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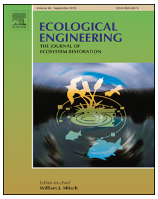

Wastewater treatment by *Lemna minor* and *Azolla filiculoides* in tropical semi-arid regions of Ethiopia

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ABSTRACT

In this study, wastewater blended from textile, distillery, and domestic sources at a corresponding volumetric ratio of 3:1:18, was treated using *Lemna minor* and *Azolla filiculoides* for 28 days in a batch system installed in a shade house. Analysis of variance between the two macrophytes showed no statistical differences in removals of all tested parameters ($p < 0.05$) except for the biochemical oxygen demand where removal was higher in the *L. minor*. Electrical conductivity, pH, total dissolved solids, the studied heavy metals, and sulfate met the agricultural reuse and discharge limits. The removal of chemical oxygen demand by *A. filiculoides* (96%) was slightly higher than the *L. minor* (92%), but the biochemical oxygen demand removal by *L. minor* (92%) was significantly higher than *A. filiculoides* (90%). Despite the high removals of chemical and biochemical oxygen demands, total phosphorus and total nitrogen attained, the concentrations were found exceeding the discharge and agricultural reuse limits. Finally, while the number of total coliform in both macrophyte populated chambers were too numerous to count, the number of colonies of fecal coliform were 400 in the *L. minor* and 267 in the *A. filiculoides* treatments. In conclusion, the higher removals of all the studied parameters in the macrophyte populated chambers compared with the control might be attributed to the contributions of the macrophytes.

1. Introduction

Urbanization and industrialization are the most noticeable pathways of development dynamics and are key determinants of population concentration in specific areas (Fan et al., 2016) and not only serve as the hubs of economic powers and opportunities, but also center of anthropogenic driven pollutions and environmental degradation (Fan et al., 2016). One of the most pronounced issues is pollution attributed to the discharge of poorly treated/untreated industrial and domestic wastewaters (Jørgensen et al., 2009). As the quantity of wastewater increased and its characteristics become more complex, the carrying capacity of the receiving environment eventually decreased to level where natural treatments are insufficient. Subsequently, the environmental problems begin to arise, and more stringent effluent standards enforced. Conventional wastewater treatment processes have emerged around the world to overcome the issues (Hastie, 1992). These centralized treatment systems basically involve physicochemical and biological unit processes and deploy high technologies, skilled manpower, energy, and chemical-intensive operations arranged named as primary, secondary and tertiary treatment systems. However, the costs of such treatment systems have been increased even in the absence of further improvements in treatment complexity and have reached levels where the industries, municipalities, and societies cannot afford (Ciria et al., 2005). Furthermore, production of chemically contaminated sludge and disposal costs are other limitations of the environmentally unfriendly and unsustainable conventional treatment systems (Birame, 2012; Smith and Moelyowati, 2001).

Affordable alternative treatment systems that can provide reliable treatment of multiple contaminants are the prior need of the industries and communities, particularly in the developing world (Sekomo et al., 2012). Application of ecologically engineered natural treatment systems is one of the promising options (Bolton, 2009). Because such systems are reported to be cost-effective, energy-efficient/solar driven and environmentally sound and viable option for developing countries (Gupta et al., 2012). In this regard, literature on the wastewater treatment capabilities of macrophytes such as water hyacinth, Pennywort, water lettuce, water ferns and duckweeds have proven that primary, secondary, and tertiary effluent standards can be achieved (Sooknah and Wilkie, 2004). Nevertheless, the systems are not spread in the developed world due to a poor understanding of the operating

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mechanisms and principles (Al-Nozaily et al., 2000a). Free-floating aquatic macrophytes, because of their high productivity and ease of stocking and harvesting are suitable for phytoremediation wetlands (Sooknah and Wilkie, 2004; Srivastava et al., 2008). For instance, striking qualities of duckweed species to remove nutrients, explosive reproduction capacity and heavy metal's sequestration ability from wastewaters and creation of habitat for aquatic organisms capable of metabolizing wastewater organics are published in the literature such as Louis et al. (2005). In addition, species of this plant were reported to be very effective for the removal of soluble salts, organic matter, algal abundance and coliform densities (El-Kheir et al., 2007) and can tolerate elevated organic loading as well as high concentrations of micronutrients (Hasan and Chakrabarti, 2009). Furthermore, such systems were reported for their capacity to reduce odors and inhibit mosquito breeding besides to the production of useful biomasses (Gupta and Prakash, 2014).

According to the literature, microbial transformation and uptake; macrophyte assimilation, absorption into organic and inorganic substrate materials; and volatilization are some of the primary mechanisms for nutrient removal (Stewart et al., 2008). Plants play an important role in providing surface area and supply oxygen through their roots, which is essential to support the growth and activities of microorganisms and pollutant removal processes, especially nitrification, decomposition of organic matter, heavy metals and nutrients uptake and other biological mediated processes in the wetland system (Kayombo et al., 2004; Lin et al., 2002; Stewart et al., 2008). Phosphorus removal mechanisms on wetlands primarily involve adsorption, filtration, and sedimentation, besides to complexation/chemical precipitation and uptake by macrophytes (Vymazal, 2007), alga and epiphytes and incorporation by microorganisms (Gopal and Ghosh, 2008). However, Vymazal (2008) reported that the other removal mechanisms played negligible role compared to the direct plant uptake. Even though phosphorus in wetlands occurs as phosphate in organic and inorganic compounds, free orthophosphate is the only form of phosphorus believed to be utilized directly by algae and macrophytes and thus represents a major link between organic and inorganic phosphorus cycle in wetlands (Ali et al., 2013). Conversely, plant-based treatment systems are having inherent limitations. The major constraint is the implementation of such systems cannot be transferred from location to location since it highly depends on climate and types of wastewater (Kangas, 2005). Even though it is not a problem for duckweed and Azolla systems, wetland treatment systems have critics of creating habitat for mosquito breeding (Srivastava et al., 2008).

