



PM, Carbon, PAH, and Particle-Extract-Induced Cytotoxicity of Emissions from a Diesel Generator Fueled with Waste-Edible-Oil-Biodiesel

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ABSTRACT

This study used pure fossil diesel (D100) and a 20% (v/v, volume percent) waste-edible-oil-biodiesel blend (W20, 20% waste-edible-oil-biodiesel + 80% diesel) as the fuels for a generator to investigate the mass concentrations of various sized PMs (PM_{0.01–0.056} (nano particles), PM_{0.01–0.1} (ultrafine particles), PM_{0.01–1} (submicron particles), PM_{0.01–2.5} (fine particles), PM_{0.01–10} and PM_{0.01–18}) and particle-bound carbons, polycyclic aromatic hydrocarbons (PAHs), and PAHs' toxicity equivalences (BaP_{eq}) in generator emissions. The engine load was set as either 0 or 3 kW. MOUDIs and Nano-MOUDIs were used as the samplers. Human male single cells (U937) and the method MTT (3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide) were used to test the cell toxicity of particle extracts (obtained from organic-solvent extraction). The results showed that, compared with D100, using W20 effectively reduced the PM (by 21.0–72.8%), particulate EC (by 2.69–57.3%), particulate OC (by 30.8–47.5%), Total-PAHs (by 64.1–81.9%), and Total-BaP_{eq} (by 70.9–92.6%) in all sized particles emitted from the diesel generator, regardless of engine load. The reduction of PM_{0.01–18} (81.3%) was higher in the lung respirable accumulation mode particles (PM_{0.1–1}). Regardless of fuel and loading, the emitted PM_{0.01–18} exhibited a single-modal distribution and peaked in the submicron size range (0.18–0.32 μm). Compared with no engine load, with the exception of PM_{0.01–0.1} and PM_{0.01–0.056}, the OC contents of various particle sizes in PM at 3 kW engine load using D100 and W20 were all reduced (by 13.3–15.0% and 28.9–31.7%, respectively), while the EC content increased (by 27.5–29.1% and 37.9–41.4%, respectively). Moreover, the cell toxicity to U937 (per μg PM) of particle extract was higher for nano (PM_{0.01–0.056}) and ultrafine (PM_{0.056–0.1}) particles than for the other sized ones. However, compared with D100, using W20 could reduce the cell toxicity to U937 (per μg PM) of extracts from all sized particles, especially for nano and ultrafine particles (reduction = 32–46%) at 3 kW engine load.

Keywords: Waste-edible-oil-biodiesel; Nano particle; Carbon; PAH; Cytotoxicity.

INTRODUCTION

Particulate and gaseous pollutants are associated with several environmental concerns (e.g., acid rain, smog, greenhouse, and health effect). Diesel exhaust particles (DEPs) or diesel particulate matters (DPMs) frequently comprised of carbon particles, ash, and soluble organic fractions (SOFs) (Ning and Sioutas, 2010), are mostly respirable particles that have particle sizes smaller than 2.5 μm (Kittelson *et al.*, 2004; Lin *et al.*, 2008a). DEP-bound

carbons and PAHs may cause adverse health effects. The DEP-bound PAHs include high molecular weight PAHs with more carcinogenic potency (such as BaA, CHR, BbF, BaP, and IND) (Kerminen *et al.*, 1997; Soontjens *et al.*, 1997). Therefore, the exposure to the emission environment of diesel engines increases the risk of the occurrence of cardiovascular diseases, respiratory diseases, and lung cancer that negatively affect human health (Sultan, 2007; Channell *et al.*, 2012).

To alleviate the environment impacts and adverse health effects from DPM emission, although the mechanical design of diesel engines has been improved and catalytic converters have been installed, the fuel used by diesel engines is also a critical factor which influences the compositions of particle and gas pollutants emitted by diesel engines. Hence, it is increasingly attractive to use renewable energy

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instead of fossil fuel in diesel engines. Bio-diesel has similar combustion features as diesel but it is able to reduce environmental pollution, clean the air, and mitigate the greenhouse effect (US EPA, 2000). It requires no modification to the oil supply system of engines and can be used directly (Dorado *et al.*, 2003; Lee *et al.*, 2004; Raheman and Phadatare, 2004). After adding bio-diesel into diesel, the oxygen content and cetane index of an oil product can be increased (Lin *et al.*, 2008b; Tsai *et al.*, 2010, 2011a) and the sulfur content can be reduced to significantly lower the sulfur oxide emission of diesel engines (Kameda *et al.*, 2007; Refaat, 2009). Although bio-diesel is a relatively potential renewable energy, the ingredients for making bio-diesel have always been an important challenge.

Ingredients of bio-diesel include economic plants such as soy beans, rape flower, palm, and sun flower (Avellaneda and Salvadó, 2011; Lin *et al.*, 2011a), and the use of economic plants for making bio-diesel may result in food shortages as well as high costs (accounting for 75% of total production cost) (Phan *et al.*, 2008; Sérgio *et al.*, 2010; Lin *et al.*, 2011b). Furthermore, due to extreme weather conditions globally during the past decade, the imbalance of the food supply has already caused soaring prices for both food and oil; the above problem will increase the basic challenge of living. Thus, to avoid competition between food and energy, it is inevitable to use non-edible oil to replace edible oil as the ingredient in bio-diesel and recycling waste edible oil is one of the solutions.

According to the statistical data from the US Energy Information Administration, daily waste edible oil in the US reaches 100 million gallons (Radich, 2006). In Taiwan, ~77 tons of waste-edible-oil are generated each year, and only 4% of them have been used for industry (Taiwan EAP, 2007a, b). Due to the low reuse volume, most waste edible oil is illegally dumped into water and landfills and later causes serious environmental pollution. As a result, recycling waste edible oil as the ingredient of bio-diesel to be used as alternative diesel may reduce environmental pollution and lower the production cost of bio-diesel.

According to earlier studies (Refaat, 2009; Tsai *et al.*, 2010; Tsai *et al.*, 2011a), 20% is the typical volume ratio of biodiesel to petroleum diesel helpful for the reduction of gaseous and particulate pollutant emissions from diesel-engines/generators. Although the use of biodiesel may significantly reduce PM emission from diesel engines (Lin *et al.*, 2006; Lin *et al.*, 2008b; Marina *et al.*, 2012), it is necessary to evaluate the potential impact of DPM size distribution on health when using biodiesel. This study used diesel (D100) and waste-edible-oil-biodiesel (W20, waste-edible-oil-biodiesel/D100 = 20%/80% (v/v)) under different loadings (0 and 3 kW) to conduct PM sampling of the particle sizes of diesel engine generators including $PM_{0.01-0.056}$, $PM_{0.01-0.1}$, $PM_{0.01-1}$, $PM_{0.01-2.5}$, $PM_{0.01-10}$, and $PM_{0.01-18}$ with a MOUDI and a Nano-MOUDI to examine the emission characteristics of carbon content, polycyclic aromatic hydrocarbons (PAHs), and toxic equivalents (BaP_{eq}) in particles. In addition, the human male monocyte cell line (U937) and MTT (3-(4,5-dimethyl-thiazol-2-yl)-

2,5-diphenyltetrazolium bromide) assay were adopted to conduct cell toxicity tests for the organic solvent extracted materials of above sized particles.

MATERIALS AND METHODS

Instruments and Sampling Methods

The diesel engine was a water-cooled, direct injection-single cylinder four-stroke diesel engine (Model: TF110E, made by YANMAR, Japan) (natural intake) with a cylinder bore of 88 mm and strokes of 96 mm, and a displacement of 583 cc. The power generator (Model: YSG-5SEN, YANMAR) had a 100/110 V (50/60 Hz) AC output (single-phase, two-wire type), with a maximum output of 4 kW. To prevent interruption in the experiment due to damage to the diesel engine power generator, 3 kW (or 75% of max output power) was used as the load.

