Chapter

Constructed Wetlands in Wastewater Treatment and Challenges of Emerging Resistant Genes Filtration and Reloading

Donde Oscar Omondi and Atalitsa Caren Navalia

Abstract

A wetland is a unique and distinct ecosystem that is flooded by water, either permanently or seasonally, where oxygen-free processes prevail, and the primary distinctive factor of wetlands from other landforms or water bodies is the occurrence of adaptive vegetation of aquatic plants, characteristic to the unique hydric soil. A constructed wetland is an artificial shallow basin filled with substrate, usually soil or gravel, and planted with vegetation that has tolerance to saturated conditions. As much as the use of constructed wetland has been recommended in the treatment of various forms of wastewater, the system efficiency is a factor of very many natural and artificial factors, with the emerging pollutants and contaminants such as resistant genes being the most complicated contaminants to eliminate through the system. Indeed, the emerging pollutants in forms of antibiotic resistant genes (ARGs) have remained prevalent in aquatic environments such as wetlands that receive ARG-loaded sewage. Therefore, this chapter covers a discussion on constructed wetlands in wastewater treatment and challenges of emerging contaminants, such as resistant genes filtration and reloading mechanisms, and provides recommendation for the proper handling and removal of such pollutants from the wetlands' functional system.

Keywords: antibacterial resistant genes, constructed wetlands, emerging pollutants, wastewater treatment, wetlands

1. Introduction

Wetland is a unique and distinct ecosystem that is flooded by water, either permanently or seasonally, where oxygen-free processes prevail, and the primary distinctive factor of wetlands from other landforms or water bodies is the occurrence of adaptive vegetation of aquatic plants, characteristic to the unique hydric soil [1, 2]. The modified form of wetland is termed "constructed wetland." Constructed wetlands for water treatment are complex, integrated systems of water, plants, animals, microorganisms, and the environment [3, 4]. Wetlands play a number of functions, including water purification, water storage, processing and recycling of carbon and other micro and macro nutrients, stabilization of shorelines, and support of plants and animals. While wetlands are generally reliable, self-adjusting systems, an understanding of how natural wetlands are structured and how they function greatly increases the likelihood of successfully constructing a wetland treatment system [5, 6].

The cleansing of water has always occurred through natural processes as the water flows through rivers, lakes, streams, and wetlands, and in the last several decades, systems have been constructed to use some of these processes for water quality improvement [7]. Wetlands are now highly preferred as systems for improving the quality of point and nonpoint sources of water pollution, including stormwater runoff, domestic wastewater, agricultural wastewater, as well as coal mine drainage [4]. To enhance sustainability in wastewater management, the use of constructed wetlands has been applied in the treatment of different forms of wastes. Artificially created wetlands have been successful in the treatment of petroleum refinery wastes, wastes from sugar factory, leachates from landfills and composts, wastes from aquaculture systems, wastes from pulp and paper mills, and wastes that emanate from slaughter houses, textile mills, and plants that process sea food. Under the management of these wastes, the constructed wetlands can serve as the sole treatment or may be part of an integrated wastewater treatment system [8].

Antimicrobial resistance (AMR) is defined as the ability of a microbe to resist the effects of medication that was once successful and efficient in treating the microbe [9]. The term antibiotic resistance (ABR) is a subset of AMR, as it applies only to bacteria becoming resistant to antibiotics. The AR phenotypes can arise within a microorganism through the lateral and horizontal gene transfers and mutation. The mutations of the chromosomal DNA alter the existing bacterial proteins, through transformation, resulting in the creation of mosaic proteins and/or as a result of the transfer and acquisition of new genetic material between bacteria of the same or different species or genera [10]. The emerging pollutants in forms of antibiotic resistance genes (ARGs) have remained prevalent in aquatic environments such as wetlands that receive ARG-loaded sewage [11].

As much as the use of constructed wetland has been recommended in the treatment of various forms of wastewater, the system efficiency is a factor of very many natural and artificial factors, with the emerging pollutants and contaminants such as resistant genes being the most complicated contaminants to eliminate through the system [11, 12]. Moreover, some studies have reported constructed wetlands as reservoirs to various forms of resistant genes, which trap them and release them to other aquatic systems, hence contributing to their higher concentration in streams, rivers, or lakes [13]. Numerous suggestions have been provided to improve wetland's functional effect, efficiency, and predictability and provide a proper ecosystem management [6, 7]. This chapter covers a discussion on the constructed wetlands in wastewater treatment and the challenges of emerging related contaminants, such as resistant genes, and provides recommendation for the proper handling and removal of such wastes from the wetland's functional system.

