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Polycyclic Aromatic Hydrocarbons (PAH) and nitrated analogs associated with total particulate emissions from a Euro V engine fuelled with Diesel/Biodiesel Blends

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ABSTRACT

Among the new technologies developed for the heavy-duty fleet, the use of SCR (Selective Catalytic Reduction) after-treatment system in standard Diesel engines associated with biodiesel and diesel mixtures is an alternative in use to control the legislated pollutants emission. Nevertheless, there is an absence of knowledge about the synergic behavior of these devices and the ratio of fuels regarding the emissions of unregulated substances as the Polycyclic Aromatic Hydrocarbons (PAH) and Nitro-PAH, both recognized for their carcinogenic and mutagenic effects on humans. Therefore, the goal of this study is the quantification of PAH and Nitro-PAH present to Total Particulate Matter (TPM) emitted from the EuroV engine fuelled with ultra-low sulphur diesel and soybean biodiesel in different percentages. Total PM sampling was performed using a EuroV – SCR engine operating in European Stationary Cycle (ESC). The PAH and Nitro-PAH were extracted from TPM emitted using an Accelerated Solvent Extractor and quantified by GC-MS. Our results indicated that the use of SCR system and the largest fraction of biodiesel studied may have been the main factor responsible for the lowest emission of total PAH. Individual emissions among the 16 priority PAH and 3 Nitro-PAH quantified from the TPM did not show a significant difference for the studied conditions. The total equivalent quotient (TEQ) was lower when using 20% biodiesel, in comparison with 5% biodiesel on the SCR system, reaffirming the low toxicity emission with the use of biodiesel. Our data also reveal that use of SCR system, on its own, suppress the Nitro-PAH compounds. In general, the use of larger fractions of biodiesel coupled with the SCR system showed the lowest PAH and Nitro-PAH emissions, meaning lower toxicity and, consequently, a potential lower risk to human health. From the emission point of view, the results of this work also demonstrated the viability of the Biodiesel programs, in combination with the SCR systems, does not require any engine adaptation.

Keywords: Selective Catalytic Reduction (SCR); Diesel Emissions; Biodiesel; Polycyclic Aromatic Hydrocarbons (PAH); Nitro-PAH; Toxic Equivalents Quotients (TEQ).

1. Introduction

Diesel engine exhaust emissions were classified as a carcinogen by the International Cancer Research Agency (ICRA) in 2013, thereby increasing the prior challenges that policymakers in several countries face (Diaz-Sanchez et al., 1994; Lighty et al., 2000; Ravindra et al., 2008; Reşitoğlu et al., 2015; Zielinska et al., 2004)). PAH and Nitro-PAH (nitrated PAH) are the most toxic compounds among the complex mixture of gases and particles that comprise diesel engine exhaust aerosols (US HSS US, 2005; Slezakova et al., 2012; Hu et al., 2013; Wang et al., 2013; Yilmaz and Davis, 2016). Composed of two or more fused aromatic rings, these organic compounds have been tested for their carcinogenic activity by the ICRA, with Benzo[a]pyrene being classified as a class 1 carcinogen (therefore carcinogenic to humans0. Dibenzo[a,l]pyrene is considered as probably carcinogenic to humans (class 2A) and Dibenzo[a,i]pyrene and Dibenzo[a,h]pyrene as possibly carcinogenic to humans (class 2B) (Zielinska et al., 2004; WHO, 2013). When these compounds are associated with diesel/biodiesel blends exhaust particle-phase emissions, it was found that the highest concentrations were in the fine particulate matter ($PM_{2.5}$) (He, 2016; Guan et al., 2017). In addition therefore to the increased risk of developing acute and chronic cardiopulmonary diseases upon exposure to high PM 2.5 mass concentration, these findings seem to suggest an increased risk for lung cancer (US HSS US, 2005; Ravindra et al., 2008; Martin et al., 2017).

Current emission regulations implemented in the U.S. and European Union (EU), US 2010 and Euro VI, respectively, adopt a limit for NO_x and PM emission that is ten times lower than the levels allowed in 2000. Countries where these emission limits are enforced and implemented (U.S., EU, Canada, Japan and South Korea) project a 26-fold reduction in PM emissions by 2045 (Posada et al., 2016). On the other hand, Brazil, Russia, India, China, Australia and Mexico whose gross domestic product heavily depends on heavy-duty vehicle transport, have yet to fully implement the equivalent emission standards, even though the increased risks are acknowledged. These regulations require extensive deployment of advanced engine tuning, the addition of two or more after-treatment devices, and the use of low sulphur diesel content, therefore potentially fleet renovations. Instead, these countries still enforce the Euro V and equivalent regulations where SCR (Selective Catalytic Reduction) or EGR (exhaust gas recirculation) after-treatment systems remain the main strategies to reduce NOx and PM emission (Du and Miller, 2017).

