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Article

Dual Role of $PM_{2.5}$ Water-Soluble Constituents in Respiratory Viral Infection: Enhanced Cellular Susceptibility and Reduced Virion Infectivity

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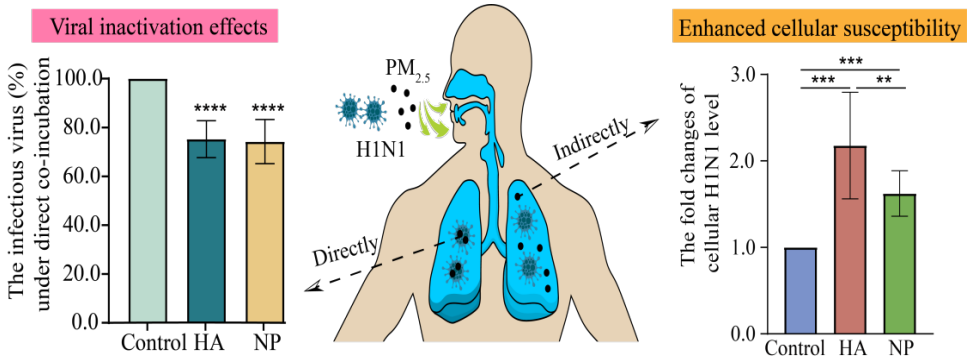
Highlights

- WSM increased cellular susceptibility to H1N1 influenza virus by 1.5- to 3.5-fold

Abstract: Fine particulate matter ($PM_{2.5}$), recognized as a critical risk factor for respiratory viral infection, frequently co-exists with respiratory viruses and elicits diverse toxic effects within host microenvironment. However, the specific $PM_{2.5}$ constituents that affect viral infection and the respective roles of host-mediated processes versus direct virion interactions have not been fully elucidated. This study investigated the impacts of water-soluble matters (WSM) from $PM_{2.5}$ collected in Guangzhou on human influenza A virus (H1N1) infection, focusing on its dual role in modulating bronchial epithelial cells (BEAS-2B) susceptibility and altering viral activity. The results demonstrated that WSM exposure potentiated cellular H1N1 infection level by 1.5- to 3.5-fold, accompanied by 30–40% defense-related signaling (e.g., MxA) reduction. It also perturbed the inflammatory response, including increased IL-6 and IL-8 (20–40%) and 50% reduction in TNF-α. Spearman analysis showed viral infectivity was associated with MxA, IL-6, and TNF-α levels, among which only MxA displayed a dose-dependent inhibitory trend. These results suggested that WSM primarily enhanced infection by suppressing antiviral defenses. Interestingly, WSM also exhibited direct viricidal effect by reducing 25% infectious H1N1 virions after short-term co-incubation and thereby partially modulated the overall viral infectivity in BEAS-2B cells. Further analysis implicated heterogeneous constituents in viral infection outcomes, with heavy metals (e.g., As, Cd) and Na^+ exerting dual effects, both enhancing cellular viral infection and directly reducing virion infectivity. These findings establish a link between prevalence of respiratory viral infection and $PM_{2.5}$ chemical constituents, highlighting the need for public health-guided mitigation strategies.

- H1N1 infection was mainly inversely correlated with key antiviral mediator MxA
- WSM directly reduced H1N1 virion and yet potentiated cellular infection
- As, Cd, Na^+ had dual effects, rising infection level and reducing virion infectivity



1. Introduction

Epidemiological evidence has linked fine particulate matter ($PM_{2.5}$, particles with aerodynamic diameter less than 2.5 μm) to increased susceptibility to numerous human respiratory viruses, including human *influenza viruses* [1,2], *severe acute respiratory syndrome coronavirus 2* (SARS-CoV-2) [3], *respiratory syncytial virus* (RSV) [4], and *adenoviruses* [5]. Among these pathogens, influenza virus remains a major public health burden, responsible for 0.66 million annually deaths [6]. Furthermore, the impact of $PM_{2.5}$ on influenza infection appears to vary by subtype, with influenza A virus (IAV) showing a higher infection risk than influenza B virus [7,8]. Mechanistically, inhaled $PM_{2.5}$ dissolves in the pulmonary lining fluid and releases water-soluble fractions. These components elicit oxidative stress, inflammatory responses, and immune dysregulations [9,10], thereby creating a permissive environment for viral infection. In addition to the indirect host-mediated processes, direct contact between these soluble constituents and virions can disrupt viral integrity and infectivity [11,12], potentially influencing the ultimate cellular infection outcomes. Despite these insights, previous studies have primarily focused on the physical properties of $PM_{2.5}$, such as size and surface area [13–15], while the extent to specific $PM_{2.5}$ chemical constituents how to modulate influenza viral infectivity, particularly that of IAV, remains largely unexplored.

