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REMOVING HEAVY METALS BY DIATOMS *NITZSCHIA PALEA* AND *NAVICULA INCERTA* IN THEIR AQUEOUS SOLUTIONS

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Abstract

Different concentrations 0.5, 1, 2 ppm from heavy metals Cd, Ni, Pb were prepared and adding *Nitzschia palea* and *Navicula incerta* within the optimum growth conditions in pH 8.5 and Temperature 25. The results showed the ability of both diatoms to remove metals from the aqueous solution. The results were showed the highest percentage removal of heavy metals was in lead in ratio reaches to 70.4, 83.3 and 78% for the concentrations 0.5, 1 and 2 ppm. Also, testing the ability of both diatoms in removing heavy metals by using immobilized lived cells diatoms. Lived *N. incerta* and *N. Palea* were mixed and immobilized in calcium-alginate beads produced beads of calcium alginate with the entrapped diatoms cells. The results showed optimum removal of heavy metals by immobilized diatoms in pH 8.5 in room temperature, So, the results showed the high decreasing of concentration 0.5, 1 and 2 ppm were 0, 0.02, 0.08 ppm respectively, in percentage removal reached to 100, 97, 96% for lead and 91, 94.6, and 94.5% for Cd and for Ni was 89, 90, and 93.6% respectively. Our results indicated that the Biosorption of heavy metals by immobilized diatoms was more effective than done as in free diatoms cells.

***Key words*:** Diatoms, heavy metals, removing, immobilize calcium alginate.

Introduction

The strategies to reduce heavy metal solution are using microorganisms and microalgae, due to their ubiquitous occurrence in nature, have been studied extensively in this regard. They can sequester heavy metal ions by adsorption and absorption, as do by other microorganisms. Diatoms are essential members of the phytoplankton communities in the marine and fresh water environment and very often form a dominant component of macro and micro plankton (de Vargas *et al.,* 2015; Massana *et al*., 2015, Fogg, 1982)**.** Using of diatoms for metal removal has the potential to achieve greater performance at a lower cost than conventional wastewater treatment technologies. This is consistent with the recent trend for growing interest in biosorbent technology for removal of trace amounts of toxic metals from dilute aqueous waste by diatoms (Chong, 2000)**.** Exposure of *Nitzschia palea* to cadmium decreased extracellular polysaccharide by 52.8 % and increased 6 times the amount of frustulins (Santos *et al.*, 2013). For *Navicula*, Cd effect the cell more than lead and nickel , because it`s affects the photosynthesis and reduces the primary productivity of phytoplankton even at small levels could reach to 0.2 mg/l (Koivisto *et al.,* 1992). Lead was found to be concentrated in cell sectors with polyphosphate bodies of diatoms *Nitzschia* at low levels also detected in the cytoplasm, also detected in *Nitzschia* cell wall at high levels could reach to 25 ppm. Nickel was very inhibitory to percentage of *N. palea*, it`s inhibit *N. palea* swaying. Growth inhibition in algae after exposure to 'heavy' metals has been attributed to inhibition of the function of photosynthetic pigments, to enzyme inhibition, uptake of nutrients or damage of cell membrane (De Filippis *et al*., 2007). While *in Navicula sp*. was found that the nickel concentrations as low as (l mg/litre) inhibit biomass accumulation and growth rates of *Navicula* cultures. The metal-binding of biosorbents could done by Adsorption (non-metabolic process) and Absorption (metabolic process). Immobilization it's the use of microalgae in biotechnology has been increased in recent years (Tampion & Tampion, 1987). Biosorption of heavy metals by living immobilized prokaryotic and eukaryotic microalgae cells, using various immobilizing material, is an additional option. Generally immobilized cells are more efficient in the removal of heavy metals compared to free living cells. Calcium alginate as example in immobilized cells is nontoxic and let different type of microorganisms to grow inside (Malik, 2004). Calcium alginate as example in immobilized cells is nontoxic and let different type of microorganisms to grow inside. The transparency of small calcium-alginate beads is enough to permit the growth of immobilized microalgae (Hertzberg and Jensen, 1989). Additionally it is an easy, cheap and feasible technique to be used in research laboratories (Papagregoriou, 1987).