2. Wetlands as functional systems

Wetlands are ecotones/transitional areas between land and water, with indistinct boundaries between the wetland area and uplands or deep water [14]. The definition expansion of the term wetland covers a broad range of systems that range from marshes, bogs, swamps, wet meadows, tidal wetlands, floodplains, and ribbon (riparian) zones along stream channels. However, all wetlands, whether they are natural or artificial, freshwater, or salty, pose a single characteristic or numerous characteristics, and they occur within the surface or near-surface water, whether they are permanently or temporarily submerged under water [15]. In most wetlands, hydrologic conditions are such that the substrate is saturated long enough during the growing season, a mechanism that creates oxygen-poor conditions in the substrate, limiting the vegetation to those species that are adapted to low-oxygen environments [16].

Wetlands provide a number of functions and benefits. Wetland functions are inherent processes occurring in wetlands; wetland values are the attributes of wetlands that society perceives as beneficial [17]. The wetland hydrology is generally one of slow flows with either shallow waters or saturated substrates, which allows sediments and other pollutants, including emerging contaminants to settle as the water passes through the wetland system. The occurrence of slow flows provides prolonged contact times between the water and the surfaces within the wetland [15]. The wetland treatment mechanisms are anchored on the complex mass of organic and inorganic materials, with diverse opportunities for gas/water interchanges, which foster a diverse community of microorganisms that break down or transform a wide variety of substances [7]. Within the wetland's ecosystems, there are dense growths of vascular plants adapted to saturated conditions, which slow the water, create microenvironments within the water column, and provide attachment sites for the microbial communities as well as other contaminants. The litter that accumulates as plants die back in the fall creates additional material and exchange sites and provides a source of carbon, nitrogen, and phosphorous to fuel microbial processes [18].

Even though, not all wetlands can perform all functions and values, majority of them provide several benefits. When subjected to appropriate ecological management without any threats, majority of wetlands can provide the following:

I. Water quality services

II. Flood storage services under excessive precipitation and the desynchronization of storm

III. Nutrients and other materials cycling services

IV. Habitat for fish and wildlife

V. Services for passive recreation, such as bird watching and photography

VI. Services for active recreation, such as hunting education and research

VII. Services for esthetics and landscape enhance merit

2.1 Constructed wetlands

A constructed wetland is an artificial shallow basin filled with substrate, usually soil or gravel, and planted with vegetation that has tolerance to saturated conditions. Water is then directed into the system from one end and flows over the surface (surface flow) or through the substrate (subsurface flow) and gets discharged from the other end at the lower point through a weir or other structure, which controls the depth of the water in the wetland [11]. Several forms of constructed wetlands have been introduced, including surface flow wetlands, subsurface flow wetlands, and hybrid systems that integrate surface and subsurface flow wetland types [6, 19]. Constructed wetland systems can also be combined with conventional treatment technologies to provide higher treatment efficiency [8]. The choice of constructed wetland types depends on the existing environmental conditions and how appropriate they are for domestic wastewater, agricultural wastewater, coal mine drainage, and stormwater [6].

Constructed wetlands have been widely used in the treatment of primary or secondary domestic sewage effluents, and others have been used to treat domestic wastewater and have also been modeled to handle high organic loads associated with agriculture or domestic wastewater [5]. A large number of constructed wetlands have also been built to treat drainage from active and abandoned coal mines [20]. The constructed wetland technology has recently been used in the control and management of stormwater flows, and its application in reducing the impacts by stormwater floods within urban areas is expanding globally [21]. The constructed wetland technology is not only preferred in stormwater flow control but also in the treatment of wastewater, and its preference is based on its low cost, low energy requirement, and need for minimal operational attention and skills. Due to its numerous merits and high sustainability potential, there is an increasing extensive research on its practical application to expand the knowledge on its operation and to provide more insight on its appropriate design, performance, operation, and maintenance for optimum environmental benefits. Even though the constructed wetlands are sturdy and effective systems, their performance depends on the periodic improvements to handle emerging contaminants such as antibiotic and antibacterial resistant genes, and for them to remain effective, they must be carefully designed, constructed, operated, and maintained [11, 12].

