Heavy Metal Concentration in Flesh Muscle of Selected Salted Fish and Health Risk Assessment among Adults in Fishing Villages in Malacca

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ABSTRACT: Salted fish is one of Malaysian popular food products and consumed widely as a delicious complementary dish among locals. This food may be contaminated with heavy metals due to water sources contamination or cross contamination during processing. This research was conducted to determine the lead (Pb), chromium (Cr), arsenic (As) and cadmium (Cd) concentration in flesh muscle of selected salted fish and to assess the health risk of adults in a fishing village in Malacca. A total of 118 respondents were randomly recruited based on inclusion criteria and interviewed to obtain socio-demographic information as well as salted fish frequency intake by using pre-tested questionnaires. Four types of salted fish namely *kembong (Psettodescrumei)*, *bulu ayam (Thryssamystax)*, *gelama (Decapterus russelli)* and *parang* (*Makrochirichthys makrochirus*) were analysed using Inductively Coupled Plasma Mass Spectrometer (ICP-MS) to determine the heavy metals concentrations. Health risk were calculated for Hazard Quotient (HQ) for non-carcinogenic and Lifetime Cancer Risk (LCR) for carcinogenic health effects. Results showed that heavy metals detected in flesh muscle of salted fish ranged as follow: Cr (9.137 mg/kg) >Pb (2.580 mg/kg) > Cd (0.367 mg/kg) >As (0.213 mg/kg) respectively. As compared to the Food Regulation 1985, Cr and Pb concentration exceeded the permitted level of 1 and 2 mg/kg respectively. The calculated health risk due to consumption of selected salted fish showed that HQ and LCR fall under acceptable risk for all fish type. Heavy metals were detected in salted fish in this study which indicated certain levels of

contamination to our food sources. Public should be aware for consuming this kind of food because they might cause the accumulation of heavy metals in the body which may cause related chronic diseases with prolonged consumption.

Keywords: heavy metals, salted fish, food contamination, health risk assessment, Malaysia

Introduction

Salted fish is one of Malaysia's traditional preserved foods which is consumed together with rice, dishes and vegetables. To prepare a salted fish, fresh gutted fish were cured in salt or salt brine and then left to dry in the hot sun for several days. These desiccated fish can be kept for several months and can be used to prepare various type of food. Several studies indicated that fish and seafood are contaminated with pollutants *via* contamination of the fish natural habitat. One of the pervasive forms of marine pollution is heavy metals contaminations because the metallic elements do not disintegrate rapidly in marine environment. Metals which contaminated fish can be taken up from water, food, sedimentation, and suspended particulate materials (Agusa *et al*., 2005).

Fishes have been on the top level of food chain for its ability to bio accumulates heavy metals in the flesh muscle and other fish organs. The safety consumption needed to take into consideration as human consumes fish as their main protein sources (Lihan *et al*., 2006). Heavy metals such as lead (Pb), chromium (Cr), arsenic (As) and cadmium (Cd) has been recognised as strong biological poison due to their persistent nature and cumulative action (Carbonell *et al*., 2009). Cr, As and Cd are classified as carcinogenic and teratogenic agents (ATSDR, 2007). Pb on the other hand is classified as very toxic that even low dosage of Pb may contribute to mental retardation and disrupting the learning process of children (CDCP, 2002). This study aimed to determine the heavy metals concentration in popular salted fish and calculate the health risk of consuming this polluted food.

Materials and methods

This cross-sectional study was carried out among adults in fishing villages in the coastal of Kuala Sungai Baru in Alor Gajah district, Malacca. A total of 118 permanent residents of the villages aged between 18 to 59 years old and consumed salted fish at least once a week were recruited as respondents through random sampling. Those of immune compromised group such as pregnant women and cancer patients were excluded. Data collection was carried out between March to May 2014.