Natural treatment systems such as ecologically engineered wetlands have been considered as more holistic and sustainable approaches for wastewater treatment in the developing world (Sekomo et al., 2012). However, results on the treatment efficiencies of such systems are not comparable; because different species and operational conditions were applied to unlike wastewater types at various climatological settings (Hasan and Chakrabarti, 2009). Furthermore, wetland systems are more suitable for the tropical and subtropical regions (Brix and Schierup, 1989), but the prevalence of mixed wastewaters and lack of experiences on design and management are some of the factors that have been constrained the application. For instance, the physicochemical characteristics of the strong textile and distillery wastewaters were reported poor in supporting the growth of macrophytes and microalgae, and hence could not be treated by them (Ansari et al., 2012). In addition, most of the research works were confined to specific wastewaters and plant species in laboratories under controlled conditions for a short experiment period (Mishra and Tripathi, 2008). In this study, the suitability and efficacy of *Azolla filiculoides* and *Lemna minor* in treating the strong textile and distiller wastewaters blended with domestic effluents have been evaluated under batch experiment in an open-field semi-arid environment for 28-days of incubation period. This experiment was done following the preliminary treatment of the industrial wastewaters by physical blending with domestic wastewater (Amare et al., 2017b). The research was conducted from August 18 to September 14, 2015.

2. Methods and materials

The wastewaters used in this experiment were textile, distillery and domestic wastewaters blended at a volumetric ratio (3:1:18, respectively) determined experimentally (Amare et al., 2017b). Blending was perfumed for neutralization and biological treatability enhancement of these waste streams without any chemical additives. The ratio was determined based on the daily wastewater generation of the factories which was 3:1 [textile: distillery], domestic wastewater was gradually added until a constant pH value within the range of 6.5–7.5 attained which is considered to be suitable for most macrophytes' survival and growth (Amare et al., 2017a). Free-floating aquatic macrophytes duckweed (*L. minor*) and water fern (*A. filiculoides*) were used to treated the blended wastewater. The experiment was conducted in a grass-covered shade house in an open field. The experiment was performed in chambers of length (180 cm), width (70 cm) and depth (50 cm) constructed from wooden frames lined with geomembrane polyvinyl. Each chamber was filled with the blended wastewater leaving a 10 cm of a freeboard. A total of six chambers populated with *A. filiculoides* (3) and *L. minor* (3) and three unplanted chambers were used as controls. Healthy 200 g of fresh weight from an acclimatized *A. filiculoides* and *L. minor* were transferred to each treatment chamber after cleaning with tap water. Treatment chambers and macrophytes were assigned randomly and each treatment, including the control, was triplicated. Rainwater was used to compensate the water loss by evaporation.

Grab wastewater samples were collected simultaneously from every treatment unit and transferred immediately to the laboratory for physico-chemical analysis and heavy metal determination in seven days interval for 28 consecutive days (optimal growth period of the macrophytes). The total concentrations of heavy metals were analyzed using an Atomic Absorption Spectrometer (Varian AA240FS). In addition, Five-day Biochemical Oxygen Demand (BOD_5), Chemical Oxygen Demand (COD) and Kjeldahl nitrogen were determined using the 5-days BOD test at 20 °C, Open Reflux Method, and the macro-Kjeldahl method respectively. Concentrations of nitrate, nitrite, phosphate, and sulfate were determined using a multiparameter photometer (Palintest wagtech Photometer 7100 model).

Similarly, temperature, pH, electrical conductivity (EC) and total dissolved solids (TDS) were measured on site using calibrated portable multi-function meters (Wagtech CyberScan CON410). Furthermore, turbidity meter (Wag-WT-3020) and Hach (HQ40d model) field kits were used to measure the turbidity and dissolved oxygen respectively. Each equipment was calibrated before use following standard procedures. A reagent blank and duplicate samples were performed in parallel for each analysis. Besides, standard samples of known concentration were incorporated within each batch of samples analyzed for heavy metals. Digital dual incubator microbiological testing was carried out using twin incubators (Hach portable incubator model) for the simultaneous incubation of fecal and total coliforms. Incubation was run for 24 h in digitally controlled temperatures preset at 37 ± 0.5 °C for total coliforms and 44 ± 0.5 °C for fecal coliform counts. Sampling and sample handling were performed following APHA (1999) procedures. Laboratory analysis results were summarized as mean values and errors represented by the standard deviation. Removal efficiencies (R) in percent were calculated based on the equation used by Bokhari et al. (2016) as follows:

$$R = 1 - \frac{C_t}{C_i} \times 100$$

Where C_i represents the initial concentration (mgL^{-1}), and C_t denotes concentration (mgL^{-1}) at the time of interest. One-way analysis of variance (ANOVA) was employed to identify differences in mean concentrations of contaminants among *A. filiculoides* and *L. minor*