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Role of Environmental Biotechnology in Remediation of Heavy Metals by Using Fungal-Microalgal Strains

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Abstract: Bioremediation is a branch of biotechnology that employs the use of living organisms, like microalgae and fungi, in the removal of contaminants, pollutants, and toxins from soil, water, and other environments. The experiment was designed to evaluate the efficiency of microorganisms to remove heavy metals by using, two fungi (*Aspegillus niger* and *Candida albicans*) with two microalgae (*Scenedesmus quadricauda* and *Tetradesmus nygaardi*), in removing heavy metals from liquid media during study period (20 days). For this study, cadmium and lead were selected by different concentrations (5, 15, 35, and 50ppm) of such heavy metals. The results indicate that fungi and microalgae effectively removed a significant amount of heavy metals. With respect to Pb and Cd, the maximum removal of lead for all concentrations (5-50ppm) were, (94, 90, 86.28 and 81.6%) respectively, and maximum cadmium removal were (88, 86.66, 84.57 and 79%) recorded by consortium culture of S*cenedesmus quadricauda* and *Tetradesmus nygaardi* on day 20th of the experiment. Statistically there were significant difference ($p \leq 0.05$) between control and all treatments for both tested heavy metals.

Keywords: Bioremediation, Environmental biotechnology, Fungi, Heavy metals, Microalgae.

Introduction

Heavy metals are generally present in form of hydroxides, sulfates, sulfides, phosphates, carbonates, silicates and organic compounds (Singh *et al.*, 2011). Even though they are present in their elemental and metallic form, but they are mobilized by anthropogenic activity or natural phenomenon (Salem *et al.*, 2000). When toxic heavy metal concentrations are high, they cannot be biodegraded, and their accumulation has been linked to significant disorders and diseases (Jackson *et al.*, 2001). Metal toxicity was related to a various human disease such as liver and kidney damage, cancer, skin sores, mental retardation leading to disability, and birth defects. Heavy metals (HMs) can be hazardous to biological systems when they are present in excess, and because they are non-biodegradable, they can often accumulate (Fairbrother *et al.*,

2007). HM contamination is an obtrusive and persistent issue that poses a hazard to environmental safety and human health (Mishra *et al.*, 2019). Anthropogenic activities play a important role in the global dispersion of HM in soil, water, and the atmosphere (Vareda *et al.*, 2019).

Heavy metals are present in considerable amounts in wastewater, particularly those from industrial sources, which can pass though the food chain in to human and animals. Microorganisms (Fungi and algae) recently have been described as bio-sorbents for removing heavy metals from wastewater at a low cost and in an environmentally favorable manner (Bai & Abraham, 2003; Elizabeth & Anuradha, 2000; Veglio & Beolchini, 1997). In environments contaminated by heavy metal resistant microorganisms could exist. The effectiveness and resistance of microbes to remove heavy metals varies significantly (Igiri *et al.*, 2018). Fungi absorb metal ions and chelate them on the cell surface which allowing them to tolerate heavy metal compounds (Anahid *et al.*, 2011; Gadd & White, 1993). As the result of tolerance mechanism, some algae are highly susceptible to accumulating heavy metals, and many algae produce phytochelatins and metallothionein, which can attach to heavy metals and transport them into vacuoles (Chekroun & Baghour, 2013).

Heavy metal detoxifications are often accomplished through biotransformation and compartmentalization into inactive form of metals (Nies, 1999; Nies *et al.*, 1989). Metals must be managed safely and sustainably in order to repair the ecosystem. Biosorption, bioaccumulation, bioleaching, and bio-immobilization are all ways for decontaminating the environment with heavy metals that are used in fungal-mediated decontamination (Goutam *et al.*, 2021). The importance of microalgae and fungi in heavy metal remediation (Cd and Pb) is highlighted in this research.

Materials & Methods

Experimental set-up and microbial cultivation

Fungi, *A. niger* (ATCC 16404), *and C. albicans* (ATCC 10231), and microalgae, *T. nygaardi* (MZ801740) and *S. quadricauda* (MZ801741), were used as test organisms in order to remove heavy metals (HMs). To study the role of fungi and microalgae in remediation of HMs (Pb and Cd), a pure mixed cultures of *A. niger* with *C. albicans* (F1+F2), *S. quadricauda* with *A. niger* (A1+F1), *S. quadricauda* with *Candida sp*.(A1+F2) and *S. quadricauda* with *T. nygardi* (A1+A2) was used.

The fresh culture of selected fungi in this study have been sub cultured individually onto slants containing solidified Sabouraud's Dextrose Agar (SDA) and incubated for seven days at 25± 1 °C for *A. niger*. Spores of *A. niger* was suspended from each culture slant in 10 ml distilled sterile water. Spore suspensions have been diluted with double sterile water to get similar cell counts of 3×10^4 CFU. ml^{-1}. Also, the fresh culture of *C. albicans* have been sub cultured individually onto slants containing solidified SDA and incubated for 24 hrs. at 37°± 1°C, yeast cells have been suspended in 10 ml sterile distilled water. Yeast cells were diluted with double sterile water to acquire similar cell counts of 2×10^4 CFU.ml^{-1}. The cells of *S. quadricauda* 6.8×10^4 CFU. ml^{-1}. and *T. nygaardi* 7.2×10^4 CFU. ml^{-1}. were cultured in BG11 broth medium in distilled water with

light-emitting diode (LED) lamps at ambient temperature, constant pH (7.5 ± 0.3), constant temperature of 25 ± 1°C, and at constant light intensity (Razzak *et al.*, 2013).

