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Investigating the Purification of Contaminated Water Supplies by Heavy Metals Such as Cupper and Cadmium Using Diatom Algae

Sara Saadatmand[1] & Atefeh Niazi[1]

[1] Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran

Correspondence: Sara Saadatmand, Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran. Tel: 98-224-486-5323. E-mail: s_saadatmand@srbiau.ac.ir

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Abstract

Using copper and cadmium decontaminating plants has been one of the most important ways in purification of water supplies in recent years. The present study was conducted to investigate the possibility of using monocellular diatom alga (*Nitzchia*) to decontaminate water from copper and cadmium heavy metals. So far, the researchers used four different copper and cadmium heavy metal consistencies of 0.5, 2, 8 and 16 ppm to treat alga. Together with investigating the concentration of the metals absorbed by alga after 14 days of incubation, its growth, chlorophyll a, carotenoids, soluble sugars, and superoxide dismutase and catalase enzymes were also studied. The results then proved a high potential for algae to decontaminate water from Cu and Cd, while the top decontamination rate was found at the highest primary concentration (16 ppm). And also at all treatments, except 0.5ppm and 2 ppm, a descending order in copper growth was apparent, while for chlorophyll a, although it increased in all copper treatments, it had a descending order in all cadmium treatments. The concentration of the carotenoids was highly irregular, although the highest amount was at 8 ppm. A growth was also apparent in reductant glucose measures and the activity of catalase and super oxide dismutase enzymes.

Keywords: diatom, *Nitzchia*, heavy metals, copper, cadmium

1. Introduction

More than 70% of the world surface is covered by water sources such as oceans and seas that are different from lakes, rivers and streams in some ways or another, although, life starts in all of those ecosystems (brine and freshwater) from their producer plants and continues to other creatures. Algae, not different from the other members, are the most simplistic chlorophyll containing members of those ecosystems. Although unlike the other plants, algae have no roots, leaves or pedicels. Such a primary construction is called thallus with the monocellular types not larger than some microns as the smallest member.

Heavy metals are natural substances having a density of more than 5 g cm^{-3}. Accordingly, 53 out of 90 known chemical elements are considered as heavy metals (Weast, 1984; Schytzendubel & Polle, 2002), although not all of them are biologically important. Based on their solvency rate in different physiologic conditions, living cells might have access to 17 heavy metals. According to Greger (1999), because these metals do not exist in the environment, they can't be eliminated from nature. Although there are such natural reasons as volcanic activities that might increase these heavy metals in the environment, there are also some other unnatural reasons caused by human activities such as mining, burning fossil fuels, metal industries, phosphate fertilizers, pesticides, sewage and waste materials that contaminate water, earth and air (Porida et al., 2003; Nalimova et al., 2005; Sebastiani et al., 2004). As a result, filtering these contaminants by different methods, finding new organisms having the ability to save bigger and bigger consistencies of these contaminators inside, and keeping them in safe places is highly important.

Microalgae are aquatic organisms with molecular mechanisms, the thing that let them separate necessary heavy metals from those with less importance. Different decontamination processes conducted by algae, with a more concentration on those containing metallothioneins or phytochelatins are investigated here. As a result, microalgae are known as an acceptable technologic organism to purify water contaminated by heavy metals (Perales-Vela et al., 2006).

Diatoms are monocellular, scarcely thalloid, materials having brown, golden or golden brown colors. They are

members of an important group known as epipelic and phytoplankton that live at both freshwater and brine. Diatoms that have siliceous walls, a high density and consequently sink in water, use such methods and characteristics as spiral movements, very small cell dimensions, or having oil and fat in their cell construction to help them stay on top of water, and cell frills such as chitin complements (such as *Thalassiosi rafluvitalis*) to increase friction and decrease sinking in water (McCormick, 1994). Nowadays, diatoms are known as biological indicators of water quality and as an acceptable organism at ecosystems by which we can identify environmental disorders (Lenat et al., 1994).

