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DOI 10.26873/SVR-744-2019 **Original Research Article**

BIOREMEDIATION OF A PESTICIDE AND SELECTED HEAVY METALS IN WASTEWATER FROM VARIOUS SOURCES USING A CONSORTIUM OF MICROALGAE AND CYANOBACTERIA

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Abstract: The presence of organophosphate pesticides and heavy metals in water are known to be toxic to aquatic organisms. Bioremediation makes use of naturally-occurring organisms to remove pollutants from the environment. This study explored the potential of using a consortium of microalgae and cyanobacteria (*Chlorella vulgaris*, *Scenedesmus quadricuda* and *Spirulina platensis*) to remove the organophosphate pesticide malathion and the heavy metals cadmium, nickel and lead from water samples taken from varying combinations of urban wastewater and agricultural drainage water in Egypt. The fastest algal growth observed in this study was in a treatment containing the microorganismal consortium, malathion and heavy metals cultured in water samples taken from agriculture drainage and urban wastewater. Microalgae in this study were able to remove malathion from samples of wastewater with up to 99% efficacy and were able to bioaccumulate nickel at up to 95% efficacy. Moreover, microalgae demonstrated the ability to uptake lead and cadmium at up to 89% and 88% efficacy respectively. The results from this study suggest that a consortium of Chlorella vulgaris, *Scenedesmus quadricuda* and *Spirulina platensis* can be effective in remediating the pesticide malathion and the heavy metals cadmium, lead and nickel from wastewater.

Key words: bioremediation, wastewater, microorganisms, microalgae, pesticides, heavy metals

Introduction

Effluents from urban and agro-industrial wastewater are known to cause considerable contamination of rivers, lakes and seas around the world (1). Such contamination can come in the form of heavy metals, pesticides and fertilizers. One particularly toxic pesticide which is found in water sources across the globe is malathion which belongs to the organophosphate group. Malathion is a non-systemic, broad-spectrum pesticide that is used to control insects

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on vegetables, fruits and crops as well as household insects and animal parasites (2). However, malathion has been reported to have numerous deleterious effects amongst humans and animals including hepatotoxicity (3), human breast carcinoma (4), genetic disruption (5), and damage of normal hormonal activity (6). In addition to pesticides, heavy metals are also recognized as pollutants that are both commonly occurring and persistent in the aquatic environment. Heavy metals are typically introduced into water bodies via wastewater which includes industrial effluents. Of the heavy metals commonly found in water bodies, lead, cadmium and nickel are reported to be some of the most hazardous (7). In particular, through the processes of bioaccumulation and biosorption, concentrations of heavy metal ions can increase across the aquatic food chain and can be transferred to humans posing a considerable risk to health (8). Due to their toxicity and tendency to accumulate in water, pesticides and heavy metals occurring in high levels can become severely toxic to all living creatures. In fish, bioaccumulation of metals has been shown to cause either high mortality or result in many biochemical and histological changes in the surviving fish (9).

Traditional wastewater treatment processes include a combination of physical and chemical methods such as chemical precipitation, activated sludge process, carbon adsorption and microfiltration. Such techniques are used to eliminate inorganic phosphates, organic wastes, and toxins. Unfortunately, such treatment methods can be expensive to implement and moreover can be inefficient and non-environmentally friendly. In contrast, bioremediation provides an alternative method of eliminating contaminants from the environment. Most organophosphate compounds are decomposed by microorganisms in the environment as a source of carbon or phosphorus or both (10-12).

Photoautotrophic microorganisms, including green algae, are used in wastewater remediation processes due to their ability to accumulate deleterious heavy metals from aqueous effluents (13). Moreover, they have the potential to utilize organic carbon and light simultaneously as sources of energy. This provides microalgae with an essential competitive advantage over bacteria and fungi in removing organic pollutants (14). Microalgae and cyanobacteria associate with other aerobic or anaerobic microorganisms to form microbial groups that live symbiotically in a community-defined consortium. This consortium of algae and bacteria can act in a synergistic way to break down organic and inorganic pollutants much more effectively than individual microorganisms (15). For these reasons, microalgae have been used in various studies on remediation of pollutants, for both organic compounds and heavy metals. Algae are capable of the uptake and elimination of organic pollutants via both biosorption and/or metabolization (16). The major advantage of algae-mediated bioremediation is that it can achieve various goals of wastewater treatment simultaneously such as correction of pH, reduction of total dissolved solids (TDS) and removal of both chemical oxygen demand (COD) and biological oxygen demand (BOD) (17-19). Furthermore, the primary mechanism in algae-mediated remediation results in the considerable production of oxygen through photosynthesis. This natural generation of oxygen further assists in the degradation of contaminants and decreases the requirement for the utilization of mechanical aerators in traditional wastewater treatment processes (20). In addition to its extensive capacity at bioremediation, the consequent biomass created by the use of algae could be used for the formation of a wide range of value-added products. This combined method of biomass production and wastewater treatment has been termed the "integrated cultivation process"(21,22).