As an additional strategy to reduce pollutants from diesel vehicles, many countries like Canada, Australia, Brazil, China and Germany are promoting the development, production and use of alternatives fuels (biofuels and natural gas) seeking a better balance to fuel economy and thereby improving green freight programs in the long term (Du and Miller, 2017). Biodiesel is already used in several countries and seems to be a promising alternative, as it can be used in diesel engines without major modifications. Despite the high cost of production when compared to diesel, biodiesel can qualitatively and quantitatively reduce regulated pollutant emissions (Ratcliff et al., 2010; He et al., 2010; Tadano et al., 2014; Sadiktsis et al., 2014). Several researches successfully demonstrated a consistent reduction for hydrocarbons (HC), total particulate matter (PM) and carbon monoxide (CO) emissions while recognizing an increase in nitrogen dioxide (NO_x) when biodiesel is used (Ravindra et al., 2008; Sadiktsis et al., 2014; Borillo et al., 2015; He, 2016). On the other hand, research evidence on the influence of alternative fuels on PAH and Nitro-PAH emissions are sparce. Guarieiro et al. (2014) concluded that the biodiesel additions decrease PAH emissions, which is in agreement with the conclusions made by Yilmaz and Davis (2016), He et al. (2010) and Lim et al. (2014). Westphal et al. (2013) tested PAH emissions using a turbo-intercooled engine with 6 cylinders operating in the European Stationary Cycle (ESC) fuelled with hydrotreated vegetable oil and jatropha methyl ester. The authors also demonstrated a slight reduction in PAH emission when low percentages of biodiesel was added and showed that toxicological effects depends on the biodiesel origin. Casal et al. (2014) tested a Euro III engine fuelled with standard diesel (B0), B5 (5% biodiesel) and B20 (20% biodiesel) and concluded that biodiesel mixed with diesel increases the production of PAH and Alkyl PAH in the engine exhaust emissions.

Changes in fuel alone are however not sufficient to meet the new or the old standards and use of after-treatment systems is crucial. The SCR system is still the most widely used, due to its efficiency and selectivity to reduce NO_x emissions, (Tadano et al., 2014). The injection of urea and a catalyst ensures reductions of up to 90% in NO_x emissions, through a reduction reaction of NO_x and NH_3 resulting in nitrogen and water (Amanatidis et al., 2014; Bacher et al., 2014). Nevertheless, SCR technology is not without its challenges, such as the emission of NH_3, stoichiometric disproportion of urea consumption, cost, and deactivation of the catalyst by deposition (Cheruiyot et al., 2017). The best reported SCR efficiency are achieved at high engine loads and temperatures (approximately 400 ºC) (Cheruiyot et al., 2017). However, the added urea increases the probability of unintended formation of Nitro-PAH thought PAH nitration (Liu et al., 2015).

The combination of low sulphur fuels (ultra-low sulphur diesel-ULSD), new engine technologies, after-treatment systems (SCR, EGR, DOC – Diesel oxidation catalyst, DPF – Diesel particulate filter) and the use of biofuels are the current strategy to achieve the regulatory reductions worldwide (Ratcliff et al., 2010; Carrara and Niessner, 2013; Hu et al., 2013; Sadiktsis et al., 2014; Reşitoğlu et al., 2015; He et al., 2016). However, there is insufficient knowledge and some disagreement in open literature with regards to the potential synergistic effect on unregulated emissions by vehicles equipped with SCR and the use of different biodiesel blends.

This paper describes the PAH and Nitor-PAH emission concentrations from an engine, which is in compliance with the standard emissions determined in PROCONVE P7/Euro V. Furthermore, the potential synergistic effect of the SCR after-treatment and the use of diesel S-10 and different soybean biodiesel blends on emissions have been investigated. Those pollutants are not regulated worldwide, even though they are proven to present several harmful effects on human health.

2. Materials and methods

2.1. Sampling

The engine emission tests were performed at the Lactec Institute's Laboratory for Vehicular Emissions in partnership with the Federal University of Paraná, Curitiba, Brazil. The tested engine is currently used in trucks and buses around the world, and has an individual four-valve cylinder head, cross-flow arrangement, common rail injection and SCR after-treatment system. The test engine fulfils the European Union Rregulation (EC - Nº 595/2009 of the European Parliament and of the Council of 18 June 2009) requirements for EuroV emission standards with a urea-SCR system and is in accordance with the P7 phase of the PROCONVE (Vehicular Air Pollution Control Program) in Brazil. The engine details are specified in Table 1.

Table 1. Engine specifications, model 2012.

Specifications

Emission	EuroV "Heavy Duty"/Proconve P7
Configuration	4 cylinders, inline
Displacement	4.8 litters
Bore x Stroke	105 x 137 mm
Combustion System	Direct injection
Injection System	Common Rail Electronic
Aspiration	TGV Intercooler
Power Output	187 hp (139,7 kW) 2200 rpm
Peak Torque	720 Nm (73 kgf.m) 1200~1600 rpm
After-treatment	SCR

The engine works in conjunction with a dynamometer and a data acquisition system, both from AVL (Graz, Austria) to perform the emission and engine parameters measurements. The dynamometer can absorb power up to 440 kW at 6,000 rpm and a torque of 2.334 N m at 1.200−1.600 rpm. The European Steady Cycle (ESC) was selected as driving cycle for the dynamometer operation to allow PM sampling. It was designed to achieve high load factors and very high temperature on the exhaust gas (Ericsson, 2001; Mock et al., 2012). Sixty-five percent of the calculated emission responds to load and engine speed above 50%. That is conservative and captures most of the stop-and-go conditions of the drive cycle experienced by urban busses in rush hours (Tadano et al., 2014).

The PM sampling was performed using an AVL dilution system (Smart Sampler SPC 472 - Graz, Austria). This system prevents filter saturation, reduces the exhaust temperature and adjusts the dilution ratio. The sampling equipment has two filter supports, arranged in sequence, to achieve higher PM retention.

