

Bioremediation of pesticide Glyphosate (Ground-UP SL) and Remove Cd, Cr elements from polluted aquatic media by using *Chlorococcum humicola*, *Chlorella vulgaris* algae

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ABSTRACT

Within this study, the effectuality of green algae cells represented by the *Chlorococcum humicola* and *Chlorella vulgaris* species have been tested for the remediation of Glyphosate pesticide and the removal of elemental Cd and Cr from a polluted aquatic medium by using different components of pesticides and elements (25, 50, 75, 100) mg/ L (2.5, 5, 7.5, 10, 12.5, 15, 20, 25) (1, 2, 3, 4, 5) mg/L. The exposure time that is applied in the study is 3, 6, 9 and 12 days under laboratory conditions of 25 °C with lighting intensity of 3000 Lux and 16 hours lighting: 8 darkness in the middle of the static and dynamic (shaker) medium at a speed of rotation 150 rpm.

The end results showed that the higher capacity of cells *C. humicola* algae was able to destroy the pesticide at a rate of 78.5% dynamic medium and 61.1% for the static medium compared to the *C. vulgaris* algae. This recorded the highest rate of the destroyed comprising of 17.7% for the dynamic medium and 15.3% for static medium. As for the results of removing heavy elements, we found that the efficiency of the first algae of Cd element recording 99.5% in the dynamic medium and 98.80% for the static medium at a concentration of 12.5 mg/L. While the Cr element was recorded with 78.7% of the dynamic medium and 76.6% for the static medium at a concentration of 1 mg/L. In retrospect, the removal results for the second algae were lower. The highest removal rate for the Cd element appeared at a concentration of 2.5 mg/L which was 86.7% for the dynamic medium and 75.7% for the static medium, whereby the Cr element was 72.7% and 71.07% for the concentration of 1 mg/L in the dynamic and static medium respectively.

The conclusion of this study is that *C. humicola* algae can be mostly characterized by a higher capacity in order to destroy the Glyphosate pesticide as well as the removal of high concentrations of Cd compared to Cr, and with high efficiency in the dynamic medium more than the static medium in the most treated.

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1. INTRODUCTION

Environmental science applications emphasize on the importance of using microorganisms from algae, fungi or bacteria in the biotreatment of heavy metals pollution or pesticide residues in aquatic media or soils under various environmental and climatic conditions. This is in due cause of the great potential of these

microorganisms to adapt for these variables and endurance to broad ranges. In addition to their appearance, many of them have the efficiency and ability to accumulate, destroy and remove the various pollutants that these mediums are exposed to especially if this pollution is complex with diverse references. The ease of obtaining any biomass of cells and

filament or cellular colony of these microorganisms and their isolation and development is in the pure individual or common culture (1-4)

The Applied studies in this field have also indicated the significant difference in the material cost required by chemical, physical or mechanical treatments. Moreover, they are specialized to treat one aspect of the problem compared to the vital methods in which it can treat more than a pollutant at the same time by using serial processors or a common farm or an orthogonal level based on the nature of the distribution of the organism in the aquatic body within the surface layer or water column or the benthic region (5-8)

Microorganisms are characterized including algae with a great potential to destroy, remove or adsorption of various pollutants (pesticides and heavy metals) as indicated by researchers (9-11) to possess such organisms for effective and specialized chemical aggregates in their cells and it can be associated with the polluting agent. Also, it can either accumulate or convert it to a less toxic compound and effect or store it in a part of the cell or destroy it into a simple prototype using these ingredients for mineral recharge or precipitation in the water bottom after they form common chelating compounds. These effective groups include alcohol, diphenyl ethers, phosphate, amines, carboxylates and others. (12-13)

Therefore, many experiments have pointed to the effect of increased biomass, the quality of the algae organism or the rest of the microorganisms that were used and the nature of their cellular construction in the process of bio-adsorption, removal and destruction. Whether it was using a damp or dry mass of algae or other biota, it was the result of ionic exchange and correlation patterns between these effective sites and

the pollutant materials in the environmental medium (14-16)

The biosorption process in algae of heavy metals and organic chemical compounds, including pesticides is followed by several activities comprised of ion, surface complexation, adsorption, sorption precipitation and exchange. This has given them a wide range of treatments and applications biosorption of various water pollutants, whether using their living or dried cells in the form of powders of different algae types (17-18)

2. MATERIALS AND METHODS

2.1.Collection of samples and selection of sites.

