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DACCIWA

"Dynamics-aerosol-chemistry-cloud interactions in West Africa"

Deliverable

D2.4 Toxicological effects of air pollution

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1 Introduction

Our objective was to characterize the toxicity of different size-fractions collected in dry and wet seasons during 2 consecutive years in 4 different locations in Abidjan (Cote d'Ivoire) and Cotonou (Benin).

The toxicity was characterized by the release of pro-inflammatory mediators. It is known that *in vivo* the main short term effect of PM exposure is an inflammation. This inflammation results from the activation of macrophages and epithelial cells that line the respiratory tract and are the first cell types encountered by PM in the lungs. PM exposure triggers the production and the release by cells of pro-inflammatory mediators that are chemoattractant for immune inflammatory cells. The production of such mediators (cytokines) results from cell activation due to an oxidative stress.

In DACCIWA, the target cell was a human bronchial epithelial cell line (NCI-H292) and the pro-inflammatory response was evaluated by measuring the release of 2 different cytokines IL-6 and IL-8 after 24h exposure to particles at 3 different concentrations. Before we have evaluated if these doses induced or not a cytotoxicity. In addition we measured the production of intracellular reactive oxygen species in order to evaluate whether an oxidative stress occurs.

Toxicological data were further put in perspective with the chemical composition of the different size-fraction of the different sites.

2 Materials and methods

2.1 Sampling sites

Samplings have been done in Cotonou (Bénin) and Abidjan (Cote d'Ivoire). In each town, there was a traffic site characterized by 2 wheels in Cotonou (CT) and old cars in Abidjan (AT). In addition in Abidjan, 2 other sampling sites were used to collect PM from domestic fire (ADF) and from waste burning (AWB).

4 campaigns were realized on each site to collect samples in the dry season (January 2016 and 2017) and wet season (July 2015 and 2016).

Collection of the aerosols was done over 3h during the period where emissions are the most important and were performed during 3 consecutive days as shown in the table below.

Table 1: Date and schedule of PM samplings during the 4 campaigns

	WET SEASON					
	CAMPAIGN 1 – July 2015			CAMPAIGN 3 – July 2016		
	1IN1	1IN2	1IN3	3IN1	3IN2	3IN3
ADF	9:35AM-12:20PM 20/07/2015	9:39AM-12:30PM 21/07/2015	8:32AM-11:04AM 22/07/2015	9:50AM-11:30AM 04/07/2016	9:03AM-10:15AM 05/07/2016	8:35AM-9:34AM 06/07/2016
AT	7:10PM-10PM 20/07/2015	4:11PM-7:31PM 21/07/2015	6:23PM-8:24PM 22/07/2015	2:56PM-5h57PM 04/07/2016	1:53PM-4:50PM 05/07/2016	1:25PM-4:25PM 07/07/2016
AWB	2:28PM-5:03PM 20/07/2015	1:07PM-4PM 22/07/2015		8:45AM-12:01PM 07/07/2016	8:34AM-12:02PM 08/07/2016	12:27PM-4:45PM 08/07/2016
CT	7:47AM-10:47AM 27/07/2015	7:40AM-11:05AM 28/07/2015	7:47AM-11:27AM 29/07/2015	11AM-2PM 11/07/2016	9:28AM-12:29PM 12/07/2016	9:52AM-01:05PM 13/07/2016

	DRY SEASON					
	CAMPAIGN 2 – January 2016			CAMPAIGN 4 – January 2017		
	2IN1	2IN2	2IN3	4IN1	4IN2	4IN3
ADF	9:01AM-12PM 07/01/2016	9:04AM-12:04PM 08/01/2016	8:46AM-11:46AM 09/01/2016	10:11AM-12:34PM 10/01/2017	9:25AM-12:22PM 11/01/2017	10AM-1PM 12/01/2017
AT	1:57PM-06:30PM 07/01/2016	3:45PM-6:45PM 08/01/2016	4:02PM-7:02PM 09/01/2016	3:30PM-6:30PM 10/01/2017	3PM-6PM 11/01/2017	4:22PM-7:22PM 12/01/2017
AWB	9:30AM-12:30PM 10/01/2016	12:55PM-3:55PM 10/01/2016	12:53PM-3:53PM 11/01/2016	10:27AM-1:30PM 13/01/2017	2:11PM-5:30PM 13/01/2017	10:30AM-1:30PM 14/01/2017
CT	9:03AM-12:03PM 13/01/2016	8:14AM-11:15AM 14/01/2016	8:37AM-11:37AM 15/01/2016	9:55AM-12:56PM 05/01/2017	9:06AM-12PM 06/01/2017	9:20AM-11:20AM 07/01/2017
				3:48PM-6:33PM 05/01/2017	3:30PM-6:36PM 06/01/2017	

Particles were sampled using a 5 stage impactor (Dekati) allowing to collect particles >2.5 µm to <0.2 µm on 5 stages equipped with polycarbonate filters (nuclepore). The upper stages (1 and 2) were used to reconstitute the coarse fraction (<2.5 µm - 1 µm), the stage 3 and 4 to reconstitute the fine fraction (1 – 0.2 µm) and the last one to recover the ultrafine fraction (<0.2 µm).

To reconstitute the particle suspensions needed for cell exposure, particles have to be detached from the filter and resuspended in the culture medium used for cells. Filters are cut in small pieces and introduced in an Eppendorf with the RPMI culture medium and submitted to a sonication allowing the detachment of particles. We assumed a total extraction allowing to consider the mass of particles deposited on filters to calculate the concentrations of particles in the suspensions. In parallel blank filters were treated in the same way and were used as negative controls.

Filters of the first dry season (campaign 1) were not used as some filters were inserted in the impactor upside down preventing the detachment of particles from the filters. For the campaign 2, particles of each of the 3 consecutive days were tested separately. However the particle mass being sometimes too low for some size fractions, we were unable to do replicates of the experiments. This is the reason why for the following campaigns, according to the collected masses, we decided to pool the particles of the 3 consecutive days while still separating the 3 size fractions.

Table 2: concentration of particle suspensions after their detachment by sonication: campaign 2

	ADF			AT			AWB			CT		
	REF	Mass mg	[c] µg/µL	REF	Mass mg	[c] µg/µL	REF	Mass mg	[c] µg/µL	REF	Mass mg	[c] µg/µL
G	IN1 1 ADF	0,128	0,08				IN1 1 AWB	0,2725	0,30	IN1 1 CT	0,544	0,32
	IN1 2 ADF	0,1245					IN1 2 AWB	0,6423		IN1 2 CT	0,415	
F	IN1 3 ADF	0,1075	0,06				IN1 3 AWB	0,1535	0,13	IN1 3 CT	0,279	0,22
	IN1 4 ADF	0,106					IN1 4 AWB	0,2455		IN1 4 CT	0,372	
UF	IN1 5 ADF	0,3625	0,11				IN1 5 AWB	0,33	0,10	IN1 5 CT	0,696	0,20
G	IN2 1 ADF	0,3005	0,16	IN2 1 AT	0,26767	0,20	IN2 1 AWB	0,3565	0,25	IN2 1 CT	0,6715	0,35
	IN2 2 ADF	0,191		IN2 2 AT	0,344		IN2 2 AWB	0,3845		IN2 2 CT	0,388	
F	IN2 3 ADF	0,1565	0,20	IN2 3 AT	0,096	0,13	IN2 3 AWB	1,015	0,43	IN2 3 CT	0,7843	0.69
	IN2 4 ADF	0,4295		IN2 4 AT	0,29533		IN2 4 AWB	0,266		IN2 4 CT	1,297	
UF	IN2 5 ADF	0,4285	0,13	IN2 5 AT	0,48617	0,15	IN2 5 AWB	0,4253	0,13	IN2 5 CT	0,487	0,14
G	IN3 1 ADF	0,71	0,33	IN3 1 AT	0,24567	0,22	IN3 1 AWB	0,2725	0,15	IN3 1 CT	0,553	0,38
	IN3 2 ADF	0,2655		IN3 2 AT	0,421		IN3 2 AWB	0,1905		IN3 2 CT	0,577	
F	IN3 3 ADF	0,2775	0,38	IN3 3 AT	0,131	0,19	IN3 3 AWB	0,1415	0,14	IN3 3 CT	0,223	0,20
	IN3 4 ADF	0,852		IN3 4 AT	0,434		IN3 4 AWB	0,271		IN3 4 CT	0,37	
UF	IN3 5 ADF	1,27767	0,38	IN3 5 AT	0,335	0,10	IN3 5 AWB	0,257	0,08	IN3 5 CT	0,52	0,15

Table 3: concentration of particle suspensions after their detachment by sonication: campaign 3. For AT, AWB, CT, particles from the 3 days were pooled in order to have enough particles to perform experiments in triplicate.

