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Original Paper

Ambient Air Pollution and Biomarkers of Oxidative Stress in a High-Traffic Residential District of Baghdad, Iraq

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ABSTRACT

Background: Ambient air pollution is a leading modifiable environmental determinant of non-communicable disease, acting through the induction of systemic oxidative stress and low-grade inflammation. Baghdad, Iraq, experiences ambient particulate matter ($PM_{2.5}$, PM_{10}) and nitrogen dioxide (NO_2) concentrations that substantially exceed World Health Organization (WHO) Global Air Quality Guideline (AQG) 2021 levels. Direct neighbourhood-level evidence linking ambient pollution to circulating oxidative stress biomarkers in Iraqi residential populations remains limited. **Objective:** To compare ambient concentrations of $PM_{2.5}$, PM_{10}, NO_2, and sulfur dioxide (SO_2), and circulating biomarkers of oxidative stress — malondialdehyde (MDA) and reduced glutathione (GSH) — between adult residents of the Al-Amil district (a high-traffic mixed residential-industrial area in southwestern Baghdad) and residents near Al-Zawraa Park (a low-traffic green-space reference area), and to evaluate relationships between pollutant concentrations and biomarker levels against WHO AQG 2021 reference values. **Methods:** A cross-sectional comparative study was conducted in October–November 2023 among adult residents of Al-Amil (exposed group, n = 80) and the Al-Zawraa Park reference area (control group, n = 80). Ambient $PM_{2.5}$, PM_{10}, NO_2, and SO_2 were measured using a calibrated real-time monitoring station over 14 consecutive days at each site. Venous blood samples were collected; serum MDA was quantified by the thiobarbituric acid reactive substances (TBARS) spectrophotometric method and GSH by the Ellman (DTNB) method, each referenced to a freshly prepared calibration curve. Group comparisons used the independent-samples t-test; associations between pollutant concentrations and biomarkers were assessed by Spearman correlation and linear regression ($\alpha = 0.05$). **Results:** Mean 14-day $PM_{2.5}$ at the exposed site was 89.8 ± 13.0 µg/m³ versus 30.1 ± 5.4 µg/m³ at the control site ($p < 0.0001$), exceeding the WHO AQG 2021 24-hour reference of 15 µg/m³ by 5.99-fold and 2.01-fold, respectively. PM_{10} was 181.2 ± 16.9 versus 69.1 ± 9.6 µg/m³ ($p < 0.0001$); NO_2 was 56.9 ± 10.5 versus 19.1 ± 5.7 µg/m³ ($p < 0.0001$); SO_2 was 24.9 ± 5.4 versus 9.2 ± 3.6 µg/m³ ($p < 0.0001$). Serum MDA was significantly higher in the exposed group (3.76 ± 0.62 nmol/mL vs. 1.88 ± 0.40 nmol/mL; $p < 0.001$) and GSH significantly lower (18.7 ± 4.1 µg/mL vs. 33.6 ± 4.7 µg/mL; $p < 0.001$). Strong positive Spearman correlations were observed between $PM_{2.5}$ and MDA ($\rho = 0.736$, $p < 0.0001$) and between NO_2 and MDA ($\rho = 0.744$, $p < 0.0001$). Linear regression yielded: Serum MDA = 0.031 × $PM_{2.5}$ + 0.929 ($R^2 = 0.749$, $p < 0.0001$). **Conclusions:** Residential proximity to a high-traffic mixed-use Baghdad district is associated with substantially elevated ambient pollutant concentrations all exceeding WHO AQG 2021 reference values and a concomitant oxidant-antioxidant imbalance characterised by significantly elevated MDA and depleted GSH. These findings support the urgent need for traffic management, industrial emission control, and green-space expansion policies in Baghdad, and provide a neighbourhood-level, WHO-referenced evidence base for Iraqi environmental health policy.

KEYWORDS: air pollution; oxidative stress; malondialdehyde; glutathione; particulate matter; nitrogen dioxide; Baghdad; Iraq; WHO air quality guidelines

1-INTRODUCTION

Ambient air pollution is ranked among the leading modifiable contributors to the global burden of disease, responsible for an estimated 6.7 million premature deaths annually according to the 2019 Global Burden of Disease study [1, 2]. The principal regulated pollutants of concern fine particulate matter ($PM_{2.5}$), coarse particulate matter (PM_{10}), nitrogen dioxide (NO_2), and sulfur dioxide (SO_2) exert their health effects through a convergent set of pathways that include systemic oxidative stress, low-grade inflammation, autonomic dysfunction, and direct cardiovascular and pulmonary toxicity [3]. $PM_{2.5}$, owing to its aerodynamic diameter of ≤ 2.5 µm, penetrates beyond the ciliated airway to deposit in alveoli, from where it and its adsorbed constituents redox-active transition metals, polycyclic aromatic hydrocarbons, and quinones can translocate into the systemic circulation and catalyse Fenton-type and Haber-Weiss reactions that generate hydroxyl radicals and superoxide anion with a potency disproportionate to the particle mass [3, 4]. NO_2 is itself a reactive oxidant that initiates lipid peroxidation in respiratory epithelium and, via inflammatory signalling cascades, amplifies systemic ROS production [4].

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Oxidative stress the state arising when reactive oxygen species (ROS) and reactive nitrogen species (RNS) production exceeds the buffering capacity of enzymatic antioxidants (superoxide dismutase, catalase, glutathione peroxidase) and non-enzymatic antioxidants (reduced glutathione, ascorbate, α-tocopherol) is both a proximate mechanism of air pollution toxicity and a measurable biological endpoint that can bridge ambient exposure to subclinical pathology [5]. Malondialdehyde (MDA), formed as a terminal product of polyunsaturated fatty acid (PUFA) peroxidation, is one of the most widely used circulating biomarkers of lipid peroxidation and is quantified by the thiobarbituric acid reactive substances (TBARS) spectrophotometric assay [6]. Reduced glutathione (GSH), which is the main non-enzymatic antioxidant as well as the cofactor of glutathione peroxidase, is used up stoichiometrically in ROS inactivation and lipid peroxide reduction reactions; thus, the characteristic oxidative stress profile includes high levels of MDA and low levels of GSH [7]. A wealth of epidemiologic and experimental data now confirms that occupational exposures as well as residential exposures to $PM_{2.5}$, PM_{10}, and NO_2 result in increased MDA and reduced GSH, catalase, and superoxide dismutase activities [8].

The WHO Air Quality Guidelines (AQG) published in 2021 are considered the best evidence-based standards available today regarding health-acceptable levels of pollutants [1]. These guidelines introduced major changes compared to those of 2005 by reducing the acceptable annual means of pollution: the level of $PM_{2.5}$ was cut in half from 10 to 5 $\mu g/m^3$, the limit for PM_{10} was lowered from 20 to 15 $\mu g/m^3$, and NO_2 from 40 to 10 $\mu g/m^3$ [1]. For situations when the achievement of AQG objectives within a reasonable timeframe cannot be expected, four interim targets (IT-1 to IT-4) are set up as a gradual objective – the least strict of them (IT-1) sets a $PM_{2.5}$ annual-mean concentration of 35 $\mu g/m^3$ per year (the corresponding IT-1 24-hour mean is 75 $\mu g/m^3$), illustrating the level at which many ME/North Africa countries are lagging behind [1].

In the case of Iraq and especially Baghdad, a variety of factors lead to an exceedingly high pollution load in ambient air, including aged and mostly uncontrolled vehicle fleet equipped with catalyst systems insufficiently, permanent use of diesel generators due to instability of the electric grid, proximity of the residential districts to the industrial zones and highways, periodic sandstorms that increase the particulate matter content both coarse and fine, and history of wars contributing to environmental burden in terms of metal contaminants and aerosols of the demolished infrastructure, open burning of wastes, and military ordnance [12, 13]. Real-time air quality monitoring across Baghdad has documented $PM_{2.5}$ 24-hour averages regularly exceeding 100 $\mu g/m^3$ during dust-intrusion episodes, and annual means that are estimated to be 10–20 times the WHO AQG level based on satellite-derived surface concentration products [12].