For PAH and Nitro-PAH analysis, total PM was collected on two borosilicate glass fiber filters, 70 mm of diameter, coated with fluorocarbon (Pallflex T60A20 fiberfilm). The filters were weighted prior and after the sampling using a Sartorius micro-balance (MSA2.7S-000-DF - Goettingen, Germany) following the NIOSH method 5000 and were stored below -18 ºC in cleaned glass containers until analysis. The NO_x

emissions were measured in-line by a SESAM i60 FT, Fourier transform infrared (FTIR) multi-component measurement system from AVL (Graz, Austria). Table S2 shows the experimental conditions set for the FTIR analysis.

2.2. Fuels

Ultra-Low Sulphur Diesel (ULSD – maximum of 10 ppm of sulphur) and soybean biodiesel were used to prepare two mixtures: B5 (ULSD with 5% of biodiesel) and B20 (ULSD with 20% of biodiesel). The B5 and B20 fuels were collected in amber glass bottles and stored protected from light at 25 °C. The fuel samples were previously characterized according to methods and essays described on American Society for Testing and Materials (ASTM) in Lacaut – Automotive Fuels Laboratory (certified on ISO 9001, ISO 14001 and ISO 17025) of the Federal University of Paraná, Curitiba, Brazil. Table 2 shows the fuel properties of the reference diesel fuels and the biodiesel blend used in this research, as tested, according with Standard ASTM Test Methods.

Table 2. Fuel Properties of B5 and B20 blends.

Property	B5	B20	Method
Sulphur, mg/kg	4	6	ASTM D5453
Cetane number	53.8	51.0	ASTM D6890
Flash point (°C)	45.5	70.5	ASTM D93
Viscosity at 40 °C (mm^2 s^{-1})	3.0	3.2	ASTM D445
Specific mass at 20 °C (kg m^{-3})	830.5	848.1	ASTM D4052

2.3. Extraction

All the sampled filters were extracted, using an Accelerated Solvent Extractor (ASE – Dionex, USA), with dichloromethane and methanol (4:1) at a pressure of 1.500 psi and temperature of 120 °C in three static extraction cycles of 5 minutes each. The total extracted volume was concentrated to near dryness with a slight nitrogen flow and recovered with 1.5 ml of dichloromethane. This procedure has been optimized, using different temperatures (100, 120, 150 and 180 °C) and extraction cycles (1, 2 and 3 cycles), based on the EPA' Standard Operation Procedure (California Standard Operation Procedure, SOP No. 144 2006 for the determination of PAH in particulate matter using GC-MS (gas chromatography with mass spectrometry). To assess the extraction efficiency, Naphthalene-d8 and Benz[a]anthracene-d12 were used as recovery standards. The recovery, in percentage, of Naphtalene-d8 was slightly lower (65-80%) than the recovery of the Benz[a]anthracene-d12 (84-114%).

2.4. Analysis

Samples and standards were analysed in triplicate, using a gas chromatograph (Perkin Elmer Clarus 680) coupled to a mass spectrometer (Perkin Elmer Clarus SQ 8 T Perkin Elmer - Waltham, USA). A fused silica capillary column (MS-5 30 mx 0.25 mm x 0.25 mm) from Sigma Aldrich (St. Louis, USA) was used to separate the PAH and Nitro-PAH. Helium was used as carrier gas at a constant flow rate of 1.0 ml min^{-1}. The volume injected was 1.5 µl in splitless mode with a pressure pulse. The oven temperature was programmed as follows: 1 minute at 40 °C, heated at a rate of 10 °C min^{-1} to 200 °C and maintained for 5 min, heated in sequence at a rate of 6 °C min^{-1} to 240 °C and maintained for 10 minutes and, finally heated to 300 °C at a rate of 10 °C min^{-1} and maintained for 5 minutes. The injector temperature, GC-MS interface and detector were maintained at 300, 270 and 260 °C, respectively. The mass spectrometer emission current was set at 350 µA, the electron energy at 70 eV (nominal) and analysis occurred with SIM (selected-ion monitoring) mode.

Acenaphthene-D10, Phenanthrene-D10 and Perylene-D12 (Sulpeco Analytical - Bellefonte, USA) were used as internal standards. Sixteen PAH recognised by USEPA (United States Environmental Protection Agency) as priority pollutants were analysed: Naphthalene, Acenaphthene, Acenaphthylene, Anthracene, Phenanthrene, Fluorene, Fluoranthene, Benzo[a]anthracene, Chrysene, Pyrene, Benzo[a]pyrene, Benzo[b]fluoranthene, Benzo[k]fluoranthene, Dibenzo[a,h]anthracene, Benzo[g,h,i]perylene, Indeno[1,2,3-c,d]pyrene . In addition, Benzo[e]pyrene, Perylene, 1-Nitronaphtalene, 2-Nitrofluorene, 3-Nitrofluoranthene, 1-Nitropirene and 7-Nitrobenz[a]anthracene were also analysed (standards solutions supplied by Dr. Ehrenstorfer - Augsburg, Germany). The list with all investigated compounds with their respective molecular mass, quantification ion and quantification limits (LOQ.) can be found in Table S1 of the supplementary material. The method accuracy was verified using the reference material SRM 1650b from NIST (National Institute Standards and Technology).

The PAH and Nitro-PAH concentrations were tested for significant differences using Analysis of Variance (ANOVA). For this purpose "R" software was used and significance was determined at a 95% confidence level.

