RESEARCH ARTICLE

PERFORMANCE OF A SMALL-SCALE CONSTRUCTED WETLAND FOR THE REMOVAL OF ANTIBIOTICS AND ANTIBIOTIC RESISTANT BACTERIA FROM HOSPITAL WASTEWATER

Mesenbet Merkeb[1], Birtukan Getnet[2] and Adey Feleke[2]*

ABSTRACT: Hospital wastewater is a reservoir for high load of antibiotics, heavy metals and antibiotic-resistant bacteria which are of public health concern and should be removed. Horizontal subsurface-flow constructed wetland (HSCW) is one of the most important green and environment-friendly technologies for removal of pollutants from wastewater. This study explored the efficiency of HSCW for removing antibiotics, antibiotic resistant bacteria and heavy metals from hospital wastewater of Tikur Anbessa Specialized Referral Hospital. Wastewater samples were taken periodically for five months in 2018 and fed into the influent of a HSCW in the CNCS, Addis Ababa University, with a flow rate of 0.72 L/hr and hydraulic retention time of 4 days. Influent and effluent samples were collected and analyzed for BOD, COD, Nitrogen and Phosphorus species, heavy metals, antibiotics and antibiotic resistant bacteria. The average BOD and COD values of the raw wastewater from the hospital were 243 and 1,312 mg/L, respectively, and mean total nitrogen and total phosphorus were 62.0 and 6.4 mg/L, respectively. Removal of TP was highest in cells planted with *P. karka* (95%) than the cell planted with the combination of the two plants (74%). Highest removal of TN was observed in the cell planted with *T. latifolia* (94%) than with combined plants (86%). *P. karka* showed high removal efficiency for Nickel (73%) and Zinc (86%). Heterotrophic bacteria exhibited resistance to ciprofloxacin and cefotaxime antibiotics but counts decreased in all cells of the CW compared to the influent. The removal efficiency observed for ciprofloxacin and cefotaxime was generally low in all cells, and requires further study for process optimization. The mean concentration of ciprofloxacin and cefotaxime residue in the influent were 1.23 ppm and 17 ppm, respectively, and were very high compared to literature reports. Strong positive correlation ($r^2=0.98$, P=0.11) was found between cefotaxime concentration and bacterial count resistant to cefotaxime in the influent and the water treated with *T. latifolia* and strong negative correlation in unplanted control, *P. karka* and the combined plants ($r^2=-0.746$, p=0.464). It is concluded that HSCW pretreatment schemes should be in place for removal of hospital wastewaters containing antibiotics and heavy metals.

[1] College of Natural and Computational Sciences, Debre Markos University, Debre Markos.
[2] Department of Microbial, Cellular and Molecular Biology, College of Natural and Computational Sciences, Addis Ababa University, P.O. Box 1176, Addis Ababa, Ethiopia. E-mail: adey.feleke@aau.edu.et
*Author to whom all correspondence should be addressed

INTRODUCTION

Hospital wastewater contains a great variety of micro-contaminants such as pharmaceuticals, heavy metals, disinfectants and pathogenic microorganisms in addition to the priority pollutants (Amouei *et al*., 2015; Hunachew Beyene and Getachew Redaie, 2011; Sintayehu Fekadu *et al*., 2015). Among these pollutants, antibiotics and heavy metals pose a great threat to the ecosystem, mainly because of the absence of regulatory process to monitor their removal (Mondal and Sinha, 2014). A large amount of antibiotic residue is released from hospitals since 90% of the antimicrobials are excreted in their active form via the urinary and digestive systems (Chunhui *et al*., 2016; Barancheshme and Munir, 2018). If not properly treated, hospital wastewater may be a potential source for the spread of antibiotic resistant bacteria (ARB) and resistance genes (Lien *et al*., 2016). Antibiotic residues within the environment exhibit negative influence on aquatic and terrestrial organisms, resulting in a series of potential ecological hazards, such as spread of mobile genetic elements carrying antibiotic resistant genes (Chunhui *et al*., 2016; Lien *et al*., 2016). Continuous exposure to antibiotics via water in an environment leads to the occurrence of antibiotic resistant bacteria (Kim *et al*., 2018), including pathogens which results in increased treatment costs, disease spread and duration of illness posing a serious challenge to the future of infection treatment (Feleke Moges *et al*., 2014; Crouch *et al*., 2015). Conventional wastewater treatment technologies do not sufficiently remove pharmaceuticals, their metabolites and degradation byproducts from wastewater, and thus allow them to reach surface, marine, ground, and drinking water (Shraim *et al*., 2017).

Heavy metals are categorized under micro-pollutant group within the hospital wastewater that cause risk to public health and the environment because they are not biologically degradable and transformable (Akin, 2016). Among the different heavy metals, cadmium, lead and mercury are the most dangerous and potentially toxic to living organisms (Yamina *et al*., 2014). Most of the heavy metals are toxic at low concentrations with a higher chance of integrating into the food chain where they accumulate and cause damage to living organisms (Ayangbenro and Babalola, 2017). When heavy metals are transferred into food chains and accumulate in vital organs, like liver, kidneys, and bones, there is an immediate threat to human health resulting in numerous serious health disorders (Sardar *et al*., 2013; Hu *et al*.,

2017). Hospitals are one among the main sources of heavy metals within the environment and are also recognized as a source of mercury discharge to the wastewater system (Kaur *et al*., 2014).

In most cases of the developing world, hospital wastewater is mixed with urban sewer systems without pretreatment (Ort *et al*., 2010) and use conventional treatment processes in municipal wastewater treatment plants. However, municipal systems are not generally designed to remove pharmaceutical wastes (Al Qarni *et al*., 2016). Constructed wetlands can facilitate removal of pharmaceuticals through natural processes involving plants, microorganisms, solid matrix components and sunlight (Li *et al*., 2014). Compared to conventional treatment, constructed wetlands provide low-cost alternative processes which make them suitable for wastewater treatment because they depend on natural processes, are less costly to construct, operate, and maintain (Mburu *et al*., 2013; Vymazal, 2011).

