ORIGINAL ARTICLE

The Effect of Age and Body Mass Index on Dietary Health Risks Assessment of Heavy Metals From *Channa Striatus* Fish Intakes From Paddy Field

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ABSTRACT

Introduction: Trace heavy metal accumulation in pesticide-treated paddy fields was shown to be associated with its concentration in soil and irrigation water, which was affected by soil organic matter and soil/water pH present in the environment. Fish raising and paddy growing are commonly found at pesticides-treated farmland as sources of income and dietary protein intake. Considering the increasing number of metabolic disorders among the elderly from rural communities, the purpose of this study is to determine the concentration of heavy metals (lead and arsenic) in the *Channa Striatu*s by examining the effects of age and body mass index (BMI) on the assessment of human dietary health risks in a rural agricultural community. **Methods:** Two kilograms of *Channa Striatus* were collected during the paddy harvest season to examine the heavy metals and 120 farmers were recruited to examine their dietary intakes of *Channa Striatus* to estimate the potential dietary health risks, that include non-carcinogenic (Hazard Quotient, HQ) and lifetime cancer risk (LCR). **Results:** Findings show that both lead and arsenic were found not to exceed the permitted level in the *Channa Striatus.* However, the estimated non-carcinogenic health risks (HQ) and carcinogenic health risks (LCR) of heavy metals dietary intake of *Channa Striatus* were found to increase proportionally with age and BMI. **Conclusion:** Although lead and arsenic residues found in fish were lower than the permitted levels, lifetime accumulative of low levels consumption of the contaminated fish that increased with ages and BMI are of concern for its possible chronic health risks.

Keywords: Fish; Heavy metals; Carcinogenic health risk; Non-carcinogenic risks, Dietary health risk

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INTRODUCTION

Decades ago, the Malaysian government initiated the National Agricultural Policy that accelerates the transformation of smallholder agriculture into an industrial agricultural setting. Rice is a primary food crop in the country, as nine out of every ten Malaysian eat rice. Apart from rice, the community from this industrial-based rice farming village consume fishes caught from the rice-fish system. Among all the fishes, *Channa striatus* (Haruan fish) (Figure 1) is an indigenous, predatory freshwater fish that enters rice fields naturally via nearby streams and is a favorite by most farm communities. The fish production might vary significantly from 1.5 to 174kg/ha/season. This supply of animal protein is critical for the farm community's diet.

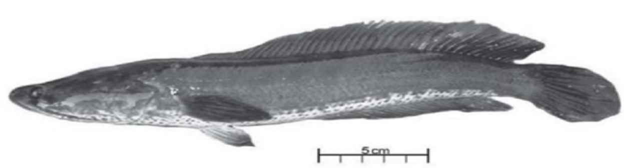

Figure 1 : *Channa striatus* (Haruan fish)

Channa striatus provides a high level of amino acid and contained polyunsaturated fatty acid, which helped to regulate prostaglandin synthesis and wound healing (2). Other beneficial aspects such as anti-inflammatory, anti-microbial, anti-nociceptive, and anti-cancer properties have also been highlighted by past studies (1,3). Even though *Channa striatus* fish appears to have wide-ranging medical uses, intensive farming has resulted in a mixture of contaminants entering the fish's body through direct intake of water and food into the digestive tract or porous gill surface membranes (4).

Chemical pesticides and heavy metals are among the contaminants widely found in the soil and water from the pesticide-treated farmland. For instance, heavy metals are commonly found in inorganic fertilizer and are the result of the long-term application of wastewater and animal manure to agricultural soils (5-6). The accumulating residues of heavy metals in the environment have alarmed the farming community, as *Channa striatus* is a fish that is frequently captured directly from rice field rivers. Given that *Channa striatus* is an essential source of protein in the agricultural community's daily diet, the harmful heavy metals found in the fish may work against their good effects.

Certain heavy metals have been linked to obesity and obesity-related comorbidities as a result of prolonged heavy metals exposure (7,8). Toxic metals may contribute to obesity by interfering with various aspects of metabolism, such as by substituting for essential micronutrients and vital metals, or by inducing oxidative stress. Another study suggested that oxidative stress is associated with heavy metal exposure and aging (9). Additionally, it is well established that with increased age, an increasing number of senescent cells accumulate in tissues and organs. Senescent cells can secrete immunostimulatory factors, initiate inflammatory responses, and impair the viability of surrounding tissues and cells (10).

