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Research Article

Comparative nutrient removal efficiency of three Cyperus species in vertical flow type of constructed wetlands, Sebeta, Ethiopia

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Abstract: *River water pollution is increasingly widespread in and around Addis Ababa and can lead to problems with users if not properly treated. Constructed wetlands are a promising solution and are being used by several countries. In this study three Cyperus species (Cyperus alternifolius, Cyperus papyrus and Cyperus usitatus) and substrate only were investigated for their removal efficiencies of TSS, BOD, NH_4-N, NO_3-N, TP and total coliforms (TC) from polluted river water in a free vertical surface flow (VSSF) wetland system. The maximum NO_3-N, BOD, TSS, NH_4-N and TP removal efficiency by C. cyperus were 95.5%, 78.5%, 76.1%, 68.2% and 66%, respectively. C. alternifolius was superior in removal efficiency compared with other treatments but C. papyrus was the highest in TC removal (92.2%). Treatment with substrate only has the lowest removal efficiency. No significant differences were observed in the removal efficiency of Cyperus species and substrate only among 3, 5 and 7 days of hydraulic retention time. Apart from nutrient removal, constructed wetland cells purified and improved the colour of the wastewater which is an added advantage to change the appearance of polluted rivers. C. alternifolius and C. papyrus have a higher nutrients, TSS and BOD removal efficiencies and can be considered from a treatment perspective in constructed wetland. Further research is required to select multipurpose wetland plants with high wastewater removal efficiency, and potential as livestock feed which was not determined in this experiment.*

Keywords: Hydraulic retention time, Organic matter, Surface flow, Wastewater, Wetland plants
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1. Introduction

The centrality of water in our lives such as social, economic, political and spiritual cannot be overestimated (Töpfer, 2003). According to UNEP‘s (United Nations Environment Programme) Global Environment Outlook Reports, global freshwater consumption rose six-fold between 1900 and 1995 more than twice the rate of population growth (UNEP, 1999). However, only less than 1 percent of the world’s freshwater is accessible for direct human uses and water resources are being used faster than they are being replenished. The depletion and pollution of the planet‘s limited supply of freshwater are becoming life-threatening crises. David *et al.* (2022) also noted that freshwater scarcity resulting from rapid population rise and economic growth is now a global crisis. Hence, wastewater remediation and recycling have proven crucial for adequate water supply and to address the increased demand for clean freshwater.

Infrastructure development, land conversion, intensive land use and massive deposition of pollutants in water threaten all ecosystem functions that produce our freshwater resources (Verhoeven *et al*., 2006). Because of the slow development of ecosystems, most of these rapid and dramatic changes are irreversible (Anker, 2002). Managing water to optimize available resources and their use is a growing concern in developing countries. In Ethiopia, for example, growing population with increasing development needs coupled with inadequate waste disposal system is the major cause of aquatic ecosystem degradation. Most industrial effluents and domestic wastes are simply discharged into rivers and wetlands without pre-treatment. Although it is known that the use of wastewater treatment plants and the implementation of a proper waste disposal system can reduce freshwater pollution, their application is unlikely in less developed countries.

However, constructed wetlands can be used and are a cost-effective wastewater treatment technology suitable for developing countries (Denny, 1997). Constructed Wetlands (CWs) appear as one of the most promising eco-tech treatment methods, which have been attracting increasing worldwide interest (Alexandros, 2016). Xiuwen *et al*. (2022) reported the ecological restoration and decontamination performance of constructed wetlands. Constructed wetlands also have significantly lower total lifetime costs and often lower capital costs than conventional treatment systems (ITRC, 2003). Compared to conventional systems, natural systems can be operated with less electricity and less labor (USEPA, 2000). There is growing evidence that constructed wetlands is particularly important for treating wastewater and protecting water quality in catchments, rivers, and lakes (Denny, 1997; Verhoeven *et al*., 2006; Cui *et al*., 2010). Many industries in developing countries use conventional wastewater treatment systems to treat their wastewater before release into the environment (Zhang *et al*., 2014). In sub-Saharan Africa, for instance, some constructed wetlands have been operational in South Africa (Wood, 1990), Kenya (Nyakang'o, 1997; Raymer, 2006), Uganda (Okurut *et al*., 1999; Kyambadde *et al*., 2004) and Tanzania (Mashauri *et al*., 2000).

