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ARAŞTIRMA MAKALESİ RESEARCH PAPER

Comparison Of Heavy Metal Absorption Of Some Algae Isolated From Altınapa Dam Lake (Konya)

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Abstract: Today, due to the intense pollution in water resources, clean water shortage is experienced in most regions. In order to remove the pollution from waters, many studies are carried out using algae with high metal absorption capacity. In our study, benthic algae samples from different habitats (epipelic, epiphytic and epilitic) were taken from the Altınapa Dam Lake in Konya, in order to study the potentials of heavy metal removal. Some microalgae were isolated from the samples and heavy metal absorption capacities were examined among the pure cultures obtained. Biosorption properties were tried to be determined in samples taken at 5, 10, 20, 45, 60, 90 and 120 minutes by exposing Chlorochytrium paradoxum, Haematococcus lacustris and Scenedesmus circumfusus species to heavy metals of Cu and Mn. Scenedesmus circumfusus species reached the best absorption capacity for both Cu and Mn metals. According to this research, the algae we studied are of the opinion that they can be used to increase the efficiency and selectivity of heavy metal biosorption.

Keywords: Algae, biosorption, heavy metal, isolation.

Altınapa Baraj Gölü'nden (Konya) İzole Edilen Bazı Alglerin Ağır Metal Absorpsiyonlarının Karşılaştırılması

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Öz: Günümüzde su kaynaklarında meydana gelen yoğun kirlilik nedeniyle, çoğu bölgede temiz su sıkıntısı yaşanmaktadır. Sularda oluşan kirliliğin giderilmesi için ağır metal absorpsiyon kapasitesi yüksek olan alglerin kullanıldığı birçok çalışma yapılmaktadır. Araştırmamızda ağır metal giderimi potansiyellerini çalışabilmek için, Konya' da bulunan Altınapa Baraj Gölü'nden değişik habitatlardan (epipelik, epifitik ve epilitik) bentik alg örnekleri alınmıştır. Alınan örneklerden bazı algler izole edilmiş ve elde edilen saf kültürler arasında ağır metal absorpsiyon kapasiteleri incelenmiştir. *Chlorochytrium paradoxum, Haematococcus lacustris ve Scenedesmus circumfusus* türleri Cu ve Mn ağır metallerine maruz bırakılarak 5. 10. 20. 30. 45. 60. 90 ve 120. dakikalarda alınan örneklerde biyosorpsiyon özellikleri tespit edilmeye çalışılmıştır. Hem Cu Hem Mn metallerinde en iyi absorblama kapasitesine *Scenedesmus circumfusus* türü ulaşmıştır. Bu araştırmaya göre; çalıştığımız algler, ağır metal biyosorpsiyonunun etkinliğini ve seçiciliğini artırmak için kullanılabilir olduğu düşüncesindeyiz.

Anahtar kelimeler: Ağır metal, alg, biyosorpsiyon, izolasyon.

INTRODUCTION

Surface water sources such as lakes, streams, dams and agricultural waters; It is very important for public health, the continuity of the aquatic ecosystem and agricultural activities (Noori et al., 2018). Trace element pollution in the aquatic environment has become a global concern due to its potential harmful effects on aquatic ecosystems and human health. Heavy metals can contaminate water bodies through natural deposits, mining waste, industrial and agricultural activities. The water contaminated with trace metals may not be suitable for human and animal drinking, irrigation, protection of aquatic ecosystems, recreation and aquaculture (Kibria, 2016).

