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Phytoremediation Potentials of *Azolla filiculoides* L. and *Lemna minor* L. for Heavy Metals from Soft Drink Factory Effluent: The Case of Hawassa Millennium Pepsi Cola Factory, Hawassa, Ethiopia

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ABSTRACT: Heavy metal pollution is increasing in the aquatic resources of Hawassa City, Ethiopia, with the expansion of industrial activities. Existing wastewater treatment technologies for the remediation of heavy metals are costly and don't provide a satisfactory solution. Therefore, the aim of this study was to investigate the phytoremediation potential of *Azolla filiculoides* and *Lemna minor* for six heavy metals (Cr, Co, Ni, Zn, Cd, and Pb) when exposed to the soft drink factory effluent. The experiment was conducted for 14 days. The levels of pH, temperature, EC, TSS, TDS, COD, and BOD were examined on 0, 7, and 14 days. Heavy metal concentrations in the effluent and plants were analyzed using inductively coupled plasma mass spectrometry (ICP-MS). The percentage of heavy metal removal and bioconcentration factor (BCF) were calculated using the standard formula. The average concentrations of heavy metals in both macrophyte treatments were in the following order: Zn > Pb > Cr >Ni >Co > Cd. ANOVA revealed significant differences ($p< 0.05$) between the two macrophytes in the removal of all tested parameters. The maximum removal was revealed for Cr (97.52%) by *L. minor* compared to *A. filiculoides* (90.04%). The BCF values for *L. minor* and *A. filiculoides* were in the following order: Zn > Co > Ni > Cd > Pb > Cr and Co > Zn > Cd >Ni > Cr > Pb, respectively. In conclusion, both macrophytes were good phytoremediation candidates for the treatment of soft drink factory effluent. Further investigation is needed to enhance the performance of the treatment systems.

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Ethiopia's rapid growth and industrialization have adverse effects on water quality and aquatic life diversity (Zinabu and Zerihun, 2002). Hawassa City is one of the fastest-growing cities in Ethiopia in terms of population and industrial development (ETIDI, 2014). There are many industries, such as Hawassa Textile, Tabor Ceramics, ETAB soap, BGI brewery, Hawassa Millennium Soft Drink, etc. (Zinabu and Zerihun, 2002). Some of them release their untreated or partially treated effluents into Boga and Boietcha streams that enter the Tikur Wuha River, which is the only visible inflow into Lake Hawassa (Girma *et al.*, 2014). Earlier studies found that the soft drink factory wastewater originates from different individual processes such as employees' sanitary waste, bottle washing, product filling, heating or cooling, soft drink manufacturing, and sanitizing floors, including piping networks (Agana *et al.*,2013; Haroon *et al.*, 2013). Previous studies reported that wastewater generated by the soft drink factory from bottle washing constitutes almost 50% of the total wastewater (Abdelfatah *et al.*, 2017; Abrha and Chen, 2017). Likewise, the effluent

