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Preliminary Investigation of Bioaccumulation of Microcystins in Hypereutrophic Irrigation Ponds Case Study – the Jordan Valley

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ABSTRACT

Microcystis blooms and the related toxin known as microcystin-LR (MC-LR) put the safety of human water consumption and global irrigation practices in jeopardy. MC-LR is widely distributed in various environments, including water, sediments, plants, and other aquatic organisms. The use of water-containing microcystins for agricultural purposes may have to be restricted despite the limited availability of clean water resources. Accordingly, the present work aimed to determine the MC-LR concentrations and recognize the environmental parameters that initiate the growth of toxic cyanobacteria and MC-LR occurrence in 20 irrigation ponds in the Jordan Valley area. The irrigation ponds studied were found in a hypereutrophic condition, with high levels of N:P ratio and low transparency. These cause inseparable effects such as cyanobacterial bloom and MC-LR occurrence. The investigated ponds were classified as hypereutrophic according to General Quality Index (GQI), with two different types of algae covering the surface. The first was the *Lemna sp.* or duckweeds (Family Araceae) which are free-floating masses, and the second was the cyanobacteria algal bloom. Unpaired t-tests were performed and showed that the concentrations of MC-LR in pond water abundant with cyanobacteria algal bloom in September 2021 were significantly higher ($P = 0.7906$) than in June for the same year (0.3022 ± 0.0444 and 0.1048 ± 0.0171 ppb, respectively). Two methods for extracting MC-LR were used and showed a significant difference in MC-LR concentration in ponds with an abundance of cyanobacteria algal blooms (0.2273 ± 0.0356 ppb) compared to the ponds with an abundance of *Lemna sp.* or duckweeds collected in June 2021 (0.1048 ± 0.0171 ppb). Despite all of the efforts made by Jordan Valley farmers to prevent or limit the mass growth of cyanobacteria and its consequences for the eutrophication process in their irrigation ponds through the use of fish breading and chemicals such as copper sulfate, this environmental problem is still harming their crops and irrigation methods and requires immediate government assistance.

Keywords: Jordan Valley, eutrophication, cyanobacteria, microcystin-LR, irrigation ponds, wastewater.

INTRODUCTION

The use of treated wastewater (TWW) for agricultural purposes has grown over the past few years due to many factors, such as climate change, increasing population numbers, and limited groundwater resources. Jordan and many other countries are increasing their reliance on treated wastewater as a non-conventional resource for irrigation purposes. Jordan is mainly affected by climate change, with a marked decrease in precipitation over the past few decades and by the negative impact of drought and water shortage on crop growth and productivity of major agronomic crops. In addition, the overdraft of the groundwater resources due to low recharge rates directly influences groundwater quality and quantity deterioration.

In Jordan, numerous wastewater treatment plants were constructed to serve and treat the wastewater generated by major cities in Jordan. Khirbet As-Samra Wastewater Treatment Plant (As-Samra WWTP) was built in 1986 with a 67,000 m^3/day capacity. Wastewater is treated using the natural stabilization ponds technique, where the anaerobic, facultative, and maturation ponds cover a broad area of 180×10^3 m^2. However, after two decades of using this type of treatment, an increase in the salinity and pollution indicators like nitrate lead to groundwater deterioration by exceeding the maximum permissible limits for drinking purposes. In 2005, As-Samra WWTP was upgraded to mechanical operation, and the new plant was constructed in 2008. About 75% of the annual national budget for treated wastewater is produced by As-Samra-WWTP, which receives wastewater from Amman, Russeifa, and Zarqa. The plant, which is located about 30 kilometers northeast of Amman, is Jordan's largest WWTP. The plant's capacity exceeds the daily wastewater inflow of 367,000 m^3. The treated effluent is discharged into Wadi Dhuleil, followed by Wadi Zarqa. The effluent flows mix with the Seil Zarqa's natural runoff. The flow of Wadi Zarqa is impounded and held for several weeks about 35 kilometers downstream at the King Talal Reservoir (KTD). After that, water drains from the dam towards the Zarqa River until it is diverted into the Abu Zeighan area and connected with the King Abdullah Canal (KAC), as shown in Figure 1.

The mixed water in the canal represents 99% from Zarqa River and 1% from KAC, distributed to farmers by the Jordan Valley Authority according to the lot area. The water is then collected in artificial ponds constructed by the farmers. These ponds are mainly 20 to 50 m in length and 3 to 5 m in depth, as shown in Figures 2A to F. The farmers in the Jordan Valley suffer from the eutrophication process and the formation of dense algae blooms in the ponds. This problem has existed for many decades, with many attempts from the farmers themselves to reduce it. Some farmers use fish breeding as an option, while others clean and eliminate eutrophication by using copper sulfate ($CuSO_4$), which binds to algal cells, causing cell lysis and releasing cellular contents into the pond. Along the flow from the source As-Samra WWTP to the collected water in the irrigation ponds, the water quality deteriorates due to the illegal dumping of untreated wastewater and the irrigation return flow along Wadi Dhuleil, Wadi Zarqa, and Zarqa River (Al Kuisi et. al, 2014). As a result, nutrient loads are increasing, especially nitrate, phosphate, and pathogens such as *Escherichia coli*. Nutrients are required for plant

