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Microbial desalination cell treated spent geothermal brine as a nutrient medium in hydroponic lettuce cultivation: Health risk assessment

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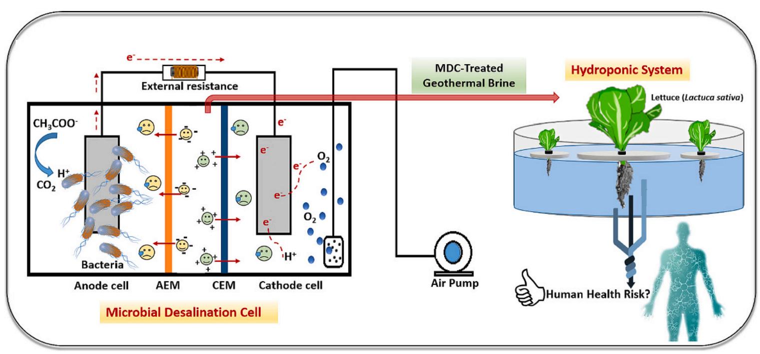
HIGHLIGHTS

- Microbial desalination cell process was performed for spent geothermal water treatment.
- Highest removal efficiency of 67.3 % was obtained for arsenic using the MDC system.
- Lettuce was effectively cultivated in hydroponics using treated geothermal water.
- Health risk was insignificant with a low health risk index and hazard quotient.
- Treated wastewater reusability in hydroponic system is a sustainable alternative.

GRAPHICAL ABSTRACT



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ABSTRACT

The scarcity and contamination of freshwater resources are extremely critical issues today, and the expansion of water reuse has been considered as an option to decrease its impact. Therefore, the reuse of microbial desalination (MDC)-treated spent geothermal brine for agricultural purposes arises as a good solution to prevent water contamination and provide sustainable water usage. In this study, the potential of treated spent geothermal water from MDC system as a nutrient solution for the hydroponic cultivation of lettuce was evaluated. The effects of different water samples (Hoagland solution (R1) as a control, MDC-treated water (R2), 1:1, *v/v* mixture of MDC-treated water and Hoagland solution (R3), 4:1, v/v mixture of MDC-treated water and Hoagland solution (R4), and tap water (R5)) on lettuce growth were considered. The application of R3 and R4 samples for hydroponic lettuce cultivation was promising since the lettuce plants uptake sufficient nutrients for their growth and productivity with low toxic metal concentrations. In addition, the chlorophyll-a, chlorophyll-b, and carotene contents of lettuce were in the range of 1.045–2.391 mg/g, 0.761–1.986 mg/g, and 0.296–0.423 mg/g in different water samples, respectively. The content of chlorophyll-a was highest in R1 (2.391 mg/g), followed by R3 (2.371 mg/g). Furthermore, the health risk assessment of heavy metal accumulations in the lettuce plants cultivated in the various water samples was determined. Results showed that heavy metal exposure via lettuce consumption is unlikely to suffer noticeable adverse health problems with values below the permissible limit value.

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1. Introduction

Water shortages and contamination present substantial health and environmental problems for an essential portion of the global population due to the lack of necessary infrastructure for effective wastewater treatment (Unfried et al., 2022). Moreover, water scarcity causes serious challenges for various sectors of water consumption, including industry, domestic, and agriculture, with the agricultural sector being the most distressed one that utilizes vast amounts of available water resources (Liu et al., 2022). In this regard, the utilization of treated wastewater has been commonly recommended for its environmental advantages, particularly in areas facing water scarcity. Namely, wastewater contains essential resources, including water, energy, and nutrients required to maintain the increasing population (Panhwar et al., 2022). Therefore, future wastewater infrastructure should be constructed as centralized, semi-centralized, decentralized, or combined processes per community requirements and constraints to operate as integrated resource recovery industries and wastewater treatment plants. Overall, the solution to the rising clean water crisis may lie in efficiently treating contaminated water resources for recycling and improving the supply of clean water with adequate management. Currently, geothermal water is a promising renewable energy source, gaining considerable interest in Türkiye and globally (Mott et al., 2022). The energy produced from geothermal water is utilized in various applications like a greenhouse, aqua-culture pond heating, agricultural drying, electricity production, and balneology (Islam et al., 2022). However, these applications cause the formation of a huge amount of saline spent geothermal wastewater, which is widely disposed into agricultural areas accidentally or deliberately (Dhar et al., 2020). The varied and concentrated ionic content of geothermal water results in harmful effects on agricultural crops. Namely, geothermal waters can be characterized by various physicochemical parameters depending on their hydrogeological properties, characteristics of the rocks involved, the depth at which resources occur, and the source of water supply. Geothermal waters may contain significant amounts of neutral species, cations, and anions (Haklıdır and Şengün, 2020). In particular, geothermal water supplies include inherent boron complexation with Cd, Cu, Ni, and Pb metals, which is more hazardous than those metals alone. The high heavy metal, anion, and cation concentrations could be toxic to plants causing chlorosis and necrosis of shoots, root growth inhabitation, presence of burns, and reduction of photosynthesis (Hua et al., 2021). These parameters largely determine the technology to be used in geothermal water treatment in regard to the relevant limits that are dictated by the final use. Among the ions that are present, boron content is critical in geothermal waters, which contain higher concentrations than seawater and brackish water. Boron in geothermal resources has been found due to natural sources including soils containing borosilicate and borate, volcanic activities, mineral dissolution from soils and rocks, and anthropogenic sources like detergents, boron-containing cleaning products, borosilicate glass, fertilizers, semiconductors, and flame retardants production (Kartikaningsih et al., 2016). Overall, the treatment of spent geothermal brine is not only essential for safe disposal but is fast becoming a requirement to tackle water scarcity. Therefore, using treated spent geothermal brine may be a promising solution to prevent environmental contamination and provide clean water for agricultural applications. Treated spent geothermal water use in agriculture also represents a considerably important option for clean water sustainability, saving it for industrial applications and drinking supplies.

However, the main problem with water reuse is the wastewater quality, namely the presence of pathogenic organisms, heavy metals, organic contaminants, and pharmaceutical product residues, which represent a hazard to the general public's health when reused without adequate treatment (Mannina et al., 2022). Systems for treating wastewater vary between biological and physicochemical processes, two polarized approaches. The selection of treatment techniques is significantly affected by investment and operating costs, wastewater resources and quality, and planned water reuse, among other factors. Compared to biological processes, chemical-based methods, such as oxidation processes, have been demonstrated to be more effective in removing organic complexes and resistant chemicals from industrial waste effluents (Brillas, 2022). However, their utilization in wastewater treatment is problematic due to their high chemical and energy needs (Saravanan et al., 2022). Conversely, biological methods are reliable, effective, and economical when appropriate bacterial cultures and processing conditions are performed. Recently, the microbial desalination cell (MDC) process has been studied to desalinate salty waters while simultaneously treating organic matter containing wastewater and producing energy and is considered a promising option to decrease dependence on fossil fuel energy and clean water for various purposes (Goren et al., 2023; Sadeq and Ismail, 2023). On the other hand, there is no comprehensive research on the utilization of the MDC-treated effluents in agriculture.

Moreover, achieving sustainable crop production under the prevailing water-shortage conditions needs sustainable agricultural methods that decrease water consumption and enhance yield per unit of water utilization. The inadequacy of agricultural land in urban communities is another limitation to sustaining crop production for these heavily populated settlements. In this context, hydroponic crop cultivation has emerged as a modern technique crucial for more substantial water usage efficiency. The hydroponic cultivation method is basically the soil-free cultivation of crops under a controlled environment, and it utilizes water and nutrients to enhance faster growth, superior water use efficiency, and improve productivity (da Carvalho et al., 2018). Namely, continuous crop production throughout the year becomes possible by cultivation under soil-free and regulated environmental conditions, even in places where crop cultivation would ordinarily be unfeasible (Jung and Kim, 2020). To date, several investigations have been conducted on the reutilization of treated wastewater in hydroponic cultivation methods for the growth of plants (Afonso et al., 2023; Okasha et al., 2022; Wiegmann et al., 2023). For instance, the hydroponic cultivation of tomato plants using municipal wastewater was investigated with contaminant removal (Rana et al., 2011). The authors reported that >75 %-nitrate, 61.4 %-Chemical Oxygen Demand (COD), and 72 %-Biological Oxygen Demand (BOD) removal efficiencies were obtained in all treatments. In a separate study, barley fodder production was investigated in various water resources (treated sewage wastewater, tap water, and mixed wastewater-tap water) using a hydroponic cultivation system (Al-Karaki, 2011). Results proved that the plants cultivated in wastewater more efficiently than those cultivated with tap water in terms of water use, namely, the water consumption was 1.26 m^3 for wastewater and 1.56 m^3 for tap water to cultivate 1 ton of barley fodder. Similarly, lettuce cultivation was studied in the hydroponic system using anaerobically treated domestic wastewater as a nutrient solution (Lee et al., 2021). Lettuce produces greater yields when grown hydroponically than it is grown on soil since it is so adaptable. Consequently, lettuce is one of the most important crops that is grown the most in hydroponic systems (Cometti et al., 2013; Gillani et al., 2023; Paulus et al., 2012).

On the other hand, the accumulation of heavy metals is key concern with wastewater irrigation of vegetables. Although the amounts of heavy metals found in secondarily treated effluent from non-industrial operations are often low, vegetables can still absorb heavy metals, even from deposits on portions exposed to air from contaminated areas (Adrover et al., 2013). Chronic exposure to heavy metals can have negative health effects on both humans and the various animals that ingest them. Therefore, it is crucial to analyze the chemical components of wastewater and evaluate the nutrients and heavy metals stored in the plants cultivated using the treated wastewater in order to determine the agricultural reuse of wastewater as a nutrient solution in hydroponic systems.