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from similar activities in the Hawassa Millennium Soft Drink Factory (HMSDF) is released into the nearby Boga and Boietcha streams that flow into the Tikur Whuha River, which enters Lake Hawassa (Behailu *et al*., 2015; Girma *et al.,* 2014). Behailu *et al*., (2010) reported that the composition of HMSDF effluent consisted of heavy metals, organic materials, and nutrients. Moreover, Zinabu (2003) presented that Lake Hawassa and the Tikur Wuha River are exposed to toxic substances from point and non-point sources of pollution, and HMSDF is recognized as a point source polluter. Furthermore, Adepoju-Bello *et al.,* (2012) presented toxic metals such as Pb, Cd, Ni, Cr, and Zn found in commonly consumed soft drinks in Nigeria. Heavy metal concentrations are potentially hazardous, mainly because they cannot be degraded biologically and are simply changed from one oxidation state to another (Gisbert *et al*., 2003). As a result, a large portion of these harmful metals become hazardous to animals and human health (Lone *et al.,* 2008). Continuous efforts have been made to develop physicochemical technologies (i.e., reverse osmosis, ion exchange, electrochemical treatment technologies, adsorption, etc.) capable of removing heavy metals from polluted environments (Memon and Schroder, 2009). Nevertheless, these treatment technologies are used to remove heavy metals and organic pollutants from wastewater, they are costly, time-consuming, environmentally destructive, and mostly inefficient (Memon and Schroder, 2009). As a result, an ecologically friendly and cost-effective treatment technique is required for the remediation of heavy metal-polluted wastewater (Sivakumar *et al.,* 2013). Phytoremediation is a plant-based approach that involves the use of plants to extract and remove pollutants or lower their bioavailability in water and soil (Berti and Cunningham, 2000). Therefore, the primary objective of the present study was to investigate the phytoremediation potential of *A. filiculoides* and *L. minor* for heavy metals such as Cr, Co, Ni, Zn, Cd, and Pb when exposed to the soft drink factory effluent. These plants have been selected as agents of choice for the phytoremediation of the effluent because of their small size, high growth rates, short life spans, and easy maintenance (Körner and Vermaat, 1998). So far, no significant research has been conducted at the Hawassa Millenium Pepsi Cola Factory on the phytoremediation of the effluent using *A. filiculoides* and *L. minor* plants for improving water quality as well as aquatic environment management. Therefore, the suitability and efficacy of *A. filiculoides* and *L. minor* for removing some heavy metals and other physicochemical parameters from the soft drink factory effluent were investigated in this research for 14 days under indoor environmental conditions.

MATERIALS AND METHODS

Description of the Study Area: Hawassa Millennium Pepsi Cola Factory (HMPCF) is found in the industrial zone, on the eastern edge of Hawassa city (the capital of Sidama Reginal State), 275 km south of Addis Ababa (the capital city of Ethiopia). Geographically, it is located at 7^{o} 03`19" N latitude and $38^{o}$28`23" E longitude. HMPCF was established in 2007 and is situated at 1708 meters above sea level. The annual precipitation varies from 800 mm to 1000 mm with a mean precipitation of 900 mm, and the annual mean temperature varies from 20.1 to 25^{0}C (CAA, 2004). According to Bereket (2020), HMPCF is producing 36,000 bottles (300 ml per bottle) per hour of soft drinks like Pepsi, Mirinda Orange, Mirinda Apple, etc. Bottle washing is performed by washing machines that wash 48,000 bottles per hour (Bereket, 2020). The wastewater from bottle washing is almost 50% of the total wastewater generated, which is about 1.25 liters of wastewater per 1 liter of soft drink produced. All effluents from HMPCF discharge into the nearby Boga and Boietcha streams that enter the nearby Tikur Wuha River, which finally enters Lake Hawassa (Girma *et al.,* 2014; Behailu *et al*., 2015). Lake Hawassa is one of the dominant surface water bodies in the city.

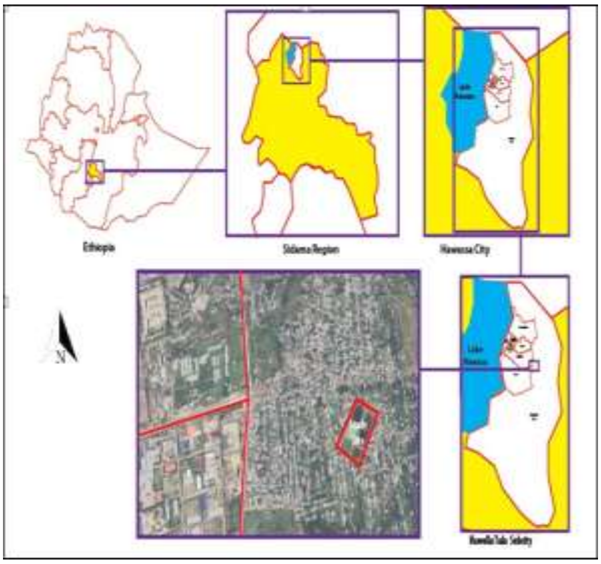

Fig.1. Location of Hawassa Millennium Pepsi Cola Factory (HMPCF)

