AMERICAN UNIVERSITY OF BEIRUT

# ASSESSMENT OF REACTIVE OXYGEN SPECIES (ROS) IN FINE PARTICULATE MATTER (PM2.5) AT DIFFERENT SITES IN BEIRUT

by
ABDEL AZIZ MOHAMMAD KORDIEH

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# ABSTRACT
# OF THE THESIS OF

Abdel Aziz Mohammad Kordieh for Master of Science
Major: Chemistry

Title: Assessment of Reactive Oxygen Species (ROS) in Fine Particulate Matter (PM2.5) at different Sites in Beirut

Shortness of breath, coughing, sneezing, runny nose, and eye, nose, throat, and lung irritation are just a few short-term health impacts that can result from exposure to fine particles (PM2.5) (particles' aerodynamic diameter, <2.5 μm) present in the atmosphere. Long exposure to these particles can impair lung function and cause illnesses like asthma and chronic heart diseases. Along with their direct impact on human health, PM2.5 induce oxidative potential (OP) and reactive oxygen species (ROS) presence in ambient air. This can cause a disturbance in the balance of ROS inside the body which is known as "oxidative stress". To assess PM2.5 and its particle-bound ROS activity in the city of Beirut, an annual study is conducted at different areas. This study includes the measurement of PM2.5 concentrations along with ROS analysis to identify the toxicity of PM2.5. Moreover, a comparison is conducted to show different site contributions with the appointment of potential sources that induce ROS production in these locations. The effects of seasonal variation and different weather conditions on the concentration of ROS in PM2.5 are also evaluated. The levels of PM2.5 were averaging between 13.3 and 18.3 $\mu g/m^3$ at different sites. And the ROS levels were varying between 0.9 and 1.3 $nmol/m^3$. Results highlight the harmfulness of pollution and present evidence for the great need for mitigation.

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# ABBREVIATIONS

| | |
|---|---|
| AA | Ascorbic Acid |
| A.U. | Arbitrary Unit |
| AUB | American University of Beirut |
| $DCFH_2$-DA | 2′,7′-dichlorodihydrofluorescin diacetate |
| $DCFH_2$ | 2',7'-dichlorodihydrofluorescein |
| DCF | Dichlorofluorescein |
| DHE | Dihydroethidium |
| DI | Deionized Water |
| DMPO | 5,5-dimethyl-1-pyrroline-*N*-oxide |
| DNTB | 5,5'-dithio-bis-[2-nitrobenzoic acid] |
| DTT | Dithiothreitol |
| DT | Downtown |
| EPA | Environmental Protection Agency |
| ESR | Electron Spin Resonance |
| HRP | Horseradish Peroxidase |
| LOD | Limit of Detection |
| LOQ | Limit of Quantification |
| OP | Oxidative Potential |
| PM | Particulate matter |
| PM2.5 | Fine particulate matter |
| PM10 | Coarse particulate matter |
| PM0.25 | Ultra-fine Particulate matter |
| PMF | Positive Matrix Factorization |

| | |
|---|---|
| ROS | Reactive Oxygen Species |
| $R^2$ | Correlation Coefficient |
| SLF | Surrogate Lung Fluid |
| Std | Standard Deviation |
| TA | disodium terephthalate |
| TFE | 2, 2, 2-trifluoroethanol |
| VOC | Volatile Organic Compounds |
| WHO | World Health Organization |
| WSOC | Water-Soluble Organic Compounds |

# CHAPTER I

# REVIEWING THE OXIDATIVE POTENTIAL OF FINE PARTICULATE MATTER: ROS MEASUREMENT METHODS AND SOURCE APPORTIONMENT

## A. Introduction

Particulate matter, often known as PM, describes the airborne solid and liquid droplets. Dust, grime, soot, and smoke are few examples of those particles that are large enough to be visible to the human eye. There are three main types of PM that vary with their sizes. Particles that can be inhaled and typically have a diameter of 10 micrometers or less are referred to as PM10 and called “coarse particulate matter”, while inhaled particles with 2.5 microns’ diameter are referred to as PM2.5 “fine particulate matter”. And the smallest out of these are the particles with diameter of 0.1 microns and known as “ ultrafine particles”.[1, 2] Studies have shown that exposure to PM2.5 can have a serious impact on human health, including respiratory problems, an increase in the morbidity and mortality, and cardiovascular diseases.[3-6] Still, the mechanisms causing PM-related health consequences, are not well known. Growing data from research on human biomarkers, animal models, and DNA methylation suggests that exposure to PM might cause oxidative stress in the body, suggesting one potential cause of PM toxicity.[6-8] Oxidative stress was typically linked to lung cancer, cardiovascular disorders, and respiratory system and airway inflammation.[9, 10] Reactive oxygen species (ROS) can cause oxidative stress when their concentration exceeds the body's antioxidant capacity. This results in a change in the cellular redox state, which in turn can cause or aggravate respiratory tract and cardiovascular system inflammation, chemically alter DNA, proteins, and lipids, and cause cell and tissue damage or

death.[6, 11] Reactive Oxygen Species (ROS) are oxygen-containing molecule with one or more unpaired electrons that is highly reactive, such as superoxide radical ($O_2^-$), hydroxyl radical (•OH), and hydrogen peroxide ($H_2O_2$). These species may enter the body through the inhalation of PM that has ROS directly bound to it and is called "particle-bound ROS" or through the catalytic production of ROS in vivo as a result of cellular redox reactions triggered by certain inhaled PM components and it's called PM "Oxidative Potential (OP)".[12] Particle-bound ROS are known to be short-lived and trickier to measure as the lifetime of ROS normally varies between few minutes to a day or more, and a delay of few hours in the measurement may differ significantly.[13, 14]

This review recaps the methods that are used to quantify atmospheric ROS and potential sources related to particle-bound ROS and oxidative potential of PM. Also, it includes recommendations for the usage of the methods while highlighting some limitations associated with these methods.

## B. Methodology

### *1. Search method*

The search was conducted on PubMed and Web of Science databases using the following terms: ("ROS") OR ("reactive oxygen species") AND ("atmospheric") OR ("air pollution") AND (source) AND ("particulate matter") OR ("PM").

### *2. Inclusion criteria*

Articles were included if authors measured PM2.5 and analyzed ROS along with potential source apportionment.

### *3. Exclusion Criteria*

Articles were excluded if measurements didn't include PM2.5, or ROS analysis. Additionally, articles were excluded if authors didn't appoint the potential ROS sources. Non-English articles were also excluded.

### *4. Study Selection and Data Extraction*

The title and abstract of each article were screened by Abdel Aziz Kordieh and Fatima Hajj Moussa separately. Records that were eligible were then full text screened. Data was extracted from the remaining included articles (methods of ROS quantification and the outcome sources). Both reviewers discussed and cross-validated the extracted data.

## C. Results

### *1. Included Studies*

The search yielded 387 articles from both databases. One additional article was included by hand-searching the references. After removing duplicates, 293 articles were screened for title and abstract. The remaining 92 articles were then full text screened to end up with 51 included articles in this review. A PRISMA diagram showing the selection procedure is represented in Figure 1.

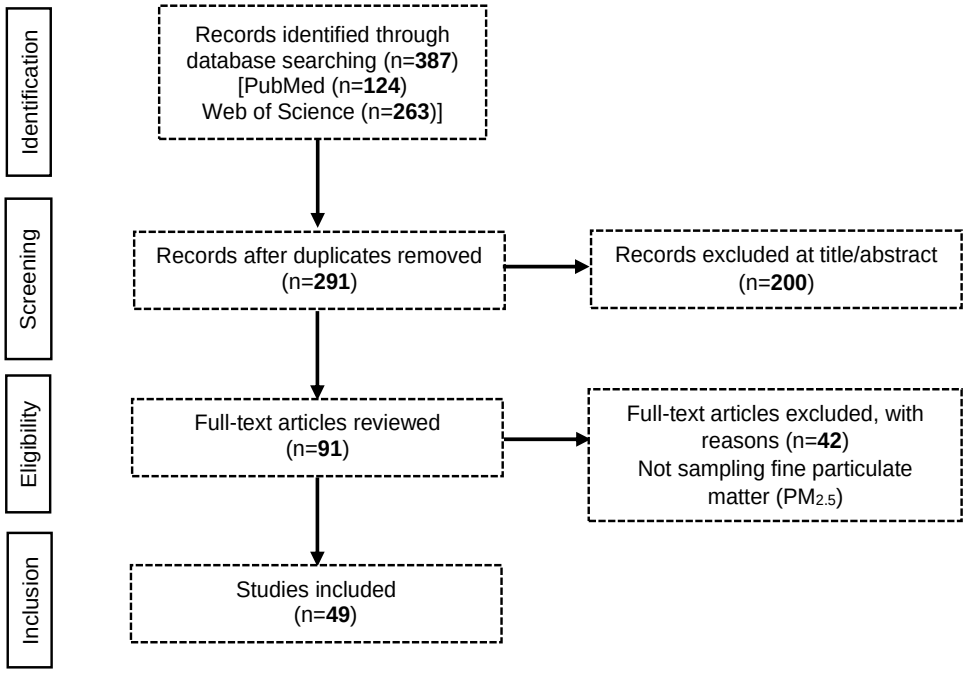



**Figure 1: PRISMA diagram showing the selection procedure of the articles included in the study.**

### *2. Analytical Methods for ROS quantification*

a. Dithiothreitol (DTT) assay:

The Dithiothreitol (DTT) assay method was found to be the most common for OP measurement as it was used in 70% of the studies.[3, 15-43] It is considered an acellular method since the method is purely chemical and no cells are involved.[15] DTT is a chemical substitute for cellular reductants like NADH or NADPH, which induce oxidative stress through reducing oxygen $O_2$ to superoxide anion $O^{\cdot}_2$.[31] DTT is oxidized by the reactive species in PM to DTT-Disulfide, and the residual DTT is treated with DTNB (Ellman's reagent, 5,5′-dithio-bis-(2-nitrobenzoic acid)) to generate $TNB^{2-}$(2-nitro-5-thiobenzoate) ion. Typically, depletion of DTT is detected as a decrease in the absorption of light at 412 nm. The ability of aerosol redox active species to catalytically transfer electrons from DTT to oxygen is quantified by the antioxidant loss rate and OP

is determined by linear regression. The DTT OP is either normalized to the sampling volume ($DTT_v$), or normalized to the PM mass ($DTT_m$) and expressed in $nmol \cdot m^{-3} \cdot min^{-1}$ and $nmol \cdot \mu g^{-1} \cdot min^{-1}$ respectively.[3, 17]

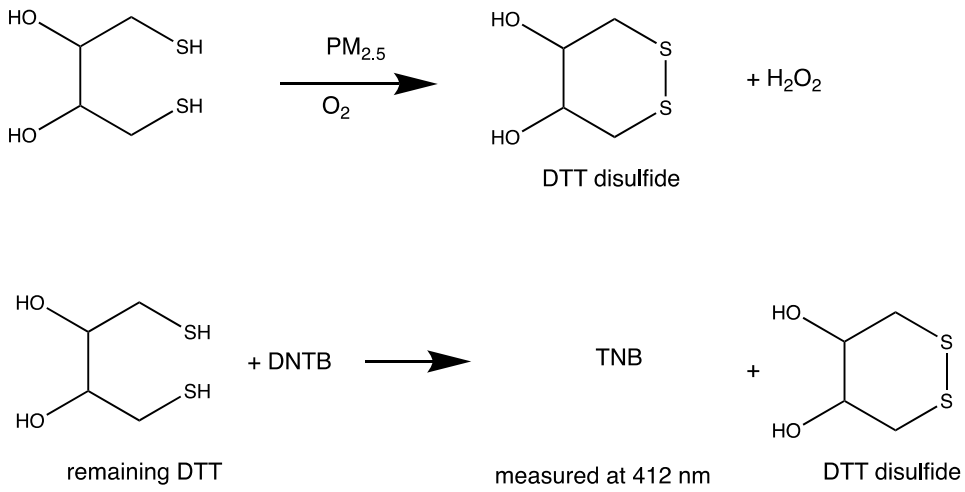



**Figure 2: DTT probe reaction with PM2.5**

b. Ascorbic Acid (AA):

Ascorbic acid (AA) is an assay that works in a similar way to DTT, and it's usually coupled with the DTT assay.[31, 33, 36, 43] While DTT is considered a chemical substitute for cellular reductants, the physiological antioxidant AA stops the oxidation of lipids and proteins in the lung lining fluid. In a similar way to DTT, the PM redox active species catalyze the formation of ROS by transferring an electron to oxygen molecules, oxidizing AA to dehydroascorbic acid.[43] The depletion of the antioxidant is detected at a wavelength of 265 nm.[31, 33, 36, 43]

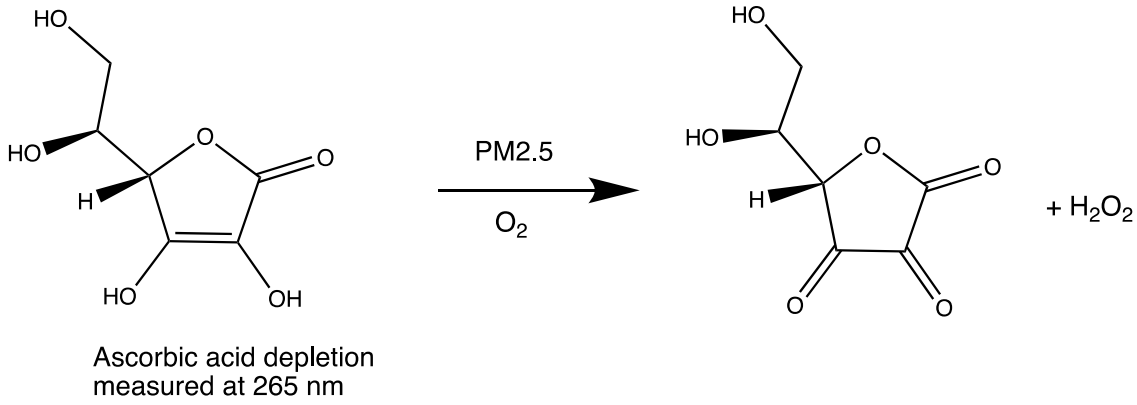



**Figure 3: AA probe reaction with PM2.5**

c. Electron spin resonance (ESR):

Electron spin resonance (ESR) assay measures the oxidative potential in accordance with the ability of PM to generate hydroxyl radical.[32, 33]The PM collected is mixed with hydrogen peroxide and 5,5-dimethyl-1-pyrroline-N-oxide (DMPO) spin trap. Signal from the DMPO-OH quartet is measured and the results are reported in arbitrary units (A.U.) per ambient air volume or per PM mass.[32, 33]

d. Dihydroethidium (DHE):

Fluorescent spectroscopy is one of the common methods used for the quantification of ROS.[44, 45] DHE is used as a fluorescent probe to detect and quantify cellular ROS. Hydroethidine (HE) is oxidized by $O_2^-$ to the fluorescent ethidium ($E^+$).[46] When superoxide is utilized as the oxidant, HE is quickly converted to fluorescent $E^+$. This is not the case when $O_2$, $H_2O_2$, HOCl, or $ONOO^-$ were used.[46] Superoxide is perhaps the oxidizing species that DHE preferentially assesses, however other oxidizing species can still have an impact.[45, 46] The fluorescent signal of DHE staining is measured at 488/620 nm (Em/Ex) using flow cytometry.[45, 47] DHE values are then normalized to control and reported in A.U.

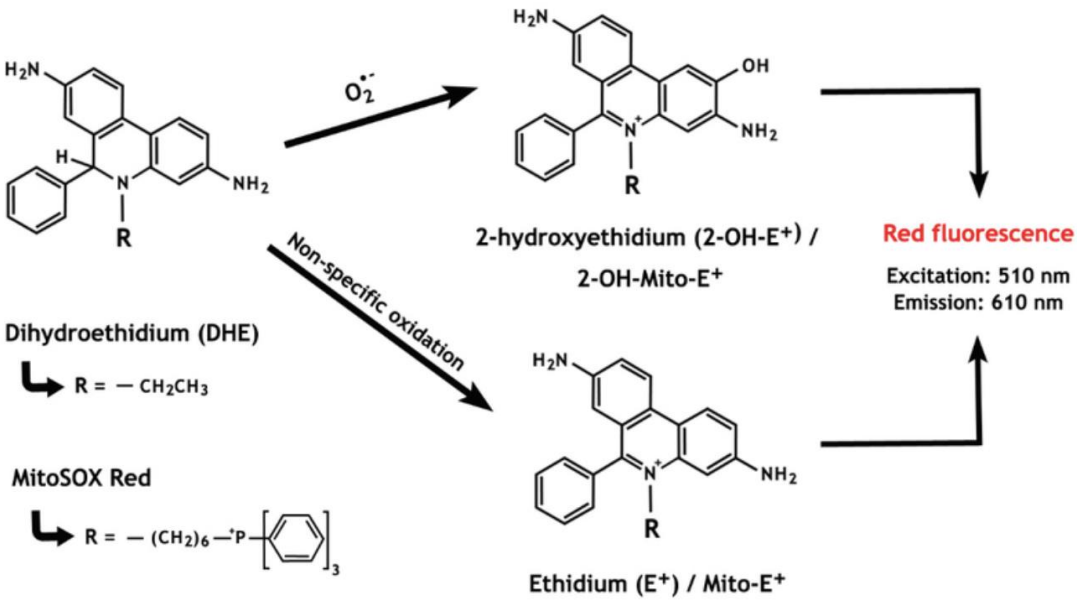



**Figure 4: DHE probe reaction with ROS**

Balbin Villaverde, Ana Izabel & Netherton, Jacob & Baker,. (2019). From Past to Present: The Link Between Reactive Oxygen Species in Sperm and Male Infertility. Antioxidants. 8. 616. 10.3390/antiox8120616.

e. Dichloro-dihydro-fluorescein diacetate ($DCFH_2$-DA) assay:

The most common fluorescent probe for ROS quantification is DCFH. [18, 40, 41, 45, 48-62] This probe is non-targeted and can be used for both cellular and acellular ROS analysis.[48] It is also known to be a positive fluorogenic probe as DCFH-DA is non-fluorescent, however, after being exposed to oxidants, DCF (the final product) shows a good fluorescence.[63] DCFH-DA undergoes deacetylation as a first step to form DCFH. In the second step, DCFH undergoes a two-electron oxidation to form the DCF fluorescent product. When DCF is excited, it exhibits strong fluorescence at a wavelength of 485–500 nm, and it emits at a wavelength of 515–530 nm.[63] The reactions of this probe are detailed in chapter II.

f. Surrogate Lung Fluid (SLF):

The Surrogate Lung Fluid (SLF) solution could potentially represent the lung fluid's redox chemical environment.[64] PM is reacted with 2, 2, 2-trifluoroethanol (TFE) in order to increase the water solubility of the particles.[65, 66] According to Vidrio et al., TFE could boost the generation of •OH by 80±40% compared to the same sample extracted by SLF without TFE.[67] Afterwards, SLF and disodium terephthalate (TA) are added, TA will then react with OH radicals produced forming TAOH which is stable and fluorescent. TAOH is detected on a fluorometer with Ex and Em wavelengths of 320/420 nm respectively. [65, 66]

### *3. Source apportionment*

The sources of ROS in the studies were appointed based on three main criteria: Statistical analysis, by comparison between different sites, or by a combination between both.

a. Statistical Analysis

This method of analysis is totally based on mathematical models and mostly Positive Matrix Factorization (PMF).[3, 28] PMF is a mathematical receptor model developed by the Environmental Protection Agency (EPA) that offers scientific validity for the establishment and execution of air and water quality regulations as well as environmental forensics.