According to the literature, MDC-treated geothermal brine could be a promising solution considering the hypothesis that it will enhance water quality. Therefore, this study proposed to investigate lettuce cultivation using MDC-treated spent geothermal water with various dilutions. Moreover, the heavy metal, anion, and cation contents of the hydroponically cultivated lettuce were considered to evaluate their health risks. To our best knowledge, no previous investigations have applied hydroponic cultivation system using MDC-treated geothermal brine to minimize the utilization of freshwater resources. The main objectives of the study were as follows: (i) to evaluate the simultaneous spent geothermal water treatment and wastewater treatment with energy production performance of the MDC process, (ii) to evaluate the heavy metal removal performance of MDC-system, (iii) to assess the heavy metal concentration in treated wastewater and plant tissues (iv) to evaluate the suitability of the MDC effluent as a promising nutrient medium to replace commercially available nutrient solutions for hydroponic systems, (v) to compare the potential of lettuce growth between treated spent geothermal water and a control medium (Hoagland solution and tap water), (vi) to assess the health risk related to the consumption of lettuce cultivated in the hydroponic system using the MDC effluent as a nutrient solution.

2. Materials and methods

2.1. MDC system set-up

The experimental set-up of the MDC reactor with an aeration unit (Fig. 1) is reported in detail in our previous paper (Goren and Okten, 2021a). Basically, the bioreactor has a compact construction with independent Plexiglass cells and gaskets for an optimum airtight seal. In this study, the lab-scale bioreactor used as the MDC system showed a three-chamber configuration: the desalination cell (cell volume: 270 mL) flanked by the cathode and anode cell (volume of both cells: 270 mL) and disconnected from them using an anion exchange membrane (AEM, Membrane International Inc.) and a cation exchange membrane (CEM, Membrane International Inc.) with both active surface areas of 90 cm^2. The cathode and anode electrode materials (36 cm^2) were composed of carbon plates (Goodfellow Ltd., England) and both connected to the copper wire, acting as an electron conductor from the anode to the cathode cell. Under the closed-circuit mode, the 100 Ω external resistor was used in the system to sustain the constant electron transfer. Lastly, the bioreactor was tightened using O-rings with stainless steel bolts to prevent water leakage from the system and air intrusion from the environment.

Industrial wastewater was collected from a yeast production facility's wastewater treatment plant in İzmir. The COD and pH values for the yeast wastewater were 5516 mg/L and 7.91, respectively, and anion, cation, and heavy metal concentrations are reported in Table 1. Before using the wastewater in the experiments, it was filtered through a coarse filter to remove coarse solids. The anaerobic sludge was also obtained from the primary clarification tank of the yeast wastewater treatment plant. After the sludge sample was taken, it was stored in amber bottles at 4 °C to prevent the microorganisms in it from dying or losing their activation, and it was brought to room temperature before use in the experiments to enable the microorganisms to become active. For operation, the anode cell was fed with a mixture of anaerobic sludge from the primary clarification tank and yeast industry wastewater as substrate with a volumetric ratio of 1:1, and then this mixture was constantly stirred at 250 rpm to prevent agglomeration and provide homogeneity of the solution. Moreover, the anode cell was heated at a mesophilic temperature of 40 °C to provide optimum conditions for anaerobic microbial consortium growth. Namely, at mesophilic temperatures, the metabolic rate of microorganisms increases resulting in better substrate degradation rates, accelerated electron generation by microorganisms, increasing current production, and hence improving desalination efficiency (Zábranská et al., 2000). Therefore, the mesophilic anolyte solution temperature of 40 °C yielded the best treatment efficiency. A cathode cell was filled with 100 mM potassium phosphate buffer solution (PBS, Sigma Aldrich) and oxygenated with a constant airflow rate of 2 L/min. Namely, acidified water, microalgae-containing solutions, phosphate buffer, ferricyanide, non-buffer saline solution, and sodium chloride solutions were previously utilized as the catholyte (Ebrahimi et al., 2018). On the other hand, our previous study proved that the MDC using phosphate buffer achieved the highest removal performance, showing that the combination of low pH variation due to buffer capacity, high conductivity, and minimum internal resistance had the greatest benefit in contaminant removal (Goren and Okten, 2022). The ion transfer in PBS solution was more effective than in acidified water and tap water solution, resulting in a great performance of non-buffer saline catholyte despite pH imbalance. The desalination cell was filled with spent geothermal brine (pH: 8.04 and electrical conductivity (EC): 1760 μS/cm) from a geothermal power plant, and the temperature was set to 40 °C, mimicking spent geothermal brine occurrence in field conditions for a real-scale application. Based on our preliminary experimental runs, considering the effective arsenic removal, the operational time of the MDC system was specified as 3 d. Consequently, the MDC bioreactor was conducted at a temperature of 40 °C in both the anode and desalination cells, an operating time of 3 d, a mixing ratio of 250 rpm in the anode cell, and an airflow rate of 2 L/min in the cathode cell in the batch operation mode. Water samples for triplicated physicochemical analysis were gathered daily from each cell. Yeast wastewater, spent geothermal brine, and MDC-treated spent geothermal brine quality results are reported in Table 1.

2.2. Cultivation of lettuce and hydroponic system set-up

The lettuce seeds (*Lactuca sativa* L.) were first rinsed with pure water (PW) three times, soaked for 20 min in pure water (PW), wrapped in

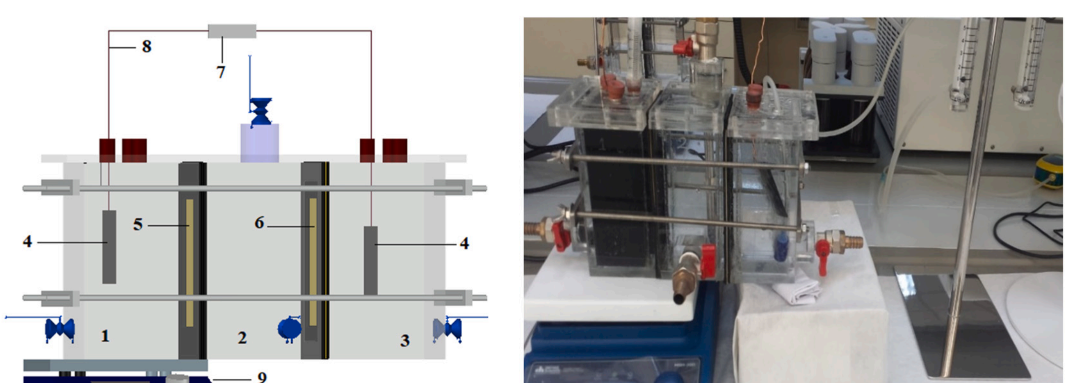


Fig. 1. Schematic description and actual view of three-chamber lab-scale MDC bioreactor: (1) Anodic cell, (2) Desalination cell, (3) Cathodic cell, (4) Carbon plate anode and cathode, (5) Anion exchange membrane, (6) Cation exchange membrane, (7) External resistance, (8) Copper wire, (9) Stirrer.

Table 1
Physicochemical properties of yeast wastewater, spent geothermal brine, and MDC-treated geothermal brine.

Water type											
Geothermal brine				Yeast wastewater				MDC-treated geothermal brine			
Ion	mg/L	Ion	mg/L	Ion	mg/L	Ion	mg/L	Ion	mg/L	Ion	mg/L
K^+	26.79 ± 0.31	F^-	7.96 ± 0.03	K^+	868 ± 2.1	F^-	0.20 ± 0.02	K^+	28.7 ± 4.69	F^-	4.17 ± 0.42
NH_4^+	1.0 ± 0	Si	45.04 ± 0.2	NH_4^+	452 ± 0.76	Si	66.0 ± 0.92	NH_4^+	10.4 ± 6.88	Si	42.17 ± 0.36
Na^+	440.3 ± 0.12	Br	N.D.	Na^+	1608 ± 1.52	Br	N.D.	Na^+	476 ± 23.3	Br	0.97 ± 0.02
Ca^{2+}	23.77 ± 1.08	Al	0.0157 ± 0.11	Ca^{2+}	299 ± 0.1	Al	0.075 ± 0	Ca^{2+}	11.4 ± 5.21	Al	0.009 ± 0.01
Mg^{2+}	6.83 ± 0.43	As	0.1997 ± 0.02	Mg^{2+}	77.5 ± 1.1	As	0.007 ± 0.13	Mg^{2+}	3.23 ± 1.86	As	0.065 ± 0.08
Mn^{2+}	N.D.	B	10.28 ± 0.24	Mn^{2+}	0.183 ± 0.1	B	0.142 ± 0.25	Mn^{2+}	N.D.	B	9.16 ± 0.06
NO_3^-	0.31 ± 0.01	Cu	0.0104 ± 0.01	NO_3^-	25.6 ± 0.49	Cu	0.003 ± 0.16	NO_3^-	2.80 ± 0.44	Cu	0.134 ± 0.02
Cl^-	192.7 ± 1.36	Cr	0.002 ± 0.03	Cl^-	1573 ± 0.54	Cr	0.325 ± 0.02	Cl^-	293 ± 45.8	Cr	0.002 ± 0
SO_4^{2-}	164.3 ± 1.08	Fe	1.35 ± 0.02	SO_4^{2-}	1117 ± 2.27	Fe	0.571 ± 0.33	SO_4^{2-}	80.3 ± 29.9	Fe	0.61 ± 0.39
PO_4^{3-}	N.D.	Li	2.399 ± 0.15	PO_4^{3-}	7.68 ± 0.2	Li	N.D.	PO_4^{3-}	11.1 ± 4.18	Li	1.63 ± 0.21