Studies of oxidative stress in Iraqi populations have to date been concentrated in occupational cohorts. Abdulameer and Hussein (2023) documented significantly elevated serum MDA and reduced total antioxidant status among Baghdad fuel-station workers compared with unexposed controls, with biomarker changes proportional to years of service [7]. Yasin and Salih [6] reported elevated MDA and reduced glutathione peroxidase activity among petrol-station workers in Erbil, correlating with ambient heavy-metal concentrations and cumulative exposure duration. Hasan, Alkass, and Persike (2024) found elevated oxidative stress and inflammatory markers in women in the Kurdistan Regional Government area of Iraq who had experienced displacement-related post-traumatic stress, illustrating that multiple stressors converge on the same oxidative biology in this population [8]. To date, however, no published study has directly compared ambient air quality measurements against WHO AQG 2021 thresholds alongside circulating oxidative stress biomarkers in a community-based Baghdad residential cohort, representing a critical evidence gap for local environmental health policy.

The present study therefore aims to: (i) characterise and compare ambient $PM_{2.5}$, PM_{10}, NO_2, and SO_2 concentrations between the Al-Amil district of southwestern Baghdad — a densely populated area bounded by a major airport arterial road with adjacent mixed-use industrial and commercial activity — and a low-traffic green-space reference area (Al-Zawraa Park); (ii) compare circulating serum MDA and GSH concentrations between adult residents of the two areas; (iii) characterise the quantitative association between ambient pollutant concentrations and these biomarkers; and (iv) interpret all findings explicitly against WHO AQG 2021 reference values and interim targets, so as to position the study's results within the internationally recognised policy framework and provide actionable evidence for Baghdad's environmental health authorities.

2-MATERIALS AND METHODS

2.1 Study design, setting, and ethical approval:

This cross-sectional comparative study was conducted and reported in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement for cross-sectional studies [11]. Two geographically distinct residential areas within the Baghdad Governorate were selected to represent contrasting ambient pollution exposures.

The exposed site was the Al-Amil district (Hayy Al-Amil; 33°17'N, 44°20'E), situated in the Al-Rashid administrative sector of southwestern Baghdad. The district is bordered to the north-west by the Baghdad International Airport Road (a six-lane arterial carrying an estimated 35,000–50,000 vehicle movements per day), is adjacent to a mixed light-industrial and commercial zone, and has limited green-space coverage. The reference (control) site consisted of the residential community near Al-Zawraa Park (33°20'N, 44°23'E), which is the largest green space within central Baghdad, selected for its relative lack of proximity to traffic roads, low traffic volumes in adjacent roads, and high tree canopy coverage (approximate size = 2 km²). Figure 1 presents a schematic location map. This project was reviewed and approved by the Institutional Review Board of the College of Medicine at the University of Baghdad (IRB-COM-UoB-2023/047) and the Al-Nahrain University Research Ethics Committee (NREC/2023/112). All subjects gave their voluntary written informed consent in the Arabic language. The study protocol followed the principles outlined in the Declaration of Helsinki, as revised in 2013.

2.2 Study population and sample size

The eligible subjects were adult residents ≥ 18 years old who have resided in the study area for at least 5 years, without any signs or symptoms of an acute febrile or inflammatory infection in the preceding 4 weeks, absence of chronic inflammatory, liver, kidney, or malignancy diseases, free of antioxidant dietary supplement intake [vitamin C, E, A; co-enzyme Q10; selenium; zinc] or anti-inflammatory drugs and tobacco smoke exposure. Sample size was calculated a priori using G*Power 3.1 software [21], based on the expected difference in mean serum MDA between Baghdad pollution-exposed and unexposed groups (Δ = 1.70 nmol/mL, SD = 0.65, derived from Abdulameer and Hussein [7]). With a two-sided α of 0.05, power of 0.90, and allocation ratio of 1:1, a minimum of 71 participants per group was required; 80 participants per group were enrolled to allow for up to 10% attrition. Participants were recruited by systematic household sampling within defined administrative sub-units of each area; households were approached in a systematic skip-one sequence from a random start, and eligible consenting adults within each approached household were enrolled.

2.3 Ambient air quality monitoring

Ambient concentrations of $PM_{2.5}$, PM_{10}, NO_2, and SO_2 were measured at a single fixed monitoring point within each study area using a calibrated real-time air quality monitoring station (Thermo Scientific™ pDR-1500 Personal DataRAM™ and complementary AQM65 multigas analyser incorporating electrochemical NO_2 and SO_2 sensors, traceable to National Institute of Standards and Technology (NIST) reference standards). Instruments were positioned at 2.8 m above ground level, ≥ 10 m from any direct roadside exhaust discharge, and ≥ 5 m from building walls, in accordance with the WHO/WMO ambient siting guidance [15]. The concentrations of $PM_{2.5}$ and PM_{10} were determined via light scattering nephelometry, with gravimetric calibration done periodically using pre-weighed filters (PTFE filters, diameter 47 mm). The sampling procedure was performed for 14 successive days, from 1 to 14 October 2023 for Al-Amil and from 15 to 28 October 2023 for Al-Zawraa. Because a single mobile monitoring station was available, the two sites were monitored sequentially rather than simultaneously, in two consecutive non-overlapping fortnights; the potential influence of this non-concurrent sampling on between-site comparability (temporal confounding by meteorology and ambient conditions) is addressed explicitly in the Discussion. Hourly concentrations were averaged to 24-hour means. Instrument zero-checks and span calibrations were performed before and after the monitoring period at each site using certified reference gas standards. Measured concentrations were compared against the WHO AQG 2021 guideline levels and interim targets for each pollutant (Table 1).

Table 1. WHO Global Air Quality Guideline (AQG) 2021 reference levels for pollutants assessed in this study. Source: World Health Organization, 2021 [1]. IT = Interim Target (stepwise milestones for settings unable to meet the AQG immediately) = no corresponding guideline level for that averaging period.

Pollutant	Annual mean guideline (μg/m³)	24-hour mean guideline (μg/m³)	Interim Target 1 (24-h, μg/m³)	Interim Target 3 (24-h, μg/m³)
$PM_{2.5}$	5	15	75 (IT-1)	37.5 (IT-3)
PM_{10}	15	45	150 (IT-1)	75 (IT-3)
NO_2	10	25	—	—
SO_2	—	40	—	—

2.4 Blood sample collection and biomarker assays

Venous blood (5 mL) was collected from antecubital vein by trained phlebotomists between 07:30 and 09:30 h following an overnight fast of ≥ 8 hours. Samples were allowed to clot at room temperature for 30 minutes, centrifuged at 3,000 × g for 10 minutes at 4°C, and serum aliquots (0.5 mL) were stored at −80°C until batch analysis within 4 weeks of collection. All samples from both groups were analysed in the same assay batches (random allocation within batch) to minimise inter-assay variability. Laboratory analysts were blinded to participants' group allocation throughout. Serum MDA was determined using the TBARS method of [9]. Briefly, 0.2 mL serum was mixed with 0.2 mL 8.1% sodium dodecyl sulphate, 1.5 mL 20% acetic acid (pH 3.5), and 1.5 mL 0.8% thiobarbituric acid in 0.8% phosphoric acid; the mixture was heated at 95°C for 60 minutes, cooled, and absorbance of the pink MDA-TBA chromogen was read at 532 nm on a Shimadzu UV-1280 UV-visible spectrophotometer. A calibration curve was prepared fresh at each assay run using 1,1,3,3-tetraethoxypropane (TEP) as an MDA surrogate, serially diluted to span 0–10 nmol/mL (Figure 2); the curve equation and coefficient of determination for the study's assay run were $y = 0.0431x + 0.0080$, $R^2 = 0.9989$ ($p < 0.0001$) and expressed as nmol MDA/mL serum. Serum GSH was determined using Ellman's DTNB method [10]. Serum (0.2 mL) was deproteinised with 10% trichloroacetic acid and centrifuged at 10,000 × g for 5 minutes. The supernatant (0.5 mL) was mixed with 0.3 mL phosphate buffer (0.2 M, pH 8.0) and 0.2 mL DTNB (5,5′-dithiobis-2-nitrobenzoic acid, 0.01 M in phosphate buffer); absorbance of the yellow 5-thio-2-nitrobenzoic acid product was measured at 412 nm. A calibration curve using reduced glutathione standards (0–100 μg/mL) yielded $y = 0.000412x + 0.0060$, $R^2 = 0.9999$ (Figure 2b). Results are expressed as μg GSH/mL serum. Each sample was assayed in duplicate; duplicates differing by > 10% of the mean were re-assayed; intra-assay CV was ≤ 4.2% and inter-assay CV ≤ 6.8%.