Since fish is one of the best indicators of heavy metal pollution, the concentration of heavy metals such as lead (Pb) and arsenic (As) in *Channa striatus* from rice field fisheries is of interest in this context. Given the cumulative dietary health risks, the study's objective is to determine whether obesity and aging are risk factors for increased dietary health risks associated with *Channa striatus* consumption in the farm community.

METHODS AND MATERIALS

Study Location

AKuala Selangor, located in the North-West Selangor Integrated Agricultural Development Area, has one of Malaysia's highest rice yields among the country's eight granary areas.

Channa striatus (Haruan Fish) Collection and Preparation

During the paddy harvest season, two kilograms of fish were collected along trenches that connected five different rice fields. The fish were collected using a landing net, as is customary in the farm community. Fish were washed in aqueous water and stored in a clean polyethylene bag in an icebox. The fish were then frozen at -20°C at the Environmental Health Laboratory. The following day, the fish was thawed to room temperature in preparation for the extraction process. Stainless steel knives were used to remove the fish muscle, which was then homogenized and weighted. After the process was completed, fish samples were oven-dried to constant weight for 48 hours at 80°C in acid-washed Petri dishes. After cooling the fish samples in desiccators, they were ground to a fine powder using a porcelain pestle and mortar.

Heavy Metals (Lead and Arsenic) Analysis

Five (5) grams (g) of fish were duplicated and microwaved in a closed vessel with 3ml of ultra-pure nitric acid (64 percent). The procedure took approximately ten minutes, and the entire sample was diluted with deionized water, filtered through 0.45m Whatman filter paper, and stored for ten minutes in a 15ml centrifuge tube. The final step is to determine the concentration of heavy metals using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (Model: ELAN 9000 Perkin Elmer ICP-MS) (11). The calibration solutions for the elements were prepared by diluting stock solutions of 30g/kg, 50g/kg, 100g/kg, 300g/kg, and 500g/kg, and the solution for the multi-element calibration standard was prepared by adding 5% of HNO3 multi-element calibration standard to each element. The purpose of this study was to determine the presence of heavy metals such as lead (Pb) and arsenic (As) in fish samples.

Community Survey (Questionnaire)

A total of 120 farm communities responded to a questionnaire concerning dietary health risks associated with *Channa striatus* intakes in order to further estimate the non-carcinogenic health risks (Hazard Quotient, HQ) and carcinogenic health risks (Lifetime Cancer Risk, LCR) associated with dietary intake. To estimate the dietary health risks from consuming contaminated fish found in the rice field fisheries, a face-to-face community survey session was performed by randomly interviewing 120 farm communities. The inclusion criteria included villagers between 20 to 70-year-old, who lived in the village for at least five years and had consumed *Channa striatus* in their everyday dishes.

Dietary Health Risk Assessment

Food safety affects us all, and in our daily lives, we want to avoid food that puts our health at acute or chronic risks. As proposed by USEPA dietary health risk assessment, we use Hazard Quotient (HQ) to estimate non-carcinogenic and Lifetime Cancer Risk (LCR) to calculate the potential cancer risks via dietary intake of Channa striatus.

HQ (12) is used to determine the ratio of the consumed dose of a toxic metal via an Oral Reference Dose. HQ values above (>) 1 mean that the contaminated food intake has likely to have noticeable harmful effects on the exposed population. The higher the HQ value is, the higher the probability of the hazard risk of the human body will be.

$$HQ_{ingestion} = ADD/RfD \text{ ---- Equation 1(a)}$$

HQ = Hazard Quotient (unitless)
ADD = Average Daily Dose (mg/kg-day)
RfD = Reference dose (mg/kg-day)

$$ADD = \frac{(Cf \times Ri \times Fi \times EF \times ED)}{(BW \times AT)} \text{ --- Equation 1(b)}$$

Cf (heavy metals) = Concentration (lead and arsenic)
Ri (Fish Ingestion Rate)
Fi (Fraction Ingestion Contamination)
EF (Exposure Frequency) = Total consumption
AT = Average time
ED = Exposure duration
RfD = Oral Reference Dose = 0.002[a] for Lead and 0.0003[b] for Arsenic

a Oral reference dose of lead, reference from Ecotoxicology and Environmental Safety
b Oral reference dose of arsenic, reference from Integrated Risk Information System (IRIS) USEEPA

Lifetime Cancer Risk (LCR) (12) is used to estimate the product of the lifetime average daily dose (LADD) and the cancer slope factor (CSF). LCR at the threshold value of more than (>) 10^{-4} indicates that 1 in every 10,000 population will likely to cause cancer, dietary LCR reaching this threshold range shall be in particular concern as it is likely to have chronic diseases.

