Chapter 17

Effective control of waterborne pathogens by aquatic plants

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The role of aquatic plants in treating wastewater is well established. Recent studies have shown that aquatic plants possess potential to remove pathogens from wastewater. High removal (90%) of pathogenic microbes such as *Enterococci*, *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Clostridium perfringens*, *Staphylococcus aureus*, and *Salmonella* have been achieved using aquatic plants viz. *Typha latifolia*, *Cyperus papyrus*, *Phragmites mauritianus*, *Pistia stratiotes*, *Lemna paucicostata*, *Spirodela polyrhiza*, and *Eichhornia crassipes*. Pathogen removal by aquatic plants mainly occurs by attachment of pathogens to plant roots followed by filtration or toxicity exerted by exudates produced by them. Effective control of pathogens in constructed wetlands has been achieved because of various physical, chemical, and biological processes operating in them. Constructed wetlands with aquatic plants have shown high efficacy in treating pathogen-contaminated water. The exact mechanism of pathogen removal by these plants needs to be evaluated so that their role in phytoremediation technologies can be emphasized.

1. Introduction

Water contamination has increased tremendously throughout the globe. The presence of pathogenic organisms has significantly increased in the municipal and domestic wastewater in last few decades (Pandey et al., 2014; Ramírez-Castillo et al., 2015). Pathogens are the microscopic biological organisms capable of causing diseases. These mainly include bacteria, viruses, fungi, protozoa, helminths, and worms (Olaolu et al., 2014). The harmful effects from pathogens range from mild illness to chronic severe sickness. Waterborne pathogens cause diseases that prove fatal and result in million of deaths annually (Awuah et al., 2001; WHO, 2012). According to an estimate, diseases caused by waterborne pathogens cause about 1.4 million deaths in children

every year (Pandey et al., 2014). About 1407 species of pathogens infecting humans have been reported worldwide (Ashbolt, 2004; Jin et al., 2018). These mainly include 538 species of bacteria, 208 types of viruses, 57 species of parasitic protozoa, and several fungi and helminth species (Table 17.1). Some of the pathogens commonly found in wastewater include strains of *Shigella*, *Salmonella*, *Leptospira*, *E. coli*, *Mycobacterium*, *Vibrio*, *Pseudomonas*, *Staphylococcus*, protozoa *Giardia*, *Cryptosporidium,* cysts of *Entamoeba histolytica*, etc. (Cabral, 2010; Makvana and Sharma, 2013).

TABLE 17.1 Different types of pathogens found in wastewater.

Type of organism	Pathogenic species
Bacterial species	*Mycobacterium tuberculosis* Mycobacterium avium-intracellulare *Salmonella* spp. *Aeromonas hydrophila* *Yersinia enterocolitica* *Shigella* *Vibrio cholerae* *Helicobacter pylori* *Tsukamurella* *Cystoisospora belli* *Escherichia coli* *Campylobacter jejuni*
Helminths	*Ancylostoma duodenale* (hookworm)
Intestinal worms	*Schistosoma mansoni* *Schistosoma japonicum* *Schistosoma haematobium* *Diphyllobothrium latum*
Fungi	*Candida albicans*
Viruses	*Enterovirus* *Adenovirus* *Rotavirus* *Reovirus* *Parvoviruses* *Coronaviruses* (SARS) *Polyomavirus*
Protozoans	*Microsporidia* *Acanthamoeba* spp. *Cryptosporidium parvum* *Cyclospora cayetanensis* *Giardia lamblia* *Entamoeba histolytica* *Balantidium* cell

Intake/ingestion of contaminated water leads to waterborne infection in humans. The pathogenic microbes infect the gastrointestinal tract and cause diseases such as cholera, typhoid, paratyphoid fever, diarrhea, giardiasis, infectious hepatitis, and amebic/bacillary dysenteries. The severity of the pathogen infection depends on the dose and the susceptibility of the host (Ramírez-Castillo et al., 2015). Most of the pathogens live for short period of time outside the human body. Transfer of resilient bacterial cysts, oocysts, and direct pathogen through contaminated water causes infection in humans.

Realizing the health risk caused by consumption of water contaminated with pathogens, various technologies for treatment of wastewater have been developed. These technologies proved useful in improving the quality of drinking water by reducing the number of pathogenic microbes via physical and chemical treatment methods (Scott et al., 2004). Water treatment approaches aim to improve quality of water by removing pathogen contamination (Zhang and Farahbakhsh, 2007). Treatment efficacy is widely measured using the log removal value (LRV). Treatment should be done in a way that removes maximum number of pathogens followed by disinfection so that high decontamination can be achieved. Pathogen treatment is done mainly by two processes—removal and inactivation (disinfection). In most of the technologies, pretreatment is done using coarse filters (gravel, sand) or removal of turbidity. This method reduces protozoa concentrations (LRV 2—3) effectively. Pretreatment is generally followed by clarification process done by means of flocculation or coagulation. This is generally followed by sedimentation.

