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

4

5 Guilherme C. Borillo<sup>1</sup>, Yara S. Tadano<sup>2</sup>; Ana Flavia L. Godoi<sup>1</sup>; Theotonio Pauliquevis<sup>3</sup>;  
6 Hugo Sarmiento<sup>1</sup>; Dennis Rempel<sup>4</sup>; Carlos I. Yamamoto<sup>5</sup>; Mary R. R. Marchi<sup>6</sup>; Sanja  
7 Potgieter-Vermaak<sup>7,8</sup>; Ricardo H. M. Godoi<sup>1\*</sup>

8

9 \*Corresponding Author: Environmental Engineering Department, Federal University of  
10 Parana, 210 Francisco H. dos Santos St., Curitiba, PR, 81531-980 Brazil. Tel.: +55 41  
11 3361-3482. E-mail address: [rhmgoi@ufpr.br](mailto:rhmgoi@ufpr.br) (R. H. M. Godoi)

12

13 1- Environmental Engineering Department, Federal University of Paraná, Curitiba, PR, Brazil.

14 2- Mathematics Department, Federal University of Technology of Paraná, Ponta Grossa, PR,  
15 Brazil.

16 3- Universidade Federal de São Paulo, Diadema, Brazil

17 4- Institute of Technology for Development, Lactec, Curitiba, PR, Brazil.

18 5- Chemical Engineering Department, Federal University of Paraná, Curitiba, PR, Brazil.

19 6- Analytical Chemistry Department, Institute of Chemistry, São Paulo State University -  
20 UNESP, Araraquara, SP, Brazil.

21 7- Division of Chemistry and Environmental Science, School of Science and the Environment,  
22 Manchester Metropolitan University, Manchester M15 6HB, United Kingdom.

23 8- Molecular Science Institute, School of Chemistry, University of the Witwatersrand,  
24 Johannesburg 2000, South Africa.

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26

27

28 ABSTRACT

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

53

54

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

58

59

60 **1. Introduction**

61

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

80 Current emission regulations implemented in the U.S. and European Union (EU),  
81 US 2010 and Euro VI, respectively, adopt a limit for NO<sub>x</sub> and PM emission that is ten  
82 times lower than the levels allowed in 2000. Countries where these emission limits are  
83 enforced and implemented (U.S., EU, Canada, Japan and South Korea) project a 26-fold  
84 reduction in PM emissions by 2045 (Posada et al., 2016). On the other hand, Brazil,  
85 Russia, India, China, Australia and Mexico whose gross domestic product heavily  
86 depends on heavy-duty vehicle transport, have yet to fully implement the equivalent  
87 emission standards, even though the increased risks are acknowledged. These regulations  
88 require extensive deployment of advanced engine tuning, the addition of two or more  
89 after-treatment devices, and the use of low sulphur diesel content, therefore potentially  
90 fleet renovations. Instead, these countries still enforce the Euro V and equivalent  
91 regulations where SCR (Selective Catalytic Reduction) or EGR (exhaust gas  
92 recirculation) after-treatment systems remain the main strategies to reduce NO<sub>x</sub> and PM  
93 emission (Du and Miller, 2017).