	ADF			AT			AWB			CT		
	REF	Mass mg	[c] µg/µL	REF	Mass mg	[c] µg/µL	REF	Mass mg	[c] µg/µL	REF	Mass mg	[c] µg/µL
G	IN1 1 ADF	0,132	0,10	IN1 1 AT	0,59	0,24	IN1 1 AWB	0,157	0,11	IN1 1 CT	0,31	0,15
	IN1 2 ADF	0,163		IN1 2 AT	0,138		IN1 2 AWB	0,164		IN1 2 CT	0,148	
F	IN1 3 ADF	0,196	0,28	IN1 3 AT	0,079	0,06	IN1 3 AWB	0,1275	0,09	IN1 3 CT	0,151	0,12
	IN1 4 ADF	0,658		IN1 4 AT	0,108		IN1 4 AWB	0,154		IN1 4 CT	0,201	
UF	IN1 5 ADF	0,984	0,29	IN1 5 AT	0,213	0,06	IN1 5 AWB	0,1659	0,05	IN1 5 CT	0,323	0,10
G	IN2 1 ADF	0,086	0,07	IN2 1 AT	0,2937	0,18	IN2 1 AWB	0,165	0,10	IN2 1 CT	0,2055	0,12
	IN2 2 ADF	0,131		IN2 2 AT	0,2605		IN2 2 AWB	0,1215		IN2 2 CT	0,152	
F	IN2 3 ADF	0,1665	0,26	IN2 3 AT	0,0645	0,06	IN2 3 AWB	0,0765	0,08	IN2 3 CT	0,1765	0,10
	IN2 4 ADF	0,616		IN2 4 AT	0,13		IN2 4 AWB	0,169		IN2 4 CT	0,119	
UF	IN2 5 ADF	0,8008	0,24	IN2 5 AT	0,1716	0,05	IN2 5 AWB	0,2333	0,07	IN2 5 CT	0,3638	0,11
G	IN3 1 ADF	0,116	0,08	IN3 1 AT	0,689	0,31	IN3 1 AWB	0,1545	0,10	IN3 1 CT	0,2615	0,14
	IN3 2 ADF	0,138		IN3 2 AT	0,2456		IN3 2 AWB	0,132		IN3 2 CT	0,161	
F	IN3 3 ADF	0,129	0,25	IN3 3 AT	0,0765	0,06	IN3 3 AWB	0,251	0,13	IN3 3 CT	0,1075	0,09
	IN3 4 ADF	0,629		IN3 4 AT	0,089		IN3 4 AWB	0,129		IN3 4 CT	0,166	
UF	IN3 5 ADF	0,749	0,22	IN3 5 AT	0,2205	0,06	IN3 5 AWB	0,153	0,05	IN3 5 CT	0,417	0,12

Concentration when the 3 DAYS are pooled

	Mass mg	[c] µg/µL		Mass mg	[c] µg/µL		Mass mg	[c] µg/µL
1 AT	2,2168	0,25	1 AWB	0,8940	0,10	1 CT	1,2380	0,14
2 AT			2 AWB			2 CT		
3 AT	0,547	0,06	3 AWB	0,907	0,10	3 CT	0,921	0,10
4 AT			4 AWB			4 CT		
5 AT	0,6051	0,06	5 AWB	0,5522	0,05	5 CT	1,1038	0,11

Table 4: concentration of particle suspensions after their detachment by sonication: campaign 4. For AT, AWB, CT, particles from the 3 days were pooled in order to have enough particles to perform experiments in triplicate.

	ADF			AT			AWB			CT-M		
	REF	Mass mg	[c] µg/µL	REF	Mass mg	[c] µg/µL	REF	Mass mg	[c] µg/µL	REF	Mass mg	[c] µg/µL
G	IN1 1 ADF	0,151	0,18	IN1 1 AT	0,299	0,19	IN1 1 AWB	0,362	0,23	IN1 1 CT	0,738	0,53
	IN1 2 ADF	0,388		IN1 2 AT	0,263		IN1 2 AWB	0,322		IN1 2 CT	0,843	
F	IN1 3 ADF	0,385	0,44	IN1 3 AT	0,334	0,15	IN1 3 AWB	0,214	0,14	IN1 3 CT	0,415	0,29
	IN1 4 ADF	0,929		IN1 4 AT	0,126		IN1 4 AWB	0,206		IN1 4 CT	0,462	
UF	IN1 5 ADF	2,758	0,81	IN1 5 AT	0,452	0,13	IN1 5 AWB	0,456	0,13	IN1 5 CT	0,938	0,28
G	IN2 1 ADF	0,152	0,15	IN2 1 AT	0,16	0,12	IN2 1 AWB	0,604	0,37	IN2 1 CT	0,769	0,43
	IN2 2 ADF	0,294		IN2 2 AT	0,2		IN2 2 AWB	0,519		IN2 2 CT	0,525	
F	IN2 3 ADF	0,336	0,58	IN2 3 AT	0,056	0,06	IN2 3 AWB	0,306	0,21	IN2 3 CT	0,316	0,26
	IN2 4 ADF	1,402		IN2 4 AT	0,123		IN2 4 AWB	0,327		IN2 4 CT	0,477	
UF	IN2 5 ADF	2,92	0,86	IN2 5 AT	0,337	0,10	IN2 5 AWB	0,911	0,28	IN2 5 CT	0,89	0,26
G	IN3 1 ADF	0,239	0,25	IN3 1 AT	0,18	0,12	IN3 1 AWB	0,22	0,13	IN3 1 CT	0,438	0,28
	IN3 2 ADF	0,499		IN3 2 AT	0,191		IN3 2 AWB	0,166		IN3 2 CT	0,406	
F	IN3 3 ADF	0,253	0,47	IN3 3 AT	0,107	0,08	IN3 3 AWB	0,259	0,20	IN3 3 CT	0,188	0,17
	IN3 4 ADF	1,143		IN3 4 AT	0,134		IN3 4 AWB	0,337		IN3 4 CT	0,312	
UF	IN3 5 ADF	2,891	0,85	IN3 5 AT	0,383	0,11	IN3 5 AWB	0,231	0,07	IN3 5 CT	0,805	0,24

	CT-E		
	REF	Mass mg	[c] µg/µL
	IN1 1 CT	0,633	0,44
	IN1 2 CT	0,692	
	IN1 3 CT	0,235	0,12
	IN1 4 CT	0,132	
	IN1 5 CT	0,778	0,23
	IN1 1 CT	0,741	0,47
	IN1 2 CT	0,659	
	IN1 3 CT	0,246	0,16
	IN1 4 CT	0,229	
	IN1 5 CT	1,372	0,40

DRY SEASON - CAMPAIGN 4 – JANUARY 2017 **POOL 3 DAYS**

	Mass mg	[c] µg/µL		Mass mg	[c] µg/µL		Mass mg	[c] µg/µL		Mass mg	[c] µg/µL
1 AT	1,2930	0,14	1 AWB	2,1930	0,24	1 CTM	3,7190	0,41	1 CTS	2,7250	0,45
2 AT			2 AWB			2 CTM			2 CTS		
3 AT	0,88	0,10	3 AWB	1,649	0,18	3 CTM	2,17	0,24	3 CTS	0,842	0,14
4 AT			4 AWB			4 CTM			4 CTS		
5 AT	1,172	0,11	5 AWB	1,598	0,16	5 CTM	2,633	0,26	5 CTS	2,15	0,32

2.2 Cell cultures

NCI–H292 cells are human bronchial epithelial cells coming a 32 years old woman. They derived from a lung mucoepidermoïd carcinoma. Cells are grown in RPMI culture medium (Gibco) containing 10 % fetal bovine serum (FBS, Gibco), 1 % glutamax (Gibco), 1 % Penicillin-Streptomycine (Gibco), 1% HEPES (Gibco) and 0,5% amphothericin B (Sigma). NCI-H292 cells are seeded at 40 000 cellules/cm², in 96- well and 24- well plates. 48h later. Cultures are rinsed with culture medium and treated with particle suspensions at 1, 5 and 10 µg/cm², in replicates, for 24h.

2.3 Viability assay

The viability of NCI-H292 cells was evaluated using the WST-1 (Roche) assay. The assay is based on the cleavage of tetrazolium salts to formazan by cellular enzymes. A reduction in the number of viable cells results in a decrease in the overall activity of mitochondrial dehydrogenases, and the formation of formazan is directly proportional to the number of metabolic active cells in the culture. Cells were seeded in 96 well plates at 40,000 cells/cm² (64 000 cells/mL) 48h before particle exposure. After 24h exposure to particles, cells were rinses with PBS 1X and exposed to WST-1 100µL/well at a final dilution of 1:10 for 4 h. The formazan dye produced from WST-1 is quantified by measuring the absorbance at 450nm using a microplate reader at 450 nm (ELx808, BioTek).

2.4 Evaluation of the oxidative stress

The probe CM-H_2DCF–DA (dihydrochlorofluoresceine di acetate) (Fisher Scientific®) was used to measure the intracellular redox state. CM-H_2DCFDA is a chloromethylated derivate of the H_2DCF probe that was modified to improve its cell retention. In presence of intracellular ROS, the probe is oxidized and becomes fluorescent. The fluorescence intensity is directly proportional to intracellular ROS amount.

Cells were seeded in 96 well plates at 40,000 cells/cm² (64,000 cells/mL) 48h before particle exposure. After rinses, cells are incubated with 25 µM CM-H2DCFDA (1.73 mM in DMSO) for 1h. After 2 rinses with Hanks Balanced Salt Solution, cells are exposed to particles. A positive control is done with H_2O_2 at 1 mM. Fluorescence intensity is measured over 4 hrs with a plate reader (Flexstation3, Molecular Devices) with λ excitation 485 nm, λ emission 520 nm.

2.5 Measurement of cytokine release

Cytokine release was measured by ELISA using DuoSet ELISA Development System kits (R&D System) according to the manufacturer instructions. Cells were seeded in 48 well plates at 40,000 cells/cm² (64,000 cells/mL) 48h before particle exposure. Before particle exposure, cells are rinsed. Culture medium (supernatants) are recovered after the 24h exposure of cells to particles and were used for the ELISA assay.

