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An Investigation of Mine-Derived Metals in the Context of Sustainable Technology

Melvin Minala[1], Aezeden Mohamed[2*]

[1]Department of Civil Engineering, Papua New Guinea University of Technology, Lae, Papua New Guinea University, Papua New Guinea.

[2*]Department of Mechanical Engineering, Papua New Guinea University of Technology, Lae, Papua New Guinea University, Papua New Guinea. E-mail: aezeden.mohamed@pnguot.ac.pg

ABSTRACT

Mining industry remains one of the top sources of various array of wastes with respect to their makeup and extent of contamination by heavy metals.

The aim of this investigation was to assess the build-up of heavy metals in the liver, kidney and muscles of *N. berneyi* and *N. ater*, while only muscles for *O. niloticus*. These three fish species were collected from Bige dredging site off the Ok Tedi River in Papua New Guinea.

The tissue samples were analysed by atomic absorption spectroscopy (AAS) at National Measurement Institute in Sydney, Australia.

The order of accumulation bymuscles of*O.niloticus* and tissues (kidney, liver and flesh) of *N. berneyi* were in the following sequence; Zn greater than Cu greater than Pb greater than Cd (Zn > Cu > Pb > Cd). Flesh and liver of *N. ater* accumulated in the same order but was slightly different in kidney, that is, Zn greater than Cu greater than Cd greater than Pb (Zn>Cu>Cd>Pb). The order of accumulation by tissues were liver>kidney> flesh.

The tissue metal concentrations of this study were compared with Ok Tedi'smonitoring data,Australian Market Basket, the United States Total Diet Studies, the local Fly and Strickland Rivers Market Basket Study, and the Porgera-Lagaip-Strickland-Lake Murray (PLSML) study.

KEYWORDS

Aquatic Environment, Contamination, Fish Edibility, Heavy Metals, Mining.

Introduction

The OK Tedi copper mine (hereafter referred to as OTML) locate in Papua New Guinea (PNG). The PNG government has approved the mine to discharge about 60 Mt (Million tonne) of waste rock (non-valuable rocks after mining) and 30 Mt of mill tailings (effluent of process waste streams consisting of various chemicals and fine sediments) directly into the OK Tedi River annually (Figure 1).

According to Marshall (2006), approximately 45% of the waste rocks remain within the creeks nearest to the mine, also tributaries to the OK Tedi River. The remainder with most of the tailings is carried down to the OK Tedi River. This then meets with the Fly River (the biggest river in PNG) and finally to the Gulf of Papua (Marshall, 2006) (see Figure 1).

This continuous discharge of waste rocks and mill tailings has several environmental consequences. One of the main impacts of riverine disposal of waste rocks and tailings was the

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aggradation (build-up of sediments) of the river-bed channel (the width of a river where flow occurs).

In this context, aggradation refers to the elevation of the river bed (OK Tedi River) with deposition of coarse sediments sourced from the waste rocks and the mine's tailings, thus reducing the water depth and subsequent overbank flooding.

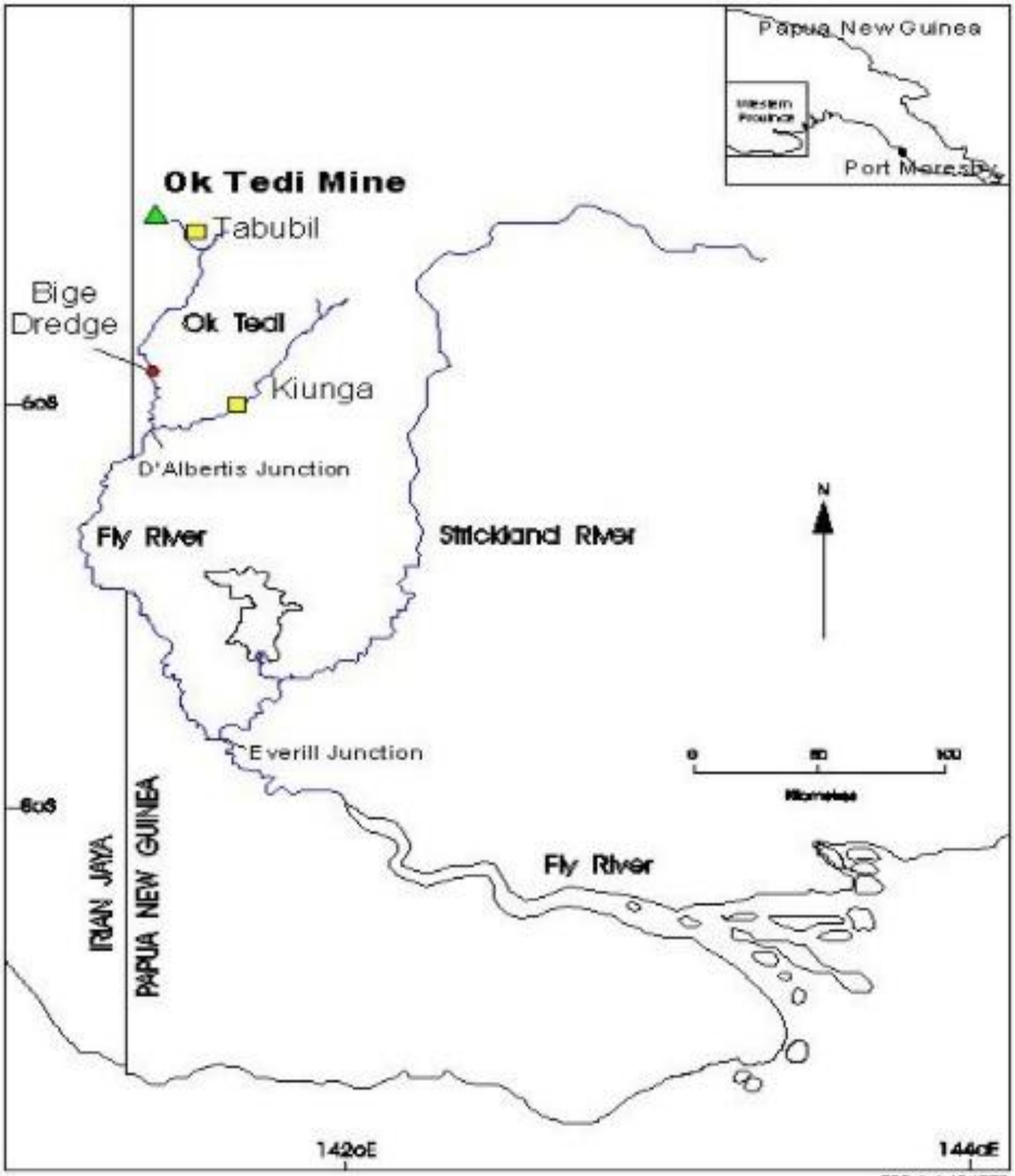

Figure 1. Map showing Mt Fubilan, Tabubil, OK Tedi, and the Fly River within the Western Province of Papua New Guinea (OTML Environment Department)

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Impacts of River Bed Aggradation

According to Swales et al. (2000), river bed aggradation causes the channel to become shallower. Consequently, water velocities (the speed at which water flows at a time in a particular direction) increase. This is because the channel becomes smaller while the volume of river water remains unchanged.

Increased velocity is unlikely to be conducive for fish species adapted to deep, slow-flowing water as more typical of the Fly River's lowland reaches. Speed previously has been shown to influence biomass (combined species weight in a system), richness (diversity of species), mean fish size, and density of fishes (Swales et al., 2000).

River bed aggradation also results in overbank flooding under high flows with subsequent loss of riparian (close to the river edge) channel forest vegetation as it becomes inundated (covered-under or flooded) by water and sediment (Storey et al., 2009).

Therefore, habitats and conditions that depend on the health of the vegetation near the river-banks are lost. Foliage also tends to stabilize banks. As a result of dieback, there is less root structure and greater bank instability (Storey et al., 2009).

These effects can be seen in reaches with high aggradation along with the river system (OK Tediand Fly), especially at banks where erosion is actively taking place. These sections also collapse continually.

