Biosorption of Cadmium, Lead and Nickel in their Aqueous Solution by
*Nitzschia palea* and *Navicula incerta*

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**Abstract:** Powder of dried of *N. palea* and *N. incerta* were mixed together in equal ratio and used as different grams 0.2, 0.4, 0.6, 0.8 and 1 g were added to the selected heavy metals Ni, Cd and Pb in concentrations 0.5, 1, 2 ppm which recorded after 1, 4 and 6 h to determined the contact time of high percentage removal. The results showed the optimum removal percentage for cadmium was in pH 8 with room temperature 25±2°C. High decreased in cadmium ions with a range between 0.003-0.04 mg/L for the concentration 0.5 ppm with percentage removal reached 90.7-99.4%. Biosorption of lead indicated high removing in the concentrations 2 ppm with percentage removal 91.5-96.6%. As well as for nickel, the data showed the high decrease in the concentration 2 ppm with percentage removal reached 93-96.5%. FTIR technology was depended for detecting the active groups on cells surfaces for the two diatoms which responsible for biosorption of lead, nickel and cadmium.

**Key words:** Powder, dry diatoms, heavy metals, biosorption, immobilize calcium alginate, FTIR

**INTRODUCTION**

One of the most interesting pollution is polluted with heavy metals. The term heavy metal is widely used and refers to metals with an atomic density which reached >5 g/cm³. Sometimes the term toxic heavy metals are used to indicate on the impact of these elements on the environment specifically on their effect on the biota. When heavy metals show toxic effects on living organisms, they are termed toxic heavy metals. Some of them such as nickel, copper and zinc are at very limiting concentrations, essential for life (also termed trace elements or microelements) because they act an important role in the metabolic processes which are taking place in living cells (Haferburg and Kothe, 2007). The search for new technologies to remove hazardous metals from wastewaters has focused attention on the metal binding abilities of different biological materials instead of the existing conventional physicochemical methods (Pavasant et al., 2006; Wenzel, 2009). Among the biological material, microalgae have proved to be advantageous because they present several advantages, i.e., economic regeneration, metal recovery potentiality, lesser volume of chemical and/or biological sludge to be disposed off, high efficiency in dilute effluents and large surface area to volume ratio (Echeveste et al., 2012; Dao and Beardall, 2016). Toxicity of heavy metals like Pb, Cd, Ni, Cu, Hg, Zn, etc. which enter the natural water bodies in various ways, to both freshwater and marine flora and fauna has long been recognized. Microalgae are very sensitive to different changes in their surroundings. Diatoms have high response to the trace levels of various organic and inorganic pollutants concentration including exposure to heavy metals in which changes that take place in their metabolism. One strategy to reduce heavy metal solution is to use microorganisms. 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. This ability may be induced in response to stress by toxic heavy metal exposure (Rai et al., 1981). The use of microalgae or diatoms for removing heavy metal has a greater performance at a lower cost than other conventional of water treatment. 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 et al., 2000). Biosensors of microalgae havin various algal species as cyanobacteria, chlorophyta and diatoms and the advantages of these biosensors in comparison to the chemical and physical analyses are discussed by Brayner et al. (2011). The process of heavy metals biosorption by living immobilized eukaryotic microalgae cells and prokaryotic using various immobilizing compounds or material is an additional option for removal efficiency. Removing heavy metals by
immobilized cells are more efficient in treatment processes in compared to the free living cells (Malik, 2004) that’s could be done by using immobilized cells of microalgae biomass is more efficient. The conditions which supporting microalgae in their processes and maintenance their growth was: light, temperature and pH. Removal of the metals from contaminated sites with high concentrations of metals can be achieved using dried (no active) biomass as biosorbents (Louseti et al., 2009). It should be noted that, the microalgae biomass differ largely in their binding sites for various heavy metals (Micheletti et al., 2008; Mistras et al., 2011). The metal-binding sites of biosorbents (diatoms or algae) depend on the composition of the cell wall of these organisms. 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. Additionally, it is an easy, cheap and feasible technique to be used in research laboratories (Papagregoriou, 1987).