Water-soluble matters (WSM), accounting for 50–70% of $PM_{2.5}$ mass, comprises a complex mixture of organic chemical compounds, microbial-derived fragments, inorganic salts, metal oxides, and unidentified components [16,17]. However, the toxic potencies of WSM constituents display considerable variability, largely affected by their intrinsic chemical characteristics. For instance, recent studies have shown that certain chemical components such as transition metals (e.g., Cr, V, Ni), and certain organic matters (e.g., lipids, proteins, and aromatics) can facilitate the infectivity of SARS-CoV-2 pseudo-virus and IAV [18–20]. In contrast, some WSM constituents including metal oxides (e.g., Ag, Cu, Zn, Ti) and inorganic ions (e.g., NH_4^+, Cl^-) have been proposed as antiviral agents through direct interactions with virions [21,22]. These seemingly contradictory findings indicate a composition-dependent bioactivity of WSM that can either enhance or suppress the viral infection. Therefore, further research is essential to identify the key bioactive components within WSM and to elucidate their respective roles in viral infection.

In this study, we systematically evaluated the impacts of $PM_{2.5}$ water-soluble constituents on IAV infectivity using a dual-role strategy that considered both cellular responses and direct contact with virions. Our specific objectives were to: (1) assess the impacts of WSM on defense, inflammatory responses, and viral infectivity, and to analyze the interrelationships among these effects; (2) examine the direct virucidal potential of WSM following its co-incubation with IAV virions; and (3) identify critical chemical constituents driving the observed changes in viral infectivity via partial least squares regression and machine learning.

2. Materials and Methods

2.1. Preparation of $PM_{2.5}$ Extracts

The twelve ambient $PM_{2.5}$ samples were collected over a 24 h period in Guangzhou, China, using a high-volume air sampler (Tisch, Cleves, Ohio, USA) operated at a constant flow rate of 1.13 m^3 min^{-1} and equipped with quartz filters (Whatman, Piscataway, NJ, USA). Following sampling, each filter was wrapped in pre-baked aluminum foil and stored at −20 °C until further analysis. $PM_{2.5}$ samples were subsequently extracted following a previously established protocol [20]. Briefly, the filter membranes were cut into pieces and subjected to three sequential ultrasonication steps in 30 mL of Milli-Q water. The extracts were analyzed for inorganic ions, metals, and water-soluble organic carbon (WSOC) concentrations, then dried, weighed, and reconstituted in ultrapure water at a concentration of 25 mg/mL for subsequent exposure experiments. Detailed information on the sampling, as well as environmental and meteorological data (e.g., $PM_{2.5}$, NO_2, O_3, SO_2, temperature, wind speed, wind

direction, and relative humidity) are summarized in Table S1 of the Supplementary Materials.

2.2. Chemical Components Analysis

Quantitative analysis of inorganic ions was conducted using ion chromatography (883 Basic IC Plus, Metrohm, Herisau, Switzerland), targeting six cations (Li^+, Na^+, NH_4^+, K^+, Mg^{2+}, Ca^{2+}) and seven anions (F^-, Cl^-, Br^-, NO_2^-, PO_4^{3-}, NO_3^-, and SO_4^{2-}). The concentrations of eighteen metal elements (Al, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Cd, Sn, Sb, Ba, Hg, and Pb) were quantified using inductively coupled plasma-mass spectrometry (ICP-MS, Thermo Scientific, Waltham, MA, USA). Inorganic carbon in the extracts was removed through acidification with phosphoric acid, after which WSOC was measured using a total organic carbon analyzer (TOC-V, Shimadzu, Kyoto, Japan). The concentration of water-soluble organic matters (WSOM) was estimated based on an organic matter-to-organic carbon (OM:OC) conversion factor of 1.8 [23]. The concentration of endotoxin in the extracts was also quantified using a kinetic chromogenic limulus amebocyte lysate assay (Genscript, Piscataway, NJ, USA). Notably, because microbial components (e.g., endotoxin) were carbon-containing organic compounds [24], in our subsequent model analysis, WSOM was considered to include these fragments. The levels of ions, metals, WSOM level were normalized to the total WSM mass. Detailed data for individual components, originally reported in our previous study [20], are summarized in Table S2.