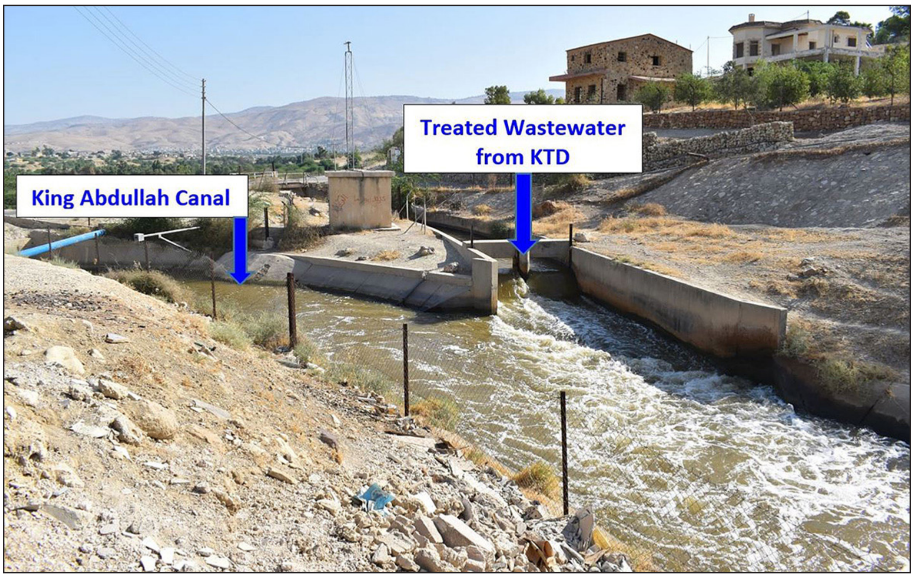


Figure 1. Mixing water for irrigation

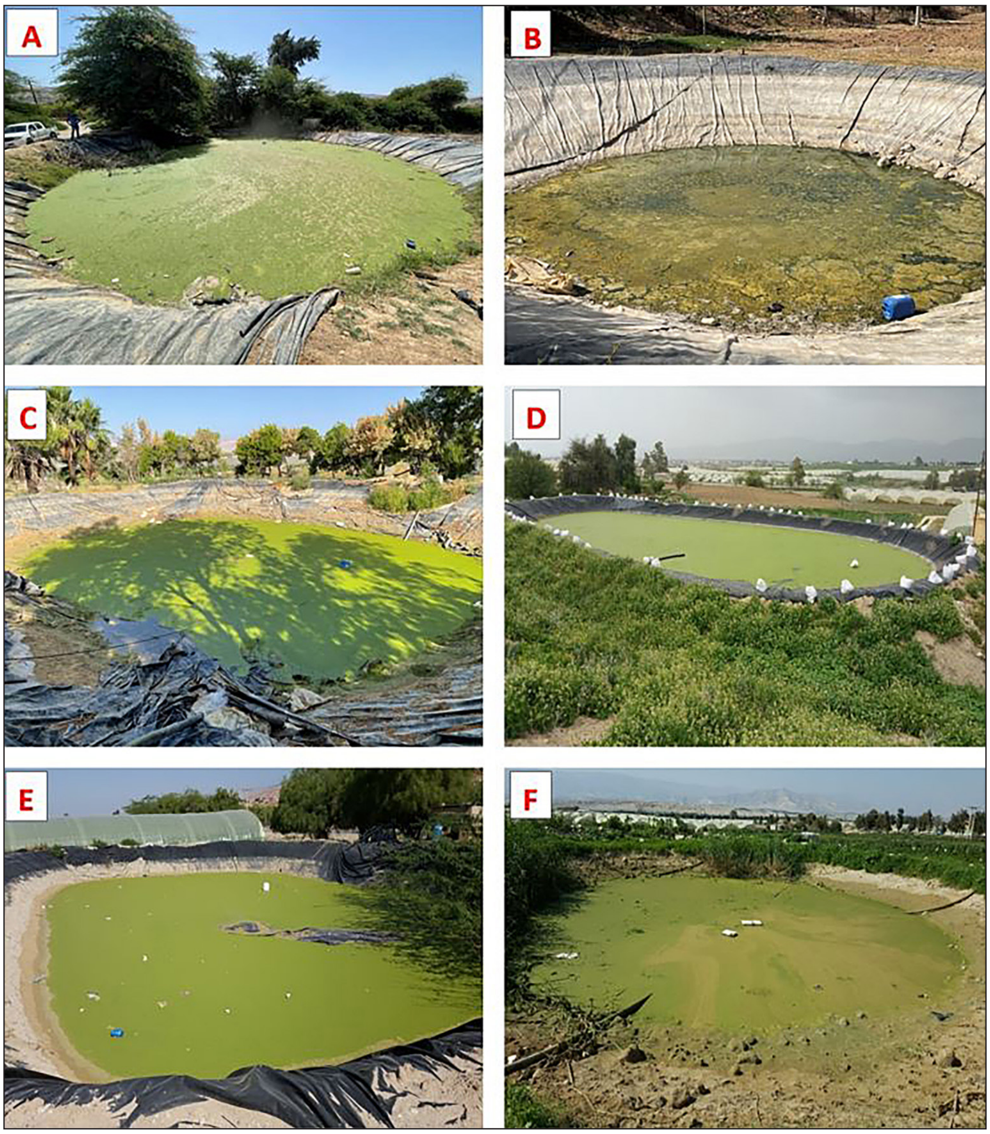


Figure 2. (A-F) Hypereutrophic irrigation ponds covered with algal blooms

growth, but an excess of nutrients in water can have numerous harmful negative health and environmental consequences. This overabundance of nutrients accelerates the eutrophication process for water in the ponds (Graham et. al, 2020).

Algae thrive in eutrophic wasters feeding on nutrients and growing into mats covering the water surface. The overgrowth of the green algal mats causes a foul odor in the water, blocks sunlight, and even releases toxins. Furthermore, decaying mats of dead algae can cause bad tastes and odors in water; their decay by bacteria consumes dissolved oxygen from water, resulting in the formation of anoxic "dead zones" and mass fish kills.

Cyanobacteria form dense blooms globally, present in fresh to saline water and even in extreme conditions like deserts and glaciers (Bacu and Zaho, 2022). Cyanobacteria and their natural products, cyanotoxins, are increasing in abundance with eutrophication. Therefore, it represents a concern for human and environmental health (Passos et al., 2022). Toxic microcystins (MCs) are commonly found in 40–75% of cyanobacterial blooms. Surveys in different parts of the world have revealed that between 25% and 75% of cyanobacterial blooms are toxic (Sivonen and Jones, 1999, Mutoti et al., 2022). Global warming, which directly affects the hydrologic cycle resulting from growth rates and bloom formation, is reported by the Intergovernmental Panel on Climate Change (IPCC). In addition, it is projected that by 2050, an increase and the extent of harmful cyanobacterial blooms will contaminate lakes by at least 20% (UNESCO, 2020).

Cyanotoxins are intracellular toxins found within living cells. Depending on the nature of the toxin and the growth stage, they are only released into the water to form dissolved toxins during cell senescence, cellular damage, and lysis, or through water treatment processes such as algaecide application rather than through continuous excretion (Babica et al., 2006). Microcystins are monocyclic heptapeptide toxins named after their two variable amino acids. The amino acids leucine (L) and arginine (R) are found in Microcystin-LR (Carmichael et al., 1988). Because of the toxicity and risks associated with MCs, a total MC-LR of 1 g/L is recommended as a drinking water guideline (WHO, 2018).

According to research and field observations, anthropogenic nutrient concentrations, rising temperatures, and high atmospheric CO_2 supplies will increase cyanobacterial dominance in many aquatic ecosystems. Cyanobacteria have efficient CO_2 and nutrient uptake mechanisms, are light and UV radiation resistant, and are highly adaptable, allowing them to benefit from changing environmental conditions in aquatic environments (Paerl et al., 2014). The most significant variation in cyanotoxin concentration in aquatic ecosystems is caused by variation in cyanobacterial biomass: the more cyanobacterial biomass, the higher the toxin concentration. CO_2, temperature, nutrients, and light influence cyanobacterial biomass (Kardinaal and Visser, 2007).

Few studies were done in Jordan to investigate the occurrence of cyanobacteria in irrigation water. However, two studies conducted in 2006 and 2013 confirm the presence of cyanobacteria in King Talal Dam. Microcystis aeruginosa is the main cyanobacterial species that produce microcystin LR; it forms extensive blooms in the King Talal reservoirs. This initial study indicated that the time of the seasonal maximum and intensity of microcystins blooms in King Talal Dam (KTD) vary from month to month, with a peak reached in summer (Al-Jassabi and Khalil, 2006). Another research was conducted for microcystin LR occurrence in six Jordan dams. The result showed that KTD, the primary source of irrigation water in Jordan Valley, has the highest amount of microcystin LR with an average concentration of 5 µg/L (Ayyash, 2013). No study has quantified microcystin production by cyanobacteria in Jordan Valley irrigation water ponds. Therefore, this study aims to investigate the accumulation of microcystin (MCs) and their concentrations in irrigation water ponds.

METHODOLOGY

Study area

The Jordan valley area is a low-lying strip that extends along Jordan's west border from northern Jordan near lake Tiberius to the southern shore of the Dead Sea in the South Shona area. As it is warmer than its surroundings and has fertile soil, Jordan valley is used for agricultural production, named Jordanian basket food. The total cultivated land is about 350 Km^2, covering only 8.5% of the country's total area and 65% of total food production (DOS, 2020). Water resources in the Jordan

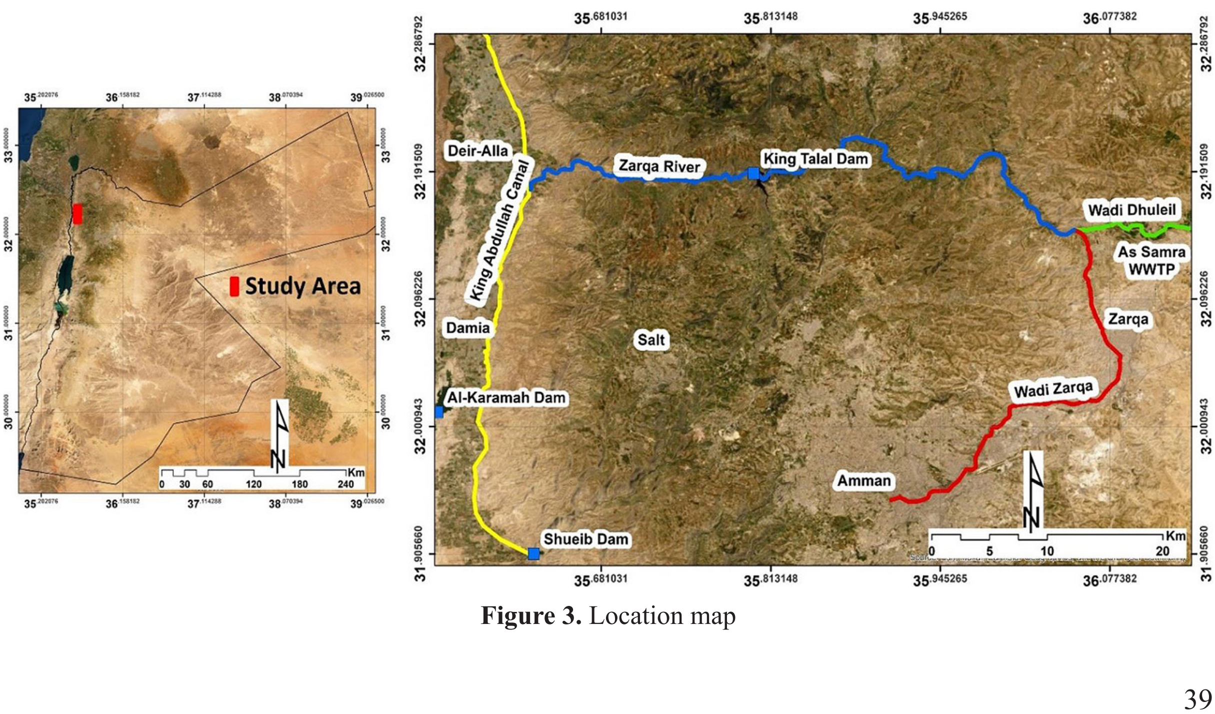


Figure 3. Location map

Valley are mainly groundwater from wells and surface water from KAC and KTD. The study area is located in the middle of the Jordan Valley. It extends between Deir-Alla and Karamah villages (Fig. 3). The Jordan Valley is subdivided into farm units (FU), and the number of these FU exceeds 5700. Each FU has its designation number, representing the water source for irrigation. The study area includes 1672 FU irrigated from a mixture of KTD water and KAC (Fig. 1).

