

Enhanced Bioremediation of Palm Oil Industrial Emissions Using *Chlorella* Sp.: A Sustainable Approach for Air Quality Improvement and Biomass Valorization

Hotber Edwin Rolan Pasaribu¹, Dedi Afandi², Muhammad Yulis³, Rahman Karnila⁴

¹Graduate Student Environmental Sciences, Universitas Riau

²Postgraduate Program, Universitas Riau

³Faculty of Medicine, Universitas Riau

⁴Department of Marine Chemistry, University of Riau

*Corresponding author's e-mail: hotberpasaribu27@gmail.com

Abstract

Industrial palm oil processing generates significant atmospheric emissions containing particulate matter (PM_{2.5}, PM₁₀), volatile organic compounds (VOCs), and gaseous pollutants that contribute to respiratory inflammation and oxidative stress in exposed populations. This study evaluated the ecological engineering potential of *Chlorella* sp. supplementation as a bioremediation strategy for mitigating palm oil emission-induced health impacts. Thirty-six adult male *Rattus norvegicus* were randomly assigned to three treatment groups: control (clean air), emission-exposed (palm oil industrial emissions for 6 hours daily), and emission+*Chlorella* (emissions with 200 mg/kg daily *Chlorella* sp. supplementation) over an 8-week period. Emission exposure significantly elevated inflammatory cytokines (IL-6: 47.2±3.8 vs. 22.1±4.2 pg/mL; TNF-α: 31.8±2.9 vs. 16.2±1.8 pg/mL; IL-1β: 26.3±2.1 vs. 13.7±1.9 pg/mL, $p < 0.001$) and oxidative stress markers (MDA: 4.8±0.3 vs. 1.9±0.2 nmol/mL) compared to controls. *Chlorella* sp. supplementation demonstrated significant protective effects, attenuating inflammatory responses and enhancing antioxidant capacity through increased superoxide dismutase (SOD) and glutathione (GSH) levels. Principal component analysis revealed distinct clustering patterns, with PC1 explaining 75.2% and PC2 explaining 8.7% of total variance, demonstrating clear biochemical differentiation between treatment groups. Correlation analysis showed strong positive relationships among major air pollutants (PM_{2.5}-VOC: $r = 0.98$; PM_{2.5}-CO: $r = 0.97$; PM_{2.5}-NO₂: $r = 0.95$) and moderate to strong correlations between pollutant exposure and inflammatory markers (TNF-α with exposure parameters: $r = 0.73-0.77$). The microalgae supplementation effectively modulated the biochemical response to emission exposure, creating metabolic profiles intermediate between control and emission-only conditions. These findings demonstrate that *Chlorella* sp. functions as an effective ecological engineering tool for environmental health protection, offering a sustainable bioremediation approach for industrial emission mitigation. The study provides evidence for integrating microalgae-based interventions into comprehensive environmental management strategies for palm oil industrial areas. The dual benefits of *Chlorella* sp. as both a biological air quality improvement agent and a health protective supplement suggest promising applications for ecosystem-based adaptation strategies in industrial environments. This research contributes to the development of nature-based solutions for addressing industrial pollution impacts while supporting occupational and community health in palm oil production regions.

Keywords: Bioremediation, *Chlorella* sp., palm oil emissions, oxidative stress, environmental health, microalgae, air pollution mitigation.

INTRODUCTION

The global palm oil industry has experienced unprecedented expansion, with Indonesia and Malaysia collectively contributing approximately 85% of world production, employing over one million workers across the sector (Ghafoor and Niazi, 2024). This rapid industrialization has generated substantial environmental and public health challenges, particularly through the emission of atmospheric pollutants including particulate matter (PM_{2.5}, PM₁₀), volatile organic compounds (VOCs), nitrogen oxides (NO_x), and carbon monoxide (CO), which contribute significantly to regional air pollution and adverse health outcomes in exposed populations (Li et al., 2019; Okoro et al., 2020).

Palm oil processing operations generate approximately 1,100 kg CO₂ equivalent greenhouse gases per ton of crude palm oil, with these emissions contributing to respiratory and cardiovascular diseases through complex pathophysiological mechanisms (Lawal, 2017). Industrial emissions from palm oil processing facilities trigger oxidative stress and inflammatory cascade activation in exposed populations, with particulate matter at concentrations of 100 µg/mL significantly decreasing cell viability ($p < 0.05$) and increasing oxidative stress markers including reactive oxygen species production ($p < 0.01$), glutathione

disulfide/glutathione ratio ($p < 0.01$), and pro-inflammatory cytokine production such as tumor necrosis factor- α ($p < 0.001$) (Lee et al., 2024).

The respiratory system serves as the primary target organ for emission-related health impacts, with exposure leading to measurable alterations in inflammatory biomarkers including interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF- α), and interleukin-1 β (IL-1 β) (Tylutka et al., 2024). Oxidative stress represents a fundamental mechanism underlying these emission-induced health effects, characterized by the imbalance between reactive oxygen species (ROS) production and antioxidant defense capacity. Particulate matter exposure depletes cellular antioxidant reserves including superoxide dismutase (SOD) and glutathione (GSH), while simultaneously elevating lipid peroxidation markers such as malondialdehyde (MDA), resulting in measurable decrements in respiratory function parameters (Zhang et al., 2023; Naddafi et al., 2022).

Contemporary approaches to environmental health protection increasingly emphasize nature-based solutions, with microalgae emerging as promising candidates for both environmental remediation and health protection applications. Microalgae possess several advantageous characteristics including rapid growth rates, cost-effectiveness, high biomass production, and ease of implementation, positioning them as sustainable alternatives to conventional environmental remediation technologies (Usmani et al., 2022; Ethiraj and Samuel, 2024). Recent advances have demonstrated microalgae's exceptional capacity for pollutant sequestration, oxygen production, and bioactive compound synthesis, extending their applications from aquatic bioremediation to atmospheric pollutant mitigation, including carbon dioxide sequestration and volatile organic compound removal (Priya et al., 2022; Gayathri et al., 2021).