MATERIALS AND METHODS

Metals: Lead A standard solution of 1000 parts per million of lead, nickel and cadmium were prepared by dissolving 1.342 g of PbCl₂ in one litter of de-ionized distilled water, 1.8 g of CdCl₂·H₂O was dissolved in one litter of de-ionized distilled water. And for nickel which prepared by dissolving 5.4 g of Ni(NO₃)₂ in 1 L of de-ionized distilled water. All above were prepared according to the following equation:

\[ \text{CIV} = \text{C}_2 \text{V}_2 \]

Measuring the concentration of heavy metals was by flame atomic absorption spectrophotometer in absorption wavelength 540 nm.

Powder of dried diatoms: The two species of diatoms were dried by Lyophilized methods were mention by Ratti (2008), each dried diatoms were tested in heavy metals and salinity removal in different grams. Using dried diatoms in lyophilized method to produce particles have very high surface area, minimized in contamination. The excellent shape of the product is maintained and quality of the rehydrated products are excellent (Ratti, 2008). The basic conditions used in lyophilized were temperature -40°C and pressure 0.12 mill-bar. Dry diatoms with particles size (50-45 μm) which measured by (FTIR) and kept in poly ethylene containers in refrigerator for used later (Vecchio, 2010).

Testing the ability of dry diatoms to remove heavy metals: The experiment was done in room temperature in 250 mL poly ethylene containers by added different grams of diatoms powder to the 100 mL of metal solution in different pH (5, 7 and 8.5). The containers were put in vibrating incubator with speed 60 cycles/min for 3 h in T 250°C and then filtered to analyzing by atomic absorption. Calculating the percentage of Ni, Pb and Cd removal by equation of Vieira was mentioned by Uten (1978):

\[ \text{Removal} \% = \frac{A - B}{A} \times 100 \]

A = The initial concentration  
B = The final concentration

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 4-6 h 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. The 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 (dried). Forcing the mixture through a sterile Pastive pipette into a growth medium fortified with calcium (3/4 f-strength medium with 0.07 M CaCl₂) produced beads of calcium alginate with the entrapped algal cells. Bead diameter was regulated in the range of 0.5-2.0 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. Diatoms were immobilized in calcium-alginate beads following Moreno-Garrido et al. (2005). 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 et al., 2005).

Fourier Transformation Infrared (FTIR): This technique used for clear the binding process of heavy metals ions into the active groups in the surfaces cells wall of adsorbents which important in biosorption and translate how the metals could binds by tested in spectrophotometer (Shimazon FTIR-800). The 100 mL of Pb, Ni, Cd solutions with 20 mg/L were prepared according to the basic solution of each metals (1000 mg/L) and kept in 250 mL plastic containers, added 1 g of dry diatoms for each container of metals and kept in pH 8. The
bottles or containers were sealed tightly and placed in vibrating incubator with speed 60 cycle/min for 3 h in 25°C. The samples were filtered by 0.45 micron filter papers finally, dried the diatom suspensions which binding the heavy metals and smash well and analysis by infrared device according to Naja et al. (2005).

RESULTS AND DISCUSSION

Powder of dried of *N. palea* and *N. incerta* were mixed together in equal ratio and used as different grams 0.2, 0.4, 0.6, 0.8 and 1 g were added to the selected heavy metals Ni, Cd and Pb in concentrations 0.5, 1, 2 ppm which recorded after 1, 4 and 6 h to determine the contact time of high percentage removal. The results showed the optimum removal percentage for cadmium in pH 8 with room temperature 25±2°C, adsorption reactions are generally, exothermic and the extent of adsorption increases with decreasing temperature (Table 1). Haluk (2011) was recorded in his study the maximum biosorption capacity for Ni and Pb was obtained at 25°C and found to decrease as the temperature was increased to 40°C. The results showed that decreased in cadmium ions with a range between 0.003-0.04 mg/L for the concentration 0.5 ppm with percentage removal reached 90.7-99.4% in the concentration 1 ppm it’s decreasing ranged reached to 0.1-0.06 mg/L with removal percentage reached 87-94% also, the percentage removal for 2 ppm was 90-97% with decreasing reached to 0.1-0.06 mg/L. So, the best contact time occurring in which highest percentage removal 100% was 4 h from (0.2 g) in 0.5 ppm because the biosorbent of heavy metals by dried diatoms would depended on the initial concentration for metal in liquids and removal ratio for sorption could increased by increasing the initial concentrations until saturated all active sites in the biosorbent (Mehta and Gaur, 2005).

