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Assessment of the Accumulation Performance of Mo, Pb, and Cu in the Acidic Water of Copper Mine with *Lemna minor* and *Lemna gibba*

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Abstract: Pollutants accumulate in aquatic habitats as a result of mining activities. *Lemna gibba* and *Lemna minor* were used in this study to examine the accumulation capacities of Mo, Pb, and Cu in the acid fluids of the copper mining. Duckweed family includes water plants like *L. minor* and *L. gibba*. These are tiny, delicate, free-floating aquatic plants. Two reactors were assigned to *L. gibba* and *L. minor*, respectively. Throughout the course of eight days, these plants and reactor water were gathered every day. The acid mine water's pH, temperature, and electric conductivity were also tested every day. The *L. gibba* and *L. minor* were cleaned, dehydrated, and burned in a drying oven for a whole day at 300 ^{o}C. ICP-MS (Inductively Coupled Plasma Mass Spectroscopy) was used to determine the Mo, Pb, and Cu content of the plant and water samples. The concentrations of Mo, Pb, and Cu in the acid mine fluids of the copper mining were 30±4, 260±12, and 15535±322 µg L^{-1}, in that order. When it came to extracting Mo, Pb, and Cu from the acidic fluids of the copper mining, L. gibba and L. minor performed well and efficiently compared to control samples; they did so 29 and 177 times for Mo, 30 and 109 times for Pb, and 495 and 1150 times for Cu. All things considered, the findings point to the possible application of *L. gibba* and *L. minor* in environmental rehabilitation by demonstrating their ability to efficiently extract Mo, Pb, and Cu from acid mine fluids.

Keywords: acid mine waters; heavy metals; accumulation; *Lemna gibba*; *Lemna minor*; water treatment

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1. Introduction

Acid mine drainage, or AMD for short, is a common occurrence in both current and abandoned mines, whether open-pit or underground. Sludge ponds, quarries, pit lakes, tailing dumps, tailing dams, stockpiles, iron ore haul highways, and mine waste dumps are additional locations where AMD can be discovered [1,2]. Because of its high pH and heavy metal content, AMD is challenging to cure and presents serious risks on human health and the environment. If AMD is discharged without receiving the appropriate care, long-term environmental problems and damage may arise [3–5]. Particularly in sulfide mines, acidic water is produced as a byproduct of mining and subsequent processes. This water can have a drop in heavy metal ion concentration from thousands or hundreds of milligrams to a few milligrams. Because heavy metals pollute the air, land, and water, as well as taint food and drink, they can cause a wide range of illnesses in both people and animals. Therefore, it is a significant task and a known scientific truth that heavy metals may be removed or reduced from the air, soil, and water. One of the hardest challenges in contemporary mining has been acid mine waters [6].

Heavy metals have large atomic weights and a density five times higher than that of water [7]. They were divided into two categories by Gergen and Harmanescu [8]. Rai et al. [9] claim that metals including Mo, Pb, Cu, Cd, Hg, Ni, As, Au, Ag, and Cr have contaminated soil, water, and air widely and have no good effect on plants or animals. Animals and plants require some metals (Fe, Cu, Zn, Co and Mn), although high amounts of these metals can be harmful. Therefore, one of the primary issues affecting aquatic plants and animals is still heavy metal contamination [10]. Certain metals include As, Tl, Hg, Pb, Cr, and Cd are an especially concerning for public health because of their high hazardous levels [7]. Mo is an essential element for plant health, animal and human [11,12]. Yet, as with all elements, exposure to high doses of Mo can be detrimental to animal, human and plant health

[13]. The distributions of Mo in freshwater systems about the environment, human health, and water supply have received relatively little study. According to Smedley and Kinniburgh [14], the majority of natural waters have Mo contents of no more than 10 µg/L. The maximum permitted concentrations in drinking water are 0.01 mg/l for lead and 0.015 mg/l for copper, as per US EPA [15] and the World Health Organization [16].

