Studies on poly aromatic hydrocarbons in wood smoke and smoked fish

Mohan I.M. Lokuge[1*], Y. Sulthanbawa[2] and A. Bamunuarachchi[3]

[1]136/1, Borala Road, Weligama, Sri Lanka.
[2]Industrial Technology Institute, Colombo-07, Sri Lanka.
[3]Department of Food Science and Technology, University of Sri Jayewardenepura, Sri Lanka.

Abstract

Fish smoked using wood smoke is popular all over the world although there is evidence to show that wood smoke contains mutagenic and carcinogenic poly aromatic hydrocarbons (PAH). In Sri Lanka, sufficient studies have not been carried out to determine the PAH emission of the existing smoking methods. This study investigates whether the existing smoking methods and wood species used for smoking are safe in relation to the PAHs present. In this study, PAH emissions of five wood species, namely, Mango, Jak, Coconut Shells, Cinnamon and Madan (*Zyzigium*) and the PAH content of fish smoked using two of the above wood species (Cinnamon and Jak) were measured by HPLC. The smoking was carried out using a locally built smoker which simulated the smoking practices in rural areas. Smoke generated by all five wood species contained PAHs in large quantities and high levels were also detectable in the smoked fish. Findings of this study reveal that the local fish smoking practice of directly exposing to flue gas generates large amounts of toxic PAHs and can hence be considered as extremely unsafe in relation to PAH toxicity.

Keywords: Carcinogenic, HPLC, PAH, Smoked fish, Wood Smoking

---

*Corresponding author - Email: mohan.lokuge@gmail.com

Introduction

Food smoking is one of the oldest food technologies used by man for over 10,000 years, even before the dawn of recorded history. Smoking has since been widely used for its special organoleptic properties like taste, flavour and colour due to impregnation of aromatic compounds, as well as for its bacteriostatic and antioxidant properties (Joint FAO/WHO Food Standards Programme 2006). World smoked food (mainly fish) consumption has shown a continuous increase irrespective of cultural and other differences. In Europe, more than 15% of meat and fish is consumed as smoked products (Jonsson, 2003).

Sri Lanka also has a long history of smoking food. People used this method to preserve fish, meat and some other foods such as Jak fruit (*Artocarpus heterophyllus*) breadfruit (from *Artocarpus nobilis* species). Smoking is carried out by exposing the food item to direct smoke by placing it on a wooden rack built over the kitchen hearth ("*Atuwa*" in Sinhalese) or by hanging over burning wood. Fish smoking is carried out mostly in areas around inland water reservoirs and rarely on the coastal belts. The "Vadda" hunters (the tribes native to Sri Lanka) practice smoking as a preserving method for meat.

A number of researchers have found that wood smoke and smoked food contains PAHs, the largest class of chemical compounds that have been identified as highly carcinogenic and mutagenic for human beings. (Stolyhwo and Zdzislaw, 2004; Simko, 2002; Joint FAO/WHO Food Standards Programme 2006; Sonia *et al.*, 2005). Incomplete combustion of organic materials produces PAHs (Kim-Oanh *et al.*, 2005 and Rehwagen *et al.*, 2005) and amounts are directly proportional to the burning temperature and the disposition of PAHs, depending on the velocity of flue gas, molecular weight of the PAH and the stability of the molecule. The pyrolysing temperature has a direct relationship with the production of PAHs (Kim-Oanh *et al.*, 2005). Some PAHs that are found in wood smoke are categorized as not harmful when considered alone, have synergistic effects on carcinogenic and mutagenic PAHs. These compounds enter into our bodies by inhalation, ingestion or penetration and are then distributed to various organs in the body where they interact with aryl hydrocarbon hydroxylase; in this process a dominant role is played by cytochrome P450 (Simko, 2002). The EU Scientific committee on food has identified 15 PAHs (Joint FAO/WHO Food Standards Programme 2006), called priority pollutants (Busetti *et al.*, 2006), as potentially carcinogenic, genotoxic/mutagenic compounds (NIOSH analytical Manual, 1998; Joint FAO/WHO Food Standards Programme 2006; Sonia *et al.*, 2005; the Official journal of the European Union 2005).

The carcinogenicity of PAHs depends on their structure and on the biological mechanisms and pathways they undergo inside the body; hence, various PAHs are toxic to different extents (Jonsson, 2003). PAHs are classified into two types called light (gaseous) and heavy (particulate) based on its molecular masses. The light PAHs are considered to be less carcinogenic but there is no agreement on this among all researchers. Among all those, priority pollutant PAHs, Benzo (a) pyrene, Phenantherene and Anthracene are particularly significant in their toxicity (BGIA, 2006). According to some other researchers B (a) Anthracene, B (b) Fluoranthene, B (a) Pyrene and Chrysene are regarded as potential carcinogens for man (NIOSH analytical manual, 1998). It is believed that PAH toxicity occurs through more than one mechanisms, whereby the toxic action depends on the concentration, exposed time and the particular PAH compound. Among the PAHs with harmful effects, BaP has been chosen as the indicator (Anon, 2001) component (marker) for smoked foods, with the maximum permissible level recommended by EU for BaP being 0.03 ìg/kg. It should noted, however, that the contribution of BaP to the total PAHs in smoked products lies within a range of 1 to 20%. The toxicity of other PAHs should also be considered to obtain a complete picture of the threat of toxicity. The European Union (Official journal of the European Union 2005) has set a limit of 5 ug/kg (wet weight basis) for muscle meat of smoked fish and fishery products, excluding bivalve molluscs, and for smoked meat and meat products.

A large number of factors are known to affect the PAH content in wood smoke. These include the method of Smoking, Wood Source, Temperature, Moisture content of wood, Type and structure of Smoker, Smoking duration, Rate of Air Flow and the Density of smoke. (Joint FAO/WHO Food Standards Programme 2006 and Kim- Oanh *et al.*, 2005). Since all PAHs present in the wood smoke are highly hydrophobic (Busetti *et al.*, 2006) in nature (e.g. the solubility of BaP in water is 1.5×10-8 mol/dm^3), they accumulate in the food material preferentially in the lipid fraction (Stolyhwo and Zdzislaw, 2004). The method used for the determination of PAH in wood smoke and smoked fish, therefore, has the following three basic steps (Anon, 2001); these are, (1) Extraction and isolation of PAHs from the sample matrix, (2) Clean up of impurities and fractionation into sub-groups, (3) Identification and quantitative determination. Since PAHs with lower molecular weights exist primarily in the gaseous phase (Rehwagen *et al.*, 2005), it is necessary to combine a particulate filter (usually a glass fiber filter) with an adsorbent cartridge (usually XAD-2 resin or polyurethane foam) for collection of PAHs. Since a few PAHs are known to be susceptible to oxidation by ozone and other oxidants present in air during the collection process, it is necessary to apply measures to eliminate such destructive factors. Extraction of PAHs from sample matrices was carried out mainly

by soxlet-extraction, Ultra sound-sonication, and partitioning with a suitable solvent or a solvent mixture.