Material and Methods

Organisms and Media: Species of diatoms *Nitzschia palea* and *Navicula incerta* were isolated from the Sawa lake sample by Serial dilution which proposed by (Kumar *et al*., 2005). And for pure culture from microbial pollution could be used agar plating method

(Patterson, 1983). F/2 medium was the growth culture for both diatoms (Guillard, 1975 and 1962), One of the most components of F/2 medium which consist of Combine equal parts of Salt Solution I (NaCl 20.758g/l, KCl 587g/l, $NaHCO_3$ 0.170g/l, NaBr 0.0746 g/l, H_3BO_3 0.0225 g/Land Salt Solution II ($MgCl_2$•$6H_2O$9.395 g/L, $CaCl_2$•$2H_2O$1.316 g/L, $SrCl_2$•$6H_2O$0.0214 g/L which dissolved in 950 of deionized water (dH_2O) to make the artificial seawater. Used 1 ml of the final trace metal solution to F/2 medium, also, added one ml of F/2 vitamin solution to make final volume up to 1 L with filtered seawater (or artificial seawater) and adjust to pH 8 using 1N HCl or 1N NaOH.

Metals: Lead A standard solution of 1000 parts per million of lead, nickel and cadmium were prepared by dissolving 1.342 g of $PbCl_2$ in one litter of de-ionized distilled water, 1.8 g of $CdCl_2.H_2O$ was dissolved in one litter of de-ionized distilled water. And for nickel which prepared by dissolving 5.4 g of Ni $(NO_3)_2$ in one litter of de-ionized distilled water. All Above were prepared according to the following equation: C1V1 =C2 V2 …….Fifty ml of each selected diatoms was cultured with medium which containing lead, cadmium and nickel separately to test their ability to remove these metals. Three replicates were used for each concentration and counting diatoms cells by Haemocytometer which was used to calculate the number of blood cells in the blood. A certain size of sample is placed on the surface of each lobby of my reaction Slide, and examine under the microscope after placing the cover of the slide. The expression of the result by (cell/ml), and used the squares according to the following steps: (Hadi, 1981). Growth rate and doubling time were calculated from the equation by Reynolds (1984). Measuring the concentration of heavy metals was by flame atomic absorption spectrophotometer in absorption wavelength 540 nm.

Immobilizing diatoms Sterile solutions of sodium alginate were prepared as follows: Na-alginate (1.5 g) was dissolved in distilled water (66 ml) by slow stirring for 4 - 6h, and a solution of NaCl (2 g) in distilled water (1 L) was added. The pH was adjusted to 7.5–8.0 by addition of 0.1 M NaOH. 50 mM aqueous tris buffer at pH 7.5–8.0 was used instead of water for the dissolution of the alginate. The sterile solution was mixed thoroughly (gentle stirring) with drops of a very concentrated suspension of diatoms (live). Forcing the mixture through a sterile Pastor pipette (Hertzberg, 1989) into a growth medium fortified with calcium (3/4 f-strength medium with 0.07 M $CaCl_2$) produced beads of calcium alginate with the entrapped algal cells. Bead diameter was regulated in the range of 0.5 to 2.0 mm. The beads remained in the calcium-enriched medium for at least 30 min. To secure gel hardening and were then transferred to the standard medium for growth. Cells were immobilized in calcium-alginate beads following Moreno-Garrido (1997). Beads were kept in 250 ml spherical flasks containing 100 ml of f/2 medium with around 50 ml of beads. Samples were taken regularly till day 17 after the beginning of the test and fixed with formalin. Known volumes of beads were dissolved by soft sonication with known volumes of tri-sodium citrate (3% w/v) and cells number was counted (Moreno-Garrido, 2005).

Testing the ability of immobilized Diatoms to remove heavy metals ions. The experiment was done in 25ºC temperatures in 250 ml poly ethylene containers by added beads of diatoms to the 100 ml of metal solution. The Containers were putted in vibrating incubator with speed 50 cycles per minute for 3 hours in T 25 ºC and then filtered to analyzing sample after 1h, 4h and 24h by atomic absorption. Calculating the percentage of Ni, Pb and Cd removal by equation of (Vieira, 2000) was mentioned in (Uten, 1978).

