

## Assessment of Sediment Dredging Effectiveness by Considering Overlying Water and Pore Water Heavy Metals in Lake Mogan, Turkey\*

### Mogan Gölü (Türkiye)'nde Sediment Tarama Etkinliğinin Sediment Üstü Su ve Sediment Gözenek Suyu Ağır Metalleri Bağlamında Değerlendirilmesi

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**Abstract:** Dredging, a practice intended to increase the recreational value of aquatic systems at risk of eutrophication, has been implemented in Lake Mogan. This study aimed to determine a) Heavy metal concentrations (Hg, As, Cd, Cr, Pb, Ni, Cu, and Zn) in the overlying water and sediment pore water, and b) Pore water toxicity levels of some of these metals (Cu, Ni, Pb, Zn) following dredging in Lake Mogan. Surface sediment samples were taken from two stations in the lake between May and November 2020 to obtain overlying water and pore water values. The first station was chosen specifically because it receives wastewater from mineral processing facilities and nearby households; and the second station. After all, it receives household and industrial waste and agricultural wastewater. The findings indicate that a) Zn had the highest concentrations during the study period, and Pb the lowest. Overlying water concentrations were found to be within the acceptable limits outlined by the "Water Quality Control Regulations". Pore water Zn concentrations for May ( $330.50 \pm 8.72 \mu\text{g L}^{-1}$ ) and pore water Cu concentrations for November at the second station ( $12.85 \pm 4.35 \mu\text{g L}^{-1}$ ) were higher than the recommended WQC limits. b) Since no values greater than 1 were found in the pore water for IWCTU (Interstitial Water Criterion Toxic Units) and NI (Nemeraw index) values for Ni, Cu, Zn, and Pb, no serious levels of pollution were present for these four metals; however, Zn had the greatest potential risk of toxicity. Moreover, the total IWCTU and NI values indicate that both the toxicity of the metals and the degree thereof increased in May. The fact that pore water heavy metal concentrations in Lake Mogan were generally higher than those in the overlying water indicates strong metal adsorption in the sediment and suggests that, at least in terms of reducing surface sediment heavy metals, dredging activities in the lake are not very effective.

#### Keywords

- Sediment dredging
- overlying water
- pore water
- heavy metal
- Lake Mogan

**Özet:** Ötrofikasyon riski altındaki sucul sistemlerin rekreatif değerlerinin artırılmasına yönelik bir yönetim aracı olan dredging, Mogan Gölü'nde uygulanmıştır. Bu çalışmada Mogan Gölü'nde sediment tarama ertesisi; a) Sediment üstü su (SÜS) ve sediment gözenek suyunda (SGS) ağır metal konsantrasyonlarının (Hg, As, Cd, Cr, Pb, Ni, Cu ve Zn), b) SGS'de bazı ağır metallerin (Cu, Ni, Pb, Zn) toksisitesinin belirlenmesi amaçlanmıştır. Bu amaçla 2020-Mayıs ve Kasım aylarında iki istasyondan (Özellikle maden işleme tesisleri ve evsel kaynaklı atık suların ulaştığı I. istasyon; Evsel, endüstriyel atıklar ile tarımsal faaliyet kaynaklı atık suların ulaştığı II. istasyon) SÜS ve SGS eldesi için de yüzey sedimentleri örneklenmiştir. Bulgular doğrultusunda; a) Çalışma periyodunca Zn en yüksek, Pb ise diğer metallerle göre daha düşük değerlerde seyretmiştir. SÜS verileri 'Su Kirliliği Kontrolü Yönetmeliğinde' verilen limit değerler

#### Anahtar kelimeler

- Sediment tarama
- sediment üstü su
- sediment gözenek suyu
- ağır metal
- Mogan Gölü



içerisinde bulunmuştur. SGS-Zn; Mayıs-II. istasyonda ( $330.50 \pm 8.72 \mu\text{g L}^{-1}$ ) ve SGS-Cu; Kasım-II. istasyonda ( $12.85 \pm 4.35 \mu\text{g L}^{-1}$ ) pore water için önerilen (WQC) limit değerleri aşmıştır, b) SGS-Ni, Cu, Zn ve Pb'ya ilişkin IWCTU (Interstitial Water Criterion Toxic Units) ile NI değerleri, 1'den büyük bulunmadığı için, dört metal açısından gölde ciddi bir kirlenme olmadığı, ancak Zn'un toksik etki riskine sahip en potansiyel metal olduğu belirlenmiştir. Ayrıca toplam IWCTU ve NI verileri, Mayıs ayında metallerin toksisite ve toksisite derecesinin arttığına işaret etmektedir. Mogan Gölü'nde genel olarak SGS-ağır metal konsantrasyonlarının SÜS'e ilişkin değerlerden daha yüksek saptanması, sedimentin metalleri kuvvetlice adsorbladığını ve tarama girişiminin en azından yüzey sedimentinin ağır metal düzeyleri bağlamında çok etkin olmadığını ortaya koymuştur.