The aim of this study was to evaluate the growth of a combined consortium of microalgae and cyanobacteria (*C. vulgaris, S. quadricuda* and *S. platensis*) in the presence of the pesticide malathion and the heavy metals cadmium, nickel and lead, as well as their efficacy in removing these pollutants from contaminated water taken from different wastewater sources.

Material and methods

Isolation of microorganisms, enrichment and acclimatization

The algal strains *Chlorella vulgaris* and *Scenedesmus quadricuda* and the cyanobacteria *Spirulina platensis* were isolated from different water samples collected from Lake Burullus in the north of the Egyptian delta in July 2016. Subsamples of the collected water were placed on sterile agar plates that were formed by the addition of 1.5% agar to BG-11 medium. Axenic strains of microalgae were obtained through continuous sub-culturing in BG11 agar plates supplemented with ampicillin and kanamycin. Fluorescent lamps were used for illumination throughout the culture process (23). Algal strains were identified through electrochemical analysis in the Institute of Biotechnology, Universidad Nacional Autónoma de México (UNAM), Mexico (24). The cultured consortium of microorganisms was then incubated with the pesticide malathion and the heavy metals cadmium (Cd), nickel (Ni) and lead (Pb). Agricultural drainage water was collected from a known source of water emanating from agricultural lands whereas urban wastewater was collected from a known source of water emanating from urban areas in Kafr El Sheikh Governorate.

Chemicals

Malathion was obtained from the Kafr El Zayat Pesticide Company in Egypt (Malathion 98% active ingredient) and Cd, Ni and Pb were obtained in pure powder form from Sigma Aldrich® in Egypt.

Experimental design and sampling

The study used eight glass aquaria (20 cm x 40 cm x 30 cm) representing seven treatments and one control (Table 1) in three replicates. All aquaria were filled with 10 L of water from either urban wastewater alone, agricultural drainage water alone, or a combination of both, according to the treatment in question. Microorganisms were batch-cultured in the seven treatment aquaria whereas no microorganisms were added to the control. The control treatment consisted solely of pesticides and heavy metals in the test water. The study used an inoculum of microorganisms at a concentration of approximately 8 x 10^4 cell/ml. Different combinations of Cd, Pb, Ni and malathion, were then added to aquaria, according to treatment type, at concentrations of 5, 10, 10 and 25 ppm respectively. To maintain the optimum condition for the culture of microorganisms throughout the experiment, the flasks were incubated at 23 ± 1 °C in a culture room under a 24 hour photoperiod by using a 25-watt bulb in each aquarium. In addition, aquaria were supplied with continuous aeration. Samples of test water were taken at different periods throughout the duration of the experiment (0, 1^{st}, 3^{rd}, 7^{th}, 14^{th}, 21^{st}, 28^{th} day) for the quantification of microorganismal growth (cell count) as well as for the analysis of heavy metals and physicochemical parameters. Malathion residues were prepared for quantification by centrifuging the algal culture filtrates.

Measurement of microorganismal growth

The growth of microorganisms in the seven treatments was quantified in terms of cell count by using a T80 UV-visible spectrophotometer (OD600 PG Instruments, United Kingdom).

Analysis of malathion concentrations

For malathion analysis, 2 ml samples were taken every four days from each glass aquarium and were placed in glass tubes. These samples were then extracted twice with equal volumes of 1:1:1 hexane-acetone-dichloromethane as the extracting reagent. The mixture was homogenized for three minutes using a vortex mixer. The extracting reagent with residual malathion was filtered, dried by use of anhydrous sodium sulfate and followed by filtration through Whatman GF/B glass-fiber paper. This operation was performed sequentially, and the filtrates were mixed. The filtrate was then evaporated till dryness and resolved in HPLC-grade dichloromethane (50 μL) for analysis. Malathion residues were quantified using trace gas chromatography coupled to a Polaris Q Thermo Finnigan mass spectrophotometer (GC–MS) using the EPA8141 method under the subsequent conditions: equity column-5; 30 m x 0.25 mm ID; 0.25 µm, temperature of oven (120 °C) for three minutes and increased at a rate of 5 °C per minute till 270 °C; temperature of injector (250 °C); injection volume 1 µl, MSD detector; scan range 45–450 amu; helium flow 30 cm/s (120 °C), transfer line (320 °C), splitless (0.3 minute), splitless liner, and double taper (25). The kinetics of the removal of malathion was conducted for a period of 54 hours.