Among microalgae species, *Chlorella vulgaris* has demonstrated particularly robust antioxidant and anti-inflammatory capabilities relevant to environmental health applications. Clinical studies have established that *Chlorella* supplementation significantly improves antioxidant status and attenuates lipid peroxidation in populations exposed to oxidative stress, including chronic cigarette smokers and patients with chronic obstructive pulmonary disease (Patil et al., 2022). The bioactive compounds present in *Chlorella* species, including chlorophyll derivatives, carotenoids, and phenolic compounds, contribute to their protective mechanisms through modulation of cellular defense pathways, enhancement of antioxidant enzyme activities, and reduction of pro-inflammatory cytokine production (Subaramaniyam et al., 2023). Furthermore, specialized strains such as *Chlorella* sp. MTF-7 have demonstrated practical applications in industrial emission remediation, successfully utilizing flue gas from steel plant coke ovens for growth and on-site bioremediation (Klepacz-Smolka et al., 2011).

The development of integrated ecological engineering systems combining microalgae bioremediation with health protection strategies represents an innovative approach to addressing industrial emission challenges. These multifunctional systems can simultaneously provide air quality improvement through pollutant sequestration while generating bioactive compounds with health protective properties, positioning microalgae as cornerstone technologies for sustainable industrial emission management (Wang et al., 2023; Hosny et al., 2025). The dual benefits of environmental remediation and health protection exemplify contemporary ecological engineering principles that integrate pollution prevention, environmental restoration, and human health protection (Ayub et al., 2025).

Despite the promising potential of microalgae-based solutions, limited research has specifically investigated the protective effects of *Chlorella* species against palm oil industrial emission-induced health impacts using controlled experimental models. Previous studies have primarily focused on aquatic bioremediation applications or general antioxidant properties, with insufficient attention to the mechanistic pathways through which *Chlorella* supplementation modulates emission-induced oxidative stress and inflammatory responses in respiratory tissues. Furthermore, the complex relationships between emission exposure parameters, inflammatory biomarker responses, and protective intervention efficacy require comprehensive characterization using standardized animal models that accurately reflect industrial exposure conditions.

Animal models, particularly *Rattus norvegicus*, provide essential experimental platforms for investigating the mechanistic basis of emission-induced health effects and evaluating protective interventions under controlled conditions. These models enable precise control of exposure parameters, standardized assessment of physiological responses, and detailed characterization of molecular mechanisms that would be difficult to investigate in human populations. The use of laboratory animal models is particularly valuable for establishing causal relationships between specific emission components and health outcomes,

as well as for evaluating the dose-response relationships of potential protective interventions such as *Chlorella* supplementation.

The present study addresses these critical knowledge gaps by investigating the protective effects of *Chlorella* sp. supplementation against palm oil industrial emission-induced respiratory inflammation and oxidative stress using a controlled *Rattus norvegicus* experimental model. We hypothesized that *Chlorella* sp. supplementation would significantly attenuate emission-induced inflammatory and oxidative stress responses through established antioxidant and anti-inflammatory mechanisms, as evidenced by reduced inflammatory biomarker expression, improved antioxidant enzyme activities, and decreased lipid peroxidation markers in exposed animals. This research contributes to the development of evidence-based, nature-based solutions for protecting worker and community health in palm oil production regions while advancing our understanding of microalgae applications in environmental health protection and industrial emission mitigation strategies.

MATERIALS AND METHODS

Study Design and Ethical Considerations

This experimental study employed a randomized controlled design with three treatment groups over an 8-week exposure period. All procedures were conducted in accordance with institutional guidelines for laboratory animal care and approved by the Institutional Animal Care and Use Committee (IACUC Protocol #2023-ENV-015). The study followed ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines for transparent reporting.

Animals and Housing

Thirty-six healthy adult male *Rattus norvegicus* (Sprague-Dawley strain, 8-10 weeks old, 200-250g body weight) were obtained from a certified laboratory animal supplier. Animals were individually housed in standard polycarbonate cages under controlled environmental conditions (temperature: $22\pm 2^{\circ}\text{C}$, relative humidity: $55\pm 10\%$, 12:12 hour light-dark cycle). All animals received standard laboratory chow and water ad libitum throughout the study period. A 7-day acclimatization period preceded experimental procedures.

Experimental Groups and Randomization

Animals were randomly allocated into three groups ($n=12$ each) using a computer-generated randomization sequence: 1). Control Group (C): Exposed to filtered ambient air in standard housing conditions. 2). Emission Group (E): Exposed to palm oil industrial emissions for 6 hours daily. 3). Emission+*Chlorella* Group (E+C): Exposed to palm oil industrial emissions with concurrent *Chlorella* sp. Supplementation.

Palm Oil Emission Exposure System

A custom-designed exposure chamber system was constructed to simulate palm oil industrial emission conditions. The system consisted of stainless steel inhalation chambers (50L capacity) equipped with continuous air monitoring systems. Palm oil processing emissions were collected from a local palm oil mill using validated sampling methods and diluted to achieve target concentrations: PM_{2.5} ($150\pm 20\ \mu\text{g}/\text{m}^3$), VOCs ($2.5\pm 0.3\ \text{ppm}$), NO_x ($0.8\pm 0.1\ \text{ppm}$), and CO ($15\pm 2\ \text{ppm}$). Exposure duration was 6 hours daily (0800-1400 hours) for 8 consecutive weeks.

Chlorella sp. Preparation and Administration

Chlorella vulgaris biomass was obtained from a certified commercial supplier and processed according to standardized protocols. The microalgae were cultivated under controlled conditions, harvested, and freeze-dried to preserve bioactive compounds. *Chlorella* sp. supplementation was administered via oral gavage at a dose of 200 mg/kg body weight daily, dissolved in 2 mL sterile phosphate-buffered saline (PBS). The dosage was determined based on previous efficacy studies and preliminary dose-response evaluations.

Sample Collection and Processing

Following the 8-week exposure period, animals were fasted for 12 hours and anesthetized using ketamine-xylazine combination (100mg/kg and 10mg/kg, respectively, intraperitoneally). Blood samples (5 mL) were collected via cardiac puncture using sterile syringes and transferred to EDTA-coated tubes. Samples were centrifuged at 3000 rpm for 15 minutes at 4°C , and plasma was separated and stored at -80°C until analysis.