A set of questionnaire was used to obtain information on socio-demographic, salted fish frequency intake, other sources of heavy metal exposure, and also general health information from each respondent through face to face interview. The species of salted fish samples were selected based on the finding from the interviews. Four most popular salted fish species, namely *kembong (Psettodescrumei), bulu ayam (Thryssamystax), gelama (Decapterus russelli) and parang (Makrochirichthys makrochirus)* were collected from local market where the respondents purchased their food supply. About one kilogramme sample of each species were randomly collected from the market. The salted fish samples were placed in sterile plastic bags and put in a cool box with ice packs for temporary storage. The samples were brought to the laboratory to be digested by dry ashing method. One gram of gutted and cleaned flesh muscle of each fish samples were placed in a high form porcelain crucible before put into a muffle furnace. The furnace temperature was slowly increased from room temperature to 450 ºC in one hour. The samples were ash for about 4 hours until a white or grey ash residue were obtained. Five mL of nitric acid (HNO_3) were mixed with the residue and heated slowly to dissolve the residue. The solution was filtered using 0.45 µm flter paper and transferred to a 25 mL volumetric flask and made up to volume. Ten mL from the solution was transferred into centrifuge tubes and proceed to heavy metals detection by ICP-MS. Ten mL of standard solution was prepared and diluted to 5 ppb, 10 ppb, 20 ppb, 50 ppb, 100 ppb and 300 ppb by using $M_1V_1=M_2V_2$ formula. The standard solutions and a blank were analysed with ICP-MS to obtain the standard curve value of ±0.999 prior to the samples analysis.

Health risk of respondent was calculated using Hazard Quotient (HQ) and Lifetime Cancer Risk (LCR) (Luchenko, 2010) based on the concentration of heavy metals detected in the fish. The HQ and LCR were calculated as follow:

Hazard Quotient (HQ) = Average Daily Dose (ADD) ÷ RfD

Lifetime Cancer Risk (LCR) = Lifetime Average Daily Dose (LADD) x q*

ADD and LADDwere calculated as follow:

ADD/LADD (mg/kg-day) = (C x IR x EF x ED) ÷ (BW x AT)

Where, C =Average lead concentration in the freshwater fish (mg/kg); IR = Ingestion rate of freshwater fish (kg/day), EF = Exposure frequency (days/ years), ED = Exposure duration (years), BW = Average body mass over the exposure period (kg), AT = Averaging time (for ADD, AT = 20 years or 7300 days; for LADD, AT = 70 years or 25500 days), Oral RfD = Oral reference dose of each heavy metal (As = 3×10^{-4}, Cd = 5×10^{-4}, Cr = 3×10^{-3}, Pb = 0.075 mg/kg^{-day}), q* = Cancer Potency/Cancer Slope (As = 1.5, Cd = 6.1, Cr = 0.42).

HQ in which if the ratio is greater than 1 showed that the respondents might posed health effects. In cases where the non-cancer HQ does not exceed unity (HQ < 1), it was assumed that no chronic risks are likely to occur at the site (Mallory, 2010). The risk acceptability for carcinogenic health effects are $>10^{-4}$ = clearly unacceptable, 10^{-6} to 10^{-4} = acceptable, and $<10^{-6}$ = clearly acceptable.

The procedure in conducting this research was approved by Ethics Committee for Research involving Human Subjects of Universiti Putra Malaysia. All respondents were briefed about the study and inform consents were obtained prior to data collection.

Results and discussions

As shown in Table 1, majority of the respondents were Malay with more than half were female. They were almost equally representing the young and mature adults and majority of them were married. More than 80% of the respondents received education at least up to secondary school and majority of them were employed. Fifty six percent of them earned monthly income below the poverty line of RM850 (Zainal Azman, 2013). About 59% of the respondents have household of less than 5.

Table 1: Socio-demographic information of respondents (N=118)

Variable	**Frequency**	**%**
Gender		
Male/Female	47 / 71	39.8 / 60.2
Age (years)		
18-35	58	47.2
36-55	54	45.7
>55	6	7.1
Race		
Malay / Non-Malay	116 / 2	98.3 / 1.7
Marital Status		
Single	36	30.5
Married	77	65.3
Separated / Widowed	5	4.2
Educational Level		
No formal education	12	10.2
Primary	7	5.9
Secondary	85	72.0
Tertiary	14	11.9
Employment		
Government	17	14.4
Private	31	26.3
Self employed	23	19.5
Housewife	4	3.4
Not working / Others	43	36.4

