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Sampling and Analysis of Petroleum Coco-Biodiesel Blend-Fueled PUV Exhaust for NO_2 and Particulate PAH, Elemental Composition and Toxicity

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The polycyclic aromatic hydrocarbon (PAH), elemental composition and toxicity of particulate matter from the exhaust of public utility vehicles (PUVs) fueled by commercial petroleum coco-biodiesel blend, were examined and compared with that from the exhaust of PUVs fueled by unblended petroleum diesel. FAME (Fatty Acid Methyl Ester) analysis of the commercial diesel/biodiesel blend and of a pure biodiesel sample was done to ascertain the presence of biodiesel in the commercial blend. NO_2 from the exhaust vapor phase was likewise also compared for the two types of fuel. PAHs in particulate matter obtained by filter sampling and from tailpipe soot were analyzed by GC-MS (gas chromatography mass spectrometry). NO_2 was analyzed by visible spectrophotometry, XRF (x-ray fluorescence spectrometry) was used for elemental analysis, and toxicological analysis was done using the Zebrafish Egg Assay Test. A general tentative conclusion from the results point to minimal difference between pure diesel and the 1% diesel/biodiesel blend (when used as fuel for PUVs) in terms of engine exhaust impact on air quality, except perhaps for PAH levels to some extent.

Keywords: *diesel/ biodiesel blend, exhaust particulates, PAH, elements, NO_2, toxicity, PUV*

INTRODUCTION

The Philippine Biofuels Act mandates all gasoline and oil users and oil companies to blend fuels with biodiesel or bioethanol. A minimum of 1% biodiesel and 5% bioethanol by volume in diesel and gasoline fuels was required by the bill which was signed into law January 2007. In the Philippines, biodiesel is primarily obtained from the transesterification of coconut oil into methyl esters of medium carbon chains. Among the benefits of biodiesel blended fuels are better combustion, less pollution, and more engine power. Biodiesel is a renewable and biodegradable alternative fuel which is claimed to be of negligible sulfur content.

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Although there have been reported benefits with the use of biodiesel, the nature of the exhaust released to the environment should be studied in detail. Motor vehicle emissions are a major anthropogenic source of air pollution. Several researches have studied regulated pollutants, such as carbon monoxide, nitrogen oxides, sulfur oxides, particulate matter, and total hydrocarbons (Lenner, 1967; Schmitz et al., 2000; Zielenska et al., 2008). Increasing interest is on the study of polycyclic aromatic hydrocarbons (PAHs) due to its toxic properties. The presence of PAHs are identified in the environment: water, soils, ambient air, sediments, dust, and food sources (Liu et al., 2005; Watanabe and Hirayama, 2001; Wu et al., 2010; Loncar et al., 2005; Kalaitzoglou et al., 2004; Yunker et al., 2002). PAHs are products of incomplete combustion, and vehicle emissions are said to be one of the primary sources. This study aims to compare the profile from the commercially available biodiesel formulation with regular diesel and determine the effects on the quality of emissions.

EXPERIMENTAL

FAME (Fatty Acid Methyl Ester) Analysis. For the FAME analysis, Method EN 14331 (Pauls, 2011) was slightly modified to optimize clean-up (to improve the baseline of the biodiesel blend), as follows: 2 mL of the biodiesel blend was subjected to sulfur removal by activated copper and subjected to gradient elution on a silica column. 7.5 g of silica gel (230 mesh) was placed in a column and eluted with 30 mL of hexane for cleanup. Three fractions were collected by consecutively eluting the column with 30 mL of hexane, 20 mL of hexane:diethyl ether (93:7) and 20 mL of hexane:diethyl ether (50:50). The FAME content of the biodiesel blend was collected in the last solvent system composed of hexane:diethyl ether (50:50). The extract was concentrated using a rotary evaporator. 100 ppm of the extract in hexane was prepared and analyzed in Shimadzu 14B GC-FID with a Supelco Equity 5 capillary column. GC-FID parameters for CME detection were optimized to detect the peaks and improve resolution. The parameters that were varied were split flo, column flow, and column program rate.

The optimized parameters are as follows: Column initial temperature was 50 ^{o}C and the final temperature was 280 ^{o}C. Column initial time was 1.5 min and the final time was 10 min. The program rate was set to 15 ^{o}C/min. The detector and injector temperatures were set to 280 and 300 ^{o}C, respectively.