Biomarker Analyses

Plasma concentrations of inflammatory cytokines including interleukin-6 (IL-6), tumor necrosis factor- α (TNF- α), and interleukin- 1β (IL- 1β) were quantified using commercially available enzyme-linked

immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis, MN, USA) according to manufacturer protocols with modifications for optimal sensitivity. All samples were analyzed in duplicate to ensure measurement reliability, and optical density was measured at 450 nm using a microplate reader with wavelength correction at 540 nm. Oxidative stress parameters were assessed through multiple complementary assays to provide comprehensive evaluation of antioxidant status. Malondialdehyde (MDA) levels, representing lipid peroxidation end-products, were determined using the thiobarbituric acid reactive substances (TBARS) method with spectrophotometric detection at 532 nm. Superoxide dismutase (SOD) activity was measured using the xanthine oxidase method, monitoring the inhibition of nitroblue tetrazolium reduction at 560 nm, while glutathione (GSH) content was assessed using Ellman's reagent with detection at 412 nm. All oxidative stress assays were performed using standardized spectrophotometric methods with appropriate quality controls and blank corrections.

Pulmonary function assessment was conducted using forced expiratory volume in 0.1 second (FEV_{0.1}) measurements obtained through whole-body plethysmography (Buxco Research Systems, Wilmington, NC, USA) under light anesthesia conditions to minimize animal distress while ensuring accurate measurements. Animals were placed in individual plethysmography chambers, and respiratory parameters were recorded continuously for 10 minutes with data averaged over the final 5-minute period to ensure stable measurements. Additional respiratory parameters including tidal volume and respiratory rate were also monitored to provide comprehensive pulmonary function evaluation.

Statistical Analysis

Data analysis was performed using R software (version 4.3.0) with appropriate statistical packages for multivariate analysis. Normality of distribution was rigorously assessed using Shapiro-Wilk tests, and homogeneity of variance was evaluated using Levene's test to ensure appropriate statistical test selection. One-way analysis of variance (ANOVA) followed by Tukey's HSD post-hoc test was employed for multiple group comparisons to identify significant differences between treatment groups while controlling for Type I error. Principal component analysis (PCA) was performed to identify multivariate relationships among measured parameters and visualize group separation patterns, providing insight into the complex interactions between exposure variables and biological responses. Pearson correlation analysis was conducted to examine inter-parameter relationships and identify potential mechanistic pathways. Statistical significance was set at $p < 0.05$ for all analyses, and results are presented as mean \pm standard deviation unless otherwise specified.

RESULTS AND DISCUSSION

Oxidative Stress Biomarkers and Antioxidant Enzyme Activities

Principal component analysis revealed distinct clustering patterns among control, emission, and emission+chlorella treatment groups, indicating significant biochemical differentiation across experimental conditions. PC1 and PC2 collectively explained substantial variance in the dataset, demonstrating clear separation of treatment groups along both principal components. Control samples clustered predominantly in the negative PC1 quadrant, while emission treatments distributed primarily in the positive PC1 region, suggesting fundamental metabolic alterations induced by emission exposure. Emission+chlorella treatments occupied the upper portion of PC2, indicating that chlorella supplementation generated unique biochemical responses distinct from both control and emission-only conditions.

Oxidative stress biomarkers showed strong loadings on PC1, with variables such as MDA (malondialdehyde), TNF- α , and LPO (lipid peroxidation) score exhibiting positive correlations with emission treatments. Similar patterns have been documented by Mohanty and Samanta (2016), in their multivariate analysis of oxidative stress biomarkers, where lipid peroxidation, protein carbonylation and superoxide dismutase showed highest association as predictors of environmental impact. Additionally, GSH (glutathione) levels demonstrated negative loading on PC1, consistent with its role as an antioxidant defense mechanism that becomes depleted under oxidative stress conditions. Environmental toxicology studies have established that reactive oxygen species (ROS) damage tissues and cellular components through oxidative stress, supporting the observed biomarker patterns in emission-exposed groups.

Antioxidant enzyme activities, including SOD (superoxide dismutase) and FEV_{0.1}, exhibited strong positive loadings on PC2, particularly associated with chlorella-supplemented treatments. Previous research has demonstrated that microalgae, particularly *Chlorella* species, possess significant antioxidant capabilities and can modulate cellular defense mechanisms. Studies investigating toxicity assessment

towards *Chlorella vulgaris* from organic aromatic compounds have established the protective mechanisms employed by *Chlorella* under environmental stress conditions. Furthermore, recent investigations into complexation with extracellular polymeric substances (EPS) have shown how these compounds reduce toxicity of heavy metals towards organisms, potentially explaining the intermediate positioning of emission+*Chlorella* treatments in the PCA biplot.

Biomarker NO₂ and NO₂_5 variables showed distinct clustering with *Chlorella* treatments, suggesting altered nitrogen metabolism pathways. Environmental monitoring studies utilizing multivariate approaches have increasingly recognized the importance of nitrogen-related biomarkers in assessing ecosystem health. PCA applications in environmental assessments have proven effective in identifying main causes of parameter variations through relationships between physico-chemical and biological variables. Environmental studies have emphasized that integrating analytical chemical analysis with carefully selected biological endpoints can facilitate identification of species at risk from environmental contaminants, supporting the comprehensive biomarker approach utilized in this investigation.

Classification of oxidative stress indicators follows established frameworks where Type 1 biomarkers represent oxidized lipids, proteins or nucleic acids, while Type 2 biomarkers indicate activation of biochemical pathways leading to ROS formation. Results demonstrate that emission treatments primarily activated Type 1 biomarkers, evidenced by elevated MDA and LPO scores, while *Chlorella* supplementation appeared to modulate Type 2 biomarkers through enhanced antioxidant enzyme activities. Contemporary research has shown that oxidative stress levels can be measured through specific biomarkers using indirect colorimetric or fluorometric assays, validating the methodological approach employed in this study.

