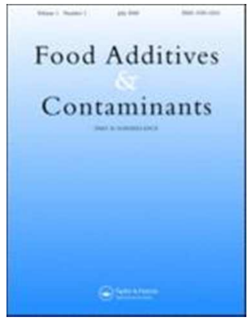

Screening of polycyclic aromatic hydrocarbons occurrence in Cambodian smoked fish

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Abstract:	More than 85% of the population in Cambodia is strongly dependent on agriculture, from which freshwater aquaculture is one of most important sources of food production. The smoked fish represents important source of nutrients for Cambodian population, however can also lead to excessive intake of PAH. A field survey was conducted among selected smoked fish producers near to Tonle Sap river in Kampong Chang province, Cambodia. The study revealed that a maximal residue limits (MRL) for a sum of 4 PAH given by EC 1881/2006BaP was exceeded form 2 to 50 times. Such burden can lead to increased risk of development of carcinogenic diseases.

Screening of polycyclic aromatic hydrocarbons occurrence in Cambodian smoked fish

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Abstract:
More than 85% of the population in Cambodia is strongly dependent on agriculture, from which freshwater aquaculture is one of most important sources of food production. The smoked fish represents important source of nutrients for Cambodian population, however can also lead to excessive intake of PAH. A field survey was conducted among selected smoked fish producers near to Tonle Sap river in Kampong Chang province, Cambodia. The study revealed that a maximal residue limits (MRL) for a sum of 4 PAH given by EC 1881/2006BaP was exceeded form 2 to 50 times. Such burden can lead to increased risk of development of carcinogenic diseases.

Key words:
Smoked fish, PAH, aquaculture, Tonle Sap, Cambodia

Introduction
Cambodia has very few income generating possibilities beyond its natural resources and its economy is almost fully dependent on agriculture, forestry and fisheries (FAO, 2002). Fish and fisheries in Cambodia is essential for providing food security to the people (FAO & MRC, 2003). More than 85% of the population in Cambodia is strongly dependent on agriculture, from which freshwater aquaculture is one of most important sources of food production (FAO, 2011; Hortle, 2007). Year production of fish is about 514 000t life weight and about 470 000t is dedicated for consumption. Although there is a marked taste preference for fresh fish inland species by the Cambodian population, large quantities of freshwater fish, and to a lesser extent marine species, are processed for human and animal consumption (Doulman & Officer, 1993). The annual fish meat and fish products consumption is about 33.8kg per person. Average income of animal protein from fish meat is 18.3kg per year and person, what is around 80% of total animal protein income for Cambodians (FAO, 2011; Hortle, 2007). Fish processing provides a continuous source of protein throughout the year (Tickner, 1996). Since in very short peak period of fish as being caught it is necessary to process fish quickly by using a simple processing methods (Eong & Hariono, 2003; FAO, 2011). Processing involves a range of basic but effective preservation techniques. These techniques include sun-drying, salt-drying, smoking and steaming (Ahmed, Navy, Vuthy, & Santos, 1999; Doulman & Officer, 1993; Hortle, Lieng, & Valbo-Jorgensen, 2004). Fresh fish meat contain up to 80% of water by mass and it is considered as highly perishable material, which results in an extremely short shelf-life when left unprocessed (Bala & Mondol, 2001). Smoking as a conservation procedure was found approximately 10 000 years ago when man hung a catches over the fire, probably as a protection from canines. From this time, smoking started to be widely used not only for special organoleptic profiles of smoked products, but also for the inactivating effect of smoke (and heat) on enzymes and microorganisms (Essumang et al., 2014; Šimko, 2002). Due to low living standard and low level of industrialization resulting in lack of access to electricity, which affecting 66% of the population in Cambodia, based on the data from The World Bank (2014), smoking as one of the oldest preservation method is still widely used in the country (Stołyhwo & Sikorski, 2005). Small-scale fish processing including products like fish paste, fish sauce, dried salted fish, and smoked fish are very common in Cambodia. These traditional ways of fish processing are well adapted to irregularity of the seasonal fish catch. Since in very short peak period it is necessary to process the fish quickly by using just a simple techniques achievable by a rural family (Cambodia HARVEST Program, 2012; Eong & Hariono, 2003). Traditional