The enormous number of variables in complicated analytical data sets are reduced b y this model to combinations of species known as source types and source contributions , allowing it to assess a variety of environmental sampling data sets. The source types

are then recognized by aligning them to measured profiles. [68] Users of the PMF provide files with information about the origins, uncertainties, and concentrations of sample species. The model determines source contributions, source profiles, or fingerprints, and source profile uncertainty. The uncertainty weighted difference between the observed and anticipated species concentrations is kept to a minimum, and the outputs of the PMF Model are required to offer positive source contributions.[68]

b. Comparison of sites:

Another method used to get an indication about the major pollutants is choosing multiple sites with different major pollutants. An example would be choosing 3 sites: one on a side road, one in an industrial region, and a background site. Then a comparison would be conducted between sites determining the effect of vehicular emissions and industries on the pollutants being measured. [55]

c. Combination of both methods:

Some Studies prefer to do a comparison between different sites with different major pollutants and then confirm the results with PMF results. This gives more validation and confirmation for the source apportionment results.[69]

d. Main sources:

The main sources found to have a direct correlation with the ROS concentration are mainly biomass burning and vehicular emissions as main sources, and water-soluble organic compounds (WSOC) and metals are the main chemical species explaining the ROS pattern.

## D. Discussion

### *1. ROS measurement methods*

The most convenient method for the measurements of PM oxidative potential and the most used is DTT. As this method is considered reliable, easy to conduct, non-targeted, repeatable, and reproducible. [23]It would be a great fit to do both AA and DTT methods as DTT simulates reductant species in cells, while AA is an antioxidant that simulates the respiratory track lining fluid. Thus, a combination of these 2 methods would show a clear idea about the OP of PM in the atmosphere.[70]

ESR would be a very good option if the target of the study is to measure the OH radicals present in the atmosphere. This is due to the spin trapping process which deals perfectly with the short-lived radicals. This method's main disadvantage is that it's targeted towards specific radicals; those which react with the spin traps, so if the study targets a specific type of ROS, this method would be a perfect fit.[33]

DCFH probe is the most common when it comes to measuring particle-bound ROS. This method is the most applicable, easy to perform, repeatable, and reproducible. [52]Moreover, DCFH method is fast to perform in order to prevent any loss of the short-lived reactive species. The main disadvantage is the photo and auto oxidation of the probe. This problem can be solved by a good optimization of the method and timing the method perfectly. It also has an edge on the ESR method, since this one is non-targeted for a specific species, while ESR focuses on the spin trap which reacts with one species rather than all the others.[53]

## *2. Source apportionment methods*

For source apportionment, the method depends on many variables including number of data points and if the points are enough to use PMF or any statistical analysis software. That is why a lot of papers go for the comparison between sites method, as it is simpler, requires less data points, and has minimal limitations. Additionally, if the study includes enough data, it would be great to compare sites to get an idea about the sources and then confirm and validate the comparison with statistical analysis.

## *3. Sources*

A large portion of the studied papers directly correlates between biomass burning generally and water-soluble organic compounds (WSOC) emissions in specific and ROS concentrations.[17, 27, 29, 52, 56] Biomass burning is known to be one of the main sources of WSOC. [71] The presence of WSOC in the atmosphere directly influences PM OP and ROS concentrations, as it was noticed in several studies that WSOC are directly correlated with higher ROS concentrations. [72]

Vehicular emissions are also considered a main contributor to the ROS concentrations. [61] The correlation between vehicles and ROS concentration is direct, but it is still not fully understood how these vehicular emissions influence ROS and OP. One explanation would be the direct influence of vehicles on the high emissions of Volatile Organic Compounds (VOC), and VOC undergoes some photochemical reactions that result in secondary organic aerosols including WSOC that can affect the ROS concentrations.[73] Another would be the emissions of some metals that can be involved in Fenton's reactions (figure 5) and cause more production of ROS. Fenton's

reaction is one of the main known reactions that forms reactive radicals outside and inside our bodies.

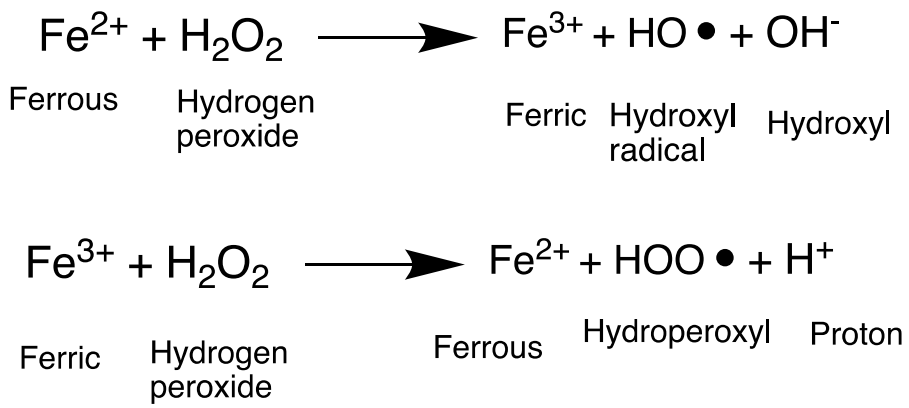



**Figure 5: Fenton Reaction and OH radical formation**

https://www.chemistrylearner.com/fenton-reaction.html

## E. Conclusion

DCFH probe is the most convenient for non-specific particle bound ROS quantification, since it's reproducible, rapid, easily performed, very sensitive, and allows online measurements. ESR is recommended for targeted analysis with a specific spin trap towards targeted compounds. DTT probe is the most common to use when targeting PM oxidative potential as this probe is sensitive and non-targeted.

Vehicular emissions and biomass burning are determined to be the main contributors to the high OP of PM2.5 followed by other emission sources. Also, the concentration of chemical components of PM2.5 (mainly WSOC and few transition metals) may govern the oxidative potential pattern of PM2.5.

# CHAPTER II

# ASSESSMENT OF REACTIVE OXYGEN SPECIES (ROS) IN FINE PARTICULATE MATTER (PM2.5) AT DIFFERENT SITES IN BEIRUT

## A. Introduction

### *1. Background*

Scientific research has been showing evidence constantly linking air pollution with adverse health effects due to the presence of several categories of toxic pollutants. Long and short exposure to different pollutants can cause severe health issues including premature death and fatal diseases such as cancer. [74] It is expected that in around twenty-five years, exposure to air pollutants is expected to be the leading cause of premature mortality surpassing malaria and water contamination. [75] Scientists have drawn an extensive amount of attention to air pollution, and tremendous efforts have been made worldwide to reduce it, particularly since the Environmental Protection Agency (EPA) was founded in 1970. This agency has defined standards or limits for six main pollutants, in which if these limits are exceeded it can be a serious issue for human health. These six pollutants are Carbon Monoxide (CO), Sulfur Dioxide ($SO_2$), Nitrogen Dioxide ($NO_2$), Lead (Pb), ozone ($O_3$), and Particulate Matter (PM). Out of these pollutants, PM has received a lot of attention due to the strong association between this pollutant and respiratory, cancer, and cardiovascular diseases. [76, 77]

PM consists of solid and liquid droplets suspended in air with their size varying between nano to few micrometers. PMs are classified according to their diameter. Particles with a diameter between 2.5 and 10 μm are known as PM10 or coarse particulate matter, while these varying between 0.25 and 2.5 μm are referred to as

PM2.5 or fine particulate matter. Those with a diameter less than 0.1 μm are called Ultra-Fine particles (UFP). Each of these types has its sources, chemical combinations, lifetime, and different health impacts.

Fine particles are known till today as the most impactful on our health out of the three categories. This is mainly due to the long lifetime of these particles as that allow them to travel long distances up to 1000 km more than coarse particles. [78] Moreover, their small diameter allows them to travel deep inside the respiratory system into the lungs and deposit on in the respiratory bronchioles and the alveoli and damage the gas exchange process. [79, 80]

Although the mechanism by which fine particles impact the human body is still unclear, reactive oxygen species and oxidative potential of the fine particles are known to have a direct influence on the mitochondrial matrix and DNA of the body cells through oxidative stress and cause inflammations and cell death. [81] ROS either forms outside the body and are inhaled directly and this type is called “particle-bound ROS” or forms inside the body through several reactions including Fenton’s reaction and this type is called PM oxidative potential.