The size distributions of particle-bound PAHs that were emitted from the generator using different fuels were measured using a micro-orifice uniform deposit impactor (MOUDI) and a Nano-MOUDI (with 0.01–18 μm aerodynamic diameters). The flow rates of the MOUDI and Nano-MOUDI were set to 30 and 10 L/min, respectively; these two samplers used 37 and 47 mm quartz filters (Pall Ltd., USA), respectively. The impactors in the MOUDIs and Nano-MOUDI separated the particulate matter into 13 size ranges (at 50% efficiency) with the following equivalent cutoff diameters 0.01–0.018, 0.018–0.032, 0.032–0.056, 0.056–0.1, 0.1–0.18, 0.18–0.32, 0.32–0.56, 0.56–1.0, 1.0–1.8, 1.8–3.2, 3.2–5.6, 5.6–10, and 10–18 μm . The particles were divided into six size groups: $PM_{0.01-0.056}$ (nano particles: $0.01 \mu\text{m} < D_p < 0.056 \mu\text{m}$), $PM_{0.01-0.1}$ (ultrafine particles: $0.01 \mu\text{m} < D_p < 0.1 \mu\text{m}$), $PM_{0.01-1}$ (submicron particles: $0.01 \mu\text{m} < D_p < 1 \mu\text{m}$), $PM_{0.01-2.5}$ (fine particles: $0.01 \mu\text{m} < D_p < 2.5 \mu\text{m}$), $PM_{0.01-10}$ ($0.01 \mu\text{m} < D_p < 10 \mu\text{m}$), and $PM_{0.01-18}$ ($0.01 \mu\text{m} < D_p < 18 \mu\text{m}$). The pure petroleum diesel that was utilized in the experiments (D100) was purchased from CPC (Taiwan) and the waste-edible-oil-biodiesel was manufactured by the Taiwan NJC Corp. The oil properties of tested fuels are listed in Table 1.

Prior to use, the quartz fiber filters were heated for 2.5 h at 900°C to reduce their carbon blank. This process minimized the background concentration of carbon in the quartz fiber matrix, as it could influence the analysis. The treated paper filters were stored in a dry chamber at a temperature of $25 \pm 3^\circ\text{C}$ and a humidity of $45 \pm 5\%$ for one day both before and after sampling. A five-digit electronic balance (model HM-202; A & D Co. Ltd., Japan) that was accurate to within 0.01 mg was used to weigh the paper filters before and after sampling. The filter blanks were determined to correct all PM and particle-bound PAH concentrations.

Carbon Analysis

The carbon contents (elemental carbon (EC) and total carbon (TC)) of the particles that were collected by quartz filters were analyzed using a total organic carbon analyzer (TOC-5000A; Shimadzu Corp., Japan) that was equipped with a suspended solid measuring (SSM) instrument. To

Table 1. Properties of fossil diesel and waste-edible-oil-biodiesel.

Item	Fossil diesel	Waste-edible-oil-biodiesel	Test method
Density, at 15°C (g/cm ³)	0.830	0.882	CNS 12017
Heating value (Cal/g)	11035.7	9478.5	
Cetane index (CN)	56	46	ASTM D976
Viscosity, at 40°C (cSt)	2.66	3.60	ASTM D445
C (wt%)	86.1	78.9	
H (wt%)	13.9	11.8	
O (wt%)	~0*	9.3	

* obtained by $O \text{ (wt\%)} = 100\% - C \text{ (wt\%)} + H \text{ (wt\%)}$ assuming that the content of other components is negligible.

make the carbon measurements, the samples were placed in a sample boat, and were then manually pushed into a 900°C burner that was filled with oxygen to ensure complete combustion. After CO₂ and H₂O had been formed, the H₂O was separated using a draining device, and the CO₂ content was determined using a non-dispersive infrared (NDIR) gas analyzer. Finally, data processing and calculations were conducted to determine the carbon content of the sample. One quarter of each filter was heated in an oven at 350°C for 100 min to expel the OC content and was then placed in the elemental analyzer to determine the EC content; another quarter of each filter was fed directly into the elemental analyzer without pre-treatment to measure the TC content (Lin, 2002). The OC value was obtained by subtracting the EC content from the TC content. An organic analytical standard (OAS) (model B2038; Elemental Microanalysis Ltd., U.K.) that consisted of purified urea was used as the routine working standard for determining the carbon content.

PAH Analysis

The extraction of PAHs from the paper filters and the glass sleeves that were used in the sampling was performed using the *Soxhlet*-extraction method. The extraction solvent was a mixture of n-hexane and dichloromethane (1:1 volume ratio). The extraction reflux time was 24 h. After extraction, the extract was concentrated to 2 mL using highly pure nitrogen gas. The extract was then poured into a purification tube that contained pretreated silica gel (dried at 105°C for 8 h and then activated with distilled water for 24 h) and n-hexane to remove moisture and highly polar substances. The purified solution was further concentrated to 1 mL using a nitrogen gas stream and then stored in brown sample vials for the subsequent identification of 15 PAHs using a gas chromatograph/mass selective detector (GC/MSD; model: GC 6890N/HP 5973; Hewlett-Packard, Wilmington, DE, USA). The GC/MSD, equipped with a capillary column (HP Ultra 2, 50 m × 0.32 mm × 0.17 μm) and an automatic sampler (HP-7683), was operated under the following conditions; GC/MSD injection volume of 1 μL, splitless injection temperature of 310°C, and ion source temperature of 310°C. The oven temperature increased from 50°C to 100°C at a rate of 20 °C/min, and then from 100°C to 290°C at a rate of 3 °C/min; this temperature, 290°C, was maintained for 40 min.

The 15 identified PAH species were four 4-ring (fluoranthene (FL), pyrene (Pyr), benzo(a)anthracene (BaA),

chrysene (CHR)), six 5-ring (cyclopenta(c,d)pyrene (CYC), benzo(b)fluoranthene (BbF), benzo(k)fluoranthene (BkF), benzo(e)pyrene (BeP), benzo(a)pyrene (BaP), perylene (PER)), four 6-ring (indeno(1,2,3,cd)pyrene (IND), dibenzo(a,h)anthracene (DBA), benzo(b)chrycene (BbC), benzo(ghi)perylene (BghiP)), and one 7-ring (Coronene (COR)) PAH compound. The 4-ring and 5-/6-/7-ring PAHs are referred to as having a middle molecular weight (MMW) and a high molecular weight (HMW) PAHs, respectively. Table 3 lists the calculated equivalent toxicity (BaP_{eq}) of each PAH species by multiplying the PAH concentration by its corresponding Toxicology Equivalent Factor (TEF) recommended by Malcom and Dobson (1994).

Preparation of Particle Extracts

The DPM of D100 and W20 were extracted by 1:1 (v/v) n-hexane/dichloromethane. Subsequently, the organic solvent that was used to extract the particles was evaporated in a stream of nitrogen. The residues that were obtained after the mixed solvent was evaporated were then resuspended in dimethyl sulfoxide (DMSO) and stored in a freezer at –80°C until the cytotoxicity assay was conducted using porcine human male monocytic cell strain (U937) as an *in vitro* screening system.

MTT Assay

The MTT assay is an MTT-based colorimetric assay, which is simple, fast, economic, and free of radioactive elements. The MTT assay is commonly used to analyze indicators of cell survival, toxicity, proliferation, and activation (Mosmann, 1983; Cory *et al.*, 1991). The MTT reagent (thiazolyl blue tetrazolium bromide) is a water-soluble, yellow tetrazolium salt. If it reacts with the dehydrogenase of the mitochondria in living cells, then the tetrazolium ring is eliminated from the reagent, forming a purple insoluble formazan crystalline precipitate.