While the river banks can create sections that can behave similar to backwaters (area of the river where the flow is usually slow, stable, and tend to be opposite the current, usually at bends and corners), these features appear to be only temporary. This means that they only last for hours to days and collapse again, especially under high channel migration rates. Besides, the backwaters also seem to have increased water velocities. The combined effects of this create an unsuitable habit for fish.

These adverse effects of river bed aggradation have implications on the OTML environmental values.

OTML Environmental Values

The above stated adverse effects of river bed aggradation have either directly or indirectly affected the OTML Environmental Regime's environmental values. (regulatory document highlighting fundamental environmental values identified by the state for OTML to comply with, non-compliance means it's a breach of a statute). OTML environmental values are as follows (OTML Regime, 2001):

a) After allowing the total suspended solids to settle, the mine's water quality downstream must be drinkable.
b) The resources that can be sourced from the water along the OK Tedi and Fly Rivers below the mine's operation must be available for community use/consumption.

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c) The edibility of aquatic resources, including fish, must not be compromised with the river system (OK Tedi/Fly), including the Gulf of Papua.
d) The resources sourced from land on the floodplains of the OK Tedi/Fly River system below the mine operation must be available for use by local inhabitants.
e) The mine operation must not have detrimental impacts on the crops /resources taken from the surrounding bushes for human consumption
f) The villagers downstream of the mine at lower OK Tedi and along the Fly River must safely navigate (using canoes/ dinghies) across/along the river channel. The same applies to the commercial ships that come to Kiunga from Port Moresby.

When inundated with water and sediment, the subsequent loss of riparian vegetation will detrimentally (adversely) affect the above-stated values as long as aggradation remains an issue. Also, the decrease in water depth due to river bed aggradation will remain another contributing factor affecting the above matters.

To mitigate these issues, a trial dredging (act of removing particles/materials from the river bed) program commenced in March 1998 at Bige, with approval from the mine management. Bige is located approximately 110km downstream of the OK Tedi Mine (Figure 2).

Bige Dredging Operation

The primary intention or reason for undertaking dredging of sediments on the OK Tedi river bed at Bige is to help recover the observed dieback of forest on the floodplain in the lower OK Tedi region. This is achieved as dredging enables the river's bed levels to be reduced and also minimizes the frequency of flooding over the banks.

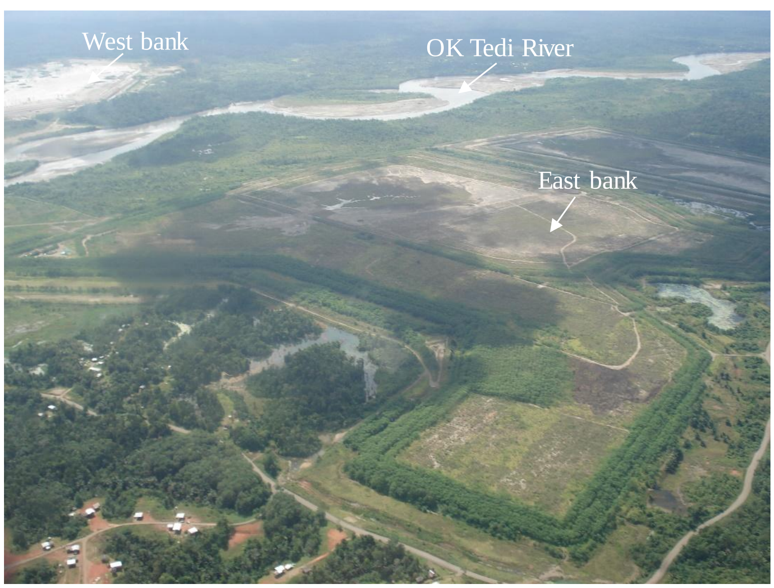

Figure 2. Map showing the east and west bank sediment stockpiles and other associated locations at OTML'sBige operations – modified (OTML environment department)

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In the dredging process, the sand from the mining operation is captured inside a dredged opening. Through a suction pump, it is transported to stockpiles located on either side of the river. This takes place at a minimal rate of ten million cubic meters per year. The dredging operation location is at Bige village in the lower section of the OK Tedi River.

When the dredging operation started in 1998, they used the Eastern bank stockpile to place the sands taken out from the river. In 2004, the placement of the sand shifted to the West bank stockpile. Several cells were developed at the east bank stockpile and referred to as A, B, C, 1, 2, 3, and 4 (Figure 2).

The Challenge

At Cell four of the East Bank stockpile, an incidental pool was created at the surface of the stockpile, which was found to harbor *Oreochromis niloticus* species, commonly known as tilapia. How these tilapias came to inhabit this habitat is uncertain.

While this uncertainty still exists, people living around there occasionally catch tilapia for household consumption. There is also another pool at the edge which receives the off flowing water from the stockpile. Which also inhabits varieties of fish species (especially catfish) that have been put in there intentionally. People also fish here for household consumption.

This poses the question of whether or not the fish if consumed, are safe for human health concerning their tissue metal concentration as the sediments are rich in particulate. Dissolved metals dredged out from the impacted OK Tedi River.
Thus, it was hypothesized that the heavy metal content (specifically copper, cadmium, lead, and zinc) of the tissues of fish species inhabiting these particular pools are expected to be somewhat higher than their natural metal level content. If confirmed, this increment was to be compared against the fish edibility guidelines set by relevant international food regulatory bodies to see whether it is above or below the accepted limit.

Rational

Several authors have identified fish as beneficial to human health, according to Daviglus et al. (2002). Two vital fatty acids provided by fish consumption are DHA (docosahexaenoic acid) and EPA (eicosapentaenoic acid). These acids help to reduce the level of cholesterol, which would otherwise lead to heart disease problems. Another essential acid provided by fish, omega-3 polyunsaturated fatty acids, as discussed by Daniels et al. (2004), improves brain development and visual system for growing babies in the womb. Besides, Morris et al. (2003) remarked that the risk of Alzheimer's disease could be reduced by including fish in the diet.
As indicated in the literature, the rate of global fish consumption is increasing annually. In 2002, the World Health Organisation (WHO) compared the annual global fish consumption in the early 1960s and 1999. In the former year, the fish consumption rate was 9kg per capita while the latter was 16.3kg. The FAO (2010) estimates a very similar trend in global fish consumption. It is now evident that including fish in regular dietary intake is a common and popular choice.

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However, if the fish consumed is contaminated with toxic substances (like heavy metals), it can pass onto humans when they eat them (fish). This is outlined by Mahaffey (2004) that heavy metals can enter the human body through consuming fish and can become a public health hazard throughout the world. For this reason, it is necessary to investigate the level of heavy metals in the fish tissues in a particular locality to assess whether it is safe for people to consume fish (Herreros et al. 2008).

The Objectives

Since consuming fish is an essential component in people living in many rural parts of PNG, including Western and Bige areas, it is essential to make sure that the food source is fit for human consumption. Therefore, the purposes of this investigation were to:

a) Collect flesh, liver, and kidney samples of fish
b) Assess the levels of heavy metals, particularly copper, cadmium, lead, and zinc
c) Compare the results against food edibility standards

Literature Review

This literature review begins with the concept of sustainability in the mining industry. Further, it discusses the current understanding of heavy metals and their bioavailability. It also discusses the importance of fish consumption and its treat if contaminated with heavy metals. In the discussion of fish consumption and heavy metals, the most recent papers reviewed here are from 2000 and beyond.

Sustainability

According to the UN (1987) report, sustainability or sustainable development is defined as the "development that meets the needs of the present without compromising the ability of future generations to meet their own needs." This report has enabled the world to incorporate sustainable development principles in various life walks, including politics, economy, and technology.

Sustainable development in the mining industry involves the application of new principles and techniques related to issues. The optimization of production processes to improve energy efficiency, decrease the unit consumption of energy and resources used for the production, reduction of harmful impact on the environment. In particular, the removal of emission of hazardous substances into the atmosphere or aquatic systems increases the reuse level of by-products and waste (Peng et al., 2015: 1931). Such actions necessarily involve high costs for companies.

