

Exposure to Automobile Pollutants and Sperm Quality among Mechanics in Brazzaville (Congo)

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How to cite this paper: Diambalou, R.C.D., Dzamba, P.J.I.M., Kaya-Ongoto, M.D. and Abena, A.A. (2023) Exposure to Automobile Pollutants and Sperm Quality among Mechanics in Brazzaville (Congo). *Open Journal of Air Pollution*, **12**, 121-141. https://doi.org/10.4236/ojap.2023.124008

Received: September 13, 2023 Accepted: November 13, 2023 Published: November 16, 2023

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Abstract

Atmospheric pollution is today at the heart of all debates because of its potentially harmful effects on the environment, the climate, and human health; it currently constitutes a real public health problem. However, the increase in infertility around the world has led scientists to look for a link between air pollution and fertility. This study consisted of evaluating the short-term influence of automobile pollution on the semen parameters of mechanics in Brazzaville. A cross-sectional, analytical, prospective study between two groups (G1, exposed people and G2, unexposed people) was carried out in Brazzaville on 228 patients, i.e., G1 with 76 subjects and G2 with 152 subjects, between June 2020 and September 2022, a period of 27 months, in order to evaluate, on the one hand, the quality of sperm in men exposed to automobile pollution according to WHO recommendations and, on the other hand, the quality of the air by a colorimetric method, punctual on a Dräger tube coupled with a Dräger Accuro pump. The concentrations of automotive pollutants measured (CO, CO₂, NO₂, SO₂) during this study were all above the 2021 air quality standards required by WHO. These results made it possible to establish a statistically significant link between air pollution and abnormal spermogram parameters, notably mobility, count, and morphology for the spermocytogram. Exposures to automobile pollutants can influence sperm quality, which is consistent with the results of our study. We observed an alteration in the morphology, mobility, and concentration of spermatozoa.

Keywords

Exposure, Automobile Pollutants, Sperm Quality

1. Introduction

In recent decades, air pollution has become a major concern across the world because of its potentially harmful effects on human health. According to WHO estimates, the combined effects of outdoor and indoor air pollution are responsible for around seven million premature deaths each year and lead to the loss of hundreds of millions of years of healthy life around the world. This disease burden falls mainly on low- and middle-income countries [1]. Acute and chronic exposure to air pollution has been associated with a wide range of non-communicable diseases, such as respiratory pathologies [2] [3] and cardiovascular diseases, including myocardial infarction, heart failure, hypertension, and stroke [4] [5] [6]. At the same time, epidemiological studies have described the involvement of air pollution in neurodevelopmental disorders and neurodegenerative diseases such as Parkinson's disease and Alzheimer's disease [7], as well as in bladder and breast cancers [8] [9]. Likewise, the results observed in a large number of studies have also led to the question of the effect of air pollution on other systems, in particular the reproductive system, which is responsible for cases of 'infertility. Said infertility is defined as the inability of a couple to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse [10] [11]. The prevalence of infertility among couples of childbearing age ranges between 12.6% and 17.5% worldwide, with relatively higher prevalence rates in some regions such as the Americas, Western Pacific, Africa, and Europe [12]. Until a few years ago, male involvement in infertility was little known throughout the world, especially in Africa. Furthermore, numerous publications have reported the existence of a decline in sperm quality in men over recent decades [13] [14]. A meta-analysis of more than 185 studies showed that sperm counts declined in Western countries by 50% to 60% between 1973 and 2011 [15].

Currently, scientific debates are focused on the causes of this decline; we know that many factors could have a negative influence on sperm quality, such as lifestyle habits, obesity, and environmental factors [16]. Many authors have established a link between air pollution and reproduction in general and with sperm quality in particular. The different existing studies are not always comparable because their methodology is not necessarily similar, in particular in terms of the pollutants studied, the population, the methods of exposure, etc. [17]. However, most of these studies seem to be in favour of a negative impact of these pollutants on human reproduction, in particular on the outcome of pregnancy, with an increase in the rate of miscarriages, problems with foetal development, premature births, pregnancy complications, and intrauterine foetal deaths [18] [19] [20] [21]. In male subjects, studies carried out in this direction have concluded that air pollution has a harmful effect on sperm quality. This involves a reduction in the mobility of spermatozoa, their concentration, and their morphology [17] [22] [23]. Air pollution today has societal, environmental, and medical impacts. In the Republic of Congo, no study has been carried out in this direction. This study aims to evaluate the short-term influence of automobile pollution on the semen parameters of mechanics.