Three stations were selected to collect and, study-targeted fungi from their natural environment (Tigris River water) in the Al-Doura area to the Al-Muthanna Bridge area and then entering river the city of Al-Kadhimiya in the province of Baghdad. The sites were chosen on the grounds of the diversity of human activities, facilities and the small factory that throw their residues into the river which have led to different sources of pollutants rather than distances. The algae were collected from three different locations (near Al-Dora refinery, opposite the city of Al-Kadhimiya, and the city of Medicine). For the purpose of sample classification, the sources were used (19-20).

2.2. Isolation and culture of algae

The algae were isolated by using the dilution (21) and the method of streaking (22) that was applied at semi-solid media by adding a (1.5 – 2) % agar to the liquid media. After the growth of the algae at the incubator, the Petri dishes and tubes were investigated at a temperature of 25 °C and lighting system of (6-18) lighting-darkness. The intensity of lighting

was 3000 Lux for 7 days with the daily shake for the tubes until they got one type of algae for all samples.

For the purpose of purifying the isolations, the (23) method was followed through to reach the axenic culture of the targetted algae through the isolations obtained in the dilution and streaking approach. This was so that they can reach an appropriate size of (240–500) ml to perform the purification process by eliminating fungi and bacteria that may be associated with the algae species. The process took 50 ml of isolated cultures putting them in darkness for 24 hours. As well as, 10 ml of the culture was pulled after the incubation in the darkness by a sterile absorbent and added the same liquid medium in which the solitude was cultured with the addition of drops of broth nutrient agar and incubated again in the dark 2-3 hours. After that, the centrifugal device was used at 3000 RPM/min for two minutes and the sediment was kept and the suspension was discarded. The sediment was washed by the sterile medium and the washing process was repeated from 12 to 15 times. After that part, each sample of sediment was cultured on the center of the solid nutrient agar which was prepared by dissolving 15 grams of nutrient agar per liter of distilled water.

2.3. Preparing the culture media algae:

The modified structural culture media Chu13 was prepared according to (24-25) methods in the form of a stock solution with distilled water, then the autoclave was sterilized at a temperature of 121 °C and pressure of 1.5 ATM for 15 min keeping it in the refrigerator until used. Whereas, the phosphate-containing solutions K_2PO_4 , have been sterilized by filtering through the diameter of 0.45 μm to avoid the deposition of phosphate salts on the walls of the preparation bottles while sterilizing them in the autoclave(26). All

the stock solutions were left to cool at room temperature and then preserved in the refrigerator until used. Solid culture was also prepared with the addition of 1.5-2 % agar to sterilize liquid media and was dissolved with indirect heating at 45-50 °C (within water bath). It was again sterilized in autoclave and poured into dry and sterile Petri dishes in a sterile room near the flame of the benzen lamp, and left to cool at a free degree in the room keeping it upside down at 4°C temperature.

2.4.Preparation of heavy metal and pesticide solutions

Ca and Cr metal solutions were prepared by dissolving 3.7 g of Cr and K_2CrO_4 were considered a source of it, whereas Cd was prepared by dissolving 1.8 g/L and using $CdCl_2 \cdot H_2O$ as its source. A pesticide (Ground-up SL) was taken as liquid concentrated with an active substance of 480 mg or equal (36% w/v), from the production of VAPCO. The concentrations were prepared according to the following formula: $C1V1 = C2V2$.

2.5. Calculation of the percentage of pesticide destruction and removal of heavy metals for elected algae in static and dynamic medium

This experiment was estimated by preparing the broth culture media CHU13 for *C. humicola* and the medium BG11 for the algae *C. vulgaris*. The stock solution was diluted to each metal in the culture media to prepare ascending concentrations (2.5, 5, 7.5, 10, 12.5, 15, 20, 25) (1, 2, 3, 4, 5) mg/L for Ca and Cr respectively and ascending concentrations of the pesticide (25, 50, 75, 100) mg/L. 100 ml of culture media with heavy metal transferred into glass bottles with the capacity of 250 ml to ensure good ventilation.