94 As an additional strategy to reduce pollutants from diesel vehicles, many countries  
95 like Canada, Australia, Brazil, China and Germany are promoting the development,  
96 production and use of alternatives fuels (biofuels and natural gas) seeking a better balance  
97 to fuel economy and thereby improving green freight programs in the long term (Du and  
98 Miller, 2017). Biodiesel is already used in several countries and seems to be a promising  
99 alternative, as it can be used in diesel engines without major modifications. Despite the  
100 high cost of production when compared to diesel, biodiesel can qualitatively and  
101 quantitatively reduce regulated pollutant emissions (Ratcliff et al., 2010; He et al., 2010;  
102 Tadano et al., 2014; Sadiktsis et al., 2014). Several researches successfully demonstrated  
103 a consistent reduction for hydrocarbons (HC), total particulate matter (PM) and carbon  
104 monoxide (CO) emissions while recognizing an increase in nitrogen dioxide (NO<sub>x</sub>) when  
105 biodiesel is used (Ravindra et al., 2008; Sadiktsis et al., 2014; Borillo et al., 2015; He,  
106 2016). On the other hand, research evidence on the influence of alternative fuels on PAH  
107 and Nitro-PAH emissions are sparse. Guarieiro et al. (2014) concluded that the biodiesel  
108 additions decrease PAH emissions, which is in agreement with the conclusions made by  
109 Yilmaz and Davis (2016), He et al. (2010) and Lim et al. (2014). Westphal et al. (2013)  
110 tested PAH emissions using a turbo-intercooled engine with 6 cylinders operating in the  
111 European Stationary Cycle (ESC) fuelled with hydrotreated vegetable oil and jatropha  
112 methyl ester. The authors also demonstrated a slight reduction in PAH emission when  
113 low percentages of biodiesel was added and showed that toxicological effects depends on  
114 the biodiesel origin. Casal et al. (2014) tested a Euro III engine fuelled with standard  
115 diesel (B0), B5 (5% biodiesel) and B20 (20% biodiesel) and concluded that biodiesel  
116 mixed with diesel increases the production of PAH and Alkyl PAH in the engine exhaust  
117 emissions.

118 Changes in fuel alone are however not sufficient to meet the new or the old  
119 standards and use of after-treatment systems is crucial. The SCR system is still the most  
120 widely used, due to its efficiency and selectivity to reduce NO<sub>x</sub> emissions, (Tadano et al.,  
121 2014). The injection of urea and a catalyst ensures reductions of up to 90% in NO<sub>x</sub>  
122 emissions, through a reduction reaction of NO<sub>x</sub> and NH<sub>3</sub> resulting in nitrogen and water  
123 (Amanatidis et al., 2014; Bacher et al., 2014). Nevertheless, SCR technology is not  
124 without its challenges, such as the emission of NH<sub>3</sub>, stoichiometric disproportion of urea  
125 consumption, cost, and deactivation of the catalyst by deposition (Cheruiyot et al., 2017).  
126 The best reported SCR efficiency are achieved at high engine loads and temperatures  
127 (approximately 400 °C) (Cheruiyot et al., 2017). However, the added urea increases the

128 probability of unintended formation of Nitro-PAH through PAH nitration (Liu et al.,  
129 2015).

130 The combination of low sulphur fuels (ultra-low sulphur diesel-ULSD), new  
131 engine technologies, after-treatment systems (SCR, EGR, DOC – Diesel oxidation  
132 catalyst, DPF – Diesel particulate filter) and the use of biofuels are the current strategy to  
133 achieve the regulatory reductions worldwide (Ratcliff et al., 2010; Carrara and Niessner,  
134 2013; Hu et al., 2013; Sadiktsis et al., 2014; Reşitoğlu et al., 2015; He et al., 2016).  
135 However, there is insufficient knowledge and some disagreement in open literature with  
136 regards to the potential synergistic effect on unregulated emissions by vehicles equipped  
137 with SCR and the use of different biodiesel blends.

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

144

## 145 **2. Materials and methods**

### 146 **2.1. Sampling**

147

148 The engine emission tests were performed at the Lactec Institute's Laboratory for  
149 Vehicular Emissions in partnership with the Federal University of Paraná, Curitiba,  
150 Brazil. The tested engine is currently used in trucks and buses around the world, and has  
151 an individual four-valve cylinder head, cross-flow arrangement, common rail injection  
152 and SCR after-treatment system. The test engine fulfils the European Union Regulation  
153 (EC - N° 595/2009 of the European Parliament and of the Council of 18 June 2009)  
154 requirements for EuroV emission standards with a urea-SCR system and is in accordance  
155 with the P7 phase of the PROCONVE (Vehicular Air Pollution Control Program) in  
156 Brazil. The engine details are specified in Table 1.