2. Methods

This study was approved after review by the Health Sciences Research Ethics Committee (HSREC). Informed consent was obtained from participants before enrollment in the study.

2.1. Description of the Study Site and Type

This study was done in Brazzaville, the political capital of the Congo, which extends from north to south over an area of 264 km². It represents the most populous city in the country, with 2,552,813 inhabitants in 2021. It has nine districts with a demographic density of 9670 inhabitants per km². Our analyses were carried out within the garages for air analyses and at the National Public Health Laboratory in the reproduction unit for semen analysis. This is an analytical, prospective cross-sectional study between two groups, an exposed group G1 and a control group G2, ranging from June 2020 to December 2022, *i.e.*, 30 months.

2.2. Sampling and Eligibility Criteria

In total, 228 male patients, including 76 patients from group G1 made up of mechanics and 152 patients from group G2, as well as 27 automobile garages, constituted our sample. The sampling was obtained by simple random sampling. Before obtaining informed consent, all participants were informed of the objectives of the study. Patients eligible for this study had: 1) for group G1, an age between 18 and 50 years, practicing for at least one year, and consenting to the study; 2) for group G2, the patients were all coming, aged between 18 and 50 years, living a certain distance from garages and major avenues, being non-smokers, having no known fertility problems, and consenting to the study.

2.3. Data Collection

2.3.1. Epidemiological Investigation

After obtaining informed consent, a standardised questionnaire on a survey form made it possible to collect sociodemographic data, medical or surgical history, lifestyle habits, and various semen parameters. The anonymity of each participant was guaranteed by the allocation of a code and an identification number.

2.3.2. Biological Investigation

It was carried out at the National Public Health Laboratory and concerned semen analysis and the spermogram.

2.4. Sperm Analysis

The spermogram was performed according to WHO recommendations [24]. To do this, the sperm was collected by masturbation in a sterile receptacle after a period of sexual abstinence of 3 to 5 days. The ejaculates were then maintained at 37°C for 30 minutes until complete liquefaction of the sperm. Subsequently, the physicochemical and microscopic characteristics of the sperm were evaluated.

2.4.1. Physicochemical Characteristics of Sperm

In this work, 4 physicochemical characteristics of sperm were evaluated. These parameters were assessed with the naked eye. These are sperm volume, viscosity, appearance of the ejaculate and sperm pH.

Spermatic volume: This volume was evaluated from a 10 mL graduated cylinder, referring to the standard of between 1, 5 and 6 mL.

Viscosity: The measurement of viscosity was assessed by observing the way in which the sperm flowed from the end of the glass rod after being dipped into the vial containing the sperm, using the Hotchkiss method. Subsequently, the viscosity is judged normal if the drop stretches at the end of the wand.

Appearance of the ejaculate: using the macroscopic observation, the liquefied sample was judged to have a milky appearance.

Spermatic pH:

This parameter was measured using a pH indicator strip on which a drop of sperm was placed. Note that this takes place less than 30 minutes after ejaculation without exceeding one hour. The pH is considered normal if its value is between 7.2 and 8.

2.4.2. Microscopic Characteristics

Sperm mobility: Motility was assessed by direct examination by placing a drop of 10 L of sperm between the slide and coverslip. It was expressed as a percentage and classified into each of the movement categories, namely progressive mobility, non-progressive mobility, and immobility.

- Progressive mobility (a + b): for sperm that move in a straight line, zigzag, or in large circles at fast speed (a) and slow speed (b). This mobility must be greater than 32%.
- Non-progressive mobility (c): for spermatozoa that move but whose movements do not allow real movement, weak beats barely moving the head or flagellum beats only.
- Immobility (d): the spermatozoa do not make any movement. The immobility rate must be <58%. Sperm count: This number is assessed by counting the spermatozoa on a hemocytometer (Thomas cell) after immobilisation with a Ringer's solution containing 1% formalin.

2.4.3. Sperm Concentration

In the case of low concentration, we count all the rooms. With X, the number of

spermatozoa is counted. This concentration is defined by parameters such as azoospermia, oligospermia, and polyspermia.