In Ethiopia, hospital wastewater management is often poor unlike the health care management system. The poor attention given to hospital wastewater management creates a potential risk to public health through the dissemination of pollutants particularly antibiotic residues, heavy metals and ARGs to the environment, animals and communities. Pretreatment of hospital wastewater before it joins the main sewer system is obligatory. When pretreatment is unavailable or malfunctioning, antibiotic residues, heavy metals and ARGs may persist in the treated water and eventually accumulate in the receiving water bodies that are used for irrigation of fresh produces. The case of Tikur Anbessa Specialized Referral Hospital is not different from this scenario. The primary treatment system located in the vicinity of the hospital is malfunctioning which results in the wastewater directly joining the main sewer line heading to Kality treatment plant. Appropriate on-site treatments of hospital effluents prevent antibiotic resistant pathogens from combination with the resident microorganisms in domestic wastewater treatment plants and also reduce long-term problems of antibiotic resistance in pathogens, which are currently worldwide concerns and also to prevent water bodies from eutrophication due to high nutrient and organic load from hospitals. This study aimed to assess the performance of a horizontal subsurface flow constructed wetland for the treatment of hospital wastewater, focusing on removal of selected heavy metals, antibiotics and antibiotic resistant bacteria.

MATERIALS AND METHODS

Wetland set-up and wastewater sampling

The constructed wetland was located in the premises of the College of Natural and Computational Sciences and consisted of four cells planted with *Typha latifolia*, *Phragmites karka*, combined *T. latifolia* and *P. karka*) and unplanted control (Fig. 1). The wetland was constructed as a horizontal subsurface flow with dimensions of 2 m × 0.68 m × 0.45 m of length, width and height. During construction, a slope of 1% at the bottom of each bed was maintained to ease the circulation of water from the inlet to the outlet.
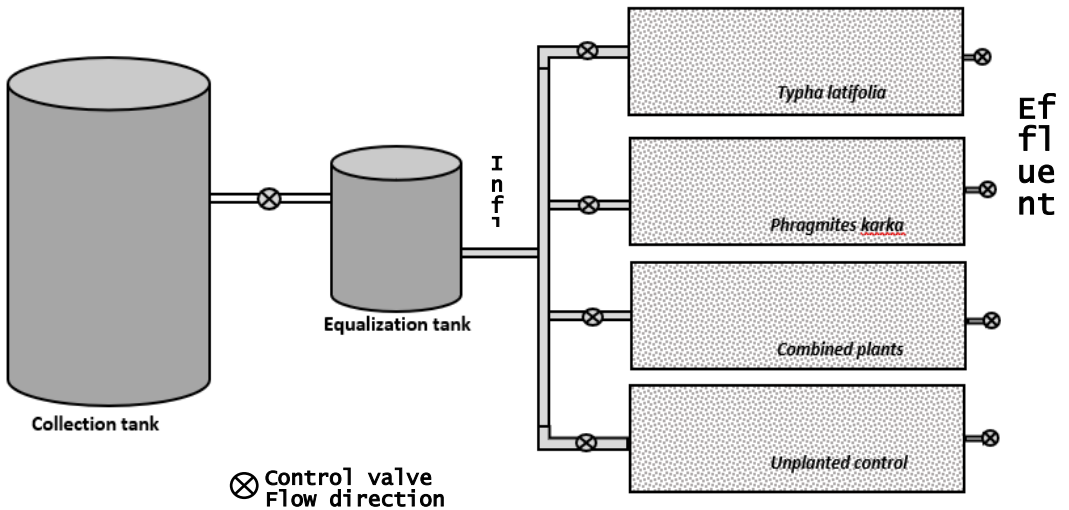

Fig. 1 Schematic depiction of horizontal subsurface flow constructed wetland used in the study.

Wastewater samples were collected from Tikur Anbessa Specialized Hospital four times from February to June 2018 to be used as feed for the constructed wetland. The samples were brought using 20 L capacity jerry cans cleaned and disinfected with 70% ethanol and samples were transported at ambient temperature. Raw wastewater was put in a 10,000 L collection tank and was periodically released through a gate valve to an equalizer tank which then flowed into each cell at an optimized flow rate of 0.72 L/hr that meets the hydraulic retention time (HRT) of four days. The raw wastewater, the influent (wastewater that enters the cells) and effluent (water that comes out from the cells after the retention time) was taken for various analyses described in the sections below.

Nutrients, Carbon and heavy metal analyses

Physico-chemical, nutrients and heavy metals in the influent and effluent were analyzed from duplicate samples using the following methods. Total nitrogen (TN) was measured by using Semi-Micro-Kjeldhal method as in protocol #APHA 4500B (APHA, 1998). Total phosphate (TP) was analyzed using vanadomolybdophosphoric acid based on protocol #APHA 4500C (APHA, 1998), at JIJE Analytical Testing Services Laboratory, Addis Ababa.

Ammonia (NH_3) nitrogen was analyzed according to Nessler method (HACH Method 8038, 1999) in Addis Ababa University (AAU). Briefly, the sample was diluted 1:100 with deionized water and three drops of mineral stabilizer (potassium sodium tartrate, sodium citrate and demineralized water) was added to the sample and deionized water as blank. The mixing cylinder was inverted several times to mix using stopper on the mixing cylinder. Three drops of polyvinyl alcohol water were added to each cylinder and mixed well. One ml of Nessler reagent (sodium hydroxide, sodium iodide, demineralized water and mercuric iodide) was added to each mixing cylinder and mixed well. After 1 minute of incubation, the sample was measured using spectrophotometer (HACH, DR/2010) at 425 nm.

COD was measured by using Manganese III Digestion Method (HACH, DR/ 2000 and DR/3000 instrument) according to HACH instructions and BOD analyses were carried out using titration method in AAU. The analyses of the following heavy metals: Lead (Pb), Zinc (Zn), Cadmium (Cd), Cobalt (Co), Nickel (Ni), Chromium (Cr) and Mercury (Hg) were conducted using Air-Acetylene flame method as in protocol number APHA 3111C (APHA, 1998) at JIJE Analytical Testing Service Laboratory, Addis Ababa.