Particle-bound ROS are reactive species that form in ambient air and then penetrate into the body causing an imbalance between oxidants and antioxidants in the body cells leading to “oxidative stress”. One of the widely used criteria to determine the toxic potential of airborne particulate matter is particle-bound ROS and the improvement of public health and risk assessment strategies depends significantly on its temporal variability. [82]

## *2. The current pollution situation in Lebanon*

Due to its enclosed geography, the Mediterranean area is renowned for having high pollution episodes. It is vulnerable to aerosol buildup due to its location between the Saharan desert of Africa and the very densely populated and highly industrialized European landmass. Additionally, due to its closeness to the three continents (Africa, Asia, and Europe), it serves as a meeting point for the air masses in this area. As a result, the Mediterranean region is distinguished by a high level of humidity, extended summers, and stagnant winds that come from eastern Europe. Additionally, during the fall and spring seasons, the eastern side gets impacted by several events of particulate matter dust storms from the Saharan and Arabian deserts, making it a contentious area in terms of pollution. [56]

A large portion of Lebanon’s residents live in Beirut the country's capital, which is geographically located between the Mediterranean shore in the west and Mount Lebanon in the east. The city's roads are frequently clogged with heavy traffic throughout the day, and construction projects are a common occurrence in the heavily populated area. To support the growing city population, new residential buildings are being built. The problem of air quality and pollution is evolving heavily especially in Beirut the last few years. This is mainly due to the economic crisis the country is facing that leads to severe shortage in electricity, resulting in heavy usage of personal diesel generators to compensate for the current shortage. This is along with the extensive vehicular emissions due to the development of the population in the city and the usage of old cars causing the emissions to get higher.

### *3. Study Objective*

Given the current situation, a lot of impactful changes happened in the city regarding air quality measures. Since the last PM annual averages in different sites in the Beirut capital was conducted around 10 years ago,[55] it is very important to repeat this study and take it further in terms of assessing the chemical content, the sources and the assessment of particle bound ROS. For this reason, the study will include winter and summer PM2.5 mass averages over two sites, one in the American University of Beirut (AUB) for reference and one in Downtown (DT). In addition, PM bound ROS will be assessed. Further studies in the way and not included in this thesis include an additional site at the Makassed General Hospital and the polycyclic aromatic hydrocarbon (PAHs) and WSOC analysis as well as ROS measurements in the three sites.

## B. Methodology

### *1. Sampling sites*

The sampling is conducted at three different sites in Beirut. The first is at the American University of Beirut's chemistry building rooftop (33°54'03.8"N 35°28'50.7"E), characterized by the dense vegetation cover in comparison to other sites. Whereas the second site is in downtown (33°54'00.3"N 35°30'31.0"E) which is characterized by the heavy presence of diesel generators. The third site is in Makassed General Hospital (MGH) (33°52'36.8"N 35°30'13.9"E) in an urban region with heavy traffic and high vehicular emissions.

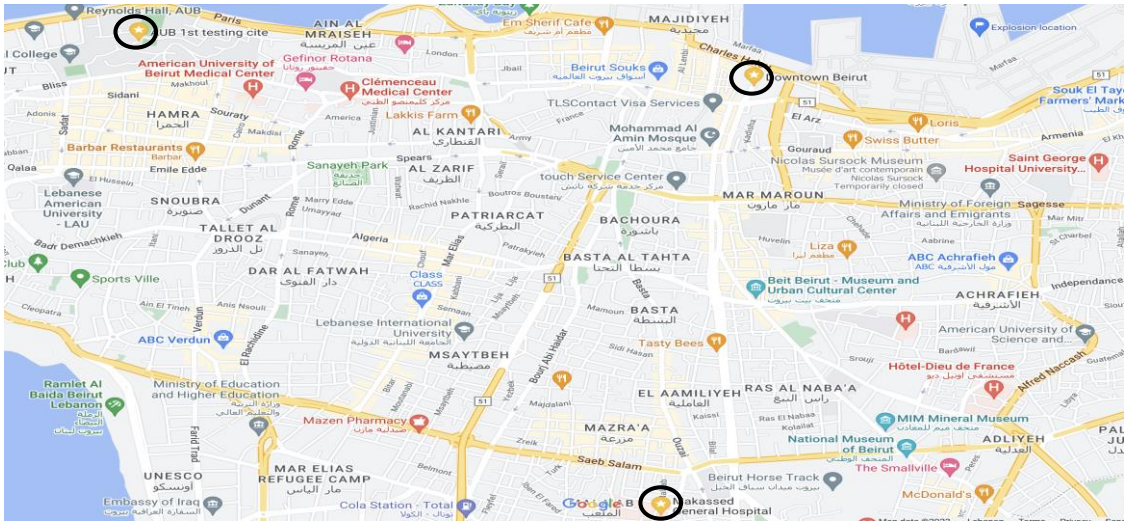



**Figure 6: The Three sampling sites in Beirut**

## *2. Sample collection*

Sampling was done every sixth day for a duration of 24 hours using two low volume samplers working simultaneously at a constant flow rate (16.7 L/min). The low volume samplers are composed of two pumps and two Harvard cartridges (*CHEMCOMB 3500*) supplied with PM2.5 impactors. Two samples were collected using two different types of filters. One is a PTFE Teflon membrane filter for the collection of PM2.5 and the other is a laminated Teflon filter for the collection of ROS. The laminated filter contains a supporting protective layer in order to prevent the ROS from reacting or escaping through the filter layers.

**Figure 7: CHEMCOMB 3500 and Teflon filter used**

### *3. Gravimetric analysis*

The membrane filter was weighed pre and post sampling using a micro balance (Balance XPR2U) to get the mass of PM2.5 collected on the filter.

### *4. ROS analysis*

a. Method selection

For this study, the target is a method to detect non-targeted particle-bound ROS trapped on a filter. According to the evaluation of the methods in the first chapter, the best method for non-targeted particle-bound ROS analysis is using fluorescent probe DCFH. Since this probe doesn't target any specific ROS species, and the method is the most common, stable, highly sensitive, repeatable, and reproducible. The main disadvantage of using this probe is the photo and auto oxidation of the fluorescent DCF, but this problem can be solved with the appropriate timing and light optimization.

The $DCFH_2$-DA probe is deacetylated as a first step as shown in figure 6 either with a strong base or by esterase. [63]

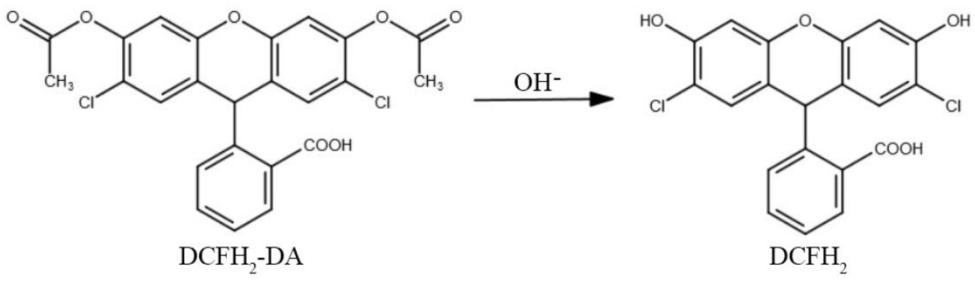



**Figure 8: Deacetylation of $DCFH_2$-DA**

The deacetylated product 2',7'-dichlorodihydrofluorescein ($DCFH_2$) then undergoes two consecutive one-electron oxidation reactions producing fluorescent DCF as presented in figure 7. [63] DCFH is a positive fluorogenic probe, as the initial DCFH doesn't fluorce, while after deacetylation and oxidation it will form DCF which is characterized by a strong intensity fluorescence at 485-500 nm. [63]

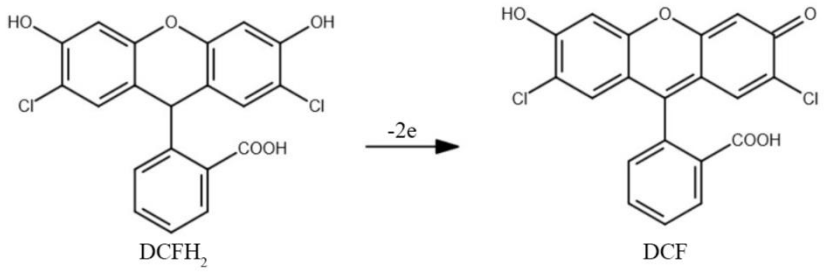



**Figure 9: DCFH2 oxidation**

DCF's existing form is strongly pH dependent as the lactonic form of DCF is obtained with ring closure dominance at pH <5. The lactonic form is not fully conjugated and is not fluorescent as shown in the figure 8 below. For this reason, a

phosphate buffer is added to maintain basic pH in which the conjugated fluorescent form of DCF dominates. [83, 84]

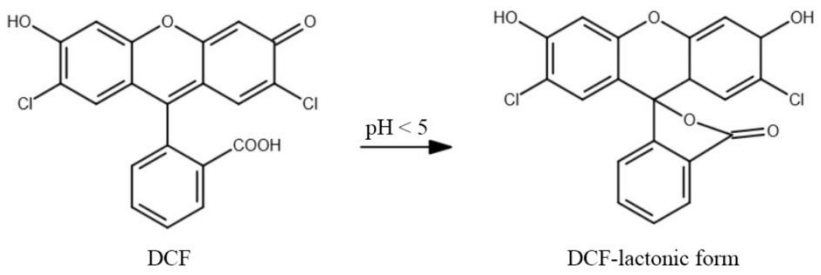



**Figure 10: DCF lactonic and conjugated forms**

b. Materials

Ethanol, horseradish peroxidase (HRP) (52 units/mg), potassium phosphate monobasic, and dibasic were purchased from Sigma-Aldrich. And 2',7'-dichlorofluorescin diacetate ($DCFH_2$-DA) was obtained from Molecular Probes (product code D399). The laminated Teflon filters (47 mm diameter) was bought from SterliTech.