Most of the modern day water treatment technologies include primary and secondary processes. The primary treatment processes include removal or inactivation of pathogens (disinfection). The chlorination, ozonation, and exposure to UV radiation are some of the effective methods used for disinfection (Johnson et al., 2010; Gomes et al., 2019). Heat treatment kills pathogens by exceeding thermal tolerance. Oxidation using chlorine gas and sodium hypochlorite removes pathogens by killing them. Oxidation disrupts the organic structure of the pathogen. Exposure to UV radiation disrupts the genetic material of the microbes and restricts their replication. The secondary processes generally followed after primary treatment includes flocculation, coagulation, and sedimentation.

Membrane filtration has emerged as a viable water treatment approach over the last decade. Use of microfiltration (MF) and ultrafiltration (UF) membranes showed great potential for removal of pathogens (Hai et al., 2014). A significant removal of pathogens viz. virus, bacteria, and protozoa could be achieved by these filtration system methods (LRV of 1—2 as prescribed by the World Health Organization). MF removes algae, protozoa, and many bacteria effectively. LRV between 4 and 7 has been achieved for *Giardia* and *Cryptosporidium* using a 0.1 μm membrane. Low virus removal is achieved by using MF. This is because of pore size considerations. The virus species are strongly associated with particles. Effective virus removal is achieved using

UF. The pathogen removal efficiency in all the cases depends upon the quality of the water and other parameters.

Apart from the physical and chemical treatment methodologies, the biological techniques such as biological reactors and filters were also evaluated for their efficacy to treat pathogen-contaminated water (Chaudhry et al., 2015; Sharma and Bhattacharya, 2017). Both dispersed growth and attached growth biological wastewater treatment systems assist in removal of pathogens but require high hydraulic retention time (HRT) (average time water molecules stay in the system) and continuous feeding of organic matter and nutrients. Moreover, the attachment of the microbes to the support medium in these systems is influenced by media composition, cell–cell interaction, and polymer molecules (Sehar and Naz, 2016).

Most of the treatment processes are expensive, complex to operate, and require huge setup, hence become unaffordable for developing countries. Membrane filtration techniques though are inexpensive, readily available, and disposable but assist in achieving effective pathogen removal at a small scale. Because of these limitations, research studies in the recent years have focused on developing alternate cost-effective sustainable technologies for wastewater treatment. Phytoremediation has emerged as an eco-friendly, cost-effective technology that possesses great potential to remove various contaminants (organic, inorganic, and biological) from water at a time (from a single go). The role of terrestrial and aquatic plant species in cleaning the environment via removal of contaminants has been well established (Conesa et al., 2012; Das, 2018; Mahajan and Kaushal, 2018). Aquatic plants in particular have shown great capacity to treat water contaminated with inorganic and organic contaminants (Zimmels et al., 2004; Schröder et al., 2007; Dhir et al., 2009, 2013; Shah et al., 2014; Saha et al., 2017; El-Din and Abdel-Aziz, 2018). Recent studies demonstrated that aquatic plants are also capable of treating water contaminated with pathogens (Reinoso et al., 2008; Kipasika et al., 2016; Srivastava et al., 2017).

2. Removal of pathogens by aquatic plants

Aquatic plants irrespective of algae, macrophytes showed good potential to remove pathogens from water. Some studies proved that algae treat bacteria-contaminated water (Abdel-Raouf et al., 2012). Algae such as species *Rhizoclonium implexum* (a freshwater species) when used in the quantity of 150 and 200 g showed maximum reduction of 100% of fecal coliforms from 7 L of water. Residence time of 6 days provided 100% reduction of all fecal indicator bacteria. Present study established that algae can prove effective for reduction of coliform bacteria from municipal wastewater. Algal-based wastewater treatment system can prove as a good alternate for treatment of pathogen-contaminated wastewater (Ahmad et al., 2014). The amount of biomass played a crucial role in removal of pathogens by algae (Ansa et al., 2012a).