$$LCR = LADD \times CSF \text{ --- Equation 2(a)}$$

LCR = Lifetime cancer risk
LADD = Lifetime average daily dose
CSF = Cancer Slope Factor= 0.042[a] for Lead and 1.5[b] for Arsenic

a Cancer slope factor of lead (13)
b Cancer slope factor f arsenic, reference from Integrated Risk Information System (IRIS) USEEPA

$$LADD = \frac{(Cf \times Ri \times Fi \times EF \times ED)}{(BW \times AT)} \text{ --- Equation 2(b)}$$

ETHICAL CLEARANCE

This study was approved by Ethics Committee for Research Involving Human Subjects, Universiti Putra Malaysia (Ref No. UPM/TNCPI/RMC/JKEUPM/1.4.18.2)

RESULTS

Background of Local Farm Community

The farm community who claimed to use pesticides in their rice farmland share a similar socio-demographical background as in average age, educational level, income, and their Body Mass Index (BMI) were randomly recruited as shown in Table I. Besides, Table II summarizes their fishing activity and their fish dietary background.

Table I: The Socio-demographic and Occupational Background of Local Villagers (N=120)

		Male (n=63)	Female (n=57)
Age (Mean ± SD)		46.63 ± 14.41	44.51 ± 14.40
Education (%)	Primary school	20.6	14.0
	Secondary school	44.4	35.1
	Diploma	20.6	21.1
	Degree	7.9	15.8
	Others	6.3	14.0
Income (%)	RM3,000	33.3	14.0
	<RM3,000	66.7	86.0
Body Mass Index (BMI) (%)	Underweight	-	1.80
	Normal	63.5	45.6
	Overweight	33.3	50.9
	Obese	3.2	1.8
Occupational (%)	Farmer	91.2	65.1
	Non-farmer	8.8	34.9
Pesticide usage (%)	Yes	91.2	65.1
	No	8.8	34.9

Table II: Fishing Activity and Fish Dietary Background among Local Villagers (N=120)

		Percentage (%)
Experience of catching fish from trenches (%)	Yes	68.3
	No	31.7
Eating frequency (Consumption per year) (%)	6 times	38.3
	11 times	13.3
	12 times	17.5
	36 times	25.0
	48 times	3.3
	96 times	1.7
	288 times	0.8
Fish size per serving (Mean ± SD)		1.90 ± 0.63

Heavy Metals (Lead, Pb and Arsenic, As) Concentration levels in body *Channa striatus*

By using the One-sample t-test, we compare the average of heavy metals contained in the fish body with the allowable dietary intake standard as established by the Malaysia Food Act 1983. Even though the concentration of Pb is higher than the As level found in the fish, there is a significant difference from these heavy metals levels in the fish as compared with the allowable dietary intake standard.

Table III: Comparison of Heavy Metals (Lead and Arsenic) Concentration levels in Body Fish

Heavy metal	Mean (SD) (µg/kg)	Standard[a] (µg/kg)	*p*-value
Lead (Pb)	8.28 (1.73)	2000	<0.001**
Arsenic (As)	5.90 (0.16)	1000	<0.001**

[a] Food Act Malaysia 1983
** *p*-value is significant at 0.001

Non-carcinogenic (HQ) Dietary Health Risk Assessment on Age and Body Weight

By utilizing Equation (1), the potential non-carcinogenic health risk of heavy metals lead and arsenic via dietary *Channa striatus* intake was estimated. Table IV tabulated the hazard quotient (HQ) based on lead and arsenic in fish, respectively.

Considering the lead and arsenic contained in *Channa striatus*, both their HQ value show less than one at all ages and BMI range among the farming community. Nevertheless, a similar dietary health risk trend was found across different age ranges and BMI groups. For instance, HQ for lead and arsenic is higher among the elderly, especially at the age of 51 – 70 years old. The farmer who is obese and overweight reported having higher $HQ_{lead\ \&\ arsenic}$ as compared to farmers who are either healthy body weight or underweight. Overall, arsenic has an increased HQ value as compared to lead in *Channa striatus.*

Table IV: Estimation of Hazard Quotient (HQ) value based on Heavy Metals contained in *C. Striatus* fish via dietary intake