The objectives of the present study were to determine the PAH content of wood smoke and smoked fish in Sri Lanka by fabricating a low cost smoker that simulates the traditional fish smoking practice using wood for generating smoke, screening the smoke from commonly used wood species for their content of PAHs and by determining the PAH content and accumulation in smoked fish under existing smoking practices in Sri Lanka.

Materials and Methods

A low-cost wood smoker was fabricated for the purpose of smoke trapping and fish smoking. It consisted of two units, namely, a smoke generator and a smoking chamber, connected to each other through a horizontal l metal pipe which was fixed on one end to the smoke generator and on the other side to the bottom of the chamber. The connecting pipe was flexible allowing the adjustment to the distance between the two chambers (Fig. 1).

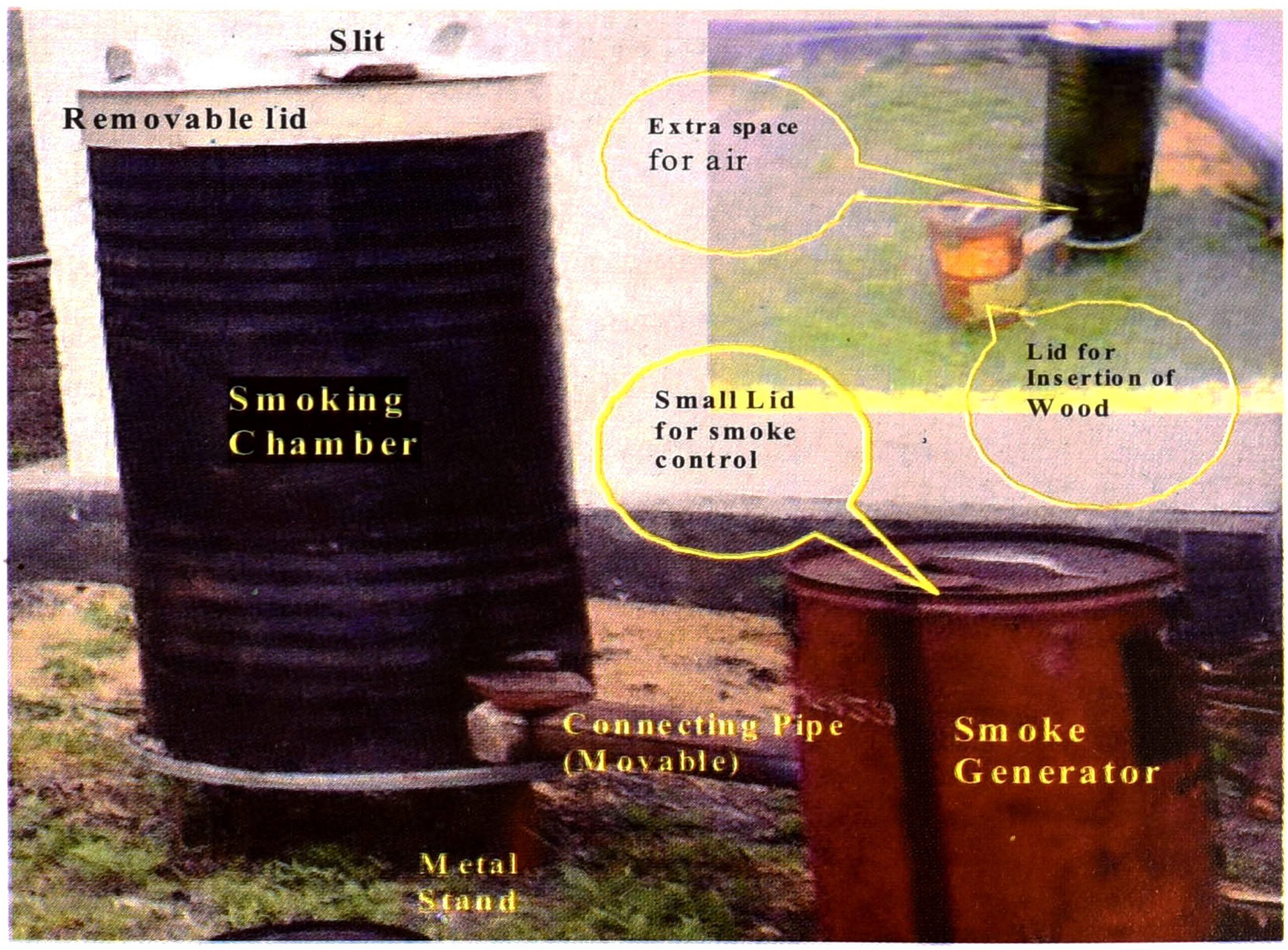


Fig.1. Low Cost Smoker used for PAH trapping.

The smoking chamber was placed on a metal platform and the top of the chamber closed with a removable lid which contained an adjustable aperture. This aperture and the distance between two barrels were used to adjust the smoke flow rate and temperature, respectively. The fish were stacked inside the smoking chamber on a removable metal rack with openings that allowed free circulation of smoke. The woods were selected on the basis of the abundance, popularity, and also represented five different plant families.

PAHs in the wood smoke - both in vapour and aerosol - were collected onto to glass fiber filters and sorbent tubes, respectively, using sampling pump. The PAHs collected in this manner were subjected to an extraction procedure and estimated by High Performance Liquid Chromatography, using UV and fluorescence detectors simultaneously.

Sampling of smoke

Equipment and Materials: Glass fiber filter papers (0.45 ìm, 25 mm), Cellulose filter papers, Sorbent tubes with washed XAD-2 resin (front 100 mg, back 50 mg) Personnel sampling pump with max flow rate 2 l/min, Air Flow meter, PVC tubing, Aluminium foils, Plastic funnels, Culture tubes with PTFE lids.

***Smoke Sampling Method*:**

Sampling pump, sorbent tube and filter paper with cassette were connected using PVC tubing at the shortest possible distance (sequence for assembling was Filter Paper, Sorbent Tube, Flow Meter, and Sampling Pump respectively). After adjusting flow rate to a suitable value, the flow meter was removed from the assembly. Samples of smoke were collected separately for each of the selected wood species. The funnel was fixed with the cellulose filter paper inserted into the smoke chamber through the slit on the top and placed just above the sample rack. When sufficient smoke started to generate, (after measuring the temperature) the pump was switched on allowing the smoke to percolate through the filter papers and sorbent tube. Throughout the period of sampling, temperature of smoke at the collecting point was maintained below 60 ^{0}C by adjusting the distance between smoker and smoke generator as well as by sprinkling water on the burning wood. As soon as the collecting process was completed, the filter paper was removed from the cassette, folded and transferred to a culture tube with a PTFE lid .These samples wrapped in aluminium foil were stored at 0 °C. The Sorbent were inserted into culture tubes, covered with aluminium foil and stored at 0 °C.