Wastes generated in regions with high industrial and urban development are important sources of pollution for aquatic environments (Çoğun & Kargın, 2020). Water pollution, which has a negative impact on the quality of life of the society, is becoming threatening to human health day by day (Gemici et al., 2019). To provide a locally controlled water supply, wastewater recycling offers great environmental benefits. Recycling of water prevents the consumption of water from ecosystems and the reduction of environmental pollution and the accumulation of pollutants in our ecosystem. With the scarcity of fresh water resources and the pollution in the ecosystem, industrial wastewater increases the importance of wastewater treatment. Extensive research is being conducted to develop an effective technology to overcome the toxicity and negative environmental effects of heavy metals and ionic forms (Nazal, 2019). Algae are eutrophic and eukaryotic organisms that play an important role in the food, pharmaceutical, cosmetic, fuel and textile industries. These are an essential part of our ecosystem and can help control the growing pollution problem. It is known that algae are a good indicator of pollution level in aquatic ecosystems (Dora et al., 2010). These biological properties make algae a very good "early warning system". Moreover, green algae have also been reported to be suggested for phytoremission of some elements (Gomes & Asaeda 2013; Olal, 2016; Sahu et al., 2020). Algae can be used to improve the efficiency and selectivity of heavy metal biosorption (Cheng et al., 2019). With the excessive contamination of aquatic ecosystems with human-induced pollutants such as heavy metals and the increase in potential toxic effects of these pollutants on living things, studies on metal accumulation in algae gain importance day by day. Due to the fact that algae are important parts of the aquatic food web, the number of studies to evaluate the toxic damage of heavy metals to these creatures has increased in recent years (Şentürk, 2018a).

The health effects of various heavy metals have raised awareness of the health risk associated with heavy metals worldwide. For this reason, researchers around the world have worked on different technologies to remove heavy metals from the environment. Biosorption has emerged as a promising alternative to traditional methods (Richards et al., 2019). In addition to biosorption, different physicochemical mechanisms include microprecipitation, chelation, ion exchange, and physical adsorption. Many studies are trying to identify suitable biosorbents that can effectively remove heavy metals. These biosorbents typically include agricultural waste, plant-based adsorbents, algae, fungi, and bacteria (Poo et al., 2018). Industrial and urban wastewater can be used as a food source for algae production. Wastewater is an ideal environment for algae growth as they contain high amounts of nitrogen and phosphorus (Knezevic, 2016).

Among the biosorbents, algae-based biosorbents have been the focus of attention due to their renewability, superior uptake capacity, absence of toxic waste generation, low cost, time and energy savings, year-round occurrence, ease of use, and great abundance in the sea. Following the biosorption process, the remaining algal biomass can be dumped into municipal landfills with a much lower environmental footprint (Yu et al., 2017; Poo et al., 2018).

In this study, it is aimed to compare the copper (Cu) and Manganese (Mn) metal absorption capacities of the microalgae samples taken from Altınapa Dam Lake in Konya. The advantages of algae such as having high metal holding capacity from wastewater and being economical and easily available are taken into consideration. *Scenedesmus circumfusus* Hortobágyi, *Chlorochytrium paradoxum* (G.A.Klebs) G.S. West, *Haematococcus lacustris* (Girod-Chantrans) Rostafinski microalgae species obtained through isolation from the samples we took from Altınapa Dam Pond were exposed to heavy metal solutions and metal uptake ability in waste water treatment was investigated.

MATERIAL AND METHOD

Sampling, Species Isolation, Cultural Conditions and Species Identification: Samples were taken from Altınapa Dam Lake in May and June 2019. Sediment, plant and stone samples taken from the benthic region of Altınapa Dam Lake were brought to the laboratory under appropriate conditions in lake water with sterile bottles. After, plant and stone samples were washed with distilled water, the samples were taken into a petri dish by scraping.

The preparation for morphological determination was prepared and examined directly under a microscope. For the isolation process, the dilution method was applied to obtain monocultures from mixed samples scraped into the petri dish (Rippka, 1988). In this method, mixed cultures taken from natural habitats were placed in a petri dish and diluted with sterile water. This dilution was continued until the cells were freely visible in one form in the mixture. Then each cell was isolated individually by working under an inverted microscope with the help of a pasteur pipette. Once single cells were obtained, the cells were transferred to BG-11 medium used for growth. The pH of the medium was adjusted to 6.8. Cultures were grown at 25 ° C under a fluorescent light intensity of 3000 lux in sterile shake flasks containing 100 mL of BG-11. It was incubated for 15-20 days in a suitable incubator for photosynthesis on a 12 hour light and 12 hour dark cycle. Then cell numbers, cell layouts and cell shapes were checked. Morphological characterization Prescott, (1973) and John et al., (2002) methods.