To perform the MTT assay, first, the cultivated U937 cell culture fluid was moved to a well plate, with the number of cells controlled at 4×10^4 cells in each well. Subsequently, 1 μL of the particle extracts pre-treated by DMSO was added to each well. Then, the fluid was transferred to a 37°C, 5% CO₂ incubator for 24 hours. Afterwards, 10 μL of the MTT reagents was added to each well before the fluid was placed in the incubator for one hour. The mitochondria of the living cells restored the MTT reagent to form purple-blue insoluble Formazan crystallizations at the bottom of the well. Next, after the reaction, the well

plate was placed in a centrifugal machine (model CS-6R, Beckman Coulter Inc., Fullerton, California, USA), which was operated at 1200 rpm. A straw was used to remove the remaining fluid from the wells, leaving the Formazan crystals. Then, 100 μ L of DMSO was added to each well to dissolve the crystals. Finally, an ELISA Reader (Multiskan Spectrum model, Thermo Electron Co., Vantaa, Finland) was used to measure the Formazan crystallization OD (optical density) at a wavelength of 550 nm. The obtained ratio of the OD of the sample group to that of the negative control group (1 μ L pure DMSO addition) was the cell viability rate of the samples. Bleomycin (BLM) may cause pulmonary cells fibrosis (Schein and Winokur, 1975) and pulmonary macrophage apoptosis (Zhao *et al.*, 2004), although it is a glycopeptide antibiotic commonly used as an anticancer drug. In this study, 75 U/mL BLM addition was used as the positive control group to evaluate the cytotoxicity of DPM organic solvent extracted materials. Six tests were carried out on each group of samples.

A larger OD value corresponds to a higher cell viability rate, because only the mitochondria of living cells have active dehydrogenase. Hence, the Formazan crystallization output is proportional to the number of living cells. Eqs. (1) and (2) yield the cell viability and death rates, respectively.

$$\text{Cell viability rate (\%)} = \frac{\text{OD}_{\text{sample}}}{\text{OD}_{\text{control}}} \times 100\% \quad (1)$$

$$\text{Cell death rate (\%)} = 100\% - \text{Cell viability rate (\%)} \quad (2)$$

RESULTS AND DISCUSSION

DPM Emission Characteristics of Bio-Diesel Generators

The concentrations of $\text{PM}_{0.01-0.056}$, $\text{PM}_{0.01-0.1}$, $\text{PM}_{0.01-1}$, $\text{PM}_{0.01-2.5}$, $\text{PM}_{0.01-10}$, and $\text{PM}_{0.01-18}$ emitted from the diesel engine generator using D100 and W20 at loadings of 0 and 3 kW are listed in Table 2. When the diesel engine generator used D100 or W20 at 0 kW, the emitted $\text{PM}_{0.01-10}$ was mainly contributed by the accumulation mode (shown as $\text{PM}_{0.1-1}$) (77% and 71%, respectively). Compared to D100, at 0 kW, the use of W20 reduced the concentrations of $\text{PM}_{0.01-0.056}$, $\text{PM}_{0.01-0.1}$, $\text{PM}_{0.01-1}$, $\text{PM}_{0.01-2.5}$, $\text{PM}_{0.01-10}$, and $\text{PM}_{0.01-18}$ by 72.2%, 72.8%, 58.7%, 54.4%, 53.9%, and 54.4%, respectively. Under loading with 3 kW, 74.2% of $\text{PM}_{0.01-10}$ was contributed by $\text{PM}_{0.1-1}$ when using D100, while when W20 was used, the value was 70%. At 3 kW, when W20 was used in place of D100, the concentrations of $\text{PM}_{0.01-0.056}$, $\text{PM}_{0.01-0.1}$, $\text{PM}_{0.01-1}$, $\text{PM}_{0.01-2.5}$, $\text{PM}_{0.01-10}$, and $\text{PM}_{0.01-18}$ were reduced by 45.7%, 44.0%, 27.5%, 25.8%, 21.8%, and 21.0%, respectively. The results reveal that with/without loading, the use of W20 in place of D100 effectively reduced the emission of various sized PM emission from the diesel engine generator. The reduction of $\text{PM}_{0.01-10}$ mainly results from the reduced contribution of accumulation modes ($\text{PM}_{0.1-1}$) (respectively accounting for 81.3% and 88.7%). This phenomenon is possibly associated with the increase of oxygen content in fuel while adding biodiesel to enhance the combustion of fuel and reduce the formation of DPMs (Lin *et al.*, 2010; Lin *et al.*, 2012). Also,

Lee *et al.* (2011) indicated that biodiesel had straight chain hydrocarbons and did not provide the original PAHs or initial radicals for the formation of aromatic rings that were critical in the formation of SOF and PM.

Regardless of loading, when using D100 and W20, the distributions of $\text{PM}_{0.01-18}$ were similar, peaking at 0.18–0.32 μ m (Fig. 1). The major contribution of $\text{PM}_{0.01-18}$ was mainly from submicron particles smaller than 1 μ m (about 73–83%) (Fig. 2). It is known that during operation, diesel engines emit fine and ultrafine particles in large volume (Harris and Maricq, 2001; Jacobson and Seinfeld, 2004; Rose *et al.*, 2006). As suggested by Palmgren *et al.* (2003), the particles emitted from diesel engines may exhibit a nucleation mode smaller than 30 nm and an ultrafine mode between 30–100 nm in size distribution. Turrio-Baldassri *et al.* (2004) concluded that a large amount of particles emitted from a bus engine fueled with rapeseed-biodiesel were in the range 0.06–0.3 μ m. Chien *et al.* (2009) indicated that the particulates emitted from diesel engines were primarily inhalable particulates, in which approximately 80% of particulates were smaller than 1.0 μ m for all tested fuels. In this study, replacing D100 with W20 at 0 and 3 kW effectively reduced the accumulation mode particles ($\text{PM}_{0.1-1}$) (that can easily enter the human respiratory system) in DPMs, and thus the use of bio-diesel could reduce the health hazards of DPMs on humans.

Carbon Content and Concentration Levels in DPMs

At 0 and 3 kW, when the diesel engine generator used W20 in place of D100, the OC and EC concentrations in various sized PMs were also effectively reduced (Table 2). At 0 kW, such emission reductions of EC for $\text{PM}_{0.01-0.056}$, $\text{PM}_{0.01-0.1}$, $\text{PM}_{0.01-1}$, $\text{PM}_{0.01-2.5}$, $\text{PM}_{0.01-10}$, and $\text{PM}_{0.01-18}$ were 55.9%, 57.3%, 56.1%, 52.4%, 51.0%, and 51.5%, respectively, and those of OC were 35.0%, 36.4%, 47.8%, 41.4%, 41.2%, and 41.5%, respectively; the data at 3 kW were 44.7%, 49.4%, 7.69%, 6.14%, 3.59%, and 2.60%, respectively for EC, and 38.5%, 42.6%, 37.5%, 38.9%, 31.7%, and 30.8%, respectively for OC. In comparison to the loading at 0 kW, the use of D100 or W20 at 3 kW increased emission concentrations of EC but decreased those of OC of all sized PMs. Compared with no engine load, despite using D100 or W20 at 3 kW engine load, the reduction (26–82.9%) of particulate OC and the increase (51.8–90%) of EC were both significant for all sized particles. Kweon *et al.* (2002) pointed out that the EC content in PM emitted by diesel engines increased (from 10% to 90%) with the increase of loading, but the OC content in PM decreased with increasing loading (from 80% to 10%). The study of Sharma *et al.* (2005) also contested the increase of EC and the decrease of OC of particles emitted by diesel engines with the increase of engine loading. Zhang *et al.* (2009) suggested that the EC content of PM tied to engine speed. The EC of micro particles may be absorbed by toxic chemical substances in the environment and when they enter human and animal bodies with a respiratory mechanism, health hazards will increase (Jacobson, 2001).

Jacobson (2001) concluded in a study that the OC of suspended particles in the atmosphere reduced the

Table 2. PM mass and particle-bound carbon content in DPMS emitted from diesel-engine generator fuelled with D100 and W20 under 0 (unload) and 3 kW loads.