When the pH value was increased from pH 3.0-8.0, there was an increase in Cd²⁺ sorption which could be attributed to the increase in electrostatic attraction between positively charged Cd²⁺ ions and negatively charged binding sites of functional groups present on the cell surface such as carboxylate, phosphate and amino groups (Adhiya et al., 2002). Solisio et al. (2008) was found the sorption efficiency of Cd with *S. platensis* was at pH 8.0. However, there was a decrease in the uptake of Cd at alkaline pH 10 which could be attributed to the formation of a metal complex such as Cd-OH which might have competed with functional binding sites for metal ions and reduced the availability of Cd sorption (Rao et al., 2005; Kumar et al., 2006). Table 2 represents biosorption of lead by dried mix diatoms.

The results indicated to removing it in concentrations 0.5, 1 and 2 ppm with percentage removal 83.6-97, 88.6-91.5 and 91.5-96.6%, respectively and about the amount of residue of the concentrations 0.5, 1 and 2 was 0.08-0.01, 0.1-0.05 and 0.1-0.06 mg/L, respectively. The best contact time occurring in which the highest percentage removal 100% was 4 h from (0.2 g) in 0.5 ppm. The present results were agreed with the study of Quintelas et al. (2007).

Table 1: Biosorbent of Cd ions by dried diatoms

| Percentage removal | Residue concentration (mg/L) | Percentage removal | Residue concentration (mg/L) | Percentage removal | Residue concentration (mg/L) | Cd
<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>88.5</td>
<td>0.230</td>
<td>84</td>
<td>0.160</td>
<td>98.2</td>
<td>0.009</td>
<td>1H</td>
</tr>
<tr>
<td>96.5</td>
<td>0.070</td>
<td>92</td>
<td>0.080</td>
<td>100</td>
<td>0.000</td>
<td>4H</td>
</tr>
<tr>
<td>97</td>
<td>0.060</td>
<td>100</td>
<td>0.000</td>
<td>100</td>
<td>0.000</td>
<td>6H</td>
</tr>
<tr>
<td>94</td>
<td>0.120</td>
<td>92</td>
<td>0.080</td>
<td>99.4</td>
<td>0.083</td>
<td></td>
</tr>
<tr>
<td>91.5</td>
<td>0.170</td>
<td>85</td>
<td>0.150</td>
<td>90</td>
<td>0.050</td>
<td>1H</td>
</tr>
<tr>
<td>94</td>
<td>0.120</td>
<td>97</td>
<td>0.030</td>
<td>99</td>
<td>0.004</td>
<td>4H</td>
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<tr>
<td>94.5</td>
<td>0.110</td>
<td>100</td>
<td>0.000</td>
<td>100</td>
<td>0.000</td>
<td>6H</td>
</tr>
<tr>
<td><strong>93.3</strong></td>
<td><strong>0.133</strong></td>
<td><strong>94</strong></td>
<td><strong>0.060</strong></td>
<td><strong>96.3</strong></td>
<td><strong>0.018</strong></td>
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</tr>
<tr>
<td>94.5</td>
<td>0.110</td>
<td>86</td>
<td>0.140</td>
<td>74</td>
<td>0.130</td>
<td>1H</td>
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<tr>
<td>96.5</td>
<td>0.070</td>
<td>66</td>
<td>0.040</td>
<td>98</td>
<td>0.010</td>
<td>4H</td>
</tr>
<tr>
<td>100</td>
<td>0.000</td>
<td>100</td>
<td>0.000</td>
<td>100</td>
<td>0.000</td>
<td>6H</td>
</tr>
<tr>
<td>97</td>
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<td>94</td>
<td>0.060</td>
<td>90.7</td>
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<td>87</td>
<td>0.130</td>
<td>88</td>
<td>0.060</td>
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<tr>
<td>97.5</td>
<td>0.050</td>
<td>87</td>
<td>0.130</td>
<td>99.6</td>
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<tr>
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<td>87</td>
<td>0.130</td>
<td>100</td>
<td>0.000</td>
<td>6H</td>
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<tr>
<td><strong>96.5</strong></td>
<td><strong>0.070</strong></td>
<td><strong>87</strong></td>
<td><strong>0.130</strong></td>
<td><strong>95.9</strong></td>
<td><strong>0.020</strong></td>
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<tr>
<td>86</td>
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<td>91.5</td>
<td>0.170</td>
<td>98</td>
<td>0.020</td>
<td>95</td>
<td>0.020</td>
<td>4H</td>
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<tr>
<td>93.5</td>
<td>0.130</td>
<td>100</td>
<td>0.000</td>
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<td>6H</td>
</tr>
<tr>
<td><strong>90.3</strong></td>
<td><strong>0.193</strong></td>
<td><strong>91.67</strong></td>
<td><strong>0.083</strong></td>
<td><strong>93.3</strong></td>
<td><strong>0.0333</strong></td>
<td></td>
</tr>
</tbody>
</table>

