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Phycoremediation of Thallium Toxic Metal Present in Gallery Water of an Abandoned Mine Area by Algae *Cladophora fracta*

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Highlights:

- Uptake of thallium by *Cladophora fracta* in mine water was determined.
- The accumulation of thallium by *Cladophora fracta* was 900% at 120 min
- It was determined that *Cladophora fracta* can be used in phycoremediation

ABSTRACT:

In this study, phycoremediation of thallium toxic metal present in galery water of an abandoned mine area was investigated by using *Cladophora fracta*. Within the scope of the study, a reactor containing *Cladophora fracta* was used and it was determined whether the *Cladophora fracta* accumulated thallium depending on time. Additionally, the bioconcentration factor was calculated. According to research findings; the accumulations of thallium by *Cladophora fracta*, compared with uncontaminated alga, were 225% at 5 min, 450% at 10 min, 550% at 20 min, 575% at 40 min, 700% at 60 min, and 900% at 120 min, respectively. BCF values were between 1000-5000. This indicated that *Cladophora fracta* had bioaccumulation potential. As a result, this research carried out in mining area has documented the phycoremediation of thallium in gallery water of an abandoned mine area.

Keywords:

- Algae
- Metal
- Phycoremediation
- Thallium
- Toxic

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INTRODUCTION

Mining, which is an important industry branch worldwide, makes a significant contribution to economy of many countries as it generates billions of dollars annually. But mining is not considered an environmentally sustainable industry (Kamal et al., 2017). Increasing mining operations cause concern for the environment and health (Palmer et al., 2015). The mining industry affects the environment in the following ways: expose toxic elements, increase the risk of contamination of nearby ground and surface waters, remove topsoil, disrupt the existing ecosystems, damages landscapes by creating erosion and deplete surrounding freshwater sources (Kamal et al., 2017). Many of the concerns for health and environment are related to mine water issues (Palmer et al., 2015). Mining industry waters includes various heavy metals some has properties of toxicity/poisonous. Metals have become a dominant pollutant of water due to the continuous growth in industrialization and urbanization (Mwandira et al., 2020). Heavy metal pollution can have a negative effect on freshwaters (e.g. rivers, lakes, dams, and underground aquifers (Zvinowanda et al., 2009). Toxic heavy metals accumulate when they pass body and cause health problems (Mwandira et al., 2020). Heavy metal toxicity can lead to the following health problems: lower energy levels and central nervous function, reduced mental and lungs, kidneys damage to blood composition, and other vital organs (Amarasinghe and Williams, 2007; Zvinowanda et al., 2009).

Thallium (Tl) is a rare heavy metal. Tl averages 0.490 mg per kg in the Earth's crust (Peter and Viraraghavan, 2005). The USEPA guideline for Tl in drinking water are at 0.5 (objective) and 2.0 µg/L (Belzile and Chen, 2017). Tl is emitted anthropogenically via the mining (Turner et al., 2013). Tl has been listed as a priority pollutant worldwide (Li et al., 2020a). It is more toxic than mercury, cadmium, and lead, even when present at very trace levels in waters (Liu et al., 2018; Liu et al., 2019). Tl is toxic to plants, animals and humans (Turner et al., 2013). Exposure to thallium can lead to acute or chronic poisoning (Peter and Viraraghavan, 2005). Thallium can affect the respiratory, cardiovascular and gastrointestinal (Wang et al., 2020). The use of thallium compounds in industrial activities can pose a threat to human health and the environment. Therefore, new technologies should be developed to control thallium pollution (Li et al., 2020b). The main thallium removal technologies reported includes solvent extraction (Chung et al., 2003; Rajesh and Subramanian, 2006; Hassanien et al., 2017), adsorption (Wan et al., 2014; Huangfu et al., 2017; Zhang et al., 2018), ion exchange (Li et al., 2017), coagulation (Huangfu et al., 2017), chemical oxidation and precipitation (Liu et al., 2017; Li et al., 2019; Li et al., 2020b; Li et al., 2020a; Wang et al., 2020). However, these technologies have various disadvantages. Among the treatment methods, biological technologies stand out with many advantages. A technique used in the removal of heavy metals in aquatic or terrestrial environments is bioremediation (Lovley and Coates, 1997; Malik, 2004; Li et al., 2015). Bioremediation is the process of reducing the concentration of pollutants by microorganisms or converting them into less hazardous products. Bioremediation technique uses natural biological mechanisms to destroy dangerous pollutants, using microorganisms and plants or their products to return contaminated environments to their original state (Ayangbenro et al., 2017). The availability of various organisms such as bacteria, fungi, algae and plants has been reported for the biological treatment of contaminants (Karigar et al., 2011). The conventional techniques are often more expensive and ineffective for removal of metal pollution (Ayangbenro et al., 2017).

