

Review

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Review

Antiviral Phytoremediation for Sustainable Wastewater Treatment

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Abstract

Presence of pathogenic viruses in wastewater pose a potential threat to public health. Conventional treatment methods often yield moderate viral reduction and toxic byproducts, whereas advanced technologies are underutilised due to their high cost and energy demands. Antiviral phytoremediation emerges as an affordable, eco-friendly and sustainable approach for removing viruses. However, recent bibliometric analysis on wastewater treatment methods from 1976–2025 revealed that only ~0.4% of total literature (~23,000) was related to antiviral phytoremediation suggesting critical knowledge gaps persist. This critical review provides insights into viral removal mechanisms, recent advancements, practical applications, and challenges and opportunities. Antiviral phytoremediation offers a promising multilayer of viral removal mechanisms (i.e., sorption/filtration, rhizosphere-mediated inactivation, internalization, and intracellular degradation mechanisms). Hybrid systems integrating constructed wetlands (CWs) with complementary technologies could achieve high removal efficiencies (i.e., ~3.0–7 log₁₀ reductions) compared to standalone CWs (i.e., ~1–3 log₁₀). Although phytoremediation efficiency is moderate for viruses (i.e., ~45–84%) relative to heavy metal removal (i.e., ~70–100%), emerging technologies (i.e., CRISPR gene editing, engineered microconsortia, and biosensors) offer promise for enhancement, which is still at proof-of-concept levels. Hybrid antiviral phytoremediation approaches provide sustainable infrastructure supporting public health, climate adaptation, and pandemic preparedness.

Keywords: phytoremediation; viral removal; wastewater treatment; hybrid systems; sustainable remediation

1. Introduction

Human pathogenic viruses, including enteroviruses, noroviruses, and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), persist in wastewater collection systems and are distributed through multiple environmental pathways (Figure 1), substantially threatening public health [1,2]. Millions of viral particles from infected individuals enter municipal sewer systems daily, where they survive for extended periods ranging from hours to months, depending on the viral strain, temperature, and organic content. These viruses can contaminate receiving water bodies, agricultural soils, and seafood production systems [3,4].

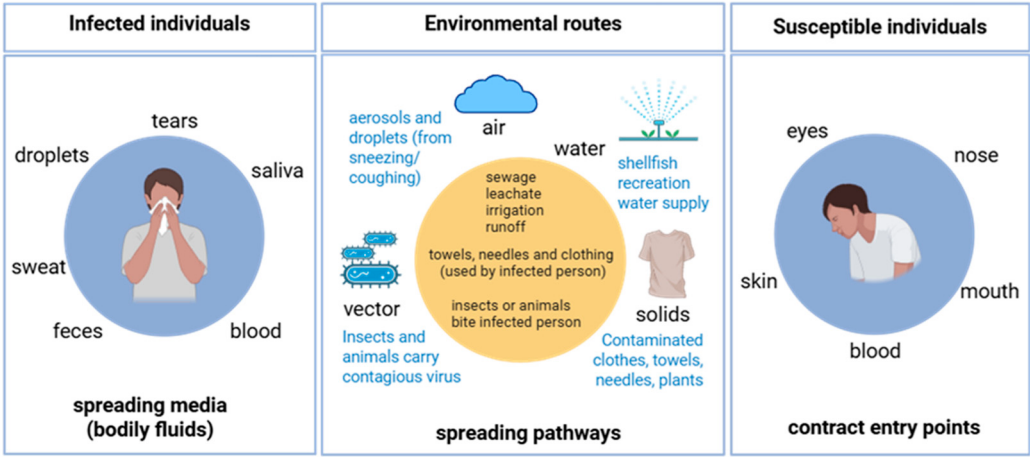


Figure 1. Transmission pathway of pathogenic enteric viruses. Viruses transmit from infected to susceptible individuals through environmental routes. Transmission occurs when bodily fluids (sweat, droplets, tears, blood, feces, saliva) spread via different pathways (air, water, solids, vectors). Susceptible individuals may contract infections through multiple entry points (eyes, nose, mouth, blood, skin).

Gastrointestinal viruses, particularly norovirus, adenovirus, and hepatitis A virus, are highly resistant to traditional disinfectants (e.g., chlorination), ultraviolet (UV) irradiation, and selected advanced treatment processes [5,6]. Advanced technologies, such as membrane bioreactors (MBRs) and advanced oxidation processes (AOPs), yield relatively higher viral inactivation than traditional technologies, but the levels of treatment are still virus specific. The efficiency of MBRs for the removal of different viral pathogens depends on log₁₀ removals of ~1–7 along with published log₁₀ removals of ~4–6 for adenovirus and ~5–6 for norovirus genogroup II [7,8]. The removal efficiency translates into ~97–100% removal efficiency which indicates significant but not complete viral removal.

The widespread deployment of advanced treatment systems in resource-constrained regions is severely limited by (1) high capital expenditure, (2) substantial energy requirements (~0.4–5.0 kWh/m³ of treated wastewater), and (3) technical complexity, which demands skilled operators and sophisticated tracking infrastructure [9,10]. These barriers are acute in low-income regions, where wastewater infrastructure development lags population growth and climate variability exacerbates water stress.

Documented disease outbreaks linked to wastewater reuse disproportionately affect farmworkers, vulnerable consumers, and communities near disposal sites, with children and immunocompromised populations experiencing elevated morbidity and mortality [11].

Detection of SARS-CoV-2 ribonucleic acid (RNA) in wastewater samples from diverse geographic regions has shown the utility of wastewater-based epidemiology for disease surveillance [12–14] and has provided compelling evidence for integrating antiviral phytoremediation into post-treatment polishing steps [15].

Nature-based approaches, notably constructed wetland systems (CWs), are highly effective at removing conventional pollutants (i.e., nutrients and pathogens) and emerging micropollutants (pharmaceuticals and microplastics) across all locations and climates [16,17]. Chemical disinfection methods, particularly chlorination, produce potentially carcinogenic water chlorination products including trihalomethanes (THM) and haloacetic acids (HAA) that might persist in treated effluents and receiving waters, thus posing threats to human health, aquatic organisms and ecosystem integrity [18].

Plants are the functional units of constructed wetlands, and have various complementary removal pathways through phytoremediation [19,20]. These pathways encompass (1) rhizofiltration, that is oxidation and immobilisation by adsorbing biofilms of contaminants on the root surfaces, (2) phytoextraction, or selective uptake of metals and transport into harvestable biomass [21], (3) phytodegradation, involving enzymatic breakdown of contaminants within plant tissues, (4)

rhizodegradation, where the rhizospheric microbial consortia degrade contaminants stimulated by plant root exudates [22–24]. Model macrophyte species, which include *Lemna minor* and *Phragmites australis*, have shown contaminant removal efficiencies of ~51–100% for heavy metals (concentration-dependent), ~70–99% for excess nutrients (nitrogen and phosphorus) and ~70–100% for culturable microbial pathogens in the possible configurations of constructed wetlands [25–27]. Elevated removal efficiencies are attributed to bioactive secondary metabolites produced, especially polyphenols, terpenoids, and organic acids that (a) have direct antimicrobial properties, or (b) stimulate the increase in beneficial rhizospheric bacteria capable of antagonising pathogenic microorganisms [28,29].

Plants have innate viral defence mechanisms such as non-host resistance pathways and salicylic acid [SA]-dependent inducible defences that inhibit the replication and movement of the virus within plant tissues [30–32]. Preliminary studies indicate that specific macrophytes have enhanced antiviral properties. *Pennisetum purpureum*, for example, effectively removes excess nutrients in constructed wetlands [33], whilst *Perilla frutescens* produces bioactive phytochemicals which include caffeic acid derivatives and polyphenols that target viral capsid proteins that at the same time modulate host immune signaling and show efficacy against SARS-CoV-2 *in vitro* [34,35].

To contextualize this review both within and among the existing literature, we performed a comprehensive bibliometric analysis of ~23,000 peer-reviewed publications on wastewater treatment systems within the 1976 to 2025 time-frame. Bibliometric analysis was performed through the online Dimensions platform [36]. Dimensions was chosen for this bibliometric evaluation based on the authenticity and comprehensive nature by which manuscripts, grants, patents, and policy documents are indexed across Google Scholar, Web of Science, and Scopus [37,38]. The insights revealed by this bibliometric survey unveiled a striking gap in knowledge: of the ~23,000 articles identified, only about 81 dealt with plant-based viral removal mechanisms, which reflects a representation of a little less than about 0.4% of the total articles. The phytoremediation literature base is primarily focused on heavy metal juggling (52% of the articles) or bacterial pathogen manipulation (34%), while antiviral phytoremediation is apparently underdeveloped considering the increasing evidence of viral contamination of wastewaters throughout the world gained through surveillance mechanisms (Figure 2). The asymmetrical representation of the forms of phytoremediation is alarming as it points to major voids existing regarding the mechanistic understandings of plant-viral interaction mechanisms among the enveloped and non-enveloped classes of viruses while highlighting the critical need for field-scale testing of hybrid antiviral platforms. Among the key issues of research that have arisen from this literature gap for these forms of phytoremediation are: 1) identifying the plant exudate-rhizospheric microbiota co-metabolic interaction mechanisms, 2) systematically comparing co-metabolic interactions of differing species under different standardized conditions, 3) harmonization of the method for assays of viral measurements among the research teams, 4) field-scale evaluations of the hybrid treatment configurations, 5) targeted genetic engineering of plant genetic traits to develop preferred antiviral phenotypes, and 7) techno-economic and life cycle assessment analyses of treatments used regarding their application to low-income settings. This critical review provides the insights gained from the researches representative of (1) the mechanistic pathways underlying antiviral phytoremediation applied to the molecular and ecosystem scales, (2) the strategies for system designs of phytoremedial platforms with reference to integrating disparities with the complementary technologies for vaunted viral removal, (3) the applications that have been implemented and the documented field-scale successes reached that indicate their operational feasibility, and (4) the ceaseless barriers that remain and the confirmed, evidence-based research directions that should be sought to globalize the use of antiviral phytoremediation.

By integrating perspectives from plant biology, environmental microbiology, wastewater engineering, and systems ecology, this review positions antiviral phytoremediation as a technically feasible, economically scalable, and environmentally resilient part of nature-based infrastructure supporting fair public health preparedness, climate adaptation, and environmental justice across diverse socioeconomic contexts.

