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Screening of PAHs concentrations in traditionally smoked fish products from Cambodian rural areas

Master thesis

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Declaration:

“I Tereza Slámová hereby declares that this thesis entitled Screening of PAHs concentrations in traditionally smoked fish products from Cambodian rural areas, is my own work and all the sources have been quoted and acknowledged by means of complete references.”

In Prague ……….........

(Signature of student)

B. Sc. Tereza Slámová

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Abstract:

More than 85% of the population in Cambodia is strongly dependent on agriculture, from which freshwater fish meat and products are one of the most important sources of food production. Smoked fish represents important source of nutrients for Cambodian population can, however, also lead to excessive intake of PAHs produced during traditional smoking of fish. Traditional technique of smoked fish product had not been fully investigated, therefore a field research was conducted among selected smoked fish producers near Tonle Sap river in Kampong Chang province, Cambodia, and questionnaire survey within 5 provinces (Kampong Chhnang, Battambang, Siam reap, Kampong Cham) in Tonle Sap area, Cambodia. During the research, 23 samples from 10 producers were collected. Extraction of completely homogenized samples by Soxtec apparatus was followed by pre-cleaning by gel permeable chromatography (GPC). Analytes in recupered eluate were evaluated by high pressure liquid chromatography with fluorescence detector (HPLC-FD) for analysis. Identification based on comparison of retention times with standards, quantification was performed by method of external standard. The study revealed that maximal limits (ML) for a sum of 4 PAHs and BaP given by EC 1881/2006 were exceeded between 2 to 60 times (34.25μg.kg^{-1} to 597.75μg.kg^{-1}) and 2 to 50 times (4.58μg.kg^{-1} to 119.45μg.kg^{-1}), respectively. Such burden can lead to increased risk of development of carcinogenic diseases and other diseases related to PAHs exposure. Further investigation and research focused on concentration on the amount of PAHs per gram of fish consumed or fat content is recommended.

Key words: pollutants, freshwater fish, food chain, Cambodia

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List of the abbreviations used in the thesis:

BaP	Benzo[a]pyrene
DNA	Deoxyribonucleic Acid
DoF	Department of Fisheries
EC	European Commission
EFSA	European Food Safety Authority
EPA	Environmental Protection Authority
FAO	Food and Agriculture Organisation
FLD	Fluorescence Detectors
GC	Gas Chromatography
GPC	Gel Permeation Chromatography
HPLC – FD	High Pressure Liquid Chromatography
IARC	International Agency for Research on Cancer
IQ	Intelligence quotient
LPG	Liquefied Petroleum Gas
LSF	Liquid Smoke Flavourings
MRC	Mekong River Committee
MRL	Maximal Residue Limits
MS	Mass Spectrometry
NCD	Noncommunicable diseases
PAHs	Polycyclic Aromatic Hydrocarbons
PPRIS	Pulp and Paper Resources and Information Site
SCF	Scientific Committee on Food
UV	Ultraviolet
WHO	World Health Organisation

Introduction

Fish and fisheries are essential for providing food security and income to the Cambodian people (FAO and 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). Yearly production of fish is about 514,000 t life weight and about 470,000 t is dedicated for consumption. Fish meat and product consumption is about 33.8 kg per person and year. Average income of animal protein from fish meat is 18.3 kg per year and person, what is around 80 % of total animal protein income for Cambodians (FAO, 2011; Hortle, 2007). Therefore fish products provide a continuous source of protein throughout the year. Fresh fish meat contains up to 80 % of water by mass and it is considered a highly perishable material, which results in an extremely short shelf-life when left unprocessed. As there is a very short peak period when fish is caught, it is necessary to process it quickly and in a basic way. These basic but effective preservation techniques are mainly focused on decreasing water activity to prevent spoiling. The processes include sun-drying, salt-drying, smoking and steaming. Smoking as a conservation procedure has been used since about 10,000 years ago when man hung catches over the fire, probably as protection from canines. Since that, 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., 2001; Šimko, 2002). Due to lack of access to electricity, which affects 66 % of the population in Cambodia (The World Bank, 2014), smoking as one of the oldest conservation methods is still widely used in the country. Traditional fish processing establishments are classified as small-, medium- and large-scale (Eong and Hariono, 2003) according to number of workers and volume of production. Traditional ways of processing fish are well adapted to irregularity of the seasonal fish catch. The preservation effect is generally attributed to antioxidant and antimicrobial properties of phenolic compounds contained in smoke. Traditional smoking involves treating of pre-salted or sundried, whole, eviscerated or filleted fish with smoke. The smoke is produced by smouldering wood and shavings or charcoal in a fireplace, directly below the hanging fish or fillets, laid out on mesh trays. The rate of deposition of different components depends on temperature, humidity, flow rate, and density of the smoke, water solubility and volatility of particular compounds, as well as shelf life, and wholesomeness of the product (Borgstrom, 2012 Stołyhwo and Sikorski, 2005). However, traditional smoked foods are associated with potential health hazards that may be caused by carcinogenic components of smoke – mainly PAHs (Polycyclic aromatic hydrocarbons) (Alomirah et al., 2011; Stołyhwo and Sikorski, 2005). Generation of wood and charcoal smoke during curing is a typical example of incomplete combustion, and it is known that polycyclic aromatic hydrocarbons (PAHs) are generated and released into the smoked products, particularly during the traditional uncontrolled smoking technique. Polycyclic aromatic hydrocarbons (PAHs) comprise the largest class of chemical compounds known to be cancer causing agents. They are a group of hydrophobic compounds consisting of two or more fused aromatic rings. PAHs are found in water, air, soil and therefore also in food. PAHs are ubiquitous environmental pollutants, and they can originate from the environment, as well as be generated during preparation of food, as a result of some heat processes such as smoke drying or smoking. Although there are sources concerning the problematic of PAHs in Europe and some developing countries, there is lack of information about the carcinogenic potential and occurrence of PAHs in traditionally smoked fish products in Cambodia, and traditional smoking of fish in general.

2 Literature review

2.1 Smoking technology

Smoking is one of the oldest food preserving technologies. It has been used by mankind for over 10,000 years. It is believed that man would hang his catch over the fire as a protection against canines and subsequently the preserving effect of smoke was probably discovered (Šimko, 2002; 2009). The first evidence of smoking as a technological process dates back 90,000 years to Poland where the oldest smoking house was discovered by archaeologists in a Stone Age colony located in Zwierzymec, near Krakow (Möhler, 1978). Since that time, smoking has been widely used not only for its special organoleptic properties of smoked products but also because of its useful inactivation effect on enzymes and microorganisms (Šimko, 2002).

Nowadays, smoking is still widely used and not only in fish processing. According to Stołyhwo and Sikorski (2005) in Europe about 15 % of the total quantity of fish for human consumption is offered on the market in the form of either cold- or hot-smoked products. Since the beginning of traditional, uncontrolled burning of biomass, smoking techniques have been increasingly improved, and various and specific procedures have been developed related to different regions and cultures for treating meat and fish products (Šimko, 2005; Ledesma et al., 2016). Currently, we suppose, the technology is mainly used to enrich the foods with specific taste, odour, and appearance, as there is a high demand for it on the market (Šimko, 2005; Burt, 1988; Hui et al., 2001; McGee, 2004). On the other hand, the role of the preservative effects is on its descend with regard to the latest trends in alternative preservation procedures. It is assumed that the technology is today applied in many forms to treat 40 – 60 % of meat products (Sikorski, 2004) and 15 % of fish (Stołyhwo and Sikorski, 2005).

2.1.1 Smoke generation

Smoke generation is a result of thermal degradation of wood, followed by the oxidation of some of the products of pyrolysis under limited oxygen supply. Generation of wood smoke during curing is a typical example of incomplete combustion (Phillips, 1999; Stołyhwo and Sikorski 2005). In general, smoke is a polydisperse mixture of liquid and solid components with diameter of 0.08 - 0.15 µm in the gaseous phase of air, carbon oxide, carbon dioxide, water vapour, methane, and other gases (Stołyhwo, and Sikorski, 2005; Šimko, 2005). Smoke has a variable composition which depends on various conditions, such as procedure and temperature of smoke generation, origin and composition of wood, water content in wood, etc. (Šimko, 2002; Sikorski, 2004; Stołyhwo, and Sikorski, 2005). Among hundreds of components, wood smoke also contains at least 100 polycyclic aromatic hydrocarbons (PAHs) and their alkylated derivatives (Stołyhwo and Sikorski, 2005). PAHs play an important role in soot formation, but they can appear adsorbed on soot surface as well as in the gas phase due to their different volatility and molecular weight. The rate of deposition of different components depends on temperature, humidity, flow rate, and density of the smoke, water solubility and volatility of particular compounds, as well as on surface properties of the fish (Borgstrom, 2012). Composition of the smoke and conditions of treatment affect the sensory quality, shelf life and wholesomeness of the product. Suitability of smoke for treating fish and meat depends primarily on the contents of phenols, since they are mainly responsible for imparting the desirable sensory properties to the products and are valuable as antioxidants (Stołyhwo and Sikorski, 2005). The smoke produced at 650 –700 °C is richest in components such as phenols able to impart desirable organoleptic properties of treated products.

2.1.2 Wood and its role in smoking

The smoke for smoking of food develops due to partial burning of wood, predominantly hardwood (beech, hickory and oak) but also softwood (pine and fir). Basic structural materials of wood cells; cellulose and hemicellulose are aggregates of sugar molecules (linear polysaccharides) which when burnt effectively, caramelize, producing carbonyls which provide most of the colour components and sweet scented aromas (McGee, 2004; Rowell, 2005; Garcia-Perez, 2008). Also lignin, the wood cells bonding glue, a highly complex arrangement of interlocked phenolic molecules, produces a high amount of distinctive aromatic product when burnt, as smoky, spicy, and pungent antimicrobial compounds like guaiacol, syringol, PAHs and phenols (Obiedziński and Borys, 1977; Maga, 1988; Hui et al., 2001; McGee, 2004; Klemm et al., 2005; Garcia-Perez, 2008).

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 (Kawamoto et al., 2007; Nakamura et al., 2008; Garcia-Perez, 2008). Hardwoods instead of softwoods have been recommended, however, dry woods generate more PAHs because of their higher smoke generation temperature (Guillen et al., 2000; SCF, 2002). According to Šimko (2005) wood smoke of different trees may impart different flavour to smoke-cured fish due to their specific ratios of components.

The thermal degradation of hemicelluloses, cellulose, and lignin of wood proceeds at 180 – 300, 260 – 350, and 300 – 500 °C, respectively (Stołyhwo and Sikorski, 2005). Nakamura et al. (2008) and Garcia-Perez (2008) found that softwood produces higher PAHs than hardwood when burnt at temperatures above 400 °C because of its high lignin content. For instance according to Sun et al. (2004) and PPRIS (2010), lignocelluloses compositions of hardwoods is cellulose (40– 50 %), hemicelluloses (25 – 35 %) and lignin (20 – 25 %) and for softwoods cellulose (45 – 50 %), hemicelluloses (25–35%) and lignin (23–35%)(Sun and Cheng, 2002). Thus, softwood with higher lignin content would produce higher levels of PAHs at temperatures from 500 to 900 °C (Nakamura et al., 2008; Garcia-Perez, 2008).

2.1.3 Smoking process

Smoke-curing is a method of fish preservation carried out through a combination of drying, depositing naturally produced chemicals such as phenols, aldehydes, acetic acids and a range of polycyclic aromatic hydrocarbons (PAHs) resulting from the thermal breakdown of wood (Kramlich et al., 1980). The main purpose of smoke-curing is to preserve the food, partly by drying and partly by transferring anti-microbiological compounds such phenols (Šimko, 2005) carbonyls, organic acids and their esters, lactones, pyrazines, pyrols, and furan derivates (Maga, 1987) on a food surface with their subsequent migration into a food bulk (Šimko, 2002). The rate of smoke deposition depends on temperature, humidity, volatility, and velocity of a smoke stream (Šimko, 2005). When wood starts to dry in some parts of the fire pyrolysis starts, while complete combustion occurs in the parts nearest the flames (Ledesma et al., 2016). After generation, smoke is driven by aerosols into a kiln, while its temperature is going down, accomplished by partial condensation of smoke components (especially compounds with high boiling point) in pipes, walls and smoking chamber, conveying PAHs to the fish products being smoked, which eventually become contaminated. Smoking is still widely used in fish processing, and it involves using either modern controlled methods, or traditional uncontrolled kilns.

During traditional smoking, several processes occur at the same time. Traditional smoking involves treating of pre-salted, sundried, 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. There are numbers of classifications of smoking technology, according to temperature of smoke, placement of foodstuff and location of fire, or structure of smokehouse. In this thesis we have focused on traditional direct cold or hot smoking as described by Ledesma et al., 2016.

Cold-smoking technology

During cold smoking, fish or meat products are hung from shelves placed above the heat, located in a grilled floor through which the smoke passes. Smoking chambers are usually large. When burning finishes, the fire is not poked and the smoke cools. Cold smoking, with smoke temperatures between 15 and 25 °C is used mainly for aromatization of uncooked sausage, raw hams, and fermented thermally untreated salami (Šimko, 2005; Stołyhwo and Sikorski, 2005). This low smoke temperature is obtained by regulation of air (Woods, 2003).