smoking involves treating of pre-salted, whole, eviscerated or filleted fish with wood smoke. The smoke is produced by smouldering wood and shavings or sawdust in the oven, directly below the hanging fish or fillets, laid out on mesh trays (Stołyhwo & Sikorski, 2005). This method make use of traditional kiln with wood burning temperature between 300 and 700°C with corresponding oven´s temperature equal to 80°C (Essumang, Dodoo, & Adjei, 2013; Nti, Plahar, & Larweh, 2002). The rate of deposition of different components depends upon the temperature, humidity, flow rate, and density of the smoke, the water solubility and volatility of the particular compounds, as well as shelf life, and wholesomeness of the product (Foster, 1957; Stołyhwo & Sikorski, 2005).
The wholesomeness of smoked fish products using the traditional kiln depends on used type of firewood, used temperature, time of smoking, type of kiln, proximity of product from the fire, species used for smoking and fat content of the fish (Akpambanga et al., 2009; Essumang et al., 2013; Farhadian et al., 2010; Knize et al., 1999; Perelló et al., 2009; Philips, 1999). Potential health hazards associated with smoked foods may be caused by carcinogenic components of wood smoke – mainly Polycyclic Aromatic Hydrocarbons PAH (Alomirah et al., 2011; Stołyhwo & Sikorski, 2005). Polycyclic aromatic hydrocarbons comprise the largest class of chemical compounds known to be cancer causing agents (Alomirah et al., 2011; Šimko, 2002). They are a group of hydrophobic compounds consisting of two or more fused aromatic rings. PAHs are ubiquitous environmental pollutants generated during incomplete combustion. Consequently, they are also present in wood smoke used for curing which is a typical example of this process. The generated PAH are undoubtedly released into the various smoked products (Essumang et al., 2014; Philips, 1999; Stolyhwo & Sikorski, 2005). PAHs produced in wood smokes are known to originate from the thermal pyrolysis (depolymerisation) of lignin and subsequent condensation of the lignin components in lignocelluloses at temperatures above 350°C (Garcia-Perez, 2008; Kawamoto et al., 2007; Nakamura et al., 2008). Moreover, PAHs may also be formed directly in food as a result of some heat processes such as smoke drying or smoking (Afolabi et al., 1983; Alomirah et al., 2011).
Although there are studies from Europe and some developing countries concerning the carcinogenic potential and occurrence of PAHs in food products, there is a lack of information about PAHs occurrence in foodstaff particularly smoked fish in Cambodia. Thus the objective of this study is the investigation of traditional fish smoking process in fishing region near the Tonle Sap river in Cambodia with emphasis on PAHs screening in smoked fish.

Materials and Methods

Site area description and sampling procedure

The study was conducted among small-scale producers of smoked fish along to Tonle Sap river near the Kampong Chhnang city in Kampong Chang province about 95km north from the capital Phnom Penh as presented in Fig.1. The Tonle Sap wetlands in Cambodia are part of the Mekong watershed and are one of the most productive fisheries in Southeast Asia. Tonle Sap supports local livelihoods through fishing and over 90% of Cambodian livelihoods are dependent on these wetlands. Fisheries are the main industry and source of household income, especially for poor villagers, approximately 85% of total fish catch comes from inland fisheries in Cambodia. In addition, the wetlands provide two-thirds of peoples' dietary protein (Kanchanaroek, Termansen, & Quinn, 2013). The Kampong Chang is a province with highest per capita consumption of fresh fish and processed particularly smoked fish in Cambodia. The total consumption of fresh fish and processed fish in the province is almost 120 kg per annum (Ahmed et al., 1999). Further, the area near the Kampong Chhnang city along to the Tonle Sap river is famous for high concentration of smoked fish producers and that is way it was selected for our study. Totally 23 smoked fish samples from 10 producers were collected from the sampling area (see Fig. 1) in November 2014. The weight of each sample was 100 g. The samples were collected using vinyl gloves and the samples were placed in appropriately labeled clean plastic bags. Immediately after sampling the bags were frozen at -20°C and then transported to the Czech University of Life Sciences Prague, Czech Republic for further laboratory analyses.