157

158 **Table 1. Engine specifications, model 2012.**  
Specifications

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Emission	EuroV "Heavy Duty"/Proconve P7
Configuration	4 cylinders, inline
Displacement	4.8 liters
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

---

159

160 The engine works in conjunction with a dynamometer and a data acquisition  
161 system, both from AVL (Graz, Austria) to perform the emission and engine parameters  
162 measurements. The dynamometer can absorb power up to 440 kW at 6,000 rpm and a  
163 torque of 2.334 N m at 1.200–1.600 rpm. The European Steady Cycle (ESC) was selected  
164 as driving cycle for the dynamometer operation to allow PM sampling. It was designed  
165 to achieve high load factors and very high temperature on the exhaust gas (Ericsson, 2001;  
166 Mock et al., 2012). Sixty-five percent of the calculated emission responds to load and  
167 engine speed above 50%. That is conservative and captures most of the stop-and-go  
168 conditions of the drive cycle experienced by urban busses in rush hours (Tadano et al.,  
169 2014).

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

174 For PAH and Nitro-PAH analysis, total PM was collected on two borosilicate  
175 glass fiber filters, 70 mm of diameter, coated with fluorocarbon (Pallflex T60A20  
176 fiberfilm). The filters were weighted prior and after the sampling using a Sartorius micro-  
177 balance (MSA2.7S-000-DF - Goettingen, Germany) following the NIOSH method 5000  
178 and were stored below -18 °C in cleaned glass containers until analysis. The NO<sub>x</sub>

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

182

## 183 2.2. Fuels

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

193

194 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 <sup>2</sup> s <sup>-1</sup> )	3.0	3.2	ASTM D445
Specific mass at 20 °C (kg m <sup>-3</sup> )	830.5	848.1	ASTM D4052

195

## 196 2.3. Extraction

197 All the sampled filters were extracted, using an Accelerated Solvent Extractor  
198 (ASE – Dionex, USA), with dichloromethane and methanol (4:1) at a pressure of 1.500  
199 psi and temperature of 120 °C in three static extraction cycles of 5 minutes each. The total  
200 extracted volume was concentrated to near dryness with a slight nitrogen flow and



201 recovered with 1.5 ml of dichloromethane. This procedure has been optimized, using  
202 different temperatures (100, 120, 150 and 180 °C) and extraction cycles (1, 2 and 3  
203 cycles), based on the EPA' Standard Operation Procedure (California Standard Operation  
204 Procedure, SOP No. 144 2006 for the determination of PAH in particulate matter using  
205 GC-MS (gas chromatography with mass spectrometry). To assess the extraction  
206 efficiency, Naphthalene-d8 and Benz[a]anthracene-d12 were used as recovery standards.  
207 The recovery, in percentage, of Naphtalene-d8 was slightly lower (65-80%) than the  
208 recovery of the Benz[a]anthracene-d12 (84-114%).

209

#### 210 **2.4. Analysis**

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

224 Acenaphthene-D10, Phenanthrene-D10 and Perylene-D12 (Sulpeco Analytical -  
225 Bellefonte, USA) were used as internal standards. Sixteen PAH recognised by USEPA  
226 (United States Environmental Protection Agency) as priority pollutants were analysed:  
227 Naphthalene, Acenaphthene, Acenaphthylene, Anthracene, Phenanthrene, Fluorene,  
228 Fluoranthene, Benzo[a]anthracene, Chrysene, Pyrene, Benzo[a]pyrene,  
229 Benzo[b]fluoranthene, Benzo[k]fluoranthene, Dibenzo[a,h]anthracene,  
230 Benzo[g,h,i]perylene, Indeno[1,2,3-c,d]pyrene . In addition, Benzo[e]pyrene, Perylene,  
231 1-Nitronaphtalene, 2-Nitrofluorene, 3-Nitrofluoranthene, 1-Nitropirene and 7-  
232 Nitrobenz[a]anthracene were also analysed (standards solutions supplied by Dr.  
233 Ehrenstorfer - Augsburg, Germany). The list with all investigated compounds with their  
234 respective molecular mass, quantification ion and quantification limits (LOQ.) can be

235 found in Table S1 of the supplementary material. The method accuracy was verified using  
236 the reference material SRM 1650b from NIST (National Institute Standards and  
237 Technology).