Analysis of heavy metal concentrations

The heavy metals Ni, Pb and Cd were quantified by first gently evaporating 200 ml of water from each aquarium till dryness. The residues were then dissolved in 5 ml concentrated nitric acid (HNO_3). Subsequently, 5-10 drops of hydrogen peroxide (H_2O_2) were added in order to ensure the completion of the process of digestion. The dried residue was eluted in 1 ml HNO_3, (26) and then the concentrations of heavy metals were quantified using an atomic absorption spectrophotometer (GBC Avanta E, Victoria, Australia; Ser. No. A5616).

Physicochemical analysis of water samples

Analysis was carried out for various water quality parameters such as temperature, pH, electrical conductivity (EC) and total dissolved solids (TDS). Temperature and pH were measured using a glass electrode pocket pH and temperature meter (Digital Mini-pH Meter, Model 55, Fisher Scientific, USA) whereas TDS and EC were measured using a salinity-conductivity meter (YSI EC300, YSI Corp., Yellowstone Springs, Ohio, USA**.**

Statistical analysis

The data were tested for normality of distribution, linearity and homogeneity of variance. The final concentrations of malathion, Ni, Pb and Cd were analyzed using general linear models (SAS Statistical Analysis Software). The physicochemical parameters were analyzed using a one-way ANOVA. The level of significance was set at $P \leq 0.05$.

Results and discussion

Effect of malathion on growth of tested algal strains

Growth of the microorganismal consortium was observed across all treatments (Fig. 1). However, the highest level of growth was observed in the treatment containing both the pesticide malathion and the heavy metals cadmium, lead and nickel in the combined water from agriculture drainage and urban wastewater (treatment 'PHMAU' [pesticides and heavy metals with microorganisms in agriculture drainage water and urban wastewater]; Fig. 1). The largest increase in the number of cells throughout the experiment was observed in this treatment, reaching a maximum level of 15 x 10^6 cells/ml in day 28 of the experiment. The second highest level of microorganismal growth was observed in the treatment only containing malathion in urban wastewater ('PMU' [pesticides with microorganisms in urban wastewater]). Peak algal growth in this treatment reached 12 x 10^6 cells/ml in day 28 of the experiment. On the other hand, the lowest growth of tested algal strains during 28 days of exposure was recorded in the treatment containing just microorganisms in agricultural drainage water ('MA').

The high level of algal growth in the presence of heavy metals and pesticides observed in this study corresponds with other studies conducted elsewhere. The study by (27) found that microorganisms can indeed utilize a wide range of organic pollutants, including pesticides, as an energy source for their growth and simultaneously mineralize and degrade the compounds. Similarly, results from the study by (28) which studied the growth of *C. vulgaris* in the presence of the organophosphate pesticide dimethoate reported enhanced microalgal growth and higher protein and chlorophyll content. The stimulatory effect of malathion on the growth of microalgae reported in our study corroborates with findings in (29) 2016 This study which examined the growth response of *S. quadricuda* and *C. vulgaris* in the presence of malathion reported a considerable increase in cell count in water containing malathion. In addition, (30) studied the impact of malathion on the growth of *Aspergiullus oryzae.* They reported accelerated growth of this microorganism when malathion was added to the culture water.

The stimulatory effect of malathion on microorganismal growth could be a result of the increase of the available phosphorus due to the breakdown by the microalgae (11). Moreover, the accelerated growth observed in the microorganisms that were cultured in the presence of malathion could be attributed to their ability to use this compound as a sole source of phosphorus in the absence of inorganic phosphate from the growth medium (31).

Effect of heavy metals on growth of the microorganismal consortium

he highest level of microorganismal growth observed in this study was in the treatment containing both the pesticide malathion and the heavy metals cadmium, lead and nickel in the combined water from agriculture drainage water and urban wastewater ('PHMAU'; Fig. 1). The second highest level of microorganismal growth observed in the treatments containing heavy metals was in the treatment consisting of just heavy metals in the sample containing only urban wastewater ('HMU'; Fig. 1). In this treatment, microorganismal growth reached a peak of 9 x 10^6 cells/ml at the end of experiment. The lowest level of growth was observed in the treatment containing microorganisms alone in agricultural drainage water ('MA'; Fig. 1).

The results from this study are in line with findings from a study by (32) which investigated the effects of mercury (Hg), Cd and Pb on the growth of *S. quadricuda*. The authors of this study observed enhanced algal growth in the presence of Pb and Cd ions at concentrations similar to those used in our study (5-20 ppm). However, this study also reported a deleterious effect of Hg on algal cells at any concentration. As suggested in this paper, the reason for this accelerated algal growth in the presence of Pb and Cd could be attributed to a phenomenon involving heavy metals resulting in an increase in chlorophyll content.