Fish smoking procedure

The traditional smoking technique of fish producers around Tonle Sap lake in Kampong Chhnang district take place in typical smoking house. Used technology may be classified as direct and hot smoking method (Ledesma et al., 2016). The typical dimensions of the smoking house are 2000 – 3000 mm x 3000 – 6000 mm x 1000 – 3000 mm, width, length and height, respectively. While the whole house is typically constructed by wood or bamboo and the roof is covered by metal sheet. The locally available firewood is used as a source of heat and smoke. The wood is burned on the ground in the place that is considered as the fire place (see Fig. 2. A). First floor of racks for fish smoking is about 0.6 m up to 1 m above the fire place (Fig. 2. B) and based on the capacity of each smoking house those floors are repeated two, three or more times. The smoking racks are made by bamboo sticks and steel rods. The typical smoking procedure starts with fish washing and fish cleaning including removing the entrails. Afterword the fish are grouped and stick together by a skewer. Those fish are then placed on the smoking racks and turned regularly every one hour. The total time of smoking differ based on fish species and can vary from one up to four or five days. Occasionally the fish are covered by paper cardboard to reduce the smoking time and improve final color of the fish.

Secondary data collection

To obtain a supplementary data to the final PAHs concentrations of the fish samples a semi-structured personal interviews and questionnaire survey was conducted among all smoked fish producers included to this study. The number of respondents was equal to 10 producers of smoked fish from the target area. The respondents were asked to answer questions related to the used fish pre-treatments before the smoking process, used technique of fish smoking, type firewood used for smoking and fish species used for smoking. All data were collected in local units and names as well as all interweaves and questionnaires were conducted in Khmer language with support of Cambodian specialist from the Royal University of Agriculture in Phnom Penh. Collected data were translated to English and scientific names and processed by descriptive statistical methods.

Determination of four priority PAH in fish samples

The amount of selected PAHs was determined by standard operational procedure as follows. Briefly, homogenized fish sample (5 g) was mixed with 10 g of Na_2SO_4 and extracted for 16 h in Soxtec by 50 ml of hexane:dichlormathane mixture (1:1) at 109°C. The solvent was then evaporated and the sample was redissolved in 10 ml of chloroform and pre-cleaned by gel permeation chromatography (GPC). Collected fraction was evaporated to dryness and redisslоved in 0.5 ml of acetonitrile and proceeded to further analysis. The content of PAH was finally analysed by high pressure liquid chromatography with fluorescenece detector (HPLC−FD). The pre-cleaned sample (20 µL) was injected to HPLC and eluted at 30 °C with flow 0.5 mL.min^{-1} with the following gradient of mobile phase: water (A) and acetonitrile (B) time 0-2 min A (25%)+B (75%); 10-21 min A(0%)+B(100%); 22 min A (25%)+B (75%) mL. Total time of analysis was 27 min. Analysis was performed on Waters PAH C18 (250 x 2.1 mm) column with 5 µm particle size. Finally, PAH were identified by comparison of sample chromatogram with standards. Quantification was performed by the method of external standard.

Results and Discussion

In general, the amount of the four priority PAH in all samples (Table 1) highly exceeded maximal residue limits (MRL) given by EC 1881/2006. Currently the limits for smoked fish are 2 and 12 µg.kg^{-1} for benzo[a]pyrene (BaP) and sum of 4 PAH, respectively. Such high amounts of PAH have been rarely reported in smoked fish before, however, it corresponds to the values reported for smoking under uncontrolled technological conditions so typical for households and developing countries (Šimko, 2002).