Using living green plants, phytoremediation is one of the most economical and environmentally beneficial techniques for the in-situ removal or restoration of heavy metals from water and soil [17–23]. Aquatic macrophytes gather pollutants and metals during the rhizofiltration stage [24]. Because *Lemna* sp. grows more quickly and is easier to harvest, many scientists favor it for phytoremediation research [25–27]. Among aquatic macrophytes, it is the most efficient plant in eliminating metals and pesticides due to its rapid growth and ability to float on the water [28–30]. It also grows well in a variety of climates, has a long storage capacity, quick reproduction rates, low cost, and little volume of biological and chemical sludge [27,30–32]. The ideal temperature and pH ranges for *Lemna* sp. rapid growth are 5–25 oC and 4–9, respectively, according to Khataee et al. [31].

One of the main factors degrading water and soil is high metal pollution from acid mine fluids. Since heavy metals can bioaccumulate in plants and other species in the receiving ecosystems into which these fluids are discharged, the water must be treated before being released back into the natural environment. This study used *L. minor* and *L. gibba* feed in the acidic fluids of the copper mining in low pH circumstances to explore the accumulation performances of Mo, Pb, and Cu. To determine the accumulation capacity of both plants in the acidic waters, daily measurements of changes in Mo, Pb and Cu were made in *L. minor* and *L. gibba*. The amount of acid mineral water that these plants extracted from metals was also assessed in the study.

2. Material and Methods

The Maden Cu mining's natural setting served as the study setting. The climatic parameters for this experiment were: average daily, average global radiation of 480±32Wm^{-2}, hours of sunny days of 13.8±0.6, temperature of 23.6±7.2 ^{o}C and relative humidity of 28.6±3.2%.

2.1. The Study Area

The investigation was carried out in the Maden, Elazig, Turkey, Cu mining area, which is situated at N38.388434° and E39.671450° (Figure 1). Mining has a lengthy history in this region, going back to prehistoric times around 2000 BC. massive sulfide ore (it is about average 6.5% Cu tenor and 6.1 million tons reserve) during 1968 and 1939, were mined from Anayatak and its adjacent deposits. Modern Cu production was started in 1939 by Etibank. One of Turkey's biggest copper-producing regions, the Maden copper deposit spans various geographic regions, including Anayatak, Weis, Mızırtepe, Kısabekir, and Hacan. Black smoker, which is derived from hydrothermal vents on the seafloor, is thought to be associated to these deposits [33,34]. There are also significant reserves of Ni, Cu, Au, Co, and Ag in these deposits. A frequent water effluent from mining operations is seen at the mine site all year long and is released into the Maden Stream.

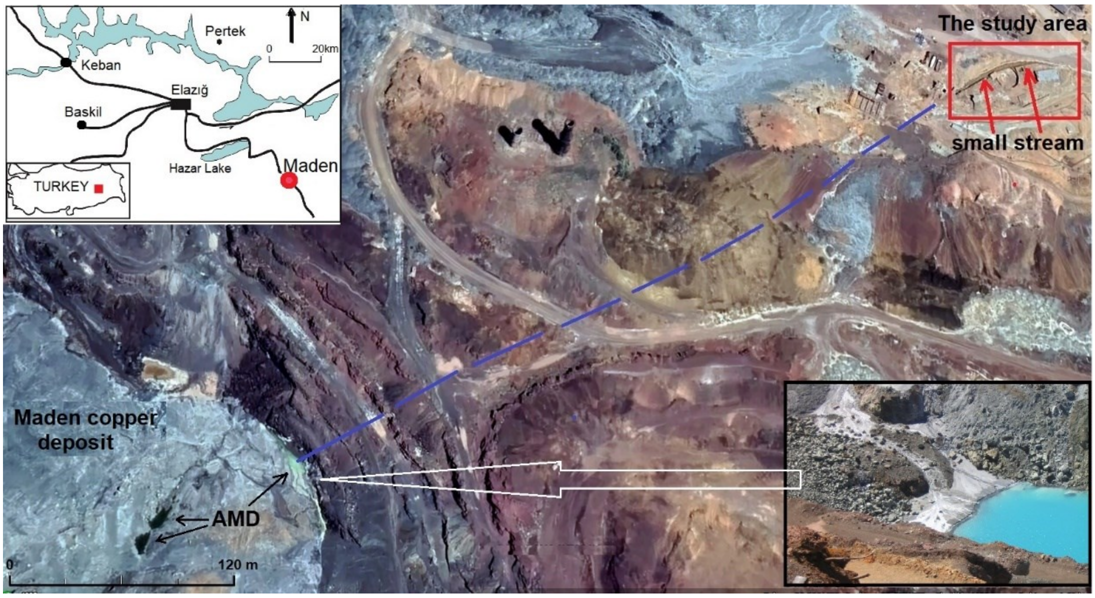

Figure 1. The experimental setup of this study (taken from Sasmaz Kislioglu, [35]).