Effluent and plant sample collection: One hundred liters of grab effluent samples were collected from the outlet of HMPCF, stored in five (20- liter Jerry cans) previously cleaned by washing in non-ionic detergent followed by distilled water, and transported immediately to the laboratory. Free-floating species of aquatic macrophytes previously identified as *A.filiculoides* L. and *L. minor* L. by botanists at the Norwegian University of Life Sciences (NMBU) during practical coursework were used as experimental plants in this study. These two plant species were collected from the shore of Lake Hawassa and carefully washed with tap water to remove 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 70cm (length), 40cm (width), and 30cm (depth), were used for the experiment. Three sets of experimental containers, having nine glass aquariums: 3 for (*A. filiculoides*), 3 for (*L. minor*), and 3 unplanted as controls, were arranged in the laboratory. Each experimental aquarium was filled with 10 liters of the factory effluent and 100g of healthy fresh weight of *A. filiculoides* and *L.minor* plants. The effluent level in the aquariums was checked throughout the experiment, and tap water was used to compensate for the water loss by 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 of the phytoremediation experiment of *A. filiculoides* (A), *L.minor (*B) in the biology laboratory

Plant and effluent samples collection for Analysis: One hundred gram 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 and air dried for one 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). The analysis was carried out for heavy metals, namely Cr, Co, Ni, Zn, Cd, and Pb, in the Norwegian University of Life Science (NMBU) soil and water laboratory, 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 factory effluent were measured on 0, 7, and 14 days, according to the standard methods for the examination of water and wastewater (APHA, 2017; 2005). 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.

Determination of Heavy metal Removal Efficiency of the Plants: The removal of heavy metals was determined by quantifying the concentration of metals left in the medium after incubation with plants. Removal efficiencies R (%) were calculated based on equation 1 as described by Bokhari *et al.*,(2016) as follows:

$$R (\%) = (C0 - Ct / C0) *100 \quad (1)$$

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 the Bioconcentration Factor (BCF): The b*ioconcentration factor was calculated by formula 2 below, as described by Zayed *et al.,* 1998:

$$BCF= (C_{pf} / C_{is}) \quad (2)$$

Where; **Cpf**= Heavy metal accumulated concentration in plant tissue after treatment, mg/kg; $\mathbf{C_{is}}$ = 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 spread sheet. Values were expressed as mean ± SD. One-way analysis of variance (ANOVA) was employed to identify differences in mean concentrations of contaminants among *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 at $P<0.05$.

RESULTS AND DISCUSSION

Characteristics of physicochemical parameters in the effluent: The characteristics of physicochemical parameters such as PH, temperature, EC, TSS, TDS, COD, and BOD in the soft drink factory effluent on 0, 7, and 14 days of treatment are shown in Table1. Among the seven physicochemical parameters analyzed in the HMPCF initial effluent samples, four were above the EEPA (2003) discharge limits. These parameters include EC (1651.2 µS/cm), TDS (1159.2 mg/L), COD (380.5 mg/L), and BOD (252.2 mg/L). In the cases of the effluent treated by the macrophytes, all the values decreased with the treatment duration, with the highest reduction on the 14^{th} day (Table 1). This indicates the capacity of the plants to absorb organic pollutants from the effluent. In the current study, the value of pH in both *A. filiculoides* and *L. minor* treatment systems was less than the control after 14 days of treatment, indicating a declining pattern in both plant treatment systems compared to the control (Table 1). The results of the present study revealed almost a similar decline in pH value as reported by Nayyef and Amal (2012), who reported a reduction in pH from 8.9 to 7.7 in the wastewater treated by *L. minor* plants. Daud *et al*., (2018) also reported a reduction of pH from 7.9 to 6.8 from the treatment of wastewater using *L. minor* plants after the 15-day experimental period. Furthermore, Mousa (1994) stated that *A. filiculoides* plants grow well in wastewater with a pH value ranging from 7.1 to 9.0. One way ANOVA in the current study revealed that the value of pH in both species of macrophyte treatment systems was significantly different ($p < 0.05$) compared to the control. According to Laffoley *et al.,* (2017), the reduction in 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.