The weather in the Jordan Valley is very hot and dry in the summer and mild, with little rainfall in winter. The maximum temperatures can exceed 50 °C in summer, while the average yearly temperature is about 28 °C. Rainfalls occur in the winter between October and May, with an average of 75 mm. The evaporation rate in the study area is more than 2500 mm, which directly influences the soil moisture from rainfall, which is inadequate to sustain non-irrigated agriculture. Therefore, the study area depends on the blended water from two KAC and KTD and is used for cultivation. While greenhouses are used primarily for cultivating tomatoes, paprika, peppers, and cucumbers, open fields are cultivated with eggplants, cauliflower, okra, and potatoes. Fruit trees and palms are grown in the Al Remal and Muaddi areas. Soil texture in the study area is mainly clay loam, loam, silt loam, and sandy loam (Al Kuisi et al., 2008).

Water sampling

Twenty pond water points were selected and sampled for water quality characteristics and microcystins (MCs) measurements, as shown in Figure 4. Forty water samples were collected during two sampling campaigns in June 2021 and September 2021. The filtration process was carried out for the water samples using fast filter papers (400µm) from Albet company. Parallel to that, two samples were collected from each pond in polyethylene bottles with zero headspaces appropriately coded before use and rinsed 2-3 times with the same water before collecting. EC (µS/cm), pH, DO (mg/L), and temperature (°C) parameters were measured in the field using WTW-portable instruments. Before each field trip, all portable equipment was checked and calibrated in buffer solutions. Moreover, all the bottles used in the collected sample were cleaned and washed many times in distilled water.

Water analysis (major and trace elements)

The major cations and anions were determined using the titrimetric method and Ion Chromatography (Shimadzu) and a flame emission photometer at the University of Jordan laboratories, following the method outlined by Arnold et al. 2017. Table 1 shows the estimated detection limits for each constituent. To assess the analytical precision, five randomly selected water samples were analyzed 2-3 times. For each sample, an overall precision, expressed as a percentage of relative standard deviation (RSD), was calculated. The analytical precision for cations and anions is less than 5%, and the charge balance error (CBC) was calculated and confirmed to be less than 5%. Unfiltered water samples for heavy metal analysis

Table 1. Analytical methods used for measuring parameters

Parameter	Unit	Analytical method	Detection limits	Reference & method number
EC, pH– value, DO Temp.	µS/cm mg/L ˚C	Field EC, pH, DO, T –meter WTW instrument	0.1 µS/cm +/- 0.005/ +/-0.01/0.1 0.01 mg/L +/-0.1 ˚C	Standard Methods, 23th edition 2510 B
HCO_3^-	mg/L	Titrimetric method	0.1	In house standard operating procedure
Ca^{2+}, Mg^{2+}	mg/L	Titrimetric method	0.1	In house standard operating procedure
Cl^-, NO_3^-, SO_4^{2}	mg/L	Ion chromatograph	0.01, 0.292 and 0.04	Standard methods, 23th edition 4110 B
PO_4^{3-}	mg/L	Stannous chloride	0.001	Standard methods, 23th edition 4500 D/P
Na^+, K^+	mg/L	Flame photometer JANEWAY-410	0.1	Standard method 23th edition 3500
Cr, Cu, Fe, Mn, Pb	mg/L	Perkin Elmer Analyst 200 (atomic absorption spectrometer)	0.05, 0.05, 0.05, 0.05, 0.01, 0.5, 0.1, 0.1, 0.05	Standard methods, 23th edition 3125 A
BOD and COD	mg/L	OxiTop WTW (BOD) Wet chemistry method	0.1 0.1	Standard methods, 23th edition 5210 B Standard method 5210 for BOD and 5220 for COD

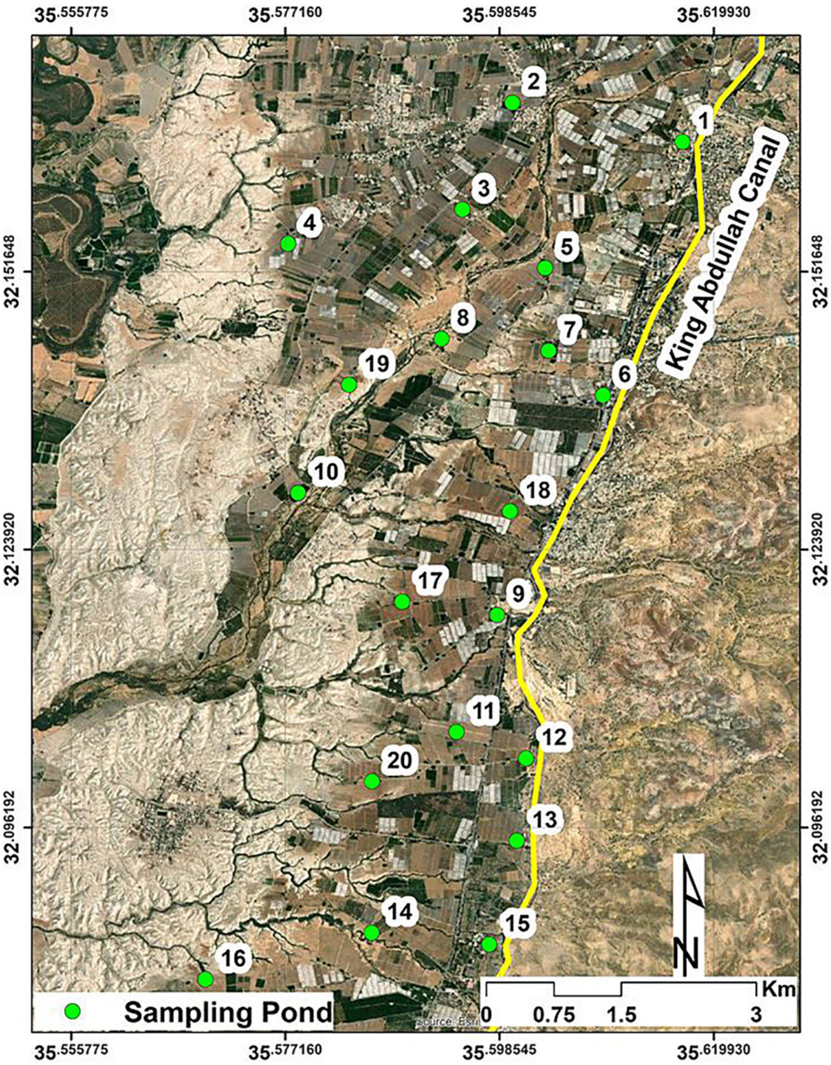


Figure 4. Sampling irrigation ponds

were placed in 100 mL polyethylene bottles that had previously been treated for three days with a 50% (V/V) nitric acid solution in double-deionized water. To avoid chemical precipitation, unfiltered water samples for trace element analysis were placed in 500-mL polyethylene bottles and acidified to pH 2 with 0.5 mL concentrated analytical-grade HNO_3. Atomic Absorption Spectroscopy was used for the analysis of Cr, Fe, Mn, Pb, and Cu (Table 1).

Determination of Microcystin by the ELISA method

Water samples from irrigation ponds were prepared for ELISA assay as described by Sheng et al. (2007) and Trifirò et al. (2016). In brief, water samples were collected from the site and filtered using a 45 μm filter. Then, the filtrate was stored in a glass container and stored at 4 °C directly. The containers were covered with foil to prevent MCs photodegradation. After arriving at the lab, each water sample was divided into two parts. One part was stored at 4 °C and analyzed using ELISA within three days of the collection (Method 1 water sample preparation for quantifying only extracellular Microcystin concentrations). The other part was stored at –20 °C for at least 24 hours before analysis to measure the total MCs in water (Method 2 water sample preparation for quantifying total microcystin concentration). MCs concentration was determined for all water samples using a Microcystin-LR ELISA method (Zeck et al., 2001). This method is based on monoclonal antibody anti-microcystin that binds MC-LR or MC-enzyme conjugate (MC-LR-Px). Samples were analyzed in duplicates using the microcystin-LR ELISA kit (Abnova company, Taiwan, Catalog Number KA 1496) according to the manufacturer's instructions and read at 450 nm using a microplate photometer (Biosan). A calibration curve was generated using the standard solutions in the kit to determine the equivalent concentration of MC in each sample. Values lower than 0.01 µg/L were considered below the limit of detection (LOD).