2.2. Plant and Water Samples

According to *the East Aegean Islands and Flora of Turkey* [36]. *L. minor* and *L. gibba* belong to duckweed family and are the member of the Lemna genus [37]. They are divided into five genera such as Lemna, Spirodela, Landoltia, Wolffiella and Wolffia. These asexually reproducing floating aquatic plants develop offspring directly from their parents, without the need for a seed stage [38]. Water and plants were collected every day for eight days in a row, with *L. minor* and *L. gibba* kept in separate reactors. The acidic mine water's pH, electric conductivity, and temperature were tested every day using sterile plastic bottles to gather the samples.

The chemical composition of acidic water may vary due to extensive mineralized wallrock in the Cu mining area. These variables may affect the T ^{o}C, pH of the water and EC (electric conductivity). The temperature, pH, and electrical conductivity were recorded using an Orion conductivity electrode. With an ICP-MS, cation and anion analyses (such as carbonate, nitrate, sulfate, and fluoride) were carried out. In addition,

2.3. Analytical Method

L. gibba and *L. minor* were cultivated independently in two natural pools prior to being moved to separate reactors. The plants were brought from Botanical Garden in the Istanbul University. As detailed by Tatar and Obek [22], each reactor contained 500 grams of plants, with dimensions of 70x35x30 cm (Figure 2), *L. gibba* in one reactor and *L. minor* in the other. Throughout the experiment, the plants were fed with fresh water by the reactors, which continuously flowed 1.28 L sec^{-1} of acid mineral water (Figure 2). From each reactor, about 50 grams of plant material were taken out every day for eight days. *L. gibba* and *L. minor* were observed to change color from green to yellow towards the end of the experiment. Possibly due to widespread heavy metal concentrations in water, toxic effects began to appear on plants (Figure 3). After being collected, it was firstly cleaned with tap water, after that, rinsed with distilled water, and then, dried for 24 hours at 60 ^{o}C in a laboratory oven. Then, the dried plants were reduced to ash for 24 hours at 300 ^{o}C to produce ash samples. These samples were then digested for one hour in HNO_3, and then for another hour at 95 ^{o}C in a mixture of HNO_3: H_2O: HCl (1:1:1) by taking one gram of the ash sample. Lastly, ICP-MS methods were used to examine all samples for Mo, Pb and Cu.

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Figure 2. *L. gibba* and *L. minor* replaced to n each reactors in the acidic mine water,.