One of the main factors which probably contributed to high levels of PAH in fish is the smoking technique. For example the samples smoked traditionally in kilns have been reported to contain increased levels of BaP (50 µg.kg^{-1}) compare to fish from smokehouses supplied with conditioned wood smoke from external generators (BaP content 0.1 µg.kg^{-1}) (Stołyhwo & Sikorski, 2005).The second important factor is smoking time, the longer the sample is smoked the higher amount of the PAH can be expected (Essumang et al., 2013; Varlet et al., 2007). In our studies only in one case were the samples smoked for less than 24 hours (Table 2) the other were usually exposed to the smoke for 1 to 2 days and in extreme cases for 5 days. This is considerably longer period compare to usually reported smoking times which ranges between 2-12 hours (Bannerman, 2001; Essumang et al., 2013; Stołyhwo & Sikorski, 2005). Furthermore, a smoking temperature together with the distance of the product from the fire have influence on the final PAHs content as well (Ledesma et al., 2016). All producers in the study have used traditional direct and hot smoking method. As it is obvious from the Fig. 2 the fish was placed directly under the fire place heated by firewood. Based on the results reported by Ledesma et al. (2016) the most typical distance between the product and the fire ranged between 2 and 10 m, which is considerably higher than the distances used in smoking houses investigated in this study.

The type of the wood used for the smoking can also significantly influence PAH content in fish (Stumpe-Vīksna, Bartkevičs, Kukāre, & Morozovs, 2008). In general, the soft wood due to its' high content of resin is not recommended for the smoking. The producers in this study usually reported the mix firewood purchased on the local market to be used for smoking (Table 2). It was not possible to identify the exact species of wood used in those mixtures. Hence we cannot exclude that some wood species with higher resin content were used. Moreover in one case the wood from rubber tree (*Hevea brasiliensis*) was reported as a main source of firewood. This firewood is not suitable for smoking due to high content of gums and resins.

The lowest amount of BaP was determined in sample no. 9, the highest in no. 23. The MRL for BaP was exceeded from 2 to 60 times. Similary the lowest amount of the ∑PAH 4 was found in sample no.9 and the highest in sample 23. Thus the MRL amount was exceeded from 2 up to 50 times.

Fish species used for smoking in the target area and their frequency as were reported by each producer are summarized in Table 3. The most typical species used for smoking is *Micronema bleekeri* followed by *Henicorhynchus caudimaculatus, Kryptopterus hexapterus, Ompok bimaculatus,* and *Belodontichthys truncatus.* Concerning the species tested, the samples from *Henicorhynchus caudimaculatus* contained one of the highest amounts of PAHs while samples from *Belodontichthys truncatus* and *Micronema bleekeri* usually contained the lowest amount of them. The higher concentration of PAH in fishes was found in those made by producers 5 – 9, who usually declared longer drying time (Table 2).

It seems that differences in PAH content are probably caused by the combination of two factors – 1) smoking time; 2) amount of fat in the fish sample, as it is known that the more fat in the fish, the higher concentration of PAH from smoke is absorbed. Moreover, pyrolysis of fat in fish with high lipid content significantly contribute to the final content of PAH in samples (Essumang, Dodoo, & Adjei, 2012). However it must be stated that the fat content of smoked fish species was not measured in this preliminary study and will be subjected to investigation in next study.

Conclusions

The smoked fish represents important source of nutrients for Cambodian population, however can also lead to excessive intake of PAH. This study was conducted among selected smoked fish producers near to Tonle Sap river in Kampong Chang province, Cambodia. The study revealed that a maximal residue limits (MRL) for a sum of 4 PAH given by EC 1881/2006BaP was exceeded form 2 to 50 times. Such burden can lead to increased risk of development of carcinogenic diseases. To decrease this risk, local population should be educated to use better smoking techniques or at least change of smoking parameters, which would lead to safer food.