2.6 Size speciated chemical aerosol composition

To allow size speciated characterization of the chemical composition of the particles, four campaigns of measurements have been performed, respectively, in July 2015 (07/20-26 period-wet season), January (01/7-15 period-dry season) and July 2016 (07/4-13 period-wet season), and January 2017 (01/5-14 period-wet season) at Abidjan Domestic Fires site (ADF), Abidjan Waste burning site (AWB), Abidjan Traffic site (AT) and Cotonou Traffic site (CT). During each campaign, two aerosol cascade impactors were used in parallel to the one devoted to toxicological studies (see above) for at least 3 hours per day over three days. The first cascade impactor, mounted with four quartz fiber filters (QMA, Whatman) including three filters of 47 mm and one of 70 mm was dedicated to carbonaceous aerosol measurements (black carbon (BC), organic carbon (OC), and water soluble

organic carbon (WSOC). The second impactor operating with three Teflon filters (Zefluor, Pall Corporation®) of 25 mm, was used to measure aerosol mass, soluble mineral ions and trace elements. In this report, only results for BC, OC, aerosol mass and soluble mineral ions will be presented.

Aerosol mass concentrations were obtained with a Sartorius microbalance (model MC21S, precision 1 µg) placed under a controlled temperature and humidity atmosphere (Person & Tymen, 2005). The filters are weighed before and after sampling. Result of a gravimetric measurement consists of the average of 2 to 4 weightings with a standard error of 5% of aerosol mass.

Carbonaceous aerosol was obtained from a thermal analysis with a two-step method adapted from Cachier et al. (1989. Two aliquots of the same filter are separately analysed. One portion is directly analysed for its total carbon content (TC). The other portion is first submitted to a pre-combustion step (2 h at 340°C under pure oxygen) in order to eliminate OC, and then analysed for its BC content. Organic carbon (OC) concentrations are calculated as the differences between TC and BC. Note that the aerosol carbon content is quantified by a non-dispersive infrared (NDIR) detector with G4 ICARUS instrument with a detection limit of the order of 2 µg C/cm^2. Uncertainty is of the order of 5% for TC, whereas in the range of 5-20%, for BC and OC, depending on the sites (Hitzenberger et al., 2006).

Inorganic ions (Na^+, NH_4^+, K^+, Mg^{2+}, Ca^{++}, SO_4^{2-}, NO_3^- Cl^-) are analysed using ion chromatography (IC) analyzer, following the analytical protocol described in Adon et al., (2010). Briefly, water extraction of half of each teflon filters is performed by a 10 min long sonication in plastic vials with 10 ml or 6 ml of purified water (Milli-Q® water with a controlled resistivity of 18.2 MΩ). After removal of the filters, vials are stored at + 4°C, and the chromatographic analysis is carried out immediately or in the days following the extraction. Cations are analyzed with Dionex DX-100 and anions with Dionex DX-500 with a detection limit of 1 to 6 ppb depending on ionic species. Uncertainties in the range of 1-50% may be indicated also depending on ionic species.

Note that results will be presented for ultra fine (UF: <0.2µm), fine (F: from 0.2 to 1µm) and coarse (C: >1µm) fractions.

Moerover, in the following, DUST, POM and WS values are also presented. Dust is the water soluble dust fraction which is obtained following Sciare et al. (2003) methodology from non sea-salt calcium ion concentrations given by our IC analysis. POM is the particulate organic matter (POM) values which are obtained from our OC concentrations and a chemical closure following Guinot et al. (2007) methodology (note that POM/OC are in the range of 1.2 to 2.1 depending on our sites). Finally WS is the sum of all ionic species.

3 Results

3.1 Cell viability

Cell viability was measured after 24h of exposure of the bronchial cells to 3 different doses (1, 5 and 10 µg/cm²) of coarse, fine or ultrafine particles. We used the WST-1 assay that measures the mitochondrial activity that reflects the metabolic capacity of cells. Whatever the campaign, the size fraction and the dose, we never observed a loss of viability. The figure 1 shows the results obtained with the samples of the campaign 1 as an example. Considering these results, this concentration range was used to evaluate the other endpoints (oxidative stress and cytokine release).

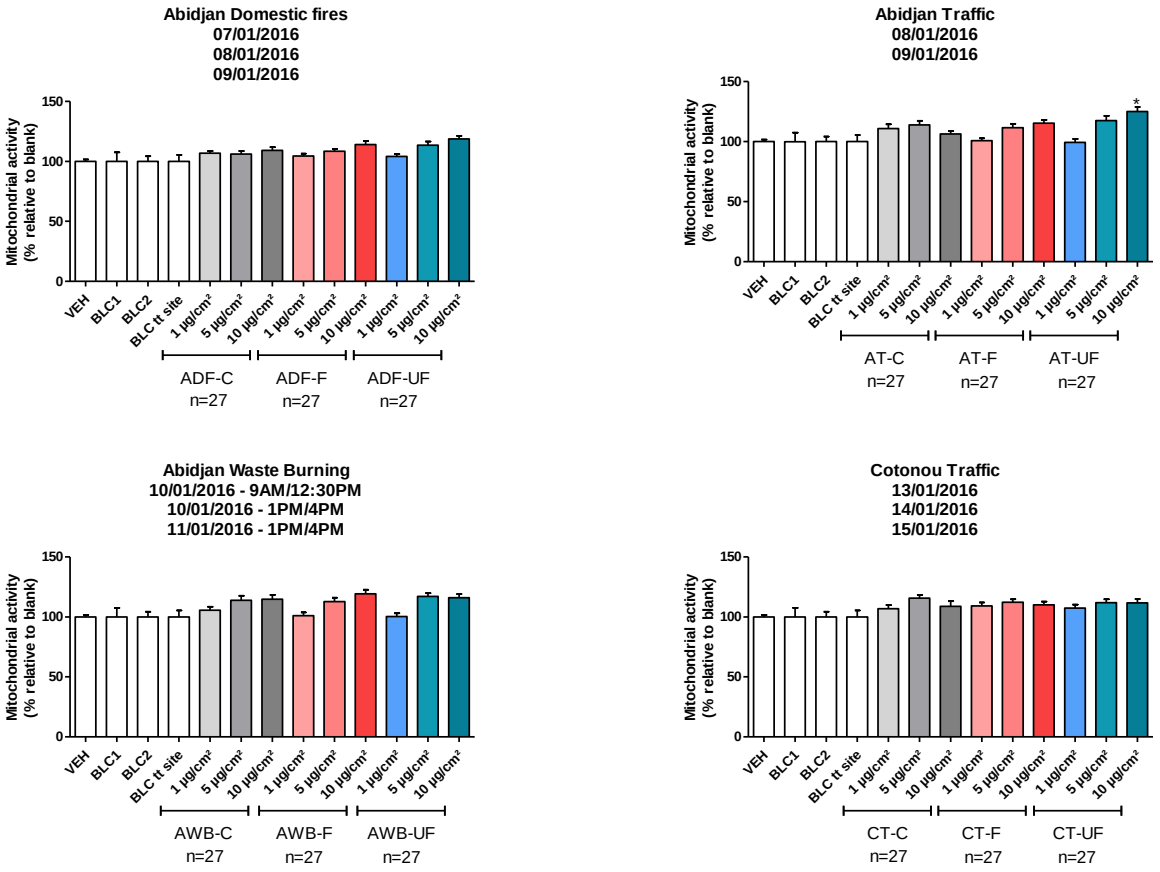


Figure 1: Viability of human bronchial epithelial cells (NCI-H292 cell line) exposed for 24h to the 3 size fractions (Coarse, Fine and Ultrafine) of particles sampled during the dry season (January 2016) in the 4 sampling sites. n correspond to the number of replicates (9 technical+ 3 biological).

3.2 Evaluation of the oxidative stress

In order to evaluate the oxidative stress, we used a probe that detects the presence of intracellular reactive oxygen species (ROS) in the cytoplasm of cells. This probe is trapped in cells that are exposed for 4 hrs to particles. If ROS are produced the probe becomes oxidized and fluoresces. The amount of fluorescence is measured over 4 h using a plate reader detecting fluorescence. Generally, we did not observe an important oxidative stress in exposed cells. Results were not consistent through the campaigns. When positive results were observed it was for the samples from Abidjan Domestic Fires but only for the campaign 3, and for Cotonou whatever their particle size as illustrated in the figure 2 and 3 respectively from campaign 3 (wet season). For this site positive results were observed for the campaign 2 and 3 but not for the 4th one.