Hot-smoking technology

During the hot-smoking technology chamber is heated by the burning of wood. Once placed inside the chamber, the fish or meat products are heated and dried by embers of burnt wood. Sawdust is then introduced into the chamber and the fire is stocked with the aim of producing a large amount of smoke (Möhler, 1978). Temperatures of 130 °C in the smoke and 80 °C in the fish meat are needed in hot smoking (Ahmad, 2003; Möhler, 1978), although some authors mention lower temperatures, between 55 and 80 °C (Woods, 2003). Either for aromatization and thermal treatment of ham, salami, sausages, etc., hot-smoking is used (Šimko, 2005). In hot-smoking, the process may be carried out in different stages, during which the temperature of the smoke ranges from about 40 – 100 °C and with temperature in the centre of the product reaching up to 85 °C (Stołyhwo and Sikorski, 2005).

2.2 Fish smoking in Cambodia

Cambodia has very few income generating possibilities beyond its natural resources and is economically almost fully dependent on agriculture, forestry and fisheries (FAO, 2002). Fish and fisheries in Cambodia are essential for providing food security to the people (FAO and 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). Yearly production of fish is about 514,000 t life weight and about 470,000 t is dedicated for consumption. Although there is a marked taste preference for fresh inland species of fish by the Cambodian population, large quantities of freshwater fish, and to a lesser extent marine species, are processed for human and animal consumption (Doulman and Officer, 1993). Fish processing provides a continuous source of protein throughout the year (Tickner, 1996). Recent amount estimates of proteins obtained from fresh fish and fish products make up to 37 % of the total protein intake (see Figure 1) and 76 % of the animal protein intake (see Figure 2) (Vilain et. al., 2016).

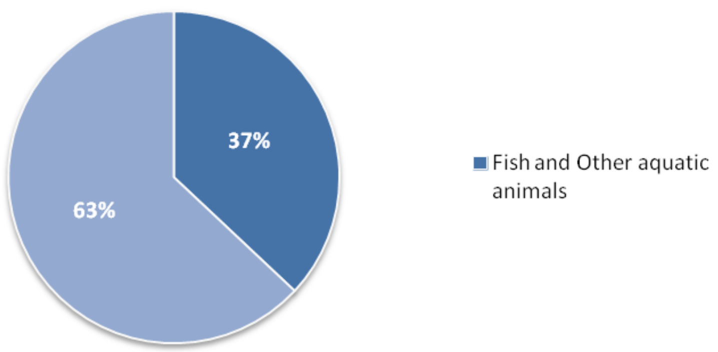

Figure 1 Contribution of fish, fish products and other aquatic animals on total protein intake in Cambodia. (Source: Vilain and Baran, 2016).

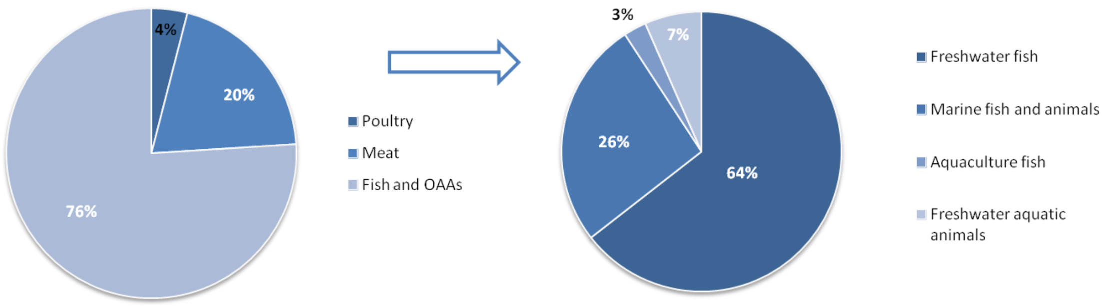

Figure 2 Total animal protein intake and breakdown on fish sub-group contribution on total animal protein intake. (Source: Vilain and Baran, 2016).

Even in relatively less important fishing areas with seasonal availability, fish and other aquatic animals are an essential part of the diet, contributing up to 56 kg per person per year (FAO, 2011). Since there is a very short peak period when fish is caught, it is necessary to process it quickly and in a basic way (Eong and Hariono, 2003; FAO, 2011). Fresh fish meat contains up to 80 % of water by mass and it is considered a highly perishable material, which results in an extremely short shelf-life when left unprocessed (Bala and Mondol, 2001). Processing involves range of basic but effective preservation techniques mainly focused on decreasing water activity to prevent spoiling. These techniques include sun-drying, salt-drying, smoking and steaming (Ahmed et al., 1999; Doulman and Officer, 1993; Hortle et al., 2004). Due to lack of access to electricity, which affects 66 % of the population in Cambodia, based on the data from The World Bank (2014), smoking as one of the oldest preservation methods, is still widely used in the country (Stołyhwo and Sikorski, 2005).

2.2.1 Traditional Khmer smoking technique

A great amount of Cambodia's inland freshwater fish production is sold through small village and town markets. The traditional fish processing establishments are classified as small-, medium- and large-scale (Eong and Hariono, 2003) according to the number of workers and production. Traditional ways of processing fish are well adapted to irregularity of the seasonal fish catch (Cambodia HARVEST project, 2016). During the peak season, thousands of people travel to the Tonle Sap, the Mekong and other waterways to trade rice for fish, to fish by their own or to buy small-sized/low value fish to produce fish products (Nam et al., 2009). Due to seasonality of the fish capture, limited use of ice or electricity for conservation, number of techniques and fish processing have developed, therefore the consumption of processed fish is expected to be high in Cambodia (FAO, 2011).

Cambodians are considered one of the highest per capita consumers of freshwater fish in the world (Nam et al., 2009; Baran, 2010). Eong and Hariono (2003) reported that about 60 % of total fish were consumed fresh, 18 % were fermented, 13 % salted-dried, 5 % smoked, 2 % fish sauce and 2 % other derived products (see Figure 3).

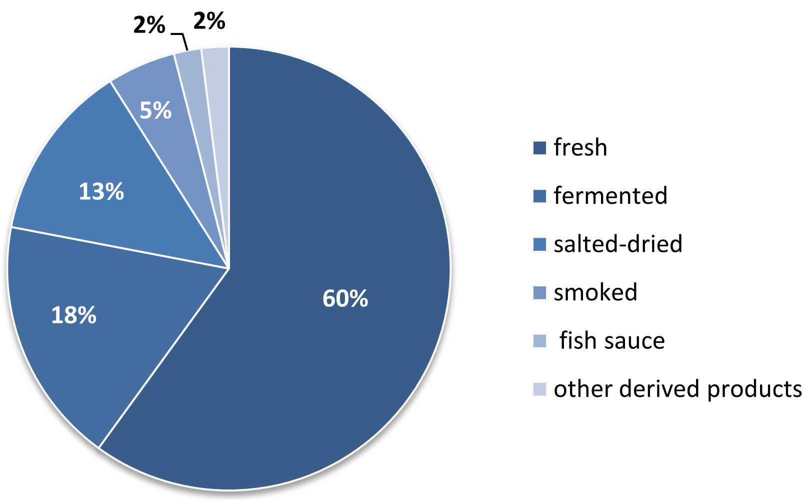

Figure 3 Reported form of consumption of total fish consumption in Cambodia. (Source: Eong and Hariono, 2003).

The wholesomeness of smoked fish products using the traditional kiln depends on type of wood used for the smoking process, temperature used, duration of smoking, type of kiln used, proximity of the fish from the fire, type of fish being smoked and fat content of the fish. In traditional Cambodian smoking is possible to regulate all these parameters to give quality smoke-cured fish product besides of the smoking temperature due to the lack of temperature regulating system on the traditional kiln and little knowledge on the control of smoking temperature to meet quality standards. This makes it difficult to effectively control smoking temperature, hence the release of toxins like PAHs into the fish product (Philips, 1999; SCF, 2002).

Used technology of traditional smoking of fish may consider Khmer traditional smoking as “Wild”, described by Šimko (2005) as: “smoking under uncontrolled technological conditions and nonexistent legislative measures, which is typical especially for households and developing countries, leads to enormous PAHs contents in smoked foods” (Alonge, 1987; Alonge, 1988; Afolabi et al., 1983).

Figure 4 Example of traditional smoking kiln, Kampong Chhnang province.

Although the final product is of low quality, it is a way of handling a large amount of fish during the peak period. In general, there are two groups of traditional smoking fish processors: small-scale and medium-scale. Small-scale fish processing is an activity of households which only produce for family consumption. These are people living near a river, fishing lots, lakes and also people who live in upland areas (Nam et al., 2009). Medium-scale is usually fish processing done by households, which work by using family labour, their relatives, and some hired labourers during the peak period (Nam et al., 2009; Eong and Hariono, 2003). Their location is usually near fishing lots, fishing villages and landing places. The fishing calendar is divided into two seasons: open (October-May) and closed (June-September). The ones fishing in small-scale, have an open access during the whole year, with imposing restrictions mainly on fishing efforts, for example type, number, size, and mesh size of gear. Whereas middle and large-scale fishers are allowed to fish only in the open season and it requires license, issued by the Department of Fisheries (DoF) (MRC, 2017).

Besides traditional smoked fish ("trey Cha-ar") Cambodians generally produce also dry salted fish, "Pho-ork" (fermented fish) or fish sauce. Since there is a market for sun-dried fish as animal food, its production has also expanded in the last few years and it is exported to Vietnam (Nam et al., 2009).

Smoked fish is a popular product that is mostly made by women involved in small- or medium-scale fish processing. Main smoking activities take place from March to April (Khiang et al., 2003). As a primary source of fuel wood for smoking fish serve flooded forests that surround the Tonle Sap Lake. In practice, the family-scale fishermen sell their catch to middlemen who collect the fish at the fishing ground, in case the market is too far from their house. And some of them sell their catch directly to consumers at their place or bring it to the landing place and then sell it to wholesalers (MRC, 2017).

The used fish must be fresh. Any size and weight of fish can be used, but each batch to be smoked must be of similar type and size. The fish must be well prepared by cutting and cleaning. Before smoking, fish is usually cleaned and head and guts are often removed because of the aesthetic, sapidity and contamination reasons (Kawarazuka, 2010; Vilain and Baran, 2016). Once the fish are gutted, the family thread them by dozen onto skewers, and then smokes them over an open fire – a task requiring all family members. The fish are skewed using small bamboo sticks about 20 cm long and 3 mm in diameter. The prepared skewers on a bamboo frame are placed over a low fire to dry out in the fish smokehouse. They are placed in vertical rows and a 1 cm gap is left between each skewer to allow the smoke to circulate. On average 100 - 150 skewers of fish are placed in the smokehouse together. The fish are smoked for 5 to 6 hours. Once the fish are smoked, they must be dried again by smoking or sun dried 2 to 3 times a week (MAP, 2010). During the high season one family can smoke about 200 to 300 fish per day, in total weight around 30 kilograms (Phnom Penh post, 2011).

2.3 Polycyclic aromatic hydrocarbons

Polycyclic aromatic hydrocarbons (PAHs) are a class of ubiquitous ecotoxicants that are harmful to human health, with some known to be carcinogenic (Vazquez Troche et al.,2000; Kishikawa et al., 2003; Janoszka et al., 2004; Davina and Yusty, 2005; Okuda et al., 2006; Tfouni et al., 2007; Essumang et al., 2012). PAHs are a class of organic compounds consisting of 2 to 7 fused aromatic rings in a linear, angular, or clustered arrangement. PAHs origin is generally defined either as anthropogenic such as exhaust of motor vehicles, petroleum refineries, heating in power plants, combustion of refuse, deposition from sewage, oil/gasoline spills, tobacco smoke, and coke production (Christensen and Bzdusek, 2005; Moon et al., 2006). Or as incomplete combustion or thermal decomposition (pyrolysis) of wood (Conde et al., 2005; Djinovic et al., 2008; Gomes et al., 2013; Hitzel et al., 2013; Ledesma et al., 2016; Lorenzo et al., 2010; Pöhlmann et al., 2013; Rey-Salgueiro et al., 2008; Škaljac et al., 2014; Wretling et al., 2010). PAHs are lipophilic in nature and usually accumulate in the fatty tissues of organisms and consequently in the food chain (Pensado et al., 2005; Shadi et al., 2012, Bansal and Kim, 2015). The formation of PAHs is known to occur through pyrolysis of fat at temperatures above 200 °C (SCF, 2002), and it is highly stimulated at temperatures over 700 °C (Bartle, 1991). However formation of PAHs during biomass combustion should be also taken into account (Ledesma et al., 2016). PAHs can be also found as tertiary tar products formed during biomass pyrolysis (Basu, 2010). Pyrolysis products can be classified as solids (mostly char or carbon), liquids (tars, heavier hydrocarbons and water) and gases (carbon dioxide - CO_2, water - H_2O, CO – carbon oxide, C_2H_2 - acetylene, C_2H_4 - ethylene, C_2H_6 - ethane, C_6H_6 - benzene, etc.).

They are of significant concern primarily because of their ubiquitous presence in the environment and well-recognized carcinogenicity, teratogenicity and mutagenicity (Tobiszewski and Namiesnik, 2012). According to the Scientific Committee on Food (2002), 15 PAHs ‘‘show clear evidence of mutagenicity/genotoxicity in somatic cells in experimental animals in vivo. They may be regarded as potentially genotoxic and carcinogenic to humans’’; their carcinogenicity depends on their structure (Bartoszek, 2002). PAHs of molecular mass below 216 Da are regarded as light and not carcinogenic.

