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RISK PROFILE AND SEMI QUANTITATIVE RISK PROBABILITY OF AFLATOXIN B1 FROM *Aspergillus flavus* IN A DRIED SALTED FISH IN SEVERAL REGIONS OFJAVA

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Abstract

This study presents a semi-quantitative risk analysis, which determines the probability of aflatoxin B1 exposure from *Aspergillus flavus* in dried salted fish from the results of research conducted by Indriati about the prevalence of aflatoxin B1 in commercial dried fish from some regions of Java. Samples were randomly collected from retailers in Java, such as Banten, DKI Jakarta, West Java, Central Java and East Java, to obtain an approximate level of aflatoxin B1 exposure into Indonesian consumers. The occurrence of the probability of aflatoxin B1 risk from *Aspergillus flavus* was calculated by statistical, probability approach in @risk version 7.0 software with Monte Carlo simulation. The results of this study showed that the consumption of salted fish was about 3.7 g/capita/day. Hence there are risks of 7.74 cfu/g *A. flavus* exposure and 0.7291 ppb aflatoxin B1 exposure in 1 g of a salted fish taken from sampling locations. However this value is still categorized as low risk level.

Keywords: risk analysis, *Aspergillus flavus*, mycotoxin, aflatoxin B1, dried salted fish

1. Introduction

Fish is an important marine natural resource, which is used as food that contains high nutritional value for human needs. However, fish is easily decomposed compare to meats, fruits, and vegetables. There are several ways to process fresh fish, either modern or traditional, which plays an important role in preservation. In Indonesia, roughly 50% of fish is traditionally processed. One of the most important fish product in Indonesia is salted fish. Salting is a preservation process of decaying bacteria, which usually conduct by the addition of 15-20% salt into to surface of fish (Siregar, 2004).

However, other biological contaminants may occur during raw materials preparation, processing, storage, and marketing (Wang & Liu, 2007). *Aspergillus flavus* is a biological contaminant in food products, especially in tropical region. This biological contamination has the ability to grow and live in a wide temperature range, can be mesophilic and thermophilic between 10-48°C, with a_w 0.8 and pH 2 (Makfoed, 1993; Kulshrestha, et al., 2008). Therefore, this organism is easy to grow in tropical country, such as Indonesia which has humidity and warm climatic condition (Maryam, 2007). A. *flavus* may also produce toxin as a secondary metabolites, such as aflatoxin B1 (Lanyasunya, Wamae, Musa, Olowofeso & Lokwaleput, 2006). Chronic exposure of aflatoxin B1 may affect to malnutrition and hepatocellular carcinoma (IARC, 1988). It was noted that people who experience chronic exposure to aflatoxin at high levels have a risk of having hepatocellular carcinoma three times greater than those without exposure (Yenny, 2006).

There were several reports that stated *A. flavus* contamination in fisheries products, nuts and cereals. In India, Prakash (2011) found that salted fish samples were positively contaminated by *A. niger, A.flavus, A. fumigatus, Absidia, Aerobasidium, Alternaria, Cladosporium*. In the same country, Sam, Jeyasanta, and Edward (2015) found that salted fish were contaminated by 23 species of fungi i.e. *A. flavus, A. fumigatus, A. terreus, A. niger, Absidia sp., Rhizopus*