2.3. Quantification of Antiviral, Inflammatory Responses, and Influenza Viral Infectivity in BEAS-2B Cells

Human bronchial cells (BEAS-2B) were cultured in RPMI 1640 medium (Gibco, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (Excell, Suzhou, China), 1% penicillin-streptomycin (Gibco, Grand Island, NY, USA) at 37 °C with 5% CO_2. Cell viability upon exposure to WSM (0, 25, 50, 100, and 200 μg mL^{-1}) was assessed using the Cell Counting Kit-8 (CCK-8). Exposure to 100 μg mL^{-1} WSM caused only a minimal viability reduction (<10%), while 200 μg mL^{-1} resulted in a more substantial decrease of approximately 20%. Consequently, the concentration of 200 μg mL^{-1} was selected for subsequent experiments. Cells were exposed to 200 μg mL^{-1} WSM for 72 h, followed by RNA extraction and qPCR analysis of interferon-related genes (type I, II, III interferon, receptors), interferon-stimulated genes (IFITM3 and MxA), and inflammatory cytokines (IL-6, IL-8, and TNF-α). Gene expressions levels were normalized to reference gene GAPDH and are shown as fold changes relative to control.

For the cellular viral infectivity assay, BEAS-2B cells were pre-exposed to 200 μg mL^{-1} WSM for 24 h and then co-exposed to H1N1 (MOI = 0.5, 4.3×10^4 copies/mL) for 48 h, allowing multi-cycle of viral replication. Cells infected under identified conditions without WSM pretreatment served as control. Total RNA was then extracted from the cell lysates according to the manufacturer's instructions (Vazyme Biotech, Nanjing, China). The load of H1N1 in BEAS-2B cells was quantified by absolute quantitative qPCR targeting viral hemagglutinin (HA) and nucleoprotein (NP) genes, which serve as markers for viral entry and replication processes, respectively. The effect of WSM on cellular viral infectivity was defined as fold changes in viral gene copy numbers, compared to the control group. Details of qPCR conditions and primers are provided in Text S1 and Table S3.

2.4. Assessment of Direct Effects of WSM on Influenza Viral Infectivity after Co-Incubation

To determine appropriate exposure conditions, two preliminary experiments were conducted using a sample representing the average $PM_{2.5}$ mass concentration. H1N1 (4.3×10^4 copies/mL) was co-incubated with WSM at concentrations of 0, 25, 50, 100, and 200 μg mL^{-1} in Dulbecco's Modified Eagle Medium (DMEM) at 37 °C for 72 h. Time-dependent effects were evaluated by treating virus with 200 μg mL^{-1} WSM for 0, 1, 2, 5, and 10 h. Preliminary results indicated that WSM reduced viral infectivity in a dose- and time-dependent manner, with approximately 20% reduction after 5 h treatment at 200 μg mL^{-1} WSM. Based on these results, H1N1 was then incubated with all WSM samples at 200 μg mL^{-1} for 5 h in DMEM. The virus-WSM mixture was then used to infect the Madin-Darby Canine Kidney (MDCK) cells for 4 h to prevent multi-cycle replication, with untreated virus serving as the control. The MDCK cell line was selected for this study owing to its high susceptible to viral infection and attenuated interferon responses compared to primary epithelial cells [25], which aligns with its well-established model for quantifying influenza virus. The infectious viral load was quantified by absolute quantitative qPCR to evaluate the direct effects of WSM on viral infectivity.

2.5. Chemical Components of WSM Associated with Influenza Viral Infection

A partial least squares (PLS) regression model was established using SIMCA 14.1 (Umetrics, Umeå, Sweden) to examine the relationship between influenza viral infectivity and proportions of chemical components. The model performance was evaluated by a seven-cross fold cross-validation and a 999-time permutation test. Compounds significantly associated with viral infection levels were identified based on a variable importance in projection (VIP) score greater than 1 and the regression coefficient (β), which indicated the direction of association ($\beta > 0$ for positive, $\beta < 0$ for negative). The detailed description of PLS regression model is presented in Text S2.

Random forest (RF) regression was applied to identify the critical components linked to influenza viral infectivity, using variables prescreened by the PLS regression. The hyperparameters of model were optimized via a comprehensive grid search coupled with three-fold cross-validation. Predictive performance was evaluated by the coefficient of determination (R^2), root mean square error (RMSE) and mean absolute error (MAE), with all analyses implemented in Python (v3.10.9). The SHapley Additive ExPlanation (SHAP) approach, rooted in the coalitional game theory, was employed to quantify each feature contribution to predictions in RF model. The optimization details for model are provided in Text S3.