Phycoremediation is used for the removal or biotransformation of pollutants, including heavy metals from wastewater (Podder and Majumder, 2016). While microalgae or macroalgae are growing they could able to remove heavy metals from wastewater (Ahmad et al., 2013; Samal et al., 2020).

Algae, due to their negatively charged cell surfaces and their large cell surface to volume ratio, display ideal properties for intra- and/or extra-cellular adsorption of heavy metals (Wilde and Benemann, 1993; Li et al., 2015). Live algae possess intracellular polyphosphates which participate in metal sequestration, as well as algal extracellular polysaccharides that serve to chelate or bind metal ions (Gardea-Torresdey et al., 1998). Leaching water containing thallium from both abandoned and operated mines must be rationally treated before reaching the environment. Ecofriendly and cost-effective technologies have to be developed for the removal of the poisonous heavy metal thallium. Therefore, in this study biotechnological application for remediation of poisonous metal thallium in an abandoned mine area gallery water (MAGW) is assessed by the usage of macroalgae *Cladophora fracta*.

MATERIALS AND METHODS

Study Area

The study area is located in Elazig, Turkey (Figure 1). MAGWs are discharged to the Keban Dam Lake. The discharge of the MAGWs adversely affects water quality and causes environmental problems in Fırat River and Karakaya Dam Lake. Therefore, it is necessary to treat the MAGWs.

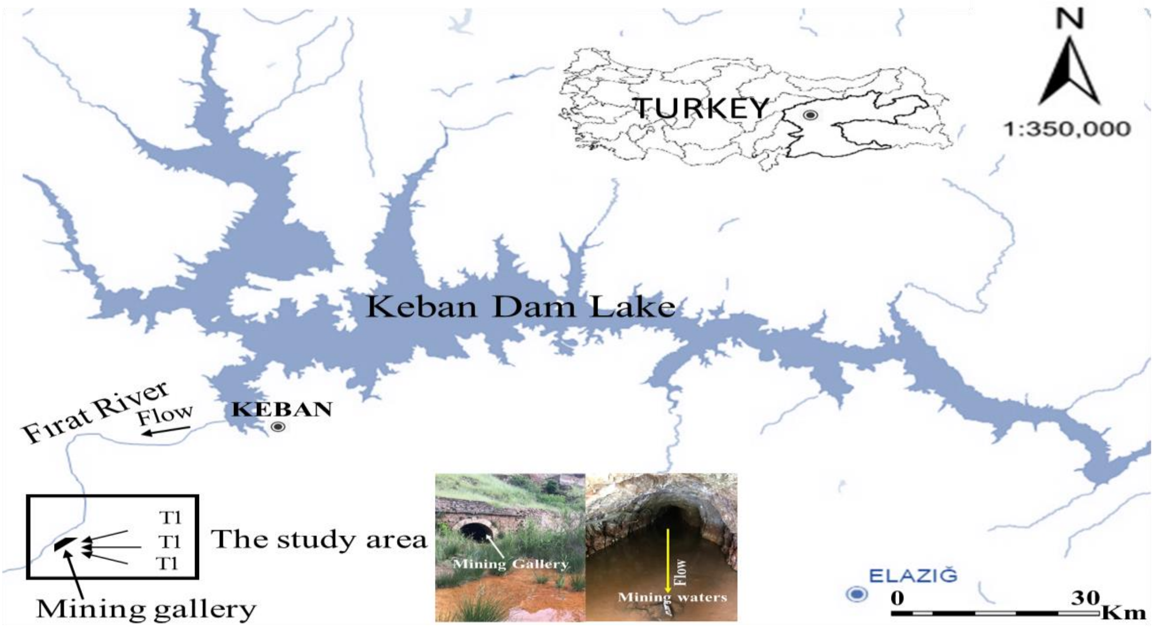

Figure 1. The Study Area

Sampling and analysis

Freshwater algae (*Cladophora fracta*) are collected from Lake Hazar (Elazig, Turkey). The reactors' size used in the study was 51x37x27cm (Figure 2). Algae were added to the reactors. The study period was 7 days. 100 gr algae was harvested first in minutes and then daily. MAGWs were taken as 250 ml. The reactors were put into the MAGWs in the real outdoor condition. Algae were dried and pulverized. The analysis procedure for algae as follows: the algae sample was cold leached with HNO_3. Aqua Regia solution of equal parts of concentrated HCl, HNO_3, and DI H_2O were added to each sample to leach in a heating block of the hot water bath. The sample was made up to volume with dilute HCl before being filtered. Then, algae and MAGWs were analyzed by ICP/MS (ICP/MS-Perkin-Elmer ELAN 9000) in a laboratory with ISO 9001:2008 accreditation. In algae and MAGWs, thallium concentrations were determined.