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sp., Mucor sp., Penicillium, Fusarium moniliformis, A. oryzae, Trichoderma sp., Geotrichus candidus, A. sulphureus, A. terricola, A. awamori, A. flavipes, A. versocolor, A. tamari, Eurotium sp., Alternaria, A. parasiticus, A. sydowii, A. ochraceus. In Africa, there are several studies that found contamination in salted fish products. Suleiman (2014) found that salted fish produced in Sudan was contaminated by *A.niger, Alternaria,* and *Penicillium.* Seila (2014) revealed that salted fish produced in Kenya were clearly contained A*. niger, A.flavus, A. fumigatus, Fusarium, Trichoderma, Rhizopus, Penicillium,* and *Mucor*. In addition, research conducted by Ayotunde, Ada, Udeh and Otu (2016) found that there were contamination of *A. flavus, Penicillium and Mucor* on salted fish of catfish, indigo, cod and lemuru from Nigeria. In the Ismailia City Egypt, Ahmed, Ismail and Abd-El-Rahman (2004) found that 50 out of 60 (83.3%) salted fish analysed were contaminated by *Aspergillus spp. Aspergillus niger* and *Pennicilium verrecosum* were the most predominant fungi strains in investigating samples. Ismail (2013) found salted fish from Egypt contaminated *A. amstelodami, A. chevalieri, A. nidulans, A. niger, A. terreus, P. chrysogenum, P. verrucosum, P.restrictum, Alternaria, Cladosporium herbarum*. Meanwhile in Indonesia, Kamil, Putra, Sidar , Setyaningsih and Rahayu (2016) found aflatoxin B1 contamination reached up to 75.81 ppb in salted fish samples from Kenjeran - Surabaya and Beringharjo - Yogyakarta markets. Moreover, Indriati, Hermana, Hidayah and Rahayu (2017) was also found several types of fungi, which potentially produce aflatoxin. However, further studies regarding food contamination by *A. flavus* and aflatoxin B1 in salted fish products in Indonesia are still limited, especially aspects of risk exposure. This study presents a semi-quantitative risk analysis, which determines the probability of aflatoxin B1 exposure from *A. flavus* in salted fish from research finding conducted by Indriati et al. (2017).

2. Materials and Methods

2.1. Data Source

Samples were grouped based on the salt content. Low salt content (0-5 %): anchovy (*Stolephorus sp*.) and whipfin silverbiddy (*Gerres filamentosus*) , moderate salt content (6-10%) : commerson's anchovy (*Stolepohorus commersonii*) and medan anchovy (*Stolephorus bataviensis*), high salt content (>10 %): moonlight gourami (*Trichogaster microlepis*) and snakehead fish (*Channa striata*).

2.2 Semi-Quantitative Risk Analysis of *A. flavus* and Aflatoxin B1

Risk profile is arranged accordingly referring to Codex Alimentarius (1999) consists of:

2.2.1.Hazard identification;

Contains various information relating to the characteristics of *A. flavus* and Aflatoxin B1, the prevalence of *A. flavus* and Aflatoxin B1 in fishery products, cases of *A. flavus* and Aflatoxin B1 contamination in salted fish in Indonesia. These data are secondary data derived from literature studies.

2.2.2.Hazard characterization;

Contains a variety of clinically related information about *A. flavus* and Aflatoxin B1 infections, virulence factors *A. flavus* and Aflatoxin B1, transmission lines of *A. flavus* and Aflatoxin B1 in humans. These data are also obtained from secondary data.

2.2.3. Exposure Assessment and Risk Characterization;

A stage that contains information about the probability of *A. flavus* and aflatoxin B1 in salted fish and contains information related to the risk of consumers affected by liver cancer due to consuming 1 serving of contaminated salted fish *A. flavus* and aflatoksin B1. These data are obtained from the primary data that is the prevalence data and the level of *A. flavus* and aflatoxin B1 contamination of salted fish samples that have been obtained.

Moreover, semi-quantitative risk characterization was calculated according to World Health Organization (2011) method, by 10,000 times Monte Carlo literation using @Risk software simulation. This semi-quantitative analysis defined the risk of consumers that consumes 1 g salted fish contaminated with *A. flavus* and aflatoxin B1.

3. Results and Discussion

Indriati *et al.* (2017) found that 11 of 77 salted fish samples were contaminated with *A. flavus* (Figure 1). Dominant contamination was found from samples taken from West Java (6 samples). There were differences in positive hit between the contamination of *A. flavus* and aflatoxin B1, particularly in the samples from West Java. It was detected that only 2 out of 5 positive *A. flavus* samples were contaminated by Aflatoxin B1 from Bandung retailers. Therefore, the presence of *A. flavus* was not directly related to the occurrence of aflatoxin B1.

3.1. Hazard Identification

The contamination of fungi on food has received special attention because of its ability to damage the nutritional value of materials and produce secondary metabolites that are toxic for human health ((Lanyasunya, Wamae, Musa, Olowofeso, & Lokwaleput, 2006). This toxigenic shell is very easy to grow due to high humidity and temperature, so the contamination of mycotoxins in food is difficult to avoid (Maryam, 2007).