Breakdown of malathion by the consortium of microorganisms in wastewater

This study measured the growth potential of a consortium of microorganisms in water from different sources as well as their capability to biologically breakdown the pesticide malathion. The highest level of malathion removal by the microorganismal consortium was observed in the treatment with pesticides, heavy metals and microorganisms in the combined water from agriculture drainage and urban wastewater ('PHMAU'; 99% [24.75 mg/L] Fig. 2), followed by the treatment containing just pesticides and microorganisms in urban wastewater ('PMU'; 95% [23.5 ± 0.1 mg/L]) and the treatment containing pesticides and microorganisms in agricultural drainage water ('PMA'; 85% [21.25 mg/L]). In comparison, malathion removal in the control treatment ('PHAU') was just 15% (3.75 ± 0.1 mg/L; Table 2).

The biggest advantage in using microorganisms for the degradation of organic compounds is the synergism with bacteria. They can degrade symbiotically wherein algae provide oxygen for the bacteria via photosynthesis and in turn uptake carbon dioxide released from the heterotrophic bacteria. The considerable capacity of the consortium of microorganisms used in this study to degrade malathion may be attributed to synergistic interactions whereby the overall degradative efficiency is increased (33). For example, (34) found that *Chlorella* spp. were capable of metabolizing up to 99% of the organophosphate pesticide fenamiphos in just 4 days making *Chlorella* a potent resource for fenamiphos removal. Similarly, (35) reported that *S. quadricuda* can effectively break down the fungicides dimethomorph and pyrimethanil as well as the herbicide isoproturon. The biodegradation observed in these studies as well as the study in question could be driven by enzymes produced by these microorganisms; enzymes such as cytochrome P450 (recorded in *Chlorella* spp.) which may assist in breaking chemical bonds in the pesticide molecules (36-40).

Bioaccumulation of heavy metals by the consortium of microorganisms in wastewater

This study investigated the bioaccumulation of Cd, Ni and Pb at concentrations of 5, 10 and 10 ppm respectively in a consortium of three different microorganisms and in different types of wastewater. The highest level of Pb removal was observed in the treatment with microorganisms, heavy metals and pesticides in the combined water from agricultural drainage and urban wastewater (‘PHMAU’; 89% [8.9± 0.02 mg/L], Fig. 2) followed by the treatment with only heavy metals and microorganisms in urban wastewater (‘HMU’; 88% [8.8 ± 0.05 mg/L]) and the treatment with heavy metals and microorganisms in agricultural drainage water (‘HMA’; 87% [8.7 ± 0.04 mg/L]). In contrast, Pb removal in the control treatment (‘PHAU) was just 1% (0.1 ± 0.02 mg/L; Table 2). Moreover, the three treatments containing microorganisms, HMA, HMU and PHMAU, showed the highest levels of Ni removal, 95% (9.5± 0.07 mg/L), 93% (9.3 ± 0.06 mg/L) and 91% (9.1 ± 0.02 mg/L), respectively in comparison with the control PHAU treatment where Ni removal was just 2% (0.2 ± 0.01 mg/L). Cadmium removal was highest in the treatment PHMAU, followed by HMA and HMU with removal of 88% (4.4 ± 0.025 mg/L), 86% (4.3 ± 0.015 mg/L) and 78% (3.9 ± 0.02 mg/L) respectively in comparison with the control PHAU treatment 3% (0.15 ± 0.015 mg/L; Table 2).

In accordance with our results, (41) reported that *Chlorella* spp. were efficient in removing Cd and Ni from growth medium under laboratory conditions with a removal potential ranging from 70% to 95%. Similarly, (16) found that *C. vulgaris* was effective in removing Cd, Pb and Hg. A study by (42) demonstrated that the cyanobacteria *Spirulina platensis* can tolerate Cd at concentrations up to 100 mg/L and was able to remove Cd at a rate of ~98.04 mg/L. (43) reported a high tolerance of *Scenedesmus* spp. to Ni, Cd, copper (Cu), and zinc (Zn) at relatively low concentrations of 2-5 mg/L but higher tolerance of lead up to 30 mg/L. The biosorption of various heavy metals such as Pb, Cd, Cu, Zn by various strains of microalgae could be attributed to their capacity to carry out ion exchange. Microalgae can hold mobile metal ions (e.g., potassium, sodium, magnesium and calcium) in their structure by binding them to acid functional groups (44, 45). Therefore, ion exchange could be the primary mechanism through which microalgae uptake heavy metals (46, 47). Furthermore, algal cell walls are surrounded by a three-dimensional network of macromolecules (proteins and polysaccharides) which carries negatively charged functional groups (e.g., hydroxyl, carboxyl, phosphate or amine groups, etc.) that play a crucial role in chemical binding with metal ions and are responsible for the biosorption ability of microalgae. Due to the cationic form of major metal ions in water solution, they are adsorbed to the algal cell walls (48-51).