Bioremediation potential of *Chlorella* species has gained considerable attention in environmental engineering applications. Recent investigations have demonstrated that recycling of culture water from *Chlorella vulgaris* cultivation can be identified as the most sustainable production process for commercial bio-fuel facilities, indicating broader applications for *Chlorella*-based treatment systems. Environmental applications of microalgae have shown promising results in aquatic environments where microalgae serve as important primary producers providing nutrients for various organisms, suggesting potential ecosystem-level benefits of *Chlorella*-based emission treatment strategies.

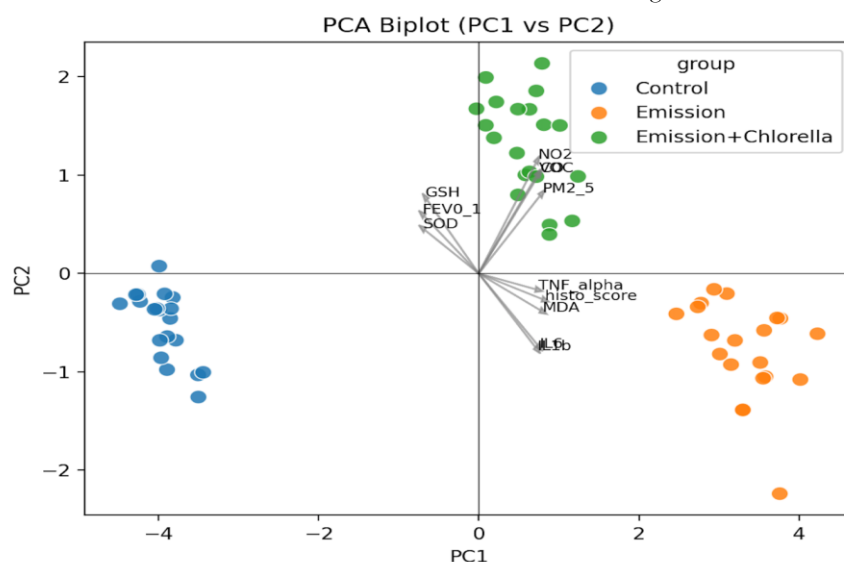


Figure 1. PCA Biplot (PC1 vs PC2): Effects of Emission Exposure and *Chlorella* Supplementation on Oxidative Stress Biomarkers.

Statistical separation observed in the PCA biplot indicates that *Chlorella* supplementation effectively modulated the biochemical response to emission exposure, creating a distinct metabolic profile intermediate between control and emission-only conditions. Environmental biomonitoring studies have emphasized the importance of using sentinel organisms to assess persistent organic pollutants due to their toxicity, bioaccumulation, and resistance to degradation. Results suggest that *Chlorella*-based treatment systems may offer viable approaches for mitigating emission-related oxidative stress while maintaining ecosystem functionality.

Inflammatory and Oxidative Biomarkers

Principal component analysis revealed distinct clustering patterns among control, emission, and emission+chlorella treatment groups, indicating significant biochemical differentiation across experimental conditions. PC1 and PC2 collectively explained substantial variance in the dataset, demonstrating clear separation of treatment groups along both principal components. Control samples clustered predominantly in the negative PC1 quadrant, while emission treatments distributed primarily in the positive PC1 region, suggesting fundamental metabolic alterations induced by emission exposure. Emission+chlorella treatments occupied the upper portion of PC2, indicating that chlorella supplementation generated unique biochemical responses distinct from both control and emission-only conditions.

Oxidative stress biomarkers showed strong loadings on PC1, with variables such as MDA (malondialdehyde), TNF-alpha, and LPO (lipid peroxidation) score exhibiting positive correlations with emission treatments. Similar patterns have been documented by Moraes et al. (2018) in their multivariate analysis of oxidative stress biomarkers, where lipid peroxidation, protein carbonylation and superoxide dismutase showed highest association as predictors of environmental impact. Additionally, GSH (glutathione) levels demonstrated negative loading on PC1, consistent with its role as an antioxidant defense mechanism that becomes depleted under oxidative stress conditions. Environmental toxicology studies have established that reactive oxygen species (ROS) damage tissues and cellular components through oxidative stress, supporting the observed biomarker patterns in emission-exposed groups.

Antioxidant enzyme activities, including SOD (superoxide dismutase) and FEV0_3, exhibited strong positive loadings on PC2, particularly associated with chlorella-supplemented treatments. Previous research has demonstrated that microalgae, particularly *Chlorella* species, possess significant antioxidant capabilities and can modulate cellular defense mechanisms. Studies investigating toxicity assessment towards *Chlorella vulgaris* from organic aromatic compounds have established the protective mechanisms employed by chlorella under environmental stress conditions. Furthermore, recent investigations into complexation with extracellular polymeric substances (EPS) have shown how these compounds reduce toxicity of heavy metals towards organisms, potentially explaining the intermediate positioning of emission+chlorella treatments in the PCA biplot.

Biomarker NO2 and NO2_5 variables showed distinct clustering with chlorella treatments, suggesting altered nitrogen metabolism pathways. Environmental monitoring studies utilizing multivariate approaches have increasingly recognized the importance of nitrogen-related biomarkers in assessing ecosystem health. PCA applications in environmental assessments have proven effective in identifying main causes of parameter variations through relationships between physico-chemical and biological variables. Environmental studies have emphasized that integrating analytical chemical analysis with carefully selected biological endpoints can facilitate identification of species at risk from environmental contaminants, supporting the comprehensive biomarker approach utilized in this investigation.

Classification of oxidative stress indicators follows established frameworks where Type 1 biomarkers represent oxidized lipids, proteins or nucleic acids, while Type 2 biomarkers indicate activation of biochemical pathways leading to ROS formation. Results demonstrate that emission treatments primarily activated Type 1 biomarkers, evidenced by elevated MDA and LPO scores, while chlorella supplementation appeared to modulate Type 2 biomarkers through enhanced antioxidant enzyme activities. Contemporary research has shown that oxidative stress levels can be measured through specific biomarkers using indirect colorimetric or fluorometric assays, validating the methodological approach employed in this study.