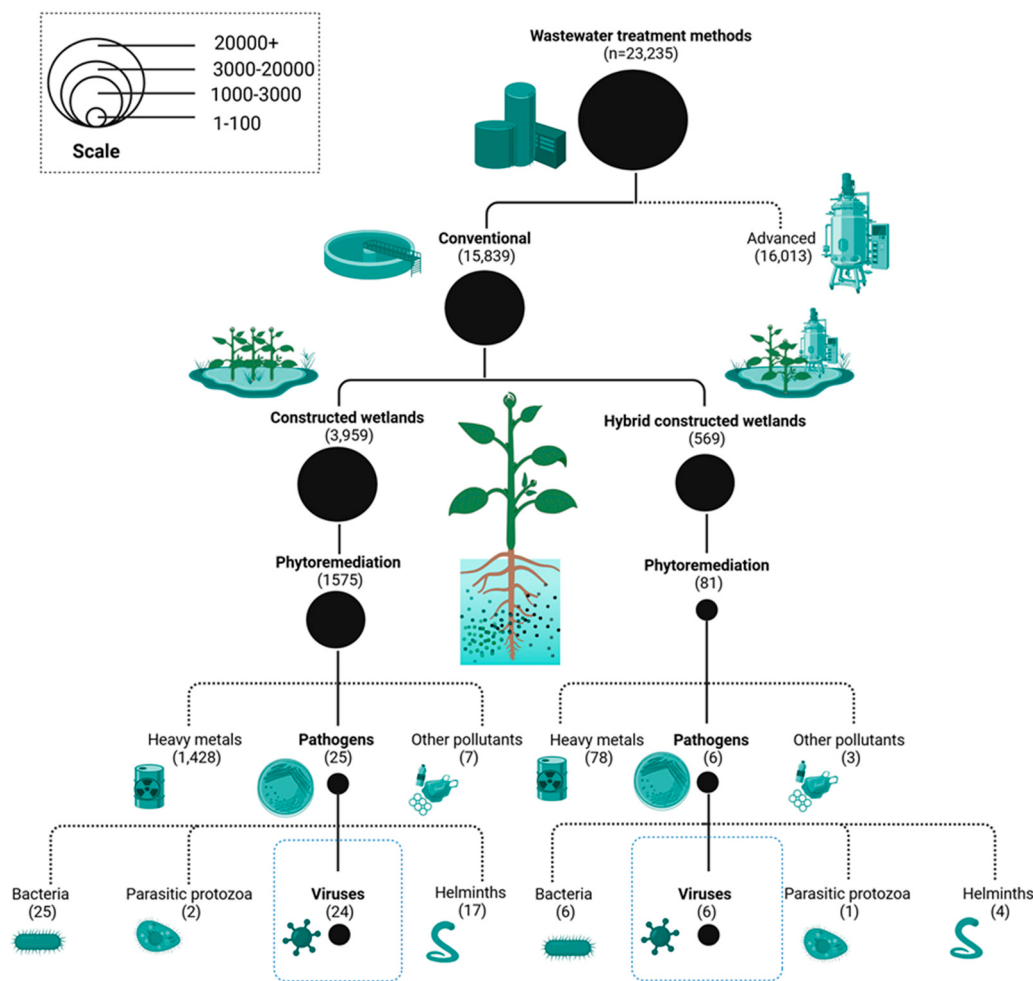


Figure 2. Studies on the phytoremediation of viruses and other pathogens from wastewater over five decades (1976–2025). Conventional and advanced wastewater studies have evolved and become interconnected, leading to overlaps between treatment domains. Most studies (>90%) identified microbial contaminants as waterborne pathogens and emphasized their combined treatments, resulting in overlaps when categorizing them (i.e., bacteria: 25, viruses: 24, parasitic protozoa: 2, and helminths: 17, totaling 25). Data from the Dimensions Research Database (<https://app.dimensions.ai>) as of 31/03/2025 showed an evolution combining conventional and advanced methods, recognizing hybrid treatment as promising for microbial contaminants.

2. Mechanistic Basis of Antiviral Phytoremediation

2.1. Phytoremediation Mechanisms

Phytoremediation in planted treatment systems runs through multiple, well-established pathways that function concurrently within the plant–soil–microbe continuum: rhizofiltration (physical interception and interfacial partitioning), phytostabilization (immobilisation within the rhizosphere), rhizodegradation/phytodegradation (microbially mediated and plant-enzymatic transformation), and phytoextraction/phytovolatilization (uptake, sequestration, and release of certain volatile species). The relative contributions of these mechanisms are influenced primarily by hydraulics, redox conditions, and microbial community composition, rather than only by the pathogen or contaminant class [39–41].

Rhizofiltration

Root mats, periphytic biofilms, and porous media act as tortuous filters that remove suspended and colloidal matter through straining, attachment, and gravitational sedimentation. Concurrently, dissolved and nano-colloidal species partition to organic and mineral surfaces via electrostatic, hydrophobic, and cation-bridging interactions [39,40]. The efficiency of contact is governed

hydraulically: multi-port dosing, baffling, and media resistant to compaction maintain adequate home time and prevent short-circuiting; alternating day dosing in vertical subsurface-flow (VSSF) beds re-oxygenates pores and mitigates clogging, thus sustaining interception stability over time [41,42]. Plant traits reinforce these barriers. A high fine-root surface area and vigorous periphyton growth expand the active surface area, but root exudates modulate the near-root charge and pH, enhancing the sorption and co-precipitation of particulate and dissolved species [43,44].

Phytostabilization

Phytostabilization reduces contaminant mobility and bioavailability through sorption to roots and media, precipitation reactions (e.g., phosphate- or carbonate-mediated), and organic ligand complexation within the rhizosphere (Pilon-Smits 2005; Ruttens et al. 2011). Plants with deep or fibrous root systems create extensive sorptive matrices and microzones characterised by distinct redox potentials and pH conditions that favour the immobilisation of heavy metals. Root cell walls and vacuoles also serve as sinks for ions and polar organic compounds [45]. Phytostabilization efficacy is enhanced by soil amendments such as phosphate and biochar, and, where appropriate, microbial inoculants that reinforce root-associated microbial consortia. Routine tracking is essential to ensure the stability of immobilised fractions under seasonal wetting–drying cycles [46,47].

Rhizodegradation and phytodegradation

The rhizosphere is a chemically reactive and microbially rich zone where plant exudates, including organic acids, amino acids, and phenolics, serve as substrates for heterotrophic microorganisms and modulate the local redox conditions. This environment fosters division-of-labour microbial consortia that can transform dissolved organic compounds and co-metabolise diverse contaminants (rhizodegradation) [23,48,49]. Biofilms on roots and media secrete enzymes, such as oxidases, peroxidases, and hydrolases, which accelerate the depolymerisation and humification of organic matter. Oxygen release via aerenchyma creates mixed redox mosaics that support coupled nitrification–denitrification and sulfur/iron cycling [39,40]. Within plant tissues, endogenous enzymes, including peroxidases, dehalogenases, and nitrilases (via phytodegradation), further transform the absorbed organic compounds. Some transformation products are sequestered in cell walls or vacuoles, completing partial mineralisation across plant and microbial compartments [20,44]. Where coloured dissolved organic carbon (DOC) or recalcitrant micropollutants limit biodegradation, staging a photochemically active free-water cell upstream can pre-oxidise chromophores, thus enhancing downstream biodegradability without compromising rhizosphere function [50].

Phytoextraction and phytovolatilization

Phytoextraction involves the removal of dissolved ions and certain polar organic compounds via root uptake, followed by intracellular binding to organic acids and thiol-rich peptides, and compartmentalisation within vacuoles or deposition in cell walls [20,41,51]. Periodic harvesting exports sequestered contaminants and promotes belowground turnover and exudation. Species showing high growth rates, extensive aerenchyma, and dense fine-root systems sustain greater contaminant fluxes without excessive clogging [40,44]. Phytovolatilization contributes to a limited set of volatile elements and compounds, notably mercury (Hg) and selenium (Se) species, through biochemical reduction or transformation processes that generate volatile forms released via transpiration [52]. The desirability of phytovolatilization depends on the specific compounds involved and the regulatory policies in place. Most engineered systems prioritise retention and in situ transformation over atmospheric transfer [20,39].

2.2. Antiviral-Specific Mechanisms

Antiviral phytoremediation functions as a multi-barrier system in which plants and their associated microbiomes attenuate viral contaminants through four interconnected pathways: sorption–filtration, rhizosphere-mediated inactivation, viral internalisation, and intracellular degradation (Figure 3). This framework extends classical phytoremediation ideas to encompass

viruses by adapting plant-driven mass transfer, surface interactions, and immune-like responses to reduce the number of infective particles in complex wastewater matrices [23,53,54].

Sorption–filtration at plant, biofilm, and substrate interfaces

Sorption–filtration is the initial barrier, intercepting virions on high-surface-area matrices, including roots, biofilms, and porous substrates [55,56]. Hydrophobic interactions, electrostatic forces, and cation bridging immobilise viral particles, but size exclusion within tortuous pore networks physically retards their transport [39]. In constructed wetlands, plant-supported biofilms and mineral surfaces provide dominant specific surface areas similar to the mechanisms of immobilising dissolved metals via sorption and complexation [57,58]. The performance depends on the hydraulic retention time and the interfacial surface area. Field and pilot studies have consistently shown longer retention times and denser rootbiofilm matrices correlate with enhanced pathogen attenuation. But clogging and hydraulic short-circuiting reduce contact efficiency, helping with viral breakthrough [59]. Across systems, viral reductions attributable only to physical barriers typically range from ~1–3 log₁₀ and vary with virion surface chemistry, ionic strength, and organic matter content, highlighting the necessity of downstream biological mechanisms [60].

Rhizosphere-mediated inactivation

The rhizosphere acts as a chemically reactive zone where plant exudates and microbial metabolites, such as reactive oxygen species and lytic enzymes, can damage viral capsids and genomes. Root-derived phenolics and flavonoids could directly inhibit viral attachment and fusion, and modulate microbial consortia, providing a biochemical complement to physical capture [23,28,61,62]. Engineered and naturally assembled plant–microbe consortia enhance these effects through division-of-labour metabolism and enzyme secretion, amplifying viral inactivation within the root boundary layer [49,63]. Non-enveloped viruses, characterised by rigid icosahedral capsids and exceptional environmental stability, generally require stronger rhizosphere chemistry and prolonged contact times to achieve attenuation comparable to that of enveloped viruses [8,64,65].

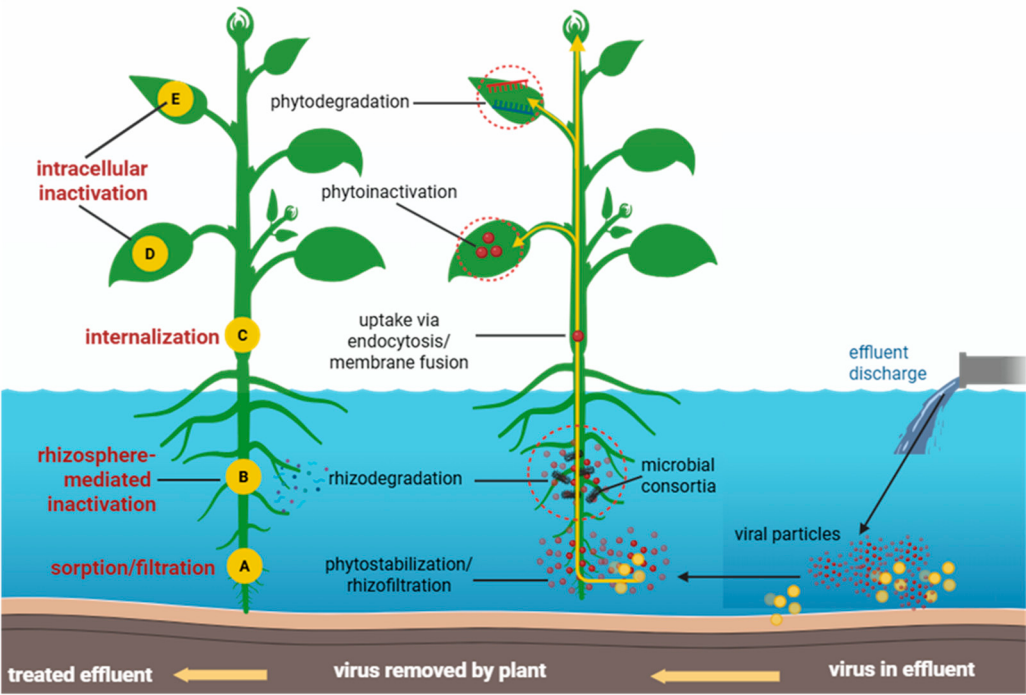


Figure 3. Conceptual antiviral phytoremediation of viral pathogens in wastewater. Effluents with infectious viral particles could be rendered inactivated/degraded via a series of complex removal mechanisms. Active/partially active viruses initially encounter sorption/filtration (phytostabilization/rhizofiltration) followed by subsequent reduction involving rhizosphere mediated inactivation (microbial consortia/root exudes trigger

rhizodegradation), internalization (viral particle uptake via endocytosis/membrane fusion), and intracellular inactivation (phytoinactivation and phytodegradation).

