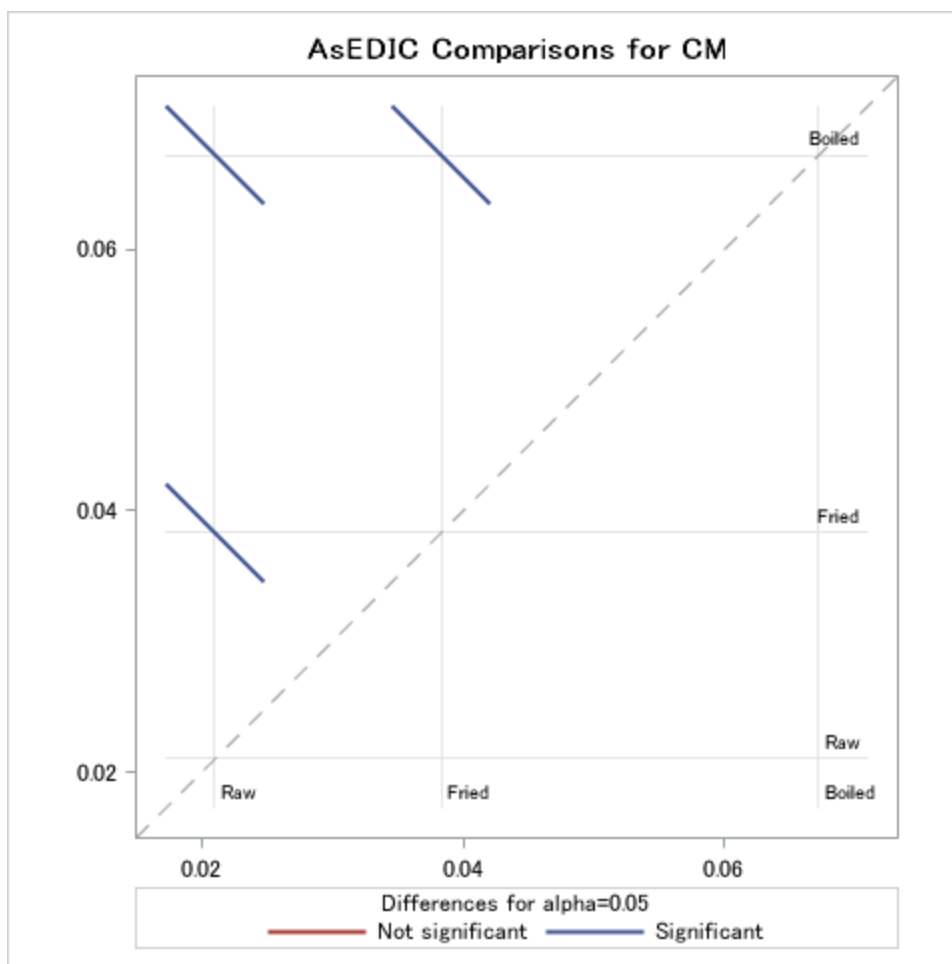


Figure 4. 2: Comparison of EDIs of the As in children in raw and cooked Common carp muscles

The mean EDI for As ranged from 0.037 to 0.119 g Kg⁻¹ BW day⁻¹ for adults, and 0.021 to 0.067 g Kg⁻¹ BW day⁻¹ for children. The As recommended daily intake set by joint FAO/WHO expert committee on food additives (JECFA) is 0.13 g/kg BW for fish and fish products. The current results showed that As EDI in both raw and cooked fish were within the recommended daily intake. Hg EDI mean for boiled fish was significantly higher compared to those of raw fish and fried fish for both adults and children respectively (Figure 4.3 and 4.4). The mean EDI for Hg ranged from 0.002 to 0.007 g Kg⁻¹ BW day⁻¹ for adults and 0.003 to 0.010 g Kg⁻¹ BW day⁻¹ for children.