Heavy metal analysis

$PbCl_2$ and $CdCl_2$ were used to prepare (1000 ppm) stock solution of Pb and Cd in deionized water. In 500 ml conical flasks of sterilized Saboraud's dextrose broth (SDB) medium of fungi and BG11 medium of algae, stock solution of heavy metals was added. On SDB medium that contained separately (5, 15, 35, and 50 ppm) for both heavy metals, all microbial strains (fungal and microalgal) isolates were streaked.

These flasks were put on a shaker at 150 rpm at 28°C and inoculated with 50ml of freshly prepared spore suspensions of both isolates (Anwer & Merkhan, 2013; Sadettin & Dönmez, 2006 2007). Heavy metals (Cd and Pb), were measured before and after treatment using an atomic absorption spectrophotometer (AAS Perkins Elmer USA 1100D) (APHA, 2012). The percentage of removal of metal concentrations was calculated as follows (Al Ahmed, 2014).

$$Removal\ \% = \left[\frac{Initial\ conc. - Final\ conc.}{Initial\ conc.}\right] * 100$$

For estimation of chlorophyll *a* 10 ml of culture was taken from each flask of sample and centrifuged at 3000 rpm for 5 min and the supernatant was discarded, the cell suspended with 5 ml of diethyl ether. Absorbance value of supernatant was measured using UV-spectrometer at 660 nm and 643nm (Becker, 1994; Castañeda *et al.*, 2018).

Statistical analysis

Statistical analysis was conducted for the data using a software program (SPSS version 26) and Excel spreadsheets. Two-way ANOVA (Analysis of variance). A post hoc test (multi comparisons Duncan test) was applied to determine significant differences at 5%. All data are expressed as Mean ± SE (Marcello pagano 2018).

Results

The mean value of concentrations heavy metals Pb, and Cd were (5, 15, 35, and 50ppm) by using a pure mixed culture of *A. niger* with *C.albicans* (F1+F2), *S.quadricauda* with *A. niger* (A1+F1), *S.quadricauda* with *C. albicans* (A1+F2) and *S.quadricauda* with *T. nygaardi* (A1+A2) (Table 1a &b).

On day 20th of experiment the concentrations of Pb reduced to (0.56, 1.95, 5.9 and 10 ppm) by mix culture of (F1+F2)(Fig. 1), whereas (0.52, 1.89, 5.2 and 9.5 ppm) by (A1+F1) (Fig. 2) and decreased to (0.56, 2 5.7, and 10.4 ppm) by (A1+F2) respectively(Fig. 3), and (0.3, 1.5, 4.8 and 9.2 ppm) by (A1+A2) respectively (Fig. 4), that comprised to (88.8, 87, 83.14 and 80%), (89.6, 87.4, 85.1 and 81%), (87, 86.66, 83.71 and 79.2%) and (94, 90, 86.28 and 81.6%) respectively (Fig. 9). The concentrations of Cd decreased to (0.86, 2.64, 6.4 and 11.2 ppm) by mix culture of (F1+F2) respectively (Fig. 5), while (0.71, 2.3, 5.89 and 10.9 ppm) by (A1+F1) respectively (Fig.6), (0.8, 2.5, 6 and 11.1 ppm) by(A1+F2) respectively (Fig. 7), and (0.6, 2, 5.4 and 10.5 ppm) by (A1+A2) respectively (Fig. 8), that comprised to (82.8, 82.4, 81.71 and 77.6%), and (88, 86.66, 84.57 and 79%) respectively (Fig. 10).

Heavy metals can bind in the microorganism, used functional groups on their cell walls. In general, the fungal group has the largest surface area and consequently the most

cell wall material available with functional groups to bind a greater number of metals (Dhankhar & Hooda, 2011; Igiri *et al.*, 2018). Fungi have a significant role in the reduction and removal of heavy metal concentrations in the soil and aquatic environment because they have a number of processes that make them effective in the process of reducing heavy metal concentrations, such as external fungal adsorption on the walls or the formation of heavy complexes that are sedated and store metals within their cells (Baldrian, 2003; Guibal *et al.*, 1992). Pande *et al.* (2022) showed, the capability of fungi to effectively absorb mercury, cadmium, and lead in an aquatic environment was recognized by the fungal as well as variable fungus that bioaccumulates heavy metals. Fungi contains several components, including polysaccharides and proteins. The latter is composed of phosphates and amino acids, and also secondary groups such as carboxyl and hydroxyl (Sharma, Giri*, et al.*, 2022; Sharma, Naruka*, et al.*, 2022). All of these chemicals have an effect on heavy element molecule binding (Sharma *et al.*, 2021; Veglio & Beolchini, 1997).

The potential of *Coprinopsis atramentaria* has been researched to bioaccumulate 94.7% of Pb at 800 mg/l Pb and 76.6% of Cd at 1 mg/l Cd. It has therefore been demonstrated to be an effective heavy metal ion accumulator for mycoremediation (Lakkireddy & Kües, 2017). According to Park *et al.* (2005), dead biomass of fungi from *Aspergillus niger*, *Saccharomyces cerevisiae*, and *Rhizopus oryzae*, *Pencillium chrysogenum, Candida sphaerica* produces biosurfactants can change dangerous Cr (VI) into less toxic or harmless Cr, *with* removal efficiencies of lead with the percentage of 79%.

Table (1a): Bioremediation of heavy metals by using different microbial stains.