Microcystin-LR concertation was measured in the pond water using the ELISA technique. The water samples were grouped into two groups: the first group collected from water ponds with an abundance of duckweeds (n=12), and the second group collected from water ponds with abundant cyanobacteria algal bloom (n=8). The water samples were prepared in two methods: in the first one, MCs concentration in water but not in cyanobacteria (extracellular MCs) was extracted and measured. In the second method, the cyanobacteria were analyzed to measure the MCs inside cyanobacteria and in the water (total MCs).

MC concentration results were analyzed using GraphPad Prism 7 Software. All data were checked for normal distribution, and an unpaired t-test was used to compare between two groups. All the results were expressed as the mean and standard error of the mean (SEM), and a P-value less than 0.05 was considered statistically significant.

Identification of cyanobacteria

For microscopic examination of water samples, samples were collected in a 2Lsterilized plastic container and transported in an ice box. The salinity and dissolved oxygen were measured for these samples. However, the samples were characterized by high turbidity. The identification and examination of the microorganisms collected from the ponds were performed on the same day. The identification of cyanobacteria is based on the methods of Komarek et al., 2014. It has been identified and investigated using a Leica DM750 microscope with a magnification of up to 100× connected to a digital camera and binocular stereomicroscope (S6 D Leica).

RESULTS AND DISCUSSION

Jordan, like many other countries located in an arid region with low precipitation and high evaporation rates, causing groundwater abstraction to very high levels, which are reflected in water availability and quality of the groundwater resources in the country (Hyarat et al., 2022). Due to the limited surface water resources in the country, agricultural activities rely on using treated wastewater for irrigation purposes. However, the eutrophication process in the Jordan valley is one of the most critical threats to the ecosystem's health and water quality deterioration. Despite the efficiency of the wastewater treatment in Jordan, which resulted in low nutrient concentrations, the eutrophication of the irrigation ponds still represents a major headache for farmers and the ecosystem. The presence of nitrogen and phosphorus accelerates algal growth, and thus, biomass increases. Their excessive concentrations may cause adverse effects on the aquatic strata.

Hydrochemical results

Anthropogenic pollution plays a vital role in defining the water quality in the Jordan Valley ponds. This leads to a deterioration in pond water quality, as defined by physical and chemical parameters. Table 2 summarizes 20 physicochemical parameters measured for the 20 water samples collected from the irrigation ponds along with the corresponding Jordanian maximum permissible limits for irrigation (JMPLI) purposes (JMPLI, 2009).

The water ponds were depleted with dissolved oxygen, with DO values ranging from 1.6 to 5.6 mg/L and an average value of 2.28 mg/L. At the surface of the water ponds, gas bubbles were escaping, mostly CO_2 gas, due to the decaying of the organic matter in the bottom of the ponds and the photosynthesis from the algae forming the surface bloom. The JMPLI has proposed EC standards for irrigation water between 700 and 3000 µs/cm. The value of EC measured in the ponds ranged between 2020 and 2290 µs/cm, which classifies them as slight to moderate restriction according to the recommendation of the JMPLI for irrigation purposes. Due to geological factors and biological activity, the pH of natural waters is usually alkaline to slightly acidic. Table 2 shows the pH values for the irrigation ponds varying between 7.06 and 9.13, indicating their alkalinity status, and lie within the recommended pH range (6-9) proposed by JMPLI, except for two samples that are more than pH 9. The reported elevation in pH has been suggested by Zepernicj et al. (2021) to favor the growth of cyanobacteria such as *Microcystis aeruginosa*, and against the development of diatoms due to

Table 2. Water quality parameters for irrigation ponds in Jordan Valley (N = 40)

Variable	Mean	Minimum	Maximum	Std. Dev.	JMPLI (2009)
EC μS/cm	2068	2020.00	2290.00	165.16	700 - 3000
pH value	7.85	7.06	9.13	0.52	6-9
Temp. °C	24.48	21.70	26.20	1.18	
DO (mg/L)	2.28	1.2	5.8	2.23	5
Ca^{2+} (mg/L)	106.40	84.16	136.27	12.87	230
Mg^{2+} (mg/L)	43.62	26.00	60.75	9.78	100
Na^{+} (mg/L)	204.94	173.29	256.00	16.80	230
K^{+} (mg/L)	20.90	14.20	28.30	3.20	50
HCO_3^{-} (mg/L)	302.88	207.40	353.92	35.30	400
Cl^{-} (mg/L)	348.88	268.00	499.84	49.14	400
SO_4^{2-} (mg/L)	139.53	69.00	239.40	34.88	500
NO_3^{-} (mg/L)	35.76	14.95	95.80	17.43	30
PO_4^{3-} (mg/L)	6.14	1.86	8.15	1.510	30
Cu (ppm)	0.24	0.043	0.639	0.155	0.2
Fe (ppm)	0.077	0.003	0.611	0.150	5
Pb (ppm)	0.002	0.001	0.003	0.001	5
Mn (ppm)	0.155	0.013	0.016	0.002	0.2
Cr (mg/L)	0.002	0.001	0.003	0.001	0.1
BOD (mg/L)	15.03	5.00	40.00	7.93	15
COD (mg/L)	24.51	9.00	59.00	11.21	50
SAR	4.24	3.53	4.92	0.058	6

Note: JMPLI* – Jordanian maximum permissible limits for irrigation.

their inability to synthesize their frustules at such alkaline pH levels.

Table 2 shows the cation concentrations for calcium (Ca^{2+}), magnesium (Mg^{2+}), Sodium (Na^{+}), and potassium (K^{+}). Values for Ca^{2+} varied between 84.17 and 136.27 mg/L, with an average of 104 mg/L. All samples lower than the upper limit (230 mg/L) were proposed for Ca^{2+} by the JMPLI standard. Values for Na^{+} varied between 173.3 and 256 mg/L, indicating that 10% of the ponds had Na^{+} concentrations higher than the JMPLI standard limitation (230 mg/l). Mg^{2+} concentrations varied between 26 and 60.750 mg/L, and all of these values fall within the proposed limitation value (100 mg/L) as per JMPLI's standards. As for the concentrations of K^{+}, their values were between 14.2 and 28.3 mg/L, complying with JMPLI's 50 mg/L limitations, and considered suitable for irrigation purposes.

Table 2 shows the reported concentrations for anions, including bicarbonate (HCO_3^{-}), chlorine (Cl^{-}), sulfate (SO_4^{2-}), nitrate (NO_3^{-}), and phosphorus (PO_4^{3-}). The concentration of Cl^{-} varied between 268 and 499.84 mg/L, with only 10% of the samples exceeding the JMPLI's upper limit for irrigation usage. The measured concentration of SO_4^{2-} and HCO_3^{-} varied between 69 and 239.4 mg/L for SO_4^{2-} and between 207.4 and 353.92 mg/L for HCO_3^{-}. All measured samples had suitable concentrations for irrigation usage and complied with JMPLI's proposed upper limitation of 500 ppm and 400 mg/L for SO_4^{2-} and HCO_3^{-}, respectively JMPLI (2009).

NO_3^{-} concentrations varied between 14.95 and 95.80 mg/L, as shown in Table 2. 58% of the measure samples had NO_3^{-} values exceeding JMPLI's upper limitation standard equal to 30 mg/L. When compared with PO_4^{3-} concentrations (Table 2), all samples complied with JMPLI's proposed upper limitation (30 mg/L), and their values ranged between 1.86 and 8.16 mg/L.

Comparing the measured values of the heavy metals (Cu, Fe, Pb, Mn, and Cr) with JMPLI's guidelines (JMPLI, 2009), all samples showed concentrations below the highest permitted values for irrigation water (Table 2).

The measured BOD values for the irrigation ponds varied from 5 to 40 mg/L, with a mean value of 15.03±7.93 mg/L, exceeding JMPLI's upper limitation (15 mg/L) for agricultural usages (JMPLI, 2009). 65% of the measured BOD values complied with JMPLI's standards. In comparison, the COD values varied between 9 and 59 mg/L in the water ponds, with a mean value equal to 24.51±11.21 mg/L, only 10% of the ponds failed to comply with JMPLI's upper limitation, and their reported values exceeded the 50 mg/L standards (JMPLI, 2009).