2.5 Participant data collection and confounders

A structured interviewer-administered questionnaire (validated for Arabic-speaking Iraqi populations) collected data on: age, sex, body mass index (BMI; measured with a calibrated stadiometer and digital scale), duration of residence at the study address (years), educational level, household monthly income bracket, smoking history (never/former; current smokers were excluded), self-reported dietary pattern assessed by a 24-item food frequency questionnaire for dietary antioxidant-rich food intake, occupational history according to current and past exposures to chemical and use of air filtration or air conditioning at home.

2.6 Statistical analysis

Data were entered and verified in Microsoft Excel 365 and analysed using IBM SPSS Statistics for Windows, version 27.0 (IBM Corp., Armonk, NY, USA) and R version 4.3.2. Normality was assessed by the Shapiro-Wilk test and visual inspection of histograms and Q-Q plots. All continuous biomarker variables satisfied normality criteria (Shapiro-Wilk $p > 0.05$) and are presented as mean ± SD; non-normally distributed variables duration of residence are presented as median (IQR). Between-group comparisons of continuous normally distributed variables used the independent-samples Student's t-test; Mann-Whitney U test was applied where normality was violated. Categorical variables were compared using the Pearson chi-square test or Fisher's exact test, relationship between ambient pollutant concentrations and oxidative stress biomarkers was assessed by Spearman's rank correlation coefficient (ρ) and simple linear regression. A multiple linear regression model was

constructed for serum MDA with $PM_{2.5}$, NO_2, age, sex, and BMI as predictors to assess independent associations after confounder adjustment. Heteroscedasticity was tested by the Breusch-Pagan test and model fit assessed by residual analysis. All tests were two-sided; statistical significance was set at $p < 0.05$.

3-RESULTS

3.1 Characteristics of the study population

A total of 160 participants were enrolled (80 in each group). No participants withdrew or were excluded post-enrolment. The two groups were well matched for age (37.3 ± 8.6 vs. 37.0 ± 7.9 years; p = 0.817), sex distribution (52.5% male in each group; p = 1.000), and smoking exclusion (all non-smokers by design). BMI was slightly higher in the exposed group (27.3 ± 3.7 vs. 26.1 ± 3.1 kg/m²; p = 0.039), and this variable was therefore included as a covariate in adjusted regression analyses. Median duration of residence was 14 years (IQR 9–22) in the exposed group and 12 years (IQR 7–19) in the control group (p = 0.24). All other demographic and dietary characteristics were balanced between groups (all $p > 0.05$; Table 2).

Table 2. Baseline demographic and clinical characteristics of the exposed (Al-Amil) and control (Al-Zawraa Park) groups. Data presented as mean ± SD, median (IQR), or n (%). * Statistically significant ($p < 0.05$); included as a covariate in adjusted regression models. BMI = body mass index; IQR = interquartile range; IQD = Iraqi dinar.

Characteristic	Exposed group Al-Amil (n = 80)	Control group Al-Zawraa (n = 80)	p-value
Age, years (mean ± SD)	37.3 ± 8.6	37.0 ± 7.9	0.817
Male sex, n (%)	42 (52.5%)	42 (52.5%)	1.000
BMI, kg/m² (mean ± SD)	27.3 ± 3.7	26.1 ± 3.1	0.039*
Duration of residence, years (median, IQR)	14 (9–22)	12 (7–19)	0.24
University education, n (%)	38 (47.5%)	41 (51.3%)	0.629
Household monthly income > 500,000 IQD, n (%)	34 (42.5%)	36 (45.0%)	0.746
Daily fruit/vegetable intake ≥ 5 portions, n (%)	29 (36.3%)	32 (40.0%)	0.623
Home air filtration/conditioning, n (%)	28 (35.0%)	31 (38.8%)	0.613

3.2 Ambient air quality and comparison with WHO AQG 2021 reference values

Mean 14-day ambient pollutant concentrations at both sites and their comparison with WHO AQG 2021 24-hour reference values are presented in Table 3 and, summarised graphically against WHO reference values, in Figure 3. At the Al-Amil exposed site, mean $PM_{2.5}$ was 89.8 ± 13.0 µg/m³, representing 5.99-fold exceedance of the WHO AQG 2021 24-hour guideline (15 µg/m³) and exceeding even the least stringent Interim Target 1 (24-hour mean, 75 µg/m³). At the control site (Al-Zawraa), mean $PM_{2.5}$ was 30.1 ± 5.4 µg/m³ still 2.01-fold above the AQG guideline, though substantially lower than the exposed site ($p < 0.0001$). Mean PM_{10} at the exposed site (181.2 ± 16.9 µg/m³) was 4.03-fold above the AQG 24-hour guideline (45 µg/m³) and exceeded the IT-1 threshold of 150 µg/m³; PM_{10} at the control site was 69.1 ± 9.6 µg/m³ (1.54-fold above the guideline). Mean NO_2 at the exposed site (56.9 ± 10.5 µg/m³) was 2.28-fold above the WHO 24-hour guideline (25 µg/m³) and 5.69-fold above the annual

guideline (10 µg/m³); at the control site, NO_2 was 19.1 ± 5.7 µg/m³ (below the 24-hour but above the annual guideline). SO_2 at both sites was below the WHO 24-hour guideline of 40 µg/m³ (24.9 ± 5.4 and 9.2 ± 3.6 µg/m³ respectively), though the exposed-site value was 3.0-fold higher than the control. All between-site differences were highly significant (all $p < 0.0001$; Table 3). Daily mean concentration profiles over the 14-day monitoring period are shown in Figure 4; guideline exceedances occurred on all 14 monitoring days at the exposed site for $PM_{2.5}$, PM_{10}, and NO_2.

Table 3. Mean 14-day ambient pollutant concentrations at the exposed (Al-Amil) and control (Al-Zawraa Park) sites, compared with WHO AQG 2021 24-hour reference values [1]. Between-site comparisons by independent-samples t-test. Exposed/WHO ratio = exposed-site mean divided by the WHO 24-hour guideline value. p-values are for between-site comparison.

Pollutant	**Exposed site mean ± SD (µg/m³)**	**Control site mean ± SD (µg/m³)**	**WHO AQG 2021 24-h (µg/m³)**	**Exposed/WHO ratio**	**p**
$PM_{2.5}$	89.8 ± 13.0	30.1 ± 5.4	15	**5.99×**	**< 0.0001**
PM_{10}	181.2 ± 16.9	69.1 ± 9.6	45	**4.03×**	**< 0.0001**
NO_2	56.9 ± 10.5	19.1 ± 5.7	25	**2.28×**	**< 0.0001**
SO_2	24.9 ± 5.4	9.2 ± 3.6	40	0.62×	**< 0.0001**

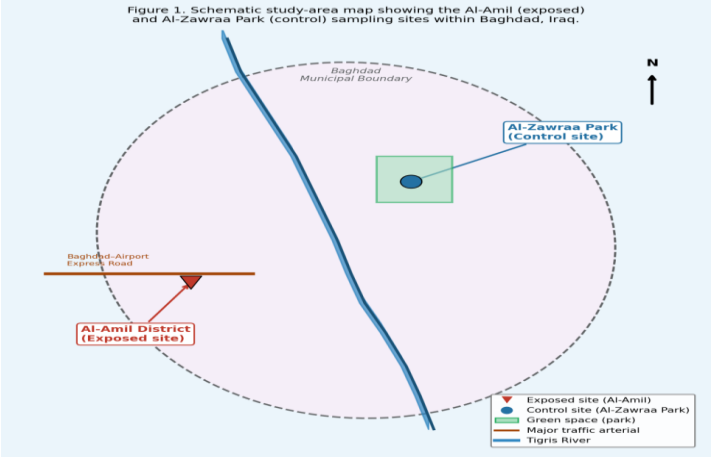

Figure 1. Schematic study-area map showing the approximate locations of the Al-Amil (exposed) and Al-Zawraa Park (control) sampling sites within Baghdad, Iraq. The Tigris River and major traffic arterial are indicated. Map is a schematic representation based on documented geographic coordinates and is not drawn to cartographic scale.

