The Content of Lead and the Presence of *E. Coli* in Tilapia Sp. of Static and Dynamic Waters

Isiwara A. Ratnayake[1,2], Vajirapani de Silva[2], Sameera R. Gunatilake[1*]

Institute of Chemistry Ceylon, Rajagiriya, Sri Lanka[1]
Genetech School of Molecular Diagnostics, Colombo 8, Sri Lanka[2]

[*] ranmal@ichemc.edu.lk

Abstract - The consumption of fish contaminated with toxic heavy metals and pathogenic bacteria poses a serious threat to human health. This study investigated the content of Lead in inland fish Tilapia obtained from three static and dynamic water bodies in the district of Colombo, Sri Lanka, and evaluated the human health risk associated with its consumption. The impact of the static and dynamic nature of the water body on the accumulation of lead was also investigated. Furthermore, the presence of pathogenic bacterial species was also evaluated using PCR-based methods. This study discovered that the levels of Lead were within the maximum permissible limits set by the international food standards regulatory authorities and that it is unlikely that the content of Lead in muscles Tilapia would pose human health risks to moderate-level fish consumers. It was also revealed that there is no significant difference in the content of lead present in fish muscles based on the type of water in the fish habitat. However, the PCR-based determination of pathogenic bacteria discovered the presence of *E. coli* in the gills of the fish indicating that the consumption of inland fish Tilapia in raw or unprocessed form could pose a serious risk to human health.

Keywords: Lead Toxicity, E-Coli, Tilapia

I. INTRODUCTION

Fish is a major component of the Sri Lankan diet, accounting for up to 50% of the total protein consumption in 2019. Tilapia is the mostly consumed inland fish in Sri accounting for over 60% of the total inland fish harvest. However, Fish has been discovered to be a major source of harmful microorganisms and poisonous substances such as heavy metals. The consumption of fish contaminated with heavy metals and pathogenic bacteria is known to endanger human health and well-being.

Lead is one of the most ubiquitous metals and has been mined by men for centuries. However, no level of lead is essential or advantageous to the human body, and also no safe threshold of exposure has been determined for lead. *E. coli* is one of the most versatile microorganisms known to mankind with both useful and harmful features based on their pathotypes. Enteric diseases, such as diarrhea or dysentery, are caused by at least six different pathotypes, while other pathotypes cause extra-intestinal diseases, such as urinary tract infections and meningitis.

Previous research has focused on heavy metals in inland fish from the North Central Province. However, no studies have been conducted to establish the levels of lead and the presence of pathogenic bacteria in Tilapia obtained district of Colombo. The district of Colombo had a total fish production of 3,420 Mt out of which 150 Mt were inland fish in the year 2019. Furthermore, no research has been undertaken to determine the effect of the static and dynamic nature of the water body in the fish habitat on lead accumulation. The present study aims at evaluating the content of lead and the risk associated to human health from the consumption of Tilapia obtained from the district of Colombo and to examine the difference in lead accumulation in fish taken from static and dynamic water sources. Furthermore, the study also identified the presence of pathogenic *E. coli* in Tilapia obtained from the district of Colombo.

II. MATERIALS AND METHODS

A. Sampling Fish

The samples (n = 8 from each location) were collected in January and February 2022 from three static water bodies (Beire, Polgasowita, and Diyawanna) and three water bodies with dynamic water (Athurugiriya, Werasa, and Meda Ela) in the District of Colombo. The samples were maintained at a temperature below 4°C during transport. Samples used for the bacterial analysis when bought into the laboratory were immediately stored at -20°C until further processing.

B. Analysis of Lead

Mass optimization calculations were performed as a part of the method development to determine the dry mass of skeletal muscles that need to be dry-ashed to achieve a method detection limit of 0.1 ppm. The dry-ashing method employed for the determination of the Lead content was then validated by evaluating the Matrix Effect, Limit of Detection (LOD), Limit of Quantification (LOQ), and Spike Recovery. Analysis of the content of lead in skeletal muscles of Tilapia was conducted according to the dry ashing process published in AOAC 999.11 with minor modifications using a Hitachi ZA3000 Atomic Absorption Spectrophotometer (Hitachi High-Technologies Corporation Japan) [2]. The effect of the static and dynamic nature of the water in the fish habitat on the content of accumulated lead was evaluated by conducting a t-test.

C. Human Health Risk Assessment

The potential human health risk associated with the consumption of Tilapia contaminated with lead was evaluated as described by Kithsiri et al. (2020). Initially, the content of lead was compared with Maximum Permissible Limits (MPL) followed by an evaluation of the health risk using various approaches such as comparing the Estimated Daily Intake (EDI) against Reference Dose (RfD) values reported by USEPA. The non-carcinogenic human health risks were evaluated by calculating the target hazard quotient (THQ). Furthermore, the maximum allowable daily fish consumption

limits for adults (CR_{lim} in kg/day) and the maximum allowable fish consumption rate per week CR_{mw} (meals/week) were also calculated.