3. Results and Discussion

3.1. Water-Soluble Matters Disrupted Antiviral and Inflammatory Responses, and Enhanced Influenza Viral Infectivity

To investigate the associations between WSM-induced host cellular responses and IAV infectivity, we first assessed the antiviral and inflammatory responses in BEAS-2B cells following 72 h of WSM exposure. WSM markedly suppressed interferon responses by 30% to 40%, particularly reducing expression of the interferon-stimulated gene MxA, relative to untreated control (Figure 1). This observation aligns with previous evidence demonstrating that ambient particle extracts collected from Colombia and Bengaluru inhibited IFN-β mediated antiviral defenses, thereby potentiating *avian influenza viruses* H5N1 *and* SARS-CoV-2 infection [26,27]. Concurrently, WSM differently regulated pro-inflammatory cytokine induction, with 20% to 40% elevation in IL-6 and IL-8, and a 50% reduction in TNF-α level. Reported pro-inflammatory potency of $PM_{2.5}$ extracts in epithelial cells have yielded inconsistent results across studies, which may be attributable to divergent exposure conditions, varied emission sources, and distinct cellular response patterns [10,28]. These results collectively suggested that WSM exposure elicited diverse cytotoxic effects, impairing interferon-mediated antiviral defenses and disrupting inflammatory responses, thereby potentially establishing a permissive environment for respiratory virus infection.

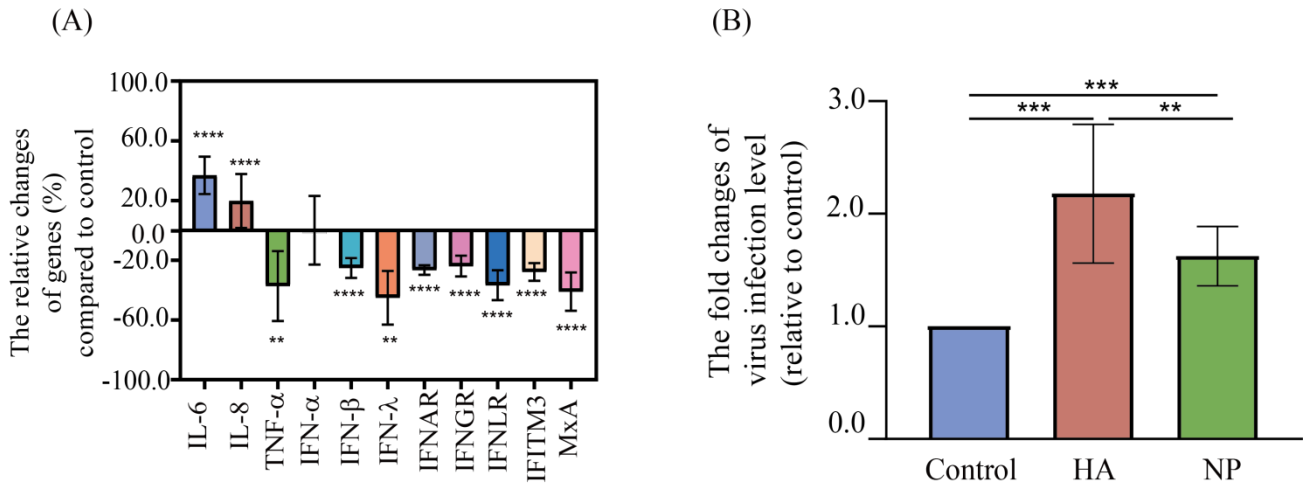


Figure 1. WSM exposure suppressed antiviral defense, disrupted inflammatory response, and increased cells susceptibility to influenza virus infection. (**A**) The relative expression levels of interferon-related genes and inflammatory cytokines. (**B**) The relative fold changes of influenza virus infection level in BEAS-2B cells following exposure to WSM. Data are presented as mean ± SD. ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ (two-tailed *t* test with Wilcoxon test).

We next evaluated the impacts of WSM exposure on cellular susceptibility to influenza viral infection. BEAS-2B cells were pre-exposed to WSM for 24 h prior to co-exposed with viruses for an additional 48 h. As illustrated in Figure 1, WSM significantly enhanced the expression of viral HA and NP genes, with copy numbers increasing by 2.2 ± 0.6, and 1.6 ± 0.3-fold, respectively, compared to H1N1 alone infected. The disproportionately greater induction of HA suggested that WSM predominantly potentiated initial viral attachment, a process mediated by binding of viral hemagglutinin to host receptors [29]. While prior studies have reported that water-soluble fractions from traffic emission source or ambient $PM_{2.5}$ can effectively facilitate H3N2 infection [14,30], our findings demonstrated that chemically distinct aqueous extracts from Guangzhou $PM_{2.5}$ exert similar facilitative effects on H1N1 infectivity, thereby extending these observations to different viral subtype and pollution profile. At the same time, it is important to note that the $PM_{2.5}$ aqueous extracts in this study are regionally specific. Our $PM_{2.5}$ samples were collected from Guangzhou, a traffic- and industry-dominated megacity in southern China [31]. In contrast, $PM_{2.5}$ from other regions strongly influenced by sources like dust storms and residential coal/biomass burning contains higher levels of crustal metals and polyaromatic hydrocarbons-related substances [32,33]. Such compositional differences may in turn modify the magnitude of the pro-infectivity effect [28,34].