Determination of ciprofloxacin and cefotaxime in the influent and effluent

The analytes for the quantitative determination study were ciprofloxacin and cefotaxime. The level of ciprofloxacin and cefotaxime in the raw wastewater, influent and effluents from each constructed wetland cell was determined using High Performance Liquid Chromatography (Agilent 1200 series, Agilent Technologies, Germany) in the Department of Chemistry, AAU. The samples collected from each cell using sterilized bottles were kept at −20°C until subject to analysis. Stock solutions of ciprofloxacin and cefotaxime were prepared as 1 mg/mL and 0.2 mg/mL, respectively. Standard curves were done using a dilution series of 0.3125 ppm, 0.625

ppm, 1.25 ppm, 2.5 ppm, 5 ppm, 10 ppm, 20 ppm and 40 ppm.

Extraction of the target analytes from the samples was done following the salting out assisted liquid-liquid extraction method described by Teshome Gezahegn and colleagues (2019). Chromatographic separation was performed on C18 analytical column (ZORBAX Eclipse XDB, 150 mm × 4.6 mm id, particle size 5 m, Agilent Technologies) with column temperature of 35°C. Data acquisition from triplicate samples and processing were performed with LC Chemstation software (Agilent Technologies). The mobile phase composition was methanol and water (75:25 v/v) which was pumped at a flow rate of 2.2 ml/min. The detection wavelength of ciprofloxacin and cefotaxime was 278 and 254 nm, respectively with a band width of 4 nm and the injection volume was 20 µl. Peak area was used as response of HPLC with run time of 15 minutes.

Quantification of resistant bacteria for four antibiotics

The following antibiotics were used for the sensitivity testing: ciprofloxacin (4 µg/mL), gentamycin (16 µg/mL), cefotaxime (64 µg/mL) and doxycycline (16 µg/mL). The choice of these antibiotics was based on availability for the tests and frequency of application (commonly used) in the hospital. Antibiotic susceptibility test was performed based on standard reference values according to National Committee for Clinical and Laboratory Standards, now Clinical and Laboratory Standards Institute (CLSI) described in (NCCLS, 2015). The influent and effluent samples were serially diluted and plated on nutrient agar containing the above-mentioned antibiotics in duplicates. One hundred micro litres of diluted sample was spread on to antibiotic containing plate and incubated for 24 hours at 37°C. The media coated with antibiotics were used to determine the number of antibiotic resistant bacteria.

The percentage removal was calculated by the ratio between CFU/mL in treated and in initial waste water.

$$\% \text{ Removal} = \left(1 - \frac{(CFU/mL) \text{ in treated waste water}}{(CFU/mL) \text{ in initial waste water}} \times 100\right)$$

Statistical analysis

Statistical analyses were performed using the software SPSS Version 20. The data was analyzed through one-way analysis of variance (ANOVA). Pearson correlation analyses were conducted to study correlations between antibiotic residue concentration and antibiotic resistant bacteria. Averages and standard deviations were calculated with Microsoft Excel.

RESULTS

Wastewater characterization and treatment performance of the constructed wetland

The influent wastewater from the equalization tank had a mean BOD and COD of 161.69 mg/L and 681 mg/L, respectively. After treating using different plants and combination, the mean BOD and COD of the effluent of wetland cell planted with *T. latifolia* was 97.13 mg/L and 179 mg/L, resulting in a 40% and 76% efficiency of *T. latifolia* in BOD and COD removal (Table 1). Comparative analysis showed that there is no significant (P=0.05) variation between the influent and treated effluents with regard to the concentrations of organic carbon in the form of BOD and COD.

Total phosphorous (TP) of the influent was 5.61 mg/L and upon treatment with *T. latifolia*, the mean concentration was reduced to 0.69 mg/L resulting in 88% removal (Table1). Treatment with *P. karka* reduced the total phosphorus to 0.28 mg/L, resulting in 95% removal. Treatment using the combined plants reduced the TP to 1.43, mg/L while the unplanted control had 3.43 mg/L TP. The removal efficiency of the combined plants treatment was 74% and the unplanted control had 39%. Comparative analysis showed that the concentration of TP in the influent was significantly (P=0.05) the highest of all, except the unplanted control effluent.

Soluble reactive phosphorous (SRP) concentration of the influent wastewater was 4.01 mg/L, which decreased to 0.33 mg/L and 0.02 mg/L upon treatment with *T. latifolia* (92%) and *P. karka* (99%), respectively. Treatment with the combined plants decreased the SRP to 0.83 mg/L with a removal efficiency of 79%. The effluent from the unplanted control had SRP of 2.86 mg/L with a removal efficiency of 29% (Table1).

The mean concentration of total nitrogen (TN) in the influent wastewater was 48.76 mg/L. The effluent treated by *T. latifolia* and *P. karka* had mean TN concentrations of 3.33 and 5.72 mg/L, with removal of 98 and 88% respectively (Table 1). The mean TN concentration of water treated using the combined plants was 6.93 mg/L while the water from untreated control had a mean TN of 24.03 mg/L (Table 1). The combined and control had removal efficiency of 86 and 51%, respectively. Comparative analysis using ANOVA showed that concentration of TN in the effluent treated with *T. latifolia* was significantly the lowest of all the other effluents including TN of the influent wastewater.

Regarding ammonia (NH$_3$), the mean concentration of the influent was 22 mg/L and effluent water treated by *T. latifolia* and *P. karka* had NH$_3$ concentrations of 0.33 mg/L and 0.66 mg/L, respectively. The mean of NH$_3$ concentration of water treated using the combined plants were 1.33 mg/L and water from the untreated control had a mean concentration of 5.33 mg/L (Table 1). The efficiency of the treatment system for the removal of NH$_3$ planted with *T. latifolia* and *P. karka* was 98% and 97%, respectively. The combined and the control had removal efficiency of 94 and 76%, respectively (Table 1). Comparative analysis using ANOVA showed that ammonia concentration in the influent was significantly higher than all except the unplanted control effluent.