2.2 Components of constructed wetland

Constructed wetland is a system that puts together different units that work together to ensure that its intended purpose is achieved. Constructed wetland systems entail a properly designed and constructed basin that holds water, a substrate that provides filtration pathways, habitat/growth media for the needed organisms, and also communities of microbes and aquatic invertebrates, which in most cases develop naturally. Most importantly, constructed wetlands also hold vascular plants whose nature depends on the intended purification role and efficiency. The efficiency of the constructed wetlands in waste treatment depends on the interaction and maintenance of these components [22].

In a constructed wetland system, natural geochemical and biological processes within a wetland realm are involved in the treatment of metals, explosives, and other contaminants that exist within the water. Normally, there are three primary components in a constructed wetland. Constructed wetland has an impermeable layer (generally clay). It also has a gravel layer that acts as a substrate needed for the provision of nutrients and support to the root zone. It also has an above-surface vegetation zone [16]. The impermeable layer within the constructed wetland system prevents infiltration of wastes down into underground aquifers. The gravel layer and root zone comprise of a layer where water flows and bioremediation and denitrification occur. The above-ground vegetative layer contains the well-adopted plant material. Within the wetlands, both the aerobic and anaerobic processes occur, and these can be divided into separate cells [5, 16]. Groundwater can be made to flow through pumping or naturally by gravity through the wetland. Within the anaerobic cells, plants and other natural microbes are involved in the degradation of the contaminant. The aerobic cell performs the work of further improving the water quality through continued exposure to the plants and the movement of water between cell compartments. The use of straw, manure, or compost with little or no soil substrate has been beneficial in the wetlands constructed primarily for the removal of metals. However, for wetlands constructed to treat explosives-contaminated water, certain plant species are used to enhance the degradation through a process termed phytoremediation [23].

2.2.1 Water

Wetlands are formed on substrates that are fully or partially submerged in water, where a relatively impermeable subsurface layer prevents the surface water from seeping into the ground [1, 2]. These conditions can be created with few modifications to form a constructed wetland. A constructed wetland can be built almost anywhere in the landscape by shaping the land surface to collect the surface water and by sealing the basin to retain the water [7]. Hydrology that enhances the linking of all the functions in a wetland system stands as the most important design factor to be considered in constructed wetlands, as it is often the primary factor in the success or failure of most constructed wetlands. Therefore, planning and putting up of constructed wetlands require the contribution of a qualified hydrologist to ensure that all the hydrological requirements and conditions are taken care of [24]. Even though the hydrology of most constructed wetlands is very much similar to the other surface and near-surface water, it does differ in several important respects. Small changes in hydrology can have fairly significant effects on a wetland's functionality and its treatment effectiveness and efficiency. Indeed, due to the large surface area of the water and its shallow depth, a wetland system interacts strongly with the atmosphere through rainfall and evapotranspiration. This (the combined loss of water by evaporation from the water surface and loss through transpiration by plants) and the density of the vegetation of a wetland strongly affect the constructed wetlands' hydrology. This can be experienced through the obstruction of water flow paths as the water finds its sinuous way through the network of stems, leaves, roots, and rhizomes, and it can also occur through the blockage of exposure to wind and sun [7, 24, 25]. Water always acts as a vehicle for delivering the pollutants to the system and also for discharging the untapped pollutants away from the system [24].

2.2.2 Substrate

Substrates for constructed wetlands can come in the form of sediment or litter. Substrates used to construct wetlands include soil, sand, gravel, rock, and organic materials such as compost [26]. Due to low water velocities and high productivity typical of wetlands, the sediment and litter accumulation occurs within the wetlands. The substrates, sediments, and litter have numerous functions that are beneficial to the efficiency of the constructed wetlands. They provide support to many of the living organisms in wetlands, and the substrate permeability also affects the movement of water through the wetland and provides numerous chemical and biological processes, many of which are microbial in nature and also enhance the transformation of pollutants within the substrates. The substrates also provide storage for many contaminants, and the accumulation of litter increases the amount of organic matter in the wetland, which provides sites for material exchange and microbial attachment. Through this process, carbon source is realized as well as the energy source that drives some of the important biological reactions in wetlands.

Flooding of the constructed wetlands with water has a contribution in its functional mechanism. The physical and chemical characteristics of soils and other substrates are altered when they are wholly or partially under water. For example, under saturated substrate, the water replaces the atmospheric gases within the pore spaces and the microbial-driven metabolism results in the consumption of the available oxygen. Therefore, since oxygen is consumed more rapidly than it can be replaced by diffusion from the atmosphere, the substrates change to anoxic condition (without oxygen). Such conditions become significant in the removal of pollutants such as nitrogen and metals. However, substrates can also act as reservoirs for most contaminants, with high concentration of emerging contaminants such as resistant genes being detected in the constructed wetland substrates [27, 28].