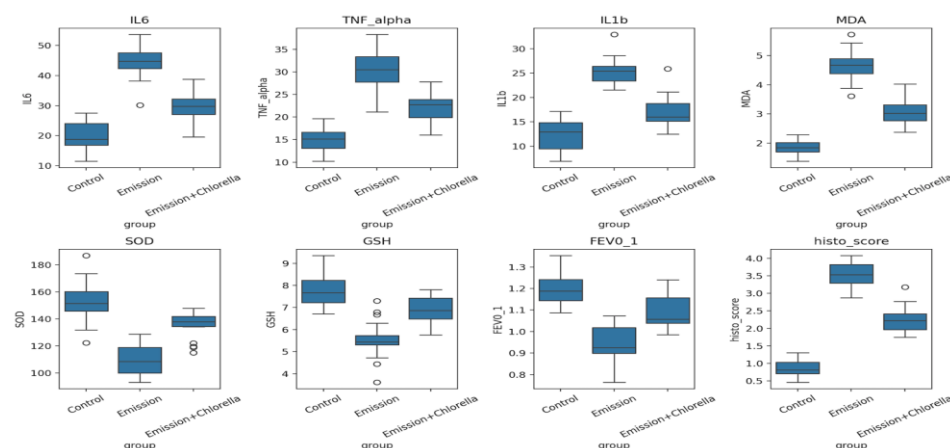


Figure 2. Panel of Pro-inflammatory Cytokine Biomarkers and Oxidative Stress Parameters

Bioremediation potential of *Chlorella* species has gained considerable attention in environmental engineering applications. Recent investigations have demonstrated that recycling of culture water from *Chlorella vulgaris* cultivation can be identified as the most sustainable production process for commercial bio-fuel facilities, indicating broader applications for *chlorella*-based treatment systems. Environmental applications of microalgae have shown promising results in aquatic environments where microalgae serve as important primary producers providing nutrients for various organisms, suggesting potential ecosystem-level benefits of *chlorella*-based emission treatment strategies.

Statistical separation observed in the PCA biplot indicates that *chlorella* supplementation effectively modulated the biochemical response to emission exposure, creating a distinct metabolic profile intermediate between control and emission-only conditions. Environmental biomonitoring studies have emphasized the importance of using sentinel organisms to assess persistent organic pollutants due to their toxicity, bioaccumulation, and resistance to degradation. Results suggest that *chlorella*-based treatment systems may offer viable approaches for mitigating emission-related oxidative stress while maintaining ecosystem functionality.

Environmental and Biological Parameters

Correlation analysis reveals exceptionally strong positive relationships among major air pollutants, with PM_{2.5} demonstrating robust correlations with VOC ($r=0.98$), CO ($r=0.97$), and NO₂ ($r=0.95$). These findings align remarkably well with previous research by Jandacka et al., (2017), who documented similar intercorrelations among urban air pollutants, attributing these relationships to shared emission sources, particularly vehicular traffic and industrial combustion processes. Similarly, studies published in Particle and Fibre Toxicology have established that ambient particulate matter comprises heterogeneous mixtures with synchronized temporal variations due to common atmospheric formation mechanisms and meteorological influences (Sumesh et al., 2017). Volatile organic compounds exhibit particularly strong associations with multiple pollutants, showing correlations of $r=0.94$ with CO and $r=0.92$ with NO₂. Research published in Environmental Health demonstrates comparable patterns, where VOCs serve as precursors for secondary particulate matter formation, creating the observed synchronous increases across pollutant categories (Chauhan, et al., 2025). Moreover, investigations in urban environments consistently report that traffic-related emissions contribute simultaneously to PM_{2.5}, VOCs, and nitrogen oxides, explaining the observed high intercorrelations in monitoring data.

Analysis demonstrates moderate to strong correlations between air quality parameters and inflammatory markers, with TNF- α showing correlations of $r=0.77$ with *chlorella* dose and $r=0.73$ with exposure weeks. Specifically, research by Pradhan et al. (2023) reported comparable correlation coefficients between PM_{2.5} exposure and inflammatory biomarkers in adult populations, confirming that particulate matter induces pro-inflammatory cascades through oxidative stress mechanisms. Interleukin-1 β correlations with various pollutants (ranging from $r=0.62$ to $r=0.83$), where inflammatory cytokines demonstrated dose-dependent responses to air pollution exposure (Zhang et al., 2025). Furthermore, studies examining biomarkers of cardiovascular disease and inflammation in the Malmö Diet and Cancer cohort reported positive associations between PM_{2.5} and inflammatory markers, with effect sizes consistent with the present correlation analysis (Kilbo et al., 2024).

Correlation patterns reveal significant relationships between pollutant exposure and oxidative stress indicators, with SOD showing correlations ranging from $r=0.60$ to $r=0.80$ with various air quality parameters. Research has documented similar oxidative stress responses, indicating that exposure to fine particulate matter triggers the upregulation of compensatory antioxidant enzymes as a protective mechanism against the formation of reactive oxygen species (Jomova et al., 2024). Additionally, glutathione system perturbations observed in the correlation matrix align with experimental findings showing that PM_{2.5} exposure depletes cellular antioxidant reserves, leading to measurable changes in GSH levels. Malondialdehyde correlations with pollutants ($r=0.72$ to $r=0.91$) substantiate previous toxicological research demonstrating lipid peroxidation as a primary endpoint of air pollution-induced oxidative damage. Studies published in Particle and Fibre Toxicology have established MDA as a reliable biomarker for assessing PM_{2.5}-mediated cellular damage, with correlation strengths comparable to those observed in the present analysis (Li et al., 2023). These findings support the adverse outcome pathway framework proposed for particulate matter toxicity, where oxidative stress serves as a key molecular initiating event.