c. DCFH probe preparation

Ethanol was used to prepare 125 µM $DCFH_2$-DA solution. A solution of 40 mL of 0.01 M aqueous NaOH was added to 10 mL of the prepared $DCFH_2$-DA solution for deacetylation. The activated DCFH solution was maintained in a dark room for 30 min along with being wrapped in aluminum foil to avoid photo-oxidation. Then, 50 mL DCFH solution was mixed with 200 mL of 0.25 mM phosphate buffer (pH=7.1), which was prepared by combining monobasic and dibasic potassium phosphate. To amplify

the fluorescence signal, 2.4 mg of horseradish peroxidase was added. The final volume of 250 ml working solution had a concentration of 5 μM of DCFH. A linear calibration curve ranging from 1 x 10-7 to 5 x 10-7 M was prepared using hydrogen peroxide ($H_2O_2$) as an equivalent of ROS. The calibration curve showed linearity with correlation coefficient ($r^2$) = 0.999.

d. Fluorescence measurement

Thermo Fisher Scientific Multimode Microplate (96-well multiple plate) reader is used as a fluorimeter. Excitation and emission wavelengths were 490 and 510 nm, respectively**.**

e. Probe optimization and validation

The full optimization and validation of the ROS method is mentioned in detail in chapter III.

## C. Results and Discussion

### *1. PM2.5 variation*

a. Comparison of PM2.5 Concentrations at different sites

The average mass concentration of PM2.5 fractions collected during clear and rainy days at AUB and DT are shown in Figure 9. Rainy days are defined in this study as the days with rainfall of 2.5mm or more, this is following the definition of the Indian Meteorological Department (IMD). [85]

The total PM2.5 average mass concentration was 18.3 μg/$m^3$in DT, which is higher than that at AUB which averaged at 13.3 μg/$m^3$.

As can be seen from figure 9 below, PM2.5 mass concentration in DT was greater than that at AUB by 35% in rainy days and by 51% in clear days. The PM2.5 concentration in DT and AUB averaged at 21.9 $\mu g/m^3$ and 14.5 $\mu g/m^3$, respectively.

Since the study is done over a year with 24-hour duration for each sampling session, these results can be compared to the annual and daily PM2.5 exposure guidelines recommended by WHO. According to the updated recommendations, 24-hour average exposures should not exceed 15 $\mu g/m^3$ more than 3–4 days per year, and annual average PM2.5 concentrations should not exceed 5 $\mu g/m^3$. The averages in both AUB and DT exceeded the annual limit of WHO by 290% and 438% respectively. The daily limits were also exceeded as more than 15 days throughout the study recorded a 24-hour PM2.5 concentrations higher than 15 $\mu g/m^3$.

This is in accordance with our expectations, as the current situation entails the constant operation of diesel generators and the presence of heavy vehicle emissions during the rush hours in the morning and afternoon at the two sites. The evolving presence of diesel generators in the last few years due to the shortage of electricity and the effect of vehicular emissions which are present near the two sites have had an impactful effect on the Mass concentration of PM2.5 which is significantly above the WHO limits in both cases. Important to mention that the economic crisis has also affected the renewal of the traffic fleet and has increased the average age of cars on the streets. The average car fleet was measured to be 19 years of age in 2015. [86]

b. <u>Dust storm events:</u>

During the period of sampling in DT, the region went through two dust storm events in the month of April. Figure 11 shows how the trends of PM2.5 during the event

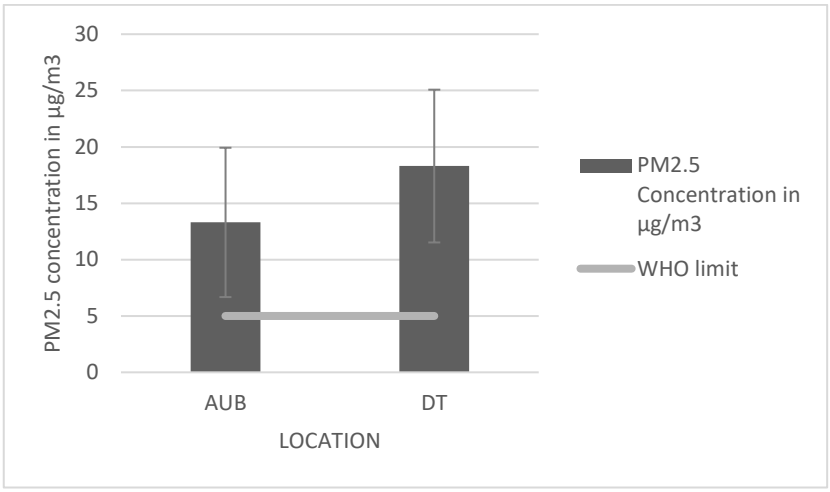
compared to normal clear and rainy days in DT. The dust storms were followed and tracked on hysplit website.

As can be seen in figure 11, dust days were found to have higher PM mass concentrations than clear and rainy days. This is expected due to the fact that dust storms originating from nearby deserts carry a large amount of Fine PM with it, because of the long lifetime of PM2.5 and its ability to travel for long distances. This is consistent with other studies done in the literature that shows how dust events contribute to rising ambient PM mass concentrations. [26, 56, 87]

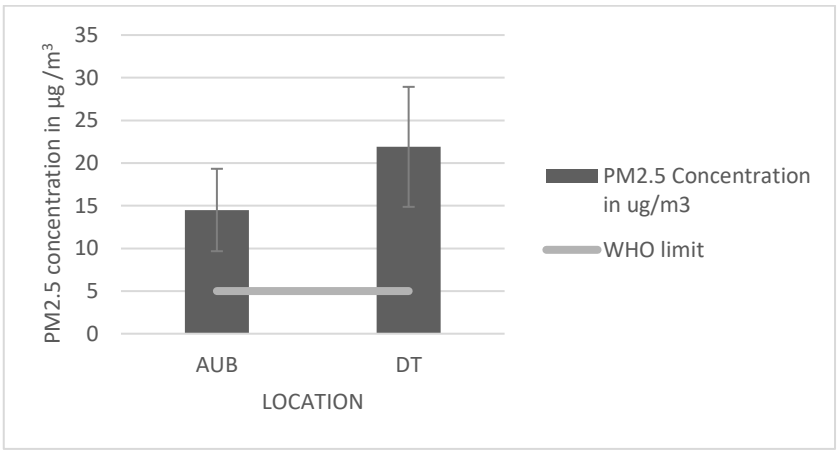
c. Seasonal variations:

A clear trend is shown in Figure 11 shedding the light on how the seasonal variations affect Fine PM. PM2.5 average concentrations decreased both in DT and AUB during rainy days compared to normal clear days. This has been attributed to the ability of the rain to deposit PM and as such the amount of particulate pollutants in the atmosphere decreases.[88] In addition, if rain is accompanied with wind, PMs are further diluted in the atmosphere and lead to lower concentrations. [88]

(a) Total PM2.5




(b) During clear days




(c) During rainy days

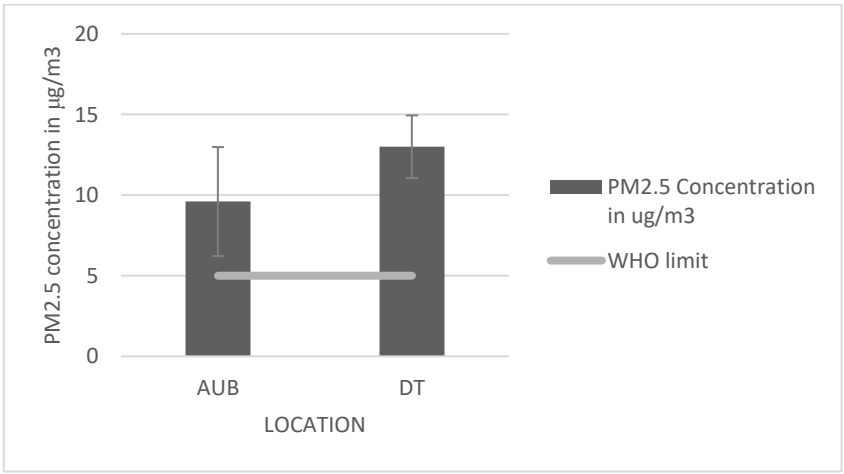



**Figure 11: Average Fine PM mass concentrations (µg/m3) – AUB vs DT, rainy and clear days.**

## *2. ROS variation*

a. Comparison of particle-bound ROS Concentrations at different sites

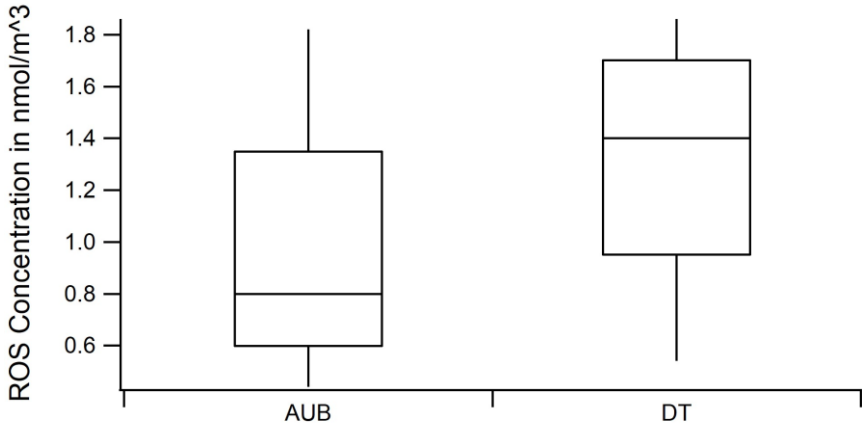
The overall ROS concentration averaged at 1.3 nmol/$m^3$ in DT which was higher than that in AUB standing at 0.9 nmol/$m^3$.