2.2.3 Vegetation

Constructed wetlands can work with both the vascular plants (the higher plants) and nonvascular plants (algae), and the photosynthesis process by algae increases the dissolved oxygen content of the water which in turn affects nutrients and metals [18, 29]. Constructed wetlands also attract large organisms such as birds which can feed on contaminants. Additionally, they form attachment surfaces for other protozoans and other microorganisms such as zooplanktons, phytoplanktons, and bacterioplanktons which also aid in the elimination of pollutants and contaminants [30, 31]. Vegetation acts as the main trapping and retention points for most contaminants. Studies have continued to detect a high concentration of emerging contaminants such as resistant genes within the root systems of most constructed wetland vegetations [11, 32].

2.2.4 Other life-forms

Constructed wetlands' performance is also a factor of other life-forms. Organisms within the wetlands include microorganisms and other larger animals. The regulation functions by the microorganisms and their metabolism processes are the fundamental functions of the wetlands systems [33]. The microorganisms are varied in species and possess the required adaptions to drive the functions of the wetland systems. The known significant microorganisms include bacteria, yeasts, fungi, protozoa, and rind algae. The biomass generated from these microbes (microbial biomass) forms a major useful sink for organic carbon and many nutrients. Additionally, the microbial activities also transform a great number of organic and inorganic substances into innocuous or insoluble substances as well as alter the reduction/oxidation (redox) conditions of the substrate, and thus not only affect the processing capacity of the wetland but also enhance the recycling of nutrients. Some microbial transformation processes are aerobic as they require free oxygen to occur, while others are anaerobic as they occur under the absence of free oxygen. However, most of the bacterial species are also facultative anaerobes in nature. These groups are capable of functioning under the constructed wetland conditions of either aerobic or anaerobic in response to changing environmental conditions [6, 34].

The level of water within a constructed system is crucial to the microbial activities, and microbial populations undergo adjustments to changes in the water delivered to them. Populations of microbes can rapidly expand under the condition of suitable energy-containing materials. However, when environmental conditions become unsuitable, many microorganisms become dormant and can remain dormant for years [35]. The microbial community of a constructed wetland can be affected by toxic substances, such as pesticides and heavy metals, and care must be taken to prevent such chemicals from being introduced at damaging concentrations. The biodiversity with the constructed wetlands is rich, and this is based on the favorable habitat that the system provides to different forms of organisms, which range from animals to plants, including invertebrates and vertebrates. The invertebrate animals, which include insects and worms, contribute to the treatment process by actively fragmenting detritus and consuming organic matter [36].

Additionally, the larvae of many insects are also aquatic and they undertake the consumption of a significant amount of material during their larval stages, which may last for several years in most insect species. The invertebrates also perform a number of ecological roles; for example, dragonfly nymphs have been confirmed to be important predators of mosquito larvae which results in biocontrol of malaria in most waterlogged areas. Despite invertebrates being the most important animals as far as water quality improvement is concerned, constructed wetlands also harbor a variety of amphibians, turtles, birds, and mammals, all of which are important in the systems' ecological balancing [37].

3. Constructed wetlands for wastewater treatment

The mechanisms that are available to improve water quality within a constructed wetland system are numerous and often interrelated. The mechanisms involve the settling of suspended particulate matter; the filtration and chemical precipitation through contact of the water with the substrate and litter; chemical transformation; adsorption and ion exchange on the surfaces of plants, substrate, sediment, and litter; the breakdown and transformation of pollutants by microorganisms and plants uptake; and transformation of nutrients by microorganisms and plants as well as the predation and natural die-off of pathogens [36]. The removal can be undertaken biologically through microbiological degradation through catabolism and anabolism, protozoic predation and digestion, and through plant uptake and storage; chemically through adsorption (ionic and covalent) oxidation, reduction, and UV degradation and physically through filtration and settlement, which filters some materials and degrades others [38–40].