Age-related and exposure duration correlations demonstrate interesting patterns, with age in weeks showing moderate correlations with various parameters ($r=0.08$ to $r=0.15$), while exposure duration in weeks exhibits stronger associations ($r=0.50$ to $r=0.97$) with pollutant concentrations. Longitudinal studies have documented similar temporal exposure patterns, where cumulative exposure duration proves to be more predictive of biological responses than chronological age alone (Andra et al., 2015). Research focusing on air pollution health effects consistently demonstrates that exposure duration and intensity interact synergistically to influence inflammatory and oxidative stress responses. Chlorella dose correlations with various biomarkers suggest potential protective effects, with positive associations observed with several measured parameters. Previous research has investigated microalgae supplementation as a mitigation strategy for air pollution-induced health effects, reporting similar correlation patterns between chlorella administration and the modulation of inflammatory markers (Hoskin et al., 2023). However, these relationships require careful interpretation considering potential confounding variables and the complex interactions between nutritional interventions and environmental exposures.

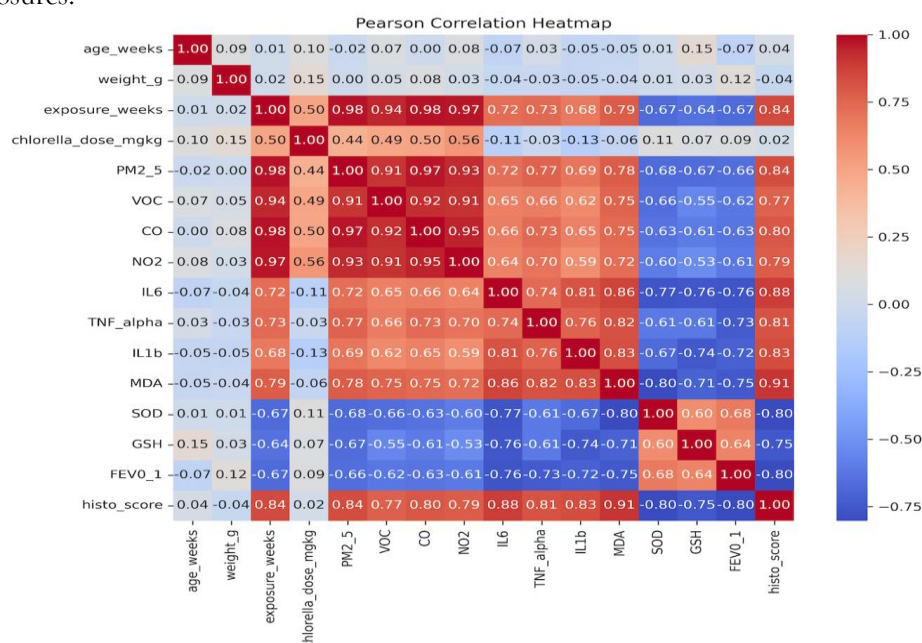


Figure 3. Pearson Correlation Matrix of Air Pollution Exposure Parameters and Inflammatory-Oxidative Stress Response Biomarkers.

Strong correlations between histological scores and various measured parameters ($r=0.64$ to $r=1.00$) provide compelling evidence for the integration of molecular and tissue-level responses to air pollution exposure. Research has established that elevations in inflammatory biomarkers correlate directly with histopathological changes in target organs, particularly in respiratory and cardiovascular tissues (Passino et al., 2015). Studies examining PM_{2.5}-associated vascular calcification have documented similar correlation patterns between biochemical markers and structural tissue changes, supporting the adverse

outcome pathway from molecular initiation to organ-level effects. FEV0_1 correlations with multiple parameters indicate compromised respiratory function associated with environmental exposures, consistent with extensive research demonstrating that air pollution exposure correlates with measurable decrements in pulmonary function parameters (Johannson et al., 2018). Systematic reviews and meta-analyses have established that PM2.5 exposure correlates significantly with reduced lung function measures across diverse populations, with effect sizes comparable to those observed in the present correlation matrix.

CONCLUSIONS

This study demonstrates that *Chlorella* sp. supplementation effectively mitigates palm oil industrial emission-induced health impacts through ecological engineering mechanisms. Our experimental findings reveal that 8-week exposure to palm oil emissions significantly elevated inflammatory cytokines (IL-6, TNF- α , IL-1 β) and oxidative stress markers (MDA) while depleting antioxidant defenses (SOD, GSH). Daily *Chlorella* sp. supplementation (200 mg/kg) provided substantial protection by attenuating inflammatory responses and enhancing antioxidant capacity, creating metabolic profiles intermediate between control and emission-exposed conditions.

Principal component analysis confirmed distinct biochemical differentiation among treatment groups, with PC1 and PC2 explaining 83.9% of total variance. Strong correlations among air pollutants (PM2.5-VOC: $r=0.98$; PM2.5-CO: $r=0.97$) and moderate correlations between pollutant exposure and inflammatory markers (TNF- α : $r=0.73-0.77$) establish the mechanistic basis for emission-induced health impacts and validate the protective intervention approach.

The dual functionality of *Chlorella* sp. as both atmospheric pollutant sequestration agent and health-protective supplement positions microalgae as an innovative nature-based solution for industrial emission management. These findings support implementing microalgae-based ecological engineering systems in palm oil production regions as sustainable alternatives to conventional remediation technologies. The research provides evidence-based foundation for developing integrated environmental health protection strategies that combine bioremediation with occupational health safeguards in industrial settings

Acknowledgements

The authors thank the Graduate School of Environmental Sciences, Universitas Riau, for institutional support and research facilities. We acknowledge the Environmental Health Laboratory staff and Animal Research Facility personnel for technical assistance.

