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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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Article

Accumulation Assessment of Mo^{4+}, Pb^{++}, and Cu^{++} in the Acidic Water of Copper Mines with *Lemna minor* and *Lemna gibba*

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Abstract: Pollutants accumulate in aquatic habitats due to mining activities. The Duckweed family includes water plants such as *Lemna gibba* and *Lemna minor*, which are tiny, delicate, free-floating aquatic plants. *L. minor* and *L. gibba* were used in this study to examine the accumulation capacities of Mo^{4+}, Pb^{++}, and Cu^{++} in acidic fluids from copper mining. Two reactors were assigned to *L. gibba* and *L. minor*, respectively. These plants and the reactor water were gathered daily for 8 days. Acid mine water pH, temperature, and electric conductivity were also tested daily. *L. gibba* and *L. minor* were cleaned, dehydrated, and burned in a drying oven for a whole day at 300°C. ICP-MS (Inductively Coupled Plasma Mass Spectroscopy) was used to determine the Mo^{4+}, Pb^{++}, and Cu^{++} content of plant and water samples. Mo, Pb, and Cu concentrations in copper mining acidic fluids were 30±4, 260±12, and 15535±322 µg L^{-1}, respectively. Regarding Mo, Pb, and Cu extraction from copper mining acidic fluids, *L. gibba* and *L. minor* performed more efficiently than control samples, gathering 29 and 177 times more for Mo, 30 and 109 times for Pb, and 495 and 1150 times for Cu, respectively. Considering these findings, *L. gibba* and *L. minor* are good plants for rehabilitating polluted waters and can efficiently remove 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 (AMD) is produced in numerous closed, abandoned, and operating mines worldwide. Regarding heavy metals (i.e., copper (Cu), lead (Pb), zinc (Zn), and cadmium (Cd)) and metalloids (i.e., arsenic (As)), AMD is a major environmental problem in the mining industry [1–9]. It is typically characterized by low pH and high salinity. Low pH dissolves heavy metals in the host rock due to sulfide mineral oxidation. Additionally, micro-organisms play a significant role in AMD development in situ [10–14]. In unsaturated zones, low pH results from the chemical reactions between oxygenated rainfall and pyrite [15,16].

Heavy metals have larger atomic weights and a density five times higher than water [17]. They were divided into two categories by Gergen and Harmanescu [18]. Rai et al. [19] claim that metals including Mo, Pb, Cu, Cd, Hg, Ni, As, Au, Ag, and Cr widely contaminate soil, water, and air, adversely affecting plants and animals. Although animals and plants need metals (Fe, Cu, Zn, Co, and Mn), but high amounts can be harmful. Therefore, heavy metal contamination is one of the primary issues affecting aquatic plants and animals [20]. Certain metals, including As, Tl, Hg, Pb, Cr, and Cd, are especially concerning for public health due to their highly hazardous levels [7]. Mo is an essential element for plants, animals, and humans [21,22]. Large amounts of waste and waste generated due to mining activities still constitute a permanent source of pollution for surface and groundwater in the region. This situation causes water quality to reach alarming levels, negatively affecting aquatic ecosystems [23]. Appropriate Cu content is essential for plant health and the nutrient supply to humans and animals. Certain plant species collect high quantities of Cu in their tissues and are highly tolerant of elevated Cu concentrations. All plants naturally contain lead (Pb), although its exact function in metabolism is unknown. Researchers have concluded that a concentration of 2–6

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μg/kg is adequate if plants require Pb. Consequently, Pb has garnered attention as a significant metallic compound with the potential to contaminate the environment and harm plants [24]. However, exposure to high doses of Mo, Cu, and Pb can be detrimental to animal, human, and plant health [25]. Yet, as with all elements, exposure to high doses of Mo, Cu and Pb can be detrimental to animal, human and plant health [26]. Mo distributions in freshwater systems related to the environment, human health, and water supply have received relatively little study. According to Smedley and Kinniburgh [26], most natural waters have Mo contents of no more than 10 μg/L^{-1}. The maximum permitted concentrations in drinking water are 0.01 mg/l for lead and 0.015 mg/l for copper, as per the US EPA [27] and World Health Organization [28].

