

Urban Wastewater Management: Microbial Ecology, Public Health Implications, and Sustainable Reuse

ABSTRACT

Wastewater, a reservoir of pathogenic microorganisms and antibiotic resistance genes (ARGs), poses a threat to public health and the environment. This review explores microbial diversity, health risks, and treatment strategies in urban wastewater management. Wastewater contains organic and inorganic constituents including pathogenic bacteria, viruses, parasites, and ARGs. The global burden of diseases transmitted through contaminated wastewater, including intestinal and skin infections, and waterborne illnesses, is substantial. The COVID-19 pandemic has highlighted the importance of wastewater surveillance for detecting SARS-CoV-2. Hospital and pharmaceutical wastewater are hotspots for antimicrobial-resistant bacteria and ARGs. Conventional wastewater treatment processes, such as activated sludge treatment and disinfection, are not always effective in removing microbial contaminants. Innovative treatment methods like membrane filtration, advanced oxidation processes, and constructed wetlands can improve elimination of pathogens and antibiotic resistance genes (ARGs). Repurposing treated wastewater for agricultural irrigation can help mitigate freshwater shortages while minimizing hazards from untreated wastewater release. This review emphasizes the need for a comprehensive approach to urban wastewater management, integrating treatment technologies, monitoring strategies, and risk assessment to mitigate microbial hazards and promote sustainable water reuse practices. — Repeat sentence

Keywords: Wastewater, Pathogens, Wastewater treatment plant, Sewage, Antimicrobial resistant genes, Diseases, Microbiota.

1. INTRODUCTION

The global scarcity of freshwater resources is a significant concern, primarily because of increasing population and anthropogenic activities. Earth's water comprises 97% seawater, 22% groundwater, and 1% ice in glaciers and polar caps. After the hydrological cycle, the available freshwater is less than 1%, with half of it being present in rivers, lakes, and wetlands (Amoah et al., 2022; Kaur et al., 2020; Toze, 1999).

Although freshwater makes up 75% of Earth's surface, it is often "available in the wrong location, at the wrong time, or of inadequate quality." The freshwater supply is affected by population growth, urbanization, pollution, and climate change. The UN predicts that by 2050,

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half of the global population will face water scarcity (Blumenthal et al., 2000; Kumar & Pal, 2018).

The various compounds released from industrial settings are wastewater pollutants (Table 1 and Table 2). When these pollutants enter the environment, they can have detrimental effects and deplete the resources. Biodegradable pollutants only have short-term negative effects on the environment. Pollutants come in different forms with distinct characteristics, such as heavy metals, synthetic chemicals, and nonbiodegradable polymers, which show minimal absorption (D. D. Mara et al., 2007; Petterson et al., 2001; Yan et al., 2018). The environment has become increasingly polluted with increasing water contamination and damage to ecosystems. Environmental pollutants are a burden to future generations and have absorption properties. Problems arise when pollutants exceed the capacity of the environment to accommodate them, as has been observed with increasing carbon dioxide concentrations (Shuval et al., 1997).

2. CONSTITUENTS OF WASTEWATER

Water usage generates wastewater containing a diverse array of organic, inorganic, and synthetic materials, including dirt, grit, nutrients, oil, chemicals, metals, and waste from plants and animals. The inorganic components of wastewater include salt, metals, and sediment. Organic substances, which are typically biodegradable, consist of bodily and food waste that bacteria can decompose, thereby reducing the oxygen available to other organisms. Consequently, the amount of organic matter in wastewater can be assessed by measuring Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) (Carducci et al., 2018; Westrell et al., 2004; Yapo et al., 2014).

Wastewater environments act as reservoirs for both pathogenic and non-pathogenic bacteria. Notable pathogens include parasitic worms, eggs, enteric bacteria, viruses, and protozoans. Most human pathogens found in wastewater originate from fecal matter, which constitutes a significant portion of the domestic sewage. Furthermore, industrial waste from animal processing can introduce harmful microorganisms into the environment (Lu et al., 2020; D. Mara & Sleight, 2010; Tajima et al., 2007). The health of individuals is significantly threatened by a wide range of diseases that are prevalent in wastewater streams. Wastewater can become contaminated with human pathogens such as bacteria, viruses, parasitic protozoans, and helminths from a variety of sources. Enteric pathogens may be introduced into wastewater through human and animal feces, as well as from water used in household activities such as bathing or laundry. Furthermore, wastewater from the food processing sector may contain zoonotic enteric pathogens that affect livestock and poultry, thereby posing a threat to human health (Lu et al., 2020; Tajima et al., 2007).

2.1 Wastewater Microbiota

Domestic wastewater (DW) consists of grey water, human waste, and feces, along with water from enterprises and sectors. Each gram of feces contained up to 10¹² microorganisms, with 9% of the wet weight being bacterial. Domestic water contains volatile acids, fatty acids, carbohydrates, proteins, peptides, and biodegradable amino acids. Traditional activated sludge processes use microbial metabolism to eliminate contaminants or to convert them into harmless substances (Cheng et al., 2012; Crockett, 2007; Loge et al., 2002). Fecal contamination is the primary source of waterborne pathogens in aquatic environments. Such exposure can lead to a variety of harmful enteric bacterial, viral, and parasitic infections in the human body, and their presence is linked to numerous microbiological disorders (Crockett, 2007; Pires et al., 2017; Seib et al., 2016). The World Health Organization (WHO) estimates that more than five million people die each year from illnesses linked to poor water, sanitation, and hygiene. The most significant threat to human health is drinking water contaminated with

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human or animal waste, which leads to microbiological pollution (Abbaszadegan et al., 1997; Aw et al., 2022; Lira et al., 2020; Okoh et al., 2007).

To assess the sanitary quality of water, specific indicator microorganisms that may indicate the presence of harmful pathogens have been identified. Identification of heterotrophic bacteria is crucial for evaluating water quality and monitoring changes during treatment and distribution (Curtis, 2003; Pires et al., 2017). Two temperatures are recommended for incubation microbial cultures: 22° C and 37° C. Bacterial populations that grew at 22°C were included in the viable plate counts. Most microorganisms that thrive at 37° C are parasitic or potentially parasitic, originate from sources such as sewage, excretions from warm-blooded animals, heavily contaminated soil, and swimming pools. Unlike benign saprophytic microbes, these microorganisms require careful handling (Blatchley et al., 2007; Ford & Colwell, 1996).