Bold values are significance
As well as for nickel, the data showed the decreasing for 0.5, 1 and 2 ppm were 0.1-0.02, 0.06-0.002 and 0.1-0.07 mg/L, respectively with percentage removal to the same concentrations was 78.6-96, 93.6-96.8 and 93.6-96.5%, respectively (Table 3). The best percentage removal was 96.8 from (0.4 g) in 1 ppm. Our results indicated to the most metal removal was cadmium followed by and lead finally nickel (Cd>Pb>Ni). These results were agreed with Cherifi et al. (2016) who studied the biosorbent of Cd and Pb by diatoms (Navicula) and observed efficiency of biosorption metals high ratio. The lower sorption of nickel in comparison to lead and cadmium could be related to the fact that nickel has much lower selectivity coefficient for bonds with aminophosphoryl, than lead which features the highest one reflected in the highest values of binding.

Nickel belongs to the intermediate metals with high affinity not only to phosphoryl, -SO3-, R-NH2 and Rz-NH but mainly to -COO groups which it likely shares with lead (Sari and Tuzen, 2007).

**Immobilized the dry diatoms by calcium alginate:** Powdery mixed of diatoms *N. incerta* and *N. paelea* were added to calcium alginate and used as beads for
Fig. 1: The spectrum of adsorption heavy metals by the dried diatoms

Table 4: Immobilized dry diatoms to remove Cadmium ions in Temp 25, pH 8.5

<table>
<thead>
<tr>
<th>Metal/ml</th>
<th>0.5 ppm</th>
<th>%</th>
<th>1 ppm</th>
<th>%</th>
<th>2 ppm</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.04</td>
<td>92</td>
<td>0.07</td>
<td>93</td>
<td>0.12</td>
<td>94.00</td>
</tr>
<tr>
<td>4</td>
<td>0.009</td>
<td>98</td>
<td>0.08</td>
<td>97</td>
<td>0.09</td>
<td>95.50</td>
</tr>
<tr>
<td>24</td>
<td>null</td>
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<td>0.03</td>
<td>97</td>
<td>0.06</td>
<td>97.00</td>
</tr>
<tr>
<td></td>
<td>0.0245</td>
<td>97</td>
<td>0.0433</td>
<td>96</td>
<td>0.09</td>
<td>96.00</td>
</tr>
</tbody>
</table>

Table 5: Immobilized dry diatoms to remove Nickel ions in Temp 25, pH 8.5

<table>
<thead>
<tr>
<th>Metal/ml</th>
<th>0.5 ppm</th>
<th>%</th>
<th>1 ppm</th>
<th>%</th>
<th>2 ppm</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.03</td>
<td>94</td>
<td>0.08</td>
<td>92</td>
<td>0.32</td>
<td>98.50</td>
</tr>
<tr>
<td>4</td>
<td>0.03</td>
<td>94</td>
<td>0.07</td>
<td>93</td>
<td>0.15</td>
<td>92.50</td>
</tr>
<tr>
<td>24</td>
<td>0.02</td>
<td>96</td>
<td>0.07</td>
<td>93</td>
<td>0.07</td>
<td>96.50</td>
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<td>0.02667</td>
<td>95</td>
<td>0.07333</td>
<td>93</td>
<td>0.18</td>
<td>92.50</td>
</tr>
</tbody>
</table>