The present study revealed that the temperature of the effluent in *A. filiculoides* and *L. minor* treatment systems during the treatment period ranged from 22.5^0C to 27.33^0C (Table 1). According to (Culley *et al.,* 1981; Singh, 1977), this range is within the tolerable limit for both *A. filiculoides* and *L. minor* plants. However, a significant reduction in the effluent temperature in both plant treatment systems was revealed after the 14-day treatment period (Table 1). The value of temperature in both macrophyte treatment systems and its value in control were significantly different ($p<0.05$) during the experimental period. The result of the present study was in agreement with the value reported by Amdebrihan (2017) for the temperature (27.5^0C) of HMPCF effluent. Nayyef and Amal (2012) reported a reduction in water temperature from 29°C to 24°C in the *L.minor* wastewater treatment system. 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 indicated 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 water and prevented light penetration, reducing the water temperature.

The results of the present study revealed that the initial concentration of EC in the effluent was higher than the value reported by Bokhari *et al.* (2016) for EC (1133 µS/cm) from wastewater treatment by *L.minor* in

Islamabad, but it was lower than the value reported by Amdebrihan (2017) for EC (1851.2 µS/cm) from HMPCF effluent. However, after 14 days of treatment, the value of EC significantly reduced to (434.8 ± 0.08) µS/cm and (404.5±0.78) µS/cm in the *A. filiculoides* and *L.minor* treatment systems, respectively (Table1). The value of EC in both treatments and its value in control were statistically different (P<.0.05). As presented by Murphy (2007), the reduction in EC in the treatment systems in the present study might be due to the reduction in the mobility of ions because of the removal of dissolved solids such as chloride, nitrates, phosphates, sodium, magnesium, and calcium by the aquatic plants from the factory effluent.

As indicated in Table 1, after 14 days of treatment, the value of TSS in the soft drink factory effluent significantly reduced to the minimum value (22.13 ± 0.77 and 18.01 ± 0.82) mg/L in *A.filiculoides* and *L. minor* treatments, respectively (Table 1). Consequently, the mean value of TSS in both macrophyte treatment systems and its value in control were significantly different (p<0.05) during the experiment. In agreement with Iqbal's (1999) suggestion, a reason for the reduction in the TSS from the effluent due to treatment by plants might be due to the biodegradation and sedimentation of organic particles in both plant treatment systems.

In the present study, after 14 days of treatment, the concentrations of TDS, COD, and BOD in both macrophyte treatment systems revealed a declining pattern, indicating the capacity of these plants to absorb organic pollutants (Table 1). Furthermore, in the *L. minor* treatment system, there was a slightly higher reduction in the concentrations of TDS, COD, and BOD compared to *A. fliculoides* treatments (Table 1). One way ANOVA revealed that the values of TDS, COD, and BOD in both macrophyte treatment systems were statistically different (p<0.05) compared to the control. The results of the present study revealed that the average concentrations of TDS, COD, and BOD in the effluent were lower than reported by Amare *et al.*, (2018): TDS, COD, and BOD (2500, 7133, and 5550) mg/L and (2500, 10940, and 7250) mg/L in *A.filiculoides* and *L.minor* treatments, respectively. As Anil *et al*., (2013) reasoned out, the reduction in TDS, COD, and BOD was attributed to the capacity of the plants to absorb inorganic and organic ions. The current investigation found that *L.minor* treated effluent had a slightly greater reduction in the values of all investigated physicochemical parameters than *A. filiculoides* (Table 1). In general, after the treatment time, the values of all evaluated physicochemical parameters treated by both plants were below the Ethiopian discharge limit.

Table.1. Physicochemical parameters measurements of the soft drink factory effluent during the experiment period.

