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Evaluation of Heavy Metal Removal Efficiency of *Azolla filiculoides* and *Lemna minor* in Tertiary Institution Referral Hospital Effluent at Hawassa City, Ethiopia

[*1]DENBERE, BZ; [2] ELIAS, D; [1] SOLOMON, S; [3]THOMAS, R

*[*1]College of Natural and Computational Sciences, Department of Biology, Hawassa University, Ethiopia*
[2]College of Natural and Computational Sciences, Department of Aquatic Science, Fisheries and Aquaculture, Hawassa University, Ethiopia
[1]College of Natural and Computational Sciences, Department of Biology, Hawassa University, Ethiopia,
[3]Faculty of Environmental Sciences and Natural Resource Management, Norwegian University of Life Sciences (NMBU), Ås, Norway

**Corresponding Author Email: denbereb97@gmail.com*
**ORCID: https://orcid.org/0009-0004-6031-7461*
**Tel: +251912094349*

Co-Authors Email: edadebo@yahoo.com; sorsasota@yahoo.com; thomas.Rohrlack@nmbu.no

ABSTRACT: Healthcare wastewater discharged without proper treatment is an emerging environmental issue. Existing wastewater treatment technologies for the remediation of heavy metals are costly, time consuming, and don‟t provide a satisfactory solution. Therefore, the objective of this paper was to investigate the heavy metal removal efficiency of *Azolla filiculoides* and *Lemna minor* for six heavy metals (Cr, Co, Ni, Zn, Cd, and Pb) from a tertiary institution referral hospital effluent at Hawassa City, Ethiopia, using appropriate standard procedures. The average heavy metal concentrations in both macrophyte treatments were as follows: Zn > Ni > Co > Cr > Pb > Cd. ANOVA showed significant differences ($p < 0.05$) between the two macrophytes in removing all evaluated factors. The maximum removal was revealed for Zn (98.35%) by *L. minor* compared to *A. filiculoides* (94.13%). The BCF values for *L. minor* and *A. filiculoides* were in the following order: Cd > Pb > Zn > Co > Ni > Cr and Cd > Pb > Zn > Cr > Co > Ni, respectively. Finally, both macrophytes were effective phytoremediation choices for the treatment of hospital effluent.

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Hospitals contribute significantly to the healthcare system by integrating diverse components and providing ongoing services to address complex human health concerns (Kumarai *et al.*, 2020). Hawassa University Referral Hospital is the only referral hospital in the Sidama region of Ethiopia, with more than 500 beds and serving a population of about 18 million per year (Ebrahim *et al.*, 2018). Hence, it uses a huge amount of water for sanitation and many other activities (Hunachew and Getachew, 2011). Previous studies reported that hospital wastewaters contain heavy metals, antibiotics, disinfectants, X-ray contrast agents, pharmaceuticals, substances with genotoxic and cytotoxic activity, and enteric pathogens (Hunachew and Getachew, 2011; Ortolan *et al.*, 2007; Galvin *et al.*, 2007). Heavy metals are toxic to humans and other organisms and may end up in surface water, where they may influence the aquatic ecosystem and interfere with the food chain (Pauwels and Verstraete, 2006). The demand for the conservation of water resources has increased interest in heavy metal removal techniques

**Corresponding Author Email: denbereb97@gmail.com*
**ORCID: https://orcid.org/0009-0004-6031-7461*
**Tel: +251912094349*

like reverse osmosis, ion exchange, electrodialysis, and adsorption (Matheickal and Yu, 1999). Likewise, waste stabilization ponds are frequently employed for wastewater treatment in many parts of Ethiopia (Adane and Tassisa, 2013). Unfortunately, each of these techniques has high energy costs and limited capabilities (Miretzky *et al.*, 2004)

In contrast, phytoremediation, the process of using plants for the uptake of heavy metals, nutrients, and organic pollutants, has been considered an encouraging technology (Ugya *et al*., 2015). Moreover, it is a cost-effective, low-impact, aesthetically pleasing, and environmentally sound remediation technology to treat metal-polluted sites (Parmer and Singh, 2015). Various aquatic macrophytes have been proposed as agents of choice for the phytoremediation of wastewater because of their features, including their very high growth rates, easy maintenance, and ability to remove various pollutants (IWSD, 1996). Likewise, the potential of free floating aquatic macrophytes for the removal of heavy metals has been studied in different works (Muthusaravanan *et al.,* 2018; Maine *et al.,* 2001). Many plant species, e.g., duckweeds (*Lemna sp*. and *Spirodella sp*.), water ferns (*Azolla sp*.), water hyacinths (*Eichhornia sp*.), and water lettuces (*Pistia sp*.), are known for heavy metal removal from aquatic media (Upadhyay *et al.,* 2007). Among them are the water fern (*A. filiculoides*) and duckweed (*L. minor*), which can bind organic substances and heavy metals from contaminated aquatic ecosystems (Khellaf and Zerdauoi, 2009; Salman *et al.*, 2012). Both macrophytes were reported as very successful plants for phytoremediation due to their low cost, ability to grow under different climatic conditions, and fast reproduction rates (Khellaf and Zerdauoi, 2009).