Table 6: Immobilized dry diatoms to remove lead ions in Temp 25, pH 8.5

<table>
<thead>
<tr>
<th>Metal/ml</th>
<th>0.5 ppm</th>
<th>%</th>
<th>1 ppm</th>
<th>%</th>
<th>2 ppm</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.004</td>
<td>99</td>
<td>0.08</td>
<td>92</td>
<td>0.07</td>
<td>96.50</td>
</tr>
<tr>
<td>4</td>
<td>null</td>
<td>100</td>
<td>0.06</td>
<td>94</td>
<td>0.04</td>
<td>98.00</td>
</tr>
<tr>
<td>24</td>
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<td>100</td>
<td>0.07</td>
<td>95</td>
<td>0.03967</td>
<td>98.00</td>
</tr>
</tbody>
</table>

removing Ni, Pb, Cd in and chosen pH 8.5 for the beads maintenances. This was considered as biofilter for heavy metals removal. The result showed high removing rate in all concentrations and in all metals (Table 4-6).

Removal percentages for concentrations 0.5, 1 and 2 were Cd 97, 69 and 96%, respectively and lead were 100, 95 and 98%. Either for nickel the rates were 95, 93 and 92%. The most high percentage removal was in lead followed by cadmium and followed by nickel. Present results were agreed with Dungwa who indicated that the sorption of Cd$^{2+}$, Ni$^{2+}$, Pb$^{2+}$, Zn$^{2+}$, Fe$^{3+}$ and Cr$^{6+}$ by a dried diatom Nitzschia closterium was investigated in a batch system. The experiments have been performed for the chosen temperature of 25°C and operational conditions such as constant agitation and pH 8 except for Cd where at pH 2.0. The sorption of all metal ions rapidly reached equilibrium within 100 min. The maximum sorption capacities of the various metal components on N. closterium biomass could be prioritized in order from high to low as: Pb$^{2+}$>Cd$^{2+}$>Ni$^{2+}$>Zn$^{2+}$>Cr$^{6+}$>Fe$^{3+}$.

Karem et al. characterize the ability of the dried diatom Planorhidiuim lanceolatum to biosorb Cadmium (Cd) from aqueous solutions he noticed the maximum uptake of metals was obtained at pH 8.0 for Cd. An increase in the biosorption of P. lanceolatum was observed with an increase in temperature from 15-250°C.

All the experiments for heavy metals removal indicated that the dry mass of diatoms either immobilized or as free without immobilizing by calcium alginate or other bindings were the most effective in removing metals during few 1-24 h that’s could because the dry diatoms were not affected by toxic metals, not required culture media or purifications, it’s could used for many times in removing process, also is stored for a long time without any corruptions and the most useful as excellent filters because the high surfaces.

**Active groups for N. incerta and N. pala**: FTIR technology was depended for detecting the active groups or active sites on cells surfaces for the two diatoms (Fig. 1). Detecting the specific groups which responsible for biosorption of lead, nickel and cadmium that represented by (amid I and II)-NH$_2$ and -SH (Sulphydryl)
functional groups in amino acids and glycoprotein of
*N. palea* where’s -CONH₂, -CONH, alcohol, Carboxtic
carbonyl groups were found in lipids acids and
polysaccharids in cell wall of *N. incerta*. Cadmium and
lead uptake clearly binding with -NH₂ and -SH functional
groups. It was similar to the results which get by
Alejandro *et al.* and Zhang *et al.* This technological
process had a quick detect on the active groups which
needs very small amount of sample also, the machine not
corrupt the sample (Kumar *et al.*, 2009) (Fig. 1).

**CONCLUSION**

Powder of dried *N. palea* and *N. incerta* was showed
the highest percentage removal for Cd, Pb and Ni within
few hour’s efficiency. Immobilizing powdery mixed of
dried diatoms *N. incerta* and *N. palea* by calcium alginate
was used as beads (biofilter) to remove metals recorded
the highest percentage removal than other method.

**RECOMMENDATIONS**

Both diatoms with good efficiency to remove heavy
metals. 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. A study would suggest
enhancing the scientific attention towards using powder
of dry diatoms in the probable controlling other pollutants
such as heavy metals

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using a metal-based titration technique. J. Colloid
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