For comparison, FAME analysis was also done on a pure biodiesel sample (CME or coconut methyl esters) synthesized from commercial coconut cooking oil, using a two-stage (acid-base catalyzed) method (Freedman et al., 1984; Araneta et al., 2004) as follows below:

Commercial coconut cooking oil (500 mL) was heated to 35 ^{o}C, then mixed with 40 mL absolute methanol. Addition of 0.5 mL 95% H_2SO_4 followed by 1 hr heating at 35 ^{o}C and standing overnight at room temperature, converted free fatty acids (FFA's) potentially present in the oil to methyl esters. To this mixture (containing esterified FFA's) was added 41 mL of $NaOCH_3$ solution (prepared by dissolving 7.75 g NaOH in 300 mL absolute methanol), followed by heating to 55 ^{o}C, addition of another 41 mL of $NaOCH_3$ solution, and stirring for 2 hours at 55 ^{o}C. Overnight standing in a separatory funnel allowed the separation of glycerol (bottom layer) from the CME (top layer). The CME in the separatory funnel was repeatedly washed with water until neutral pH was achieved.

PAH Collection, Extraction and Analysis. *Filter Particulate Samples (from the same vehicle).* Exhaust particulate samples were obtained from an engine of a public transportation jeepney in the University of the Philippines campus plying the Ikot route. Diesel and diesel/biodiesel blend were separately used as fuels on different sampling dates. Particulates were collected on preweighed PTFE filters with 1 μm pore size. The filter was placed in an aluminum open face sampler positioned 15 cm away from the mouth of the exhaust tailpipe of a public utility jeepney. Sampling

was done as the vehicle plied the Ikot route for 20 minutes. The filters were weighed, kept in glass jars, and placed in cold storage before extraction.

PAH was extracted according to EPA method 3550B with slight modifications. The filters were extracted with 40 mL hexane:dichloromethane (50:50) in an ultrasonic bath for 30 minutes. The extracts were then filtered and concentrated into approximatedly 1 mL in a rotary evaporator. A column was prepared according to EPA method 3630C for silica gel cleanup with slight modifications. 10 g of activated silica in dichloromethane was placed in a column and topped with 1-2 cm of anhydrous sodium sulfate. The column was pre-eluted with 40 mL hexane. The crude extract was loaded to the column and 25 mL of hexane was added to elute the non-PAH fraction. 25 mL of hexane:dichloromethane was added to elute the PAH fraction. The collected PAH fraction was concentrated by nitrogen blowdown into exact volume. The extract was analyzed in Shimadzu GC-MS DB-5 capillary column. PAHs were determined by Selected Ion Monitoring.

Tailpipe Soot Samples (from different vehicles). Particulate samples were collected from exhaust tailpipes of public utility vehicles (PUVs) that are stationed in Pantranco, Philcoa and SM North terminals in Quezon City, Philippines. Samples were differentiated according to the type of fuel used (diesel or diesel/biodiesel blend). The samples were placed in glass jars previously washed free of organic material. Samples were then stored under nitrogen gas, sealed with Teflon and kept at 4°C until analysis.

Cleanup parameters for PAH were modified to speed up extraction using SPE, from 24 hours soxhlet extraction to 30 minutes using sonication and SPE (Xie et al., 2003). A 0.1 gram sample of soot was weighed and extracted with 30 mL hexane:dichloromethane (DCM) (50:50) in an ultrasonic bath for 30 minutes. All trials were done in triplicate. The soot extracts were concentrated in a rotary evaporator to a volume of 1 mL. A 3 mL solid phase extraction (SPE) silica tube (Supelco) was first cleaned with 10 mL DCM followed by 10 mL hexane. The flow rate in the manifold was maintained at 1 mL/min. The 1 mL sample extract was loaded to the SPE column and the non-PAH fraction was discarded. 3 mL of 20% DCM in hexane was then used to elute the PAH fractions. Nitrogen blowdown was used to concentrate the final extract to 1 mL. The extracts were stored at 4°C until analysis.

The analyses of PAHs were carried via splitless mode on a Varian 4000 GC-MS. Optimized parameters for PAH detection (to detect all the peaks and improve resolution) are as follows: injector temperature set at 280°C, transfer line at 280°C, ion trap at 150°C, and the ion source at 230°C. The column temperature program was set as follows: initial temperature at 55°C, hold at 1 minute; ramp of 30°C/min to 140°C; ramp of 5°C/min to 240 °C, hold at 5 minutes; and ramp of 8 °C/min to 300 °C, hold at 12 minutes. PAHs were identified by the NIST Library Search Software built-in the Varian 4000 GC-MS, and validated using the retention times of standards.