So far, there has been no significant work done on the phytoremediation of hospital effluent using *A. filiculoides* and *L. minor* for improving water quality and the proper management of the aquatic environment at Hawassa University Referral Hospital and other health care centers in Hawassa City. Therefore, the objective of this paper was to evaluate the heavy metal removal efficiencies of two aquatic macrophytes (*A. filiculoides* and *L. minor)* from tertiary institution referral hospital effluent at Hawassa City, Ethiopia.

MATERIALS AND METHODS

Description of the Study Area: Hawassa University Referral Hospital (HURH) is located in the south western part of Hawassa City (the capital city of Sidama Region), 275 km south of Addis Ababa (the capital city of Ethiopia). Geographically, the city is located at 7^{o} 03`19” N latitude and 38$^{o}$28`23” E longitude. It is situated at 1708 meters above sea level. The annual mean precipitation was 900 mm, and the annual mean temperature varies from 20.1 to 25^{0}C (CAA, 2004). HURH is situated on the eastern shore of Lake Hawassa (a freshwater lake) in Hawassa City. According to Hunachew and Getachew (2011), the wastewater generated in the HURH has reached 143,285 liters per day, and it enters the waste stabilization pond for treatment. However, the present waste stabilization pond is unsuitable for providing the necessary degree of pollution treatment, and there is concern that the lake would be significantly harmed (Hunachew and Getachew, 2011; Sewhunegn, 2012). Finally, the hospital effluent discharge is then released into the vicinity of Lake Hawassa, which is an ecologically sensitive area. Lake Hawassa is the only dominant surface water body in the city (Hunachew and Getachew, 2011).

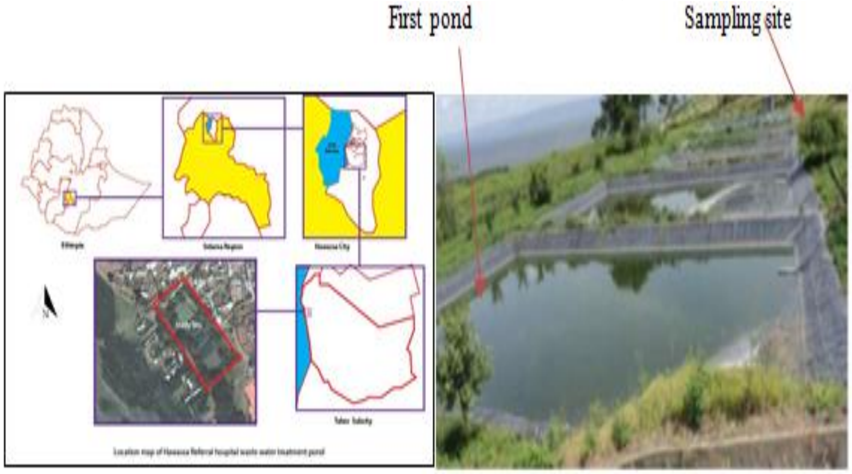

Fig.1. Location of Hawassa University Referral Hospital waste treatment pond (the right side photo taken by camera)

Effluent and plant sample collection: One hundred liters of grab effluent sample were collected from the outlet of HURH effluent and stored in five (20-liter Jerry cans) previously cleaned by washing in non-ionic detergent followed by distilled water, and transported to the laboratory. Two species of aquatic macrophytes (*A. filiculoides* and *L. minor*) were used as experimental plants in this study. These two plant species were collected from the shoreline of Lake Hawassa and thoroughly cleaned with tap water to eliminate insect larvae and epiphytes. Then the two plant species were separately placed into plastic containers filled with tap water, and Hoagland nutrient solution (1%) was added for one week to allow them to grow and acclimate to the existing environment in the laboratory.

Experimental Setup: In this study, glass aquaria with 70 cm (length), 40 cm (width), and 30 cm (depth) were used for the experiment. The treatment system contains three sets of experimental containers: having nine glass aquariums, three for *A. filiculoides,* three for *L. minor*, and three unplanted, as controls, were arranged in the laboratory. Each experimental aquarium was filled with 10 liters of hospital effluent and 100 gm of healthy, fresh weight *A. filiculoides* and *L. minor* plants. The effluent level in the aquariums was monitored throughout the experiment, and tap water was utilized to make up for water loss due to evaporation. The experimental duration was 14 days and was conducted from November 26 to December 9, 2018, in the Biology Department Laboratory, Hawassa University, Ethiopia.