Smoking of Fish

Materials: Tilapia fillets, individual wood species dried and cut into small pieces, Wooden dishes (String hopper dishes).

Smoking Method: The Tilapia (*Tilapia mosambica* sps:) fillets were smoked by placing them on the dishes inside the smoker on the top-most shelf; marinating was not done to avoid interferences to analysis. Temperature was maintained below 60^0C throughout the smoking process to avoid cooking. The smoking was continued until the colour of fish turned into a golden yellow colour. After several trials, it was found that smoking of fish samples could be achieved after 3 1/2 hrs. The smoked samples were kept frozen until analyzed.

Extraction and Analysis

Materials: SS EPA 610 PAH standard mix (Supelco) 1×1ml (4S-8743}, Benzo (a) pyrene solution 200 ìg/ml(Supelco),(4-8665), 1×1ml, Naphthalene (analytical grade), Chrysene (analytical grade), Acetonitrile (HPLC grade) obtained from Merk India, Cyclohexane (Analytical grade), Acetone (A/R) Double distilled water, Anhydrous Sodium Sulphate (AR), Column Silica - Merk India, Glass wool, Alu-foils, Ethanol (Analytical Grade), Potassium Hydroxide (AR), Altec Florisil cartridges (Phase: Florisil, End Capped, Particle size 75-150 ìm, Pore size; 85A^0). Ultrasonic Bath, Rotary evaporator, Centrifuge, Heating Mantles, Air circulating Oven, Weighing Balance(Analytical), Electric Blender, Refrigerators (-10^{0}C and standard type), 50 ml Burettes, Water condensers and PVC tubing,10 ml, 25 ml volumetric flasks, Amber coloured glass vials with PTFE lids (10 ml, 1 ml), Round bottom flasks and connectors (50 ml and 25 ml), Separating Funnels.

Extraction Method for Smoke

Ten ml of cyclohexane was added to the culture tubes containing smoke trapped glass fiber filters and to culture tubes containing the glass pieces from the sorbent tubes. The tubes were sonicated for about 1hour in an ultra sound bath and centrifuged. The extracts of filter papers and sorbent tubes were mixed because light and heavy PAHs not determined separately. The extraction was carried out twice and all the extracts collected to get the final solution. Anhydrous Sodium sulphate was added to the solvent extracts and after shaking, the solution was transferred to a round bottom flask; the anhydrous sodium sulphate residue was rinsed with 5 ml of cyclohexane and added to the round bottom flask. The extracts were concentrated to about 2 ml and passed through a florisil cartridge (previously activated by passing cyclohexane). The cartridge was further washed with 2 ml of cyclohexane and this was also added to the sample solution. This

solution was concentrated to near dryness using a Rotary evaporator and solvent exchanged into Acetonitrile. This solution diluted suitably was used for HPLC analysis. A sorbent tube and a glass fiber filter paper obtained without exposing to wood smoke and extracted as above for wood smoke sampling to be used as blank sample.

Extraction Method for Smoked Fish

Smoked fish samples were blended using an electric blender and weighed portions saponified with ethanolic KOH for 2 hours. This solution was allowed to cool and was transferred into a separating funnel and extracted with cyclohexane 50 ml in three steps (as 20 ml, 20 ml and 10 ml respectively). This solution was further concentrated to about 4 ml and passed through previously activated set of columns consisted of purified silica and fluorisil cartridge. This extract was then concentrated to near dryness and solvent exchanged into acetonitrile. This final solution suitably diluted with acetonitrile was used for HPLC analysis. Non-smoked fish (same species and equivalent weight) sample saponified and extracted using same procedures were used as for sample blanks.

Standard solutions preparation

PAH standard (EPA 610) was used for the PAH determinations while other PAH standards were used for spiking and identification purposes. Four standard solutions were prepared by diluting the PAH stock solution. The Stock solution and working standards stored in a (-10^{0}C) refrigerator, during the period of analysis.

Validation of methods by determining recovery of added PAH

Non-smoked filter paper fortified with PAH standard solution by adding 20 il of stock solution through a syringe. Allowed to dry in the dark for half an hour and stored in a culture tube overnight and this was extracted using the same analytical procedure used for the smoke collected filter papers. The final solution of extract adjusted to 10 ml and recovery percentage determined by comparing with the standard solutions.

Limit of Detections (LOD):

Generally this defines as the signal to Noise ratio, which is at least to be 3:1 (maximum possible signal and minimum possible noise) (Busetti *et al.*, 2006). The minimum concentration (Standard solution-01) used here provided sufficient LOD for many of PAHs among 16 PAH standard solution and all the samples were prepared in such a manner that their concentrations were higher than LOD (Michael Nemcik, Thermo electron corporation n.d.).

HPLC Analysis

Thermo Finnigan Spectra HPLC System (USA) controlled by a computer was used for analysis, which consisted of following principal devices, Photo Diode Array Detector (UV6000LPC). Programmable Fluorescence Detector, Auto Sampler, Temperature controllable column oven, Pump System with gradient capability, online degassing Unit. The HPLC column used was and Altima HP, C18, 5 ìm, 250 mm×4.6 mm ID. The mobile phase was a mixture of Acetonitrile with water .The ratio of the two changed through a gradient change program. The flow rate was maintained at 0.95 ml /minute and the injection volume was 10 ìl (through auto sampler); the temperature of the analysis was Ambient (23-24 ^{0}C). Detection was carried out using UV absorbance 254 nm, 230 nm and 270 nm (Simultaneous) and Fluorescence detector used with a wavelength program.

The standard and blank chromatograms were overlaid and it was found that there were no significant interferences from the blanks.

Table 1. Wave length (λ) program for FLD.