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 [29–35]. Aquatic macrophytes gather pollutants and metals during the rhizofiltration stage [36]. *Lemna* sp. grows quickly, is easy to harvest, and many scientists favor it for phytoremediation research [37–39]. Among aquatic macrophytes, it is the most efficient plant for eliminating metals and pesticides due to its rapid growth and ability to float on water [40–42]. It also grows well in various climates and has a long storage capacity, quick reproduction rates, low cost, and little volume of biological and chemical sludge [39,42–44]. According to Khataee et al. [43], the ideal temperature and pH ranges for *Lemna* sp. rapid growth are 5–25°C and 4–9, respectively. Animals feed on *L. minor*, which is rich in protein (30% mass), minerals, and vitamins [45,46], but poor in fiber [47]. Eutrophication has caused *Lemna* to spread excessively, making it a scourge in many areas. An excessive amount forms a thick mat on aquatic bodies that impede movement, harbors dangerous wildlife, and blocks sunlight from reaching the photosynthetic species below. Consequently, the surrounding water is not oxygenated properly. In addition to being widely accessible, this plant material has potential as a long-term biosorbent for handling toxic-contaminated wastewater [48,49]. In this study, the daily accumulation of Mo, Pb, and Cu in *L. gibba* and *L. minor* grown was examined in the acidic mineral waters of the Maden copper deposit. At the same time, we calculated how many liters of water these plants cleaned of metals in a week. Additionally, we also evaluated harvested biomass with higher metal concentrations.

2. Material and Methods

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

2.1. The Study Area

The investigation was conducted in the Maden Cu mining area in Elazig, Turkey, which is situated at N38.388434° and E39.671450° (Figure 1). Mining has a lengthy history in this region, dating to prehistoric times at around 2000 BC. Massive sulfide ore (reserves of 6.5% Cu tenor and 6.1 million tons on average) was mined between 1968 and 1939 from Anayatak and its adjacent deposits. Modern Cu production began in 1939 by Etibank. One of Turkey's largest copper-producing regions, the Maden copper deposit spans various geographical regions, including Anayatak, Weis, Mızırtepe, Kısabekir, and Hacan. Black smoker, which is derived from hydrothermal vents on the seafloor, is thought to be associated with these deposits [50]. There are also significant reserves of Ni, Cu, Au, Co, and Ag in these deposits. Throughout the year, frequent water effluent from mining operations is seen at the mine site, which is released into the Maden Stream.

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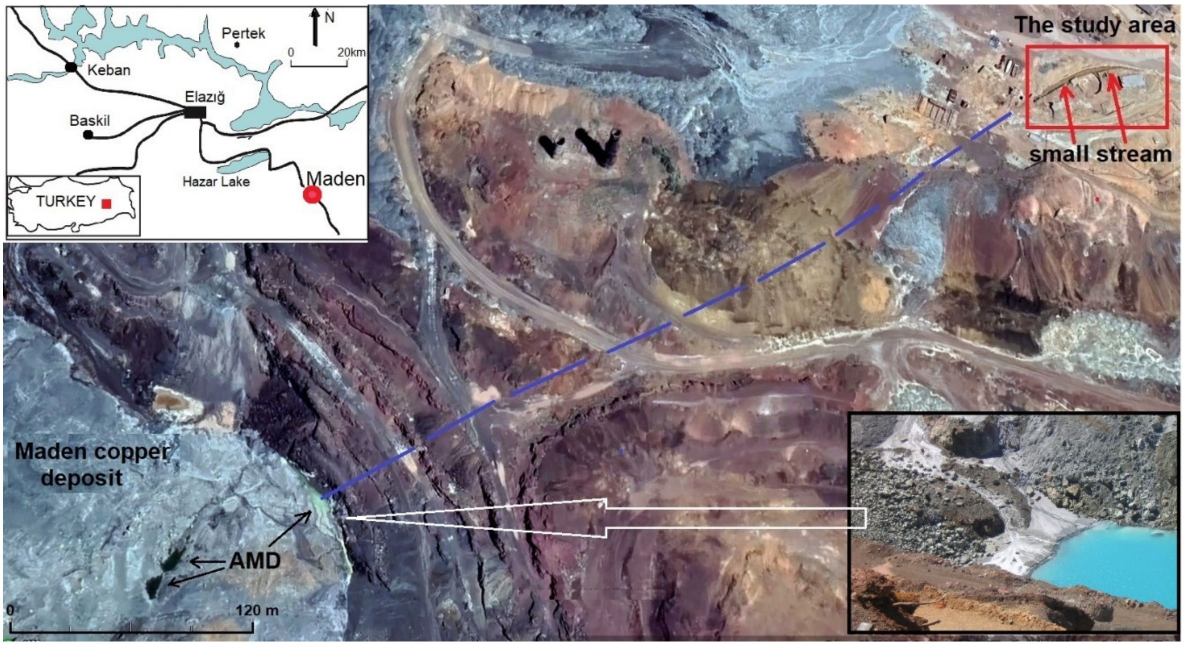