Monthly Income		
≤ 850	67	56.1
> 850	51	43.9
Household		
≤ 5	73	59.3
> 5	45	38.1

The heavy metals concentration of each salted fish studied were shown in Figure 1. The highest heavy metal detected was Cr followed by Pb in three out of four species of salted fish samples. When compared with Malaysian Food Regulation (1985) permitted level of heavy metals in fish, Cr and Pb exceeded the level of 1 mg/kg and 2 mg/kg, respectively. As and Cd concentration detected in the fish samples were not exceed the permitted level of 1 mg/kg for both heavy metals, Cr was found the highest in the salted fish samples may due to this particular heavy metal was a major contaminant of water bodies and highly lethal because of its ability to accumulate in our body (Bhatkar, 2011).

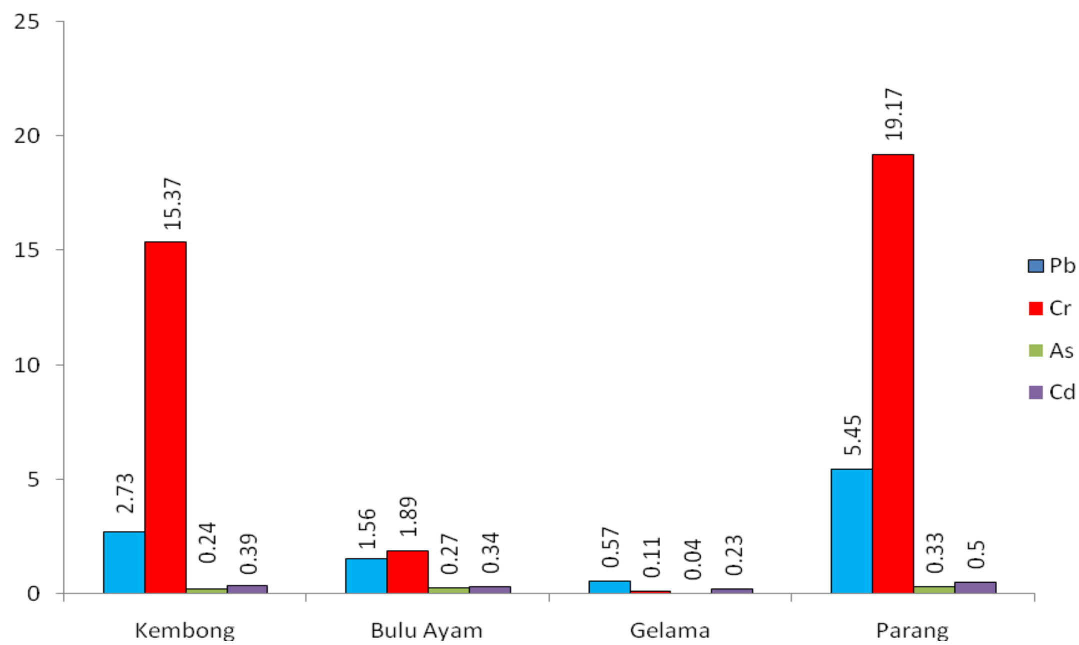

Figure 1: Heavy metals concentration (mg/kg) in each species of salted fish

The calculated Average Daily Dose (ADD), Lifetime Average Daily Dose, Hazard Quotient (HQ) and Lifetime Cancer risk (LCR) were presented in Table 2. HQ indicated the non-carcinogenic health effects while LCR indicated the carcinogenic effects of consuming food which contaminated with the heavy metals. The HQ was less than one which signified that all the respondents posed an acceptable risk condition for non-carcinogenic health risk. This study revealed that all respondents fall under clearly acceptable and acceptable risk towards the carcinogenic health effects (LCR). It might be due to the low level of heavy metals concentration detected in salted fish sample in study areas and the lifetime average daily dose (LADD) of the respondents and unlikely to cause adverse health effects to respondents as the salted fish was only taken in a very small amount to be added in the daily meals. Hence, the carcinogenic risk faced by the respondents considered as acceptable risk. This study result was similar with Amirah *et al.* (2013) who addressed the heavy metals contaminations in fish resulted in HQ less than 1. This study was also supported by Yujun *et al*. (2011) who suggested people would not experience significant health risks from intake of individual metals through fish consumption.