However, the implementation of practices to improve environmental quality is necessary to achieve sustainable development. Sustainable development contributes to economic growth while reducing natural resource management (El-Haggar, 2007: 1). The mining industry uses various technologies depending on the production process applied (milling of ores with various physical

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and chemical methods to extract precious metals like copper or gold). It may also affect the environment to a different extent, polluting the air, soil, and water (PPTS, 2006: 9).

One of the reasons for incorporating the principles of sustainable development in the mining industry is to minimize a negative environmental impact (e.g., heavy metal contamination of fish in aquatic systems) of the industry, which is a challenge to technical sciences. In the field of sustainable waste management, technological processes should be carried out in such a manner to reduce/mitigate adverse environmental impacts. This is achieved by adopting new and cutting-edge technology in engineering and sciences, like the multi-million-kina Bige dredging project along the Ok Tedi River undertaken by OTML.

Heavy Metals

Several definitions for heavy metals, but look at a few for this discussion. First, Csuros and Csuros (2002) defined a heavy metal as "a metal with a density greater than $5g/cm^3$ (i.e., specific gravity greater than 5)". Second, the term heavy metal is defined as a group name for metals and semimetals (metalloids) associated with contamination and potential toxicity or ecotoxicity.

Just recently, Ali & Khan (2018) have proposed a general meaning for heavy metals as "naturally occurring metals having an atomic number greater than 20 and an elemental density greater than $5g{\cdot}cm^3$".

So, from these definitions, it is clear that heavy metal is a metal element and can contaminate or behave as a toxic substance. Now, where do these heavy metals come from? The below paragraph describes the sources of heavy metals.

They are established that the sources of heavy metals are of two origins, according to Zukowska and Biziuk (2008); "the natural; and anthropogenic."

Examples of natural sources include volcanic eruptions, landslides, and surface runoffs. This also provides dust particles that may carry heavy metals and sinks into wetland systems. The anthropogenic sources include agricultural activities, logging, manufacturing industries, and the extractive industries, including the mining industry.These man-induced activities, particularly the mining industry, are rated as the top sources of heavy metals because they discharge many heavy metals into the aquatic environment (EPA, 2007).

As indicated by EPA (2007), toxic substances contained within the rock and tailings are more easily released once these substances are processed and left in mine pits. Underground workings, waste rock piles, and tailings impoundments or discharged into river systems (as is the case with Ok Tedi Mining) where they exposed to the environment.

Acid mine drainage (AMD) occurs when the sulfide mineral in the waste stream reacts with oxygen from the atmosphere or water, thereby creating an acidic condition in the aquatic system. Under this acidic condition, metals tend to leach out and immobilize. Thus, AMD can speed up toxic metals into the marine environment (EPA, 2007).

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Metals are released into the environment in their elemental state. They are free to react with any ligands, which means that they can be quickly taken in by living organisms' bloodstream. The biological system of an organism can depurate or remove metals. However, as more metals are taken in, this natural system is overcome, and metals tend to accumulate in the organisms (Ebrahimpour et al., 2011). Humans are at greater risk of consuming these organisms (e.g., fish or prawns) contaminated with heavy metals.

Heavy metals can be either essential or no-essential. Virtual means the metal has a known function in the biological system in living organisms, including human beings (Neff, 2002). Non-essential means a metal has no known role in living organisms.

Examples of essential metals include copper, zinc, and iron, necessary for fish metabolism (Bohn et al. 2001). Examples of non-essential metals include cadmium, arsenic, and mercury. These are harmful even at low concentrations.

Some of these metals (copper, cadmium, lead, and zinc), both essential and non-essential, are discussed in the subsequent subsections.

- **Copper**

As discussed above, copper (Cu) is an essential element. Cu is a constituent in fish, found in several enzymes (approximately 30) and glycoproteins. It takes part in the destruction and production of organic substance and energy, respectively. Cu also performs other functions in the biological system. It includes facilitating the absorption of iron from the gastrointestinal or digestive system. It helps maintain myelin in the nervous system. It constituted an essential element when bone and brain tissues formed involve haemoglobin production.
Cu is bound to ceruloplasmin in the blood and is transported to the liver, kidney, heart, Central Nervous system (CNS), bone, and muscle for storage (UNITAS, 2000).

In general terms, fish exposed to low Cu levels will regulate the metal, with excess Cu being stored in internal organs (i.e., liver and kidney as seen in the Fly River monitoring data).

Levels in the muscle do not increase due to this ability to regulate (although a minor increase may occur as fish acclimate to higher ambient levels). If defines mechanisms are overcome by excessively high external Cu levels, the regulatory ability is overcome, and toxicity occurs (UNITAS, 2000).

- **Cadmium**

Cadmium (Cd) is a non-essential metal, meaning it does not play any crucial biological role in an organism. It is known for causing cancer, as confirmed by some studies that found out that employees exposed to cadmium were seen to have higher Cd levels in their bodies. The metal is also known for destroying essential body organs such as kidneys, lungs, and intestines (ATSDR, 2009).

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If a mother has elevated levels of cadmium, it can pass onto her breastfeeding child. Cd's harmful effects on developing fetus in animals when pregnant include: causes detrimental effects on behaviour and learning, causes fetal metabolism to be abnormal, causes the weight of the fetal to be lower, and it deforms the formation of skeletons.

Cd can also detrimentally affect human reproductive systems, causing the newborn child's weight to be lower than the expected average and reduces the count of sperm (EPA, 2007).

Although Cd is non-essential metal, the process through which the metal take into the human and animal bloodstream is very similar to that of essential metals like iron. In such cases, the assimilation accelerates by a lack of Cd, iron, and protein in the diet (Goyer and Clarksom 2001).

The concentration of Cd in the environment can be increased by both natural and anthropogenic releases or discharges. Human-made sources include mining, applying fertilizers in the Agriculture sector, and taking waste sludge from sewage treatment systems and placing them on agricultural land (ATSDR 2003).

Cd is discharged into the environment at an estimated figure of thirty thousand tons annually, of which about four thousand to thirteen thousand tons are coming from man-made sources only (ATSDR 2003).

- **Lead**

Lead (Pb) is a non-essential metal and so does not perform any important biological function in organisms when assimilated. Like other metals, it has its source from both nature and anthropogenic, therefore, it is present in all compartments of the environment.

From the two sources, the anthropogenic activities remain the leading sources of Pb emission with activities such as mining, agriculture, manufacturing and burning of fossil fuels. Especially with mining industry, certain activities associated with the industry which enhance the release of the metal (including other metals as well) into the environment, according to Cobbina et al., (2015) include: transporting of ore, exploring for mineral, mineral smelting, refineries, discharge of tails, andused or wastewaters.

Pb is known for causing many health and environmental problems even at very low concentrations (Ekpo et al., 2013). Once the metal has entered human body and starts to accumulate, it first replaces the calcium in the bones and consequently causes a lot of health problems includingcauses the intellect not to work properly, causes kidney to fail,interferes with foetus resulting in miscarriage, “hypertension”(Jilai, 2014), causes cancer especially in the lung (EPA, 2007), Pb accumulation can even lead to death (Jilai, 2014), poisonousor harmful to brain cells and the nerves, children exposed to lead can suffer from abnormal and reduced physical and mental growth” (ATSDR, 2007), diminishes the intellectual power, “associated with attention deficit hyperactivity disorder and antisocial behaviour” (Bellinger, 2008), and “an endocrine disrupting chemical, with the potential to alter hormone function” (Wide, 1980 and Dearth et al, 2002).

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- **Zinc**

Zinc (Zn) is an essential metal and is found in all breathing creatures (Eisler 1993), and is plentiful in the natural environment. Naturally, Zn makes up about 20-200 parts per million "in the earth's crust". Zn does not exist on its own as an element, like Zn^+, but it exists as a compound with other elements (for examples as "Zinc oxide or sphalerite (ZnS)" (ATSDR 2004).

Zinc is added further into the environment through man-made activities (just like other metals), according to (ATDSR 2004), includes: "mining, smelting of zinc, lead and cadmium ores, steel production,coal burning andburning of wastes."