Figure 1. The experimental setup of this study (adapted from Sasmaz Kislioglu, 51).

2.2. Plant and Water Samples

According to *the East Aegean Islands and Flora of Turkey* [52], *L. minor* and *L. gibba* belong to the Duckweed family and are members of the Lemna genus [53]. They are divided into five genera: *Lemna, Spirodela, Landoltia, Wolffiella,* and *Wolffia*. These asexually reproducing floating aquatic plants develop offspring directly from their parents without a seed stage [54]. Water and plants were collected daily 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 daily using sterile plastic bottles to gather samples. The chemical composition of acidic water may vary due to extensive mineralized wall rock in the Cu mining area. These variables may affect the T^{o}C, pH of the water, and EC (electrical conductivity). The temperature, pH, and electrical conductivity were recorded using an Orion conductivity electrode. Cation and anion analyses (such as carbonate, nitrate, sulfate, and fluoride) were conducted using an ICP-MS.

2.3. Analytical Method

L. gibba and *L. minor* were cultivated independently in two natural pools prior to moving to separate reactors. The plants were brought from the Botanical Garden at Istanbul University. As detailed by Tatar and Obek [34], each reactor contained 500 grams of plants, with dimensions of 70 x 35 x 30 cm (Figure 2), with *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 had a continuous flow of acidic mineral water at 1.28 L sec^{-1} (Figure 2). About 50 grams of plant material were removed daily from each reactor for eight days. *L. gibba* and *L. minor* changed color from green to yellow toward the end of the experiment. Possibly due to widespread heavy metal concentrations in water, toxic effects appeared on plants (Figure 3). After collection, the plants were cleaned with tap water, rinsed with distilled water, and dried for 24 hours at 60^{o}C in a laboratory oven. The dried plants were then 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 another hour at 95^{o}C in a mixture of HNO_3: H_2O: HCl (1:1:1) with one gram of the ash sample. Lastly, ICP-MS methods were used to examine all samples for Mo, Pb, and Cu. Figs. 4, 5, and 6 provide dry-weight values. Standard solutions for each element were created from stock solutions (E. Merck, Darmstadt, Germany). Since Ir's mass equals the mass of every element under study, an internal standard of 10 mg L^{-1} Ir (E. Merck, Darmstadt, Germany) was used. The water samples were prepared with Merck's concentrated HNO_3.

