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Antibiotic resistant bacteria removal of subsurface flow constructed wetlands from hospital wastewater

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ABSTRACT

Eight horizontal subsurface flow pilot scale artificial wetlands were constructed to evaluate their effectiveness in the removal of antibiotic-resistant bacteria from hospital wastewater. A total of 40 composite samples were collected from the inlet and outlet of wetlands, transported and processed for enumeration of indicator organisms, bacteriological identification and susceptibility testing following standard procedure. The treatment wetlands achieved 7.1 logs10 and 5.1 logs10 removal of total and fecal coliforms. Among the total samples, 159 bacterial isolates were detected, of these 45 (28.3%) were from hospital wastewater and 114 (71.7%) from the outlets of wetlands. The most frequently isolated bacteria from hospital wastewater samples were found to be *Staphylococcus sp* 12 (26.6%) followed by *E.coli* 11(24.4%), *Klebsiella sp* 9(20%) and *Shigella sp* 5(11.1%). Similarly, the overall isolates in treated wastewater samples were *E.coli* 45(39.5%), *Staphylococcus* (35.1%) and *Klebsiella sp* 35 (30.7%). Among bacterial isolates from hospital wastewater, 100% of *Salmonella* isolates were found to be resistant to ampicillin and 75% to doxycycline, erythromycin, ceftazidime, cefoxitin, and chloramphenicol. *E.coli* was also found to be 81.8% resistant to ampicillin and 72.7% to cotrimoxazole and amoxicillin-clavulanic acid. Significantly ($P < 0.005$) higher removal (80.8% to 93.2%) of antibiotic-resistant bacteria was recorded in vegetated wetlands. In contrary, no significant difference between the vegetated and non-vegetated wetlands was observed in the removal of indicator and pathogenic bacteria. Constructed wetlands could help to solve the problem of the cost-effective disposal of hospital wastewater and be promoted in a strategy to reduce water pollution in low-income countries like Ethiopia.

1. Introduction

In developing countries, the release of wastewater pollutants into the environment without treatment is an extensive practice, thus rendering the contamination of lakes, rivers, groundwater, estuaries, and ponds [1]. This practice could make the water bodies a reservoir of multidrug-resistant microorganisms which can contain genes that can be transmissible and exhibit better survival potential for very long time over a wide range of difficult environmental conditions [2]. A number of reports indicated that antibiotic-resistant species are ubiquitously found in various environmental compartments of surface waters, treated wastewater, groundwater, sediments, soils and drinking water [3–6]. The major sources of such contamination are due to the release of untreated sewage from municipal services, hospital, industrial, agricultural and veterinary activities [6,7].

A huge amount of water consumption in hospitals releases a significant volume of wastewater loaded with complex mixtures of chemical and biological substances such as heavy metals, disinfectants, reagents, detergents, radioactive markers, hormones, pharmaceuticals, endocrine disrupting compounds and microorganisms [8–10]. The growing application of antimicrobial drugs to treat infectious diseases in these setups can also lead to the release of antibiotic residua in their wastewater [11,12]. Hospital effluent could enhance the number of resistant bacteria in the environment by introduction and selection of resistant bacteria as well as inhibiting the growth of susceptible bacteria [13]. This happens due to those antibiotics, which are mostly released into the environment at low concentration, exert high selective pressure on bacterial communities and, consequently, accelerating their resistance [2]. Researchers indicated that wastewater discharged from hospital contains higher amounts of antibiotic-resistant bacteria, genes, and residual antibiotics than municipal wastewater [14,15]. Thus the discharge of hospital effluent without treatment might cause a massive

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health impact through the dissemination of infectious agents and antibiotic-resistant microbes that result in outbreaks of communicable diseases and diarrhea epidemics such as cholera, typhoid fever, dysentery and gastroenteritis [16].

Antibiotic resistance remained the most important challenge throughout the world [17,18]. It became an emergent healthcare crisis due to the fact that the currently existing antimicrobial drugs couldn't effectively work against the disease agent and the incidence of multidrug-resistant genes in both developed and developing countries. Its impact in developing countries is extremely serious where the infectious disease burden is high and access to effective diagnostic and treatment options in health institutions with enough manpower is a fundamental problem. In these countries, overuse, misuse, and damping of antibiotics is a common practice [19]. It is difficult to evaluate and substitute antibiotics to which resistance is developed by newly fabricated ones within a short period of time. These make the treatment option limited, increasing costs of treatment, an extra length of stay in the hospital, therapeutic failures, and death [18,20].

Conventional wastewater treatment plants (WWTP) play a crucial role in the reduction and removal of many pollutants including organic matters, pathogenic microorganisms, and even antibiotics. However, such treatment processes serve as collection points for resistant organisms and antibiotics from various sources as well as enhance the proportion of bacteria resistant to antibiotics by creating a suitable environment for the horizontal gene transfer via conjugation, transduction, and transformation [21,22]. Thus WWTPs have to be considered as a hot-spot site for the dissemination of antibiotics, antibiotic-resistant bacteria and genes which can survive long, harsh routs and final disinfection processes into surface and drinking waters [23,24].