- Azoospermia: total absence of sperm in the ejaculate;
- Oligospermia: sperm concentration less than 15 million/mL of ejaculate;
- Polyspermy: sperm concentration greater than 200 million/mL of ejaculate.

2.4.4. Vitality

It represents the percentage of live sperm. The eosin dye and nigrosin, acting as fixatives, allowed the evaluation of the vitality of the spermatozoa. Thus, when reading the smear, the dead spermatozoa turn pink and the live ones are white. The percentage of live sperm at ejaculation must be \geq 58%.

2.5. Environmental Investigation

As recently reported in our previous study on automobile pollutants in ambient air in Brazzaville (Diakouka *et al.* [25]), this time our samples were taken from different automobile garages in the districts of Brazzaville. For each district, 4 garages were selected, and 4 samples were taken per garage for two seasons, one in the rainy season and the other in the dry season, over the course of two years (2020 and 2022), *i.e.*, a total of 144 samples for the 9 districts.

Assessment of Pollutants

An air sampling campaign for the quantitative evaluation of the concentrations of gaseous automobile pollutants (NO₂, SO₂, CO, and CO₂) was evaluated at garages in Brazzaville. Sampling was done using an exhaustive method. In this study, samples were taken using the spot method using a measuring system on Dräger reagent tubes (Image 1), coupled with a Dräger Accuro sampling pump (Image 2). To avoid their degradation, the tubes were kept in the containers and stored in the refrigerator, the recommended temperature being between 0 and 10°C. These tubes were then transported to the different sites by car in transport coolers. The method for measuring the concentration of pollutants is based on a colorimetric reaction (in the Dräger tube) that takes place in the presence of specific pollutants. Dräger reagent tubes, or gas detection tubes, are filled with chemical reagents that absorb and react with the target gas or vapour being measured. After suction of air by the pump, the reading was taken immediately in a clear place, protected from sunlight, on white paper from the scale engraved on the tube and in comparison to a non-contact tube. In the absence of standards and regulations on air quality in Congo, our results were appreciated according to the WHO 2021 air quality standards.

2.6. Statistical Analysis

The data has been processed using Excel 2016 software (Microsoft Corporation, USA). This tool was used for the statistical analysis and design of graphs. The Census and Survey Processing System (CSPro) 4.1 software was also used for data entry. The Chi-squared test and Fisher were used for the comparison of qualitative variables and the student for the comparison of quantitative variables.



Image 1. Dräger tube.



Image 2. Dräger accuro pump.

3. Results

3.1. Samples

Our samples consisted of 228 subjects (76 cases and 152 controls) for the year 2020; we recorded 4 losts to follow-up during the year 2022, thus reducing the sample size to 224 subjects (74 cases and 150 witnesses), *i.e.*, one case for two controls. In percentage terms, the sample is distributed as follows: 66% of controls, 33% of cases, and 1% of those lost (**Figure 1**). In our study, the largest numbers of subjects were identified in Poto-Poto and Ouénzé, both among cases and among controls (**Table 1**). Based on age, in 2020 as in 2022, the most representative age group was 36 to 46 years old, followed by 26 to 35 years old for both cases and controls, with an average age of 37 ± 7 years for the year 2020 and 38 ± 8 years for the year 2022 (**Table 2** and **Table 3**).

3.2. Identification of Risk Factors Associated with Investigations

Concerning the risk factors, only alcohol consumption was statistically significant (p < 0.05) both in 2020 and in 2022, at the respective rates of 11.8% among cases and 28.3% among controls for the year 2020 compared to 12.2% among cases and 28,7% among controls for the year 2022 (**Table 4**).

	2	020	2	2022	L	osts
	Cases	Controls	Cases	Controls	Cases	Control
M - 1- (1 (1- (1 (7	8	7	8	0	0
Makélékélé	9%	5%	9%	5%		
D	6	8	6	8	0	0
Bacongo	8%	5%	8%	5%		
D. C. D. C.	17	37	17	36	0	1
Poto-Poto	22%	24%	23%	24%		
	10	14	9	14	1	0
Moungali	13%	9%	12%	9%		
o (16	19	16	18	0	1
Ouenzé	21%	13%	22%	12%		
m 1	9	17	9	17	0	0
Talangaï	12%	11%	12%	11%		
	5	20	5	20	0	0
M'filou	7%	13%	7%	13%		
	2	15	2	15	0	0
Madibou	3%	10%	3%	10%		
	4	14	3	14	1	0
Djiri	5%	9%	4%	9%		
Total	76	152	74	150	2	2

 Table 1. Distribution of the sample by district.