## 1. INTRODUCTION

When heavy metals are deposited in the sediment through natural or anthropogenic means redissolve into the water, they can further increase heavy metal concentrations in the water. While a portion of heavy metals entering aquatic systems disperses in the water, another portion form solid compounds with carbonates, sulfates, and sulfur, sink to the bottom and collect in the sediment. Sediment metals directly threaten detrital and deposit-feeding benthic organisms and, reaching higher up the food chain, become a long-term source of pollution (Pulatsü and Topçu, 2015).

Pore water plays a more important role than sediment in the heavy metal cycle in aquatic ecosystems. Tang et al. (2016) report that pore water plays a bridging role in the exchange of heavy metals between sediment and overlying water. Other researchers have considered the idea that metal ions in pore water may have direct biological effects. Pore water heavy metals are found either in the dissolved or particulate phase; these phases are in a state of interaction with each other and with the overlying water of the sediment-water interface. Dispersion and mobilization of metals in the pore water play a significant role in the metal cycle at the sediment-water interface (Zhu et al., 2016).

The mobilization of pollutants is one of the most important environmental risks of dredging. If pore water metal concentrations are greater than those in the overlying water, the concentration gradient will result in the release (transport) of dissolved metal from the sediment into the overlying water. Other factors affecting dissolved metal release are pH, redox potential, level and composition of organic matter, bottom oxygen concentration, depth of penetration, benthic organism activities, and bacteria (Ni et al., 2017; Zhang et al., 2020).

As a physical method of controlling eutrophication in lakes, dredging can be defined as the removal of bottom sediment from rivers, lakes, coastal waters, and seas. Among the factors increasing its effectiveness is its application in relatively shallow lakes, those with low sedimentation speed, organically rich sediments, relatively low catchment area/surface area (10/1), or long hydraulic retention periods. The decision whether or not to dredge depends on sediment composition, pollutant types, sediment layer depth, thickness, volume, distance from where the next step in the process will take place (the dumping area), and equipment on hand. Dredged lake sediment is generally transferred to wetland or coastal ecosystems, used in agriculture or used for land reclamation in wetland areas/habitats (Pulatsü et al., 2015).

Peterson (2007) mentions several disadvantages to dredging, such as its expense, temporary release of phosphorus from the sediment, increase in phytoplankton fertility, odor problems, a temporary decrease of organisms that nourish benthic fish, release of toxic material into the overlying water, and its environmental impact on the area to which dredged sediment is transferred.

Heavy metal concentrations in the water and sediment of a body of water vary according to the number of pollutants entering the receiving environment and their removal through various methods. After measuring overlying water heavy metal concentrations, the next step in studying heavy metal pollution in aquatic ecosystems is measuring their pore water concentrations and determining their

toxicity. Pore water heavy metal toxicity measurement is a method used in sedimentological studies of both lake and river ecosystems (Tang et al., 2016; Zhu et al., 2016; Ji et al., 2018).

Lake Mogan, located in Gölbaşı Special Conservation Area, is an important recreation area because of its proximity to Ankara capital city of Turkey. Its near surroundings play host to various activities such as housing, industry, and tourism. Numerous studies have been conducted on its surface water and sediment heavy metal levels (Benzer et al., 2013; Topçu and Kaya 2017; Küçükosmanoğlu and Filazi, 2020).

For many years, certain lake management practices have been implemented in this shallow eutrophic lake, under intense pressure from urban-industrial pollution. As a management tool often used in aquatic ecosystems at risk for eutrophication, dredging has also been practiced periodically in Lake Mogan. The local government reports that dredging mud from the lake bottom started in 2017 and ended in November 2018, with a total of 3,100,000 m<sup>3</sup> of sediment removed.