These bottles were then sterilized for 15 minutes by the autoclave, and were left to cool to 45 °C. The culture media was inoculum by the seed culture at 20 ml in

100 ml (27) then the inoculated media with minerals and control treatment were incubated in the incubator at static medium within the shaker incubator of the dynamic medium at 150 RPM/min. The tests were conducted in controlled conditions at 25 °C and pH= 7 and the brightness of 3000 Lux with 16 hours of lightening and 8 hours of darkness. Then the biomass were separated from the static and dynamic medium every 3 days for 12 days using Millipore filtration paper (0.45 µm). The residues of herbicides Glyphosate were estimated in the broth medium by High Performance Liquid chromatography SYKNM S4011Gmbh, Origin: Germany in Laboratory of Pollutant Treatment Centre/environment and Water Department, Ministry of Science and Technology) as stated in (28) The remaining concentration of heavy metals in the centre were estimated for all experiments using the atomic spectrophotometer (Shimadzu AA-7000, Origin: Japan) in Central Service Laboratory/ College of Education for Pure Science (Ibn Al-Haitham) /University of Baghdad, as reported in the method of APHA (1998).

In order to determine the percentage of pesticide destruction and heavy metals removal, the following formula has been applied:

Percentage of damage and removal for pesticide and metal = (concentration before treatment – concentration after treatment / concentration before treatment * 100 (28)

2.6.Statistical analysis

The results are analyzed according to the complete random design (C.R.D.) The Complete Randomized Design used the SAS (2010) whereby the averages were compared with a test application Least Significance Difference (L.S.D) and the significance was

determined by applying ANOVA at the probability level ($p \leq 0.05$).

3. Results and discussion

3.1.Studied algae genus

Five genus of local algae were diagnosed as follows: *Chlorococcum humicola*; *Chlorella vulgaris*, *Scenedesmus sp.*; *Diatom*; *Chlamydomonas sp.* Those algae were repeated presence in three location:, after isolation and cultured on solid media, two pure isolation of *Chlorococcum humicola* and *Chlorella vulgaris* whose efficacy was tested in destroying pesticide (Glyphosate), adsorption and removal of heavy elements (Cr and Cd).

3.2.Percentage of pesticide destroying in a suspension algae culture

The results of table (1) indicated the destruction of the pesticide Glyphosate for the *C. humicola*, which was the highest rate of destruction of the static medium 61. 1% at concentration of 25 mg/L on the twelfth day and the lowest rate of destroying was 30.7% at the concentration of 100 mg/L. The outcome showed that the static medium was the highest rate of destruction of 78.5% when the concentration of 25 mg/L on the twelfth day and the lowest rate of destruction was 37.3% at concentration 100 mg/L. The results of the destruction of algae *C. vulgaris* were the highest rate of destruction at the static medium 15.3% at concentration of 25 mg/L on the twelfth day and the lowest rate of destruction was 8.3% at the concentration of 50 mg/L at the third day. In the meantime, the results of the dynamic medium were 17.7% higher than the 25-day concentration and the lowest rate of destruction was 9.2% at the concentration of 50 mg/L for the third day. In retrospect, there were no destruction ratios at concentration 75 and 100 mg/L due to the non-tolerance of algae for these

concentrations, which represent the highest recommended concentrations where a few absorption values have appeared within these concentrations.

It was statistically found that there were significant differences for the two algae between the two concentrations and day, except for algae *C. vulgaris* and we noted the lack of significant difference between the two medium in two concentrations of 75 and 100 mg/L. This is

due to the under study susceptibility of algae to the production of antioxidant enzymes (C-P lyase), which broke the strong binds between carbon and phosphate (C-P) in pesticide Glyphosate and its use as a source of phosphorous, as well as because of its containment high fat compared with other algae (29-30) This has led to their high tolerance of the pesticide up to 100ppm and these conclusions are consistent with the views of the researchers (31-35)

Table (1) Percentage of destroying of Glyphosate pesticide in the static and dynamic medium and of algae isolation *C. humicola* and *C. vulgaris*.