The presence of algae leads to increase in pH and oxygen concentration. Increase in both the parameters proves detrimental to fecal coliforms (Davies-Colley et al., 1999; Awuah et al., 2001). Increase in the concentration of dissolved oxygen increases the rate of decay of fecal coliforms (Curtis et al., 1992). It is supposed that in the presence of light, toxic forms of oxygen molecules are produced (peroxides and singlet oxygen) (Curtis et al., 1992). The concentration of these molecules increases with increased dissolved oxygen (DO) concentration. The toxic forms of oxygen are injurious to bacteria. They damage the cytoplasmic membrane of the bacteria (Awuah, 2006). The damage of fecal coliform increases with changes in pH. Research studies have shown that algal toxins play a role in the inactivation of fecal coliforms. It has been observed that algae produce toxins when grown in waste stabilization ponds (Oudra et al., 2000). Cyanobacteria (or blue-green algae) *Synechocystis* sp. produced toxin microcystin in waste stabilization ponds. The toxin proved toxic for the fecal bacteria. Studies have shown that green algae release some substances that prove harmful to fecal coliforms, thus contributing to their removal (Ansa et al., 2012b). Increased inactivation of fecal coliforms was also noted in darkness with increased chlorophyll-a concentration (Ansa et al., 2012b). The studies lead to the conclusion that some substance in the algae might be contributing to the inactivation of the fecal coliforms.

Realizing the potential of algae in removal of pathogens from water, algal turf scrubbers (ATS) were designed by engineers. They represent ecologically engineered systems that utilize the growth of filamentous algae on a submerged surface to remediate polluted water. They use energy from the sun for running the process. These scrubbers showed an ability to remove or degrade a variety of pollutants from water including nutrients, metals, and organic chemicals. ATS also showed ability to treat water polluted with pathogenic bacteria. These scrubbers showed an ability to trap pathogenic bacteria through adhesion onto mucilaginous algal filaments. Algal turf could remove *Flavobacterium columnare* and *E. coli* from the water column with an average 3.6 log reductions in *F. columnare* and 2.6 log reduction with *E. coli* over a 24 h period (Rains, 2016). Some studies have also reported that high rate algal ponds reduce the level of pathogenic bacteria significantly from water.

The pathogen removal in macrophyte (*Pistia stratiotes* and *L. paucicostata*) and algal-based wastewater treatment systems was evaluated. The batch scale studies indicated that all treatment systems irrespective having duckweed, lettuce, and algae showed pathogen removal and biochemical oxygen demand (BOD) reduction over a period of 29 days under tropical conditions (temperature ranged between 28.3 and 30°C, pH between 4.5 and 5). High pH levels around 10.5 and DO levels of about 20 mg/L and decrease in the fecal enterococci population from 1.18×10^5/mL to values below 100/mL in all treatment systems were observed in the algal-based system (Awuah et al., 2001).

High removal of pathogens such as *Salmonella* spp., *E. coli*, and other fecal coliforms from wastewater has been achieved using aquatic plant species, viz. *T. latifolia*, *C. papyrus*, *C. alternifolius*, *P. mauritianu*s, *Iris pseudacorus*, and *S. lacustris* (Molleda et al., 2008; Katsenovich et al., 2009; Leto et al., 2013; Abou-Elela et al., 2014). Duckweed ponds have shown significant reduction in the number of total coliforms (57%), fecal coliforms (62%), and coliphage (38%) (Karpiscak et al., 1996). Studies indicate that effective removal of waterborne pathogens by aquatic plants can be achieved in constructed wetlands (Stefanakis and Akratos, 2016).

The removal of pathogens by aquatic plants is supposed to occur via different mechanisms: (1) The chemical substances, i.e., antimicrobial compounds, produced by roots of aquatic plants reduce the survival of pathogens such as bacteria (Sundaravadivel and Vigneswaran, 2001; Stottmeister et al., 2003). (2) Plants regulate the supply of oxygen to roots. Oxygen is crucial for the activity and metabolism of microorganisms such as protozoa, nematodes, bacteria, and viruses (Stottmeister et al., 2003; Vymazal, 2005). (3) Extensive roots of macrophytes or biofilms formed on the plant material/basal media provide surface area for attachment/adherence of microorganisms including bacteria such as *E. coli* (Stevik et al., 2004; Stott and Tanner, 2005). (4) The secondary defensive compounds release on wounding of plants during pathogen or insect attack proves also affect the survival of pathogens (Taiz and Zeiger, 2006). The submerged plants and their associated biofilms form "sticky traps" which are capable of trapping a considerable number of organisms (Stott and Tanner, 2005; Weber and Legge, 2008; Kadlec and Wallace, 2009).

The capacity of the aquatic plants in removing parasites is variable and depends upon the properties such as surface area, unique biofilms, and water quality (Hogan et al., 2013). Temperature, pH, salinity, and pathogen−sediment interaction are some other factors that also affect the removal of pathogens (microbes) (Stott, 2003; Stevik et al., 2004; Searcy et al., 2006; Hogan et al., 2013).