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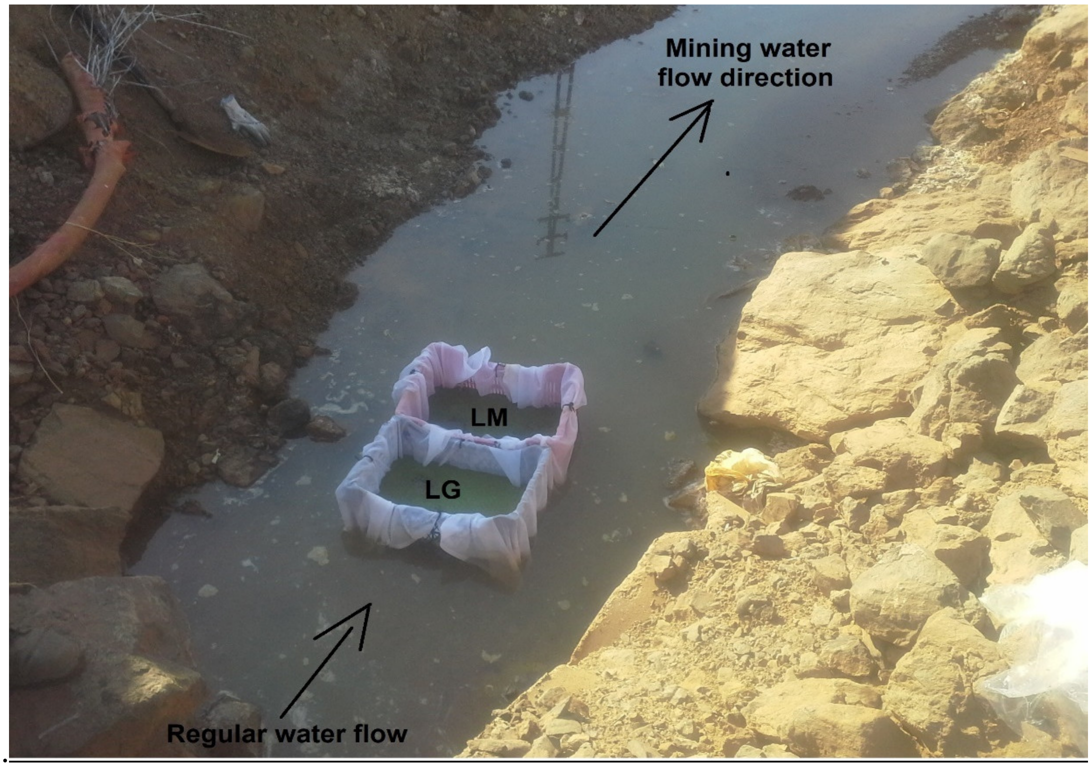

Figure 2. Reactors assigned to *L. gibba* and *L. minor* in acidic mine water (adapted from Sasmaz Kislioglu, 51).