		Physicochemical parameters (mean ± SD)						
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	8.36±0.01	27.33±0.12	1651.23 ± 0.86	75.33 ± 2.05	1159.23± 0.78	380.47 ±0.73	252.2 ± 0.78
	7	7.14±0.03	25.40±0.12	953.2 ± 0.89	43.13 ± 1.63	695.3 ± 0.98	165.3±1.69	115.33 ± 0.75
	14	6.54±0.01	23.33± 0.04	434.8 ± 0.08	22.13 ± 0.77	346.27 ± 0.26	98.33 ±0.84	67.17 ± 1.31
Lemna minor	0	8.36±0.01	27.33 ±0.12	1651.23 ± 0.86	75.33 ± 2.05	1159.23±0.78	380.47±0.73	252.2 ± 0.78
	7	7.05±0.01	24.67 ± 0.38	814.35 ± 0.25	35.37 ± 0.84	565.33 ± 0.85	145 ± 0.51	106.13 ± 0.83
	14	6.41±0.01	22.5± 0.08	404.8 ± 0.78	18.01 ± 0.82	325.12 ± 0.82	87.47±0.78	56.5 ± 0.65
Control	0	8.36±0.01	27.33±0.12	1651.23 ± 0.86	75.33 ± 2.05	1159.23±0.78	380.47±0.73	252.2 ± 0.78
	7	7.89±0.01	26.4 ±0.08	1532.13 ± 1.59	65.33 ± 0.69	1145.2 ±0.81	345.3 ±2.66	203.53 ± 0.54
	14	7.25±0.01	25.2 ±0.08	1498.2 ± 0.32	57.2 ± 0.73	1098.21±0.74	298.43±0.54	189.13 ± 0.55
EEPA, 2003		6-9	40	1000	100	1000	250	80

Note; *Day 0 = before treatment, Day 7=mid time of the treatment, 14 = after treatment*
EEPA, 2003= Ethiopian Environmental Protection Authority discharge limit

The removal efficiency of plants for physicochemical parameters: The percentage removal efficiencies of the two species of macrophytes for physicochemical parameters and the reduction in control are presented in Table 2. In this study, the level of pH in both macrophyte treatment systems exhibited a declining pattern during the treatment period (Table 2). Nevertheless, after 14 days of treatment, the *L. minor* treatment system showed a relatively higher reduction in pH (23.33*%)* compared to *A. filiculoides* (21.76%) treatments (Table 2). Simultaneously, the pH value of the control was reduced by 13.27% (Table 2). Accordingly, the percentage reduction of pH in both treatments and the control was statistically different (p<0.05). The result of the current study revealed that the percentage reduction of pH was higher than reported by Nayyef and Amal (2012), who stated a 13.4% reduction of pH from wastewater treated by *L.minor*. In the present study, a relatively higher reduction in the effluent temperature (17.54%) was revealed in the *L.minor* treatment system compared to the *A.filiculoides* (14.31%) treatment (Table 2). Whereas, in the control, the reduction in the effluent temperature was 7.78% in the same treatment period. Accordingly, the percentage reduction of the effluent temperature in both treatments and the control was statistically different (p<0.05). The result of the present study is in agreement with Nayyef and Amal (2012), who reported a 17.2% reduction in temperature in wastewater treated by *L.minor* plants.

The result of the present study displayed a significant reduction in the level of EC in both macrophyte treatment systems (Table 2). Consequently, after 14 days of the treatment, a relatively higher reduction in EC (75.48%) was revealed in the *L.minor* treatment system compared to the *A.filiculoides* treatment (73.65%) (Table 2). At the same time, the reduction of EC in control was 9.27% (Table 2). Therefore, the reduction of EC in the treatments and the control was statistically different ($p<0.05$). In the current study, the value of EC reduction was higher than reported by Amare *et al.*, (2018), who reported a reduction of EC of 69% (*A. filiculoides)* and 68% (*L. minor)* from blended wastewater. As indicated in Table 2, the results of the TSS removal efficiency of both macrophytes increased with treatment time. After 14 days of the treatment period, *L.minor* showed a relatively higher percentage removal of TSS (76.17%) compared to *A.filiculoides* (70.63%) treatment system (Table 2). Whereas, in the control, the percentage removal of TSS was 23.94% during the same time (Table 2). The TSS removal in treatments and control was statistically different ($p<0.05$). The result of TSS removal in the present study was higher than that reported by Al-Sabunji and Al-Marashi (2002) and Nayyef and Amal (2012), who reported a (45% and 38%) reduction of TSS by *L. minor* plants in wastewater treatment, respectively. Furthermore, the results of the present study revealed that *L. minor* displayed a relatively higher percentage removal of TDS, COD, and BOD compared to *A.filiculoides* treatment after 14 days of treatment (Table 2). Whereas, in control, there was no significant reduction in TDS, COD, or BOD from the effluent. Consequently, the percent removal of TDS, COD, and BOD in both treatments and the control was statistically different ($p<0.05$). Results of the present study revealed that the removal efficiencies of both macrophytes for TDS, COD, and BOD were in agreement with some previous studies: Amare *et al., (*2018) reported the removal of 70% of TDS in *A. filiculoides* and 68% in *L. minor* treatment systems. Likewise, COD removal efficiencies were comparable with the results of Korner *et al*., (2003), who reported (50% to 95%) COD removal in the *L.minor* treatment systems. Nevertheless, it was lower than presented by Amare *et al., (*2018) (96% and 92%) for COD removal in *A.filiculoides* and *L.minor* treatments, respectively. Likewise, the BOD removal in this study was lower than reported by Amare *et al*., (2018), who presented a BOD removal of 92% and 90% in *L.minor* and *A. filiculoides* treatments, respectively.