NO_2 Determination. Nitrogen dioxide (NO_2) was determined with the use of a passive sampler as described in a study of Quirit *et al* (Quirit et al., 2010). with slight modifications. NaI-NaOH-methanol impregnating solution (100 μL) was pipetted on filter paper (Whatman 40) and assembled into the passive sampler. NO_2 is reduced by NaI to nitrite (NO_2^-) ions. Samples were collected from different vehicles using diesel or diesel/iodiesel blend as fuels. Passive sampler was attached to the tailpipe while the jeepney was moving. The filters were transferred to a vial and extracted with 5 mL of ultrapure water. An aliquot (100 μL) of this solution was diluted to exactly 5 mL with the Griess-Saltzmann absorbing solution (Lodge, 1989). The absorbance was read at 540 nm. A calibration curve was plotted from Griess-Saltzmann reaction with freshly prepared $NaNO_2$ standard solutions equivalent to a range of 0.2–2 μL NO_2/mL absorbing solution (Lodge, 1989).

Elemental Analysis. Elemental analysis of pelletized soot samples was done by x-ray fluorescence spectrometry, with a Spectro Xepos benchtop XRF spectrometer with molybdenum, corundum & graphite targets. 1.000 g sample was mixed with 3.9000 g of Licowax binder and pressed into a pellet using 15 tons pressure.

Toxicity Testing. Soot (1.0 gram) was weighed and extracted with 30 mL hexane:dichloromethane (DCM) (50:50) in an ultrasonic bath for 30 minutes. All trials were done in triplicate. The extracts were concentrated in a rotary evaporator to a volume of 1 mL. A 3 mL solid phase extraction (SPE) silica tube (Supelco) was first cleaned with 10 mL DCM followed by 10 mL hexane. The flow rate in the manifold was maintained at 1 mL/min. The 1 mL sample extract was loaded to the SPE column and the non-PAH fraction was discarded. DCM (3 mL of 20% solution in hexane) was then used to elute the PAH fractions. The extracts were evaporated to near dryness and diluted to 10 mL of 1%DMSO. This was referred to as the DMSO stock solution (per soot sample) and labeled 1:0 in the toxicity test solutions. 50%, 33%, 25% and 20% dilutions of the stock solution were also prepared and labeled 1:1, 1:2, 1:3 and 1:4 solutions, respectively. The 1:0 to 1:4 solutions were tested for toxicity by the Zebrafish Egg Assay (Nagel, 2002).

RESULTS AND DISCUSSION

Commercial Diesel/Biodiesel Blend Comparison with Synthesized Pure CME Biodiesel. Figures 1A and 1B show FAME chromatograms from pure CME (coconut methyl esters) biodiesel and the diesel/biodiesel blend, respectively. Peaks for the two types of sample show the same retention times and comparable normalized areas, as quantified and shown in Table 1.

Table 1. FAME Chromatogram Retention Times and Normalized Areas (for pure CME and Diesel/Biodiesel Blend).

Methyl Ester	Retention time	CME (%Normalized area)	Diesel/Biodiesel Blend (%Normalized area)
Caprylic	12.05	8.26	4.86
Capric	14.5	7.49	13.82
Lauric	16.65	51.42	46.75
Myristic	18.67	18.12	20.49
Palmitic	20.87	8.47	9.33
Oleic	23.34	6.25	4.77

The procedure employed for Figure 1B is a simple cleanup procedure for extracting the fatty acid methyl esters from the complex matrix of the diesel/biodiesel blend. The chromatogram shows the presence of the unresolved complex mixture (UCM), typical of organic matter from petroleum sources, in the form of a "hump" along the baseline. The success of the extraction method is shown by the major peaks eluting within, but distinguishable from, the UCM complex, and mirroring the pure CME peaks shown in Figure 1A.