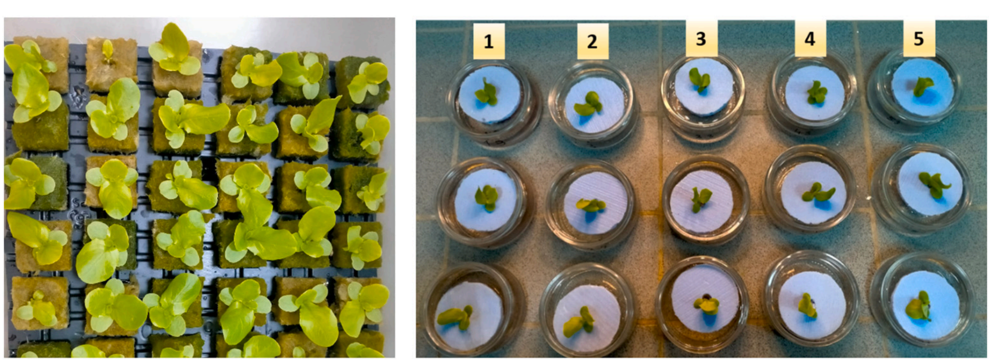
N.D.: Not detected.

moisturized towels, and kept in the refrigerator at a temperature of 4 °C for 24 h before the sowing process. Then, in a controlled environment (22.5–27 °C) sprouting greenhouse, a total of moisturized 100 lettuce seedlings were placed on Grodan Rockwool cells (52.5 × 31.5 × 4 cm) and cultivated using commercial nutrient solution (EC: 500 μs/cm and pH: 6.5) in containers for two weeks (Fig. 2a). After two weeks in the sprouting container, the seedlings in the Rockwool cells were removed and transplanted to the hydroponic systems. Furthermore, the germination rate of seeds is calculated by dividing the number of seeds that sprout by the total number of seeds started, and then multiplying that number by 100 to get a percentage, and the germination rate was found as 65 %.

Cylindrical glass cells with an operated total volume of 120 mL, 8 cm in diameter, and 3.5 cm in height were used as a lab-scale fed-batch hydroponic system (Fig. 2b). Each hydroponic cell was equipped with floating Styrofoam drilled in the middle to place the lettuce plants and three lettuce plant was separately placed per each condition. Then, 14-day-old lettuce seedlings were placed into these holes. Finally, the hydroponic cells were fed with 120 mL of specified water samples (Hoagland solution (R1) as a control, MDC-treated water (R2), 1:1, *v*/v mixture of MDC-treated water and Hoagland solution (R3), 4:1, v/v mixture of MDC-treated water and Hoagland solution (R4), and tap water (R5) for perpetual rinsing of the roots. Water samples were replaced with fresh samples once a week. Essentially, since the volume of water used in the experiments was small, the sampling time interval for heavy metal, anion, and cation analyses was determined as once a week in order not to reduce the sample amount. On the other hand, physicochemical parameters were taken daily since they were analyzed directly without wasting the solution. Additionally, preliminary studies have shown that after a one-week growth period, the ions and metals needed for the effective growth of plants in the solutions decrease. For this reason, the samples were renewed weekly as stated, and during this process, the ions and heavy metals in the samples were analyzed. The physicochemical analysis results of utilized water samples are reported in Section 3.2. Experiments were performed, allowing air circulation at room temperature (21–25 °C) under an appropriate location, gathering natural sunlight irradiation with a photoperiod of 16 and 8 h of light and darkness in the laboratory for an operating time of four weeks with three replications. All water and lettuce samples were analyzed at the end of the four-week experiments.

2.3. Analytical methods

The pH, dissolved oxygen (DO), and EC values of the raw and utilized water samples in the hydroponic system were measured daily using a portable pH meter (Hach HQ11D). The COD of the anolyte was measured using the standard closed reflux method (APHA, 2007). Moreover, water samples were analyzed before being utilized in hydroponic lettuce cultivation and weekly periods to evaluate the heavy metals, anions, and cations concentrations. In addition, the lettuce growth parameters, such as leaves number and length, root length, and wet weight, were measured at the beginning, weekly periods, and end of the experiments. The dry weight (oven-dried at 60 °C for 24 h) of the lettuce samples was determined at the end of the runs. The raw and utilized lettuce samples were also processed for anion, cation, and heavy metal analysis. Anions and cations concentrations were determined using ion chromatography (IC, Dionex ICS-5000), while the heavy metal concentrations were measured by an Inductively Coupled Plasma–Optical Emission Spectrometer (ICP-OES, AGILENT 5110). Firstly, the 0.2 g of oven-dried lettuce samples were grained and powdered. Then, powdered samples were mixed with 1 mL H_2O_2 and 10 mL HNO_3 and digested with a microwave device (MARS 6) at a temperature of 200 °C for 30 min. Finally, the microwave-digested samples were filtered and measured for heavy metal and ion concentrations using ICP-OES and IC,



(a) (b)

Fig. 2. (a) Acclimatized lettuce and (b) fed-batch hydroponic system set-up for control and different water samples.

respectively. All analyzes were performed in triplicate, and average data were reported. Moreover, three samples were gathered from the same biomass and/or water sample, and these samples were analyzed. Then, their average values were reported, and standard deviation values were calculated in this approach. Standard deviation values for water and lettuce biomass analysis were in the range of 0.01–2.27 mg/L and 0.02–1.36 mg/kg, respectively. The confidence of the analysis revealed a 95 % confidence.

For the carotene and chlorophyll analysis, the 0.25 g of lettuce leaves were extracted in 50 mL of methanol (purity, 80 %) using a pestle and mortar and then the solution was filtered with a coarse filter. Total chlorophyll content was evaluated by measuring the absorbance of the filtered solution at a λ_{max} of 645 and 663 nm using a UV-spectrophotometer (Shimadzu, UV-2600). In addition, the carotene content was evaluated by measuring the absorbance of the solution at a λ_{max} of 470 nm (Ahmed et al., 2021). The chlorophyll-a, chlorophyll-b, and carotene contents were determined using the following equations:

$$\text{Chlorophyll} - \text{a}\ (\text{mg/mL}) = (12.7 \times \text{Abs663}) - (2.69 \times \text{Abs645}) \tag{1}$$

$$\text{Chlorophyll} - \text{b}\ (\text{mg/mL}) = (22.9 \times \text{Abs645}) - (4.68 \times \text{Abs663}) \tag{2}$$

$$\text{Carotene} = ((1000 \times \text{Abs470}) - (1.82 \times \text{Ch} - \text{a}) - (85.02 \times \text{Ch} - \text{b}))/198 \tag{3}$$

2.4. Irrigation water quality assessment

The water quality of MDC-treated geothermal brine was evaluated in terms of its suitability for irrigation purposes. According to Appendix 7 of the Wastewater Treatment Plants Technical Operation Communique (Turkish Official Turkish Official Gazette, 2010), quality of irrigation water was evaluated in three categories: Quality I, Quality II, and Quality III. Water of Quality I indicated that it would not damage crops or soil media (Table 2). Water of Quality II had various drawbacks and should be used with caution, whereas Quality III water had substantial adverse effects on the soil media.

Moreover, the Regulation on minimum requirements for water reuse is indicated as one of the implementing acts for the realization of the first circular economy Action Plan for the EU (EU Commission, 2015). The regulations regarding water reuse are continued in the second circular economy Action Plan for the EU, which also emphasizes the possibility of using reclaimed water from urban wastewater treatment plants as a source of not only water but also nutrients, which should be directed to agriculture (Commission, 2020). The requirements applicable to reclaimed water for agricultural irrigation are categorized into reclaimed water quality classes as class A (all food crops consumed raw where the edible part is in direct contact with reclaimed water and root crops consumed raw), B (food crops consumed raw where the edible part is produced aboveground and is not in direct contact with reclaimed water, processed food crops, and nonfood crops), C (food crops consumed raw where the edible part is produced aboveground and is not in direct contact with reclaimed water, processed food crops, and nonfood crops), and D (industrial, energy, and seeded crops). Furthermore, irrigation water quality guidelines and standards for wastewater reuse in agriculture were reported by (Jeong et al., 2016) in more detail considering different countries.

Table 2
Assessment of the physicochemical parameters for irrigation water quality (Turkish Official Gazette, 2010).