Researchers found that seagrass meadows act as efficient systems in reducing the potentially pathogenic bacteria, viz. *Enterococcus* by 50%. Studies indicated that release of oxygen via photosynthesis in seagrasses proves toxic to pathogens. The chemicals isolated from leaf blades of seagrasses are supposed to kill or inhibit growth of numerous bacterial pathogens. Natural biocide produced by seagrasses removed microbiological contamination (Lamb et al., 2017). Studies have proved that eelgrass beds having *Zostera* sp. filter and trap potentially harmful bacteria from coastal waters (Webb et al., 2019).

Recent studies have indicated that xylem tissue in plants possesses capacity to remove biological contaminants such as pathogens. Xylem is a porous material and acts as membrane comprising of nanoscale pores (few nanometers to around 500 nm). Xylem conducts, uptake of fluids from their roots to

shoots, can prove feasible as filter where the flow rates of sap are driven by gravitational pressure. These pores act as ideal system for filtering out pathogens and hence can prove as inexpensive water filtration devices. The xylem filter could effectively filter out bacteria such as *E. coli* from water with rates exceeding 99.9% (Boutilier et al., 2014). Xylem filters fabricated with inexpensive, biodegradable, and disposable materials could prove as ideal materials for filtering pathogens from water (Boutilier et al., 2014).

3. Role of wetlands in treating pathogen-contaminated wastewater

Constructed wetlands (CWs) have proved as the most efficient wastewater treatment systems because of their capacity to remove various pollutants. Effective treatment of wastewater released from different sources such as agriculture, food processing, tannery, stormwater runoff, landfill leachate, acid mine drainage, municipal discharges, and domestic sources has been achieved using wetlands (Headley et al., 2004; Calheiros et al., 2007; Ahmed et al., 2008; Vymazal, 2010; Gruyer et al., 2013; Zurita and Carreón-Álvarez, 2014; Zhang et al., 2014; Maiga et al., 2017; Almuktar et al., 2018). Easy handling, operation using solar energy, self-organization, increased treatment capacity over time, and high levels of treatment with less maintenance establish them as ideal wastewater treatment systems (Hench et al., 2003). Recent studies indicated that wetlands act as excellent biofilters and reduce significant levels of microbes such as bacteria from wastewater (Gersberg et al., 1989; Gopal and Ghosh, 2008; Meng et al., 2014; Alexandros and Akratos, 2016). Effective removal of pathogens (rates up to 99%) has been achieved using constructed wetlands and hence considered as the best wastewater treatment systems (Stefanakis and Akratos, 2016; Donde and Xiao, 2017). Significant removal of various types of pathogens, i.e., bacteria, viruses, protozoan, and helminths, has been noted in constructed wetlands (Al-Gheethi et al., 2018). Effective removal of fecal coliforms (90%−98%), MS2 coliphages (67%−84%), and protozoa (60%−100%) from wastewater has been reported in wetland wastewater treatment systems (Smith et al., 2005; Ávila et al., 2013; García et al., 2013; Marín et al., 2015). High removal capacity of eggs from parasitic organisms (nearly 100%) has also been noted in wetland treatment processes (Ávila et al., 2013). Bacteria and virus removal as high as $<1 \log_{10}$ has been reported in vegetated wetlands. Significant reduction of waterborne bacterial pathogens namely *Salmonella*, *Shigella*, and *Vibrio* (by average of 94%, 87%, and 94%) from domestic wastewater has been reported from a constructed wetland in different seasons of the year (rainy, winter, and summer) (Makvana and Sharma, 2013).

Wetlands act as biofilters. They remove pathogens such as bacteria, virus, protozoa, and helminths via a combination of physical, chemical, and biological processes taking place in the wetland (Kadlec and Knight, 1996;