Figure 1. Satellite image of Altınapa Dam Lake.

Heavy Metal Absorption Measurements of Cultured Species: Distilled water of the appropriate pH value was used for the solution in which the algae will be studied, and a buffer solution was prepared to adjust the pH of the environment. An appropriate amount of undissolved MnSO4 (Merck) and CuSO4 (Merck) was used to prepare the copper and manganese stock solution (1000 mg/L). It was mixed with the Britton-Robinson (B-R) buffer solution to make the stock solution suitable. In order to prepare the Britton-Robinson (BR) buffer solution, 2.3 mL of glacial acetic acid, 2.7 mL of phosphoric acid and 2.4720 g of boric acid are added to 1 liter of water and 100 ml of this mixture is added to 2M NaOH and the pH of the stock solution is 2.0. and between 8.0 (Inam & Toprak, 2005). Experimental groups were formed by taking 100 ml of algae cultures into flasks. In these experimental groups formed from live algae cultures, the pH amounts were fixed to pH 7.04 in the Manganese test and to 6.36 in the Copper test. pH measurements were made with a Hach-Lange brand pH meter.

Measurements were made at 5, 10, 20, 30, 45, 60, 90 and 120 minutes. At each measurement, 15 ml of algae culture was taken and filtered on Whatman GF / C branded filter paper with a pore opening of 0.125 µm, and heavy metals in this filtrate were measured in the ICP-OES device.

The formula for the retention efficiency of metals is given below (König-Peter et al., 2015).

$$\% \text{ removal efficiency} = C_o - C_{remaining} / Co*100$$

C_o: Origin metal concentration (mg/L)

$C_{remaining}$: Metal concentration remaining in solution at the end of the process (mg/L)

Table 1. Technical information about the device.

ICP-OES working conditions	
Device	ICP-OES (Varian-Vista, Australia))
RF power	0.7-1.5 kW (1.2-1.3 kW for axial)
Plasma gas flow rate (Ar)	10.5-15 1/min (radial) 15 (axial)
Auxiliary gas flow rate (Ar)	1.5’’
Detection height	5-12 mm
Copying and reading time	1-5 s (max, 60 s)
Copying	3 s (max, 100 s)

The wavelengths of the elements measured in the device are Cu 327.395 nm and Mn 257.610 nm, respectively. The graphs of the statistical analysis showing the Cu and Mn absorption capacities of the algae and their removal efficiency were created in the SPSS 21 program.

RESULTS

To calculate the level of Cu and Mn absorption, all three experimental groups were prepared in the same way. 15 ml samples were taken for each measurement from three separate solutions prepared in *Scenedesmus circumfusus* Hortobágyi, *Chlorochytrium paradoxum* (G.A.Klebs) G.S. West, *Haematococcus lacustris* (Girod-Chantrans) Rostafinski species isolated from Altınapa Dam Lake and filtered through filter paper. The samples were taken at 5, 10, 20, 30, 45, 60, 90 and 120. minutes and measurements were performed.

Copper; highly toxic to fish, invertebrates and amphibians. It can reduce the growth of aquatic organisms such as fish and damage the gill and kidney. Cu is an essential trace element for humans, but high levels of Cu or Cu poisoning can cause liver cirrhosis (liver diseases) and, in extreme cases, death of humans (Kibria, 2016).

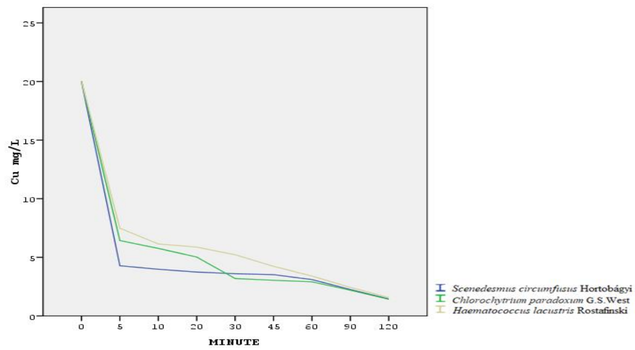

Figure 2. Cu absorption capacity of algae.