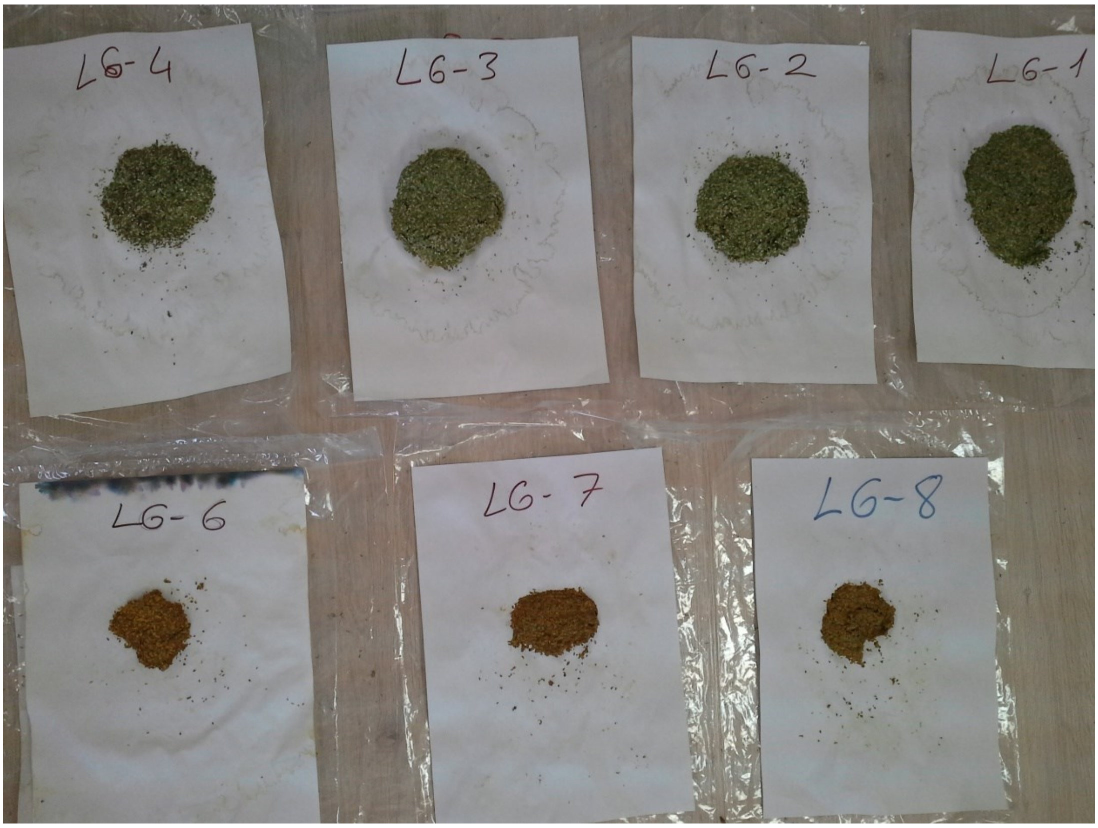

Figure 3. *L. gibba* and *L. minor* were observed to change color from green to yellow towards the end of the study.

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3. Results and Discussion

3.1. Mo, Pb and Cu in acidic mining water

The principal anion and cation results of the acid mine water's physicochemical characteristics are displayed in Table 1. The temperature varied from 18.6 to 24.8 °C (mean: 22.6 ± 1.2 °C); the pH of the water altered to 5.84 from 5.62 (mean: 5.76±0.14); and the EC values were between 2.64 and 2.38 mS cm^{-1} (mean: 2.55 ± 0.08 mS cm^{-1})(taken from 35). Throughout the eight-day experiment, daily field samples of water were collected. Table 1 displays the average concentrations of Mo, Pb, and Cu in the acid mine water, which were found to be 30±4, 260±12, and 15535±322 µg L−1, respectively ($p < 0.5$). The chemistry of acidic mine water is greatly influenced by several factors, including its distance from the recharge area, the length of time it spends in the flow system, the volume of acid mine water flowing through it, and the long-term rock-water interaction. According to the measured data, the chemistry and physicochemical properties of the waters originating from the ore location are generally comparable. Significant pollution near the Maden stream is caused by heavy metal pollution in the land and water.

The mean values of Mo, Cu and Pb in the acid mine fluids exceeded the US EPA's [15] and ATSDR's [32] limit levels, as indicated in Table 1. The research area's acid mine water included varying quantities of Mo (28.4 to 31.6 µg L^{-1}). Most natural waters have Mo concentrations of around 10 µg/L or less [14]. The research area's average Mo value was higher than the WHO-established threshold levels (10 mg L^{-1}) for drinking water [13] (Table 1). Average Pb levels in these natural waters according to US EPA [15] have been recorded as 10– 15 µg/L [16]. The environment's soil and water are contaminated by the mine's leaky water. It is extremely difficult to clean these contaminated soils and waterways [39–41]. According to Ning et al. [42], the average readings of WHO [16] for heavy metal levels were not as high as those found in the water surrounding Pb resources. A median Mo content of 0.5 mg/L was reported by Reimann and de Caritat [43] for streamwaters worldwide. The estimates for world rivers are 0.11-8.63 (mean 1.21 mg/L) [44] and around 0.42 mg/L [45]. Rivers from India can contain up to 20 mg/L [46] and up to 8.6 mg/L [47].