	PM (n = 3)						EC (n = 3)						OC (n = 3)						TC (n = 3)					
	Unload		3 kW		Unload		3 kW		Unload		3 kW		Unload		3 kW		Unload		3 kW		Unload		3 kW	
	D100	W20	D100	W20	D100	W20	D100	W20	D100	W20	D100	W20	D100	W20	D100	W20	D100	W20	D100	W20	D100	W20		
Concentrations (mg/m³)																								
PM ₁₈	28.7 (±3.05)	13.1 (±0.56)	61.4 (±3.18)	48.5 (±2.96)	6.48 (±1.18)	3.14 (±0.39)	30.8 (±3.65)	30.0 (±4.06)	12.4 (±2.74)	7.26 (±0.66)	18.5 (±6.43)	12.8 (±2.50)	18.9 (±3.00)	10.4 (±0.29)	49.2 (±4.26)	42.8 (±3.33)								
PM ₁₀	28.4 (±3.02)	13.1 (±0.49)	60.9 (±3.50)	47.6 (±2.29)	6.37 (±1.19)	3.12 (±0.39)	30.6 (±3.57)	29.5 (±4.21)	12.3 (±2.71)	7.23 (±0.67)	18.3 (±6.58)	12.5 (±2.36)	18.7 (±2.99)	10.4 (±0.29)	48.8 (±4.54)	42.0 (±3.16)								
PM _{2.5}	27.2 (±3.13)	12.4 (±0.57)	57.8 (±2.93)	42.9 (±0.69)	5.97 (±1.29)	2.84 (±0.27)	29.3 (±3.56)	27.5 (±4.20)	12.0 (±2.78)	7.03 (±0.64)	17.5 (±6.29)	10.7 (±1.38)	17.9 (±3.17)	9.87 (±0.42)	46.7 (±4.25)	38.2 (±2.93)								
PM ₁	23.9 (±2.66)	9.88 (±1.49)	48.8 (±2.90)	35.4 (±0.55)	5.17 (±1.11)	2.27 (±0.10)	24.7 (±3.40)	22.8 (±4.24)	10.8 (±2.79)	5.64 (±1.44)	14.6 (±5.73)	9.12 (±1.91)	15.9 (±3.30)	7.91 (±1.46)	39.4 (±4.62)	31.9 (±2.33)								
PM _{0.1}	2.17 (±0.58)	0.59 (±0.16)	3.61 (±1.71)	2.02 (±0.42)	0.75 (±0.21)	0.32 (±0.09)	1.62 (±0.39)	0.82 (±0.23)	0.22 (±0.03)	0.14 (±0.03)	1.29 (±0.84)	0.74 (±0.11)	0.97 (±0.19)	0.46 (±0.12)	2.91 (±1.22)	1.56 (±0.34)								
PM _{0.056}	1.98 (±0.63)	0.55 (±0.15)	3.22 (±1.70)	1.75 (±0.40)	0.68 (±0.21)	0.30 (±0.09)	1.41 (±0.39)	0.78 (±0.23)	0.20 (±0.03)	0.13 (±0.02)	1.17 (±0.82)	0.72 (±0.12)	0.87 (±0.20)	0.43 (±0.11)	2.58 (±1.21)	1.49 (±0.33)								
Contents (%)																								
PM ₁₈	-	-	-	-	22.6 (±4.72)	23.92 (±3.3)	50.1 (±6.94)	61.8 (±8.59)	43.3 (±5.18)	55.4 (±4.98)	30.0 (±9.28)	26.5 (±3.93)	65.8 (±5.92)	79.3 (±2.87)	80.1 (±2.91)	88.2 (±4.94)								
PM ₁₀	-	-	-	-	22.4 (±4.74)	23.9 (±3.32)	50.1 (±7.03)	62.1 (±8.79)	43.4 (±5.15)	55.4 (±5.01)	29.9 (±9.44)	26.3 (±4.11)	65.8 (±5.94)	79.3 (±2.93)	80.1 (±2.97)	88.4 (±4.99)								
PM _{2.5}	-	-	-	-	21.9 (±4.94)	22.9 (±2.55)	50.9 (±7.02)	64.2 (±9.19)	44.0 (±5.36)	56.6 (±4.37)	30.4 (±9.74)	24.9 (±3.30)	65.9 (±6.23)	79.4 (±2.46)	81.3 (±3.36)	89.1 (±5.94)								
PM ₁	-	-	-	-	21.6 (±4.14)	23.0 (±3.24)	50.7 (±7.16)	64.4 (±11.8)	45.0 (±6.95)	57.1 (±5.71)	30.0 (±10.6)	25.8 (±5.43)	66.6 (±7.41)	80.0 (±2.52)	80.7 (±4.90)	90.2 (±6.59)								
PM _{0.1}	-	-	-	-	34.6 (±0.89)	54.0 (±1.66)	44.7 (±9.91)	40.3 (±3.09)	10.3 (±3.64)	23.9 (±2.46)	35.9 (±6.09)	36.5 (±2.54)	44.91 (±3.4)	78.0 (±1.94)	80.6 (±4.67)	76.9 (±1.11)								
PM _{0.056}	-	-	-	-	34.2 (±0.63)	54.1 (±1.18)	43.7 (±10.3)	44.3 (±3.07)	9.93 (±4.08)	23.6 (±2.60)	36.3 (±6.46)	41.0 (±3.51)	44.1 (±4.37)	77.7 (±2.20)	80.0 (±5.06)	85.4 (±0.57)								

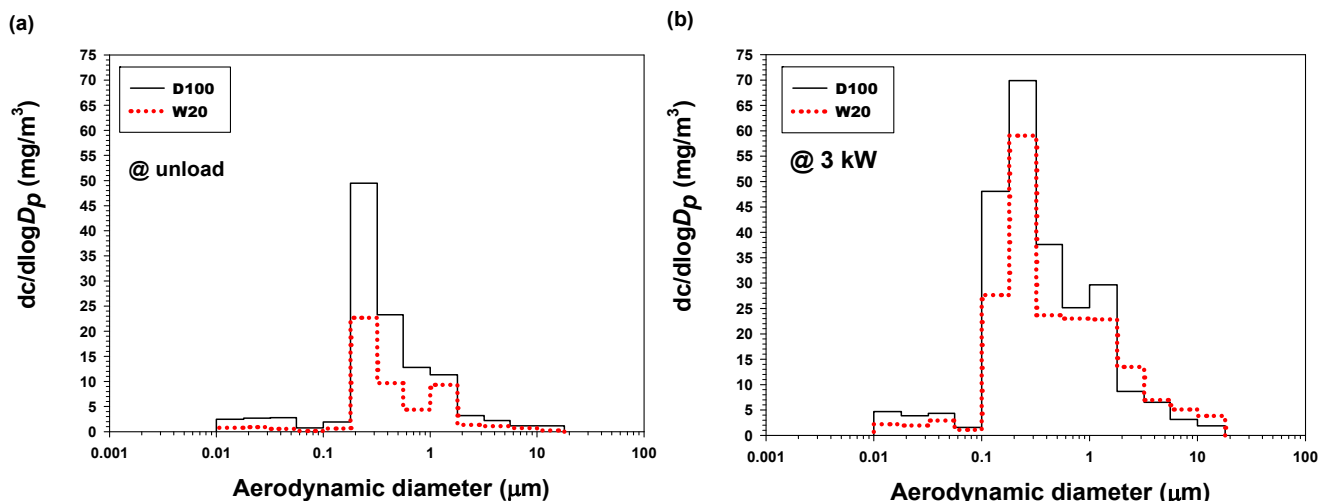


Fig. 1. Size distribution of DPMS emitted from diesel-engine generator fuelled with D100 and W20 under (a) 0 (unloaded) and (b) 3 kW loads.