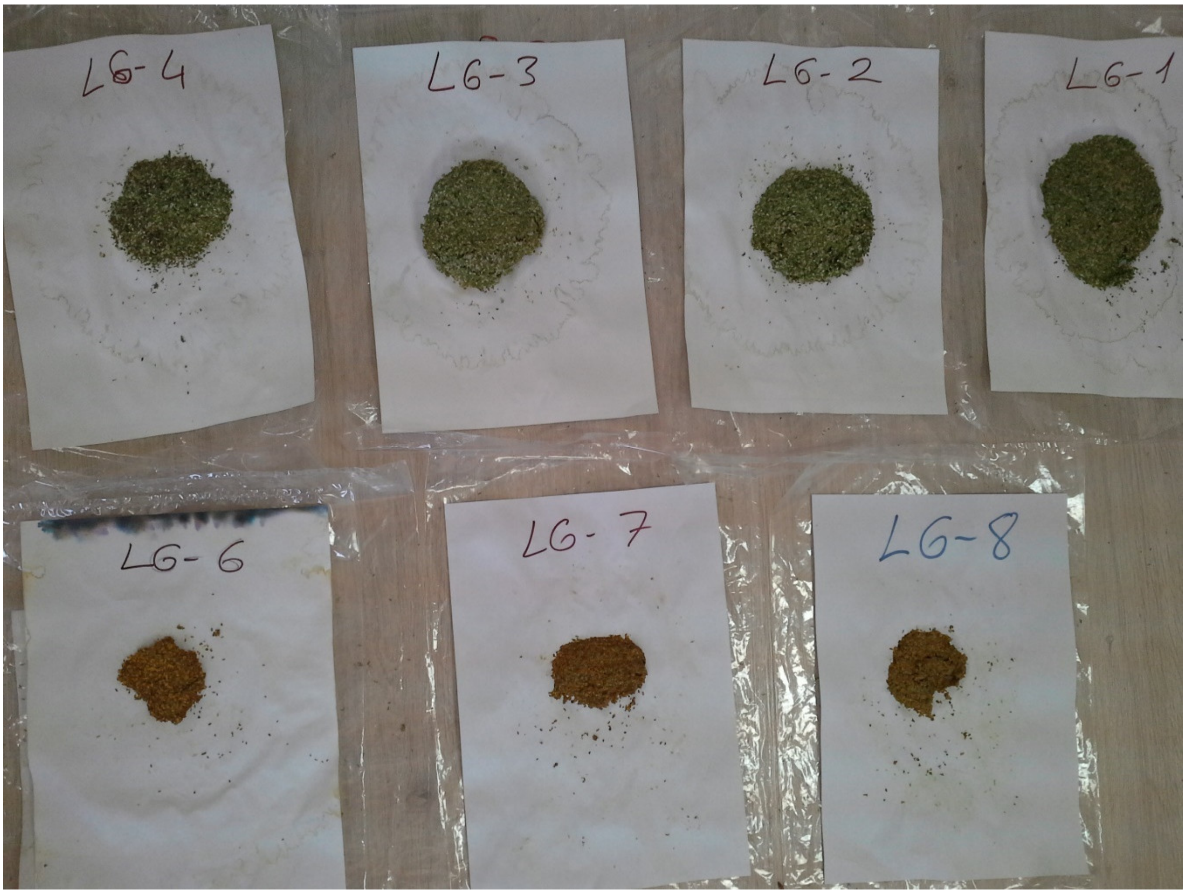

Figure 3. *L. gibba* and *L. minor* changed color from green to yellow toward the end of the study.

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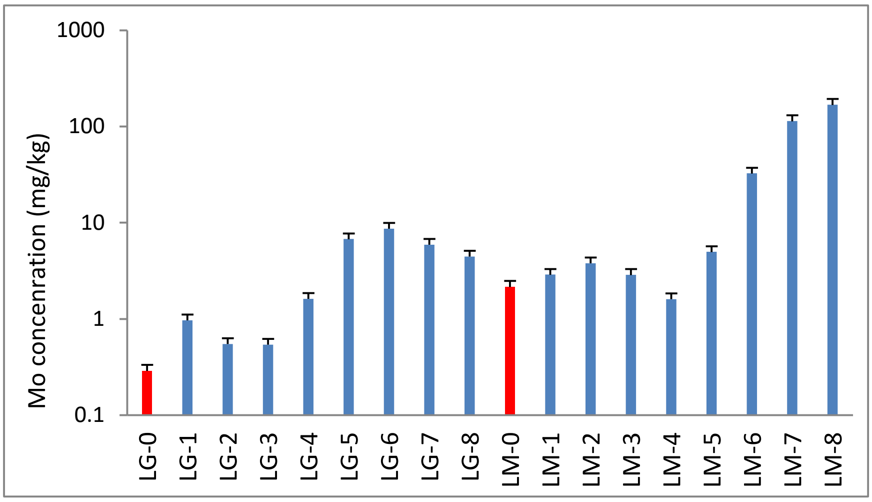

Figure 4. Mo accumulation ratios by *L. gibba* (LG) and *L. minor* (LM).

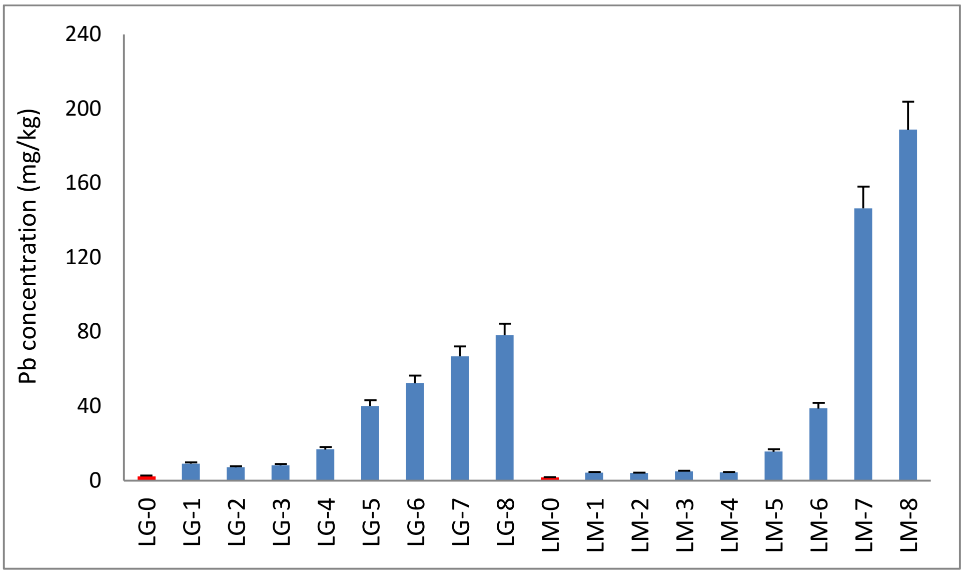

Figure 5. Pb accumulation ratios by *L. gibba* (LG) and *L. minor* (LM).