Viral internalization (cell entry and early destabilization)

Following surface capture, some virions could be internalised into root tissues via endocytic and non-endocytic pathways [66,67]. Enveloped viruses display greater susceptibility to plant-mediated internalisation and destabilisation, largely due to their fragile lipid envelopes, but non-enveloped viruses, protected by strong icosahedral capsids, exhibit greater persistence and resistance to internalisation and early chemical degradation [8,64,68]. Plant chitinases and β -1,3/1,6-glucanases (glycoside hydrolase family 19 [GH19]), along with lectins that recognise N-acetyl-glucosamine motifs, can target glycoprotein domains on enveloped viruses during or after entry, helping with particle disassembly [23,66]. Heterologous expression of antiviral membrane proteins (e.g., IFITM3 [Interferon-Induced Transmembrane Protein 3]) may enhance uptake-linked destabilisation by modulating late endosomal lipid dynamics in model plants, suggesting the potential for engineered improvements to this barrier [69,70].

Intracellular inactivation (genome silencing and proteolysis)

Once internalised, plant antiviral responses operate on multiple fronts. Small RNA pathways (RNA interference; *RNAi*) mediate post-transcriptional silencing of viral genomes, while the ubiquitin–proteasome system and macroautophagy (autophagy-related *atg*-dependent) help to degrade viral proteins and particles [71–73]. Phytochemicals, such as the bioflavonoid rutin from *Ocimum basilicum* (via phytoinactivation), bind to structural proteins across diverse viruses with favourable binding energies (-7 to -10 kcal·mol⁻¹), inhibiting attachment, fusion, and genome release [74]. Peroxidases and proteases also contribute to capsid degradation, particularly under oxidative conditions [23,49]. Owing to the pH and temperature tolerance of non-enveloped viruses and their resistance to lipid-targeting phytochemicals, achieving ~ 1 – 3 log₁₀ removal typically requires the concerted action of all four barriers combined with enough retention time, highlighting the importance of designing antiviral phytoremediation as a sequential coupled system [75,76]. From a systems perspective, intracellular pathways establish the ceiling for true viral inactivation (loss of infectivity), but upstream barriers regulate the encounter frequency and loading of the cellular machinery. Designs incorporating high-surface-area capture, chemically active rhizospheres, and enough retention time before intracellular processes could yield the most reliable viral removal performance across virus classes and seasonal variations [39,77].

2.3. Comparative Analysis of Viral Particles and Heavy Metals

Removal mechanisms

Plants and plant–microbe systems remove heavy metals and viruses through superficially similar stages: interception at interfaces, transformation in the rhizosphere, and organism-level processing. However, the underlying physicochemical and biochemical mechanisms differ significantly, with important implications for system design (Table 1). Heavy metals, primarily existing as ions or complexes, are predominantly removed via sorption and complexation to roots, biofilms, and mineral media [78]. Subsequent uptake, chelation (e.g. by organic acids and phytochelators), and vacuolar sequestration stabilise metals within plant tissues, culminating in phytostabilization and phytoextraction [79,80]. Viruses (nanoscale colloids composed of proteinaceous capsids with or without lipid envelopes) are first immobilised through electrostatic and hydrophobic interactions, followed by inactivation mediated by exudate chemistry and microbial enzymes [81,82]. A subset undergoes internalisation and intracellular degradation [83].

These differences arise from particle identity: metals are elemental and cannot be degraded, only transformed in speciation and sequestered [84], but viral particles are supramolecular assemblies whose infectivity can be neutralised through capsid or envelope damage and genome cleavage [82]. Achieving “true removal” of metals focuses on preventing remobilization (e.g., via pH stability), but viral removal emphasises the loss of infectivity in addition to physical capture [85,86]. Structurally,

the strong icosahedral capsids of many non-enveloped viruses confer resistance to desiccation, pH fluctuations, and moderate oxidants, but lipid envelopes are more labile [87]. This contributes to the generally higher susceptibility of enveloped viruses to rhizosphere chemistry and intracellular defences compared to their non-enveloped counterparts [77]. At the interface scale, the adsorption parameters also differ. For model bacteriophages and coronaviruses, the Freundlich adsorption coefficients range from $\sim 2 \times 10^3$ – 2.7×10^5 mL·g⁻¹ and vary with ionic strength, organic matter content, and temperature [88]. Divalent metal cations show more predictable surface complexation trends, primarily governed by pH and competing ligands [89]. Therefore, design strategies emphasise charge neutralisation and aggregation aids (e.g. biofilm matrices and upstream destabilisation) for viruses, but pH and redox control, alongside ligand management, predominate for metals.

Factors influencing removal efficiency

Pilot- and field-scale macrophyte systems commonly report ~ 1 – 2 log₁₀ reductions in viral load attributable to physical capture alone, with higher total reductions achieved when rhizosphere inactivation and intracellular degradation pathways are activated (Table 1) [90]. Plant traits that increase the active surface area, such as fine-root density and healthy periphyton growth, combined with hydraulics that prevent short-circuiting, enhance encounter rates and viral attenuation [91]. Temperature fluctuations influence virion partitioning and adsorption efficiency, contributing to seasonality in virus removal, a phenomenon that is less pronounced in metals [92]. For heavy metals, removal efficiencies are often higher and more consistent under comparable hydraulic retention times, often reaching ~ 70 – 100% in well-maintained wetlands or rhizofiltration units if metal speciation favours sorption and that plant uptake and compartmentation capacities are not saturated [93]. Virus removal is more sensitive to virion architecture (enveloped vs. non-enveloped), dissolved organic carbon (DOC), and competing colloids. For heavy metals, removal efficiencies are often higher and more consistent under comparable hydraulic retention times, often reaching ~ 70 – 100% in well-maintained wetlands or rhizofiltration units if metal speciation favours sorption and that plant uptake and compartmentation capacities are not saturated [93]. Virus removal is more sensitive to virion architecture (enveloped vs. non-enveloped), dissolved organic carbon (DOC), and competing colloids. Chemical amendments can help to narrow this performance gap. Polyphenols, such as rutin, bind to viral surface proteins with favourable free energies (-7 to -11 kcal·mol⁻¹), promoting virion aggregation and enhancing capture efficiency. Upstream charge destabilisation methods, including electrocoagulation and optimised flocculation, further increase early-stage retention within root-associated matrices [83,94,95]. Maintaining young, metabolically active plant stands with a balanced nutrient status and moderate redox heterogeneity sustains both antiviral metabolite flux and metal-binding capacity [96–98].

Design considerations for combined virus and metal removal

When wastewater has both viruses and dissolved metals, the system design must integrate complementary removal barriers while minimising interference (Table 1). Organic acids and flavonoids that help with viral inactivation also chelate metals and influence speciation; generally, this enhances metal immobilisation but may increase competitive adsorption with natural organic matter, requiring careful control of influent chemistry and retention time [99,100]. Similarly, dense biofilms that capture virions provide ligands for metal binding. However, operational challenges include avoiding clogging while preserving a high surface area [80,101]. Operational tracking should reflect these differences. Viral removal requires infectivity assays or confirmed surrogates to confirm true inactivation, but metal removal tracking focuses on dissolved and particulate speciation and plant tissue burdens to assess stability against re-mobilisation [75,102]. For example, macrophyte beds based on *Pistia stratiotes* achieve ~ 0.5 – 1.0 log₁₀ reduction of bacteriophage surrogates per pass while simultaneously lowering the dissolved metal concentrations [83,103–105]. Coupling with polishing steps such as ultraviolet (UV) irradiation, ozone, or ferrate increases viral removal to ~ 3 – 7 log₁₀, with metal removal maintained or enhanced [106–108]. Cost and reliability considerations often favour modular hybrid systems. Downstream activated carbon or nanofiltration units provide resilience during peaks in organic load or viral spikes and control trace organics that may interfere

with adsorption and enzymatic activities. These technologies have also been established for treating metal-bearing effluents [109,110]. Adaptive tracking and rule-based operational control, such as flow equalisation, intermittent aeration, and targeted coagulant dosing, support sustained performance under seasonal stressors [111–114].

Emerging innovations for enhanced removal performance

Three promising innovation streams address the differential removal challenges posed by viruses and heavy metals. First, engineered plant–microbe consortia that partition antiviral and metal-binding functions can amplify rhizosphere chemistry without compromising stability. Examples include *Streptomyces* and *Bacillus* species, which provide complementary lytic enzymes alongside exopolysaccharide-rich periphyton that enhance metal capture and complexation [115,116]. Second, targeted host enhancement through the choice of genotypes showing high fine-root areas and the introduction of traits that bolster antioxidant and proteostasis pathways can improve intracellular viral degradation while maintaining metal tolerance [117,118].

Table 1. Virus and heavy metal removal mechanisms in plant-microbe rhizosphere system.

Mechanism/Parameter	Virus (Antiviral Mechanism)	Heavy Metal (co-Removal)	Performance Metrics	Critical Variables	Example Systems	Ref.
Primary removal	electrostatic/hydrophobic adsorption; aggregation; enzymatic inactivation	ion exchange; surface complexation; chelation; precipitation	virus: ~1–2 log ₁₀ capture, up to 7 log ₁₀ with polishing; metal: ~70–100% removal	charge density, DOC, pH, root potential, ionic strength	<i>Pistia stratiotes</i> , <i>Typha latifolia</i> , <i>Phragmites australis</i> beds	[80,81,90]
Rhizosphere biochemistry	exudate oxidation, proteolysis; polyphenol virion destabilization ($\Delta G \approx -10$ kcal/mol)	organic acid complexation; phytochelatin synthesis; redox cycling	inactivation rate: $k = \sim 0.02\text{--}0.07$ h ⁻¹ (25°C); infectivity loss: 65 ± 12% (48 h)	root activity, flavonoid flux (~0.8–1.5 mg g ⁻¹ DW), microbial profile, T-sensitivity	natural/ changed wetlands	[83,96,119]
Particle stability	capsid/envelope disruption, genome cleavage; enveloped viruses removed ~2–5× better	speciation-dependent stability; vacuolar sequestration post-uptake	enveloped removal: >90%; RNA decay: ~65–84% (~48–72 h)	temperature, pH, oxidative potential, virion charge	macrophyte–biofilm systems	[83,87]
Adsorption/partitioning	Freundlich $K_n = \sim 2 \times 10^3\text{--}2.7 \times 10^5$ mL g ⁻¹ ; mean capture: 58 ± 20%	surface complexation on log $K = \sim 4\text{--}8$ (pH-driven)	capture efficiency: ~58 ± 20% (n=16); $K = \sim 10^3\text{--}10^4$ mL g ⁻¹ for bacteriophages	ionic strength, DOC competition, surface pKa, hydrophobicity	rhizofiltration, periphyton-root systems	[88,89]
HRT requirement (d)	~3–6 d for viral attenuation; ideal ~5–10 d	~2–4 d for metal sorption equilibrium	virus: ~1–2 log ₁₀ per stage; metal: ~70–100% removal	flow uniformity, aeration regime, recirculation, temperature effects	hybrid wetland + UV, VSSF units	[93,106]
Chemical aids	polyphenols (ΔG); electrocoagulation (EC) → ~2–3× capture boost	biochar, zeolite, Fe(OH) ₃ , molecular imprinted polymers composites	virus capture: +1–1.5 log ₁₀ gain with EC; metal removal: +15–30% with media	coagulant dose (FeCl ₃ ~5–20 mg/L), pH, oxidation reduction potential (ORP)	modular wetland-filter hybrids	[109,120,121]
Microbial contribution	lytic enzymes, quorum-regulated	extracellular polymeric	+0.5–1.0 log ₁₀	microbial diversity,	engineered consortia	[41,116,122]