Up to now, the PAHs content in foods has been commonly considered as not affected by environmental factors and additional operations, e.g., cooking, or even packaging. However, photodegradation of PAHs by UV light is possible and formation of oxidative products (such as aromatic alcohols, ketones, quinones, and ethers) has already been proven (Bernstein, et al., 1999; Šimko, 2002). And in spite of the decreased BaP content, the total toxicity of the PAHs might be even elevated due to the presence of oxidized PAHs compounds (Law et al., 2002). Also as Lijinsky, (1991) and Knize et al., (1999) stated, simple processing practices are known to result in a significantly reduced contamination of foods by PAHs as well as by other undesirable contaminants. This may include selecting preferentially lean meat and fish, avoiding contact of foods with flames for barbecuing, using less fat for grilling, and, in general, cooking at lower temperature for a longer time. Broiling (using of heat source above the product) instead of grilling can significantly reduce the levels of PAHs.

2.3.1 PAHs Determination

Currently, chromatographic techniques, mainly gas chromatography (GC) and high performance liquid chromatography (HPLC), are dominant effective analytical tools capable of separating individual isomers of PAHs fraction to be isolated from both, smoked fish meat and liquid smoke flavourings (LSF) matrix (Bartle, 1991; Stahl et al., 1988; Šimko, 2005; Tamakawa, 1996; Chiu et al., 1997; Guillén et al., 2000 a, b ; Šimko, 2002; Tamakawa, 2004; Jira, 2004; Šimko, 2005).

Following foodstuff homogenization, contemporary analytical procedures based on extraction of hydrocarbons from the matrix, clean-up procedure, separation by gas chromatography (GC) or high performance liquid chromatography (HPLC) are used. With following detection and quantification by mass spectrometry (MS) or fluorescence detectors (FLD), respectively, make it possible to determine individual PAHs in smoked foods at concentrations of the order of 0.1 $\mu g.kg^{-1}$ or even $0.01 \mu g.kg^{-1}$. Because of very low contents of individual PAHs in foods, of the order of $1 \mu g.kg^{-1}$, and the requirement to determine BaP, with a reproducibility not lower than 48 % of the value tolerated in the products (SCF, 2002). The efficiency of extraction of PAHs depends on the polarity of the solvent, on the nature of the matrix, and on the preparation of the sample (Jarvenpaa et al., 1996; Moret et al., 1999; Wang et al., 1999). It has been shown by Grimmer and Böhnke (1975), that alkaline hydrolysis of samples, previously extracted with boiling methanol, increased (about 3-fold) the total recovery of PAHs from meat. On the other hand, prolonged alkaline hydrolysis may lead to some loss of BaP due to degradation (Takatsuki et al., 1985). There is a significant correlation between the fish lipid content and the total PAHs levels.

Nowadays, there is still no official procedure accepted by all concerned organisations, which would solve difficulties associated with quantitative isolation of PAHs from the food material, clean-up of the extract without significant loss of the analyte, separation of all individual PAHs contained in the purified extract, detection of the separated components, unequivocal identification of the PAHs, and quantification of the identified compounds.

2.3.2 Derivates of PAHs

According to current knowledge, some PAHs are able to interact in organisms with enzymes to form PAHs dihydrodiol derivates. These reactive products are considered ultimate carcinogens that are able to form covalent bounds with proteins and nucleic acids. In general, DNA bounds are thought to initiate cell mutation which results in malignancy (Bartle, 1991; IARC, 1987; Stahl and Eisenbrand, 1988; Rogan et al., 1993). The most potential mutagenicity was observed in PAHs fractions isolated from smoked fish, treated before smoking with nitrites in an acid solution (Kangsadalampai et al., 1997).

2.4 Health risks related to PAHs

Carcinogenic risks are estimated as incremental probability of an individual to develop cancer over a lifetime as a result of exposure to a potential carcinogen (IELCR, or just carcinogenic risk) (Essumang et al., 2010). According to WHO Noncommunicable Diseases (NCD) Country Profiles (2014) 13 % of Cambodian population suffer from cancer (see Figure 5).

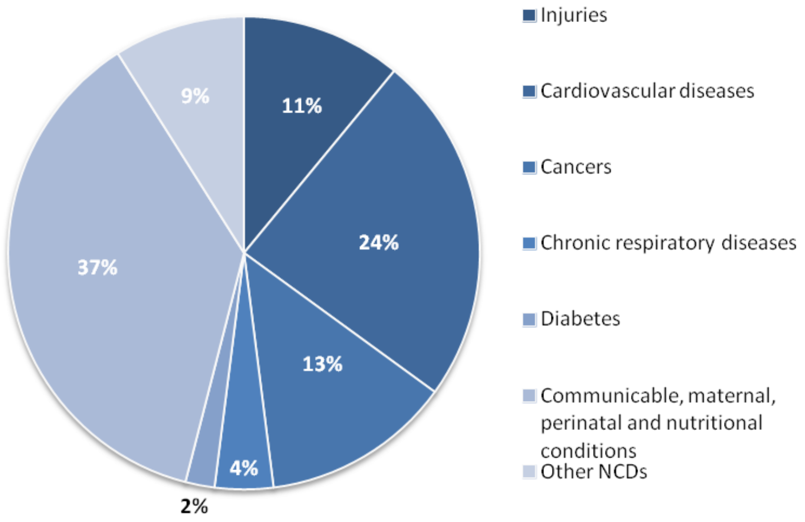

Figure 5 Proportional distributions of causes of death in Cambodia. (Source: NDCs Country Profiles, 2014).

Potential health hazards associated with smoked foods may be caused by carcinogenic components of wood smoke – mainly PAHs and derivatives of PAHs. Carcinogenic, mutagenic and bioaccumulative effects of PAHs have been reported by several institutions concerned with public health, food security and safety as: Food and Agriculture Organization of the United Nations (FAO), the World Health Organization (WHO), the International Agency for Research on Cancer (IARC), the European Scientific Committee on Food (SCF), the European Food Safety Authority (EFSA) and the US Environmental Protection Agency (EPA) (SCF, 2002; WHO, 2006). Very mutagenic and carcinogenic is BaP; it has been accepted as a marker of carcinogenic PAHs in wood smoke, smoked products, and environmental samples (Stołyhwo and Sikorski, 2005). Recently discussed consequences on health after PAHs exposure (Shen et al., 2008) were reported as growth retardation, low birth weight, small head circumference, low IQ, damaged DNA in unborn children and disruption of endocrine systems, such as estrogens, thyroid and steroids. Skin changes (thickening, darkening, and pimples) and reproductive-related effects such as early menopause due to destruction of ova; have also been identified with exposure with PAHs (Essumang et al., 2011).

Due to binding to cellular macro-molecules in mammalian cells including DNA, where PAHs undergo metabolic activation to diol, and epoxides, thereby causing errors in DNA replication and mutations that initiate the carcinogenic process (Rodriguez et al., 1997; Schoket, 1999; Lightfoot et al., 2000). According to the latest classification on carcinogenicity of PAHs by IARC monograph, it has been established that benzo[a]pyrene is defined as carcinogenic (group 1), dibenz[a,h]anthracene is probably carcinogenic (group 2A), whereas naphthalene, benzo[a]anthracene, chrysene, benzo [b]fluoranthene, benzo[k]fluoranthene, benzo[j]fluoranthene and indenol[1,2,3-c,d]pyrene are classified as possible human carcinogens (group 2B), (IARC, 2012). As well as wood smoke has been classified by the IARC (2012) monograph as certainly carcinogenic (group 1).

2.5 PAHs contents in smoked fish

In smoked fish, Grimmer and Böhnke (1975) found about 100 PAHs and their alkylated derivatives. Thus it is a very rich mixture of compounds that are similar in chemical character, difficult to analyze, especially if also accompanied by other nonpolar components. Fish, too, contains naturally occurring hydrocarbons, such as squalene $C_{30}H_{50}$, that is abundant, e.g., in some fish oils. These hydrocarbons present in some fish oils behave the same during the procedures and thus complicates further steps of analysis (Chen and Lin, 1997; Reinik et al., 2007; Lund et al., 2009). Normal content of benzo[a]pyrene in smoked fish is between 0.1 and $1 \mu g.kg^{-1}$ (Gómez–Guillén et al., 2009). Residual PAHs concentrations in smoked foods are highly variable and result from use of different smoking methods. Traditional direct smoking, in which the smoke is generated in the same chamber where the product is processed, exposes it to higher PAHs content than indirect smoking which uses a separate chamber for smoke generation (Akpambang et al., 2009). The highest concentration of PAHs in smoked products is immediately after finishing of smoking, and then it decreases due to light decomposition and interaction with present compounds (Šimko, 2005; Dennis et al., 1984). However, PAHs also penetrate into smoked products, where they are protected from light and oxygen, and after some time, the concentration stabilizes at a certain constant level (Šimko et al., 1992). Commission Regulation (EC) No 1881/2006 sets maximum levels for certain contaminants in foodstuffs. According to this regulation, since 1st September 2014 limits of benzo[a]pyrene for muscle meat of smoked fish and smoked fishery products, smoked sprats and canned smoked sprats, and bivalve molluscs (smoked) were defined as following: 2.0 $\mu g.kg^{-1}$; 5.00 $\mu g.kg^{-1}$; 6.00 $\mu g.kg^{-1}$ of benzo[a]pyrene and 12.0 $\mu g.kg^{-1}$; 30.0 $\mu g.kg^{-1}$; 35.0 $\mu g.kg^{-1}$ sum of benzo[a]pyrene, benz[a]anthracene, benzo[b]fluoranthene and chrysene, respectively (see Table 1). Fat binding PAHs are capable of accumulating in food chain (Roeder et al., 1998; McLachlan, 1997) therefore; the amount of PAHs per gram of fish consumed is a very important data to help advise on long-term implication on human health.

Table 1 Maximum levels for certain contaminants in foodstuffs set by Commission Regulation (EC) No 1881/2006. (Source: Commission Regulation (EC) No 1881/2006, 2016).

Type of products/Type of contaminant	muscle meat of smoked fish	smoked fishery products	smoked sprats and canned smoked sprats and bivalve molluscs (smoked)
Benzo[a]pyrene	2.0 $\mu g.kg^{-1}$	5.00 $\mu g.kg^{-1}$	6.00$\mu g.kg^{-1}$
chrysene, benzo[a]pyrene, benz[a]anthracene and benzo[b]fluoranthene	12.0 $\mu g.kg^{-1}$	30.0 $\mu g.kg^{-1}$	35.0 $\mu g.kg^{-1}$

3 Aims of the thesis

Fish and fish products are the second main dietary compound of the Cambodian diet; due to its affordability and protein and essential micronutrients supply not widely available elsewhere in the diet. Smoking as a conservation procedure is one of the oldest mankind has used. The preservation effect is generally attributed to the antioxidant and antimicrobial properties of phenolic compounds. However, generation of wood smoke during curing is a typical example of incomplete combustion, and undoubtedly polycyclic aromatic hydrocarbons (PAHs) are generated and released into various smoked products. Polycyclic aromatic hydrocarbons (PAHs) comprise the largest class of chemical compounds known to be cancer causing agents. The thesis objective was to monitor a traditional way of smoking fish products in Cambodian rural areas and evaluate amounts of carcinogenic compounds in traditionally smoked fish products in Cambodia.

4 Material and Methods

The thesis was based mainly on field research and laboratory analyses. The first part of the thesis served to introduce the necessary background for the field research part. The second part evaluated results obtained during the field research according to selected criteria. Samples were transported to the Czech University of Life Science in Prague, faculty of Tropical AgriScience and laboratory analyses focused on potential PAHs content and data evaluation was carried out. Gathered data were analyzed. Extraction of completely homogenized samples by Soxtec apparatus was followed by pre-cleaning by gel permeable chromatography (GPC). Analytes in recupered eluate were evaluated by high pressure liquid chromatography with fluorescence detector (HPLC-FD) for analysis. Identification was based on comparison of retention times with standards. Quantification was performed by the method of external standard. Steps described below from 4.4.2 to 4.4.3 were taken in certified laboratory of National Veterinary Institute in Prague.

4.1 Site area description

Wetlands of the Tonle Sap area in Cambodia are part of the Mekong watershed with one of the most productive fisheries in Southeast Asia. Fisheries in this location 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 et al., 2013). Location of villages where producers were questioned is presented in Figure 6 below.