Time /min	0.00	20.50	32.0	50.0-55.0
λ / nm (Ex; & Emi)	220;326	244,360	254,420	250,496
PAH Detection	Naphthalene	Phenan	Chrysene, BaP, BbF, BkF	I(1,2,3-cd) Py

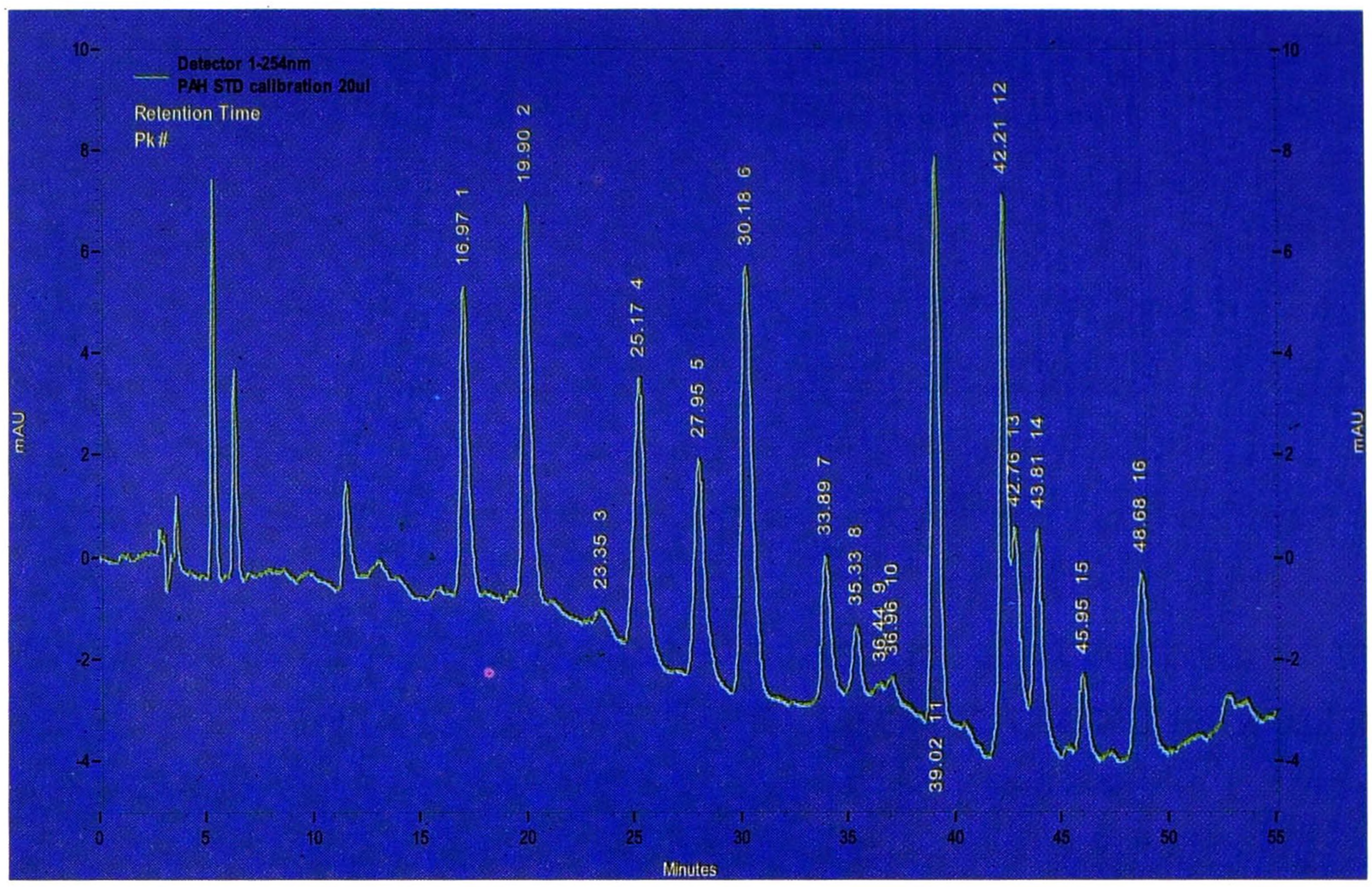
Results and Discussion

Detection of PAH: While the UV Detector (254 nm) could detect all 16 PAHs in the standard mixture, the fluorescence detector (FlD) could resolve 12 PAHs, namely Naphthalene, Fluorene, Phenantherene, Anthracene, Fluoranthene, Pyrene, Benzo(k)F, Benzo(b)F, B(a)P, Dibenso(a,h)A, B(ghi)P, Indenol(123-cd)pyrene, but not Acenaphthene, Acenaphthylene, B(a)anthracene and Chrysene (Fig. 2). Among the non-detected PAHs Acenaphthylene has been shown to be not detectable by FlD due to lack of fluorescence activity of this molecule. Other three PAHs may not have detectable due to the limitations in the FlD and the wavelength used by the program. This can be further explained as FlD is greater in its sensitivity, using single wavelength FlD cannot obtain broader spectra. This problem could not be resolved because of the narrow time gap between the emergence of the PAHs and the slight changes in the retention times by changing the FlD wavelengths, could affect the detection of the more important PAHs. The conditions and parameters used were sufficient, however, to determine the most important Bap (Kim- Oanh, *et al.*, 2005 and Department of Environment and Conservastion n.d.) and other potentially genotoxic, mutagenic and carcinogenic PAHs effectively.

The results (Table 2) showed that all the wood species studied, contained light PAHs in large quantities and significant amounts of the heavy PAHs, all of which far exceeded the limiting levels. BaP was detected, however, in only some of the wood species and the content varied with the smoking time and other non-controllable parameters. Among the light PAHs detected naphthalene, acenaphthylene and Phenanthrene were prominent. This can be accepted because there was no way of controlling the wood burning temperature and the effect of other critical parameters (like humidity and wind speed) on the formation of PAHs. Because of the low molecular weights, light PAHs can pass with wood smoke for longer distances and get deposited easily. In the extracting process, the low hydrophobic nature (Jonsson, 2003) and the cavitation effect of the extracting solvent towards light PAHs, it can be expected to extract with greater efficiency, when sonication is applied (Ping Sun *et al.*, 2006). Among the five woods studied, Naphthalene contents were much higher in smoke produced from cinnamon (3118 ig/liter).This wood is well known for consisting of a large number of volatile organic materials and the burning of these compounds may have generated the lighter PAHs in large quantities. Jak wood (1075 ì g/liter) and Zyzigium (968 ì g/liter) also contained significantly high amounts of naphthalene.

Table 2. PAH content of wood smoke and smoked fish samples (N.D: Not detected).

