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Efficiency assessment of rhizofiltration by *Mentha aquatica* L. of polluted water from urban rivers

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ABSTRACT

Urban rivers in Bosnia and Herzegovina are under the influence of industrial as well as communal loads resulting in high concentrations of heavy metals as well as faecal bacteria, and investigating how this could be managed is essential. *Mentha aquatica* is a widely growing plant with the potential to be used in pollution management. In this study, a setup of artificial ponds using water from urban rivers was used to evaluate the efficacy of *M. aquatica* in rhizofiltration systems. During the 15-d trial various physico-chemical and microbiological parameters of water, as well as the removal rate of heavy metals and faecal bacteria, were evaluated. The chemical oxygen demand, dissolved oxygen, and pH values were reduced after the introduction of *M. aquatica* plants. Nitrite, nitrate, orthophosphate, and total phosphorus were also decreased after 15 d post *M. aquatica* introduction for highly polluted water. Absorption of lead and cadmium by *M. aquatica* and 45% and 100% pathogen removal ratio were recorded. The results obtained from this study suggest that *M. aquatica* has the potential to remove heavy metals and pathogens from polluted river ecosystems and could be considered for phytoremediation purposes through the process of rhizofiltration.

Keywords: Phytoremediation; Wastewater treatment; River; Heavy metals; Pathogens; *Mentha aquatica*

1. Introduction

The presence of heavy metals and pathogens in aquatic environments raises concerns for the scientific community as well as public health organizations worldwide. Rapid industrialisation and urbanisation have contributed to high pollutant loads in urban rivers, and have been recognised as the main sources of heavy metals and organic pollutants [1–3]. Through ingestion of fish inhabiting such polluted rivers, contamination of the food chain by heavy metals can cause bioaccumulation of toxic metals in human tissues resulting in different health problems in humans. Long-term exposure to heavy metals and organic pollutants in the environment is a real threat to living organisms [4–6]. Technologies used for the removal of these pollutants from wastewater before they enter the aquatic ecosystems include reverse-osmosis, ion-exchange, electrodialysis, adsorption, etc. Most of these technologies are very expensive, energy-intensive, and

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metal-specific. Therefore, environmentally friendly remediation technologies have been gaining attention among researchers worldwide.

Many researchers have reported that phytoremediation is a promising technology for the remediation of wastewater [7,8]. Phytoremediation consists of a group of technologies based on the use of natural processes of plants and their associated rhizosphere microorganisms, to reduce, remove, break, or immobilize pollutants [9,10]. The process is sustainable with low costs of maintenance and low energy consumption [11]. Plant species selected for phytoremediation must have the ability to accumulate either a specific or wide range of pollutants and metals transported in the aboveground parts [12,13], they should be native with a quick growth rate, high biomass yield, and high tolerance to heavy metals [14–16]. In aquatic systems, best rhizofiltration and phytoextraction is the most common way to remove heavy metals [17,18], persistent organic pollutants [19,20], and inorganic pollutants such as ammonia, nitrate, and nitrite [21,22]. Among other species, *M. aquatica* L. has been used in phytoremediation due to its high potential for the reduction of pollutants from the wastewater as demonstrated for some related plant species [23] and can be used as a bioindicator of the pollution level of water and soil [22,24].

Therefore, this study aims to determine the efficiency of *M. aquatica* for the phytoremediation of inorganic and heavy metal pollutants in aquatic ecosystems. The present study also aimed to shed further light on the role of macrophytes in aquatic ecosystems and suggest a practical and cost-effective approach for the treatment of wastewater. The novelty of the presented work lies in the interdisciplinary assessment of the phytoremediation potential of *M. aquatica* by not only assessing the ability to remove the heavy metals but also in addition faecal bacteria that can pose a great threat in freshwater ecosystems.

2. Material and methods

2.1. Water and plant sample collection

Specimens of *M. aquatica* L. were collected from the unpolluted water spring of Perućica creek at the "Skakavac" locality (43°56′ 54″ N, 18°27′ 00″ E). Plants were washed thoroughly with running tap water and rinsed with distilled water to remove any pollutants that may affect the research. The experiment is followed by acclimatization in distilled water for one week in the greenhouse at 20°C, with a 16/8 h (light/dark) photoperiod.