Decamp and Warren, 2000; Werker et al., 2002; Kuschk et al., 2012; Jasper et al., 2013). The removal of pathogens in wetlands occurs because of filtration by plant roots, attachment to plant roots, plant microbe interaction within biofilms, adsorption to the soil/media/organic matter, sedimentation, and predation by microorganisms (Jiménez, 2007; Alufasi et al., 2017). Other mechanisms involved in removal of pathogens include (1) exposure of pathogens to biocides excreted by macrophyte roots, (2) natural die-off, (3) predation by nematodes, protozoa, and rotifers, and (4) oxidation and exposure to UV radiation (Kadlec and Wallace, 2008). The root zone (or rhizosphere) has been considered as active zone of constructed wetlands because of the presence of macrophytes growing there. Various physical, chemical, and biological processes take place in this zone. These processes are induced by the interaction of plants, microorganisms, the soil, and pollutants. This is referred as root zone technology (Preetha and Senthil, 2009; Makvana and Sharma, 2013). Biofilms present in the plant roots are believed to supply a more effective substrate for removal of bacteria through various methods such as mechanical filtration, sedimentation, adsorption, die-off, predation, and antibiotic excretion (Soto et al., 1999; Karathanasis et al., 2003) Removal (83%−94%) of pathogen especially coliform and enteric bacteria by surface flow constructed wetlands has been reported earlier (Perkins and Hunter, 2000). Studies have indicated that beds and stand of reeds reduce pathogens such as *Salmonella*, *Enterococci*, and *E. coli* by mechanisms such as predation by protozoa and exposure to antibiotic excretions from the roots of plants (Perkins and Hunter, 2000; Healy and Cawley, 2002; Nielsen, 2007).

The presence/absence of plants also affects pathogen removal in wetlands (Arias et al., 2003; Vymazal, 2005). Generally high removal of pathogens has been reported in wetlands that possess aquatic vegetation (Brix, 1997). A significant reduction in fecal coliform bacteria has been reported in constructed wetlands planted with aquatic species (Thurston et al., 2001). High removal of pathogens such as *E. coli* and *Salmonella typhimurium* has been reported from wetlands having aquatic plant*s*. About 99% removal of coliforms, *E. coli*, and *Streptococci* has been reported from a lagoon having plantations of water hyacinth in Malaysia. About 94%−99% removal of total coliform, fecal coliform, and Enterobacteriaceae has been reported in constructed wetlands located at the Czech Republic (Ottoval et al., 1997). Field studies reported greater reduction of indicator bacteria, bacteriophage, and viruses from vegetated wetlands (Karpiscak et al., 1996; Mandi et al., 1996; Green et al., 1997; Quinonez-Diaz et al., 2001). The removal of pathogens in these wetlands may be due to an increased competition for nutrients or trace elements with natural microorganisms, attack by lytic phage and bacteria, or predation by nematodes, protozoa, or ciliates. About 99% (equivalent to 2 $\log_{10}$ reduction) removal of fecal coliforms has been reported from a wetland planted with *Phragmites australis* after a retention time of 1.7 days (Rivera et al., 1997). A significant reduction of 98% and 93% in total and fecal

coliforms has been noted in a wetland system having multiple aquatic plant species such as bulrush, cattail, black willow, and cottonwood (Karpiscak et al., 1996). High removal of pathogens has also been reported from a vegetated constructed wetland in Spain (Molleda et al., 2008). Removal of waterborne plant pathogens such as *Pythium ultimum* and *Fusarium oxysporum* from the greenhouse wastewater has been reported (Gruyer et al., 2013). The development of microbial biofilm around the filter media and the production of cell wall−degrading enzymes assist in the removal of pathogens. The removal efficiency of 99.9% for *E. coli*, total coliforms, 97.0% for fecal streptococci, 100% for *C. perfringens*, and 100% in the case of *Giardia* cysts, *Cryptosporidium* oocysts, and helminth eggs has been noted in wetlands (Molleda et al., 2008).

Plants irrespective of free-floating, planted, or emergent assist in removal of pathogens from wetlands (Vymazal, 2013). They help in the removal of pathogens by mechanical filtration and adsorption processes. The removal of pathogens by plants occurs by different mechanisms: (1) influencing supply of oxygen to the microorganisms present in the root zone (Stottmeister et al., 2003; Vymazal, 2005), (2) adsorbing microbes to biofilms on the rocks, media, and plant roots (Stevik et al., 2004; Stott and Tanner, 2005), (3) excreting toxic antimicrobial substances by roots that prove toxic to pathogens (Sundar-avadivel and Vigneswaran, 2001; Stottmeister et al., 2003), and (4) predation by nematodes and protists (Gerba, 2000; Vymazal, 2005; Kuschk et al., 2012; Wu et al., 2016). The pathogens get attached to the root of aquatic plants, and roots filter out pathogens from wastewater. Biofilms around plant rhizosphere zone support proliferation of bacterial populations. Photosynthates and other compounds released by plant roots provide nutrients for these rhizosphere microorganisms. These microbes produce antibiotic compounds that lead to pathogen removal. Compounds produced by algae have been shown to inhibit or restrict the growth of bacteria.