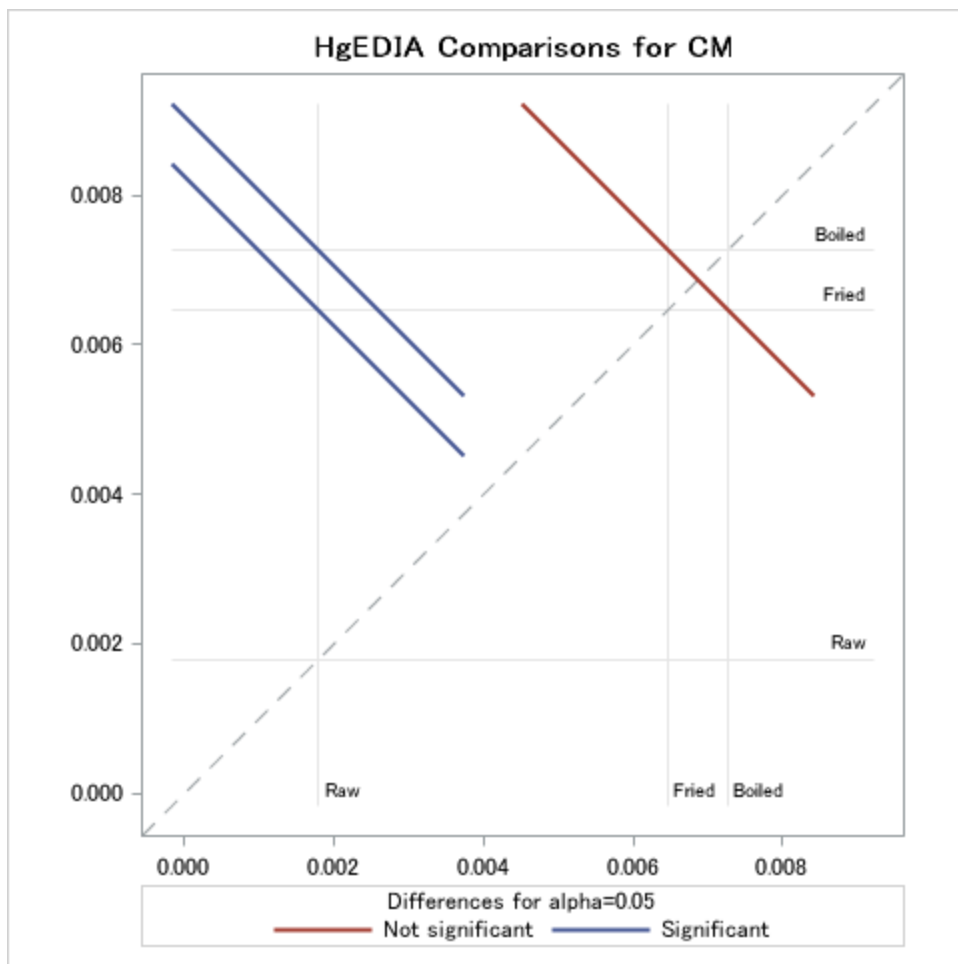


Figure 4. 3: Comparison of EDIs of the Hg in adult population/groups in raw and cooked Common carp muscles

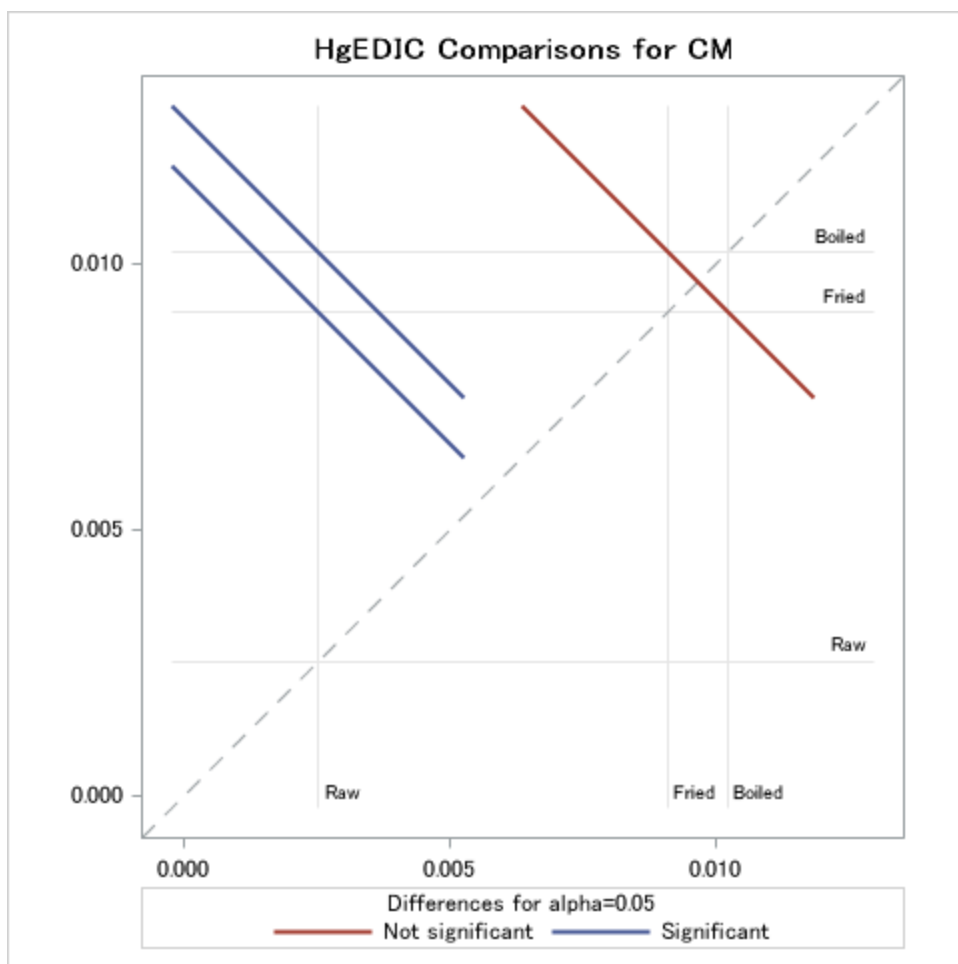


Figure 4. 4: Comparison of EDIs of the As in children in raw and cooked Common carp muscles

4.3.2 Target Hazard Quotient

Table 4.2 presents the calculated target hazard quotient (THQ) of As and Hg for adults and children consuming Common carp fish. From the results cooking methods had a significant effect ($P < 0.05$) on both As and Hg THQs for adult and children (Figures 4.5 and 4.6). The mean THQ of As was significantly higher in boiled fish followed by fried fish. The raw fish had the lowest mean THQ of As for both adult and children.