Classification and hydrochemical facies

Piper diagram

The Piper trilinear diagram (Piper, 1944) is a graph used to evaluate water quality through the hydrochemical properties, especially the major cations and anions, as shown in Figure 5. The piper diagram analysis clearly shows the presence of two distinguishable hydrochemical facies, the mixed type (CaMgCl) and the NaCl type, representing two alkaline earth water types, one with prevailing chloride and another dominated by sodium and chloride. Plotting the chemical data from the water samples in the study sites indicates that 70% of groundwater belongs to the Na-type, while only 30% fits within the "no dominant type" water class. In comparison, the "Cl-type" of the anion triangle predominated in 70% of the water samples.

Sodium adsorption ratio (SAR)

Another index of water quality for irrigation usage is the Sodium adsorption ratio (SAR), which measures the high proportion of Na^+ relative to other cations such as Mg^{2+} and Ca^{2+} in soil indirectly through quantitative chemical analysis of water in contact with the soil sample. The value of SAR is calculated using the following Equation (Suarez et al., 2006).

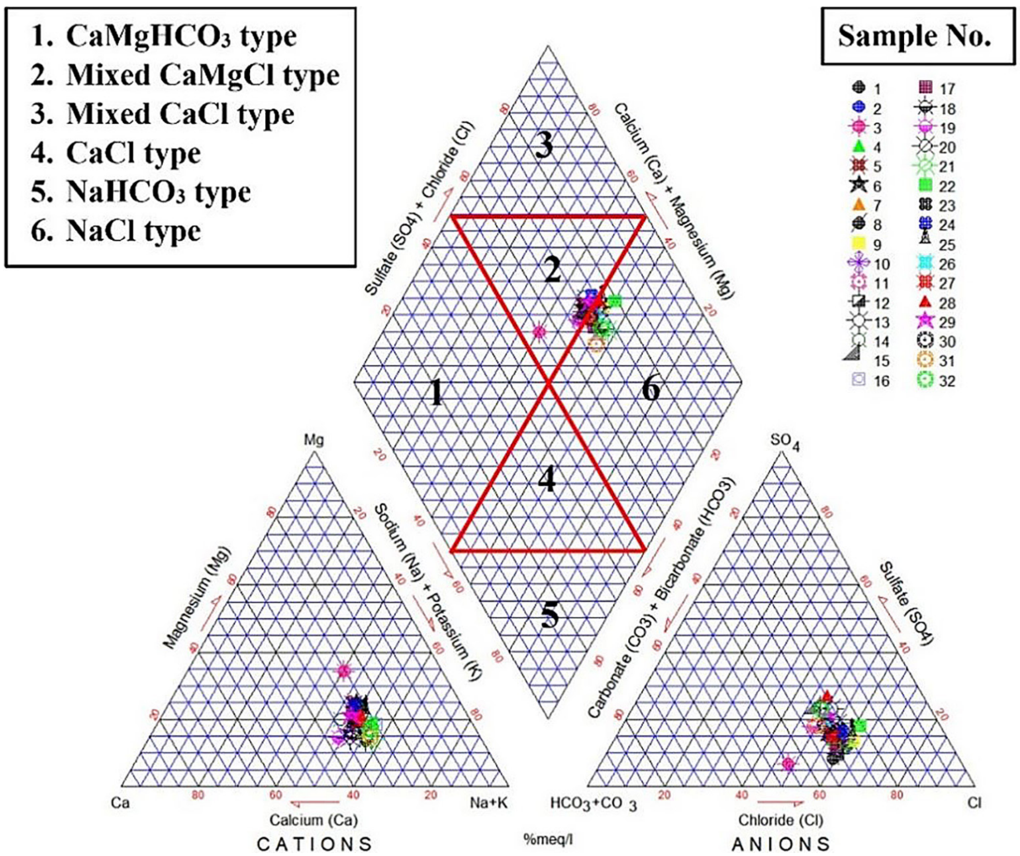


Figure 5. Water classification according to Piper diagram

Table 3. Sodium adsorption ratio (SAR) according to Sumner, 1993

Sodium hazard class	SAR (meq/L)	Remark
C1	0–10	Excellent (little or no hazard)
C2	10–18	Good (appreciable hazard but can be used with appropriate management)
C3	18–26	Doubtful (unsatisfactory for most of the crops)
C4	>26	Unsuitable (unsatisfactory for all the crops)

$$SAR = \frac{Na^+}{\sqrt{\frac{1}{2}(Ca^{2+} + Mg^{2+})}} \quad (1)$$

where: Na^+, $Mg^{2+,}$ and Ca^{2+} concentrations expressed in (meq/L).

The average SAR value of the collected water samples was 4.24 and ranged between 3.53 and 4.92. Indicating that water in all samples is suitable for irrigation purposes, and can be classified within the "Excellent/C1" category in terms of SAR as shown in Table 3.

Algal classification and abundance

Morphological analysis of water samples indicated the presence of *Lemna sp.*, commonly known as duckweeds (Family Araceae), which are present as free-floating masses in the hypereutrophic ponds, as shown in Figure 6. In addition, cyanobacterial algal blooms were observed. In all studies of water ponds, the most commonly observed genera of toxigenic cyanobacteria detected in irrigation ponds from the Jordan Valley were*: Mougeotia sp., Oedogonium sp., Chlorella sp.,* and *Anabaena sp.*, as shown in Table 4.

Other algae populations are dominated by harmful algal species altered by eutrophication. Growing public safety and environmental concerns concerning cyanobacterial blooms have emphasized the crucial need for practical, environmentally safe, and long-term remedial strategies (Westrick et al., 2010). Thus, the toxicity and the microcystin content of two types of algae, *Lemna* sp. or duckweeds (Family Araceae), and the cyanobacteria blooms present in irrigation water bonds were analyzed.

For phytoplankton data analysis, potentially toxic cyanobacteria species were pooled into the main genera: *Aphanizomenon* (*A. flosaquae*, *A. gracile*, and *A. issatschenkoi* (=*Cuspidothrix issatschenkoi*), *Dolichospermum* (*D. affine*, *D. circinale*, *D. crassum*, *D. flosaquae*, and *D. lemmermannii*), *Microcystis* (*M. aeruginosa*, *M. flosaquae*, *M. viridis*, and *M. wesenbergii*), and *Woronichinia* (*W. compacta* and *W. naegeliana*). It can be observed that the predominant species were the *M. aeruginosa* and the *Anabaena sp.*, which are the most widespread, proliferating in the majority of all high trophic water ponds. Microcystis blooms occur all over the year in the irrigation ponds, and during summer (May to November), thick scums of the algae tend to accumulate with thickness exceeding 10 cm.

Microcystin (MC-LR) concentrations in water ponds

The concentrations of microcystin measured in the water ponds varied significantly across time and place. Figure (7) shows the average concentrations of microcystin-LR in the studied water ponds measured using the two analytical methods described in the methodology section for the 20 water ponds; extracellular MC-LR and Total MC-LR.

The calculated average concentrations of microcystin for extracellular have increased from 0.156 ppb in June to 0.271 ppb in September 2021 as presented in Figure 8, while it increases from 0.158 ppb in June to 0.315 ppb in September 2021 for total microcystin-LR as shown in Figure 9.

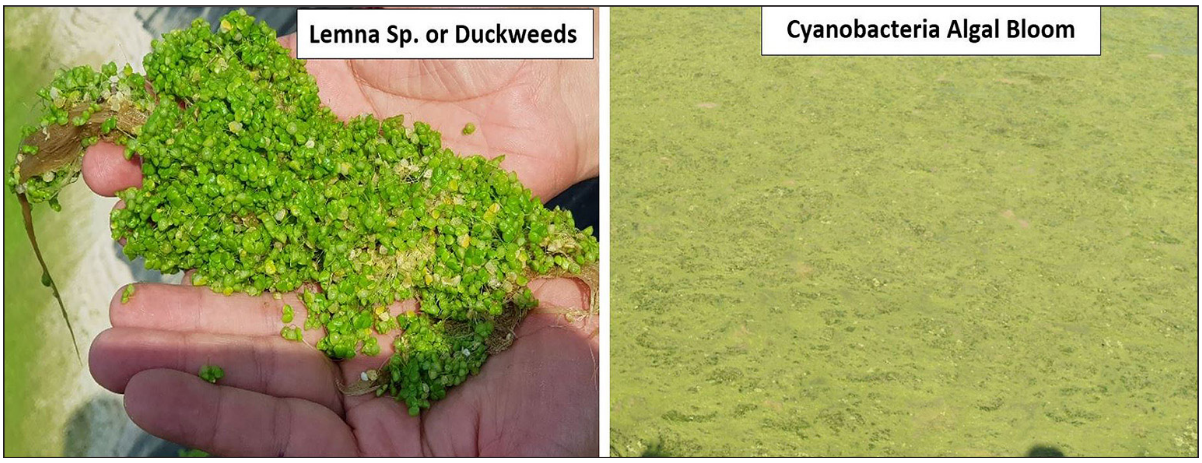


Figure 6. Blooms of algae in irrigation ponds

Table 4. Eutrophication-tolerant organisms identified from the irrigation ponds in the study area

