Selected non-essential and trace elements concentration in trash fish and aquafeeds

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Abstract. Some elements in feeds for aquaculture would raise health issues in fish. The objective of this study was to assess and compare the non-essential and trace elements in trash fish (collected during March 2018 from the fish landing port of Lembaga Kemajuan Ikan Malaysia (LKIM) in Pulau Kambing, Terengganu, Malaysia) and aquafeeds (10 samples from the market in Malaysia). Non-essential (As, Cd and Pb) and trace elements (Cu, Fe, Mn, Se and Zn) concentration in the trash fish and aquafeeds were determined using inductively coupled plasma mass spectrometry (ICP-MS). Hg was analyzed by Direct Mercury Analyser (DMA). The Standard Reference Material (SRM) 2976 was used in elements recovery and method validations. The concentrations of non-essential and trace elements, except for the Cu concentration, in trash fish and aquafeeds varied with significant differences (One-way ANOVA, LSD; $p<0.05$). The result showed higher Cd and Pb concentrations in aquafeeds than in trash fish, while the As and Hg concentrations in trash fish were higher than in aquafeeds. The concentrations of Fe, Mn and Zn in trash fish were higher than in aquafeeds, but not the concentrations of Cu and Se. The concentrations of elements in the samples from this study were within the European Union (EU) upper tolerable limits.
Key Words: aquafeeds, non-essential elements, trace elements, trash fish.

Introduction. In recent years, degradation of ecosystems or overfishing caused the reduction of wild fish, therefore aquaculture became important (Wong et al 2016). In the 1970's, about 3 million tons of fish were produced by aquaculture and reached 80.1 million tons by 2017, where the Asian region dominates this sector, accounting for 91.9% of the global productions (FAO 2019a). As fish is a major source of protein, therefore producing feed for fish farming is also important (Ikem & Egilla 2008). In accordance with the increasing aquaculture farming, the demand of the fish feed also increased. Trash fish is one of the major feed sources, as well as commercial feed such as pellets, also known as aquafeed, both for marine or freshwater species and in floating or sinking forms (Merican & Sanchez 2016).

Trash fish consists of marine fish and molluscs with no market value as human food, due to their low protein, and are discarded as by-catch, with potential environmental and aesthetical problems (Huntington & Hasan 2009; Nunoo et al 2009). Commonly used trash fish are small and medium pelagic fish species, such as anchovy, pilchards, herring, sardines, mackerel, capelin, sand eel, menhaden, lizard fish and pony fish (Nunoo et al 2009). Besides pelagic fish species, small shrimp and squid can also be used as trash fish (Nunoo et al 2009). In Asian finfish mariculture, in particular groupers, barramundi, snappers, pompano and cobia, the rapidly growing farming is still largely dependent on trash fish feed (De Silva & Hasan 2007).

Aquafeed is generally formulated feed, obtained by adding multiple ingredients to the regular nutrition. The required ingredients in order to ensure a good nutrition to the cultured species are: proteins, multi-vitamins, multi-minerals, binder agents, moisture and ash. In aquafeed production, fish meal is the main ingredient, as source of proteins (Bostock et al 2010; Cheng et al 2014). Besides having high protein content, fish meal also has a balanced composition of essential amino acids, digestible energy, minerals, and vitamins (Cheng et al 2014). However, the concern of the rising prices due to the scarcity of fishmeal forced the major industrial aquafeed manufactures to identify and evaluate alternate protein sources such as: soybean, corn, rice and wheat (Bostock et al 2010). Fish meal is an excellent source of dietary elements, thus replacing fish meal with other protein sources requires careful consideration of dietary mineral levels and of possibly antagonistic interactions that might necessitate additional elements supplementation in fish diets (Hardy 2001). However, using trash fish in fish meal and aquafeed production amplifies the risk of contamination of the human food chain with mercury, heavy metals, plastics, organic pollutants, hydrocarbons and other harmful substances, due to the environmental pollution (Mok et al 2014; Wong 2017).