Although the present data indicated that health risk is unlikely to be happen, a certain amount of heavy metal contaminant were found in all salted fish samples. Even though the heavy metals contaminated salted fish were consume at small amount, the fact that salted fish is one of most favourable condiment make it to be consume continuously. There was no guarantee that the risk will remain at acceptable level in the future.

Continuous intake of heavy metals contaminated salted fish may cause accumulation which later can cause adverse health effects. Previous researches have proven that long-term, low level exposure to heavy metals could cause several health effects. A study conducted by Lustberget *et al.* (2002) stated that an increase exposure to Pb were associated and may give rise to cardiovascular events. The observed increase in Peripheral Arterial Disease (PAD) prevalence occurred at Pb levels much lower than current safety levels used by environmental and occupational regulatory agencies (Acean *et al*., 2004).

Table 2: Calculated ADD, LADD, HQ and LCR according to each heavy metal

Salted fish species / Heavy metal	**ADD**	**HQ**	**LADD**	**LCR**
Kembong				
Pb	1.92 ×10-7	5.48×10^{-5}	-	-
Cr	1.06×10-6	3.53×10^{-4}	3.03×10^{-7}	1.27×10^{-5}
As	1.70×10^{-8}	5.68×10^{-5}	4.90×10^{-9}	2.44×10^{-7}
Cd	2.80×10^{-8}	2.81×10^{-5}	8.00×10^{-9}	4.89×10^{-8}
Bulu ayam				
Pb	1.09×10^{-7}	3.12×10^{-4}	-	-
Cr	2.67×10^{-7}	8.87×10^{-5}	7.60×10^{-8}	3.19×10^{-6}
As	1.60×10^{-8}	5.44×10^{-5}	5.00×10^{-9}	2.34×10^{-7}
Cd	2.20×10^{-8}	2.16×10^{-5}	6.00×10^{-9}	3.76×10^{-8}
Gelama				
Pb	1.20×10^{-9}	3.41×10^{-7}	-	-
Cr	1.50×10^{-9}	4.90×10^{-7}	4.00×10^{-10}	1.76×10^{-8}
As	1.00×10^{-10}	3.85×10^{-7}	$<10^{-11}$	1.60×10^{-9}
Cd	4.00×10^{-10}	1.15×10^{-7}	$<10^{-11}$	7.00×10^{-10}
Parang				
Pb	5.70×10^{-9}	1.62×10^{-6}	-	-
Cr	2.08×10^{-8}	6.94×10^{-6}	5.90×10^{-9}	2.50×10^{-7}
As	4.00×10^{-10}	1.24×10^{-6}	$<10^{-11}$	1.60×10^{-9}
Cd	6.00×10^{-10}	5.66×10^{-7}	$<10^{-11}$	1.00×10^{-9}

HQ: Acceptable (< 1) LCR: Acceptable (10^{-6} to 10^{-4}), Clearly Acceptable (< 10^{-6})

The risk assessment done by Budiati (2010) showed that a pattern of skin changes was the most characteristic effect of long-term oral exposure to inorganic arsenic. Renal tubular dysfunction and consequent proteinuria are generally accepted as the main effects following long-term, low-level exposure to cadmium. Long-term renal tubular dysfunction may also lead to abnormalities of calcium metabolism which the symptoms include weak bones that lead to deformities, especially of the spine, or to more easily broken bones (Cheng and Gabos, 2007). Experimental studies have suggested that low-dose exposure to cadmium may affect life cycle related diseases and reproductive toxicity include endocrine disrupting effects (Kaji *et al*., 2002).

Our results showed that there was an evidence of Cr, As, Cd and Pb in every salted fish sample collected, therefore the public should aware of what they eat. This finding has the limitation by only calculated the health risk based on one food type which was the salted fish and other possible food which may also contaminated with heavy metals were not considered. This study was also not taking the biological sample as a better indicator for heavy metal actual exposure level.

Conclusion

Heavy metals were detected in all salted fish samples which Cr and Pb exceeded the Malaysian Food Regulatory limit. However calculated health risk indicated by HQ and LCR showed that the respondents fall under acceptable risk which depicted that they are unlikely to be affected by non-carcinogenic or carcinogenic health effects by consuming heavy metals polluted salted fish.

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