JACK Smoked fish µg/100g	Cinnamon Smoked Fish µg/100g	Jack Wood µg/l	Mango µg/l	Coconut Shell µg/l	Cinnamon µg/l	Madan µg/l	SAMPLE / PAH
27236.73	397345.49	1074.55	254.28	36.89	3117.65	967.51	**NAPH**
220.93	7106898.21	8416.84	N.D	19.35	114.80	80.85	**ACY**
N.D	N.D	N.D	N.D	N.D	N.D	N.D	**ACE**
74787.15	85126.08	303.75	34.66	1.49	5.00	10.14	**FLU**
23957.60	66164.53	66.99	N.D	3.69	132.47	89.47	**PHE**
2180.63	19463.39	20.76	97.67	73.70	36.72	84.19	**ANT**
204930.60	183499.16	4.77	12.14	16.29	10.23	5.96	**FTH**
3148765.39	3148765.39	57.34	49.70	26.10	11.57	79.06	**PYR**
N.D	N.D	61.12	24.79	4.96	32.60	47.65	**BaA**
N.D	N.D	N.D	N.D	N.D	N.D	N.D	**CHRY**
2405.40	N.D	9.04	11.58	27.40	N.D	68.73	**BbF**
41.94	1126.36	N.D	N.D	N.D	N.D	8.60	**BkF**
38.77	2033.87	1.35	N.D	N.D	0.48	11.77	**BaP**
N.D	N.D	N.D	N.D	3.96	N.D	35.93	**DaA**
183050.38	50297.56	0.00	3598.99	10.78	N.D	84.71	**BghiP**
12406.47	7133.47	25.37	N.D	1.36	N.D	22.93	**Icd-123P**



No	Retention Time	Name
1	16.972	Napt
2	19.898	Acy
3	23.345	Ace
4	25.173	Flu
5	27.953	Phe
6	30.182	Anth
7	33.888	Fluor
8	35.33	Pyrene
9	36.443	B(a)a
10	36.962	Chry
11	39.017	B(b)f
12	42.208	B(K)fl
13	42.763	B(a)p
14	43.81	Di(a)a
15	45.95	B(g,h,i)p
16	48.68	In(123-cd)p

Fig. 2. Standard Chromatogram for EPA 16 PAHs (at 254nm UV-DAD).

Coconut shell smoke also consisted of many PAHs but the amounts were lower than that of Zyzigium. Most significantly, coconut smoke did not contain BaP under the optimized conditions. Among those five wood types mango wood can be considered the safest for smoking purposes under the optimized conditions because it was free from most of the harmful PAHs. Jak wood smoke contained higher amounts of light PAHs and also the heavy PAHs (even under the optimized conditions). This higher PAH content in Jak smoke might have resulted due to the higher content of resinous compounds present in this wood. According to the results, mango, cinnamon and coconut shell smoke were lower in heavy PAHS. The Jak wood and Zyzigium wood smoke contains significant amount of heavy PAHs.

Smoked fish from Jak wood and cinnamon contained significant amounts of harmful PAHs where the levels detected were far higher than the maximum levels permitted in the EU and other countries (Sonia *et al.*, 2005). The BaP contents of Cinnamon and Jak smoked fish were,2034 ìg/100g and 39 ìg/100g respectively and these are much higher than the globally accepted limits. Besides this, both smoked fish samples contained other harmful PAHs also in greater quantities. It is likely that these woods contain large amount of resinous organic compounds and incomplete combustion may produce these high quantities of PAHs. This aspect, however, needs further study and there is a need to compare with the smoked products from other wood species before reaching any conclusions.

It is not possible to establish a direct relationship between the PAH content of the smoke from different wood species and the PAH content of the smoked fish. This is because the volumes and density of the smoke varies considerably over the relatively long period over which the fish is subjected to the process. The higher PAH content of smoked fish – when compared to the smoke – is expected because the fish samples accumulate them during the process of smoking. Other studies have also found that the direct exposure to smoke in fish products brings about higher concentrations of PAHs (Joint FAO/WHO Food Standards Programme, 2006 and Simko, 2002). The lightest PAHs, namely, Naphthalene, Acenaphthelene and Anthracene found in relatively large quantities in both wood smoke and in smoked fish. Smoked fish contained heavy and light PAHs in very large quantities compared to PAHs found in the respective wood smoke. The results obtained in this study, tallies with the work done by other researchers in the same field (Kim-Oanh *et al.*, 2005).

The co-complexity of fish texture, moisture, and fat content and surface area will also affect the retention of PAH, unlike with smoke trapping filter papers. In addition smoking of fish carried out for a long periods (several hours continuously) whereas PAH collecting from smoke was done only for about 30 to 60 minutes and the possible variations of PAH deposition with time was not analyzed under this study. It is also possible that the PAH deposition rate increases with time because of textural changes that occur in the fish due to heat and temperature effects. When smoking is continued for a period of time, the fat present in fish will melt and the hydrophobic PAHs can easily be trapped in the molten fat under these conditions. The factors that govern the quality and composition of smoke can very significantly in smoking for long periods. These variations cannot be captured by just sampling smoke for a short period of time where the critical parameters affecting the smoking were not controlled. For these reasons, the PAH deposition on smoked fish will not be proportionate to the PAHs contents found on wood smoke even when using the same wood species. These heavy PAHs tend to form larger particles, which are less capable of moving with flue gas effectively under low temperatures and hence the temperature was controlled by increasing the distance between two units suitably, but the heavy PAHs found on the wood smoke were significantly higher than the accepted limits. Among five wood species used for the study, Mango and Coconut shell smoke showed lower PAHs compared to other three woods. B(a) P content was higher in *Zyzigium* (Madan in Sinhalese) while Mango and Jak wood smoke contained lesser amounts. In this study coconut husk and other parts were not considered because they are not commonly used for fish smoking and also due to the previous research work where they have reported that BaP content of coconut husks were significantly higher (Rodrigo *et al.*, 1999).

Conclusion

In this study, an attempt was made to modify the smoke generation and application in order to produce smoked fish free from PAHs. The results show, however, that PAHs were produced in harmful quantities. Generally, in village level smoking, fish was exposed to direct flue gas (smoke) for several days in order to produce taste, flavour and also for the removal of moisture. According to our results, it could be stated that such products can be considered as extremely unsafe as PAH levels are very high. It should be noted here that international limits for smoked fish are based on sophisticated smoke houses, where many parameters that affect the generation of PAHs can be controlled effectively and therefore these smoker chambers can produce smoked fish with PAH levels far below the harmful range (about 0.1 ug/kg BaP). The direct exposure method has many limitations (Joint FAO/WHO Food Standards Programme, 2006) and it is not possible to reduce PAH emission or the deposition on fish during the smoking by using such simple methods. Smoke cannot be successfully filtered simply by using filter cloths or any such materials to trap PAHs, because smoke contains sticky materials such as tars, resins and fly ash particles, which can block the filtering media and reduce the efficiency or sometimes may stop the process by blocking the smoke passage. The other major obstacle is that the application of extreme conditions to remove PAHs may also remove the flavouring constituents in the wood smoke so that the whole process becomes meaningless.

A Smoking Method (indirect) for reducing exposure to PAHs

We wish to suggest an alternative low cost method to obtain PAH free smoking in which a heating method, including fuel is used to heat the wood. The wood expected to be used for smoking must be heated to pyrolizing temperature to obtain smoke rich in the chemicals that provide flavour and taste. The advantages in this method include more control over the heating process, and that uniform and clean smoke, free of ash and sticky matter free, can be provided throughout the period of smoking. The most prominent advantage is that the smoke can be filtered to remove PAHs easily because the tannins and tar content is minimal and hence there will no problem of blockages in the filtering media. Smoke can be filtered using filter cloths and activated charcoal to eliminate PAHs (Zhou *et al.*, 2005) from the smoke passing through the unit (Fig. 3). This suggested method also has some indirect benefits when compared to local smoking methods. Traditional smoking methods not only produce un-healthy products but releases large amount of PAH compounds to the environment. When using the proposed, the fire-source can be adjusted to complete combustion where the major emissions are only Carbon Dioxide and Water.