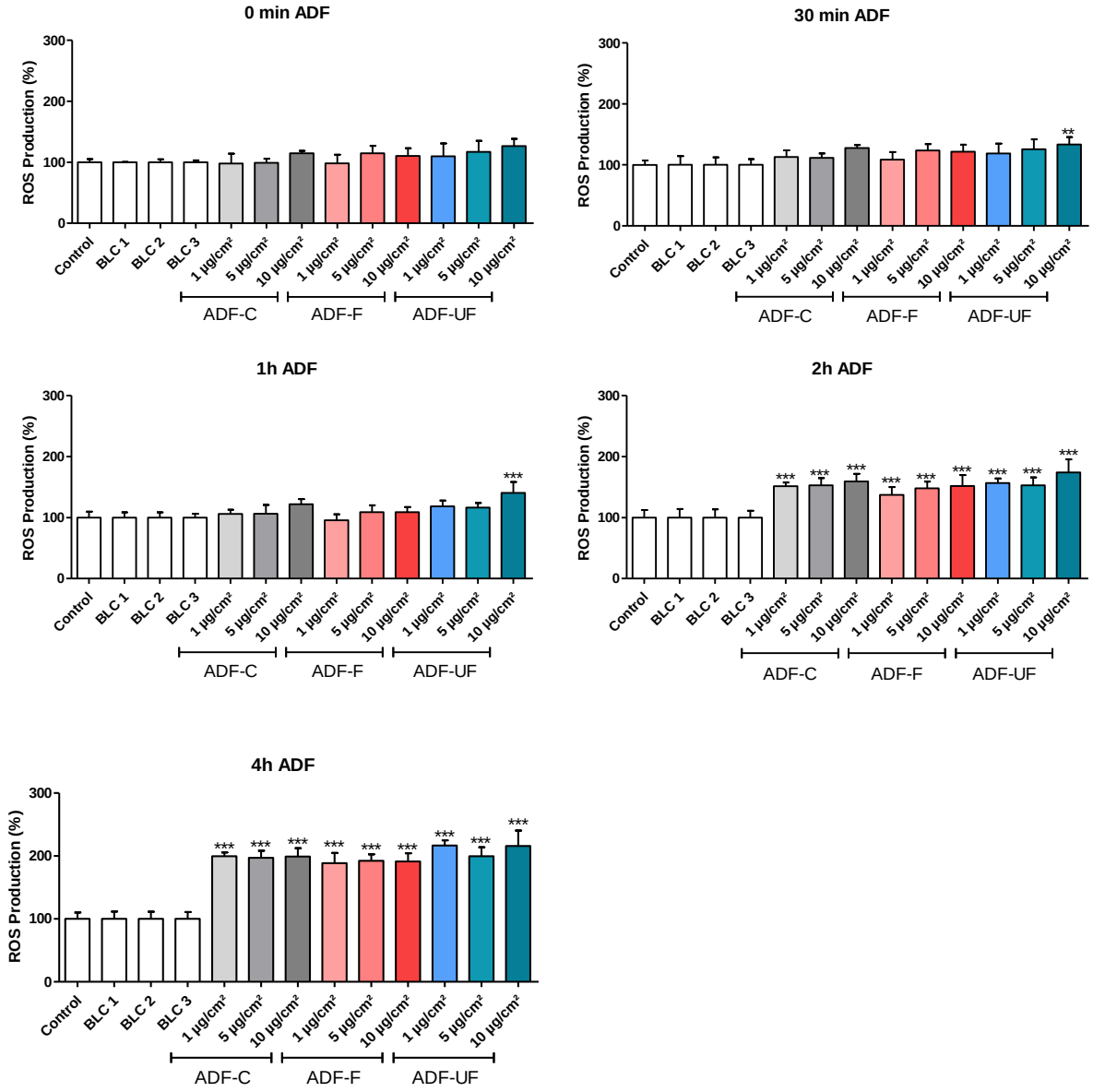
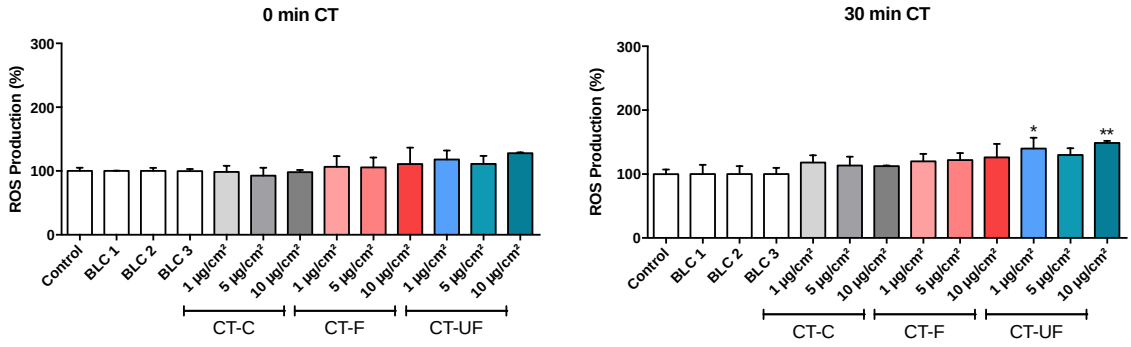


Figure 2: **Reactive oxygen species production in** human bronchial epithelial cells (NCI-H292 cell line) exposed to the 3 size fractions (Coarse, Fine and Ultrafine) of particles sampled during the wet season (July 2016) in Abidjan Domestic Fires site. Cells were loaded with the CM-H_2DCF for 40 min and further exposed to particles. The fluorescence indicating the oxidation of the probe was measured over 4 h of treatment. Control correspond to untreated cultures and the blanks (BLC) correspond to blank filters 1, 2 and 3 for Coarse, Fine and UltraFine fractions respectively. Results are expressed relatively to their respective blank.



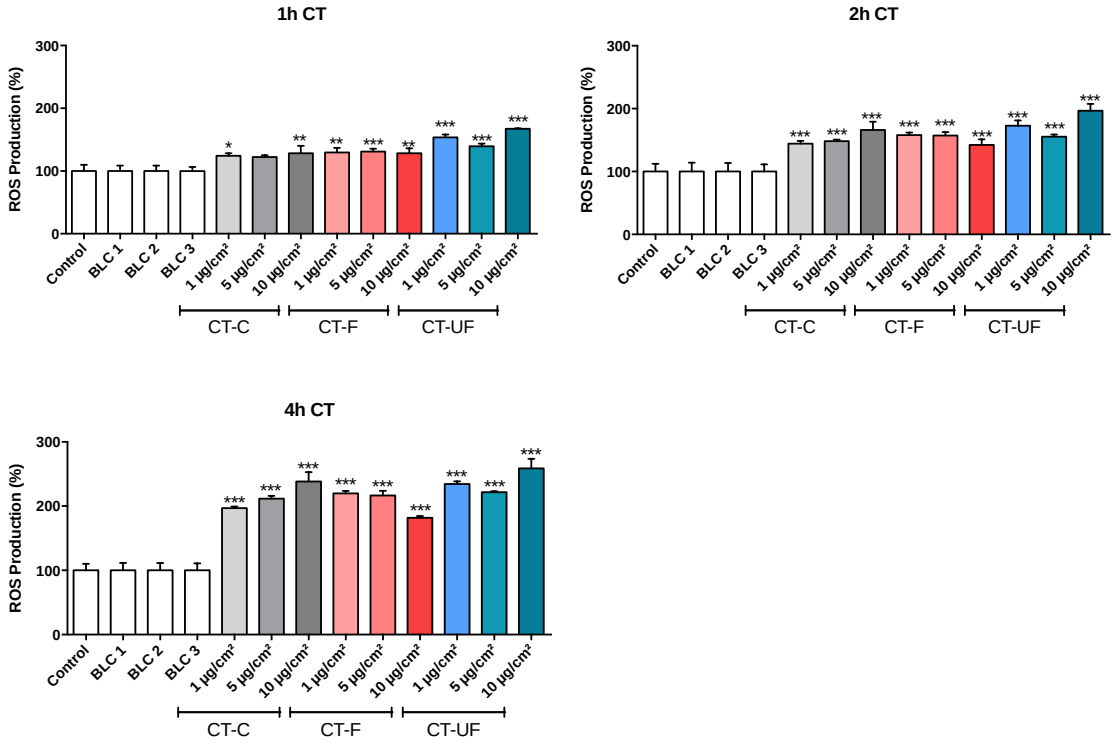


Figure 3: **Reactive oxygen species production in** human bronchial epithelial cells (NCI-H292 cell line) exposed to the 3 size fractions (Coarse, Fine and Ultrafine) of particles sampled during the wet season (July 2016) in Cotonou Traffic site. Cells were loaded with the CM-H_2DCF for 40 min and further exposed to particles. The fluorescence indicating the oxidation of the probe was measured over 4 h of treatment. Control correspond to untreated cultures and the blanks (BLC) correspond to blank filters 1, 2 and 3 for Coarse, Fine and UltraFine fractions respectively. Results are expressed relatively to their respective blank.

3.3 Pro-inflammatory response: Measurement of cytokine release

The pro-inflammatory response was characterized by the release of pro-inflammatory mediators in the culture medium of cells exposed for 24 h to different doses of the 3 size-fractions. Two cytokines were measured: IL-6 and IL-8. The results are summarized in the following figures showing cytokine release for the 3 campaigns for all the sites and the size fractions. Two different illustrations were done: one allowing analysing the trends through the campaigns whereas the other allowing identifying differences between the sites for a given season.

TEMPORAL TRENDS

The figure 4 shows **IL-6** release by bronchial epithelial cells and data are organized to see if results are consistent or not *through the campaigns*.

For Abidjan Domestic Fires (ADF):

The Coarse fraction had no effect whatever the season except a significant effect at the highest dose for the 1st dry season (campaign 2).

The Fine fractions sampled during the campaigns 2 and 3 induced IL-6 release. The effect observed at the highest concentration for the dry season was not confirmed at the next dry season.

The UltraFine fraction sampled during the campaign 2 induced a high dose-dependent IL-6 release that was not confirmed at the next dry season. During the wet season, this fraction also induced IL-6 but to a lower extend.

To sum up, when effects were observed for the F and UF fractions during the 1st dry season they were not confirmed during the 2nd dry season. These two fractions sampled during the wet season are reactive.

For Abidjan Traffic (AT):

The Coarse fraction had no effect whatever the season.

The Fine fractions induced IL-6 release for the campaigns 2 and 3; the effect observed at the 1st dry season at the highest concentration was not confirmed at the 2nd dry season.

The UltraFine fraction induced a strong dose-dependent IL-6 release during the 1st dry season that was not confirmed during the 2nd dry season. The induction of IL-6 release by this fraction sampled during the wet season was less important.

To sum up, when effects were observed with the F and UF fractions during the 1st dry season they were not confirmed during the 2nd one. These two fractions sampled during the wet season are reactive.

For Abidjan Waste Burning (AWB):

The Coarse fraction had no effect whatever the season.