Physicochemical parameters of water samples

The highest pH observed in this study was in the PMA treatment (8.517 ± 0.076) whereas the lowest pH recorded (7.966 ± 0.079) was in the PHAU treatment. Overall, there was a significant increase in pH in treated water. This could be attributed to the photosynthetic activity of algae resulting in the consumption of high quantities of bicarbonates and formation of a high level of carbonates thereby raising the pH (52). The highest levels of conductivity were observed in the PMA treatment (2339 ± 264.7 µS/cm) and the lowest levels were observed in the PHAU treatment (851.0 ± 18.57 µS/cm). Conductivity increased markedly towards the end of the experiment in treatments containing microorganisms, in particular the PMA treatment (3040 µS/cm). Higher conductivity in the presence of microorganisms coincides with the results of (53). The highest levels of TDS recorded in this study were in the PMA treatment (1171 ± 133.0 ppm) and the lowest levels were recorded in the PHAU treatment (425.9 ± 9.433 ppm). In the HMU treatment, there was a slight increase in TDS (590.0 ± 23.79 ppm). Overall, there was a significant increase in TDS in treated water compared to untreated water. There were no significant differences in temperature between experimental treatments. The reading of temperature ranged from 16.61 ± 0.81 °C in the PHAU treatment to 16.81 ± 0.805 °C in the PMA treatment (Table 3). Temperature is a critical factor as algal productivity is accelerated by increasing temperature up to an optimum temperature which varies between algal species. Optimum temperature differs with limited nutrients or light conditions and growth often decreases when algae are subjected to a sudden change of temperature (54).

Table 1: Various treatments of the different sources of wastewater representing seven treatments and one control

Treatment	
1	Pesticides with microorganisms in urban wastewater (PMU)
2	Pesticides with microorganisms in agricultural drainage water (PMA)
3	Heavy metals with microorganisms in urban wastewater (HMU)
4	Heavy metals with microorganisms in agricultural drainage water (HMA)
5	Pesticides and heavy metals with microorganisms in agricultural drainage water and urban wastewater (PHMAU)
6	Microorganisms in urban wastewater (MU)
7	Microorganisms in agricultural drainage water (MA)
8	Pesticides and heavy metals in agricultural drainage water and urban wastewater (PHAU) (Control)*

* Initial concentrations for experimental water used: Cd, Ni and Pb were found to be 0.036, 0.2and 0.025 ng/mL respectively and 0.0012 ng/mL malathion

Table 2: Removal (%) of malathion and heavy metals using a consortium of microorganisms (the microalgae *Chlorella vulgaris*, *Scenedesmus quadricuda* and cyanobacteria *Spirulina platensis*) tested in wastewater obtained from different sources with concentrations of 25 ppm of the pesticide malathion and the heavy metals cadmium (Cd), nickel (Ni) and lead (Pb) at concentrations of 5 ppm of Cd, 10 ppm of Ni and 10 ppm of Pb after 28 days of treatment. The data are means ± standard error of three replicates

	Malathion		*Cd*		*Ni*		*Pb*	
Treatment	*mg/L*	*Removal (%)*	*mg/L*	*Removal (%)*	*mg/L*	*Removal (%)*	*mg/L*	*Removal (%)*
PMU	1.25 ± 0.1	95 ± 0.4						
PMA	3.75 ± 0.75	85 ± 0.3						
HMU			1.1 ± 0.02	78 ± 0.4	0.7 ± 0.06	93 ± 0.6	1.2 ± 0.05	88 ± 0.5
HMA			0.7 ± 0.015	86 ± 0.3	0.5 ± 0.07	95 ± 0.7	1.3 ± 0.04	87 ± 0.4
PHMAU	0.25 ± 0.125	99 ± 0.5	0.6 ± 0.025	88 ± 0.5	0.9 ± 0.02	91 ± 0.2	1.1 ± 0.02	89 ± 0.2
PHAU	21.25 ± 0.1	15 ± 0.4	4.85 ± 0.015	3 ± 0.3	9.8 ± 0.01	2 ± 0.1	9.9 ± 0.02	1 ± 0.2