Figure 10 shows the box plot of ROS concentrations at AUB and DT. The $25^{th}$ and $75^{th}$ percentiles are shown at the bottom and top of the box, respectively. The whiskers show the lowest and highest concentrations, while the band near the middle of the box represents the median or $50^{th}$ percentile.

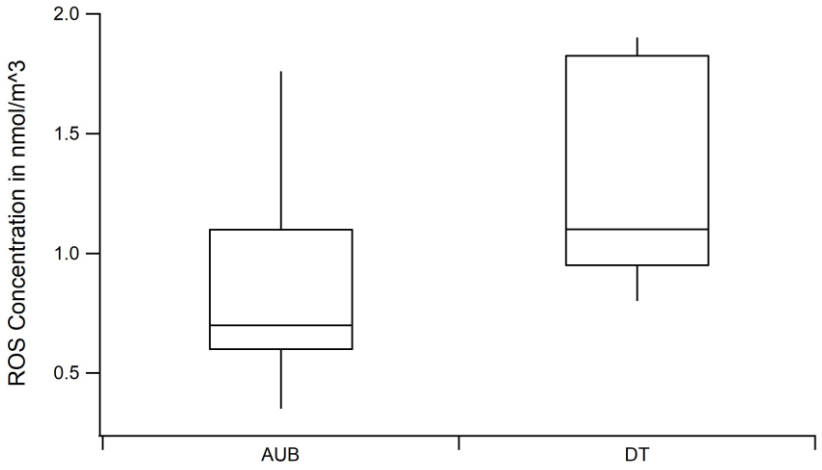
Both sites reported slight differences in ROS average concentrations in clear days as it varied between 0.9 and 1.1 nmol/$m^3$ in AUB and DT, respectively. It seems that vehicular emissions and diesel generators are contributing similarly to the ROS concentration in normal clear days.

However, the levels of ROS at AUB and DT differed significantly during rainy sessions. While AUB's average ROS concentration decreased to 0.6nmol/$m^3$, the levels at DT got higher to average at 1.5 nmol/$m^3$. This probably shows that wind direction and cold weather have made the collected PMs more loaded with ROS components that originate from diesel generators situated in close proximity to the PM samplers. This hypothesis will be confirmed with chemical analysis study that is still work in progress.

(a) Total ROS




(b) During clear days




(c) During rainy days

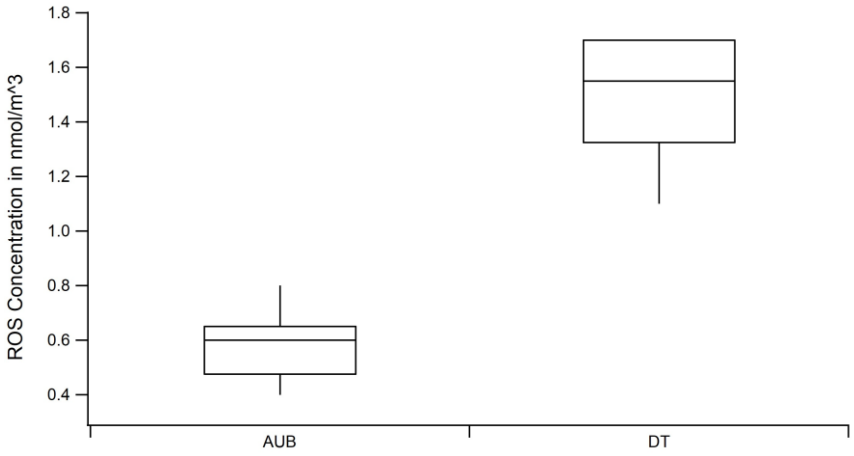



**Figure 12: Box plots presenting ROS concentrations (nmol/m$^3$) – AUB vs DT, total, rainy, and clear days.**

b. Dust storm events:

ROS concentration was observed to be much lower during dust events (0.6 nmol/$m^3$) compared to normal clear (1.1 nmol/$m^3$) and rainy days (1.5 nmol/$m^3$). This is also in agreement with a previous study done previously in our lab, in which we showed a clear decreasing trend in the ROS concentration of PM2.5 during dust storms in comparison to normal days. [56] This decrease is mostly associated with PM2.5 being mainly formed of crustal materials rather than vehicle and other fuel burning sources.

c. Seasonal variations:

ROS concentration at AUB followed the same trend of PM2.5, where the values decreased during rainy days. But the opposite happens with the ROS concentration in DT. This is as mentioned above probably due to the higher contribution of diesel generators in rainy days.

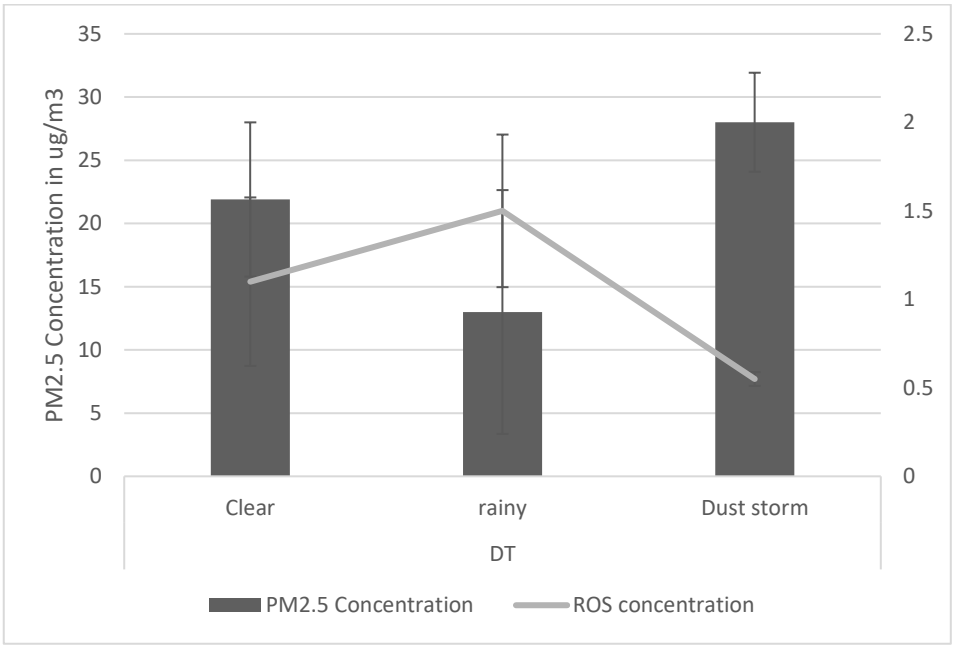



**Figure 13: ROS and PM2.5 average concentrations Variation during different events**

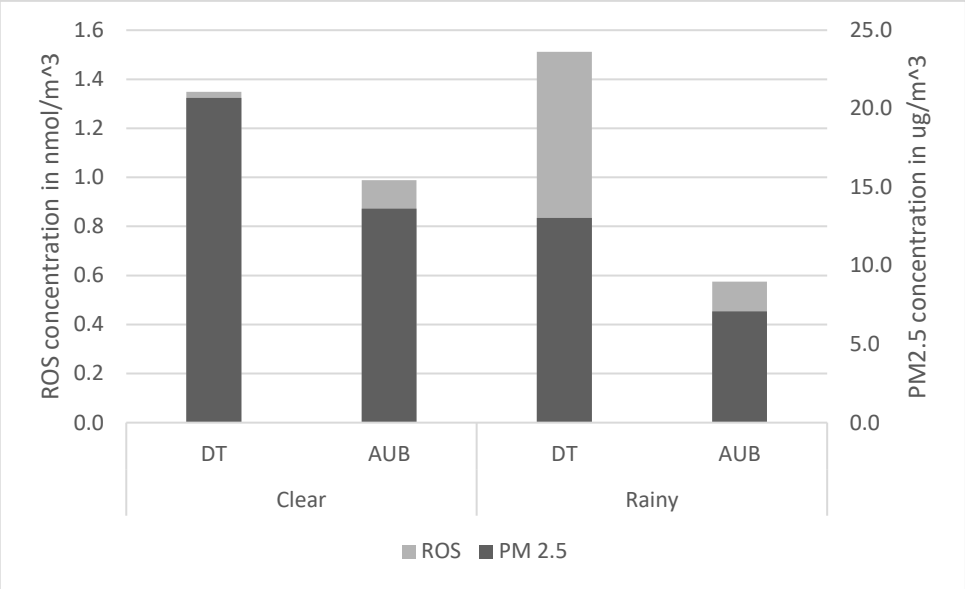



**Figure 14: Average concentrations of ROS and PM2.5 during clear and rainy days in AUB and DT**

### *1. Discussion*

These results imply that the levels of PM2.5 in Beirut are above the limits set by WHO by a large margin ranging between 300% and 440% in AUB and DT, respectively.