	proteases (<i>Bacillus</i>); ROS generation	substance matrix, siderophore secretion, biosorption	increment; peroxidase activity ↑25–60%.	nutrient ratio (C:N:P ≈ 100:10:1), rhizosphere age	
Seasonal sensitivity	strong T-dependence (−0.3 log ₁₀ per 10°C drop); dissolved organic carbon (DOC) competition	moderate; resilient under redox/pH shift	winter: retains ~70–85% of summer rate with thermal buffering	temperature, DOC level, biofilm maturity, flow fluctuation	aerated/intermittent-flow constructed wetland systems [91,92,123]
AI control	adaptive flow/dosing for dual targeting; real-time viral prediction (~12–18 h lead time)	dynamic ligand control via real-time speciation	±10% variance reduction under fluctuating loads	pH/ORP sensors, metabolite biosensors, AI feedback	smart AI-integrated wetlands [114,124]

Third, upstream physicochemical aids tailored to viral colloids, such as mild electrocoagulation for charge neutralisation, reduce biological stage loading and stabilise performance under cold or high-DOC conditions while remaining compatible with metal removal pathways [125–127]. Looking forward, artificial intelligence (AI)-guided tracking and adaptive control of flow, aeration, and dosing parameters can optimise coupled system performance across seasonal and influent variability, enhancing both viral inactivation and metal immobilisation [124]. Collectively, these approaches translate mechanistic contrasts into complementary barriers that achieve high and stable removal efficiencies across various classes of contaminants.

3. Recent Advancements in Antiviral Phytoremediation

3.1. Plant Selection and Optimization

Strategic choice of antiviral plants via mechanisms to traits

Selecting plants for antiviral phytoremediation should be grounded in the four-barrier framework outlined in previous Section 2 (i.e., capture, rhizosphere inactivation, internalisation, and intracellular degradation) and mapped to specific plant traits (Table 2). These include a high fine-root specific surface area to enhance sorption–filtration, metabolically active rhizospheres that release phenolics and flavonoids and support dense periphyton to promote chemical and enzymatic inactivation, tissues exhibiting strong endocytosis and proteostasis for efficient internalisation and intracellular degradation, and shoot–root architectures compatible with target hydraulics and redox zoning [56,83,128]. Systems maintaining young, rapidly growing stands with vigorous root turnover and periphyton renewal provide more stable viral attenuation across seasons than overaged canopies with diminished belowground activity [40,129]. Seasonal variability and influent chemistry further refine the plant-selection process. Species that sustain exudation and peroxidase/oxidase activity at lower temperatures (°C) and tolerate dissolved organic carbon (DOC) fluctuations without impairing biofilm health mitigate performance decline during winter and high DOC shocks [130,131]. Matching species to hydraulic regimes is essential; for example, VSSF wetlands benefit from species that maintain porosity and resist clogging under intermittent loading, but free-water surface beds prioritise emergent canopies that enhance light and oxygen inputs for oxidative chemistry [132–134].

High-performance antiviral plant species

Evidence from bench, pilot, and field studies has identified macrophytes that combine extensive, cleanable surface areas with chemically active rhizospheres as high-performing (Table 2). *P. australis*, *Typha* spp., *Cyperus* spp., and selected aromatic or medicinal species, such as *Ocimum basilicum* and *Strobilanthes cusia*, showed superior antiviral phytoremediation potential [135,136]. *P. australis* consistently supports diverse, functionally rich rhizobacterial communities linked to pathogen degradation while preserving hydraulic conductivity in VSSF beds. Other macrophytes, such as *Typha* and *Cyperus*, provide comparable periphyton support and tolerate nutrient and DOC variability typical of municipal influents [137,138]. Certain medicinal plants confer distinct antiviral advantages through exudates or tissue-bound polyphenols and alkaloids. For example, rutin from *O. basilicum* exhibited favourable binding free energies (−9.7 to −10.9 kcal·mol^{−1}) against multiple viral

surface proteins, promoting virion aggregation and helping with capture within root and biofilm matrices [74]. *S. cusia* produces tryptanthrin, which inhibits the coronavirus NL63 protease at low micromolar concentrations [139,140]. Interplanting these species with structural macrophytes, such as *Phragmites*, could result in a division of roles, with large surface areas for virus capture and enhanced rhizosphere chemistry, resulting in improved and consistent virus removal [138,141].

Selection criteria and screening workflow

A strong plant selection workflow integrates (i) desk-based pre-screening, (ii) bench assays, and (iii) pilot verification of the selected plant. Pre-screening filters candidate species based on local availability, invasiveness risk, hydraulic compatibility (root porosity, aerenchyma), and evidence of antiviral metabolites or vigorous rhizosphere metabolism [142,143]. Bench assays measure barrier-aligned metrics, including short-term sorption coefficients ($\text{mL}\cdot\text{g}^{-1}$) on roots and periphyton, exudate-driven loss of infectivity for enveloped versus non-enveloped viral surrogates, early internalisation markers such as viral RNA decay within root tissues over ~24–72 h, and indicators of intracellular degradation, including peroxidase, protease, and RNase activities, alongside stress-response gene expression under realistic ionic strength and DOC levels [66,144,145]. Pilot verification in the intended hydraulic configuration should confirm (a) the stability of log reductions across seasons and loading rates, (b) maintenance requirements, such as harvesting frequency and clogging control, and (c) ecological safeguards to ensure no pathogen amplification or spread of non-native taxa. Comparative tests among *Phragmites*–*Typha*–*Cyperus* mixtures typically reveal complementary hydraulic and rhizosphere chemistries. Including a minority fraction (~10–30% stem density) of antiviral-rich aromatic species can enhance inactivation without compromising hydraulic conveyance [143].

Optimization strategies and pretreatment integration

Three consistent optimisation strategies can improve plant-mediated antiviral performance. First, maintaining young, metabolically active stands through staged planting and periodic harvesting preserves a high root-specific surface area and fresh periphyton [146]. Second, stabilising rhizosphere chemistry by ensuring a balanced nutrient supply and moderate redox heterogeneity, such as intermittent aeration in VSSF wetlands, sustains enzyme production and reactive oxygen species critical for capsid and envelope damage [147–149]. Third, pairing plants with gentle pretreatment methods that enhance early viral capture without damaging roots or biofilms, such as mild electrocoagulation or optimised flocculation to neutralise charge and aggregate virions before root zone contact, further improves performance [150–152]. In systems experiencing frequent viral loads and DOC spikes, interplanting antiviral-rich species (i.e., *O. basilicum* and *S. cusia*) with structural macrophytes (Table 2) and incorporating upstream destabilisation steps (iron-based coagulants or low-dose electrocoagulation) can increase first-barrier retention and reduce the intracellular processing burden [150–152]. Maintaining system flexibility for seasonal rebalancing through transient aeration and selective harvesting keeps the plant–microbe assembly near ideal performance [130,153].

3.2. System Optimization

Hydraulics and contact optimization

Virus attenuation in planted treatment systems is limited by contact efficiency; thus, optimising the flow architecture to maximise the uniform interaction between influents and active interfaces including roots, biofilms, and media is critical (Table 3). In VSSF beds, the influent should be evenly distributed via multiple ports or perforated headers, with hydraulic loading rates (HLR) matched to bed conductivity and maintained porosity to prevent short circuiting. In free-water surface (FWS) cells, the installation of baffles and staged islands can straighten flow paths and reduce wind-driven recirculation, which dilutes root zone contact [59,154,155].

Table 2. Plant traits and viral removal in constructed wetland systems.

Scientific Name (Common Name)	Functional Traits (Key Mechanisms)	Optimal Configurations	Viral Removal Performance (log ₁₀ Reduction)	Co-Removal Benefits	Critical Constraints	Ref.
Monoculture systems						
<i>Phragmites australis</i> (Common reed)	fine root area (>300 cm ² g ⁻¹); dense periphyton; strong O ₂ transfer; high porosity (~25–35%)	VSSF (intermittent loading); baffled FWS	1.2 ± 0.3 log ₁₀ (capture-dominant); field stability ~60–75%	high N/P removal (~70–99%); stable heavy metal uptake (~70–90% Zn/Cu)	seasonal dormancy (winter); requires periodic harvest (~2–4× yr ⁻¹); establishment time 4–6 wks	[40,138,156]
<i>Ocimum basilicum</i> (Sweet basil)	high phenolic/flavonoid exudates (0.8–1.5 mg g ⁻¹ DW); elevated oxidase/peroxidase activity	horizontal/free-water flow with aeration; mixed beds (2:1 ratio)	2.3 ± 0.4 log ₁₀ (chemical inactivation); +40% for enveloped viruses	volatile oil antimicrobial effects; phenolic anti-biofilm agents; biomass valorization potential	high T-sensitivity (~20–30°C ideal); short lifespan (requires replacement ~2–3× yr ⁻¹)	[83,139,140]
<i>Strobilanthes cusia</i> (Assam indigo)	indole alkaloid production (Tryptanthrin 10–50 µM IC ₅₀); elevated RNase/protease activity	floating macrophytes; warm shallow beds (~20–28°C)	+0.7 ± 0.2 log ₁₀ gain over baseline (intracellular enzymatic defense)	medicinal/commercial value co-product potential; strong nucleic acid hydrolysis capability	tropical requirement (dies <10°C); limited geographic deployment; alkaloid bioaccumulation risk	[104,139,140]
<i>Pistia stratiotes</i> (Water lettuce)	extensive adventitious root system; rapid biomass production; high transpiration	floating-bed systems; rhizofiltration units	~0.5–1.0 log ₁₀ per pass; ~3–5 log ₁₀ in CWs–UV hybrid (high sorption capacity, K = ~10 ³ –10 ⁴ mL g ⁻¹)	high heavy metal uptake (~70–85%); scalable for rapid deployment	Invasive potential (requires containment); sensitive to low DOC/high shear; capture-dominant mechanism	[83,157,158]
Optimized polyculture systems						
<i>Phragmites</i> + <i>Typha</i> + <i>Ocimum</i> (triculture)	trait complementarity: max surface area + diverse exudate chemistry + functional redundancy	coupled VSSF–free-surface system	2.8 ± 0.5 log ₁₀ reduction; 85 ± 10% infectivity loss (capture–inactivation synergy)	superior stability; buffering seasonal/load variations; showed performance over 3+ yrs	higher complexity in operation and maintenance (O&M); longer initial establishment (~8 wks); requires strict nutrient control	[138,143,159]

Design heuristics recommend targeting a municipal-strength wastewater HRT that secures physical barrier removal of ~1–2 log₁₀ in the initial stage, followed by an additional ~0.5–1.0 bed volume for rhizosphere chemical and intracellular viral attenuation [128,160,161]. When the influent organic matter is elevated or the temperatures are low, incorporating a ~20–40% HRT safety margin or a compact pre-treatment stage (see *Pretreatment, polishing, and seasonal adaptation*) stabilises upstream capture and mitigates desorption risk [123,162]. Because viral removal scales with the

interfacial area, selecting plant species and media that maintain a high specific surface area, such as fine roots and cleanable media, and avoiding compaction are paramount [163]. Operational strategies should maintain a high “contact efficiency” throughout the system lifespan. Alternating-day dosing in VSSF systems re-aerates pores and supports biofilm recovery, rotating inlet zones minimise localised clogging, and annual tracer tests reassess effective HRT. If the effective HRT falls below about ~70% of the design or home time variance goes up, remedial actions such as hydro-flushing, media scarification, or partial media replacement should be undertaken before an irreversible performance decline occurs [164].