Mentha aquatica plants were introduced into constructed artificial ponds under three levels of pollution, using sampled water from polluted and unpolluted sites of the Miljacka river (Localities: Hreša – unpolluted spring of river Miljacka; Bentbaša – upper stream of the river the Miljacka; Otoka – middle stream of river Miljacka). The upper stream locality is under mild anthropogenic influence (few restaurants and small communities), while the middle stream locality is under the heavy anthropogenic influence (considered the most polluted site of river Miljacka). Water sampling followed the standard protocols for water and wastewater examination [25], sampled water was stored in polyethene containers and transported to the Laboratory of the Faculty of Science, University of Sarajevo, under controlled conditions (+4°C). In the construction of the experimental pond bed, rock samples corresponding to the water sampling site were used for the construction of experimental ponds, respectively.

2.2. Experimental setup

The experimental system was constructed under controlled conditions with three replicates per sampling site. Artificial ponds consisted of a small basin (41 cm upper, 34 cm lower diameter, and 29 cm depth), the bottom was layered with a corresponding bedrock sample (8 cm in depth) and basins were filled with 10 L of sampled water. From the acclimatized plants, specimens of uniform size (approximately 1,200 g) were selected and placed in an experimental pond, by submergence of the roots into the water. To prevent algal proliferation, the ponds were covered with aluminium foil throughout the experiment duration [26]. Ponds with no plants were used as control.

The water samples were collected on days 0, 5, 10, and 15 d after the introduction of plants to the constructed ponds, and physico-chemical and microbiological parameters were monitored. Loss of water, from the ponds, due to sampling and evapotranspiration was substituted with distilled water up to the original water level from the beginning of the experiment [26].

2.3. Analysis of physico-chemical parameters

Initial physical, chemical, and microbiological parameters, of sampled water, were analysed before the plant was introduced into the ponds. Selected physico-chemical parameters were monitored according to standards: pH (BAS ISO 10523:2013), dissolved oxygen (DO) (BAS ISO 8467:2002), ammonium NH_3–N (BAS ISO 7150/1:2002), nitrate NO_3–N (ISO 7890-3:1988), nitrite NO_2–N (JUS H.Z1.137:1985), orthophosphate (ISO 6878:2004), total phosphorus (ISO 6878:2004), chemical oxygen demand (COD) (BAS ISO6060:2000), and biochemical oxygen demand (BOD) (BAS ISO 5815-1).

2.4. Analysis of microbiological parameters

Water samples were analysed for the number of aerobic heterotrophic bacteria, total coliforms, and faecal coliforms using the membrane filtration (MF) technique (0.45 μm pore-size membrane filters; Millipore Corp., Berdford, MA) according to Standard Methods for the Examination of Water and Wastewater [25]. Microbiological analysis was conducted at 0 and 5, 10, and 15 d after the introduction of *M. aquatica* plants into the ponds. A total number of aerobic heterotrophic bacteria was assessed after 7-day incubation at 23°C on R2A agar (Sigma-Aldrich, Switzerland). A total number of coliform bacteria was recorded after 24 h incubation at 35°C ± 0.5°C on Endo agar (Sigma-Aldrich, Switzerland). For faecal coliforms, inoculated plates of mFC agar (Sigma-Aldrich, Switzerland) were incubated at 44°C ± 0.5°C for 24 h, after which the number of bacteria colonies was recorded.

All results were expressed as colony-forming units (CFU) per 100 mL. Further confirmation of coliform and faecal coliform bacteria included inoculation of well-isolated colonies in Lauryl Tryptose broth and EC (*Escherichia coli*) broth. Identification of bacterial strains was performed using standard microbiological methods: indole, methyl red, voges proskauer, citrate, russells double sugar, oxidase tests, and motility determination.

2.5. Analysis of heavy metals concentration

For the water samples lead (Pb) and cadmium (Cd) content was determined using graphite furnace atomic absorption spectrometry (GFAAS). Water samples were filtered, followed by digestion with 10 mL of concentrated analytical grade nitric acid (Merck, Germany) to 250 mL of the water sample. The solutions were evaporated in a crucible to approximately 5 mL, then filtered into 20 mL standard flask and made up to the mark with distilled water [27]. The detection limits of the instrument (AAS, PerkinElmer, model 3110) for Cd and Pb were 0.1 and 0.08 mg/L, respectively.