Based on the main cations and anions (Ca–Mg–HCO_3; Ca–Mg–Fe–SO_4; Na–F–NO_3), the waters in the research region were divided into three groups. The water kinds in the aquifer were identified by using Piper's [48] triangular drawing approach. Over 90% of the cations in the aquifer are found in the examined fluids, with Ca, Mg, Fe, Na, S, K and Mn being the most common. In the waters of the research area, bicarbonate and sulfate constituted 85–90% of all anions, making them the main anion species. Ca-Mg-Fe-Na-SO_4 HCO_3 water is one possible classification for the acid mining water in the Maden Cu mining.

Table 1. Physicochemical characteristics, cation major anion and trace element results the acid mine water [35].

Parameter	T (°C)	pH	EC (mS cm^{-1})	HCO_3^- (mgL^{-1})	NO_3^- (mgL^{-1})	SO_4 (mgL^{-1})	F^- (mgL^{-1})	Ca (mgL^{-1})	Mg (mgL^{-1})	K (mgL^{-1})	Na (mgL^{-1})	Fe (mgL^{-1})
DL	-	-	-	-	-	-	-	0,05	0,05	0,05	0,05	10
Mining water	22.6±1.6	5.76± 0.1	2.55± 0.2	282±16	1.86± 0.06	128±8	0.41±0.1	482±24	426±18	5.80± 0.3	115± 6	118±7
Parameter	Mn (mg L^{-1})	S (mg L^{-1})	P (µg L^{-1})	B (µg L^{-1})	Zn (µg L^{-1})	Cr (µgL^{-1})	Ni (µg L^{-1})	Co (µg L^{-1})	As (µg L^{-1})	Mo (µg L^{-1})	Pb (µg L^{-1})	Cu (µg L^{-1})
DL	0,05	1	10	5	0,5	0,5	0,2	0,02	0,5	0,1	0,1	0,02
Mining water	6.4± 0.3	670±28	236± 12	850±45	2852± 84	202± 16	965± 58	1766±72	193±12	30±4	260±12	15535±322

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3.2. Lemna gibba and Lemna minor

Cleaning and restoring contaminated areas can be done affordably, effectively, sustainably, and economically with phytoremediation. But before building a decontamination system, knowledge regarding the effects of heavy metals on plant physiology should be acquired to optimize the system [49]. The uptake process of Mo, Pb, and Cu can be impacted by variables such the metal's bioavailability, the contaminant's chemical characteristics, organic matter contents, plant species, phosphorus, pH, and environmental factors of the contaminated environment [50]. Numerous aquatic plants are employed successfully for the monitoring of contaminated settings and are recognized as heavy metal pollution indicators [51]. Due to their ability to accumulate in biological systems, heavy metals like Mo, Ag, Pb, Au, Cu, As, Co, Hg, Zn, Tl and Cd are hazardous and poisonous.

Prior to the commencement of the experimental investigation, it was found that *L. minor* (LM-0) and *L. gibba* (LG-0) had Mo levels of 2.16 and 0.29 mg kg^{-1}, respectively ($p < 0.05$) (Figure 4). These values are regarded as the values of the control group for these plants. A total of 2.89 and 0.97 mg kg^{-1} of Mo were collected by *L. minor* and *L. gibba* on the first day of the experimental investigation. Over the first five-6 days of the experiment, both plants' absorption of Mo from acidic mining water either marginally increased. On the fifth and sixth days, *L. gibba* and *L. minor* removed 84 and 77 times more Mo compared to the control from acid mine water. *L. gibba* showed outstanding Mo accumulation ability between day 5 and day 7. *L. minor* showed a high ability to accumulate rapidly after the 5th day until the end of the experiment, and on the 8th day it accumulated 169 ppm Mo, which corresponds to an approximately 77 times Mo accumulation compared to the control sample.

Even though the acidic mining water utilized in the study contained low values of Mo (30 µg L^{-1}), by the end of the study. *L. gibba* removed molybdenum in 274 L of acidic mineral water at the end of the 6th day of the study, and *L. minor* accumulated molybdenum in 5561 L of acidic mineral water at the end of the 8th day.