The levels of PM2.5 at AUB are mostly impacted by the high traffic emissions in the area and AUB's power plant releases. Moreover, the main sources that were clearly identified around the site of DT are traffic emissions and diesel generators.

Additionally, ROS levels were similar in AUB and DT in clear days. The main deriving factor for the ROS concentrations are the secondary organic aerosols, volatile organic compounds, and metals. Further chemical analysis of WSOC and metal content are required to decipher the main reasons behind the difference between the ROS levels in winter and summer. Moreover, rain and wind play a role in decreasing the amount of PM2.5 and ROS in the atmosphere by diluting some and depositing some others.

Dust storms are also playing a factor in increasing Fine PM concentration as they bring a lot of traveling Fine PM to the region, while reducing the amount of ROS probably due to the chemical composition of the crustal PM2.5.

## F. Summary and Conclusion

In this study, the current situation of air quality in Beirut was tested. The levels are high and surpassing WHO recommended limits. The main causes of high PM2.5 levels are vehicular and diesel generators emissions. Particle-bound ROS levels were also tested in this study for the first time in Lebanon. The levels of ROS also showed a correlation with both vehicular emissions and diesel generators.

Additionally, PM2.5 and ROS showed a clear trend during dust events and seasonal changes (rainy and clear days). Dust storms tends to increase PM2.5 concentrations while lowering the ROS amounts throughout the event. Also, the levels of PM2.5 and particle-bound ROS decreased during rainy days in comparison to normal clear ones.

Till now we sought to gain a better understanding of the situation of air pollution in the city. A follow-up will be the third site measurements and analysis of both ROS and PM2.5. This will be completed along with the analysis of WSOC at the 3 sites in order to get a better understanding about the origin and main sources of the analyzed ROS levels. In addition to chemical analysis, statistical analysis using PMF will be performed for a potential source apportionment to identify the main contributors to the PM and ROS levels in Beirut area.

# CHAPTER III

# OPTIMIZATION OF REACTIVE OXYGEN SPECIES ANALYSIS METHOD AND QUALITY CONTROL

## A. Introduction

DCFH is one of the most used probes for ROS measurements. The main disadvantage of using DCFH is the photo and auto oxidation of the fluorescent DCF. For this reason, a series of experiments have been performed to maximize our optimization in two ways. First, to ensure the stability of the fluorescent DCF so it auto-, and photo- oxidations are minimized. Several experiments were done comparing different timings and different conditions for this purpose. Second, to ensure that the full DCFH solution was oxidized into DCF and will give a full response to the added ROS; not just a partial response due to incomplete reaction of DCFH.

Along with this, and since we are measuring particle-bound ROS which are short-lived and fast to react in the atmosphere, we had to make sure that we are not losing any of the ROS collected on the filter and at the same time we had to know that we are measuring ROS over the whole period of 24 hours and not just for the last hour. This is due to some apprehensions regarding the high reactivity of particle-bound ROS, that most of what's collected on the filter is being lost during the sampling process. And what is being collected is just what has been collected in the last hour or few of sampling. Thus, the quality control experiments were a necessity to validate our ROS sampling period.

## B. Method optimization

### *1. Storage Temperature*

The storage temperature was chosen to be 18$^{o}$C over room temperature due to the instability and faster auto oxidation of the DCF compound.

### *2. Storage duration*

Four experiments were done using known amount of $H_2O_2$ and under the same conditions. The storage duration was varied between 30, 60, 90, and 120 minutes in order to compare reactivity. The first 3 at 30, 60, and 90 minutes showed very similar results. While the last one at 120 minutes showed a significantly higher result than the others with an indication that the best suitable timing for the storage duration is up to an hour and a half, after that oxidation of the probe interferes with our results.

### *3. Filters reactivity*

Since the study includes collecting filters from different sites in Beirut, the experiment was chosen to be started 30 minutes before the arrival of the filter. This will ensure the full time needed for the activation of DCFH while minimizing the waiting time of. The filter before being introduced to the probe. Thus, the amount of ROS lost during the collection duration is maximally reduced. The filter would be collected directly into a vial and stored in ice until it reaches the lab and gets analyzed.

An experiment was done in AUB to compare the results of directly adding a filter and another one after 30 minutes waiting time in ice. The results showed around 0.2% difference in concentration of ROS on the filter which is considered insignificant.

### *4. Photo oxidation*

The solution and reacting samples were all wrapped with aluminum foil, and the experiment was performed in a dark room to minimize photo-oxidation.

Additionally, all samples were contained in a dark box while on their way for analysis on the microplate reader.

A calibration curve with the final optimized conditions is shown below with $r^2$=0.999.

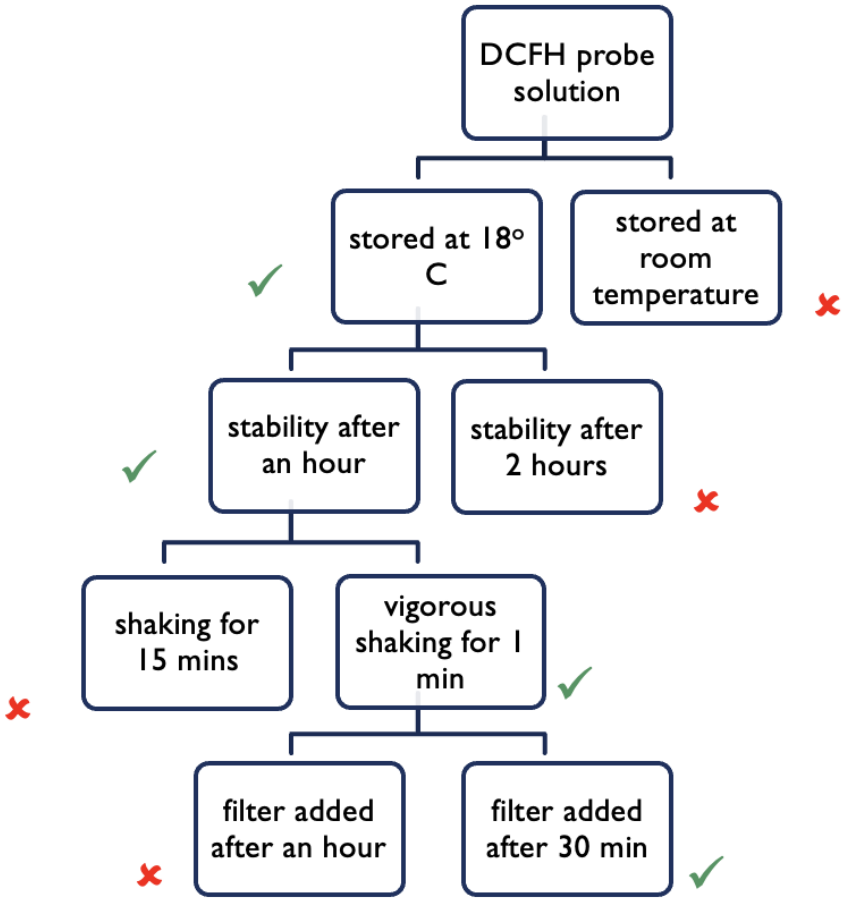



**Figure 15: The optimization process of the DCFH experimental setup to minimize photo- and auto-oxidation.**

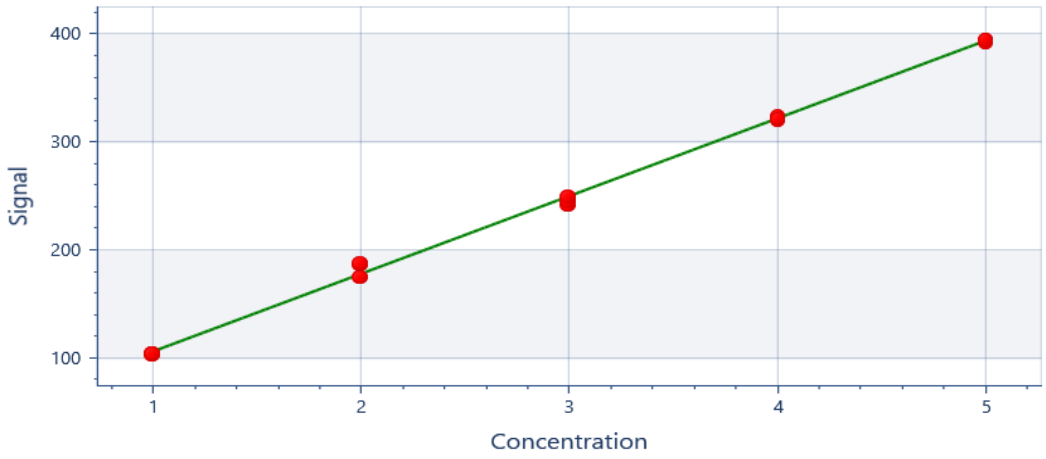



**Figure 16: Calibration curve presenting H2O2 concentration vs fluorescent signal after full optimization process.**

## C. Method Validation

This method was proven to be repeatable and reproducible with the limit of detection (LOD) and limit of quantification (LOQ) noted below.

### *1. Limit of detection (LOD)*

It is the lowest quantity measured that differs in a significant way from the blank. LOD is determined by the equation (3s/m), where s is the standard deviation of a low concentration point and m is the slope of the calibration curve. Our LOD was determined to be $0.244 * 10^{-7}$ mol/L which is less than our lowest calibration curve point standing at $1 * 10^{-7}$ mol/L.