The Fine fractions induced IL-6 release for the campaigns 3 and 4 but without exhibiting a dose-dependent response.

Only the UltraFine fraction of the wet season (campaign 3) was active in inducing IL-6 release

To sum up, F and UF particles of this site have mainly showed effects when sampled during the wet season.

For Cotonou Traffic (CT):

The Coarse fraction showed whatever the season a significant effect on IL-6 release at the highest concentration.

The Fine fractions induced IL-6 release when sampled during the campaigns 2 and 3. The effect observed during the 1st dry season that was not confirmed during the 2nd dry season.

The UltraFine fractions induced a clear IL-6 release especially at the highest concentration when sampled during the campaigns 2 and 3. The effect observed during the 1st dry season was not confirmed during the 2nd dry season.

To sum up, all the size-fractions of this site exhibited a pro-inflammatory effect when sampled during the 2 1st campaigns.

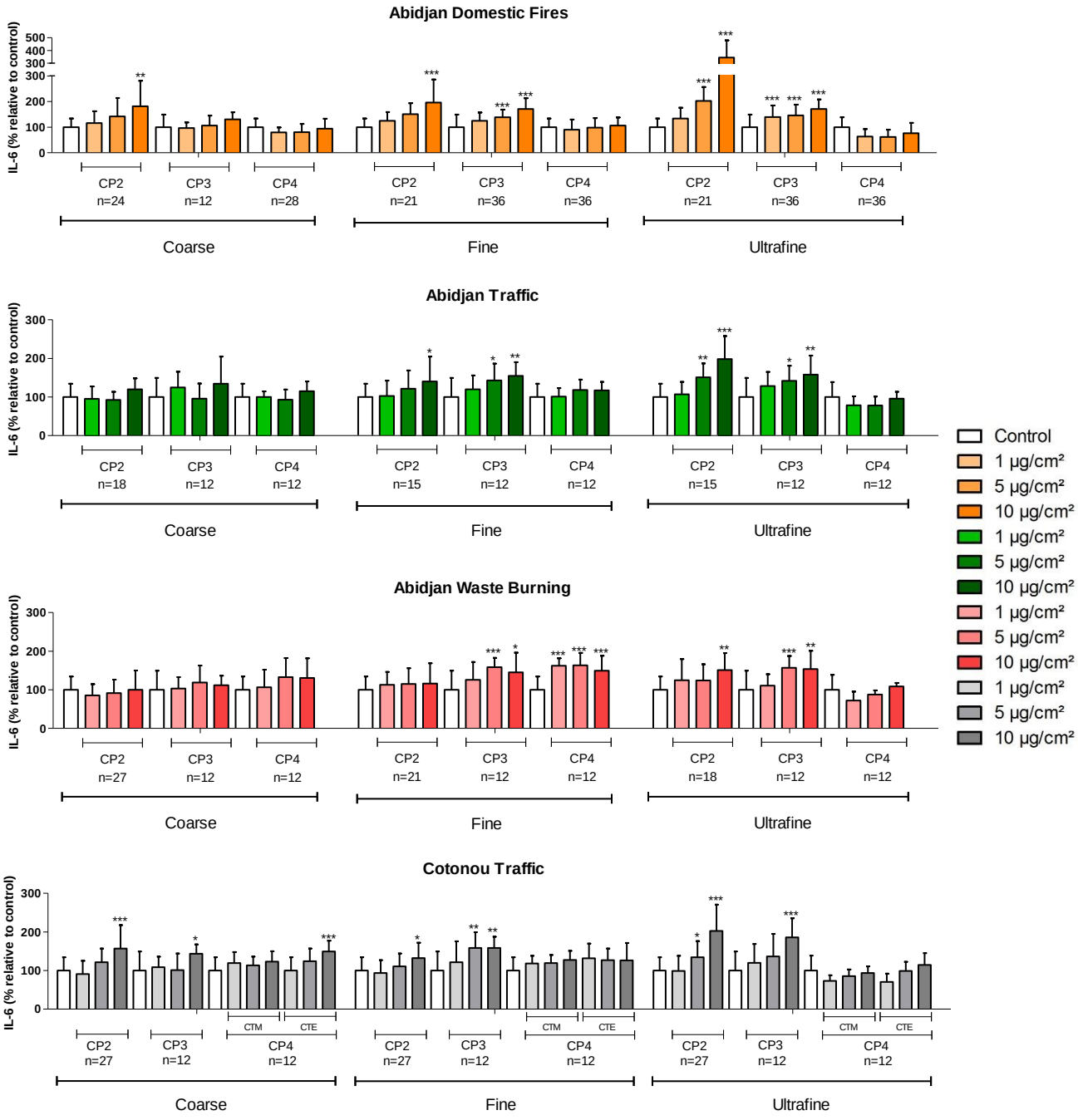


Figure 4 : Measurement of IL-6 release by human bronchial epithelial cells exposed for 24h to 1, 5 ou 10 µg/cm² of the Coarse, Fine and UltraFine fractions sampled in the 4 sites during the 3 campaigns. Results are shown by site. n correspond to the number of replicates (technical+ biological).

The figure 5 shows **IL-8** release by bronchial epithelial cells and data are organized to see if results are consistent or not *through the campaigns*.

For Abidjan Domestic Fires (ADF):

The Coarse fraction induced pronounced IL-8 release only during the wet season (campaign 3).

The Fine fractions had no effect whatever the season.

The UltraFine fraction induced a dose-dependent IL-8 release during the 1st dry season, and it was not confirmed for the 2nd one. It had also an effect when sampled during the wet season but without a dose-dependent effect.
To sum up, effects were mainly seen during the wet season what is different to what was observed for IL-6 release.

For Abidjan Traffic (AT):

The Coarse and Fine fractions had no effect whatever the season.

The UltraFine fractions sampled during the 2 dry seasons induced IL-8 release but the dose-dependency was only observed for the 1st one.
To sum up, when effects were observed it was only for the dry season and the UltraFine fraction.

For Abidjan Waste Burning (AWB):

The Coarse fraction had an effect on IL-8 release only at the highest concentration for the 2nd dry season –campaign 4).

The Fine fractions had no effect whatever the season.

The UltraFine fraction exhibited a dose-dependent effect for the campaigns 3 and 4.
To sum up, particles from this site had limited reactivity except the UltraFine fraction.

For Cotonou Traffic (CT):

The Coarse and Fine fractions had no effect whatever the season.

The UltraFine fractions triggered IL-8 release only during the dry seasons
To sum up, only UltraFine particles from this site exhibited reactivity when sampled during the dry season.

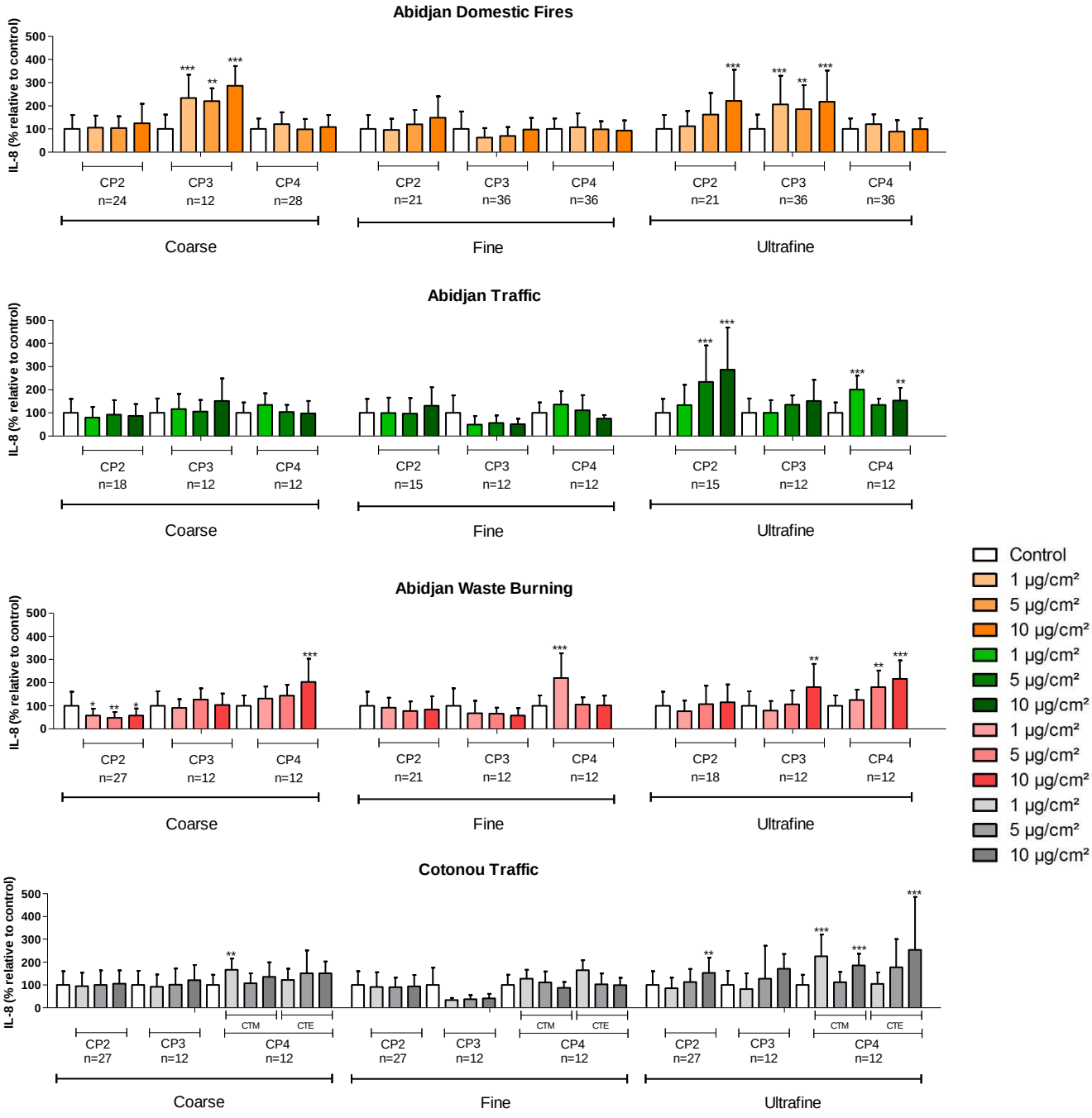


Figure 5 : **Measurement of IL-8 release** by human bronchial epithelial cells exposed for 24h to 1, 5 ou 10 µg/cm² of the Coarse, Fine and UltraFine fractions sampled in the 4 sites during the 3 campaigns. Results are shown by site. n correspond to the number of replicates (technical+ biological).