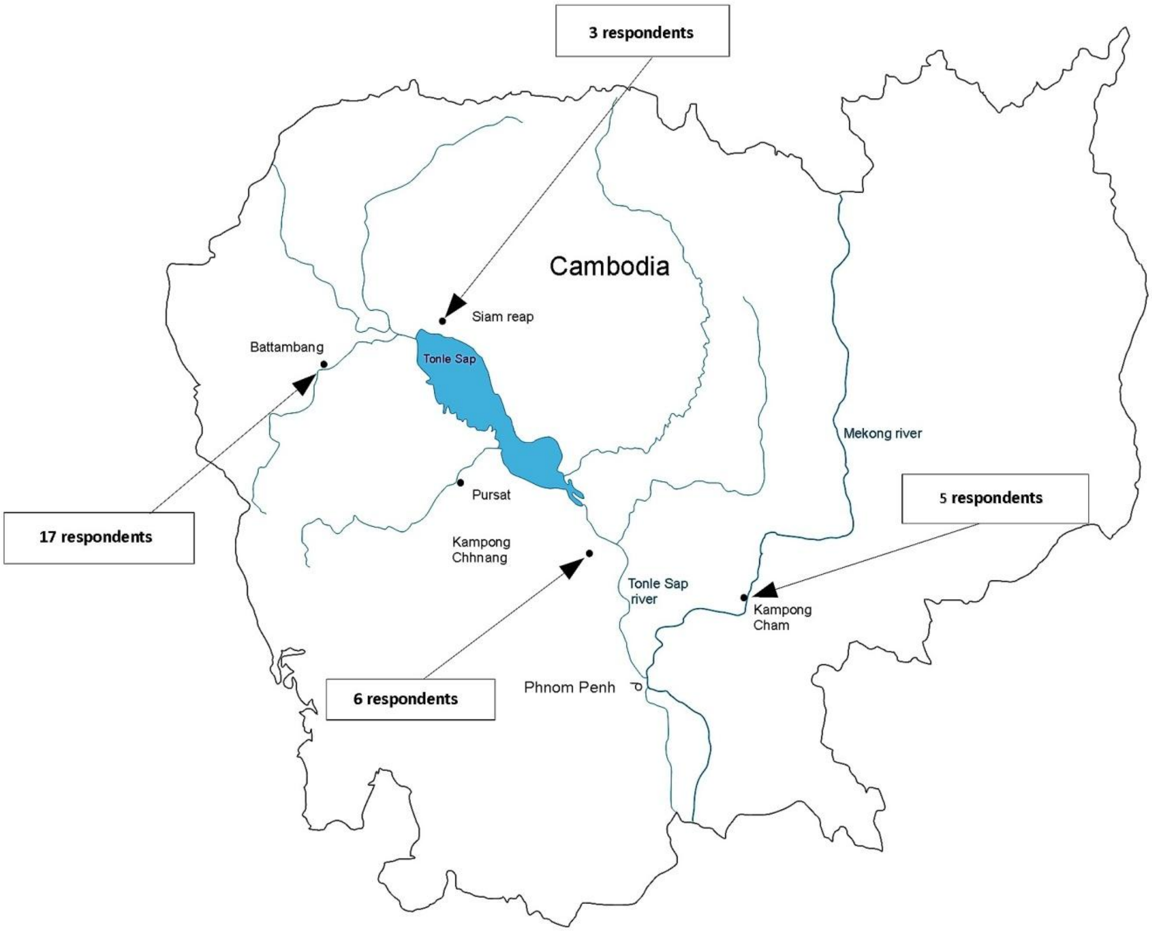

Figure 6 Location and number of respondents during questionnaire survey, Tonle Sap area.

Namely Kandal village, Psear Chhnang village – Kampong Chhnang province, about 95 km north from the capital Phnom Penh; Chom Karisy village, Odombong village, Norea village, Takok village, Lor Eit village – Battambang province approximately 300 km northwest direction from the capital Phnom Penh; Orn Long village, Psar Kheang village, Kampong Mkeak – Siam Reap province about 300 km to the north around Tonle Sap lake from capital Phnom Penh; Spean Trong village – Kampong Cham province placed about 100 km from the capital city Phnom Penh. Sample collection was located along Tonle Sap river near the Kampong Chhnang city in Kampong Chhnang province about 95km north from the capital Phnom Penh as presented in Figure 7.

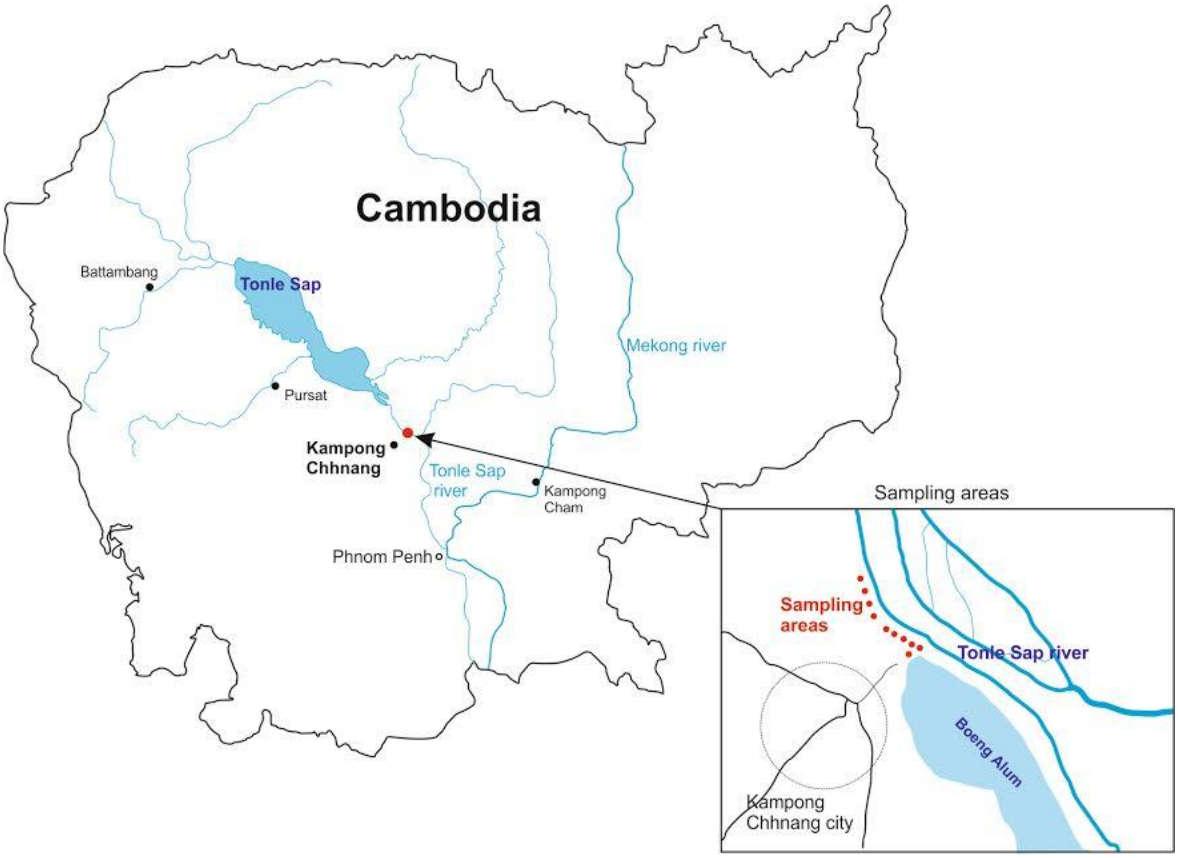

Figure 7 Site area of sample collection, Kampong Chhnang province, Cambodia.

The Kampong Chhnang province is among the highest per capita consumption of fresh fish and particularly smoked fish processing in Cambodia. Total consumption of fresh fish and processed fish in the province is almost 120 kg per year (Ahmed et al., 1999). Further, the area near the Kampong Chhnang city along Tonle Sap river is famous for high concentration of smoked fish producers. Hence this area was selected for our sample collection. Figure 7 above text describes area where specifically sample collection was placed in Kampong Chhnang province.

4.2 Questionnaire survey

About 84 % of Cambodian population is considered as rural (Hortle, 2007). At least 45 % of the population is working full time in fisheries or fisheries-related activities and dependent on these wetlands hence improving local people livelihoods (Nam and Bunthang, 2011). To gather a supplementary relevant data to evaluate the final PAHs concentrations of the fish samples personal interviews and questionnaire survey were conducted. Questionnaire survey was conducted among small-scale producers of smoked fish products in Cambodia in eleven villages within Tonle Sap area of 4 provinces with history of smoking. This survey was driven among all producers of smoked fish products visited within this field research. Number of respondents was equal to 31 producers included in research in the targeted area (Figure 6). Questions in the survey were related to location of the producer, source of fish, fish species used for smoking, pre-treatment used before the smoking procedure, technique used for fish smoking, type of fire woods used period of smoking and main production, storage and selling practices (see Appendix I). All data were collected in local units and names as well as all interviews and questionnaires were conducted in Khmer language held by Cambodian student from the Royal University of Agriculture in Phnom Penh (Figure 8). Gathered data were translated to English and scientific names and processed.

Figure 8 Questionnaire survey conducted by Cambodian student, Sukunthia Chhong.

4.3 Sample collection

During period from August till October 2016 samples were collected and field research was conducted among small-scale producers of smoked fish in Cambodia. Totally 23 samples from 10 producers were collected directly from smokehouses. The samples were collected using vinyl gloves and placed in clean, properly labelled plastic bags and vacuum closed (see Figure 9). Each of the samples weighted approximately 100 g. Immediately after sampling the bags with samples were frozen in freezer at -20 °C and then transported to Czech University of Life Sciences Prague, Czech Republic for further laboratory analyses.

Figure 9 Vacuum packaging of the samples.

Fish species were determined as 3 samples of Siamese mud carp (*Henicorhynchus caudimaculatus*) (Sauvage, 1881) which belongs to order Cypriniformes, family Cyprinidae; 5 samples of *Phalacronotus bleekeri* (Bleeker, 1846) belonging to order Siluriformes, family Siluridae; 3 samples of *Belodontichthys truncatas (*Kottelat and Ng, 1999) belonging to order Siluriformes, family Siluridae; 3 samples of Lesser bighead carp (*Thynnichtys thynnoides*) (Bleeker, 1852) which belongs to order Cypriniformes, family Cyprinidae; 2 samples of *Paralaubuca typus* (Bleeker, 1864) belonging to order Cypriniformes, family Cyprinidae; 3 samples of *Kryptopterus hexapterus* (Bleeker, 1851) belonging to order Siluriformes, family Siluridae; one sample of *Ompok bimaculatus* (Bloch, 1794) belonging to order Siluriformes, family Siluridae Table 2.

Table 2 Number of sampled species.

Fish name	Number of samples per species
Henicorhynchus caudimaculatus	*3*
Phalacronotus bleekeri	*5*
Kryptopterus hexapterus	*3*
Belodontichthys truncatus	*3*
Thynnichtys thynnoides	*3*
Osteochilus lini	*3*
Ompok bimaculatus	*1*
Paralaubuca typus	*2*

4.4 Determination of PAHs in smoked fish sample

4.4.1 Sample preparation

Prior to extraction, whole sample of fish (approximately 50 g per sample) was homogenized in a mortar with about 10 ml of liquid nitrogen until a completely dry homogenate was obtained. Fish samples were kept in amber bottles and refrigerated at temperatures below 4 °C prior to analysis (see Figure 10). Fish samples were than extracted in Soxtec.

Figure 10 Sample preparation, prior to extraction. A – Laboratory equipment used, B – Homogenate obtained, C – Amber storage bottles.

4.4.2 Sample extraction

Fat was extracted using a solvent and the Randall modification of the Soxhlet method. The Soxtec apparatus (see Figure 11) consisted of a six thimbles in one line. The three-step extraction procedure consists of boiling, rinsing and recovery. Typically solvent recovery is 80 %, with only ~50 ml solvent used per sample.

We put 5 g of completely homogenized sample into the beaker. After addition of 10 g of dehydrogenized sodium sulphate in mortar we thoroughly grinded the sample. This modified sample was then transported to the paper Soxtec cartridge, pluged with glass wool and placed for 16 hours into Soxtec. Extraction went in 50 ml of hexane: dichloromethane (1:1) mixture at temperature 109 °C. The sample was then submerged in boiling solvent prior to rinsing in cold solvent, reducing the time needed for extraction. The solvent dissolves fats, oils, pigments and other soluble substances. After the extraction, the solvent was evaporated and redissloved in 10 ml of chloroform into volumetric flask. The resulting fat residue was weighed back after drying. All the chemicals were of trace analysis purity purchase from Sigma Aldrich (CZ).

Figure 11 Soxtec apparatus used for fat extraction.

4.4.3 Sample pre-cleaning

Redissolved solvent was pre-cleaned using gel permeable chromatography (GPC). The 2 ml of diluted extract was injected on GPC colon to clean off the lipids. Fractions 24 - 32 ml were collected. This fraction was then evaporated at rotation vacuum vaporizer at maximal temperature 40 °C till dryness. Pre-cleaned extract was redissolved in 0.5 ml of acetonitrile and preceded to further HPLC-FD analysis.

4.4.4 Determination of PAHs using HPLC-FD method

Final analysis of PAHs content was done by high pressure liquid chromatography with fluorescence detector (HPLC-FD). Analysis was performed on Waters PAH C18 (250 × 2.1 mm) column with 5 µm particle size. The pre-cleaned sample 20 µl was injected to HPLC and eluted at 30 °C with flow 0.5 $mL.min^{-1}$ with following gradient of mobile phase: water as eluent A and acetonitrile as eluent B. During period 0 - 2 min the ratio of eluents was A (25 %) + B (75 %); from 10 to 21.5 min A (0 %) + B (100 %) and from 22 min A (25 %) + B (75 %) ml. Total time of analysis was 27 minutes. Finally, PAHs were identified by comparison of retention times of analytes with standards by fluorescence detector. Quantification was performed by the method of external standard. Results were presented in $\mu g.kg^{-1}$ of sample. Sum PAHs (PAHs4) was calculated as total of concentration of benzo[a]pyrene, chrysene, benzo[a]anthracene and benzo[b]fluoranthene according to the Appendix of Commission Regulation (EC) No 1881/2006 see Table 1.