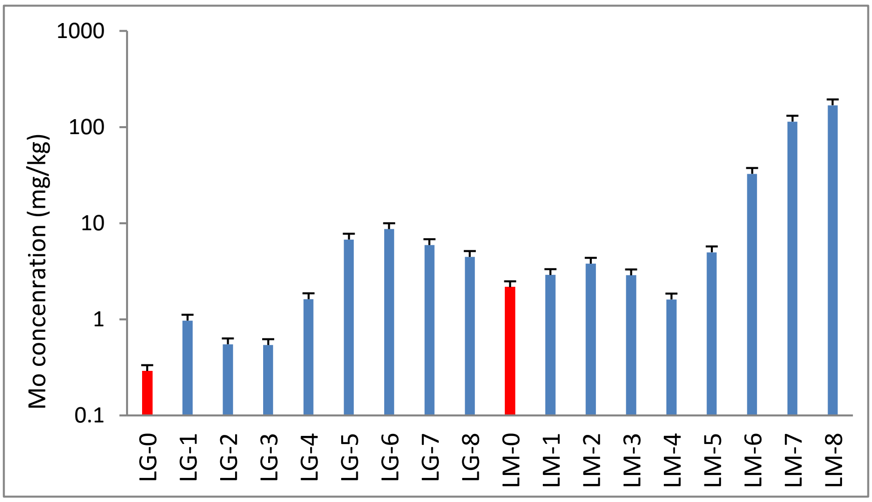

Figure 4. Mo accumulation ratios by *L. gibba* and *L.minor*.

Both *L. minor* and *L. gibba* showed comparable increases in Pb accumulation throughout the course of the first five days of the study. Both *L. minor* and *L. gibba* showed limited, comparable increases in Pb accumulation throughout the first five days of the experiment. Both plants showed a linear and extremely high accumulation ability from the fifth to the eighth day. On days 5 and 8, *L. gibba* accumulated 30 times (78.2 mg kg^{-1}) and 109 times more Pb (189 mg kg^{-1}) from acidic water, respectively, compared to the control samples of each plant (Figure 5).

By the end of the 8-day trial, Pb had been extracted to acidic mining water of 291 L and 720 L, respectively, by *L. gibba* and *L. minor*, despite the low content of lead (260 µg L^{-1}) in the acidic mine water used for the study.

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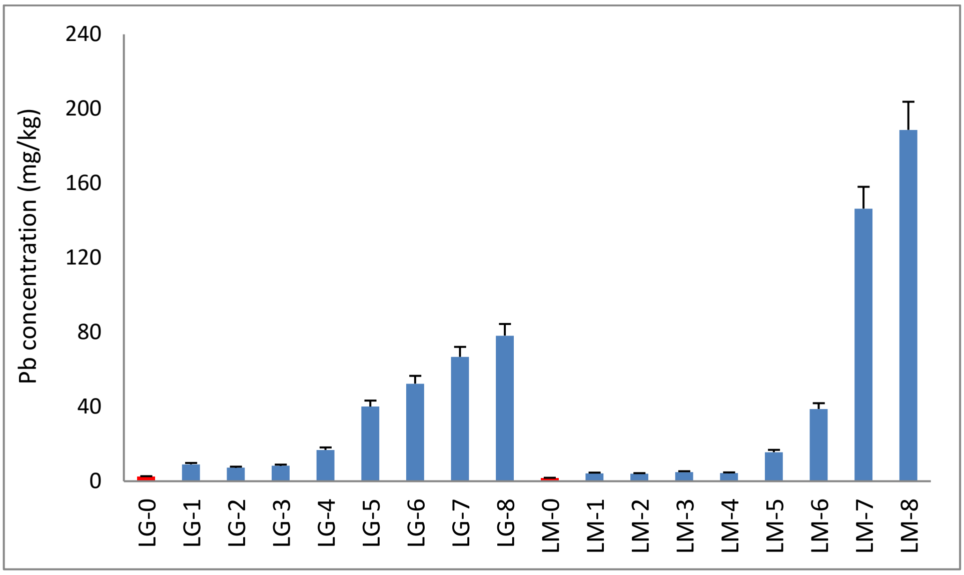

Figure 5. Pb accumulation ratios by *L. gibba* and *L. minor*.