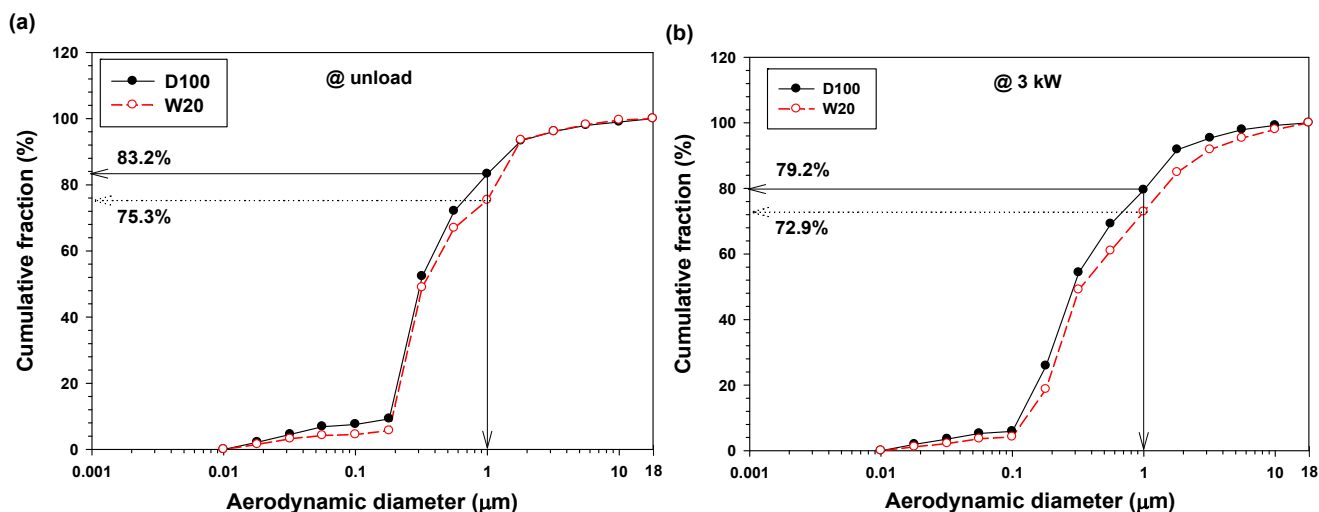


Fig. 2. Accumulation rate of DPMS emitted from diesel-engine generator fuelled with D100 and W20 under (a) 0 (unloaded) and (b) 3 kW loads.

visibility and contained carcinogenic substances that negatively affected human health. Watson *et al.* (2001) contested that the OC of micro particles in the atmosphere is mainly composed by carbon-containing organic matter (with complicated structures) such as aliphatics, aromatic hydrocarbon, organic acids. It is a serious health concern when the OC content of micro particles in the atmosphere is high. Canesi *et al.* (2008) demonstrated that in mussel hemocytes, like in mammalian cells, nanosized carbon black (NCB) exposure could induce inflammatory processes.

In this study, compared to without-loading (0 kW), the loading at 3 kW, the use of D100 or W20, the OC contents were mostly reduced in sized PM (except $PM_{0.1}$ and $PM_{0.056}$). For the use of D100 and W20, the reductions of PM OC contents were 13.3–15.0% and 28.9–31.7%, respectively, while the PM EC contents increased by 27.5–29.1% and 37.9–41.4%, respectively (Table 2). This result proves that the waste-edible-oil-biodiesel used in this study

can be used as an alternative fuel for diesel generators to dramatically reduce the emission of particle-bound OC and EC.

Total-PAHs and Total-BaP_{eq} Concentrations in DPMS

According to Table 3, the use of W20 instead of D100 at 0 kW, the Total-PAHs concentrations of $PM_{0.01-0.056}$, $PM_{0.01-0.1}$, $PM_{0.01-1}$, $PM_{0.01-2.5}$, $PM_{0.01-10}$, and $PM_{0.01-18}$ were reduced by 71.4%, 69.6%, 82.2%, 81.3%, 81.2%, and 81.3%, respectively, and the corresponding Total-BaP_{eq} concentrations were reduced by 64.2%, 56.6%, 78.3%, 76.7%, 76.3%, and 76.3%, respectively. Under 3 kW, the reductions were 82.9%, 82.7%, 59.3%, 57.3%, 57.1%, and 56.7%, respectively for Total-PAHs concentration, and 92.9%, 92.5%, 68.9%, 64.8%, 62.5%, and 61.7%, respectively for Total-BaP_{eq} concentration. Therefore, with or without engine loading, the 20% addition of bio-diesel into petroleum diesel (W20) effectively reduced Total-PAHs (by 56.7–82.9%)

and Total-BaP_{eq} (by 56.6–92.9%) concentrations of micro particles emitted by the generator. Accordingly, the use of waste edible oil bio-diesel effectively reduced the emission of DPM-bound PAHs from the diesel engine generator.

When W20 was used instead of D100 at 0 kW, the Total-PAHs concentrations of PM_{0.01–0.1} and PM_{0.01–0.056} were reduce by 74.3% and 76.0%, respectively, with corresponding Total-BaP_{eq} concentration reductions of 76.9% and 81.6%, respectively. At 3 kW, the reductions were 81.9% and 81.6%, respectively for Total-PAHs concentration, and the reductions of corresponding Total-BaP_{eq} concentrations

reached 92.5% and 92.6%, respectively. In general, diesel and gasoline-powered engines may emit large amounts of PAHs that partition between gas and particle phases. Zielinska *et al.* (2004) concluded that particle-bound semivolatile PAHs and nonvolatile four- to six-ring PAHs dominated the submicrometer particles that were collected on 0.1–0.18, 0.18–0.32, and 0.32–0.56 µm MOUDI stages. Miguel *et al.* (1998) found that significant fractions of diesel-derived PAHs were present in both the ultrafine size mode ($D_p < 0.12$ µm) and the accumulation mode (0.12 µm $< D_p < 2$ µm) in a tunnel. In 2005, Miguel *et al.* (2005)

Table 3. Concentrations of tested 4–7 ring PAHs in DPMs emitted from diesel-engine generator fuelled with D100 and W20 under 0 (unload) and 3 kW loads.