This study aimed to a) measure overlying water and pore water heavy metal concentrations (Hg, As, Cd, Cr, Pb, Ni, Cu, and Zn), and b) determine the toxicity of certain heavy metals (Cu, Ni, Pb, Zn) in the pore water of Lake Mogan after dredging, a management practice meant to increase a lake's recreational value, was implemented there.

The study findings are significant because they put forth the question of whether Lake Mogan's sediment functions as a source and/or a trap for heavy metals. Moreover, it is thought that the findings of the study, the first to be conducted following dredging in the lake, will provide insight regarding the efficacy of the practice in lake management.

## **2. MATERIALS and METHODS**

### **2.1. Material**

#### **2.1.1. The study area**

The study area, Lake Mogan, is an alluvial set lake fed mostly through precipitation, and by more than five streams of various sizes. It is located in the Lower Ankara River Basin, 20 km south of Ankara on the Ankara-Konya highway. Lake Mogan is on the edge of the Gölbaşı Special Conservation Area, and at the same time, is one of Turkey's important Ramsar candidate wetlands (Anonymous, 2016).

#### **2.1.2. Research station selection**

Two suitable stations representative of pollutant sources were selected in the littoral zone of Lake Mogan for the collection of bottom sediment samples. The first station was selected especially for its location in an area reached by wastewater from mineral processing facilities and domestic wastewater; and the second station for its proximity to wastewater from domestic and agricultural sources. The study area and locations of the research stations are shown in Figure 1.

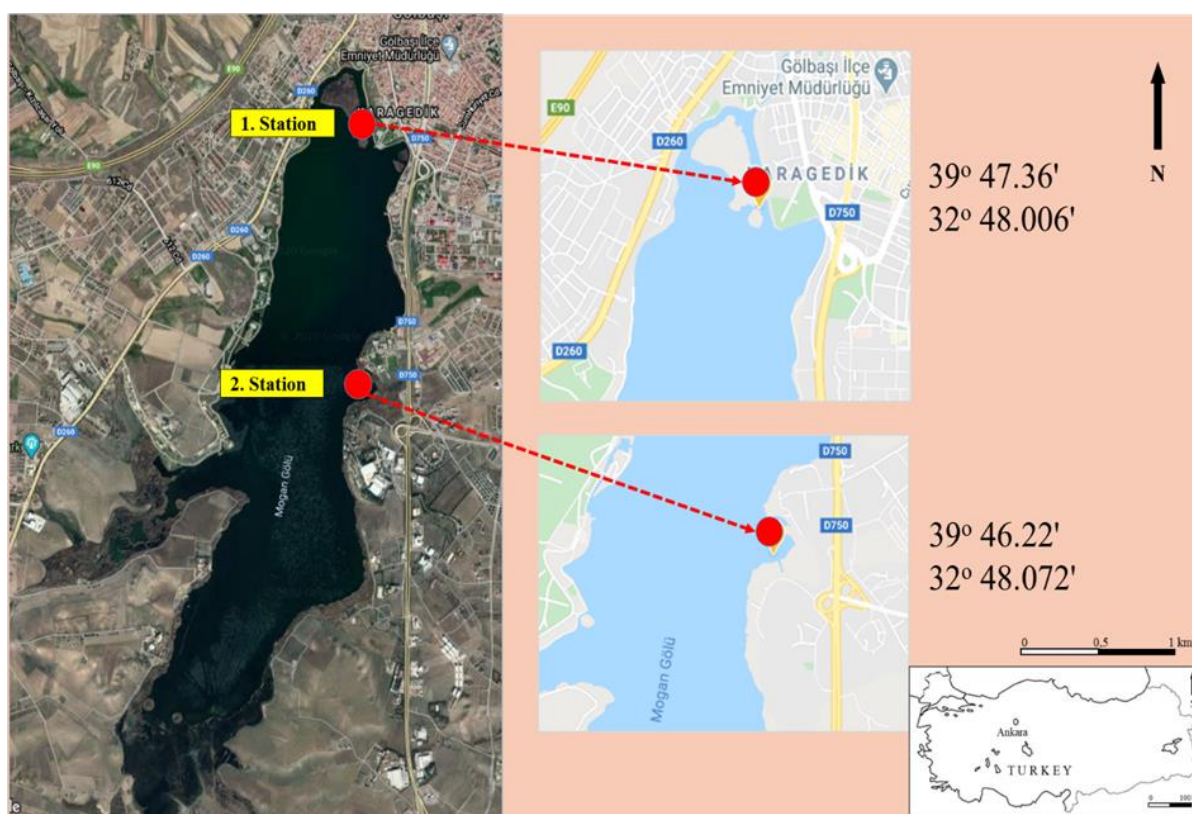


Figure 1. Research area and location of stations

### 2.1.3. Methods

In this study, overlying water and surface sediment samples were collected at the stations in Lake Mogan's littoral zone in May and November 2020.