These include *Vibrio*, *Pseudomonas*, *Plesiomonas*, *Escherichia*, *Serratia*, *Shigella*, *Enterobacter*, *Klebsiella*, *Proteus*, *Bacillus*, *Corynebacterium*, *Acinetobacter*, *Flavobacterium*, *Alcaligenes*, *Arthrobacter*. These microbes, including *Klebsiella*, *Pseudomonas*, *Aeromonas*, and nontuberculous mycobacteria, are prevalent and can be detrimental to human health (Lira et al., 2020; Trang et al., 2007).

4 Add Picture

Coliform levels in water treatment plants are among the most reliable indicators of treatment process effectiveness. This category includes *Enterobacter*, *E. coli*, *Klebsiella spp.*, and *Citrobacter spp.* To conduct a quantitative microbial risk assessment, it is essential to understand the populations of enteric microbes as well as their growth and decay rates in different water disposal systems. Research has also been conducted on the concentrations of harmful viruses and their degradation rates in seawater samples taken from various depths, the presence of fecal indicator bacteria in different types of wastewaters, and the potential for these bacteria to multiply in gray water (Lira et al., 2020; Pires et al., 2017; Seib et al., 2016).

Both human and animal waste contain significant levels of total coliforms (TC), intestinal coliforms, and *E. coli*, with some originating from feces. Although many coliform bacteria and indicator species are not harmful, their presence in water suggests the existence of potentially harmful organisms. Coliforms ferment lactose and produce gas within 48 hours at 37°C. Fecal coliforms (FCs) are naturally found in the digestive systems of humans and other warm-blooded animals. However, they can sometimes be associated with pathogens that cause diseases in humans, such as cholera (*Vibrio cholerae*) and typhoid fever (*Salmonella typhi*), as they are excreted in the feces (Anfruns-Estrada et al., 2017; Becerra-Castro et al., 2015; Pires et al., 2017; Seib et al., 2016; Ye et al., 2016).

FCs are rod-shaped gram-negative bacteria that do not form spores and can thrive in both aerobic and anaerobic environments. They possess the ability to ferment lactose, produce acid and gas after 48 h at 44°C, and they test negative for oxidase. The presence of bile salts and similar surface chemicals can promote bacterial growth. FCs are utilized as proxies to assess the risk of human infection and serve as the primary microbial indicator species for monitoring sewage treatment systems, aiding in determining the microbiological safety of water resources (Pires et al., 2017; Seib et al., 2016; Shen et al., 2017).

Sewage exposure significantly increases the risk of gastrointestinal infections and related complications. Fecal streptococci encompass various species, including *S. faecalis*, *S. gallinarum*, *S. arium*, *S. faecium*, *S. bovis*, and *S. equines*, which are typically found in the digestive systems of warm-blooded animals. Among these, *S. faecalis* and *S. faecium* are more closely associated with humans than other *Streptococcus spp.* (Guo et al., 2021; Lira et al., 2020; Yin et al., 2019).

Although various species have been detected in human waste, they have appeared less frequently. Although some streptococcal species are more commonly found in different animals, determining the origin of fecal contamination solely by identifying fecal streptococcal species is not feasible. The detection of *Salmonella*, *enterococci*, *E. coli*, and other fecal indicator bacteria in Apulian aquifers indicates significant fecal pollution. This contamination could increase the risk associated with fresh produce meant for raw consumption during the early stages of production. Bacteriophages such as those found in sewage water can be identified in any environment that supports bacterial growth. Numerous studies have demonstrated that bacteriophages are reliable indicators of enteric viruses in humans (Delforno et al., 2017; Guo et al., 2021; Lira et al., 2020; Yin et al., 2019).

Effluents from domestic and industrial wastewater treatment plants often contain harmful bacteria and parasites. In all biological wastewater treatment systems, bacteria constitute the majority of the microbial population and are typically found at concentrations of approximately 10⁶ bacteria/ml. Pathogens are released by individuals who are either ill or asymptomatic. The presence of enteric pathogens in wastewater is influenced by local infection rate, socioeconomic status of the population, and per capita water consumption (Delforno et al., 2017; Guo et al., 2021; Lira et al., 2020; Nakayama et al., 2017; Yin et al., 2019).

Wastewater is known to contain several significant viral pathogens, primarily enteric viruses, such as hepatitis A, rotavirus, norovirus, adenoviruses, astroviruses, and other enteroviruses. These viruses typically replicate in the intestines of infected individuals, although they can also affect other tissues. Specific illnesses caused by these viruses often depend on their replication sites within the host. Consequently, enteric viruses can cause a variety of human diseases, including gastrointestinal disorders, encephalitis, hepatitis, and cardiogenic shock. Infected individuals can excrete these viruses in large quantities, ranging from 10⁵ to 10¹¹ virus particles per gram of feces. Zoonotic viruses such as animal adenovirus, Nipah virus, and hepatitis can also be transmitted through industrial waste from slaughterhouses. Additionally, harmful plant viruses, such as pepper mild mottle virus and tobacco mosaic virus, have been detected in human feces and wastewater. Owing to their frequent presence in wastewater, adenoviruses and noroviruses are often considered common viruses. Cultivating these viruses, particularly norovirus, is challenging and requires advanced three-dimensional cell culture techniques to produce fully differentiated enterocytes that are essential for infection and replication. It has been suggested that fecal bacteriophages, including somatic and F-specific coliphages (viruses that infect enteric bacteria), can serve as indicators of harmful viruses (Hill, 2003; Topp et al., 2009).


Table 1. Key Pollutants and Their Origins in Water Contamination (Singh et al., 2020).

Source of pollutants	Source of release
Organic water	Household waste, plant and animal degradation, human excretion, and factory runoff water
Pesticides	Insecticides and other chemicals used to kill insects on cropland
Microorganisms	Hospital waste, domestic sewage discharge,
Toxic heavy metals	Hospital waste, domestic wastewater
Fertilizers	sewage discharge
Radioactive substances	mining industries

2.2 Bacteriophage ecology in Wastewater

Bacteriophages are viruses that infect prokaryotic cells. These phages are obligatory parasites that lack their own metabolic processes and rely on the metabolic machinery of the host cell for reproduction. Phage activity is recognized as a significant factor shaping microbial communities in natural aquatic environments, especially in marine environments. In artificial biological wastewater treatment systems, phage concentrations are estimated to range from 10^8 to 10^9 particles/mL, surpassing levels found in any other studied ecosystem. Despite this prevalence, phages remain somewhat enigmatic, akin to dark matter, and their ecological roles still need exploration, partly because of current scientific limitations. Traditional methods for phage testing rely on pure cultures of either the phages themselves or their potential hosts, making them culture-dependent (Liu et al., 2021).