Table 4. 2: Target hazard quotient (THQ) of As and Hg in Common carp for Adults and Children in the Hex River Catchment in Rustenburg (**Mean $\pm$ SE**)

Parameters	Raw fish	Boiled fish	Fried fish
As (Adults)	0.13 $\pm$ 15.90	0.39 $\pm$ 15.90	0.23 $\pm$ 15.90
As (Children)	0.07 $\pm$ 8.94	0.22 $\pm$ 8.94	0.13 $\pm$ 8.94
Hg (Adults)	0.002 $\pm$ 4.651	0.008 $\pm$ 4.651	0.007 $\pm$ 4.651
Hg (Children)	0.003 $\pm$ 6.542	0.011 $\pm$ 6.542	0.010 $\pm$ 6.542

The mean THQs of As was less than 1, varying from 0.125 to 0.389 for adults and 0.070 to 0.224 for children. Hg mean THQs for boiled fish samples obtained for both adults and children in this study were significantly higher ($P < 0.05$) compared to those of fried and raw fish and varied from 0.002 to 0.008 for adults and 0.003 to 0.011 for children (Figures 4.7 and 4.8). However, the mean THQ of Hg for both raw and cooked fish of less than 1 were within the range recommended by USPEA.

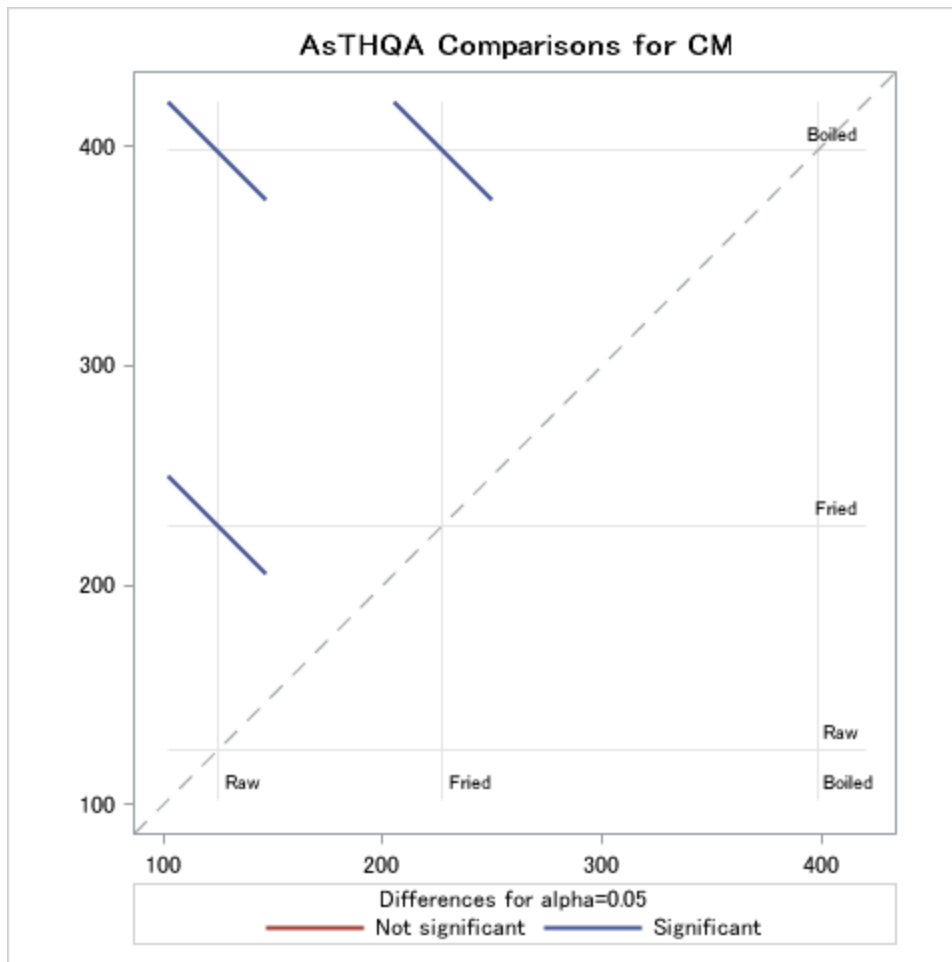


Figure 4. 5: Comparison of THQ values of the As in adults in raw and cooked Common carp muscles