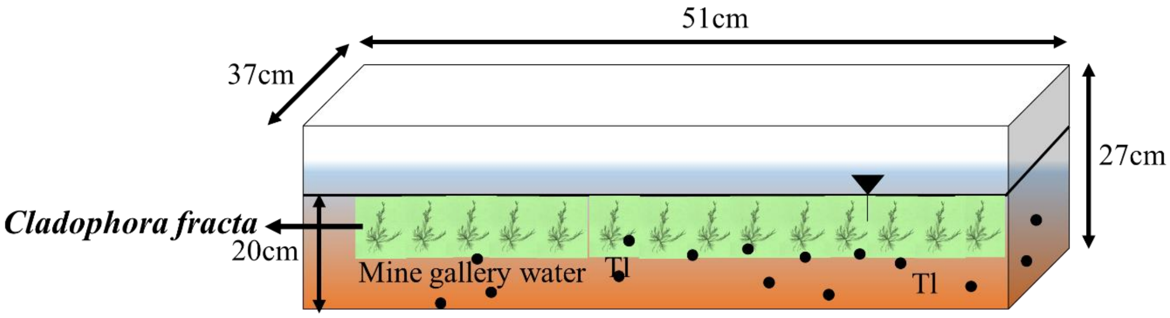

Figure 2. The Reactors

BCF (bioconcentration factor)

BCF is the rate of thallium value in algae to thallium value in MAGWs. It is an indicator of the bioaccumulation potential of thallium by *Cladophora fracta*. BCF is calculated as follows;

$$BCF = C_{CF}/C_{MW} \quad (1)$$

where C_{CF} is the thallium value in the *Cladophora fracta* and C_{MW} is the thallium value in mine gallery water.

Uptake of thallium by *cladophora fracta*

The uptake of thallium by *Cladophora fracta* is calculated as follows;

$$AC\ (\%) = \frac{C-C0}{C0} x100 \quad (2)$$

where, AC= accumulation capacity (%), C=thallium values in algae (mg/kg), and C_o = uncontaminated algae (mg/kg)

Statistical analysis

The statistical analysis was carried out using IBM SPSS Statistics 21 program (USA). The results were analyzed by a Pearson test to determine the relationship between thallium in *Cladophora fracta* according to different times.

RESULTS AND DISCUSSION

EC and pH values, concentrations of Tl were determined in the MAGWs. The EC value ranged from 2.32 to 2.54 mS/cm (average EC=2.41 mS/cm). pH value of the gallery water ranged from 7.38 to 7.49 (average pH = 7.44). The average of Tl concentration in MAGWs was determined as 1.81±0.09 µg/L. The *Cladophora fracta* was investigated for the bioaccumulation of thallium in MAGWs. The value of thallium in *Cladophora fracta* was determined in the uncontaminated area. The determined value was used as control values. Thallium accumulation by *Cladophora fracta* is shown in Figure 3.

Compared to control (0.04±0.02 mg/kg), maximum value of thallium by *Cladophora fracta* in reactors was 900% (0.4±0.02 mg/kg) for 120min and minimum accumulation of thallium was determined as 225% (0.13±0.01 mg/kg) for 5 min.

The accumulation of thallium concentrations by *Cladophora fracta* increased continuously for 120 min. The concentrations of thallium by *Cladophora fracta*, compared with uncontaminated alga (0.04 mg/kg), were 3.25 times at 5 min, 5.5 times at 10 min, 6.5 times at 20 min, 6.75 times at 40 min, 8 times at 60 min and 10 times at 120 min, respectively. Thallium accumulation were 120 min> 60 min > 40 min > 20 min > 10 min > 5 min, respectively (Fig 3a). According to Figure 3b, compared to control (0.04±0.02 mgkg), maximum accumulation of thallium by *Cladophora fracta* in reactors was 5025% (2.05 mg / kg) for on 5 day and minimum accumulation was 3575% (1.47 mg/kg) for on 7 day.