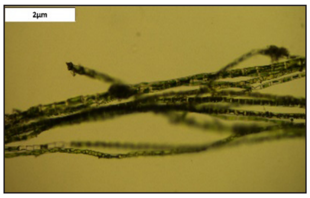
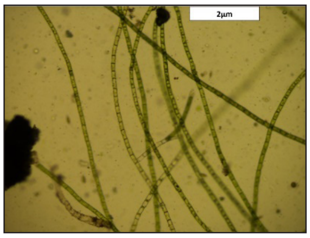
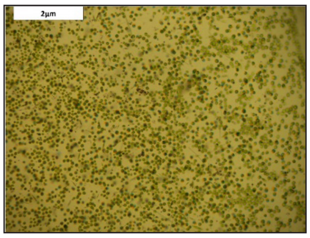
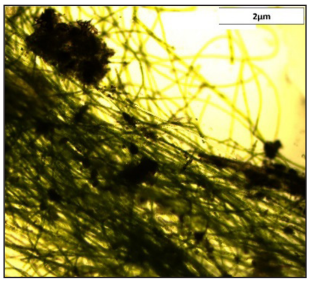
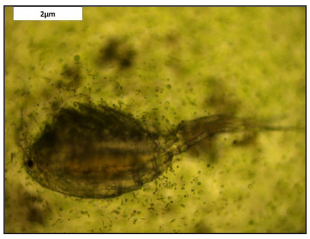
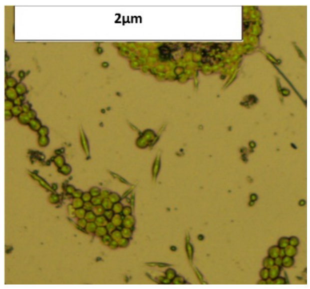
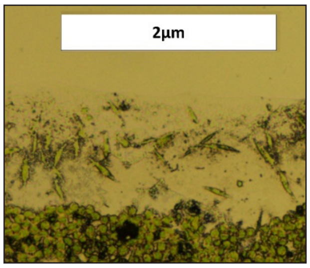
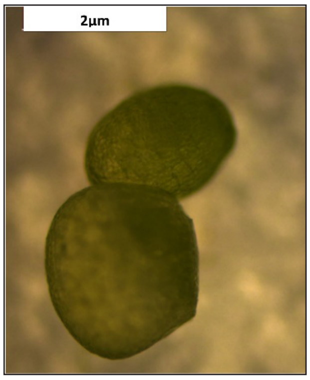
Phylum	Family	Name	Description	Occurrences	Bio indicator values	Picture
Charophyta (Green algae)	Zygnemataceae	*Mougeotia* sp.	It is a genus of green macroalgae that has slimy unbranched long filamentous. They mostly exist as benthic which occur in running and stagnant waters	Large populations of *Mougeotia* sp. are found in low-quality water, eutrophic water	A good indicator for acidic water bodies (decomposition of algae produce carbon dioxide and leading to decreased pH). Also, it is a pollution tolerant species, can be found in environments with heavy metal pollution and low pH conditions.	
Chlorophyta (Green algae)	Oedogoniaceae	*Oedogonium* sp.	Genus Oedogonium is unbranched, long, attached, and multicellular filaments of green algae. Common in stagnant freshwater bodies as free-floating algae.	Stagnant Freshwater	They can tolerate a relatively wide range of pH (7.3-9.6), temperatures, and can survive in nutrient-rich environments (Pikosz and Messyasz, 2015). It is s used for wastewater purification.	
Chlorophyta (Green algae)	Chlorellaceae	*Chlorella* sp.	*Chlorella* sp. is known as seaweed. Its genus of single-celled micro-green algae. It has rounded cell shaped without flagella .	Common in freshwater. Grow in aquatic and terrestrial environments	They are inhabitants of freshwater with high nutrient content. Used in bioremediation process of nutrient-enriched wastewater effluents.	
Cyanobacteria (commonly known as blue-green algae)	Nostocaceae	*Anabaena* sp.	Planktonic beadlike shaped, long uniseriate filamentous cyanobacteria.	Grow on the surfaces of water bodies	Can tolerate polluted water. Effective *bio-indicator* for heavy metals pollutants.	
Arthropoda		*Ostracoda*	Cosmopolitan crustaceans with an oval hinged bivalve shell		Ostracods associated with *Oedogonium sp.* Ostracod can tolerate a wide range of environmental conditions	
Arthropoda		*Calanoid copepod*	Planktonic tiny crustaceans with a tear-drop shaped body and long antennae. They live in diverse aquatic habitats		*Calanoid sp.* together with *Navicula sp* and green algae Occur in eutrophic water.	
Ochrophyta	Naviculaceae	*Navicula* sp.	Benthic boat-shaped diatoms.	Found in marine and freshwater environments.	Used as bioindicator for water quality and organic pollution in aquatic bodies (Taş et al., 2015; Yusuf, 2020)	

Table 4. Cont. Eutrophication-tolerant organisms identified from the irrigation ponds in the study area

Ochrophyta	Bacillariaceae	*Nitzschia* sp.	Benthic diatoms with elliptical cell shape and spherical apices. Found in standing water associated with *Chlorella sp and Navicula sp.*	Found in marine and freshwater environments.	Considered an effective water quality bio-indicator (Maznah and Mansor, 2002; Trobajo et al., 2009)	
Tracheophyta	Araceae	*Lemna sp.* (Common name Duckweed)	A genus of Eutrophic green plants. Characterized by two light green cells with oval appearance and hair-like root.	Found free-floating on surface of eutrophic water pond	It can tolerate a polluted water, and is used for wastewater treatment in Jordan at Khirbat AS-Samra waste water treatment plant (Shammout et al., 2008)	 Close-up of leaves

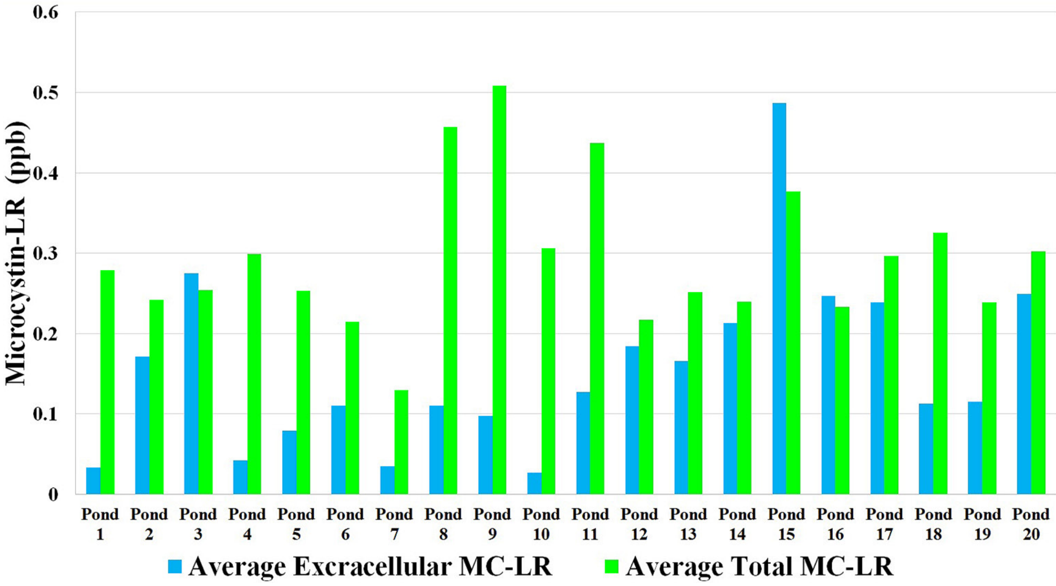


Figure 7. Average Microcystin-LR (MC-LR) concentrations measured in water ponds

Figure 10 shows the measured concentrations of MC-LR in the water collected from irrigation ponds during the two periods (June and September 2021) and from ponds with abundant algal blooms (*Lemna* sp. and Cyanobacteria). The concentration of MC-LR was significantly higher (P = 0.57) in pond water abundant with *Lemna sp.* in September 2021 than in June for the same year (0.3022±0.044 and 0.1048±0.0171 ppb, respectively). The same results were found when comparing the two methods of MC-LR extraction. Furthermore, there was a significantly (P = 0.003) lower concentration of MC-LR in the ponds with an abundance of *Lemna* sp. (0.1048 ± 0.01707 ppb) compared to the ponds with an abundance of Cyanobacteria Algal bloom collected in June 2021 (0.2273 ± 0.03563 ppb). Opposite, the ponds with abundant *Lemna* sp. showed no significant difference (P = 0.79) when compared with the ponds with abundant Cyanobacteria algal bloom when sample collection was conducted during the two different periods (June and September), as indicated in Figure 10.