SPATIAL TRENDS

The figure 6 shows **IL-6** release by bronchial epithelial cells and data are organized to see if differences are observed *between the sampling sites* at a given season.

For the dry season (campaigns 2 and 4)

The Coarse fraction induced IL-6 release at the highest concentration when sampled in Cotonou (CT) during the 2 campaigns and in Abidjan domestic fires (ADF) of the 1st campaign.

The Fine fractions did not induce the same effects during the 2 dry seasons: a significant effect was observed at the highest concentration for ADF (campaign 2) and AWB (campaign 4).

The UltraFine fraction induced dose-dependent IL-6 release whatever the sites during the 1st dry season but these results were not confirmed during the following dry season.

To sum up, it was rarely observed consistent results between the samples collected during the two dry seasons making impossible to draw conclusions on the differences of reactivity between the sites.

For the wet season (campaign 3) :

Only the Coarse fraction sampled in Cotonou induced IL-6 release at the highest concentration.

The Fine and UltraFine fractions induced a dose-dependent IL-6 release whatever the site.

To sum up, the Fine and UltraFine fractions sampled during the wet season exhibit ability to induced IL-6 release whatever the site.

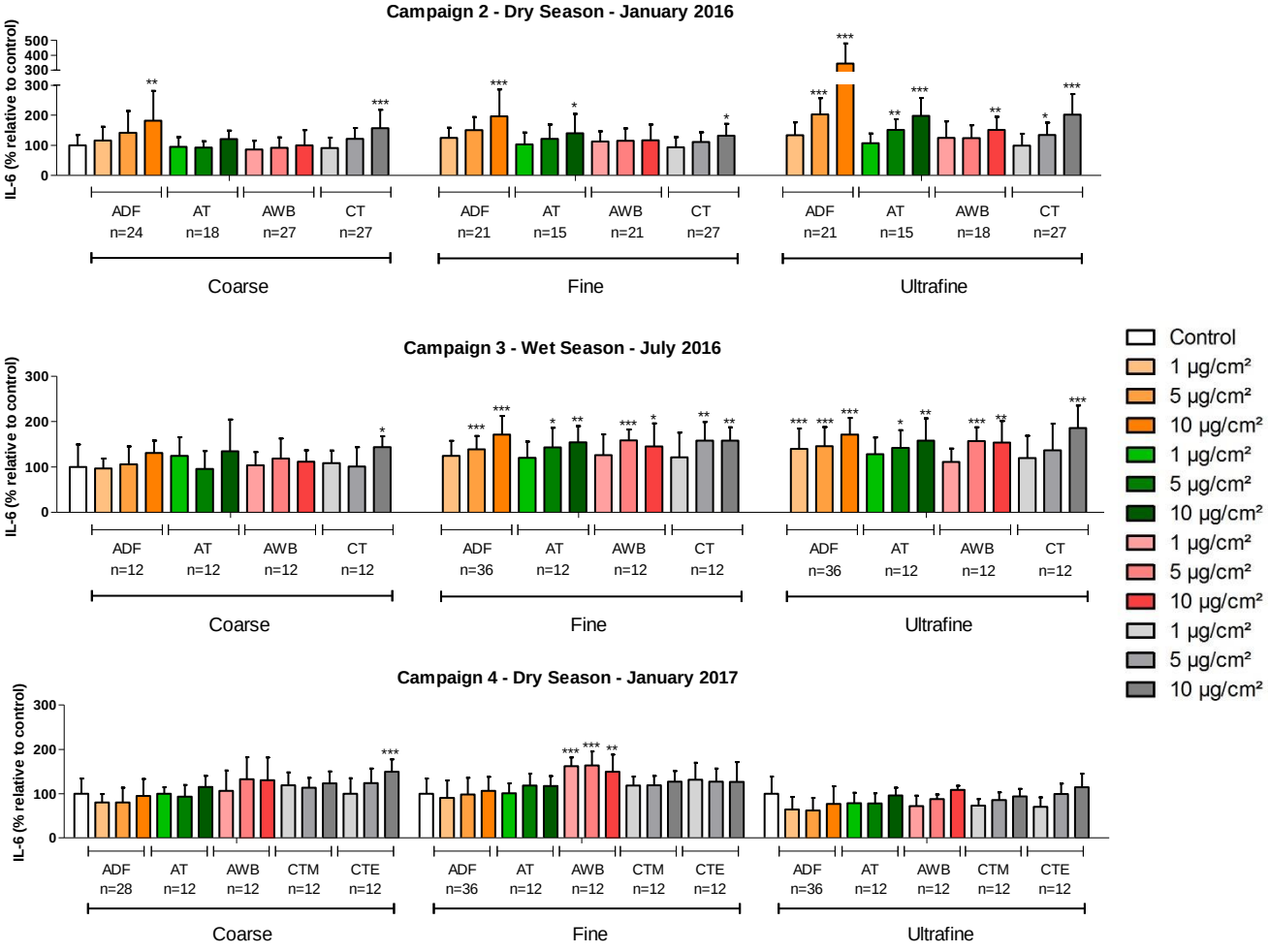


Figure 6 : Measurement of IL-6 release by human bronchial epithelial cells exposed for 24h to 1, 5 ou 10 µg/cm² of the Coarse, Fine and UltraFine fractions sampled in the 4 sites during the 3 campaigns. Results are shown by site. n correspond to the number of replicates (technical+ biological).

The figure 7 shows **IL-8** release by bronchial epithelial cells and data are organized to see if differences are observed *between the sampling sites* at a given season.

For the dry season (campaigns 2 and 4)

The Coarse fraction induced IL-8 release only at the highest concentration for AWB campaign 4.

The Fine fraction had no effect during the 2 dry seasons whatever the site.

The UltraFine fraction from ADF and AT induced a dose –dependent IL-8 release during the 1st dry season whereas the effects were induced by particles from CT and AWB during the 2nd dry season

To sum up, when IL-8 releases were observed they were not confirmed for the two dry seasons.

For the wet season (campaign 3) :

Only the Coarse fraction from induced IL-8 release.

The Fine fraction never induced dose-dependent IL-8 release whatever the site.

The UltraFine fraction from ADF and AWB induced IL-8 release.

To sum up, whatever the campaigns, the UltraFine fraction is the only fraction being reactive but exhibiting a different reactivity according to the site and the season.