L. gibba regularly showed significant increases in copper accumulation throughout the experiment and accumulated 9866 ppm Cu on the last day of the experiment. This corresponds to a 495-fold copper accumulation compared to the control group. *L. minor* showed incredible accumulation ability during the first four days of the experiment, and at the end of the 4th day, 12668 ppm copper was accumulated by this plant. This indicates 1150 times more accumulation compared to control samples. Between the 5th and 8th days, the accumulation values of *L. minor* decreased due to the plant being sufficiently saturated with copper (Figure 6).

At the end of the study, *L. minor* and *L. gibba* accumulated, respectively, copper in 634 L and 815 L acidic mine water, despite the high amount of copper in acid mine water (15535 µg L^{-1}) of the research region..

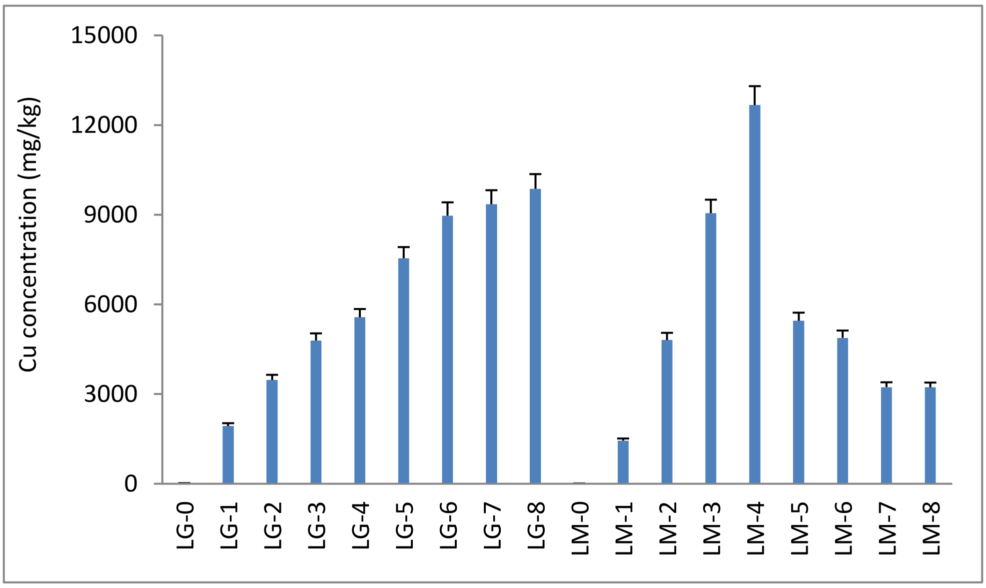

Figure 6. Cu accumulations by *L. minor* and *L. gibba*.

Sasmaz et al. [21] examined the metal accumulation rates and the best time to harvest in gallery water using plants such as *L. minor* and *L. gibba* in waters from the Keban Pb-Zn mine. The pH of gallery water is 7.36 and has a neutral composition. It was observed that both plants achieved higher accumulations in acidic waters than in neutral mineral waters of Pb-Zn mining, Keban. The study determined the best time to harvest by monitoring daily changes in the amounts of metals in both plants. Based on the acquired data, *L. gibba* and *L. minor* accumulated Pb and Cu at 2888 and 3708 times and 108 and 147 times, respectively, greater than those found in the gallery water.

Sasmaz [35]) examined the Ag, Au and As accumulation performances with the same plants in acidic mineral water in the same experiment setup. In comparison to control samples of these plants, *L. minor* and *L. gibba* showed effective and high abilities in accumulating As, Au and Ag from the acidic mine water of Cu mining area; respectively, 30 and 907 times for As; 336 and 394 times for Au; and. 240 and 174 times for Ag