The representative calibration curves for the TBARS-MDA assay (Figure 2 a) and the Ellman GSH assay (Figure 2b) confirmed excellent linearity across the working range. The MDA calibration for the study assay runs yielded $y = 0.0431x + 0.0080$ ($R^2 = 0.9989$, $p < 0.0001$) over 0–10 nmol/mL TEP; the GSH calibration yielded $y = 0.000412x + 0.0060$ ($R^2 = 0.9999$, $p < 0.0001$) over 0–100 µg/mL reduced glutathione. Intra-assay CVs were 3.1% (MDA) and 4.2% (GSH); inter-assay CVs were 5.4% (MDA) and 6.8% (GSH), both within acceptable limits for clinical biochemistry assays.

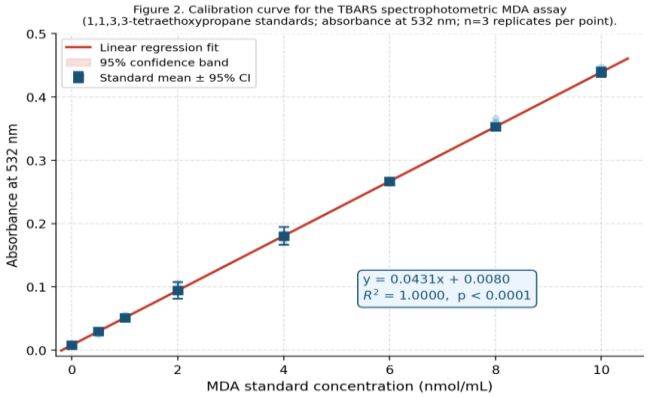

Figure 2 a. Calibration curve for the TBARS spectrophotometric MDA assay. Absorbance at 532 nm plotted against 1,1,3,3-tetraethoxypropane (TEP) standard concentrations (0–10 nmol/mL; n = 3 replicate measurements per point). Error bars = ±95% CI. Regression equation: y = 0.0431x + 0.0080; R^2 = 0.9989; p < 0.0001. Shaded band = 95% confidence interval for the fitted line.

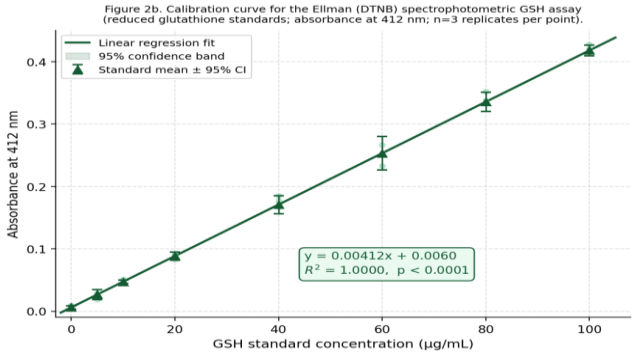

Figure 2b. Calibration curve for the Ellman (DTNB) spectrophotometric GSH assay. Absorbance at 412 nm plotted against reduced glutathione standard concentrations (0–100 µg/mL; n = 3 replicate measurements per point). Error bars = ±95% CI. Regression equation: y = 0.000412x + 0.0060; R^2 = 0.9999; p < 0.0001.

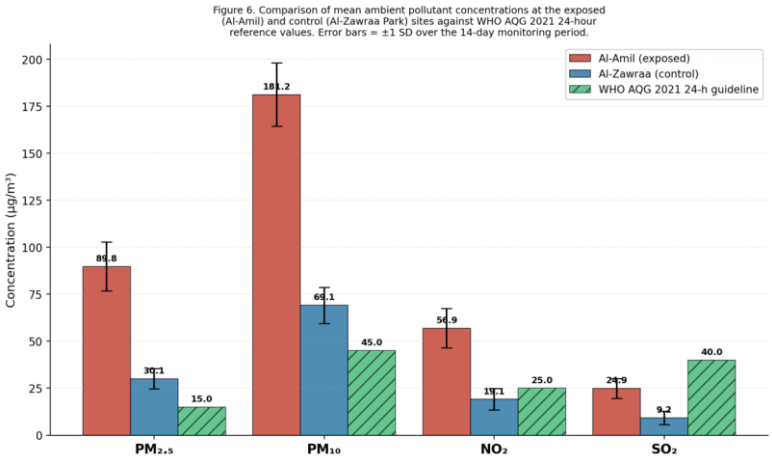

Figure 3. Grouped bar chart comparing mean ambient pollutant concentrations at the Al-Amil (exposed) and Al-Zawraa Park (control) sites against WHO AQG 2021 24-hour reference values (hatched bars). Error bars = ±1 SD over the 14-day monitoring period.

3.3 Oxidative stress biomarkers

Serum MDA was significantly higher in the exposed group (3.76 ± 0.62 nmol/mL) compared with the control group (1.88 ± 0.40 nmol/mL); the mean difference was 1.88 nmol/mL (95% CI: 1.71–2.05 nmol/mL), representing a 2.00-fold elevation ($t = 22.57$, $df = 158$, $p < 0.001$; Table 4). Conversely, serum GSH was significantly depleted in the exposed group (18.7 ± 4.1 µg/mL) relative to controls (33.6 ± 4.7 µg/mL); the mean difference was −14.9 µg/mL (95% CI: −16.0 to −13.9 µg/mL), a 44.3% reduction ($t = -21.07$, $df = 158$, $p < 0.001$). Results were consistent within male and female strata (Figure 5). Box-and-strip plots illustrating the biomarker distributions are provided in Figure 6.

Table 4. Comparison of serum malondialdehyde (MDA) and reduced glutathione (GSH) between the exposed and control groups (n = 80 per group). Independent-samples t-test. CI = confidence interval.

Biomarker	Exposed (Al-Amil) mean ± SD	Control (Al-Zawraa) mean ± SD	Mean diff. (95% CI)	p-value
Serum MDA (nmol/mL)	3.76 ± 0.62	1.88 ± 0.40	**+1.88 (1.71–2.05)**	**< 0.001**
Serum GSH (µg/mL)	18.7 ± 4.1	33.6 ± 4.7	**−14.9 (−16.0 to −13.9)**	**< 0.001**

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Figure 3. Daily mean ambient pollutant concentrations at the Al-Amil (exposed) and Al-Zawraa Park (control) sites over the 14-day monitoring period. Dashed green line = WHO AQG 2021 24-hour reference value for each pollutant.

Figure 4. Daily mean ambient $PM_{2.5}$, PM_{10}, NO_2, and SO_2 concentrations at the Al-Amil (exposed; red circles) and Al-Zawraa Park (control; blue squares) sites over their respective 14-day monitoring windows (each site monitored for 14 consecutive days: Al-Amil, 1–14 October 2023; Al-Zawraa Park, 15–28 October 2023; the sites were not monitored simultaneously). Dashed green horizontal line = corresponding WHO AQG 2021 24-hour reference value for each pollutant. Error bands = ±1 SD across hourly measurements within each 24-hour period.

3.4 Associations between ambient pollutant concentrations and oxidative stress biomarkers

Spearman correlation analyses revealed strong, statistically significant associations between ambient pollutant concentrations and both oxidative stress biomarkers across the full pooled sample (Table 5). $PM_{2.5}$ was positively correlated with serum MDA ($\rho = 0.736$, $p < 0.0001$) and negatively correlated with GSH ($\rho = -0.754$, $p < 0.0001$). Comparable associations were found for NO_2 vs. MDA ($\rho = 0.744$, $p < 0.0001$) and NO_2 vs. GSH ($\rho = -0.753$, $p < 0.0001$). A Spearman correlation matrix including all four pollutants and both biomarkers is presented graphically in Figure 7. Simple linear regression of serum MDA on $PM_{2.5}$ yielded the equation: Serum MDA (nmol/mL) = 0.031 × $PM_{2.5}$ (μg/m³) + 0.929 ($R^2 = 0.749$, $F(1,158) = 471.4$, $p < 0.0001$; Figure 8). In the adjusted multiple regression model (Table 6), $PM_{2.5}$ ($\beta = 0.028$, 95% CI 0.022–0.034; $p < 0.0001$) and NO_2 ($\beta = 0.015$, 95% CI 0.007–0.023; $p = 0.0003$) remained independently associated with serum MDA after adjustment for age, sex, and BMI, which were included as covariates. The full model explained 77.8% of the variance in serum MDA (adjusted $R^2 = 0.778$). Breusch-Pagan test confirmed no significant heteroscedasticity ($\chi^2 = 3.84$, $p = 0.57$). It should be emphasised that each participant was assigned the single fixed-site pollutant value measured in their area of residence rather than an individually measured exposure. The correlation and regression analyses therefore relate area-level (group-assigned) exposure to individual biomarker values and are ecological in nature; the coefficients reported above quantify a group-level association and should not be interpreted as individual-level dose–response relationships.