Phages are typically confined to specific bacterial species or strains. However, wastewater treatment facilities consistently yield polyvalent phages that can infect a wide array of hosts. Moreover, some phages had a relatively high abundance of host targets. Consequently, complex networks depict phage-host interactions within BWT ecosystems. Identifying phage host infections under in situ conditions remains a challenge (Tang et al., 2022).

The success of wastewater treatment depends largely on various bacteria, each with distinct functions. These include heterotrophs that break down organic materials, nitrogen-fixing bacteria, specialized ammonia-oxidizing bacteria (AOB), phosphorus-removing bacteria, such as phosphate-accumulating organisms (PAOs), and denitrifying bacteria involved in nitrogen elimination. These functional groups collaborate to remove pollutants. Although both living organisms and non-living factors are crucial for maintaining consistent pollutant removal efficiency, the impact of phage activity on bacterial communities is significant (Liu et al., 2021).

3. EPIDEMIOLOGICAL EVIDENCE IN RELATION WITH WASTEWATER

Wastewater-based epidemiology has emerged as a significant method for monitoring public health and identifying disease outbreaks. This approach analyzes sewage samples to detect pathogens, chemicals, and biomarkers that provide insights into population health trends. Studies have demonstrated the efficacy of wastewater surveillance in detecting and monitoring infectious diseases, including COVID-19, poliovirus, and antibiotic-resistant bacteria. Researchers have successfully employed wastewater monitoring to identify SARS-CoV-2 RNA in sewage days before clinical cases emerged, facilitating early warning systems and targeted responses. Furthermore, wastewater epidemiology has proven advantageous in assessing drug use patterns, environmental pollutants, and antimicrobial resistance genes among populations. This non-invasive, cost-effective approach complements traditional epidemiological methods by providing real-time data that can inform public health decisions and resource allocation.

3.1 Detection of Covid-19 strains in Wastewater

At this crucial moment, it is essential to prevent the disease from spreading, as SARS-CoV-2 represents a major risk to global health. Implementing effective preventive measures is vital to address this pressing problem, particularly given the scarcity of vaccines and antiviral medications. The fastest way to measure viral ribonucleic acid is through reverse transcriptase polymerase chain reactions (RT-PCR); however, it is advisable to carefully choose primers and probes (Brumfield et al., 2022; Gallardo-Escárate et al., 2021). The coronavirus SARS-CoV-2, which causes COVID-19, leads to severe acute respiratory syndrome and has a profound impact on public health and economic activities. As the virus replicates in human enterocytes, its genetic material is excreted in feces, making wastewater surveillance an

effective, non-invasive, and cost-efficient early warning system for epidemiological monitoring. This discussion also addresses the detection of SARS virus as both an antiviral disinfectant and a method for identifying SARS-CoV-2 in municipal wastewater. To detect ribonucleic acid, it must be concentrated using techniques, such as polyethylene glycol precipitation, ultrafiltration, and ultracentrifugation. The various identification methods include high-throughput screening, recombinase polymerase amplification, loop-mediated isothermal amplification, helicase-dependent amplification, nucleic acid sequence-based amplification, reverse transcriptional amplification, and biosensor assays. (Acosta et al., 2022; Naughton et al., 2023; Vo et al., 2022). Ribonucleic acid from active SARS-CoV-2 eventually contaminates wastewater as it is expelled through human feces, saliva, and sputum. Scientists have discovered SARS-CoV-2 RNA in sewage samples collected from wastewater. This discovery has led to the idea of using water-based epidemiology or the monitoring of wastewater as a means to track the spread, persistence, and detection of SARS-CoV-2 in regions where medical diagnostic testing is limited (Figure 1). Water-based epidemiology, which entails the examination of water samples, is an effective approach for identifying and isolating microbes to monitor public health (Acosta et al., 2022). The findings of this study indicate that water-based methodologies can be utilized to identify hotspots within neighborhoods or communities. Consequently, water-based epidemiology may serve as a cost-effective early warning system to detect pandemics before their widespread dissemination, ascertain the causes of ongoing epidemics, and monitor disease prevalence. Numerous academic and commercial laboratories have developed substantial expertise in the analysis of water samples for viral detection (Acosta et al., 2022; Naughton et al., 2023; Vo et al., 2022).

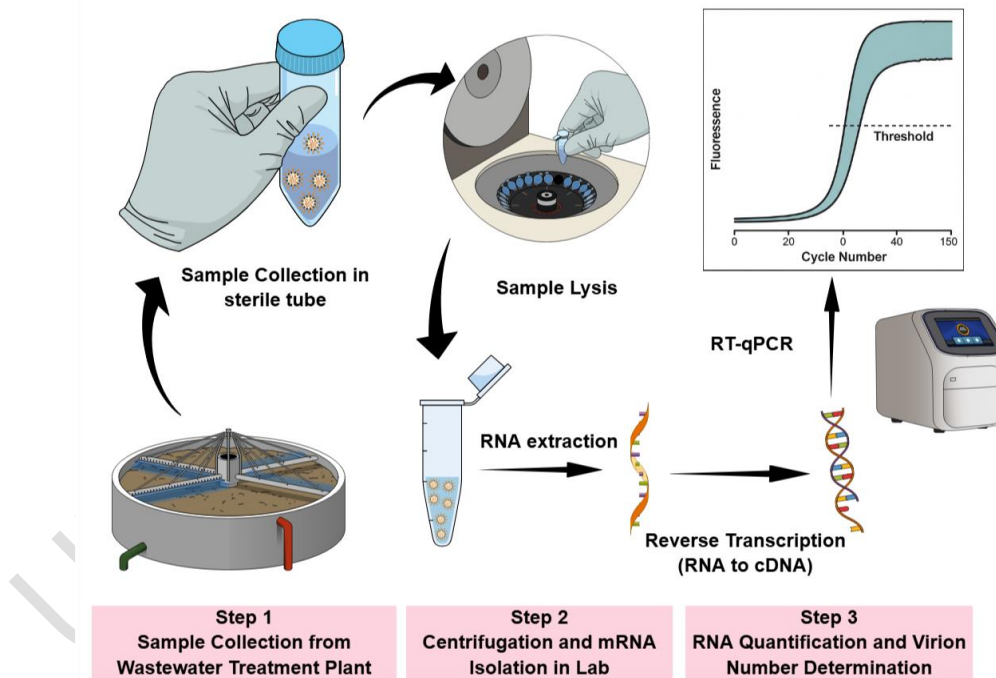


Figure 1. Quantification of Covid 19 virions in city wastewater. A. Collection of samples from the wastewater treatment plant. Centrifugation of the sample and extraction and purification of RNA in the laboratory c. Quantification of RNA and determination of virion number using RT-qPCR.