CWs remove indicator, pathogenic and antibiotic-resistant bacteria more significantly than other conventional WWTPs [25]. The removal mechanisms mostly caused by filtration, sedimentation, adsorption, aggregation, natural die-off, influence of toxins from plants and other microorganisms, unfavorable water chemistry, biofilm interaction, exposure to biocides and oxidative damage [26,27]. Competition with the consortium of organisms surrounding them and predation also play a great role in the destruction of many bacteria [28]. Their removal also appears to be correlated with suspended solid removal and hydraulic residence time [27,28].

Owing to the current expansion of hospitals and health centers by the Federal Ministry of Health (FMOH) of Ethiopia, the environmental and public health consequences of the wastewater released in these healthcare setups will be immense unless proper wastewater treatment system is in place. Most of the health institutions constructed in Ethiopia do not have reliable treatment plants. The available few treatment systems are not designed considering the complex nature of healthcare wastes, and; therefore, they are not effective in treating at least indicator microorganisms [29,30].

There are several hospital wastewater treatment technologies throughout the world [8]. However, due to their high cost and complexity of operation and maintenance, it would be difficult to make them practical in countries like Ethiopia. Beyene and Redaie [29] recommended that constructed wetland systems might be one of the most appropriate treatment systems in developing countries like Ethiopia. Even if, different constructed wetland designs and plant species were used as effective treatment options for municipal and industrial wastewaters, their application in the treatment of wastewaters generated from healthcare institutions has not been thoroughly tried [28]. There is also a lack of information whether these kinds of treatment plants are effective to remove drug-resistant microbial strains by using different kinds of wetland plant species and substrates. Therefore, the current study aims to evaluate the potential of subsurface flow constructed wetland treatment systems to remove antibiotic resistant species from hospital wastewater.

2. Materials and methods

2.1. Description of study area

The study was conducted from September to December 2017 in the compound of Hawassa University Referral Hospital, Southern Ethiopia. It is located 7.06 latitude and 38.48 longitude with an elevation of 1697 m above sea level. The average annual rainfall, temperature, and humidity were 945 mm, 19.5°c, and 70–80%, respectively. The hospital has been working as a teaching institute and a referral center for the community of the region and other neighboring areas. At the time of data collection, the hospital had around 300 beds in 6 wards and the number of patients visited per day was ranged from 200 to 350. The quantity of wastewater generated has been reached to 143,285 liters per day [29]. The wastewater generated in the hospital has passed through a septic tank pretreatment before it inters to the oxidation ponds for final treatment. The wastewater is then released into an ecologically sensitive area (Lake Hawassa) which is located adjacent to it.

2.2. Experimental setup

Eight horizontal subsurface flow pilot Scale wetlands were constructed in the compound of Hawassa University Teaching Referral Hospital to determine their effectiveness in improving the quality of septic effluent passing through them. Each bed was 4 m long, 1.2 m wide and 0.6 m deep with gravel and broken brick media as substrate. The bottom slope of each cell was 0.5% to allow easier water collection. The internal bottom part was lined with polyethylene sheet in order to avoid percolation of wastewater into the groundwater and to protect the wastewater from being eroded out of the system. Coarse sized gravel with a diameter of 40–50 mm was used at 50 cm long of the inlet and 30 cm of the outlet area of the wetlands in order to prevent clogging and facilitate wastewater distribution. The treatment zone of five wetlands was packed with the gravel of 20–25 mm in diameter at a depth of 45 cm. The other three wetlands were packed with the broken brick of 20–25 mm diameter of the same depth [28,31]. The upper top layer (15 cm) of all wetlands was filled with gravel of 5–10 mm to provide better rooting of plants. The top surface of the media was leveled for easier planting and root maintenance. There was additional 10 cm freeboard for water accumulation (Figs. 1 and 2).

2.3. Plant material and experimental start-up

Four gravel bed wetlands were planted with aquatic tropical plants of Typha domingensis, Cyperus Papyrus, Dark Green Bulrush (Scirpus atrovirens) and Sugarcane (Andropogoneae Saccharum) and two broken brick bed wetlands were planted with Typha domingensis and Cyperus Papyrus. The remaining two from both media left unplanted to act as the control. Plants of different species were selected based on efficiency in removing different pollutants reported in literature and abundance in the local area [28,31,32]. The experiment basically consisted of a simultaneous comparison of numerous wetlands with different plants and substrate media. Partially treated wastewater from the hospital was pumped to temporary collection tank and released into wetlands. Both planted and unplanted wetlands were operated under continuous flow conditions with an equal hydraulic flow rate of 165.75 l/day and a hydraulic residence time of 4 days (Table 1).

2.4. Sampling and sample collection

A total of 8 composite untreated hospital wastewater samples were collected from the two outlets of temporary collection tank (four times from each) and a total of 32 composite samples were collected from the outlets of wetlands (four times from each) at a monthly interval for bacteriological analysis, identification and susceptibility testing.