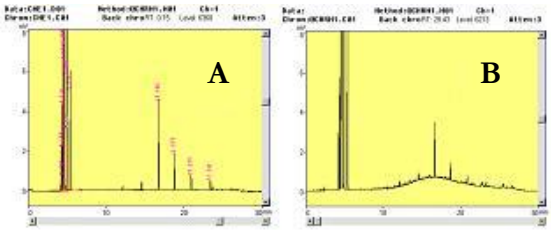


Figure 1. *GC-FID chromatogram of (A) pure cocomethyl ester (CME) sample and (B) FAME extract from diesel/ biodiesel blend.*

Both of the chromatograms show the third peak as the major peak. Based on literature, the major peak is due to the presence of lauric acid methyl esters and usually accounts for the

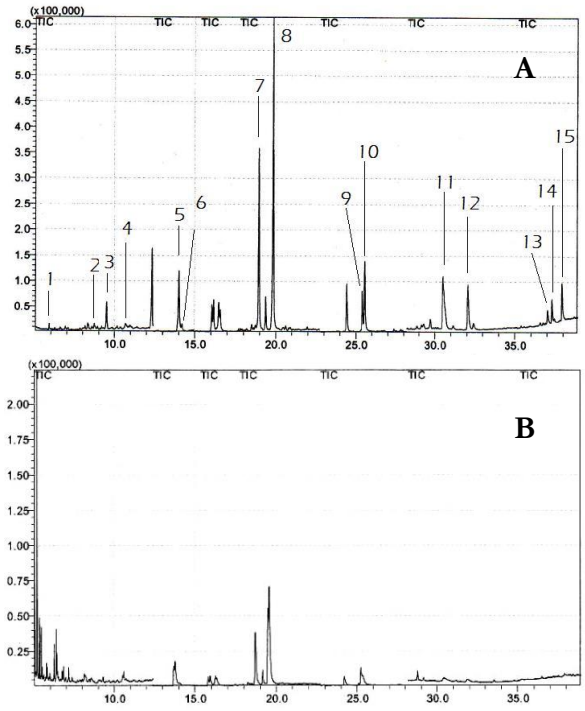


Figure 2. *GC-MS chromatogram of PAHs in tailpipe soot. The peaks correspond to the ff: (1) naphthalene [NAP], (2) acenaphthylene [ANY], (3) acenaphthene [ACP], (4) fluorine [FLU], (5) phenanthrene [PHEN], (6)anthracene [ANT], (7) fluoranthene [FLT], (8) pyrene [PYR], (9) benzanthracene [BaA], (10) chrysene [CHR], (11)benz(b)fluoranthene [BbF], (12) benzo(a)pyrene [BaP], (13) indenopyrene [IPY], (14) dibenzanthracene [DBA], and (15) benzo(ghi)perylene [BgP] (A); GC-MS chromatogram of PAHs in filter particulate (B).*

highest percentage in coconut methyl esters (Beare-Rogers et al., 2001). The FAME content of the diesel/biodiesel blend is an indication of the source of the biodiesel. In the Philippines, biodiesel is primarily obtained from the transesterification of coconut oil, and thus contains methyl esters of medium carbon length.

Comparison of Filter Particulate and Tailpipe Soot Samples. Figures 2A and 2B show samples of GC-MS chromatograms of tailpipe soot and filter particulate samples, respectively. The retention times are similar, but there is a striking difference in the size of the peaks, with much smaller peaks for the filter particulate sample. This was due to the much smaller sample size obtained using filter sampling. Figures 3A and 3B compare PAHs obtained from the two types of samples. In Figure 3A, 1d and 2d samples are tailpipe soot samples, while the sample labeled MJ Diesel is a filter particulate sample (MJ stands for Mang Jesus, the name of the vehicle driver). The three samples were obtained from three different vehicles, all of which used diesel fuel. Similarly, 1b and 2b samples are tailpipe soot samples and the sample labeled MJ Biodiesel is a filter particulate sample, in Figure 3B (from three different vehicles which used the diesel/biodiesel blend fuel). It can be seen that the PAH profiles are similar for the two types of samples (filter particulate and tailpipe soot), supporting the qualitative picture seen in Figures 2A and 2B. Hence, samples used for subsequent PAH and elemental analysis, and toxicity testing, were all tailpipe soot samples. This was due to the ease of sampling and the greater amounts obtained for tailpipe soot samples (10 to 100 times more compared to the filter particulate samples).

