Analytical Method Development, Validation, and Application for Supercritical Fluid Extraction of Selected Polynuclear Aromatic Hydrocarbons from the Fish *Oreochromis niloticus* ("Tilapia")

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Abstract

Polynuclear aromatic hydrocarbons (PAHs) are ubiquitous environmental pollutants which accumulate in the fatty tissues of aquatic organisms. A Supercritical Fluid Extraction (SFE) method to extract selected PAHs (phenanthrene, fluoranthene, and pyrene) loaded onto *Oreochromis niloticus* ('tilapia') fish samples by simulated sorption process was developed and validated.

Method development results showed that extracts were efficiently cleaned using H_2SO_4 and florisil. The amount of PAH sorbed onto the fish (sorption experiments) increased with contact time, i.e., 29.51%, 35.43%, and 41.24% for 30 min, 1 hr, and 2 hrs, respectively. The corresponding % recoveries using SFE method increased from 72.43% for 30 min contact time to 81.31% for 1 hr contact time, but decreased to 71.61% for 2 hrs contact time. The decrease in overall SFE % recovery in the 2-hr contact time could be attributed to the degradation of the analytes into their metabolites.

Method validation results showed an overall % recovery of 85.06% (10.50% RSD) for SFE and 74.37% (13.79% RSD) for Soxhlet extraction, indicating that the developed SFE method is more accurate and more precise than Soxhlet method.

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The validated method detected all the selected PAHs in the edible and inedible parts of "tilapia" from Pulangi IV Reservoir of Maramag Bukidnon (concentration range: 0.0058 – 0.0643 μ/g dry basis). The PAH levels in the inedible part were relatively higher than in the edible part. Of the PAHs tested, only fluoranthene in the inedible part of fish was below the US EPA maximum permissible level for the protection of human health.

I. Rationale

Aquatic environments are sinks for polynuclear aromatic hydrocarbons (PAHs) and other pollutants from diverse sources. PAHs are ubiquitous in distribution, particularly in aquatic habitats near heavily populated areas where atmospheric deposition, terrestrial runoff, and boating activity is high (Adams, 2002). Because of their persistence and carcinogenic properties, routine determination and monitoring of PAHs in environmental samples is essential. Fast analytical methods with sufficient accuracy, specificity, and sensitivity are required to detect their presence at very low quantities in biological and other specimens.

Method validation is an integral part of the development of an analytical method. It is the process of proving that an analytical method is acceptable for its intended purpose (Green, 1996). For SFE methods, two important validation parameters usually evaluated are accuracy (expressed as percent recovery) and precision (expressed as percent relative standard deviation, %RSD). The accuracy is determined by extracting a standard reference material (SRM), and calculating the percent recoveries (and precision) relative to the certified values (Capangpangan, 1996). In the absence of an SRM, the accuracy of a method can also be determined by comparing the percent recovery results from the method with in-house results from an existing method that is known to be accurate (Green, 1996), e.g. sonication or Soxhlet extraction method.

This study aimed to develop and validate a supercritical fluid extraction (SFE) method to extract selected PAHs (phenanthrene, fluoranthene, and pyrene) from *Oreochromis niloticus* ("tilapia"). PAHs were allowed to sorb onto live fish samples (instead of direct spiking) to simulate the actual sorption of the analytes in real samples. Laboratory-spiked samples have the disadvantage of giving higher but inaccurate recoveries, which are not reflective of real samples where the analytes are native, and where stronger matrix-analyte interactions exist between the analytes and the active sites in the matrix. High Performance Liquid Chromatography (HPLC) with UV detector was employed for detection and quantitation of the analytes. Percent recovery results from the SFE method were validated against those obtained from the conventional Soxhlet extraction method. The developed and validated method was used to extract the selected

PAHs from real fish samples obtained from Pulangi IV Reservoir in Maramag, Bukidnon.

II. Review of Literature

Polynuclear Aromatic Hydrocarbon Pollutants and Fish as Biomonitor of Pollution

Polynuclear aromatic hydrocarbons are pollutants which are strongly implicated in the degradation of human health in some cities. The larger PAHs have been found to bioaccumulate in the fatty tissues of some marine organisms; they have been linked to the production of liver lesions and tumors in some fish. Although they constitute only about 0.1% of airborne particulate matter, their existence as air pollutants is of concern since many PAHs are carcinogenic, at least in test animals (Baird, 1995).

PAHs are ubiquitous in distribution. They are found throughout the environment: in the air, water, and soil. They can occur in the air, either attached to dust particles or as solids in soil or sediment. They enter the environment mostly as releases to air from volcanoes, bush and forest fires, residential burning, and exhaust from automobiles and trucks. They can also enter surface water through discharges from industrial effluents, municipal wastewater, and improper disposal of used motor oil. They can be released to soils at hazardous waste sites if they escape from storage containers (Agency for Toxic Substances and Disease Registry, 1995).

The movement of PAHs in the environment depends on properties such as how easily they dissolve in water and how easily they evaporate into the air. PAHs in general, do not easily dissolve in water. They are present in air as vapors or stuck to the surfaces of small solids dissolved in water. They can travel long distances before they return to earth in rainfall or particle settling. Some PAHs evaporate into the atmosphere from surface waters, but most stick to solid particles and settle to the bottom of rivers or lakes (Agency for Toxic Substances and Disease Registry, 1995).

Figure 1 shows how pelagic organisms such as fish participate on the fate of PAH in the aquatic environment. The reversible arrows signify equilibrium of PAH between water and the indicated medium. PAH adsorbed on particulate matter may enter water through atmospheric deposition which in turn may be suspended in water or settle to the bottom as sediment (Cunningham *et al.*, 2003).

Fish are at or near the top of aquatic food webs and therefore can have a significant impact on other aquatic populations. Fish are also caught for human consumption, and investigations on the bioaccumulation of toxic chemicals and

the associated potential for human health impacts from consuming contaminated fish are often the primary objectives in fish studies. Because fish populations respond to changes in oxygen, light penetration, food supplies, temperature, pH, salinity, and the presence of pollutants, the species of fish present, their numbers, and their distribution are all indicators of water quality and the general health of the ecosystem (Rogalla, 1996; Henry *et al.*, 1998).