Constructed wetland treatment technology incorporates the principal components of wetland ecosystems that promote degradation and control of contaminants by plants, degradation by microbial activity, and increased sorption, filtering, and precipitation [38–40]. The treatment need dictates the nature of technology required and requires proper selection of designs, such as surface or subsurface flow, single or multiple cells, and parallel or series flow. Putting up of constructed wetland systems are sometimes part of a treatment train that integrates processes in series such as settling ponds, oil/water separators, and physical/chemical treatment methods. The removal mechanisms within the constructed wetlands can act uniquely, sequentially, or simultaneously on each contaminant group or species [3, 4]. For instance, the volatile organic compounds (VOCs) in contaminated groundwater are primarily eliminated through the integrative physical mechanism of diffusion-volatilization. Further to this, mechanisms such as adsorption to suspended matter, photochemical oxidation, and biological degradation may also play a role. Within a constructed wetland treatment system, physical removal mechanisms of contaminants include settling, sedimentation, and volatilization. Gravitational settling is responsible for most of the removal of suspended solids. The most effective treatment wetlands are those that foster these mechanisms.

3.1 Merits and demerits of constructed wetlands in waste handling

The long-term effectiveness of constructed wetlands to contain or treat some contaminants is not well known. Wetland aging may contribute to a decrease in contaminant removal rates over time. However, constructed wetlands are a cost-effective and technically feasible approach to treating wastewater and runoff for several reasons [41].

Constructed wetlands' demerits outweigh the merits. Some of the merits are that they can be less expensive and more affordable to build than other forms of treatments, their cost of operation and maintenance (required supplies and energy) are low, and the operation and maintenance only require periodic and not continuous on-site labor. Furthermore, the constructed wetlands are able to tolerate fluctuations in flow, they sustainably facilitate water recycling and reuse, they provide favorable habitat for many wetland organisms, and the system can be built to fit harmoniously into the landscape. Constructed wetlands have the ability to provide numerous benefits in addition to water quality improvement, such as wildlife habitat that supports tourism and other sporting, and they enhance the esthetic enhancement of open spaces. Therefore, due to all the above economic, ecological, and esthetic benefits, constructed wetlands are environmentally sensitive treatment approaches that are viewed with favor by the general public [42].

The use of constructed wetlands is also subject to limitations that are associated with the use and putting up of the system. Compared to conventional wastewater treatment systems, constructed wetlands generally require larger land areas. Even though wetland treatment may be economical relative to other options, this only applies to where land is available and affordable. The constructed wetland's performance efficiency may be less consistent as compared to the conventional treatment. The treatment efficiency of constructed wetlands may vary; this variation may be seasonal in response to changing environmental conditions, including rainfall and drought or spatial in relation to the existing weather conditions in different places. While the average performance over the year may be acceptable, but due to such fluctuations in performance efficiency, wetland treatment cannot be relied upon if the effluent quality must meet stringent discharge standards at all times. The biological components are always sensitive to toxic chemicals, such as ammonia, and other pesticides that are periodically flushed or surged by the flowing water, and this may temporarily reduce treatment effectiveness and reduce the efficiency. For proper survival and improved efficiency, constructed wetlands also require a minimum amount of water. While wetlands can tolerate temporary drawdowns, they cannot withstand complete drying and some plants in it can also not tolerate complete submergence [1]. The use of constructed wetlands for wastewater treatment and stormwater control is a fairly recent development. There is yet no consensus on the optimal design of wetland systems, nor is there much information on their long-term performance. Furthermore, its ability and potential to eliminate emerging contaminants such as resistant genes have not been fully realized [32].

4. Constructed wetlands and drug-resistant bacteria and related genes

Antibiotic-resistant genes (ARGs) originate from hospitals, wastewater treatment plants effluents and sewage sludge, and animal slurry in farmland. Soils, surface water (e.g., seas and rivers), and sediments are contaminated by these large arrays of antibiotic resistance genes [43]. Resistant genes are the major courses of antibiotic resistance, which is one of the upcoming crucial concerns to global health care with considerable effect in rising morbidity, mortality, and costs associated with major public health problems. Antimicrobial resistance occurs naturally over time, usually through genetic changes. However, the misuse and overuse of antimicrobials is accelerating this process [44]. Horizontal and lateral gene transfers have greatly contributed to the increasing number of drug-resistant pathogens within the environment (**Figure 1**).

Antibiotic resistance has the potential to affect people at any stage of life as well as the health-care, veterinary, and agriculture industries, making it one of the world's most urgent environmental and public health problems. [45]. Its chain of spread spans from contaminated wastewater discharges from the hospitals to the consumption of contaminated food material (**Figure 2**). The occurrence of antibiotic-resistant genes in the environment is considered one of the most urgent threats to modern health care and environmental quality and safety. It is often assumed that the abundance and diversity of known resistance genes are representative also for the non-characterized fraction of the resistome in a given environment [46]. Antibiotic resistance genes are ubiquitous in the environment, which has led to the suggestion that there is a high risk these genes can cause in the spread of the disease [46, 47].