#### 2.1.4. Overlying water sampling:

Due to the shallow depth at the first station, sediment samples were obtained by suctioning water directly above the sediment. As the water at the second station was deeper, sediments there were obtained using a Ruttner Water Sampler.

#### 2.1.5. Sediment pore water extraction and analysis:

The pore water was extracted from the sediment particles by centrifuging them at 5000 rpm for ten minutes. The clear portion of the water that collected in the top of the tubes was drawn out with a pipette and filtered through a 0.45  $\mu\text{m}$  membrane with a vacuum filtration apparatus (Eckert et al., 2007). The Hg, As, Cd, Cr, Pb, Ni, Cu, and Zn levels in the pore water samples were then measured with an ICP-OES instrument specified in Table 1. Heavy metal analyses were performed by Middle East Technical Central Laboratory.

Table 1. ICP-OES operating conditions.

RF power	1300 W
Plasma gas flow rate	18 L/min
Auxiliary gas flow rate	1.2 L/min
Nebulizer gas flow rate	0.92 L/min
Sample uptake rate	1 ml/min
Number of replicates	3
Spray chamber	Cyclonic
Nebulizer	Glass Meinhard concentric
Isotopes	Cr 52, Ni 62, Cu 63/65, Zn 66, As 75, Cd 111/114, Pb 206/207/208

**2.1.6. Analysis of the overlying water:** The Hg, As, Cd, Cr, Pb, Ni, Cu, and Zn levels in the overlying water were measured with an ICP-OES instrument. Limit values are as follows: Hg: 0.04 µg L<sup>-1</sup>; As: 1 µg L<sup>-1</sup>; Cd: 0.01 µg L<sup>-1</sup>; Cr: 0.1 µg L<sup>-1</sup>; Pb: 0.03 µg L<sup>-1</sup>; Ni: 0.2 µg L<sup>-1</sup>; Cu: 0.1 µg L<sup>-1</sup>; Zn: 1 µg L<sup>-1</sup>.

**2.1.7. Pore (interstitial) water criterion toxic units (IWCTU):** IWCTU was calculated taking the equation below, reported by Lourino-Cabana et al. (2011) and Zhu et al. (2016), as a basis:

$$IWCTU = \frac{C_{i,IW}}{FCV_i} \quad (1)$$

Here;

$C_{i,IW}$ : The concentration of the *i*th heavy metal in pore water

$FCV_i$ : Hardness-dependent final chronic values for the metals:

$$FCV_{Cu} = 0.960[e^{0.8545 \ln(\text{hardness})} - 1.465]$$

$$FCV_{Ni} = 0.997[e^{0.8460 \ln(\text{hardness})} + 1.1645]$$

$$FCV_{Pb} = 0.791[e^{1.273 \ln(\text{hardness})} - 4.705]$$

$$FCV_{Zn} = 0.986[e^{0.8473 \ln(\text{hardness})} + 0.7614]$$

The  $IWCTU > 1$  indicates potential risks of toxicity to aquatic organisms. The  $IWCTUs$  can directly reflect the pollution level of heavy metals in pore water (Tang et al., 2016; Ji et al., 2018).

$$NI = \left[ \frac{(IWCTU)_{\max}^2 + (IWCTU)_{\text{mean}}^2}{2} \right]^{1/2} \quad (2)$$