PAHs	PAHs Concentrations (µg/m ³) (n = 3)											
	D100						W20					
	PM ₁₈	PM ₁₀	PM _{2.5}	PM ₁	PM _{0.1}	PM _{0.056}	PM ₁₈	PM ₁₀	PM _{2.5}	PM ₁	PM _{0.1}	PM _{0.056}
FL	31.7 (±8.23)	30.7 (±7.74)	27.2 (±6.14)	22.8 (±3.18)	5.63 (±1.25)	4.77 (±1.35)	3.93 (±0.13)	3.81 (±0.13)	3.46 (±0.13)	2.86 (±0.21)	1.32 (±0.05)	1.18 (±0.03)
Pyr	35.6 (±15.7)	34.4 (±14.7)	29.5 (±10.6)	24.0 (±7.70)	5.71 (±3.78)	4.84 (±3.49)	3.97 (±0.09)	3.86 (±0.10)	3.56 (±0.12)	2.89 (±0.27)	1.14 (±0.08)	1.01 (±0.05)
CYC	7.46 (±2.09)	7.25 (±2.27)	6.86 (±2.52)	6.12 (±2.50)	0.70 (±0.21)	0.53 (±0.22)	0.96 (±0.10)	0.93 (±0.08)	0.90 (±0.08)	0.77 (±0.14)	0.10 (±0.01)	0.09 (±0.01)
BaA	3.19 (±1.53)	3.12 (±1.55)	3.02 (±1.57)	2.69 (±1.33)	0.19 (±0.06)	0.17 (±0.06)	0.27 (±0.02)	0.27 (±0.02)	0.26 (±0.03)	0.21 (±0.03)	0.03 (±0.002)	0.03 (±0.001)
CHR	9.92 (±4.41)	9.71 (±4.54)	9.04 (±4.63)	7.98 (±3.72)	0.78 (±0.15)	0.60 (±0.28)	2.19 (±0.63)	2.11 (±0.59)	1.93 (±0.52)	1.60 (±0.35)	0.61 (±0.15)	0.57 (±0.15)
BbF	5.22 (±2.03)	5.13 (±2.04)	4.94 (±2.05)	4.31 (±1.39)	1.41 (±0.39)	1.24 (±0.33)	1.86 (±0.29)	1.85 (±0.29)	1.69 (±0.43)	1.39 (±0.63)	0.38 (±0.21)	0.26 (±0.19)
BkF	5.81 (±2.60)	5.77 (±2.62)	5.31 (±2.78)	4.30 (±2.28)	0.61 (±0.23)	0.47 (±0.16)	2.33 (±0.74)	2.28 (±0.73)	2.13 (±0.62)	1.83 (±0.46)	0.45 (±0.12)	0.37 (±0.11)
BeP	3.63 (±1.48)	3.62 (±1.48)	3.35 (±1.35)	2.91 (±1.13)	0.26 (±0.06)	0.22 (±0.004)	0.65 (±0.14)	0.64 (±0.14)	0.61 (±0.16)	0.51 (±0.21)	0.07 (±0.05)	0.04 (±0.01)
BaP	9.50 (±2.43)	9.43 (±2.42)	8.92 (±2.40)	7.82 (±1.15)	0.49 (±0.34)	0.39 (±0.39)	2.77 (±1.36)	2.74 (±1.34)	2.62 (±1.37)	2.23 (±0.94)	0.20 (±0.07)	0.17 (±0.07)
PER	4.48 (±2.41)	4.32 (±2.39)	3.57 (±2.41)	2.93 (±1.69)	0.91 (±0.71)	0.71 (±0.49)	1.62 (±1.45)	1.61 (±1.45)	1.21 (±0.81)	1.02 (±0.64)	0.11 (±0.05)	0.09 (±0.04)
IND	2.00 (±1.33)	1.97 (±1.34)	1.90 (±1.36)	1.78 (±1.31)	0.58 (±0.27)	0.35 (±0.18)	0.99 (±0.17)	0.97 (±0.18)	0.92 (±0.25)	0.79 (±0.34)	0.14 (±0.12)	0.06 (±0.05)
DBA	4.54 (±1.19)	4.49 (±1.22)	4.22 (±1.41)	3.56 (±1.08)	1.11 (±0.18)	1.00 (±0.21)	1.19 (±0.43)	1.18 (±0.43)	1.05 (±0.22)	0.79 (±0.29)	0.14 (±0.15)	0.05 (±0.02)
BbC	6.23 (±2.44)	5.87 (±2.28)	5.30 (±2.04)	3.41 (±1.25)	2.05 (±0.42)	1.79 (±0.34)	4.35 (±0.07)	4.34 (±0.06)	3.69 (±1.04)	2.50 (±1.55)	0.76 (±0.72)	0.33 (±0.28)
BghiP	6.21 (±2.33)	6.19 (±2.32)	5.74 (±2.06)	4.52 (±1.17)	1.16 (±0.34)	1.02 (±0.33)	1.04 (±0.39)	1.03 (±0.39)	0.99 (±0.35)	0.82 (±0.14)	0.11 (±0.06)	0.07 (±0.04)
COR	3.60 (±0.54)	3.42 (±0.50)	2.96 (±0.37)	2.65 (±0.27)	1.70 (±0.36)	1.53 (±0.35)	0.93 (±0.09)	0.88 (±0.08)	0.75 (±0.05)	0.67 (±0.04)	0.42 (±0.04)	0.37 (±0.03)
MMW-PAHs	80.4 (±24.2)	77.9 (±22.7)	68.7 (±17.4)	57.5 (±10.4)	12.3 (±4.90)	10.4 (±4.78)	10.4 (±0.66)	10.0 (±0.59)	9.21 (±0.50)	7.57 (±0.36)	3.10 (±0.27)	2.79 (±0.22)
HMW-PAHs	58.7 (±17.3)	57.5 (±17.2)	53.1 (±17.7)	44.3 (±11.3)	11.0 (±1.70)	9.24 (±1.13)	18.7 (±4.07)	18.5 (±4.04)	16.6 (±1.68)	13.3 (±3.27)	2.89 (±1.35)	1.91 (±0.55)
Total-PAHs	139 (±37.7)	135 (±36.1)	122 (±32.1)	102 (±18.2)	23.3 (±5.69)	19.6 (±5.74)	29.0 (±4.53)	28.5 (±4.43)	25.8 (±1.93)	20.9 (±3.62)	5.99 (±1.52)	4.70 (±0.42)
BbF+BaP+DBA	19.2 (±5.63)	19.0 (±5.65)	18.1 (±5.80)	15.7 (±3.49)	3.01 (±0.56)	2.62 (±0.58)	5.81 (±1.69)	5.77 (±1.67)	5.36 (±1.28)	4.41 (±1.04)	0.72 (±0.31)	0.47 (±0.13)
Total-BaP _{eq}	16.7 (±4.57)	16.5 (±4.61)	15.6 (±4.84)	13.5 (±3.07)	1.99 (±0.24)	1.69 (±0.41)	4.64 (±1.83)	4.60 (±1.81)	4.31 (±1.59)	3.56 (±1.07)	0.46 (±0.21)	0.31 (±0.03)

Table 3. (continued).