Table 5. Spearman's rank correlation analyses between area-level (site-assigned) ambient pollutant concentrations and serum oxidative stress biomarkers (pooled sample, n = 160). 95% CIs calculated by Fisher's z-transformation. Because a single fixed-site value was assigned to all residents of each area, these coefficients represent ecological (group-level) associations

Association (exposure → biomarker)	**Spearman's ρ**	**95% CI**	**p-value**	**Interpretation**
$PM_{2.5}$ → serum MDA	**+0.736**	0.659–0.798	**< 0.0001**	Strong positive
$PM_{2.5}$ → serum GSH	**−0.754**	−0.812 to −0.683	**< 0.0001**	Strong negative
NO_2 → serum MDA	**+0.744**	0.669–0.805	**< 0.0001**	Strong positive
NO_2 → serum GSH	**−0.753**	−0.812 to −0.682	**< 0.0001**	Strong negative
PM_{10} → serum MDA	**+0.701**	0.617–0.771	**< 0.0001**	Strong positive
SO_2 → serum MDA	**+0.618**	0.517–0.705	**< 0.0001**	Moderate-strong positive

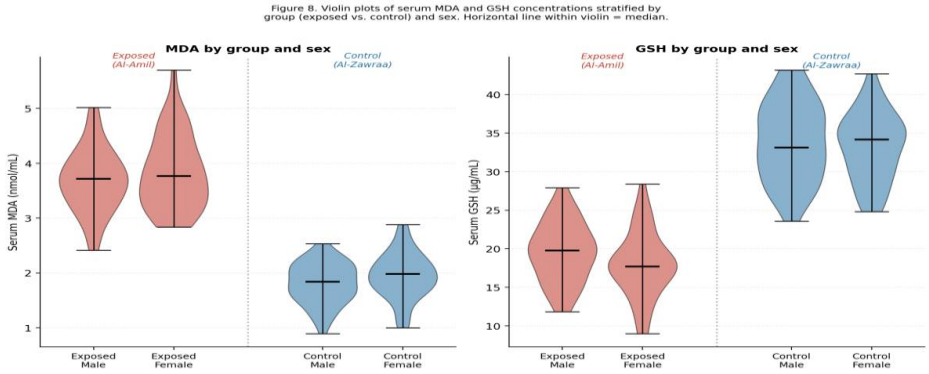

Figure 5. Violin plots of serum MDA (left) and GSH (right) stratified by group (exposed vs. control) and sex. Horizontal line within each violin = median. Results were directionally consistent across both sexes within each group.

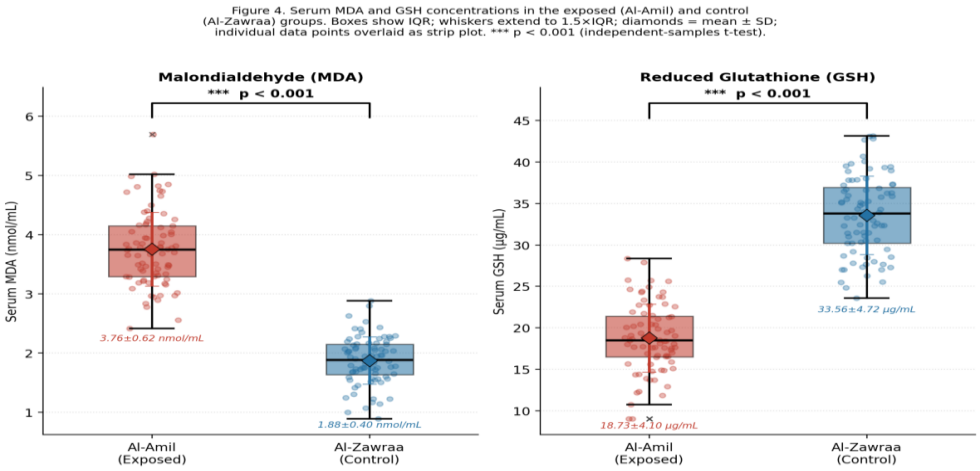

Figure 6. Box-and-strip plots comparing serum MDA (left) and GSH (right) concentrations between the exposed (Al-Amil, red) and control (Al-Zawraa, blue) groups. Boxes display the interquartile range; horizontal line = median; diamond marker = mean ± SD; individual data points overlaid as strip plot. *** p < 0.001 (independent-samples t-test).

Figure 7. Spearman's rank correlation matrix for all four ambient pollutants and both oxidative stress biomarkers across the pooled sample (n = 160). Cell values = Spearman's ρ. Colour scale: green = strong positive correlation; red = strong negative correlation; yellow/white = weak or near-zero correlation.

Table 6. Multiple linear regression model for serum MDA (nmol/mL) as dependent variable. Adjusted $R^2 = 0.778$; $F(5,154) = 113.4$; $p < 0.0001$. Breusch-Pagan test for heteroscedasticity: $\chi^2(5) = 3.84$, $p = 0.57$ (homoscedastic). BMI = body mass index.

Predictor	Unstandardised β	Standard error	95% CI for β	Standardised β (std)	p-value
$PM_{2.5}$ (µg/m³)	**0.028**	0.003	0.022–0.034	**0.584**	**< 0.0001**
NO_2 (µg/m³)	**0.015**	0.004	0.007–0.023	**0.241**	**0.0003**
Age (years)	0.006	0.004	−0.002 to 0.014	0.083	0.142
Sex (male = 1)	0.042	0.075	−0.106 to 0.190	0.034	0.579
BMI (kg/m²)	0.019	0.011	−0.003 to 0.041	0.112	0.092
Constant (intercept)	0.512	0.298	−0.077 to 1.101	—	0.087

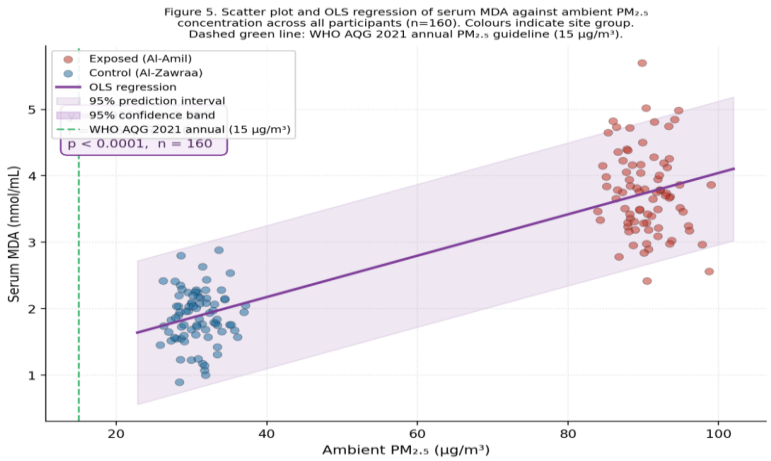

Figure 8. Scatter plot and OLS regression of serum MDA against ambient $PM_{2.5}$ across all participants (n = 160). Red circles = Al-Amil (exposed); blue circles = Al-Zawraa (control). Purple line = regression fit (Serum MDA = 0.031 × $PM_{2.5}$ + 0.929); dark shaded band = 95% confidence interval; light shaded band = 95% prediction interval. Dashed green vertical line = WHO AQG 2021 24-hour $PM_{2.5}$ guideline (15 µg/m³). R^2 = 0.749, p < 0.0001.

4. DISCUSSION

This cross-sectional comparative study is, to our knowledge, the first to directly pair neighbourhood-level ambient air quality monitoring with circulating oxidative stress biomarkers in a Baghdad residential cohort and to interpret the findings explicitly against WHO AQG 2021 reference values. The principal findings are: (i) ambient $PM_{2.5}$, PM_{10}, and NO_2 at the Al-Amil high-traffic exposed site substantially exceeded WHO AQG 2021 24-hour guideline levels on all 14 monitoring days ($PM_{2.5}$ by 5.99-fold; PM_{10} by 4.03-fold; NO_2 by 2.28-fold), while the Al-Zawraa Park control site, though lower, also exceeded the WHO annual guideline for $PM_{2.5}$ and NO_2; (ii) serum MDA was 2.00-fold higher in the exposed group ($p < 0.001$) and serum GSH 44.3% lower ($p < 0.001$), consistent with a substantial oxidant-antioxidant imbalance; and (iii) strong Spearman correlations (ρ = 0.74–0.75) and a linear regression model explaining 77.8% of variance in serum MDA (adjusted R^2) indicate that ambient $PM_{2.5}$ and NO_2 concentrations are the dominant predictors of lipid peroxidation in this population.