Table 1. Concentration of organic matter, Nitrogen and Phosphorus and efficiency of the wetland.

| | **Mean concentration (in mg/ml) and percent removal efficiency** | | | | | |
	BOD (RE)	**COD (RE)**	**TP (RE)**	**SRP(RE)**	**TN (RE)**	**NH$_3$(RE)**
Influent	161.69	681.67	6.00a	4.00c	48.66e	22.00g
T. latifolia	97.00 (40%)	179.00 (74%)	0.67b (68 %)	0.33c (92%)	3.33f (94%)	0.33h (98%)
P. karka	66.67 (59%)	146.33 (79%)	0.00b (95%)	0.00d (99%)	6.00e (88%)	0.67h (97%)
Combined	73.00 (55%)	147.00 (78%)	1.33b (74 %)	1.00c (79%)	7.00e (86%)	1.33h (94%)
Control	73.00 (55%)	97.00 (86%)	3.33a (39%)	2.67c (29%)	24.00e (51%)	5.33g (76%)

*Within each column, the mean values with different letters are significantly different at p<0.05

Heavy metal concentration in hospital wastewater and its removal

Zinc and Nickel were the only detected metals in the wastewater with samples among the range of metals screened including Lead, Cadmium, Chromium and Cobalt (Table 2). The average concentration of Zinc in the raw wastewater was 0.28 mg/L while nickel had an average concentration of 0.08 mg/L in the raw wastewater. There was no significant (P=0.05) variation between the influent and treated effluents with regard to the concentrations of Zinc and Nickel.

The efficiency of *T. latifolia*, *P. karka* and the combined plants in removing Zinc was 84, 86 and 65%, respectively while the unplanted control had 34% removal efficiency. The efficiency of *T. latifolia* and *P. karka* in removing Nickel was 59 and 73%, respectively. The combined cell showed an increased concentration of Nickel compared to the influent and hence had the lowest efficiency (below zero), while the unplanted control had 41% removal efficiency (Table 2).

Table 2. Mean concentrations and percentage efficiency of the constructed wetland for the removal of Zinc and Nickel.

Constructed wetland Cell	Mean concentration (RE %)	
	Zinc	**Nickel**
Influent	0.33	0.09
T. latifolia	0.05 (84%)	0.04 (59%)
P. karka	0.045 (86%)	0.02* (73%)
Combined	0.11 (65%)	0.16 (−78%)
Control	0.22 (34%)	0.05 (41%)

*The concentration of Nickel in the treatment cell using *P. karka* in some of the triplicate samples was below the detection limit.

Quantity and removal of antibiotic residue

The mean concentration of ciprofloxacin in the influent was 1.23 ppm which decreased to 1.13 and 1.16 ppm upon treatment with the combined plants and *P. karka*, respectively (Table 3). The concentration of ciprofloxacin in the outlet of the wetland planted with *T. latifolia* and the unplanted control had 1.2 ppm. In all the plant types, the removal efficiency of ciprofloxacin was below 10% (Table 3).

The other analyzed antibiotic residue, cefotaxime, was found to be present in the influent with an average concentration of 17 ppm which decreased by more than 50% to 6.66 ppm and 6.33 ppm upon treatment with *T. latifolia* and the unplanted control, respectively. The concentration of cefotaxime in the outlet of the wetland planted with *P. karka* and combined plant had 12.33 and 11.3 ppm, respectively. The highest performance was observed with *T. latifolia* (61%) and control (62%) relative to the other treatment cells (Table 3).

Table 3. Removal of ciprofloxacin and cefotaxime antibiotics from the hospital wastewater using the plants *T. latifolia*, *P. karka* and their combination in the constructed wetland.

	Mean and SD of antibiotic drug (ppm)		Removal efficiency (%)	
	Ciprofloxacin	**Cefotaxime**	**Ciprofloxacin**	**Cefotaxime**
Influent	1.23±0.05	17±7.5		
T. latifolia	1.20±0.1	6.66±2.3	3	61
P. karka	1.16±0.05	12.33±4.1	5	28
Combined	1.13±0.05	11.33±1.1	8	33
Control	1.2±0.0	6.33±2.5	2	62

Removal of antibiotic-resistant heterotrophic bacteria

Ciprofloxacin- resistant heterotrophic bacteria in the influent was 1.66E+05 CFU/mL. Maximum removal efficiency was obtained with *T. latifolia*; with 79% removal efficiency in the effluent. After being treated by *P. karka*, ciprofloxacin resistant bacteria colony count decreased to 4.99E+04 with a removal efficiency of 70%. The constructed wetland cell with mixed plants

and the unplanted control had 48 and 75% removal efficiency for ciprofloxacin resistant bacteria (Fig. 2). The efficiency of *T. latifolia*, *P. karka*, combined plants and unplanted control in removing cefotaxime resistant bacteria was 96, 92, 95 and 86%, respectively.
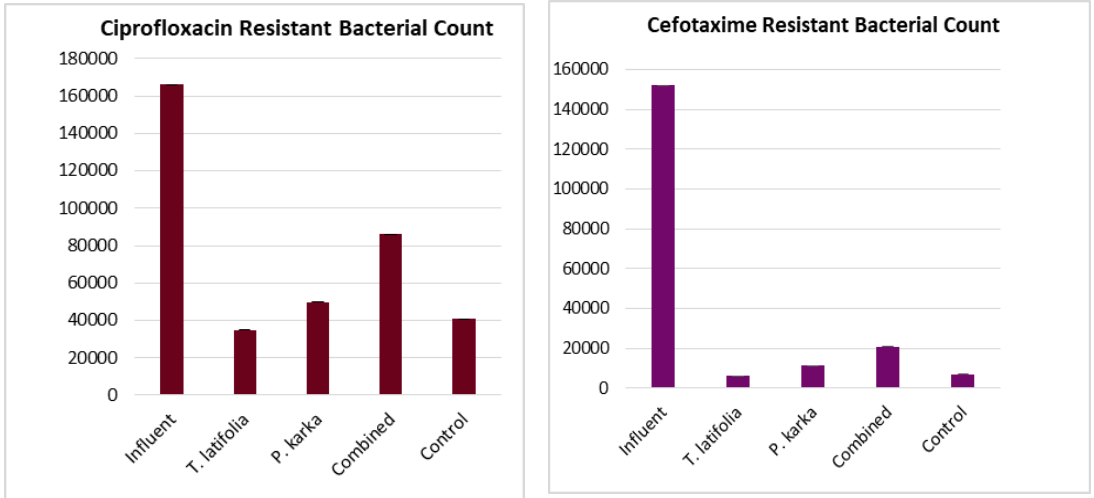

Fig. 2. Mean count of heterotrophic bacteria resistant to ciprofloxacin (left) and cefotaxime (right) in the influent and effluent samples.