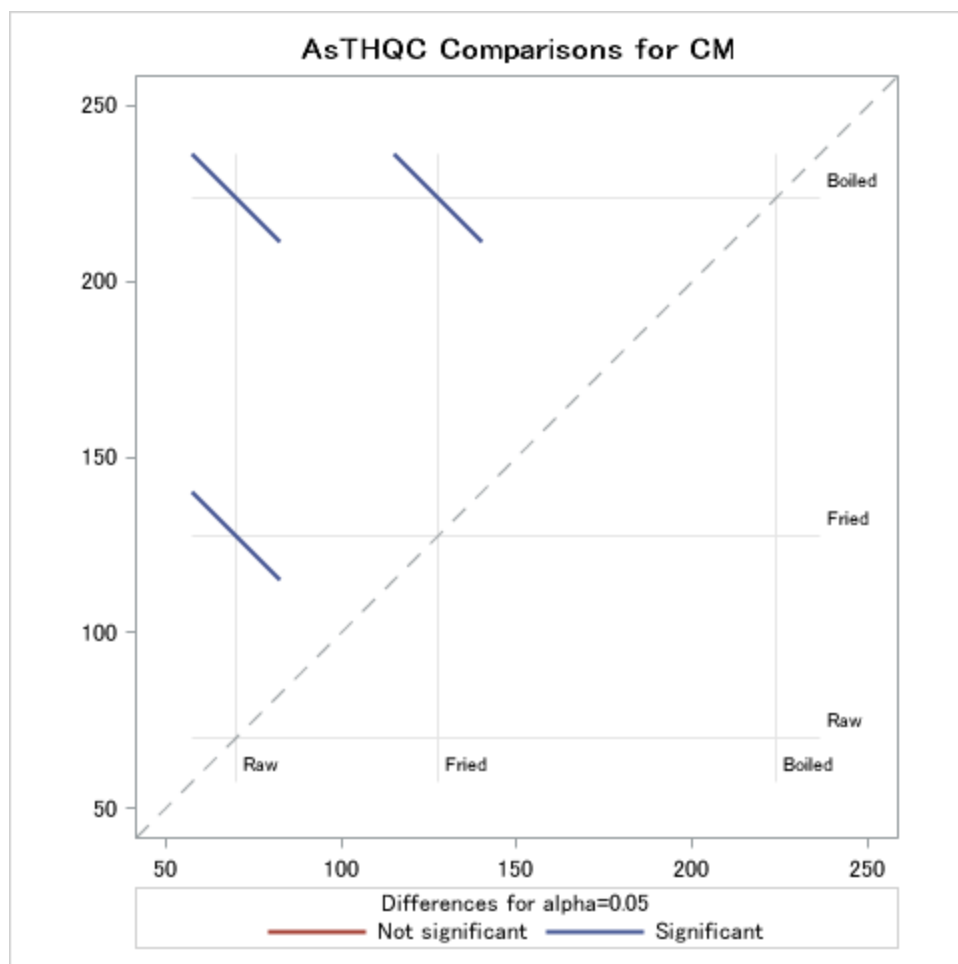


Figure 4. 6: Comparison of THQ values of the As in children in raw and cooked Common carp muscles