Rhizosphere chemistry and redox management

Sustaining a metabolically active and chemically reactive rhizosphere is the second key optimisation lever, as it helps with capsid and envelope damage and accelerates the intracellular antiviral response (Table 3). Intermittent aeration in VSSF systems using duty cycles that maintain dissolved oxygen (DO) levels above 2 mg·L⁻¹ near inlets while preserving anoxic zones deeper in the bed could support oxidative enzymes and reactive oxygen species generation without inhibiting denitrification or causing biofilm overoxidation [48,165,166]. In FWS cells, shallow shelves combined with emergent canopies enhance light and oxygen penetration, promoting peroxidase activity and exudation, and providing a habitat for periphyton development [167]. Nutrient balance critically influences antiviral metabolite flux and microbial consortia dynamics. Maintaining carbon:nitrogen:phosphorus (C:N:P) ratios prevents carbon starvation, which suppresses enzyme production and avoids eutrophic blooms that can smother roots. Temperature-responsive regulation in beneficial *Pseudomonas* spp. and quorum-sensing mechanisms in biofilms further modulate in situ metabolite release [168–171]. Plant immune signalling pathways, such as salicylic acid/NPR1 and redox cues, potentiate rhizosphere responses under stress, linking systemic acquired resistance with localised oxidative bursts and enzymatic defences [172–175]. During cold seasons, stabilising metabolism by increasing water levels (thermal buffering) and reducing HLR to preserve contact time compensates for slowed reaction rates [123,176]. Tracking should prioritise functional indicators over surrogate parameters. Routine physicochemical profiling (DO, oxidation-reduction potential [ORP], pH, and conductivity) should be complemented by periodic assays of peroxidase and protease activities or proxies for exudate production. Declines in enzymatic activity justify stand rejuvenation via selective harvesting and short-term aeration changes to prevent downstream performance loss [177].

Biomass and media maintenance

Optimized phytoremediation systems maintain an actively renewing surface characterized by abundant fine roots, refreshed periphyton, and unclogged pore networks (Table 3). Staggered harvesting, which involves removing ~20–40% of the aboveground biomass per event two to four times annually depending on the growth rate, promotes root turnover and exudation while preventing thatch accumulation [178,179]. Rapid removal of senescent litter is essential, as its decomposition elevates dissolved organic carbon (DOC) levels and fosters anoxic mats that impair viral capture and enzymatic activity [180,181]. Media stewardship is also important. Use well-graded aggregates resistant to compaction that provide tortuous flow paths and minimise fines (<1–2 mm), except in thin reactive layers [182]. When the head loss goes up, back-flushing with clean water or air-pulse scouring during offline periods may restore the permeability. Persistent clogging near inlets requires dosing line rotation, raising or flipping near-surface media, or replacing the top ~10–15 cm of media to restore hydraulic conductivity and root penetration [183,184]. Excessive biofilm accumulation may slough during load shocks, releasing colloids and viruses. Moderate shear stress via pulsed dosing and avoidance of abrupt changes in the hydraulic loading rate mitigate the risk of sloughing. Recurrent sloughing events require shortening of the dosing pulses, increasing rest intervals, and assessing nutrient balance. Sometimes, a small upstream roughing filter captures sloughed material before it enters the planted beds [183,184].

Pretreatment, polishing, and seasonal adaptation

Gentle pretreatment stabilises the initial viral capture barrier without damaging the plants or biofilms (Table 3). Practical options include (i) low-dose coagulant addition (e.g. iron salts optimised by zeta potential) to neutralise the charge and promote virion aggregation and (ii) mild electrocoagulation in compact tanks operated at conservative current densities. Both approaches could enhance early retention within the root and biofilm matrices, reducing the intracellular processing burdens, particularly under high DOC or low-temperature conditions [121,185–187]. For stringent compliance, especially when non-enveloped viral surrogates dominate or during peak viral loads, macrophyte stages should be paired with compact polishing units such as ultraviolet (UV) irradiation (with confirmed fluence), ferrate or ozone micro-dosing, or short granular activated carbon (GAC) contactors. Hybrid treatment trains show additive viral inactivation relative to individual barriers [188,189]. Oxidant placement is critical, and oxidants should be kept downstream of plants to avoid phytotoxicity. If upstream stabilisation is necessary, low doses with short contact times and rapid quenching should be used [190,191]. Explicit seasonal operating envelopes can enhance the resilience of the system. In summer, the HLR within capacity should be increased, biomass removal intervals should be shortened, and brief daytime aeration should be added to suppress anoxic mats [192,193]. In cold-temperate climates, coupling wetlands with low-enthalpy geothermal systems supports year-round function and reduces the energy penalties associated with greenhouse enclosures [194,195].

3.3. Hybrid Systems and Technology Integration

Rationale and design principles

Hybrid systems may integrate plant antiviral mechanisms (i.e., sorption/filtration, rhizosphere inactivation, viral internalisation, and intracellular degradation) with on-site biophysiochemical barriers and/or other treatment methods to enhance log removal, stabilise performance under cold or high DOC conditions, and reduce spatial footprints for urban applications. Typically, vertical or subsurface constructed wetlands are preceded by charge-destabilisation units such as electrocoagulation or optimised coagulant dosing, and followed by ultraviolet (UV) irradiation or granular activated carbon (GAC) polishing. Membrane bioreactors (MBRs) serve as alternative polishing steps when stringent reuse standards are applied [7,196]. This design logic aligns with mechanistic complementarity: pretreatment enhances early virion capture, planted-vegetation beds help with biochemical inactivation, and polishing secures complete loss of infectivity, especially in non-enveloped viruses [75,197]. Across pilot studies, hybrid plant-based treatment systems have consistently outperformed single-stage planted systems under varying influent qualities.

Table 3. Design and operational drivers of viral attenuation in constructed wetlands.

Factor/Strategy	Target Parameters	Key Action/Specification	Performance Metric	Mechanistic Rationale	Ref.
Hydraulic loading rate (HLR)	HLR & distribution uniformity	VSSF: 0.05–0.15 m ³ m ⁻² d ⁻¹ ; perforated manifold dosing	~1–2 log ₁₀ removal; HRT = ~4–15 d	maximizes root–water contact and filtration efficiency; prevents short-circuiting	[59,154,198]
Flow configuration	flow pattern & dead-zone control	baffled FWS/staged islands; dispersion index >0.7	HRT efficiency ~70–95%; channeling causes up to ~30% loss	promotes plug flow (extended home time); increases uniform virion–biofilm interaction	[161,199]
Hydraulic retention time (HRT)	retention stability & redundancy	design: ~4–10 d (+20–40% safety margin for low T)	stable up to 3 log ₁₀ removal	sustains contact time for adsorption/inactivation kinetics; reduces desorption risk	[123,162]
Rhizosphere aeration	intermittent air cycles & DO	ON/OFF ~1–2 h cycles; DO > 2 mg L ⁻¹ at inlet.	enzyme gain ~20–45%; redox	boosts oxidative/enzymatic antiviral activity	[165,200]

			maintained (+50 to +200 mV)	(peroxidases, ROS); prevents anoxic clogging	
Redox / nutrient balance	C:N:P Ratio & ORP	C:N:P ≈ 100:10:1; ORP target: +100– +250 mV	infectivity loss 2.5 ± 0.4 log ₁₀	optimizes synthesis of antiviral exudates and enzymatic function; stabilizes microbial consortia	[169,171]
Temperature buffering	seasonal heat retention	raise water depth ~10–20% (winter); optional geothermal loop (<10°C differential)	keeps ~70– 85% of summer rate; viral loss: – 0.3 log ₁₀ per 10°C drop	counteracts T- dependent reduction in enzymatic/adsorption kinetics; ensures year- round stability	[123,176]
Biomass management	harvest fraction & frequency	remove ~20–40% biomass ~2–4× yr ⁻¹	+0.5–0.8 log ₁₀ improvement post-harvest	renews roots and exudation capacity (young plants are more active); prevents DOC release from senescence	[178,179]
Pretreatment (chemical)	charge neutralization & aggregation	FeCl ₃ 5–20 mg L ⁻¹ or EC ~1–2 mA cm ⁻²	+1–1.5 log ₁₀ viral gain (primary capture); +20% DOC tolerance.	strengthens primary capture by neutralizing negative virion charge; flocculation enhances settling/adsorption	[121,185]
Polishing/disinfection	secondary oxidation	UV ~30–60 mJ cm ⁻² ; ferrate ~0.5–1 mg L ⁻¹ ; ozone ~0.2–0.5 mg L ⁻¹	~5–7 log ₁₀ total removal; low phytotoxicity	eliminates residual, recalcitrant infectivity (non-enveloped viruses); ensures safety for reuse standards	[188,191]

For example, macrophyte–UV sequences convert stable ~2–4 log₁₀ reductions in the planted stages into ~3–7 log₁₀ overall removal, while reducing land requirements relative to plant-only configurations [56,193]. These benefits are maximised when upstream mixing and contact are well-engineered, and downstream polishing units are sized to residual pathogen loads rather than influent peaks [201,202].

UV and advanced oxidation process (AOP)-enhanced macrophyte systems

UV is a compact and energy-efficient polishing step that inactivates RNA and DNA viruses primarily via nucleic acid photodamage. Performing these systems depends on the delivered fluence, water transmittance, and shielding effects of particulates and coloured dissolved organic matter [203,204]. Typical UVC doses ranging from ~10–48 mJ·cm⁻² achieve complete inactivation of many enveloped viral surrogates and large reductions in more resistant non-enveloped viruses. For example, SARS-CoV-2 exhibits ~50% reduction within 1.4 min at laboratory irradiance levels [197–199]. When combined with planted beds, UV treatment addresses residual virions that persist following rhizosphere-mediated inactivation, effectively ensuring thorough removal [56,205]. Advanced oxidation processes (AOPs), applied pre- or post-UV, further improve performance by degrading UV-absorbing chromophores and reducing DOC, which competes for adsorption sites on roots and periphyton. Constructed wetland–ultraviolet (UV)–hydroxyl radical (HO·) pretreatment shows the concurrent removal of pesticides and total organic carbon (TOC), illustrating the enhancement of mass transfer and enzymatic efficacy downstream [206]. Designers must consider light-absorbing humic substances and melanoidins that reduce UV penetration and photochemical quenching in specific matrices. In such cases, modest coagulation or roughing filtration upstream of UV reactors can restore the transmittance [199,200].