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Based on the observation that viral HA was markedly more affected than NP in BEAS-2B cells exposed to WSM, we examined the associations of WSM-induced antiviral and inflammatory responses with HA level. Spearman correlation analysis revealed that key antiviral mediator MxA was inversely correlated with HA level ($r = -0.39$, $p < 0.05$, Figure 2). Furthermore, MxA exhibited a dose-dependent inhibitory on cellular HA level, suggesting that suppression of MxA expression may contribute to the enhanced H1N1 viral infectivity [35,36]. Although IL-6 and TNF-α also showed a negative correlation with HA level ($r = -0.47$ and -0.27, respectively, both $p < 0.05$, Figure 2), neither cytokine showed an obvious dose-dependent pattern with HA. While seeming contradictory to elevation in both IL-6 and HA levels, this negative association suggested that IL-6 signaling may play a balancing role against influenza viral infection in exposed cells, which aligned with previously reported limited or suppressive effects IL-6 on influenza virus infection [14,37,38]. The compositional heterogeneity of WSM may modulate this inverse relationship, with distinct constituents differently stimulating IL-6 induction (e.g., via aromatics) [39] while promoting respiratory virus infection (e.g., via trace metals, lipids and proteins) [19,20], therefore causing their nonparallel enhancement. Importantly, MxA is widely recognized as a central effector of interferon-induced antiviral response, and its reduction substantially weakens innate defense against viral infection [36]. Multiple rescue experiments, including MxA knockdown and overexpression, have causally linked MxA expression to the restriction of influenza virus infection [40]. Therefore, we inferred that the enhanced susceptibility to influenza virus following WSM exposure is primarily a consequence of impaired intrinsic immunity, resulting from WSM-induced compromise of critical antiviral defense responses, rather than to pro-inflammatory signaling.