4. TYPE OF INFECTIONS

Wastewater-based illnesses, caused by exposure to untreated or inadequately treated sewage, can lead to a range of gastrointestinal, respiratory, and skin infections, highlighting the critical importance of proper sanitation and water treatment systems in protecting public health.

4.1 Intestinal infections

Research has established a connection between diarrheal diseases and the reuse of partially or fully treated wastewater. ~~Meta-analysis~~ data revealed that family members and farm workers exposed to sewage used for irrigation had a combined proportion of 1.65 (95% CI: 1.31, 2.06) for contracting waterborne illnesses such as diarrhea. The primary transmission method involves direct contact between agricultural workers and sewage. Additionally, exposure to pathogens can occur through interactions with soil contaminated by wastewater, especially when wastewater is untreated or only partially treated. Soils irrigated with wastewater contain higher levels of pathogens than the average microbiota (Farhadkhani et al., 2020; Khan et al., 2013). Studies have shown that irrigated soils have a higher presence of *Bacillus spp.*, *Pseudomonas spp.*, *Enterobacteriaceae*, *Staphylococcus spp.*, and *Clostridium spp.* A significant number of farmers reported working without shoes (93%), handling contaminated soil with their hands (86%), and placing their contaminated hands in their mouths (53%). These are the primary risk pathways linked to the soil. The nutrient-rich nature of wastewater, which can encourage bacterial growth, may also contribute to the increasing incidence of diarrhea. Risk models that focus only on pathogens in the effluent from liquid wastewater treatment plants and overlook their potential regrowth might lead to an underestimation of the risks (Bonetta et al., 2016). Numerous epidemiological studies have focused on resource reuse, linking diarrheal diseases to direct contact with wastewater, particularly among agricultural workers. This is attributed to the potential for increased human-to-human viral transmission within households. This finding is crucial because inadequate domestic hygiene has been associated with an increase in childhood diarrheal diseases (Alexandrino et al., 2004; Nasser, 2016).

4.2 Skin infections

Exposure to wastewater can lead to various skin issues, including rashes, allergic reactions, hives, and fungal infections affecting toes and fingernails. Deep systemic dermatitis and other fungal infections can cause skin problems (Kesari et al., 2021; Knudsen et al., 2008). Among the 235 farmers who used wastewater for irrigation or aquaculture, the incidence of dermatitis was recorded at 3.0 (95% CI: 1.1-7.7). Skin infections were more prevalent not only through direct contact but also when living with someone who reused wastewater. Factors such as occupation, immune status, frequency of exposure, and absence of protective clothing increase susceptibility to the effects of wastewater (Trang et al., 2007).

Table 2. Toxicological Profile of Waterborne Heavy Metals and Associated Disease (Jaishankar et al., 2014; Mohammad Ali et al., 2021)

Heavy metal Pollutants of Water	Disease caused by them
Arsenic	manifestations of skin, lung, bladder, kidney, and other cancers
Cadmium	gastrointestinal problems and psychological disorders
Chromium	lung cancer, toxic nephritis, liver damage, stomach ulcers, nausea, vomiting, fever, diarrhoea, gingivitis, bronchitis, and pneumonia

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Iron	Genetic disorder, haemorrhagic necrosis
Lead	kidney damage, low birth weight and early birth, and stomach pain Miscarriage, Hypertension
Mercury	Memory loss, Down's syndrome, Defective reproductive system
Copper	Agitation, Dizziness, Insomnia, Anxiety
Nickel	Asthma, Heart disorders, Respiratory failure

5. WASTEWATER-BASED ILLNESSES

Wastewater is home to a variety of pathogenic microorganisms, such as bacteria, viruses, and protozoa, which can lead to numerous waterborne illnesses like cholera, typhoid fever, hepatitis A, and giardiasis when people encounter polluted water sources (Table 3 and Figure 2).

5.1 Campylobacteriosis

Campylobacteriosis, attributable to the bacterium *Campylobacter*, is the leading cause of diarrheal illness. Clinical manifestations, including fever, bloody diarrhea, abdominal cramps, nausea, and vomiting, typically emerge 2–5 days after exposure to the bacterium. However, some individuals may remain asymptomatic. In certain cases, *Campylobacter* can invade the bloodstream of individuals with compromised immune systems, leading to potentially life-threatening infections (Alexandrino et al., 2004; Nasser, 2016).

5.2 Cryptosporidiosis

Cryptosporidium parvum is a microscopic parasite responsible for various illnesses. It has a protective coating that makes it extremely resistant to chlorine, enabling it to survive outside of the host for extended periods. Cryptosporidiosis, commonly referred to as "crypto," is the leading waterborne disease. Although some individuals may remain asymptomatic, most experience symptoms such as diarrhea, loose or watery stools, abdominal pain, nausea, and mild fever (Ajonina et al., 2012; Castro-Hermida et al., 2008; Hamilton et al., 2018; Razzolini et al., 2020; Xiao et al., 2001).

5.3 *Escherichia coli* Diarrhea

The condition referred to as "diarrhoeagenic *E. coli*" is caused by *E. coli* bacteria, with the *E. coli* O157:H7 strain being particularly significant. Transmission can occur through the fecal-oral route or through the ingestion of contaminated water. Most individuals experience symptoms, such as fever, nausea, vomiting, diarrhea, bloody or watery stools, and abdominal cramps, although some may remain asymptomatic. Around 2-7% of those infected with *E. coli* O157:H7 develop hemolytic uremic syndrome, which can lead to kidney failure and, in rare cases, death. This syndrome is more prevalent among children, the elderly, and individuals with a weakened immune system. Certain *E. coli* serotypes are believed to cause chronic diarrhea in HIV patients (Adefisoye & Okoh, 2016; Bibbal et al., 2018; Ibekwe et al., 2002; Muniesa et al., 2006; Okoh et al., 2010; Yang et al., 2014).