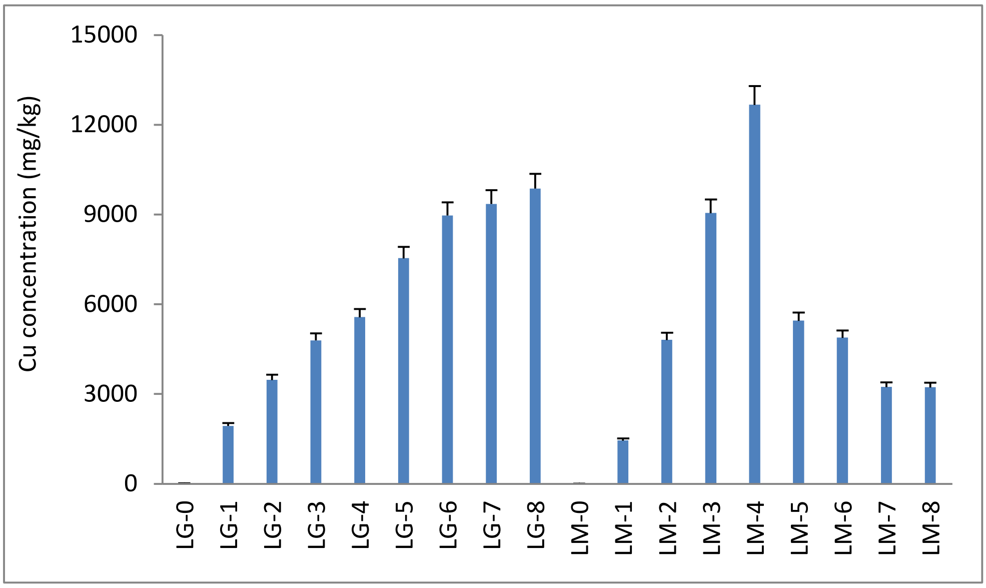

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Figure 6. Cu accumulations by *L. gibba* (LG) and *L. minor* (LM).

3. Results and Discussion

3.1. Mo, Pb, and Cu in Acidic Mining Water

The principal anion and cation results of acidic 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 from 5.84 to 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}, referenced from Sasmaz Kislioglu [51]). Throughout the eight-day experiment, daily field water samples of water were collected. Table 1 displays the average concentrations of Mo, Pb, and Cu in acidic mine water, which were 30±4, 260±12, and 15535±322 µg L^{-1}, respectively ($p < 0.05$). The chemistry of acidic mine water is influenced by several factors, including its distance from the recharge area, the length of time dedicated to the flow system, the volume of acidic mine water flowing through it, and long-term rock–water interaction. According to the measured data, the chemistry and physicochemical properties of water originating from the ore location are generally comparable. Significant pollution near the Maden stream is caused by heavy metal pollution on land and in the water.

Table 1. Physicochemical characteristics, including cation, anion, and trace element results in acidic mine water [51].

Parameter	T	pH	EC	HCO_3	NO_3^-	SO4	F	Ca	Mg	K	Na	Fe
	(°C)		(mScm^{-1})	(mgL^{-1})	(mgL^{-1})	(mgL^{-1})	(mgL^{-1})	(mgL^{-1})	(mgL^{-1})	(mgL^{-1})	(mgL^{-1})	(mgL^{-1})
Detection Limit	-	-	-	-	-	-	-	0,05	0,05	0,05	0,05	10
Mining water	22,6	5,76	2,55	282	1,86	128	0,41	482	426	5,8	115	118
	±1.6	± 0.1	± 0.2	±16	± 0.06	±8	±0.1	±24	±18	±0.3	±6	±7
Parameter	Mn	S	P	B	Zn	Cr	Ni	Co	As	Mo	Pb	Cu
	(mgL^{-1})	(mgL^{-1})	(µgL^{-1})	(µgL^{-1})	(µg L^{-1})	(µgL^{-1})	(µgL^{-1})	(µgL^{-1})	(µgL^{-1})	(µg L^{-1})	(µg L^{-1})	(µg L^{-1})
Detection Limit	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