Parameter	Irrigation water quality classifications and limitations		
	Quality I (None)	Quality II (Minimum-Medium)	Quality III (High)
Salinity			
EC (dS/cm)	< 0.7	0.7–3	>3
Toxicity of ions			
Na (mg/L)	< 3	3–9	> 9
Cl (mg/L)	< 140	140–350	> 350
B (mg/L)	< 0.7	0.7–3	> 3
pH	6.5–8.4		

2.5. Health risk assessment

The human health risks from the consumption of lettuce cultivated with various water samples were assessed. Possible health risk values were evaluated considering bio-accumulated heavy metals (HM) such as arsenic (As), boron (B), copper (Cu), chromium (Cr), iron (Fe), lithium (Li), and nickel (Ni). For the HM risk evaluation, daily intake rate (DIR) by humans, health risk index (HRI), and hazard quotient (HQ) values were calculated using Eqs. (4, 5, 6) as follows (Cao et al., 2014; Sibuar et al., 2022):

DIR (Daily intake rate) is one of the HM exposure pathways through the ingestion of lettuce, which evaluates the average daily loading of HM into the human body of an indicated body weight. The DIR is mainly related to concentrations of HM in plant tissues, the body weight of the consumer, and the amount of consumed plants.

$$\text{DIR}\ (\text{mg/kg.d}) = \frac{C_{HM} \times CF \times IR}{BW} \tag{4}$$

where C_{HM} is the heavy metal concentration in lettuce, CF is the conversion factor that is calculated using ratio between fresh plant weight to dry weight (dry-weight/fresh-weight), IR is the daily intake of lettuce (kg/day), assumed as 0.05, and BW is the average body weight for adults (kg), assumed as 75 (Goren et al., 2022).

The HRI (Hazard Resilience Index) is basically the ratio between the DIR of the lettuce and the reference dose (RfD. mg/kg/d) of the selected HMs that assess the non-carcinogenic health risk.

$$\text{HRI} = \frac{\text{DIR}}{\text{RfD}} \tag{5}$$

where RfD is an assessment of the minimum daily human exposure that is expected to occur without causing a considerable lifetime toxicity risk for non-carcinogenic impacts (U.S.E.P.A, 2007). Furthermore, the HRI < 1 demonstrates that the exposed contamination is safe from the human health risk that occurs from HM consumption.

$$\text{HQ} = \frac{EF \times ED \times IR \times C_{HM} \times F}{RfD \times BW \times ET} \tag{6}$$

The HQ is a ratio between HM concentration and the RfD, ED is an exposure duration (ED, 70 years), EF is an exposure frequency (EF, 104 d/year), ET is an average exposure time (ET, 25550 years), IR is ingestion rate, F is the unit conversion factor (F), and BW is the average body weight (BW, 75 kg) (Hope and Stock, 1998). The HQ value is a unitless index that demonstrates the problem level but does not assess the risk. The HQ < 1 and 1 ≤ HQ ≤ 5 denote that HM exposure via lettuce consumption is unlikely to suffer noticeable adverse health problems and refer that HM exposure is considerable to certain adverse health effects, respectively.

3. Results and discussions

3.1. Treatment efficiency of MDC

Geothermal brine treatment in MDC system was performed under optimum operating conditions such as anode and cathode electrode surface are of 36 cm^2, air flow rate of 2 L/min, activated sludge to yeast wastewater ratio of 1:1 (270 mL:270 mL), and catholyte solution of PBS (pH: 6.5), according to our previous studies (Goren and Okten, 2022; Goren and Okten, 2021b). In this study, we focused on the HM removal performance of the MDC system (Fig. 3).

The lowest removal efficiency was observed for B, most probably since the B in the solution occurred in neutral form, which prevents the

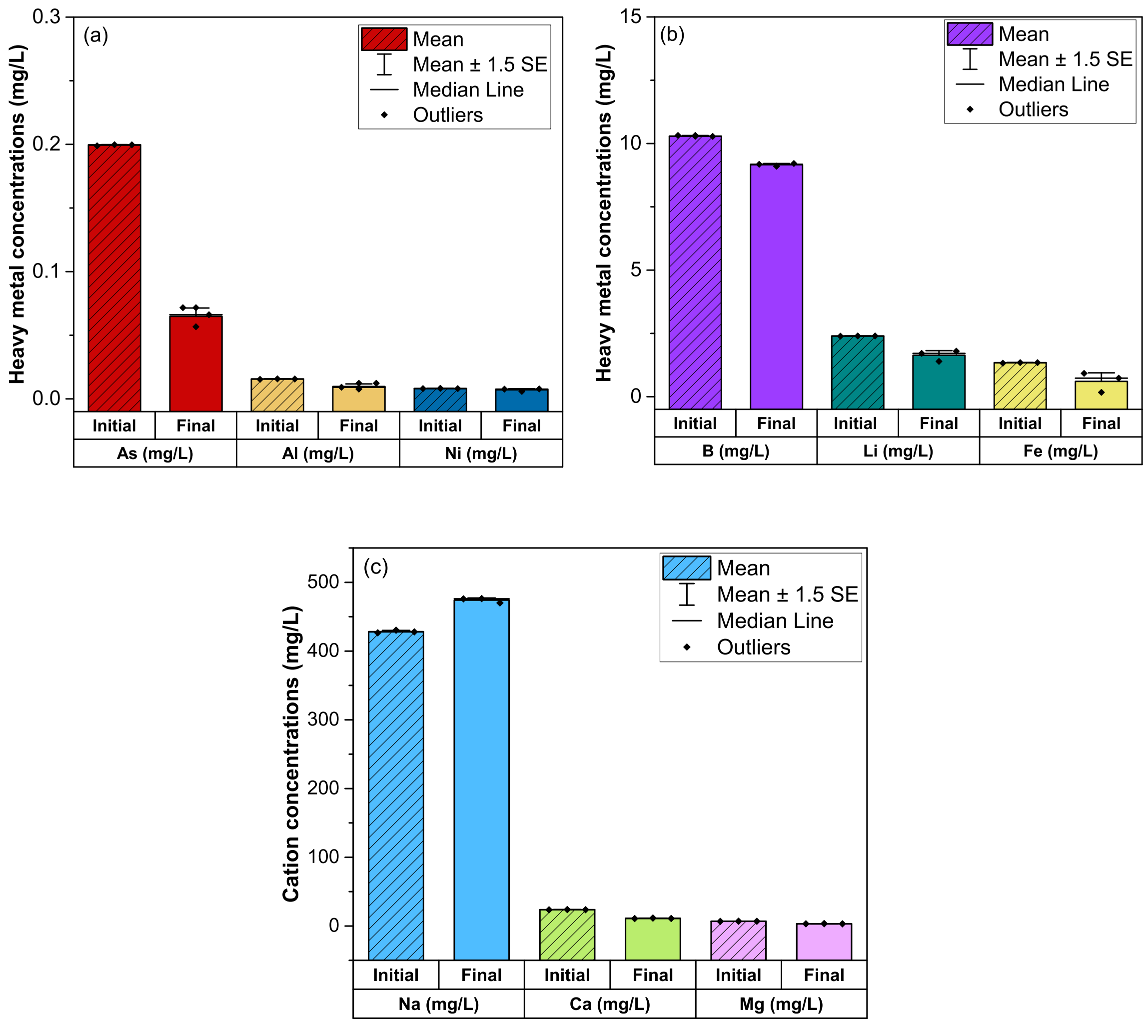


Fig. 3. Heavy metal and cation concentration in spent geothermal brine and MDC-treated brine: (a) As, Al, and Ni metals, (b) B, Li, and Fe metals, and (c) Ca, Na, and Mg ions.

transfer of B from desalination to anode cell through AEM. Namely, the initial B concentration was decreased from 10.28 to 9.16 mg/L at the end of the operational time of 3 days. On the other hand, the highest removal efficiency of 67.3 % ($C_{f\text{-}As}$: 0.065 mg/L) was achieved for As because of the effective transfer of positively charged As ions from desalination to the cathode cell through CEM. Furthermore, under the same operating conditions, the removal efficiencies were 32 % ($C_{f\text{-}Li}$: 1.63 mg/L), 42.7 % ($C_{f\text{-}Al}$: 0.009 mg/L), and 54.8 % ($C_{f\text{-}Fe}$: 0.61 mg/L) for Li, Al, and Fe, respectively, while there was no significant removal for Cu, Cr, and Ni metals. The MDC system's COD removal and electrical potential were also measured at the end of the experiment. Maximum COD removal efficiency was 68.8 % in 3 days. The initial electrical potential of the system was increased from 600 to 620 V at an operational time of 3 days. There was no change in electrical potential value in 3 days since no heavy metal inhibitory effect was observed on microbial activity. In addition, these results showed that the electrical potential may increase with increasing operating time owing to improving microbial activity. For instance, the highest electrical potential of the MDC system was reported as almost 900 V at an operational time of 12 days in our previous study (Goren and Okten, 2022). Similarly, the COD removal performance of the system increased with the increment in operating time. Consequently, results revealed that the MDC system is a sustainable and relatively effective system for spent geothermal brine treatment owing to its HM removal performance, particularly As removal, and energy self-sufficiency. On the other hand, water quality levels revealed that the MDC-treated water was in the range of Quality II and Quality III considering Cl^- and B concentrations. Therefore, further solutions should be performed for significantly effective HM removal and produce Quality I irrigation water using the MDC system. HM removals can be considerably increased with advanced optimization studies in the MDC system. For instance, studies on HM removal from waters using an MDC system may increase the practicability of the MDC in real applications under continuous operating mode with increasing contaminant removal performance.

3.2. *Water quality in the hydroponic system*

Initially, the MDC-treated water was investigated with the objective of assessing the efficiency of the MDC bioreactor and the applicability potential of performing the treated geothermal brine in agricultural irrigation, considering permissible limit values (Table 2). Results revealed that the MDC-treated water is suitable for utilization in irrigation, however, the utilization field should be specified and monitored to prevent or eliminate the risk of salinity related to the high concentration of Na^+ in the MDC-treated water. The quality parameters reported in this study and the other reported parameters in literature only on the quality of wastewater for utilization in soil irrigation (WHO, 2006); on the other hand, there are no specified limit values for hydroponic systems.