PAHs	PAHs Concentrations ($\mu\text{g}/\text{m}^3$) (n = 3)												TEF ^a
	3 kW												
	D100						W20						
	PM ₁₈	PM ₁₀	PM _{2.5}	PM ₁	PM _{0.1}	PM _{0.056}	PM ₁₈	PM ₁₀	PM _{2.5}	PM ₁	PM _{0.1}	PM _{0.056}	
FL	60.4	57.9	50.8	43.5	16.9	14.3	28.0	26.0	23.0	19.8	7.01	6.00	0.001
	(±9.67)	(±9.64)	(±10.5)	(±9.67)	(±4.04)	(±3.69)	(±9.15)	(±8.16)	(±6.97)	(±5.31)	(±2.11)	(±1.88)	
Pyr	51.0	49.2	43.9	37.6	12.5	10.5	26.3	24.9	22.4	19.3	6.13	5.23	0.001
	(±11.4)	(±11.5)	(±12.1)	(±11.1)	(±2.84)	(±2.61)	(±9.12)	(±8.45)	(±7.28)	(±5.27)	(±2.03)	(±1.92)	
CYC	14.8	13.7	9.6	6.3	3.26	2.69	2.55	2.43	2.04	1.75	0.58	0.50	0.1
	(±17.2)	(±15.9)	(±9.79)	(±5.01)	(±2.07)	(±1.66)	(±1.24)	(±1.26)	(±1.29)	(±0.90)	(±0.04)	(±0.06)	
BaA	2.57	2.46	2.13	1.83	0.71	0.60	1.49	1.44	1.37	1.14	0.18	0.15	0.1
	(±1.72)	(±1.65)	(±1.37)	(±1.12)	(±0.32)	(±0.26)	(±1.45)	(±1.27)	(±1.16)	(±1.07)	(±0.02)	(±0.03)	
CHR	16.8	16.2	13.7	11.0	2.95	2.18	12.4	11.5	9.93	8.33	3.18	2.82	0.01
	(±2.52)	(±2.05)	(±1.78)	(±1.55)	(±1.01)	(±1.22)	(±5.86)	(±5.49)	(±5.12)	(±3.74)	(±1.71)	(±1.42)	
BbF	11.4	11.2	10.6	10.1	7.75	6.46	4.79	4.59	4.00	3.26	0.81	0.62	0.1
	(±10.1)	(±10.1)	(±10.0)	(±10.1)	(±11.1)	(±9.15)	(±4.64)	(±3.69)	(±3.57)	(±2.20)	(±0.35)	(±0.06)	
BkF	16.7	16.1	14.9	12.7	5.13	4.31	7.76	7.04	5.90	4.91	2.02	1.73	0.1
	(±0.27)	(±0.28)	(±0.23)	(±0.91)	(±3.69)	(±3.12)	(±2.99)	(±2.45)	(±2.11)	(±1.69)	(±0.73)	(±0.60)	
BeP	3.02	2.96	2.68	2.35	1.39	1.20	1.63	1.57	1.44	1.17	0.21	0.18	0.01
	(±1.26)	(±1.30)	(±1.20)	(±1.23)	(±1.24)	(±1.00)	(±1.69)	(±1.69)	(±1.48)	(±0.99)	(±0.06)	(±0.06)	
BaP	9.91	9.80	9.24	7.95	2.86	2.43	6.69	6.33	5.60	4.78	0.98	0.85	1
	(±4.55)	(±4.48)	(±4.30)	(±3.52)	(±2.06)	(±1.75)	(±3.14)	(±2.89)	(±2.64)	(±2.25)	(±0.44)	(±0.40)	
PER	34.2	33.2	25.4	18.2	14.8	11.5	3.69	3.59	3.09	2.78	0.44	0.39	0.001
	(±25.1)	(±24.6)	(±18.8)	(±19.4)	(±21.2)	(±16.4)	(±4.58)	(±4.59)	(±4.25)	(±3.38)	(±0.13)	(±0.08)	
IND	12.6	12.3	10.3	9.35	8.56	7.69	1.85	1.82	1.72	1.55	0.39	0.34	0.1
	(±10.1)	(±10.0)	(±8.09)	(±7.10)	(±7.26)	(±6.82)	(±1.37)	(±1.38)	(±1.30)	(±0.73)	(±0.12)	(±0.15)	
DBA	22.5	22.4	20.7	19.2	18.5	15.5	2.50	2.45	2.17	1.70	0.35	0.27	1
	(±18.8)	(±18.8)	(±17.2)	(±15.7)	(±15.5)	(±13.1)	(±4.56)	(±4.56)	(±4.78)	(±0.90)	(±0.18)	(±0.02)	
BbC	44.1	43.5	42.2	41.2	38.7	32.1	7.65	7.49	6.20	5.49	1.44	1.13	– ^b
	(±50.6)	(±50.8)	(±51.1)	(±51.7)	(±52.1)	(±43.1)	(±8.60)	(±8.07)	(±7.43)	(±6.24)	(±1.11)	(±0.23)	
BghiP	8.84	8.74	8.09	7.48	6.67	6.04	2.88	2.80	1.85	1.40	0.31	0.26	0.01
	(±6.76)	(±6.68)	(±6.54)	(±6.56)	(±6.50)	(±6.10)	(±3.06)	(±3.09)	(±2.73)	(±2.89)	(±0.05)	(±0.07)	
COR	12.7	11.8	9.99	8.81	5.57	4.72	5.56	5.15	4.32	3.78	2.38	2.05	0.001
	(±2.27)	(±2.15)	(±1.74)	(±1.50)	(±0.55)	(±0.35)	(±1.25)	(±1.04)	(±0.85)	(±0.63)	(±0.53)	(±0.51)	
MMW-PAHs	131	126	111	93.9	33.0	27.6	67.7	63.8	56.6	48.6	16.5	14.2	
	(±24.7)	(±24.4)	(±25.2)	(±23.0)	(±7.65)	(±7.28)	(±25.2)	(±23.3)	(±20.5)	(±14.6)	(±5.86)	(±5.25)	
HMW-PAHs	191	186	164	144	113	94.7	47.6	45.3	38.4	32.6	9.92	8.32	
	(±108)	(±108)	(±101)	(±102)	(±106)	(±86.3)	(±41.3)	(±32.6)	(±29.4)	(±25.7)	(±1.68)	(±1.58)	
Total-PAHs	321	312	274	238	146	122	115	109	95.0	81.1	26.4	22.5	
	(±109)	(±109)	(±97.5)	(±94.8)	(±102)	(±82.4)	(±70.2)	(±69.9)	(±62.7)	(±45.2)	(±6.66)	(±6.82)	
BbF+BaP+DBA	43.8	43.4	40.6	37.2	29.1	24.4	14.0	13.4	11.8	9.74	2.14	1.74	
	(±27.0)	(±26.9)	(±25.6)	(±24.6)	(±22.6)	(±18.5)	(±9.69)	(±9.60)	(±7.19)	(±5.04)	(±0.64)	(±0.46)	
Total-BaP _{eq}	38.7	38.2	35.1	31.5	24.1	20.3	11.3	10.7	9.46	7.90	1.79	1.50	
	(±26.1)	(±25.9)	(±23.3)	(±20.7)	(±16.8)	(±14.0)	(±10.0)	(±10.0)	(±5.58)	(±3.32)	(±0.52)	(±0.51)	

^a Malcom and Dobson, 1994; ^b No TEF has been suggested.

investigated the particle size distribution down to 10 nm for 12 USEPA priority PAHs collected by MOUDI/Nano-MOUDI samplers from the ambient air at the central area of Los Angeles. They found bi- or tri-modal size distributions for the same PAH species, and the major peaked mostly in the 0.056–0.1 μm range, and two minor ones in the 0.010–0.018 and/or 0.18–2.5 μm ranges. Lin *et al.* (2008a) also found that the average particulate total-PAHs in five samplings displayed a tri-modal distribution with a major peak in the *Aitken* mode (0.032–0.056 μm), and about half of the particulate total-PAHs were in the ultrafine size range.

Cell Toxicity of Organic Solvent Extracted Materials in DPMs

This study used human male monocytic cell line (U937) to expose to DMSO (dimethyl sulfoxide) in organic solvent extracted materials from sized particles for 24 hours before taking samples. The MTT assay was then introduced to analyze the survival rate of U937 in each PM organic solvent extract to examine the cell toxicity of organic solvent extracted materials from sized PMs, when D100 and W20 were used in the diesel-engine generator at 0 and 3 kW. As shown in Fig. 3, at 0 kW, the use of D100

exhibited a higher cell toxicity of U937 in organic solvent extracted materials from all sized PMs than the use of W20, but the BLM toxicity is low. Compared to D100, the use of W20 reduced the cell toxicity of organic solvent extracted materials in sized PMs by 28–73%. At 0 and 3 kW, the use of W20 place of D100, with the exception of PM_{1.8–3.2} and PM_{10–18}, reduced cell toxicity of U937 in PM organic solvent extracted materials (by 5–71%). In addition, when D100 is used at 3 kW, the cell toxicity of organic solvent extracted materials in nano particles (PM_{0.01–0.018}, PM_{0.018–0.032}, and PM_{0.032–0.056}) was higher than that of BLM

value; also, the cell toxicities of all sized PMs increased at the higher engine loading when using W20, suggesting that the increase of generator engine load increase the cell toxicity of DPMs. The results suggest that the W20 bio-diesel is useful to reduce the cell toxicity of DPMs. PAHs are one of those associated with the toxicity of DPMs. Schirmer *et al.* (1998) reported that cell toxicity caused by PAHs in PM correlated with water or fat solubility because PAHs with a higher ring number have a lower water solubility that can prevent them from receiving cell membrane damage. Body fluids outside of the pulmonary epithelial cells of