Zinc has a multitude of biological functions in human body. It is an important constituent of over 100 enzymes involved in a variety of fundamental metabolic processes. It is major constituent of the choroid region of the eye, a dark layer involved in absorbing excess light within the eye. It is involved in the production and function of several hormones. It plays an important role in reproduction and sexual maturation. Zinc is present in the plasma and in erthrocytes, leukocytes and platelets (Ansari et al).

Most common zinc compounds are not particularly toxic but some zinc salts may be carcinogens (Ansari et al).

Heavy Metal Bioaccumulation in Fish

A bioavailable metal means that a portion of the metal, mostly in its elemental state (like Cu^{2+}, Cd^+), can be assimilated by an organism directly or facilitated by other ligands (as a compound). In other words, the fraction of metal that can be taken up by an organism by active or passive mechanisms is termed the 'bioavailable' fraction. Bioavailable metals may consist of many species and forms, which will affect whether uptake is via passive or active means.

The different metal species may be dissolved, associated with particulate matter, or in colloidal form. Various chemical forms arise from the equilibria between metal ions and inorganic and organic ligands naturally present in the aquatic environment.

Bioaccumulation occurs when an organism's metal intake is greater than its capacity to remove metals out from the body. In other words, bioaccumulation is defined, according to Burgess (2005), as "the net uptake of contaminants overtime in an organism experiencing continual exposure." The author further stated that "the rate of metal uptake which is greater than the rate of elimination in body tissue explains why metal bioaccumulation occurs in organisms as they grow older."

Metal uptake and accumulation in an organism in an "aquatic food web" is determined by several factors. Weiner et al. (2003) and Das et al. (2003a) described these factors as either "biological or environmental" or a combination of both. They listed them as: "age, body size, dietary preference, trophic position, gender, metabolic rate, and geographic diversity."

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Deducing from the list of factors, the larger the fish and feeding at higher trophic levels bioaccumulate. Biomagnify more metal than smaller fish at lower trophic levels (Weiner et al. 2003). Dorea et al. (2006) attributed "variations in metal levels in fish" to differences in feeding strategies, mobility, foraging locations as well as migratory behaviours.

Methodology

Study Area

The location where this investigation was conducted is near a village called Bige. Bige is located downstream of the Ok Tedi River, approximately 106kilometers downstream from the Ok Tedi Mine. The location where OTML's dredging activity is taking place, a mitigation strategy to combat river bed aggradation. The subsequent overbank flooding into the floodplains, causing detrimental environmental impacts.

This place is precisely described, according to the Minerals Council of Australia (2000) as below.

"In this region, landslides occur on almost a daily basis. This is attributed to two forces, besides gravity, that generates the high energy, erosional landscape; the high torrential rainfall and the high frequency of earth tremors and earthquakes. The earthquakes often measure more than seven on the Richter scale, and they regularly trigger massive rockfalls and landslides."

This place is no different from the rest of PNG, "having an equatorial climate with nearly uniform temperatures throughout the year, averaging 260C" (Clarke, 1996). "The region where the mine is located in one of the wettest areas in the world experiencing extremely high rainfall of over 10,000 mm per year which evenly distributed throughout the year" (Murray et al., 2000).

Field Sample Collection

Samples were collected from two different pools (Pool 1 and 2) on separate dates, respectively.

Using a standard gill net over a double 12hr period, nineteen catfish (6 *N. ater* and 13 *N. berneyi*) were collected from pool one on the 12thto 13thSeptember, 2013. Bank 1 receives off-flowing water from the high-risk sediment stockpile. It has riparian vegetation and aquatic plants growing on the sides and sparsely in the middle respectively.

From pool 2, 67tilapias (*O. niloticus*) were caught using hook and line on the 19thof September, 2013. Bank 2 is right on top of the sediment stockpile and comparatively highly contaminated than pool 1. No growth of riparian vegetation; however, only a few aquatic plants are growing scattered.

Laboratory Sample Collection

The methodological approaches used to collect data for this study were selected based on those that would bring systematic answers to the primary research question. Thus, two well-practiced approaches were applied. They were tissue metal analysis and Market Basket Survey (MBS).

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- **Tissue Metal Analysis**

The tissues collected include flesh (F), liver (L), and kidney (K). Collection of L and K depended on size. Replicates per species from each site absolutely depended on the availability.

Following tissue removal (i.e., flesh, liver, and kidney), samples (flesh) were retained as they would be further tested using the Market Basket approach.

Dissections were done at the OTML Biology laboratory in Tabubil. Individual length and weight measurements were recorded, and fish were frozen in individual zipped plastic bags.

All dissecting equipment (e.g., scalpels and forceps) were dipped into the 5% acid solution after each dissection or when a different part of a fish is cut or touched (e.g., changing from cutting the skin to cutting flesh).

The acid solution releases any metals that are attached to the dissecting equipment, which was then rinsed away with deionized water. Rinsing with deionized water was done three times before commencing a new dissection.

To reduce contamination further, a separate scalpel blade and plastic forceps were used for each tissue type. It was also preferable to use different instruments to cut away the skin and cut the tissues used for metal determinations.

The flesh samples were dissected before the kidneys and liver for adult fish as the latter organs usually have higher metal concentrations than flesh. Gloves were worn for all dissections and rinsed with deionized water between dissections or changed if contamination occurred.

The specimens were thoroughly washed with deionized water. Using a clean, sharp scalpel, the rear portion of the fish was removed by cutting from either in front of or behind the dorsal fin to behind the anus, taking care not to penetrate the gut cavity. If the specimen had a forked tail, this was also removed.

The specimen was rinsed in deionized water and then placed in a 'Whirlpak' sample bag. The samples collected were weighed on the Mettler AE 160 balance, ensuring that the plastic bag's weight has been allowed.

A sample of 5 grams of wet weight is required for tissue digestion. If the weight was less than this, one or more specimens of the same species were dissected and combined with the first sample until the required weight was achieved. Once the required weight was achieved, the 'Whirlpak' bag was sealed, labeled, and stored in the freezer.

In the appropriate field or data book, indications were made regarding fish that have been combined to produce a single sample.

If the fish had scales, it was removed those on the dorsal surface where the flesh sample was to be taken from. The exteriors of the fish were washed with deionized water to remove any slime and

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dirt. A square or rectangular portion of the dorsal skin was cut, taking care not to penetrate the flesh. The skin was carefully sliced and peeled away from the flesh.

Using a different scalpel, a block of flesh was cut. The flesh was removed using an extra pair of plastic forceps to those used during the skin removal process. The flesh was well rinsed using deionized water before putting it into a pre-labelledWhirlpak plastic sample bag.

The sample bag was sealed tightly and stored in a larger bag with other samples from the same site. All samples were preserved in the freezer awaiting subsequent steps.

With deionized water, the ventral surfaces of the fish were washed to remove any slime and dirt. The fish was split along the ventral surface to expose the internal organs if it was a catfish, blade run from the operculum and the pectoral fin and around the pelvic fin on either side.

In some species, particularly *O. niloticus*, the kidney and liver are reduced or diffused, dissecting them out very difficult and impossible. The liver is generally located near the pharynx. In contrast, the kidneys are usually located posteriorly on the body cavity's dorsal surface.

Care was taken not to damage any other organs (particularly the digestive system) while dissecting the liver and kidney. These may be a source of metal contamination if they are damaged.

The organ was rinsed well with deionized water before putting it into a pre-labelledWhirlpak plastic sample bag. The sample bag was tightly sealed and stored in a larger bag with other samples from the same site. Samples were preserved in the freezer awaiting further treatments.

- **Digestion of the Tissue Samples**

Samples were dried entirely, and to achieve homogenization, the samples were grounded using a mortar and a pestle.
In freeze-drying, the partial pressure of moisture in the sample compartment was maintained at a superficial level. A combined effect of "cold finger" condensing, which is operated at less than -50°C and a high vacuum of less than 25T(3Pa), achieves this.