The mean values of Mo, Cu, and Pb in acidic mine fluids exceeded the US EPA's [27] and ATSDR's [44] limit levels, as shown in Table 1. The research area's acidic 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 [26]. The research area's average Mo value was higher than the WHO-established threshold levels (10 mg L^{-1}) for drinking water [23] (Table 1). According to the US EPA [25], the average Pb levels in these natural waters have been recorded as 10–15 µg/L [28]. Water leaks from the mine contaminate the environment's soil and water, and cleaning these contaminated soils and waterways is difficult [55,56]. According to Ning et al. [57], the average WHO readings [28] 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 [58] for stream waters worldwide. The estimates for world rivers are between 0.11 and 8.63 (mean 1.21 mg/L) [50] and around 0.42 mg/L [59,60]. Rivers in India contain up to 20 mg/L [61] and 8.6 mg/L [62].

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 using Piper's [63] triangular drawing approach. Over 90% of the cations in the aquifer were found in the examined fluids, with Ca, Mg, Fe, Na, S, K, and Mn being the most common. In the research area

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waters, bicarbonate and sulfate were the main anion species, constituting 85–90% of all anions. Ca–Mg–Fe–Na–SO_4 HCO_3 water is one possible classification for Maden Cu mine acidic water.

3.2. Lemna gibba (LG) and Lemna minor (LM)

Cleaning and restoring contaminated areas can be done affordably, effectively, sustainably, and economically with phytoremediation. Before building a decontamination system, knowledge regarding heavy metal effects on plant physiology should be acquired to optimize the system [64]. The uptake process of Mo, Pb, and Cu can be impacted by variables such as the metal's bioavailability, the contaminant's chemical characteristics, organic matter contents, plant species, phosphorus, pH, and contaminant-specific environmental factors [65]. Numerous aquatic plants have been successfully employed to monitor contaminated settings and are recognized as heavy metal pollution indicators [66]. Heavy metals such as Mo, Ag, Pb, Au, Cu, As, Co, Hg, Zn, Tl, and Cd are considered hazardous and poisonous for their ability to accumulate in biological systems.

Prior to commencing the investigation, we 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 were considered the control group values of these plants. On the first day, 2.89 and 0.97 mg kg^{-1} ($p < 0.05$) of Mo were collected from *L. minor* and *L. gibba*. During the first five to six days of the experiment, both plants' absorption of Mo from acidic mining water marginally increased. On the fifth and sixth days, *L. gibba* and *L. minor* removed 84 and 77 times more Mo than the control from acidic mine water. *L. gibba* showed outstanding Mo accumulation ability between days 5 and 7. *L. minor* accumulated rapidly after the fifth day until the end of the experiment. On the eighth day, it accumulated 169 ppm Mo, which corresponds to approximately 77 times more Mo accumulation than the control sample. To determine how much water the *Lemna minor* plant cleaned on the eighth day, the control concentration (2.16 mg kg^{-1}) was subtracted from the eighth day *Lemna minor* concentration (169 mg kg^{-1}). Then, the resulting value was divided by the Mo value (30 μg L^{-1}) in one liter of water (=169.000-2.160/30) to determine how much water (5561 L) the plant cleans of Mo. *L. gibba* removed molybdenum from 274 L of acidic mineral water at the end of the sixth day of the study.

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

Despite the low lead content (260 μg L^{-1}) of acidic mine water used in this study, *L. gibba* and *L. minor* extracted Pb from 291 L and 720 L, respectively.

L. gibba regularly showed significant increases in copper accumulation throughout the experiment, accumulating 9866 ppm ($p < 0.05$) on the last day of the experiment. This increase corresponds to a 495-fold copper accumulation compared to the control group. *L. minor* showed substantial accumulation ability during the first four days of the experiment. By the end of the fourth day, it had accumulated 12668 ppm of copper. This value indicates 1150 times more accumulation than the control samples. Between the fifth and eighth days, *L. minor* accumulation levels decreased because the plant was sufficiently saturated with copper (Figure 6).

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

Plants such as *L. minor* and *L. gibba* were used by Sasmaz et al. [33] to determine metal accumulation rates and optimal harvesting times in gallery water from the Keban Pb–Zn mine. The pH of gallery water is 7.36 and has a neutral composition. Both plants achieved higher accumulations in acidic waters than in neutral mineral waters of the Keban Pb–Zn mine. They determined optimal harvesting times by monitoring daily changes in the metallic concentrations of 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 greater than those found in gallery water, respectively.