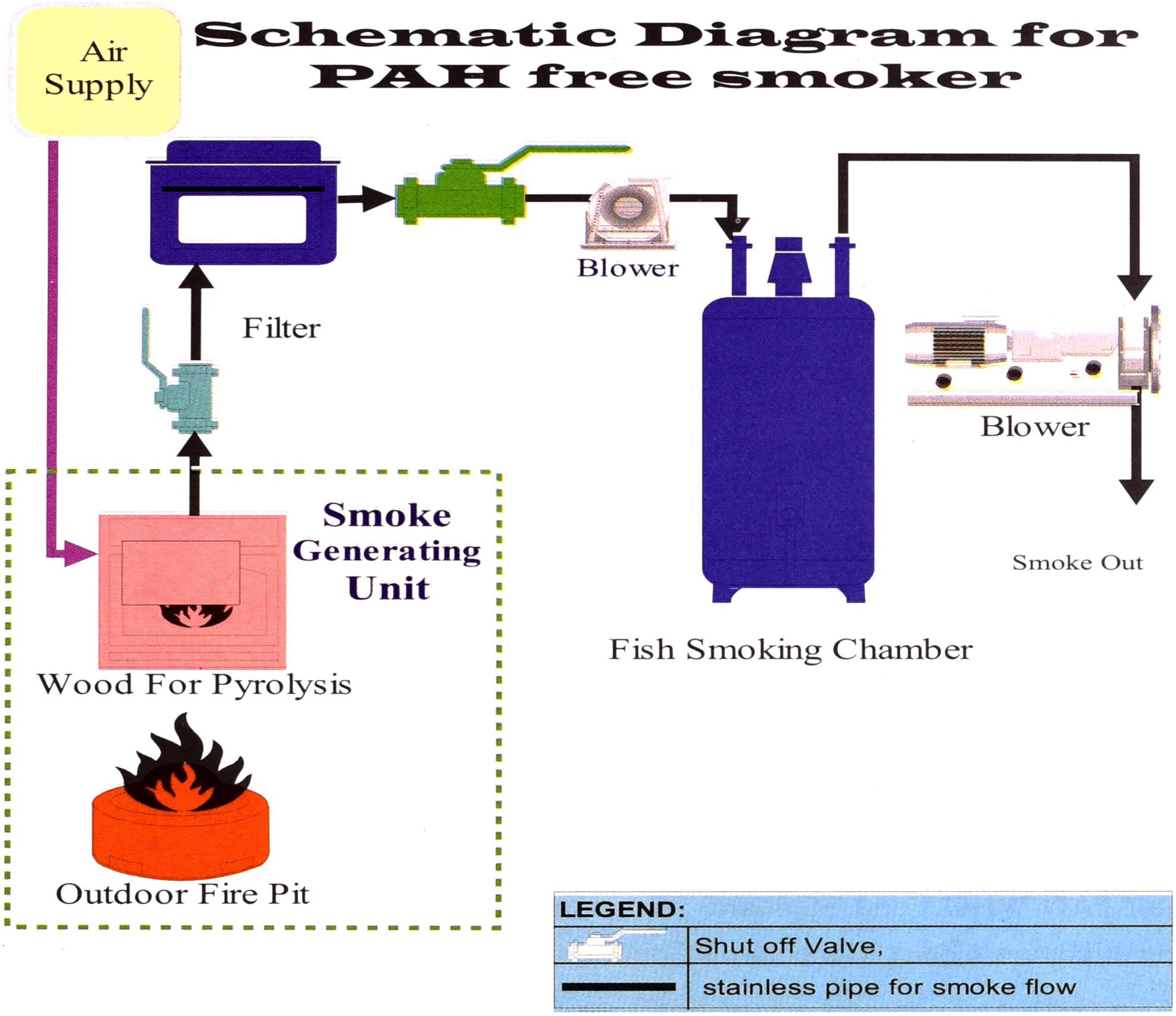


Fig. 3. Suggested Smoker for low PAH emission.

Acknowledgement

The authors acknowledge and thank Dr. K.K.D.S. Ranaweera, Head of Department and all staff members at the Department of Food Science and Technology, University of Sri Jayewardenepura for providing resources, suggestions and valuable comments and Dr. Mahanama and all staff members at the Department of Chemistry, University of Colombo for providing an opportunity to use the HPLC instrument and necessary resources and the Industrial Technology Institute, Sri Lanka for providing financial assistance and its resources for the research.

References

Anon. (2001) "Ambient Air Pollution by Polycyclic aromatic Hydrocarbons; official publications, EuropeanUnion.http://europa.eu.int/comm/environment/pubs/home.htm

Anon. Ambient air quality research project. Internal Working Paper 3 (2001), Department of Environment and Conservastion, (NSW); www.environment.nsw.gov.au.

Application Note 95; Poly Aromatic Hydrocarbon determination by reverse phase highperformance Chromatography; Dionex Limited; http://www.dionex.com (accessed February 11, 2007).

BGIA - Institute for Occupational Safety and Health (2006). Round Robin test for polycyclic Aromatic Hydrocarbons. http:www.kantonslabor-bs.ch/e-infos_berichte.cfm/ labor (accessed February 16, 2006).

Busetti, F., Heitz, A., Cuomo, M., Badoer, S. and Traverso, P. (2006). Determination of sixteen polycyclic aromatic hydrocarbons in aqueous and solid samples from an Italian wastewater treatment plant. *Journal of Chromatography* **1102**: 104–115.

Chromatography database. (2004). Altec associations Inc. http:// www.chromatography.com (accessed February, 12, 2006).

Joint FAO/WHO Food Standards Programme. (2006). Discussion Paper on PAH contamination, Codex Alimentarius Commission, Rome, Italy,

Jonsson, G. (2003). Determination of PAH metabolites in fish bile. Department of Chemistry, University of Bergen, Norway.

Kim- Oanh, N.T., Albina, D.O., Li, P. and Xiaoke, W. (2005). Emission of particulate matter and polycyclic aromatic hydrocarbons from selected cookstove –fuel systems in Asia. *Journal of Biomass & Bioenergy* **28**: 579-590.

Measurement Of Polycyclic Aromatic Hydrocarbons In Cigarette Smoke. http://www. phenomenex.com (accessed February 22, 2006).

Nemcik, M. "Low level PAH analysis using the finnigan Surveyor HPLC system with PDA detection. Thermo electron corporation." http://www.thermo.com. (accessed February 7, 2006).