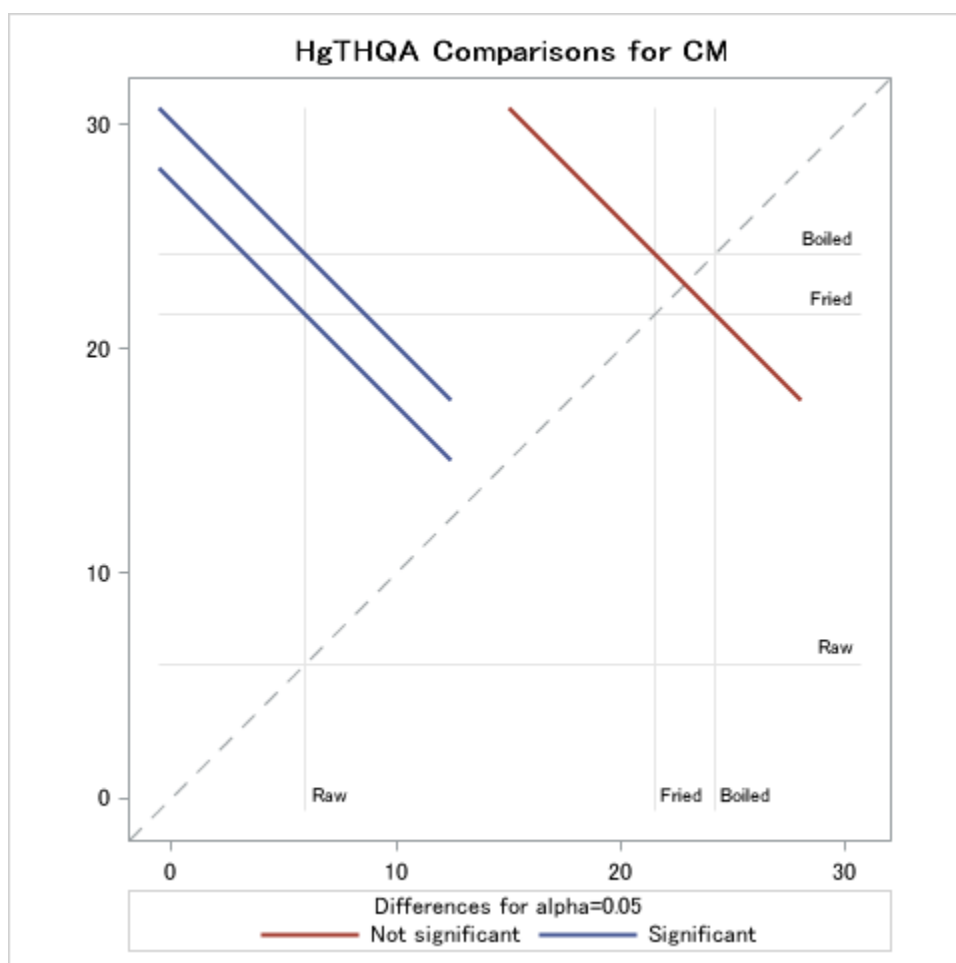


Figure 4. 7: Comparison of THQ values of the Hg in adults in raw and cooked Common carp muscles

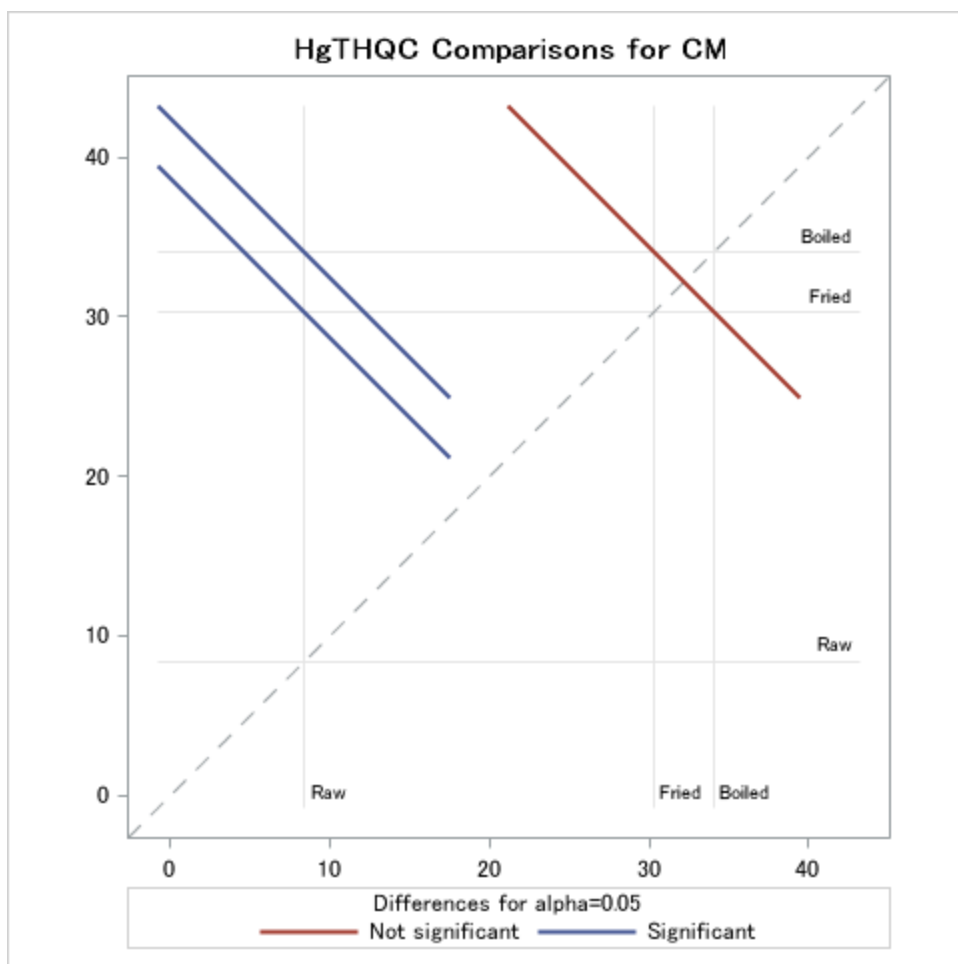


Figure 4. 8: Comparison of THQ values of the Hg in children in raw and cooked Common carp muscles

4.3.3 Carcinogenic risk

The mean carcinogenic risk (CR) of As for adults and children are presented in Table 4.3. Carcinogenic risk for Hg was not calculated since Hg currently does not have a predetermined cancer slope factor (CSF). The mean CR level of As for boiled fish was observed to be significantly higher than those of raw and boiled fish in both adults and children respectively (Figures 4.9 and 4.10). Mean CR levels for As in adults and children ranged from 0.056 to 0.179 $\text{g Kg}^{-1} \text{ day}^{-1}$ and 0.032 to 0.101 $\text{g Kg}^{-1} \text{ day}^{-1}$. The standard limit set by the South African government is 10^{-5} (0.00001) based on fish and fish products. All the results obtained in this study for raw, boiled and fried fish exceeded the standard limit for CR in both adults and children.