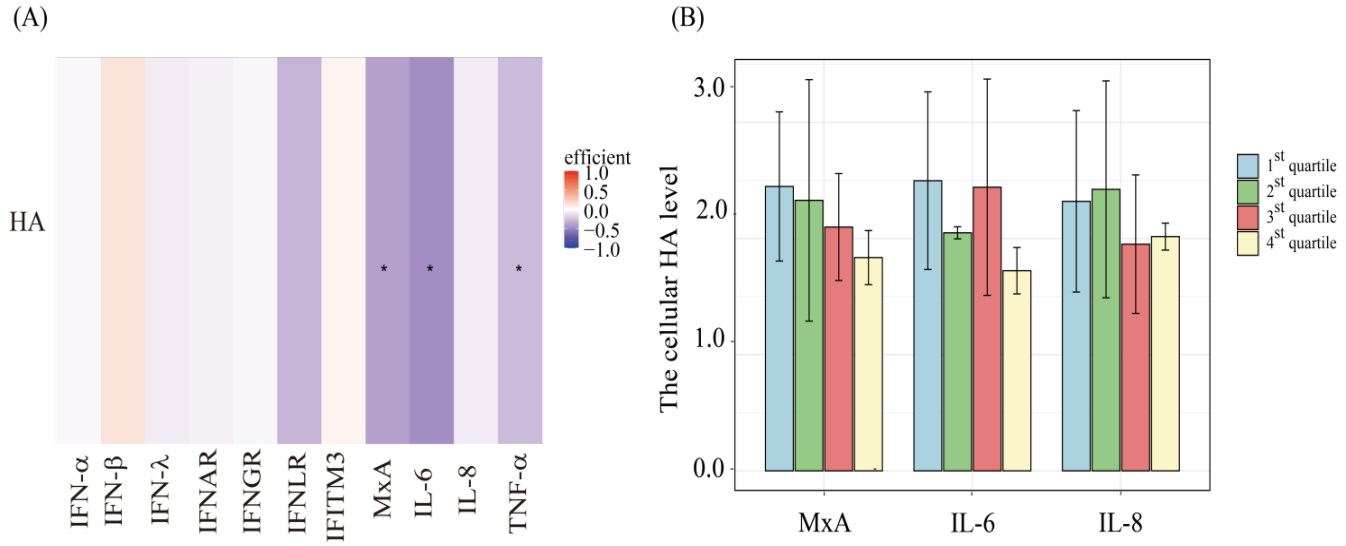

Figure 2. (**A**) Spearman correlation analysis between viral infectivity and expression of inflammatory and interferon-related responses. The asterisk (*) indicates a significant correlation ($p < 0.05$). (**B**) MxA, IL-6, and IL-8 expression across four quantiles show a monotonic dose-response or non-monotonic relationship with cellular HA level. The x-axis shows the three cellular endpoints (MxA, IL-6, and IL-8). For each endpoint, the four bars (blue to yellow) represent the mean fold change relative to control for the 1st-4th quantiles following WSM exposure. Error bars indicated standard deviations across all WSM samples.

3.2. Direct Antiviral Activity of Water-Soluble Matters against Influenza Virus

In our co-exposure model, WSM constituents were incubated simultaneously with influenza virions, enabling direct chemical-viral interactions. Previous studies using a pre-exposure design, where only cellular responses were involved, reported the influenza infection levels 1.5- to 2-fold higher than those observed in our experiment [14,41]. Hence, we hypothesized that chemical-viral interaction may alter H1N1 infectivity, ultimately modulating severity of cellular infection level. To test this hypothesis, we first conducted a cell-free incubation assay, in which H1N1 virions were co-incubated with WSM for 5 h. As shown in Figure 3, WSM treatment markedly reduced the number of infectious virions, with 25% reduction in HA and NP gene copies, relative to the control, suggesting that direct virucidal potential of $PM_{2.5}$ aqueous extracts on H1N1. Although limited reports have shown that standard reference materials extracts, such as diesel exhaust particles (DEP) and urban dust, can degrade IAV nucleic acids through co-incubation with the plasmid DNA mimicking influenza viral genome [42], our results provided direct evidence that aqueous extracts from real-world ambient $PM_{2.5}$ can reduce infectious influenza virion infectivity. This finding was corroborated by research in which aqueous species from engine exhaust exhibited virucidal impacts on SARS-CoV-2 infection [43].

Together, these findings highlighted the overlooked virucidal potential of $PM_{2.5}$ chemical constituents, providing a new insight into the mechanism underlying $PM_{2.5}$-induced modulation of respiratory virus infectivity.

The observed antiviral effects of WSM are likely attributable to its chemical compositions, comprising a mixture of water-soluble ions, metals, and various organic matters. Such compositional diversity enables multiple chemicals-virions interaction, as supported by previous studies demonstrating that specific constituents can reduce viral particles through distinct processes. For instance, humic-like substances, have reported to disrupt the functional integrity of viral enveloped glycoproteins, thereby rendering respiratory virus noninfectious [44]. Similarly, certain metal or metal oxides (e.g., Ag, Fe, Cu, Ti) can directly reduce infectivity of virions by releasing metal ions that oxidatively damage viral macromolecules, including nucleic acid, proteins, and lipids [45,46]. These findings suggested that the anti-influenza virus activity of WSM likely results from the combined actions of diverse constituents operating through different mechanisms. However, further investigations are warranted to pinpoint the critical compounds responsible for these virucidal effects.

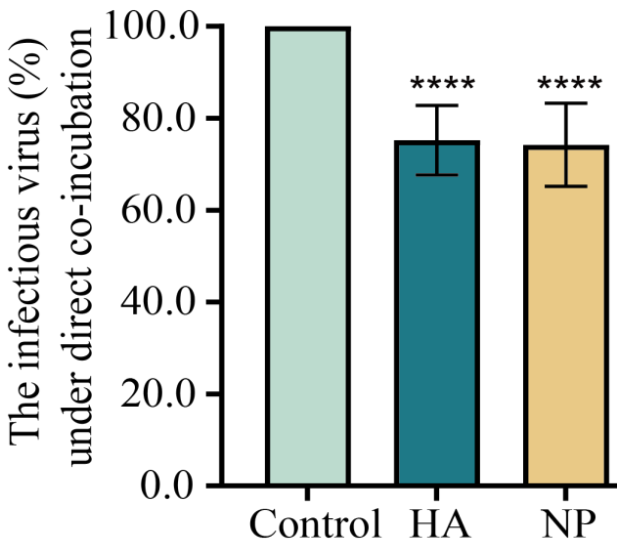

Figure 3. WSM exhibited direct virucidal effects against influenza viral infectivity. Data are presented as mean ± SD (**** $p < 0.0001$, two-tailed t test with Wilcoxon test).