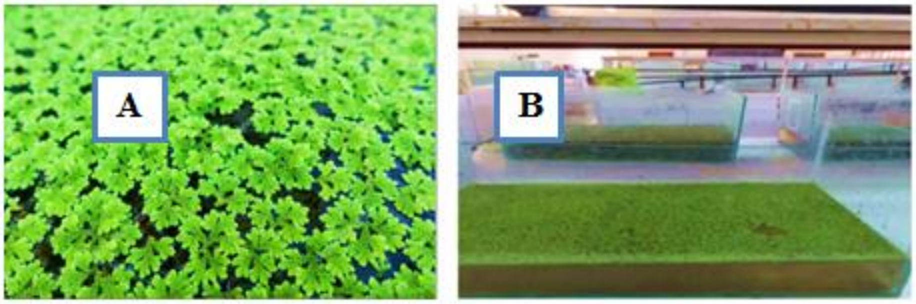

Fig.2. Photo taken from the phytoremediation experiment of *A. filiculoides* (A), *L. minor* (B) in the biology laboratory

Samples collection for analysis: One hundred grams fresh weight of *A. filiculoides* and *L. minor* samples were collected from each treatment glass aquarium on 0, 7, and 14 days (a total of nine samples from each species) and washed with tap water followed by distilled water, then air dried for a day. The dried plant samples were separately packed in clean plastic bags and labeled. Similarly, 50 ml of effluent samples (a total of nine samples) were collected from each plant species treatment system and the control on 0, 7, and 14 days to estimate the composition of heavy metals. All the effluent samples were filtered through Whatman No. 42 filter paper and preserved with 0.3 ml of 2% nitric acid. Finally, all the plant and effluent samples were kept in the refrigerator at 4^0C for two days and packed in an icebox filled with ice bags to be transported to the Norwegian University of Life Science (NMBU) soil and water laboratory in Norway for heavy metal analysis.

Analysis of heavy metals in plants and effluent samples: At NMBU, A. filiculoides and *L. minor* plant samples were first washed with ultrapure water and then freeze-dried. The plants were decomposed by the Ultraclave Milestone microwave digestion system (260 ^{0}C and pre-pressurized up to 50 bar) for 1 hour. Two mL of deionized water and 5 mL of ultrapure HNO3 were added to 0.25g of plant samples in Teflon tubes and diluted up to 50 mL with deionized water. After cooling, each sample was analyzed using ICP-MS (Agilent 8800 QQQ), according to the National Institute of Standards and Technology (NIST), Standard Reference Material 1575a. Similarly, the effluent samples were also analyzed using similar equipment, ICP-MS (Agilent 8800 QQQ). All dilution factors were according to NIST Standard Reference Material 1643e. trace elements in water. Heavy metals such as Cr, Co, Ni, Zn, Cd, and Pb were analyzed in the soil and water laboratory at the Norwegian University of Life Science (NMBU) in Norway.

Physicochemical parameters measurement and analysis: Temperature, pH, electrical conductivity (EC), total suspended solids (TSS), total dissolved solids (TDS), chemical oxygen demand (COD), and biological oxygen demand (BOD) in the hospital effluent were measured on 0, 7, and 14 days, according to the standard methods for the examination of water and wastewater (APHA, 2017). EC, TDS, temperature, and pH were measured in situ using portable Hanna (HI991300) waterproof pH, TDS, EC, and temperature meters. Before the measurement, the portable field instrument was calibrated using buffers of pH (at pH 4.0, 7.0, and 10.0) and potassium chloride solution for pH and EC, respectively. The gravimetric method was used to determine TSS. BOD_5 and COD were determined using the 5-day BOD test at 20 °C and the Open Reflux Method, respectively. The BOD and COD analyses were conducted at Hawassa University, Department of Biology Laboratory, Ethiopia.

Method for Determination of Heavy Metal Removal Efficiency of the Macrophytes: The removal of heavy metals was determined by quantifying the concentration of metals left in the medium after incubation with plants. The removal efficiencies R (%) were computed using equation 1 as published by Bokhari *et al.* (2016), as follows:

$$R(\%) = \left(C0 - \frac{Ct}{C0}\right) * 100 \quad (1)$$

Where, **R** = Removal efficiency of plants; **C0 =** Residual concentration of metal at the beginning of the experiment; **Ct =** Residual concentration of metal at time t.

Method for Determination of Bio concentration Factor (BCF): The bioconcentration factor was calculated by formula 2 below, as described by Zayed *et al.*, (1998):

$$BCF = \left(\frac{Cpf}{Cis}\right) \quad (2)$$

Where; **Cpf**= Heavy metal accumulated concentration in plant tissue after treatment, mg/kg; **Cis** = Initial concentration of metal in external solution ($mg \cdot L^{-1}$);

Data Analysis: The data was entered and analyzed using SPSS software version 20.0 and an Excel spreadsheet. Values were expressed as mean ± SD. A one-way analysis of variance (ANOVA) was used to determine differences in mean contaminant concentrations between *A. filiculoides* and *L. minor* treatments and controls. Tukey's post hoc test was carried out to perform pairwise comparisons of the mean concentrations. The differences were statistically significant when $P<0.05$.