3. Results and Discussion

Particle emissions from a EURO V engine, operating with and without selective catalytic reduction after-treatment, and testing ULSD with 5% of biodiesel (B5) and ULSD with 20% of biodiesel (B20), were analysed for their PAH and Nitro-PAH content. The results of the different PAH and Nitro-PAH detected are presented in Tables 3 and 5, respectively. We reported in previous published work (Borillo et al., 2015) that the SCR after-treatment reduced NO_x emissions (90% reduction observed for both: B5 and B20), as it was expected, and therefore this parameter was used to evaluate the performance efficiency of the SCR system.

3.1. PAH concentrations in PM exhaust emissions

Table 3 displays the identity of the PAHs emitted from a Euro V engine operating with an ESC cycle, using two different fuels and conditions, as well as their respective concentrations (in $\mu g\ kWh^{-1}$). Of the 23 PAHs, eight were not detected or were found below the quantification limit (Anthracene, Chrysene, Benzo[k]fluoranthene, Benzo[e]pyrene, Benzo[a]pyrene, Perylene, Indeno[1,2,3-cd]pyrene, Dibenzo[a,h]anthracene - Table S1).

Table 3 – PAH concentrations in PM exhaust emissions, in µg kWh^{-1}.

PAH (µg kWh^{-1})	B5 SCR-off			B5 SCR-on			B20 SCR-off			B20 SCR-on		
	Average	SD (Min – Max)	n	Average	SD (Min – Max)	n	Average	SD (Min – Max)	n	Average	SD (Min – Max)	n
Naphthalene *	0.076	-	1	0.036	0.010 (0.028 - 0.043)	2		< LoQ.			< LoQ.	
Acenaphthylene	0.047	-	1		< LoQ.			< LoQ.		0.043	0.004 (0.040 – 0.046)	2
Acenaphthene	0.138	-	1	0.075	0.014 (0.065 - 0.084)	2		< LoQ.			< LoQ.	
Fluorene	0.841	0.045 (0.809 - 0.873)	2	0.845	0.240 (0.476 - 1.100)	5	0.759	0.144 (0.619 - 0.987)	5	0.867	0.060 (0.824 - 0.908)	2
Phenanthrene	0.786	0.072 (0.679 - 0.875)	5	0.642	0.081 (0.577 - 0.755)	4	0.669	0.124 (0.512 - 0.876)	8	0.658	0.076 (0.604 - 0.711)	2
Fluoranthene	0.317	0.247 (0.136 - 0.622)	5	0.319	0.124 (0.184 - 0.444)	5	0.225	0.128 (0.106 - 0.388)	9	0.128	0.016 (0.105 - 0.139)	4
Pyrene	0.738	0.559 (0.284 - 1.400)	5	0.724	0.283 (0.388 - 1.000)	5	0.587	0.314 (0.289 - 0.993)	9	0.307	0.043 (0.246 - 0.345)	4
Benzo[a]anthracene *	0.085	-	1	0.081	0.011 (0.074 - 0.089)	2		< LoQ.			< LoQ.	
Benzo[b]fluoranthene *	0.152	0.021 (0.122 - 0.173)	4	0.229	0.122 (0.143 - 0.315)	2	0.128	0.014 (0.109 - 0.150)	9	0.121	0.014 (0.105 - 0.130)	3
Benzo[g.h.i]perylene	0.188	0.012 (0.178 - 0.201)	3	0.183	0.002 (0.181 - 0.185)	2	0.176	0.002 (0.174 - 0.179)	3		< LoQ.	
∑PAH (particles)		**3.37 ± 0.62****			**3.13 ± 0.42****			**2.54 ± 0.39****			**2.12 ± 0.11****	
PAHsC - Possibly carcinogenic for Humans*		0.313 (9.2 %)			0.347 (11.1 %)			0.128 (5.0 %)			0.121 (5.7 %)	

n – Number of valid samples; <LoQ. – Below quantification limit; SD – Standard Deviation; * IARC – group 2B; ** Uncertainties propagation for PAH sum (estimative).

Considering the sum of all PAHs, the B5 SCR-off condition had the highest value among the sampling conditions (3.37 ± 0.62 µg kWh^{-1}), followed by B5 SCR-on (3.13 ± 0.42 µg kWh^{-1}), B20 SCR-off (2.54 ± 0.39 µg kWh^{-}1) and the B20 SCR-on (2.12 ± 0.11 µg kWh^{-1}).

The results indicate therefore a slight reduction (7% and 16% respectively for B5 and B20) in total PAH emissions when the after-treatment technology is applied. Lee et al. (2015) described a moderate decrease of particle emissions for EURO V vehicles equipped with SCR system, in agreement to Mayer et al. (2007) and Czerwiski et al. (2011), who reported a reduction of 15% in particle concentration. Pietkäinen et al. (2015) studied the PM emissions from a turbocharged, intercooled, common rail, 4-cylinder non-road engine with SCR system and also reported a decrease in total particle number when the SCR converter was used, but there was an increase of nanoparticles, increasing the risk to human health. These findings therefore suggest that the SCR system reduces total particle emissions and thus it is possible that this may be the reason for the observed decrease in PAH emission.