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

241

242

### 243 **3. Results and Discussion**

244

245 Particle emissions from a EURO V engine, operating with and without selective  
246 catalytic reduction after-treatment, and testing ULSD with 5% of biodiesel (B5) and  
247 ULSD with 20% of biodiesel (B20), were analysed for their PAH and Nitro-PAH content.  
248 The results of the different PAH and Nitro-PAH detected are presented in Tables 3 and  
249 5, respectively. We reported in previous published work (Borillo et al., 2015) that the  
250 SCR after-treatment reduced NO<sub>x</sub> emissions (90% reduction observed for both: B5 and  
251 B20), as it was expected, and therefore this parameter was used to evaluate the  
252 performance efficiency of the SCR system.

253

#### 254 **3.1. PAH concentrations in PM exhaust emissions**

255 Table 3 displays the identity of the PAHs emitted from a Euro V engine operating  
256 with an ESC cycle, using two different fuels and conditions, as well as their respective  
257 concentrations (in µg kWh<sup>-1</sup>). Of the 23 PAHs, eight were not detected or were found  
258 below the quantification limit (Anthracene, Chrysene, Benzo[k]fluoranthene,  
259 Benzo[e]pyrene, Benzo[a]pyrene, Perylene, Indeno[1,2,3-cd]pyrene,  
260 Dibenzo[a,h]anthracene - Table S1).

261

**Table 3 – PAH concentrations in PM exhaust emissions, in  $\mu\text{g kWh}^{-1}$ .**

PAH ( $\mu\text{g kWh}^{-1}$ )	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.	
<b><math>\Sigma</math>PAH (particles)</b>	<b><math>3.37 \pm 0.62^{**}</math></b>			<b><math>3.13 \pm 0.42^{**}</math></b>			<b><math>2.54 \pm 0.39^{**}</math></b>			<b><math>2.12 \pm 0.11^{**}</math></b>		
PAHsC - Possibly carcinogenic for Humans*	0.313 (9.2 %)			0.347 (11.1 %)			0.128 (5.0 %)			0.121 (5.7 %)		

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

**264**

**265**

**266**

267           Considering the sum of all PAHs, the B5 SCR-off condition had the highest value  
268 among the sampling conditions ( $3.37 \pm 0.62 \mu\text{g kWh}^{-1}$ ), followed by B5 SCR-on ( $3.13 \pm$   
269  $0.42 \mu\text{g kWh}^{-1}$ ), B20 SCR-off ( $2.54 \pm 0.39 \mu\text{g kWh}^{-1}$ ) and the B20 SCR-on ( $2.12 \pm 0.11$   
270  $\mu\text{g kWh}^{-1}$ ).

271           The results indicate therefore a slight reduction (7% and 16% respectively for B5  
272 and B20) in total PAH emissions when the after-treatment technology is applied. Lee et  
273 al. (2015) described a moderate decrease of particle emissions for EURO V vehicles  
274 equipped with SCR system, in agreement to Mayer et al. (2007) and Czerwinski et al.  
275 (2011), who reported a reduction of 15% in particle concentration. Pietkäinen et al. (2015)  
276 studied the PM emissions from a turbocharged, intercooled, common rail, 4-cylinder non-  
277 road engine with SCR system and also reported a decrease in total particle number when  
278 the SCR converter was used, but there was an increase of nanoparticles, increasing the  
279 risk to human health. These findings therefore suggest that the SCR system reduces total  
280 particle emissions and thus it is possible that this may be the reason for the observed  
281 decrease in PAH emission.