Constructed wetlands, though designed to remove and eliminate pollutants from wastewater, can also be the hot spots for horizontal or vertical gene transfer, enabling the spread of antibiotic resistance genes between different microorganisms. Antibiotic resistance occurs due to changes or mutations in the DNA of the microorganism, or due to the acquisition of antibiotic resistance genes from other microbial species through gene transfer. The transfer of genetic materials between unrelated individuals is termed horizontal gene transfer, while the transfer of genetic materials from parent to their offspring is termed vertical gene transfer [48]. Horizontal gene transfer is the major source of ARGs as well as the emergence of pathogenic forms

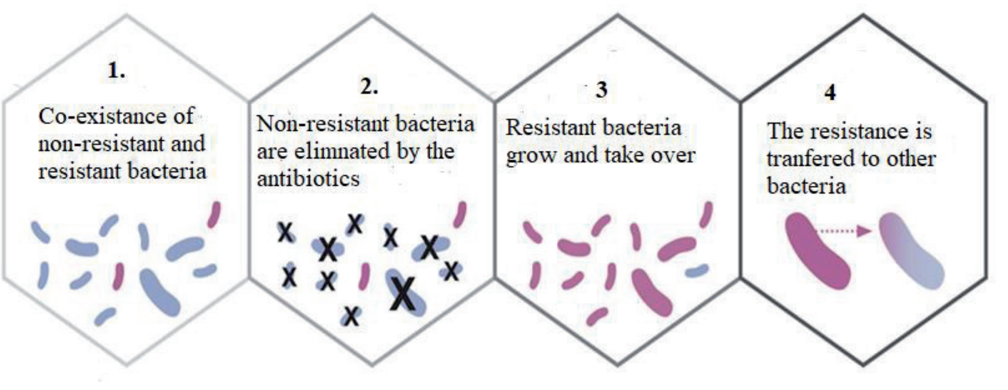

Figure 1.
The transfer of resistant genes between resistant and nonresistant microbes.

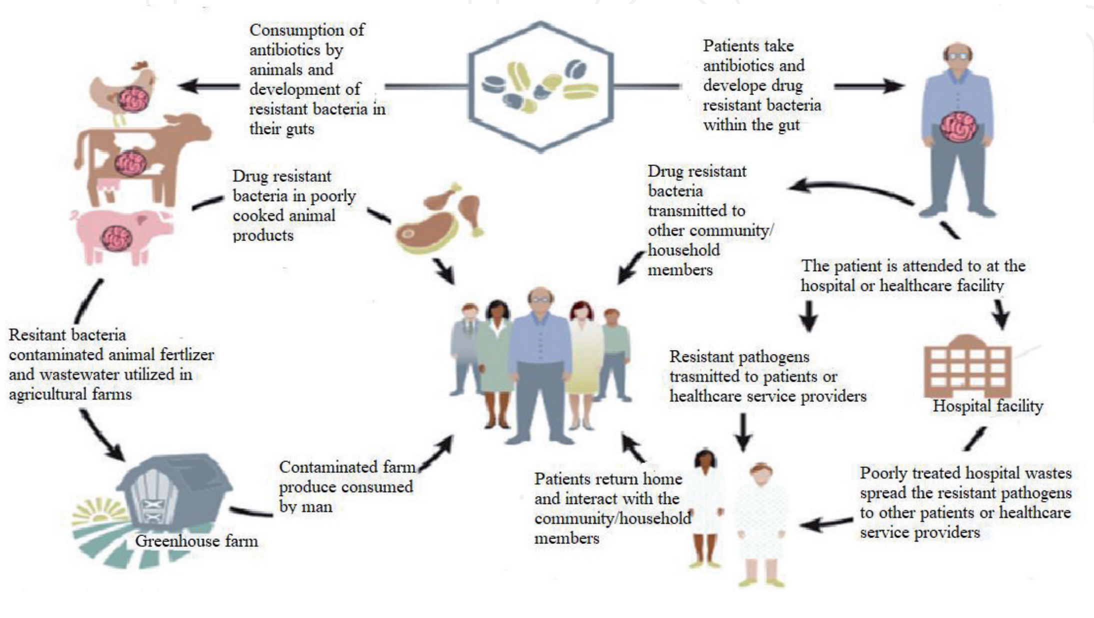

Figure 2.
Resistant genes contamination pathways.

of microorganisms with new virulence [11, 12]. Constructed wetlands being the reservoir for various strains and species of microorganisms may provide the media for such transfers to occur, hence contributing to problems of drug resistance [11]. There is rising concern due to the wide presence of antibiotics in the constructed wetlands, as it not only causes serious toxic effects on organisms but also promotes the spread of antibiotic-resistant genes (ARGs), even with low concentrations in the environment. ARGs being spread through horizontal or vertical gene transfers can also be spread and maintained in microbial populations, even without selection pressure from antibiotics, and wetlands systems provide favorable transfer grounds [12].