The ion and HM concentrations of water samples used for lettuce cultivation are reported in Table 3. The suitability of water samples was compared with the R1 sample as a control (commercial hydroponic nutrient solution). In R2, R3, R4, and R5 samples, the Al, Cr, Cu, and Fe concentrations were found to be almost the same as the values in the control sample. On the other hand, compared with R1 sample the B concentrations were 8.9, 21.1, and 40 times high in R3, R4, and R2 samples, respectively. These results revealed that although B concentrations are high in MDC-treated (R2) and diluted MDC-treated waters (R3 and R4). On the other hand, considering that B is a beneficial micronutrient for plants, even if the R3 and R4 samples were found as Quality II and Quality 3, these samples could be utilized in the cultivation of plants with high B demand. For instance, B is a crucial micronutrient for plant cultivation, and its necessity depends on plant type; there is a broad spectrum of sensitivity (avocado, lemon: 0.5 mg/L; apple, pear, plum, walnut: 1 mg/L; cotton, potato, tomato, sunflower: 2 mg/L; alfalfa, oat: 3 mg/L; asparagus, bean, rice, onion, palm: 4 mg/L) (Hilal et al., 2011). On the other hand, since the MDC-treated water (R2) has a considerably high B concentration (9.12 mg/L), it is unsuitable for direct plant cultivation. In order to overcome this drawback, it has been reported in our previous studies that the B concentrations will be reduced by operating the MDC treatment system for a longer time (Goren and Okten, 2021b). Furthermore, the Na^+ and Ca^{2+} concentrations in MDC-treated water (R2) were significantly higher than that for the R1 control sample. This problem can be eliminated by reducing the ion load as a result of the dilution of the R2 sample. Furthermore, the Cl^- concentrations in all water samples were higher than that for the R1 sample. The Cl^- concentrations of samples were in the range of 59.5–293 mg/L. The lowest Cl^- concentration of 59.5 mg/L was observed for the R3 sample, while the highest Cl^- concentration of 293 mg/L was obtained using the R2 sample. The lettuce planted using the R2 sample was presented a low growth rate since it exposed to ionic stress due to the accumulation of Cl^- in its tissues, which has a detrimental effect on its DNA, cell membrane structure, and enzyme activity (Li and Li, 2017). The salt stress can also disturb the uptake of other mineral ions, lead to nutrient starvation, and decrease the physiological activity of plants (Tavakkoli et al., 2011). Moreover, the high Cl^- concentrations in the rhizosphere decreases water potential, which results in a lower amount of water available to plants (Acosta-Motos et al., 2017). To balance high salinity, plants lower their osmotic potential, which is their strategy of adaption to salinity stress. Overall, these results provided that when the R3 and R4 samples were evaluated in terms of HM and ion concentrations among the utilized water samples, they provided similar physicochemical properties to the control sample (R1).

According to previous findings, the EC and pH are key factors that determine the chemical species, nutritional availability, and composition of the nutrients in the liquid fertilizers (Majid et al., 2021). The growth and production of the plant are adversely affected by hydroponics strengths that are either excessively high or low (Suhandy, 2014). Therefore, the pH, EC, and DO parameters were measured daily in all hydroponic systems during the whole cultivation period and are presented in Fig. 4. The pH and EC values were considerably changed during the experiments in all cases, while there was no significant change in DO concentrations in all cases, except the hydroponic system performed using the R2 water sample. According to Domingues et al. (2012) (Domingues et al., 2012), the continual changes in EC and pH during the growth cycle could be linked to nutrient absorption by plants. The initial pH values were in the range of 6.32–8.22. In all hydroponic systems, an increase in pH was observed on the 1st day and continued to the 7th day. The increase in pH could be explained by the acidic elements found in the solutions; when these elements are absorbed by plants, a natural response raises pH, causing pH upswings. Once lettuces were planted, a continuous and incremental elevation in pH was seen throughout the cultivation period in all cases, and this was prevented by replacing the solutions on a weekly period. Furthermore, in all hydroponic systems, an increase in EC was observed and continued to the 7th day. A similar trend was observed weekly after freshwater sample addition in all cases. The most significant increase was observed in the R2 sample. The EC of water samples (R1: 900 μS/cm, R3: 1100 μS/cm, and R5: 1300 μS/cm) in the final duration of lettuce growth ranged from 900 to 1300 μS/cm, which was below or equal to the suitable EC value of 1000–1300 μS/cm for lettuce growth (Sapkota et al., 2019). On the other hand, the EC values in R2 and R4 utilized hydroponic systems were above 1300 μS/cm. Namely, the highest EC value measured in the effluent was 2200 μS/m, and thus effluent salinity was not suitable for lettuce production. The increase in the EC value is most likely due to water loss from the solutions by transpiration or evaporation. Moreover, the amount of DO in plant growth showed that it is crucial for root formation and growth (Suyantohadi et al., 2010). The DO concentrations in R1, R3, R4, and R5 were uniform during the operational time of 14 days. However, the DO concentration decreased during the 5 days operation and then increased at the end of the experimental run using the R1 sample. Namely, the DO concentration decreased from 11 to 7.5 mg/L after 5 days in the R1-containing hydroponic system. In addition,

Table 3
Heavy metal, anion, and cation concentrations of utilized waters for lettuce cultivation.

	Parameter (mg/L)	R1	R2	R3	R4	R5
Heavy metals	Al	0.012 ± 0	0.009 ± 0.002	0.0119 ± 0	0.011 ± 0.001	0.0122 ± 0.002
	As	N.D.	0.065 ± 0.008	0.024 ± 0.001	0.052 ± 0.012	N.D.
	B	0.229 ± 0.02	9.16 ± 0.056	2.039 ± 0.124	4.838 ± 0.31	0.105 ± 0.032
	Cr	0.008 ± 0.001	0.002 ± 0	0.0008 ± 0	0.0005 ± 0	0.0008 ± 0
	Cu	0.049 ± 0.001	0.134 ± 0.019	0.053 ± 0.006	0.092 ± 0.013	0.0026 ± 0
	Fe	0.939 ± 0.015	0.61 ± 0.39	0.672 ± 0.021	0.634 ± 0.16	0.015 ± 0.001
	Li	0.0001 ± 0	1.63 ± 0.21	0.295 ± 0.014	0.793 ± 0.25	0.049 ± 0.036
	Ni	N.D.	0.007 ± 0.001	N.D.	0.0053 ± 0	0.0011 ± 0
	Na^+	2.98 ± 0.13	476.6 ± 23.38	97.2 ± 3.18	248.7 ± 1.334	111.4 ± 1.42
	NH_4^+	6.58 ± 0.045	10.4 ± 6.88	6.55 ± 0.35	6.80 ± 0.53	0.24 ± 0.034
Cations	K^+	68.8 ± 2.31	28.7 ± 4.69	53.9 ± 0.036	46.1 ± 1.52	4.76 ± 0.002
	Mg^{2+}	18.4 ± 1.046	3.23 ± 1.86	13.4 ± 0.021	10.55 ± 1.16	15.3 ± 1.16
	Ca^{2+}	60.9 ± 1.38	11.4 ± 5.21	56.1 ± 1.18	41.7 ± 1.37	169.9 ± 2.45
	F^-	<0.03	4.17 ± 0.42	0.8 ± 0.001	2.06 ± 0.23	0.1 ± 0
	Cl^-	1.75 ± 0.002	293 ± 45.8	59.5 ± 0.21	159.9 ± 3.17	236.6 ± 0.994
	Br	<0.15	0.97 ± 0.02	<0.15	0.26 ± 0.002	N.D.
Anions	NO_2^-	<0.15	0.31 ± 0.05	N.D.	0.20 ± 0.001	0.18 ± 0.21
	NO_3^-	350.6 ± 2.014	2.80 ± 0.44	257.6 ± 1.52	160.7 ± 2.31	15.2 ± 1.04
	SO_4^{2-}	54.1 ± 0.54	80.3 ± 29.9	69.0 ± 2.07	71.4 ± 1.52	55.7 ± 2.35
	PO_4^{3-}	35.6 ± 0.63	11.1 ± 4.18	27.3 ± 2.16	17.8 ± 1.02	N.D.

N.D.: Not detected.