Elements contained in food can be described as non-essential and trace elements. Non-essential elements are such as arsenic (As), cadmium (Cd), mercury (Hg) and lead (Pb). These elements are known to be not beneficial in daily activities of fish but they are found in fish and also in almost all organisms and feed. General lack of information and regulation concerning the presence of non-essential elements in feeds can cause possible short and long term effects on human health, therefore trade restrictions can be necessary (De Silva & Hasan 2007).

Trace elements such as: copper (Cu), iron (Fe), manganese (Mn), selenium (Se) and zinc (Zn) are components of body fluids, cofactors in enzymatic reactions and structural units of non-enzymatic macromolecules (Watanabe et al 1997). Elements deficiencies, although easily avoidable, are frequent in aquaculture fish diets (Hardy 2001). Dietary elements deficiencies fall into 3 general categories: (1) those which affect hard tissue mineralization; (2) those which affect other specific tissues; and (3) those which do not affect specific tissues (Hardy 2001). In order to maintain the balance of the trace elements in fish, it is necessary to determine their concentration in aquafeeds.

The purpose of this study was to determine and to compare the concentrations of non-essential and trace elements in trash fish and aquafeeds, according to the guideline levels.

Material and Method

Specimens collection. Trash fish was collected from the fish landing port, Lembaga Kemajuan Ikan Malaysia (LKIM) in Pulau Kambing, Terengganu, Malaysia in March 2018. Also, 10 samples of aquafeeds were collected from the available markets in Malaysia or imported from various countries such as China, Japan and Vietnam, besides of local made products. All production companies were kept confidential in this research and the products were categorised into aquafeeds for marine and freshwater species. The sampling of trash fish and aquafeeds was triplicated.

Precautions taken to avoid contamination. A few precautions were necessary to avoid contamination during the experiment. Deionized water was used to prepare all aqueous solutions. All glassware and polyethylene containers in contact with the samples were previously soaked in 1% of nitric acid for overnight and rinsed with deionized water. Metal boats that were used for the Hg content determinations were soaked in water with detergent over the night. The next day, the metal boats were rinsed with Milli-Q water and put in a furnace at 70°C for 3 h, before adding the samples.

Assessment of mercury (Hg). The samples were dried in an oven at 40°C until they were completely dried before being grinded using mortar and pestle. A quantity of 15 mg of each sample was then retained in the sample boats, for both trash fish and aquafeeds. Next, each sample was analyzed by Direct Mercury Analyzer (DMA) for about 8 min at 550°C to measure the total Hg concentration (Ikem & Egilla 2008). The mercury analyzer was calibrated using the Hg standard solution (Wako Pure Chemical Industries Ltd., Tokyo, Japan).

Assessment of non-essential and trace elements. The trash fish and aquafeed samples were mashed using mortar and pestle, while a ceramic knife was used to mash trash fish. About 7 mL of 65% concentrated nitric acid and 1 mL of 30% concentrated of hydrogen peroxide was added into a TFM (tetrafluoromethoxil) vessel with a sample of approximately 1 g (Jaafarzadeh et al 2015). The vessel was closed and was introduced into the rotor segment. After that, the vessel was tightened by using the torque wrench. The segment was inserted into the microwave cavity and the temperature sensor was connected. The microwave program was run at 200°C for 30 min (Ikem & Egilla 2008).

The rotor was cooled down until the solution reached room temperature. The vessel was opened and the solution was transferred into a 25 mL volumetric capacity flask with filter paper. Then, the vessel was rinsed with double deionized water to obtain a final volume of 25 mL. The samples were kept in a refrigerator at 4°C (Jaafarzadeh et al 2015). Finally, the samples were analyzed using ICPMS for the assessment of As, Cd, Cu, Fe, Mn, Pb, Se and Zn.

The ICP-MS was calibrated using the multi-element standard solution W-X (Wako Pure Chemical Industries Ltd., Japan), with a suite of elements having concentrations ranging from 0 to 10 μg mL^{-1}.