The potential of the rhizobacterium can be enhanced through inoculation of antagonist bacteria. The bacteria promote rhizosphere competence and improve pathogenic bacteria removal from wastewater. In a horizontal sub-surface flow constructed wetland system planted with *P. australis*, the inoculation of the strain *Saccharomyces cerevisiae* PFH_1 remarkably improved the inactivation kinetics of the pathogenic bacterium *Salmonella typhi* in the plant rhizosphere. The single inoculation of the bacteria improved the kinetics of *S. typhi* inactivation by approximately log 1 unit while multiple inoculations brought enhancement in the rate of inhibition by log 2 units. Thus this strategy proved useful in enhancing the removal of pathogenic bacteria (Saad et al., 2019).

Vegetation plays an important role in filtering contaminants from wetlands (Vacca et al., 2005). Fibrous roots of plants construct pathogen filtration system. Plants build biofilm along the roots (VanKempen-Fryling and Camper, 2017). The pathogens attach to biotic plant roots. Vegetation increases the

surface area of the substrate for microbial attachment. The microbial communities in the biofilms help in the transformation of the contaminant. Vegetation provides biomass uptake and adsorption of nutrients (O'Geen and Bianchi, 2015). The aeration and oxygen level around plant roots assist in effective pathogen removal. Vegetated systems reported high DO levels than the unplanted control (Sharma and Brighu, 2016). More DO around the root zone reduces the pathogenic activity but increases the removal efficiency. Fibrous roots enable good aerated conditions required for removal of pathogens (Sharma and Brighu, 2016). Improved aeration in the root zone provides additional removal efficiency and helps to oxidize pathogens present on the root surface, thereby decreasing the pathogen load (Sharma and Brighu, 2016). High levels of DO around plant roots facilitate removal of microbes such as *E. coli* (Avelar et al., 2014).

Studies reported that inactivation of bacteriophage in wetlands has been caused because of secretion of microbial metabolites or the presence of proteolytic substances released by microbes or plants. Certain bacteria release proteolytic enzymes that are capable of destroying the protein capsid of the viruses. The substances that enhance inactivation of viruses might also be responsible for reduction in other pathogens. Thus antiviral properties of aquatic plants could be another possible mechanism causing inactivation of virus in vegetated wetlands. Besides this, enhancement in the bacterial population present in rhizosphere of vegetated wetlands might also be responsible for inactivation of viruses.

Predation is another mechanism that plays an important role in the removal of bacteria, protozoan (oocysts), and fecal coliforms from wastewater in constructed wetlands (Mandi et al., 1993; Green et al., 1997). Predators involved in removal of pathogens in wetlands mainly include nematodes, copepods, rotifers, and protozoa (Decamp and Warren, 1998). Bacteriovorous activity of protozoa and ciliates such as *Paramecium* spp., *Oxytrichids*, *Halteriain*, *Plagiopyla*, and *Casmomorphin* has been reported in wetlands (Green et al., 1997; Decamp and Warren, 1998). Studies indicate that besides the zooplanktons, rotifers also help in the removal of pathogens such as *Giardia* cysts and *Cryptosporidium* via grazing because of their presence in high amounts (Fayer et al., 2000).

The environmental variables such as sunlight, temperature, and pH regulate the removal of viral and bacterial pathogens from water. Temperature plays an important role in the reduction of enteric bacteria and viruses (Quinonez-Diaz et al., 2001; Winward et al., 2008). Studies report that high temperatures improve pathogen removal capacity by approximately 1 $\log_{10}$ unit (Ulrich et al., 2005). The increase in temperature also supports the predator activity of grazing protozoa (Weber and Legge, 2008). The constructed wetlands have a pH range between 5.5 and 7.5. The low pH contributes to high removal of bacteria such as *Salmonella* (Pundsack et al., 2001). The dissociation

of carbonate and bicarbonate ions in water increases pH, thereby causing death of fecal bacteria.

Sunlight also contributes to the inactivation of pathogens. It mediates the process via three different mechanisms. This includes photolysis of chemical contaminants, absorption of UV-B radiation by DNA, and formation of reactive oxygen species (ROS). The damage to cellular DNA occurs by formation of pyrimidine dimer (Davies-Colley et al., 1999). Indirect damage occurs when sensitizers absorb light and produce ROS. These ROS cause damage to pathogens. Superoxide radical is one of the ROS produced by sensitizers that cause inactivation of MS2 coliphage (Kohn and Nelson, 2007). High light intensity, i.e., wavelength more than 450 nm, causes damage to fecal bacteria because of increase in the level of dissolved oxygen and humic substances. Exogenous and endogenous substances (such as humic acid) absorb light energy and transfer it to other molecules leading to formation of ROS. Consequently, ROS react with pathogens and cause death because of cell damage (Davies-Colley et al., 1999; Muela et al., 2002). The process is known as photooxidation. The photolysis process is affected by change in pH of water. Hydroxyl radical (OH) gets quenched by inorganic carbon under alkaline conditions and results in the formation of carbonate radical (CO_3^-). High levels of dissolved oxygen affect the process of photolysis by removing intermediate triplet states or enhancing formation of O_2. The high oxygen concentration, i.e., high levels of dissolved oxygen present in wastewater, inactivates pathogens (Kohn and Nelson, 2007).