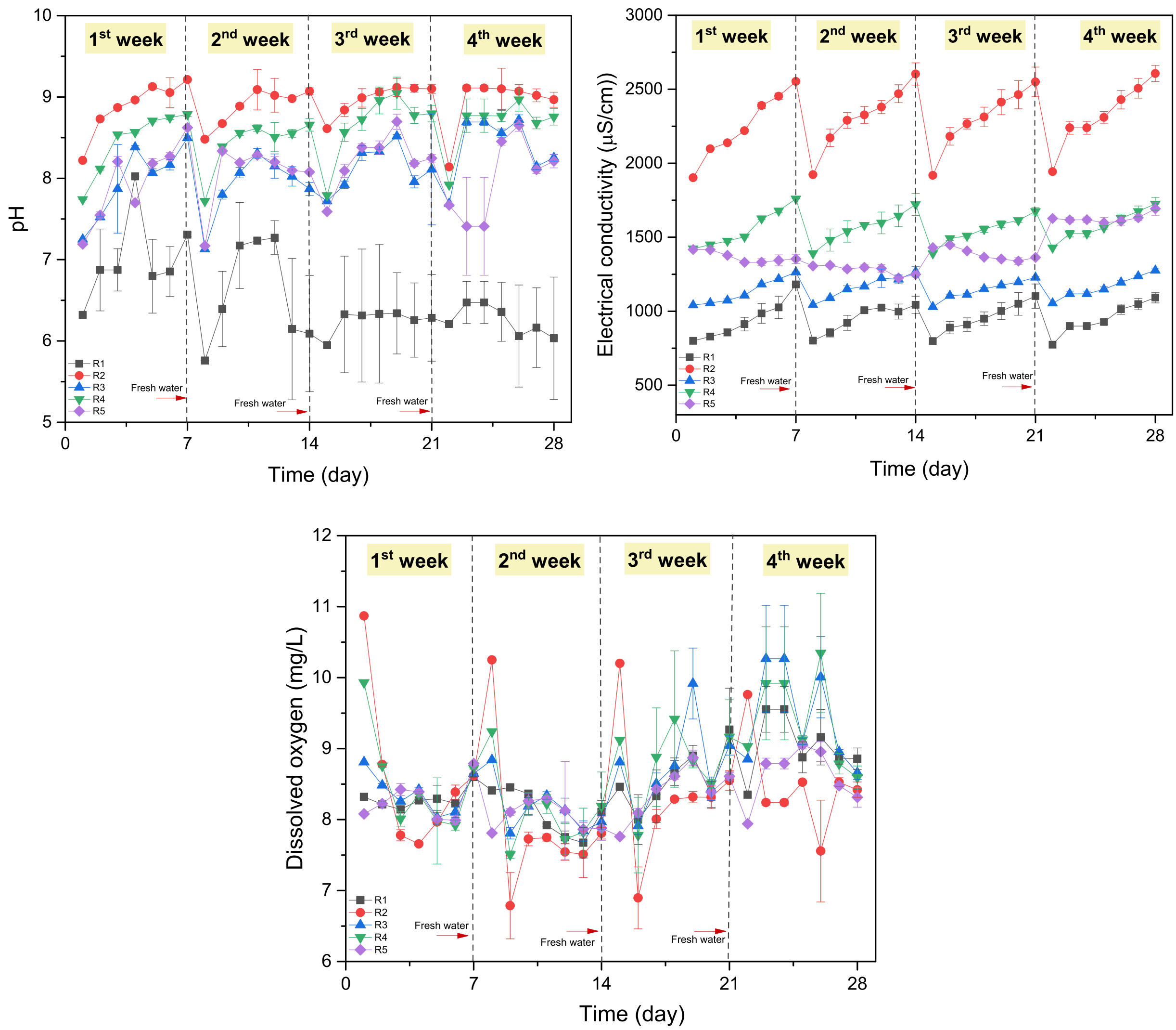


Fig. 4. Daily change in pH, EC, and DO concentrations in hydroponic systems for various water samples.

an increase in DO was observed in almost all samples after the 3rd week owing to the DO production via photosynthesis of lettuce plants and continued at the end of the cultivation period (Abu-Shahba et al., 2021). Overall, the DO concentrations in all cases were suitable for effective lettuce growth. For instance, Suyantohadi et al. (2010) reported that the optimum DO concentrations are in the range of 7–8 mg/L for leafy plants, while the minimum DO requirement is suggested as 2.1 mg/L (Ang et al., 2022; Suyantohadi et al., 2010).

3.3. Growth potential of lettuce in various water samples

The type of water utilized as a nutrient solution for lettuce cultivation considerably affects the growth parameters of plants. Therefore, the effects of various water sources on the lettuce plants were investigated in this study. The lettuce plants were exposed to R1, R2, R3, R4, and R5 for 30 days, and the impacts of water sources on lettuce growth and tolerance were monitored (Table 4 and Fig. 5). The white root color, green leaves color, and fresh root and leaves formation in lettuce plants were observed using R3, R4, and R5 samples compared with the R1 control sample. On the other hand, the application of R2 sample showed considerable morphological or physiological inhibition impacts on lettuce. The wilting and browning symptoms started emerging from the second week of the cultivation and continued till fourth week. While the main leaves and roots of lettuce cultivate in R1 was greater and had numerous sprouted-leaves and roots, the main leaves and roots of the lettuce cultivated in R2 sample had minor sprouted-leaves. Expose to MDC-treated water (R2) resulted with brown-black root color, green-yellow leaf color, and no production of fresh leaves and roots. The reason for these adverse effects in the lettuce cultivated in the R2 sample could be due to the relatively higher HM concentrations, nutrient deficiency, and pH imbalance (Ahmed et al., 2021; Amori et al., 2022; Lee et al., 2021). Moreover, these symptoms could be explained by the NO_2^- and NH_4^+ toxicity in lettuce as proved by Hoque et al. (2007) (Hoque et al., 2007). The authors investigated the toxic effects of NO_2^- and NH_4^+ on particularly lettuce plants and reported that the adverse effect of NO_2^- on lettuce growth was higher than that for the NH_4^+. Namely, the excess amount of NO_2^- and NH_4^+ in water samples may cause retardation in leaf and root production, discoloration, and chlorosis. Furthermore, the relatively lowest DO concentration was observed in the R2 sample compared to the other samples and control. Krishnasamy et al. (2012) (Krishnasamy et al., 2012) reported that the low DO content in media may prevent or inhibit plants' nutrient uptake performance and indirectly affect growth. In addition, the pH and EC values are important water quality parameters in lettuce cultivation applications. The optimum pH and EC values for effective lettuce cultivation were reported as in the range of 5.5–6.5 and 1.0–1.3 mS/cm, respectively (da Silva et al., 2018; Sace and Natividad Jr, 2015; Sapkota et al., 2019). Utilization of water or nutrient solutions with higher pH values may result in the

Table 4
The tolerance of lettuce in different water samples in a four-week cultivation period.

Parameter	Raw lettuce	R1	R2	R3	R4	R5
Lettuce root color	White	White	Brown-black	White	White	White
Lettuce leaf color	Green	Green	Green-yellow	Green	Green	Green
New root and leaf	–	Yes	No	Yes	Yes	Yes
Chlorophyll a (mg/g)	2.136	2.391	1.045	2.371	2.290	1.842
Chlorophyll b (mg/g)	1.776	1.986	0.761	1.953	1.819	1.690
Carotene (mg/g)	0.485	0.423	0.296	0.408	0.418	0.314
Actual photo of lettuce plants in different samples						

precipitation of macro and micronutrients, while at a pH in the range of 5.5–6.5, nutrients are excessively absorbed by lettuce. Therefore, the adverse symptoms were significantly observed on lettuce plants cultivated in the R2 sample due to its high pH (9.0) and EC values (2.5 mS/cm), which were inhibitory for lettuce growth.

Furthermore, the chlorophyll and carotene contents of the lettuce plants in different water samples were measured since their concentrations present information on lettuce productivity, viability, environmental quality, and phytosanitary. The chlorophyll-a, chlorophyll-b, and carotene contents of lettuce were in the range of 1.045–2.391 mg/g, 0.761–1.986 mg/g, and 0.296–0.423 mg/g in different water samples, respectively. The carotene content observed in this study was greater than that reported in the literature such as 0.066–0.165 mg/g (Mampholo et al., 2016), which reveals the advantageous effect of utilization of the MDC-treated water with directly and different dilution ratios as a nutrient solution. Similarly, the range of total chlorophyll content measured in this study (1.81–4.38 mg/g) was higher than that reported in the literature such as 1.04–1.18 mg/g (Ahmed et al., 2021), and 0.52–0.79 mg/g (Fallovo et al., 2009). Namely, the total chlorophyll content in lettuce was moderately affected by nutrient composition of the utilized water samples. At the end of the four-week period, there was no considerable difference between lettuce cultivated in R3 and R4 samples and those cultivated in R1 control, in relation to the carotene and chlorophyll contents. The content of chlorophyll-a was highest in R1 (2.391 mg/g) followed by R3 (2.371 mg/g) for both water samples. However, the lowest chlorophyll-a content of 1.045 mg/g, chlorophyll-b content of 0.761 mg/g, and carotene content of 0.269 mg/g was obtained using R2 sample most probably due to the inadequate growth of lettuce related to high HM, NO_2^-, NH_4^+ concentrations, and unsuitable operational conditions (pH, EC, and DO). Similar results were observed for the R5 sample related to the limited nutrient content of the tap water. On the other hand, the greater chlorophyll content observed in the R1 sample relative to the R2 sample was not completely signified in the lettuce productivity performance. These findings may reveal that lettuce growth has complex features that are related to various operational parameters such as light irradiation, combined effects of nutrients, enzymatic activity, DO content, and CO_2. Namely, the chlorophyll content in plants is not the individual parameter that controls plant yield and growth (Sapkota et al., 2019).

Moreover, the cultivation of lettuce with various water samples was assessed for four-week, considering the length of leaves and roots, width and number of leaves, the surface area of leaves, and wet weight, as presented in Fig. 5. As expected, the lettuce growth was dwarfed in all water samples when compared with the R1 control. For instance, the growth of the lettuce in the R2 sample was insignificant compared with the R1 control sample. The length of roots and leaves was 3.77 ± 1.59 and 5.40 ± 1.12 cm for R2, whereas that of R1 (control) was 6.77 ± 0.85 and 15.4 ± 0.7 cm in four-week cultivation, respectively. Similarly, the wet weight and number of leaves were 1.29 ± 0.75 g and 5 for the R2 samples, while that of the R1 was 2.56 ± 0.26 g and 8, respectively. Moreover, after for week of cultivation, the leaf length, root length, and number of leaves of R3 sample was 13.4 cm, 6.87 cm, and 7, respectively. Overall, the deterioration of the lettuce plant in R2 is mainly due to the accumulation of HM, NO_2^-, and NH_4^+.