Metal concentrations	*A. niger*× *C. albicans* (F1+F2)		*S. quadricauda* × *A. niger* (A1+F1)	
	Pb	Cd	Pb	Cd
5ppm	3.023±0.13^{d}	3.295±0.148^{d}	2.973±0.154^{d}	3.235±0.132^{d}
15ppm	9.975±0.13^{c}	11.075±0.148^{c}	9.652±0.154^{c}	10.887±0.132^{c}
35ppm	22.579±0.135^{b}	22.854±0.155^{b}	21.183±0.161^{b}	22.473±0.137^{b}
50ppm	29.309±0.127^{a}	33.54±0.145^{a}	28.254±0.151^{a}	33.367±0.129^{a}

Table (1b): Bioremediation of heavy metals by using different microbial stains.

Metal concentrations	*S. quadricauda* × *C. albicans* (A1+F2)		*S. quadricauda*×*T. nygaardi* (A1+A2)	
	Pb	Cd	Pb	Cd
5ppm	3.025±0.147^{d}	3.248±0.153^{d}	3.025±0.147^{d}	3.248±0.153^{d}
15ppm	9.985±0.147^{c}	10.75±0.153^{c}	9.985±0.147^{c}	10.75±0.153^{c}
35ppm	22.383±0.153^{b}	22.252±0.159^{b}	22.383±0.153^{b}	22.252±0.159^{b}
50ppm	29.105±0.143^{a}	33.121±0.15^{a}	29.105±0.143^{a}	33.121±0.15^{a}

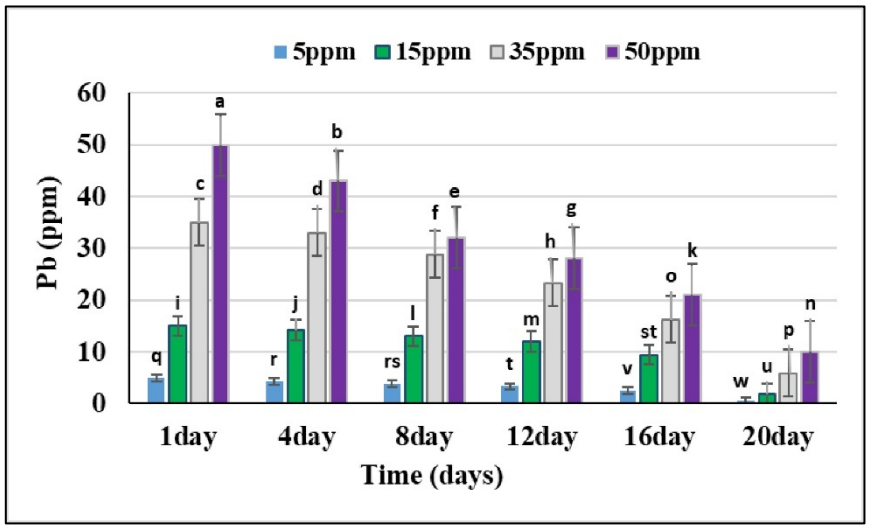

Fig. (1): Effect of *A. niger* with *C. albicans* on Pb concentrations.

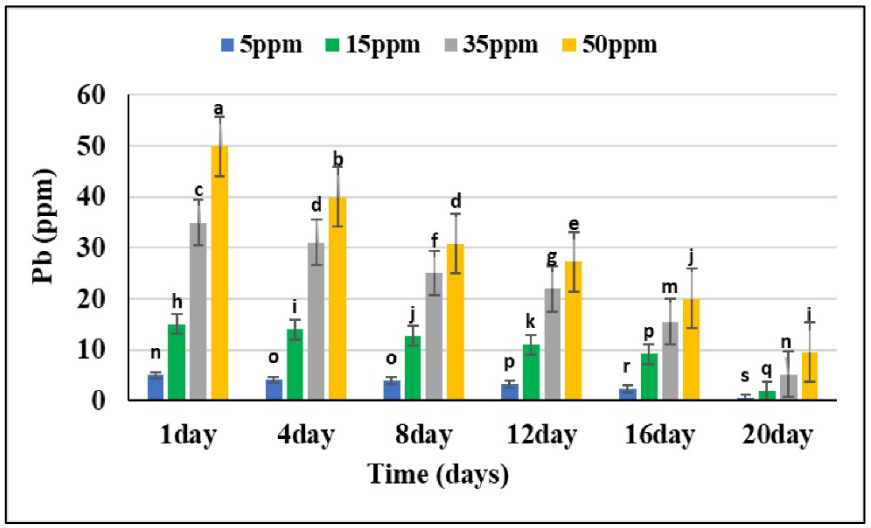

Fig. (2): Effect of *S. quadricauda* with *A. niger* on Pb concentrations.

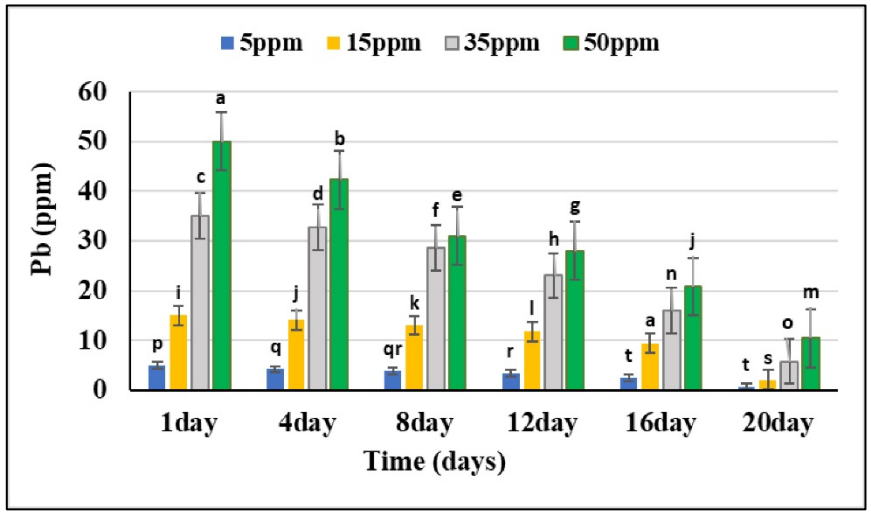

Fig. (3): Effect of *S. quadricauda* with *C. albicans* on Pb concentrations.