5.4 Encephalitis

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West Nile virus (WNV) and St. Louis encephalitis are acute viral diseases transmitted through infected mosquito bites, particularly from *Culex* species. These mosquitoes lay eggs in water bodies polluted with sewage, and their populations grow when adults feed on human blood, facilitating transmission between mosquito vectors and avian reservoir hosts. Mosquitoes carrying the virus transmit the virus to susceptible birds through saliva during feeding. Birds harbor the virus in their bloodstream for one–four days before developing lifelong immunity. A sufficient number of mosquitoes must feed on infected birds to ensure that some survive long enough to process blood meals and feed on other hosts. Humans do not reach the infectious virus levels and cannot sustain the reservoir-host cycle. Most individuals remain asymptomatic, and their illness is typically mild. However, it can be severe in young children, the elderly, and in those with compromised immune systems. No vaccine is currently available for encephalitis (Sahoo et al., 2021).

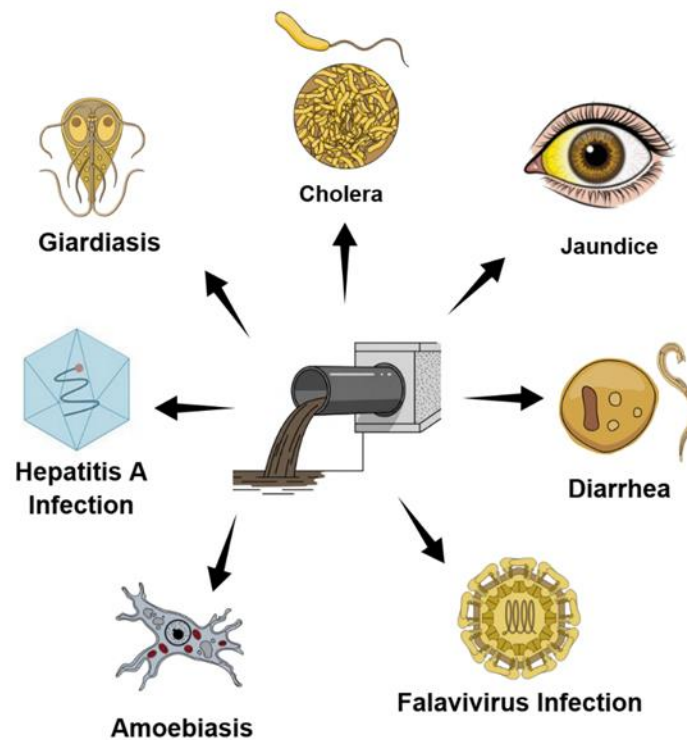


Figure 2. Disease Burden Associated with Untreated Wastewater

5.5 Gastroenteritis

Viral gastroenteritis, commonly referred to as the "stomach flu," is an illness that can be caused by Norwalk viruses and similar types of viruses, including caliciviruses, rotaviruses, adenoviruses, astroviruses, and influenza viruses. Symptoms such as vomiting, nausea, fever, headache, and stomach pain typically appear one–two days after infection and can last from one to ten days, depending on the specific virus involved. Infants, young children, the elderly, individuals with disabilities, and those with weakened immune systems may experience severe illness if they are unable to consume enough fluid to offset the loss caused by vomiting or diarrhea (Birkhead et al., 1989; Medema & Schijven, 2001; O'Reilly et al., 2007).

5.6 Giardiasis

Giardia intestinalis, also referred to as *Giardia lamblia*, is a single-celled parasite responsible for this disease. Its protective shell enables it to endure the host for a long duration. Giardiasis is a prevalent waterborne illness globally, with parasites found all over the world. Although some people do not show symptoms, the majority experience symptoms such as diarrhea, loose or watery stools, abdominal cramps, or an upset stomach (Castro-Hermida et al., 2008; Hamilton et al., 2018; Medema & Schijven, 2001).

5.7 Hepatitis A

Hepatitis A primarily affects the liver, causing symptoms such as jaundice, fatigue, abdominal pain, nausea, vomiting, diarrhea, and fever. These symptoms are more common in adults than in children. Approximately 15% of those infected experience symptoms that persist or recur over a period of 6–9 months (Fenaux et al., 2019).

5.8 Leptospirosis

Leptospirosis is a disease caused by bacteria belonging to the genus *Leptospira*. Its clinical manifestations include erythema, skin rashes, fever, persistent headaches, chills, muscle pain, diarrhea, hepatitis, conjunctival suffusion, and pelvic discomfort; in some cases, it may be asymptomatic. Without appropriate treatment, leptospirosis can lead to renal impairment, meningitis, liver failure, respiratory complications, and, although rarely, death (Lu et al., 2020; Tajima et al., 2007).

5.9 Methemoglobinemia

Methemoglobinemia, commonly known as "blue-baby syndrome," poses a significant risk to infants in their early months if they consume water with high nitrate levels. It is well established that improperly constructed septic systems in sandy soils can lead to nitrate contamination of the groundwater. Mothers who drink water with elevated nitrate concentrations may inadvertently expose their breastfed infants to nitrate poisoning. This condition can be fatal within a few days, manifesting with symptoms such as respiratory distress and cyanosis. The gastric pH of infants differs from that of adults and older children. In the stomach of an infant, nitrate is converted into nitrite, which impairs the oxygen-carrying capacity of the blood, resulting in poisoning. The U.S. Environmental Protection Agency has set the maximum allowable nitrate concentration in drinking water as 10 mg/L of nitrogen or 45 mg/L of nitrate. Boiling water does not remove nitrates; instead, it increases their concentration. (Lu et al., 2020; Tajima et al., 2007).

5.10 Poliomyelitis

Poliomyelitis is caused by a virus. While most people exposed to it remain asymptomatic, some may experience symptoms, such as sore throat, fever, nausea, vomiting, abdominal pain, constipation, and occasional diarrhea. Paralysis occurs in less than 1% of infected patients. Although most patients eventually recover and regain some muscle function, paralysis or weakness persisting a year after infection is usually permanent (Mehndiratta et al., 2014).