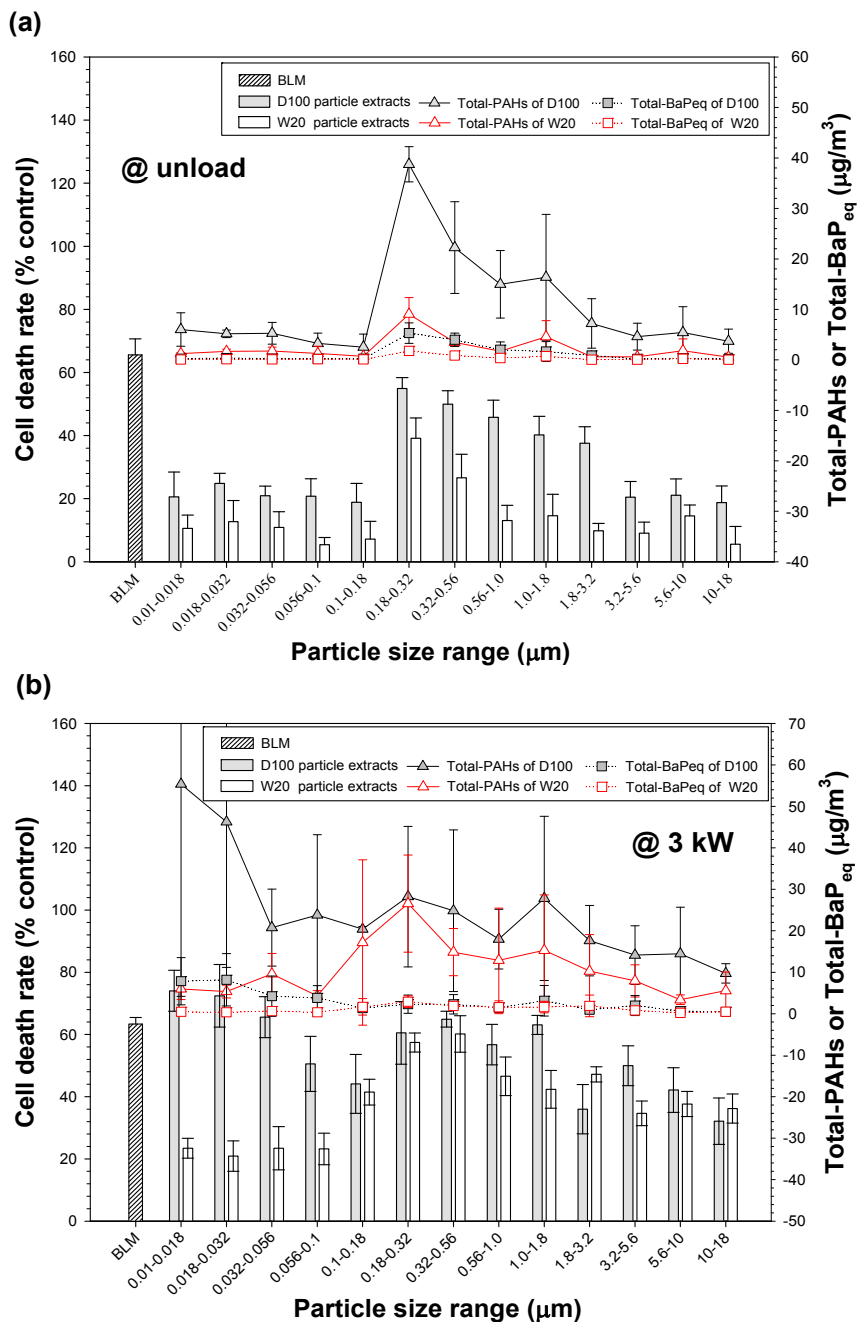


Fig. 3. PAH-associated BaP_{eq} and extract cytotoxicities (cell death rates) of DPMs emitted from diesel-engine generator fuelled with D100 and W20 under (a) 0 (unloaded) and (b) 3 kW loads. Cells ($4 \times 10^4/100 \mu\text{L}/\text{well}$) were treated with $1 \mu\text{L}$ of bleomycin (BLM, 75 U/mL) or particle extracts, and incubated in CO₂ incubator at 37°C for 24 h.

human bodies contain fat and non-water soluble content beneficial to the solubility of PAHs with high ring numbers and therefore, the reduction of the contents of PAHs with a high ring number in PM or PAHs with high toxic equivalents will reduce health hazards. The use of bio-diesel to reduce PAHs emission of diesel engines and the toxicity of emitted micro particles was also reported in earlier works (Bünger *et al.*, 2006; Di *et al.*, 2009; Tsai *et al.*, 2011b). Recent studies have found that smaller particles pose more threats to the damages and pathological change of pulmonary cells than larger ones; the smaller the particles are, the larger the total surface area and the more the toxic substance carried with (such as metal elements, endotoxins, and water soluble ion contents) (Lin *et al.*, 2005; Shima *et al.*, 2006; Chuang *et al.*, 2010a, b; Kim *et al.*, 2011; Hsieh *et al.*, 2011). In this study, however, for both D100 and W20 cases, the cell death rates induced by organic solvent extracted materials of submicron particles were higher than those of nano ($PM_{0.01-0.056}$)/ultrafine ($PM_{0.056-0.1}$) and micron particles at unload, and a similar tendency was also observed at 3 kW when using W20 (Fig. 3). Interestingly, the magnitudes of cell death rates per PM mass were in order nano/ultrafine > micron > submicron particles for both D100 and W20 cases at unload and 3 kW conditions (Fig. 4). The fact that nano/ultrafine particles have higher total surface area than larger particles should be responsible for their higher unit-mass cell death rates, but the higher unit-mass cell death rates for micron-sized particles than for submicron particles was partially attributable to mass effect because the mass of submicron particles were noticeably greater than those of micron-sized particles (Fig. 1). Nevertheless, at both 0 and 3 kW, the use of W20 in place of D100 lowered the unit-mass cell death rates of organic solvent extracted materials of most sized PMs. The results of this study show that the proposed blend (20% waste-edible-oil-biodiesel + 80% diesel) is promising for use as an alternative fuel for diesel

generators to reduce the risk of DPMs on human health.

CONCLUSIONS

The results showed that compared with D100, using W20 could effectively reduce the PM (21.0–72.8%), particulate EC (2.69–57.3%), particulate OC (30.8–47.5%), Total-PAHs (56.7–82.9%), and Total-BaP_{eq} (56.6–92.9%) in all sized particles emitted from the diesel generator, regardless of engine load. The reduction of $PM_{0.01-18}$ (81.3%) was higher in the lung respirable accumulation mode particles ($PM_{0.1-1}$). The reduction of PM_{10} emission was mainly contributed by the accumulation mode particles (88.7%). Compared with no engine load, despite using D100 or W20 at 3 kW engine load, with the exception of $PM_{0.01-0.1}$ and $PM_{0.01-0.056}$, the OC contents of sized PMs were all reduced (reductions = 13.3–15.0% and 28.9–31.7% for using D100 and W20, respectively), but the EC content increased (by 27.5–29.1% and 37.9–41.4%, respectively). Regardless of fuel and loading, the emitted $PM_{0.01-18}$ exhibited a single-modal distribution and peaked in the submicron size range (0.18–0.32 μm). Moreover, the cell toxicity to U937 (per μg PM) of particle extract (of organic solvent) was higher for nano ($PM_{0.01-0.056}$) and ultrafine ($PM_{0.056-0.1}$) particles than for the other sized particles. Compared with D100, using W20 could reduce the cell toxicity to U937 (per μg PM) of extracts from all sized particles, especially at 3 kW engine load for nano and ultrafine particles (reduction = 32–46%).

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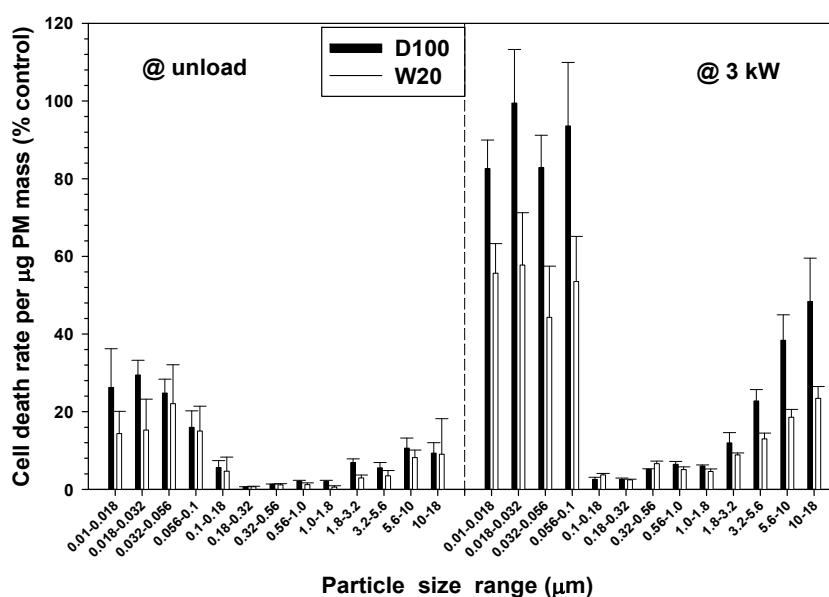


Fig. 4. Unit mass cytotoxicity (cell death rate per unit mass) of DPMs emitted from diesel-engine generator fuelled with D100 and W20 under (a) 0 (unloaded) and (b) 3 kW loads.

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