The leaf and root samples were dried in an oven at 70°C for 24 h. Approximately 0.2 g of the dried leaf and root samples, were treated individually with 8 mL HNO_3 (65% Merck) and 10 mL H_2O_2 (30% Merck) and then mineralized using a Berghof MWS-2 microwave digestion system. After 40 min of digestion, the samples were cooled for 30 min, and the clear solutions were filtered and filled to a final volume of 50 mL with distilled deionized water. Metals concentrations in the final solutions were analysed by flame atomic absorption spectrometry (FAAS), using Perkin Elmer M 3110 spectrometer. Determination of elemental concentrations in leaves and roots samples was performed using the method of calibration curve according to the absorber concentration. The metal concentrations were reported as mg/kg dry weight.

The translocation factor (TF) was calculated to estimate the transfer of heavy metals from the roots to the leaves according to the concentration of heavy metals in leaves and roots [28] according to the equation:

2.6. Data analysis

Descriptive statistics were performed by Statistica 10 software package (StatSoft. Inc). Experimental data were presented as mean values ± standard deviation (S.D.). To verify the statistical significance of the difference among various treatments, the data were analysed using a one-way analysis of variance and the Tukey HSD post hoc test ($p < 0.05$). The translocation factor which is a ratio of the concentration of metals (mg/kg) in the plant shoots and leaves to that of the metals in roots (mg/kg) was evaluated.

$$\mathrm{TF} = \frac{\text{Metal concentration}\left(\text{shoots}\right)}{\text{Metal concentration}\left(\text{roots}\right)}$$

3. Results

3.1. Removal efficiency of heavy metals and faecal pathogens (sampling site Hreša)

Mentha aquatica rhizofiltration efficacy in low-level polluted water was assessed in a pond containing water samples from the Hreša sampling site (upper stream locality) through analysis of physico-chemical, microbiological parameters and heavy metal content (Table 1). The phytoremediation capacity of *M. aquatica* was estimated according to the content of metals in a shoot, root, and translocation factor (Figs. 1A and 2A).

Table 1
Effect of *Mentha aquatica* planted in constructed wetland on physico-chemical and microbiological parameters and heavy metal content in water from upper stream of Miljacka river (Hreša locality)

Parameter	Unit	Days after *Mentha aquatica* planted in constructed wetland			
		0	5	10	15
pH		7.356a ± 0.040	6.450b ± 0.062	6.766c ± 0.015	6.823c ± 0.030
Dissolved oxygen	(mg/L)	8.673a ± 0.037	8.270b ± 0.030	6.840c ± 0.095	6.706c ± 0.140
Ammonium NH_3–N	(mg/L)	0.024a ± 0.019	0.230b ± 0.014	0.300c ± 0.011	0.910d ± 0.035
Nitrite NO_2–N	(mg/L)	0.007a ± 0.000	0.017b ± 0.000	0.018b ± 0.000	0.022c ± 0.001
Nitrate NO_3–N	(mg/L)	0.183a ± 0.005	0.331b ± 0.010	0.357c ± 0.040	0.442d ± 0.002
Orthophosphate	(mg/L)	0.014a ± 0.000	0.065b ± 0.004	0.070b ± 0.001	0.078c ± 0.002
Total phosphorus	(mg/L)	0.170a ± 0.010	0.186a ± 0.005	0.287b ± 0.003	0.291b ± 0.010
COD	(mg/L)	75.830a ± 0.855	53.383b ± 0.433	55.773b ± 0.971	30.590c ± 0.375
BOD	(mg/L)	1.366a ± 0.057	2.540b ± 0.115	9.323c ± 0.115	14.366d ± 0.315
Cadmium (Cd)	(µg/L)	1.910a ± 0.013	1.632b ± 0.115	0.973c ± 0.015	0.147d ± 0.054
Lead (Pb)	(µg/L)	1.355a ± 0.011	1.188b ± 0.017	0.984c ± 0.016	0.751d ± 0.0165
Aerobic heterotrophic bacteria	CFU/100 mL	1,281.33a ± 1.52	871.00b ± 1.00	667.66c ± 3.05	551.66d ± 1.52
Total coliform bacteria	CFU/100 mL	362.66a ± 5.85	292.66b ± 1.52	162.00c ± 1.00	8.00d ± 1.00
Faecal coliform bacteria	CFU/100 mL	324.00a ± 1.00	271.33b ± 1.00	24.00c ± 1.00	0.00d ± 0.00

Values are means (±SD) of three replicate ponds. Significant differences (indicated by different lowercase letters) within a row are based on a one-way analysis of variance with Tukey HSD test ($p < 0.05$).