What is more noticeable, however, is that the addition of 20% of biodiesel in the fuel mixture resulted in a decrease of 25% in PAH emissions in the particle-phase for SCR-off and 32% for SCR-on. Similar results were found by previous studies (Turrio-Baldassarri et al., 2004; He et al., 2010; Yilmaz and Davis, 2016; He et al., 2016; Martin et al., 2017). Our results concur with the findings of He et al. (2010), who compared several studies, concluding that, on average, a reduction of 23% in PAH emissions can be expected with a 20% addition of biodiesel to the fuel. Additionally, He et al. (2010) showed that PAH emissions from EURO II engines were two orders of a magnitude higher than those we report, therefore enlightening a decrease in PAH emissions due to the engine technology evolution.

Similarly, the PAHsC (possibly carcinogenic to humans PAH) were reduced by 59% and 65%, respectively, when B5 was replaced by B20, either with or without SCR. These results clearly demonstrate the beneficial influence on PAH emissions if biodiesel additions to fuel are made.

These results confirm the difference in combustion products between diesel and biodiesel, as highlighted by Ravindra et al. (2008), who stated that the pyrolysis and pyrosynthesis processes that diesel undergoes during combustion (in reduced oxygen conditions), will result in the formation of aromatic rings through condensation products. In the case of biodiesel combustion, PAH formation involves the thermal polymerization of the fatty acid methyl esters forming cyclohexane (Ratcliff et al., 2010), as there are no aromatic organic compounds present. Therefore, the incorporation of biodiesel in the mixture can lead to a reduction in PAH emissions due to the natural absence of PAH in biodiesel, and to its higher oxygen content. The observed result represents the different chemistry path of biodiesel combustion in relation to diesel and an improvement of the combustion process, reducing the PAH generated by incomplete combustion of fuel and lubricant oils.

On the other hand, this tendency is only observed when the B20 fuel was tested for PAHsC emissions and an increase (11%) was observed for the B5.

The average and standard deviation for the individual PAH associated with PM emission are presented in Fig 1. The major compound quantified in all the experimental conditions was Fluorene followed by Phenanthrene, Pyrene, Fluoranthene, Benzo[b]fluoranthene and Benzo[g,h,i]perylene. The latter compound presented concentrations below the LoQ for B20 during the SCR-on condition. **T**he high molecular weight PAH with three or more aromatic rings, recognized for their toxicity, contributed more to the total particle PAH emission, since they have a higher probability to condense during the combustion process (Ballesteros et al., 2010). Benzo[a]pyrene, among the most toxic PAH species, was not quantified for any of the conditions (LoQ - 3.36 ng ml^{-1}).