Cu element absorption capacities of algae are given in Table 2. In *Scenedesmus circumfusus* Hortobágyi, it was found to be 4.28 mg/L, 3.98 mg/L, 3.74 mg/L, 3.6 mg/L, 3.52 mg/L, 3.1 mg/L, 2.25 mg/L and 1.45 mg/L respectively. *Chlorochytrium paradoxum* (G.A.Klebs) G.S.West species 6.43 mg/L, 5.76 mg/L, 5.02 mg/L, 3.19 mg/L, 3.04 mg/L, 2.91 mg/L, 2.19 mg/L ve 1.42 mg/L' dir. Cu was found to be 6.43 mg/L, 5.76 mg/L, 5.02 mg/L, 3.19 mg/L, 3.04 mg/L, 2.91 mg/L, 2.19 mg/L and 1.42 mg/L respectively Chlorochytrium paradoxum (G.A.Klebs) G.S.West. Copper element in *Haematococcus lacustris* (Girod-Chantrans) Rostafinski species 7.48 mg/L, 6.14 mg/L, 5.87 mg/L, 5.22 mg/L, 4.23 mg/L, 3.4 mg/L, 2.43 mg/L and 1.57 mg/L (Table 2).

Table 2. Algae Mn and Cu element absorption capacity (mg/L).

		0'	5'	10'	20'	30'	45'	60'	90'	120'
S.C.	**Mn**	48,24	40,63	29,35	20,74	18,57	17,44	16,32	16,01	14,45
	Cu	20,02	4,28	3,98	3,74	3,6	3,52	3,1	2,25	1,45
C.P.	**Mn**	48,24	43,17	38,68	29,64	29,08	28,53	27,11	26,64	25,58
	Cu	20,02	6,43	5,76	5,02	3,19	3,04	2,91	2,19	1,42
H.L	**Mn**	48,24	45,09	40,91	32,26	31,82	30,27	29,91	29,56	28,98
	Cu	20,02	7,48	6,14	5,87	5,22	4,23	3,4	2,43	1,57

S.C.: *Scenedesmus circumfusus* Hortobágyi C.P.: *Chlorochytrium paradoxum* (G.A.Klebs) G.S.West H.L.: *Haematococcus lacustris* (Girod-Chantrans) Rostafinski.

The highest uptake was seen at 5 minutes in copper-added algae cultures. *Scenedesmus circumfusus* Hortobágyi took the heavy metal in the environment with 79%. All species have reached absorption capacities close to each other. The retention efficiency of all three algae reached 92% after 120 minutes (Figure 3).

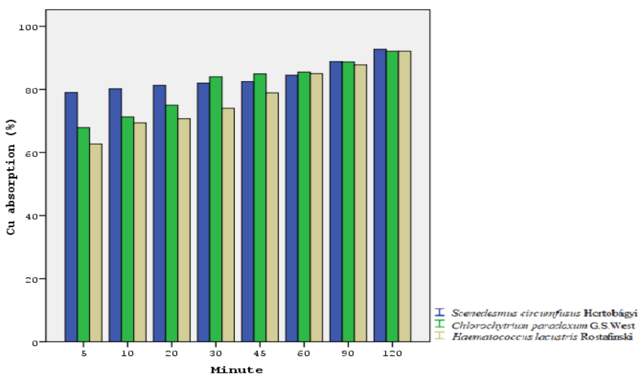

Figure 3. Cu retention efficiency of algae over time (%).