Results

Non- immobilized diatoms

Lead removal ratio increases with increased concentration of the metal. The removal percentage of lead in concentrations 0.5, 1 and 2 ppm was 70.429, 83.343 and 78% respectively as in (table 1), removal percentage could be more effective in the first life cycle (Ziames, 2014) (during the 10 days in diatoms), Also, in the last experiment days , the removal ration less than in the early days of experiment ,This may be due to the competition between diatoms on the space , nutrients and may also be due to the lack of effective sites for the binding heavy elements because the closure of some of them when removing elements (Nirmal *et al.,* 2009). In this mixing diatoms system, there were more cell numbers and more affective binding sites and more removing ration.

Table 1 : Removal percentages of lead ions by both diatoms

Removal percentage of Pb %				LSD value
Con. /day	0.5	1	2	
2	24	34	38	8.73 *
4	42	66	49.5	8.59 *
6	66	89	75.5	8.96 *
8	74	95	87	7.63 *
10	88	99.4	96	8.16 *
12	99	100	100	NS
14	100	100	100	NS
Sum	70.429	83.343	78	
LSD value	9.62 *	10.14 *	10.33 *	---

Also, the statistical analysis at ($P < 0.05$) indicated that there were significant differences among the results in the first 10 days of experiment and between them, (Table 2).

Table 2 : Both diatoms with lead ions (Slandered ± Error)

Mean ±SE				Concentration
Growth rate	**Doubling time**	**Cell/10^4**	**Removal %**	**ppm**
2.045 ± 0.06	2.928 ± 0.08	107.090 ± 5.37	70 ± 2.65	0.5
2.120 ± 0.09	3.329 ± 0.11	127.678 ± 7.75	83.342 ± 4.26	1
2.631 ± 0.06	1.524 ± 0.07	138.679 ± 7.64	78 ± 2.92	2
0.437 *	0.772 *	9.68 *	7.21 *	$LSD_{(P<0.05)}$

Cell numbers were increased in high percentage after 4 days (figure A), which mean after adapting both diatoms to the competition between the two diatoms in terms of food, active sites but, lacking nutrients in media (Chen, 1998) accumulation of waste due to metabolic processes and blocking active sites after metal absorption led to decreasing cell number especially after 8 days of growth (Table 1-3).

Growth rate decreased after 4 days of exposure because of the competitions between diatoms cells as mentioned previously (figure B). Doubling time (hour) increased rapidly in the last days of experiment (Figure C).

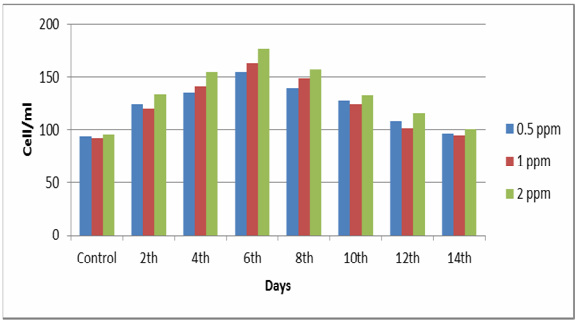

Fig. A : Biomass of *N. palea and N. incerta* in different concentrations of lead

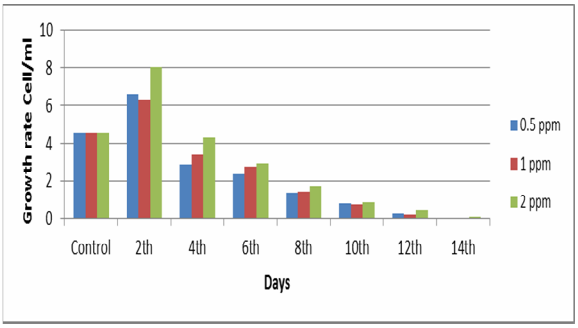

Fig. B : Growth rate of both diatoms in different concentrations of lead

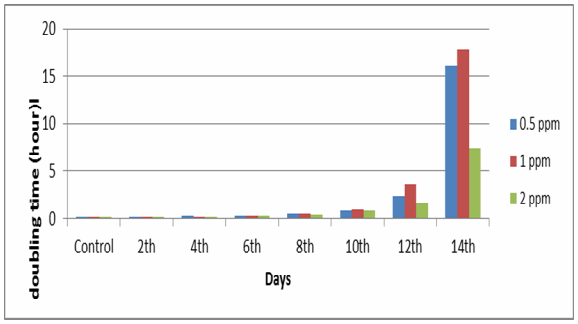

Fig. C : Doubling time of both diatoms in different concentrations of lead

The statistical analysis at (P <0.05) indicated that there were significant differences between the results of experiment, but there were no differences among them (Table 3-14).