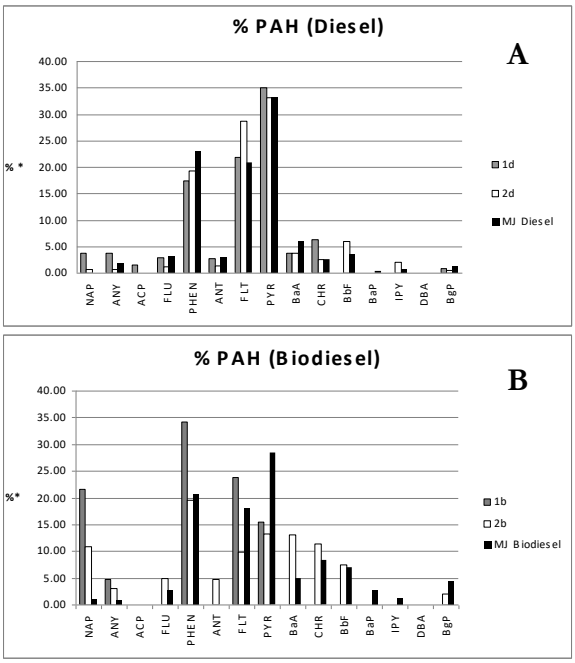
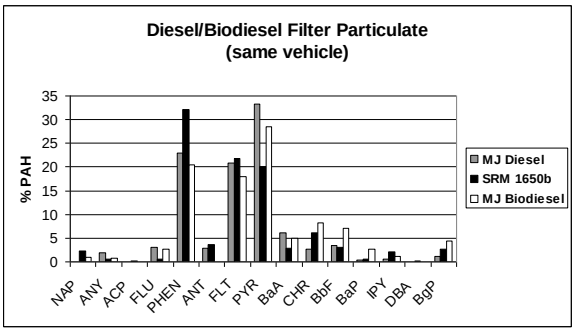


Figure 3. *Composition profile of PAH (% or g individual PAH/100 g total PAH) in particulate matter from exhaust of three diesel-fueled vehicles: 1d and 2d are tailpipe soot samples, MJ Diesel is a filter particulate sample (A); 1b and 2b are tailpipe soot samples, MJ Biodiesel is a filter particulate sample (B). The abbreviated PAH labels in the axis are fully spelled out in Figure 2.*

Comparison of Diesel and Diesel/ Biodiesel Blend Filter Particulates (using the same vehicle). Filter particulate samples from the same vehicle using two fuel types (diesel and diesel/biodiesel blend) are compared in Figure 4, in terms of %PAH. Trends for the two fuel types are comparable with SRM (standard reference material) 1650b, analyzed in the study of Oukbedane et al. (2010) and also shown in Figure 4.



% PAH: g individual PAH per 100 g total PAH

Figure 4. *Comparison of composition profile of PAH in filter particulate samples from the same vehicle when diesel-fueled (MJ Diesel sample) and diesel/biodiesel blend-fueled (MJ Biodiesel sample), and in standard reference material diesel soot (SRM 1650b).*

It can be observed that some PAHs are lowered when the fuel is switched from diesel to biodiesel. This is observed by other studies that previously investigated the effects of biodiesel blends on PAH emissions. Yang et al. (2007) found lower values for PAH with 1403 ± 227 μg bhp-h^{-1} for diesel and 1051 ± 218 μg bhp-h^{-1} for biodiesel. Other studies also confirm the significant reduction of PAHs with the increase in biodiesel blends (Chien et al. 2009). A study on soot from biokerosene and kerosene also obtained PAH reduction on biokerosene at 50% and 100% combustion (Correa and Arbilla, 2006). However, in this study, the heavier PAHs are greater in biodiesel blends, at least for the vehicle sampled. This suggests that the reduction does not apply to all the PAHs. For low biodiesel blends, Correa and Arbilla concluded that the results show great deviations (Andrade-Eiroa et al., 2000). In the Philippines, the blend consisting of 1% biodiesel and 99% petroleum diesel is currently in use in service stations.