PMU; Pesticides with microorganisms in urban wastewater, PMA; Pesticides with microorganisms in agricultural drainage water, HMU; Heavy metals with microorganisms in urban wastewater, HMA; Heavy metals with microorganisms in agricultural drainage water, PHMAU; Pesticides and heavy metals with microorganisms in agricultural drainage water and urban wastewater, PHAU; Pesticides and heavy metals in agricultural drainage water and urban wastewater

Table 3: Analysis of physicochemical parameters of the various sources of wastewater throughout the duration of the experiment

			Sample time							
Treatment	Parameter	Unit	Zero	Day1	Day3	Day7	Day14	Day21	Day28	Mean ±SEM
	pH	-	8.42	8.3	8.21	8.27	8.84	8.56	8.46	8.437±0.081
	Temperature	°C	18.7	18.8	18	17.7	16.7	14.3	13.4	16.80±0.812
PMU	EC	μS	992	1008	1123	1380	3030	2640	2340	1788±324.5
	TDS	ppm	496	504	560	690	1520	1330	1180	897.1±163.8
	pH	-	8.43	8.39	8.34	8.31	8.83	8.72	8.60	8.517±0.076
	Temperature	°C	18.7	18.8	18	17.7	16.7	14.4	13.4	16.81±0.805
PMA	EC	μS	1653	1672	1728	2120	2990	3170	3040	2339±264.7
	TDS	ppm	826	836	864	1060	1500	1590	1520	1171±133.0
	pH	-	8.45	8.46	8.51	8.44	8.54	8.55	8.46	8.487±0.017
	Temperature	°C	18.6	18.6	17.9	17.5	16.7	14.2	13.3	16.69±0.803
HMU	EC	μS	1076	1080	1096	1128	1177	1296	1404	1180±47.36
	TDS	ppm	538	540	548	564	589	648	703	590.0±23.79
	pH	-	8.5	8.48	8.5	8.45	8.52	8.54	8.45	8.491±0.013
	Temperature	°C	18.5	18.6	17.9	17.5	16.6	14.2	13.3	16.66±0.798
HMA	EC	μS	1651	1663	1684	1728	1770	1887	1986	1767±47.57
	TDS	ppm	825	832	843	864	885	944	990	883.3±23.45
	pH	-	8.37	8.42	8.5	8.11	8.40	8.58	8.49	8.410±0.057
	Temperature	°C	18.5	18.6	17.9	17.5	16.6	14.3	13.3	16.67±0.791
PHMAU	EC	μS	1001	1004	1017	1144	1789	1980	1898	1405±173.5
	TDS	ppm	501	502	509	572	896	990	949	702.7±86.76
	pH	-	8.37	8.31	8.23	8.12	8.28	8.26	8.18	8.250±0.031
	Temperature	°C	18.5	18.6	17.9	17.5	16.6	14.3	13.3	16.67±0.791
MU	EC	μS	1031	1052	1125	1247	1536	1911	2210	1445±174.4
	TDS	ppm	515	526	563	623	769	956	1110	723.1±87.81
	pH	-	8.44	8.36	8.28	8.04	8.10	8.33	8.31	8.266±0.054
	Temperature	°C	18.6	18.6	18	17.6	16.6	14.3	13.4	16.73±0.792
MA	EC	μS	997	1008	1051	1092	1236	1578	1848	1259±124.6
	TDS	ppm	498	504	525	546	618	788	925	629.1±62.39
	pH	-	7.9	8.04	8.27	7.64	7.79	8	8.12	7.966±0.079
(PHAU)	Temperature	°C	18.5	18.5	17.9	17.5	16.6	14.1	13.2	16.61±0.81
(Control)	EC	μS	806	808	819	834	857	898	935	851.0±18.57
	TDS	ppm	403	404	410	417	429	449	469	425.9±9.433

PMU; Pesticides with microorganisms in urban wastewater, PMA; Pesticides with microorganisms in agricultural drainage water, HMU; Heavy metals with microorganisms in urban wastewater, HMA; Heavy metals with microorganisms in agricultural drainage water, PHMAU; Pesticides and heavy metals with microorganisms in agricultural drainage water and urban wastewater, MU: Microorganisms in urban wastewater, MA; Microorganisms in agricultural drainage water, PHAU; Pesticides and heavy metals in agricultural drainage water and urban wastewater, EC; electrical conductivity, TDS; total dissolved solids