5.11 Salmonellosis

Salmonella typhimurium and *Salmonella enteritidis* are the most common types of *Salmonella*. Symptoms such as diarrhea, fever, and stomach pain usually start 12–72 h after infection. Most people get better without medical help, and the illness lasts approximately four to seven

days. However, babies, young children, people with disabilities, the elderly, and those with weak immune systems can get very sick if they cannot drink enough fluids to replace what they lose from vomiting or diarrhea. Without treatment, Salmonella can move from the intestines to the blood and other body parts, which can be fatal (Adefisoye & Okoh, 2016).

5.12 Shigellosis

Shigella is the bacterium responsible for shigellosis, also known as "Bacillary Dysentery." *S. sonnei* and *S. flexneri* strains were most frequently identified in shigellosis cases. While some individuals may remain asymptomatic, most experience bloody diarrhea, fever, and abdominal cramps within one–two days of bacterial exposure. Shigellosis generally resolves within 5–7 days, but it can become severe in newborns, young children, individuals with disabilities, the elderly, and those with weakened immune systems if they do not consume enough fluids to replace those lost due to diarrhea. Children under the age of two may experience seizures if they have a severe infection accompanied by high fever (Adefisoye & Okoh, 2016; O'Reilly et al., 2007).

5.13 Paratyphoid Fever

Salmonella paratyphi is a bacterium that causes infection. This infection results in fewer fatalities and fewer severe symptoms than typhoid fever (Matrajt et al., 2020).

5.14 Typhoid Fever

Salmonella typhi is a bacterium that exclusively resides in the human blood and intestines and acts as a pathogen. This infection can lead to a high fever of 104 °F accompanied by symptoms such as fatigue, coughing, headaches, abdominal pain, and reduced appetite. Some individuals may also develop rashes with flat, rose-colored spots. Antibiotic treatment generally leads to improvement within 2–3 days and fatalities are rare. Without antibiotics, fever can persist for weeks or even months, with 20% of untreated cases resulting in death due to complications. A small number of people, known as carriers, recover from typhoid fever but continue to harbor bacteria. Both carriers and those who were ill excreted *S. typhi* in their feces. Bacteria can remain in the body even after symptoms have subsided, increasing the risk of relapse or transmission to others. It is essential for individuals to complete the entire course of the antibiotics prescribed by their doctors. Patients with Typhoid Fever should refrain from preparing or serving food (Adefisoye & Okoh, 2016; Bibbal et al., 2018; Ibekwe et al., 2002; Muniesa et al., 2006; Okoh et al., 2010; Yang et al., 2014).

5.15 Yersiniosis

This condition is caused by Yersinia, a rod-like bacterium. *Yersinia enterocolitica* is associated with yersiniosis, an illness predominantly affecting young children. The clinical symptoms vary depending on the age of the individual. In children, the common symptoms include fever, abdominal pain, and frequent bloody diarrhea. In older children and adults, symptoms may include right-sided abdominal pain, fever, occasional skin rash, and joint pain. Typically, symptoms emerge 4–7 days after exposure and can last for up to 3 weeks (Aghalari et al., 2020; Fenaux et al., 2019).

Table 3. Pathogenic Microorganisms in Wastewater and Their Associated Diseases

Microorganisms present in Wastewater	Diseases caused by them
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⑧ There is no citation for table 3.

<i>Salmonella spp.</i>	Typhoid fever
<i>E. coli</i>	Haemorrhagic Diarrhoea
<i>Vibrio cholerae</i>	Cholera
<i>Yersinia spp</i>	Gastroenteritis
<i>Shigella spp.</i>	Bacillary dysentery, Shigellosis
<i>Campylobacter jejuni</i>	Diarrhoea
<i>Salmonella paratyphi</i>	Paratyphoid fever
<i>Staphylococcus aureus</i>	Skin infections
<i>Norovirus</i>	Dehydration, Malnutrition
<i>Listeria monocytogenes</i>	Septicaemia, meningitis
<i>Clostridium botulinum</i>	Foodborne infections
<i>Clostridium perfringens</i>	Abdominal pain and stomach cramps

6. DETECTION OF ANTIBIOTIC-RESISTANT GENES IN WASTEWATER

While most ecosystems generally exhibit low levels of antibiotic resistance, wastewater often contains high amounts of antibiotic-resistant bacteria and antibiotic resistance genes, particularly in clinically important pathogenic bacteria (Sharma et al., 2021). Numerous Enterobacteriaceae strains isolated from wastewater treatment facilities exhibit resistance, with some strains showing resistance to multiple drugs. These strains are resistant to non-beta-lactam antibiotics, such as trimethoprim/sulfamethoxazole, ciprofloxacin, and tetracycline, as well as beta-lactam antibiotics, such as ceftiofur, amoxicillin, and cefotaxime. Further research has identified genes in wastewater that confer resistance to antibiotics including tetracycline, methicillin, and sulfonamide. Several studies have highlighted that tetracycline resistance genes are among the most prevalent antibiotic resistance genes (ARGs) found in wastewater treatment systems worldwide (Aghalari et al., 2020; Stevik et al., 2004). Hospital wastewater can harbor pathogenic bacteria that are resistant to one or more drugs, presenting a unique risk. Hospitals and urban wastewater systems release antibiotics, such as beta-lactams, lincosamides, macrolides, quinolones/fluoroquinolones, sulfonamides, tetracyclines, dihydrofolate reductase inhibitors, nitroimidazoles, and ARGs. This discharge affects their removal by wastewater treatment plant (WWTP) effluents and influences the rivers that receive these outputs (Aghalari et al., 2020; Stevik et al., 2004). The findings indicated that while antibiotics were absent upstream of the WWTP discharge, they were present in significant quantities in the downstream river samples, with ofloxacin reaching concentrations as high as 131.0 ng/L. Additionally, ciprofloxacin and sulfamethoxazole concentrations were nearly tenfold higher downstream of the WWTP discharge than upstream. The incomplete breakdown of antibiotic compounds can create biological selection pressure that encourages the development of ARGs, effectively turning WWTPs into breeding grounds for these organisms. Throughout the wastewater treatment process, bacteria are consistently exposed to heavy metals, residual pharmaceuticals, and antibiotics, which drive the evolution of resistance genes. Nonetheless, a recent study found that mixed bacterial cultures might resist treatment. When *E. coli* was grown within a pig fecal community, both resistant and non-resistant (otherwise isogenic) focus strains demonstrated minimal selection concentrations of gentamicin or kanamycin by more than an order of magnitude (Sharma et al., 2021). In the range of gentamicin concentrations from 0 to 10 g/ml, the growth of the gentamicin-resistant focus strain was hindered owing to a greater fitness cost, indicating that resource constraints had a more significant impact on resistance phenotypes. Nevertheless, only the resistant

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strains were able to flourish at extremely high gentamicin concentrations (100 g/ml). A comparable pattern was observed at moderate kanamycin concentrations (0–20 g/ml), where the susceptible strain outperformed the resistant strain when cultured together with the pig fecal community (Stevik et al., 2004).