The prime consideration in selecting "tilapia" as test organism in this study is their geographical distribution, abundance, and availability within a practical size range. Another consideration is their ability to tolerate poor water quality.

Supercritical Fluid Extraction (SFE)

Nature and features of SFE. Supercritical fluid extraction is an isolation process, which uses a supercritical fluid as extracting solvent. A supercritical fluid is any substance at a temperature and pressure above its critical temperature and critical pressure (critical point). For example, carbon dioxide gas has a critical temperature of 31.1°C and a critical pressure of 72.8 atmospheres or 1070 psi (pounds per square inch) (McHugh and Krukonis, 1980). Thus, when carbon dioxide gas is compressed to a pressure greater than 72.8 atmospheres at a temperature above 31.1°C, it becomes a supercritical fluid. Carbon dioxide is the most popular supercritical fluid in SFE applications. **Figure 2** shows a schematic diagram of a supercritical fluid extraction system (Capangpangan, 1996).

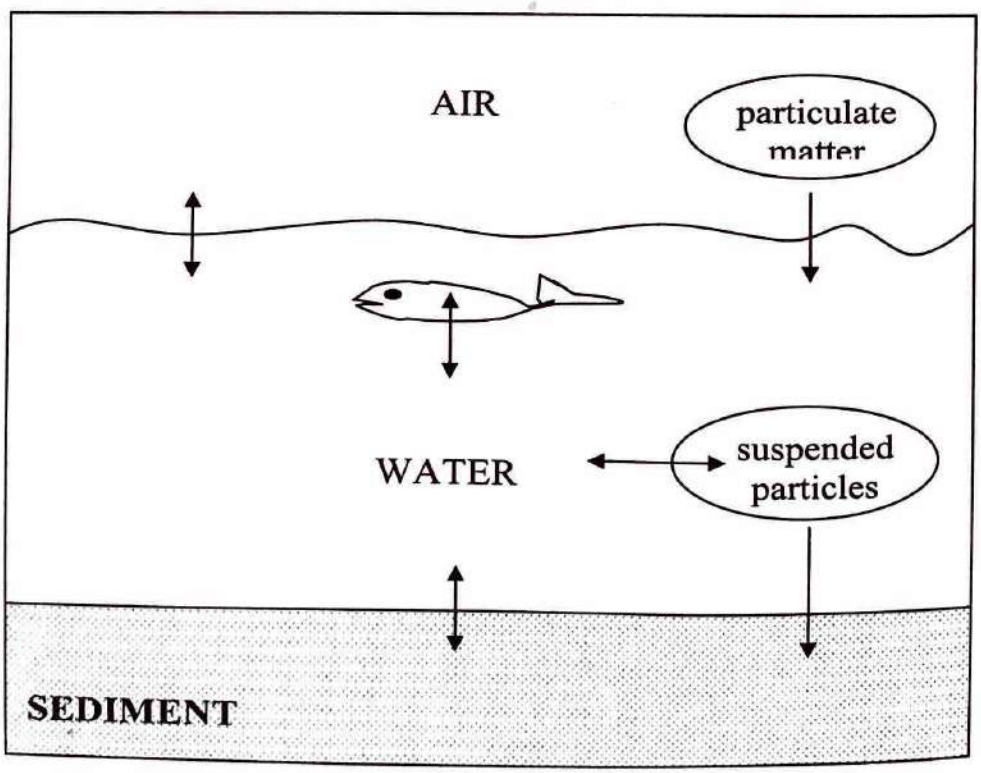

Figure 1. Movement of PAH in an aquatic ecosystem (Modified from Cunningham, *et al.*, 2003).

The increasing interest and use of SFE to extract different compounds from different matrices is mainly due to its features which other extraction methods lack. Greibrokk (1990) attributed such popularity of SFE as an extraction method to the following features:

(1) *Rapid extractions.* Due to low viscosity and high diffusivity, supercritical fluids can penetrate solid samples faster than liquids, thereby reducing the extraction time.

(2) *Selective extractions.* The solubility in the fluid is a function of fluid density. Since the density can be varied according to the pressure and temperature, selective extractions can be obtained. By adding organic solvents (either continuously or batchwise) as modifiers to the fluid, the properties of the fluid can be changed significantly, thereby adding another element of selectivity to the extraction.

(3) *No Degradation of Labile Components.* The supercritical state of the most common fluids, such as carbon dioxide, exist at temperatures close to room temperature, leading to practically no degradation of labile components, in contrast to time-consuming methods such as Soxhlet extraction.

(4) *"Clean" methods.* The most commonly used fluid for SFE is carbon dioxide. Extraction with supercritical CO_2 and its subsequent decompression back into gaseous CO_2 simply by releasing the pressure, significantly reduces the need for chlorinated and other toxic solvents in the laboratory.

(5) *On-line coupling.* The property of a compressed gas enables easy on-line coupling to analytical methods, such as Gas Chromatography (GC), Supercritical Fluid Chromatography (SFC), or High Performance Liquid Chromatography (HPLC), by placing the decompression unit (the restrictor) at the head of the collector.

(6) *Extraction yields.* The yields can be varied versus the selectivity, as with few other extraction methods. In addition to the extraction time and the fluid volume, the yield depends on the density, the temperature (at constant density), the type and amount of modifiers, in addition to sample related parameters such as water content and matrix absorptivity.

Applications of Supercritical Fluid Extraction

For the past many years, there have been increasing number of samples for which SFE has been applied. These include environmental, pharmaceutical, polymeric, natural products, and food samples. Analytes amenable to SFE range from herbicides, pesticides, polychlorinated biphenyls (PCBs), polynuclear aromatic hydrocarbons (PAHs), essential oils, to a few metal-containing compounds. For purposes of this study, only important applications relevant to the present study are cited.

The application of analytical SFE to environmental matrices has focused almost exclusively on solid matrices. Several investigations were conducted on the SFE of PAH from soils and sediments (Wenclawiak *et al.* (1992) and Dankers *et al.* (1993), as cited by Chester *et al.* (1994); Dean (1996), Plate *et al.* (1996), Bowadt *et al.* (1997) and Cordellicchio (1996) as cited by Chester *et al.* (1998); Hawthorne *et al.* (2001). PAHs were also extracted by Paschke *et al.* (1992) from diesel exhaust particulate and diesel soot using CO_2, solvent-modified CO_2, and Freon-22 as SFE extracting fluids as cited by Chester *et al.* (1994).