5 Results and Discussion

5.1 Fresh water fish smoking around Tonle Sap Lake in Cambodia

Data were obtained during questionnaire survey within field research in the Tonle Sap area within eleven villages; Kandal village, Psear Chhnang village, Chom Karisy village, Odombong village, Norea village, Takok village, Lor Eit village, Orn Long village, Psar Kheang village, Kampong Mkeak, Spean Trong village. These villages belong to four provinces; Kampong Chhnang, Battambang province, Siam Reap province and Kampong Cham province (Figure 6).

5.1.1 Source of fish for traditional smoking of fish

As we can conclude from Figure 12, distribution of four main sources of fish for smoking products is balanced. However middlemen who transport fish from fishermen to the producers were used in most cases 36 %. Consequently, was named by the respondents as a source of fish port, directly from fishermen, or at the market, 29 % > 19 % > 16 % respectively. This is in accordance with Nam et al., (2009), who described the seasonality movements of Cambodian population towards Tonle Sap area to gather fish for their own production by trading, fishing or buying.

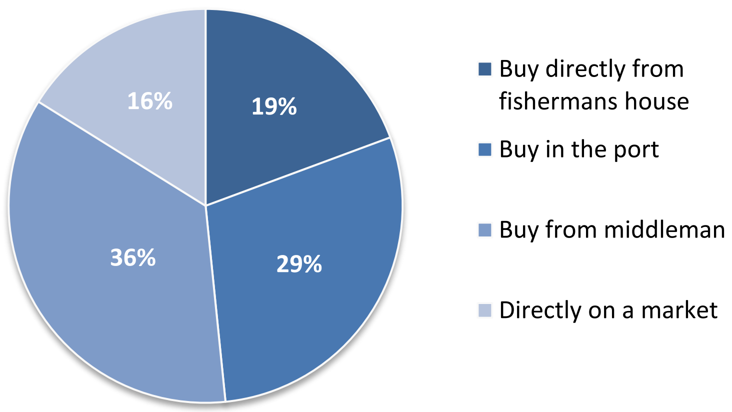

Figure 12 Source of fish for traditional smoking.

According to the data from Figure 13 describing differences within the provinces, we can conclude that provinces situated closer to wetlands as Kampong Chhnang and

Siam Reap province have higher number of respondents who bought fish for production directly from fishermen. On the contrary, areas with higher distance from water surfaces prefer markets or middlemen who brings fish from fishermen (Ahmed et al., 1999).

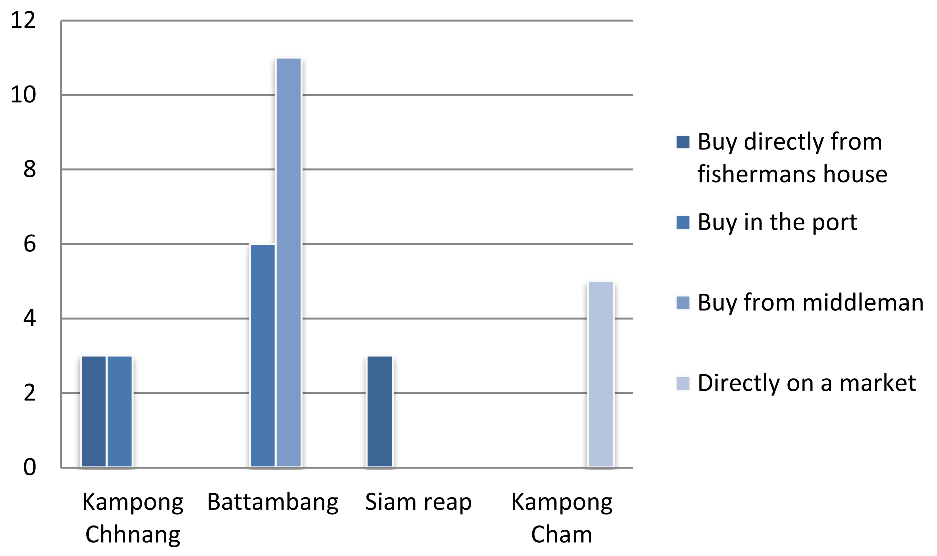

Figure 13 Source of fish for smoking within provinces.

All questioned producers, 100 %, within all provinces, process fish directly by the smokehouse or their households (Figure 14), there is no additional transportation. This practice is reasonable due to natural conditions in the area and fast spoilage of fish meat. Same as the technique used for conservation itself.

Figure 14 Traditional fish processing site (local household), Battambang province, Cambodia.

5.1.2 Traditional smokehouse

Traditional technique of smoking fish takes place in typical smokehouses along the river or close to water areas. Dimensions of these smokehouses vary according to production as described by Nam et al., 2009; medium- and small-scale. Generally the dimensions are 200 – 1500 cm × 90 – 400 cm × 70 – 400 cm, length, width and height, respectively Figure 15.

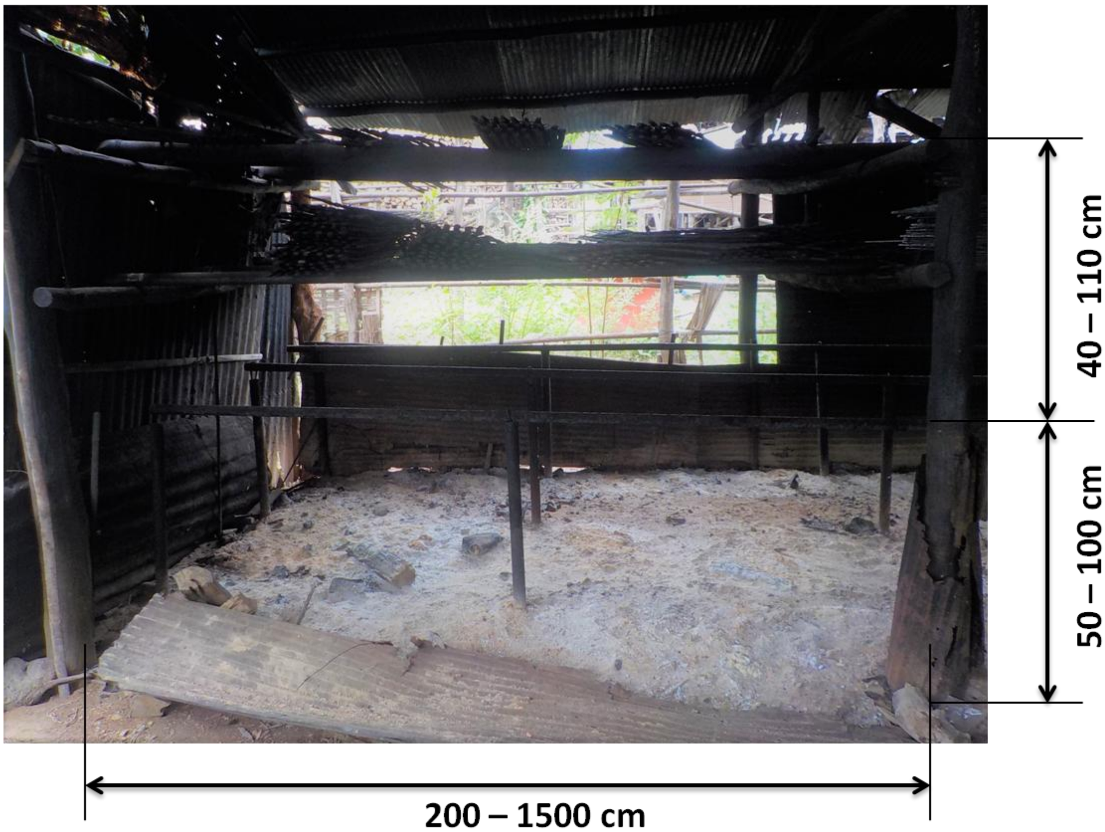

Figure 15 Dimensions of typical smoking house in Tonle Sap area, Cambodia.

Smokehouses, as results from the field research have wooden, bamboo or brick construction with or without walls made from mats from leaves or bamboo, fibre or metal sheets. However, roof, if present is made from palm leaves or metal sheets (see Figure 16). Structure and diameters may depend on family´s financial limits. Bamboo and wooden smokehouses were mostly made from material collected in the surrounding area on contrary to brick buildings for which family has to buy material.

Figure 16 Three representatives of traditional smokehouse in Tonle Sap area, Cambodia, A – metal sheet smokehouse, B – wooden smokehouse, C – brick smokehouse with metal roof.

Traditional smoking kiln consist of fire place and levels of tray, where grouped fish are placed, from one to four according to the production. Used technology may be classified as direct and hot smoking method according to definition by Ledesma et al. (2016). First level of trays is placed in distance from 50 up to 100 cm above the fire place Figure 15. Next levels of trays are usually placed in distance about 40 cm each. The trays are made from bamboo sticks or rarely from metal rods. There are basically two main dimensions used for smoking of fish, square dimension 25 - 40 × 30 – 50 cm and rectangular 15 – 30 × 80 – 250 cm width and length, respectively Figure 17.

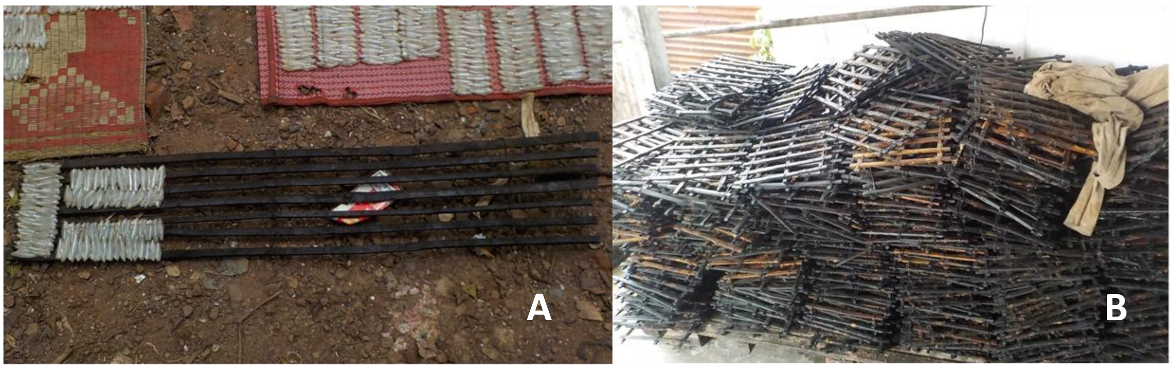

Figure 17 Two representatives of trays used for traditional smoking, Cambodia, A- Rectangular tray, B – Square tray.

5.1.3 Fuel used for traditional smoking of fish

Fires are in general placed on the ground considered a fireplace; most of the producers use net arrangement due to better circulation of hot air, which results in better quality of smoked products (see Figure 18).

Figure 18 Typical arrangement of fire used in Tonle Sap area, Cambodia.

As a main source of fuel for traditional smoking of fish were used two types of fuel; wood and charcoal (see Figure 20). In Figure 19 we can see that 74 % of respondents used as a primary source of fuel wood in comparison to 26 % who used charcoal. This results are consist with results presented by San et al., 2012, who stated that fuel wood are main source of energy (85 % in 2007) for cooking, boiling water and animal protection for majority of population in Cambodia. Charcoal, on contrary, was used in 6 % followed by LPG (liquid petroleum gas) 5.2 %. This great difference may be caused by ostensibly infinite source of fuel wood in surrounding area and higher price of charcoal compared to fuel wood.

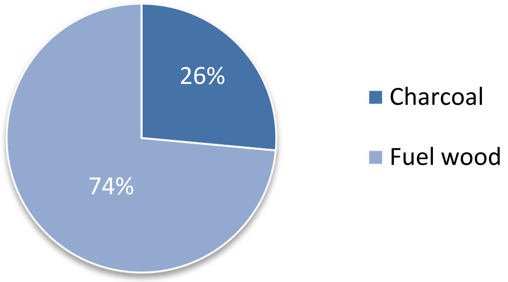

Figure 19 Type of fuel used for smoking, intargeted area.

Figure 20 Fuel wood and charcoal mostly used for smoking, targeted area.

As we can conclude from the Figure 19, 74 % of the questioned producers use wood as a fuel for traditional smoking of fish. According to the questionnaires the amount of wood used for production ranges from 0.3 m^3 to 4 m^3. In case of charcoal, the amount is from 90 to 250 kg per day in high season. Used wood is mostly local fuel wood collected in the surrounding area (see Figure 21). As other source was mentioned buying of prepared batches of wood. These batches are called in local name Kreak and they are described as a mixture of unspecified mixture of wood.