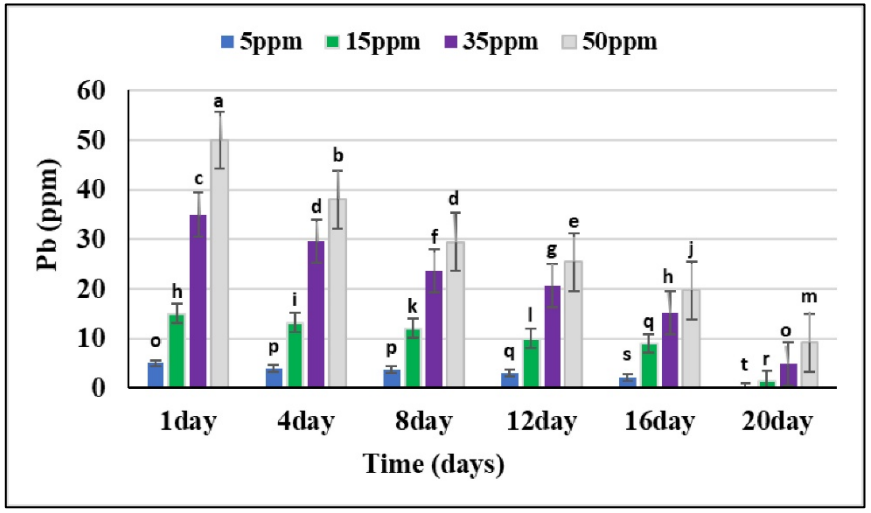

Fig. (4): Effect of *S. quadricauda* with *T. nygaardi* on Pb concentrations.

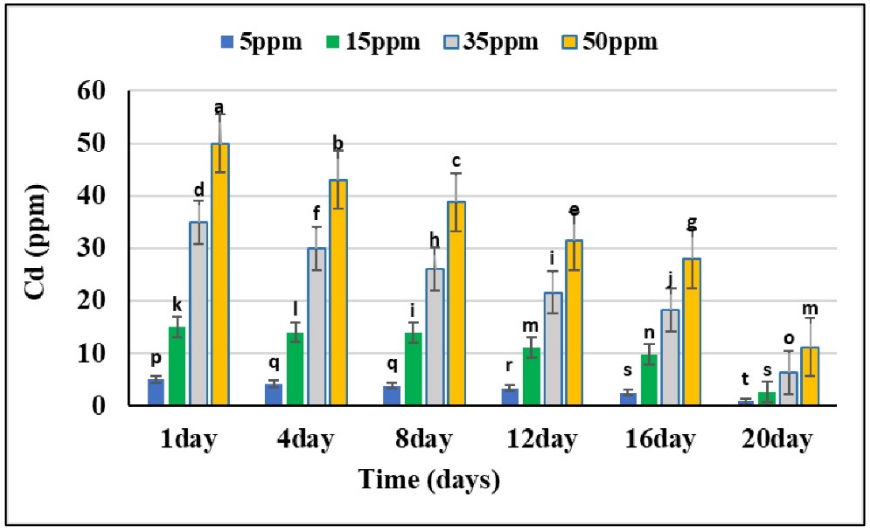

Fig. (5): Effect of *A. niger* and *C. albicans* on Cd concentrations.

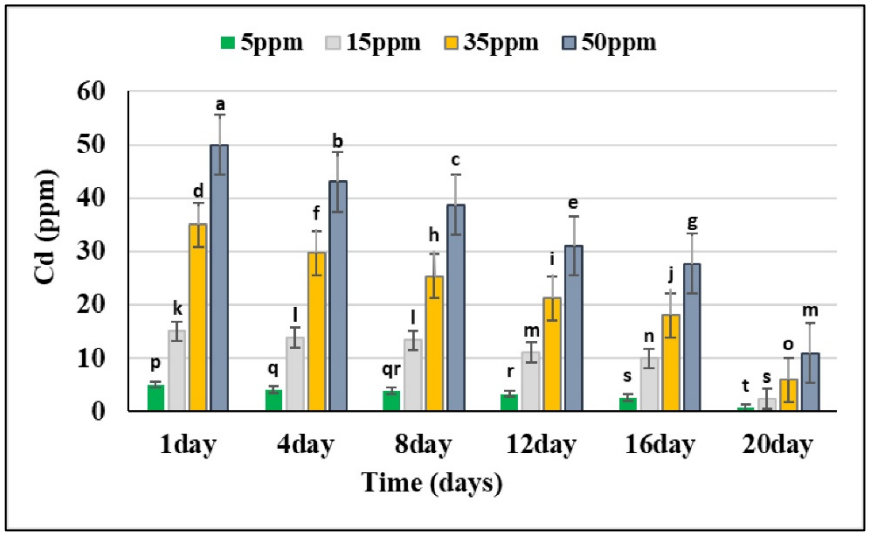

Fig. (6): Effect of *S. quadricauda* with *A. niger* on Cd concentrations.