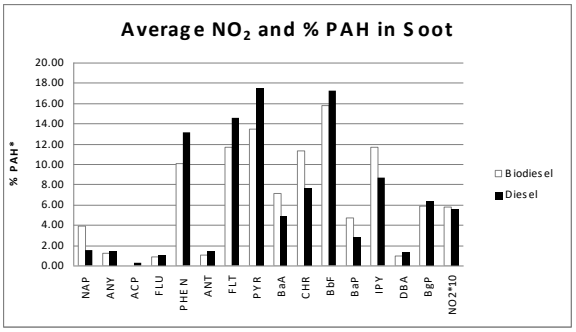
Results shown in Figure 4 and in Table 2 suggest that the most abundant PAHs are pyrene (PYR), phenanthrene (PHEN) and fluoranrthene (FLT). For the lighter PAHs, the low % composition might be due to their volatility, resulting in limited amounts in the particulate phase. This is supported by the % recoveries shown in Table 3, with a general trend of increasing % as molar mass of the PAH increases.

Table 3. % Recovery and Limit of Detection of PAH in Soot from Diesel and Diesel/Biodiesel Blend Fueled Exhaust of Same Vehicle. (The abbreviated PAH names are fully spelled out in Figure 2A.)

PAH*	Average % Recovery	Limit of Detection (ppm)
NAP	45.15	0.0011
ANY	55.95	0.0014
ACP	48.41	0.0014
FLU	60.21	0.0021
PHEN	63.85	0.0032
ANT	70.40	0.0066
FLT	85.10	0.0050
PYR	83.50	0.0030
BaA	121.74	0.0080
CHR	103.36	0.0025
BbF	142.06	0.0112
BaP	134.53	0.0011
IPY	102.70	0.0238
DBA	77.74	0.0023
BgP	118.75	0.0075

* Molar mass of PAH increases from top to bottom

NO_2, Average % Individual PAH and Total PAH in Tailpipe Soot Samples (two fuel types used by different vehicles). For the four PAHs with highest average %PAH's (Figure 5), soot samples from the diesel fueled vehicles had higher average %PAH compared to the samples from vehicles using diesel/biodiesel blend fuel. For the other eleven PAHs, five had higher average %PAH for the samples from vehicles using diesel/biodiesel blend fuel (this includes the highly toxic benzo(a)pyrene or BaP, five PAHs had comparable average %'s, and one (ACP) was detected only in diesel fueled vehicles, albeit at very low levels. Average % RSD ranged from 12.5 to 39 %.



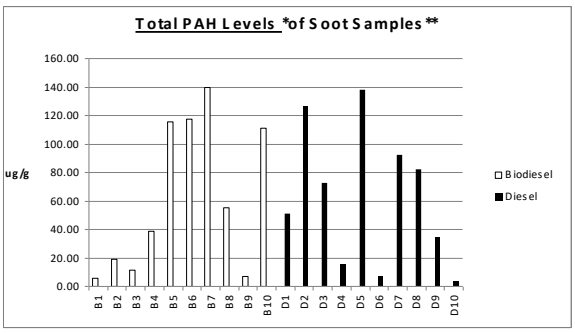
% PAH: g individual PAH per 100 g total PAH (average of 10 samples from different vehicles per fuel category)
*<u>*except*</u> for NO_2 (calculated as 10* [uL gas per mL absorbing solution])

Figure 5. *Comparison of exhaust NO_2 of and average PAH composition of tailpipe soot samples from diesel-fueled and diesel/biodiesel blend-fueled vehicles.*

NO_2 results are comparable for the two types of fuels. Literature suggests that NO_2 results could be higher in the diesel/biodiesel blend (Karavalakis et al., 2010) due to the presence of oxygenated species (the methyl esters), resulting in higher flame temperature in the combustion chamber. The average NO_2 results are actually slightly higher for vehicles using the diesel/biodiesel blend, but the standard deviations for NO_2 results of the two fuel types offset this difference (0.58 ± 0.07 and 0.56 ± 0.05 μL NO_2/mL absorbing solution, for biodiesel and diesel, respectively).

In Figure 6, Total PAH levels (in μg total PAH per g soot) are shown for individual vehicle samples. Great variability can be seen in samples from vehicles using both types of fuel and could be due to the variability in the conditions of the vehicle engines.