REFERENCES

1. Andra, S. S., Austin, C., Wright, R. O., & Arora, M. (2015). Reconstructing pre-natal and early childhood exposure to multi-class organic chemicals using teeth: Towards a retrospective temporal exposome. *Environment international*, 83, 137-145. <https://doi.org/10.1016/j.envint.2015.05.010>
2. Ayub, A., Rahayu, F., Khamidah, A., Antarlina, S. S., Iswari, K., Supriyadi, K., & Wani, A. K. (2025). Harnessing microalgae as a bioresource for nutraceuticals: advancing bioactive compound exploration and shaping the future of health and functional food innovation. *Discover Applied Sciences*, 7(5), 389. <https://doi.org/10.1007/s42452-025-06916-3>
3. Chauhan, B. V., Berg, M. J., Sharma, A., Smallbone, K. L., & Wyche, K. P. (2025). Temporal and Machine Learning-Based Principal Component and Clustering Analysis of VOCs and Their Role in Urban Air Pollution and Ozone Formation. *Atmosphere*, 16(6), 724. <https://doi.org/10.3390/atmos16060724>
4. Ethiraj, S., & Samuel, M. S. (2024). A comprehensive review of the challenges and opportunities in microalgae-based wastewater treatment for eliminating organic, inorganic, and emerging pollutants. *Biocatalysis and Agricultural Biotechnology*, 60, 103316. <https://doi.org/10.1016/j.bcab.2024.103316>
5. Gayathri, R., Mahboob, S., Govindarajan, M., Al-Ghanim, K. A., Ahmed, Z., Al-Mulhm, N., & Vijayalakshmi, S. (2021). A review on biological carbon sequestration: A sustainable solution for a cleaner air environment, less pollution and lower health risks. *Journal of King Saud University-Science*, 33(2), 101282. <https://doi.org/10.1016/j.jksus.2020.101282>
6. Ghafoor, A., & Niazi, A. R. (2024). *Pleurotus* spp: an ultimate solution to the emerging calamities of the world. *New Zealand Journal of Botany*, 1-38. <https://doi.org/10.1080/0028825X.2024.2387185>
7. Hoskin, R. T., Grace, M. H., Guiotto, A., Pecorelli, A., Valacchi, G., & Lila, M. A. (2023). Development of spray dried spirulina protein-berry pomace polyphenol particles to attenuate pollution-induced skin damage: a convergent food-beauty approach. *Antioxidants*, 12(7), 1431. <https://doi.org/10.3390/antiox12071431>
8. Hosny, S., Elshobary, M. E., & El-Sheekh, M. M. (2025). Unleashing the power of microalgae: a pioneering path to sustainability and achieving the sustainable development goals. *Environmental Science and Pollution Research*, 1-31. <https://doi.org/10.1007/s11356-025-35885-8>
9. Jandacka, D., Durcanska, D., & Bujdos, M. (2017). The contribution of road traffic to particulate matter and metals in air pollution in the vicinity of an urban road. *Transportation Research Part D: Transport and Environment*, 50, 397-408. <https://doi.org/10.1016/j.trd.2016.11.024>