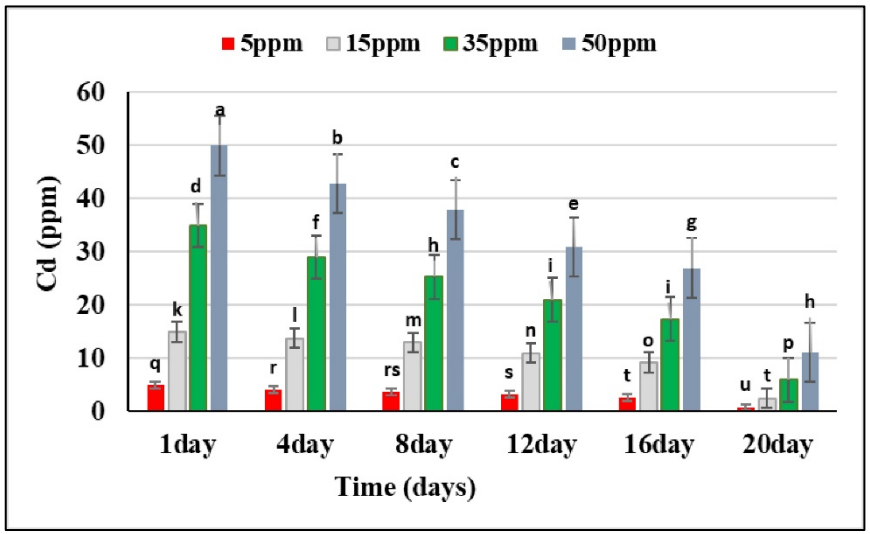

Fig. (7): Effect of *S. quadricauda* with *C. albicans* on Cd concentrations.

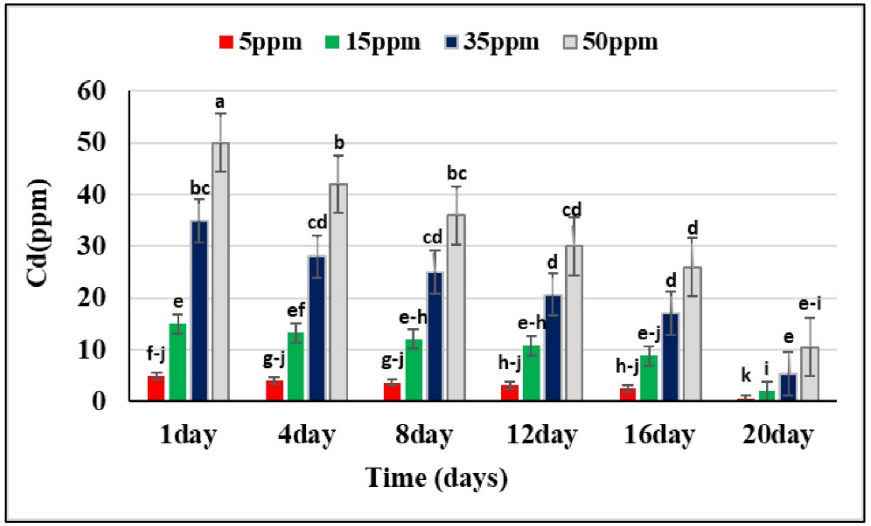

Fig. (8): Effect of *S. quadricauda* with *T. nygaardi* on Cd concentrations.