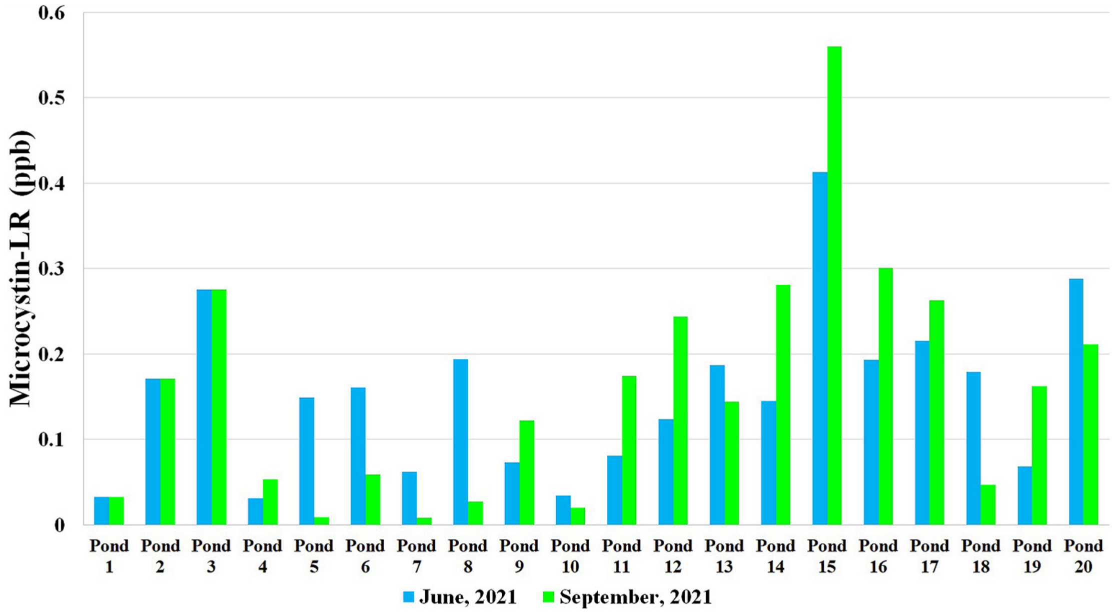


Figure 8. Extracellular MC -LR concentrations measured in water ponds

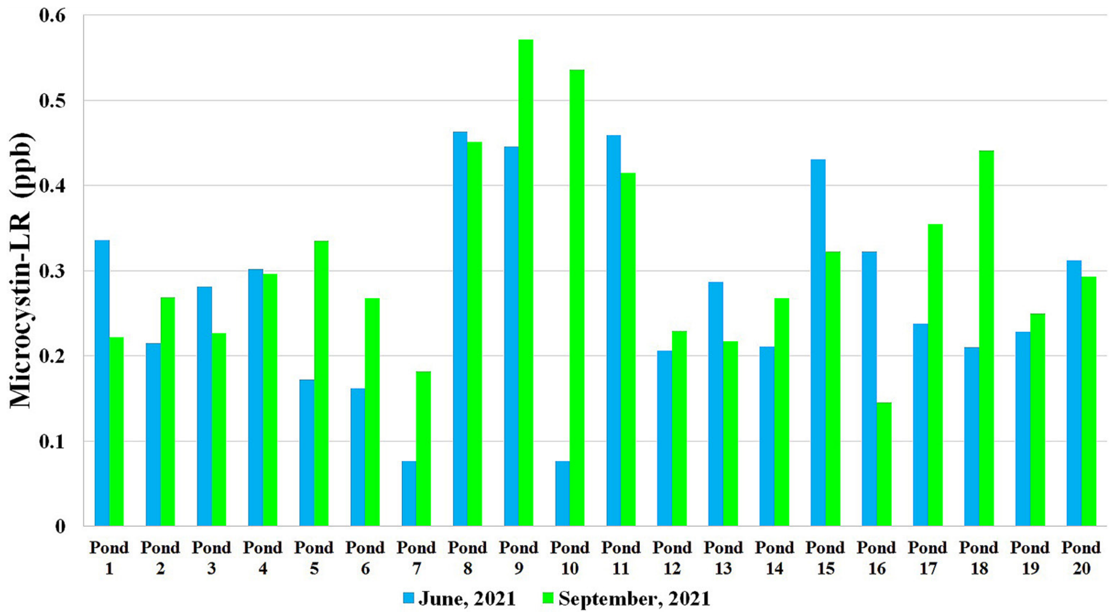


Figure 9. Total microcystin -LR concentrations measured in water ponds

Comparing the methods of MC-LR extraction (Fig. 11) showed no significant differences ($P = 0.303$) in MC-LR concentration between the water samples, indicating that the intracellular MC-LR content did not significantly affect the total concentration of MC-LR in water.

General quality index

In addition to climate change, there is a significant source of nutrient enrichment for water resources as population growth causes an increase in domestic wastewater discharges and excessive use of nitrogen and phosphorus fertilizers in agricultural activities (Havens et al., 2019). As a result of these nutrient inputs, aquatic ecosystems will eutrophicate, resulting in deterioration of water and environmental quality, algae growth, reduced water clarity, oxygen depletion, taste and odor changes, fish deaths, loss of biodiversity, including ecosystem services, and negative impacts on human health. As a result, defining the appropriate amount of nutrients to protect aquatic life, and the relationship between aquatic organisms' biological conditions and nutrient concentrations necessitates

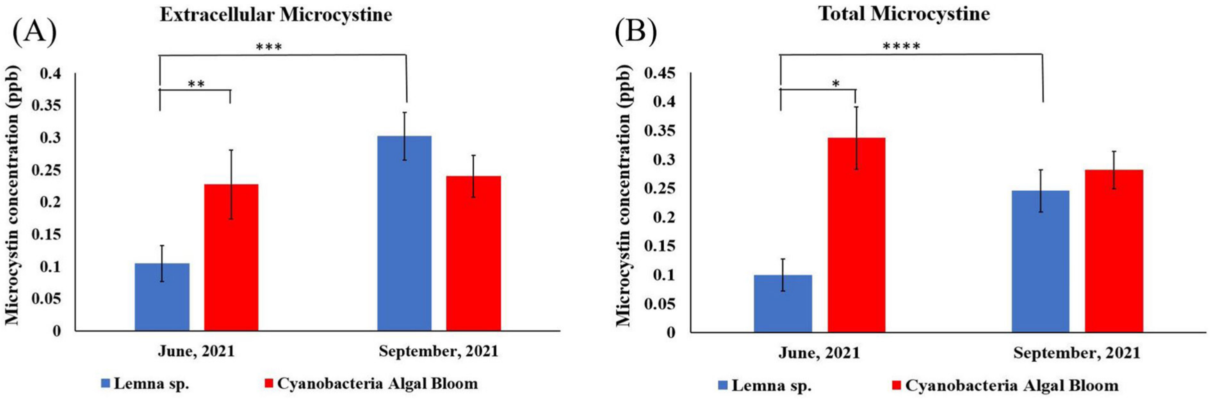


Figure 10. Microcystin LR (MC-LR) concentrations were extracted from pond water samples using two different methods and measured using ELISA. Extracellular MC-LR; from water only (A), Total MC-LR, from both water and cyanobacteria (B). Blue bars show MC concentration in pond water with abundant *Lemna sp.* (n=12), red bars show MCs concentration in pond water with abundant Cyanobacterial algal blooms (n=8). Statistical significance indicated as: *P <0.05, **P <0.01, ***P <0.001, ****P <0.0001

using various eutrophication assessment methods. After reviewing studies on eutrophication assessment for aquatic ecosystems' health and nutrient concentrations in surface water resources, eutrophication indexes are considered essential for water quality assessments. These indices are primarily based on water quality parameters such as total phosphorus, nitrogen concentrations, and the estimation of chlorophyll concentrations. However, several trophic status indicators have been developed, including dissolved oxygen (DO), dissolved inorganic nitrogen (DIN), dissolved inorganic phosphorus (DIP), total nitrogen (TN), total phosphorus (TP), Secchi disk depth (SD), chlorophyll-a (Chl-a), seagrass, macrobenthos, HAB, and benthic invertebrates (Hoyer et al., 2015).