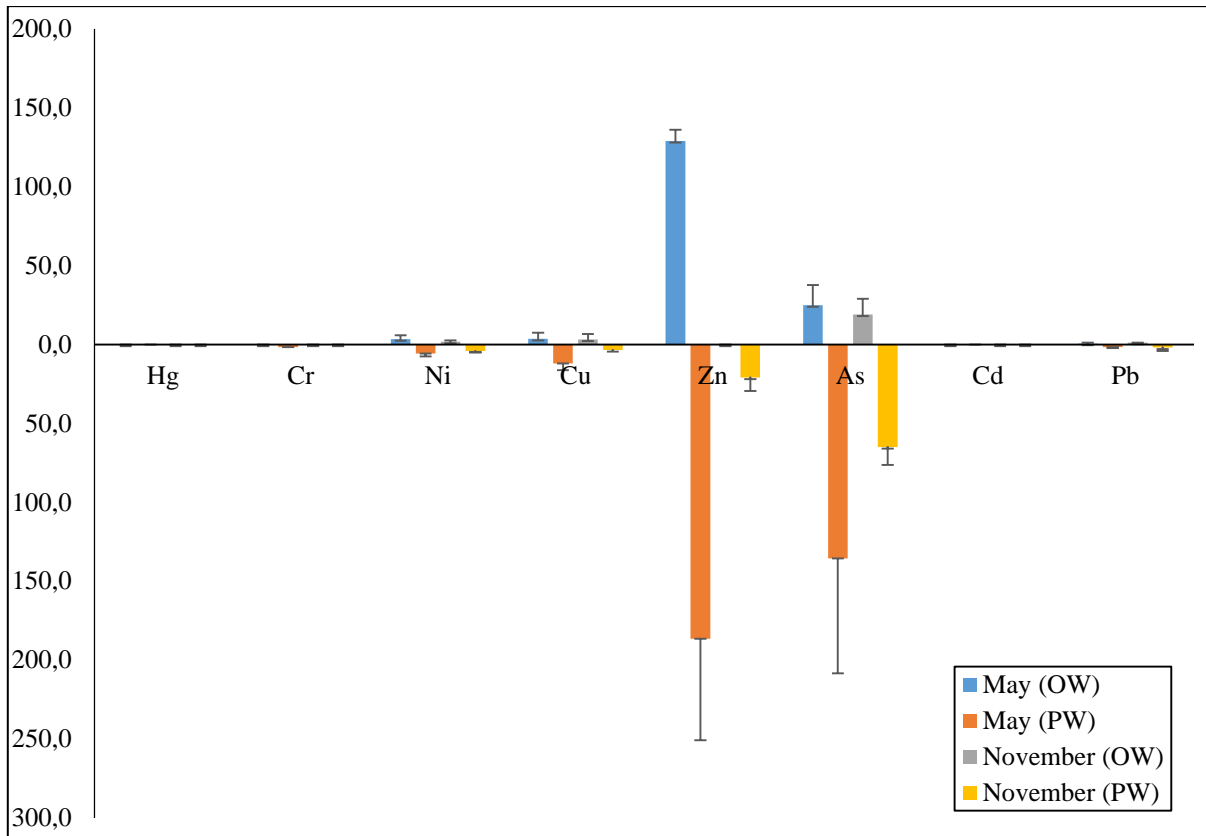
NI can reflect the impact degree of heavy metals in pore water. The NI can be divided into five classes:  $0 < NI < 1$  (no impact),  $1 < NI < 2$  (slight impact),  $2 < NI < 3$  (moderate impact),  $3 < NI < 5$  (strong impact), and  $NI > 5$  (serious impact) (Tang et al., 2016; Ji et al., 2018).