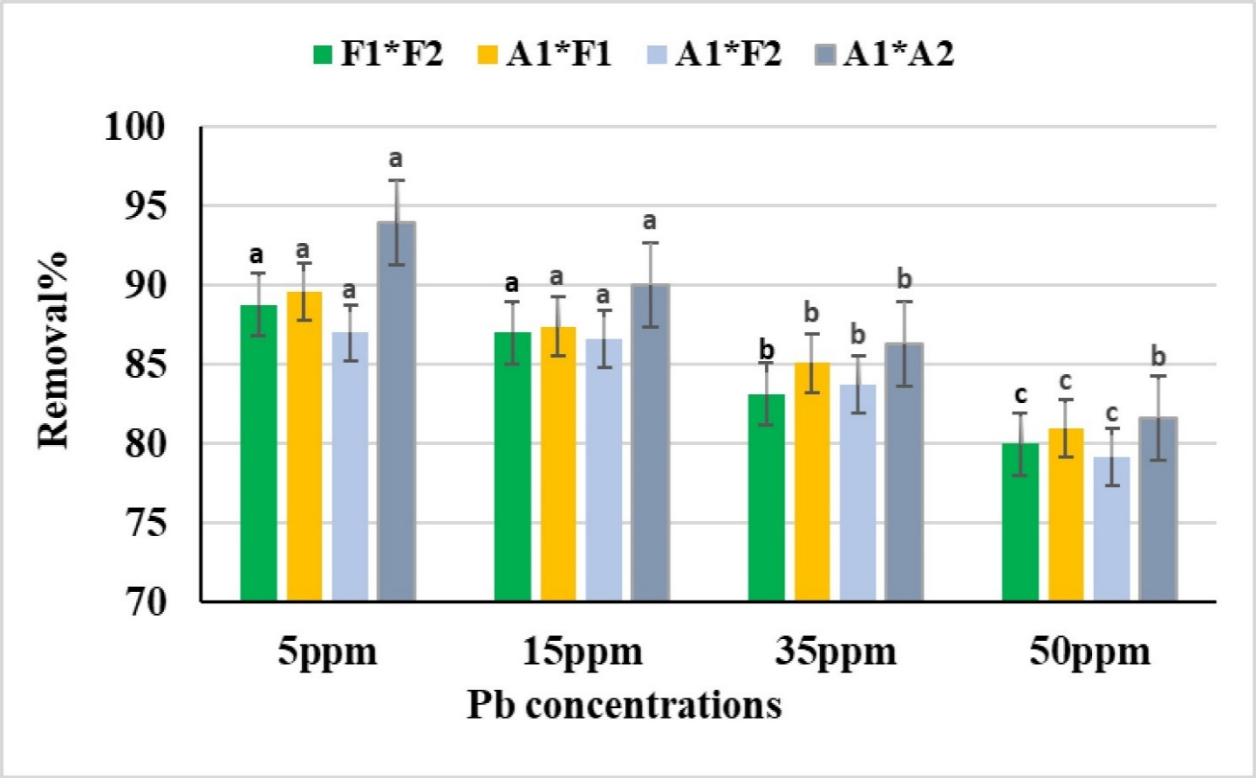

Fig. (9): Removal percent of lead when treated with different microbial cultures.

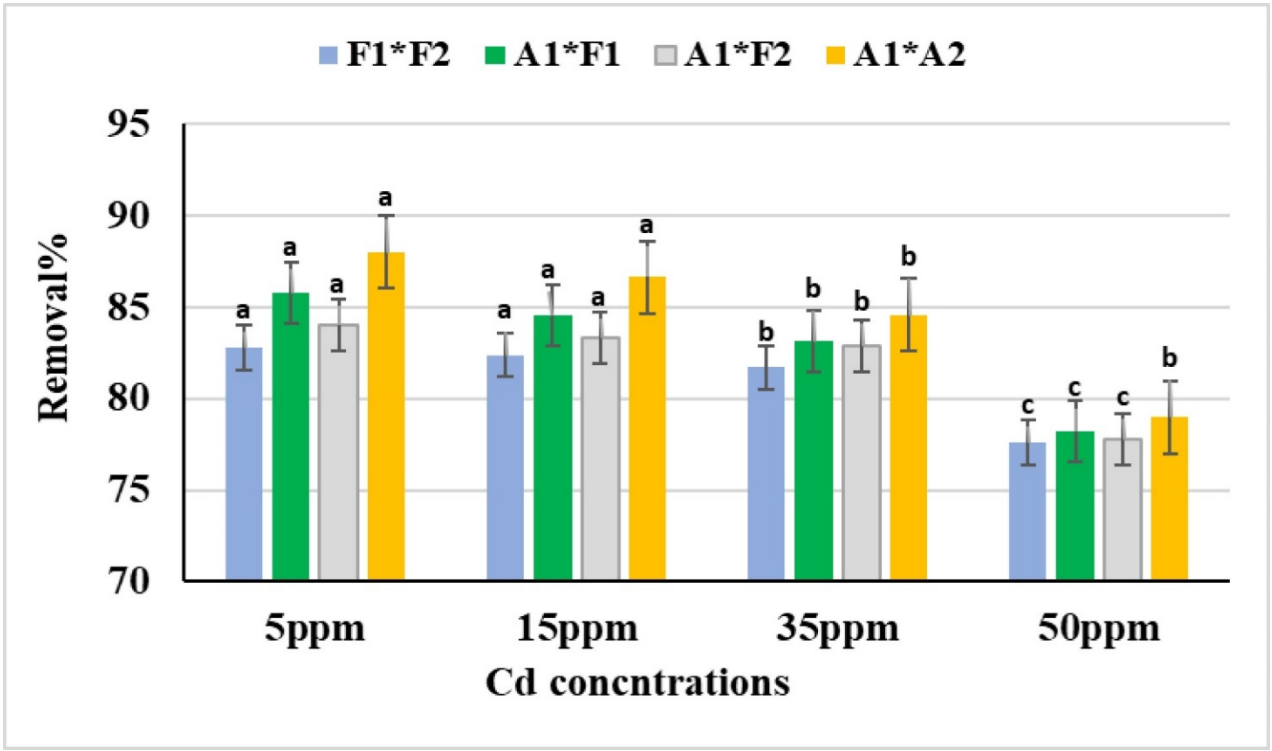

Fig. (10): Removal percent of cadmium when treated with different microbial cultures

Metal uptake by living microalgae occurs in two stages: the first is "adsorption" onto the cell surface, which occurs quickly and is largely independent of cell metabolism. The other is cell metabolism – "absorption" or "intracellular uptake." (Dwivedi, 2012). The algae have a number of characteristics which make them an ideal candidate for heavy metal remediation due to their high heavy metal tolerance, capability to grow both heterotrophically and autotrophically, large surface area to volume ratios, phytochelatin expression, phototaxy and genetic manipulation potential (Cai *et al.*, 1995). The capacity of algae to uptake and remove heavy metals is largely determined by the initial concentration of metals in the solution. Metal sorption (metal sorbed per unit biomass) rises with increasing concentration of metals in the solution as well as become saturated after a certain metal concentration in the solution (Aloysius *et al.*, 1999; da Costa & Leite, 1991; Mehta & Gaur, 2001).

The metal removal efficiency, in contrast to metal sorption, decreases as the metal concentration in the solution increases (Mehta & Gaur, 2001). This indicated that the increase of initial concentrations of heavy metal ions contributed to enhance the driving force at the solid–liquid interface to increase the adsorption capacity until the adsorption sites were saturated (Adebowale *et al.*, 2006).

Microalgae are aquatic organism with molecular mechanisms that allow them to discriminate between HMs that are crucial for growth and those that are not (Perales-Vela *et al.*, 2006). According to the present study the lower concentration (5ppm) had the highest reduction percent by all microbial cultures. While a pure mixed culture of *S. quadricauda* with *T. nygaardi* showed the best removal of heavy metals (Pb and Cd) for all concentrations (Figure 9-10).