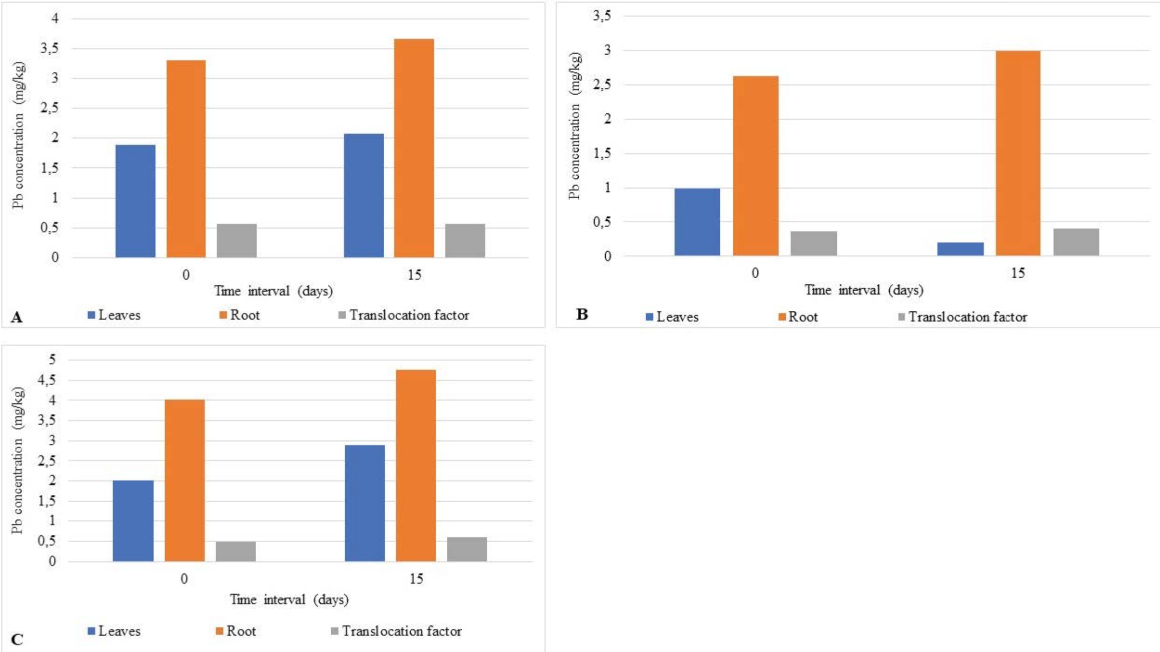

Fig. 1. Accumulation level of lead (Pb) in leaves and roots of *Mentha aquatica* (A) Hreša, (B) Bentbaša, and (C) Otoka.