Bio-removal of Cadmium by mixture of the two diatoms: The results were showed decreased in cadmium for all concentrations 0.5, 1 and 2 ppm; with removing percentage was 82, 66.286 and 79.629 % respectively (Table 3).

Table 3 : Removal percentages of Cd ions by both diatoms

Removal percentage of Cd %				LSD value
Con. /day	**0.5**	**1**	**2**	
2	32	39	38	NS
4	62	44	53	7.47 *
6	82	52	78.5	8.58 *
8	98	57	91.5	8.09 *
10	100	79	97	7.21 *
12	100	94	99.6	NS
14	100	99	99.8	NS
sum	82	66.286	79.629	
LSD value	8.68 *	8.95 *	8.26 *	---

Removing cadmium in concentration 0.5 was more than other concentration due to its ability of 90% of its ratio to binding with polysaccharide, so in little concentrations, the binding sites were more available for binding cadmium.

The statistical analysis at (P <0.05) and LSD value showed that there were significant differences between the results of removal percentage and among them (Table 4).

Growth rate of both diatoms showed negative growth because its effected by cell number, and according to the biomass of diatoms cells which decreased especially at the last period of experiment in which the number of cells less than control, that's could returned to the effects of cadmium on both diatoms on growth rate and photosynthesis, as well as, the accumulation of waste results from metabolic reactions and could changes in some conditions or pH that made

sever competition between both diatoms, Also, The competition between diatoms and the effect of diatoms cells by sudden and temporary changes in temperature or some other chemical-physical conditions have an impact on the concentration of heavy metals in effective sites of algae (Figure D) . Doubling time also showed negative data (Figure E).

Table 4 : Mixture of *N. palea* and *N. incerta* and cadmium ions

Mean ±SE				Concentration
Growth rate	**Doubling time**	**Cell/10^4**	**Removal %**	**ppm**
2.958 ± 0.09	0.596 ± 0.06	103.687 ± 4.75	82.00 ± 3.96	0.5
3.172 ± 0.12	0.550 ± 0.04	145.143 ± 7.37	66.286 ± 2.53	1
2.226 ± 0.08	0.435 ± 0.04	107.671 ± 5.29	79.629 ± 2.87	2
0.633 *	NS	11.69 *	6.02 *	$LSD_{(P<0.05)}$

Our results agreed with Katarzyna *et al.* (2015), who used mix algal culture to remove metals as Cd and Cu, and he indeed, the study showed that mixed algal population was good biosorbent of Cu and Cd ions. It was observed that spiking of different doses of cadmium (II) and copper (II) considerably affected the effectiveness and efficiency of sorption and better than used one species alone.

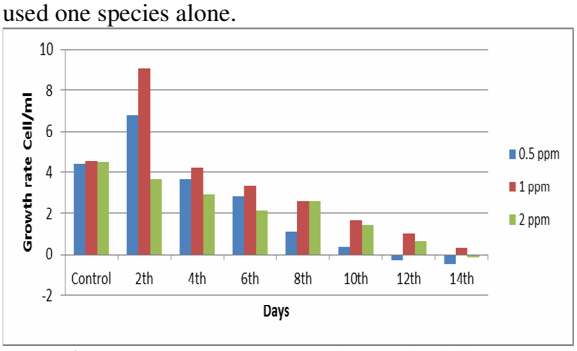

Fig. D : Growth rate both diatoms in different concentrations of Cd

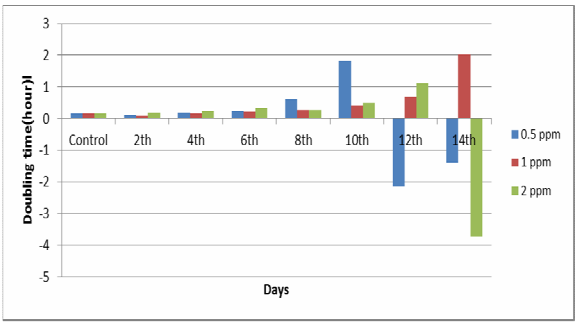

Fig. E : Doubling time for diatoms in different concentrations of Cd

Bio-removal of Nickel by mixture of the two diatoms

Nickel showed slight increased in cell number at the first 6 days of experiment, after the day 6 of experiment; nickel showed the highest decreased of cell even cell number at the end of experiment reached less than in the control (Figure F).