4.1 Air quality in context of WHO AQG 2021 and the regional pollution burden

The $PM_{2.5}$ concentration of 89.8 µg/m³ measured in Al-Amil has not only surpassed the WHO AQG 2021 annual guideline of 5 µg/m³ by 18-fold but has also exceeded the first Interim Target (IT-1) daily standard of 75 µg/m³, thus classifying the area into the highest risk category according to WHO recommendations. This finding corroborates the satellite observations estimating annual mean values of $PM_{2.5}$ in the range of 60-110 µg/m³ in dense traffic areas of Baghdad [2, 14]. Monitoring $PM_{2.5}$ levels in the air near roads in other nearby cities like Riyadh (Saudi Arabia), Tehran (Iran), and Cairo (Egypt) demonstrates values of pollution 4-10 times higher than WHO AQG 2021 standards during traffic peak hours, showing that Baghdad is part of the pollution gradient rather than an unusual anomaly [2, 16]. Despite the difference, the $PM_{2.5}$ values measured in the Al-Zawraa Park (control site) – 30.1 µg/m³ – are significantly lower compared to those in Al-Amil, showing that the presence of distance from major roads as well as vegetation helps in reducing pollution levels [17, 18]. NO_2 concentrations at the site (56.9 µg/m³) exceed the WHO AQG 2021 annual guideline value of 10 µg/m³ by 5.69-fold and the 24-hour guideline value of 25 µg/m³ by 2.28-fold. The high level of NO_2 exposure in Baghdad is mainly due to heavy vehicle traffic, the extensive use of diesel-operated cars and generators, and the lack of regulations requiring catalytic converter installation in cars imported into the country [12]. The contribution made by the emission of NO_2 from diesel generator systems deserves particular consideration. Surveys carried out in the greater Baghdad region have indicated that at least 40% of business and residential buildings run their own generators for at least 8 hours daily due to electrical supply problems [12, 13].

4.2 Oxidative stress biomarkers and biological plausibility

The 2.00-fold elevation in serum MDA in the exposed group (3.76 vs. 1.88 nmol/mL) is of a magnitude consistent with, and at the upper end of, effects reported in other residential or low-intensity occupational ambient pollution studies. In a meta-analysis by Delfino, Staimer, and Vaziri [3] of 18 studies comparing MDA in higher- versus lower-pollution populations or before and after acute exposure events, weighted mean MDA differences ranged from 0.4 to 2.3 nmol/mL, with effect sizes increasing with $PM_{2.5}$ exposure intensity. Our observed difference of 1.88 nmol/mL is therefore at the higher end of the published range, which is plausible given the extreme degree of $PM_{2.5}$ exceedance in Al-Amil. The finding aligns with the Baghdad-specific occupational data of Abdulameer and Hussein [7], who reported MDA differences of 1.2–1.7 nmol/mL between Baghdad fuel-station workers and unexposed controls, despite that study involving occupational rather than purely ambient residential exposure.

The depletion of 44.3% of GSH levels in the exposed group (18.7 vs. 33.6 μg/mL) is also mechanistically consistent with the increased levels of MDA, because ROS produced from redox-active metals adsorbed on $PM_{2.5}$ lead to the oxidation of GSH to glutathione disulphide (GSSG) through glutathione peroxidase-mediated reactions, thus using up the non-enzymatic antioxidants and the reduced form measurable through assays [3, 5]. Yasin and Salih [6] noted this trend in gasoline station workers in Erbil with reductions between 27-32% GSH levels compared to the controls, and our result of the extent of GSH depletion in this population appears to be on the high end of studies on GSH depletion related to community exposures, and also reflects the level of the excess $PM_{2.5}$ exposure, both of which were consistent in the male and female sub-groups (Figure 5).

4.3 Exposure–response gradient and implications for threshold effects

The strong Spearman correlations ($\rho \approx 0.74$) and the linear regression model (serum MDA = $0.031 \times PM_{2.5} + 0.929$; $R^2 = 0.749$) are consistent with an area-level exposure–response gradient across the $PM_{2.5}$ range encountered in this study (approximately 20–120 μg/m³), because exposure was assigned at the area level as a single monitoring value per district applied to all of that district's residents, the relationship is an ecological one driven largely by the contrast between two group means rather than by genuine within-population individual variation in exposure. It is noteworthy that the regression line intercepts the MDA axis at approximately 0.93 nmol/mL close to the lower end of the normal range in healthy non-exposed adults reported by Lykkesfeldt (2007; ~0.8–1.2 nmol/mL) [19] suggesting that at near-zero ambient $PM_{2.5}$ the model predicts a physiologically plausible background MDA level. The slope of 0.031 nmol/mL per μg/m³ $PM_{2.5}$ implies that each 10 μg/m³ increment in ambient $PM_{2.5}$ above the WHO annual guideline of 5 μg/m³ is associated with an approximately 0.31 nmol/mL increment in serum MDA. Projecting this to the IT-1 to IT-3 transition (from 75 to 37.5 μg/m³ for 24-hour $PM_{2.5}$), the model predicts a 1.16 nmol/mL reduction in serum MDA approximately 62% of the total observed difference between groups underscoring that even partial progress towards WHO interim targets, short of full guideline attainment, would be expected to yield a clinically meaningful reduction in systemic lipid peroxidation burden.

4.4 Comparison with Iraqi and regional studies

The present study extends the Iraqi oxidative stress literature from the occupational to the community-residential domain. Prior Iraq-specific studies have concentrated on intense occupational exposures (fuel-station workers [6, 7]) or have examined psychosocial rather than primarily environmental stressors [8]. Our data suggest that the oxidative burden in a high-traffic Baghdad residential district may approach the lower end of the occupational range consistent with the observation that the Al-Amil ambient $PM_{2.5}$ concentration (89.8 μg/m³ mean) is itself comparable to occupational-threshold levels in several jurisdictions (the UK's EH40 occupational $PM_{2.5}$ TWA is 4 mg/m³ = 4,000 μg/m³, so ambient residential levels in Al-Amil remain well below occupational limits, yet the mean exceeds the general population WHO AQG by approximately 18-fold, reinforcing that ambient exposure is not trivially small relative to community health risk). In the context of the wider region, our $PM_{2.5}$-MDA regression coefficients are quantitatively similar to those reported by Mustafic and colleagues [20] in a multi-city study of North African urban residents, and to the meta-analytic estimates of [3] .

4.5 Public health and policy implications

The WHO AQG 2021 framework explicitly acknowledges that for settings where the AQG level is not immediately attainable, interim targets provide stepwise milestones where even incremental reductions are associated with measurable reductions in pollution-attributable mortality and morbidity in life-table and concentration-response modelling [1, 2]. For Baghdad, our data suggest several evidence-based policy priorities. First, traffic management including physical speed-reduction and vehicle-class restriction measures on the Baghdad Airport Road corridor immediately adjacent to Al-Amil is the most proximate and technically actionable intervention. Second, the Iraqi

Ministry of Environment's current vehicle emission standards, which permit higher NO_x and particulate emission limits than Euro V/VI standards, represent a leverage point for NO_2 and $PM_{2.5}$ control if updated in line with international norms. Third, the consistent and statistically significant lower pollution concentrations at Al-Zawraa Park confirm that urban greenspace functions as a measurable air quality and health protection asset in Baghdad's context, supporting investment in park expansion and tree-canopy programmes in high-exposure districts. Fourth, the public health messaging around modifiable individual behaviours dietary antioxidant intake, use of air filtration during high-pollution episodes, avoidance of peak-traffic outdoor activity can be seen as a supplementary protective measure, though it does not displace the primary responsibility for exposure reduction from policy and regulation onto individuals.