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

Treatments	Day	pH	Temp	EC	TSS	TDS	COD	BOD
A filiculoides	7	14.59	6.72	42.27	42.75	40.02	56.52	54.32
	14	21.76	14.31	73.65	70.62	70.13	74.14	73.36
L.minor	7	15.67	9.73	50.68	53.17	51.27	61.88	58.89
	14	23.33	17.54	75.48	76.02	71.98	76.86	77.66
Control	7	5.62	3.29	7.21	13.16	1.21	9.21	19.24
	14	13.27	7.78	9.27	23.94	5.28	21.61	25.00

Heavy metals mean concentration in the effluent and plants removal efficiency: Heavy metals mean concentration in the effluent: The mean concentrations of heavy metals in the soft drink factory effluent treated by *A. filiculoides* and *L. minor* plants at different times (days) of exposure and relevant discharge limits 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. In comparison, no significant difference was observed in the control system during the treatment period. The mean concentrations of heavy metals in the effluent treated by the two species of macrophytes were in the following order: Zn > Pb > Cr >Ni >Co > Cd (Table 3). ANOVA showed significant ($p < 0.05$) differences in heavy metal concentrations between the two macrophyte treatment systems during the experimental period. In contrast, no significant difference was observed in the control system during the same treatment period. The results of the present study were in agreement with Adepoju-Bello *et al.,* (2012), who discovered heavy metals such as lead, cadmium, nickel, chromium, and zinc in regularly consumed soft drinks. Furthermore, Behailu *et al.,* (2010) reported that the effluent from the Hawassa Millennium Pepsi Cola Factory contained heavy metals, organic compounds, and nutrients. In the current study, the maximum mean concentration of heavy metals in the soft drink factory effluent was revealed for Zn in both macrophyte treatment systems (Table 3). Nevertheless, after 14 days of treatment, the concentration of Zn in *A. filiculoides* and *L. minor* treatments considerably dropped to (37.78 ±1.3 and 32.89 ±1.49) µg/L, respectively (Table 3). In contrast, the concentration of Zn in the control system didn't show a significant decrease during the same experimental period (Table 3). Results in the present study revealed that the concentrations of Zn after 14 days of treatment were 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 current study revealed that the mean concentrations of Pb, Cr, and Ni in the effluent displayed a considerable declining pattern in both macrophyte treatment systems during the experimental period (Table 3). However, after 14 days of the experiment, the *L. minor* treatment system revealed a relatively higher reduction in the concentrations of Pb, Cr, and Ni (Table 3). ANOVA showed significant ($p < 0.05$) differences between the treatments and the control. Results of the present study revealed that, after 14 days of treatment, the concentrations of Cr and Ni in both treatments were lower than reported by Amare *et al.,* (2018), who presented Cr (24) µg/L and Ni (38) µg/L in *A.filiculoides* as well as Cr (21) µg/L and Ni (30) µg/L in *L.minor* treatments (Table 3). The removal of Pb in this study was lower than reported by Bokhari *et al.,* (2016), who presented a reduction of Pb from 608 µg/L to 15 µg/L in sewage mixed industrial effluent treated by *L.minor*. Singh *et al.,* (2012) also stated that *L. minor* has the potential to remove lead from industrial wastewater. Likewise, the concentrations of Co and Cd in the *A. filiculoides* and *L. minor* treatment systems revealed a similar declining pattern after 14 days of the experimental period (Table 3). Initial Cd concentration of soft drink effluent was 0.88 µg/L in which a significant decrease ($p < 0.01$) was observed in the first seven-day interval, and reduced to 0.01 µg/L after 14 days in the *L.minor* treatments. The concentration of Cd was lower than reported by Bokhari *et al.,* (2016) (2µg/L and 3 µg/L) in sewage mixed industrial effluent and municipal effluent treated by *L.minor*, respectively. In the present study, the highest metal removal by the two macrophytes might be due to the low concentration of heavy metals in the residual effluent (Table 3). In general, results in the present study indicate that both species of macrophytes proved to be very effective in the removal of all examined heavy metals from the soft drink factory effluent.