Genome editing and synthetic biology for enhancing plant–microbe defense

Targeted genome editing and microbiome engineering offer promising routes to strengthen biological antiviral barriers, specifically rhizosphere inactivation and intracellular degradation, without compromising the hydraulic performance. CRISPR-Cas (Clustered Regularly Interspaced Short Palindromic Repeats [CRISPR]-associated [Cas] nuclease)-mediated editing in aquatic and

wetland plants has enhanced antioxidant capacities, ion transport, and exudation profiles, traits associated with improved contaminant removal and stress tolerance, thus bolstering antiviral efficacy under cold or high DOC conditions [207,208]. Concurrent advances in synthetic biology have enabled the design of engineered rhizobacterial consortia that partition functions, such as lytic enzyme secretion, reactive oxygen species (ROS) generation, and extracellular polymeric substance production, to augment virion capture and capsid damage at the root interface [209,210]. At the system scale, these enhancements can reduce the reliance on UV/GAC polishing or decrease bed volume, thus improving the feasibility of space-constrained urban catchments [209,211]. Field deployment must navigate biosafety and regulatory challenges, including genetically modified organism (GMO) approval and environmental release tracking. Emerging risk mitigation strategies include pilot studies within containment and engineered microbial kill-switch circuits [212,213].

Digital/artificial intelligence (AI) tracking and scaling feasibility

Digital tracking technologies, including optical and DOC sensors coupled with rule-based control systems, have stabilised the hydraulics and redox conditions in planted beds. Early AI-assisted operational frameworks promise predictive control of flow equalisation, aeration duty cycles, and coagulant dosing; however, successful implementation requires attention to accessibility, local operator skill sets, and cyber-physical system reliability [214]. Scaling analyses have identified major non-technical barriers, such as land scarcity, fragmented governance, and financing mechanisms. Modularization and decentralisation of wastewater treatment (DEWATS) hybrid systems address footprint constraints and enable phased implementation, but community engagement and capacity building foster public acceptance and sustainable operation [215,216]. Fair deployment requires coupling technology choices with community co-design and long-term operation and maintenance (O&M) support to prevent critical parts such as UV lamps, sensors, and control software from becoming single points of failure “minimum viable hybridization” configurations (e.g., coagulation + planted bed + compact UV reactor) often offer ideal reliability-to-cost ratios in resource-limited settings, with advanced oxidation or membrane steps reserved for applications demanding stringent water reuse standards [215,216].

4. Practical Applications and Implementation

4.1. Constructed Wetland Systems (CWs)

Wetland configurations and hydraulic design

Constructed wetlands (CWs) are engineered ecosystems that integrate capture, rhizosphere inactivation, viral internalisation, and intracellular degradation under controlled flow regimes (Table 4). FWS cells combine light exposure, oxygenation, and photochemical pathways with plant–biofilm interfaces, but subsurface-flow (SSF) beds prioritise filtration and intimate water–root contact [217]. Unit-process wetlands show phototransformation and microbial degradation can be arranged sequentially to address the co-location of pathogens and trace organics in municipal effluents [218,219]. However, densely vegetated FWS systems may suffer from canopy shading and recirculation, which suppress photolysis and reduce first barrier contact, potentially resulting in less than 1 log₁₀ removal without hydraulic correction [202]. Horizontal SSF (HSSF) wetlands provide steady filtration but are prone to anoxic conditions. Column and sand-bed studies have reported diminished adsorption and slower inactivation under anaerobic conditions, which follows weaker electrostatic fixation and reduced oxidative chemistry [81,202,220]. VSSF designs, intermittently dosed via perforated manifolds, maintain aerobic microzones, a high specific surface area, and short diffusion distances, which help prevent surface clogging and sustain enzymatic activity [221]. Consequently, field practice combines well-distributed dosing, baffling, and staged cells to maintain an effective hydraulic retention time (HRT) near the design values and stabilise contact during fluctuations in organic matter or temperature [113,202]. Design heuristics recommend sizing the initial planted stage to achieve ~1–2 log₁₀ viral reduction via capture/filtration, followed by an additional ~0.5–1.0 bed volume to support rhizosphere and intracellular processes beyond threshold

HRTs and efficiency plateaus, owing to pore saturation and biofilm maturation [67,202,222]. Cold-season operation benefits from modest HRT goes up and equalisation to counteract reduced reaction rates [223].

Substrate, vegetation, and rhizosphere dynamics

Substrate selection governs the hydraulics and microbiology of the system (Table 4). High-porosity, biofilm-supportive media increase the interfacial area and microbial colonisation, but appropriate hydraulic loading rates limit short-circuiting [224]. Steel-slag derivatives, particularly basic oxygen furnace slag, contribute to pathogen reduction via transient alkalinity (pH ≈ 10.6–11.4) and enhanced phosphorus capture; however, pH management and safety assessment are essential [225,226]. Alternate-day loading in VSSF wetlands promotes re-oxygenation and nitrification–denitrification cycling; during rest periods, redox rebounds and substrate re-oxygenation maintain enzyme activity supportive of antiviral chemistry [221].

Table 4. System configurations and innovations for antiviral hybrid wetlands.

Components	Configuration Description	Primary Antiviral Mechanisms	Demonstrated Performance	Innovation Value/Application	Ref.
FWS wetland	shallow vegetated channels (~0.3–0.6 m); open photic zone	photolysis, oxidation, biofilm sorption (low shear)	~0.5–1.0 log ₁₀ baseline; up to 2.0 with baffling	simple, low-cost system; sensitive to temperature and climate variability	[202,217]
HSSF wetland	saturated porous bed; laminar flow	filtration and anoxic biofilm degradation (stable pH)	1.0 ± 0.3 log ₁₀ (n=15); high stability across pH changes	filtration-dominant removal; good hydraulic control; limited oxidative capacity	[81,220]
VSSF wetland	intermittent dosing (alt-day); aerated percolation	adsorptive capture and oxidative decay on roots/media (high O ₂)	~2–3 log ₁₀ at HRT ~5–10 d	high efficiency (~2–3× HSSF); reduces land area; requires mechanical dosing/aeration	[59,227]
Multistage hybrid CWs	sequential VSSF–FWS or VSSF–UV trains (multi-barrier approach)	combined filtration, oxidation, photolysis, enzymatic action	~3–7 log ₁₀ total removal (highest efficacy)	meets stringent reuse standards; functional redundancy buffers system failures	[113,161]
Substrate innovation	gravel, slag, zeolite, biochar, ferric media	enhanced adsorption; pH ~10–11 microzones; ROS generation	+10–30% extra removal from reactive layers	increases specific surface area; biochar adds catalytic/adsorptive properties; controls metal mobility	[224–226]
Vegetation selection	<i>Phragmites</i> , <i>Typha</i> , <i>Ocimum</i> , <i>Strobilanthes</i> (targeted functional traits).	O ₂ release, enzyme induction, antiviral metabolite exudation	~2–4 log ₁₀ (field mean); 85–95% infectivity loss	shifts CWs from simple filtration to biochemically active reactors; cost-effective performance boost	[138,228]
Digital monitoring/AI	IoT sensors (DO, ORP, metabolites, microbial activity)	predictive control and early alerting (machine learning integration)	~12–18 h lead time before viral breakthrough prediction	improves reliability/uptime; enables adaptive dosing/flow control; important for fluctuating loads.	[229,230]
Synthetic biology integration	engineered microbial consortia & biosensors	self-regulated enzymatic capture loops;	+1–2 log ₁₀ added potential (proof-of-concept)	high potential for targeted virus/pathogen removal; highly specific	[137,231,232]

(PGPR, lytic strains)	enhanced proteolysis	mechanism; requires regulatory acceptance
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Plant selection must balance hydraulic integrity and biochemical potency. *Phragmites australis* and *Typha* spp. provide aerenchyma-driven oxygen transfer, strong root mats, and stable periphyton, but aromatic and medicinal taxa enrich the rhizosphere with polyphenols and other antiviral metabolites [138,233]. Root-associated biofilms structured by exudates (i.e., organic acids, amino acids, and fatty acids) enhance nutrient cycling and contaminant attenuation, and plant growth-promoting rhizobacteria (PGPR) further coordinate division-of-labour metabolism, including lytic enzymes relevant to viral inactivation [41,224]. Polyculture wetlands leverage complementarity and functional redundancy by mixing structural macrophytes with antiviral-rich species, thus widening seasonal operating windows and buffering influent variability (see previous Table 2) [142,234]. Cold-climate adaptations include greenhouse enclosures, choice of plant species tolerant to low temperatures, and experimental use of CRISPR-edited macrophytes with enhanced stress tolerance. However, genome-edited plants require a biosafety review before open system deployment [223,228].

Operational management and digital tracking

Operational management should maintain an actively renewing surface state characterised by staged harvesting (~20–40% of aboveground biomass per event), removal of senescent litter, and periodic media maintenance to prevent pore blockage and anoxic mats [67,235]. Routine functional tracking, including dissolved oxygen, oxidation–reduction potential, pH, conductivity, and head loss, should be complemented by periodic enzyme activity assays (peroxidase and protease) or exudate markers to detect declines in rhizosphere reactivity before performance degradation [221,224]. Machine learning (ML) models trained on multi-seasonal plant, hydraulic, and chemical data have shown high predictive accuracy ($R^2 \approx 0.85\text{--}0.95$) for nitrogen and chemical oxygen demand (COD) and benefit from virtual-sample augmentation to improve predictions under sparse field data conditions [229]. Root exudate biomarkers integrated with artificial intelligence (AI) have been proposed as early warning indicators for viral breakthrough (lead times of ~12–18 h), and field-deployable CRISPR–Cas12a assays can detect viral RNA within ~30 min to support event-driven operational controls [229,230]. These digital layers enable rule-based changes, including flow equalisation, aeration duty cycle modulation, and coagulant dosing, to have system performance despite influent variability [113,236]. Seasonal operational playbooks may enhance reliability. Winter mode reduces hydraulic loading rates by ~10–30%, increases water depth for thermal buffering, and lengthens rest periods, but summer mode tightens harvesting intervals and may incorporate brief daytime aeration to suppress anoxic mats [113].

Implementation and governance

Pilot projects in resource-limited contexts have shown the feasibility and community benefits of CWs, including livelihood co-benefits and local stewardship. Participatory projects in the Philippines illustrate how co-design and community operation and maintenance (O&M) improve system longevity and compliance [237]. Persistent adoption gaps come from regulatory exclusion, limited long-term pathogen tracking and fragmented governance. Updated guidance emphasising adaptive management and performance verification would accelerate mainstreaming [202,238]. Looking forward, synthetic biology offers tools for augmenting CW robustness. Engineered microbial consortia partition lytic enzyme production, reactive oxygen species generation, and extracellular polymer synthesis to enhance capture and inactivation, but biosensors and gene circuit sentinels provide online diagnostics [231,232]. Root exudate manipulation and microbiome engineering represent active frontiers with the potential to improve antiviral performance without compromising hydraulic performance [137]. A pragmatic implementation pathway involves minimum-viable hybridization such as low-dose coagulation upstream of planted beds combined with compact UV downstream, while concurrently developing data and governance frameworks that certify viral infectivity loss alongside conventional water quality metrics [137,138,202]. Collectively, these strategies translate mechanistic insights into deployable, resilient antiviral constructed wetlands

characterised by hydraulically disciplined, biochemically active, digitally supervised, and socially embedded systems capable of delivering $\sim 3\text{--}7 \log_{10}$ virus reduction when paired with polishing, where required [113,161,202].