### 3. RESULTS

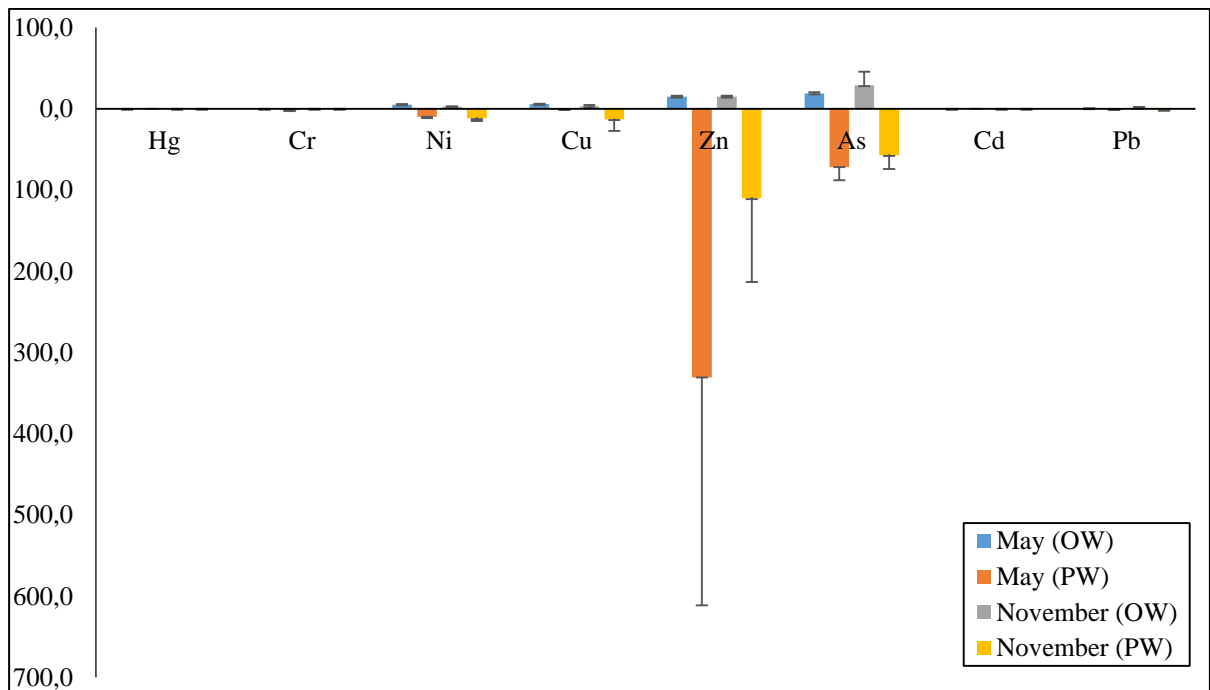
The range of overlying water and pore water heavy metal concentrations between the two months of the study for both stations are shown in Figures 2 and 3. As shown in the figures, pore water heavy metal concentrations were generally higher than those in the overlying water; only in November, the pore water concentrations at the second station were higher than those at the first station (except for Pb). In line with these findings, it appears that rainfall may have caused an increase in the transport of these metals into the lake.

In addition to the facts that pore water heavy metal concentrations were higher than those in the overlying water and the concentrations at the second station were higher than at the first station during both months of the study, Cu levels at the second station were found to be lower in May, and Pb was found to be lower at the second station in both May and November. As for Hg and Cd, no difference was detected in either pore water or overlying water concentrations at either station during both months of the study.

Irrespective of the station, the pore water heavy metal sequence for May was  $Zn > Ni > Cu > Pb$ , and for November,  $Zn > Cu > Ni > Pb$ . Using values for both months, the sequence for the first station was  $Zn > Cu > Ni > Pb$ , and for the second station,  $Zn > Ni > Cu > Pb$ . Accordingly, Zn had the highest values during the entire study period, while Pb had the lowest concentrations in comparison to the other metals (Figures 2 and 3). As shown in the figures, Hg, Cr, and Cd concentrations were below detectable limits.



**Figure 2.** Variation of overlying water (OW) and pore water (PW) heavy metal concentrations ( $\mu\text{g L}^{-1}$ ) according to months at the station I.



**Figure 3.** Variation of overlying water (OW) and pore water (PW) heavy metal concentrations ( $\mu\text{g L}^{-1}$ ) according to months at station II.

**Table 2.** Overlying water and pore water heavy metal concentrations with water quality classes and quality criteria

Metals ( $\mu\text{g/L}$ )	Min.-max. <sup>a</sup> ( $\mu\text{g L}^{-1}$ )	Min.- max. <sup>b</sup> ( $\mu\text{g L}^{-1}$ )	WQC <sup>c</sup>	Class I/II/III/IV <sup>d</sup>
Cu	3.10-5.80	0.69-12.85	9	$\leq 20/20-50/50-200/>200$
Ni	1.75-5.30	4.15-11.50	52	$\leq 20/20-50/50-200/>200$
Pb	0.61-1.38	0.75-1.88	2.5	$\leq 10/10-20/20-50/>50$
Zn	15.0-129	21.0-330.50	120	$\leq 200/200-500/500-2000/>2000$

<sup>a</sup> Overlying water<sup>b</sup> Pore water<sup>c</sup> Pore water quality criteria, Anonymous (2002)<sup>d</sup> Anonymous (2004)

Again, irrespective of the station, the sequence of overlying water heavy metal concentrations during both months of the study was found to be, from highest to lowest,  $\text{Zn} > \text{Cu} > \text{Ni} > \text{Pb}$ . The same holds true for both stations, irrespective of month. The highest concentration was found for Zn in May at the first station ( $129.0 \pm 7.07 \mu\text{g L}^{-1}$ ), and the lowest for Pb in the same month, again at the first station ( $0.61 \pm 0.57 \mu\text{g L}^{-1}$ ). These heavy metals with the highest and lowest concentrations in the rankings are in parallel with the pore water concentration sequences.