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Table 1 Determined values ($\mu g.kg^{-1}$) of four priority PAH

Sample No.	Producer	Fish scientific name	benzo[a]anthracene*	chrysene	benzo[b]fluoranthene	benzo[a]pyrene	∑PAH 4
1	*3*	*Micronema bleekeri*	13.65	23.85	3.84	6.13	47.47
2	*2*	*Micronema bleekeri*	18.1	46.88	3.68	11.32	79.98
3	*1*	*Micronema bleekeri*	31.91	43.52	15.84	36.16	127.43
4	*4*	*Micronema bleekeri*	37.86	148.37	14.91	70.48	271.62
5	*9*	*Micronema bleekeri*	111.56	162.19	8.27	63.75	345.77
6	*10*	*Henicorhynchus caudimaculatus*	113.66	161.78	16.33	108.96	400.73
7	*9*	*Henicorhynchus caudimaculatus*	174.12	236.32	17.61	93.68	521.73
8	*8*	*Henicorhynchus caudimaculatus*	208.63	220.35	32.03	108.77	569.78
9	*3*	*Kryptopterus hexapterus*	10.54	16.57	2.56	4.58	34.25
10	*1*	*Kryptopterus hexapterus*	28.58	35.91	16.24	25.23	105.96
11	*9*	*Kryptopterus hexapterus*	92.2	121.62	26.1	92.26	332.18
12	*2*	*Ompok Bimaculatus*	10.82	36.64	0.89	6.17	54.52
13	*2*	*Belodontichthys truncatus*	5.22	21.57	6.64	8.66	42.09
14	*3*	*Belodontichthys truncatus*	17.29	27.36	3.98	8.01	56.64
15	*9*	*Belodontichthys truncatus*	91.39	125.1	9.5	51.01	277
16	*6*	*Thynnichthys thynnoides*	23.7	114.26	6.08	41.63	185.67
17	*10*	*Thynnichthys thynnoides*	69.29	98.3	8.54	53.85	229.98
18	*8*	*Thynnichthys thynnoides*	119.04	139.74	23.69	81.46	363.93
19	*6*	*Osteochilus lini*	77.92	99.72	23.4	59.57	260.61
20	*10*	*Osteochilus lini*	81.52	113.91	11.04	70.28	276.75
21	*8*	*Osteochilus lini*	131.47	152.78	21.23	87.74	393.22
22	*7*	*Paralaubuca typus*	73.4	113.95	7.57	48.64	243.56
23	*5*	*Paralaubuca typus*	204.06	242.62	31.62	119.45	597.75
		median	73.4	113.95	11.04	53.85	260.61
		average±SD	75.91± 60.59	108.84± 67.89	13.55± 9.09	54.69± 36.26	252.98± 167.67

* Uncertainty of the measurement for the determined analytes (±% from the reported value) was calculated as follows benzo[a]anthracene (±26%), chrysene (±22%), benzo[b]fluoranthene (±30%), benzo[a]pyrene (±34%), ∑ PAH4 (±15%)

Table 2 Fish smoking procedure as was reported by each respondent in the study

Fish producer	Time of smoking	Fish turning frequency	Type of fire used for smoking	Fuel used for smoking
1	5 – 10 h	-	Open fire	Mixed firewood form the market; wood from: Trosek (*Peltophorum dasyrrhachis*)
2	1 – 2 d	every 1 h	Open fire	Mixed firewood form the market; wood from: Trosek (*Peltophorum dasyrrhachis*)
3	1 – 2 d	every 1 h	Open fire	Mixed firewood form the market; wood from: Trosek (*Peltophorum dasyrrhachis*), Raing (*Barringtonia acutangula*)
4	1 – 2 d	every 1 h	Open fire	Mixed firewood form the market; wood from: Trosek (*Peltophorum dasyrrhachis*), Raing (*Barringtonia acutangula*), Rubber tree (*Hevea brasiliensis*), Ampil (*Tamarindus indica*).
5	5 – 6 d	every 1.5 h	Open fire	Mixed firewood form the market , wood from: Raing (*Barringtonia acutangula*)
6	2-3 d	every 1 h	Open fire	Mixed firewood form the market, wood from: Raing (*Barringtonia acutangula*)
7	4 – 5 d	every 1.5 h	Open fire	Mixed firewood form the market , wood from: Raing (*Barringtonia acutangula*)
8	2 – 3 d	every 2 h	Open fire	Mixed firewood form the market , wood from: Raing (*Barringtonia acutangula*)
9	1 – 3 d	every 1.5 h	Open fire	Mixed firewood form the market, wood from: Raing (*Barringtonia acutangula*)
10	1 - 2 d	every 1.5 h	Open fire	Wood from: Raing (*Barringtonia acutangula*