Recovery test method validation. The precision and accuracy of analyses were determined by repeated determinations in mussel tissue, according to the biological Standard Reference Material (SRM) 2976. The recovery values percentages were obtained when compared to the certified values listed in Table 1.

Table 1

References concentration values of SRM 2976 in mussel tissue

Elements	*Certified values (mg kg^{-1} dw)*	*Measured values (mg kg^{-1} dw)*	*Recovery (%)*
		Non-essential	
As	13.30±1.80	11.9±0.3	89
Cd	0.82±0.16	0.66±0.01	80
Hg	61.00±3.60	58.40±5.50	96
Pb	1.19±0.18	1.02±0.20	86
		Trace	
Cu	4.02±0.33	3.92±0.60	97
Fe	171.00±4.90	153.20±5.80	89
Mn	33.00±2.00	27.33±0.01	82
Se	1.80±0.15	1.71±0.01	95
Zn	137.00±13.00	121.02±0.30	88

All values are expressed as mean±SE (standard error); dw-dry weight.

Statistical analysis. The data were tested for normality using a Kolomogorov-Smirnov test and for homogeneity of variance using Levene's test. The data were analyzed using one-way analysis of variance (ANOVA) with a least significant difference (LSD) follow-up test, in order to find significant differences. Different samples' effects were considered at a significance level of $p<0.05$. Statistical calculations were performed with IMB SPSS statistics 25.

Results and Discussion

Concentration of non-essential elements. The concentration of non-essential elements in trash fish and aquafeeds dry weight are shown in Table 2. In the present study, the concentrations of non-essential elements such as Cd (Non-detected–0.006 mg kg^{-1}) and Pb (0.001–0.010 mg kg^{-1}) were lower than the As (0.027–0.062 mg kg^{-1}) and Hg (0.007–0.074 mg kg^{-1}) concentrations in all trash fish and aquafeeds. The concentration of As in the trash fish and aquafeeds for marine species (0.062±0.007 and 0.047±0.006 mg kg^{-1}) were significantly higher than in the aquafeeds for freshwater species (0.027±0.004 mg kg^{-1}), while the concentrations of Cd and Pb in trash fish (non-detected and 0.002±0.001) were significantly lower than in aquafeeds for marine species (0.006±0.001 and 0.010±0.001 mg kg^{-1}) and for freshwater species (0.003±0.001 and 0.009±0.002 mg kg^{-1}). The concentration of Hg in the trash fish appeared as significantly higher (0.074±0.023 mg kg^{-1}) than in aquafeeds for marine (0.025±0.003 mg kg^{-1}) and freshwater species (0.007±0.002 mg kg^{-1}).

Table 2
The concentration of non-essential elements (mg kg^{-1} dw) in trash fish and different aquafeeds collected in Malaysia

Samples	*n*	*As*	*Cd*	*Hg*	*Pb*
		mg kg^{-1} dw (Mean±SE)			
Trash fish	9	$0.062±0.007^{a}$	ND^{a}	$0.074±0.023^{a}$	$0.002±0.001^{a}$
Aquafeeds for marine species	7	$0.047±0.006^{a}$	$0.006±0.001^{b}$	$0.025±0.003^{b}$	$0.010±0.001^{b}$
Aquafeeds for freshwater species	3	$0.027±0.004^{b}$	$0.003±0.001^{c}$	$0.007±0.002^{b}$	$0.009±0.002^{b}$

All values are expressed as mean±SE (standard error); dw- dry weight; ND -non-detected. The mean difference is significant at p<0.05 by using One-way ANOVA with LSD test. Superscript letter-significant difference between samples.