When the heavy metal levels in the overlying water are evaluated according to the “Water Quality Control Regulations” in force in Turkey, they do not exceed the acceptable limits (Table 2). However, it appears that the maximum overlying water Zn value (measured in May at the first station:  $129.0 \pm 7.07 \mu\text{g L}^{-1}$ ) exceeds the Category I value established for Zn ( $50 \mu\text{g/L}$ ) by MEPPRC (Ministry of Environmental Protection, People’s Republic of China) (2002).

Zn, observed at maximum values in the pore water during the entire study period, reached its maximum value of  $330.50 \pm 8.72 \mu\text{g L}^{-1}$  in May at the second station, exceeding the recommended limit for pore water Zn, shown in Table 2. Similarly, Cu exceeded its recommended value ( $9 \mu\text{g L}^{-1}$ ) in November at the second station, with a value of  $12.85 \pm 4.35 \mu\text{g L}^{-1}$ .

The IWCTU and NI values, calculated using equations (1) and (2), are presented in Table 4. To calculate interstitial water criterion toxic units (IWCTU), the value of  $462 \text{ mg CaCO}_3 \text{ L}$  reported by Demir et al. (2014) was used as the total hardness value for the FCV (Final Chronic Values) for each metal. Since the Hg, Cr, and Cd concentration values were below detectable limits, and as no FCV equivalent is available for As, toxicity values were not calculated for these metals.

As shown in Table 3, since IWCTU and NI values for Ni, Cu, Zn, and Pb were not greater than 1 for the selected stations and months, significant pollution from these four metals was not present in the lake; however, Zn was determined to have the highest potential risk of toxicity. Moreover, aggregate IWCTU values indicate that the toxicity of these metals increased, particularly in May. Similarly, NI values, which determine the degree of toxicity, also show that there is a greater risk of toxicity in May than in November.

#### 4. DISCUSSION

Heavy metal pollution has become a widespread problem that damages the normal functions of lakes and rivers. As their most important reserve, sediment plays an extremely important role in the transformation of heavy metals. Sediment dredging is implemented periodically in Lake Mogan, located in the Gölbaşı Special Conservation Area.

There are studies in which overlying water samples had higher heavy metal values than the surface water (Mwamburi and Oloo, 1997), and other studies indicate the complete opposite (Hou et al. 2013). In a study conducted in Lake Mogan by Küçükosmanoğlu and Filazi (2020), surface water sediment

heavy metals were reported as Fe, Cu, Cr, Zn, Pb, Ni, As, Se, and Hg, in descending order. In the present study, overlying water heavy metal concentrations showed a sequence of Zn>Cu>Ni>Pb.

Van den Berg et al. (2001) report that, while heavy metal mobilization in aquatic ecosystems depends on physical transport (e.g., advection, mixture, or diffusion), biological processes (bioturbation, etc.), or geochemical (adsorption/desorption and sedimentation/dissolution) processes, the biologically useful state for marine life generally depends on solid-phase re-sedimentation and retention. According to the researchers, dredging projects can result in the dispersion of both particulate and pore water pollutants. These projects increase the mobility of heavy metals and especially may cause the mixing of suspended solid matter with that in the dredged areas, decreased organic matter and Mn values, and increased suspended solid matter heavy metal levels. Similarly, the heavy metal concentrations measured in this study in the pore water of Lake Mogan after dredging point to the possibility of dredging as a triggering mechanism.

Fan et al. (2019) report that fundamentally, surface sediment is directly proportional with heavy metals and that clay has a large surface area and pore volume. The researchers also indicate that one reason that heavy metals are found in greater concentrations in surface sediment is that clay may promote greater heavy metal adsorption. In the present study, considering the stations and the months, the percentage of clay (45.13-87.13%) was found to be greater than the percentage of silt (12.86-54.86%) in the sediment. It is thought that the sediment's primarily clay composition played a significant role in the higher sediment heavy metal presence in comparison to the pore water and overlying water.