3.3. Chemical Components Associated with Influenza Viral Infectivity

To identify key WSM constituents associated with influenza viral infectivity, the PLS regression and explainable machine learning models were employed. The model used proportions of water-soluble ions, metal and WSOM as predictors, with fold changes of viral gene HA and NP as responses variables. The robustness and predictability of PLS regression model were confirmed through seven cross-validations and permutation test (Table S4). The significant compositions were selected based on a VIP score greater than 1 and their coefficients (β). A positive coefficient ($\beta > 0$) indicated positively enhanced cellular susceptibility to influenza infection, whereas a negative coefficient ($\beta < 0$) suggested direct virucidal activity. All screening results are summarized in Table S5.

Explainable machine learning model, trained with PLS-prescreened features, was then constructed and evaluated through R^2, RMSE, and MAE (Table S6). The predictor importance was quantified using mean absolute SHAP values. Machine learning analysis identified substantial variability in chemical constituents that were associated with influenza viral infectivity (Figure 4). Among these, water-soluble ions and metals emerged as the principal determinants, whereas WSOM exerted comparably minor influence. Specifically, water-soluble ions (e.g., NO_3^-, Mg^{2+}, Na^+, Cl^-), metals (e.g., Al, Zn, As, V and Cd) were positively linked to cellular increased HA and NP levels. Consistent with our findings, early studies using standard substances have similarly observed that water-soluble ions (e.g., Na^+, Cl^-) and trace metals (e.g., Al, As, Cd) in promotion of influenza entry and replication by suppressing antiviral defenses and host other cellular responses [34,41,47,48]. At the mechanistic level, several studies further demonstrated that environmentally relevant As and Cd exposure blunted innate defenses by disrupting type I interferon-related signaling, including suppression the MxA expression [49,50]. Such impairment is expected to render host cells more susceptible to influenza virus infection. Additionally, building upon epidemiological evidence linking secondary nitrate and V to respiratory viral infection, including influenza virus [51,52], our results provided experimental confirmation that NO_3^- and transition metals (e.g., Zn and V) in ambient $PM_{2.5}$ markedly associated with H1N1 infectivity. These findings collectively suggested that secondary nitrogen and redox-active metals play an important role in the enhancement of respiratory viral infection.

In influenza virions and WSM co-incubation system, Na^+, SO_4^{2-}, and specific heavy metals (e.g., As, Mn, Cd and Ti) were identified as negatively correlated with viral HA and NP gene levels, indicating that these constituents contributed to the virucidal potential of WSM. This is aligned with prior mechanistic studies in which individual metal-related substances (e.g., Mn, Fe, Ti, Cu, As, Sb) and salts (e.g., Na^+, Cl^-) reduce respiratory viral infectivity. Metal ions/oxides have been shown to decrease viral infectivity through oxidizing viral enveloped protein, thereby compromising virion structural stability [45,53]. In parallel, salt ions, such as Na^+, Cl^-, are proposed to decrease IAV infectivity by disruption of virion integrity [54]. Our findings extended this knowledge by demonstrating that ionic and metallic compounds in WSM

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can collectively reduce virions infectivity. Despite considerable heterogeneity among constituents affecting intracellular and extracellular infectivity, As, Cd, and Na^+ were consistently correlated with infection outcomes, suggesting a dual role in modulating viral infection. This duality may emerge from two concurrent processes: the direct antagonism of virions by chemical constituents [54], operating alongside immunotoxic effects that dysregulate host defenses [55,56], which together shape eventual infection outcomes.