Table 3 Fish species usually used for smoking in the target area (n=10)

Fish species	The frequency of species as they were named by each respondents (%)
Micronema bleekeri	100
Henicorhynchus caudimaculatus	90
Kryptopterus hexapterus	80
Ompok bimaculatus	80
Belodontichthys truncatus	70
Thynnichthys thynnoides	50
Osteochilus lini	40
Wallago attu	30

Table 4 Limit of detections, limit of quantification, recovery and repeatability.

Analyte	Matrix	LOD [µg.kg-1]	LOQ [µg.kg-1]	Recovery [%]	RSDr	Measurement uncertainty	Accreditation
Benzo[a]anthracene	smoked fish	0,025	0,05	88.7 - 80.9*	7,5	26%	Yes
chrysene		0,025	0,05	76.1 - 89.5	14,3	22%	Yes
benzo[b]fluoranthene		0,05	0,1	89.4 - 87.6	6,7	30%	Yes
benzo[a]pyrene		0,025	0,05	90.4 - 86.6	7,9	34%	Yes

*recovery values are given for concentrations of analytes ranging from 0.5-4.8 µg.kg

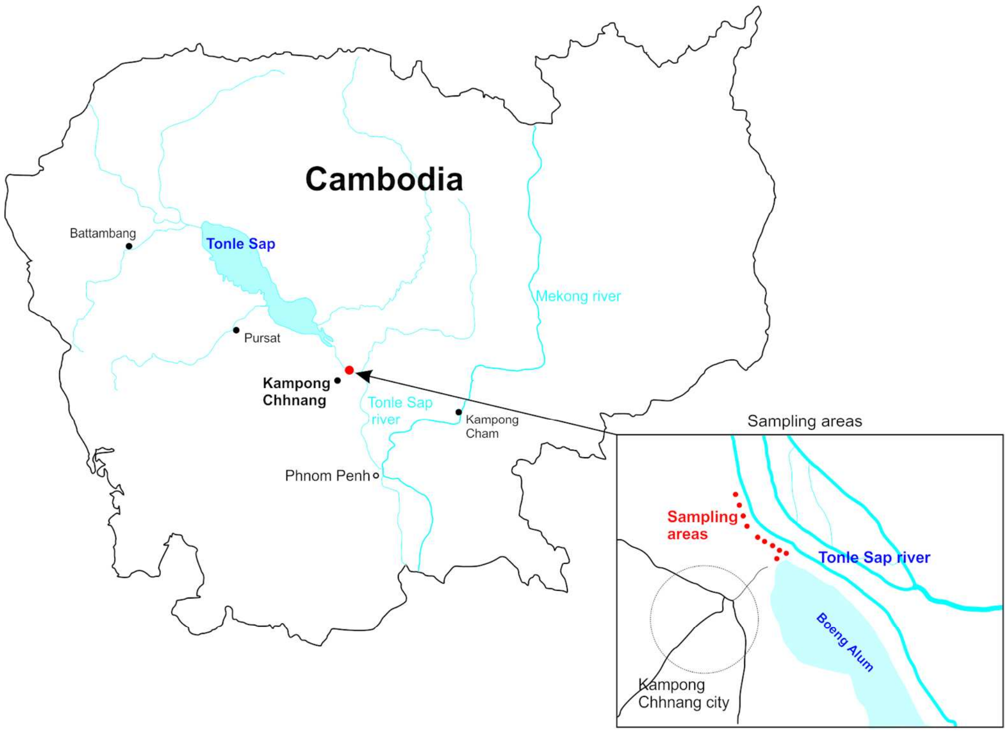

Fig. 1. Location of sampling area

144x104mm (300 x 300 DPI)