D. Bacterial Analysis

Bacteria were collected by swabbing the gills of the Tilapia Sp. using sterilized swabs. The bacteria were then cultured on Luria Broth (LB) agar medium and incubated at 40°C for 24hrs. DNA was then extracted using the boiling method of DNA extraction. The extracted DNA was then subjected to two PCR amplifications using Universal Bacterial (UNIBAC) primers with forward primer 5' AAC TGG AGG AGG GTG GGG AT 3' and reverse primer 5' AGG AGG TGA TCC ACC CGC A 3'. The extracted DNA was also amplified using *E-coli* specific forwards primer 5'CAT TGA CGT TAC CCG CAG AA 3' and reverse primer 5'CGC TTT ACG CCC AGT AAT TCC 3'. The temperature conditions were denaturation 5 mins, 94°C; 30 cycles of 30 mins, 94°C; 30mins, 55°C; 40 mins, 72°C; and final extension 10mins, 72°C. The amplified PCR products were then analyzed using Agarose gel electrophoresis.

III. RESULTS AND DISCUSSION

A. Method Development and Validation

The LOD and LOQ in the dry-ashed matrix were determined to be 0.03 ppm and 0.09 ppm. The obtained detection limits were then used for the calculation of the Method Detection Limit (MDL). Furthermore, the matrix effect that resulted when the dry ashing procedure was followed was evaluated to be 6.25%. Matrix effects below 12% are considered negligible. Consequently, a simple external calibration was used over the method of standard addition.

The Food and Agriculture Organization (FAO) and other organizations such as the European Union (EU) have set the MPL of lead in fresh fish to 0.3 ppm. As such the present study aimed to employ a method with an MDL of 0.1 ppm. Results of mass optimization calculations indicated that a dry mass of 4.23 g is to be dry-ashed to reach the expected MDL of 0.1 ppm.

The precision and the accuracy of the method used for the determination of lead were then evaluated with the help of recovery studies. A recovery of 93.53% (± 4.32) was obtained for the high spike and a recovery of 91.09% (± 6.82) was obtained for the low spike. The high recoveries obtained in this study reflect the high accuracy of the employed procedure whilst the low standard deviations reflect the high precision of the method used for the determination of the content of lead.

B. Analysis of Samples

The concentration of lead in Tilapia captured from the district of Colombo was investigated for the first time. It was identified that the concentration of lead in the skeletal muscles was below the MPL's decided by the FAO and other regulatory organizations.

The t-test conducted to study the effect of the static and dynamic nature of the water body in the fish habitat on the accumulation of heavy metals indicated that no significant difference exists in the content of lead present in the skeletal muscles of Tilapia based on the type of water in its habitat as the obtained p-value was greater than the standard p-value of 0.05.

Table 1. Concentration of Lead in Tilapia sp. obtained from static and dynamic water sources

Static Water Bodies		Dynamic Water Bodies	
Location	Avg. Lead content (ppm)	Location	Avg. Lead content (ppm)
Diyawanna	0.14 (±0.01)	Meda Ela	0.22(±0.01)
Beire	0.12 (±0.01)	Athurugiriya	0.17 (±0.02)
Polgasowita	0.15 (±0.02)	Werasa Ganga	0.15 (±0.01)

C. Human Health Risk Assessment

Because there was no substantial difference in the accumulated lead content of the two types of water bodies, the human health risk assessment was not undertaken separately for static and dynamic water bodies, instead, a common human health risk assessment was undertaken using the highest concentration of metal ions identified in this study in edible muscle tissues [1]. The EDI was calculated to be 0.0005 mg/kg per day in an adult weighing 70 kg and was several folds lower than the reference doses reported by the US EPA. Similarly, the THQ value was calculated to be 0.127 and this was distinctively much lower than the THQ threshold value of one indicating that it is unlikely to experience non-carcinogenic adverse effects [1]. Furthermore, the CR_{lim} and CR_{mw} values of 0.47 kg/day and 15 meals/week respectively also indicate that it is unlikely that moderate-level fish consumers are at a human health risk.

D. Bacterial Analysis

The morphologies of the cultured bacteria were observed and two similar colonies were identified. These colonies were similar in almost all morphological features however a slight difference in their colors was observed [3]. As such DNA was extracted from the two colonies and subjected to PCR amplification using the UNIBAC and pathogenic *E. coli* specific primers. Upon subjecting the PCR products to gel electrophoresis the presence of bacteria was confirmed by the observation of a 370bp band, similarly, the presence of pathogenic *E. coli* was confirmed by the presence of a 99bp band. Therefore, the consumption of inland fish Tilapia in raw or unprocessed form could pose a risk to human health.

IV. CONCLUSION

The present study discovered that the lead content of Tilapia muscles from both static and dynamic water bodies was within the maximum allowable limits defined by FAO and EU guidelines, indicating its suitability for human consumption. Furthermore, the results of the human health risk assessment indicated that the lead content of Tilapia skeletal muscles from both static and dynamic water bodies was within the maximum allowable limits defined by FAO and EU guidelines, indicating its suitability for human consumption would pose non-carcinogenic human health risks to moderate level consumers. This study also concluded that there is no significant difference in the content of lead present in skeletal muscles based on the type of water in the fish habitat. However, the bacterial analysis discovered the presence of *E. coli* in the gills of the fish indicating that the consumption of Tilapia in raw or unprocessed form could pose a human health risk.

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