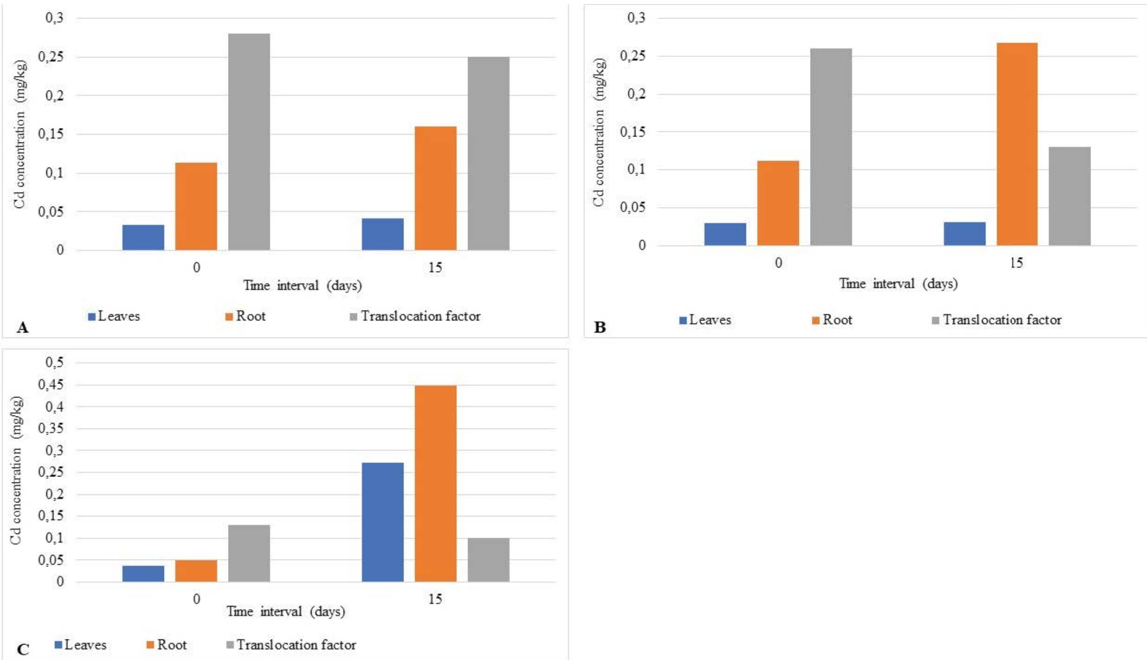

Fig. 2. Accumulation level of cadmium (Cd) in leaves and roots of *Mentha aquatica* (A) Hreša, (B) Bentbaša, and (C) Otoka.

Introduction of *M. aquatica* into the constructed ponds from the Hreša site induced a decrease in pH values up to 7.24%, with the successive reduction of dissolved oxygen through 4 sampling intervals (0, 5, 10 and 15 d) reaching a 22.67% decrease at day 15. No changes in parameters: ammonia, nitrate, nitrite, and phosphate levels were recorded. The initial COD concentration was 75.83 mg/L, while after 15 d concentration decreased to 30.59 mg/L. The values of BOD ranged from 1.36 mg/L at the beginning of the experiment to 14.36 mg/L 15 d after the inoculation of *M. aquatica*. The content of lead (Pb) in the water ranged from 1.355 to 0.751 µg/L, which indicates a decrease of 44.57% after treatment with *M. aquatica*, similarly content of Cd in water was decreased by 92.3% (Table 1).

The analysis of the total number of aerobic heterotrophic bacteria showed a significant reduction ranging from

32.02% after 5 d, 47.89% after 10 d, and 56.94% at the end of the experiment. A decrease of 97.79% for the total coliforms was recorded after 15 d. In addition, a 100% elimination of total faecal coliform bacteria was achieved after 15 d of the experiment.

The initial concentration of lead and cadmium, before the introduction, was assessed in the leaves and roots of *M. aquatica*, followed by planted of *M. aquatica* into the constructed wetland, after which the final assessment of Cd and Pb in *M. aquatica* was performed (Figs. 1 and 2). Slight elevation of Pb and Cd content was recorded for leaves and roots, compared to initial values with low translocation factors, ranging from 0.25–0.28 for Cd and 0.56–0.57 for Pb in shoots and roots, respectively.

3.2. Removal efficiency of heavy metals and faecal pathogens (sampling site Bentbaša)

During the 15-d trial, the pH values ranged from 8.04 to 6.96, including a reduced rate of 13.35% in the second pond originating from sampled water and bedrock from the Bentbaša site. The dissolved oxygen value varied from 8.76 mg/L on day 0, 7.73 mg/L on day 5, 8.34 mg/L on day 10, and dropped to 8.13 mg/L at the end of the experiment. Nitrate values increased from 0.068 to 0.56 mg/L after 15 d after the introduction of *M. aquatica* to artificial ponds. A decreasing trend in COD was recorded, while BOD increased from 1.13 mg/L at the beginning of treatment to 14.36 mg/L after 15 d of the experiment. The lead content decreased by 45.45% compared to the initial concentration, while cadmium content decreased by 96.07% (Table 2).

The absorption rate of heavy metals by *M. aquatica* is presented in Figs. 1 and 2. The lead content ranged from 0.993 to 1.199 mg/kg for the leaves and 2.634 to 2.994 mg/kg for the roots depending on the sampling day. The values of cadmium content in the leaves of *M. aquatica* ranged from an initial 0.029 to 0.035 mg/kg at the end of the trial. Cd concentrations in the roots increased from 0.111 mg/kg before the experiment to 0.268 mg/kg at the end of the experiment. TF value for the lead was 0.37 to 0.4 and 0.26 to 0.13 for cadmium indicating that most of the metals accumulate in the root.