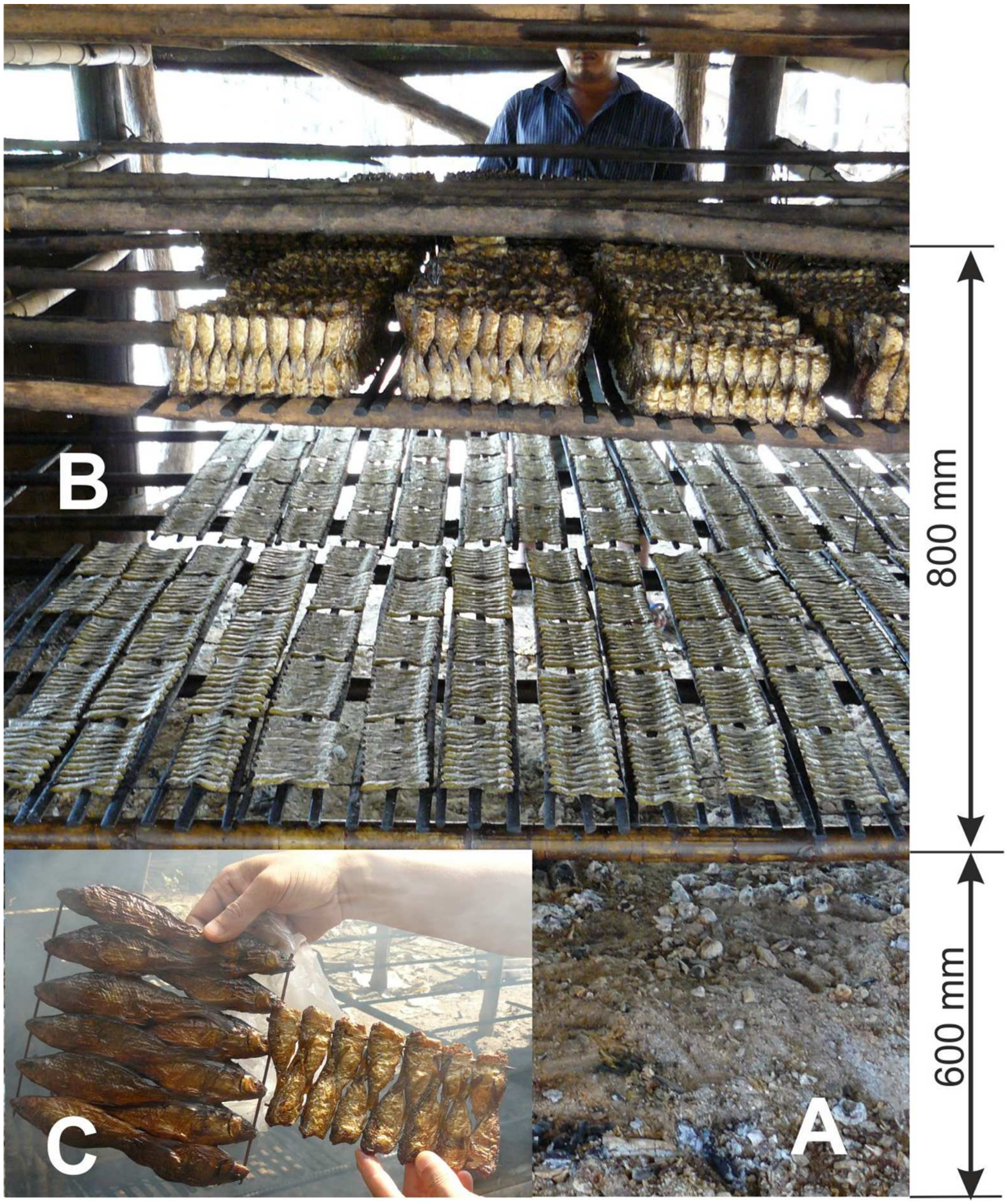

Fig. 2. Typical smoking house around Tonle Sap lake in Kampong Chhnang district, Cambodia. A - fire place, B- racks for fish smoking, C – final product: grouped smoked fish

108x130mm (300 x 300 DPI)