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

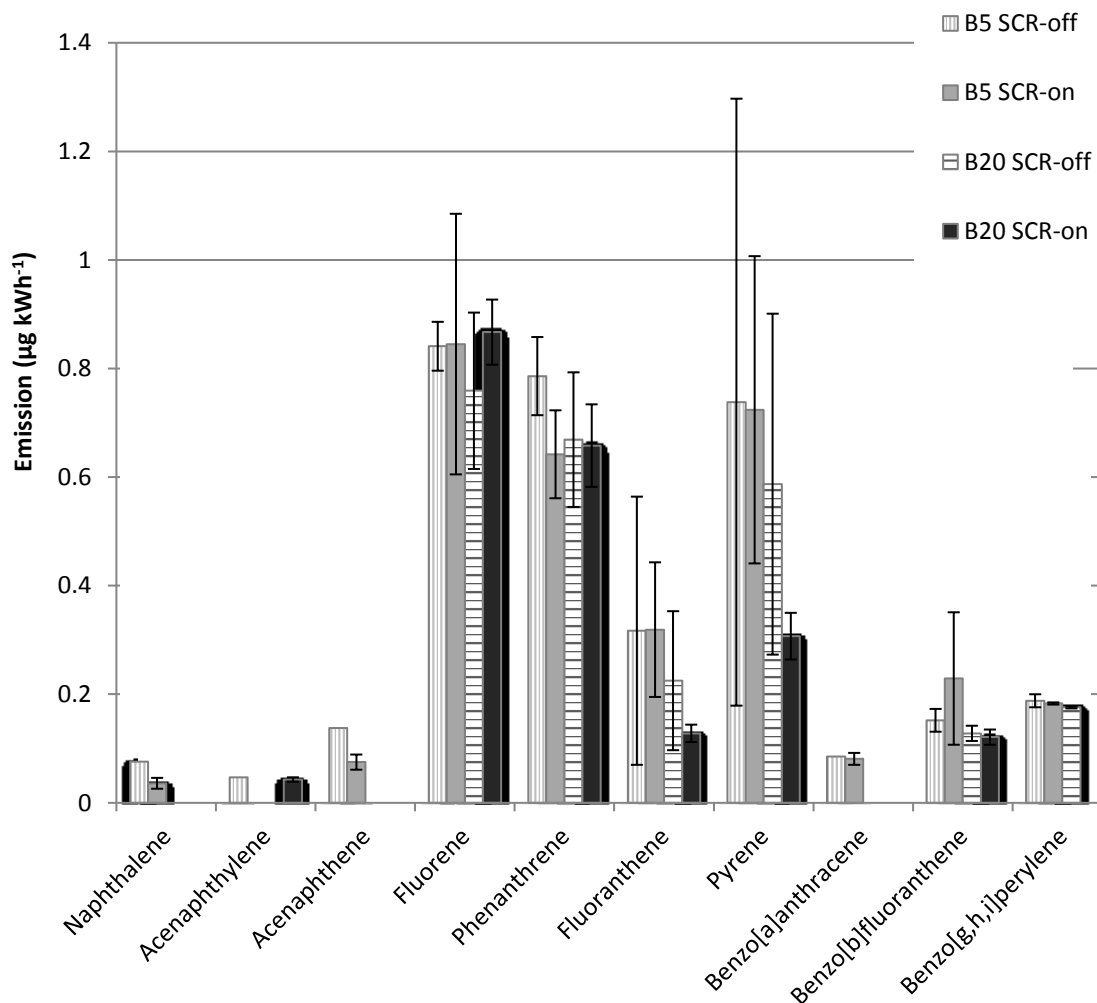
292           Similarly, the PAHsC (possibly carcinogenic to humans PAH) were reduced by  
293 59% and 65%, respectively, when B5 was replaced by B20, either with or without SCR.  
294 These results clearly demonstrate the beneficial influence on PAH emissions if biodiesel  
295 additions to fuel are made.

296           These results confirm the difference in combustion products between diesel and  
297 biodiesel, as highlighted by Ravindra et al. (2008), who stated that the pyrolysis and  
298 pyrosynthesis processes that diesel undergoes during combustion (in reduced oxygen  
299 conditions), will result in the formation of aromatic rings through condensation products.  
300 In the case of biodiesel combustion, PAH formation involves the thermal polymerization

301 of the fatty acid methyl esters forming cyclohexane (Ratcliff et al., 2010), as there are no  
302 aromatic organic compounds present. Therefore, the incorporation of biodiesel in the  
303 mixture can lead to a reduction in PAH emissions due to the natural absence of PAH in  
304 biodiesel, and to its higher oxygen content. The observed result represents the different  
305 chemistry path of biodiesel combustion in relation to diesel and an improvement of the  
306 combustion process, reducing the PAH generated by incomplete combustion of fuel and  
307 lubricant oils.