RESULTS AND DISCUSSION

Characteristics of physicochemical parameters in the effluent: The values of seven physicochemical parameters: pH, temperature, electrical conductivity (EC), total suspended solids (TSS), total dissolved solids (TDS), chemical oxygen demand (COD), and biochemical oxygen demand (BOD), in the hospital effluent were measured on days 0, 7, and 14 of treatment, as presented in Table 1. Among these parameters, two, TSS (65.73 mg/L) and TDS (421.51 mg/L), exceeded the discharge limits set by the Ethiopian Environmental Protection Authority (EEPA, 2003) in the initial effluent samples. Following treatment with *Azolla filiculoides* and *Lemna minor*, all parameter values decreased over time, with the most significant reductions observed on day 14 (Table 1). These findings highlight the potential of these aquatic plants to effectively absorb and reduce organic pollutants from hospital effluents.

In this study, the findings indicated that the initial average pH value of the hospital effluent was 7.56 ± 0.01 across both macrophyte treatment systems. Over the treatment period, the pH exhibited a declining trend (Table 1). Notably, a significant reduction in pH was observed after 14 days of treatment (Table 1). Consequently, the pH values in both macrophyte treatment systems were consistently lower than those recorded in the control group (Table 1). Statistical analysis using one-way ANOVA, followed by Tukey's post hoc test, confirmed that the pH values in the two macrophyte treatment systems were significantly different ($p < 0.05$) when compared to the control.

The findings of the present study regarding pH levels in hospital effluent are consistent with earlier research, highlighting the potential of *Azolla filiculoides* and *L. minor* in the phytoremediation of wastewater treatment. The pH value observed in this study aligns closely with the report by Simachew *et al.* (2019), who documented a pH of 7.3 in HURH wastewater. Similarly, Daud *et al.* (2018) demonstrated a comparable reduction in pH, from 7.9 to 6.8, using *L. minor* plants for wastewater treatment. This supports the idea that phytoremediation can contribute to stabilizing pH levels in wastewater systems. In agreement with Laffoley *et al.* (2017), the reduction of pH in both plant treatment systems might be due to the breakdown of organic matter through bacterial activities; as they release CO_2, it reacts with water and forms H_2CO_3. Furthermore, Mousa (1994) indicated that *Azolla filiculoides* plants can thrive in wastewater with a pH range of 7.1 to 9.0, reinforcing the adaptability of aquatic plants to varying pH conditions in polluted environments. In the present study, the results confirm the ability of the test plants to effectively reduce pH levels in hospital effluent, indicating their capacity to mitigate wastewater acidity or alkalinity through natural phytoremediation processes. These findings emphasize the utility of specific aquatic plants in improving wastewater quality while maintaining environmental pH balance.

Results of the current study revealed that the effluent temperature in *A. filiculoides* and *L.minor* treatment systems during the experimental period ranged from 19.14 ^{0}C to 22.79 ^{0}C (Table 1). According to Culley *et al.* (1981) and Singh (1977), this range is within the tolerable limit for both macrophytes. However, a significant decline in effluent temperature in both macrophyte treatments was revealed after 14 days (Table 1). As a result, the value of temperature in both macrophyte treatment systems and its value in control were significantly different ($p < 0.01$) during the experimental period. The results were in agreement with the value of temperature reported by Simachew (2021), who presented a mean value (23^{0}C) of effluent temperature from HURH wastewater. Nayef *et al.* (2012) also presented a reduction in water temperature from 29°C to 24°C in the *L. minor* wastewater treatment system. Likewise, Singh (1977) reported that the most favorable temperature for the growth of *Azolla spp.* is between 20°C and 30°C, indicating a good growth condition for these species in the current study. As presented by Kara *et al.* (2007), a reduction in the effluent temperature might be due to the floating nature of these macrophytes, which covered the surface of the water and prevented light penetration, reducing the water temperature.

The initial concentration of electrical conductivity (EC) in the hospital effluent was lower than the values reported by Hunachew and Getachew (2011) for HURH wastewater (1098 µS/cm) and Bokhari *et al.* (2016) for municipal effluent treated with *L. minor* (1133 µS/cm) (Table 1). However, a declining trend in EC concentration was observed in both macrophyte treatment systems throughout the treatment period, with the most significant reduction recorded on the 14th day of the experiment: 234.82 ± 0.32 µS/cm in the *A. filiculoides* treatment system and 214.89 ± 0.52 µS/cm in the *L. minor* treatment system (Table 1). Furthermore, the EC values in both treatments after 14 days were statistically different from those in the control throughout the study period ($P \leq 0.05$). As noted by Murphy (2007), the reduction in EC values observed in both treatment systems may be attributed to decreased ion mobility, likely resulting from the removal of dissolved solids such as chloride, nitrates, phosphates, sodium, magnesium, and calcium by the aquatic plants from the hospital effluent.