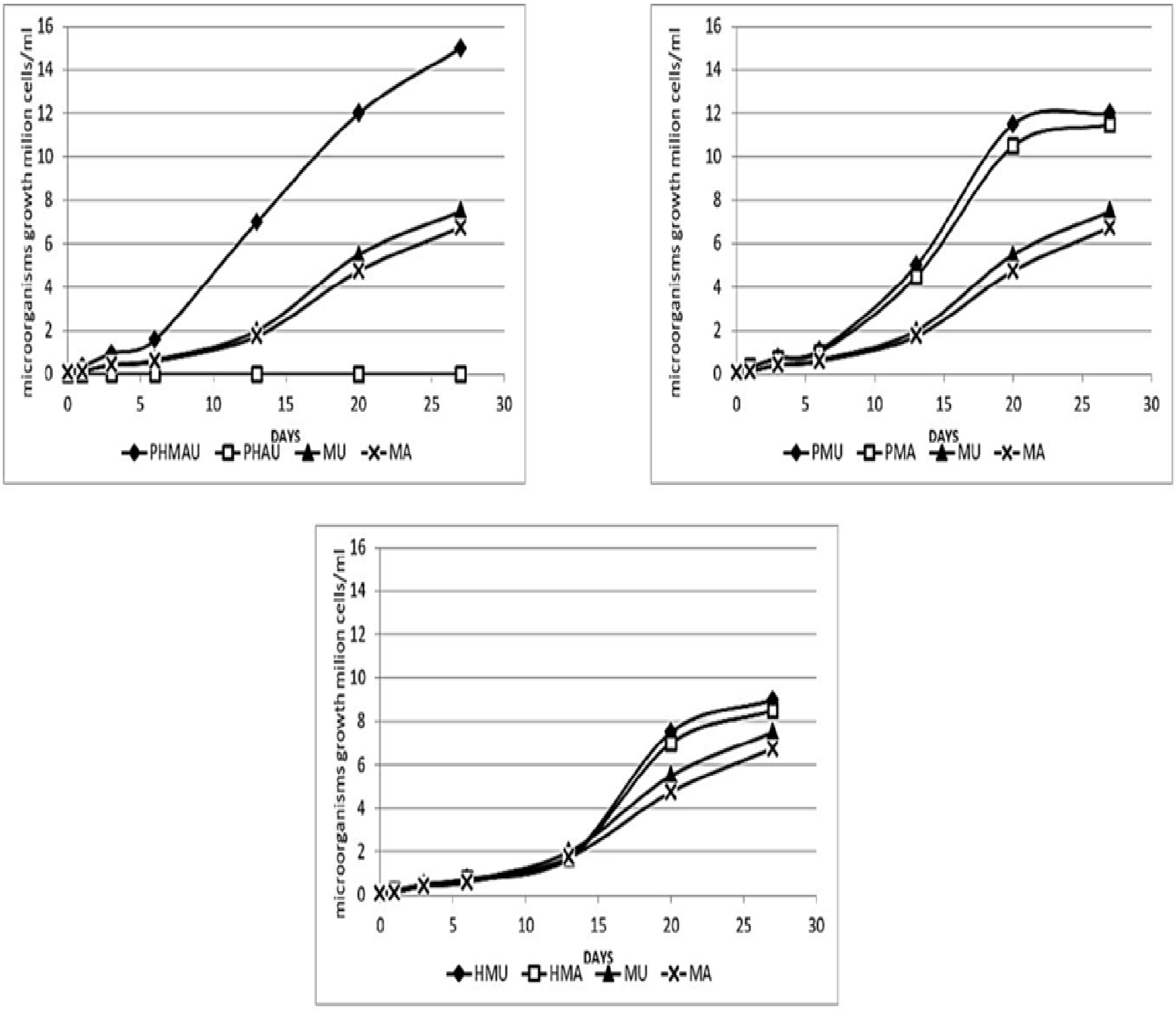

Figure 1: Growth curves of a consortium of *Chlorella vulgaris*, *Scenedesmus quadricuda* and cyanobacteria *Spirulina platensis* incubated in wastewater from different sources with heavy metals (5 ppm of cadmium, 10 ppm of nickel and 10 ppm of lead) and 25 ppm of the pesticide malathion

PMU; Pesticides with microorganisms in urban wastewater, PMA; Pesticides with microorganisms in agricultural drainage water, HMU; Heavy metals with microorganisms in urban wastewater, HMA; Heavy metals with microorganisms in agricultural drainage water, PHMAU; Pesticides and heavy metals with microorganisms in agricultural drainage water and urban wastewater, MU: Microorganisms in urban wastewater, MA; Microorganisms in agricultural drainage water, PHAU; Pesticides and heavy metals in agricultural drainage water and urban wastewater