Table 2. Distribution of the sample by age group.

	2	020	2	022
Age (year) –	Cases	Controls	Cases	Controls
	9	4	8	3
[18 - 25]	12%	3%	11%	2%
	25	53	23	48
[26 - 35]	33%	35%	31%	32%
	31	72	28	71
[36 - 45]	41%	47%	38%	47%
	11	23	15	28
[46 et plus]	14%	15%S	20%	19%
Total	76	152	74	150

DOI: 10.4236/ojap.2023.124008

			2020		2022			
	Sample	Cases	Controls	Student test	Sample	Cases	Controls	Student test
Number of observations	228	76	152		224	74	150	
Middle age	37	36	37	t = -1.623; p = 0.106	38	37	38	t = -0.978; p = 0.329
Standard deviation	7	8	7		7	8	7	

Table 3. Distribution of the samples by contribution to average ages.

Table 4. Distribution of risk factors.

Dials Eastana		2020			2022	
Risk Factors	Cases	Controls	p-Value	Cases	Controls	p-Value
Varicocele treated or not						
X.	1	5		0	5	
Yes	1.3%	3.3%	0.000	0.0%	3.3%	0.110
	75	147	0.380	74	145	0.112
No	98.7%	96.7%		100.0%	96.7%	
Cryptorchidism						
V	1	1		1	1	
Yes	1.3%	0.7%	0.616	1.4%	0.7%	0.608
	75	151		73	149	
No	98.7%	99.3%		98.6%	99.3%	
Excessive use of condoms						
X.	0	0		0	0	
Yes	0%	0%	0.360	0%	0%	0.512
NT.	76	152		74	150	
No	100.0%	100.0%		100.0%	100.0%	
Sexually transmitted infection						
V	10	14		9	14	
Yes	13.2%	9.2%	0.260	12.2%	9.3%	0 510
NT.	66	138	0.360	65	136	0.512
No	86.8%	90.8%		87.8%	90.7%	
Bilharzia						
Yes	10	0		0	0	
105	0.0%	0.0%		0.0%	0.0%	
Na	0	0		0	0	
No	0.0%	0.0%		0.0%	0.0%	

Obesity						
	16	18		13	18	
Yes	21.1%	11.8%	0.066	17.6%	12.0%	0.256
N-	60	134		61	132	
No	78.9%	88.2%		82.4%	88.0%	
Alcohol consumption						
V	67	109		65	107	
Yes	88.2%	71.7%	0.005	87.8%	71.3%	0.000
N	9	43	0.005	9	43	0.006
No	11.8%	28.3%		12.2%	28.7%	
Tobacco consumption						
17	1	1		1	1	
Yes	1.3%	0.7%	0.616	1.4%	0.7%	0.000
N-	75	151	0.616	73	149	0.608
No	98.7%	99.3%		98.6%	99.3%	
Others (surgeries, history, family, cancer)						
V	0	0		0	0	
Yes	0.0%	0.0%		0.0%	0.0%	
N-	76	152		74	150	
No	100.0%	100.0%		100.0%	100.0%	
Total	76	152		74	150	



Figure 1. Overall distribution of the sample.

3.3. Fertility Abnormalities Resulting from Exposure to Pollutants

The study on the psychochemical parameters of sperm revealed no significant differences regarding pH, colour, or viscosity. However, we noted a significant difference in sperm volume for the year 2022 (**Table 5**). The distribution of spermogram results by district in our study reported a statistically significant increase in abnormal spermogram results to 29% for cases and 14% for controls in 2020, compared to 47% for cases and 14% for controls in 2022 at Poto-Poto. In Ouénzé, we also noted that 25% of abnormal spermograms were for cases and 26% for controls in 2020, compared to 44% for cases and 22% for controls in 2022. However, we observe an increase in Makélékélé, although not significant (**Table 6**).