The initial concentration of total suspended solids (TSS) in this study was consistent with the value reported by Hunachew and Getachew (2011) for HURH effluent (60.5 mg/L) (Table 1). However, it was lower than the TSS concentration (535 mg/L) reported by Simachew (2021) for HURH effluent. Throughout the experimental period, a declining trend in TSS concentration was observed in both treatment systems, with a significant reduction recorded after 14 days (12.27 ± 0.09 mg/L in the *A. filiculoides* treatment system and 9.46 ± 0.12 mg/L in the *L. minor* treatment system) (Table 1). Consequently, the mean TSS values in both macrophyte treatment systems were significantly different from those in the control ($p < 0.05$). In agreement with Iqbal's (1999) suggestion, the reason for the reduction of TSS in the effluent treated by these macrophytes might be due to the biodegradation and sedimentation of organic particles in both plant treatment systems. The organic particles are aerobically biodegraded by microorganisms, with a portion of the degraded products being absorbed by plants (Iqbal, 1999).

The initial average concentrations of TDS, COD, and BOD in this study were lower than those reported by Amare *et al.* (2018), which documented concentrations of 2,500, 7,133, and 5,550 mg/L for TDS, COD, and BOD, respectively, and 2,500, 10,940, and 7,250 mg/L in *A. filiculoides* and *L. minor* treatment systems, respectively.

Table.1. Physicochemical parameters measurements during the experiment period

		Physicochemical parameters (mean ± SD) (n = 3)						
Treatment	Time (day)	pH	Temp.(^{0}C)	EC (µS/cm)	TSS (mg/L)	TDS (mg/L)	COD (mg/L)	BOD (mg/L)
Azolla filiculoides	0	7.56 ± 0.01	22.79 ± 0.02	898.86 ± 2.14	65.73 ± 1.36	421.51 ± 0.87	197.11 ± 1.1	47.65 ± 0.27
	7	7.04 ± 0.01	21.38 ± 0.03	453.32 ± 1.78	33.00 ± 0.31	245.24 ± 7.18	94.34 ± 0.16	34.39 ± 0.61
	14	6.53 ± 0.01	20.55 ± 0.15	234.82 ± 0.32	12.27 ± 0.09	94.97 ± 0.45	44.91 ± 0.56	17.23 ± 0.63
Lemna minor	0	7.56 ± 0.01	22.79 ± 0.02	898.86 ± 2.14	65.73 ± 1.36	421.51 ± 0.87	197.11 ± 1.10	47.65 ± 0.27
	7	7.02 ± 0.00	20.13 ± 0.01	423.06 ±0.70	23.21 ± 0.06	215.26 ± 0.43	84.07 ± 0.28	24.48 ± 0.18
	14	6.48 ± 0.04	19.14 ± 0.00	214.89 ± 0.52	9.46 ± 0.12	75.55 ± 1.58	35.79 ± 0.36	12.97 ± 0.41
Control	0	7.56 ± 0.01	22.79 ± 0.02	898.86 ±2.14	65.73 ± 1.36	421.51 ± 0.87	197.11 ± 1.10	47.65 ± 0.27
	7	7.46 ± 0.01	21.57 ± 0.03	845.34 ± 8.12	54.70 ± 1.23	384.89 ± 0.65	173.67 ± 3.02	34.44 ± 0.66
	14	7.27 ±0.06	21.21 ± 0.16	780.43 ± 8.39	41.58 ± 1.15	312.78 ± 0.51	135.01 ±1.97	28.73 ± 0.01
EEPA, 2003		6-9	40	1000	50	80	250	50

Note; Day 0 = before treatment, Day 7=mid time of the treatment, 14 = after treatment EEPA= Ethiopian Environmental Protection Authority discharge limit

However, throughout the treatment period, both macrophyte treatment systems exhibited a decreasing trend in TDS, COD, and BOD concentrations, with the most significant reduction observed after 14 days of treatment (Table 1). ANOVA analysis confirmed that the differences in TDS, COD, and BOD values between the macrophyte treatment systems and the control were statistically significant ($p < 0.05$). In agreement with Anil *et al*. (2013), the reduction in TDS, COD, and BOD was attributed to the capacity of the plants to absorb inorganic and organic ions.

Percentage Reduction Efficiency of Plants: The percentage reduction efficiencies of the two species of macrophytes for physicochemical parameters and the reduction in the control are presented in Table 2. In the present study, the level of pH in both macrophyte treatment systems exhibited a decreasing pattern with time (Table 2). Nevertheless, a significant percentage reduction in the value of pH was observed after 14 days of treatment in both the *L. minor* (14.29%*)* and *A. filiculoides* (13.62%) treatment systems (Table 2). Simultaneously, the pH value of the control was reduced by 3.84% (Table 2). Accordingly, the percentage reduction of pH in both treatments and the control was statistically different ($p < 0.05$). The findings of the present study indicate that the percentage reduction in pH was greater than that reported by Nayyef *et al.* (2012), who documented a 13.4% reduction in pH from wastewater treated with *L. minor*.