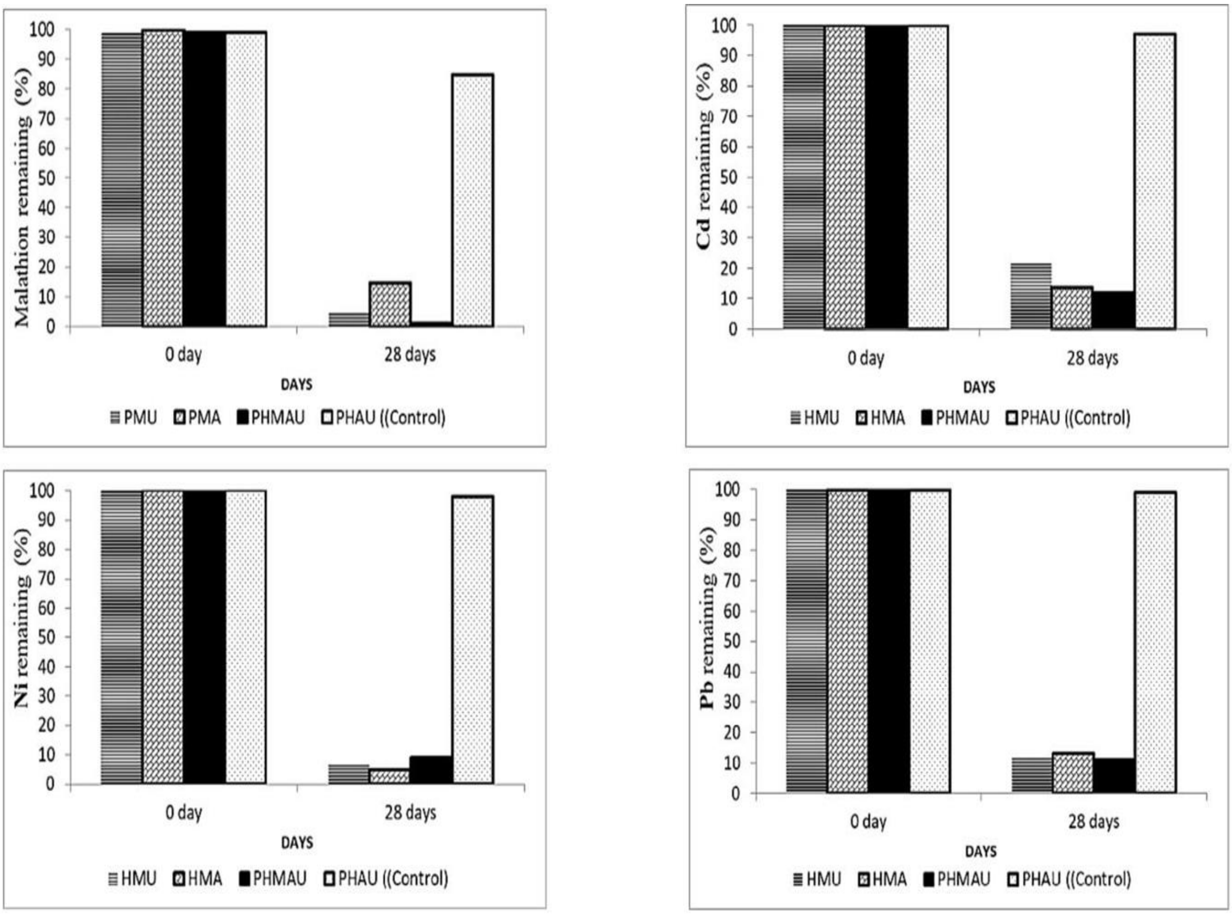

Figure 2: Residual malathion, cadmium, nickel and lead after treatment in wastewater from different sources containing a microalgal consortium (*Chlorella vulgaris*, *Scenedesmus quadricuda* and *Spirulina platensis*) for 28 days. Malathion was added at a concentration of 25 ppm in addition to 5 ppm of cadmium, 10 ppm of nickel and 10 ppm of lead

PMU; Pesticides with microorganisms in urban wastewater, PMA; Pesticides with microorganisms in agricultural drainage water, HMU; Heavy metals with microorganisms in urban wastewater, HMA; Heavy metals with microorganisms in agricultural drainage water, PHMAU; Pesticides and heavy metals with microorganisms in agricultural drainage water and urban wastewater, MU: Microorganisms in urban wastewater, MA; Microorganisms in agricultural drainage water, PHAU; Pesticides and heavy metals in agricultural drainage water and urban wastewater

Conclusion

A consortium of *Chlorella vulgaris, Scenedesmus quadricuda* and *Spirulina platensis* can be considered an effective method for remediation of the pesticide malathion and heavy metals in polluted wastewater. Moreover, heavy metals and pesticides can provide a source of energy for microorganisms thereby increasing the overall potential for degradation.

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Conflict of interest

The authors declare that they have no conflict interests.

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