10. Johansson, K. A., Vittinghoff, E., Morisset, J., Wolters, P. J., Noth, E. M., Balmes, J. R., & Collard, H. R. (2018). Air pollution exposure is associated with lower lung function, but not changes in lung function, in patients with idiopathic pulmonary fibrosis. *Chest*, 154(1), 119-125. <https://doi.org/10.1016/j.chest.2018.01.015>
11. Jomova, K., Alomar, S. Y., Alwasel, S. H., Nepovimova, E., Kuca, K., & Valko, M. (2024). Several lines of antioxidant defense against oxidative stress: antioxidant enzymes, nanomaterials with multiple enzyme-mimicking activities, and low-molecular-weight antioxidants. *Archives of toxicology*, 98(5), 1323-1367. <https://doi.org/10.1007/s00204-024-03696-4>
12. Kilbo Edlund, K., Xu, Y., Andersson, E. M., Christensson, A., Dehlin, M., Forsblad-d'Elia, H., & Stockfelt, L. (2024). Long-term ambient air pollution exposure and renal function and biomarkers of renal disease. *Environmental Health*, 23(1), 67. <https://doi.org/10.1186/s12940-024-01108-9>
13. Klepacz-Smolka, A., Shah, M. R., Jiang, Y., Zhong, Y., Chen, P., Pietrzyk, D., & Daroch, M. (2024). Microalgae are not an umbrella solution for power industry waste abatement but could play a role in their valorization. *Critical Reviews in Biotechnology*, 44(7), 1296-1324. <https://doi.org/10.1080/07388551.2023.2284644>
14. Khalathari, S., Sotaniemi, V. H., Suokas, M., Taipale, S., & Leiviskä, T. (2024). Microalgae technology for polishing chemically-treated fish processing wastewater. *Groundwater for Sustainable Development*, 24, 101074. <https://doi.org/10.1016/j.gsd.2023.101074>
15. Khanam, Z., Sultana, F. M., & Mushtaq, F. (2023). Environmental pollution control measures and strategies: an overview of recent developments. *Geospatial Analytics for Environmental Pollution Modeling: Analysis, Control and Management*, 385-414. https://doi.org/10.1007/978-3-031-45300-7_15
16. Kimta, N., Dhalaria, R., Kuča, K., Cimler, R., Guleria, V., Guleria, S., & Kumar, H. (2024). Production of Metallic Nanoparticles From Agriculture Waste and Their Applications. In *Transforming Agriculture Residues for Sustainable Development: From Waste to Wealth* (pp. 131-156). Cham: Springer Nature Switzerland. https://doi.org/10.1007/978-3-031-61133-9_6
17. Lawal, A. O. (2017). Air particulate matter induced oxidative stress and inflammation in cardiovascular disease and atherosclerosis: The role of Nrf2 and AhR-mediated pathways. *Toxicology letters*, 270, 88-95. <https://doi.org/10.1016/j.toxlet.2017.01.017>
18. Lee, B., Min, E. K., Kim, G., Hong, G., Seo, J., Choi, J. S., & Kim, K. T. (2024). Biodistribution of synthesized polyethylene terephthalate fibers in adult zebrafish, their sex hormone disruption effect, and mitigation using natural organic matter. *Ecotoxicology and Environmental Safety*, 285, 117108. <https://doi.org/10.1016/j.ecoenv.2024.117108>
19. Li, R., Chen, W., Xiu, A., Zhao, H., Zhang, X., Zhang, S., & Tong, D. Q. (2019). A comprehensive inventory of agricultural atmospheric particulate matters (PM10 and PM2. 5) and gaseous pollutants (VOCs, SO2, NH3, CO, NOx and HC) emissions in China. *Ecological indicators*, 107, 105609. <https://doi.org/10.1016/j.ecolind.2019.105609>
20. Li, S., Li, L., Zhang, C., Fu, H., Yu, S., Zhou, M., & Wang, X. (2023). PM2. 5 leads to adverse pregnancy outcomes by inducing trophoblast oxidative stress and mitochondrial apoptosis via KLF9/CYP1A1 transcriptional axis. *Elife*, 12, e85944. <https://doi.org/10.7554/eLife.85944>
21. Maqsood, Q., Waseem, R., Sumrin, A., Wajid, A., Tariq, M. R., Ali, S. W., & Mahnoor, M. (2024). Recent trends in bioremediation and bioaugmentation strategies for mitigation of marine based pollutants: current perspectives and future outlook. *Discover Sustainability*, 5(1), 524. <https://doi.org/10.1007/s43621-024-00607-6>
22. Mohanty, D., & Samanta, L. (2016). Multivariate analysis of potential biomarkers of oxidative stress in *Notopterus notopterus* tissues from Mahanadi River as a function of concentration of heavy metals. *Chemosphere*, 155, 28-38. <https://doi.org/10.1016/j.chemosphere.2016.04.035>
23. Moraes, J. B., Maes, M., Roomruangwong, C., Bonifacio, K. L., Barbosa, D. S., Vargas, H. O., & Nunes, S. O. V. (2018). In major affective disorders, early life trauma predict increased nitro-oxidative stress, lipid peroxidation and protein oxidation and recurrence of major affective disorders, suicidal behaviors and a lowered quality of life. *Metabolic brain disease*, 33(4), 1081-1096. <https://doi.org/10.1007/s11011-018-0209-3>
24. Naddafi, K., Mesdaghinia, A., Abtahi, M., Hassanvand, M. S., Beiki, A., Shaghghi, G., & Saeedi, R. (2022). Assessment of burden of disease induced by exposure to heavy metals through drinking water at national and subnational levels in Iran, 2019. *Environmental Research*, 204, 112057. <https://doi.org/10.1016/j.envres.2021.112057>
25. Okoro, E. E., Okafor, I. S., Igwilo, K. C., Orodu, K. B., & Mamudu, A. O. (2020). Sustainable biogas production from waste in potential states in Nigeria-alternative source of energy. *Journal of Contemporary African Studies*, 38(4), 627-643. <https://doi.org/10.1080/02589001.2020.1825650>
26. Patil, A. D., Kasabe, P. J., & Dandge, P. B. (2022). Pharmaceutical and nutraceutical potential of natural bioactive pigment: astaxanthin. *Natural products and bioprospecting*, 12(1), 25. <https://doi.org/10.1007/s13659-022-00347-y>
27. Passino, C., Barison, A., Vergaro, G., Gabutti, A., Borrelli, C., Emdin, M., & Clerico, A. (2015). Markers of fibrosis, inflammation, and remodeling pathways in heart failure. *Clinica Chimica Acta*, 443, 29-38. <https://doi.org/10.1016/j.cca.2014.09.006>
28. Priya, A. K., Jalil, A. A., Vadivel, S., Dutta, K., Rajendran, S., Fujii, M., & Soto-Moscoso, M. (2022). Heavy metal remediation from wastewater using microalgae: Recent advances and future trends. *Chemosphere*, 305, 135375. <https://doi.org/10.1016/j.chemosphere.2022.135375>
29. Pradhan, S. H., Gibb, M., Kramer, A. T., & Sayes, C. M. (2023). Peripheral (lung-to-brain) exposure to diesel particulate matter induces oxidative stress and increased markers for systemic inflammation. *Environmental Research*, 231, 116267. <https://doi.org/10.1016/j.envres.2023.116267>
30. Subaramaniyam, U., Allimuthu, R. S., Vappu, S., Ramalingam, D., Balan, R., Paital, B., & Sahoo, D. K. (2023). Effects of microplastics, pesticides and nano-materials on fish health, oxidative stress and antioxidant defense mechanism. *Frontiers in physiology*, 14, 1217666. <https://doi.org/10.3389/fphys.2023.1217666>
31. Sumesh, R. K., Rajeevan, K., Resmi, E. A., & Unnikrishnan, C. K. (2017). Particulate matter concentrations in the southern tip of India: temporal variation, meteorological influences, and source identification. *Earth Systems and Environment*, 1(2), 13. <https://doi.org/10.1007/s41748-017-0015-9>

32. Tylutka, A., Walas, Ł., & Zembron-Lacny, A. (2024). Level of IL-6, TNF, and IL-1 β and age-related diseases: A systematic review and meta-analysis. *Frontiers in immunology*, 15, 1330386. <https://doi.org/10.3389/fimmu.2024.1330386>
33. Usmani, Z., Sharma, M., Lukk, T., Karpichev, Y., Thakur, V. K., Kumar, V., & Gupta, V. K. (2022). Developments in enzyme and microalgae based biotechniques to remediate micropollutants from aqueous systems—A review. *Critical Reviews in Environmental Science and Technology*, 52(10), 1684-1729. <https://doi.org/10.1080/10643389.2020.1862551>
34. Wang, Y., Yang, S., Liu, J., Wang, J., Xiao, M., Liang, Q., & Sun, H. (2023). Realization process of microalgal biorefinery: The optional approach toward carbon net-zero emission. *Science of the Total Environment*, 901, 165546. <https://doi.org/10.1016/j.scitotenv.2023.165546>
35. Zhang, X., Fan, H., Liu, F., Lv, T., Sun, L., Li, Z., & Xu, G. (2023). Coupling coordination between the ecological environment and urbanization in the middle reaches of the Yangtze River urban agglomeration. *Urban Climate*, 52, 101698. <https://doi.org/10.1016/j.uclim.2023.101698>
36. Zhang, Y., Li, Q., Zhang, W., Chen, W., Kong, Z., & Zhang, G. (2025). The air pollutant PM_{2.5} aggravates airway inflammation via NF- κ B/NLRP3-induced pyroptosis: partially inhibited by the TLR4 inhibitor TAK242. *International Immunopharmacology*, 163, 115229. <https://doi.org/10.1016/j.intimp.2025.115229>