In the present study, the *L. minor* treatment system demonstrated a significantly higher reduction in effluent temperature (16.02%) compared to the *Azolla filiculoides* treatment system, which achieved a reduction of 9.83% (Table 2). In contrast, the control group exhibited a temperature reduction of only 6.94% over the same treatment period. The differences in temperature reduction among the *L. minor* treatment, *A. filiculoides* treatment, and the control were statistically significant ($p < 0.05$). These findings are consistent with the study by Nayyef *et al.* (2012), which reported a 17.2% reduction in wastewater temperature using *L. minor* plants. This comparison further highlights the efficacy of *L. minor* in reducing effluent temperature through phytoremediation processes.

The findings of the current study demonstrate a significant percentage reduction in the electrical conductivity (EC) levels across both macrophyte treatment systems (Table 2). After 14 days of treatment, the *L. minor* system exhibited a notably higher reduction in EC (76.09%) compared to the *A. filiculoides* system (73.88%) (Table 2). In contrast, the control group showed a reduction of only 13.18% (Table 2). Statistical analysis revealed that the reductions in EC between the treatment systems and the control were significantly different ($p < 0.05$). Furthermore, the EC reduction observed in this study surpassed that reported by Amare *et al.* (2018), who documented EC reductions of 69% and 68% for *A. filiculoides* and *L. minor*, respectively, in Mekele, Ethiopia. Similarly, the findings exceeded those of Rezooqi *et al.* (2021), who reported a 49% EC removal in wastewater treated with *A. filiculoides* in Iraq.

As presented in Table 2, the total suspended solids (TSS) removal efficiency of both macrophytes increased with the duration of the treatment period. After 14 days of treatment, *L. minor* demonstrated a significantly higher TSS removal efficiency (85.61%) compared to the *A. filiculoides* treatment system (81.33%). In contrast, the control system achieved a TSS removal efficiency of only 36.74% during the same period. Statistical analysis confirmed that the differences in TSS removal efficiency among the treatments and the control were significant ($p < 0.05$). Furthermore, the TSS removal efficiencies observed in this study exceeded those reported in some previous research. For instance, Al-Sabunji and Al-Marashi (2002) documented a 45% reduction of TSS using *L. minor* in wastewater treatment. Similarly, Huang *et al.* (2011) reported a 52% reduction of TSS in Taihu Lake, which is covered by floating aquatic plants. Nayyef *et al.* (2012) also noted a 38% TSS reduction in oil refinery wastewater treated with *L. minor*. These findings highlight the higher performance of *L. minor* and *A. filiculoides* in the current study.

The findings of this study demonstrated that *L. minor* achieved a significantly higher percentage removal of total dissolved solids (TDS), chemical oxygen demand (COD), and biochemical oxygen demand (BOD) compared to *A. filiculoides* after 14 days of treatment (Table 2). In contrast, the percentage decrease observed in the control group was notably lower than in the treatment groups, underscoring the critical role of the aquatic macrophytes *A. filiculoides* and *L. minor* in the removal of organic pollutants (Table 2). Furthermore, statistical analysis revealed that the percent removal of TDS, COD, and BOD differed significantly between the treatments and the control ($p < 0.05$). The removal efficiencies achieved by both macrophytes for TDS, COD, and BOD exceeded those reported in previous studies. For instance, Amare *et al.* (2018) documented TDS removal rates of 70% and 68% for *A. filiculoides* and *L. minor* systems, respectively. Similarly, the COD removal efficiencies observed in this study align with the findings of Körner *et al.* (2003), who reported COD removal rates ranging from 50% to 95% in *L. minor* treatment systems.

Table.2. Percentage reduction (R %) in physicochemical parameters by *A. filiculoides* and *L. minor* plant treatment systems and control

	Day	PH	Temp	EC	TSS	TDS	COD	BOD
A.fliculoides	7	6.88	6.16	49.51	49.79	41.82	52.14	27.83
	14	13.62	9.83	73.88	81.33	77.47	77.22	63.84
L.minor	7	7.14	11.67	52.93	64.69	48.93	57.35	48.63
	14	14.29	16.02	76.09	85.61	82.08	81.84	72.78
Control	7	2.65	5.35	5.95	16.78	8.69	11.89	27.72
	14	3.84	6.94	13.18	36.74	25.79	31.51	39.71

Heavy metals mean concentration in the hospital effluent: The mean concentrations of heavy metals in HURH effluent treated by *A. filiculoides* and *L. minor* plants at different times (days) of exposure are shown in Table 3. Analysis of variance showed significant ($p < 0.05$) variations between the initial and final concentrations of all heavy metals examined in both macrophyte treatment systems (Table 3). The mean concentrations of heavy metals in the hospital effluent treated by the two species of aquatic macrophytes were in the following order: Zn > Ni > Co > Cr > Pb > Cd (Table 3). One-way ANOVA showed significant differences ($p < 0.05$) in heavy metal concentration between the two macrophyte treatment systems after 14 days of the experimental period. In contrast, no significant difference was observed in the control system except for Cd during the same treatment period (Table 3).