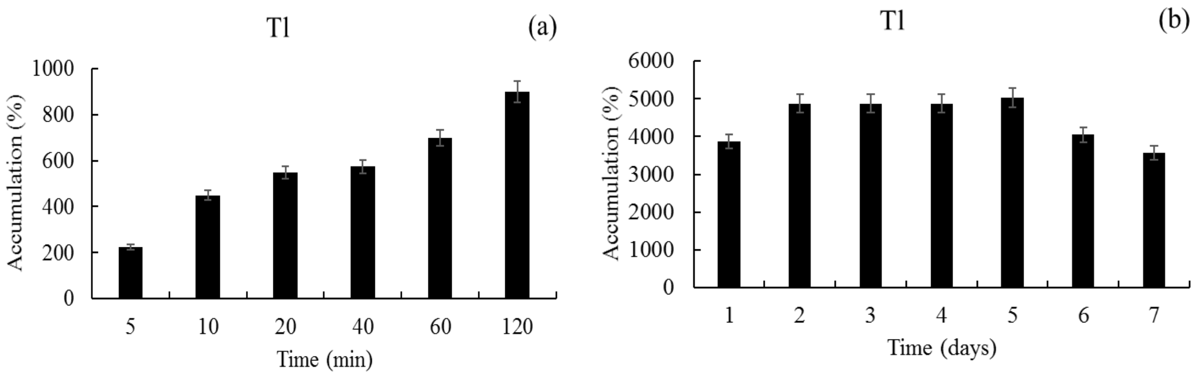

Figure 3. Accumulations of Thallium by *Cladophora Fracta*

Accumulation of thallium concentrations by *Cladophora fracta* increased for the first day 5 and showed a decreasing trend from day 5. This showed that *Cladophora fracta* tends to accumulate the thallium in MAGWs for the first day 5. The concentration of thallium by *Cladophora fracta*, compared with uncontaminated alga (0.04 mg/kg) were 39.75 times on day 1, 49.75 times on days 2, 3, and 4, 51.25 times on day 5, 41.5 times on day 6 and 36.75 times on day 7, respectively. Thallium accumulation were 5 > 4 = 3 = 2 > 6 > 1 > 7 day (Fig 3b). Thallium accumulation was high by *C. fracta*. *Cladophora* sp. is known as a good scavenger of toxic metal ions in a short time (Michalak and Messyasz, 2021). Because, *Cladophora* species are characterized by a high tolerance to toxic metal ions (Zbikowski et al., 2017). Macroalgae have developed a number of defence mechanisms against high concentrations of toxic metals (Michalak and Messyasz, 2021). Zhang et al. (2019) reported that toxic metal ions can be accumulated in the cell wall of algae during accumulation. In the literature, different Tl concentrations from our results were reported in various algae species because of the different species and conditions. In the study of Queirolo et al. (2009) on the presence of Tl in the vicinity of a mining-impacted region in Chile, Tl varied from 0.295 to 8.3 μg/g in algae (*Myriophyllum acuaticum*, *Zannichellia palustris* L.). Turner et al. (2013) reported Tl concentrations as 39.4 ± 10.8 μg/kg and 19.4 ± 2.1 μg/kg in macroalga *F. ceranoides* and *F. vesiculosus* from estuaries of southwest England, respectively. When Birungi and Chirwa (2015) used green micro-algae for adsorption and removal of Tl from eutrophic waters, the sorption capacity of algae was between 830 and 1000 mg/g. Furthermore, in the literature, high metal accumulations by various organisms were also reported. Lakra et al. (2017) reported heavy metal accumulations by *Salvinia molesta* and *Pistia stratiotes*. Topal et al. (2020) reported accumulation of precious metals by algae. Şentürk et al. (2023) reported effective bioaccumulation of heavy metals by *P. stratiotes.*

BCF values for *Cladophora fracta* are shown in Table 1.

Table 1. BCF Values of Thallium for *Cladophora Fracta*

BCF values			
Time (min)	**BCF**	**Time (day)**	**BCF**
5	71.8	1	878.4
10	121.5	2	1099.4
20	143.6	3	1099.4
40	149.1	4	1099.4
60	176.7	5	1132.5
120	221	6	917.1
		7	812.1

BCF=1000-5000; bioaccumulation. BCF<1000; non-bioaccumulation. BCF>5000; very bioaccumulation (BA, 2019). When BCF given in Table 1 were examined, according to minutes, the highest BCF value was determined as 221 for 120 min while minimum BCF value was determined as 71.8 for 5 min. BCF values for 10, 20, 40 and 60 min were lower than 1000. This indicated that *Cladophora fracta* was non-bioaccumulation potential according to minutes. BCF values were 5 min < 10 min < 20 min < 40 min < 60 min < 120 min, respectively. According to days, the highest BCF value was determined as 1132.5 for day 5. Similar bioaccumulation was reported by Turner and Furniss (2012). They reported BCF for Tl accumulated under laboratory conditions by the green macroalga, *Ulva lactuca*, of about 10^3 in both sea water and estuarine water.