The total number of aerobic heterotrophic bacteria ranged from 2,501 CFU/100 mL recorded at the beginning of the trial to 771 CFU/100 mL 15 d post the introduction of *M. aquatica* demonstrating a statistically significant removal of bacteria through rhizofiltration (Table 2).

3.3. Removal efficiency of heavy metals and faecal pathogens (sampling site Otoka)

A decrease of 9.09% in pH values was observed after 15 d of the experiment. Dissolved oxygen was reduced by 10.78%, from 8.78 mg/L at the beginning of the experiment to 7.83 mg/L at the end. The general reduction rate for nitrites was 88.17%, with a slight decrease to 86.5% by day 15, for nitrates reduction rate was much lower, only 29.52% by the end of the trial. The orthophosphate concentration levels on the last day of the trial were reduced by 88.49%. In addition, in the constructed pond with *M. aquatica*, the concentration of total phosphorus was significantly reduced (46.15%). The BOD values showed a significant decrease after *M. aquatica* phytoremediation, COD increased over time. A significant decrease in lead and cadmium content in the pond was recorded.

The microbiological analysis found that the aerobic heterotrophic bacteria were very high in water samples before

Table 2
Effect of *Mentha aquatica* planted in constructed wetland on physico-chemical and microbiological parameters and heavy metal content in water from upper stream of Miljacka river (Bentbaša locality)

Parameter	Unit	Days after *Mentha aquatica* planted in constructed wetland			
		0	5	10	15
pH		8.040a ± 0.010	6.433b ± 0.020	6.973c ± 0.015	6.966c ± 0.015
Dissolved oxygen	(mg/L)	8.763a ± 0.105	7.733b ± 0.119	8.346c ± 0.073	8.134c ± 0.007
Ammonium NH_3–N	(mg/L)	0.063a ± 0.005	0.141b ± 0.006	0.170c ± 0.004	0.418d ± 0.003
Nitrite NO_2–N	(mg/L)	0.033a ± 0.005	0.061b ± 0.005	0.069b ± 0.003	0.083c ± 0.004
Nitrate NO_3–N	(mg/L)	0.068a ± 0.003	0.056b ± 0.006	0.051b ± 0.002	0.056b ± 0.002
Orthophosphate	(mg/L)	0.030a ± 0.001	0.043b ± 0.005	0.056c ± 0.001	0.073d ± 0.001
Total phosphorus	(mg/L)	0.186a ± 0.005	0.103b ± 0.002	0.208c ± 0.002	0.254d ± 0.004
COD	(mg/L)	25.586a ± 0.500	1.973b ± 0.005	1.976b ± 0.005	17.526c ± 0.100
BOD	(mg/L)	1.133a ± 0.057	2.030b ± 0.010	6.176c ± 0.015	14.360d ± 0.060
Cadmium (Cd)	(µg/L)	2.905a ± 0.001	2.419b ± 0.032	1.947c ± 0.049	0.114d ± 0.002
Lead (Pb)	(µg/L)	0.550a ± 0.011	0.481b ± 0.022	0.358c ± 0.006	0.300d ± 0.002
Aerobic heterotrophic bacteria	CFU/100 mL	2,501.00a ± 1.00	932.00b ± 1.00	856.00c ± 1.00	771.00d ± 1.00
Total coliform bacteria	CFU/100 mL	1,511.33a ± 1.52	625.33b ± 1.52	294.66c ± 1.15	22.00d ± 1.00
Faecal coliform bacteria	CFU/100 mL	1,351.33a ± 1.52	642.66b ± 2.51	191.00c ± 1.00	10.00d ± 1.00

Values are means (±SD) of three replicate ponds. Significant differences (indicated by different lowercase letters) within a row are based on a one-way analysis of variance with Tukey HSD test ($p < 0.05$).

beginning the trial of rhizofiltration using *M. aquatica*. After 15 d a significant reduction of the total coliforms (TC) and faecal coliforms (FC) concentrations was observed. The total removal rates of 89.1% and 97.35% for total coliforms and faecal coliforms were recorded, respectively (Table 3).

The initial lead concentration in *M. aquatica* leaves was 2.005 mg/kg, which increased by the end of the second week to 2.882 mg/kg. The lead concentrations in the root ranged from 4.018 to 4.753 mg/kg. The concentration of cadmium in the leaves varied from 0.038 to 0.049 mg/kg and from 0.273 to 0.44 g/kg in the roots of *M. aquatica* (Figs. 1 and 2).