Table 1. Prevalence of *A. flavus* and Aflatoxin B1 (Indriati et al., 2017)

Location/fish species	N	*A. flavus*	Aflatoksin B1
Bandung	10	5	2
Anchovies *(Stolephorus sp.)*	2	1	0
Whipfin silverbiddy (*Gerres filamentosus*)	2	2	1
Commerson's anchovies (Stolephorus bataviensis)	1	1	1
Medan anchovy (*Stolephorus commersoniil)*	1	0	0
Snakehead fish (*Trichogaster microlepis*)	2	0	0
Moonlight gouramy (*Channa striata*)	2	1	0
Tangerang	17	1	1
Anchovies *(Stolephorus sp.)*	3	0	0
Whipfin silverbiddy *(Gerres filamentosus)*	2	0	0
Commerson's anchovies (Stolephorus bataviensis)	2	0	0
Medan anchovy *(Stolephorus commersoniil)*	4	0	0
Snakehead fish *(Trichogaster microlepis)*	2	0	0
Moonlight gouramy *(Channa striata)*	4	1	1
Cirebon	5	0	0
Anchovies *(Stolephorus sp.)*	1	0	0
Whipfin silverbiddy *(Gerres filamentosus)*	1	0	0
Medan anchovy *(Stolephorus commersonii*)	1	0	0
Snakehead fish (*Trichogaster microlepis*)	1	0	0
Moonlight gouramy (*Channa striata*)	1	0	0
Tegal	5	0	0
Commerson's anchovies (*Stolephorus bataviensis*)	4	0	0
Moonlight gouramy (*Channa striata*)	1	0	0
Pelabuhan Ratu	5	1	0
Stolephorus bataviensis	2	0	0
Stolephorus commersonii	2	0	0
Trichogaster microlepis	1	1	0
Cilacap	21	1	1
Anchovies *(Stolephorus sp.)*	6	0	0
Whipfin silverbiddy (*Gerres filamentosus*)	1	0	0
Commerson's anchovies (Stolephorus bataviensis)	3	0	0
Medan anchovy (*Stolephorus commersoniil)*	3	1	1
Snakehead fish (*Trichogaster microlepis*)	2	0	0
Moonlight gouramy (*Channa striata*)	6	0	0
Banyuwangi	2	0	0
Anchovies *(Stolephorus sp.)*	1	0	0
Whipfin silverbiddy (*Gerres filamentosus*)	1	0	0
Tuban	12	3	3
Anchovies *(Stolephorus sp.)*	3	2	1
Whipfin silverbiddy (*Gerres filamentosus*)	3	1	2
Commerson's anchovies (Stolephorus bataviensis)	1	0	0
Medan anchovy (*Stolephorus commersoniil)*	2	0	0
Snakehead fish (*Trichogaster microlepis*)	2	0	0
Moonlight gouramy (*Channa striata*)	1	0	0
Sum	77	11	7
Prevalence *A. flavus*		11/7 14,28%	7/77 9,09%

Table 2. Concentration of *A. flavus* in dried salted fish samples (Indriati et al. 2017)

Fish species	Total of *A. flavus* (Log cfu/g)	Concentration range of AFB1 (ppb)
Anchovies (*Stolephorus sp*.)	1.0-1.70	6.50 - 8.0
Whipfin silverbiddy (*Gerres filamentosus*)	1.0-1.70	10.71 - 33.56
Commerson's anchovies (*Stolepohorus commersonii*)	1	27.43 - 30.0
Medan anchovy (*Stolephorus bataviensis*)	1	27.66 - 31.20
Snakehead fish (*Trichogaster microlepis*)	1.0-1.78	16.0 - 20.02
Moonlight gouramy (*Channa striata*)	1.0-1.60	24.60 – 27.0

A. flavus has the ability to grow and live in a wide temperature range, can be mesophilic and thermophilic. The minimum growth of *A. flavus* at temperatures of 10-12 ° C, a_w 0.8 and pH 2 (Makfoed, 1993). Kulshrestha, et al. (2008) stated that the maximum growth temperature of *A. flavus* occurred at 48 ° C. The growth of *A. flavus* is largely influenced by various factors such as substrate composition, moisture content, relative humidity, temperature, and presence of competing microorganisms (Mishra & Das, 2003). *A. flavus* will show orange color if growing on AFPA selective media (Rodrigues, Venaˆncio, Kozakiewicz, & Lima, 2009).