Concentration of trace elements. The concentration of trace elements in trash fish and aquafeeds dry weight are shown in Table 3. The only element that showed no significant differences in the concentrations between trash fish and aquafeeds was Cu. Trash fish have significantly higher concentrations of Fe (0.370±0.045 mg kg^{-1}), Mn (0.008±0.003 mg kg^{-1}), Se (0.014±0.001 mg kg^{-1}) and Zn (0.143±0.026 mg kg^{-1}) than aquafeeds for marine and freshwater species. Aquafeeds for marine species have a significantly higher concentration of Se (0.020±0.001 mg kg^{-1}) than trash fish and aquafeeds for freshwater species, but showed a significantly lower concentration of Zn (0.016±0 mg kg^{-1}) than in trash fish and aquafeeds for freshwater species. Aquafeeds for marine and freshwater species had similar concentrations of Fe (0.109±0 and 0.108±0 mg kg^{-1}).

Table 3
The concentration of essential elements (mg kg^{-1}) in trash fish and different aquafeeds collected in Malaysia

Samples	*n*	*Cu*	*Fe*	*Mn*	*Se*	*Zn*
		mg kg^{-1} dw (Mean±SE)				
Trash fish	9	$0.021±0.002^{a}$	$0.370±0.045^{a}$	$0.008±0.003^{a}$	$0.014±0.001^{a}$	$0.143±0.026^{a}$
Aquafeeds for marine species	7	$0.043±0.014^{a}$	$0.109±0^{b}$	$0.003±0^{b}$	$0.020±0.001^{b}$	$0.016±0^{b}$
Aquafeeds for freshwater species	3	$0.007±0^{a}$	$0.108±0^{b}$	$0.003±0^{b}$	$0.013±0.001^{a}$	$0.175±0.080^{a}$

All values are expressed as mean±SE (standard error); dw- dry weight. The mean difference is significant at p<0.05 by using One-way ANOVA with LSD test. Superscript letter referred as significant difference between samples.

Accumulation of non-essential elements in trash fish and aquafeeds. Trash fish collected in this study were marine species, generally with high As concentrations, due to the organic As (Arsenobetaine), that is not poisonous (FAO 2019b). While NRC (2005) suggested that most of the As (inorganic) is derived from sea plants, fish products and supplemental minerals, tending to bioaccumulate in fish tissues. This may explain that aquafeeds for marine species has similar As concentrations compared to trash fish, probably due to the raw ingredients used. Deemy (2019) also suggested that As can be detected from the premix of feed in mineral supplements and mineralized salt. Thus, it is necessary to determine whether the As contained in fish feeds is in organic or inorganic forms.

The Hg concentration in trash fish is relatively higher than in aquafeeds. The accumulation of Hg in trash fish could be due to fish exposure to the polluted environments (Gomez et al 2010; Mok et al 2011). It suggested that by continuously feeding cultured fish with trash fish could cause accumulation of Hg. The Hg in aquafeeds for freshwater species has lower Hg concentrations, suggesting that Hg concentrations depend on the source of raw materials used in the fish pellets. Deemy (2019) stated that in mixed pellets the range of Hg detected concentrations was of 0–0.003 mg kg^{-1}, lower than in most of the samples tested in the current study. According to the EU permitted levels, the feed ingredients derived from fish or other aquatic animals should not exceed 0.5 mg kg^{-1} of Hg, while the fish feed should not exceed 0.2 mg kg^{-1} (EC 2002; Elliott et al 2017). This has suggested that the concentration of Hg in trash fish and aquafeeds were still within EU permitted level.

EC (2002) and Elliott et al (2017) stated that the concentrations of Cd and Pb in the premix used for feed formulation should not exceed EU permitted levels with more than 15 and 200 mg kg^{-1}, respectively, but in the completed feed for fish they should not exceed EU permitted levels with more than 1 and 5 mg kg^{-1}, respectively (EC 2002; Elliott et al 2017). It is noticed that the concentrations of Cd and Pb in trash fish are significantly lower than those in the aquafeeds for marine and freshwater species. Over 10 and 4%, respectively, in a series of 1,200 premixes tested from 2009 until 2016, showed Cd and Pb concentrations above the EU limits (Elliot et al 2017). This has suggested that the premix of the aquafeeds tested in the current study might have higher concentrations of these elements.