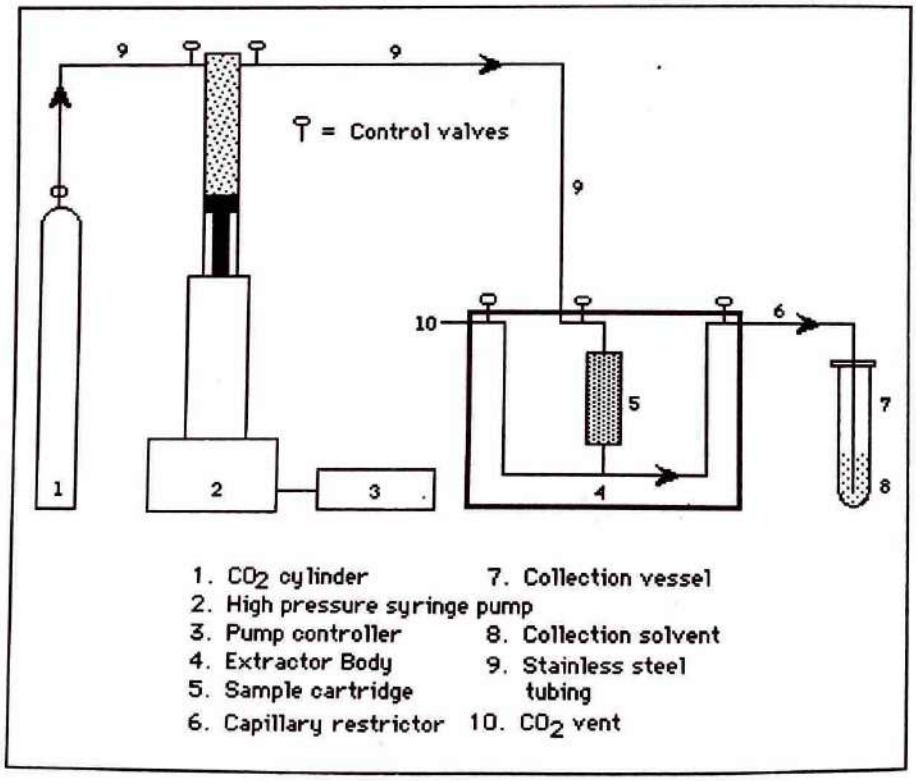

Figure 2. Schematic diagram of a supercritical fluid extraction system (Capangpangan, 1996)

A number of studies comparing SFE with other extraction methods were also conducted. Results from these studies showed that SFE is comparable to or better than the other methods in terms of extraction efficiency. As cited by Chester *et al.* (1994), for example, Burford and co-workers (1993) compared SFE with methylene chloride sonication for the removal of PAHs ranging from naphthalene to benzo[b]fluoranthene from petroleum waste sludge, urban air particulate matter, and railroad bed soil. Furthermore, as cited by Chester *et al.* (1998), Riley *et al.* (1995) examined airborne and solid wastes generated in comparing SFE with Soxhlet procedures for the extraction of organic compounds from sediments; Cordellicchio (1996) extracted PAH from marine sediment using SFE and characterized the extract with GC/MS; Lopez-Avila *et al.* (1996) compared SFE with microwave-assisted extraction, sonication, and Soxhlet methods for a number of compounds adsorbed onto soil; while Lage *et al.* (1997) found no quantitative difference between SFE and conventional liquid extraction methods for extracting PAH from water-soluble smoke.

On the other hand, Capangpangan *et al.* (1996) evaluated selected filters for collection and subsequent supercritical fluid extraction of suspended solids for trace organic analysis. The following year, Capangpangan and Suffet (1997) used SFE for the isolation of hydrophobic organic compounds from filtered suspended solids and obtained acceptable accuracy and precision.

Recently, Gonzales (2002) utilized SFE to determine selected PAHs from marine sediments in Iligan Bay. Results of the study showed that SFE gave much better performance than Soxhlet extraction based from the achieved high overall accuracy and good overall precision. In the following year, Rodilas (2003) used SFE for the analysis of selected PAHs from mussel tissues and found that SFE is more accurate and more precise than Soxhlet extraction, proving that SFE is highly acceptable for application purposes.

Soxhlet Extraction

Soxhlet extraction is by far the most widely used method for pretreatment of solid samples (Majors, 1996). In this technique, the sample contained in a porous thimble, is placed in the Soxhlet extractor (**Figure 3**) and extracted using a desired volatile solvent, which can be water-miscible or water-immiscible. Here, the solvent in the boiling flask is first vaporized. When it condenses, it drops on the solid substance contained in the thimble and extracts soluble compounds. When the liquid level fills the body of the extractor, it automatically siphons into the flask. This process continues repeatedly as the solvent in the flask is vaporized and condensed (Dean, 1995).

Soxhlet extractions are usually slow – often requiring 24 hours or more. However, the process requires little operator involvement after the sample is loaded and refluxing begins, until the conclusion of the extraction. Although

Soxhlet extraction is a low-cost method, however, the most common extractors use hundreds of milliliters of very pure solvent, which is expensive (Majors, 1996).

Because this form of extraction is one of the oldest methods, it is the standard by which many of the newer extraction technologies (such as SFE, accelerated solvent extraction, and microwave-assisted extraction) are measured (Majors, 1996).