Incubation time of *A. flavus* is around 3-7 days (Pitt & Hocking, 2009). *A. flavus* as the main producer of aflatoxin, generally producing only aflatoxin B1 (AFB1) and aflatoxin B2 (AFB2). While *A. parasiticus* produces AFB1, AFB2, AFG1 and AFG2. The difference is based on the appearance of fluorescence on thin layer chromatography plates under UV light giving blue for B and green for G. *A. flavus* has 8 hours germination time at a_w of 0.97 and temperature of 30°C.

3.2. Hazard Characterization

Mycotoxins are secondary metabolites produced by fungi. According to Rahayu, Sardjono and Samson, (2014) this metabolite is produced at the end of exponential growth, when oxygen demand is reduced in products stored for long periods of time. *A. flavus, A. parasiticus* and *A. niger* are fungi that can produce mycotoxins called aflatoxins (Rahmadi & Fleet, 2008, Wrather & Sweet, 2006). Aflatoxin is a dominant mycotoxin in foodstuffs. Until now it is known there are several types of aflatoxin, among others aflatoxin B1, B2, B3 and B4.

Aflatoxin is a compound that has a high level of potential hazards compared with other mycotoxins. According to the International Agency for Research on Cancer, chronic exposure to aflatoxins in foodstuffs is a major risk factor for the occurrence of malnutrition and hepatocellular carcinoma, especially in countries where hepatitis B infection is an endemic disease (IARC, 1988). People who experience chronic exposure to aflatoxin at high levels have a risk of having hepatocellular carcinoma, three times greater than those without exposure (Yenny, 2006). Aflatoxin has been classified as a group 1 human carcinogen by the IARC and has shown carcinogenicity in many animal species, including some rodents, non-human primates and fish (International Program on Chemical Safety (IPCS) / WHO, 1998). The P450 enzyme in the liver metabolizes aflatoxin into a reactive oxygen species (aflatoxin-8,9-epoxide), which can then bind proteins and cause acute toxicity (aflatoxicosis) or bind to DNA causing lesions over time to increase the risk of Hepatocellular carcinoma (HCC) or cancer (Groopman, Kensler and Wild, 2008). Liver cancer as a result of chronic aflatoxin exposure has been well-documented, most often shown in people with chronic hepatitis B virus (HBV) infection (Wild & Gong 2010).

The risk of liver cancer in individuals exposed to chronic hepatitis B and aflatoxin infections is up to 30 times greater than the risk of aflatoxin-exposed individuals alone (Groopman, Kensler, & Wild, 2008). Both of these HCC risk factors-aflatoxins and HBV-are common in poor countries around the world. In these countries, there are often significant urban and rural differences in aflatoxin exposure and HBV prevalence, with both these risk factors typically affecting rural populations stronger than urban population (Plymoth, Viviani, & Hainaut, 2009).

Aflatoxin also appears to have a synergistic effect on hepatitis C-induced hepatitis C liver cancer (Kirk, Bah, & Montesano, 2006; Kuang, et al. 2005; Wild & Montesano, 2009), although quantitative relationships are not as well established as in aflatoxin and HBV in inducing HCC. Other important contributing factors in the development of HCC, in addition to HBV or HCV

infection and aflatoxin exposure, are genetic characteristics of the virus, consumption

3.3. Exposure Assessment and Risk Characterization

Statistical analysis revealed that *A.flavus* prevalence was 14%, or 14 in 100 pack size of 500 g (Figure 2). Moreover, *A. flavus* concentration was found of 2.307 log cfu/g in salted fish at retailers and the probability of *A. flavus* contamination level of 1 g salted fish was 0.3206 log cfu/g or 2 cfu/g. It means that someone will have the chance to be exposed of *A. flavus* per gram of salted fish consumed by 2 cfu/g. Or the probability of the level of *A. flavus* contamination is 2 cfu/g. It is assumed that in West Java, salted fish is consumed for about 3.7 g/capita/day (Badan Pusat Statistik Provinsi Jawa Barat, 2016). Therefore, the risk characterization of *A. flavus* exposure was found 7.74 cfu/g in 1 g of salted fish, which was derived from the multiplication between probability and fish consumption value. These results may suggest that the risk of salted fish contamination level is still below the maximum limit of SNI fungus contamination, which is less than 102 cfu/g (SNI, 2009).