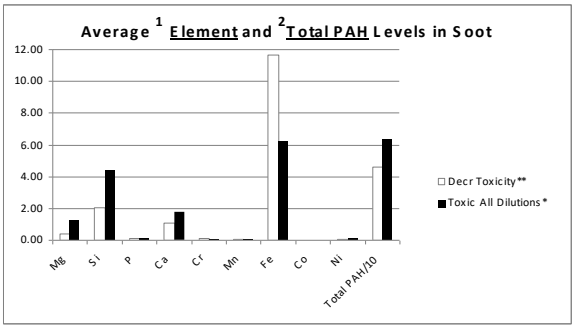
Effect of Elemental Composition and Total PAH Level on Toxicity. The zebrafish assay toxicity test for the particulates resulted in two categories for the samples (Category 1: samples which resulted in 100% toxicity to zebrafish embryos at the undiluted (1:0) and 50% (1:1) dilution levels, but starting with 1:2 down to 1:3 and 1:4 dilution levels, resulted in decreasing severity on the embryotoxic effects in the zebrafish eggs; Category 2: samples which caused 100% mortality in all tested dilution levels (1:0 to 1:4) within 24 hours). These two categories



* Total PAH Level (in ug total PAH per g soot sample)
** B1 to B10 are biodiesel samples, D1 to D10 are diesel samples

Figure 6. *Total PAH levels of tailpipe soot samples from diesel-fueled and diesel/biodiesel blend-fueled vehicles.*

were labeled "Decr Toxicity" (for Category 1) and "Toxic All Dilutions" (for Category 2) in Figure 7, where the average element and average total PAH levels were plotted for samples in the two toxicity categories. It can be seen that average total PAH and average element levels for three of the four major elements (Mg, Si, Ca and Fe) were higher for the "Toxic All Dilutions" category compared to the "Decr Toxicity" category. In contrast, the average Fe level was markedly higher for "Decr Toxicity" compared to "Toxic All Dilutions". It is to be noted that Diesel and Diesel/Biodiesel blend samples are included in both categories (see Fig. 7 footnote).



[1] g element per 100 g soot (average of 9 or 11 samples per toxicity category)
[2] ug total PAH per g soot (average of 9 or 11 samples per toxicity category)
**average of 11 samples (5 diesel & 6 biodiesel)
*average of 9 samples (5 diesel & 4 biodiesel)

Figure 7. *Average element levels and average total PAH Levels in tailpipe soot samples grouped according to zebrafish egg assay toxicity.*

CONCLUSION

The study is a preliminary attempt to determine effects of the Philippine Biofuels Act mandated into law in 2007, specifically the blending of coco-biodiesel with petroleum diesel (1% biodiesel blend), on some air quality parameters. Usual studies on diesel/biodiesel blend use controlled parameters, such as the use of a single engine under specified conditions. This study investigated samples from typical PUVs plying some Quezon City routes.

Two exhaust particulate sampling methods (filter sampling and simple tailpipe soot collection) were investigated and were found comparable in terms of %PAH composition profiles. Analysis methods were also optimized, especially clean-up methods for verification of CME in the commercial diesel/biodiesel blend, and for PAH analysis of filter particulates and tailpipe soot.

A general tentative conclusion from the results point to minimal difference between pure diesel and the 1% diesel/biodiesel blend (when used as fuel for PUVs) in terms of engine exhaust impact on air quality, except perhaps for PAH levels to some extent. For the four PAHs with highest average %PAH's, soot samples from the diesel fueled vehicles had higher average %PAH compared to the samples from vehicles using diese/biodiesel blend fuel. For the other PAHs, five had higher average %PAH for the samples from vehicles using diese/biodiesel blend fuel (including the highly toxic benzo(a)pyrene or BaP) and five PAHs had comparable average %'s. When each PAH was subjected to the *t* test for comparison of two experimental means (Skoog et al., 2004), almost all t's (absolute values ranging from 0.001 to 0.773) were less than t critical (2.09), signifying no significant difference between the means (of diesel and biodiesel blend soot) for total PAH ($t = 0.001$), and per individual PAH (except for ACP with t of 2.496). The only reason for t of ACP being greater than t critical is the almost zero % ACP of the biodiesel blend soot, compared to the very small % ACP of the pure diesel soot (see Figure 5).

NO_2 results were comparable for the two types of fuels. Toxicity results were also comparable, with both diesel and diesel/biodiesel blend samples found present in the two toxicity categories using the zebrafish assay. The PAH content seemed an important determining factor in the toxicity of the soot samples. This is seen in the significantly higher average total PAH in the "Toxic All Dilutions" category, compared to that of the "Decr Toxicity" category (Figure 7). Elemental content was determined on the untreated soot samples, while PAH was determined using the same extracts used for the zebrafish toxicity assay, hence the probable minor impact of elemental composition on toxicity.

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