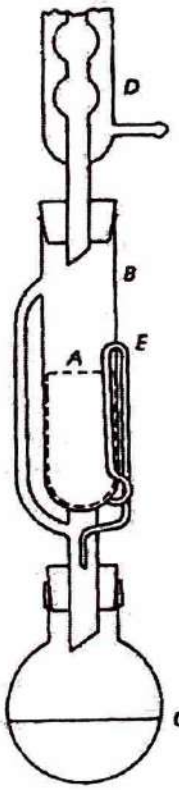

Figure 3. Soxhlet extractor. (A) porous thimble, (B) extractor body, (C) boiling flask containing the solvent, (D) condenser, (E) siphon. (Dean, 1995)

III. Objectives

The overall objective of this study was to develop, validate, and apply to real samples, an analytical method to extract polynuclear aromatic hydrocarbons from fish using supercritical fluid extraction. In achieving this overall objective, the study had to aim for the following specific objectives:

1. simulate and optimize the actual sorption of selected PAHs namely phenanthrene, fluoranthene, and pyrene by the fish *Oreochromis*

niloticus by exposing the fish at different contact times of 30 minutes, 1 hour, and 2 hours,

2. extract the sorbed PAHs by Supercritical Fluid Extraction Method [using supercritical carbon dioxide (CO_2) as extracting solvent] under optimized conditions, for subsequent quantitation by HPLC with UV detection,
3. optimize the cleanup method for the SFE extracts in order to remove co-extracted and interfering compounds.
4. calculate the percentage recoveries of the PAH analytes to determine the contact time at which recovery is maximum,
5. validate the accuracy of SFE to extract the sorbed PAHs at the optimum contact time by comparing the percent recovery results with those obtained from the conventional Soxhlet Extraction Method.
6. use the developed and validated method to extract the selected PAHs from real fish samples obtained from Pulangi IV Reservoir in Maramag, Bukidnon.

IV. Methodology

Selection of Live Fish Samples for Sorption Studies

Live samples of *Oreochromis niloticus* (**Figure 4**) utilized in the optimization of PAH sorption and in the method validation phase were obtained from the Fisheries Technology Resource Center, Mindanao State University-Naawan, Naawan Misamis Oriental. The fish samples came from the same spawning and were of the same sex (all male) to eliminate the sorption variability due to the difference in age and sex.

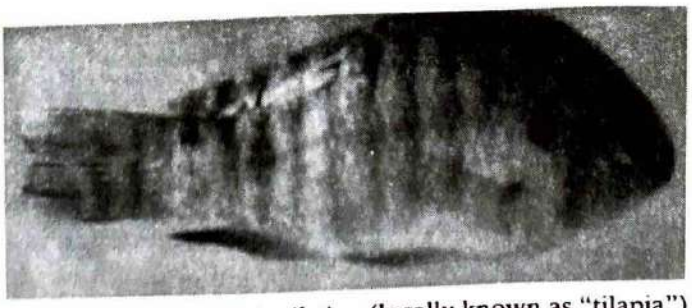

Figure 4. *Oreochromis niloticus* (locally known as "tilapia")

Preparation of Aqueous PAH Solutions for Sorption

One-liter PAH sorption solutions were prepared by spiking calculated volume of PAH stock mixture (of pre-determined concentration) into 1.0 liter of distilled water in glass tanks. The spiked volume used was pre-calculated such that the resulting concentrations of the spiked analytes (ranging from 97 ppb to 118 ppb) did not exceed 80% of their corresponding saturation levels to prevent precipitation due to temperature fluctuations.

Sorption of Analytes Onto Live Oreochromis niloticus **Samples**

Four pieces of live "tilapia" (average length = 7.7 cm , average wet weight = 6.6 g) were placed into each of 12 glass tanks or compartments containing the prepared aqueous PAH solutions (**Figure 5**). Four tanks were exposed for 30 minutes, four for 1 hour, and four for 2 hours, in order to determine the optimum contact time.

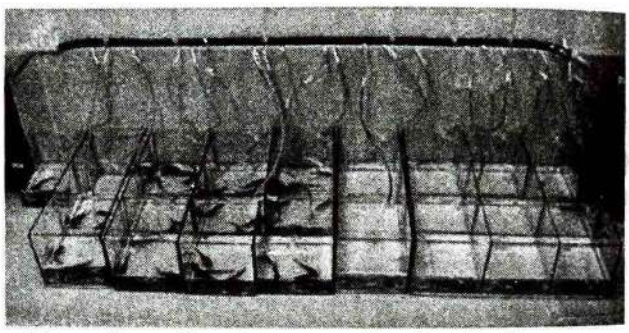

Figure 5. Sorption set-up, showing the glass compartments used in the sorption experiment.

Corresponding reference aqueous PAH solutions without fish samples were also prepared in quadruplicates. The solutions were properly aerated. After each contact period, the fish samples were collected using fish nets and stored in glass containers, and the final PAH sorption solutions were decanted into clean glass bottles. The reference and final PAH sorption solutions were then subjected to liquid-liquid extraction (LLE) to extract the analytes. The amounts of PAH anlytes sorbed by the fish samples were determined from the difference in concentrations between the reference and the final sorption solutions.

Liquid-Liquid Extraction (LLE) of Reference and Final Sorption Solutions

Five hundred milliliters of the reference and final sorption solutions were extracted with 30 mL of dichloromethane (DCM) thrice, following a modified procedure based from Method 6410B (APHA, 1995). The extracts were combined and concentrated using Kuderna-Danish (K-D) apparatus.

Supercritical Fluid Extraction of Whole Fish Samples

The fish samples were homogenized, dried using closed-jar drying method (Capangpangan and Suffet, 1996), and extracted with supercritical CO_2 using SFX 2130 System (Figure 6). The following optimized conditions (Capangpangan and Suffet, 1997) were used: Pressure = 355 atm; Temperature = 120°C; Extraction time = 5 min static, 20 min dynamic; Modifier = 250 µL each of methanol, water, and dichloromethane. Extracts were concentrated by purging with pressurized CO_2 (Montebon, 2006).

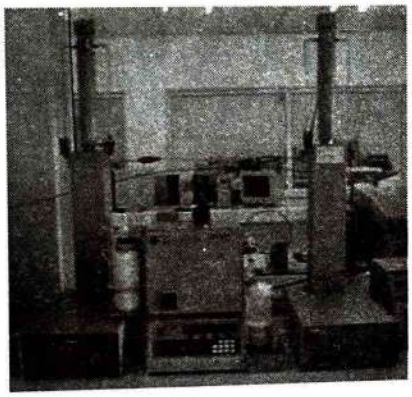

Figure 6. Supercritical Fluid Extractor (ISCO SFX 2130 System).

Soxhlet Extraction of Whole Fish Samples

Closed-jar dried fish samples were Soxhlet-extracted with 200 mL of dichloromethane for 24 hours at a rate of 4-6 cycles per hour. The extracts were concentrated using K-D apparatus.