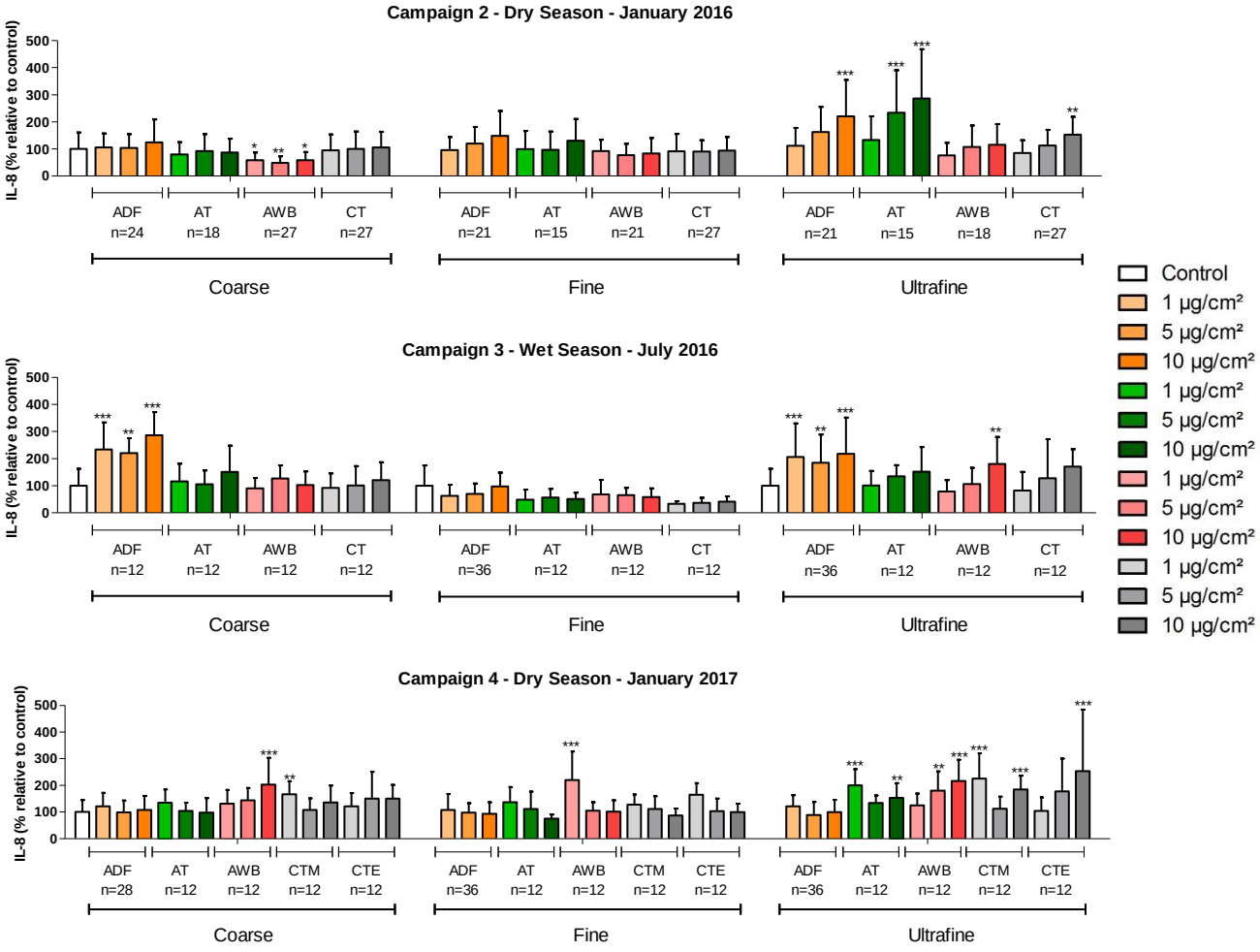


Figure 7 : Measurement of IL-8 release by human bronchial epithelial cells exposed for 24h to 1, 5 ou 10 µg/cm² of the Coarse, Fine and UltraFine fractions sampled in the 4 sites during the 3 campaigns. Results are shown by site. n correspond to the number of replicates (technical+ biological).

3.4 Size speciated chemical aerosol composition

Figure 8 shows aerosol mass concentrations for each site, and for each campaign and each aerosol size. The Abidjan Domestics fires (ADF) site has the highest mean total particulate matter (TPM) concentration comparing to other sites overall the campaigns (450 µg/m^3 versus 116 for AT, 143 for AWB and 187 for CT). ADF maximum concentrations appear in fine and ultra-fine size modes (37%

and 46% respectively) whereas the three other sites present maximum values in coarse modes (mean for these three sites: 44%, 30% and 26% respectively). We may also note that CT concentrations are higher than AT or AWB concentrations. These differences in TPM concentrations and in size speciated ratios between the sites can be explained (1) by the source specificity with more or less incomplete combustion : wood combustion and two wheel vehicle emission factors are higher than gasoline emission factors (Keita et al., 2018), (2) by the proximity between the sites and the sources: ADF site is much closer to the studied sources than traffic or waste burning sources to the other sites and (3) by the relative influence of transported sources to the studied sources such as dust and biomass burning: dust events are relatively more important in Cotonou than in Abidjan.

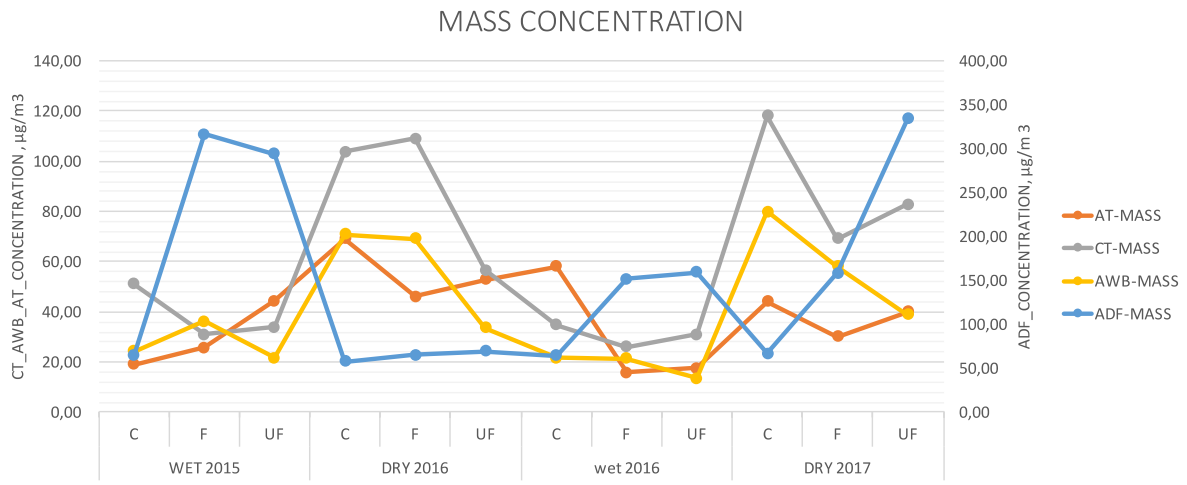


Figure 8: **Aerosol mass concentrations** in µg/m³ for each site, each campaign and each aerosol size.

These assumptions are confirmed when studying the size-speciated aerosol chemical compositions as presented by Figure 9 for each site, each campaign and each size. Aerosol components are separated into four groups: WS, BC, POM and dust. With regard to the relative contribution of each compound to TPM, POM is the largest (20-92%), followed by dust (5-70%), ions (2-45%), and BC (2 -35%) except in Cotonou where dust is the most abundant (70% against 50% for POM). The dust concentrations are higher in the F and UF modes than in the coarse mode, whatever the site considered. In the Cotonou traffic (CT) samples for example, the F mode is dominated by dust particles (up to 70%), which is much larger than in the coarse fraction, where dust accounts for 30% versus 50% for the ultra-fine fraction. These higher values in Cotonou may probably be due to long-range transported dust from the Sahelian desert, but also to local emissions including non-paved road dust emissions. There are no marked differences of dust/TPM ratios between seasons. As it may be seen in Figure 9, POM contribution is the highest at AFD site with 92% in the UF mode, 88% in the fine mode and 80% in the C mode. Such values reveal in one hand, the proximity between the domestic fires sources and the sampling site and in the other hand the specificity of the studied source. As below mentioned, POM is also the main component at AT and AWB sites with more important contributions in UF and C modes than in F mode. Black carbon (BC) contributions at AT and AWB are very similar with 20%, 6% and 7% in coarse, fine and ultrafine particles whereas there are less coarse and more ultra-fine particles in CT site (18%, 4%, 11%). A lower importance of BC may be underlined at ADF (of the order of 13%, 3% and 6% for C, F and UF particles respectively). These trends are confirmed by BC/POM ratio values which can be considered as an indicator of the source specificity. ADF site with sources with more incomplete combustions, has the lowest values whatever the sizes. Ratios in AT is higher than in CT for the coarse and fine particles. Note that this feature can be due to the relative importance of diesel vehicles in Abidjan while Cotonou vehicle park is highly constituted by two wheel vehicles with more incomplete combustion. Seasonal

variation of BC/POM ratios shows highest values for the dry season campaigns in AT, CT and AWB sites and no marked differences in ADF site. Finally, WS contribution is the lowest in ADF (6% in average) and the highest in AWB (around 20%). Note that WS is slightly higher in Cotonou (18%) than in Abidjan (16%). Seasonal variation of WS/TPM ratios shows highest values for the wet season campaigns in AT, CT and AWB sites and no marked differences in ADF site. When scrutinizing each ion contributions (data are not presented here), we may notice that sulfate is mainly retrieved in ultra-fine mode in AT, AWB and CT with a mean relative contribution of 26% except in ADF with 11%. In the opposite, nitrate is mainly retrieved in coarse and fine modes with a mean contribution of about 27% whatever the sites. Chloride ion is higher in ADF (23% of WS) and AWB (17%) than in CT (15%) and AT (13%). Note that this specie is mainly retrieved in coarse and fine particles in all the sites except in ADF with a higher contribution of UF particles.

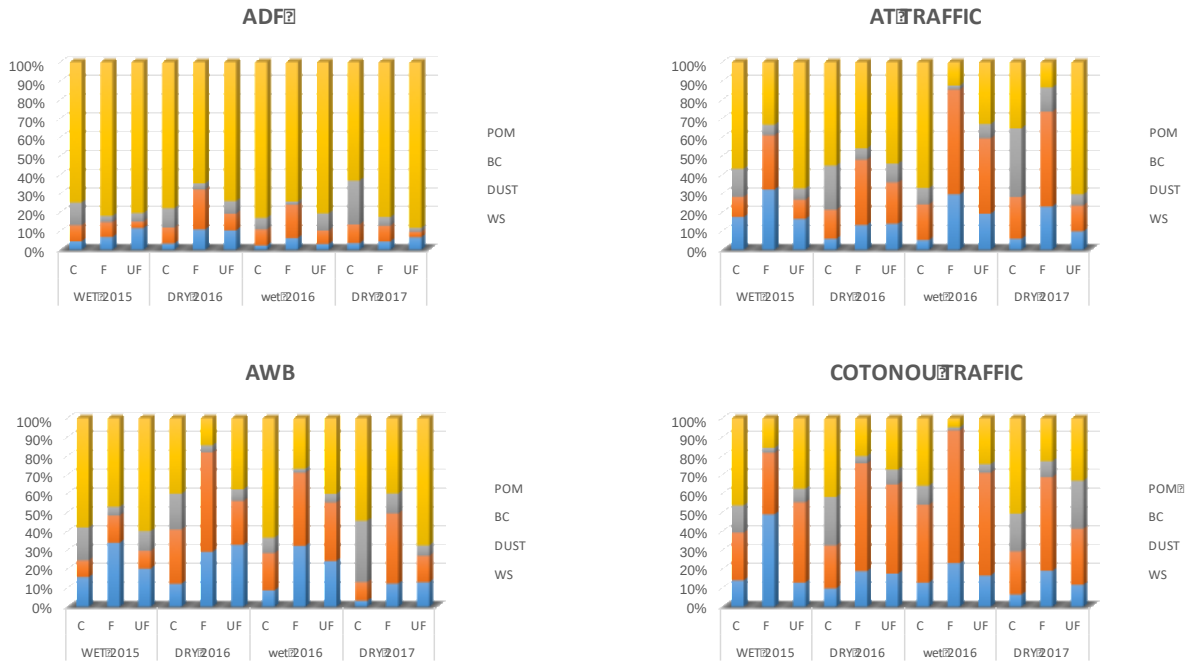


Figure 9: **Size speciated aerosol chemical composition** for each site, for each campaign and each aerosol size.