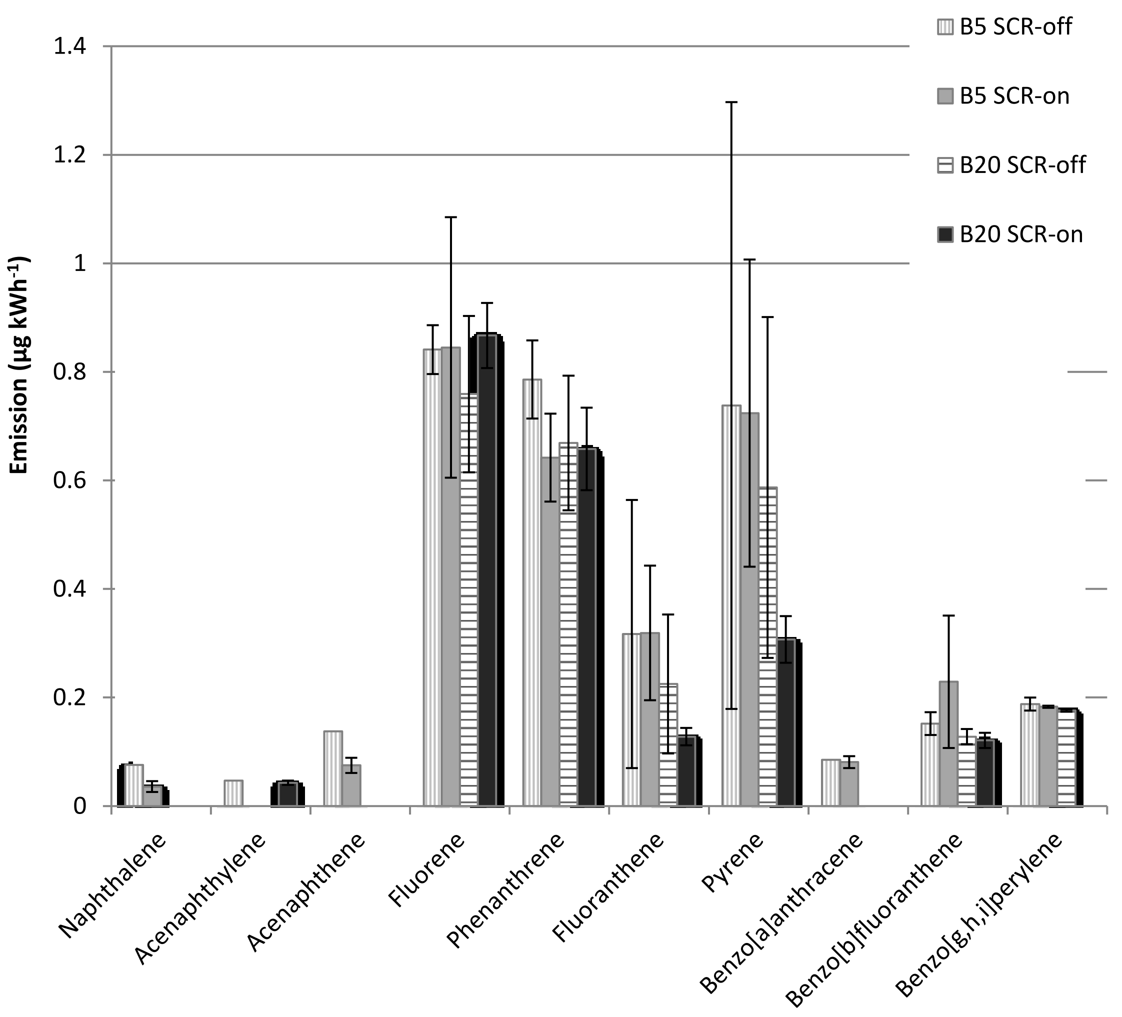

Fig 1. Average concentrations in µg kWh^{-1} (columns) and standard deviation (bars) for individual PAH associated with total PM emission for all experimental conditions.

The ANOVA test was performed for the substances that present N > 2: Fluorene, Phenanthrene, Fluoranthene, Pyrene and Benzo[b]fluoranthene. No statistical difference (95% confidence level) was found for the conditions tested, except Benzo[b]fluoranthene when comparing B5 SCR-off to B20 SCR-off and B5 SCR-on to B20 SCR-off. A similar result was obtained by Shah et al. (2012) who found no significant difference in PAH emissions considering the use of the SCR system and a low sulphur diesel. Liu et al. (2015) and Zhao et al. (2014) indicated that the Diesel Particulate Filter (DPF) is better to reduce the individual emissions of PHAs associated with PM, resulting in reductions greater than 90%. They also pointed out high tendency of engine manufacturers to couple more than one after-treatment system to achieve larger reductions in pollutants.

Considering biodiesel, Ballesteros et al. (2010) studied the particle-associated PAH emission using three diesel (ULSD)/biodiesel mixtures (B30, B70 and B100) with a 4-cylinder, 4-stroke, turbocharged, intercooled, 2.2 L Nissan diesel engine without any after treatment system operating in a transient cycle. The authors showed that the biodiesel addition decreased the low molecular weight PAH concentration (three aromatic rings) in relation to pure diesel and concluded that these emissions depend on the biodiesel origin. In agreement, no significant difference was found in the individual PAH emission for the present study. The same results were reported by Rojas et al. (Rojas et al., 2011) for 10 out the 16 priority PAH (as proposed by US EPA) when they monitored the particle emissions from a 20 year old engine fuelled with pure diesel (1000 ppm of sulphur) and B15. The authors also quantified high concentrations of Pyrene, Benzo[k]fluoranthene and Benzo[g,h,i]perylene when B15 was used. What I find missing here is talk about the PAHs which are absent when B20 is used (or at least below quantification limit). I think that is the more interesting bit. Discuss these and then also indicate what the health consequence of the reduction of these are, if any.

The beneficial effects of the biodiesel/SCR system similarly can be confirmed by the Toxicity Equivalent Quotients (TEQ) values, presented in Table 4.

Table 4 – Toxicity Equivalent Quotient of total of PAH emission in relation to Benzo[a]pyrene.

Fuel	B5 SCR-off	B5 SCR-on	B20 SCR-off	B20 SCR-on
TEQ (μg kWh^{-1})	0.029	0.036	0.017	0.014

The toxicity equivalent factors (TEF) for each PAH and the calculation procedure were the same as those proposed by Nisbet & Lagoy (1992) and US EPA (2006). The results indicate that the highest equivalent toxicity value was found in the B5 SCR-on combination and the lowest for the B20 SCR-on. The highest TEQ value, 0.036 μg kWh^{-1}, match the highest PAHsC, 0.347 μg kWh^{-1} (Table 3), for the B5 SCR-on condition, suggesting that Benzo[b]fluoranthene was mostly responsible for the increased toxicity. Shah et al. (2012) tested the influence of the SCR system on the TEQ values and concluded that the after-treatment increased the quotient due to the increase in PAH with high TEFs.

The preponderant factor leading to the reduction of the TEQ was the fuel variation. The reduction without using the SCR system from B5 to B20 was 41% and from B5 and B20 using SCR was 60%. Yilmaz et al. (2016) found low particle-bound PAH TEQ values and in the same order of magnitude for B10 and B20, comparing the pure regular diesel. The authors also reported that compared to B100, the B10 reduced toxicity by 49% and B20 by 46%. In addition to the discussion about the biodiesel life cycle and the economical/environment sustainability, this biofuel is an alternative to reduce PAH in particle-phase of Diesel engine emissions and toxicity.

3.2. Nitro-PAH associated to PM emissions

The concentration of five Nitro-PAHs associated with PM emissions from a Euro V (SCR) engine using diesel/biodiesel blends as fuels were assessed in this research and the results are shown in Figure 2 and Table 5. The 1-Nitropyrene was quantified with only one sample of each experimental condition. 2-Nitrofluoranthene and 7-Nitrobenzo[a]anthracene were below the quantification limit for all samples??. The Nitro-PAHs that were present in quantifiable amounts were XXXXXXX (name them). It is observed that 2-Nitrofluorene was up to 8.5 times higher than the other nitro-PAHs quantified and in general higher than any other individual PAH identified and quantified as indicated in Table 3. According to the World Health Organization (WHO, 2003), both 2-Nitrofluorene and 1-Nitropyrene are found in diesel combustion particulate emissions and are the major contributors to the increase in mutagenic emission potential. When considering the Nitro-PAHsC sum (Table 5), it is observed that these compounds 87 – 92% of the total nitro-PAH emissions and are therefore of concern, since these compounds are currently classified as class 2B and 2A carcinogens by the ICRA.

