

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

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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).

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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_{2.5}) (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_x 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_x 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_x) 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_x emissions, (Tadano et al.,
121 2014). The injection of urea and a catalyst ensures reductions of up to 90% in NO_x
122 emissions, through a reduction reaction of NO_x and NH₃ 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 NH₃, 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

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_x

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 ² s ⁻¹)	3.0	3.2	ASTM D445
Specific mass at 20 °C (kg m ⁻³)	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⁻¹. 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⁻¹ to 200 °C
218 and maintained for 5 min, heated in sequence at a rate of 6 °C min⁻¹ to 240 °C and
219 maintained for 10 minutes and, finally heated to 300 °C at a rate of 10 °C min⁻¹ 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_x 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⁻¹). 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.	
ΣPAH (particles)	$3.37 \pm 0.62^{**}$			$3.13 \pm 0.42^{**}$			$2.54 \pm 0.39^{**}$			$2.12 \pm 0.11^{**}$		
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 ± 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.

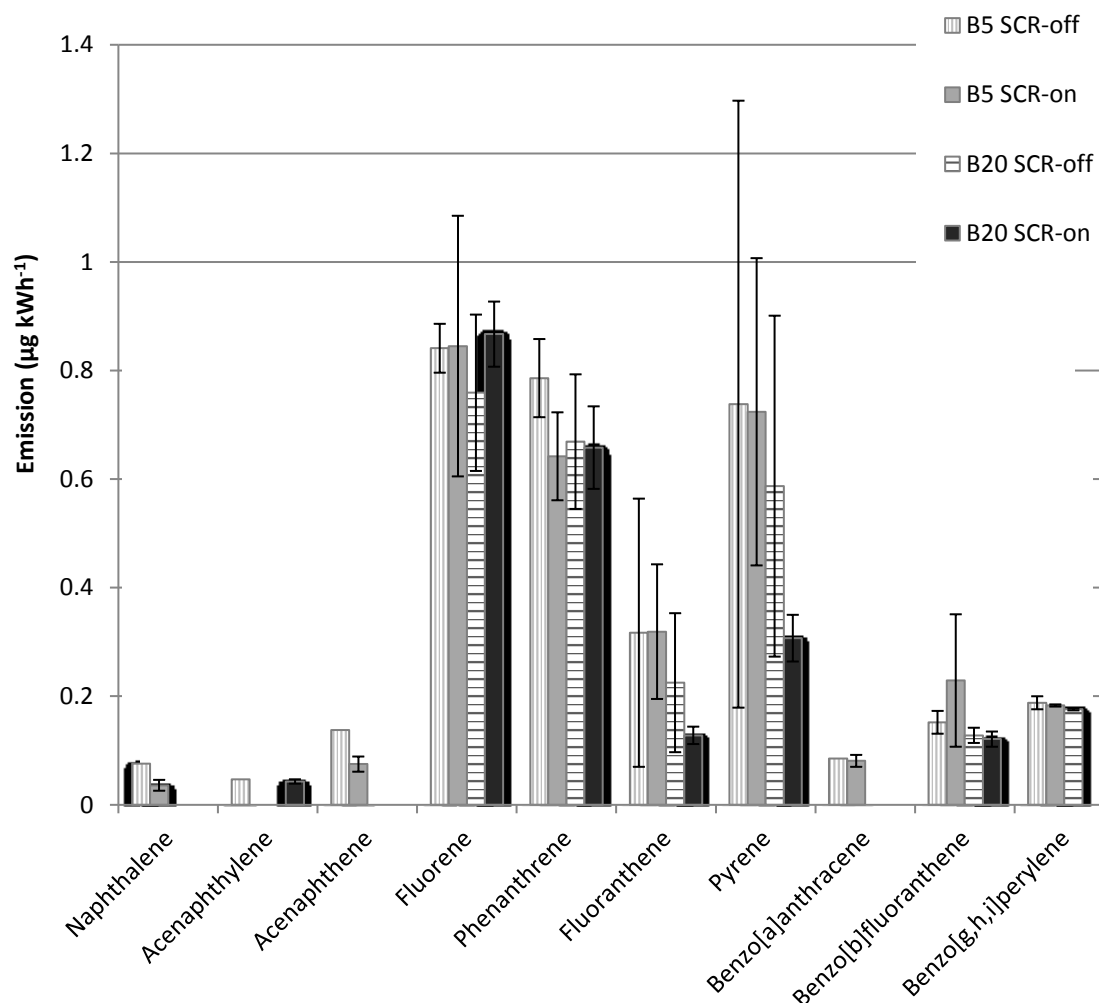
292 Similarly, the PAHsC (possibly carcinogenic to humans PAH) were reduced by
293 59% and 65%, respectively, when B5 was replaced by B20, either with or without SCR.
294 These results clearly demonstrate the beneficial influence on PAH emissions if biodiesel
295 additions to fuel are made.