Higher removal efficiency observed in the cell plant with *T. latifolia* (79%); the removal efficiency of *P. karka* and unplanted control in removing gentamicin resistant bacteria was 76%. The efficiency of *T. latifolia*, *P. karka*, combined plants and unplanted control in removing doxycycline resistant bacteria was 88, 82, 81 and 95%, respectively. Maximum removal efficiency was obtained from unplanted control and also higher removal efficiency was observed in the cell planted with *T. latifolia*, *P. karka* and the combined plants (Fig. 3).
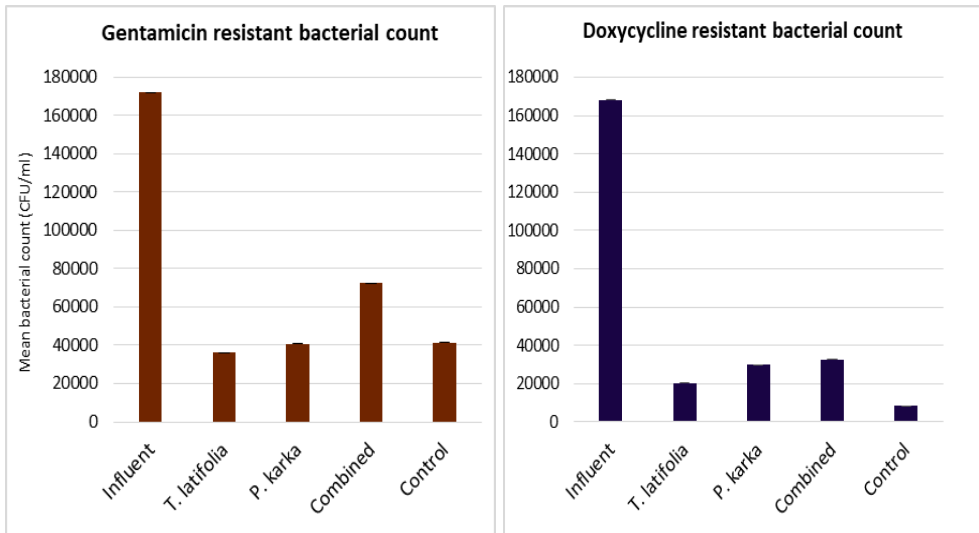
Fig. 3. Mean count of heterotrophic bacteria resistant to gentamicin (left) and doxycycline (right) in the influent and effluent samples.

Upon the comparison of colony forming units grown in media containing 4 µg/mL ciprofloxacin, the cfu/mL of the influent that grew in the presence of ciprofloxacin was significantly the highest of all the effluents from the different treatment cells. Similarly, the cfu/mL of the influent wastewater grown on 64 µg/mL cefotaxime and 16 µg/mL gentamicin was significantly higher than all the effluents (Table 4). The cfu/mL of the influent grown with 16 µg/mL doxycycline was significantly higher than the effluent treated with *P. karka*, followed by *T. latifolia*, the combined plants and the unplanted control (Table 4).

Table 4. Mean of resistant bacterial count with the total heterotrophic bacterial count in the influent and effluents in paired plating with and without the listed antibiotics.

Plant type	Log-transformed mean ± SE CFU/ml of bacteria resistant to the four antibiotics			
	Ciprofloxacin (4 µg/mL)	Cefotaxime (64 µg/mL)	Doxycycline (16 µg/mL)	Gentamicin (16 µg/mL)
Influent	5.21±0.49[a]	5.17±0.02[a]	5.21±0.04[a]	5.21±0.06[a]
T. latifolia	4.51±0.89[b]	3.71±0.15[b]	4.10±0.28[bc]	4.42±0.2[b]
P. karka	4.64±0.10[b]	4.13±0.07[b]	4.48±0.15[b]	4.45±0.24[b]
Combined	4.64±0.10[b]	3.92±0.27[b]	4.17±0.27[bc]	4.48±0.25[b]
Control	4.45±0.17[b]	3.68±0.17[b]	3.85±0.12[c]	4.39±0.24[b]

* Within each column, value with different letters are significantly different (P<0.05)

Relationship between the load of antibiotic residues and antibiotic resistant bacteria

Upon examining the presence of cross-sectional relationship between antibiotic concentration and resistant bacteria in the wastewater, ciprofloxacin and ciprofloxacin resistant bacteria in the influent had strong positive correlation (r^2=0.58, P=0.61). Strong negative correlation (r^2=−0.99, P=0.076) with the effluent treated with *P. karka* was observed, while the wetland planted with *T. latifolia* had a weak positive correlation (r^2=0.31, P=0.798) in all the cases with ciprofloxacin, the relationships were statistically insignificant (Table 5).

Regarding cefotaxime and cefotaxime resistant bacteria, strong positive (r^2=0.98, P=0.11) correlation was exhibited in the influent and the cell with *T. latifolia*, the latter being statistically significant (Table 4). Cefotaxime and cefotaxime resistant bacteria in the outlet of the wetland planted with *P. karka* and the unplanted control had strong negative (r^2=−0.977, −0.746, P=0.138, 0.464) correlation which was statistically insignificant. Strong negative correlation was observed between cefotaxime and cefotaxime resistant bacteria after treated with combined plant and was statistically significant (p=0.01).

Table 5. Correlation of antibiotics load in the water and abundance of antibiotic resistant bacteria in the constructed wetland.