Heavy metals, naturally or unnaturally, have the potential to make different forms of reactive oxygen species (ROS) (Dipierro et al., 2005; Laspina et al., 2005; Rucinska & Gwozdz, 2005; Demirevska-Kepova et al., 2004; Kopyra & Gwozdz, 2003; Milone et al., 2003; Foyer et al., 1997). In cases that an herbaceous cell can not, by increasing its antioxidant activities, stop increasing ROS, the imbalance between the creation and ROS oxidation metabolism can not be controlled and the chain reactions lead to oxidative stress (Mahalingam & Fedroff, 2003; Bolwell et al., 2002). As a result, the damage to the cells under heavy metals' stress depends on free radical, ROS, and the plants' decontamination mechanisms (Dietz et al., 1999).

Some research findings are related to oxidative damages caused by heavy metals, lipid peroxidation, or oxidative damages to nucleic acids, proteins, chlorophyll and also controlling photosynthesis. One of the most important protective mechanisms of diatoms against heavy metals' stress is chelated the metals inside cytosols using highly active ligands including phytochelatins, metallothioneins, amino acids and organic acids (Rauser, 1999; Clemens, 2001). Phytochelatins (PCs) are a group of peptides complexible to heavy metals with a general structure of (γ-GluCys)n-Gly, in which n = 2 − 11. In the presence of some heavy metals, especially cadmium, the PC of the synthase activates and makes PCs out of glutathione (Cobbet, 2000). Then, the metal complex (PC) is transferred into the vacuole. This PC-Cd transmission to the vacuole is provided by Cd^{+2}/H^{+} antiporter and an ABC transporter related to ATP which exists in tonoplast. Attaching sulfide ions into PC-Cd complexes also increases its stability (Ruley et al., 2004). Cadmium is one of the most poisonous metals and causes the highest consistencies of polypeptide PCs attached to metals. It is seen in *Thalassiosira weissflogii* sea diatoms, in which exists a cadmium transpiration of *T.weissflogii* high mineral cadmium consistencies. This transpiration is so much that more than half of the absorbed cadmium is returned to the cultivation medium. In high cadmium consistencies, PC transpiration out of the cells also appears and continues until the exterior cadmium concentration decreases, preventing cadmium and PC transpiration. It is believed that *T. weissflogii* PC complex, releases cadmium as an antitoxin mechanism. Although this complex doesn't seem to be stable in sea water and out of the cell body, PC-cadmium transpiration can be an important survival strategy that helps phytoplankton's survival in contaminated waters by heavy metals (Lee, 1996).

In addition to the effect of heavy metals on enzymatic antioxidants, heavy elements influence on a big number of compounds involving in an oxidative defensive process, while have a light molecular weight. Two different reactions are seen while open to heavy metals. Not only the presence of metallic ions decreases the storage of antioxidants having light molecular weights, an oxidative stress may also activate enzymes that take part in those antioxidants' biosynthesis (Dietz et al., 1999). Non-enzymatic antioxidant systems contain two system redox, ascorbate (ASA) and glutathione (GSH) together with tocopherol, flavonoids, carotenoids, some phenyl compositions, polyamines, and etc.

2. Methods

2.1 Diatom Samples Collection and Identification

Water samples were taken from Tehran, Iran. The samples were investigated looking for diatoms, and those containing such materials were chosen to cultivate and use in the study. To identify the live samples of diatom, they were studied by a microscope having 100 magnifying power (Figure 1).