There are different types of constructed wetland like surface flow (SF), horizontal subsurface flow (HSSF) and vertical flow (VF) which differ from one another in system layout, the removal efficiency of certain pollutants, area requirements, technical complexity, applications, and costs (Gauss, 2008). Surface flow or free water surface constructed wetland- is strongly related to natural wetlands consisting of large, shallow lagoons that contain submerged, emergent, or floating plant species. Most commonly used as tertiary treatment that is, to remove nutrients to prevent eutrophication (algae growth) in the receiving water body. SF consists of shallow basins filled with coarse sand or gravel as filter material. VF constructed wetland consists of shallow sand filter beds that provides an intermittent hydraulic loading which provides an effective aeration mechanism because pores of the filter bed refill with oxygen as a result, high nitrification rates1 can be achieved in the filters (Gauss, 2008). Although HSSF constructed wetlands are the dominant types, mainly due to higher overall costs for VF constructed wetland construction and operation, VF has higher oxygen transfer capacity compared to the horizontal flow beds (Alexandros *et al., 2014*). Alexandros (2016) also reported that HSSF constructed wetland has low nitrification capacity due to limited oxygen transfer capacity but VF has good nitrification capacity because of high oxygen transfer capacity (Alexandros, 2016). According to Sandeep *et al*. (2021) the contaminant removal efficiency of the vertical subsurface flow constructed wetlands (VSSFCW) commonly used for the treatment of domestic and municipal wastewater ranges between 31% and 99%. Generally, HSSF requires high area demand but VF requires small area, has good oxygen supply means good nitrification and high purification performance but requires higher technical demands.

It is not only the types of the constructed wetlands but also, vegetation plays a critical role in the performance of constructed wetlands and hence selection of the most efficient vegetation type is important. According to (Yuan *et al*., 2016) the roots absorb pollutants from wastewater, prevent wastewater from taking preferential paths in the substrate that can result to hydraulic short circuiting which would consequently reduce the retention time in the wetlands and also provide a large surface area for attachment of micro-organisms that degrade the organics in the wastewater but their roots. Wetland plants also had the ability to accumulate high biomass and remove nutrients and therefore have high potential in biological nutrient removal processes (Yezbie and Seyoum, 2014). The efficient treatment and recycling of wastewater will undoubtedly help in elevating the level of clean water and sanitation (Tortajada, 2020).

The study site, Sebeta is located 24 km Southwest of Addis Ababa. In the recent times there is expansion of urbanization and industrial development in and around Addis Ababa where both domestic and industrial wastes are released and damped into the rivers without prior treatment. Our personal observation also revealed that downstream inhabitants are using the water from the rivers for irrigation and watering their animals but complained that they are seeing problems on their animals. So far constructed wetlands are not applied in the area to treat wastewater. Therefore, the objective of this study was to develop a pilot constructed wetland at the National Fishery and Aquatic Life Research Center (FALRC) and to investigate the nutrient removal efficiency of three wetland plants to treat wastewater collected from the nearby river.

2. Materials and Methods

2.1. Construction of constructed wetland site

A suitable area was selected to construct the wetland based on the type and extent of wastewater effluent. Then a constructed wetland experimental site was established at NFALRC, consisting of 12 wetland cells (units), each with an area of 6 m^2 (2m*3m) (Fig. 1). The type of constructed wetland established was vertical flow type of constructed wetland, which was designed to take advantage of the chemical and biological processes of natural wetlands to remove contaminants/nutrients from wastewater (Chao *et al*., 2022). The constructed wetland system included 12 rectangular treatment cells (units), each measuring 3 m long, 2 m wide and 0.5 m deep, with a surface area of 6 m^2 and a total volume of 3 m^3. A photo indicating the experimental layout including the storage and equalizer tanks is shown in Figure 1. The cells were built with concrete bottom and walls with fitted outlets filled with layers of gravel/crushed rocks 12.5 cm, sand 27.5 cm and soil 5 cm on top (Yan *et al*., 2018; Chao *et al*., 2022). The gravels were between 5 -15 mm and the sand between 0.1 - 0.5 mm in sizes. The wastewater tanks, storage (5000 liter) and equalizer (3000 liter) were connected to the inlet and outlet controlled by a valve. Each cell gets the wastewater from the equalizer through fitted pipe and the effluents were collected through the outlets fitted just above the bottom of each cell.