Plant type	Ciprofloxacin versus resistant bacteria		Cefotaxime versus resistant bacteria	
	Pearson's Correlation	P-value	Pearson's Correlation	P-value
Influent	0.58	0.61	0.98	0.11
T. latifolia	0.31	0.798	0.998*	0.044
P. karka	−0.993	0.076	−0.977	0.138
Combined	−0.521	0.651	−1.000**	0.000
Control	-	-	−0.746	0.464

* Correlation is significant at the 0.05 level (2-tailed)
**Correlation is significant at the 0.01 level (2-tailed)

DISCUSSION

The BOD and COD values of Tikur Anbessa Specialized Hospital wastewater were above the permissible limit of the US EPA standard (30 mg/L). Much higher BOD and COD levels were detected in Hawassa referral hospital, with average influent concentrations 632±31.11 and 1388.75±206.83 mg/L, respectively (Hunachew Beyene and Getachew Redaie, 2011) which was higher than the result obtained from this study (161.69 mg/L and 681 mg/L). Such fluctuation of organic matter in hospital

wastewater depends on factors including number of patients, number of students and residents in the hospital at the particular time of the sampling.

When comparing the performance of *T. latifolia*, *P. karka* and their combination with respect to BOD and COD removal, no significant difference was found between the treatment types and with the unvegetated control. It was expected that the wetland cell with the combined plants would show higher performance in the removal of organic matter as reviewed by Vymazal (2011). Among the different plant types and their combinations, the highest organic matter removal was observed in the *P. karka* planted cell for BOD (59% RE) and the unplanted control cell for COD (97% RE). The highest removal of COD in the cell with no plantation is unusual and is in contrary to the fact that plant roots in wetlands facilitate organic matter removal through providing surface area for microorganisms, increasing uptake of nutrients and oxygen transfer to their roots (Kadlec *et al.*, 2000). The reason for the highest COD removal in the unvegetated wetland cell requires further investigation.

Despite being a referral hospital, the wastewater from Tikur Anbessa Referral Hospital had lower total Nitrogen and Ammonia when compared with Hawassa Referral Hospital which had 98 mg/L total N and 0.2 mg/L NH_3 (Hunachew Beyene and Getachew Redaie, 2011). The constructed wetland planted with *T. latifolia* showed highest total Nitrogen and NH_3 removal (98%), indicating the efficiency of this plant for the removal of Nitrogen. *T. latifolia* has long been known among the most important wetland plants and its efficiency for removal of various nutrients both in natural and constructed wetland settings were reported by various researchers (Vymazal *et al.*, 2017; Ciria *et al.*, 2005).

Total phosphorus and soluble reactive phosphorus in the current study was also much lower than that of Hawassa Referral Hospital (Hunachew Beyene and Getachew Redaie, 2011), which is accounted for by the number of patients who came to the hospital for service at the time of the sampling months. The constructed wetland cell planted with *P. karka* showed 95% removal for TN and 99% removal for SRP. Similar to *T. latifolia*, members of the genus *Phragmites* are well known by their high efficiency for the removal of organic pollutants as reported by researchers (Akratos and Tsihrintzis, 2007; Vymazal, 2001; Brix, 1993).

The concentration of Zinc and Nickel in the influent was below the standard limit set by WHO (3 and 0.2 mg/L, respectively). For the removal of Nickel, *P. karka* was found to be the most efficient with 73% removal efficiency

and the combined cell showed negative performance with increasing concentration of Nickel in the effluent. Based on the current study, it is difficult to explain the reason for such an increase of Nickel in the effluent from the combined plant cell but it is worth considering repeated measurement for Nickel.

The mean concentrations of ciprofloxacin in the influent and effluent were much higher than studies done elsewhere. In a study conducted by Le *et al*. (2016) on a hospital wastewater in Singapore, the concentration of ciprofloxacin in the influent ranged between 8.74–76.44 µg/L, with a significant reduction in the effluent ranging 1.72–4.34 µg/L. Recently, a study on the fate of fluoroquinolone from hospital wastewater which joins the municipal wastewater through sewer line and treated anaerobically revealed that ciprofloxacin in the hospital wastewater ranged from 1.3–33.9 ng/mL and decreased to 0.5–5.6 ng/mL after treatment (Rodrigues-Silva *et al*., 2019). A study by Wang *et al*. (2011) also showed that the concentration of ciprofloxacin in a hospital wastewater was 1334.10 ng/L, still much lower than the current study.

The low removal efficiency of ciprofloxacin was exhibited in all the wetland cells irrespective of plant type. The possible reason for this might be the lack of optimization of process parameters such as hydraulic retention time (HRT) for the removal of pharmaceuticals in wastewater treatment plants. Studies indicate that HRT is an essential parameter for the removal of micropollutants in different configurations of WWTPs including constructed wetlands (Boonnorat *et al*., 2019; Ejhed *et al*., 2018). Further study is required on optimizing hydraulic retention time in the framework of antibiotics removal in constructed wetland system.

The mean concentration of cefotaxime, a third generation cephalosporin, in the wastewater before and after treatment with the constructed wetland showed alarmingly high level that has not been reported elsewhere. A study by Li and Zhang (2011) reported an average concentration of 15 ng/L of cefotaxime was detected in municipal wastewater treatment systems in China, which is very low compared to the current finding. Wang *et al*., (2011) reported that the level of cefotaxime in the influent and effluent of selected WWTPs treating hospital wastewater in China had maximum concentrations of 0.82 µg/L and 0.13 µg/L, respectively, a much lower concentration compared to the current finding. The higher removal efficiency of cefotaxime observed in the unplanted control cell can be attributed to photodegradation of the drug in the absence of vegetation

coverage, as indicated in Verlicchi and colleagues (2010).

Hospital wastewater are generally characterized by high heterotrophic bacteria reaching as high as 1010 CFU/mL (Amouei *et al*., 2015). The mean concentration of total heterotrophic bacteria in the influent was lower compared to a study conducted in Mekelle hospital wastewater, which was $1.6×10^6$ CFU/mL (Tsegahun Asfaw *et al*., 2017) and Hawassa referral hospital wastewater which was $7.4×10^7$ CFU/mL (Hunachew Beyene and Getachew Redaie, 2011). The count was also lower than the concentration of total cultivable heterotrophic bacteria in raw hospital wastewater from three tertiary hospitals in central China, which ranged from $2.03×10^9$ CFU/L to $3.31×10^9$ CFU/L (Wang *et al*., 2010). According to Aali *et al*. (2014), the mean concentration of heterotrophic bacteria in hospital wastewater was $6.05×10^7$ CFU/L. The cell vegetated with *T. latifolia* showed highest performance for significant removal of total heterotrophic bacteria in this study. The effectiveness of *T. latifolia* on the removal of heterotrophic bacteria and coliforms was also indicated in a study by Kipasika and colleagues (2016).