Table 3. Heavy metals mean concentration (µg/L) during the entire treatment period

Treatments	Time (Day)	Cr	Co	Ni	Zn	Cd	Pb
		Heavy metals (mean ± SD)					
	0	26.44± 1.93	4.72 ± 0.15	13.11±1.34	135.33±4.06	0.88±0.04	33.89± 5.84
	7	9.57 ± 0.31	1.6 ± 0.04	4.98 ± 0.40	61.67± 1.36	0.43±0.01	12.44±1.09
A. filiculoides	14	2.34 ± 0.34	0.92± 0.05	2.87± 0.19	37.78± 1.34	0.03±0.02	7.04± 0.22
	0	26.44 ±1.93	4.72 ±0.15	13.11±1.34	135.33±4.06	0.88± 0.04	33.89±5.84
	7	7.31 ± 0.01	1.38± 0.29	4.03± 0.08	64.67 ± 4.72	0.09± 0.01	10.24 ±1.44
L. minor	14	1.87 ± 0.13	0.72± 0.06	1.73± 0.04	32.89± 1.49	0.01 ± 0.00	2.99± 0.42
	0	26.44 ±1.93	4.72± 0.15	13.11±1.34	135.33±4.06	0.88± 0.04	38.89±5.84
Control	7	19.40 ± 0.56	3.73± 0.28	10.17±0.24	126.89±1.49	0.76± 0.03	31.0 ±0.94
	14	17.56 ±1.49	3.53± 0.31	9.16 ± 0.24	123.5 ±1.22	0.73± 0.02	26.89±0.42
EDL		1000	1000	2000	5000	1000	500

Note; Day 0 = before treatment Day 7=mid time of treatment, 14 = after treatment

EDL (Ethiopian discharge limits)

Heavy metal removal efficiencies of A.filiculoides and L. minor: As presented in Table 4, the percentage heavy metal removal efficiencies of *A. filiculoides* and *L. minor* plants were more than 72% for all six heavy metals (Cr, Cd, Pb, Ni, Co, and Zn). The result of this study showed that *L. minor* displayed higher removal efficiency for Cr (97.5%) compared to *A. filiculoides* (91.04%) during the 14-day treatment period (Table 4). The findings of the current study for Cr were higher than reported by Maha (2012) and Sekomo *et al.,* (2012): Maha (2012) presented a removal of Cr in the range of (86.2 to 94.8) % by *L. minor* from aqueous solutions, and Sekomo *et al.,* (2012) stated that *L. minor* removed 94% of Cr from wastewater throughout the 12-day incubation period. In agreement with Greger (1999), the reduction of the concentration of chromium in the current study from the soft drink factory effluent might be due to the availability of nutrients that encourage plant development, which causes an increased number of metal absorption sites in the plants, boosting metal ion uptake. In the present study, the lowest percentage removal efficiency by *L. minor* was recorded for Zn on the 14th day of the experiment (Table 4). The result was in line with Nayyef and Amal (2012), who presented a reduction of Zn (72%) by *L.minor* wastewater treatment. Khellaf and Zerdaoui (2009) also reported that duckweed plants successfully removed 61-71% of Zn from polluted water. Whereas, Daud *et al.,* (2018) indicated that duckweed (*L. minor*) removed 83% of Zn within two weeks from landfill leachate. Nevertheless, in *A.filiculoides*, the maximum removal efficiency was revealed for Cd (96.58%), and its minimum removal was recorded for Zn (72.08%) on the 14th day of the experiment (Table 4). From the results, *L. minor* revealed a slightly higher removal efficiency for the tested heavy metals (Table 4). Therefore, the percentage heavy metal removal efficiencies of *L. minor* and *A. filiculoides* were in the following order: Cr > Cd > Pb > Ni > Co > Zn and Cd > Cr > Co > Pb >Ni > Zn, respectively (Table 4). In general, *L. minor* and *A. filiculoides* displayed higher potential for the removal of heavy metals under study compared to the control, which makes the two macrophytes suitable candidates for phytoremediation of heavy metals from the soft drink factory effluent.