In our study, the microscopic results of the spermogram reported a statistically significant increase in abnormal sperm parameters, including motility, count,

		2020			2022		
	Cases	Controls	p-Value	Cases	Controls	p-Value	
Volume							
Normal (S15 and)	75	152		72	150		
Normal (≥1.5 mL)	98.7%	100.0%	0.156	97.3%	100.0%	0.043	
Aba ann al (21 5 mJ)	1	0	0.156	2	0	0.045	
Abnormal (<1.5 mL)	1.3%	0.0%		2.7%	0.0%		
pH							
Normal [7.2.9]	69	141		65	137		
Normal [7.2 - 8]	90.8%	92.8%	0.150	87.8%	11.3%	0.062	
	7	12	0.158	9	13	0.063	
<u>Abnormal</u>	9.2%	7.9%		12.2%	8.5%		
Colour							
	74	148		70	147		
Opalescent gray	97.4%	97.4%	1 000	94.6%	98.0%	0.070	
	2	4	1.000	4	3	0.078	
Translucent	2.6%	2.6%		5.4%	2.0%		
Viscosity							
N 1	73	150		71	149		
Normal	96.1%	98.7%		95.9%	99.3%		
••• ·	3	2	0.201	3	1	0.072	
Hypo-viscous	3.9%	1.3%		4.1%	0.7%		
Total	76	152		74	150		

Table 5. Distribution of psychochemical parameters of sperm.

			2	020					2022	
	C	Cases Controls Khi-square		C	ases	Co	ntrols	Khi-square		
Makélékélé										
Normal	5	71%	7	88%	0.429	4	57%	7	88%	0 195
Abnormal	2	29%	1	13%	0.438	3	43%	1	13%	0.185
Bacongo										
Normal	3	50%	6	75%	0.224	4	67%	6	75%	0 522
Abnormal	3	50%	2	25%	0.334	2	33%	2	25%	0.733
Poto Poto										
Normal	12	71%	32	86%	0.1.62	9	53%	31	86%	
Abnormal	5	29%	5	14%	0.162	8	47%	5	14%	0.009
Moungali										
Normal	8	80%	11	79%	0.932	6	67%	11	79%	
Abnormal	2	20%	3	21%		3	33%	3	21%	0.526
Ouenzé										
Normal	12	75%	14	74%	0.000	9	56%	14	78%	0.010
Abnormal	4	25%	5	26%	0.929	7	44%	4	22%	0.018
Talangaï										
Normal	8	89%	14	82%	0.660	6	67%	14	82%	0.266
Abnormal	1	11%	3	18%	0.660	3	33%	3	18%	0.366
M'filou										
Normal	4	80%	17	85%	0.705	3	60%	18	90%	0.102
Abnormal	1	20%	3	15%	0.785	2	40%	2	10%	0.102
Madibou										
Normal	2	100%	13	87%	0.500	2	100%	14	93%	0 505
Abnormal	0	0%	2	13%	0.582	0	0%	1	7%	0.707
D'jiri										
Normal	3	75%	13	93%	0.014	3	100%	13	93%	0.600
Abnormal	1	25%	1	7%	0.316	0	0%	1	7%	0.633

Table 6. Distribution of spermogram results by district.

and morphology (spermocytogram), in cases between 2020 and 2022. We also note an increase, although not significant, in abnormal vitality to 32.9% for cases in 2020 compared to 45.9% in 2022 (**Table 7**).

		2020		2022			
	Cases	Controls	p-Value	Cases	Controls	p-Value	
Vitality							
	51	86		40	86		
Normal (≥58%)	67.1%	56.6%	0.100	54.1%	57.3%	0.642	
A has a more all (25 00()	25	66	0.126	34	64	0.642	
Abnormal (<58%)	32.9%	43.4%		45.9%	42.7%		
Numeration (per mL)							
No	67	139		54	136		
Normal ($\geq 15 \times 10^6/mL$)	88.2%	91.4%		73.0%	90.7%	0.002	
Anormal [6 - 14] × 10 ⁶ /mL	8	12	0 701	18	12		
	10.5%	7.9%	0.701	24.3%	8.0%		
	1	1		2	2		
Abnormal [0 - 5] × 10 ⁶ /mL	1.3%	0.7%		2.7%	1.3%		
Mobility [a + b + c]							
Normal (≥40%)	65	130		52	130		
Normal ($\geq 40\%$)	85.5%	85.5%		70.3%	86.7%		
	11	22	1.00	22	20	0.003	
Abnormal (<40%)	14.5%	14.5%		29.7%	13.3%		
Sperm morphology							
	75	150		67	147		
Normal (≥4%)	98.7%	98.7%		90.5%	98.0%		
	1	2	1.000	7	3	0.011	
Abnormal (<4%)	1.3%	1.3%		9.5%	2.0%		
Total	76	152		74	150		

 Table 7. Distribution of microscopic spermogram parameters.