Through sublimation, moisture from the sample was drawn to the "cold finger" condenser. Samples were frozen to achieve an effective transfer of water.

Samples were broken into small pieces while still in their plastic bags. Then poured into a mortar and grounded using a pestle. Separate mortar and pestle were used for muscle and liver, and kidney. Hand gloves were changed as well when switching from kidney/liver to grounding of muscles.

When the samples are grounded into a fine powder, they were put through a 1000µm sieve. The sieved pieces were placed in the original plastic bags and shacked several times to mix samples.

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The tissue samples were digested with concentrated nitric acid and hydrogen peroxide. The digested samples were analysed for copper, cadmium, lead, and zinc using a flame atomic absorption spectrophotometer (AAS) at NMI (National Measurement) laboratory in Sydney (Australia).

- **Market Basket Survey**

For the market basket survey, three fish samples were selected of the ones already used for the tissue dissection method, each weighing approximately 300g. They were all of the catfish species.

The flesh specimens were cooked in unsalted water, flesh and skin removed from bones, chopped, and mixed.200g of sample were preserved in OTML freeze for back-up. Cooked samples were split into two packages and labelled. Excess and bones were discarded.

Another 200g of sample were sent to Queensland Forensic Health andScientific Services (QFHSS) lab for the required heavy metal analysis.

Human Health Risk Assessment

"Consumption of fish containing high concentrations of cadmium, lead and arsenic have correspondingly been linked with detrimental health effects in adults and children as reported by several researchers" (Abernathy et al. 2003; Burger and Gochfeld 2005; Andreji et al. 2006).

Thus, communities which rely heavily on fish as daily protein requirement may be at risk from chronic to high exposure to metals as fish consumption is the primary route of exposure in humans, especially in the developing nations" (Andreji et al. 2006).

For this study, human health risk was assessed inconsistently with previous reviews recommended for OTML (OTML, 2011).

Mean concentrations for the fish were compared against same or similar food category data from the Australian Market Basket (ANZFA 1994, 1996,,1998; and FSANZ 2001, 2003), the United States Total Diet Studies (USFDA, 2000) and the local Fly and Strickland Rivers Market Basket Study (FSR MBS), (CEH, 2006) and the Porgera-Lagaip-Strickland-Lake Murray (PLSML) study (Bentley, 2005).

For fish, the USFDA (2000) data is based on marine and freshwater fishes. In contrast, data from all the other studies are for freshwater fish.

Results and Discussion

The analytical results for the fish are presented in Figures 3, 4, and 5. All fish samples had detectable concentrations for the essential metals copper and zinc. In contrast, for the non-essential metals, 24% and 21% of samples were below the detection limit of 0.01ppm for cadmium and lead, respectively.

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Unless specified, 50% of the detection limit was used to report below-detection-limit (BDL) data. This means that if the detection limit was 0.1, and a sample was detected <0.1, it is reported as 0.05 (which is 50% of the detection limit) for statistical purposes.

Concentration of Heavy Metals in Fish Tissues

Muscles of *O. niloticus* (Refer figure 3) and tissues (kidney, liver and Flesh) of *N. berneyi* accumulated heavy metals in the order Zn > Cu > Pb > Cd. Refer to figure 5. The same order of accumulation was observed in Flesh and liver of *N. ater* but slightly different in the kidney: Zn > Cu > Cd > Pb. Refer to figure 4. The tissues with abundant accumulation were liver > kidney > flesh.

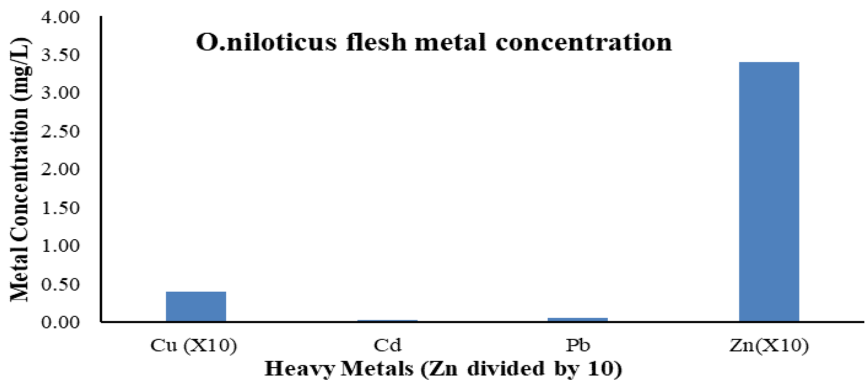

Figure 3. Median concentration of Cu, Cd, Pb and Zn in the Flesh of *O. niloticu* species from pool 2, Bige

Accumulation of heavy metal followed different trends in the studied species. Zn remained on the top while Cd concentration was the least among all species investigated. Copper always remained the second-highest accumulated heavy metal in all the different species and tissues compared.

Median heavy metal concentrations in the three fish species' muscle tissues were in the order *O. niloticus*>*N. berneyi*>*N. ater*.Median liver metal concentrations of *N. berneyi* was higher compared to their attention in *N. ater*.

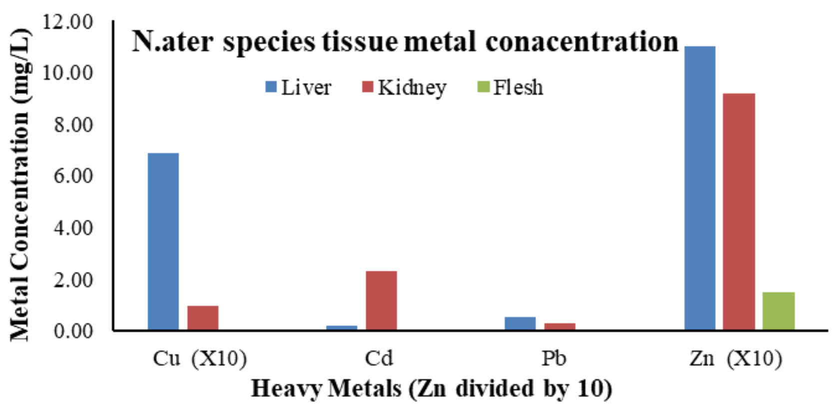

Figure 4. Median concentration of Cu, Cd, Pb and Zn in the Flesh, liver & kidney of *N. ater* species from pool 1, Bige

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Median levels of zinc and lead in the liver of *N. berneyi* were higher than their concentrations compared with N. ater. However, copper and cadmium concentrations were higher in *N. ater* than compared with *N. berneyi*.

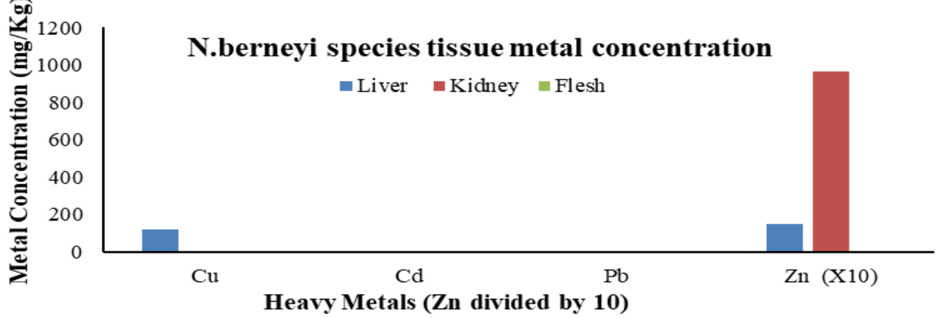

Figure 5. The median concentration of Cu, Cd, Pb, and Zn in the Flesh, liver and kidney of *N. berneyi* species from pool 1, Bige

Comparison of Metal Values and Total Diet Studies

Data generated from flesh tissue in this study is used to compare against the food edibility guidelines, as Flesh remains the edible portion of fish. For the non-essential metals, the overall mean value for cadmium is 0.02mg/kg and for Lead is 0.05mg/kg.