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

Treatments	**Time (day)**	**Cr**	**Co**	**Ni**	**Zn**	**Cd**	**Pb**
	7	82.58	77.59	58.28	54.4	51.03	58.64
A. filiculoides	14	91.04	87.68	76	72.08	96.58	78.42
	7	90.59	65.37	69.7	49.15	58.81	82.83
L.minor	14	97.52	84.16	86.96	74.15	96.91	92.43
	7	24.13	16.96	25.24	13.88	14.32	18.69
Control	14	31.63	21.46	32.7	16.79	18.04	30

Bioconcentration Factor (BCF): The bioconcentration factor (BCF) is the heavy metal concentration present in the plant biomass divided by the heavy metal concentration in the water (Zayed *et al.*, 1998). As presented in Table 5, in the *A. filiculoides* treatments, the BCF values for heavy metals ranged from (192.92 to 561.7) and (258.99 to 823.4) after a 7-day and 14-day incubation period, respectively. Whereas, in the *L. minor* treatment systems**,** the BCF value ranged between **(**126.7 and 32,544.3) as well as (170.99 and 40,064) during the same treatment period (Table 5). The highest BCF value was revealed for Zn (40,064) in the *L. minor* treatment system within the 14-day treatment period (Table 5).Therefore, *L. minor* displayed the best performance for the accumulation of Zn from the soft drink factory effluent (Table 5). The reason might be due to the importance of zinc as an essential trace element that plays an important role in the growth and development of plants. Zayed *et al*., (1998) reported that good heavy metal accumulator plants should have a BCF value of more than 1000. In this study, after 14 days of treatment, the BCF values for the tested heavy metals were in the following order: Zn > Co > Ni > Cd > Pb > Cr and Co > Zn > Cd >Ni > Cr >Pb, by *L.minor* and *A.filiculoides* treatments, respectively (Table 5). In general, both macrophytes showed the best efficiencies to remove and accumulate all the tested heavy metals in all treatments in a glass aquarium. Therefore, the result of the present study suggests that *L.minor* and *A.filiculoides* are the best candidates for the phytoremediation of all tested heavy metals in soft drink factory effluent.

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

	Time (days)	Cr	Co	Ni	Zn	Cd	Pb
	7	269.7	561.7	245.8	365.41	211.11	192.92
A. fliculoides	14	346.6	823.4	386.25	563.19	400	258.99
	7	126.7	1,928.26	401.5	32,544.30	300	239.93
L. minor	14	170.99	2,443.47	739.1	40,064.12	411.1	294.89

Conclusions Although, the findings of this study revealed that *A. filiculoides* and *L. minor* plants were quite effective in treating the soft drink factory effluent compared to the control, *L.minor* proved to be more efficient for the removal of heavy metals and organic contaminants. Therefore, it provides a deep insight into the potential of the plants to be used as suitable treatments for metal-polluted factories in the country. Furthermore, the study provides basic data for future comparisons of phytoremediation studies in countries that have soft drink factories around the world.

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