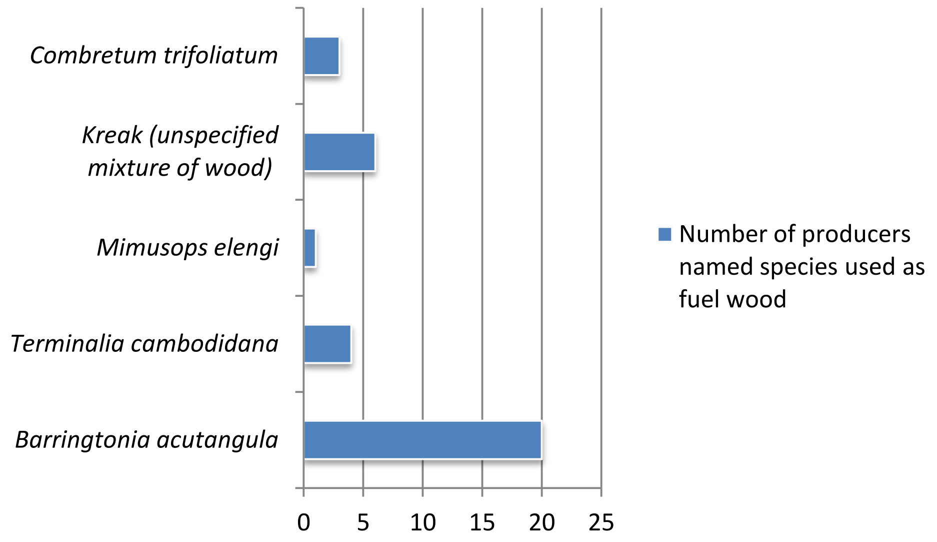

Figure 21 Plant species used as fuel named by producers, Tonle Sap area.

The lowest diversity of fuel wood used for traditional smoking of fish was presented in Siam reap and Kampong Chhnang province with clearly dominating *Barringtonia acutangula* named by locals “Deam Reang”. In both of these areas there is a long history of smoking with one of the highest concentration of smoked fish producers. Therefore preference of *Barringtonia acutangula* known for its favourable properties of smoke produced, in comparison to other plant species producing smoke (with higher probability of darker final product which is undesirable), is reasonable. On contrary, highest diversity in fuel woods was observed in Battambang province where all named species were used, with the exception of *Combretum trifoliatum* (see Figure 22).

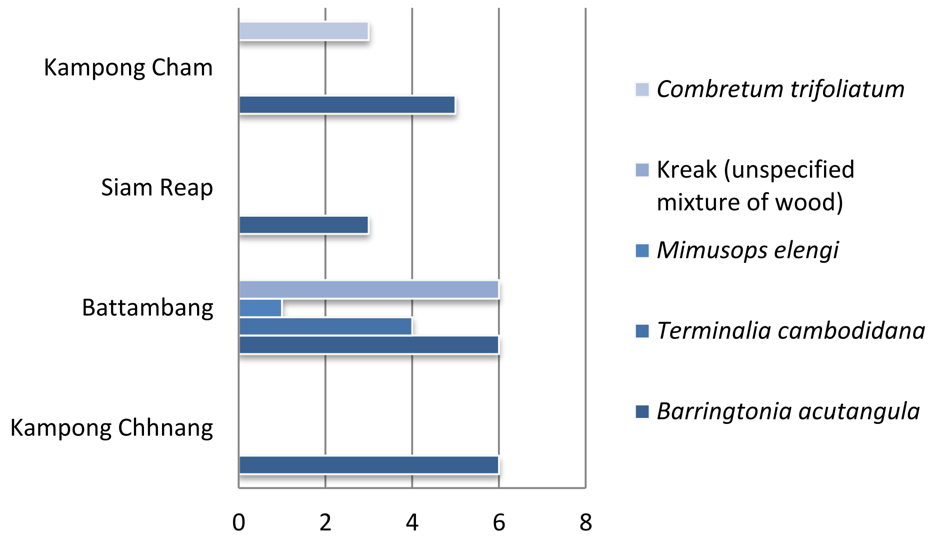

Figure 22 Plant species used as a fuel wood within the provinces, Tonle Sap area.

However as you can see in a Figure 23 in Battambang province is highest amount of producers using charcoal as main fuel for smoking. This might be a reason for higher diversity of fuel wood due to lower preference in wood for smoking.

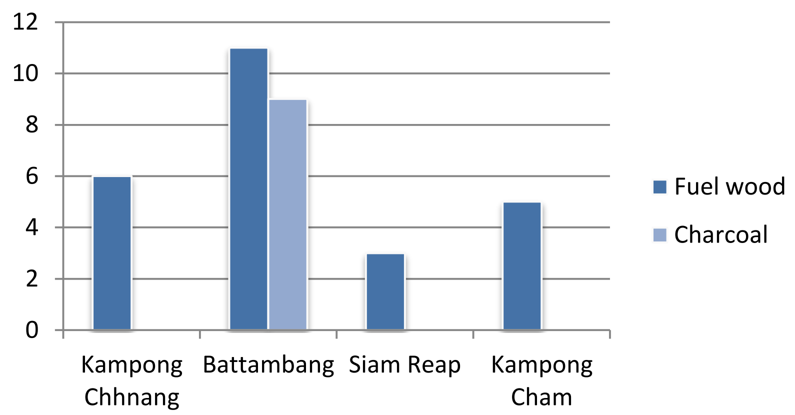

Figure 23 Comparison of fuel used for fish smoking within provinces in Tonle Sap area, Cambodia.

Other plant named by producers as fuel wood used for smoking were *Mimusonps elengi* and *Terminalia cambodiana.*

5.1.4 Fish used for traditional smoking

Fish species traditionally used for smoking named by producers in targeted area were determined as 22 fish species belonging to six different orders. Namely *Henicorhynchus caudimaculatus* (Sauvage, 1881) belonging to order Cypriniformes, family Cyprinidae; *Esomus longimanus* (Lunel, 1881), belonging to order Cypriniformes, family Cyprinidae; mixture of *Clarias batrachus* (Linnaeus, 1758), which belongs to family Clariidae, order Siluriformes; *Clarias macrocephalus* (Günther, 1864) belonging also to family Clariidae, order Siluriformes; *Channa striata* (Bloch, 1793) belonging to family Channidae, order Perciformes; *Coilia macrognhatos* (Bleeker, 1852) belonging to order Clupeiformes, family Engraulidae; *Phalacronotus bleekeri* (Bleeker, 1846) belonging to order Siluriformes, family Siluridae; *Belodontichthys truncatus (*Kottelat and Ng, 1999) belonging to order Siluriformes, family Siluridae; *Notopterus notopterus* (Valenciennes, 1848) belonging to order Osteoglossiformes, family Notopteridae; *Rasbora hobelmani* (Hamilton, 1822) belonging to order Cypriniformes, family Cyprinidae; *Wallago attu* belonging to order Siluriformes, family Siluridae; *Mastacembelus armatus* (Lacepéde, 1800), belonging to order Synbranchiformes, family Mastacembelidae; *Corica laciniata* from order Clupeiformes, family Clupeidae (see Figure 24). *Kryptopterus hexapterus* (Bleeker, 1851) belonging to order Siluriformes, family Siluridae; *Henicorhynchus lobatus* (Smith, 1945) belongs to order Cypriniformes, family Cyprinidae; *Puntioplites proctozysron* (Bleeker, 1865) belongs to order Cypriniformes, family Cyprinidae; *Thynnichtys thynnoides* (Bleeker, 1852) belonging to order Cypriniformes, family Cyprinidae; *Rasbora myersi* (Brittan, 1954) belonging to order Cypriniformes, family Cyprinidae; *Hemibagrus nemurus* (Valenciennes, 1840) belonging to order Siluriformes, family Bagridae; *Osteochilus lini* (Flower, 1935) belonging to order Cypriniformes, family Cyprinidae; *Ompok bimaculatus* (Bloch, 1794) belonging to order Siluriformes, family Siluridae; *Paralaubuca typus* (Bleeker, 1864) belonging to order Cypriniformes, family Cyprinidae; *Mastacembelus armatus* (Lacepède, 1800) belonging to order Synbranchiformes, family Mastacembelidae.

As presented in Figure 24, the most used fish species for traditional smoking in Tonle Sap area were *Clarias macrocephalus*, *Clarias batrachus* belonging to order Siluriformes and *Henicorhynchus caudimaculatus* belonging to order Cypriniformes. This might be caused by taste preferences, resistance of the species to survive harsh environment due to unique ability to breathe atmospheric oxygen and therefore persist in the mud whole dry season (*Clarias macrocephalus*, *Clarias batrachus)* or due to efficient growth rate.

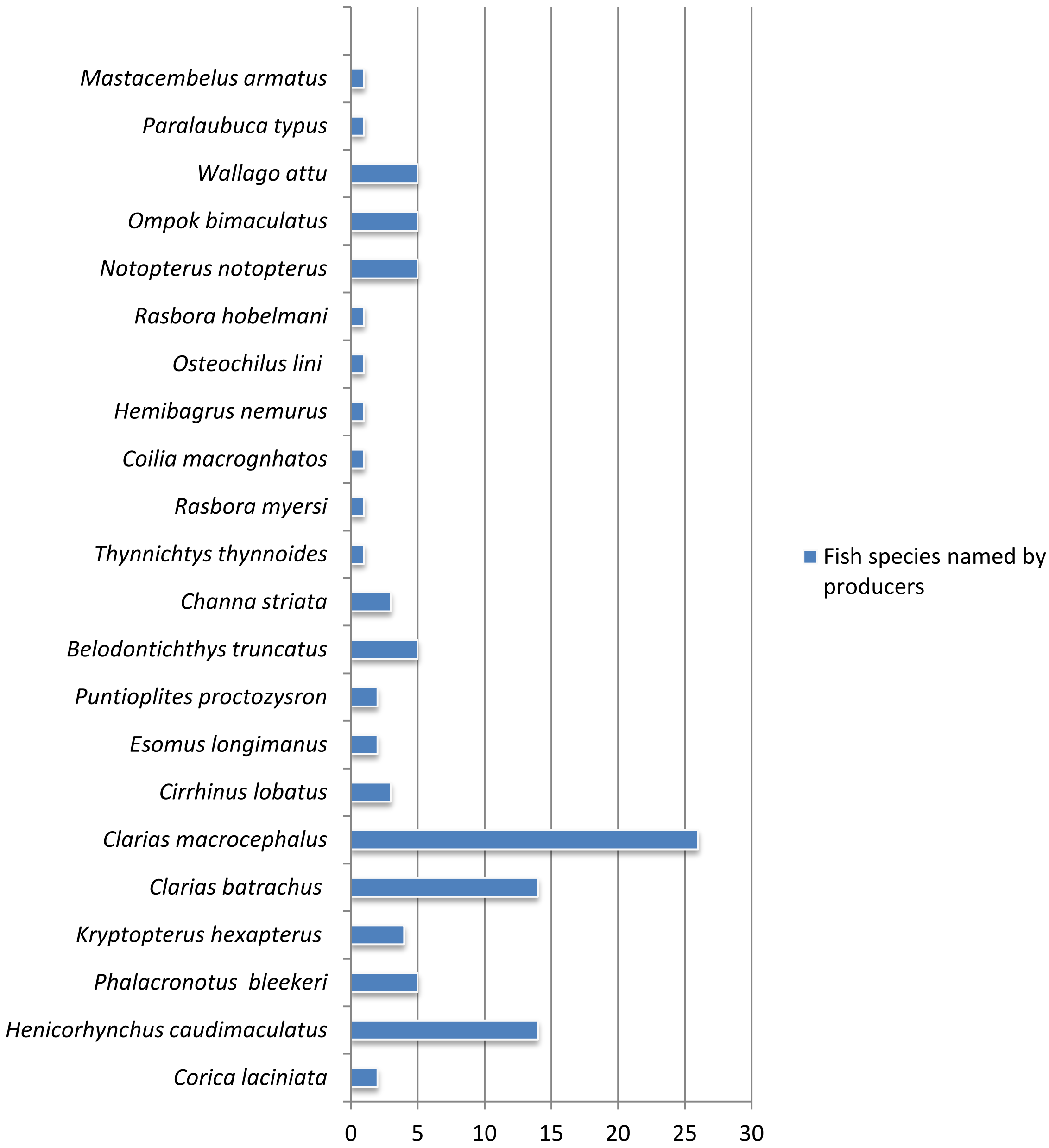

Figure 24 Fish species usually used for smoking in the targeted area (n=23).

Results in Figure 25, highlight diversity of fish species, where the highest was in Kampong Cham province. This could be caused by bigger distance from the source of fish (fishing lots, markets, etc.), higher number of respondents within province or length of production.

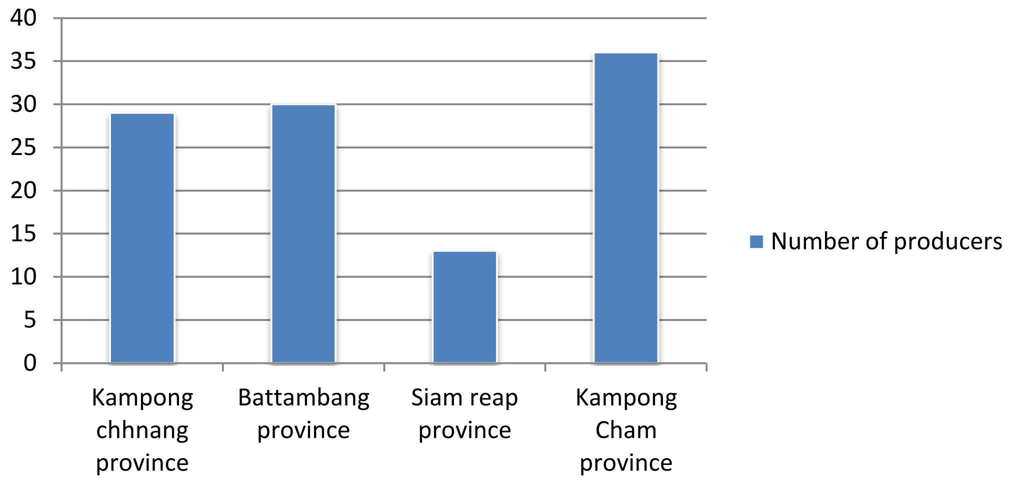

Figure 25 Fish species diversity within provinces, Tonle Sap area.