Sedimentation is another factor that removes the pathogens such as protozoans, coliforms, bacteria, and helminth eggs (Karim et al., 2004, 2008). Pathogens such as helminth eggs possess high settling velocities in comparison to other pathogens such as protozoans and bacteria and hence get removed by sedimentation process in wetlands (Sengupta et al., 2011). The presence of macrophytes enhances the sedimentation process. Other factors that influence pathogen removal include mechanical filtration, adsorption to organic matter, and adhesion to biofilm. The removal of pathogens (particularly subsurface constructed wetland) is also influenced by characteristics of the filter bed, i.e., nature of filter media, grain size. Studies have reported that wetlands constructed with peat media removed a larger amount of pathogens such as *Salmonella* in comparison to those having sand as media (Pundsack et al., 2001). High removal of 5 $\log_{10}$ units for *E. coli* and 3 $\log_{10}$ units for MS2 phage has been noted in a filter bed having fine soil (1−4 mm diameter) in a horizontal subsurface flow wetland in comparison to those having coarse soil (Ushijima et al., 2013).

The rate and mechanism of removal varies for each pathogen. The rate of removal of pathogen depends on its structure and genetic composition. Each pathogen has a different structure and genetic composition (Silverman et al., 2013; Mattle et al., 2014; Kohn et al., 2016), hence removal mechanism also varies for each species. Viruses are resistant than bacteria and hence do not get

easily removed by sunlight-mediated reactions (Sinton et al., 2002; Davies-Colley et al., 2005). Viral particles can be removed by attachment or adsorption to submerged stalks of emergent plants and biofilm layer of rhizomes and roots (Quinonez-Diaz et al., 2001; Nokes et al., 2003), while bacteria get removed via attachment to plant roots or sedimentation (Nokes et al., 2003; Wu et al., 2016).

Constructed wetlands irrespective of horizontal subsurface flow, horizontal free water surface flow, and vertical flow have shown efficacy in treating wastewaters (Arias et al., 2003; Mburu et al., 2008; Jiménez et al., 2010). Greater pathogen removal efficiency has been noted in a subsurface wetland in comparison to free water wetlands (Tables 17.2 and 17.3). Reduction of pathogens, i.e., bacteria to be as low as <1 to 3 $\log_{10}$, viruses <1 to 2 $\log_{10}$, protozoa <1 to 2 $\log_{10}$, and helminthes <1 to 2 $\log_{10}$, has been noted in subsurface wetland (Nguyen et al., 2015; Silverman et al., 2015). A better pathogen removal capacity has been reported in horizontal subsurface wetlands in comparison to free water surface flow wetlands (Wu et al., 2016). Vertical flow wetlands have aerobic environment in the water and an anaerobic environment at the bottom. The removal of the cysts to the capacity 2 $\log_{10}$ has been noted for protozoans such as *Cryptosporidium* and *Giardia* in horizontal and vertical flow subsurface wetlands (Stott et al., 2001; Falabi et al., 2002; Redder et al., 2010). According to literature reports, hybrid constructed wetland systems have also shown good efficiency in treating pathogen-contaminated wastewater (Kim et al., 2006; Barros et al., 2008; Reinoso et al., 2008). A horizontal surface flow constructed wetland treatment system has shown great potential for treating wastewater containing domestic sewage in Karachi (Mustafa, 2013). The pilot-scale constructed wetland planted with the Phragmites karka (Retz.) reduced indicator bacteria (total coliforms and fecal coliforms). The average removal of the both total and fecal coliforms ranged from 93% to 99% (Mustafa, 2013). Furthermore, Almuktar et al. (2017) assessed the possibility of recycling domestic wastewater treated by vertical flow constructed wetlands for crop irrigation.

TABLE 17.2 Comparative assessment of wetlands for removal of pathogens.

Pathogen	Reduction of pathogens ($\log_{10}$)	
	Free water surface flow wetland	Subsurface flow wetland
Bacteria	$<1-2$	$1-3$
Viruses	$<1-2$	$1-2$
Protozoa	$1-2$	2
Helminths	$1-2$	2

TABLE 17.3 Average removal of pathogens reported in wetlands.