### *2. Limit of Quantification (LOQ)*

A point can be higher than the limit of detection can be detectable but no with a reasonable accuracy. The smallest amount that can be measured accurately is called

limit of quantification and it's determined by the equation (10s/m). The LOQ in this method also was below our lowest point in the calibration curve at a value of 0.816 (E-07 M)

### *3. Repeatability*

The method is considered repeatable as its performance seven times in a short period of time, by the same person, in the same location, and with the same measurement procedure resulted in a relative standard deviation %rsd ranging between 0.45 and 1.44%.

### *4. Reproducibility*

This method was also fully performed in different days, and it was easily replicated under the optimal conditions with %rsd between 5.29 and 11.31% which is considered acceptable.

**Table 1: QA/QC**

| | |
|---|---|
| Calibration curve | 1-5 (E-07 M) |
| LOD | 0.244 (E-07 M) |
| LOQ | 0.816 (E-07 M) |
| Repeatability | (0.45-1.44) % |
| Reproducibility | (5.29-11.31) % |

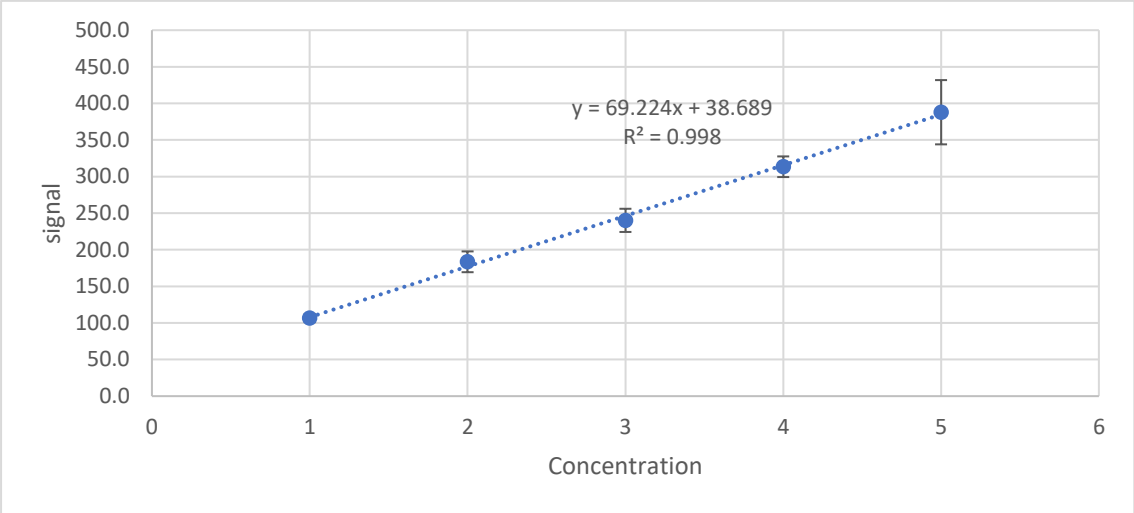



**Figure 17: A calibration curve showing 6 calibration curves variations during a period of 1 month.**

## D. Quality Control

### *1. Materials*

Used ethanol, horseradish peroxidase (HRP) (52 units/mg), potassium phosphate monobasic, and dibasic were purchased from Sigma-Aldrich. And 2',7'-dichlorofluorescin diacetate (DCFH-DA) was obtained from Molecular Probes (product code D399). The laminated Teflon filters (47 mm diameter) was bought from SterliTech.

### *2. Sampling and study design*

a. Sampling site

The sampling of particle-bound ROS for the quality control experiments and optimization were done at the background site (AUB).

b. Sample collection

Three equivalent pumps and cartridges were used to sample fine PM's ROS for different durations starting at the same time. All the cartridges were equipped with Laminated Teflon filters to capture ROS.

Cartridges one, two, and three were put to start sampling concurrently at 8:00 am with a flow rate of 16.7 L/min. Pump of cartridge one was turned off after an hour of sampling and directly analyzed for particle-bound ROS. Cartridge two was left to sample for 5 hours and then turned off and analyzed directly. While cartridge three was left to complete 24 hrs of sampling and analyzed directly after it was done.

A field blank was also used to test and eliminate any background interference. The field blank is a blank filter treated in a similar way to the sampling filters in which it is taken out to the site of sampling and placed in a cartridge, but the difference is that air is not sucked into the field blank (the pump is turned off).

c. ROS analysis

Same DCFH method was used for this experiment, as this method is direct and eliminates the possibility to lose any ROS as it's known that some particle-bound ROS are short-lived and can react quickly with the ambient air.[89] Also, this method was proven to be repeatable and reproducible along with being very sensitive and the fastest to perform under optimized conditions.[90]

### *3. Results and Discussion*

The field blank that was left in the cartridge was analyzed and showed a similar result to the normal experiment blank (the one that included only DCFH without any ROS) with a concentration around zero and below the limit of detection.

The first filter which was analyzed after one hour of sampling barely showed a detectable signal, as the collected particle-bound ROS concentration on the filter was still low at a concentration around 0.086 μM.

The second filter which was analyzed after five hours of sampling resulted in a 50% higher concentration than filter one at around 0.17 μM.

The last filter was analyzed after a full day since the sampling started, and the final concentration was 0.41 μM.

The three filters showed a detectable result upon analysis. However, a clear trend showed that the increase in the sampling time until we reach the recommended by EPA (24 hours of sampling) is showing an increasing concentration of ROS on the filter as shown in the graph below. The concentration of ROS is increasing overtime and it is directly proportional to the sampling duration. If we take the last setup as a reference, we can say that in five hours we collected around 40% of the total ROS, and in one hour 20% of the total ROS was collected. Thus, the amount of ROS is accumulating on the filter throughout the full duration of sampling, and it's not being collected in the last few hours. This trend is clearly shown in the figure below as the amount of ROS accumulating on the filter is directly proportional to the sampling duration with a correlation coefficient $r^2$=0.9951.

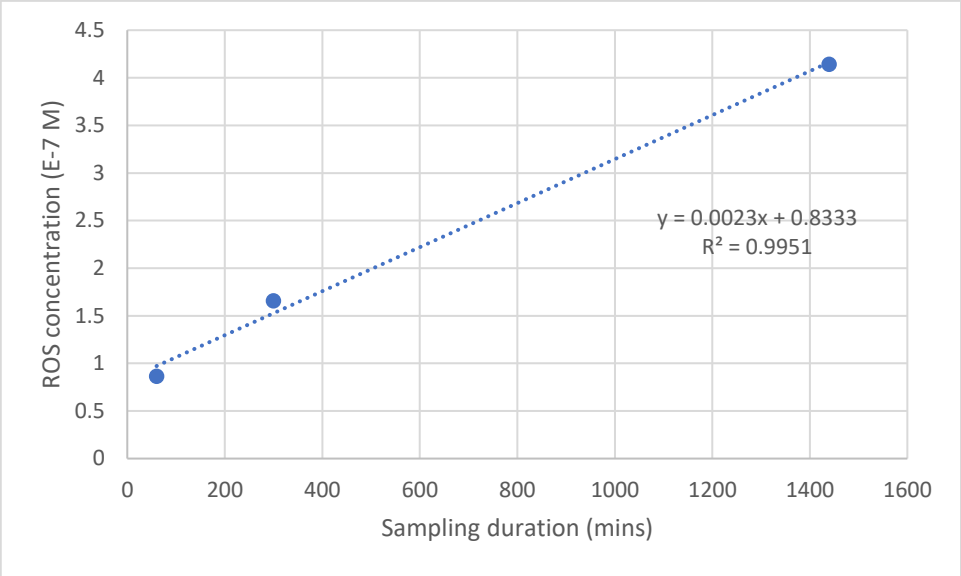



**Figure 18: particle-bound ROS variation as function of sampling duration**

## *4. Conclusion*

Given the short lifetime of particle-bound ROS and our sampling duration, it was a necessity to check if the ROS is being lost already during the sampling process. The results above showed a clear indication that the ROS is not being lost during sampling but accumulated on the filter throughout the full sampling duration.

The method showed up to be repeatable and reproducible upon the optimization of the auto-oxidation and photo-oxidation, their effect was minimized under optimal conditions.

# CHAPTER IV

# CONCLUSION

In order to assess the current situation of air pollution in Beirut, this annual study was conducted. This study included the measurement of PM2.5 and Particle-bound ROS at two different sites. The study included sampling during rainy and clear days, along with sampling during dust events. The levels of ROS also showed a clear correlation with both vehicular emissions and diesel generators. Additionally, PM2.5 and ROS showed a trend during dust events and seasonal changes (rainy and clear days). Dust storms tends to increase PM2.5 concentrations while lowering the ROS amounts throughout the event. Also, the levels of PM2.5 and particle-bound ROS decreased during rainy days in comparison to normal clear ones. A remarkable result was the increase of the ROS concentration in DT during rainy days, in which it didn't follow the general decreasing trend of toxicants during rain. This trend will be explained in the coming study as it is an interesting trend to be justified.

A follow up to this study will be the measurement of ROS and PM2.5 in the third site (MGH). This is along with the WSOC and PAHs analysis for the three sites using GC-MS. Also, statistical analysis will be done using PMF, which will include all the data for potential sources identification. Results will update the current understanding of the pollution situation in Beirut. They can also form the basis for mitigation measures in order to alleviate the health risk burden on Beirut residents.

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