Results in Figure 26 showed results of fish species diversity within villages which supports results about distribution of fish species diversity within provinces with exception of Kandal village belonging to Kampong Chhnang province with high fish species diversity.

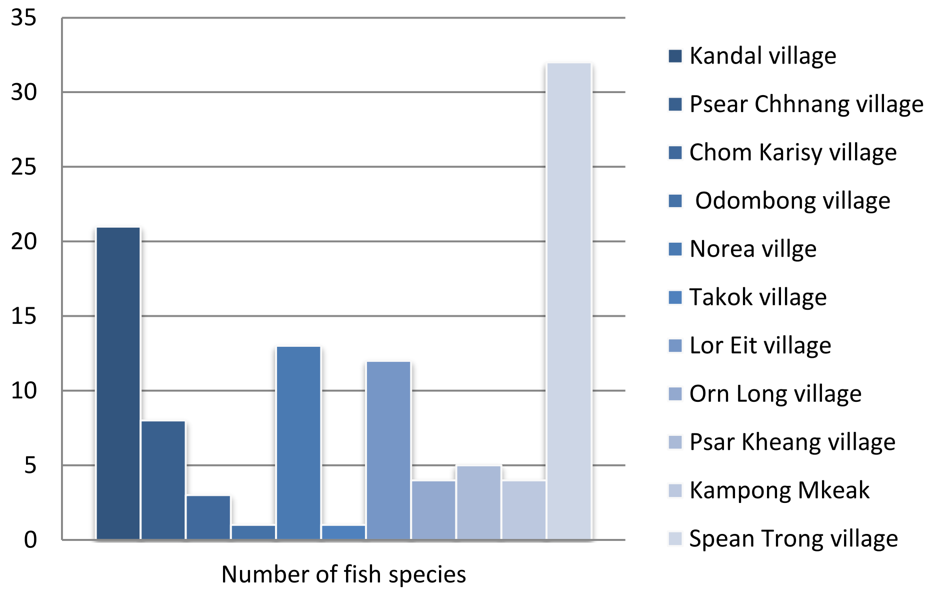

Figure 26 Fish species diversity within villages, Tonle Sap area.

5.1.5 Fish pre-treatment

According to processing, there is a difference within the provinces but moreover within fish species. Due to taste preferences and fish size we can observe different approaches of treatment before smoking.

A typical smoking procedure starts with fish washing and cleaning in fresh water or salted water to remove slime in particular species; *Clarias batrachus* and *Clarias macrocephalus* or *Channa striata*. Subsequently the cleaning consist of removing of guts, which is also a subject of species e.g. *Corica laciniata, Henicorhynchus caudimaculatus, Phalacronotus bleekeri.* Removing of head is used for small-scale fish species, where it is expected to be consumed whole, or due to taste preferences; bitterness of head. This technique is used for example in case of *Esomus longimanus* (see Figure 27 - A). During the field research a different approach was observed in case of *Clarias batrachus* and *Clarias macrocephalus* where head was not removed and its hard shell was used for spiking (see Figure 27 - B). After spiking some of the species, mainly small-scale or the fresh one, where placed on the mats, on the ground and left to sundry for 20-30 minutes. After drying grouped fish were place on smoking trays and placed into a smokehouse (see Figure 27 - C).

Figure 27 Pre-treatments used prior to traditional smoking of fish in Tonle Sap area, Cambodia; A – head and guts removing, B – Spiking on a bamboo sticks, C – placing of grouped fish after sun drying on smoking tray.

Some of the species were turned into half circles before sun drying; this technique was used mainly for better storage of the final product and visual marketing preferences (Figure 28).

Figure 28 Half circles prior to smoking made from fishes, Cambodia.

In Battambang province with strong preference of *Clarias batrachus* and *Clarias macrocephalus* (see Figure 29), a different technique is used. The frontal part of head is cut, guts taken out through the head space, and the fish is spiked with wooden sticks, cleaned with water repeatedly 3 - 4 times before they start to smoke it, to remove the slime.

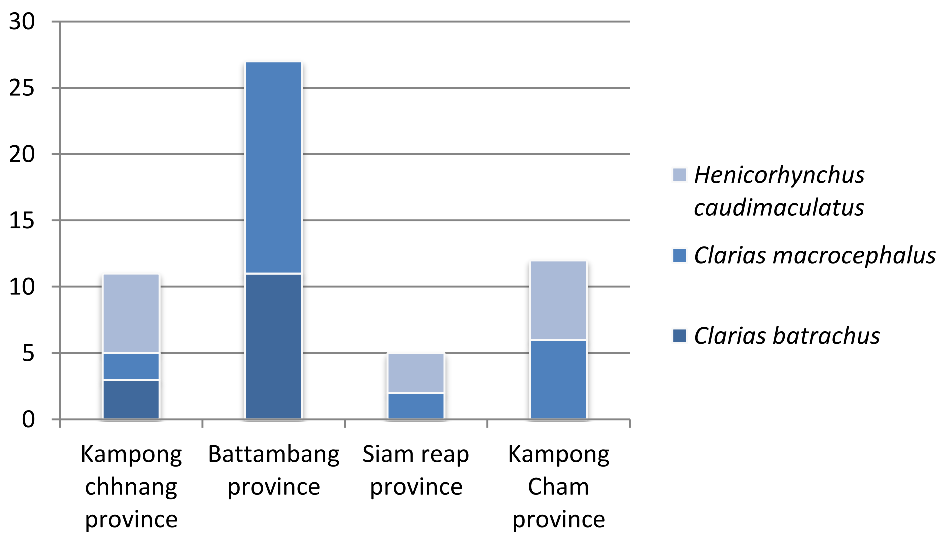

Figure 29 Distribution of three main used fish species within the provinces, Tonle Sap area, Cambodia.

Producers who do smoke *Channa striata* buy only heads, which might be due to higher price of the whole fish, size of the fish that is usually caught or cooking preferences of this particular fish based in traditional Cambodian cuisine. The heads are cut in the middle afterwards cleaned with water, opened and directly smoked on trays grouped of eight to ten (see Figure 30).

Figure 30 Smoked heads of *Channa striata* in Battambang province, Cambodia.

5.1.6 Traditional technique of smoking fish

A typical smoking procedure starts with pre-treatment as described above, after the fish are grouped and pierced together, they are placed on smoking trays and turned regularly. The time of turning ranges from 15 minutes to 3 hours. This varies according to the fish size and number of tray levels. First the grouped fish are placed in "fish scale" order once the fire is prepared, subsequently after period of 15 minutes to 3 hours fish are rotated 180°, then within the levels, if they are present (see Figure 31).

Figure 31 Typical "fish scale" order of grouped fish on smoking trays, Tonle Sap area.

The total smoking time of differs based on fish species and can vary from 1 up to 4 days as presented in Table 3. Producers also use various materials as; paper cardboard, fabrics or metal sheets, to cover fish during the smoking to reduce the smoking time and improve final colour and appearance of the fish product. The length of smoking, presented in Table 3, is considered as long in comparing to traditional European times of smoking (Essumang et al., 2013; Stołyhwo and Sikorski, 2005) this might be attributed to lower knowledge of technology and it results in higher concentration of PAHs in fish smoked products, due to time and additional burnings of artificial components material used for cover contain.

Table 3 Processing and storage of traditional smoking fish in Tonle Sap area.

Fish producer	Time of smoking	Production per day*	Main smoking season	Fuel consumption per day	Storage time	Storage facility
1	1 day	100 kg/day	I - VII	2 m3	0	N/A
2	1 day	300 – 400 kg/day	I - VII	3 m3	overnight	B
3	1 day	100 kg/day	I - VII	3 m3	< 5mth	B
4	1 day	200 - 250 kg/day	I - VII	4 m3	< 5mth	B
5	1 day	200 - 250 kg/day	I - VII	3 m3	< 5mth	B
6	1 day	700 – 800 (up 1000) kg/day	I - VII	2 m3	< 5mth	B
7	3 days	500 kg/day	IX - VI	212	3 days	NO
8	4 days	400 - 500 kg/day	IX - VI	108 kg	< 1mth	SM
9	3 days	500 – 15 000 kg/day	IX - VI	225 kg	< 1mth	SM
10	4 days	5 000 kg/day	IX - VI	160 kg	< 1mth	SM
11	4 days	500 - 1000 kg/day	I - III	90 kg	2-3 days	SM
12	3 days	80 kg/day	II - III	1m3 of wood; 45 kg of charcoal	overnight	SM
13	3 days	70 - 100 kg/day	II - III	1m3 of wood; 45 kg of charcoal	overnight	SM
14	3 days	50 kg/day	I - III	1 m3	2 days	SM
15	2,5 days	50 kg/day	I - III	0,5 m3 /day of wood	1 day	B
16	3 days	50 kg/day	IV	1 m3 / week of wood ; 45 kg/week charcoal	2 days	B
17	3 days	500 - 700 kg/day	III- IV	0,5 m3 / day of wood ; 45kg /day of charcoal	3 days	B
18	3 days	70 kg/day	X -IV	1 m3 / day of wood	2 days	SH
19	3 days	70 kg/day	X -IV	0,33 m3 / day of wood	2 days	SH
20	1 day	200 – 300 kg/day	I - VIII	2 m3 / day of wood	3 days	SH
21	2 days	300 kg/day	I - VIII	0,5 m3 / day of wood	2 days	SH
22	1 day	80 kg/day	X - XI	1 m3 / day of wood	overnight	SH
23	1 day	90 - 100 kg/day	IX - X	1 m3 / day of wood	overnight	SH
24	1 day	50 kg/day	XI - I	1 m3 / day of wood	1 day	SH
25	1 day	80 - 100 kg/day	XIII - IV	0,5 - 1 m3 / day of wood	3 days	B
26	1 day	50 kg/day	XII- IV	1 m3 / day of wood	overnight	SH
27	1 day	100 kg/day	VI - VII	2 m3 / day of wood	0	A
28	1 day	50 - 100 kg/day	VIII - XII	2 m3 / day of wood	0	A
29	2 days	50 - 70 kg/day	VIII - XII	2 m3 / day of wood	0	A
30	1 day	50 kg/day	VIII - XII	2 - 3 m3 / day of wood	0	A
31	1 day	30 - 40 kg/day	VIII - XII	2 - 3 m3 / day of wood	0	A

***** The smoking fish production is continuous however the data presented in the table show the main

The fire is mostly prepared in the fireplace in the form of several small piles from fuel (wood or charcoal), in the net position (Figure 18), to pre-heat the smokehouse. Subsequently after period from 30 minutes to 2 hours, the production of smoke starts by lowering the fire, addition of sawdust or palm husk or by covering. In general, regular flames are needed at the beginning to heat the smokehouse and then the fire is either left to finish till next rotation or kept low to produce smoke. Figure 32 represents proportion of fire appearance used by questioned producers.

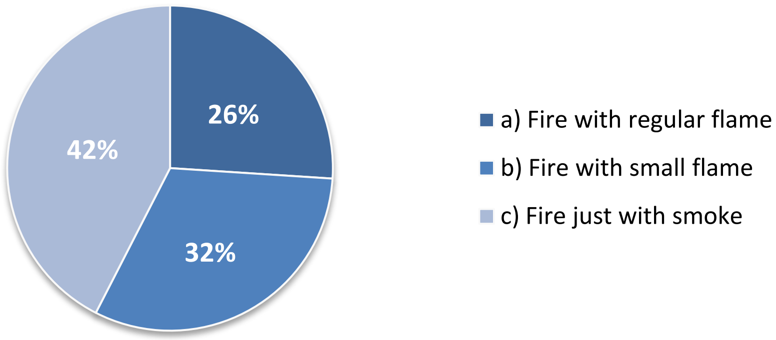

Figure 32 Appearance of the fire during traditional smoking, Tonle Sap area.

Temperature of fire or smoke is not measured. Readiness of smoked fish products by traditional technique is estimated visually by change in colour, weight loss or change in structure or texture due to lack of any temperature or humidity measuring devices. You can see smoked fish products and final traditionally smoked fish product in Figure 33.

Figure 33 Smoked fish products during process – A, final product: grouped smoked fish – B.

From results presented in Table 3 it is clear that high season and production data are not consistent and vary within producers. Producers numbered 1 - 6 belong to Kampong Chhnang province where high season was appointed equally by all interviewees from January to July, however in Siam reap province this high season of production was reported later from August to December. Daily production of smoked fish products per producer has great range from 40 kg per day to 500 – 5000 kg per day (see Table 3). This might be attributed to sample of respondents who belong to either small- or medium-scale smoked fish producers.