It is used in conjunction with aluminum and copper alloys as a component in the manganese material industry and steel production. Manganese dissolves into water as a result of various atmospheric events found naturally in soil and sedimentary masses. It is found in sediments at the bottom of lentic systems and passes from sludge to water in case of a reducing environment. Increasing manganese in water increases the turbidity of the water and the number of bacteria in it, and spoils the taste of the water. Among the discomfort caused by manganese, neurological disorders, weakening of joint and muscle tissue control, respiratory problems are seen (Bradl, 2005; Gürbüz, 2005; Kaptan, 2014, Gümüş & Akköz, 2020).

Mn element absorption capacities of algae are given in Table 3. In *Scenedesmus circumfusus*, it was found to be 40.63 mg/L, 29.35 mg/L, 20.74 mg/L, 18.57 mg/L, 17.44 mg/L, 16.32 mg/L, 16.01 mg/L and 14.45 mg/L respectively. Mn was found to be 43.17 mg/L, 38.68 mg/L, 29.64 mg/L, 29,08 mg/L, 28,53 mg/L, 27,11 mg/L, 26,64 mg/L and 25,58 mg/L, respectively in *Chlorochytrium paradoxum*. In *Haematococcus lacustris*, it was determined as 45,09 mg/L, 40.91 mg/L, 32.26 mg/L, 31.82 mg/L, 30.27 mg/L, 29.91 mg/L, 29.56 mg/L and 28.98 mg/L respectively (Table 2).

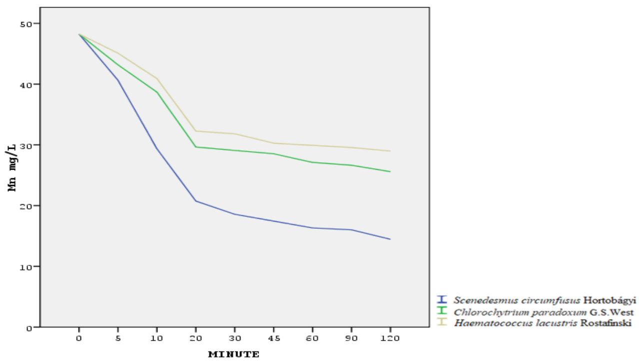

Figure 4. Mn absorption capacity of algae.

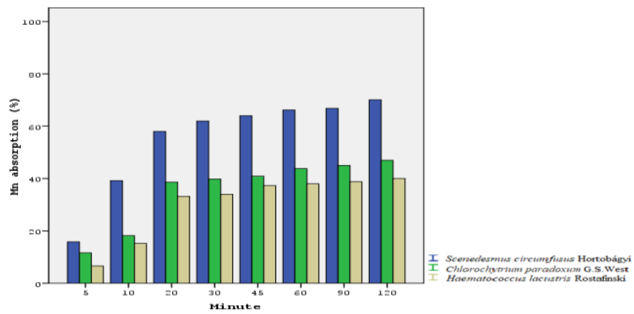

Figure 5. Mn retention efficiency of algae over time (%).

The algae cultures with added manganese have the highest intake in all three species 30 minute has also been seen. Adhesion efficiency reached 70% in *Scenedesmus circumfusus* Hortobágyi species, 47% in Chlorochytrium paradoxum (G.A.Klebs) species, and 40% in *Haematococcus lacustris* (Girod-Chantrans) Rostafinski species after 120 minutes (Figure 5). Manganese-added algae cultures could not reach the absorption capacity of copper-added algae cultures in all three species.

DISCUSSION

In the biosorption process, obtaining maximum efficiency with minimum biosorbent is important in terms of ease of operation, cost and duration. Biosorption method can be recommended as an easy method with its applicability to metal removal from wastewater compared to other methods, its suitability to the biological environment, being effective, economical and applied (Şentürk & Yıldız, 2018a). Especially algae have come to the fore in recent years.

Gokhale et al., (2008) reported that Cr (VI) ion was adsorbed around 50% in the first 5 minutes in S. platensis. In our study, Cu metal was found to be above 60% in all three algae species.