Figure 1: Wastewater treatment system (field view) at NFALRC

2.2. Nature and source of wastewater

Wastewater was collected from Sebeta River (08° 45′ 964″ N, 38° 38′136″ E) that is flowing near the constructed wetland site that receives domestic and industrial wastes from the local residents and alcohol factory located about 0.5 km above the site. The raw wastewater has dark colour with low dissolved oxygen (< 2 mg/L) and high conductivity of 1110 µScm^{-1}. The wastewater was pumped from the river into a storage tank near the constructed wetland, settled for 4 hours and then allowed to flow into the equalizer tank through a PVC pipe. Then the wastewater from the equalizer tank was distributed into each constructed cell for 20 minutes at a discharge rate of 10 L/minute.

The physicochemical characteristics of the wastewater from the source river are given in Table 1.

Table 1: Characteristics of wastewater from the source

Parameters	Mean values
Wastewater temperature ($^{\circ}$C)	14.3
Dissolved oxygen (mg l^{-1})	1.73
Conductivity (µS cm^{-1})	1616.7
pH	6.2

Total suspended solids (mg l^{-1})	910
Total dissolved solids (mg l^{-1})	508
Nitrite (mg l^{-1})	5.04
Nitrate (mg l^{-1})	30.71
Ammonium (mg l^{-1})	3.67
Soluble reactive phosphorus (mg l^{-1})	9.43
Total phosphorus (mg l^{-1})	13.38
BOD_5 (mg l^{-1})	175
Total coliforms ($CFUml^{-1}$)	$1.04*10^4$

2.3. Treatments and design

The treatments were three *Cyperus* species (*Cyperus alternifolius, Cyperus papyrus and Cyperus usitatus*) collected from Lake Ziway and substrate only (a wetland cell without wetland plant). The treatments were randomly assigned to the constructed cells each with replicates. The *Cyperus* plants were planted equidistantly at 20 cm in all the treatment cells. Dead shoots were replaced with new ones randomly until the growth stabilized in the gravel bed. The plants were allowed to grow and multiply to form a dense stand in the cells for three months with periodic application of pond water to all treatments before wastewater was introduced into the treatment cells. To evaluate nutrient removal efficiency of wetland plants, three treatment plants and substrate only in triplicate received wastewater with different retention times (HRT) 3, 5 and 7 days.

2.4. Sampling and wastewater analysis

The inflow rate of wastewater from the equalizer into the treatment cells was calculated by taking the minute taken to fill 20 liter of waste. *In situ* measurements were conducted for influent temperature, DO, pH, and conductivity using a digital probe at the source, equalizer and in the treatment cells. The influent and effluent water samples were collected with 2 liter plastic bottles from each treatment cells and immediately transferred from the site to the laboratory for further analysis. Parameters were measured *in-situ* from the source and equalizer, water samples collected from the source and equalizer and analyzed in the laboratory. From the effluents some parameters were measured *in-situ* and water samples were collected from of each treatment and replicates in triplicate, parameters were analyzed in the laboratory after 3 days, 5 day and 7 days of retention time. Parameters analyzed in the laboratory were total suspended solids (TSS), total dissolved solids (TDS), Nitrite, Nitrate, Ammonium-N (NH_4-N), soluble reactive phosphorus (SRP), total phosphorus (TP), biological oxygen demand (BOD) and total coliforms.

Wastewater samples were taken to measure five-day biochemical oxygen demand (BOD), ammonia nitrogen (NH_4-N), nitrite (NO_2), nitrate (NO_3) and total phosphorous (TP), soluble reactive phosphorous (SRP), total suspended solids (TSS), total dissolved solids (TDS) and total coliforms. The analytical methods used for the water quality analyses were in accordance with Standard Methods for the Examination of Water and Wastewater (APHA, 2005). Some parameters were measured in the field, including wastewater temperature, electrical conductivity (EC), pH and dissolved oxygen (DO) in the source and in the constructed wetland using a Hach HQ40d multi-parameter probe. Treatment efficiency was calculated as the percentage of removal for TSS, TDS, nutrients and total coliforms as follows:

$$\mathrm{TSS\%}\left(\frac{\mathrm{m}}{\mathrm{v}}\right) = 100 * (\mathrm{M2} - \mathrm{M1})/\mathrm{V} \qquad [1]$$

$$\mathrm{TDS\%} = 100 * (\mathrm{M2} - \mathrm{M1})/\mathrm{V} \qquad [2]$$

$$\text{Removal Efficiency (\%)} = [(Ci – Ce)/Ci)] *100 \qquad [3]$$

Where, M2 is the mass of the dish with dried material in gram, M1 is the mass of the dish in gram and V is the volume in mL of sample taken. Ci is the concentration of the wastewater in the influent Ce is the concentration of the wastewater in the effluent.

To determine total coliform bacteria water samples were taken from the source, equalizer and three constructed wetland with plants and from one constructed wetland structure without plant. Samples were taken from the equalizer, at day 3, day 5 and day 7. The samples were collected in 20 ml sterile test tubes covered with aluminum foil and immediately transported into microbiology laboratory for further analysis following the methods by Tekpor *et al*. (2017). To count the total coliforms samples from each site violet red bile agar medium was prepared and 1 ml of sample was transferred into 9 ml of peptone water and serially diluted by 10^{-1}, 10^{-2},

10^{-3} and 10^{-4}. From the dilution, 0.1 ml sample was transferred into sterile petri dishes using spread plate technique and incubated for 24 h at 37 °C. Finally, the numbers of colonies were counted using most probable methods (Rachma *et al*., 2020).and were expressed as colony forming unit per ml sample analyzed (CFU/ml).

2.5. Data analysis

A statistical was used to determinate how pollutant concentration varied among treatments (constructed wetland cells and hydraulic retention time). Data was assessed with one way ANOVA with a mean separation test was done to validate which *Cyperus* species and also which hydraulic retention time had significant removal efficiency than others. The results were considered significant when $P < 0.05$. All calculations were performed using SPSS version 20 for windows.

3. Results and Discussion

The effluents of the wastewater were collected from each treatment in triplicate after 3, 5 and 7 days of hydraulic retention time. The result showed that the three *Cyperus* species have highest removal efficiencies of total suspended solids (TSS), biological oxygen demand (BOD), Ammonium (NH_4^+), Nitrate (NO_3^-), Total phosphorous (TP) and total coliforms (TC) (Table 2). The ANOVA test for the removal efficiency of the treatments (three *Cyperus* species and substrate only) for three different hydraulic retention time was not significantly different ($p<0.05$) and hence mean of the three hydraulic retention time was considered as percent removal efficiency of the treatments as indicated in Table 2.

Table 2: Average ± SD of parameters in the influent and effluents with percentage removal efficiency in brackets

Parameters*	Influent	Effluent concentration in the treatments			
		C. alternifolis	*C. papyrus*	*C. usitatus*	*Substrate only*
TSS	870	232±0.02 (73.3)	263±0.03 (69.8)	311±0.07 (64.3)	636±0.12 (26.9)
BOD_5	137	36.9±8.27 (73)	56±8.19 (59.1)	63.7 ± 6.66 (53.5)	85±11.14 (38)
NH_4-N	3.18	1.19±0.17 (62.6)	1.37±0.17 (57)	1.5 ± 0.16 (52.9)	2.29±0.24 (28.1)
NO_3-N	30.67	2.1±0.79 (93.1)	3.14±0.17 (89.8)	3.34 ± 0.17 (89.1)	25.6±0.98 (16.5)
TP	12.71	5.5±1.18 (56.7)	7.11±1.43 (44)	6.87±1.08 (45.9)	11.4±0.72 (10.3)
TC	$8.6x10^3$	$1.3x10^3$±$2.6x10^2$ (85)	$1.1x10^3$±$3.4x10^2$ (88)	$2.3x10^3$±$1.9x10^2$ (74)	$3x10^3$±$5.4x10^2$ (65)

*All units are in mg L^{-1} except for TC which is in CFU mL^{-1}; TSS = total suspended solids, BOD = Biological Oxygen Demand; NH_4-N = Ammonium-N; NO_3 = Nitrate; TP = Total phosphorous; TC = Total coliform