**Table 3.** Final chronic values (FCVs), interstitial water criterion toxic units (IWCTU), total interstitial water criterion toxic units ( $\Sigma$ IWCTU) and NI values corresponding to the months-stations (M: May, N: November; Stations: I, II) for four metals.

Metals	Cu		Ni		Pb		Zn		$\Sigma$ IWCTU	NI
FCV	41.97		573.97		17.66		382.21			
Month-station	( $\mu\text{g L}^{-1}$ )	IWCTU <sub>Cu</sub>	( $\mu\text{g L}^{-1}$ )	IWCTU <sub>Ni</sub>	( $\mu\text{g L}^{-1}$ )	IWCTU <sub>Pb</sub>	( $\mu\text{g L}^{-1}$ )	IWCTU <sub>Zn</sub>		
M-I	12	0.29	5.75	0.01	1.70	0.10	186.5	<b>0.49</b>	<b>0.88</b>	0.31
M-II	0.69	0.02	9.80	0.02	0.75	0.04	330.5	<b>0.86</b>	<b>0.94</b>	<b>0.59</b>
N-I	3.50	0.08	4.15	0.01	1.88	0.11	21.00	0.05	0.25	0.05
N-II	12.85	0.31	11.5	0.02	1.18	0.10	110.0	0.29	<b>0.68</b>	0.19

Peng et al. (2006) report that sediment oxidation must be avoided to reduce the release of heavy metals from the sediment. Due to oxygen-rich conditions in the overlying water and at the sediment-water interface, it is possible that metals can be adsorbed by reducing release from the sediment or that they will settle again with Fe and Mn oxyhydroxide particles (Tang et al., 2016). In Lake Mogan, the overlying water dissolved oxygen values were found to range between 1.28 and 7.12 mg L<sup>-1</sup>, and anoxic conditions were not encountered. Moreover, the fact that pH values ranged between 7.01-7.78 seems to indicate that pH is another factor preventing the release.

The pore water heavy metal toxicity values, and parallel to these, the fact that NI values were very close to 1, point to potential heavy metal toxicity. The pore water Cu and Zn concentrations in our study were found to exceed the reference values (WQC) for sediment pore water; this finding corresponds to the values reported by Şeker (2019) for Deriner Reservoir.

The variety of pollution sources affecting the second station during our study, among which were industrial activity and agricultural and household waste, resulted in higher heavy metal toxicity values at that station than at the first station.

In recent years, various advanced techniques for dredging have been studied. Chen et al. (2019) report that, in sediments that had and had not undergone dredging in eutrophic Taihu Lake (China), they used a thin film diffusive gradient technique and a high-solubility separation technique on dissolved metals and DGT-unstable metals; according to the measurements for dredging in April and



July, there was a positive effect on the polluted sediments, and in October and January, decomposing algae found in the dredging zone after dredging decreased its effectivity. It is thought that research into the possibility of using these new techniques in Lake Mogan would result in both financial and time-saving gains.

## 5. CONCLUSION

The pore water metal concentrations in Lake Mogan reflect the lake basin's pollutant sources. In addition to anthropogenic pollutants, the possibility should not be ignored that, during different geological ages from the Triassic period (200-250 million years ago) to the current day, the existence of extreme variations in rock formations (Akyürek et al., 1997) may have affected the metallic elements in the lake water and sediment. As external pollutants continue to affect the lake, it is possible that toxicities for Zn and Cu, which were found in the pore water and at higher levels than other metals, pose a long-term risk. Our finding that pore water heavy metal concentrations in Lake Mogan were generally higher than those in the overlying water indicates that there is strong adsorption of metals in the lake. In other words, the fact that sediments function as a trap for heavy metals and promote their retention means that dredging is not very effective at reducing heavy metal contamination in the lake. Considering the findings, dredging depth stands out as the most important factor leading to the more effective utility and feasibility of sediment dredging in the lake. Moreover, performing parallel analytic chemical and eco-toxicological tests is an important further step toward evaluating heavy metal release from the lake's sediment and recommending suitable dredging alternatives to dredging depth.

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## CONFLICT of INTEREST

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

## AUTHOR CONTRIBUTIONS

All authors contributed equally.

## ETHICS

There are no ethical issues with the publication of this manuscript.

## DATA AVAILABILITY STATEMENT

The authors confirm that the data that supports the findings of this study are available within the article. Raw data that support the finding of this study are available from the corresponding author, upon reasonable request.

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