In our study, oligoasthenospermia as well as necrozoospermia constituted the most recorded spermogram abnormalities, with a statistically significant increase in 2022, *i.e.*, 9.2% for cases and 2.6% for controls with regard to oligo-asthenospermia in 2020 compared to 18.9% for cases and 2.7% for controls in 2022. Necrosospermia was observed at 9.2% for cases and 5.3% for controls in 2020, compared to 9.5% for cases and 3.3% for controls in 2022 (**Table 8**).

Our study reveals a statistically significant link between spermogram results and the duration of exposure. **Table 9** reveals that 9 (47%) of our cases with an abnormal spermogram had been exposed between the ages of 20 and 29, in 2020, compared to 14 (48%) in 2022.

	2020 (p-Va	alue = 0.227)	2022 (p-Va	alue = 0.000)
	Cases	Controls	Cases	Controls
	57	127	46	128
Normal spermogram	75.0%	83.6%	62.2%	85.3%
A	0	1	2	2
Azoospermia	0.0%	0.7%	2.7%	1.3%
Oligozoospermia	3	5	1	4
Ongozoosperima	3.9%	3.3%	1.4%	2.7%
A stan and a sub-sub-	1	6	1	6
Astenozoospermia	1.3%	3.9%	1.4%	4.0%
Necrozoospermia	7	8	7	5
Recrozoosperinia	9.2%	5.3%	9.5%	3.3%
Oligo-asthenospermia	7	4	14	4
Ongo-astrichosperinia	9.2%	2.6%	18.9%	2.7%
Oligo-asthenoteratospermia	1	1	3	1
enge astrenoteratosperinta	1.3%	0.7%	4.1%	0.7%
Total	76	152	74	150

 Table 8. Distribution of spermogram abnormalities.

Table 9. Distribution of spermogram parameters among cases according to duration of exposure.

A	2020 (p-Va	lue = 0.001)	2022 (p-Value = 0.005)			
Age (year)	Normal	Abnormal	Normal	Abnormal		
	16	1	13	2		
[0 - 9]	28%	5%	29%	7%		
[10, 10]	20	5	16	9		
[10 - 19]	35%	26%	36%	31%		
[20, 20]	21	9	16	14		
[20 - 29]	37%	47%	36%	48%		
[20, 25]	0	4	0	4		
[30 - 35]	0%	21%	0%	14%		
Total	57	19	45	29		

The analysis of the correlation coefficient shows that there is a linear link between the results of the spermogram and the environmental variables (the correlation coefficient is close to 1). However, this link is statistically significant for carbon dioxide (CO₂), carbon monoxide (CO), and nitrogen dioxide (NO₂) ($p \le$ 0.05). Furthermore, there is no link between the spermogram and the sulphur dioxide population (Table 10).

A h	Environmental parameters						
Abnormal spermogram cases	CO2	СО	NO ₂	SO ₂			
Correlation coefficient	0.88	0.92	0.95	0.55			
p-Value	0.0018	0.0005	0.0001	0.12			

 Table 10. Linear link between environmental parameters and the proportion of abnormal spermogram cases among cases.

3.4. Concentrations of Pollutants in Ambient Air

In this study, the NO₂ concentration levels were all higher than the WHO 2005 and 2021 standards (200 μ g/m³). The strongest were recorded in the 2022 dry season, with peaks in Ouénzé, Poto-Poto, and Mfilou (**Figure 2**). The highest CO₂ concentration levels were recorded during the dry season of 2022 in Poto-Poto and Ouénzé, followed by Bacongo and Moungali, all above the 2005 and 2021 standards established by the WHO at 400 μ g/m³ (**Figure 3**). CO concentrations measured during the rainy season were lower than those measured during the dry seasons, with peaks in Poto-Poto and Ouénzé, all above the WHO 2005 and 2021 standards at 10 μ g/m³ (**Figure 4**).