CONCLUSION

The results from the present study indicated the possibility of applying constructed wetland as a pretreatment scheme for wastewater from Tikur Anbessa Specialized Hospital before joining the main sewer line that channels municipal wastewater to the wastewater treatment plant in Kality. No significant difference was detected between mixed macrophytes-based and single macrophyte-based constructed wetland in terms of removal of nutrient, organic matter, antibiotics, antibiotic resistance and heavy metals. The alarmingly high level of ciprofloxacin and cefotaxime requires due attention with further studies to investigate the potential of constructed wetland for the removal of the available drug residues in hospital wastewater. The bacterial count resistant to all the four drugs was the highest in the cell with the combined plants surpassing the unplanted control cell. Further studies should be conducted to optimize the different parameters including plant materials for maximum removal efficiency of heterotrophic bacteria as well as antibiotic residues.

ACKNOWLEDGEMENTS

REFERENCES

Aali, R., Nikaeen, M., Khanahmad, H. and Hassanzadeh, A. (2014). Monitoring and comparison of antibiotic resistant bacteria and their resistance genes in municipal and hospital wastewaters. _Int. J. Prev. Med._ **5**: 887–894.

Akin, B.S. (2016). Contaminant properties of hospital clinical laboratory wastewater: A physiochemical and microbiological assessment. _J. Environ. Protect._**7**: 635–642.

Akratos, C.S. and Tsihrintzis, V.A. (2007). Effect of temperature, HRT, vegetation and porous media on removal efficiency of pilot-scale horizontal subsurface flow constructed wetlands. _Ecol. Eng._ **29**: 173–91.

Al Qarni, H., Collier, P. O'Keeffe, J. and Akunna, J. (2016). Investigating the removal of some pharmaceutical compounds in hospital wastewater treatment plants operating in Saudi Arabia. _Environ. Sci. Pollut. Res_. **23**: 13003–13014.

Amouei, A., Asgharnia, H., Fallah, H., Faraji, H., Barari, R. and Naghipour, D. (2015). Characteristics of effluent wastewater in hospitals of Babol University of Medical Sciences. Babol, Iran. _Health Scope_. **4**: 1–5. doi: 10.17795/jhealthscope-23222.

APHA (American Public Health Association) (1998). **Standard Methods for the Examination of Water and Wastewater**. 20th edition. United Book Press, Inc., Baltimore, Maryland.

Ayangbenro, A.S. and Babalola, O.O. (2017). A new strategy for heavy metal polluted environments: A review of microbial biosorbents. _Int. J. Environ. Res. Public Health_ **14**: 1–16.

Barancheshme, F. and Munir, M. (2018). Strategies to combat antibiotic resistance in the wastewater treatment plants. _Front. Microbiol._ **8**: 1–12.

Boonnorat, J., Kanyatrakul, A., Prakhongsak, A., Honda, R., Panichnumsin, P. and Boonapatcharoen, N. (2019). Effect of hydraulic retention time on micropollutant biodegradation in activated sludge system augmented with acclimatized sludge treating low-micropollutants wastewater. _Chemosphere_ **230**: 606–615.

Brix, H. (1993). Wastewater treatment in constructed wetlands: System design, removal processes, and treatment performance. In: **Constructed Wetlands for Water Quality Improvement**, (Moshiri, G.A., ed.). 1st edition. CRC Press.

Chunhui, Z., Liangliang, W., Xiangyu, G. and Xudan, H. (2016). Antibiotics in WWTP discharge into the Chaobai River, Beijing. _Arch. Environ. Prot_. **42**: 48–57.

Ciria, M.P., Solano, M.L. and Soriano, P. (2005). Role of macrophyte _Typha latifolia_ in a constructed wetland for wastewater treatment and assessment of its potential as a biomass fuel. _Biosyst. Eng._ **92**: 535–544.

Crouch, E., Dickes, L. and Kahle, A. (2015). Review on antibiotic resistance. _Adv. Pharmacoepidemiol. Drug. Saf._ **4**: 183.

Ejhed, H., Fång, J., Hansen, K., Graae, L., Rahmberg, M., Magnér, J., Dorgeloh, E. and Plaza, G. (2018). The effect of hydraulic retention time in onsite wastewater treatment and removal of pharmaceuticals, hormones and phenolic utility substances. _Sci. Total Environ_. **618**: 250–261.

Feleke Moges, Mengistu Endris, Andargachewu Mulu, Belay Tessema, Yeshambel Belyhun, Yitayal Shiferawu, Kahsay Huruy, Unakal, C. and Afework Kassu (2014). The growing challenges of antimicrobial drug resistance in Ethiopia. _J. Glob. Antimicrob. Resist._ **2**:148–154.

Hu, B., Jia, X., Hu, J., Xu, D., Xia, F. and Li, Y. (2017). Assessment of heavy metal pollution and health risks in the soil-plant-human system in the Yangtze River

Delta, China. *Int. J. Environ. Res. Public Health* **14**: 1–18.

Hunachew Beyene and Getachew Redaie (2011). Assessment of waste stabilization ponds for the treatment of hospital wastewater: The case of Hawassa University Referral Hospital. *World Appl. Sci. J.* **15**: 142–150.

Kadlec, R.H., Knight, R.L., Vymazal, J., Brix, H., Cooper, P. and Haberl, R. (2000). **Constructed Wetlands for Pollution Control: Processes, Performance, Design and Operation**. IWA specialist group on use of macrophytes in water pollution control. Scientific and Technical Report, No. 8, IWA Publishing, London.

Kaur, B., Saxena, R.K. and Kaur, R. (2014). Quantitative analysis of mercury burden in waste water released from hospital in Jhansi, U.P. *Int. J. Environ. Monit. Anal*. **2**: 328–332.