4.2. Modular and Scalable Designs

Modular treatment architectures

Modular treatment enables the assembly of plant-based units integrated with compact physicochemical steps into site-specific modules that can be scaled, duplicated, or reconfigured as load demands and reuse targets evolve [239,240]. This approach aligns with decentralised wastewater (DEWATS) strategies applicable in dense urban and dispersed peri-urban contexts, facilitating phased implementation and plug-in upgrades that reduce capital risk and accelerate service time [107]. Governance analyses highlight the role of modularity in lowering institutional barriers by enabling performance-verified subunits, such as a planted bed combined with UV as a minimum viable hybrid which communities can adopt and expand as financing and operational capacity mature [107].

The design follows the antiviral barrier framework: an initial compact charge destabilisation or roughing step enhances early virion capture; planted units provide sorption–filtration and rhizosphere inactivation; and a downstream UV or granular activated carbon (GAC) module ensures the loss of infectivity for recalcitrant, non-enveloped viruses [241–243]. Standardised hydraulic and electrical interfaces between modules, including header geometry, bypass loops, and 24 V control systems, help with field assembly and maintenance by local operators [244,245]. Module sizing targets stable partial reductions of $\sim 2\text{--}4 \log_{10}$ during the planting stage, with enough headroom for seasonal performance dips. Polishing units are sized to treat residual pathogen loads rather than influent peaks, optimising energy use and footprint [209,246].

Compact wetland designs for urban areas

Compact constructed wetlands, including intensified vertical subsurface flow (VSSF) beds, baffled FWS cells, and unit-process wetlands, maximise the specific interfacial area and narrow home time distributions, thus enhancing viral contact efficiency within limited land areas [99,209]. Urban pilot studies have shown using high-porosity media, multi-port influent dosing, and short flow paths maintains effective first-barrier capture and rhizosphere chemistry under varying organic matter loads. These effects are amplified when combined with downstream UV polishing [244,245,247]. Rooftop and podium-deck wetlands leverage gravity-fed pulsed dosing and solar exposure to support periphyton productivity, but green corridors along drainage channels and rights-of-way convert linear hydraulics into staged contact zones with minimal land-use conflicts [248,249]. To mitigate performance declines during cold seasons or high dissolved organic carbon (DOC) events, modular pre-treatment stages, such as coagulation or electrocoagulation cartridges, stabilise virion capture upstream of compact beds, but UV or GAC skids ensure regulatory compliance during peak viral loads [250]. The operations remained straightforward, with alternating day dosing preserving media porosity and oxygen supply. Quarterly tracer tests have verified that the effective hydraulic retention time (HRT) remains near the design value despite biofilm growth [251,252].

Mobile and adaptive treatment units

Trailer-mounted or containerised modular units provide rapid deployment capabilities suitable for outbreak response, festivals, or disaster relief, with flexibility for redeployment as demand shifts [244,253]. To operationalise mobile modules as decision-ready assets, rapid pathogen analytics, such as Reverse Transcription Recombinase Polymerase Amplification (RT-RPA)–CRISPR–Cas12a assays, enable viral RNA detection within ~ 30 min, integrating with low-power field readers for event-driven operational control [254–256]. These data streams support rule-based changes to flow equalisation, aeration duty cycles, and coagulant dosing, thus maintaining antiviral treatment efficacy despite episodic influent variability [216,257]. Given the variable influent chemistry in mobile deployments, interchangeable upstream destabilisation cartridges (i.e., coagulant or electrocoagulation) and

downstream UV modules preserve reliability when non-enveloped viral surrogates predominate or when coloured dissolved organics attenuate UV transmittance [169,258].

Advanced materials and bio-digital rhizosphere enhancements

Additive manufacturing (3D printing) helps to produce root-zone scaffolds and advanced media with high, cleanable surface areas and engineered pore networks that resist compaction and clogging under pulsed hydraulic regimes, thus improving initial viral capture and periphyton stability within a compact footprint [259]. Incorporating redox-active fillers or slow-release micronutrients into these scaffolds sustains enzymatic activity and reactive oxygen species generation, which are critical for capsid and envelope damage without phytotoxic effects [108,260]. The synthetic biology and biosensing layers further enhance antiviral capacity and enable closed-loop control. Engineered plant-microbe consortia partition lytic enzyme secretion, extracellular polymeric substance production, and reactive oxygen species generation, while gene circuit-based biosensors provide real-time reporting of stress or viral surrogate signals [261,262]. In decentralised contexts, these biosignals can tell simple control systems that autonomously adjust dosing or aeration, complementing modular governance frameworks [263–265]. A pragmatic implementation pathway involves “minimum-viable hybridization” constructed from standardised modules: upstream destabilisation via cartridge coagulation or mild electrocoagulation, a compact planted bed (i.e., intensified VSSF or baffled FWS), and a small ultraviolet (UV) skid. This configuration is equity-friendly, scalable, and capable of achieving $\sim 3\text{--}7 \log_{10}$ overall viral reductions when tuned to residual pathogen loads rather than influent peaks [75,205,266].

4.3. Hybrid Treatment Systems and Economic Considerations

Cost-performance and governance alignment

Hybrid treatment integrates plant-based barriers with compact physicochemical processes to convert stable partial reductions achieved in planted units, such as viral capture and rhizosphere inactivation, into compliance-grade removals while minimising land use and operational complexity [108,266]. Specifying treatment targets based on established reuse and effluent standards explains the critical final stage of polishing work by hybrid systems particularly in agricultural reuse or urban unpotable application [267,268]. In resource constrained environments, technical limitations and fragmented governance often impede the implementation of one step wastewater treatment systems. Modular hybridisation reduces these risks and shares performance requirements amongst smaller, verifiable units [267,268]. Economically, hybrid systems affect the cost curve favourably by diminishing the treatment footprint (and attending land costs) and normalising seasonal performance, negating the need for over-sizing to accommodate winter lows. In land constrained settings compact pretreatment combined with vertical subsurface flow (VSSF) constructed wetlands and ultraviolet (UV) disinfection usually minimises total lifecycle costs for a given viral log-reduction goal [205,269,270]. Governance mechanisms, for example clear reuse standards, performance-based procurement and community-based operation and maintenance (O&M) contracts enhance the feasibility still further by aligning the incentives between regulatory agencies and operators [270,271].

Plant-based and physicochemical hybrid systems

Field and pilot-scale studies have shown macrophyte treatment stages constructed with *Typha* spp. and *Phragmites* spp. could achieve $\sim 2.0\text{--}3.5 \log_{10}$ reductions in viral surrogates under optimised hydraulic conditions. Subsequent UV disinfection typically provides an additional $\sim 1\text{--}3 \log_{10}$ reduction, depending on the UV fluence and water transmittance [75,272]. Floating wetland configurations employing *Eichhornia crassipes* combined with compact UV units have shown further $\sim 1.5\text{--}2.1 \log_{10}$ reductions for norovirus surrogates while maintaining low capital expenditure (CAPEX) and enabling biomass valorization [273–275]. Where influent turbidity and coloured dissolved organic carbon (DOC) reduce UV effectiveness, upstream light-touch charge destabilisation via optimised coagulant dosing or mild electrocoagulation enhances early viral capture and restores downstream UV efficiency [276,277]. For applications requiring higher treatment assurance, electrochemically enhanced membrane bioreactors (e-MBRs) have been

reported to achieve $\sim 2.8\text{--}5.0 \log_{10}$ reductions in SARS-CoV-2 through combined filtration and in situ oxidation. When deployed as polishing units following the planting stages, e-MBRs can be downsized to treat residual viral loads, thus preserving energy efficiency [7,196]. The design sequence leverages mechanistic complementarity: upstream destabilisation for viral capture, planted beds for biochemical inactivation, and UV, advanced oxidation processes (AOP), or membrane bioreactors for losing infectivity [278].

Financing, operation and maintenance (O&M) economics, and scale-up

Hybridisation helps with stepwise financing approaches, beginning with a “minimum-viable hybrid” (e.g., coagulation cartridge, planted bed, and small UV unit) and allowing the incremental addition of modules as reuse standards tighten or influent flows go up [279]. Blended financing mechanisms, including municipal budgets combined with green bonds, microfinance for community O&M, and public-private partnerships (PPP), help bridge CAPEX gaps while securing maintenance commitments [280]. Lifecycle cost assessments should incorporate avoided land purchase costs, reductions in winter over-sizing, and revenues or cost offsets from biomass valorization products such as compost or biochar, thus improving the net present value of plant-centric treatment systems [7,196]. System reliability depends on lean O&M practices: periodic harvesting, inlet rotation, cartridge replacement for pretreatment units, UV sleeve cleaning, lamp maintenance on monthly or quarterly schedules, and simple sensor-based feedback loops (dissolved oxygen [DO], oxidation-reduction potential [ORP], head loss) to trigger rule-based operational changes [113,281–283]. Incorporating digital tracking layers can further reduce operational risks by forecasting influent perturbations and optimising set points if these tools remain accessible to local operators to prevent the introduction of new failure modes [113,284].

Equity, biosafety, and technological innovation

The fair deployment of hybrid treatment systems requires modular governance frameworks coupled with context-sensitive technology selection, including appropriately sized UV units, affordable consumables, and avoidance of dependence on reagents difficult to obtain [285–287]. Emerging synthetic biology innovations, such as engineered plant–microbe consortia that partition lytic enzymes, reactive oxygen species (ROS) generation, and extracellular polymer production, have the potential to enhance planted-stage viral removal performance and reduce polishing requirements. However, these approaches require rigorous biosafety assessments, continuous tracking, and, where appropriate, incorporating genetic kill switches [285,288,289]. Integrating biotechnological advances with pragmatic financial and operational strategies, including operator training, spare part logistics, and community stewardship, yields resilient and socially embedded wastewater treatment systems [290–292]. Collectively, these pathways enable antiviral hybrid treatment systems that are hydraulically disciplined, biochemically active, and economically viable across diverse income settings, achieving $\sim 3\text{--}7 \log_{10}$ overall viral reductions when sequenced and sized to treat residual loads rather than peak influent concentrations [278,293].

5. Challenges and Prospects

5.1. Current Challenges and Persistent Gaps

Variability of rhizosphere antiviral activity

Antiviral phytoremediation is limited by the intrinsic stability of many non-enveloped viruses and the variable nature of plant–microbe antiviral interactions. Enveloped virions are generally susceptible to oxidative chemistry and enzymatic degradation in the rhizosphere, but non-enveloped particles with strong icosahedral capsids often persist, resulting in only $\sim 1\text{--}3 \log_{10}$ reduction without supplementary treatment steps [294–296]. This structural asymmetry implies that systems relying only on physical capture mechanisms, such as roots, biofilms, and porous media, are vulnerable to desorption or downstream breakthrough under fluctuating hydraulic conditions [297–299]. The antiviral activity of the rhizosphere varies constantly and depends on environmental conditions such as temperature, nutrient supply and redox conditions. These environmental variables influence the

synthesis of antiviral metabolites and lytic enzymes, resulting in seasonal plateaux in viral inactivity despite adequate contact time [300,301]. For example, in the colder seasons and in influent with high dissolved organic carbon (DOC) there will be reduced exudation rates and enzyme turnover, reducing the strength of the intracellular degradation routes which are extra to those providing physical capture [302–304]. The diversity of viruses complicates predictive modelling and system design. The kinetics of persistence and inactivation is different between strains of virus (e.g., rotavirus vs. enterovirus) such that plant–microbe assemblages designed for one surrogate virus may perform poorly against others unless the systems are made to incorporate together multilayer effects [226,305]. These biological constraints show that hybrid systems and modes of operation should be used in winter and seasonal work rather than in single barrier effects [278,306,307].