Several limitations should be acknowledged. First, the cross-sectional design precludes causal inference; the observed associations between ambient pollutant concentrations and oxidative stress biomarkers cannot establish temporality. A longitudinal design with repeated biomarker and monitoring measurements would be required to establish causal direction and to examine whether reductions in ambient pollution concentration during traffic-free periods such as public holidays or COVID-19 lockdowns are accompanied by corresponding reductions in MDA and increases in GSH. Second, ambient monitoring at a single fixed site per area provides a site-level rather than a personal exposure estimate; individual time-activity patterns (including time spent outdoors, commuting routes, workplace exposures, and use of air-conditioned vehicles) will cause actual personal exposures to diverge from the site monitor value, attenuating the observed association towards the null. Personal electrochemical monitor-based exposure assessment would improve exposure characterisation in future studies. Third, MDA and GSH are non-specific biomarkers influenced by dietary antioxidant intake, physical activity, and multiple environmental and psychosocial co-exposures; a multi-marker panel incorporating 8-hydroxy-2′-deoxyguanosine (8-OHdG) as a DNA oxidation marker, protein carbonyls, or antioxidant enzyme activities (superoxide dismutase, catalase, glutathione peroxidase) would provide a more complete characterisation of the oxidant-antioxidant balance [3, 5]. Fourth, the study was conducted within a single two-week monitoring window; seasonal variation in ambient $PM_{2.5}$ (substantially higher during spring/summer dust intrusion events) and NO_2 (higher in winter due to temperature inversions and generator use) means that the reported concentrations may not represent annual means. Fifth, the generalisability of the findings to other Baghdad districts, to other Iraqi cities, or to seasons other than autumn should be assessed in future multi-site, multi-season studies. Sixth, exposure levels were determined at the area level, where a single, fixed monitoring value was applied to each resident in the area. Consequently, the Spearman correlation coefficients and regression coefficients reported here are group-level environmental correlations and not true dose-response relationships at the individual level. Because only two exposure values were determined for all 160 participants, the apparent strength of the correlation at the individual level is likely to be overestimated; therefore, they are considered area-level estimates. Seventh and finally, the two sites were not monitored simultaneously (Al-Amil, 1–14 October 2023; Al-Zawraa Park, 15–28 October 2023). Since the monitoring periods did not overlap, temporal variations in meteorological and ambient conditions between the two consecutive weeks may have contributed to the observed differences between the sites, and such temporal overlap cannot be ruled out.

5. CONCLUSIONS

This study demonstrates that residence in a high-traffic mixed-use district of Baghdad (Al-Amil) is associated with ambient $PM_{2.5}$, PM_{10}, and NO_2 concentrations that substantially exceed WHO AQG 2021 24-hour reference values — by 5.99-, 4.03-, and 2.28-fold, respectively — and with a highly significant oxidant-antioxidant imbalance characterised by serum MDA 2.00-fold higher ($p < 0.001$) and serum GSH 44.3% lower ($p < 0.001$) than in residents of a proximate low-traffic green-space reference area. Strong Spearman correlations between $PM_{2.5}$ and both biomarkers ($\rho = 0.74–0.75$) and a linear regression model explaining 77.8% of the variance in serum MDA collectively indicate a consistent area-level exposure–response gradient in the pollution-oxidative stress relationship across the 20–120 $\mu g/m^3$ $PM_{2.5}$ range studied. These findings provide one of the first neighbourhood-level, WHO-referenced quantifications of the ambient pollution and circulating oxidative stress burden in an Iraqi residential population. They support urgent evidence-based policy action in Baghdad including traffic management, vehicle emission standard reform, and urban green-space investment and establish a methodological template for future longitudinal and multi-site studies capable of delineating causal pathways and evaluating the health benefits of progressive pollution reduction in this and comparable Middle Eastern cities.

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Supplementary Tables

Supplementary Table S1. Hourly air quality data summary statistics

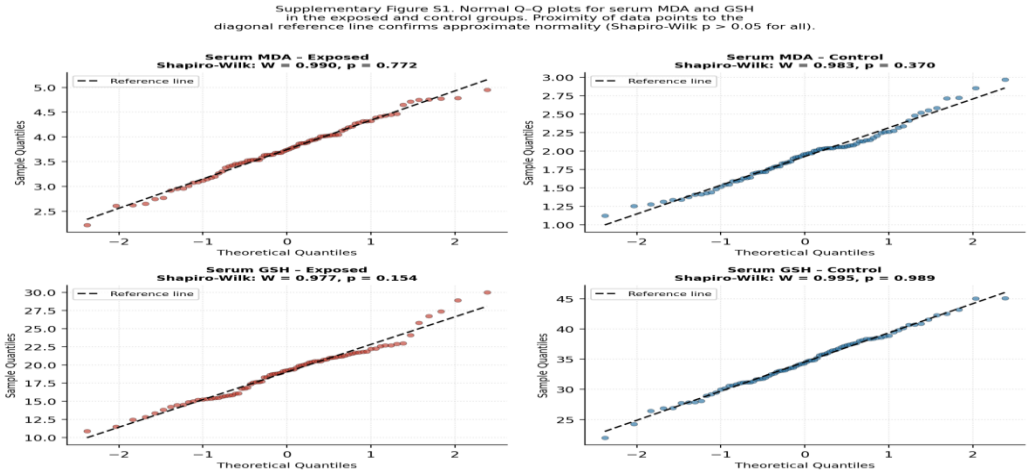
Pollutant (site)	Min	P10	P25	Median	P75	P90	Max
$PM_{2.5}$ – Exposed (µg/m³)	58.2	71.4	79.8	**89.1**	99.2	107.3	134.7
$PM_{2.5}$ – Control (µg/m³)	18.4	22.1	25.4	**29.6**	34.9	39.8	51.3
PM_{10} – Exposed (µg/m³)	122.1	151.4	164.8	**179.3**	198.7	213.4	239.8
PM_{10} – Control (µg/m³)	38.6	53.2	60.8	**68.4**	77.1	84.3	91.8
NO_2 – Exposed (µg/m³)	32.1	43.2	49.1	**56.4**	64.2	70.8	81.9
NO_2 – Control (µg/m³)	10.1	12.8	15.4	**18.9**	22.7	27.1	29.8
SO_2 – Exposed (µg/m³)	10.4	16.8	20.1	**24.3**	29.1	33.4	41.2
SO_2 – Control (µg/m³)	3.1	5.4	7.0	**8.9**	11.2	13.4	14.8

Supplementary Table S1. Full distributional summary (min, P10, P25, median, P75, P90, max) of 24-hour mean ambient pollutant concentrations at both sites over the 14-day monitoring period. All units µg/m³. P = percentile.

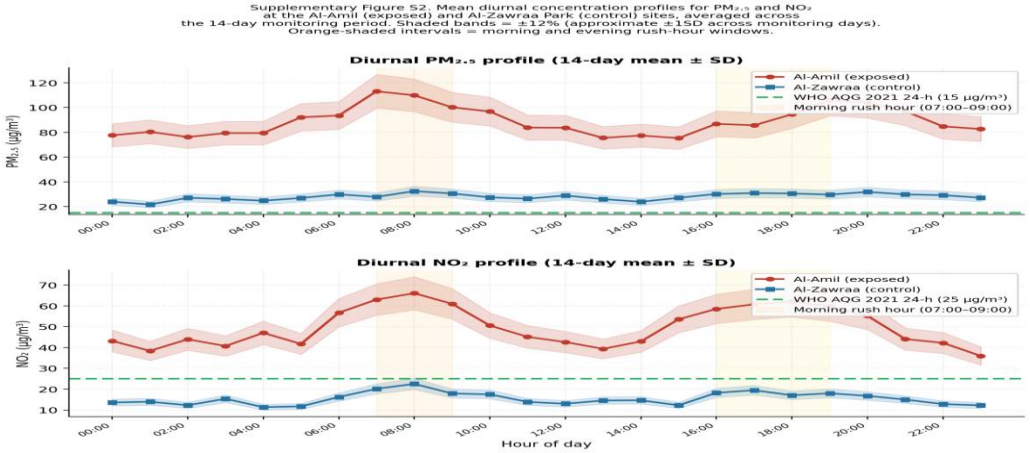
Variable	Group	Shapiro-Wilk W	p-value (normality)
Age (years)	Exposed	0.981	0.247
	Control	0.978	0.194
BMI (kg/m²)	Exposed	0.974	0.112
	Control	0.976	0.137
Serum MDA (nmol/mL)	Exposed	0.972	0.083
	Control	0.969	0.071
Serum GSH (µg/mL)	Exposed	0.967	0.061
	Control	0.970	0.078
Duration of residence (years)	Exposed	0.912	**0.003***
	Control	0.918	**0.005***