In the current study, the maximum initial heavy metal concentration in the hospital effluent was revealed for Zn in both macrophyte treatment systems (Table 3). Results revealed that the initial concentration of Zn in the present study was lower than reported by Simachew (2021) for Zn (134 µg/L); nevertheless, it was higher than reported by Hunachew and Getachew (2011) for Zn (5.62 µg/L) from HURH effluent. However, after 14 days of treatment, its concentration in *A. filiculoides* and *L. minor* treatment systems considerably reduced to 1.40 ± 0.1 and 0.38 ± 0.1 µg/L, respectively (Table 3). ANOVA showed significant differences ($p < 0.02$) between the two macrophyte treatment systems. In contrast, the concentration of Zn in the control didn't show a significant reduction during the same experimental period (Table 3). The results of the current investigation demonstrated that the reduction in the Zn concentrations after 14 days of treatment was lower than the values reported by Amare *et al.* (2018), who reported a (60 and 76) µg/L reduction in Zn concentration from the wastewater treated by *L. minor* and *A. filiculoides* plants, respectively.

The concentrations of chromium (Cr) and cobalt (Co) in the hospital effluent exhibited a significant decreasing trend in both macrophyte treatment systems throughout the experimental period (Table 3). However, the *L. minor* treatment system demonstrated a comparatively greater reduction in Cr and Co concentrations after 14 days of treatment (Table 3). ANOVA analysis indicated a statistically significant difference ($p < 0.05$) between the two plant treatment systems after 14 days, whereas no significant difference was observed in the control group over the same period. The findings of this study further revealed that the Cr and Co concentrations in both treatments were lower than those reported by Amare *et al.* (2018), who documented Cr at 30 µg/L and Co at 30 µg/L in *A. filiculoides*, as well as Cr at 21 µg/L and Co at 33 µg/L in *L. minor* treatments (Table 3).

The concentrations of Ni and Pb exhibited significant variations between the two treatments after 14 days of exposure. ANOVA analysis confirmed significant differences ($p < 0.05$) in Ni and Pb concentrations between the two macrophyte treatments. The removal efficiency of Ni and Pb observed in this study was lower than that reported by Bokhari *et al.* (2016), who achieved a reduction in Pb from 419 µg/L to 46 µg/L and in Ni from 51 µg/L to 8 µg/L using *L. minor* to treat municipal effluent. In contrast, Cd concentrations in both plant treatments remained below the detection limit on the 14th day of the experimental period (Table 3). In general, results in the present study revealed that both species of macrophytes proved to be very effective in the removal of all examined heavy metals from the hospital effluent.

Heavy metal removal efficiencies of A. filiculoides and L. minor: The highest heavy metal removal efficiencies for both macrophytes across all six heavy metals (Cr, Co, Ni, Zn, Cd, and Pb) were achieved after 14 days of treatment (Table 4). In this study, *L. minor* demonstrated the highest removal efficiency for Zn, achieving 98.35%, compared to *A. filiculoides*, which achieved 94.13% after 14 days (Table 4). Conversely, Zn reduction in the control group after 14 days was lower, at only 28.24%. The

Zn removal efficiencies observed in the present study exceed those reported by Amare *et al.* (2018), who documented 90% Zn removal by *L. minor* in wastewater, and Nayyef *et al.* (2012), who reported a 72% reduction of Zn using *L. minor* in wastewater treatment.

Table 3. Heavy metal mean concentration in the hospital effluent during the treatment (µg/L)

		Heavy metal mean concentration ± SD					
Treatments	Time (Day)	Cr (µg/L)	Co (µg/L)	Ni (µg/L)	Zn (µg/L)	Cd (µg/L)	Pb (µg/L)
A.filiculoides	0	2.20 ± 0.3	2.40 ± 0.1	5.37 ± 0.0	24.56 ± 3.5	0.02 ± 0.0	1.43 ± 0.1
	7	1.62 ± 0.2	1.86 ± 0.2	4.60 ±0.6	10.88 ± 6.9	0.01 ± 0.0	0.58 ± 0.5
	14	0.39 ± 0.1	0.51 ± 0.0	1.69 ± 0.3	1.40 ± 0.1	0.00 ± 0.0	0.03 ± 0.0
L.minor	0	2.20 ± 0.1	2.40 ± 0.1	5.37 ± 0.1	24.56 ± 4.1	0.02 ± 0.0	1.43 ± 0.1
	7	1.23 ± 0.1	1.62 ± 0.1	3.59 ± 0.2	4.73 ± 0.3	0.01 ± 0.0	0.25 ± 0.0
	14	0.23 ± 0.1	0.31 ± 0.0	1.59 ± 0.3	0.38 ± 0.1	0.00 ± 0.0	0.04 ± 0.0
Control	0	2.20 ± 0.1	2.40 ± 0.1	5.37 ± 0.2	24.56 ± 3.3	0.02 ± 0.0	1.43 ± 0.1
	7	1.91 ± 0.0	1.93 ± 0.0	4.89 ± 0.2	22.11 ± 3.2	0.01 ± 0.0	1.34 ± 0.1
	14	1.57 ± 0.1	1.70 ± 0.1	4.40 ± 0.3	18.89 ± 1.7	0.01 ± 0.0	1.26 ± 0.6
WHO(2006)		20	50	200	2000	10	200