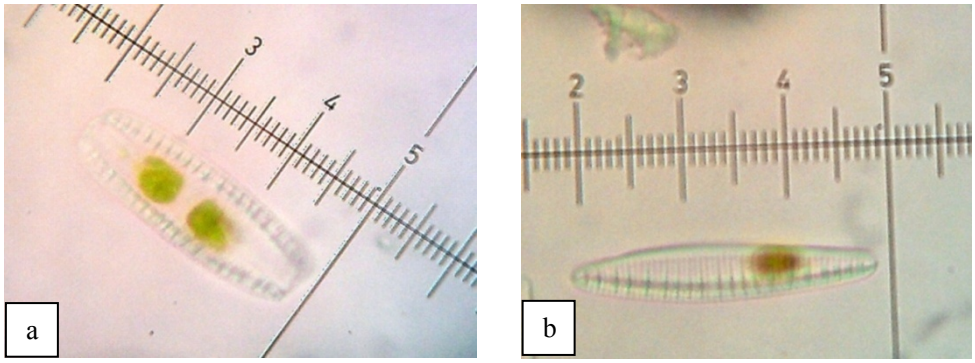

Figure 1. Diatom's microscopic image (100 times magnified), (a): valvic side image, (b): belt side image

Then, a fixed preparation was prepared using Werff's (1995) method. Here, the samples were heated for one hour at 80 ºC with 37% H_2O_2. After that, the saturated solution of $KMnO_4$ was added to form precipitation. By adding a little HCl rarified in H_2O_2, the precipitates were separated and investigated using a light microscope (Werff, 1955) (Figure 2).

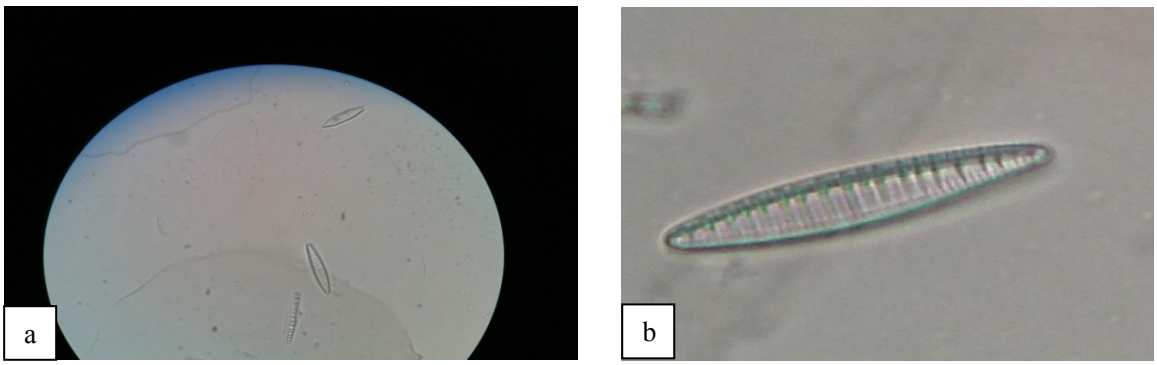

Figure 2. Diatom microscopic image, (a): microscopic image (100 times magnified), (b): details of the image presented at slide a

SEM electron microscope was used to identify diatom samples and to see the interior design of the algae wall (Figure 3).The required diatom material also was prepared for the rest of the investigation using the visual identification key of diatoms at (http://westerndiatoms.colorado.edu) *Nitzschia*.