Using the conservative approach of comparing the overall mean values, for Cd, the overall mean of 0.02 mg/kg is below the mean values of Porgera (PLSML MBS). The Australian Market Study mean but just above the Fly Rivers Region Market Basket Survey (FSR MBS) studies and FY13 mean (Table 2 and Figure 6).

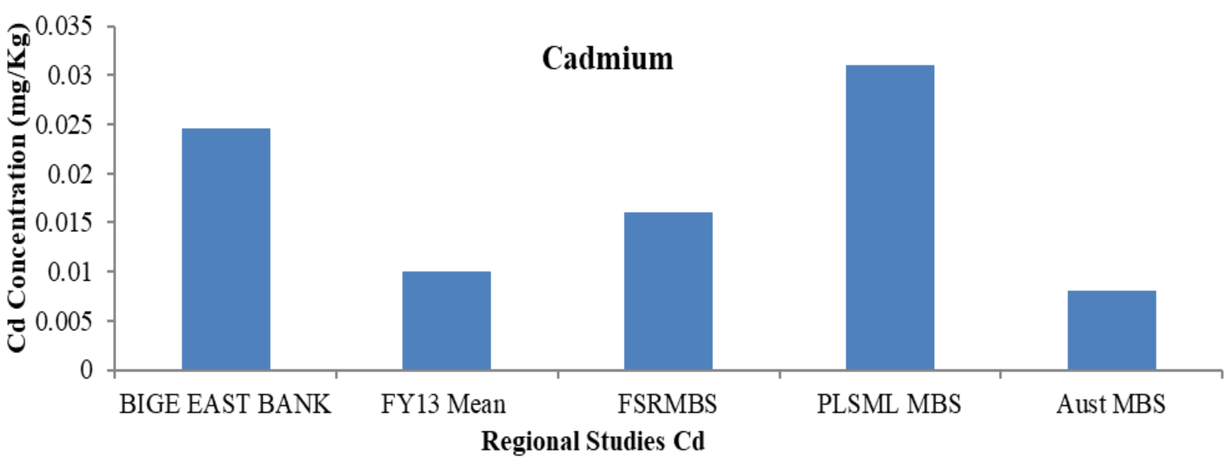

Figure 6. Zinc content in Bige samples compared with means of Australian (AMBS), United States (USFDA TDS), OTML community health study (FSR MBS), and Porgera (PLSML) market basket and total dietary studies. (Note that USFDA uses 0% of BDL while the other studies utilize 50% of BDL. USFDA database does not report mean values for cadmium and Lead in fish tissue)

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Table 1. Metal content of fish flesh compared with Australian and USFDA (all mean values as mg/kg wet weight

Location	Cadmium	Copper	Lead	Zinc
BIGE EAST BANK	0.025	3.39	0.049	30.21
FY13 Mean	0.010	0.38	0.030	11.70
FSRMBS	0.016	0.41	0.071	19.01
PLSML MBS	0.031	0.16	0.062	31.60
Aust MBS	0.008	0.73	0.011	03.93
USFDA TDS	NA	0.40	NA	19.20

Note: Non-detect values were assigned a value of DL = 50% (for AMBS comparisons) or a value of 0% (USFDA comparisons) in accord with the conventions adopted by the respective national authorities. It should also be noted that both national total diet studies report "table ready" foods as defined by the FAO/WHO Codex Alimentarius (FAO/WHO 1997).

However, this should not be a concern because of the minimal number of samples used in this study compared to FY13 and FSRMBS.

For Lead, the overall mean value of 0.05mg/kg is below the means for Porgera (PLSML MBS) and the Fly Rivers Region Market Basket Survey (FSR MBS). Still, above the Australian Market Study mean value for freshwater fish (Figure 7). However, this should not be a concern because of the differences in the detection limits used and the significant number of samples used for the AMBS.

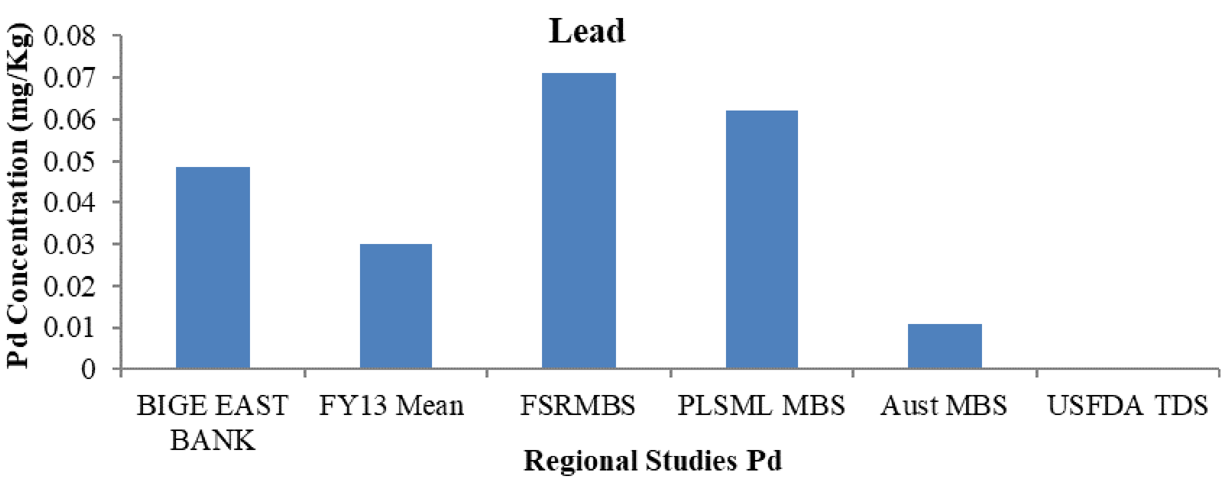

Figure 7. Lead content in Bige samples compared with means of Australian (AMBS), United States (USFDA TDS), OTML community health study (FSR MBS), and Porgera (PLSML) market basket and total dietary studies. (Note that USFDA uses 0% of BDL while the other studies utilize 50% of BDL. USFDA database does not report mean values for cadmium and Lead in fish tissue)

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For the essential metals copper and zinc, the median values are 3.38mg/kg and 30.20, respectively. For copper, the median value (3.38mg/kg) is higher than all the studies being compared (Table 2 and Figure 8). This higher reading may be attributed to the differences in the species used to calculate the median values as bioaccumulation varies between species.

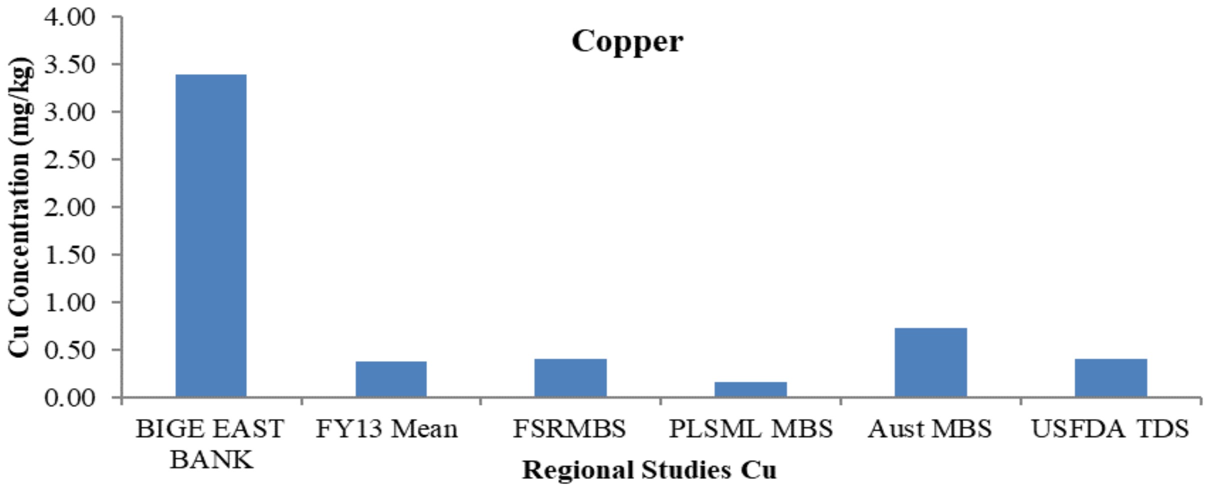

Figure 8. The copper content in Bige samples was compared with means of Australian (AMBS), United States (USFDA TDS), OTML community health study (FSR MBS), and Porgera (PLSML) market basket and total dietary studies. (Note that USFDA uses 0% of BDL while the other studies utilize 50% of BDL. USFDA database does not report mean values for cadmium and lead in fish tissue).