Cleanup and Analysis of Extracts

The concentrated extracts were reconstituted with n-hexane and extracted with 9M H_2SO_4 thrice to remove high level lipids present. Extracts were then allowed to pass through florisil column, solvent-exchanged into acetonitrile, and analyzed using HPLC.

V. Discussion of Results

Sample Chromatogram.

Figure 7 shows a sample chromatogram of a standard sample containing phenanthrene, fluoranthene, and pyrene, with 2-methylnaphthalene as internal standard.

Optimization of Cleanup Step.

An effective cleanup procedure for removing co-extracted interfering substances is a crucial step before quantitative analysis of PAHs that are present only at trace levels in the crude extracts. In this study, 9M H_2SO_4 was used to remove the high-level lipids present in the extracts of fish followed by passage of the extracts through a florisil column Recovery study for this step was done (in quadruplicates) to assess if analytes were lost or not during the process (Wang *et al.*, 1999, with some modifications). Representative chromatograms from the cleanup process are shown in **Figure 8**, while the corresponding mean percent recoveries are graphically presented in **Figure 9**.

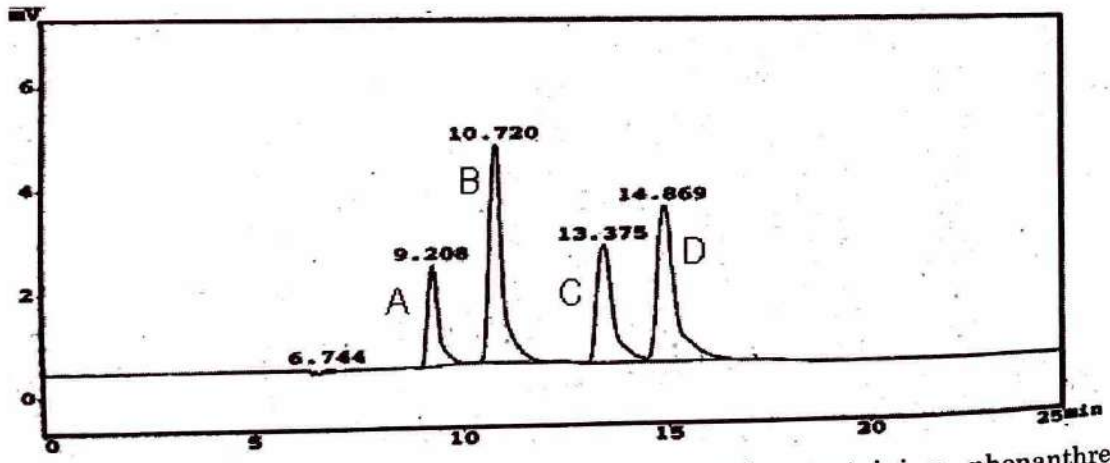

Figure 7. Chromatogram of a standard sample containing phenanthrene (B), fluoranthene (C), and pyrene (D) analytes with 2-methylnaphthalene (A) as internal standard. Column: Wakosil-II5C18AR, 7.5 mm x 250 mm (D); mobile phase: 100% methanol; flow rate: 1.0 mL/min; detector: UV at 254 nm.

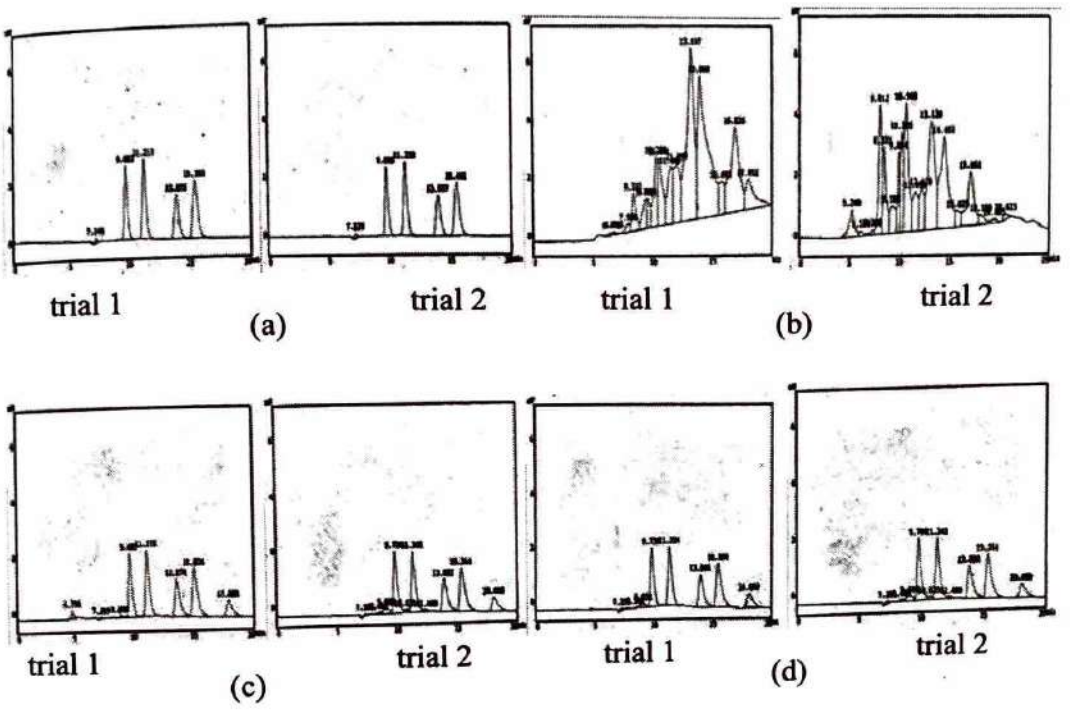

Figure 8. Chromatograms of different samples (only 2 trials shown) from the recovery study for the cleanup step. (a) reference solutions (freshly prepared and injected into HPLC system), (b) with fish fat (extract), without H_2SO_4 cleanup, with florisil column cleanup, (c) without fish fat (no extract), with H_2SO_4 and florisil column cleanup, (d) with fish fat (extract), with H_2SO_4 and florisil column cleanup.