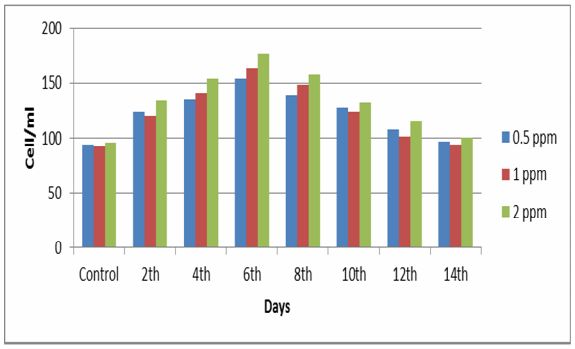

Fig. F : Biomass both diatoms in different concentrations of Nickel

The value of growth rate (cell /ml) has shown increased from the first day of experiment as in the figures (G). It's notice increased in the day 2 for all concentrations and returned to decreased, While doubling time figure (H) recorded with slight increasing during the experiment period until the last day which recorded highest increased. The statistical analysis showed there was no significant differences ($P < 0.05$) among the concentrations in growth rate and doubling time, but there were differences between them (Table 5).

For the removal percentage of nickel, the results showed the nickel was the lowest removal percentage compared with lead and cadmium but still higher than the results of using one of diatoms at alone. The percentage for the concentrations 0.5, 1, 2 ppm was 77.571, 73.857 and 69.429% (Table 6).

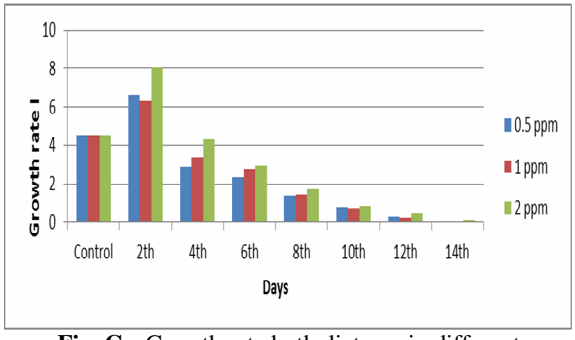

Fig. G : Growth rate both diatoms in different concentrations of Ni

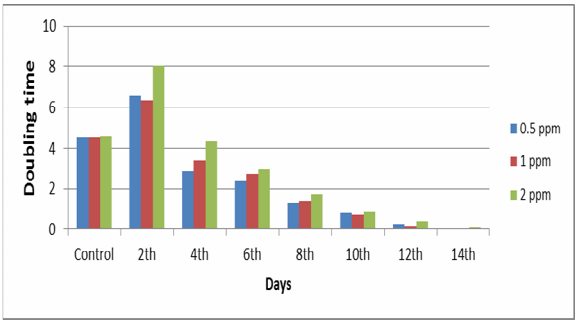

Fig. H : Doubling time for diatoms in different concentrations of Ni

Table 5 : Mixture of *N. palea* and *N. incerta* and nickel ions

Mean ±SE				Concentration
Growth rate	**Doubling time**	**Cell/10^4**	**Removal %**	**ppm**
2.190 ± 0.07	1.378 ± 0.08	116.285 ± 5.06	77.571 ± 3.15	0.5
2.156 ± 0.06	0.497 ± 0.03	122.857 ± 7.13	73.857 ± 2.86	1
2.978 ± 0.10	0.585 ± 0.05	89.107 ± 3.05	69.428 ± 2.56	2
0.588 *	0.536 *	11.85 *	6.32 *	$LSD_{(P<0.05)}$

Table 6 : Removal percentages of Ni ions by both diatoms

Removal percentage of Ni %				LSD value
Con. /day	**0.5**	**1**	**2**	
2	18	28	12	8.94 *
4	54	44	44	8.13 *
6	80	70	56	9.62 *
8	92	83	84	NS
10	99	92	92	NS
12	100	100	98	NS
14	100	100	100	NS
sum	77.571	73.857	69.429	
LSD value	9.27 *	9 .66 *	11.94 *	---

Gupta and Agrawal (2007) mentioned the effects of Nickel on culture of *N. palea and N. incerta* and indicated that, the continues decreasing of culture under exposure to different concentrations of nickel.