In the figure of the prevalence of *Aspergillus flavus* Figure (1a), a deviation of 0.3 from 1.4 (20%). It means that the deviation of the salted fish sample containing *A flavus* is large. It is also found in the concentration graphs (1b) and probability (1c) *A flavus* that has a standard deviation value above 100%. This shows the diversity of salted fish sample data tested. A varied *A flavus* concentration with a considerable range between the minimum and maximum values of 0-60 log cfu/g causes the standard deviation of probability to also be large and diverse. Of 77 samples, there were only 10 positive samples containing *A flavus.* These results may suggest that the risk of salted fish contamination level in Java Region is still low and under the maximum limit of SNI fungus contamination, which is less than 10^2 cfu/g (SNI, 2009).

Meanwhile, statistical analysis revealed that the prevalence of *A. flavus* with Aflatoxin B1 is 10% or 10 in 100 packs size of 500 g (Figure 1). *C*oncentration of Aflatoxin B1 was found 1.8939 ppb in salted fish at retailers and the probability of Aflatoxin B1 contamination level in 1 g salted fish was 0.1970 ppb. Based on these values, with the same assumption of consumer behaviour level as previously (3.7 g/capita/day), the risk probability of aflatoxin B1 exposure in 1 g of salted fish was found as much as 0.7291 ppb. This value derived from the multiplication of concentration, prevalence, and consumption level of salted fish. Several studies have been conducted regarding the effect of aflatoxin b1 dose response. Butler, Greenblatt and Lijinsky (1969) has been tested by long-term feeding in drinking water to rats, at concentrations of 1µg/ml and 3 µg/ml. Aflatoxin B1

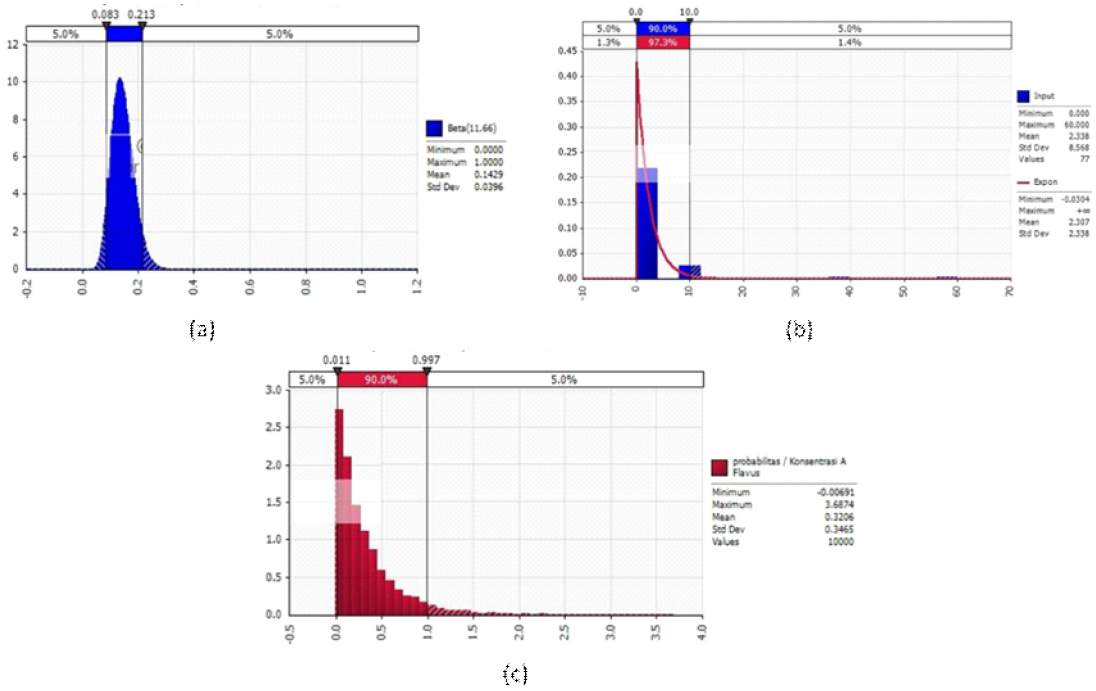

Figure 1. Prevalence (a), concentration (b), and probability (c) of *A. flavus* in salted fish at retailer level in Java Island.