Thus, as far as copper is concerned, the Fly River's fish is comparable to fish from the other regional and national studies.

For zinc, the median value (30.20 mg/kg) is higher than all the other studies compared except for Porgera (PLSML MBS), which is a little bit higher (Figure 9).

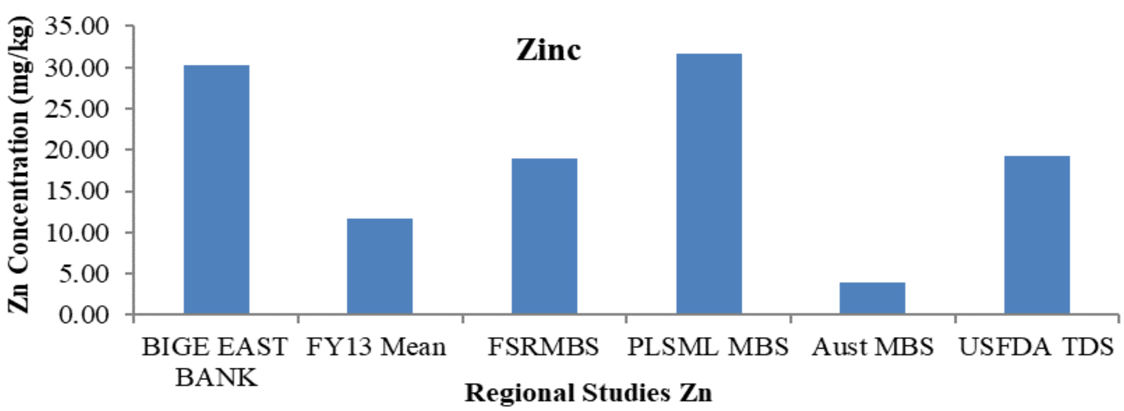

Figure 9. Zinc content in Bige samples compared with means of Australian (AMBS), United States (USFDA TDS), OTML community health study (FSR MBS), and Porgera (PLSML) market basket and total dietary studies. (Note that USFDA uses 0% of BDL while the other studies utilize 50% of BDL. USFDA database does not report mean values for cadmium and Lead in fish tissue)

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Comparison with Food Edibility Standard

This study's data were compared with relevant functional food edibility standards or guidelines, as shown in table 2. Cadmium and Lead were below their respective limits, thus does not pose any risk to the receiving ecosystem. However, copper and zinc were above the edibility guidelines compared in this study, indicating that they pose a threat to human health if consumed.

Table 2. Median concentration compared with relevant food edibility guidelines

Location	Cadmium	Copper	Lead	Zinc
Bige East Bank	0.030	4.6	0.06	36
Fish	0.050	2.0	0.30	15
Food Standard Guidelines	Cd & Pb: Europeanfoodsafety authority (EC2006). Cu & Zn: Food standard Australia New Zealand (ANZFA2001)			

Quality Control and Quality Assurance

Undertaking quality control and quality assurance (QAQC) procedures is critical in any scientific study to derive reliable, accurate, and credible data. In this study, duplicate samples were taken as a QAQC process. Same samples, according to QSLD EPA (2018), "are obtained by splitting a sample into two and may also be collected in the field by sampling at the same time and place" (QSLD EPA, 2018).

"The duplicates' assessment is commonly undertaken by expressing the duplicate results as the Relative Percent Difference (RPD). As a rule of thumb, an RPD of ≤ 20% may indicate an acceptable result for duplicate samples (Equation 1), provided the result is five to ten times the limit of reporting (LOR). In those circumstances where the result is close to the LOR, RPD may exceed 20%. However, acceptable RPD can be strongly influenced by the analyte and matrix" (QSLD EPA, 2018).

$$RPD = \frac{|c_1 - c_2|}{\left(\frac{c_1 + c_2}{2}\right)} \times 100 \quad (1)$$

From equation (1),where: RPD is relative percentage difference, C_1 is the concentration of analyte from sample 1, C_2 is the concentration of analyte from sample 2.

Table 3 shows the results of the five duplicate samples tested as part of the QAQC process in this study. The products received were well below 20 percent, indicating that the analytical laboratory's data is accurate and reliable. The three in amber suggest outlier but insignificant as most data are within the same duplicate sample's acceptable limit.Figures from 10 to 13, three dimensions graphs produced here, using Mathematica, are meant to be purely descriptive of the shapes that can be formed from equations (1) and Table 3. It has been understood that there is no significant statistical relative difference between them.

Table 3. RPD for the five duplicate samples tested, almost all results remained well below 20%
Note: Dup=duplicate

Duplicates ID	Cadmium (%)	Copper (%)	Lead (%)	Zinc (%)
Dup/1	04.1	08.0	000.0	0.3
Dup/2	00.0	13.9	000.0	5.7
Dup/3	00.0	05.7	000.0	0.0
Dup/4	00.0	02.1	107.7	2.6
Dup/5	28.6	07.4	050.0	2.5