7. HOSPITAL WASTEWATER AS A RESERVOIR OF ARGS AND ARBS

Recent studies have underscored that the discharge of wastewater from hospitals and nearby water bodies that receive untreated hospital waste is marked by high concentrations of clinically important antimicrobial-resistant (AMR) bacteria and antibiotic resistance genes (ARGs). Specifically, samples of hospital wastewater have been found to contain Enterococcus species that are resistant to vancomycin and ampicillin as well as multidrug-resistant (MDR) bacteria, including carbapenemase-producing Enterobacteriaceae and bacteria that produce extended-spectrum beta-lactamase (ESBL) (Fouz et al., 2020). Numerous studies have demonstrated that effluent samples from wastewater treated in hospitals, pharmaceutical facilities, and municipal systems exhibit high levels of various ARGs, ARBs, and antimicrobial drugs. For instance, a recent study found that sewage samples from low-income countries had significantly higher levels of ARGs than those from high-income countries. Pharmaceutical wastewater treatment plants have been identified as having high concentrations of ARGs, particularly those associated with clinically important antimicrobial drugs, such as sul1, sul2, and tet, which remain elevated throughout the treatment process and are subsequently released into the environment (Fouz et al., 2020). This is in addition to the NDM-1-producing species already identified, such as *E. coli* and *K. pneumoniae*. *Enterobacteria*, *Aeromonads*, and *V. cholerae* possess blaNDM-1-bearing plasmids that are stable, transmissible, and exhibit NDM-1 resistance. Environmental isolates of *Aeromonas caviae* and *V. cholerae* were the first to have blaNDM-1-bearing chromosomes, although most bacteria previously carried the gene on plasmids. Another study confirmed the identification of new *Acinetobacter cumulans* species in hospital wastewater. ARGs associated with resistance to clinically important drugs, including carbapenems, cephalosporins, and aminoglycosides, were found in these strains. Additionally, a *K. pneumoniae* strain that produces carbapenemase and carries blaKPC-2, which is rarely encountered in clinical settings, has been detected in wastewater treatment plant (WWTP) effluent (Bürgmann et al., 2018; Fouz et al., 2020). Another study revealed that the *mecA* gene, which encodes penicillin-binding proteins, along with the -lactamase genes blaTEM and blaCTX-M9, was present in all DNA phages isolated from river water and sewage samples (Bürgmann et al., 2018; Fouz et al., 2020). The collective findings from the reviewed studies suggest that wastewater, particularly untreated wastewater, along with hospital and pharmaceutical wastewater, may serve as an environmental reservoir for the emergence and spread of new AMR bacterial strains. This is largely attributed to the presence of diverse bacterial species and high concentrations of ARGs in these environments, which increase the likelihood of ARGs being transferred to mobile elements among closely related species. Furthermore, untreated wastewater contains substantial amounts of antimicrobial drugs, exerting a strong selective pressure that fosters the development and dissemination of AMR bacteria (Fouz et al., 2020).

8. PURIFICATION AND REMOVAL OF PATHOGENIC MICROBES FROM WASTEWATER

In rural areas, the release of wastewater, characterized by distinct wastewater containing high levels of nutrients, organic materials, and pathogens, presents a significant challenge. The lack of public sewage infrastructure, inadequate wastewater disposal methods, and limited environmental awareness are key factors contributing to the increase in diseases and bacterial contamination in both surface and groundwater sources. These environmental issues can only be addressed through the adoption of wastewater treatment (Aghalari et al., 2020; Stevik et al., 2004). A method for removing microorganisms from household wastewater introduced

through feces involves the use of multiple soil layers (MSL). The experimental setup consisted of a laboratory-scale MSL system measuring 30 cm depth, 36 cm width, and 65 cm height. In this system, soil mixture blocks (SMB) were arranged in a brick-like pattern and surrounded by permeable gravel layers (PL). The dry weight ratio of the SMB components was 7:1:1:1, consisting of sand, charcoal, sawdust, and metallic iron. Each day, 200 L water was hydraulically loaded into the system. The bacterial analysis included total bacterial counts at 22 °C and 37 °C, along with the identification of pathogenic bacteria, such as *Clostridium*, *Staphylococcus sp.*, *Pseudomonas*, and *Salmonella sp.*, as well as *Streptococci*, intestinal enterococci, and *E. coli*. *Staphylococcus spp.* showed the highest removal efficiency at 1.42 log units, whereas *E. coli* had the lowest at 1.01 log units. The average removal efficiencies for total nitrogen (TN), total phosphorus (TP), total suspended solids (SS), biochemical oxygen demand (BOD), chemical oxygen demand (COD), and total oxygen demand (TOD) were 93, 86, 81, 78, and 80%, respectively. These findings indicate that the MSL system is effective for removing organic matter, phosphate, and nitrogen. However, the reduction in bacterial indicators and pathogens remained below average (Stevik et al., 2004). Rapid industrialization and urban growth have led to the production of large quantities of wastewater containing heavy metals. Industries such as pulp and paper, distilleries, tanneries, and textiles are particularly known for generate hazardous wastewater. If released untreated, wastewater can pose significant risks to both the environment and human health. Treating such wastewater is costly and energy-intensive because of the high levels of pollutants and the waste involved. Microorganisms are essential for energy and resource cycles within ecosystems because of their ability to endure metal toxicity. Utilizing microbes for detoxification of wastewater has proven beneficial to society, leading to an emphasis on processes such as biomineralization, oxidation-reduction, bio-precipitation, bioaccumulation, biosurfactant technology, bioleaching, bio-volatilization, and biosorption. Each of these methods has unique benefits and challenges (Aghalari et al., 2020; Stevik et al., 2004). Certain bacteria, such as *Corynebacterium glutamicum* (Zn, Pb, Cd, Cr, and Co), *Aspergillus lentulus* (Cr, Cu, and Pb), *Bacillus*, *Microbacterium*, *Micrococcus*, *Shigella*, *Arthrobacter*, *Bacillus*, and *Serratia*, can compartmentalize heavy metals. Additionally, nanocomposites, such as CuFeO, FeO, Co₃O₄SiO₂, and Fe₃O₄ have been effectively utilized to detoxify wastewater owing to their unique properties (Aghalari et al., 2020; Stevik et al., 2004) (Figure 3).