NIOSH Analytical Manual. (1998). Vol. 03. Analytical Method 5506; National Institute of Occupational Safety and Health, USA.

Official journal of the European Union. (2005). Commission Regulation (EC) No 208/ 2005;http://eur-ex.europa.eu/LexUriServ/Lex Uri Serv.do?uri=OJ:L:2005:034:0043:0045: EN:PDF

Rehwagen, M., Mqller, A., Massolo, L., Olf, H. T. and Ronco, A. (2005). Polycyclic aromatic hydrocarbons associated with particles in ambient air from urban and industrial areas. *Science of the total Environment* **348**: 199-210.

Rodrigo, M.C.P., Samarajeewa, U. and Wijerathne, M.C.P. (1999). Deposition of Polycyclic Aromatic Hydrocarbons from Different Coconut Based Fuels in Smoke Curing of Copra. *Tropical Agricultural Research* **11**: 103-109.

Simko, P. (2002). Determination of polycyclic aromatic hydrocarbons in smoked meat products and smoked flavouring additives. *Journal of Chromatography* **770**: 3-18.

Sonia, M., Garcýa, F., Beatriz, C.G. and Jesus, S. (2005). Minimal clean-up and rapid determination of poly cyclic aromatic Hydrocarbons in instant coffee. *Journal of Food Chemistry* **90**: 643-647.

Stolyhwo, A.E. and Zdzislaw, S. (2004). Polycyclic aromatic hydrocarbons in smoked fish- a critical review. *Journal of Food Chemistry* **91**: 303-311.

Trabajo, MTA/MA/-039/A00. Determination of polynuclar aromatic hydrocarbons in air filter and sorbent tube method/ HPLC. Instituto nacional de Seguridal e Higiene en el.Baracaldo; Spain.

Zhou, H., Zhao-Ping, Z., Bao-Sheng, J. and Rui Xiao, Y.J.H. (2005). Experimental study on the removal of PAHs using in-duct activated carbon injection. *Journal of Chemosphere* **59**: 861-869.

Guidelines to authors

1. Introduction

Journal of the National Aquatic Resources Research and Development Agency is the official journal of the National Aquatic Resources Research and Development Agency (NARA). This journal is a continuation of the publication of the "Bulletin of the Fisheries Research Station, Sri Lanka", which was first published in 1950 under the Ministry of Fisheries. All articles published in the Journal will be peer reviewed by at least two experts in the relevant field.

2. Scope of the Journal

Manuscripts submitted as papers reporting results of original research, review articles or short communications, brief technical notes, news & announcements, pertaining either directly or indirectly to living & non living aquatic resources their utilization and management will be considered for publication in the journal. This covers a wide range of aspects including marine and inland fisheries, marine biology, post harvest technology, aquaculture, aquatic plants, ornamental fish, wetlands, conservation and management of aquatic environment, oceanography, hydrography, socioeconomic and marketing aspects related to fisheries and aquatic resources.

3. Frequency of publication

Journal of the National Aquatic Resources Research and Development Agency is a bi annual publication with two issues per volume published in months of June and December each year.

4. Submission of manuscripts

The manuscripts should be submitted in triplicate, including illustrations, on or before 15th February and 15th August of each year to reach the Editor-in-Chief:

The Editor-in-Chief,
NARA Journal,
NARA,
Crow Island,
Colombo 15, Sri Lanka
Email: editor_journal@nara.ac.lk

5. Preparation of manuscripts

The Journal will accept manuscripts in soft copy form or electronic form in MS Word format together with three hard copies. A translation of the abstract to one of the national languages (Sinhala or Tamil) should accompany the manuscript. Submission of the translated copy of the entire manuscript is encouraged and an honorarium payment for translation will be considered.

Presentation of Script

- Manuscripts should be free of grammatical and typographical errors
- Typewritten on one side of A4 size paper, leaving at least 2.5 cm margins on right and left side, and 4.0 cm at the top and bottom of the page.
- Use Font size 12 (Times New Roman).
- Double space the manuscript throughout, including references, Tables and Figure legends.
- All pages including Tables and Figures should be numbered consecutively in the upper right hand corner.

Length and Style

- The paper should be written clearly and concisely. The style of writing should conform to UK English.
- Slang, jargon, unauthorized abbreviations and abbreviated phrasings should be avoided. In general, the impersonal form should not be used.
- Research papers must not exceed 20 manuscript pages including Abstract, Text, Tables and Figures.
- Short communications must not exceed 8 manuscript pages, must have an abstract but may omit the major usual headings of full papers.
- Review articles must not exceed 40 manuscript pages and must have an abstract.

Layout of the manuscript

Manuscripts should generally be organized as follows: Title page, Abstract, Keywords, Introduction, Materials and Methods, Results, Discussion, Acknowledgements and References. (Note: Authors may opt to amalgamate Results and Discussion and Conclusions can be given as a sub-heading under Discussion.)

Title page

Title must be concise & identify the specific subject of the paper. Title page must contain the full title, the affiliation, the full address(es) of the author(s), the name, telephone & fax numbers and E-mail address of the author who will be responsible for correspondence & correction of proof. Where there is more than one author's address, add a superscript following each author's name and corresponding numeral preceding each address.

Abstract

An abstract of not more than 300 words providing a summary of the article should accompany the manuscript. It should outline objectives and methodology together with important results and conclusions. The abstract should not contain any references and it must be able to stand on its own without reference to the main text.

Keywords

Up to five keywords that could be used for indexing should be provided. Words that are used as subject headings in indexing journals are preferred.

Introduction

This should include the specific problem/s addressed, relevant previous work, aim and scope of the study and hypothesis tested.

Materials and Methods

The methods used should be described clearly and in detail to allow repetition. New methods may be described in detail with an indication of their limitations. Established methods can be mentioned with appropriate references. Statistical analyses done should be mentioned where applicable.

Results and Discussion

The results should be presented adequately and logically. Repetition of the same results in figures, tables or text should be avoided.

The discussion should clearly interpret the results and should logically relate new findings to earlier ones. Statements and conclusions not completely supported by data should be avoided. Arguments should be cohesive. Contradictory statements should be avoided.

Acknowledgements

Acknowledgements should be made for contributors of expertise and funds but not for routine help in preparing manuscripts. Assistance for collation / collection of data may also be acknowledged. If a significant part of the research was performed in an institution other than in those indicated by the authors' affiliations given in the title page, this fact should be acknowledged.

Illustrations and Tables

Illustrations should not be inserted in the text of the manuscript but supplied separately. All illustrations are considered as figures, and each graph, drawing or photograph should be numbered in sequence. Figures should be planned to fit the proportions of the printed page size of the journal (12 x 17 cm).They should be professionally prepared & submitted in a form suitable for reproduction (i.e. as camera ready copy or in digital form). The approximate position for each figure/ table should be indicated in the margin of the text. Copyright permission must be obtained for material copied from other publications.