Chlorophyll-*a* is ubiquitous in nature, plays a fundamental light harvesting role in photosynthesis, and is essential to the survival of both animal and plant kingdoms (Humphrey, 2004). During the experiment, the amount of chlorophyll-*a* content (micro-algal biomass) of *S. quadricauda* and *T. nygaardi* was increased. For all concentrations of heavy metals Pb and Cd (5, 15, 35, and 50), the starting value of chlorophyll-*a* was (0.914, 0.914 and 1.879 mg. l^{-1}) for mixed pure cultures of treatment (A1+F1), (A1+F2) and (A1+A2) respectively. Then, on the 20th day, chlorophyll-*a* content reached their maximum value for treatment (A1+F1), were (5.63, 6.02, 6.72 and 7.51 mg. l^{-1}) for Pb and (1.56, 1.63, 1.69 and 1.81 mg. l^{-1}) for Cd respectively (Table 2), whereas for treatment(A1+F2) were (5.02, 5.48, 5.72 and 5.83 mg. l^{-1}) for Pb, and (1.47, 1.53, 1.58 and 1.64 mg. l^{-1}) for Cd respectively (Table 3), maximum value of chlorophyll *a* on 20th day for treatment (A1+A2) (4.77, 5.68, 5.47 and 5.77 mg. l^{-1}) for Pb and (4.81, 4.92, 5.1 and 5.32 mg. l^{-1}) for Cd respectively (Table 4).

The maximum chlorophyll-*a* concentration was reported at the end of the treatment period, which could be due to algae's high metal consumption for metabolism and growth (Samorì *et al.*, 2013). Study revealed that chlorophyll *a* content increased with increased the concentrations of heavy metals especially Pb during the experimental period. Also, the present study showed that chlorophyll content was higher in pure mixed culture of (A1+A2) than other mixed microbial cultures in all concentration for (Pb and Cd) during 20 days of treatment. When the rate of nutrients increases, the algal cell will increase, lead to increase in algal biomass.

Current study has demonstrated that increase and decrease algal biomass is due to the quantity of nutrients in the environment and then related with the light. The decline in biomass in the environment can be attributed to the low nutritional environment because the light intensity in the environment was constant throughout the experiment (Samorì *et al.*, 2013). Some of metals (Cd and Pb) may serve as micronutrients for microalgae growth if they are present at very small concentrations (Renuka *et al.*, 2014).

Table (2): Effects of different heavy metal concentrations on chlorophyll *a* content of mixed culture *S. quadricauda*× *A. niger*.

Time	Lead Concentrations				Cadmium Concentrations			
	5ppm	15ppm	35ppm	50ppm	5ppm	15ppm	35ppm	50ppm
1day	0.914±0.03^{n}	0.914±0.03^{n}	0.914±0.03^{n}	0.914±0.03^{n}	0.914±0.05^{k}	0.914±0.05^{k}	0.9144±0.05^{k}	0.914±0.05^{k}
4day	1.125±0.03^{m}	1.242±0.03^{m}	1.473±0.03^{m}	1.601±0.03^{m}	1.203±0.05^{j}	1.221±0.05^{ij}	1.267±0.05^{ij}	1.344±0.05^{g-j}
8day	1.699±0.03^{l}	1.791±0.03^{k}	1.9082±0.03^{j}	2.077±0.03^{i}	1.293±0.05^{hij}	1.280±0.05^{ij}	1.304±0.05^{g-j}	1.457±0.05^{d-h}
12day	2.037±0.03^{i}	2.167±0.03^{h}	2.253±0.03^{e}	2.524±0.03^{e}	1.373±0.05^{f-j}	1.397±0.05^{e-i}	1.475±0.05^{d-g}	1.598±0.05^{bcd}
16day	2.456±0.03^{g}	2.9051±0.03^{f}	2.6003±0.03^{c}	2.972±0.03^{b}	1.467±0.05^{d-g}	1.541±0.05^{c-f}	1.568±0.05^{b-e}	1.728±0.05^{ab}
20day	2.837±0.03^{d}	2.9027±0.03^{c}	3.020±0.03^{b}	3.153±0.03^{a}	1.563±0.05^{b-e}	1.630±0.05^{bcd}	1.692±0.05^{abc}	1.813±0.05^{a}

Table (3): Effects of different heavy metal concentrations on chlorophyll *a* content of mixed culture *S. quadricauda*× *C. albicans*.

Time	Lead Concentrations				Cadmium Concentrations			
	5ppm	15ppm	35ppm	50ppm	5ppm	15ppm	35ppm	50ppm
1day	0.914±0.06^{n}	0.914±0.06^{n}	0.9144±0.06^{n}	0.914±0.06^{n}	0.914±0.04^{j}	0.914±0.04^{j}	0.914±0.04^{j}	0.914±0.04^{j}
4day	1.030±0.06^{m}	1.095±0.06l^{m}	1.4636±0.06^{m}	1.580±0.06^{l}	1.072±0.04^{i}	1.099±0.04^{i}	1.143±0.04^{hi}	1.201±0.04^{ghi}
8day	1.586±0.06^{k}	1.699±0.06^{k}	1.8633±0.06^{j}	2.004±0.06^{j}	1.140±0.04^{hi}	1.178±0.04^{ghi}	1.271±0.04^{fgh}	1.306±0.04^{efg}
12day	1.885±0.06^{i}	1.948±0.06^{h}	2.0505±0.06^{e}	2.297±0.06^{g}	1.387±0.04^{def}	1.411±0.04^{cde}	1.452±0.04^{bcd}	1.538±0.04^{abc}
16day	1.980±0.06^{f}	2.049±0.06^{f}	2.232±0.06 ^{c}	2.822±0.06^{d}	1.451±0.04^{cde}	1.465±0.04^{cde}	1.507±0.04^{bcd}	1.575±0.04^{abc}
20day	2.029±0.06^{c}	2.484±0.06^{b}	2.7258±0.06^{b}	3.039±0.06^{a}	1.478±0.04^{bcd}	1.533±0.04^{abc}	1.584±0.04^{ab}	1.642±0.04^{a}