Table 5 – Nitro- PAH concentrations in PM exhaust emissions, in µg kWh^{-1}.

Nitro-PAH (µg kWh^{-1})	B5 SCR-off			B5 SCR-on			B20 SCR-off			B20 SCR-on		
	Average	SD (Min – Max)	n	Average	SD (Min – Max)	n	Average	SD (Min – Max)	n	Average	SD (Min – Max)	n
1-Nitronaphthalene	0.270	0.165 (0.153-0.387)	2	0.161	0.017 (0.148 - 0.180)	3	0.193	0.036 (0.168 – 0.219)	2	0.146	0.009 (0.140 – 0.152)	2
2-Nitrofluorene*	1.34	0.771 (0.519-2.50)	5	1.22	0.309 (0.836 – 1.50)	5	1.62	0.666 (0.984 – 2.40)	5	0.624	0.222 (0.467 – 0.781)	2
1-Nitropyrene**	0.408	-	1	0.542	-	1	0.360	-	1	0.350	-	1
∑ Nitro-PAH (particles)	**2.02 ± 0.79*** **			**1.93 ± 0.31*** **			**2.17 ± 0.67*** **			**1.12 ± 0.22*** **		
Nitro-PAHsC - Possibly carcinogenic for Humans*	1.75 (87 %)			1.76 (92 %)			1,98 (91 %)			0.974 (87 %)		

n – Number of valid samples; <LoQ. – Below quantification limit; SD – Standard Deviation; * IARC – group 2B; ** IARC – group 2A; *** Uncertainties propagation for PAH sum (estimative).

Unlike the results obtained in the PAH analysis, in which the fuel had the greatest reduction influence on the total emissions, the determining factor is a combination of fuel composition and the use of the SCR after-treatment system. The SCR after-treatment resulted in a 48% reduction when the B20 fuel was used and 5 % in the case of B5. This suggests that the SCR after-treatment was more effective when larger biodiesel additions are made. The only significant reduction was observed for B20 with after-treatment in comparison to the other experimental conditions. It seems therefore that only in the case of a combination of increased biodiesel additions and after-treatment a reduction of nitro-PAH emissions are observed. This is in contrast to Martin et al. (2017) who found that biodiesel was capable of reducing overall Nitro-PAH concentrations in particle phase emissions.

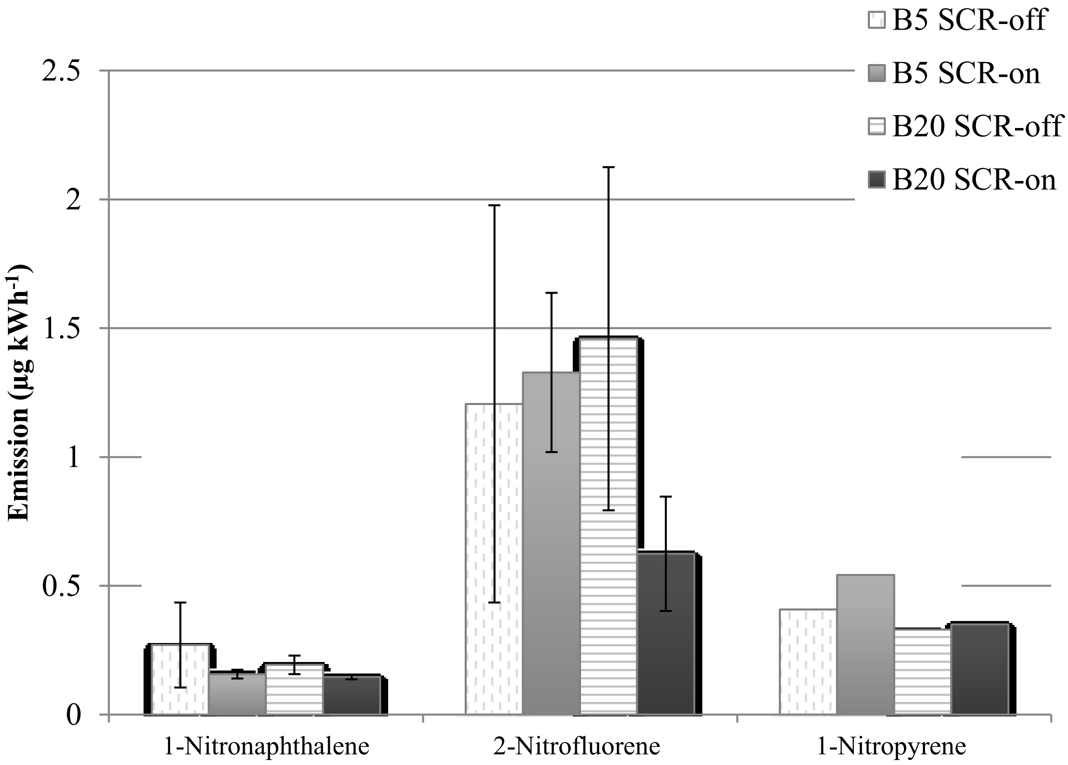

Fig 2. Average (columns) and standard deviation (bars) for individual Nitro-PAH associated to total PM emission for all experimental conditions in µg kWh^{-1}.