Kim, C., Ryu, H., Chung, E., Kim, Y. and Lee, J. (2018). A review of analytical procedures for the simultaneous determination of medically important veterinary antibiotics in environmental water: Sample preparation, liquid chromatography, and mass spectrometry. *J. Environ. Manage.* **217**: 629–645.

Kipasika, H.J., Buza, J., Smith, W.A. and Njau, K.N. (2016). Removal capacity of faecal pathogens from wastewater by four wetland vegetation*: Typha latifolia, Cyperus papyrus, Cyperus alternifolius* and *Phragmites australis. Afr. J. Microbiol. Res.* **10**: 654–661.

Le, T-H., Ng, C., Chen, H., Yi, X.Z., Koh, T.H., Barkham, T.M., Zhou, Z. and Gin, K.Y. (2016). Occurrences and characterization of antibiotic-resistant bacteria and genetic determinants of hospital wastewater in a tropical country. *Antimicrob. Agents Chemother*. **60**: 7449–7456.

Li, B. and Zhang T. (2011). Mass flows and removal of antibiotics in two municipal wastewater treatment plants. *Chemosphere*. **83**: 1284–1289.

Li, Y., Zhu, G., Ng, W.J. and Tan, S.K. (2014). A review on removing pharmaceutical contaminants from wastewater by constructed wetlands: Design, performance and mechanism. *Sci .Total Environ*. **468**: 908–932.

Lien, L.T., Hoa, N.Q., Chuc, N.T, Thoa, N.T, Phuc, H.D., Diwan, V., Dat, N.T., Tamhankar, A.J. and Lundborg, C.S. (2016). Antibiotics in wastewater of a rural and an urban hospital before and after wastewater treatment, and the relationship with antibiotic use - A one year study from Vietnam. *Int. J. Environ. Res. Public Health.* **13**: 1–13.

Mburu, N., Tebitendwa, S.M., Rousseau, D.P., Bruggen, J.J. and Lens, P.N. (2013). Performance evaluation of horizontal subsurface-flow constructed wetlands for the treatment of domestic wastewater in the tropics. *J. Environ. Eng*. **139**: 358–367.

Mondal, S. and Sinha, A. (2014). Treatment of pharmaceutical waste with special emphasis to treatment processes - A review. *Int. J. Environ. Res. Dev*. **4**: 171–176.

National Committee for Clinical and Laboratory Standards Institute (CLSI) (2015). Performance standards for antimicrobial susceptibility testing. Twenty fifth informational supplement. CLSI document M1002-S25. Clinical and Laboratory Standards Institute, Wayne, PA.

Ort, C., Lawrence, M.G., Reungoat, J., Eaglesham, G., Carter, S. and Keller, J. (2010). Determining the fraction of pharmaceutical residues in wastewater originating from a hospital. *Water Res*. **44**: 605–615.

Rodrigues-Silva, C., Porto, R.S., Sabrina, G., dos Santos, S.G., Schneiderb, J. and Rath, S. (2019). Fluoroquinolones in hospital wastewater: Analytical method, occurrence, treatment with ozone and residual antimicrobial activity evaluation. *J. Braz. Chem.*

Soc. **30**: 1447–1457.

Sardar, K., Ali, S., Hameed, S., Afza, S., Fatima, S., Shakoor, M.B., Bharwana, S.A. and Tauqeer, H.F. (2013). Heavy metals contamination and what are the impacts on living organisms. *Greener J. Environ. Manage. Public Saf*. **2**: 172–179.

Shraim, A., Diab, A., Alsuhaimi, A., Niazy, E., Metwally, M., Amad, M., Sioud, S. and Dawoud, A. (2017). Analysis of some pharmaceuticals in municipal wastewater of Almadinah Almunawarah. *Arabian J. Chem*. **10**: 719–729.

Sintayehu Fekadu, Yared Merid, Hunachew Beyene, Wondu Teshome and Solomon Gebre-Selassie (2015). Assessment of antibiotic- and disinfectant-resistant bacteria in hospital wastewater, south Ethiopia: a cross-sectional study. *J. Infect Dev. Ctries*. **9**: 149–156.

Teshome Gezahegn, Bisratewongel Tegegne, Feleke Zewge and Chandravanshi, B.S. (2019). Salting-out assisted liquid–liquid extraction for the determination of ciprofloxacin residues in water samples by high performance liquid chromatography–diode array detector. *BMC Chem*. **13**: 1–10.

Tsegahun Asfaw, Letemichael Negash, Amlsha Kahsay and Yemane Weldu (2017). Antibiotic resistant bacteria from treated and untreated hospital wastewater at Ayder Referral Hospital, Mekelle, North Ethiopia. *Adv. Microbiol.* **7**: 871–886.

Verlicchi, P., Galletti, A., Petrovic, M. and Barcelo, D. (2010). Hospital effluents as a source of emerging pollutants: An overview of micro pollutants and sustainable treatment options. *J. Hydrol.* **389**: 416–428.

Vymazal, J. (2001). **Removal of organics in Czech constructed wetlands with horizontal sub-surface flow. Transformations on Nutrients in Natural and Constructed Wetlands,** pp. 305–327. Backhuys Publishers, Leiden.

Vymazal, J. (2011). Constructed wetlands for wastewater treatment: five decades of experience. *Environ. Science Tech*. **45**: 61–69.

Vymazal, J., Březinová, D.T., Koželuh, M. and Kule, L. (2017). Occurrence and removal of pharmaceuticals in four full-scale constructed wetlands in the Czech Republic – the first year of monitoring. *Ecol. Eng*. **98**: 354–364.

Wang, Y.T., Hu, J. and Tan, Y. (2011). Determination of cephalosporin antibiotics in water samples by optimised solid phase extraction and high-performance liquid chromatography with ultraviolet detector. *Int. J. Environ. Anal. Chem*. **91**: 1267–1281.

Yamina, B., Tahar, B., Lila, M., Hocine, H. and Laure, F.M. (2014). Study on Cadmium resistant-bacteria isolated from hospital wastewaters. *Advanc. Biosci. Biotechnol*. **5**: 718–726.