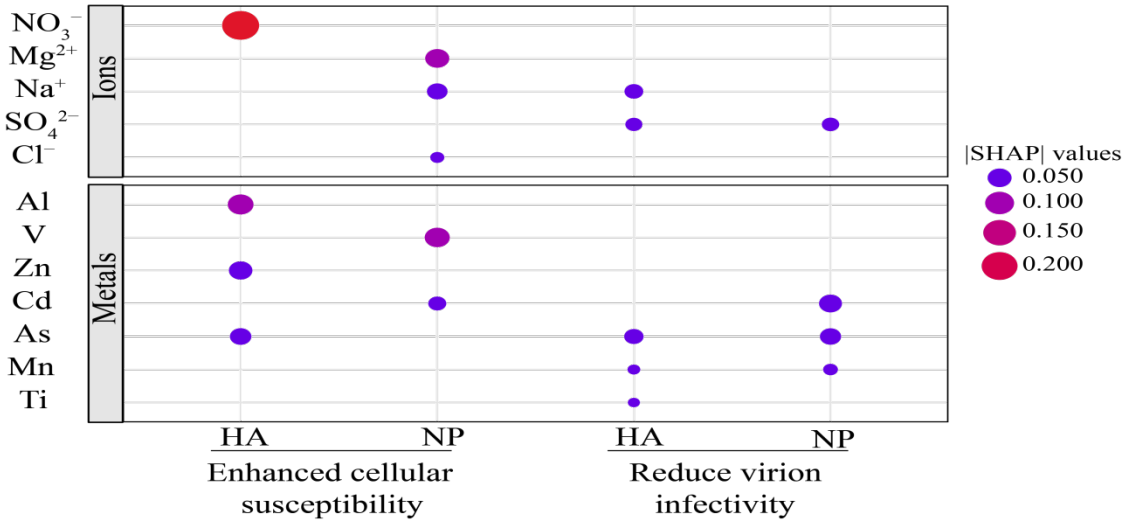

Figure 4. Machine learning model identified five key constituents correlated with intracellular and extracellular viral infectivity. Dot plot shows the mean absolute SHAP value for each feature.

4. Limitations of Study

Despite these insights, several limitations should be considered. Firstly, although our results indicated a dose-dependent negative correlation between viral HA and MxA expression, this relationship does not establish causality. Secondly, because $PM_{2.5}$ samples analyzed in this study were collected solely in Guangzhou, the results may not fully represent regional viability in chemical compositions and its effects on viral susceptibility. Subsequent studies should incorporate $PM_{2.5}$ from locations with distinct emission sources to better assess the generalizability of these findings. Thirdly, despite identifying As, Cd, and Na^+ as key constituents with dual effects on cellular susceptibility and reducing viral infectivity, the underlying molecular mechanism and the impact on virion integrity remain unclear. Future work should incorporate targeted protein-protein interaction assay and transmission electron microscopy to investigate these aspects. Fourthly, the immortalized cell lines used in this study cannot fully capture the structural and cellular complexity of the human respiratory tract, including the mucus barrier, mucociliary clearance, and cross talk with resident immune cells. Future studies employing primary human airway epithelial cultures (e.g., air-liquid interface models) and in vivo systems will be important to validate these findings under more physiologically relevant conditions.

5. Conclusions

This study elucidated the bidirectional role of $PM_{2.5}$ water-soluble constituents in regulating influenza virus infection. We found that WSM enhanced cellular susceptibility to viral infection by 1.5- to 3.5-fold, mainly driven by the suppression of interferon-stimulated gene MxA expression, with minimal contribution from inflammatory cytokines. In parallel, WSM also demonstrated direct virucidal activity, reducing viral infectivity by 25% after short-term co-incubation and thereby modifying the extent of influenza virus infection in the host cells. Machine learning model identified water-soluble ions and metals as key drivers for viral infectivity, whereas WSOM contributed little to the observed variation. Among the variable components influencing intracellular and extracellular infection, heavy metals (e.g., As, Cd) and Na^+ consistently affected both processes. These findings underscore that health risk assessment of $PM_{2.5}$ should account not only for its inflammatory potential, but also for its capacity to suppress essential antiviral defenses in promoting influenza virus transmission in polluted environments.

Supplementary Materials

The additional data and information can be downloaded at: https://media.sciltp.com/articles/others/2512221634182114/GES-25110064-Supplementary-Materials.pdf. Table S1. Samples collection and atmospheric meteorological conditions. Table S2. The proportion of water-soluble organic matters, ions, and metals in WSM. Table S3. The paired primer sequence used to qRT-PCR assays. Table S4. Summary of cross-validation and overfitting results of PLS regression. Table S5. The markedly compounds associated with viral infectivity. Table S6. Overview of machine learning model performance

after three cross-validation. References [57–62] are cited in supplementary materials.

Author Contributions

S.P.: Visualization, Writing—original draft, review & editing; J.L.: Methodology; Y.H.: Supervision, Methodology; B.G.: Investigation, Methodology; W.S.: Methodology; G.Z.: Project administration, Funding acquisition; W.S.: Methodology; B.J.: Methodology; C.P.: Methodology; J.Z.: Methodology; J.D.: Methodology; X.W.: Project administration, Funding acquisition; P.P.: Project administration, Funding acquisition; X.B.: Methodology, Writing—review & editing, Project administration, Funding acquisition. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement

Not applicable.

Informed Consent Statement

Not applicable.

Data Availability Statement

Relevant data are available upon request.

Conflicts of Interest

The authors declare no conflict of interest.

Use of AI and AI-assisted Technologies

No AI tools were utilized for this paper.

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