The recognition that the environment could serve as a source for resistance genes to human pathogens has spurred interest in investigating the distribution of resistance genes in various environments to better understand the process [10, 32]. Wastewater and wastewater treatment plants such as constructed wetlands can act as reservoirs and environmental suppliers of antibiotic resistance through filtration and load of resistant genes into the aquatic ecosystems [13]. Indeed, wastewater has been confirmed to be the major route by which the antimicrobials, ARBs, and ARGs are introduced into the natural ecosystem from the human settings. Although wastewater treatment plants such as the constructed wetlands significantly reduce the load of bacteria, the final effluents may contain ARBs, sometimes even at higher concentrations than in the raw wastewater [11, 12].

Considerable research has been conducted on the behavior and fate of ARBs and ARGs discharged from different forms of wastewater to soil through the application of animal manure wastewater irrigation and to aquatic environments through wastewater discharge and runoff. The impact of discharging ARGs in treated wastewater to aquatic systems as well as associated ARG amplification and attenuation dynamics has neither been adequately researched nor discussed. Indeed, intracellular and free ARGs in surface and groundwater can propagate through horizontal gene transfer to indigenous pathogenic microbes. Furthermore, these ARBs may eventually reach and colonize humans through multiple pathways resulting in acute infections or long-term silent colonization that can eventually evolve into an infection [13].

4.1 Challenges of emerging resistant genes in constructed wetlands

There are various routes through which the antibiotic-resistant genes can enter the environment. One major route is when the antibiotic-resistant pathogens and associated metabolites are released from hospitals through urine and feces from patients as hospital wastewater. After the release, the effluent physical chemical characteristics and the prevailing environmental factors determine the biodegradation, adsorption, and uptake processes of these drug-resistant pathogens and related genes, eventually shaping the abundance and diversity of the available drug-resistant bacteria. Similarly, antibiotics may be released into the wastewater treatment system via people taking antibiotics from home (**Figure 3**). From the wastewater treatment plants, the antibiotics can load into sludge, which are later dispersed on fields as fertilizer or released as runoff directly into the receiving surface water [49, 50]. Further to this, wastewater can also be treated by releasing it into constructed wetlands. In such cases, the constructed wetlands will be exposed to antibiotic contaminants from the wastewater. Even though the constructed wetland is expected to filter all the contaminants, including the drug-resistant pathogens and related genes, that is always not the case, the receiving effluents may still receive some amount of drug-resistant pathogens and related genes as effluent loads from the constructed wetland system. Additionally, antibiotics are also used therapeutically or as growth promoters in livestock and poultry. Antibiotics and