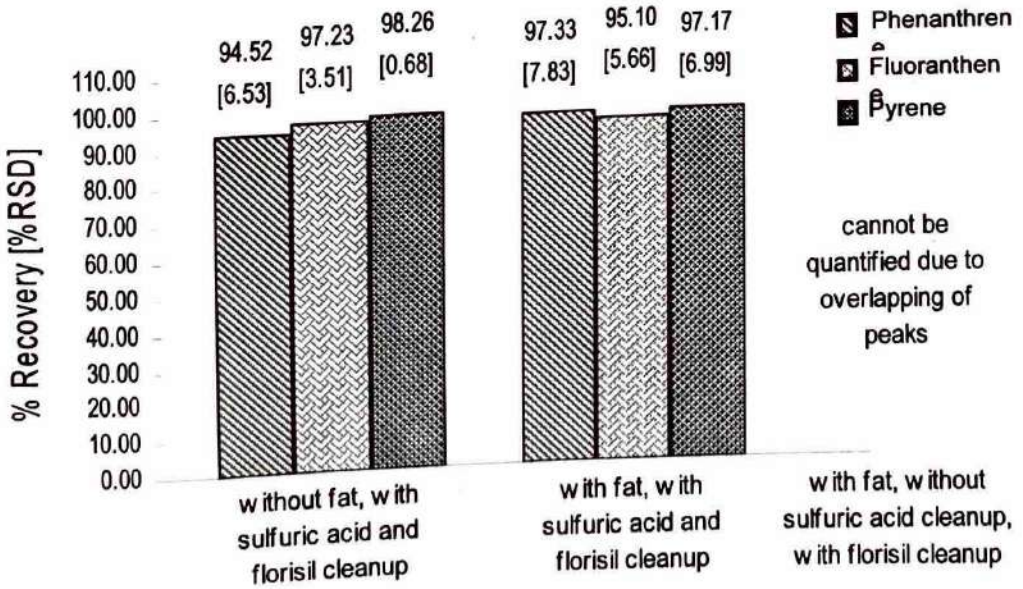

Figure 9. Graphical presentation of the mean percent recoveries for the cleanup step.

Recovery Study for the Cleanup Step

Results presented in **Figures 8** and **9** showed that the co-extracted lipids were efficiently removed by extraction with 9M H_2SO_4 before passage through florisil column. It was observed that no appreciable loss of PAHs was removed (as indicated by high percent recoveries) during the cleanup process, thus the basis of its use in this study. However, when H_2SO_4 was not employed, quantitation of the resulting chromatograms was not possible due to overlapping of peaks.

Sorption Experiments at Different Contact Times

Figure 10 shows the amount of PAHs sorbed by live fish samples exposed at different contact periods. The corresponding percentage recoveries of these analytes by SFE method is presented in **Figure 11**.

As shown in **Figure 10**, the percent and amount of analyte sorbed by the fish increased with contact time for all three analytes studied. The percent (and amount) of analyte sorbed ranged from 21.34% to 35.26% (24.01 µg to 41.65 µg) for the 30-minute contact time, 27.21% to 40.94% (30.61 µg to 48.36 µg) for the 1-hour contact time, and 33.79% to 46.20% (38.01 µg to 54.58 µg) for the 2-hour contact time.

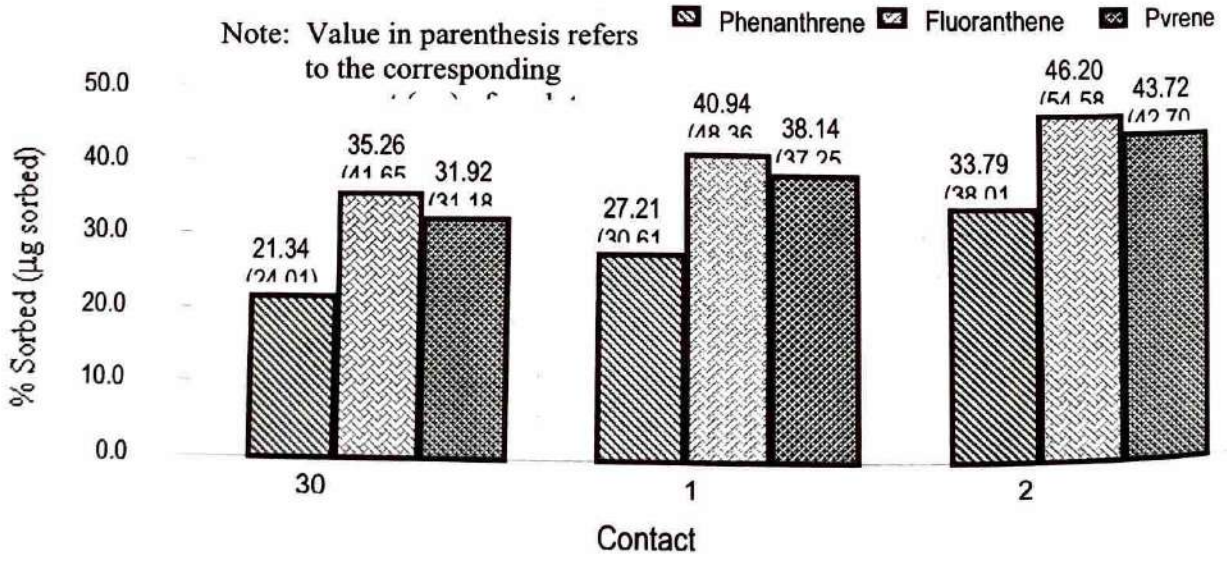

Figure 10. Graphical presentation of the amount of PAHs sorbed at different contact times.