4. Discussion

Aquatic ecosystem pollution is a consequence of human activities such as urbanization, industrialization, and agriculture-related pollution [29]. To prevent the harmful effects of such pollution, it is necessary to come up with technologies for its remediation that will not result in another type of ecosystem deterioration [30,31]. Among the available options, phytoremediation is one of the eco-friendly techniques for the clean-up of polluted aquatic ecosystems. This technique exploits plant mechanisms such as absorption of pollutants through roots, accumulation in body tissues, decomposition, and their transformation to less harmful forms [32,33]. Plant characteristics, such as rapid growth, high biomass, deep roots, easy manipulation, tolerance, and accumulation of many pollutants in aboveground parts, are necessary plant characteristics for the phytoremediation process to be efficient and sustainable [34].

In this study, following the introduction of *M. aquatica* to artificially constructed ponds containing water with different degrees of pollution, a significant reduction in physico-chemical parameters was observed. The recorded reduction in pH is possibly a result of pollutants absorption, by the plant which could affect the overall pH level, considering that plant often absorbs minerals by exchange with other molecules. A similar decrease in pH due to pollutant absorption was observed during a phytoremediation study of industrial mine wastewater using water hyacinth [35]. Many studies have shown that pH is one of the most relevant parameters in the process of removing metal ions from aqueous solutions due to its effect on metal forms in the solution and its bioavailability to the plant [36–38]. A decrease in pH can have further effects on microbial performance and influence BOD and COD [35].

In all tri artificial ponds (with different pollution degrees), a reduction in dissolved oxygen was noted at the end of the experiment (Tables 1–3), which is consistent with other studies demonstrating similar findings [39]. The decrease in the dissolved oxygen concentration is most often related to the temperature and light conditions during the experiment, as well as the fact that artificial ponds do not have a percolation system meaning any fresh water is added during the experiment.

Nitrogen and phosphorus concentration can be limiting factors in the aquatic systems, and excess of these nutrients can lead to eutrophication and other undesirable changes in the environment [40,41]. Decrease of nitrites in heavily polluted water (Otoka sampling site) through rhizofiltration using *M. aquatica* demonstrated a significant efficacy reaching a reduction of nitrate by 88.17% (Table 3), at the end of the trial. In literature, it is recorded that the efficiency of nitrogen removal by plants can range from 25% to 98%, depending on the nitrogen form [42–45], indicating that *M. aquatica* provides an effective rhizofiltration

Table 3
Effect of *Mentha aquatica* planted in constructed wetland on physico-chemical and microbiological parameters and heavy metal content in water from middle stream of Miljacka river (Otoka locality)

Parameter	Unit	Days after *Mentha aquatica* planted in constructed wetland			
		0	5	10	15
pH		8.030a ± 0.010	6.653b ± 0.015	6.756b ± 0.050	7.300c ± 0.026
Dissolved oxygen	(mg/L)	8.780a ± 0.096	7.650bc ± 0.020	7.726b ± 0.015	7.833c ± 0.015
Ammonium NH_3–N	(mg/L)	0.780a ± 0.001	0.796b ± 0.002	0.802b ± 0.002	0.811c ± 0.001
Nitrite NO_2–N	(mg/L)	0.186a ± 0.005	0.186a ± 0.005	0.034b ± 0.009	0.025b ± 0.001
Nitrate NO_3–N	(mg/L)	0.376a ± 0.015	0.351a ± 0.005	0.307b ± 0.005	0.265c ± 0.001
Orthophosphate	(mg/L)	0.113a ± 0.005	0.068b ± 0.000	0.048c ± 0.000	0.013d ± 0.002
Total phosphorus	(mg/L)	0.650a ± 0.010	0.474b ± 0.002	0.424c ± 0.005	0.350d ± 0.008
COD	(mg/L)	25.546a ± 0.011	24.126b ± 0.335	5.786c ± 0.106	1.976d ± 0.011
BOD	(mg/L)	1.140a ± 0.000	7.923b ± 0.05	14.326c ± 0.200	16.480d ± 0.157
Cadmium (Cd)	(μg/L)	4.508a ± 0.000	3.950b ± 0.045	2.247c ± 0.109	0.146d ± 0.001
Lead (Pb)	(μg/L)	1.480a ± 0.011	1.233b ± 0.004	1.115c ± 0.003	0.894d ± 0.001
Aerobic heterotrophic bacteria	CFU/100 mL	22,651.00a ± 1.00	13,401.00b ± 1.00	1,123.33c ± 1.52	914.33d ± 2.08
Total coliform bacteria	CFU/100 mL	87,201.33a ± 1.53	48,202.00b ± 2.00	31,701.00c ± 1.00	9,501.67d ± 1.53
Faecal coliform bacteria	CFU/100 mL	45,401.00a ± 1.00	35,701.00b ± 1.00	16,121.33c ± 1.53	1,201.00d ± 1.00