The General quality index (GQI) has been adapted from Acuña-Alonso et al. (2020) and used to calculate the quality index for the different irrigation water ponds according to the following mathematical form (Cordoba et al., 2010):

$$GQI = \sum_{i=1}^{n} q_i w_i \tag{2}$$

where:

$$\sum_{i=1}^{n} q_i w_i = weight\ sum \tag{3}$$

where: n – the number of both physical and chemical dependent variables for GQI calculation;
q_i – an equivalence function that transforms the concentration of variable i into a quality level ranging from zero to one hundred (with 0 representing the worst level and 100 representing the ideal level, based on the intended use of the water);
P_i – the weighting of variable i.

The sum of all weightings must equal 1 for the calculated index to be between 0 and 100 (Cordoba et al., 2010). According to Table 5, the quality level associated with

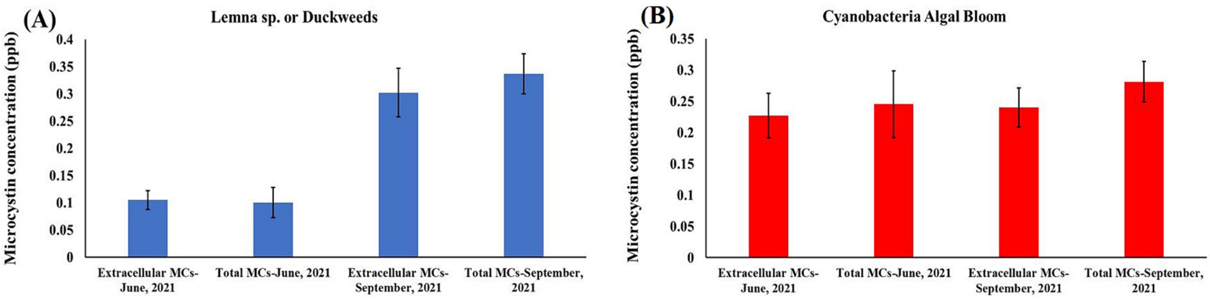


Figure 11. Microcystin-LR concentrations extracted from pond water with two different types of Algae. MC-LR from ponds with abundant *Lemna sp.* (A), and MC-LR from ponds with abundant Cyanobacteria bloom (B)

Table 5. Values for the general water quality index (GQI) adapted from Acuña-Alonso et al., 2020

GQI (Eutrophication Index)		
Qi = 100	Very good	Microtrophic
100 > Qi ≥85	Good	Oligotrophic
85 > Qi ≥ 75	Usable	Mesotrophic
75 > Qi ≥ 60	Bad	Eutrophic conditions
60 > Qi > 0;	Unacceptable	Hypereutrophic

variable *i* is excellent if Q_i = 100; very good if 100 > Q_i > 85; good if 85 > Q_i > 75; usable if 75 > Q_i >60; bad if 60 > Q_i > 0; and unacceptable if Q_i = 0 (Acuña-Alonso et al., 2020). To calculate GQI, the values of P_i and q_i were obtained according to the Jordanian maximum permissible limits for irrigation purposes (JMPLI, 2009).

Table 6. GQI variables and measurement units

Variable	Measurement unit	Weight (w_i)	Relative weight (P_i)
Electrical conductivity	µS/cm	5	0.067
pH	Integer values from 1 to 14	4	0.053
Dissolved oxygen	mg/L	10	0.133
Total phosphates	mg/L	10	0.133
Nitrate	mg/L	10	0.133
Potassium	mg/L	8	0.107
Biochemical oxygen demand	mg/L	9	0.120
Chemical oxygen demand	mg/L	9	0.120
Cu	ppm	5	0.067
Fe	ppm	5	0.067
		$\Sigma w_i = 75$	$\Sigma W_i = 1$

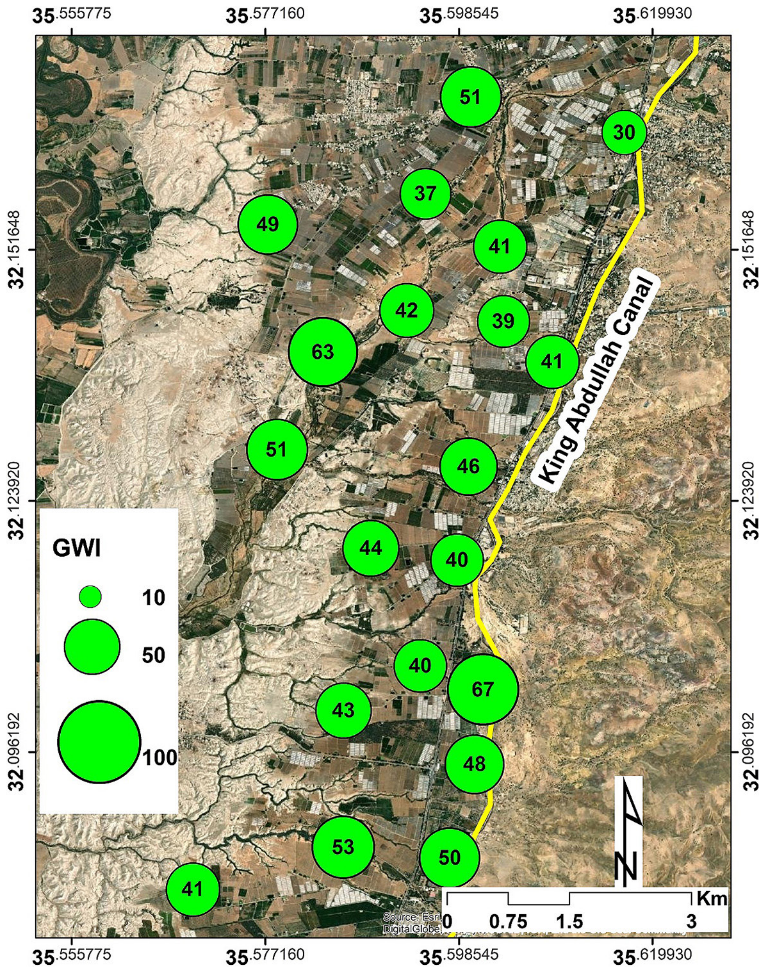


Figure 12. Calculated GQI for irrigation ponds

Ten water quality parameters are used to determine the general water quality index (GQI) Table 6: electrical conductivity (µS/cm), pH, dissolved oxygen (mg/L), total phosphorus (mg/L), nitrate (mg/L), potassium, biological oxygen demand, biochemical oxygen demand, copper, and iron. The GQI (Eutrophication status) was calculated for all irrigation water ponds and it ranged from 30 to 67 with an average value of 45 as shown in Figure 12. According to Acuña-Alonso et al. (2020) classification all irrigation ponds are classified as a hypereutrophic condition; this is clear and proven by the thick algal mat covering the irrigation ponds (see Figure 2).

CONCLUSIONS

Treated wastewater from King Talal Dam has been used intensively in the Jordan Valley for irrigation purposes. Pollutants in the dam can harm the environment and compromise water use, including irrigation. In this work, we measured the concentration of MC-LR for the irrigation water from the artificial ponds in two different periods from the Jordan Valley. Our findings from this investigation tests of 40 samples for their microcystins MC-LR and physicochemical parameters indicated that the water quality of these ponds could be used for irrigation. According to SAR values, the water of irrigation ponds is suitable for irrigation and can be classified in the 'Excellent / C1' category. However, some of the measured parameters for the water samples exceed the permissible limits of JMPLI (2009). Moreover, our findings showed that the MC-LR results are higher for the samples collected from September 2021 compared to those from June 2022. This is due to the temperature variations of the water and air and the nutrient concentrations (N:P) during the September period. Furthermore, the average concentrations of MC-LR were significantly higher in September 2021 than in June of the same year in the two methods of MC extraction. In addition, the dominant algae type in ponds significantly determined the water's MC-LR content. Nevertheless, all the irrigation ponds investigated in this study had a GQI (Eutrophication level) indicative of hypereutrophic status. Finally, it is necessary for further investigation of the irrigation ponds in the Jordan Valley and elsewhere in Jordan. On the other hand, MC-LR needs to be measured using molecular and biochemical tools such as quantitative Polymerase Chain Reaction (real-time PCR) and high-performance liquid chromatography (HPLC) to accurately estimate the microcystins and other toxin content in irrigation pond waters.

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