Note; WHO (2006), World Health Organization, standards for wastewater discharge limit

The percentage removal of chromium (Cr) observed in this study exceeded the rates reported by Amare *et al.* (2018), who documented Cr removal efficiencies of 26% and 19% using *L. minor* and *A. filiculoides*, respectively (Table 4). However, the results were lower than those reported by Maha (2012) and Sekomo *et al.* (2012), who achieved Cr removal efficiencies of 94.8% and 94%, respectively, using *L. minor* in wastewater treatment. Consistent with the findings of Greger (1999), the reduction in Cr concentration in the effluent in this study may be attributed to the presence of nutrients that support plant growth, leading to an increased number of metal absorption sites and enhanced metal ion uptake. Additionally, after 14 days of treatment, cadmium (Cd) concentrations in both plant treatment systems were found to be below the detection limit (BDL).The heavy metal removal efficiencies of *L. minor* and *A. filiculoides* followed the order: Zn > Pb > Cr > Co > Ni > Cd and Pb > Zn > Cr > Co > Ni > Cd, respectively (Table 4). These findings indicate that *L. minor* and *A. filiculoides* exhibit strong potential for heavy metal removal, making them promising candidates for the phytoremediation of hospital effluent.

Table 4. Heavy metal percentage removal (R%) in the treatments and control system

Treatments	Time(day)	Cr	Co	Ni	Zn	Cd	Pb
A. filiculoides	7	27.94	32.34	23.43	73.20	50.00	80.44
	14	81.56	78.69	68.58	94.13	BDL	97.89
L. minor	7	35.86	31.08	32.05	78.24	50.00	81.28
	14	87.90	86.76	70.01	98.35	BDL	97.66
Control	7	9.53	18.06	9.31	14.54	50.00	5.24
	14	25.03	27.77	19.54	28.24	50.00	10.36

BDL= below detection limit

Table.5. Bio-concentration factor (BCF) by *A. filiculoides* and *L. minor* macrophytes for heavy metals from the effluent during the treatment period

	Time(day)	**Cr**	**Co**	**Ni**	**Zn**	**Cd**	**Pb**
A.filiculoides	7	1601	1161	640	1709	3988	1760
	14	2020	1643	1022	2720	4890	3049
L. minor	7	1168	3224	1019	4391	10323	5606
	14	1,823	5,188	2,036	8118	22304	10027

Bioconcentration Factor (BCF): The bioconcentration factor (BCF) results indicated that in *A. filiculoides* treatments, BCF values for heavy metals ranged from 640 to 3,988 and 1,022 to 4,890 after 7 and 14 days of treatment, respectively (Table 5). In contrast, the *L. minor* treatment systems exhibited BCF values ranging from 1,019 to 10,323 after 7 days and from 1,823 to 22,304 after 14 days of treatment (Table 5). Notably, *L. minor* achieved the highest BCF value for cadmium (22,304) after 14 days of treatment (Table 5). This indicates that *L. minor* demonstrated superior performance in accumulating all tested heavy metals, with the highest accumulation observed for cadmium from the hospital effluent (Table 5). According to Zayed *et al.* (1998), an effective heavy metal accumulator plant should have a BCF value exceeding 1,000. In this study, after 14 days of treatment, the BCF values for the tested heavy metals followed the order: Cd > Pb > Zn > Co > Ni > Cr for *L. minor* and Cd > Pb > Zn >

Cr > Co > Ni for *A. filiculoides* (Table 5). Overall, both macrophytes demonstrated high efficiency in removing and accumulating all tested heavy metals in glass aquarium treatments. Therefore, the findings of this study suggest that *L. minor* and *A. filiculoides* are promising candidates for the phytoremediation of heavy metals in hospital effluent.

Conclusions: The findings of this study demonstrate that *A. filiculoides* and *L. minor* plants are effective in treating hospital effluent when compared to the control. Notably, *L. minor* exhibited superior efficiency in removing heavy metals and organic contaminants. These results provide valuable insights into the potential application of these plants as viable treatments for metal contaminated hospital effluents within the country. Additionally, this study establishes foundational data that can serve as a reference for future phytoremediation research in countries with tertiary referral hospitals worldwide.

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