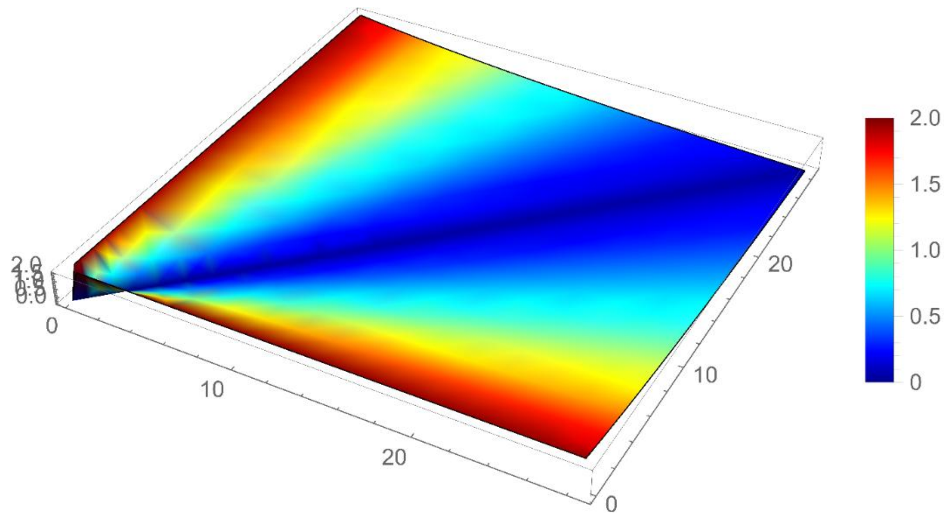

Figure 10. Relative percentage difference for Cadmium

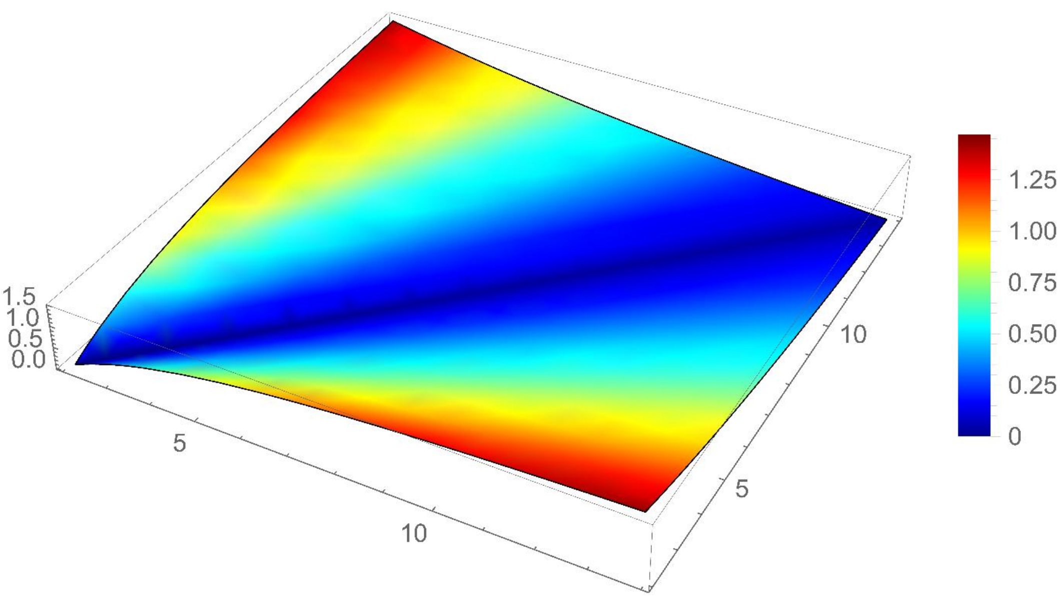

Figure 11. Relative percentage difference for Copper

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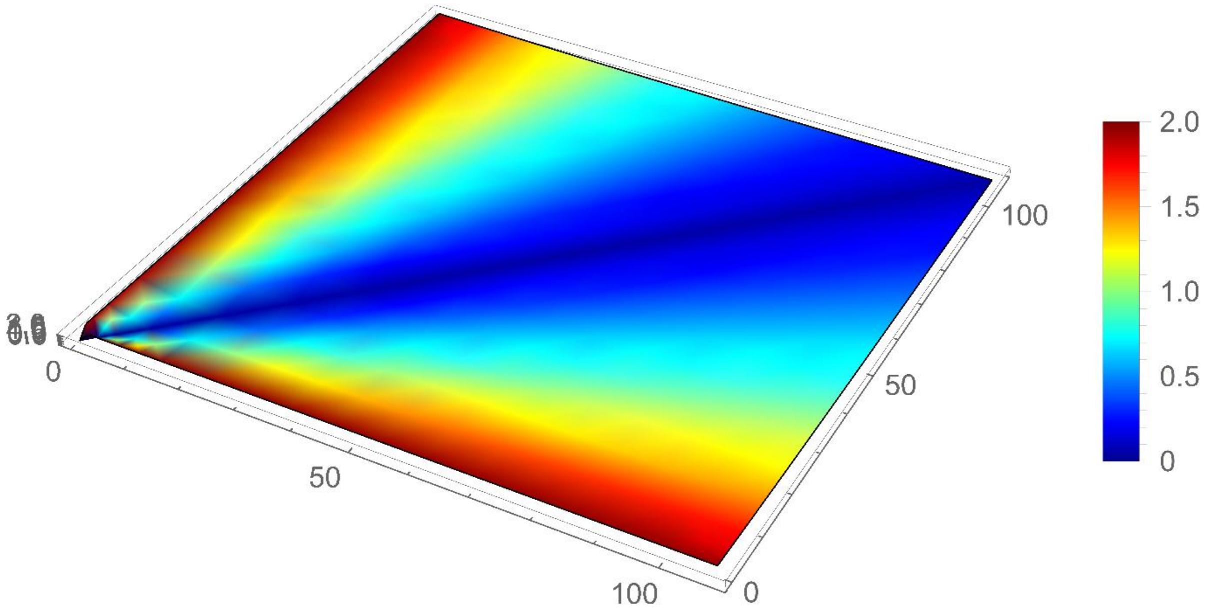

Figure 12. Relative percentage difference for Lead

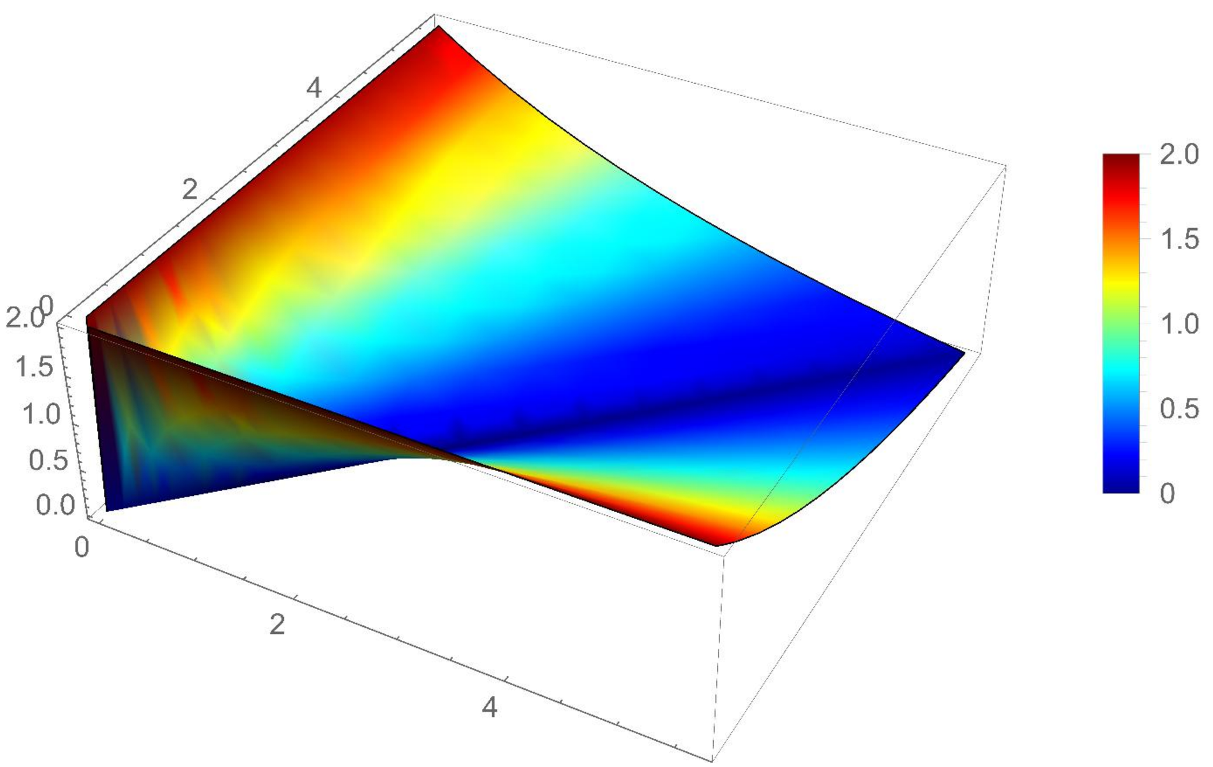

Figure 13. Relative percentage difference for Zinc

Conclusion

Including fish in the local people's diet living along the OK Tedi/Fly River corridor, including Bige area, is a regular practice. Two essential metals and two non-essential metals were investigated in this study, Cu and Zn, and Cd and Pb. The data generated for this project were

assessed for edibility standards using two criteria, the market basket. The tissue metal analysis approach, wherein median levels of the four metals were compared against means in the regional and national market basket.
Evaluation with market basket data showed that the median values in this study's samples were well within the range of mean values from the other reviews. The observation implied the metal content in Fly River fish is not different from the levels of the national studies of Australia (AMBS) and the United States (USFDTS), including the regional studies (FSR MBS and PLML MBS).

However, the increase in the copper content may imply several things. Firstly, the east bank area is a high-risk zone. Secondly, there may have been some sample contamination; the significantly large number of a single species used as different species has different sensitivities to copper.

Evaluation of the median concentration of metals in Bige East Bank Pool compared to a variety of guidelines and standards established that, except for Cu and Zn. There is no greater hazard of any effect on the well - being of the local communities around Bige if they happen to consume the fish found in the pools.

It is recommended that similar studies consider water quality parameters (i.e., pH, conductivity, etc.) as well as other environmental media, including water and sediment samples. An increased number of samples for each environmental media also recommended increasing statistical power to derive sound conclusions.

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