During our investigation, we also noted SO_2 concentrations higher than the WHO standards for 2005 and 2021 (20 µg/m³). In all districts, with a peak in the 2022 dry season in Poto-Poto, Ouénzé is followed by Djiri and Madibou (**Figure 5**).

4. Discussion

The spermogram is an essential diagnostic marker recognised as the first-line examination in the assessment of male reproductive health and the prevention or treatment of male infertility. This study included 76 cases and 152 controls in 2020 and 74 cases and 150 controls in 2022, *i.e.*, one case for two controls, all male. Four (04) lost to follow-up were recorded in this study. The largest workforce in our sample, 21% in 2020 and 22% in 2022; in some cases, 24% in both 2020 and 2022, was recruited in Poto-Poto. This can be explained by the fact that in Poto-Poto, people were more interested and receptive to information on the danger posed by ambient air pollution and therefore more able and motivated to participate in the study. The samples are entirely male in this study, which could be explained by the fact that the profession of motor mechanic is very physical and that it is therefore mainly practiced by men in our country. The few female mechanics I met during this investigation did not consent to the study.

The results linked to the age of the population studied indicate that the age group of 36 to 45 years old, followed by that of 26 to 35, were the most representative, respectively, at 38% of cases in 2020 and 31% in 2022, against 47% of controls. With an average age of 37 ± 7 years for the year 2020 and 38 ± 8 years for the year 2022. These results could be justified by the fact that the African population is young and the said profession requires a good state of health and physical endurance due to sometimes restrictive postures. These observations



2020 Rainy season 2020 Dry season 2022 Rainy season 2022 Dry season

Figure 2. Distribution of NO₂ concentrations in garages by district.



Figure 3. Distribution of CO₂ concentrations in garages by district.



Figure 4. Distribution of CO concentrations in garages by district.



Figure 5. Distribution of SO2 concentrations in garages by district.

are similar to those of Frikh *et al.*, who found an average age of 39 years for both cases and controls [26].

In this study, risk factors were sought in order to avoid confusion when interpreting the results. This study reveals that alcohol consumption was the only statistically significant risk factor in both cases (11.8%) and controls (28.3%) for the year 2020, compared to 12.2% in cases and 28.7% in controls for the year 2022. These results are in agreement with those of Concordéli *et al.*, who noted a deleterious effect of alcohol on fertility and parameters of sperm [27]. Similarly, Najapour *et al.*, revealed that lifestyle habits (alcohol, tobacco, STIs, etc.) have a negative effect on sperm quality [16]. This result can be explained by the fact that the congolese have integrated alcohol consumption into their daily lifestyle, unaware that this promotes the overproduction of reactive oxygen species, which will potentiate the effects of stress on health in general and on the reproductive system in particular.

Studies on the health effects of air pollution are difficult to interpret; existing ones are not always comparable because they do not necessarily concern the same pollutants, and their methodologies often differ in terms of populations studied, duration, and exposure period. In this study, the analysis of the macroscopic parameters of the spermogram did not reveal any significant differences with regard to the physicochemical parameters of sperm (pH, viscosity, colour, appearance, and volume) for the year 2020; however, the sperm volume was abnormally (<1.5) significant for the year 2022 at 2.7% in cases compared to 0% in controls. This result could be explained by the fact that prolonged exposure to automobile pollutants such as NO_2 and Pb leads to ulcerations in the urethra but also malformations in the genital tract, which could either obstruct the passage of sperm or promote retrograde ejaculations, the consequence of which would be the absence of sperm or the reduction in sperm volume, as revealed in the study by [28]. These results are in agreement with those found by Jurewicz in toll booth workers exposed to automobile pollutants such as NO, SO,