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⁻¹).



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

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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
Σ Nitro-PAH (particles)	2.02 \pm 0.79***			1.93 \pm 0.31***			2.17 \pm 0.67***			1.12 \pm 0.22***		
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

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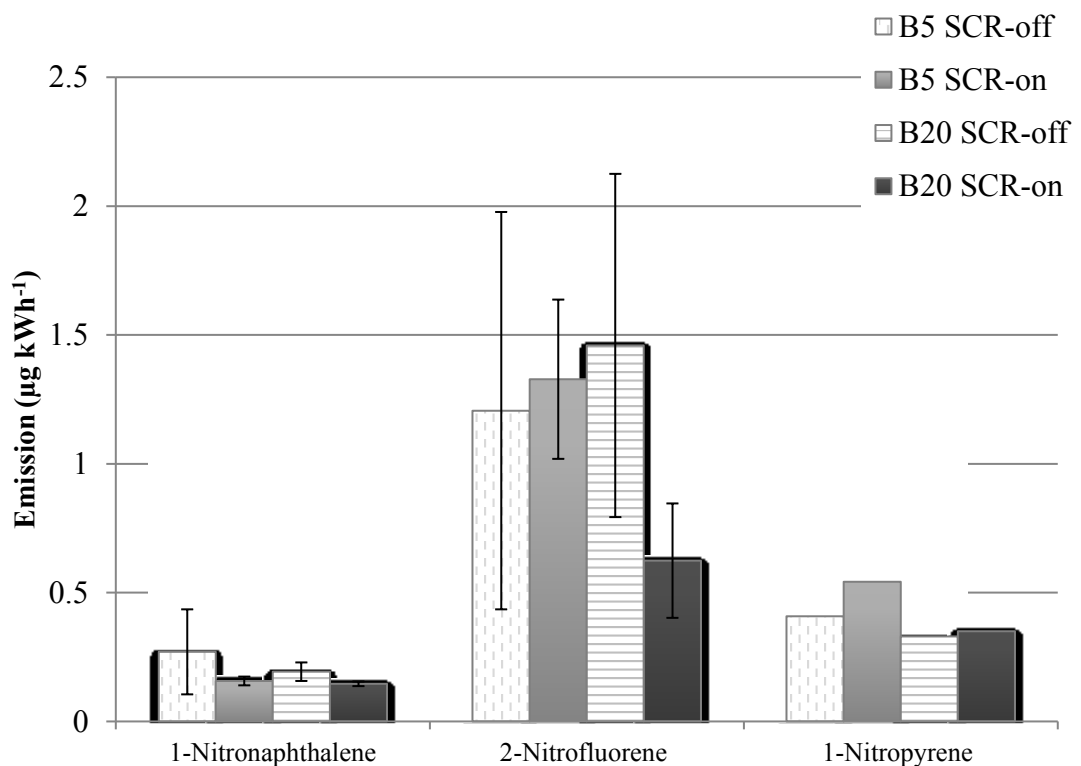
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404 Unlike the results obtained in the PAH analysis, in which the fuel had the greatest
 405 reduction influence on the total emissions, the determining factor is a combination of fuel
 406 composition and the use of the SCR after-treatment system. 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⁻¹.
 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_x 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 NO₂ 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_x. 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 NH₃, 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_x 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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