Values are means (±SD) of three replicate ponds. Significant differences (indicated by different lowercase letters) within a row are based on a one-way analysis of variance with Tukey HSD test ($p < 0.05$).

system for the removal of nitrites, especially in heavily polluted water. Similar results were recorded for phosphorous where a removal rate of 46.15% for heavily polluted water was recorded (Table 3). Considering that the rate of total phosphorus removal can vary between 20% and 90% [46–48], our results indicate that *M. aquatica* has a moderate ability to remove phosphorous from aquatic systems. The nitrate and phosphate removal rate depends on the type of constructed pond, vegetation, and substrate, including the water regime and environmental conditions [49,50].

M. aquatica was able to remove heavy metals from the test artificial ponds through rhizofiltration and metal absorption with low translocation to the shoots, similar to literature data where it was found that heavy metals decrease in water samples after treatment with *M. aquatica* in artificial pond system [51,52], as well as in treatments of wastewater [22]. The concentrations of heavy metals (Pb and Cd) were increased in the roots and leaves, at the end of the investigation period comparing the initial metal content. Considering metal translocation between the roots and leaves, the TF indicates low translocation. Low translocation of heavy metals into the upper part of the *M. aquatica* plants recorded in our study suggests that the plant is not a heavy metal accumulator, but the removal rate of heavy metals is a result of rhizofiltration. Heavy metal restriction to the roots in rhizofiltration systems contributes to the prevention of heavy metal dispersal through animals feeding on upper plant parts contributing to the prospects of using *M. aquatica* in wastewater management systems. Removal rates of waterborne pathogens by aquatic plants in artificial ponds can reach up to 99.99% [53]. Our study suggests that *M. aquatica* is an efficient rhizofiltration system for the removal of coliforms and faecal coliforms which could be related to root exudates. Reports suggest that *M. aquatica* can secret substances with antibacterial and bactericidal properties resulting in a reduction of some pathogens, such as microorganisms [54,55].

Concerning the level of pollution differences in *M. aquatica* efficacy suggest that this plant is very efficient for highly polluted water concerning the removal of faecal bacteria. The level of inorganic pollutant removal was influenced by pH, and plants' absorption of different minerals and inorganic compounds such as nitrites is often correlated to pH levels. Adding to the ability of *M. aquatica* to absorb Pb and Cd, species of the genus *Mentha* can also accumulate Ni [52]. The levels of Pb and Cd that *M. aquatica* can absorb are related to their concentration and availability in the solution, but even small dosages of lead can be lethal to some aquatic invertebrates as well as to small fish [56]. Thus the importance to manage even small pollution with heavy metals is crucial, especially in rivers providing services to locals such as fishing.

5. Conclusion

Research in this paper focused on testing environmentally friendly, efficient, and cost-effective solutions for the revitalization of aquatic ecosystems contaminated due to anthropogenic influences. The ability of *M. aquatica* to absorb heavy metals and restrict them to the roots demonstrates a good potential of this plant to be used in wastewater management as well as aquaponics for water quality management. High efficiency (99%–100%) of pathogen removal adds to *M. aquatica* value as a complete multilevel rhizofiltration system for improvement of water quality through reduction of nitrites and heavy metal content as well as the removal of faecal bacteria.

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Competing interests

The authors have no relevant financial or non-financial interests to disclose.

Author contributions

All authors contributed to the study's conception and design. Material preparation, data collection and analysis were performed by Sabina Dahija, Erna Karalija and Amir Čaušević. The first draft of the manuscript was written by Sabina Dahija and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Ethics approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Consent to participate; consent for publication

Not applicable.

Conflict of interest

The authors declare no competing interests.

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