	Male (n=63)			Female (n=57)		
	n(%)	$HQ_{arsenic}$ (mean ±SD)[a]	HQ_{Lead} (mean ±SD)[a]	n(%)	$HQ_{arsenic}$ (mean ±SD)[a]	HQ_{Lead} (mean ±SD)[a]
Age						
20-30	6(9.5)	0.19 ±0.23	0.04 ± 0.49	15 (26.3)	0.43 ± 0.45	0.09 ± 0.09
31-40	17(26.9)	0.30 ±0.29	0.06 ± 0.06	5 (8.7)	0.38 ± 0.24	0.08 ± 0.05
41-50	10(15.8)	0.18 ±0.19	0.03 ± 0.04	16 (28.1)	0.26 ± 0.25	0.05 ± 0.05
51-60	17(26.9)	0.39 ± 0.47	0.08 ± 0.10	10 (17.5)	0.46 ± 0.33	0.09 ± 0.07
61-70	13(20.6)	0.84 ± 2.36	0.17± 0.40	11 (19.3)	0.46 ± 0.43	0.09 ± 0.09
BMI						
Under-weight	-	-	-	1(1.8)	0.24 ±0.01	0.05 ± 0.01
Normal	40 (63.5)	0.05 ± 0.02	0.01 ± 0.01	26 (45.6)	0.30 ± 0.31	0.07 ± 0.06
Over-weight	21 (31.7)	0.30 ± 0.36	0.06 ± 0.07	29 (50.8)	0.48 ± 0.40	0.10 ± 0.05
Obese	2 (3.2)	0.64 ± 1.85	0.13 ± 0.39	1(1.8)	0.65 ± 0.02	0.13 ± 0.01

[a]Hazard Quotient (HQ) more than (>1) indicated that there is potential of non-carcinogenic health risks via dietary intake

Carcinogenic Dietary Health Risk Assessment on Age and Body Weight

Table V shows the mean value for LCR estimation by considering the age and BMI of an individual. Overall, the LCR of exposure to lead and arsenic among the farming community was lower at 1 x 10^{-4}, i.e. the LCR threshold range established by the United States Environmental Protection Agency (USEPA) regulatory organizations. Nevertheless, a similar carcinogenic dietary health risk trend was found across different age ranges and BMI groups. For instance, LCR for lead and arsenic increased among the elderly, especially at the age of 51 – 70 years old. Besides, the obese and overweight farmers reported having relatively higher LCR values for lead and arsenic as compared to farmers who are either healthy or underweight. Arsenic, on the other hand, has relatively higher LCR values as compared to LCR values of Pb levels.

Table V: Estimation of Lifetime Cancer Risk (LCR) value based on Heavy Metals contained in haruan (*C. Striatus)* fish via dietary intake

	Male (n=63)			Female (n=57)		
	n(%)	$LCR_{arsenic}$ (mean ±SD)[a]	LCR_{Lead} (mean ±SD)[a]	n(%)	$LCR_{arsenic}$ (mean ±SD)[a]	LCR_{Lead} (mean ±SD)[a]
Age						
20-30	6(9.5)	3.2 x 10^{-5}	1.5 x 10^{-6}	15 (26.3)	8 x 10^{-5}	2.2 x 10^{-6}
31-40	17(26.9)	7.4 x 10^{-5}	2.1 x 10^{-6}	5 (8.7)	1.3 x 10^{-4}	2.6 x 10^{-6}
41-50	10(15.8)	5.3 x 10^{-5}	2.3 x 10^{-6}	16 (28.1)	1.2 x 10^{-4}	2.5 x 10^{-6}
51-60	17(26.9)	1.2 x 10^{-4}	3 x 10^{-6}	10 (17.5)	1.2 x 10^{-4}	2.8 x 10^{-6}
61-70	13(20.6)	3.3 x 10^{-4}	3.1 x 10^{-6}	11 (19.3)	1.4 x 10^{-4}	2.9 x 10^{-6}
BMI						
Under-weight	-	-	-	1(1.8)	-	-
Normal	40 (63.5)	1.0 x 10^{-5}	2.5 x 10^{-6}	26 (45.6)	8.6 x 10^{-5}	2.1 x 10^{-6}
Over-weight	21 (31.7)	9 x 10^{-5}	2.3 x 10^{-6}	29 (50.8)	2.3 x 10^{-4}	2.3 x 10^{-6}
Obese	2 (3.2)	2.2 x 10^{-4}	1.3 x 10^{-6}	1(1.8)	1.4 x 10^{-4}	2.1 x 10^{-6}

[a]Hazard Quotient (HQ) more than (>1) indicated that there is potential of non-carcinogenic health risks via dietary intake

DISCUSSION

Intensive farming has resulted in the widespread use of chemical fertilizers by farmers, who are widely regarded as a major source of heavy metal pollution in agricultural fields (14-15). For instance, lead and arsenic are found at all altitudes in terraced rice fields as a result of chemical fertilizer and pesticide use (16). Worse yet, these heavy metals are found not only in industrial and residential rice fields but also in paddy fields that develop organically (17).