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

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



319 Fig 1. Average concentrations in  $\mu\text{g kWh}^{-1}$  (columns) and standard deviation (bars) for  
 320 individual PAH associated with total PM emission for all experimental conditions.  
 321  
 322

323 The ANOVA test was performed for the substances that present  $N > 2$ : Fluorene,  
 324 Phenanthrene, Fluoranthene, Pyrene and Benzo[b]fluoranthene. No statistical difference  
 325 (95% confidence level) was found for the conditions tested, except Benzo[b]fluoranthene  
 326 when comparing B5 SCR-off to B20 SCR-off and B5 SCR-on to B20 SCR-off. A similar  
 327 result was obtained by Shah et al. (2012) who found no significant difference in PAH  
 328 emissions considering the use of the SCR system and a low sulphur diesel. Liu et al.  
 329 (2015) and Zhao et al. (2014) indicated that the Diesel Particulate Filter (DPF) is better  
 330 to reduce the individual emissions of PHAs associated with PM, resulting in reductions  
 331 greater than 90%. They also pointed out high tendency of engine manufacturers to couple  
 332 more than one after-treatment system to achieve larger reductions in pollutants.

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

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

350

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

353

Fuel	B5 SCR-off	B5 SCR-on	B20 SCR-off	B20 SCR-on
TEQ ( $\mu\text{g kWh}^{-1}$ )	0.029	0.036	0.017	0.014

354

355

356

357

358 The toxicity equivalent factors (TEF) for each PAH and the calculation  
 359 procedure were the same as those proposed by Nisbet & Lagoy (1992) and US EPA  
 360 (2006). The results indicate that the highest equivalent toxicity value was found in the B5  
 361 SCR-on combination and the lowest for the B20 SCR-on. The highest TEQ value, 0.036  
 362  $\mu\text{g kWh}^{-1}$ , match the highest PAHsC, 0.347  $\mu\text{g kWh}^{-1}$  (Table 3), for the B5 SCR-on  
 363 condition, suggesting that Benzo[b]fluoranthene was mostly responsible for the increased  
 364 toxicity. Shah et al. (2012) tested the influence of the SCR system on the TEQ values and  
 365 concluded that the after-treatment increased the quotient due to the increase in PAH with  
 high TEFs.

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

374

375

### 376 **3.2. Nitro-PAH associated to PM emissions**

377

378           The concentration of five Nitro-PAHs associated with PM emissions from a Euro V  
379 (SCR) engine using diesel/biodiesel blends as fuels were assessed in this research and the  
380 results are shown in Figure 2 and Table 5. The 1-Nitropyrene was quantified with only  
381 one sample of each experimental condition. 2-Nitrofluoranthene and 7-  
382 Nitrobenzo[a]anthracene were below the quantification limit for all samples??. The Nitro-  
383 PAHs that were present in quantifiable amounts were XXXXXXXX (name them). It is  
384 observed that 2-Nitrofluorene was up to 8.5 times higher than the other nitro-PAHs  
385 quantified and in general higher than any other individual PAH identified and quantified  
386 as indicated in Table 3. According to the World Health Organization (WHO, 2003), both  
387 2-Nitrofluorene and 1-Nitropyrene are found in diesel combustion particulate emissions  
388 and are the major contributors to the increase in mutagenic emission potential. When  
389 considering the Nitro-PAHsC sum (Table 5), it is observed that these compounds 87 –  
390 92% of the total nitro-PAH emissions and are therefore of concern, since these  
391 compounds are currently classified as class 2B and 2A carcinogens by the ICRA.

392

393



Table 5 – Nitro- PAH concentrations in PM exhaust emissions, in  $\mu\text{g kWh}^{-1}$ .

Nitro-PAH ( $\mu\text{g kWh}^{-1}$ )	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
<b><math>\Sigma</math> Nitro-PAH (particles)</b>	<b>2.02 <math>\pm</math> 0.79***</b>			<b>1.93 <math>\pm</math> 0.31***</b>			<b>2.17 <math>\pm</math> 0.67***</b>			<b>1.12 <math>\pm</math> 0.22***</b>		
Nitro-PAHsC - Possibly carcinogenic for Humans*	1.75 (87 %)			1.76 (92 %)			1,98 (91 %)			0.974 (87 %)		