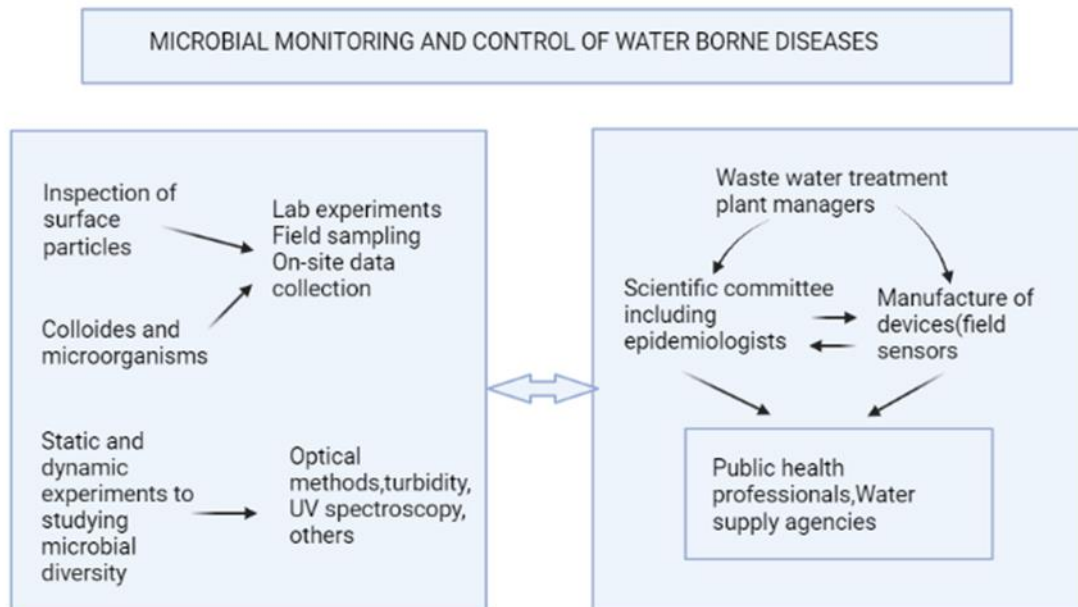


Figure 3. Microbial monitoring and control of waterborne diseases

9. WASTEWATER REUSE

One of the most pressing challenges facing the world today is the scarcity of freshwater, which affects millions of people globally. In many countries, the use of untreated wastewater in agriculture is prevalent, leading to significant environmental and public health concerns. Research has indicated that treated wastewater is more beneficial and environmentally sustainable than untreated wastewater (Table 4). The health risks associated with environmental toxicity from exposure to solid waste are substantial (Stevik et al., 2004).

Table 4. Sequential Stages of Wastewater Treatment and Their Key Processes

Steps	Treatment
Pre-treatment	Screening in general
	Screening perfectly
	Homogenization tank for flow
	removing grit
	Removal of grease
Primary treatment	Sifting
	Decantation
Secondary treatment	Physiochemical treatment
	Adipose sludge
	Long-term aeration
	removing nitrogen
	elimination of phosphorus
	Clotting
Filtration	

Like Table 3.

Household and treated wastewater contain a variety of minerals, including phosphate, nitrogen, potassium, and sulfur. Wastewater is rich in nitrogen and phosphorus, which are readily absorbed by plants, making it a preferred choice for irrigation. The nutrient-rich composition of reclaimed wastewater enhances soil fertility, reduces reliance on fertilizers, boosts crop production, and can potentially lower agricultural costs by owing to increased crop yields and improved soil quality (Sharma et al., 2021). Several technologies have been explored to convert organic matter into bioenergy, such as microbial fuel cells, biodiesel, bioethanol, and bacterial hydrogen production. Although these methods can generate electricity from wastewater, further refinement is required. Wastewater energy has the potential to produce ten times more energy than chemical, thermal, and hydraulic sources, making it an effective means of managing wastewater energy. Sludge stabilization in a wastewater treatment facility can be achieved through vermicomposting (Aghalari et al., 2020; Stevik et al., 2004).

10. CONCLUSION

Wastewater contains a variety of organic and inorganic components including pathogenic bacteria, viruses, parasites, and antibiotic resistance genes (ARGs). Exposure to inadequately

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treated or untreated wastewater can result in a spectrum of diseases, such as intestinal and skin infections, as well as other waterborne illnesses. Hospitals and pharmaceutical wastewater are hotspots for the emergence and spread of antimicrobial-resistant bacteria and ARGs. Although conventional wastewater treatment processes are not always effective in eliminating microbial contaminants, advanced treatment technologies hold promise for improving the removal of pathogens and ARGs. Reusing treated wastewater for agricultural irrigation and other purposes can help alleviate freshwater scarcity, while mitigating the environmental and health risks associated with the discharge of untreated wastewater. A comprehensive approach to urban wastewater management that integrates effective treatment technologies, monitoring strategies, and risk assessment is essential to mitigate the microbial and chemical hazards associated with wastewater and to promote sustainable water reuse practices.

By implementing essential treatment and distribution methods, rising wastewater production from municipal, industrial, and agricultural sources in developing countries, especially in India and other parts of Asia, could potentially replace freshwater in rice farming. Addressing wastewater treatment is a major challenge for many nations because the increasing presence of unwanted or unidentified pollutants significantly affects both the environment and human health. Water recycling, the most effective method for reuse, involves collecting water from various sources, treating it, and using it in industrial processes, environmental restoration, agriculture and irrigation, drinking water supplies, groundwater replenishment, and other beneficial applications. Water security, sustainability, and resilience can be enhanced by reusing water and providing alternatives to the current water sources. Although not yet fully utilized, water efficiency and reuse are powerful strategies for improving water sustainability and resilience. They can reduce vulnerability to droughts and other water supply challenges as well as decrease water withdrawals from overexploited rivers and aquifers. Furthermore, conserving and reusing water can lead to energy savings, which can help to lower greenhouse gas emissions and mitigate the effects of climate change.

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