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In the same experiment, Sasmaz Kislioglu [51] examined the Ag, Au, and As accumulations of *L. minor* and *L. gibba* in acidic mineral water. Compared to the control samples of these plants, *L. minor* and *L. gibba* showed effective and high accumulation abilities for As, Au, and Ag in the acidic water of Cu mining areas. For instance, 30 and 907 times for As, 336 and 394 times for Au, and 240 and 174 times for Ag, respectively.

For eight days, Sasmaz and Obek [67] gathered evidence of *L. gibba*'s ability to extract As, U, and B from secondarily treated urban wastewater. During the first two days of the study, *L. gibba* showed the highest uptake ratios for B, U, and As with removal rates of 40%, 122%, and 133%, respectively. These results imply that *L. gibba* may be a natural strategy for reducing the amount of these pollutants in wastewater. *L. minor* has a higher capacity for collecting lower amounts of Cr and Ni, according to Goswami and Majumder [29]. Furthermore, Au and Ag uptake from secondarily treated municipal wastewater by *L. gibba* was examined by Sasmaz and Obek [68]. Within six days of their investigation, both Au and Ag accumulated rapidly. However, Ag and Au accumulations fluctuated after day 6, perhaps because the plant had reached saturation. The greatest accumulations of Au and Ag on the fifth and sixth days of the study were 2303% and 247%, respectively. Uysal [69] investigated *Lemna*'s capacity to sorb Cr at various pH and concentration levels and found that they could still absorb Cr from water despite undergoing harmful consequences. During the 12-day experiment, Abdallah [70] noted that *L. gibba* performed 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 it can absorb these metals quickly and efficiently, according to Ucuncu et al. [71]. According to Goswami et al. [30], *L. minor* adequately corrected low-concentration As-contaminated waters. *L. gibba* and *L. minor's* effectiveness in extracting Y, La, and Ce from contaminated gallery water was ascertained by Sasmaz et al. [72]. *L. gibba* accumulated more metals than *L. minor* when compared to the control samples. *Salvinia natans* and *L. minor* are two aquatic macrophytes whose biological reactions and phytoremediation potential were examined by Leblebici et al. [73]. They discovered that *L. minor* was superior to *S. natans* as a Cd accumulator, while *S. natans* was a more effective Ni and Pb accumulator. According to Amare et al. [74], *L. minor* should be a moderate phytoaccumulator of Cd, Cu, Ni, and Cr but a high phytoaccumulator of Mn, Co, Zn, and Fe. According to Tatar et al. [75], *L. minor* has a high removal 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.

Conclusion

In this study, the daily accumulation of Mo, Pb, and Cu by *L. gibba* and *L. minor* in the Maden copper deposit's acidic mineral waters was examined. By comparing the studied plants to the control LM-0 and LG-0 samples, we observed that *L. minor* and *L. gibba* gathered 77 and 84 times more Mo from acidic mine water, respectively. After days 4 and 5, both plants showed increased Mo-accumulating abilities, which persisted until the end of the experiment. At the conclusion of the eight-day experiment, Mo in 5561 L and 274 L acidic mining water was removed by *L. minor* and *L. gibba*, respectively. After 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* showed 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 acidic mine water and 720 L of water by *L. gibba* and *L. minor*, respectively. Compared to the control sample, *L. gibba* removed Pb 495 times (9866 mg kg^{-1}) on day 8 from acidic mine water, and *L. minor* accumulated Pb 1150 times (12668 mg kg^{-1}) on day 4. At the end of the study, *L. gibba* extracted Cu from 634 L of acidic mine water. At the end of the 4-day experiment, 815 L of acidic mine water had accumulated Cu in *L. minor*. Acidic mine water contaminated with Mo, Pb, and Cu can be effectively purified using *L. minor* and *L. gibba*, which are proven to be efficient, economical, and environmentally safe. To prevent environmental damage due to high concentrations of Mo, Pb, and Cu, *L. minor* and *L. gibba's* biomass in these waters must be immediately washed with strong acids after harvest. The metals must then be collected and used to augment the national economy. We recommend implementing this technique for all mining operations utilizing acidic mine water and constructing suitable pools to enhance metal recovery procedures and supply nature with pure water.

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

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