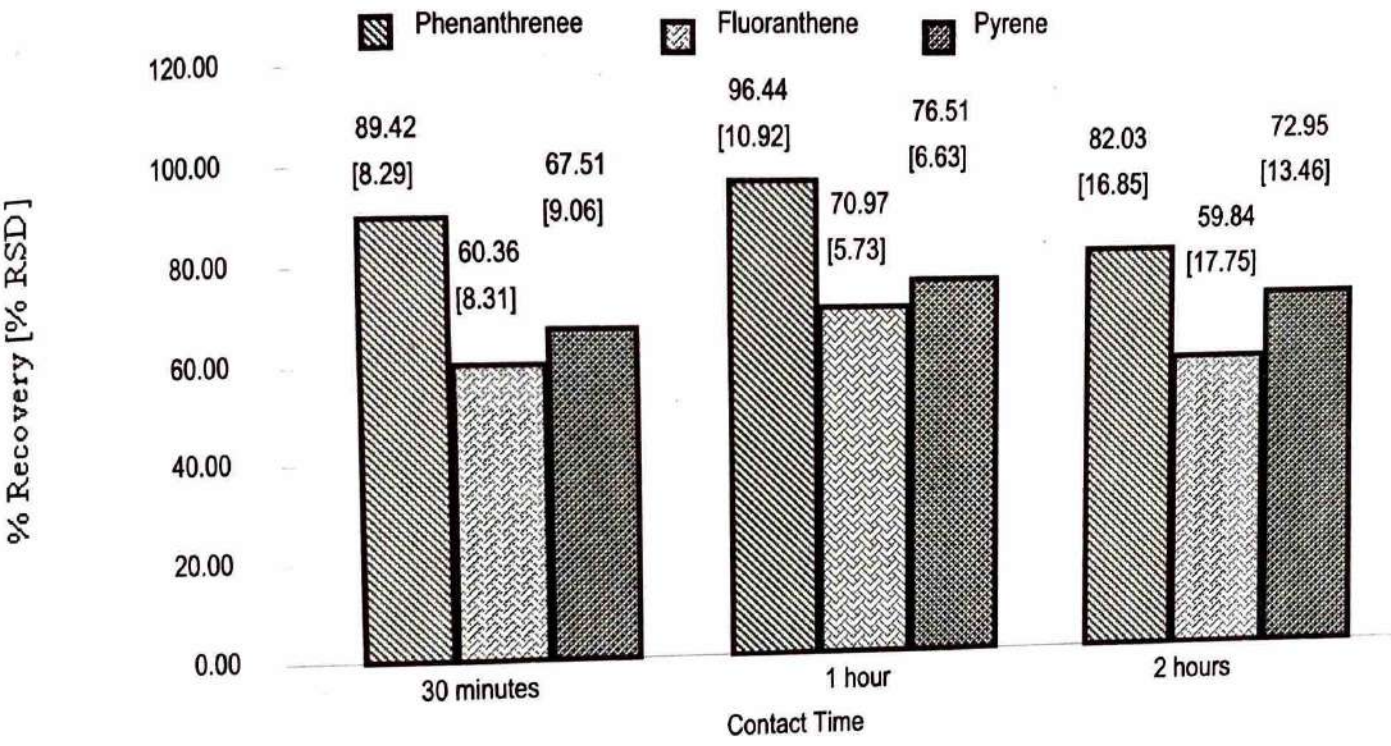

Figure 11. Graphical presentation of the percent recoveries (of PAHs sorbed by the fish at different contact times) using SFE method.

SFE of PAH Analytes Sorbed at Different Contact Times

The percent recoveries of the three analytes were highest at 1-hour contact period (**Figure 11**). The decrease in percent recoveries at the 2-hour contact time could be due to the degradation of the analytes into their metabolites, although this has to be confirmed in a separate study. According to Neff (1979), fish are able to metabolize and excrete accumulated PAHs due to their more active mode of life.

Method Validation Phase: Comparison of Extraction Efficiencies of SFE and of Soxhlet Extraction

For the method validation phase, a graphical comparison of the percentage recoveries of the sorbed analytes using SFE and Soxhlet extraction methods is shown in **Figure 12**. In this phase, analytes were previously sorbed

using the optimum contact time of 1 hour. From the graphical illustration depicted in **Figure 12**, results showed that the recoveries using SFE were higher compared to the recoveries obtained using Soxhlet extraction. In addition, % RSDs were lower using SFE. These results indicate that SFE is more accurate (high percent recoveries) and more precise (low % RSD) than Soxhlet extraction. Thus, SFE is proven to be suitable for the extraction of PAHs from fish samples.

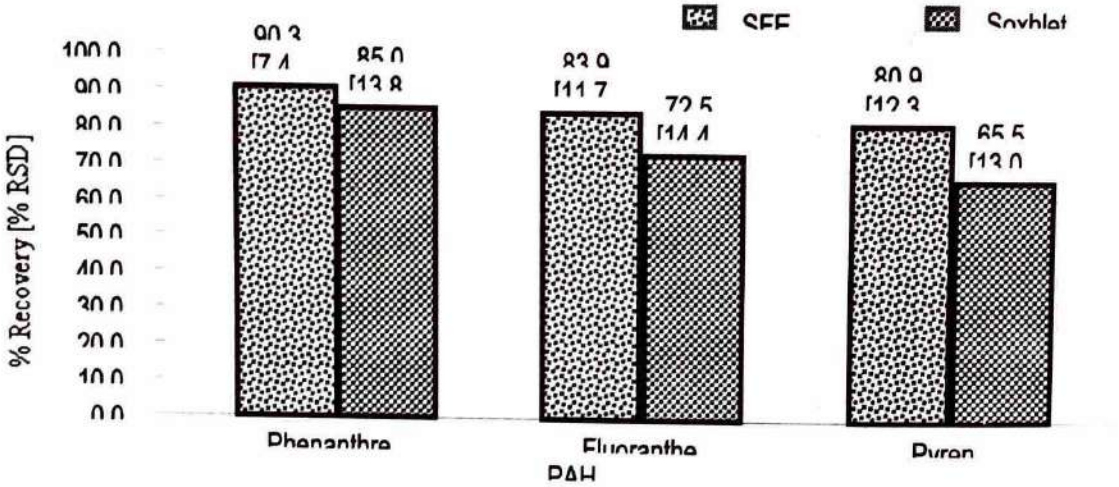

Figure 12. Graphical comparison of the percent recoveries (of PAHs sorbed by the fish) using SFE and Soxhlet extraction methods, as obtained in the method validation phase.