<i>Chlorococcum humicola</i>									
Conc. mg/L	Static medium				Dynamic medium				P-value
	3 days	6 days	9 days	12 days	3 days	6 days	9 days	12 days	
25	54.767±0.186	56.033±0.555	58.100±0.586	61.100±0.493	60.133±0.467	62.267±0.371	65.167±0.406	78.500±0.551	P<0.001
50	45.800±0.153	46.233±0.617	49.167±0.441	53.600±0.265	45.700±0.252	50.767±0.285	54.933±0.581	56.433±0.328	P<0.001
75	31.033±0.549	35.867±0.088	40.400±0.551	48.200±0.416	37±0.462	41.967±0.448	47.700±0.252	51.733±0.817	P<0.001
100	19.400±0.833	22.067±0.581	25.967±0.504	30.767±0.120	21.033±0.318	30.067±0.521	33.100±0.529	37.300±0.874	P<0.001
P value	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	----
<i>Chlorella vulgaris</i>									
Conc. mg/L	Static medium				Dynamic medium				P-value
	3 days	6 days	9 days	12 days	3 days	6 days	9 days	12 days	
25	8.333±0.318	10.733±0.176	12.800±0.252	15.300±0.321	11.033±0.219	13.767±0.291	15.767±0.291	17.767±0.219	P<0.001
50	5.733±0.088	6.767±0.176	7.833±0.145	8.333±0.203	5.800±0.173	6.933±0.318	7.767±0.418	9.200±0.208	P<0.05
75	0	0	0	0	0	0	0	0	1.00 NS
100	0	0	0	0	0	0	0	0	1.00 NS
P value	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	----

4. Percentage of removal of the Cd in a suspension algae culture

The table (2) shows that the removal ratios of the algae *C. humicola* were very high for the static and the dynamic medium of the third day of all the concentrations where they showed the highest rate of removal of the static medium 98.80% at concentration 12.5 mg/L on the ninth day and the lowest removal rate of 80.38% at

concentration 2.5 mg/L on the ninth day. While the results of the dynamic medium showed the highest removal rate of 99.55% at concentration 12.5 mg/L on the third day and the lowest removal rate of 82.44% at concentration 25 mg/L on the 12th day. Through continuous readings of pH values, the pH values were observed down to 6, resulting in a little decrease in the biomass of the high concentrations where the best

values of the removal were shown at 8-9, which showed the best biomass of algae under study. These results were consistent with the results in the researchers (36) who confirmed that the best remove for Cd, Cr and Cu when the (pH=8) because of increasing the functional groups responsible for removing these heavy elements.

We note the lack of significant differences of this alga in astatic medium of the sixth day between Cd and the medium at concentrations 12.5 and 25 mg/L. The results were consistent with the results in the researcher (37), who indicated the efficacy of alga in the removal of Cd with a concentration of 12.5 mg/L and its tolerance to the high concentrations. These results are consistent with the results of the researchers (38) where the highest removal rate of the Cd by alga *Chlorococcum* 94%. The tendency of this alga to assemble in the form of sporadic cellular groups and resistance to the influence of the element, as well as its ability to grow and adsorption, has been observed through the experiment, even in a concentration of 25 ppm.

The results of the removal of alga *C. vulgaris* where the highest removal rate of the static medium 75.48% appeared at the concentration of 2.5 mg/L on the twelfth day and the lowest removal rate 43.60% at concentration 12.5 mg/L on 12th day. While the results of the dynamic medium, where the highest removal rate of 86.73% appeared at a concentration of 2.5 mg/L on the sixth day and the lowest removal rate of 52.93% at concentration 12.5 mg/L on the 12th day. The reason for decreasing the removal ratios to decrease the growth at concentration 12.5 mg/L. These results were in line with the researchers (39) where the alga *Chlorella* sp. shown removed for Cd reached to 48.7% after a 12th day incubation period and 67% of the removed Cd was

accumulated within the cell and 25% of the removed on the surface of the living cells. (40) have been able to use seaweed and green algae, including algae *Chlorococcum* sp. and *Chlorella* sp. As a vital material for heavy metals, ions including these are Cd elements and are highly efficient.