High nutrient removal efficiencies of *Cyperus* species (for example 86-95% NO_3; 70-90% TP removal) could be as organic or inorganic form as dissolved or particulate matter. According to Maynard *et al*. (2011) the removal and retention of TP in CWs occurs as a result of several physical, chemical and biological processes such as sedimentation of particulate phosphorous (organic and inorganic), sorption and precipitation of phosphorous associated with mineral particles within the water column, phosphorous sorption in wetland soils and biological uptake by plants (Liu *et al*., 2022) and microorganisms (Reddy and DeLaune, 2008). The decomposition of biological material is one phosphorous source in wetland effluent, indicating that wetlands are ineffective for permanent phosphorous removal unless proper management of the plants is in place. This indicates that phosphorus is available in different forms either in the influent or in the substrate structures (soil, sand and gravel) that can be broken down into inorganic forms and subsequently removed through plant uptake. This study also showed higher nutrient (NO_3 and TP) removal on the 7th days of retention time than on the 3rd and 5th days, suggesting that additional residence time resulted in enhanced nutrient removal from the influent, dead plants and substrate structures.

On the other hand the main mechanism for N removal in constructed wetlands is respiratory denitrification, microbially mediated transformation of nitrate to N_2O and N_2 gasses in the absence of oxygen (Kadlec and Knight, 1996; Reilly *et al*., 2000). N removal is also possible through sedimentation, ammonia volatilization, plant uptake and algal assimilation (Reinhardt *et al*., 2006). Nitrate leaching is another mechanism for nitrogen removal in a combined sand and gravel substrate as in this study. On the other hand, N can be released during death and decay of aquatic plants, which would promote the release of organic N back into the wetland (Smith *et al*., 2006). However, in this study, N-release due to plant death was minimized by removing the dead branches, which is also a means to remove some quantity of N from the system. Our study results are consistent with reports that confirm CWs cultivated with aquatic plants were most effective at removing Nitrogen in the form of NO_3 instead of NH_4 (Phipps and Crumpton, 1994). Moreover, we observed consistently high NO_3 removal efficiency (>85%) in three, five and seven-day residence time in all types of aquatic plants used in this study. This could be due to similar hydrology with slow flow in each cell that can lead to accumulation of organic matter in the substrate column (sand and gravel) uniformly.

Apart from the nutrients, constructed wetlands are also highly effective in reducing TSS and several studies reported up to 97% TSS removal in CWs receiving influent from irrigation runoff (e.g., Kadlec *et al*., 2010). In the present study a lower removal efficiency 76% reduction in TSS was obtained in *C. alternifolius* after 7 days of hydraulic retention time. The other three treatments also showed quite reasonable reduction in TSS ragging between 56-74%. Our result on total coliform removal efficiency of *C. papyrus* from-88% and 85 by *C. alternifolis* are comparable with the reports by Fernando *et al*. (2019) who found 98.08% removal using *C. papyrus*.

In the present study, high loads of total coliforms were found in the source samples (Table 1). This is because untreated solid and liquid wastes from households and different processing industries could be released. The presence of total coliforms in the sample indicates a high probability of pathogenic microorganisms since coliforms are the most important indicator bacteria for most disease-causing microorganisms (Aghalari *et al.,* 2020). In constructed wetland system, aquatic plants can remove total coliforms (57-90%) (Dhir, 2020). During the 3rd, 5th, and 7th days, our results indicated that all treatment plants have the potential for total coliforms reduction (57.9 -92.2%) and hence, the aforementioned plants could be ideal for wastewater treatment. On the other hand, substrate only (constructed wetland without vegetation) has lowest nutrient removal efficiency but as high as 70% TC removal signifying the role of plants. Similar result is also report by Mahder (2017), Yezbie and Seyoum (2014). The reduction in coliforms could also be due to filtration, sedimentation, and adsorption ability of the selected aquatic plants. Among the three aquatic plants highest TC removal efficiency was by *C. alternifolius* up to 90% followed by *C. papyrus* 87.5% and *C. usitatus* 81%. Up to 70% total coliforms reduction efficiency of *C. alternifolius* was reported by Shahi *et al*. (2013) which is lower than the present study. But high TC reduction with *C. papyrus* up to 98% was also reported by Garcia-Avila *et al*. (2019) indicating the significance of wetland plants in wastewater treatment.