Table (4): Effects of different heavy metal concentrations on chlorophyll *a* content of mixed culture *S. quadricauda*× *T. nygaardi*.

Time	Lead Concentrations				Cadmium Concentrations			
	5ppm	15ppm	35ppm	50ppm	5ppm	15ppm	35ppm	50ppm
1day	1.879±0.06^{m}	1.879±0.06^{m}	1.879±0.06^{m}	1.879±0.06^{m}	1.879±0.06^{o}	1.879±0.06^{o}	1.879±0.06^{o}	1.879±0.06^{o}
4day	2.238±0.06^{l}	2.296±0.06k^{l}	2.373±0.06^{kl}	2.432±0.06^{k}	1.935±0.06^{no}	2.027±0.06^{no}	2.080±0.06^{mn}	2.248±0.06^{lm}
8day	2.821±0.06^{j}	3.184±0.06^{i}	3.292±0.06^{hi}	3.448±0.06^{h}	2.334±0.06^{k}	2.465±0.06^{jk}	2.627±0.06^{ij}	2.722±0.06^{i}
12day	3.125±0.06^{i}	3.825±0.06^{fg}	4.051±0.06^{de}	4.214±0.06^{d}	3.715±0.06^{h}	3.825±0.06^{gh}	3.985±0.06^{fg}	4.080±0.06^{ef}
16day	3.731±0.06^{g}	3.945±0.06^{ef}	4.623±0.06^{c}	4.806±0.06^{c}	3.955±0.06f^{g}	4.102±0.06^{ef}	4.236±0.06^{e}	4.469±0.06^{d}
20day	4.771±0.06^{c}	5.687±0.06^{a}	5.478±0.06^{b}	5.779±0.06^{a}	4.819±0.06^{c}	4.920±0.06^{c}	5.100±0.06^{b}	5.324±0.06^{a}

Conclusion

Mycoremediation and phycoremediation, which use algae and fungi to remove contaminants from the environment, they are cost effective and environmentally friendly. According to the obtained results:

1-The result showed that within 20th days of culture, the higher the efficiency of Pb and Cd reduction by mix culture of *Scenedesmus quadricauda* with *Tetradesmus nyaardi*, for all the concentrations (5-50 ppm).

2-The highest removal rate was in a concentration of (5 ppm) and the removal rate decreased with the increase in the concentration of the two heavy metals.

3-Decreases in the concentrations of both heavy metals (Pb and Cd) coincided with an increase in chlorophyll-*a* content with the highest biomass on the 20th day of the experiment 5.77 and 5.32 mg. l^{-1} respectively.

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Contributions of authors

M.Q.Q.: Laboratory analysis of the samples, Statistical analysis and writing the manuscript.
Y.A.S.: Prepare the proposal and edit the final manuscript.

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Conflict of interest

As for the requirements of the publishing policy, there is no potential conflict of interest for the authors.

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دور التكنولوجيا الحيوية البيئية في معالجة المعادن الثقيلة باستخدام سلالات الفطريات و الطحالب الدقيقة

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المستخلص

المعالجة الحيوية هي فرع من فروع التكنولوجيا الحيوية التي تستخدم الكائنات الحية ، مثل الطحالب الدقيقة والفطريات ، في إزالة الملوثات والملوثات والسموم من التربة والمياه والبيئات الأخرى .صممت التجربة لتقييم كفاءة الكائنات الحية الدقيقة لإزالة المعادن الثقيلة ، بواسطة فطران (*Aspergillus niger* و *Candida albicans*) ، واثنان من الطحالب الدقيقة *Scenedesmus quadricaua* and *Tetradesmus nygaardi* من الوسط السائل خلال فترة الدراسة (20 يوم). في هذه الدراسة تم اختيار الكادميوم والرصاص بتركيزات مختلفة (5 ، 15 ، 35 ، 50 ملغم / لتر) لكل معدن ثقيل. أشارت النتائج إلى إزالة كمية كبيرة من المعادن الثقيلة من طريق الفطريات والطحالب الدقيقة. فيما يتعلق بالرصاص والكادميوم ، كان الحد الأقصى لإزالة الرصاص (94 ، 90 ، 86.28 ، 81.6٪) على التوالي ، بينما كان الحد الأقصى لإزالة الكادميوم (88 ، 86.66 ، 84.57 ، 79٪) على التوالي في معاملة المزرعة المختلطة من *Scenedesmus quadricauda* مع *Tetradesmus nygaardi* في اليوم العشرين من التجربة. احصائيا توجد فروق معنوية ($p\leq0.05$) بين المقارنة وأعلى معاملة (50 ملغم / لتر) لكل من المعادن الثقيلة المختبرة.

الكلمات المفتاحية :المعالجة الحيوية ، التكنولوجيا الحيوية البيئية ، الفطريات ، المعادن الثقيلة ، الطحالب الدقيقة