3.4. Plant tissue anion and cation concentrations

The anion and cation concentrations for lettuce leaves cultivated in different water samples are reported in Table 5. In the R2 sample, heavy metal and ion analyzes could not be performed in the plant tissues because no growth was observed in the plant and even the plant rotted. Among the cations included in water samples (Na^+, NH_4^+, K^+, Mg^{2+}, and Ca^{2+}), the only differences in lettuce tissues were observed for Na^+ and K^+ anions, and these variances were proportional to the difference in Na^+ and K^+ concentrations in water samples. In this context, there was no significant difference for the concentrations of other anions in the lettuce tissues since there was no considerable variance between the anion concentrations in the water samples. The Na^+ content (4.18 mg/kg) in R1 cultivated lettuce tissue was almost 8.5 times lower than that for the R4 sample with 35.33 mg/kg of Na^+ content. Furthermore, the K^+ tissue concentrations were 23.88, 37.4, 66.07, and 95.68 mg/kg for R3, R5, R4, and R1 samples. These results proved that the high K^+ concentration in the R1 water sample correlated with the increase in R1 cultivated lettuce. Similarly, the accumulation of NH_4^+ by lettuce plants in all water samples was <6.25 mg/kg, which proved that the uptake of NH_4^+ by lettuce from all water samples was quite high and at the same proportion, considering the water samples' NH_4^+ concentration. In addition, the Mg^{2+} accumulation was low in all lettuce samples with a range of 6.51–8.5 mg/kg most probably due to the high Ca^{2+} concentrations in the water samples, thus, preventing or reducing the uptake rate of Mg^{2+} by lettuce (Abrahão et al., 2014; Yap et al., 2022). The cation accumulation values in lettuce tissues were reduced in the following order: $K^+ > Ca^{2+} > Mg^{2+} > NH_4^+ > Na^+$ in R1 and R3. The uptake of cations in lettuce tissues is consistent with the order of $Mg^{2+} \geq K^+ > Ca^{2+} > Na^+$, which was found by Amori et al. (2022) (Amori et al., 2022), and authors reported that this order could be changed related to the plant species, HM permissibility, and nutrient-water balance. Among the cations, the K was most effectively absorbed by lettuce plants. This may be related to the significant function K performs in a number of processes in plants, such as glucose metabolism, photosynthesis, and tolerance to biotic-abiotic pressures (Tränkner et al., 2018).

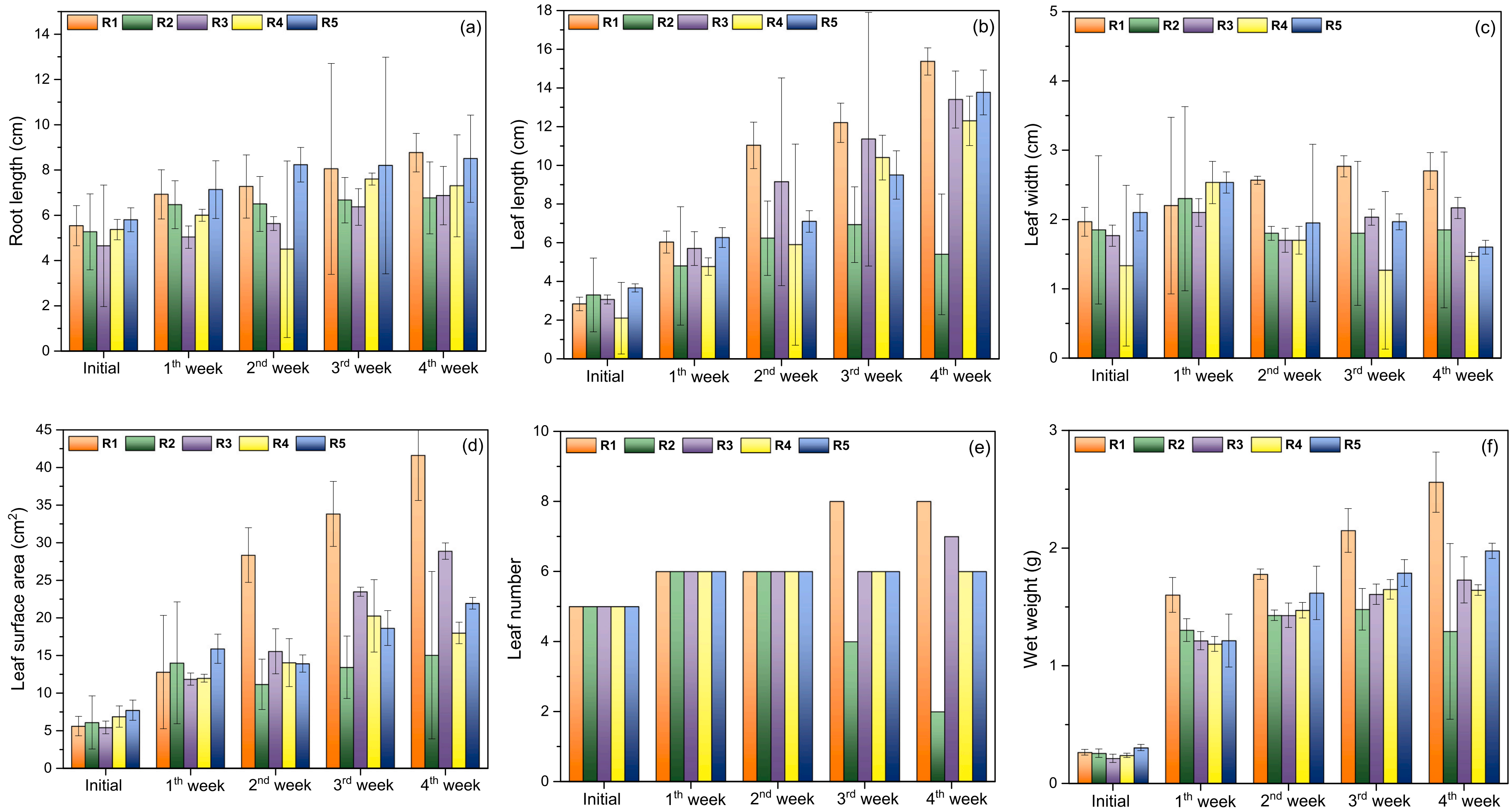


Fig. 5. Change in lettuces root (a) and leaf lengths (b), leaf width (c), leaf surface area (d), leaf number (e), and wet weight (f) in various water samples (three plant samples per each of the performed analyses were used).

Table 5
The anion and cation concentrations found in lettuce leaves for different solutions.

	Parameter (mg/kg)	R1	R3	R4	R5
Cations	Na^{+}	4.18	5.10	35.33	25.46
	NH_4^{+}	<6.25	<6.25	<6.25	<6.25
	K^{+}	95.68	23.88	66.07	37.4
	Mg^{2+}	7.82	<6.25	6.51	8.50
	Ca^{2+}	21.87	<12.5	14.27	35.69
Anions	F^{-}	1.21	1.29	1.81	1.57
	Cl^{-}	25.49	15.64	58.63	86.63
	NO_2^{-}	N.D.	N.D.	N.D.	N.D.
	NO_3^{-}	N.D.	N.D.	N.D.	N.D.
	SO_4^{2-}	17.07	12.57	19.44	22.99
	PO_4^{3-}	N.D.	<10	N.D.	N.D.

N.D.: Not detected.

Moreover, the anion concentrations were measured in lettuces tissues. The NO_2^-, NO_3^-, and PO_4^{3-} concentrations (except in R3 cultivated lettuce) were below the limits of detection for all samples. On the other hand, we observed considerable Cl^- accumulation in lettuce (15.64–86.63 mg/kg) for all water samples. The interactions between Cl and NO_3^- anions may be a major limitation, particularly in arid environments with salty water, for hydroponic plant cultivation. Namely, the high concentration of Cl^- is the main inhibition factor on NO_3^- uptake by plants, which is consistent with our results (Al Meselmani, 2022; Corrado et al., 2020). Furthermore, the absorption of NO_3^-, NO_2^-, and SO_4^{2-} depends on the presence of other elements as well as their availability in the hydroponic solution. For instance, the low concentration of SO_4^{2-} may slowed the absorption of NO_3^- and NO_2^- ions, and this impact on the assimilation process appears to be the main factor influencing the accumulation of NO_3^- and NO_2^- ions at the leaves level (Astolfi et al., 2021).