5.1.7 Selling practices of the smoke fish producers

Following Figure 34 represents percentage distribution of selling practices within all questioned producers. Over fifty percent of respondents sell their own fish products by themselves on the market (54 %), second largest group sells to customers who came individually to their house (27 %) third group consists of producers who use services of middlemen who buy their products and transport to further markets (19 %). Selling practice is highly conditioned to the distance from production site and financial resources of the producer. In some areas the service of middlemen is free of charge.

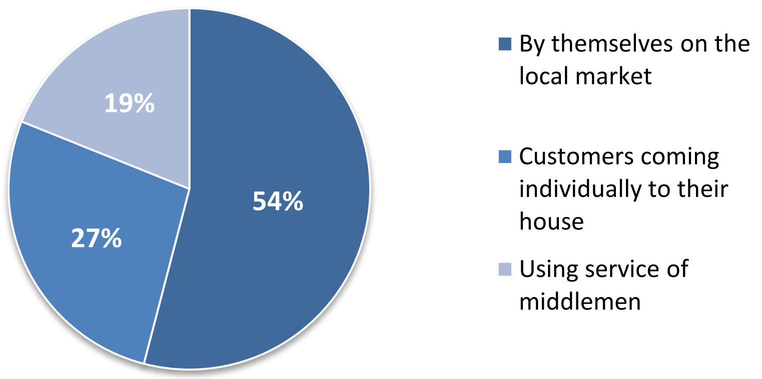

Figure 34 Selling practices in targeted area.

Service of middlemen was mainly used by producers in Battambang province which might be due to higher distances from relevant markets. On contrary, producers in Kampong Cham province were situated in proximity of the local markets and therefore only sell directly on their own (Figure 35).

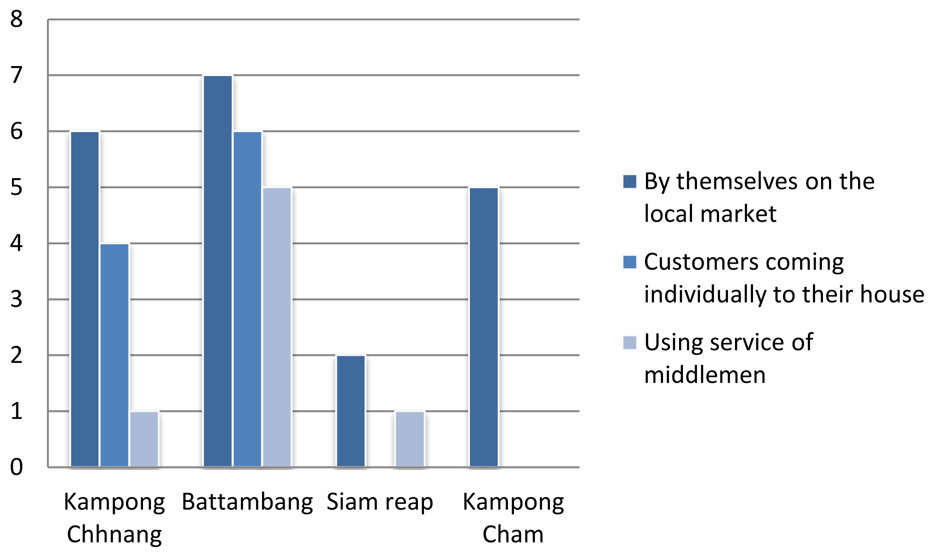

Figure 35 Selling practices within provinces in targeted area.

Most of the producers use cartoon boxes as packaging, in case they use middlemen or for transport to markets, sometimes there is a use of baskets or if the products are sold by producers they tie them on a rope and hang them around the store or at the market (Figure 36).

Figure 36 Examples of selling practice in Tonle Sap area, Cambodia.

Final product is directly sold next day; there is no additional storage time. Longer storage ranges from 2 days to 0.5 – 1 month. Fish products are stored directly on smoking trays, hung in the air or prepared in transported boxes or baskets (Figure 36). This can cause additional contamination of smoked fish products.

5.2 Concentration of PAHs in smoked fish

In general, the amount of the four priority PAHs in all samples (Table 4) highly exceeded maximum limits (ML) given by EC 1881/2006 (Table 1). Currently the limits for smoked fish are 2 and 12 $\mu g.kg^{-1}$ for benzo[a]pyrene (BaP) and sum of 4 PAHs, respectively. Such high amounts of PAHs 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 PAHs in fish is the smoking technique. For example the samples smoked traditionally in kilns have been reported to contain increased levels of BaP (50 $\mu g.kg^{-1}$) compared to fish from smokehouses supplied with conditioned wood smoke from external generators (BaP content 0.1 $\mu g.kg^{-1}$) (Stołyhwo and Sikorski, 2005). According to Šimko (2005) levels of contamination by PAHs could be significantly decreased under controlled conditions, by accepting good manufacturing practice principles or appropriate technological equipment which corresponds with Stołyhwo and Sikorski (2005).

Table 4 Determined values ($\mu g.kg^{-1}$) of four priority PAHs.

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%), ∑ PAHs4 (±15%).

The second important factor is smoking time. The longer the sample is smoked, the higher amount of PAHs can be expected (Essumang et al., 2013; Varlet et al., 2007). Producers included in the research stated only in one case that the samples were smoked for less than 24 hours (Table 5) other ones were usually exposed to smoke for 1 to 2 days and in extreme cases for 6 days. This is considerably longer period compared to usually reported smoking times which ranges between 2 - 12 hours (Bannerman, 2001; Essumang et al., 2013; Stołyhwo and Sikorski, 2005). Furthermore, a smoking temperature together with the distance of the product from the fire has influence on the final PAHs content as well (Ledesma et al., 2016).

Table 5 Smoking procedures reported by producers of sampled smoked fish products.

Fish producer	Time of smoking	Fish rotation frequecy	Fuel used for smoking
1	5 - 10 hours	N/A	Kreak (unspecified mixture of wood); fuelwood: Trosek (Peltophorum dasyrrhachis)
2	1 - 2 days	every 1 hour	Kreak (unspecified mixture of wood); fuelwood: Trosek (Peltophorum dasyrrhachis)
3	1 - 2 days	every 1 hour	Kreak (unspecified mixture of wood); fuelwood: Trosek (*Peltophorum dasyrrhachis*), Deam reang (*Barringtonia acutangula*)
4	1 - 2 days	every 1 hour	Kreak (unspecified mixture of wood); fuelwood: Trosek (*Peltophorum dasyrrhachis*), Deam reang (*Barringtonia acutangula*), Rubber tree (*Hevea brasiliensis*), Ampil (*Tamarindus indica*)
5	5 -6 days	every 1.5 hour	Kreak (unspecified mixture of wood); fuelwood: Deam reang (*Barringtonia acutangula*)
6	2 - 3 days	every 1 hour	Kreak (unspecified mixture of wood); fuelwood: Deam reang (*Barringtonia acutangula*)
7	4 - 5 days	every 1.5 hour	Kreak (unspecified mixture of wood); fuelwood: Deam reang (*Barringtonia acutangula*)
8	2 - 3 days	every 2 hours	Kreak (unspecified mixture of wood); fuelwood: Deam reang (*Barringtonia acutangula*)
9	1 - 3 days	every 1.5 hour	Kreak (unspecified mixture of wood); fuelwood: Deam reang (*Barringtonia acutangula*)
10	1 - 2 days	every 1.5 hour	Fuelwood: Deam reang (*Barringtonia acutangula*)

All producers in the research have used traditional direct and hot smoking method. As it is clearly displayed from the Figure 15, fish were placed directly above the fireplace heated by fuel wood or charcoal. 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 during this field research. Also according to Larsson et al. (1983), smoking over an open flame produces very high concentrations of PAHs compared to charcoal grilling which usually yields small amounts of PAHs. Type of wood used for smoking can also significantly influence PAHs contents in fish (Stumpe-Vīksna et al., 2008). In general, soft wood is not recommended for smoking due to its high contents of resin and lignin (Sun and Cheng, 2002). Because PAHs produced in wood smokes are known to originate from thermal pyrolysis (depolymerisation) of lignin and subsequent condensation of the lignin components in lignocelluloses at temperatures above 300 - 500 °C (Stołyhwo and Sikorski, 2005; Kawamoto et al., 2007; Nakamura et al., 2008; Garcia-Perez, 2008). Dry wood cannot be recommended either due to its higher smoke generation temperature (Guillen et al., 2000). Producers in this research usually reported "Kerak", mixed firewood purchased on local markets to be used for smoking (Table 5). Therefore it was not possible to identify the exact type of wood used in those mixtures. Hence we cannot exclude that some wood types with higher resin or lignin content or lower moisture contents were used. Moreover in one case, 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 the **∑**PAHs 4 was found in sample no.9 and the highest in sample 23. The **∑**PAHs 4 mean concentration ranges from 34.25µg.kg^{-1} to 597.75µg.kg^{-1}. This maximum mean concentration of 597.75µg.kg^{-1} was recorded for *Paralaubuca typus sample* smoked for 5 - 6 days on mixed fire wood and *Barringtonia acutangula.* Thus the ML amount was exceeded from 2 up to 50 times. Similarly the lowest amount of BaP was determined in sample no. 9, the highest in no. 23. Benzo[a]pyrene used as biomarker in monitoring carcinogenic PAHs had recorded mean concentration from 4.58µg.kg^{-1} to 119.45µg.kg^{-1}. The ML for BaP was exceeded from 2 to 60 times. These results are supported by results presented by Essumang et al. (2012) where a significantly high accumulation of PAHs was found in the smoke-cured fish as compared to the non-smoke-cured fish control samples, which showed PAHs levels, below detection. This implies a need to find alternative ways of curing fish other than the traditional smoking technique.

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

It seems that differences in PAHs content are probably caused by combination of two factors: firstly - smoking time; secondly - amount of fat in the fish sample, as it is known that the PAHs are lipophilic in nature and tend to accumulate in fatty tissue (Pensado et al., 2005; Shadi et al., 2012, Bansal and Kim, 2015), thus more fat in the fish, the higher concentration of PAHs from smoke is absorbed. Moreover, pyrolysis of fat in fish with high lipid content significantly contributes to final contents of PAHs in samples (Essumang et al., 2012). However it must be stated that fat content of smoked fish species was not measured during this field research and it would be subjected to investigation in further research.

6 Conclusions

This thesis had objective to monitor a traditional way of smoking fish products in Cambodian rural areas and evaluate amounts of carcinogenic compounds in the traditionally smoked fish products in Cambodia. The smoked fish represents important source of nutrients for Cambodian population, however can also lead to excessive intake of PAHs during traditional smoking of fish. This study was conducted among selected smoked fish producers near the Tonle Sap river in Kampong Chang province and Tonle Sap area, Cambodia and questionnaire survey within 5 provinces (Kampong Chhnang, Battambang, Siam reap, Kampong Cham) in Tonle Sap area, Cambodia. The field research renders that a maximum limit (ML) for a sum of 4 PAHs given by EC 1881/2006 was exceeded form 2 to 50 times and Benzo[a]pyrene used as biomarker in monitoring carcinogenic PAHs 2 to 60 times, with the mean value 34.25μg.kg^{-1} to 597.75μg.kg^{-1} and 4.58μg.kg^{-1} to 119.45μg.kg^{-1}, respectively. Such burden can lead to increased risk of development of carcinogenic diseases and other diseases related to PAHs exposure. To decrease this risk, local population should be educated to use better smoking techniques and improved smoking kilns or at least change of smoking parameters, which would lead to safer processed fish products. Also further investigation and research focused on concentration on the amounts of PAHs per gram of fish consumed or fat content is recommended.

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Appendices

List of appendices

Appendix I Questionnaire for Producers of smoked fish products used during survey in Tonle Sap area, Cambodia.

a)Location of collection of fresh fish (GPS)
b)Do you buy the fish for smoking or do you fish them?
c) Name mainly smoked fish species.
d)Location of fish processing
e)Way of pre-treatment's before smoking of fish

f)How do you sell the smoked fish? 1) By yourself on the local market 2) Customers coming individually to your house 3) To the middleman 4) Directly to some bigger company or supermarket

Technical part of traditional smoking process

What material do you use for smoking (charcoal, wood)?	
If wood what kind?	
For how long do you smoke fish?	
Do you use trays?	
i. If yes, do you change them regularly?	
ii. Do you change trays in some order?	

What is the amount of production per day or per batch?	
In which period of the year is main smoking season?	
How many days or month you smoking fish per year?	
How looks the fire during fish smoking?	**a) Fire with regular flame** **b) Fire with small flame** **c) Fire just with smoke**
Do you measure the right temperature?	
How you estimate or recognize that the fish is ready (already smoked)?	
Do you know how much of fire wood or charcoal you use per day or per batch in kg or in m^3?	
Do you use any additional technique?	

(use of cartoon, covering,…)	
Do you use any packaging of marketed smoked fish?	
For how many days you store your smoked fish before selling usually?	
If you store the fish, where you store them and how?	
Dimensions of smoking kiln - house (in cm)	**Length :** **Width :** **Height:** **Distance of each tray (or fish if no trays) from the fire:** **1. Tray:** **2. Tray:** **3. Tray:** **Etc.** **Dimensions of trays:**

1. Photo of smoking kiln – house 2. Photo of smoked fish (detailed and whole package)	