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

397

398

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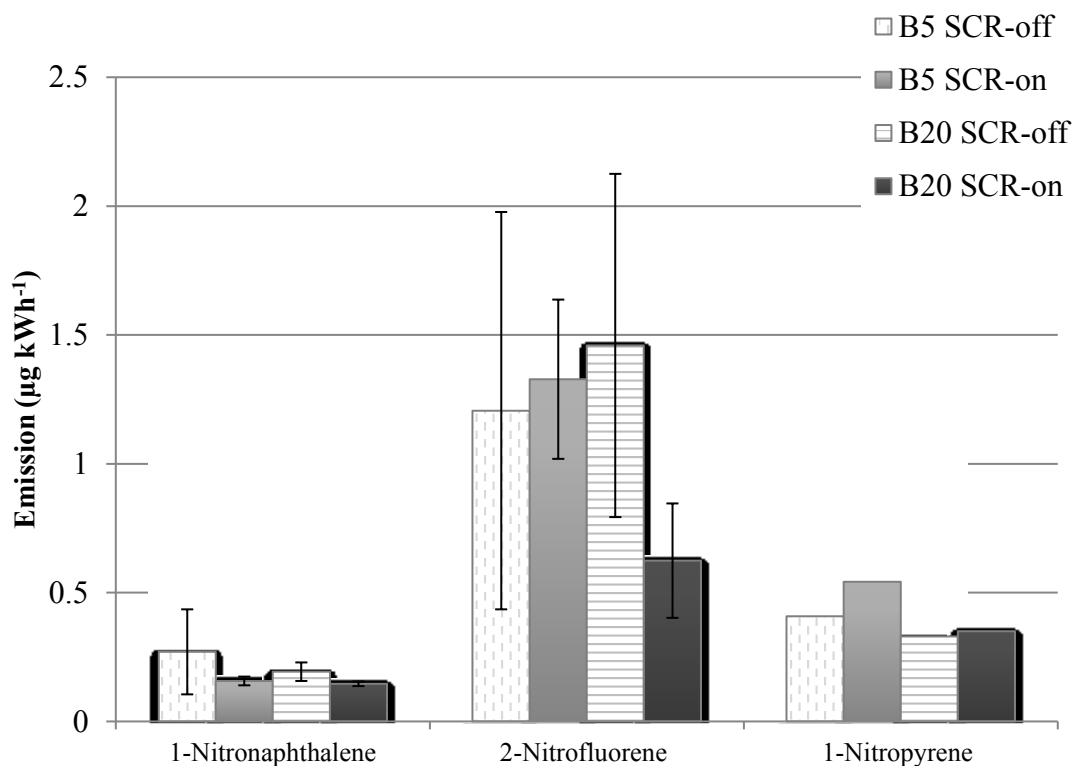
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404 Unlike the results obtained in the PAH analysis, in which the fuel had the greatest  
 405 reduction influence on the total emissions, the determining factor is a combination of fuel  
 406 composition and the use of the SCR after-treatment system.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



415

416 Fig 2. Average (columns) and standard deviation (bars) for individual Nitro-PAH  
 417 associated to total PM emission for all experimental conditions in µg kWh<sup>-1</sup>.  
 418

419

420 The analysis of variances for 1-Nitronaphthalene and 2-Nitrofluorene showed no  
 421 significant difference (95% confidence level) among the results obtained with the  
 422 addition of biodiesel in the mixture and the SCR system used. The same outcome was

423 determined for its precursor PAH, Naphthalene and Fluorene, that presented no  
424 significant difference (95% confidence level) in fuel and after-treatment variation.