Although the trend in nutrient removal efficiency of *Cyperus* species with longer hydraulic retention time (HRT) is higher, the ANOVA test showed no significant difference ($p>0.05$) between the hydraulic retention time except for TC where there was significant difference ($p=0.019$) between 3 and 7 days of HRT. On the other hand there was significant difference between the plant species and the substrate only for different parameters. For example the removal efficiency of the three plants species *C. alternifolius, C. papyrus* and *C. usitatus* were significantly different ($p<0.001$) with the substrate only for NO_3 and TP. *C. alternifolius* was also significantly different with the substrate only for NH4-N ($p = 0.005$) and TC ($p = 0.006$). *C. papyrus* was significantly different ($p=0.028$) with the substrate only for TC.

Apart from the removal of nutrients, wetland cells have also played a key role in changing the appearance of the wastewaters' colour where the change before and after the waste passed through the wetland cells was clear (Figure 2) indicating the importance of constructed wetlands for river restoration programs as most and all rivers in and around cities in Ethiopia are ugly looking.

Figure 2: Colour change in the wastewater after (left) and before (right) treated with the constructed wetland

Generally constructed wetlands are effective systems for the removal of water pollutants and are widely used for the treatment of wastewater. Aquatic plants are an important component of constructed wetlands and contribute to the purification of nutrients, such as nitrogen and phosphorus. The contribution of aquatic plants to nitrogen and phosphorus removal in CWs varies depending on the design, hydraulic conditions, substrate structure, plant species, and effluent load (Allen *et al*., 2013; Nivala *et al*., 2013). In this study, a substantial reduction in NH_4- N, NO_3 –N, SRP and TP were observed in all three aquatic plants (*C. papyrus*, *C. alternifolius* and *C. usitatus*) compared to approximately 10– 15% of plant uptake of nutrients (N and P) reported by Chan *et al*. (2008). The removal of NO_3-N in all treatment plants showed 87-95.5% reduction during the three, five and seven-day residence time. This could be due to the high plant root surface area in a sand substrate that might have created environment conducive for bacterial growth responsible for nitrification process. This finding is consistent with Bastviken *et al*. (2005), who reported that bacteria are much more abundant when they grow on surfaces than when they are suspended in water. Plant roots not only provide the necessary surfaces for bacterial growth but also release organic carbon as a carbon and energy source for heterotrophic bacteria, such as the denitrifying bacteria (Bastviken *et al*., 2005). The role of plant in nutrient removal is also reported by Hassan *et al*. (2021) who stated that plant roots transfer some oxygen to the subsurface, and dryness periods allow oxygen diffusion to subsurface. Thus, the oxygen level in wetland cell is high, which promotes the growth of aerobic bacteria enhancing aerobic degradation suitable for nitrification.

4. Conclusion and Recommendations

In this study, three *Cyperus* species (*C. papyrus*, *C. alternifolius* and *C. usitatus*) showed high removal efficiencies for TSS, BOD, TC (and nutrients (NO_3, NH_4, TP). The maximum NO_3-N, BOD, TSS, NH_4-N and TP removal efficiency by *C. cyperus* were 95.5%, 78.5%, 76.1%, 68.2% and 66%, respectively. *C. papyrus* on the other hand was the highest in TC removal (92.2%). The removal efficiency of the constructed wetland without vegetation (substrate only) was the lowest for all parameters compared with those with vegetation and was significantly different from the three *Cyperus* species. No significant differences were observed in the removal efficiency of *Cyperus* species and substrate only among 3, 5 and 7 days of hydraulic retention time. All the *Cyperus* species showed good removal efficiency but *C. alternifolius* and *C. papyrus* have a higher nutrients, TSS and BOD removal efficiencies and can be considered from a treatment perspective in constructed wetland. Apart from nutrient removal, constructed wetland cells purified and improved the colour of the wastewater which is an added advantage to change the appearance of wastewaters. Further research is required to select multipurpose wetland plants with high wastewater removal efficiency, and potential as livestock feed which was not determined in this experiment.

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Data availability statement

Data will be made available on request.

Conflicts of interest

The authors declare that there is no conflict of interest in publishing the manuscript in this journal.

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