4 Discussion

In this study we have shown that the different size fractions collected in the different sites are not cytotoxic for the human bronchial epithelial cells in the concentration range we used (1 to 10 µg/cm²). Higher concentrations were not used as they would have been not representative of a real exposure.

Oxidative stress assessed by measuring the oxidation of the DCFH probe was shown only for some samples (CT and ADF) but without consistency through the different campaigns.

As already observed in our previous studies, particles induced a dose-dependent release of pro-inflammatory mediators (Val et al) at different extend according to the samples and the size-fraction. Globally, effects are higher with the fine and ultrafine fraction but not always consistent through the different campaigns. However Cotonou exhibited a consistent effect of the coarse fraction at the highest concentration whatever the season.

In order to relate the toxicological data with chemistry, correlations studies were performed between IL6 expression and the main aerosol components. The most relevant correlations appear for

particulate organic matter which has been already seen as high inflammatory particles (Val et al., 2013). Although a health impact of ammonium sulfate and ammonium nitrate cannot be ruled out (Reiss et al., 2007), no good correlations have been noticed in our study.

Figure 10 shows the results obtained for the dry campaign in 2016 between POM and IL6 expression considering the size-fractions together. A positive significant correlation is observed for all sites except AWB.

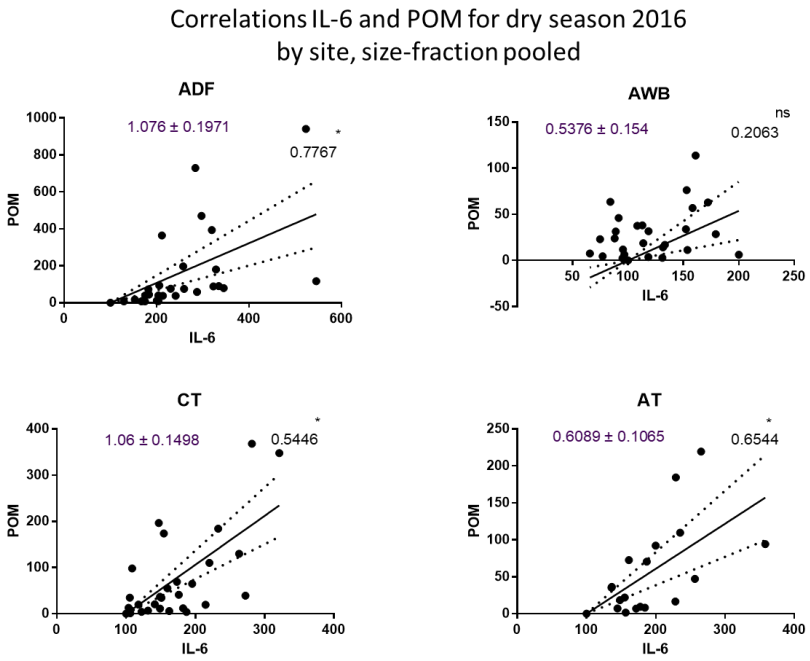


Figure 10: **Correlations between IL-6 expression in bronchial epithelial cells and POM measurement**. Results are shown for the dry season and by site. The data from the different size-fraction have been pooled. In purple the slope and in black the correlation coefficient, * when p<0.05.

In the table 4, these results are compared to the ones obtained during the wet season. Positive significant correlations are observed for the same sites. When looking at the slopes of the relationships, we may note that ADF has the highest slope values for both seasons with a very high slope in the wet season. First, this shows the importance of the inflammatory impact of POM which is predominant at the ADF site. Differences between seasons may be explained by POM characteristics there sampled. Indeed, first analysis in the UF mode of water soluble organic carbon to OC ratios (ratios which are known to be responsible of important activation of pro-inflammatory response) presents the highest values for ADF site and especially for the wet season (0.9) comparing to the dry season (0.7). Slopes for AWB and AT sites are lower than those of ADF site. This can be also explained by POM characteristics in these sites. Relative importance of water soluble OC in OC in AT and AWB in UF mode is lower than in ADF. Moreover, same seasonal variability between AT, AWB and ADF may be seen with higher slopes in the wet season such as the WSOC/OC ratio. In Cotonou however, the sampled aerosol seems to have a different behavior. In the dry season, CT slope is as important as in ADF while CT aerosol is less water-soluble in the UF mode than in ADF (WSOC/OC=0.44 instead of 0.70). Also, CT slope is higher in the dry season than in the wet season in spite of a higher UF aerosol solubility in the wet season than in the dry season (0.66 instead of 0.44). As mentioned earlier, relative importance of dust is much higher in CT than in other sites whereas less relative importance may be found for POM. POM characteristics may be perhaps different in those conditions which will be further investigated.

Table 4: Correlations IL-6 / POM by site for the dry (01/2016) and wet season (07/2016), size-fraction being pooled. *p< 0.05

	Dry season 2016		Wet season 2016	
	r	slope	r	slope
ADF	0,777*	1,076	0,442*	7,076
AWB	0,263	0,538	0,6	0,948
AT	0,654*	0,609	0,77*	0,803
CT	0,545*	1,06	0,733*	0,846

Correlations have been also done by site and size-fraction pooling the data from both the dry and wet 2016 seasons. Figure 11 illustrates results for the ultrafine fraction.

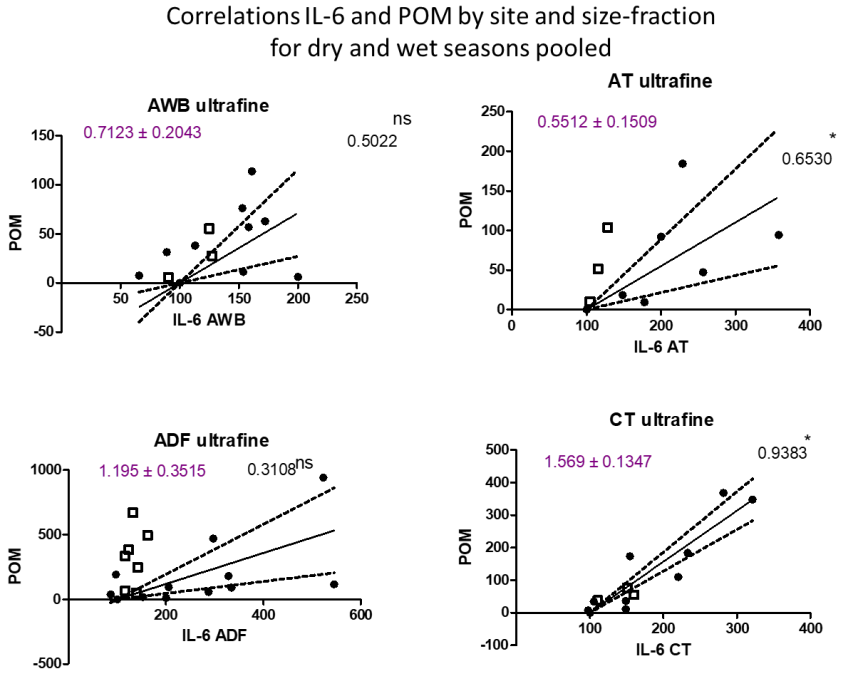


Figure 11: **Correlations between IL-6 expression in bronchial epithelial cells and POM measurement**. Results are shown by site and the ultrafine fraction. The data from the dry and wet seasons 2016 have been pooled. In purple the slope and in black the correlation coefficient, * when p<0.05.

Positive significant correlations are obtained for the two traffic sites as well as for the fine fraction as shown in the table 5, summarizing the results for the 3 size-fractions.

Table 5: Correlations IL-6 / POM by size-fraction and site, dry and wet 2016 seasons being pooled. *p< 0.05

	Coarse		Fine		Ultrafine	
	r	slope	r	slope	r	slope
ADF	0,469*	1,242	0,333	1,961	0,311	1,195
AWB	0,176	0,398	0,423	0,657	0,502	0,7123
AT	0,543	0,345	0,899*	1,046	0,653*	0,551
CT	0,630*	0,476	0,590*	0,811	0,938*	1,569

In the ultra-fine mode, CT slope is 2 to 3 times higher than AT or AWB sites while of the order of that of ADF site. Such differences cannot be directly correlated with the first results of water soluble organic fraction since CT fraction is lower than those of the three other sites. In the fine and the coarse modes, highest slopes may be observed for ADF site which is in agreement with our previous comments.

Further investigations are on-going. Our study will be completed by WSOC/OC ratio measurements in the coarse and fine modes. Also trace elements are being analyzed since they have been shown to potentially have inflammatory impacts (Huang et al., 2003).

Finally, note that once this study will be completed, and for the first time to our knowledge, IL6/POM slopes determined for each different sources will be applied to POM modelled concentration outputs, to obtain regional distributions of IL6 values for each different sources and therefore regional inflammatory impact distributions.