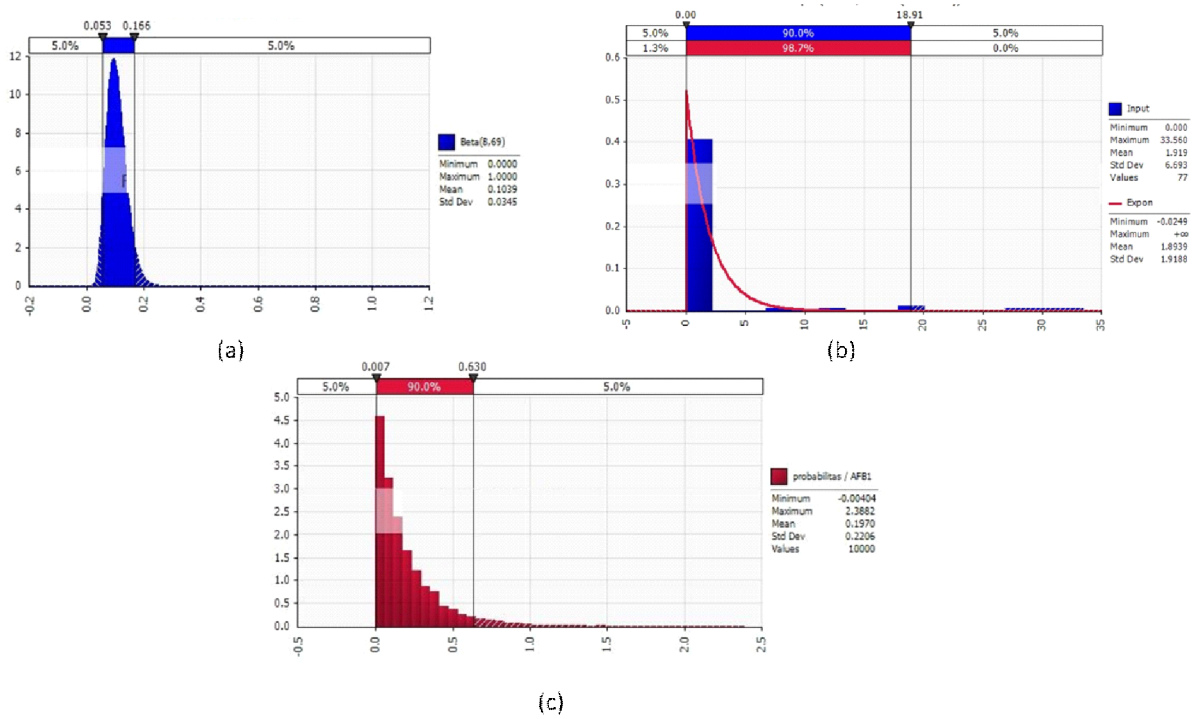

Figure 2. Prevalence (a), concentration (b), and probability (c) of Aflatoxin B1 in salted fish at retailer level in several cities in Java.

produced liver tumors in 19 of 30 rats given a total dose of 2 mg each; 3 of 10 animals receiving a total dose of 1 mg developed liver tumors.

In the figure of the prevalence of *A. flavus* Figure (2a) shows a deviation of 0.3 from 1.0 (20%). it means that the deviation of the salted fish sample containing AFB1 is large. It is also found in the concentration graphs Figure (2b) and probability Figure (2c) AFB1 that has a standard deviation value above 100%. This showed the diversity of salted fish sample data tested. A various AFB1 concentration with a considerable range between the minimum and maximum values of 0-33 ppb caused the standard deviation of probability to also be large and diverse. From total of 77 samples, there were only 7 samples containing AFB1. The risk may suggest within the low risk category, as the limit of 20 ppb in corn and bean limits products (Dharmaputra, Putri, Retnowati, & Ambarwati, 2003).

4. Conclusion

This study showed that the consumption rate of salted fish about 3.7 g/capita/day in several cities in Java had the risks probability of 7.74 cfu/g *A. flavus* exposure and 0.7291 ppb aflatoxin B1 exposure in 1 g of salted fish. These values are categorized as low risk level, below the threshold of standard limit. However, as aflatoxin B1 may accumulate in the human body, annual monitoring of *A. flavus* and its toxic contamination is needed to guarantee the food safety level in fisheries products.

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