The analysis of variances for 1-Nitronaphthalene and 2-Nitrofluorene showed no significant difference (95% confidence level) among the results obtained with the addition of biodiesel in the mixture and the SCR system used. The same outcome was determined for its precursor PAH, Naphthalene and Fluorene, that presented no significant difference (95% confidence level) in fuel and after-treatment variation.

The formation mechanism of Nitro-PAH through the nitration reaction during the combustion process in the cylinder, has being investigated by several researches. However the knowledge about this process remains inconclusive (WHO, 2003; Heeb et al., 2008; Liu et al., 2015). After the combustion, Nitro-PAH can be formed by electrophilic substitution reactions in the presence of NO_x and the combination of high temperatures. The presence of precursor PAH and long residence times inside the aftertreament system can promote the formation of these compounds (Heeb et al., 2008). In a recent study, Liu et al. (2015) examined the emission of Nitro-PAH using various types of after-treatment systems in different configurations. When testing DOC + SCR, the emissions of PAH increased compared to the results of the engine without any after-treatment system. When studying only the SCR system, a reduction in the emission of Nitro-HPAs was observed by Liu et al. (2015). However the authors also concluded that the formation of Nitro-PAH by the SCR system is unlikely, since this system significantly reduces NO_2 concentrations, decreasing their availability for the conversion of PAH into their nitrated forms. In addition, the pores of the SCR system exclude large molecules such as PAH, reducing their residence time and their interaction with NO_x. Hu et al. (2013) evaluated the use of two SCR systems' composition (vanadium and copper) after a DOC + DPF system and none appear to promote the nitration of PAH as a whole, but may lead to the selective nitration of some PAH, such as phenanthrene. Inomata et al. (2015) conclude that the SCR aftertreament suppress Nitro-PAH emission studying 4-Nitrophenol, 1-nitropyerene and 9-nitroanthracene compounds from 3 different Diesel vehicles emission with DOC, DPF and SCR as aftertreament systems.

Considering the biodiesel effect on the Nitro-PAH emissions, Ratcliff et al. (2010) and Guan et al. (2017) observed that the use of biodiesel and oxygenated fuels, respectively, significantly reduces the emission of Nitro-HPAs associated to PM with proportions above 20%. Comparing the results obtained in this study and those by Turrio-Baldassarri et al. (2004), it is concluded that despite advances in engine and fuel technologies, Nitro-PAH emissions associated with PM increased. The authors do not list any significant difference between the Nitro-PAH emissions comparing the fuel used (diesel and B20), in agreement with the present results and also with Bagley et al. [48].

Sharp et al. (2000) studied the effects of biodiesel on unregulated emissions in 3 different heavy duty vehicles (119, 205 and 176 kW) operating with a transient cycle.

Their results showed that Nitro-PAH emissions associated with PM were lower for biodiesel (B100) compared to the S500 diesel. This result was expected since biodiesel has low concentrations of aromatic compounds, the precursor PAH in its composition. He (2016) described an increase in Nitro- and Oxy-PAH when using soybean biodiesel, palm biodiesel and oxidized biodiesel from used fried oil. 1-Nitropyrene has been reported by other studies and can be considered as a diesel emission marker (Bagley et al., 1998; Karavalakis et al., 2010; He, 2016; Guan et al., 2017). Even with the injection of a urea solution and the consequent formation of NH_3, there is no favouring of the nitration process in the SCR after-treatment system. The results suggest that the opposite occurs: the SCR system combined with biodiesel addition above 20% helps to reduce Nitro-HPAs emissions by suppressing NO_x and improving the combustion process due to the higher oxygen concentration in biodiesel.

This last section I think you should tighten up and rather focus on what really contributes to your findings or showed contrast to your findings. Again, there is more on what other people did than what you did and that makes your results look insufficient and not worthy

4. Conclusion

This study brings an important contribution to a better understanding regarding the synergistic effects of new engine technologies, after-treatment systems and biofuels, on the unregulated PAH and nitro-PAH emissions from heavy-duty diesel engines still in use in most developing countries. Total PAH emission reductions were observed (up to 28%) with the replacement of diesel fuel with biodiesel, which has been ascribed to the different chemistry pathway during the pyrolysis of the biodiesel. These reductions were more pronounced if the SCR after-treatment system was used. In addition, die fuel composition also played a significant role in the amount of carcinogous PAHs emitted, with an overall 50% reduction with the B20 fuel. The results were tested against the toxic equivalent quotient (TEQ) and the lowest toxicity values were observed with the B20 SCR-on condition, followed by B20 SCR-off, B5 SCR-off and B5 SCR-on. The combination of B20 and SCR after-treatment systems resulted in a consistent reduction of Nitro-PAH emissions. There is no substantial evidence that the SCR system promotes PAH nitration. Therefore, the higher oxygen content and the absence of aromatic compounds in biodiesel together with PM emission reduction by the SCR system seem to be able to reduce human health risks associated with PAH and Nitro-PAH exposure. These findings can assist in policy making, especially setting new emission standards that include limits for persistent organic pollutants such as PAHs and nitro-PAHs. In addition, the authors recognise that the reduction of tailpipe emissions from diesel-type fuels is a challenging task and therefore hope that the data from this study could help planning the best pathway to assess and reduce vehicular emissions since it depends on fuel type, experimental approaches, engines size and technologies, operating cycles, and after-treatment systems.

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