Method Application Phase: Determination of PAH Concentrations in Fish Samples from Pulangi River

Figure 13 shows a graphical correlation of PAH concentrations (in the edible and inedible parts of fish samples obtained from the three sampling sites in Pulangi IV Reservoir (Pulangi River) in Maramag, Bukidnon) to the US EPA maximum permissible levels for the protection of human health. As shown in the figure, the concentrations of phenanthrene (ranging from 0.0355 to 0.0443 μg/g), fluoranthene (ranging from 0.0489 to 0.0643 μg/g), and pyrene (ranging from 0.0227 to 0.0329 μg/g) in the inedible part of the fish exceeded the maximum permissible level set by the US EPA for the protection of human health (phenanthrene = 0.001 ppm, fluoranthene = 0.042 ppm, pyrene = 0 ppm). In the edible part, only fluoranthene with concentration ranging from 0.0137 to 0.0184

µg/g is below the maximum permissible level set by the US EPA for the protection of human health. Phenanthrene with concentration ranging from 0.0101 to 0.0117 µg/g and pyrene with concentration ranging from 0.0058 to 0.0076 µg/g exceeded the maximum permissible level.

The very high concentrations of PAH in the inedible part is due to the bioaccumulation of the analytes in the fatty tissues. Although the concentrations in the edible part are relatively lower, results are still of concern because phenanthrene and pyrene exceeded the maximum level for the protection of human health.

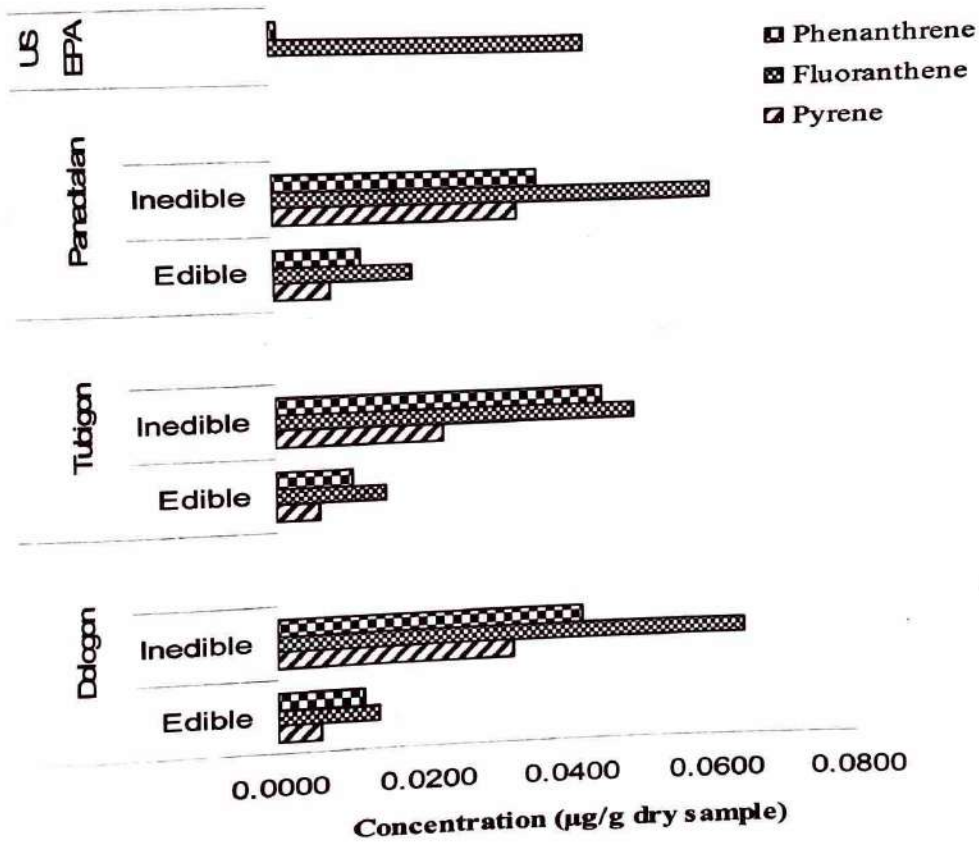

Figure 13. Graphical correlation of the PAH analytes detected in the edible and inedible parts of the fish obtained from the three sampling sites in Pulangi River of Maramag, Bukidnon to the US EPA maximum permissible levels for the protection of human health.

VI. Summary and Conclusion

Optimization experiments for the cleanup step showed that the use of 9M H_2SO_4 efficiently removed co-extracted lipids in the extracts without appreciable loss of the analytes, resulting in the accurate quantitation of the chromatograms obtained.

In the method development sorption studies, the overall percent recovery using SFE increased from 72.43% for 30 minutes contact time to 81.31% for 1 hour contact time, but decreased to 71.61% for 2 hours contact time. The decrease in overall SFE percent recovery in the 2-hour contact time could be attributed to the degradation of the analytes into their metabolites, although this has to be confirmed in a separate study.

Method validation results gave an overall percent recovery of the analytes of 85.06% with 10.50% RSD for the SFE method and 74.37% with 13.79% RSD for the Soxhlet extraction method indicating that SFE is more accurate and more precise than the conventional Soxhlet extraction method.

The developed and validated method was used to extract the selected PAHs in the edible part (consisting of flesh only) and in the inedible part (consisting of scales, bones, gills, and internal organs) in real fish samples obtained from Pulangi River of Maramag, Bukidnon. All three analytes (phenanthrene, fluoranthene, and pyrene) were detected in the three sampling sites, with concentrations ranging from 0.0058 to 0.0643 µg/g dry sample. The concentrations of phenanthrene, fluoranthene, and pyrene in the inedible part of the fish and the concentrations of phenanthrene and pyrene in the edible part exceeded the maximum permissible level set by the US EPA for the protection of human health. Only fluoranthene in the edible part is below the maximum permissible level.

VII. Recommendations

With the results obtained from this study, the following are recommended:

1. Compare the use of UV detector with that of fluorescence detector in the quantitation of PAH analytes using HPLC, to see if interferences due to fats are minimized.
2. Conduct a long-term monitoring of the PAH levels in the area considering (a) both the wet and the dry season to determine if runoffs due to rain could have contributed to the high levels of PAH detected in the area and (b) pre-harvest and post-harvest season of sugarcane to assess if the post-

harvest burning of sugarcane debris would make a significant difference in PAH levels in the area.

3. Conduct similar study for the other 13 PAHs listed by the US EPA as priority PAH pollutants which are also possibly present in the area.
4. Conduct similar study for other biological matrices. Different samples have different analyte-matrix interaction and would therefore require a separate study.

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