Accumulation of trace elements in trash fish and aquafeeds. Trace elements or micronutrients in fish nutrition have been neglected by the research. Trace elements are essential for fish and are involved in the normal metabolism and life processes: Fe and Se are needed for specific physiological functions, while Cu, Mn and Zn act as cofactors for enzyme activity (Hardy 2001). The trace element requirements in fish diet are, in mg kg^{-1}: Cu 3-5, Fe 30-170, Mn 2-30, Se 0.15 and Zn 15-80 (Chanda et al 2015). Hardy (2001) stated that aquaculture fish are at risk of Zn deficiency, mainly due to antagonistic interactions with other diet ingredients of the diet, such as phytate and high-ash levels (high calcium and phosphorus). Thus, additional Zn concentrations can be considered according to the cultured species, depending on the forms of Zn, which cause different interactions with the other ingredients (e.g. influencing their availability). Dietary Cu above the level of 15 mg kg^{-1} could reduce the feed efficiency and the fish hematocrit (FAO 1987). The dietary levels of Se above 13 mg kg^{-1} can cause high mortality (FAO 1987). Excessive levels or deficiency of dietary Fe can compromise the immune system (Lim et al 2001). It is necessary to be sure that the trace elements dosage in feed is sufficient for the fish, staying within the homeostatic limits. Nevertheless, the type of dietary trace elements source, whether of a chelated, inorganic or organic form, can influence their bioavailability in fish (Prabhu et al 2016). However, the studies on dietary trace elements in aquaculture remained scarce.

The concentration of all trace elements varied among trash fish and aquafeeds. Hardy (2001) therefore stated that NRC had suggested that a safety margin should always be made for diets with higher than minimum requirements since they include an extra amount to compensate for any losses that could occur in the processing of diets and distribution to fish. As mentioned previously by Elliott et al (2017), some of the premix in the feed industries might have higher elemental concentrations than the EU allowed limits for feed materials, therefore it is still necessary to be precautious with the usage of premix in aquafeeds formulation. Meanwhile, aquafeeds that are produced in factories had undergo several processes, including adding chemicals which limit the trace elements concentrations (Skøien et al 2016).

Trash fish vs fish pellet. From the current study it results that higher Cd and Pb concentrations were found in aquafeeds than in trash fish, while in trash fish there are higher As and Hg concentrations than in aquafeeds. The concentrations of Fe and Zn in trash fish were higher than in aquafeeds, but the concentrations of Cu and Se were lower in trash fish. Considering the concentrations of all the studied elements, this research can neither conclude in favor of the aquafeeds or in favor of the trash fish, regarding their safety and efficiency as aquaculture feed. Bunlipatanon et al (2014) also stated that the feed conversion rates (FCR) for both trash fish and fish pellet are not significantly different, but in some culture systems and in certain countries, trash fish feeding in the marine cultured fish is more cost effective. However, the quality of the trash fish is generally low and that may lead to bacteria growth in fish after feeding, which may cause sickness and disease to the cultured fish, as well as an increasing demand in the aquaculture sector and consequently increases the overexploitation of the available fish stocks (FAO 2004; Bunlipatanon et al 2014). In addition, the concentration of elements in trash fish is generally due to their diet (Hajeb et al 2010), feeding behavior (Dorea et al 2006) and living habitat (Mok et al 2011), thus it is very difficult to monitor the contamination of elements, particularly the non-essential elements in trash fish.

Conclusions. This study provides important information on the distribution of non-essential and trace elements in fish feeds such as trash fish and aquafeeds. With respect to the standard requirements for the maximum content of the non-essential and trace elements, the current samples did not exceed the limits. Nonetheless, it is suggested to use aquafeeds in aquaculture in order to avoid a possible transmission of diseases and Hg contamination due to the poor quality of trash fish. Further investigations on the mitigation or elimination of non-essential elements in the aquafeeds should be considered. In addition, as different fish species need different amounts of nutrition in their diet, self-additive supplements may be made for certain elements or nutrients in aquafeeds in order to enhance fish growth.

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