The results of table (3) showed that the removal ratios of the *C. humicola* were highest for static medium of 78.61% at the concentration 1 mg/L on the ninth day and the lowest removal rate of 30.02% at a concentration of 4, 5 mg/L on the 12th day. Thereby, the results of the dynamic medium showed the highest removal rate of 76.72% at concentration 1 mg/L for third day and the lowest removal rate was 52.51% at a concentration of 4 mg/L on the third day.

This table showed that there was a decrease of the removal ratios from concentrations (3, 4, 5) mg/L for the static medium as a result of the decrease in the biomass, and statistically there are no significant differences between the removal ratios and concentration of this algae on the third and sixth day of the removal of the dynamic medium. The final results of the removal in alga *C. vulgaris*, where the highest removal rate of the static medium 71.07% appearing at 1 mg/L on the twelfth day, and we note a significant decrease in the removal ratios of 2.77% at concentration 5-4-3 mg/L on the 12th day as a result of the decrease in the biomass. The results of the dynamic medium, where the highest removal rate of 72.79% appearing at the concentration of 1 mg/L on the ninth day and the lowest of 37.5% at the concentration of 5 mg/L. Within this table, we see a noticeable decrease for the removal ratios at 3, 4, 5 mg/L for the static medium and it was more than the dynamic medium, These results could very much be due to a lowest of cells because of the reduced value of pH into 6.2, which led to a

decrease in the numbers of cells (41) The reason behind these results could be because some of them were to die near the end of the experiment and to saturate their

effective sites exposed to some of the properties of metal particles exposed to algae (42-45)

Table (2): Percentage of removal of different metal from heavy Cd metals in the static and dynamic broth culture medium by using algae isolation *C. vulgaris* and *C. humicola*.

<i>Chlorococcum humicola</i>									
Static medium					Dynamic medium				
Conc. mg/L	3 days	6 days	9 days	12 days	3 days	6 days	9 days	12 days	P-value
2.5	92.707±0.07 2	83.300±0.096	80.380±0.202	88.360±0.096	98.33±0.17	97.80±0.11	96.73±0.13	90.64±0.05	P<0.001
5	92.243±0.14 4	87.677±0.154	94.310±0.128	97.577±0.064	97.45±0.16	99.48±0.13	98.73±0.03	96.68±0.1	P<0.05
7.5	93.587±0.16 0	89.583±0.130	96.533±0.088	97.513±0.247	98.62±0.08	98.79±0.09	99.04±0.01	96.49±0.38	P<0.05
10	92.410±0.08 9	91.570±0.055	98.563±0.066	98.283±0.139	99.07±0.02	99.33±0.05	98.67±0.03	97.45±0.02	P<0.05
12.5	93.683±0.13 4	96.600±0.115	98.800±0.175	95.410±0.040	99.55±0.27	98.44±0.06	97.63±0.05	96.78±0.165	0.071 NS
15	91.763±0.11 7	92.653±0.103	92.520±0.099	92.350±0.113	86.49±0.04	96.66±0.13	95.51±0.06	90.65±0.130	P<0.05
20	91.280±0.14 0	92.527±0.151	92.493±0.047	90.433±0.067	86.50±0.12	90.58±0.04	84.91±0.04	84.59±0.130	P<0.05
25	83.477±0.16 7	84.687±0.176	85.710±0.031	86.583±0.059	84.74±2.02	83.20±0.02	83.78±0.10	82.44±0.088	0.192 NS
P value	P<0.001	0.465	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	---

<i>Chlorella vulgaris</i>									
Static medium					Dynamic medium				
Conc. mg/L	3 days	6 days	9 days	12 days	3 days	6 days	9 days	12 days	P-value
2.5	65.813±0.12 7	72.653±0.124	74.543±0.176	75.767±0.109	84.1667±0.083	86.7300±0.025	86.5033±0.093	81.7933±0.074	P<0.001
5	60.193±0.02 0	63.733±0.015	65.627±0.128	65.433±0.093	65.6333±0.065	67.7167±0.095	77.2833±0.033	77.0633±0.021	P<0.001
7.5	56.480±0.03 2	59.473±0.126	60.390±0.031	60.557±0.172	63.4500±0.064	66.8300±0.096	68.5667±0.086	67.5633±0.104	P<0.05
10	56.273±0.03 9	59.197±0.029	61.700±0.058	58.500±0.289	60.6133±0.081	61.7467±0.096	69.0633±0.014	67.6633±0.161	P<0.05
12.5	50.500±0.05 8	51.350±0.115	54.407±0.152	43.600±0.047	55.4667±0.072	58.5433±0.038	62.4633±0.063	52.9300±0.035	P<0.05
P value	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	---

Table (3): The percentage of removal at different concentration of chrome heavy metal in the broth culture media, static and dynamic by using algae isolations *C. vulgaris* and *C. humicola*.