and Pb [29]. At the same time, the overall spermogram results reveal no significant difference in 2020. However, we note a significant difference in these results for the year 2022, at 38% among cases compared to 15% among controls. In our study, we compared the semen parameters of mechanics and controls between 2020 and 2022. The analysis of these results reported no significant difference on the different semen parameters for the year 2020; however, we observed for the year 2022: a statistically significant decrease in sperm count (24% for cases versus 8% for controls for a sperm count between 4 - 6 million and 2.7% versus 1.3% for a sperm count between 0 - 5 million); a reduction in sperm mobility (27.3% of cases compared to 13.3% of controls had total mobility < 40%) a change in the morphology of the spermatozoa (in 9.5% of cases compared to 2% for abnormal morphology, <4%). Our results are similar to those reported by [13] [23], who found in their study a significant reduction in the daily production of spermatozoa, their mobility, and their vitality, as well as morphological abnormalities reflecting an inhibition of spermatogenesis in subjects exposed to diesel exhaust gases. The results obtained in this study could be explained by the effects of oxidative stress on spermatozoa. Indeed, certain pollutants such as NO₂, O₃, etc. can easily oxidise to form reactive oxygen derivatives (R.O.S.). However, the sperm plasma membrane is very rich in polyunsaturated fatty acids, which are responsible for membrane fluidity and make it vulnerable to oxidative stress. When the quantity of R.O.S increases, we witness a degradation of polyunsaturated fatty acids, which will lead to a cascade of chemical reactions leading to oxidation or even peroxidation of lipids in the sperm, which will result in a loss of membrane fluidity and a decrease in enzymatic activity and ion channels (depolarization by exit of calcium and entry of carbonate ions). This depolarization will therefore reduce calcium flows at the level of the sperm flagellum, thus leading to a reduction in its mobility [30]. Similarly, ROS-induced protein oxidation could also damage sperm through the cleavage of polypeptide chains and accumulation of protein aggregates. Finally, R.O.S. could initiate chain reactions leading to apoptosis, in particular by altering the membrane integrity of mitochondria. This process could be accelerated by damage induced at the level of DNA and the spermatic membrane. This would favour morphological abnormalities and lead to a reduction in sperm count [30].

Ambient air analysis was evaluated in all garages selected from 2020 to 2022 in the nine districts of Brazzaville. It consisted of a quantitative evaluation of the different automobile pollutants (CO, CO₂, NO₂, and SO₂) and made it possible to assess the air quality in the selected garages. In the absence of air quality standards in Congo, our results were assessed by referring to the 2021 air quality standards established by the WHO. In this study, the concentrations of CO, CO₂, and NO₂ measured in the different garages were all higher than WHO air quality standards, with peaks in Poto-Poto and Ouénzé. With the production of these three pollutants being strongly linked to traffic, it should be noted that the nine districts of Brazzaville are inhabited and active. The concentrations of CO, CO₂, and NO₂ obtained in this study could be justified by the fact that our country, Congo, is experiencing strong growth in the automobile fleet (especially with regard to four-wheeled vehicles), due to the location of these garages, which are located on or near major roads, to the not very confined spaces of these garages, but also to the quality of the vehicles, which sometimes lack maintenance, and also to the poor quality of the fuel. These results are in agreement with those found by Bopaka *et al.*, where the concentrations of automobile pollutants were higher than WHO standards in different automobile garages in Brazzaville [2].

5. Conclusion

During this study, one of the first carried out in the Congo on the subject, the general objective was to evaluate the short-term effects of air pollution on fertility among mechanics in Brazzaville. We found that in all the garages selected during this study, the concentrations of air pollutants (NO₂, CO₂, CO, and SO₂) were all higher than the 2021 air quality standards established by WHO. The results related to the age of the population studied indicate an average age of 36 ± 8 years for the cases and 38 ± 7 years for the controls, with extremes of 18 to 50 years. This study reveals that alcohol consumption was the only statistically significant risk factor. Regarding the analysis of spermogram parameters, we noted for the year 2022 a decrease in sperm volume, mobility, and count and an increase in morphological abnormalities of the sperm (macrocephalus). Likewise, neucrosospermia as well as oligoasthenozospermia constituted the most common spermogram abnormalities. Likewise, a linear equation made it possible to establish the link between atmospheric pollution (automobile) and sperm parameters.

Recommendations

At the end of this study, we propose the following mitigation strategies: 1) To the Ministry of the Environment, to set up a permanent air quality monitoring network in order to better manage pollution levels in real time; 2) To the Ministry of Health, to promote awareness campaigns on the dangers linked to air pollution, to disseminate through advertisements on national channels and posters illustrating the risks linked to said pollution; 3) To the government, to expand the road network in order to streamline traffic and therefore reduce the level of pollution; 4) Mechanics, to research the risks linked to their professions, to ensure individual protection by wearing mechanical individual protection equipment (gloves, overalls, glasses, bibs, etc.) to improve their lifestyle habits, but above all to plan follow-up regular medical.

Conflicts of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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