In their study by Şentürk & Yıldız, (2018a), the average retention efficiency of *C. vulgaris* for Cu and Mn was 87-95% and 6-56%, respectively. These results are consistent with the algae in this study Cu and Mn retention yields. It shows that manganese and copper in the environment are taken up by two algae and that heavy metals are stored in cell components or specific metal binding proteins.

Elmacı et al., (2005) investigated the biosorption of dried *Chara*, *Cladaphora* and *Chlorella* species in diluted solutions of Zn, Cd, Co and remozal Turkish-G dyestuff and it was found that these three species could be used in heavy metal removal. In the other study, the dry mass, immobilized living and heat inactivated *Oscillatoria* sp. When the Cd heavy metal removal of the species is examined, immobilized living *Oscillatoria* sp. H1 showed the highest biosorption capacity compared to dry mass and inactive Oscillatoria (Katırcıoğlu et al., 2008)

Karaca, (2008) measured the heavy metal biosorption capacities of different algae species in water, and *Oocystis* sp. was found to be the algae species with the highest absorption capacity after Dunaliella salina.

In a similar study, Terry & Stone, (2002) evaluated the biosorption using Scenedesmus abundans in waters contaminated with Cu and Cd. In this study, *Scenedesmus circumfusus* Hortobágyi species performed better biosorption.

Considering the results of their study, Şentürk & Yıldız, (2018b) revealed that Chlorella and Scenedesmus cells are effective adsorbents in removing four heavy metals, especially Cu^{+2} ions from aqueous solutions, due to the high efficiency of Cu adsorption. The results of these reports were found to be consistent with the findings obtained in our study.

Akköz et al., (2013) examined the Cd (II) biosorption capacities in their study. They found that the Cd absorption capacity of Oocystis solitaria species was higher than that of *Scenedesmus quadricauda* species.

As a result of the measurements, it was seen that the Mn absorption capacity of *Scenedesmus circumfusus* Hortobágyi species was higher than *Chlorochytrium paradoxum* (G.A.Klebs) G.S.West, *Haematococcus lacustris* (Girod-Chantrans) Rostafinski) species. As a result, *Scenedesmus circumfusus* Hortobágyi species was found to be more useful in heavy metal removal from industrial wastewater.

The results we obtained in this study, Yong et al., (1997) shows that the effect of Cu accumulation in Scenedesmus quadricauda increases depending on the concentration, and Yan & Pan, (2002) in Scenedesmus obliguus algae are rapidly absorbed by the algae of Cu, Zn and Cd.

Studies show that the biosorption capacity increases with increasing atomic number (Akçelik, 2008; Özer & Özer, 2003). The atomic numbers of the Cu (II) and Pb (II) ions are $^{29}Cu > ^{25}Mn$. According to the data of our study, it can be said that the biosorption capacity increases with increasing atomic number.

Akköz et al., (2013) reported that heavy metal storage levels may vary among algae species. Similar results were observed in the copper deposition in *Scenedesmus circumfusus* Hortobágyi cells, and these results suggest that manganese and copper in the environment are constantly absorbed by two algae species and heavy metals are stored in cellular components or special metal binding proteins.

CONCLUSION

In this study, it has not been studied for longer periods for Cu and Mn because of the stabilization of adsorption after 120 minutes. Using such creatures to purify heavy metals from water systems is much more convenient than other methods in terms of protecting the environment and minimizing side effects. Researching new approaches to removing heavy metals from wastewater, determining the heavy metal absorption capacity of different biological materials will contribute to technologies that do not harm the environment.

In particular, microalgae are beneficial in the elimination of various areas contaminated with inorganic nutrients and heavy metals. For this reason, it would be appropriate to use 3 algae species used in the study as a biosorbent to remove heavy metal from water.

As a result of this study, it has been determined that *Scenedesmus circumfusus* Hortobágyi, *Chlorochytrium paradoxum* (G.A.Klebs) G.S.West, *Haematococcus lacustris* (Girod-Chantrans) Rostafinski species have positive results in the removal of heavy metals in the laboratory environment, so they can be used in this field. It has been determined that these algae can be useful bioremeditors for removing pollutants from wastewater.

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