Figures

Figures must be neat line drawings in black Indian ink or computer generated graphics printed with a good quality laser printer. The figure legends should be typed on a separate paper at the end of the manuscript and not included in the text or under the illustration. Legend should be written so that the general meaning of the figure can be understood without reference to the text. Name of the author and title of paper should be given in pencil at the back of each illustration. The caption of each axis of graphs should be clearly parallel to the axis.

Tables

Tables should be numbered and headed with short titles. They should be typed on separate sheets at the end of the manuscript. Symbols and abbreviations in the lettering should correspond accurately with those in the legend and text.

Photographs

Non-mounted, high quality glossy black and white photographs will be accepted. Magnification should be indicated with a scale line on the photograph. The author's name and figure number should be given on the back of each photograph.

Illustrations and photographs in colour are accepted only very exceptionally and inclusion of such material will be at the discretion of the Editorial Board.

References

- All publications cited in the text should be presented in a list of references and the papers should be arranged alphabetically by family name of the first/principal author.
- The list of references must be placed at the end of the manuscript.
- A Paper submitted by three or more authors should be cited as first/principal author *et al*. (year).
- Unpublished data and personal communications should not be listed under references but may be mentioned in the text as (Author, pers. comm). Reference to work 'in press' may be listed under references.

Journal

When journal articles are listed, the order should be;

In each reference, the initials of the author must be given after the surname. The year of publication should follow in parentheses and should be followed by the full title of the referred article. The name journal should be given in full italicised and followed by the volume number in bold type, the issue (part) number in parentheses and then the inclusive pages.

e.g.:

Standard article

Fernando, C.H. and Indrasena, W.M. (1998). The freshwater fisheries in Ceylon. *Bulletin of the Fisheries Research Station* Ceylon **20(1):** 101-134.

Books

When books are listed, the order should be: author/s, year in parenthesis, title of the book, volume number, edition, pagination/ inclusive pages, publisher and place of publication.

e.g.

Books

De Silva, S.S. and Anderson, T.A. (1995). Nutrition and feeding of fish. 1st edn, p 319. Chapman and Hall. London.

When sections of a book are listed, the order should be: author (s) of section, year, title of the section, title of the book, edition, inclusive pages, publisher and place of publication.

e.g.

Smith, I. and Chong, K.C. 1984. Southeast Asian milkfish culture: economic status and prospects. In: Advances in milkfish biology and culture, ed. Juario, J.V. and Ferraris, R.P. and Benitez, L.V., 5th edn, pp 1-20. Island Publishing House, Manila.

Electronic sources

Roshan, Pejman & Leary, Jonathan (2003) Wireless LAN Fundamentals. Sebastopol, CA : Cisco Press. [Available at: http://proquest.safaribooksonline.com/1587050773]

References to web-only journals should give authors, article title and journal name as above, followed by url in full - or doi if known - and the year of publication in parentheses.

References to websites should give authors if known, title of cited page, url in full, and year of posting in parentheses.

Agency publication

Geological Survey and Mines Bureau of Sri Lanka (1995). Geology of the country around Battulu Oya and Puttalam. Geological Survey & Mines Bureau, Colombo.

Nomenclature

Scientific names of plants and animals should be italicized. Complete scientific names should be given when organisms are first mentioned in the text and in tables, figures

and key words. In the first citation genus, species and authority may be given. e.g. *Crangon crangon* (Linnaeus*)*. In subsequent references, the generic name may be abbreviated. e.g. *C. crangon*

Footnotes

Inclusion of footnotes should be avoided and should be included only if they are indispensable.

Abbreviations and Symbols

The Standard International System of units or metric units should be used wherever possible (e.g. meter, gram, second, joule etc.). Use abbreviations of units only beside numerals (e.g. 10 g), otherwise spell out units. If measurements were made in units other than SI, equivalent value in SI units should be given in brackets e.g.1000 ft (304.3m).

Formulae and Equations

Equations should be typewritten and quadruple spaced. They should be started on the left margin and should be numbered at the right hand end.

Manuscripts will be reviewed prior to acceptance for publication and may be subjected to editorial revision in order to improve presentation. The decision of the Editorial Board on publication is final.

On publication, a PDF file containing the published article will be sent to the corresponding author together with one copy of the issue.

Authors submitting Research articles must furnish a signed statement on authorship responsibility and financial interests in the format given below.

Each author must read and sign the statement of

(1) authorship responsibility;

(2) financial disclosure.

Photocopies of this form may be used for this purpose.

1. Authorship responsibility

I/We certify that I/we have actively participated in the conception, design and execution of this work and the analysis of the data (where applicable) as well as in the writing of the manuscript, to take public responsibility for it.

I/We also certify that the material reported here in represents original work carried out by me/us.

Neither this manuscript nor one with substantially similar content under my/ our authorship has been published or is being considered for publication elsewhere, except as described in an annexure.

I/We also attest that I/we shall produce the original data upon which the manuscript is based for examination by the editors or their assignees should they request it.

2. Financial disclosure

I/We certify that I/we have no affiliation with or financial involvement in any organization or entity with a direct financial interest in the subject matter or materials discussed in the manuscript (e.g. consultancies, employment, honouraria, stock ownership).

Author's signature

I/We hereby jointly and severally take responsibility for authorship of the paper titled ..... submitted for publication in the Journal of the National Aquatic Resources Research and Development Agency of Sri Lanka and, we also certify jointly and severally agreement with the financial disclosure statement shown above.

I/We also indemnify the National Aquatic Resources Research and Development Agency and the Editorial Board of the Journal for any liability arising out of publication of the papers submitted herein.

Name:

Signature:

JOURNAL OF THE NATIONAL AQUATIC RESOURCES RESEARCH AND DEVELOPMENT AGENCY OF SRI LANKA

Volume 40 (2010)

Contents ***Page***

ISSN-1391-6246

National Digitization Project

National Science Foundation

Institute : National Aquatic Resources Research and Development Agency(NARA)

1. Place of Scanning : Crow Island, Colombo 15

2. Date Scanned : ..2017...05...19.....

3. Name of Digitizing Company : Sanje (Private) Ltd, No 435/16, Kottawa Rd,
Hokandara North, Arangala, Hokandara

4. Scanning Officer

Name : ..chamod...Lakshan.....

Signature : .....

Certification of Scanning

I hereby certify that the scanning of this document was carried out under my supervision, according to the norms and standards of digital scanning accurately, also keeping with the originality of the original document to be accepted in a court of law.

Certifying Officer

Designation : Chief Librarian

Name : B G Sunethra Kariyawasam

Signature : .....

Date : ..2017...05...19.....

"This document/publication was digitized under National Digitization Project of the National Science Foundation, Sri Lanka"