<i>Chlorococcum humicola</i>									
Static medium					Dynamic medium				
Conc .mg/ L	3 days	6 days	9 days	12 days	3 days	6 days	9 days	12 days	P-value
1	74.487±0.119	76.510±0.038	67.907±0.018	76.613±0.029	78.727±0.073	65.873±8.987	72.817±0.034	69.397±0.052	P<0.05
2	64.497±0.048	74.810±0.101	70.550±0.056	50.700±0.035	58.680±0.015	60.773±0.015	64.453±0.098	66.897±0.042	P<0.001

3	61.700±0.075	68.330±0.107	67.040±0.021	34.373±0.046	54.517±0.072	57.080±0.015	66.087±0.020	62.857±0.092	P<0.001
4	55.843±0.052	58.573±0.064	58.543±0.033	29.807±0.117	52.517±0.160	57.103±0.043	63.483±0.018	62.573±0.062	P<0.001
5	55.510±0.259	58.797±0.042	57.197±0.061	29.800±0.050	57.413±0.041	59.460±0.015	66.607±0.050	62.203±0.024	P<0.001
P value	P<0.001	P<0.001	P<0.001	P<0.001	0.449	0.547	P<0.001	P<0.001	---

Chlorella vulgaris

Conc .mg/ L	Static medium				Dynamic medium				P-value
	3 days	6 days	9 days	12 days	3 days	6 days	9 days	12 days	
1	54.26±0.021	64.733±0.037	68.09±0.017	71.073±0.022	61.450±0.260	63.500±0.265	72.797±0.054	68.690±0.121	P<0.01
2	47.723±0.03711	53.55±0.118	50.8±0.057	37.523±0.037	50.443±0.043	51.183±0.041	58.097±0.029	58.120±0.040	P<0.001
3	47.120±0.535	49.857±0.043	34.723±0.228	6.920±0.232	50.910±0.312	54.730±0.023	54.993±0.064	45.633±0.139	P<0.001
4	46.823±0.228	50.810±0.075	30.243±0.140	4.840±0.224	47.000±0.115	56.370±0.049	54.067±0.088	40.030±0.095	P<0.001
5	42.590±0.070	46.730±0.065	30.480±0.319	2.277±0.194	48.480±0.147	55.563±0.148	53.620±0.246	37.560±0.225	P<0.001
P value	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	---

The end results also showed a decrease in removal values for the twelfth day of the algae under study, and some of the concentrations may have caused cell death and lack of preparation causing the return of these cells from the element after degradation to the aquatic environment (46-48). It turns out that the increasing percentage of the removal was synchronized with the increase in the biomass, thus increasing the number of effective locations in the wall of the cells that have a desorption of these elements according to (49-51) respective, that was the most cell wall of algae contained active groups such as carboxylates, hydroxylates, Sulphur, phosphates and amines, which bind with the heavy (52). According to Garadea-Torresdey in 1990, he indicated that the carboxylates on the surfaces of the

algae cells were primarily responsible for the correlation of a large proportion of the elements and their adsorption on the surfaces, even if the cells are of a biomass that was ineffective.

CONCLUSIONS

Through the analysis of all the results, several conclusions can be drawn from this study. Most importantly, that the tested algae have been widely adapted in the resistance of pesticide use, which is encouraged to be used in the Bioremediation of such a pesticide group. The alga *C. humicola* also scored destroying, adsorption and higher removal of algae *C. vulgaris* in the static and dynamic media exposed to the pesticide as well as Cd and Cr elements. In general, the two genus of the algae

targeted by the study in the dynamic medium were greater than in the static medium, especially with the treatment by different concentrations of Cd and Cr.

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