Pathogen	Removal (%)
Total coliforms	57−90
Fecal coliforms	62−99
Giardia	98
Cryptosporidium	87
Rotavirus	99
Helminth eggs	100

Vymazal (2005) demonstrated that free water surface flow constructed wetlands that contained emergent vegetation effectively removed 95%−99% of *E. coli* and 80%−95% of fecal *Streptococci*. This is because planted systems enhance the presence of oxygen in the root zone and plants produce excretions that exert antimicrobial properties (Neori et al., 2000). Greater reduction of thermotolerant coliforms, *Enterococci*, *Salmonella*, *Shigella*, *Yersinia*, and coliphage populations has been reported in subsurface wetlands containing aquatic vegetation (Hench et al., 2003). Hill and Sobsey (2001) reported high removal of *Salmonella* from a horizontal subsurface flow wetland containing aquatic plants. In contrast, some studies reported no significant difference in the removal efficiency of pathogens such as thermotolerant coliforms, *E. coli*, somatic coliphages, and F-specific bacteriophages from planted and unplanted vertical flow constructed subsurface wetlands (Torrens et al., 2009; Lana et al., 2013). *E. coli* removal efficiency of 2.8 log units has been noted in planted CWs in southeastern Brazil as compared to 2.3 log units in unplanted CWs (Avelar et al., 2014). This is because CWs having plants show pathogen removal by several mechanisms (Weerakoon et al., 2016).

The removal mechanism of pathogens differs for free water surface and subsurface flow wetlands. The rate of removal/inactivation varies to a great extent in different zones of the wetland. This is because efficiency of pathogen removal depends on many factors. The different zones of a wetland differ in terms of properties such as oxygen concentration, depth, and amount of planted vegetation. The hydraulic characteristics such as HRT and hydraulic loading rate influence the removal of pathogens to a great extent (Garcia et al., 2003; Stottmeister et al., 2003; Vymazal, 2005). HRT has been considered as the primary factor affecting the removal of pathogens in surface flow constructed wetlands (Diaz et al., 2010). The HRT for a free water surface flow wetland depends on the flow rate, presence of plants, characteristics of the

porous media, and water depth. In most of the studies, a significant removal of pathogens has been noted after retention time of 4 days. High removal of parasites has been achieved in wetlands with long retention time (Sharafi et al., 2012). This is because longer HRT increases the rate of pathogen inactivation because of increased exposure to sunlight, availability of more time for sedimentation, adsorption to organic matter, predation, and exposure to toxins from plants (Garcia et al., 2003; Stottmeister et al., 2003; Ulrich et al., 2005; Diaz et al., 2010). Sunlight causes inactivation of virus and bacteria (Kadir and Nelson, 2014). Studies confirmed that hydraulic overloading reduces the removal efficiency of thermotolerant coliforms because of decreased ability to adsorb to the biofilm (Wu et al., 2016).

Hence pathogen removal by wetlands occurs via physical, chemical, and biological processes. The physical processes include attachment, adsorption, sedimentation, and mechanical filtration by roots (Jasper et al., 2013). The chemical processes involved in pathogen removal are oxidation and exposure to UV radiation, sunlight (photooxidation), high temperature, pH, and exposure to biocides. The biological processes involved in removal of pathogens mainly include plant microbe interaction within root biofilms, root-substrate complex, and adsorption (Morsy et al., 2007; Sleytr et al., 2007; Alufasi et al., 2017).

4. Conclusion and future studies

Aquatic plants have shown tremendous potential to remove pathogens from wastewater. Plants inactivate pathogens by mechanisms such as adsorption by plant roots, biofilm interactions, alteration in soil, or water chemistry. High removal efficiency 2 to 4 log_{10} (99% to 100%) of pathogens particularly bacteria, viruses, protozoa (cysts), and helminths (eggs) from wastewater has been noted in vegetated constructed wetlands. Predation by protozoans, adsorption to plant roots, and biofilms on the rhizosphere are the major biological mechanisms responsible for reduction in the concentration of pathogens. Toxins produced by plants and natural die-off because of starvation also contribute to removal of pathogens. Besides this sedimentation, absorption, inactivation in presence of chemical substances or sunlight, light, temperature, and pH changes are some of the other physicochemical processes responsible for removing pathogens from wastewater treated in wetlands. All the processes contributed in getting a significant reduction (90%−99%) in the concentration of pathogens from water. Field studies are required to evaluate efficiency of different macrophytes in removing waterborne pathogens and to determine the exact mechanism of pathogen removal so that large-scale phytoremediation technologies for treatment of pathogen-contaminated water can be developed.

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