425 The formation mechanism of Nitro-PAH through the nitration reaction during the  
426 combustion process in the cylinder, has being investigated by several researches.  
427 However the knowledge about this process remains inconclusive (WHO, 2003; Heeb et  
428 al., 2008; Liu et al., 2015). After the combustion, Nitro-PAH can be formed by  
429 electrophilic substitution reactions in the presence of NO<sub>x</sub> and the combination of high  
430 temperatures. The presence of precursor PAH and long residence times inside the  
431 aftertreatment system can promote the formation of these compounds (Heeb et al., 2008).  
432 In a recent study, Liu et al. (2015) examined the emission of Nitro-PAH using various  
433 types of after-treatment systems in different configurations. When testing DOC + SCR,  
434 the emissions of PAH increased compared to the results of the engine without any after-  
435 treatment system. When studying only the SCR system, a reduction in the emission of  
436 Nitro-HPAs was observed by Liu et al. (2015). However the authors also concluded that  
437 the formation of Nitro-PAH by the SCR system is unlikely, since this system significantly  
438 reduces NO<sub>2</sub> concentrations, decreasing their availability for the conversion of PAH into  
439 their nitrated forms. In addition, the pores of the SCR system exclude large molecules  
440 such as PAH, reducing their residence time and their interaction with NO<sub>x</sub>. Hu et al.  
441 (2013) evaluated the use of two SCR systems' composition (vanadium and copper) after  
442 a DOC + DPF system and none appear to promote the nitration of PAH as a whole, but  
443 may lead to the selective nitration of some PAH, such as phenanthrene. Inomata et al.  
444 (2015) conclude that the SCR aftertreatment suppress Nitro-PAH emission studying 4-  
445 Nitrophenol, 1-nitropyrene and 9-nitroanthracene compounds from 3 different Diesel  
446 vehicles emission with DOC, DPF and SCR as aftertreatment systems.

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

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

457 Their results showed that Nitro-PAH emissions associated with PM were lower for  
458 biodiesel (B100) compared to the S500 diesel. This result was expected since biodiesel  
459 has low concentrations of aromatic compounds, the precursor PAH in its composition.  
460 He (2016) described an increase in Nitro- and Oxy-PAH when using soybean biodiesel,  
461 palm biodiesel and oxidized biodiesel from used fried oil. 1-Nitropyrene has been  
462 reported by other studies and can be considered as a diesel emission marker (Bagley et  
463 al., 1998; Karavalakis et al., 2010; He, 2016; Guan et al., 2017). Even with the injection  
464 of a urea solution and the consequent formation of NH<sub>3</sub>, there is no favouring of the  
465 nitration process in the SCR after-treatment system. The results suggest that the opposite  
466 occurs: the SCR system combined with biodiesel addition above 20% helps to reduce  
467 Nitro-HPAs emissions by suppressing NO<sub>x</sub> and improving the combustion process due to  
468 the higher oxygen concentration in biodiesel.

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

473

#### 474 **4. Conclusion**

475

476 This study brings an important contribution to a better understanding regarding the  
477 synergistic effects of new engine technologies, after-treatment systems and biofuels, on  
478 the unregulated PAH and nitro-PAH emissions from heavy-duty diesel engines still in  
479 use in most developing countries. Total PAH emission reductions were observed (up to  
480 28%) with the replacement of diesel fuel with biodiesel, which has been ascribed to the  
481 different chemistry pathway during the pyrolysis of the biodiesel. These reductions were  
482 more pronounced if the SCR after-treatment system was used. In addition, die fuel  
483 composition also played a significant role in the amount of carcinogous PAHs emitted,  
484 with an overall 50% reduction with the B20 fuel. The results were tested against the toxic  
485 equivalent quotient (TEQ) and the lowest toxicity values were observed with the B20  
486 SCR-on condition, followed by B20 SCR-off, B5 SCR-off and B5 SCR-on. The  
487 combination of B20 and SCR after-treatment systems resulted in a consistent reduction  
488 of Nitro-PAH emissions. There is no substantial evidence that the SCR system promotes  
489 PAH nitration. Therefore, the higher oxygen content and the absence of aromatic

490 compounds in biodiesel together with PM emission reduction by the SCR system seem  
491 to be able to reduce human health risks associated with PAH and Nitro-PAH exposure.  
492 These findings can assist in policy making, especially setting new emission standards that  
493 include limits for persistent organic pollutants such as PAHs and nitro-PAHs. In addition,  
494 the authors recognise that the reduction of tailpipe emissions from diesel-type fuels is a  
495 challenging task and therefore hope that the data from this study could help planning the  
496 best pathway to assess and reduce vehicular emissions since it depends on fuel type,  
497 experimental approaches, engines size and technologies, operating cycles, and after-  
498 treatment systems.

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502 Technological Development (CNPq).

503

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