3.5. Heavy metal health risk evaluation

Human health risk assessment related to HM accumulations in the tissues of the lettuce plants cultivated in the various water samples was carried out. The HM risk evaluation was carried out for 5 metals, and the results are reported in Table 6. The As concentrations were below the detection limit in lettuce plants and water samples; therefore, the health risk assessment was not performed for As. Results revealed that the accumulations of HMs in the lettuce tissues were in the order of Fe > B > Cu > Cr in R1, B > Cu in R3, B > Fe > Cu > Cr > Ni in R4, and Fe > B > Cu > Cr > Ni in R5. In a separate study, the authors reported that the metals were classified based on their concentrations in the lettuce plants in the following order: Fe > B > Mn > Zn > Cu (da Carvalho et al., 2018). The uptake of HMs in lettuce may differ depending on the HM permissibility, nutrient-water balance, and microbial species in plant roots (Hu et al., 2014; Mirzaienia et al., 2017). Results revealed that the lettuce plants presented low Ni accumulation and uptake of 0.5 and 0.9 mg/kg in R4 and R5 samples, while there was no Ni accumulation in R1 and R3 most probably related to the competition with other ions. Namely, these results revealed that the Ni uptake performance of lettuce, which is produced using R1 and R3 samples may improve with a long cultivation period. Furthermore, various studies reported the antagonistic impacts of Ni on Cu absorption of lettuce plants and these findings were a good agreement with the results of this study (Cataldo et al., 1978; Zubillaga and Lavado, 2002). Namely, the Cu accumulation in lettuce was higher than that for Ni since the Ni concentrations were low in all water samples compared with Cu concentrations, which eliminated the antagonistic effect of Ni on the absorption of Cu by lettuce, enhancing Cu uptake. In all water samples, the lettuce plants showed a slightly minor affinity to accumulate Cu and Cr compared with the B and Fe metals. A similar trend was reported by Gattullo et al. (2017) (Gattullo et al., 2017). Furthermore, the low accumulation of Cu and Cr in lettuce plants could be explained by the inadequate transfer of these metals from roots to leaves (Ginocchio et al., 2002). In addition, the Fe and B accumulation tendency of plants was considerably consistent in all water samples, even though the R1 water sample B and Fe concentrations were higher than that for all of the water samples. If Fe and B metals are passively picked up from water samples, as reported by Brown et al. (2002) (Brown et al., 2002), we concluded that B and Fe

Table 6
The human health risk assessment of heavy metals in lettuce for various solutions.

Parameter	Water sample	Heavy metals				
		B	Cu	Cr	Fe	Ni
Concentration (mg/kg)	R1	51.59	8.43	0.253	75.32	N.D.
	R3	89.50	6.49	N.D.	N.D.	N.D.
	R4	330.6	13.36	1.59	144	0.918
	R5	41.76	5.72	0.684	106.9	0.497
Daily Intake Rate (DIR)	R1	0.0036	0.0006	1.76E-05	0.0052	0
	R3	0.0053	0.0004	0	0	0
	R4	0.0176	0.0007	8.45E-05	0.0077	4.88E-05
	R5	0.0026	0.0004	4.28E-05	0.0067	3.11E-05
Health Risk Index (HRI)	R1	0.0179	0.0147	0.0059	0.0075	0
	R3	0.0266	0.0096	0	0	0
	R4	0.0878	0.0177	0.0282	0.0109	0.0024
	R5	0.0131	0.0089	0.0143	0.0096	0.0016
Hazard Quotient (HQ)	R1	0.049	0.04	0.016	0.02	0
	R3	0.085	0.031	0	0	0
	R4	0.314	0.063	0.101	0.039	0.0087
	R5	0.039	0.027	0.043	0.029	0.0047
RfD (mg/kg/day)		0.2	0.04	0.003	0.7	0.02

N.D.: Not detected.

uptake was low and sufficient in the water, such that equilibrium was observed between the lettuce and water samples. These results could explain that the same order of Fe and B metals in lettuce plant tissue was achieved using different water samples (Anderson et al., 2017). Furthermore, the B concentration in the lettuce tissues (41.8–89.5 mg/kg), except for the R4 sample, fell within the range of 15–84 mg/kg reported by Sahin et al. (2017) (Sahin et al., 2017). The Cu content in the lettuce tissues was in the range of 5.72–13.36 mg/kg, which is similar to the reported range of 10.9–11.5 mg/kg (Jurga et al., 2021). Considering all water samples, the Fe content in lettuce was in the range of 75.32–144 mg/kg and consistent with the values (>100 mg-Fe/kg) found in the literature (Kleiber et al., 2015). Consequently, the nutrient composition of the B-Cu-Fe-infused lettuce used in this study may be suitable.

Moreover, the permissible limits (mg/kg) for heavy metals in the food are reported according to the EU, and on heavy metals, there are regulations for arsenic, cadmium, mercury, and lead metals. The arsenic, cadmium, mercury, and lead concentrations should be in the range of 0.1–0.2, 0.05–3, 0.1–1, and 0.02–3 mg/kg, respectively (Commission Regulation (EU), 2023). In this study, none of these metals were observed in lettuce samples and there are no limit values reported for the analyzed metals (B, Cu, Cr, Fe, and Ni).

Furthermore, the heavy metal DIR values of the lettuce were different for various water samples and HM elements (Table 6). The DIR of B was greater than the other HMs for all cases. For instance, the DIR of B by lettuce cultivated in the R4 sample was highest (0.0176 mg/kg-d) as compared to the other samples since the B accumulation in lettuce tissues was significantly great in lettuce cultivated using R4, while the DIR of Ni (4.88E-05 mg/kg-d) was significantly lowest. Namely, the DIR of B was increased using the R4 sample (4:1, *v*/v mixture of MDC-treated water and Hoagland solution) as the MDC-treated water volume in the sample was increased compared to the R3 sample (1:1, v/v mixture of MDC-treated water and Hoagland solution) with boron DIR of 0.0053 mg/kg-d. Furthermore, the DIR of Cu for all lettuce samples cultivated in different water samples was low obviously due to the minimum accumulation tendency of Cu in lettuce tissues. Similar trends were observed for the DIRs of the Cr, Fe, and Ni metals in all other cases. Overall, the DIR is crucial to calculating the level of exposure via lettuce consumption for the determination of HRI and HQ. In this study, the calculated HRI and HQ values were below 1 in all experimental runs, presenting a negligible risk and demonstrating that the exposed contamination is safe from the human health that occurs from HM consumption. The HRI results showed that the highest value was observed in the case of B as expected: it was 0.0878 in the case of lettuce grown in the R4 sample. Furthermore, the HRI of HMs decreased in the order of B > Cr > Cu > Fe > Ni in R4 cultivated lettuce, B > Cu > Cr = Fe = Ni in R3 cultivated lettuce, and B > Cu > Fe > Cr > Ni in R1 cultivated lettuce. To sum up, the HRI values of all these HMs were below the permissible limit (HRI < 1) demonstrating that the lettuce plants cultivated in all samples were nontoxic for consumption. Similar results were observed for the HQ values. The calculated HQ values were in the range of 0–0.314 in all water samples of cultivated lettuces. Results clearly proved that HM exposure via lettuce consumption is unlikely to suffer noticeable adverse health problems with values below the permissible limit value (HQ < 1). Overall, although these results indicated that the lettuce cultivated from treated wastewater samples does not significantly affect human health, prolonged intake may cause a considerable accumulation of HMs over the permissible limits. On the other hand, the prolonged consumption of treated wastewater-cultivated lettuce will not have any detrimental effects on human health because of the extremely low HRI and HQ values in lettuce tissues. Nevertheless, there were limited studies investigating the correlation between the HM concentrations in plant and cultivation samples, based on our humble opinion. Therefore, additional comprehensive studies should be performed to understand both cultivation medium and plant quality.

4. Conclusion

This study investigated the reuse potential of spent geothermal brine treated with an MDC bioreactor for hydroponic lettuce cultivation. In the MDC system, the heavy metal removal from spent geothermal brine and organic matter removal from yeast wastewater with energy production were provided simultaneously. The highest removal efficiency of 67.3 % was obtained for arsenic using MDC system, while the maximum organic matter removal efficiency and electrical potential values were 68.8 % and 620 V at an operational time of 3 days, respectively. Moreover, the effects of different water samples (Hoagland solution (R1) as a control, MDC-treated water (R2), 1:1, v/v mixture of MDC-treated water and Hoagland solution (R3), 4:1, v/v mixture of MDC-treated water and Hoagland solution (R4), and tap water (R5)) on lettuce growth were investigated. The application of R3 and R4 samples for hydroponic lettuce cultivation was promising since the lettuce plants uptake sufficient nutrients for their growth and productivity with low toxic metal concentrations. Lettuce plants indicated a white root color, green leaves color, and fresh root and leaves formation in R3, R4, and R5 samples compared with the R1 control sample, while the application of the R2 sample showed considerable morphological or physiological inhibition impacts on lettuce. Furthermore, the health risk assessment of heavy metal accumulations in the lettuce plants cultivated in the various water samples was determined. Results showed that heavy metal exposure via lettuce consumption is unlikely to suffer noticeable adverse health problems with values below the permissible limit value (HRI and HQ < 1). Overall, these results suggested that the diluted MDC-treated geothermal brine can be an attractive source of valuable water and nutrients and could be utilized as a hydroponic nutrient solution for lettuce cultivation. However, further experiments should be performed to directly utilize MDC-treated wastewater to guarantee effective pH control and heavy metal removal performance in energy-self-sufficient and simple methods for real-scale applications. Extensive research may also be required in view of the economic, environmental sustainability, and social concerns. For instance, in order to incorporate MDC technology to real scale water treatment trains, there is a need for further investigation on membrane scaling by biofilm formation and salt decomposition to enhance the performance of the MDC process. It was obvious that the factors contributing to the internal resistance such as organic and inorganic membrane fouling and inter-membrane distance had to be tackled in further studies. The development of cheap and effective electrode and membrane materials is also necessary to provide an economically sustainable process. Moreover, the life cycle assessment studies should be conducted on the MDC process considering its environmental impacts such as greenhouse gas emissions, water use, and acidification potential.

CRediT author statement

Aysegul Yagmur Goren: Conceptualization, Methodology, Writing -Review & Editing, Visualization, Formal analysis; **Deniz Naz Eskisoy:** Experimental Investigation; **Sebnem Genisoglu:** Experimental Investigation; **Hatice Eser Okten:** Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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