The minimum BCF value was determined as 812.1 for day 7. BCF values for 2, 3, 4, and 5 days were between 1000-5000. This indicated that *Cladophora fracta* had bioaccumulation potential according to days. BCF values were 7<1<6<2=3=4<5 days.

The relationship between thallium concentrations is given in Table 2 and 3.

Table 2. The Relationship Between Thallium Concentrations Detected in Algae According to Minutes

		min5	min10	min20	min40	min60	min120
min5	Pearson Correlation	1					
	Sig. (2-tailed)						
min10	Pearson Correlation	,655	1				
	Sig. (2-tailed)	,546					
min20	Pearson Correlation	-,866	-,189	1			
	Sig. (2-tailed)	,333	,879				
min40	Pearson Correlation	,961	,839	-,693	1		
	Sig. (2-tailed)	,179	,367	,512			
min60	Pearson Correlation	-,866	-,945	,500	-,971	1	
	Sig. (2-tailed)	,333	,212	,667	,154		
min120	Pearson Correlation	,961	,419	-,971	,846	-,693	1
	Sig. (2-tailed)	,179	,725	,154	,358	,512	

Table 3. The Relationship Between Thallium Concentrations Detected in Algae According to Days

		day1	day2	day3	day4	day5	day6	day7
day1	Pearson Correlation	1						
	Sig. (2-tailed)							
day2	Pearson Correlation	-,786	1					
	Sig. (2-tailed)	,425						
day3	Pearson Correlation	-,189	,756	1				
	Sig. (2-tailed)	,879	,454					
day4	Pearson Correlation	-,839	,996	,693	1			
	Sig. (2-tailed)	,367	,058	,512				
day5	Pearson Correlation	,189	-,756	-1,000**	-,693	1		
	Sig. (2-tailed)	,879	,454	,000	,512			
day6	Pearson Correlation	,614	-,971	-,891	-,945	,891	1	
	Sig. (2-tailed)	,579	,154	,300	,212	,300		
day7	Pearson Correlation	,189	-,756	-1,000**	-,693	1,000**	,891	1
	Sig. (2-tailed)	,879	,454	,000	,512	,000	,300	

**Correlation is significant at the 0.01 level (2-tailed).

There was no important correlation between 10-120 min. Correlations between 20min-5min, 20min-10min, 40min-20min, 60min-5min, 60min-10min, 40min-60min, 120min-20min and 120min-60min were found to be negative. Correlation between 5min-40min (r=0.961) and 5min-120min (r=0.961) was observed to be positive and important (Table 2). There was no significant correlation between 5-1 and 7-1 days. Correlations between day2-day1, day3-day1, day4-day1, day5-day2, day5-day3, day5-day4, day6-day2, day6-day3, day6-day4, day7-day2, day7-day3, and day7-day4 were found to be negative. Correlation between day5-day3 (r=-1.000) and day7-day3 (r=-1.000) was observed to be negative, but it was important (Table 3). Correlation between day5-day7 (r=1.000) was observed to be positive and important (Table 3). The fact that r values were close to 1 in the relationship between the Tl in algae indicated a strong relationship between these times.

CONCLUSION

Contaminated waters leaching from abandoned mine without treatment may cause a negative impact on livings, resulting in disturbance of the ecosystems. Thallium present in the leaching waters from the abandoned mine poses a great threat to water safety and undirectly food chain. Therefore, leaching water containing thallium from both abandoned and operated mines must be rationally treated before reaching the environment. Ecofriendly and cost-effective technologies have to be developed for the removal of the poisonous metal thallium. In this study, to achieve this goal, the alga-mining water system in the natural environment was used. The outcome of the assessment of biotechnological application according to bioconcentration factors has indicated bioaccumulation potentials of *Cladophora fracta* for poisonous metal thallium. According to the data obtained as a result of the study, the remarkable part of the study is that the thallium in the gallery water was accumulated in very high concentrations by the algae. Additionally, the difference of the study from other studies is that it was carried out by placing the reactor in a real mining area. In future studies, it can be determined whether thallium accumulates in abandoned mine sites by using different algae species. The size or shape of reactors can be changed or improved. In this way, the findings can be improved by comparing them with the findings in this study.

Conflict of Interest

The article authors declare that there is no conflict of interest between them

Author's Contributions

The authors declare that they have contributed equally to the article

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