Table 4. 3: Carcinogenic risk (CR) of arsenic (As) in Common carp for Adults and Children in the Hex River Catchment in Rustenburg (**Mean $\pm$ SE**)

Parameters	Raw fish	Boiled fish	Fried fish
As (Adults)	0.06 $\pm$ 0.007	0.18 $\pm$ 0.007	0.10 $\pm$ 0.007
As (Children)	0.03 $\pm$ 0.004	0.10 $\pm$ 0.004	0.06 $\pm$ 0.004

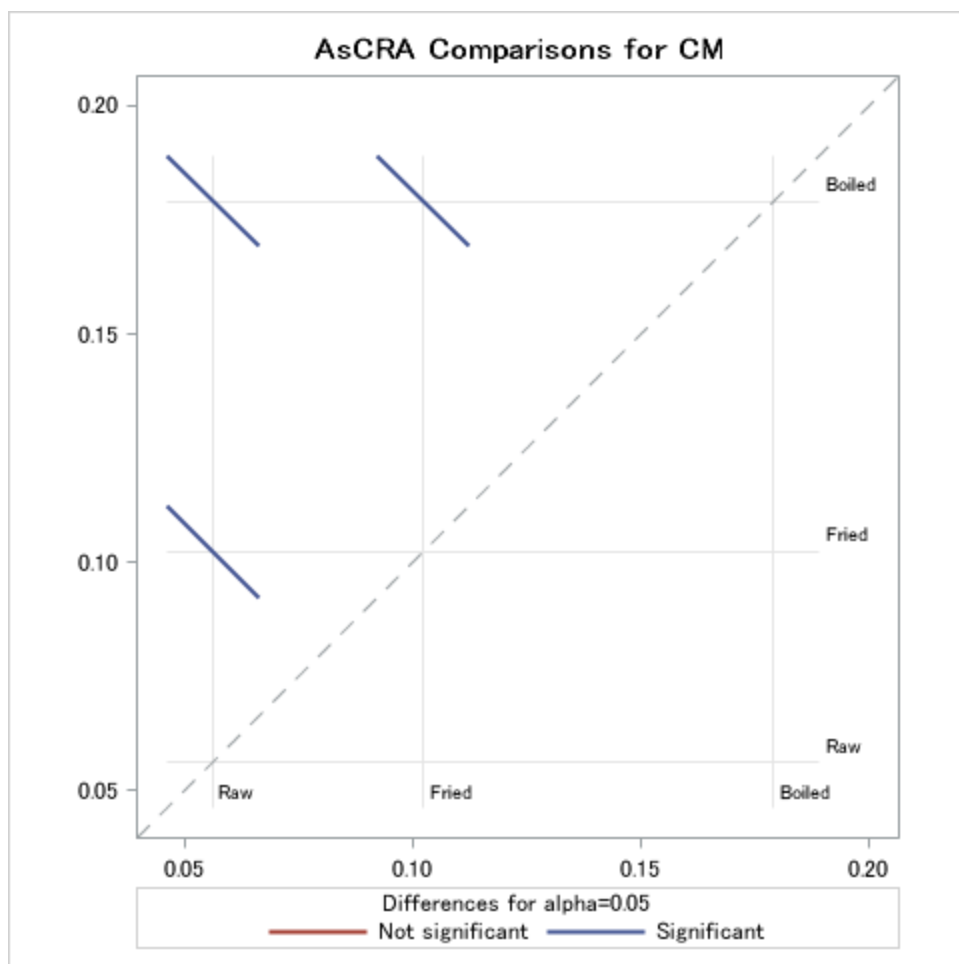


Figure 4. 9: Comparison of CR values of the As in adults in raw and cooked Common carp muscles