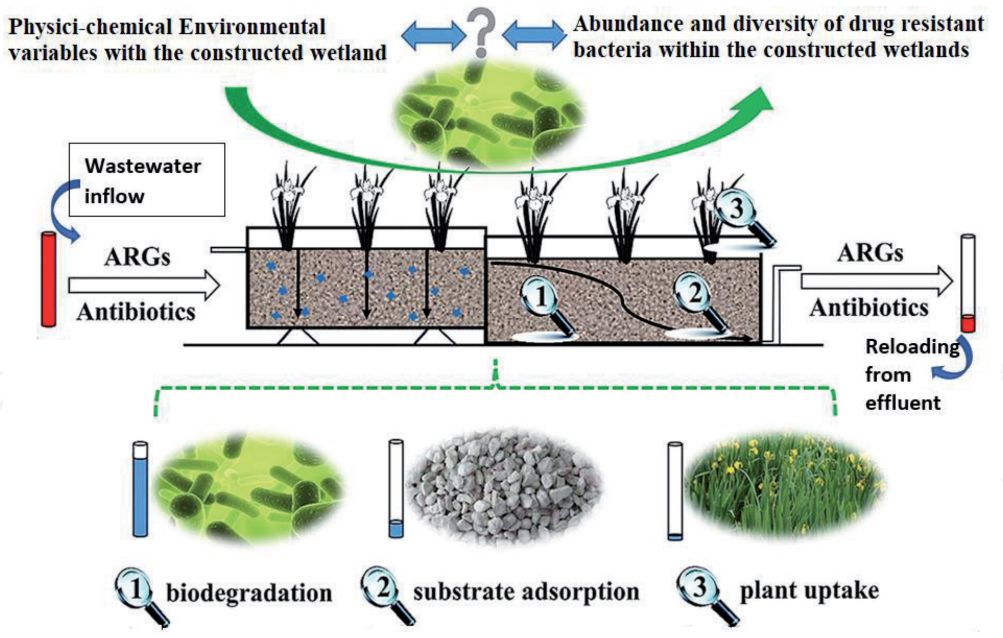

Figure 3.
Contribution of constructed wetland in the ARG removal and reloading.

their metabolites can spread through animal excrements and end up in the treatment systems such as the constructed wetlands, which can eventually release the treated effluents into the fields and groundwater, or in the case of antibiotic use in fish farms, directly into the aquatic environment. It is also worth noting that wherever antibiotics are spread, it is also likely that resistant bacteria follow the same routes of dispersal [51, 52]. Due to these interactions and movements of the drug-resistant pathogens and related genes, there have been increased levels of antibiotics, ARGs, and drug-resistant bacteria within the environment. Furthermore, the environmental bacterial flora which also harbor ARGs and potential ARGs continue to increase within the receiving aquatic environments. Therefore, these types of environments are the likely resistance hot spots where ARGs proliferate and new resistant strains are created by and transferred to other parts of the environment. Due to the increased spread of drug-resistant bacteria and related genes, the routes by which humans come into contact with these bacteria are also increasing. These may include consumption of crops grown by contaminated sludge used as fertilizer, drinking of water drawn from contaminated groundwater or surface water, and frolicking in marine water linked to contaminated surface water. When these resistant bacteria enter humans, they have the opportunity to spread their ARGs to the human microbiome and, through constructed wetlands in wastewater treatment, the cycle repeats [50, 53].

While ARGs in their environmental context may originally have had other primary functions aside from conferring resistance to antibiotics, these genes have now been recruited as resistance genes in pathogenic bacteria. Reuse of treated wastewater is increasingly seen as one of the solutions to tackle the water scarcity problem and to limit the pollution load to surface water. Yet, using reclaimed water for non-potable purposes and particularly to irrigate food crops presents an exposure pathway for antibiotics and antibiotic-resistant bacteria and genes (ARB & G) to enter the human food chain. Wastewater reuse is currently of particular concern as the potential source of selective pressure that elevates the levels of antibiotic resistance in native bacteria [54]. Aquatic ecosystems are considered important matrices for the release, mixing, persistence, and spread of ARBs and ARGs associated with horizontally transferable genetic elements [11, 12]. Presently, existing regulations give little attention to the protection and management of wetlands, making them to increasingly get exposed to resistant gene-loaded human excreta, raw sewage, untreated wastewater, and other pollutants from diverse sources, making natural and constructed wetlands to be the potential reservoirs of ARBs carrying ARGs that might spread to microbes as well as man [55].

5. Conclusion and recommendations

The knowledge on antibiotic resistance in wastewater has continued to expand, but proper management for complete elimination with zero reloading into the environment has not been achieved. Indeed, in the past few years, introduction of high-tech molecular studies has increased the understanding on this study subject. However, there are still numerous gaps on the subject, such as how active are horizontal and lateral gene transfers in wastewater, what are the specific main driving factors to the transfer mechanisms, and what is the role of the wastewater treatment plants in increasing the spread of drug-resistant microbes. Indeed, even though constructed wetlands have been commercially used to control and degrade municipal and industrial wastewater, there is need for caution on how exotic wastes such as explosives and those that harbor resistant genes are handled by these systems. With the growing concerns that environmental concentrations of antibiotics exert a selective pressure on clinically relevant bacteria, for the control of such acute strains, there is need for a major shift toward a more localized management of the water cycle, pioneering low-cost wastewater treatment technologies, and more efficient monitoring strategies based on a limited number of indicators that would facilitate the assessment of the anthropogenic impact on the water cycle. Furthermore, there is need to better understand the dispersion processes and the fate of pathogenic and antibiotic-resistant bacteria in the environment, in order to prevent risks to humans and their environment, while also controlling and reducing as much as possible the anthropogenic bacterial input into the environment.

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