Immobilized the living diatoms by Calcium Alginate.

The experiment were done in different pH 5, 7 and 8.5 in room temperature 25± ºC , it was found pH 8.5 was more suitable for diatoms living and for the calcium alginate beds to avoid corruption , also, the optimum removal was recorded in this pH. For removing lead, the results showed the high decreasing of concentration 0.5, 1 and 2 ppm were 0, 0.02, 0.08 ppm respectively, in percentage removal reached to 100, 97, 96 % respectively. Immobilized diatoms cell also showed decreasing in all concentrations (Table 7).

Cadmium removing by calcium alginate indicated decreasing reached to 91, 94.6, and 94.5 % for concentration 0.5, 1 and 2 respectively. Cell number also indicated to decrease to less than half percent (Table 8). Nickel was almost the less decreasing than the other metals reached to 89, 90 and 93% (Table 9). For nickel, the results were recorded 89, 90 and 93.6% for the concentrations 0.5, 1 and 2 ppm. Moreno-Garrido *et al*. (200) studied the removal of cadmium and copper by immobilized diatom *Tetraselmis chui* and he found 20% of cadmium was removed during 24 hour. *Lau et al.* (1998) designed a laboratory scale algal column reactor with the green microalgae C*hlorella vulgaris* with 75 ml alginate algal beads and was used to treat 30 mg/L Ni with, at the end of experiment 91% of Ni was removed. Also, Naggar (2018) also has the study of removing lead in removal percentage (100%) of Pb2+ from aqueous solution by immobilized *Gelidium amansii*.

The most conclusion was

- Removing heavy metals by mixture of the two diatoms indicated high removal percent which more than the ration in removing metals by *N. palae* or *N. incerta*, separately
- Using Calcium alginate to immobilizing lived cells as biofilter which get results with high percentages removal than in free diatoms cells

Recommendations

- Both diatoms with good efficiency to remove heavy metals and salinity, So we recommended to dependent on them in water treatment to remove different pollutants, and there need to additional studies using other species of diatoms in biosorption of heavy metals.
- Designing and improving lab adsorption unit by diatoms to work on a large scale as being used in various sites and more studies on the technique by using it on other toxicant or chemicals.

Table 7 : Immobilized diatoms cells to remove lead ions

Metal / hour	Pb								
	0.5	**Cell**	**%**	**1**	**Cell**	**%**	**2**	**Cell**	**%**
1 h	nil	25.75	100	0.06	27.91	94	0.14	33.1	93
4 h	nil		100	0.009		99	0.05		97.5
24 h	nil	16.46	100	0.009	7.58	99	0.05	11.67	97.5
Sum	0	21.105	100	0.026	17.745	97.3333	0.08	22.385	96

Table 8 : Immobilized diatoms cells to remove cadmium ions

Metal / hour	Cd								
	0.5	**Cell**	**%**	**1**	**Cell**	**%**	**2**	**Cell**	**%**
1 h	0.08	29.5	84	0.06	34.5	94	0.13	30	93
4 h	0.02			0.05		95	0.1		95
24 h	0.01	15.75	98	0.05	17.5	95	0.09	12.5	95.5
Sum	0.03667	22.625	91	0.05333	26	94.6667	0.10667	21.25	94.5

Table 9 : Immobilized diatoms cells to remove nickel ions

Metal / hour	Ni								
	0.5	**Cell**	**%**	**1**	**Cell**	**%**	**2**	**Cell**	**%**
1 h	0.01	28.18	88	0.11	29.68	89	0.14	18.5	93
4 h	0.01		90	0.09		91	0.12		94
24 h	0.01	19.5	90	0.09	10.75	91	0.12	11.8	94
sum	0.01	23.84	89.3333	0.09667	20.215	90.3333	0.12667	15.15	93.6667

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