During the course of eight days, Sasmaz and Obek [52] provided evidence of *L. gibba*'s ability to extract As, U, and B from secondary-treated urban wastewater. During the first two days of the study, *L. gibba* showed, respectively, the highest uptake ratio for B, U, and As with removal rates of 40%, 122%, and 133%. These results imply that *L. gibba* may be useful as a natural strategy to lessen the amount of these pollutants in wastewater. *L. minor* shows a higher capacity for collecting lower amounts of Cr and Ni, according to Goswami and Majumder [17]. Furthermore, the uptakes of Au and Ag from secondary-treated municipal waste water by *L. gibba* were examined by Sasmaz and Obek [52]. Within six days of the experiment, the investigation showed that both Au and Ag were accumulated rapidly. But after day six, the concentrations of Ag and Au accumulation fluctuated, perhaps because the plant had reached saturation the greatest accumulations for Au and Ag on the 5th and 6th days of the study, were noted as 2303% and 247%, respectively. Uysal [53] investigated *Lemna*'s capacity to sorb Cr at various pH and concentration levels and found that despite being subjected to harmful consequences, the plants were still able to absorb Cr from the water. During the course of the 12-day experiment, Abdallah [54] noted that *L. gibba* did remarkably well, accumulating over 84% of the Cr in the solution. *L. minor* is a viable choice for repairing habitats damaged with Pb and Cr because of its ability to absorb these metals fast and efficiently, according to Ucuncu et al. [55]. According to Goswami et al. [18], *L. minor* worked well to correct low low concentration As-contaminated waters. The effectiveness of L. gibba and L. minor in extracting Y, La, and Ce from contaminated gallery water was ascertained by Sasmaz et al. [56]. Comparing the results with the control samples, it was shown that *L. gibba* accumulated more metals than in *L. minor*. *Salvinia natans* and *L. minor* are two aquatic macropyhtes whose biological reactions and phytoremediation potential were examined by Leblebici et al. [57]. They discovered that *L. minor* was a better Cd accumulator than *S. natans*, although *S. natans* was a more effective Ni and Pb accumulator. According to Amare et al. [58], *L. minor* should be a moderately phytoaccumulator of Cd, Cu, Ni, and Cr but a high phytoaccumulator of Mn, Co, Zn, and Fe. According to Tatar et al. [59], *L. minor* has a high removed capacity for Ag, Hg, Mn, Pb, Zn, Fe, Ba, Sb, Co and P, while *L. gibba* has a good uptake capacity for Mo, Cu, Ca, Na, Mg, Se and S.

4. Conclusion

By comparing the studied plants with the control LM-0 and LG-0 samples, it can be observed that *L. minor* and *L. gibba* gathered respectively, 77 times and 84 times more Mo from acidic mine water. After days 4 and 5, both plants showed faster Mo-accumulating abilities, which persisted until the very end of the experiment. At the conclusion of the eight-day experiment, Mo in the 5561 L and 274 L acidic mining water had been removed, respectively, by *L. minor* and *L. gibba*. For eight days of the experiment, Pb was removed linearly by *L. gibba* and *L. minor* in acidic mineral water. Compared to control samples, *L. gibba* shown 30 times the Pb accumulation at the end of the experiment, and *L. minor* displayed 109 times the Pb accumulation. At the end of the experiment, Pb was also extracted from 291 L of acid mine water and 720 L of water by *L. gibba* and *L. minor*, respectively. In comparison to the control sample, *L. gibba* removed 495 times (9866 mg kg-1) on day 8 from acidic mine water, and *L. minor* accumulated 1150 times (12668 mg kg-1) on day 4. At the end of the study, *L. gibba* extracted Cu from 634 L acidic mine water. By the end of the 4-day experiment, 815 L acidic mine water had accumulated Cu in *L. minor*. Acidic mine waters contaminated with Mo, Pb, and Cu can be effectively purified using *L. minor* and *L. gibba*, which has proven to be a very efficient, economical, and environmentally safe approach. To prevent damaging the environment due to high concentrations of Mo, Pb, and Cu, after harvest, the biomass from *L. minor* and *L. gibba* growing in

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these waters must be immediately washed with strong acids. The metals must then be collected and used to boost the economy of the nation. It is recommended that this technique be implemented for all mining operations that utilize acidic mine water, and that suitable pools be constructed to enhance metal recovery procedures and supply nature with purer water.

Conflicts of Interest: The author declare no conflict of interest.

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