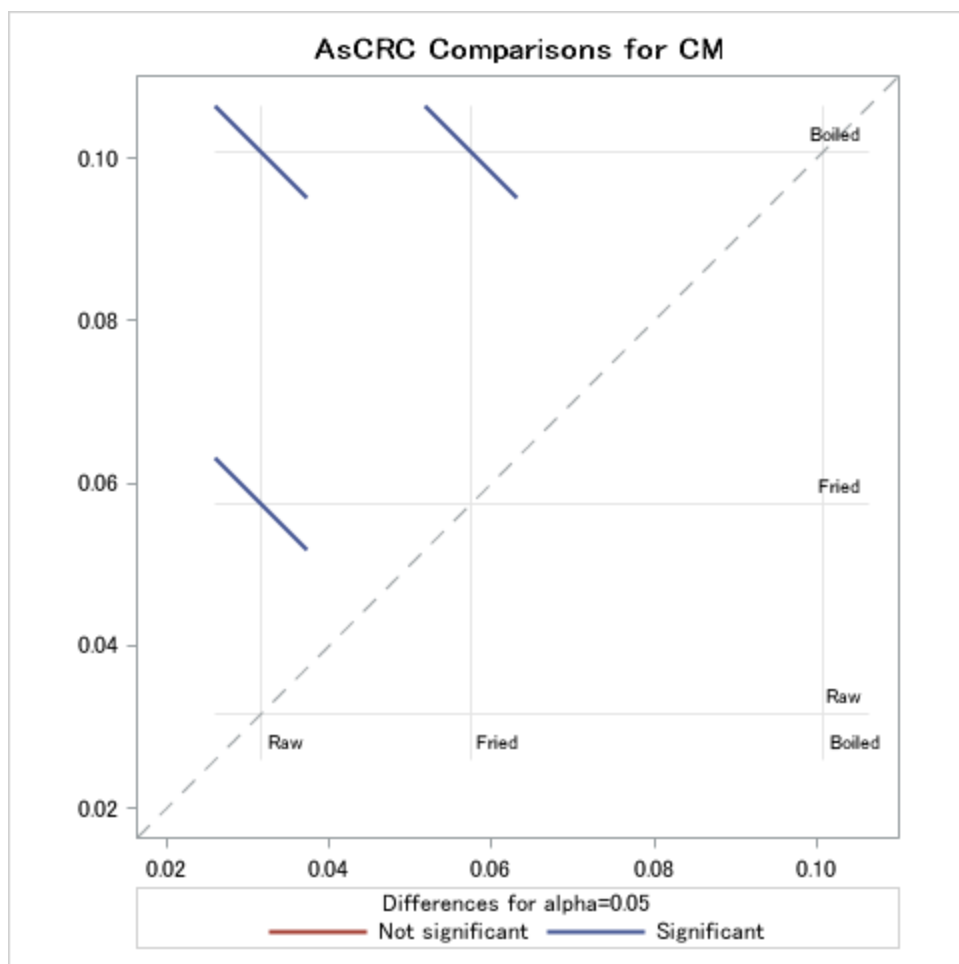


Figure 4. 10: Comparison of CR values of the As in children in raw and cooked Common carp muscles

4.4 Discussion

4.4.1. Estimated Daily Intake

The estimated daily intake for As and Hg were calculated by multiplying heavy metal concentration (g Kg^{-1}) in fish muscle with different consumption rate (g day^{-1}) for adults and preschool children divided by body weight (BW) for adults and preschool children. Rahman *et al.* (2008) reported that there are various ways for consumers to be susceptible to arsenic poisoning, the main routes being through eating, dermal contact and inhalation of contaminated substance with eating being considered as the main predisposing factor (Mondal and polya, 2008). Many scholars demonstrated that except drinking water, other products like “rice, vegetables and fish” can be accounted as possible predisposing factors for arsenic diet susceptibility (Lin *et al.*; 2004; Ohno *et al.*, 2007; Signes-Pastor *et al.*, 2008; Signes-Pastor *et al.*, 2009; Bhattacharya *et al.*, 2010).

The EDI ranges for adults and children concurred with the results obtained by Schoof *et al.* (1999) and Diaz *et al.* (2004) who obtained estimated daily intake ranging from 0.003 to 0.184 $\text{g day}^{-1} \text{Kg}^{-1} \text{BW}$ in children. On the contrary, Martin-Cid *et al.* (2007) obtained estimated daily intakes ranging from 0.011 to 0.013 $\text{g day}^{-1} \text{Kg}^{-1} \text{BW}$ for children which were higher compared to the current study. Razo *et al.* (2002) reported higher adult EDIs ranging from 0.039 to 0.394 $\text{g day}^{-1} \text{Kg}^{-1} \text{BW}$. With regards to Hg EDIs, Bassy *et al.* (2013) and Storelli (2008) reported Hg EDIs that are similar values to those obtained in the current study ranging from 0.003 to 0.006 $\text{g.kg}^{-1}\text{BW day}^{-1}$ for boiled, fried and grilled fish. The EDIs of As and Hg in the current study were generally below the recommended values of estimated weekly intake for adults and children. Nevertheless, regardless of whether on average people do not consume above acceptable limits

for estimated daily intake, those who consume too much of contaminated fish might be susceptible to the dangers associated with heavy metal poisoning (Leblanc *et al.*, 2005)

4.4.2 Target Hazard Quotient

The THQ determination has generally been viewed as a valuable approach to assess potential hazards associated with heavy metal susceptibility (Yi *et al.* 2011; Tacon and Metian, 2013). The THQs below 1 demonstrate that the assessed subject cannot be declared as problematic (Basim and Khoshnood, 2013). The THQ values cannot be multiplied, but be add, thus a THQ of 20 value is not ten times bigger than THQ 2 value (Naughton and Petroczi, 2008). From the consumer health hazard view, it is vital to characterize the THQ level of the As and Hg in various cooking methods of fish (raw, boiling and frying) so that typical THQ can be calculated. The results on THQs obtained in the current study are in agreement with the findings of Zheng *et al.* (2007) and Yi *et al.* (2011) who also obtained THQ values in marine meat products that were less than 1.0. Storelli (2008) and Mol *et al.* (2017) also obtained similar results, reported THQ values that were less than 1.0 for Hg and other metals in three types of fish: Red mullet 0.038 Hg, Whiting 0.019 Hg and Turbot 0,019 Hg from the Black Sea in Turkey. However, Islam *et al.* (2016) reported THQ values that were greater than 1.0 contrary to the results obtained in the present study. All the THQ values were less than the risk threshold of 1 for As and Hg in both adults and children in the present study, this clearly showing that the consumption of the fish in the studied area will not cause cancer to the consumers and is considered safe for consumption.