Given that the majority of individuals are exposed to heavy metals through dietary intake (17), this study examined the potential health risks associated with heavy metals through the dietary intake of *Channa striatus*. Plates of seafood, primarily fish, have contributed significantly to dietary heavy metal intake and have garnered the attention of public health practitioners in recent years (18-19). Fish can assimilate heavy metals through ingestion of suspended particulate matter in the water, through food consumption, through ion exchange across their lipophilic membranes (e.g., the gills), and through adsorption on tissues and membrane surfaces. The presence of toxic heavy metals in this aquatic biota is concerning due to the potential health risks associated with their consumption by humans. Accumulation of heavy metals in the tissues can result in chronic disease and may harm the population (19-20). Consumption of heavy metals above the recommended daily allowance has detrimental effects and can result in non-carcinogenic hazards such as neurological problems, migraine, liver, and kidney disease (19, 21).

This study showed that lead and arsenic were present in trace amounts in fish muscles, with lead-containing 0.00828mg/kg more than arsenic at 0.00589mg/kg. Both heavy metals in concentrations that do not exceed the permissible limit established in the Food Act 1983. This finding was consistent with past studies indicating that lead concentrations were generally below the permissible level (22-23). Besides, the study demonstrates that the lead and arsenic's HQ and LCR estimation via dietary intake of *Channa striatus* was found to be negative for both indices. This is consistent with past studies (24), which showed that the $HQ_{lead\ \&\ arsenic}$ in fish samples were generally less than 1. The findings of this study indicate that the LCR ranges for both heavy metals are 3.1×10^{-6} -1.2×10^{-4}. This result is consistent with past studies, which show that the $LCR_{lead\ \&\ arsenic}$ in adults are between 2.71×10^{-6}-1.04×10^{-4}, which is within the acceptable range (25).

Apart from estimating diet-related health risks, this study also estimates the potential dietary health risks based on age and BMI. This study found that HQ and LCR increase proportionately with age and BMI. Many people view aging as a toxic process, and many of the functional changes associated with aging are similar to the toxic process. Given that the cumulative health effects were known as an additive, this could be a result of bioaccumulation due to age-related heavy metals consumption (26). Another study indicated that aging is associated with physiological changes such as molecular damage and changes in tissue composition and that low-level dietary intake of contaminated food sources is causally associated with aging and subsequent chronic disease as a result of cumulative damage (27). This explains that the HQ and LCR values for lead and arsenic are higher in the elderly, particularly those between the ages of 51 and 70 in this study.

Other than the assumption that toxicity increases with age, another study suggested that an increased percentage of fat found in individuals acts as the magnet for lipophilic persistent heavy metals toxicants inside the human body (28). Additionally, a recent study indicates that cumulative exposure to heavy metals in mixtures over time is associated with obesity and the chronic conditions associated with it, such as hypertension and type II diabetes mellitus (29-30). Heavy metals may exacerbate oxidative stress by decreasing ATP synthesis and increasing lipid synthesis (31). Additionally, reactive oxygen species generated by heavy metals can impair mitochondrial function, alter the activity of enzymes such as aconitase, and disrupt the tricarboxylic acid cycle via oxidative phosphorylation (32-33). These findings are consistent with the current study, which found that obese and overweight farmers reported having higher HQ and LCR values for lead and arsenic than healthy or underweight farmers.

CONCLUSION

In conclusion, this study found that when *Channa striatus,* a commonly consumed fish species, contained trace amounts of lead and arsenic when collected from trenches connected to the rice field. *Channa striatus* had an average concentration of heavy metals of 8.28 μg/kg (Pb) > 5.90 μg/kg (As). Lead and arsenic had HQ values less than one, indicating that the non-carcinogenic dietary health risk to local villagers was negligible. Besides, lead and arsenic both had LCRs within an acceptable range ($<1 \times 10^{-4}$). Additionally, it was found that the non-carcinogenic health risk and lifetime cancer risk associated with lead and arsenic were proportional to age and BMI, whereas cumulative exposure to heavy metals may play a role in both aging and obesity, and related metabolic conditions. Even though the estimated dietary health risks are negligible and within an acceptable range, the study raises concerns among farming communities because the relatively low levels of heavy metals chronically evoke potential health risks over time, particularly among the elderly and villagers who report being overweight or obese.

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