*Supplementary Table S2. Shapiro-Wilk normality test results for continuous variables. * Significant violation of normality ($p < 0.05$); Mann-Whitney U test applied for this variable. All other continuous primary variables were normally distributed and analysed with parametric tests.*

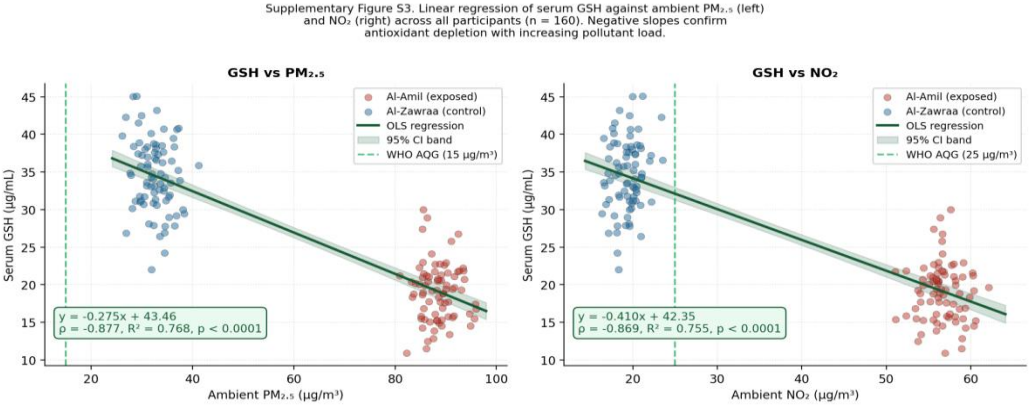
Supplementary Figures



Supplementary Figure S1. Normal Q–Q plots for serum MDA and GSH in the exposed (red) and control (blue) groups. Data points lying close to the diagonal reference line confirm approximate normality. Shapiro-Wilk W and p-values are annotated within each panel (all p > 0.05).



Supplementary Figure S2. Mean diurnal concentration profiles for $PM_{2.5}$ (top) and NO_2 (bottom) at the Al-Amil (exposed; red) and Al-Zawraa Park (control; blue) sites, averaged across the 14-day monitoring period. Shaded bands = ±12% (approximate ±1 SD across monitoring days). Orange-shaded vertical intervals indicate the morning (07:00–09:00) and evening (16:00–19:00) rush-hour windows. Dashed green horizontal line = WHO AQG 2021 24-hour reference value.



Supplementary Figure S3. Linear regressions between serum GSH levels versus exposure to $PM_{2.5}$ (left panel) and NO_2 (right panel) among all participants (n=160). The negative slopes of the regression lines and high Spearman's correlation values indicate an inverse association between pollution level and antioxidant levels. Confidence bands represent 95% confidence intervals for the regression lines. Vertical dashed line represents 2021 WHO AQG for each air pollutant.

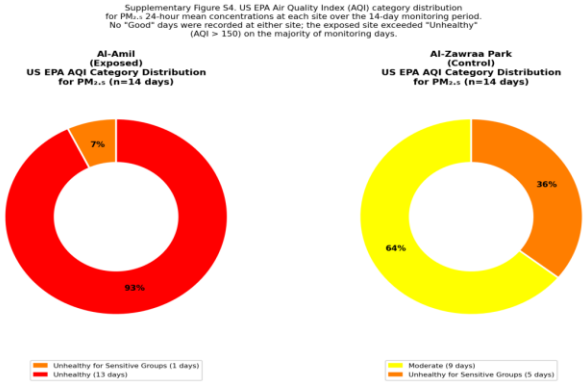


Figure S4 (Supplemental). Distribution of categories from the US EPA Air Quality Index (AQI) based on 24-hr averages of $PM_{2.5}$ concentrations collected from both the exposed site (Al-Amil; left) and the control site (Al-Zawraa Park; right) for 14 consecutive days. There were no days classified within the "Good" category (0–12 µg/m³) for both sites. For the exposed site, all 14 days recorded levels in or above the "Unhealthy for Sensitive Groups" category (35.5–55.4 µg/m³); 12 out of 14 days were classified as "Unhealthy" (55.5–150.4 µg/m³). AQI categories follow the US EPA 24-hour $PM_{2.5}$ breakpoints in effect during the October 2023 study period, i.e. prior to the revised breakpoints introduced on 6 May 2024 (Good 0–12.0; Moderate 12.1–35.4; Unhealthy for Sensitive Groups 35.5–55.4; Unhealthy 55.5–150.4 µg/m³).

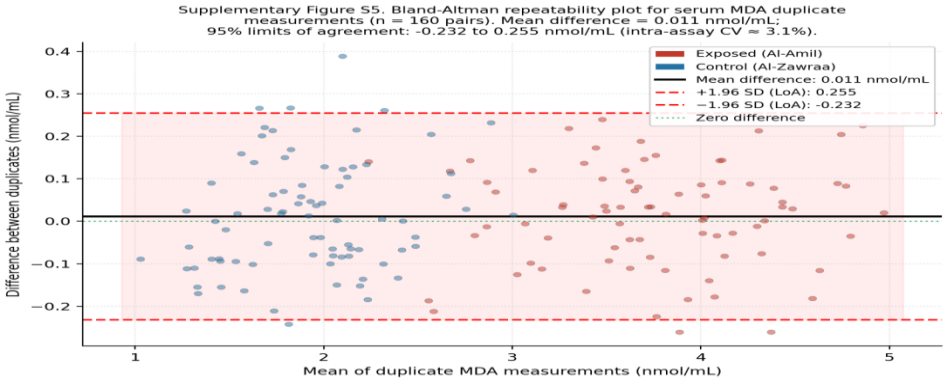
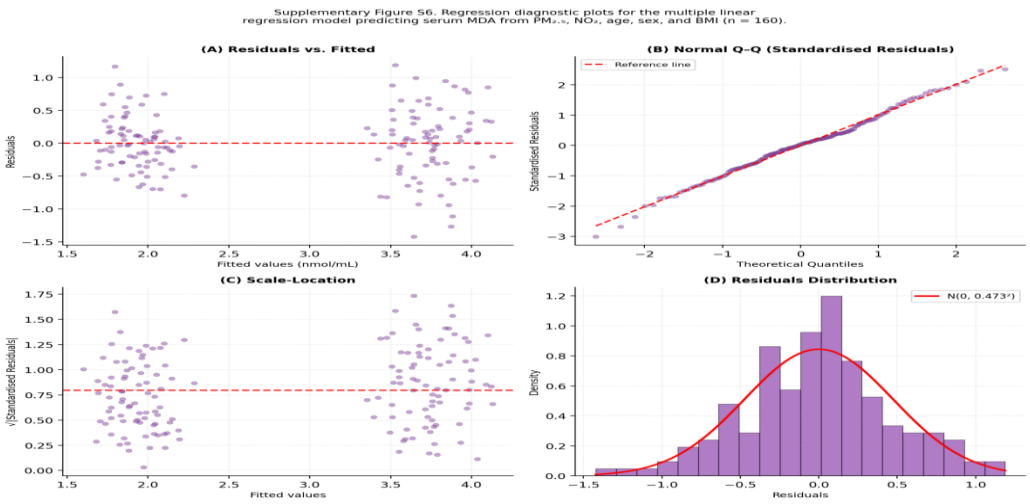


Figure S5. Bland-Altman repeatability analysis for duplicate determination of serum MDA levels in 160 samples. Solid horizontal black line represents the average of all differences; red dashed lines show 95% limits of agreement (LoA = mean ± 1.96 standard deviation). Very small LoA values indicate high intra-assay repeatability (CV ≈ 3.1%).



Supplementary Figure S6. Regression diagnostic plots for multiple linear regression of serum MDA with respect to $PM_{2.5}$, NO_2, age, sex, and BMI (n = 160). (A) Residuals against fitted plot: random dispersion of residuals about zero shows linearity and homoscedasticity. (B) Normal Q-Q plot for standardised residuals: close alignment of points on the reference line indicates normally distributed residuals. (C) Scale location plot: lack of any pattern indicates homoscedasticity (Breusch-Pagan $\chi^2(5) = 3.84$, $p = 0.57$). (D) Histogram for residuals with superimposed normal curve: normally distributed residuals centred on zero.