4.4.3 Carcinogenic Risk

All the carcinogenic risk values obtained in this study were above the recommended South African government standard limits (0.00001) for fish and fish products (GSA,2006; USEPA, 2018). Islam *et al.* (2016) also reported As carcinogenic risk values that were above the standard limits set by set by Environmental Protection Agency (EPA) in fish that were normally eaten by the communities of Bangladesh. Similarly, Ahmed *et.al.* (2015) also obtained results above the Bangladesh standard limits (10^{-6}) for carcinogenic risk in *Ailia coila*, *Gagata youssoufi* and *Mastacembelus pancalus* sampled from Buriganga River. In contrast Low *et al.* (2015) obtained carcinogenic risk values that were within the normal standard limits in Malaysia, ranging from 10^{-4} to 10^{-7} for *tilapia* fish. The observed high CR values in the current study means that people who consume fish sampled from the study area might be exposed to high chances of getting cancer despite the fact that the EDIs were within the recommended ranges. The findings obtained in the present study therefore recommend that evaluation of carcinogenic risks should not be overlooked even if expected daily intakes are within the expected ranges.

4.5 Conclusion

The bioaccessible fraction of As and Hg were all below recommended standard limits on fish, except As concentrations of boiled fish muscles. The risk assessment on estimated daily intake(EDI) were all below the recommended limits, indicating that there is no health risk to consumers and target hazard quotient THQ values of As and Hg were less than 1 which demonstrates that there is no potential health risk for the Rustenburg communities. However, the carcinogenic risk (CR) effects as a result to consumption of As in Common carp was higher than

the acceptable limits, therefore Rustenburg community is expected to experience carcinogenic health effects.

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CHAPTER 5

5.1 GENERAL DISCUSSION AND CONCLUSIONS

The idea of utilizing bioaccessibility or *in-vitro* tests as an alternative method to assess consumer health risk on fish consumption has gained popularity, after realizing that bioaccumulation (total concentration) overestimated the absorption rate of heavy metal per fish diet consumption (Yang *et al.*, 2012). Bioaccessibility has been estimated by instruments by a three compartment *in-vitro* method which is regarded as an accurate process to evaluate human risk exposure (Wang *et al.*, 2011). The research study had the major objective to determine the bioaccumulation and accessibility of arsenic (As) and mercury (Hg) using ICPMS machine.

In Chapter 3, results of the study showed no significant differences in As and Hg concentrations across all culinary treatments for bioaccumulation, while significant difference was obtained for bio accessibility. The results suggested that cooking treatments modified As and Hg concentrations in Common carp muscles when compared to raw fish muscles total concentrations (bioaccumulation) and it also modified the As and Hg bioaccessibility during *in-vitro* digestion. This might be due to the heat produced during culinary treatment on fish muscle can alter the structure of proteins, this activity can cause protein complexes of As and Hg to be less present to be solubilized while digestion is taking place.

For all fish muscle samples, cooking treatments and mixed acids digestion; Hg in fried fish muscles was very high compared to those in boiled and raw fish muscles, while As concentration was high but not significant in boiled fish compared to fried and raw fish muscles. While cooking and enzyme digestion alter the total bioaccessibility of As and Hg in the fish muscles,

with most of the As and Hg obtained in the supernatant phase for digested raw fish muscles while less As and Hg bioaccessible was obtained in the pellet for boiled and fried fish muscles. Contamination of the aquatic environment is caused by platinum mines around Rustenburg, which is a health risk to human exposed to the As and Hg from the fish in this area, based on the As estimated daily intake and non-carcinogenic risk (THQ) for both As and Hg bioaccessibility were within acceptable values, while As carcinogenic risk bioaccessibility was above acceptable limits. Since fish is preferred to be consumed cooked most of the time and cooking treatments increase the total heavy metal content of As and Hg, people consuming fish frequently at Rustenburg, that is from the Hex River, should reduce fish intake in their diet.

5.2 RECOMMENDATIONS

The information from this study can be made freely accessible to the knowledge of consumers, platinum mining industry and environmental law enforcement committee. According to the findings, , it was observed that residents in and around Rustenburg do consume fish from the Hex River catchment on regular basis, it is therefore imperative that restrictive measures should to be put in place to protect consumers from As and Hg contaminants exposure. One of the recommendation for this is the platinum mining industry near the Hex River catchment should “decrease the amount of contaminated waste discharged into the environment or to put in place measure that will monitor wastes to levels under the standard permissible limits as set within the environmental laws.

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