Engineering and operational constraints

Constructed antiviral phytoremediation systems are limited by effective contact between viruses and reactive surfaces. Even minor dead zones or hydraulic short-circuiting can reduce effective hydraulic retention time (HRT), reducing first-barrier viral removal from less than $\sim 1\text{--}2 \log_{10}$, despite favourable plant traits [278,308,309]. Clogging phenomena, including inlet obstruction, pore occlusion by fines, and biofilm aging, reduce media porosity and interfacial area, thus lowering virus capture and suppressing rhizosphere activity. Remediation of hydraulic performance typically requires disruptive media maintenance, which is rarely incorporated into routine operational planning or budgeting [40,238]. Material selection involves critical trade-offs between various factors. Alkaline industrial by-products and reactive substrates may transiently enhance pathogen reduction, but require precise pH control to avoid phytotoxic effects and regulatory non-compliance downstream [310,311]. But biologically favourable media with a high specific surface area can accelerate clogging if the dosing and harvesting protocols are not optimised to maintain a “young active surface” [113,312]. Operational discipline is paramount, and strategies such as alternating day dosing in vertical subsurface-flow beds, seasonal changes in hydraulic loading rates (HLR), selective harvesting, and tracer-based verification of HRT are rarely standardised beyond research pilots. This disconnect between laboratory-grade performance and long-term field reliability hampers confidence in antiviral phytoremediation for water reuse applications [113,313].

Tracking, standards, and knowledge gaps

Many demonstration studies have reported reductions in viral molecular signals (RNA/DNA) but have not assessed infectivity, which is the definitive measure of viral removal efficacy [314,315]. Standardised protocols that integrate surrogate virus selection, dose–response relationships, and side-by-side infectivity assays remain limited in long-term field deployments, complicating cross-study comparisons and meta-analyses [316]. Economic and governance data are also limited. Few investigations provide life-cycle cost analyses encompassing seasonal operational modes, routine media maintenance, and analytical tracking, thus obscuring budgeting and impeding adoption in decentralised systems, where modular hybrid designs are most effective [293,317]. The absence of widely accepted verification frameworks, such as minimum HRT testing, enzyme activity proxy panels, and event-driven ultraviolet (UV) backstops, further widens the gap between laboratory validation and field application [318,319]. Pathways for translating promising enhancements, such as biochar amendments that stabilise redox conditions and adsorptive capacity, or synthetic biology approaches that reinforce rhizosphere and intracellular antiviral barriers, into permitting and biosafety frameworks for open systems remain undefined [320,321]. Addressing these gaps through the development of infectivity-based tracking protocols, operational playbooks, and regulatory guidance for novel materials and engineered microbial consortia is critical for the mainstream adoption of antiviral phytoremediation technologies.

5.2. Prospects and Emerging Innovations

Genetic engineering to enhanced antiviral activities

Genetic engineering offers targeted strategies to enhance the two primary biological barriers in antiviral phytoremediation, rhizosphere inactivation, and intracellular degradation, without

compromising hydraulic performance. Building on foundational phytoremediation principles, CRISPR–Cas genome editing can modulate the antioxidant capacity, exudation profiles, and proteostasis pathways, which are critical for capsid damage and viral genome cleavage [322]. Plants engineered for increased late-endosomal restriction, reactive oxygen species (ROS) generation, and protease/ribonuclease (RNase) activity are poised to reduce the contact time required for enveloped viruses, with potential benefits extending to more resilient non-enveloped viruses. Complementary approaches include the expression of antiviral decoy receptors or lectin-like proteins that bind viral surface motifs extracellularly, promoting aggregation and capture within the root or periphyton matrix before internalisation [323,324]. The localisation of these proteins in root exudates or apoplastic spaces may minimise growth penalties while intercepting virions at critical interfaces in planted treatment systems. Translational research should prioritise bench-to-pilot pipelines that link genetic modifications to measurable barrier-specific endpoints, such as enzyme activity proxies and infectivity loss assays for both enveloped and non-enveloped surrogate viruses, under environmentally relevant conditions of ionic strength, dissolved organic carbon (DOC), and temperature [325].

Biosafety and biocontainment strategies

Genetic enhancement programs must integrate strong and effective biocontainment strategies. These include conditional containment systems, sterile triploid or polyploid cultivars, and chloroplast-targeted transgenes, which minimise gene flow without compromising plant vigour or remediation efficacy [326,327]. Spatial confinement via biofilm-centric cultivation and physical barriers can further restrict dispersal while supporting dense periphyton communities that enhance antiviral activity [328]. Risk assessment frameworks should incorporate off-target effect evaluations, ecological interaction studies, staged field releases, environmental DNA tracking, and rollback plans, treating engineered consortia as time-limited interventions subject to periodic re-licencing [329]. Ethical oversight and community consent models developed for nature-based solutions provide valuable frameworks for transparent decision-making and shared stewardship during pilot deployments [330,331]. Governance toolkits that combine technical safeguards (e.g., sterility and genetic kill-switches), operational controls (e.g., harvest scheduling and inlet rotation), and social contracts (e.g., community operation and maintenance agreements) will help with responsible scaling from greenhouse experiments to neighbourhood-scale implementations [332,333].

Advanced materials and autonomous systems

Next-generation media and root-zone scaffolds, fabricated via additive manufacturing to provide tunable porosity gradients and high, cleanable surface areas, can enhance early viral capture while resisting compaction and clogging under variable hydraulic loading [334]. Incorporating redox-active fillers or micronutrient dopants sustains enzyme production and ROS generation, thus boosting rhizosphere antiviral activity without inducing phytotoxicity [335,336]. Biomimetic self-cleaning surfaces inspired by lotus leaf morphology show promise for fouling resistance in compact pretreatment and polishing cartridges positioned upstream or downstream of the planted units [337,338]. Artificial intelligence (AI)-enabled tracking is emerging as a critical control layer for modular and decentralised treatment systems. Edge-computing sensor networks tracking dissolved oxygen (DO), oxidation-reduction potential (ORP), head loss, and proxies for exudate or enzyme activity can feed predictive models to expect clogging and viral breakthrough events, enabling condition-based maintenance that reduces downtime by ~30–60% [338,339]. Techno-economic forecasting and adaptive operational dispatch—adjusting ultraviolet (UV) or coagulant dosing based on residual rather than influent peaks—can lower operational spending while maintaining regulatory compliance during seasonal performance lows [278,340]. In land-constrained settings, intensified VSSF units, baffled free-water surface cells, and minimum-viable hybrid systems (combining destabilisation, planted beds, and small UV units) give scalable and reliable building blocks simplified failure modes, suitable for peri-urban corridors and informal settlements [99].

Policy, climate resilience and circular value

Policy alignment is important for the widespread adoption of antiviral phytoremediation techniques. Harmonised verification protocols, including minimum hydraulic retention time (HRT) testing, infectivity-based endpoints, and enzyme activity proxy panels, integrated into state manuals and international guidance documents, would bridge the gap between laboratory validation and field application [1,341]. Climate resilience requires both biological and infrastructural adaptation. Drought-tolerant species, such as *Vetiveria zizanioides* and *Pennisetum purpureum*, sustain fine-root areas and exudation during water scarcity, but salt-tolerant taxa and hybrid desalination-phytoremediation systems address the challenges of coastal intrusion [342]. Circular economy strategies, including biochar production from macrophyte biomass (i.e., surface area ~200–400 m²/g), nutrient recovery, and the development of local soil amendment markets, enhance the lifecycle value and stabilise redox and adsorption properties when applied judiciously as media amendments [343,344]. Integrated roadmaps combining technology, finance, and policy—starting with minimum-viable hybrid systems and scaling via modular blocks under community operation and maintenance (O&M) and transparent performance verification offer the most credible pathway toward fair, climate-resilient antiviral phytoremediation deployment [345].

6. Conclusions

Antiviral phytoremediation is a promising method for removing viruses in wastewater owing to its complex series of multi-defense mechanisms (i.e., sorption-filtration, rhizosphere-mediated inactivation, viral internalisation, and intracellular degradation). Collectively, these mechanisms could convert hydraulic contact into loss of viral infectivity. Enveloped virions are more susceptible to rhizosphere chemicals and intracellular defence mechanisms but non-enveloped viruses with strong capsids present problems. Planted treatment measures lead to ~2–4 log₁₀ reduction in viral numbers, while their use with UV irradiation or GAC enables ~3–7 log₁₀ reduction without unacceptable growth of treatment footprint. Efficient system design depends on even flow distribution through the bed crown, verified HRT, and active interfacial surface area for initial ~1–2 logs of viral removal. Stabilising treatment rhizosphere chemistry requires balanced C:N:P ratios, periodic aeration, and seasonal operation modes to maintain enzyme activity. Optimising the polishing step, based on residual viral load, involves calibrated fluence level UV or compact sorptive units to reduce non-enveloped viruses. For decentralised applications, a system based on minimum viable hybrid modules would use cartridge coagulation, planted beds, UV units to simplify O&M and enable incremental adoption through standard interfaces. Future goals include viral infectivity tracking protocols, establishing operational basis for equipment maintenance through seasonal variations, and integrating new materials and autonomous systems in regulatory frameworks for reliable antiviral phytoremediation. This would create a system with satisfactory laboratory and field reliability, enabling worldwide water reuse and pestilential control implementation.

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Abbreviations

AOPs	Advanced Oxidation Processes
AI	Artificial Intelligence
CAPEX	Capital Expenditure
C:N:P	Carbon:Nitrogen:Phosphorus Ratio
COD	Chemical Oxygen Demand
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
CRISPR-Cas	Clustered Regularly Interspaced Short Palindromic Repeats [CRISPR]-associated [Cas] nuclease
CWs	Constructed Wetland Systems
DEWATS	Decentralisation of Wastewater Treatment
DO	Dissolved Oxygen
DOC	Dissolved Organic Carbon
EC	Electrocoagulation
e-MBRs	Electrochemically Enhanced Membrane Bioreactors
EPS	Extracellular Polymeric Substance
FWS	Free-Water Surface (or Free-Water Surface cells/systems)
GAC	Granular Activated Carbon
GH19	Glycoside Hydrolase Family 19
GMO	Genetically Modified Organism
HAA	Haloacetic Acids
HLR	Hydraulic Loading Rate
HO·	Hydroxyl Radical
HRT	Hydraulic Retention Time
HSSF	Horizontal Subsurface-Flow
IC ₅₀	Inhibitory Concentration 50%
IFITM3	Interferon-Induced Transmembrane Protein 3
MBRs	Membrane Bioreactors
ML	Machine Learning
O&M	Operation and Maintenance
ORP	Oxidation-Reduction Potential
PGPR	Plant Growth-Promoting Rhizobacteria
PPP	Public-Private Partnerships
RNA	Ribonucleic Acid
RNAi	RNA Interference
RNase	Ribonuclease
ROS	Reactive Oxygen Species
RT-RPA	Reverse Transcription Recombinase Polymerase Amplification
SA	Salicylic Acid

SARS-CoV-	
2	Severe Acute Respiratory Syndrome Coronavirus 2
SSF	Subsurface-Flow
THM	Trihalomethanes
TOC	Total Organic Carbon
UV	Ultraviolet
UVC	Ultraviolet C
VSSF	Vertical Subsurface-Flow

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