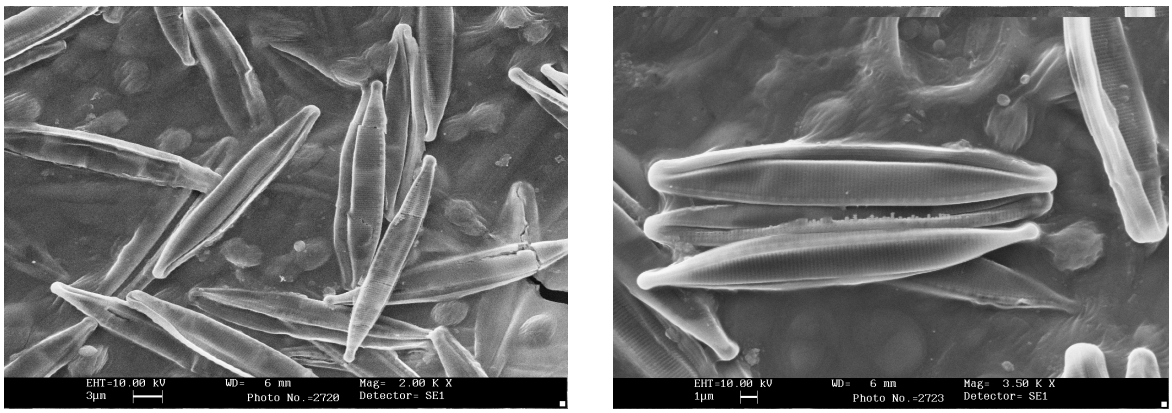

Figure 3. SEM electronic microscope image of diatoms concentration

2.2 Preparing Cultivation Setting and Samples

The Chu's cultivation medium was used to cultivate diatom samples (Bold & Wynne, 1978). The samples, then were distributed under a Laminar flow hood and among 500 ml flasks, and supplied with a 250 ml Chu's Medium sterilized liquid cultivation. After that, the flasks input were covered by cotton, while they were placed in suitable locations under 12-12 photoperiod hour and at 25 ºC to grow the samples. After 14 days, a subculture was prepared and relocated at their immediate place. This process continued till the samples became perfectly pure, ready to grow and form the major cultivations and treatments.

2.3 Copper and Cadmium Treatment

Completely pure and standard flasks were used at the major cultivation process. Here, in the presence of a control, the algae were open to four different cadmium and four copper consistencies of 0.5, 2, 8, and 16 ppm. It should also be mentioned that there were 9 repetitions for each treatment in 14 days. Then, the samples growth were compared.

2.4 Measuring the Algae Growth Rate by Spectrophotometer

There are different methods to measure the growth rate of an alga. For example, spectrophotometery method which is a completely new and more accurate one while comparing to the other methods. During the growth rate measurement at this method, some cubic centimeters (CC) of cultivation mediums are taken and their light absorption rate is investigated by spectrophotometer at 530 nm light wave. It should be mentioned that to measure the real absorption rate, the researchers needed to prepare standard solutions for spectrophotometer. Therefore, some CC of the cultivation medium was centrifuged at 2000 g spins for 10 minutes. The resulting liquid would be the standard solution. It is also clear that the samples with the higher absorption level are those having a higher growth rate. Researchers at the present study used this method after 14 days of copper and cadmium insemination to the samples.

After measuring the samples growth and investigating them by a light microscope, they were centrifuged at 1500 g spin for 5 minutes, then separated completely from the cultivation medium and kept at -20 ºC for use at comparisons.

2.5 Pigments Investigation (Examining Chlorophyll a and Carotenoids)

Arnon (1949) method was used to investigate the ingredients of chlorophylls, and Davis (1976) method was used to study carotenoids. First, 0.05 gr diatom alga was ground and homogenized with 80% acetone (adding some calcium carbonate while grinding prevents magnesium to exit from chlorophylls ingredients). Then, the resulting homogeneous combination was filtered using number 2 Wathman. To investigate chlorophyll a and carotenoids, the liquid absorption at 645, 663 and 480 nanometer wavelengths was measured in the presence of 80% acetone treatment. And then chlorophyll a and carotenoids were measured.

2.6 Measuring Reducing Sugars by Nelson and Somogyi Method Reductant

As much as 0.025 gr alga was taken, 5ml of water was added and the combination was grinded and filtered by filter paper. Then, 2 ml of the resulting liquid was moved into a test tube containing 2 ml of copper sulfate (40 gr sodium carbonate in 400 C H_2O_2 + 7.5 gr tartaric acid + 4.5 gr hydrous copper sulfate at the final volume of ml) and heated for 8 min at 100 ºC. Cu^{2+} (reduction) is the basis for glucose measurement here. Two ml of phosphomolybdic acid was added to the cool test tubes (70 gr phosphomolybdic acid and 10 gr sodium in 70 ml of 5% soda for 40 min at 40 ºC + 250 ml orthophosphoric acid) while putting them on a mixer and increasing the volume to one liter. Then, the test tubes were shook very fast till their colors turned into blue, and their absorption rate was measured at 600 nanometer using spectrophotometer. The glucose concentration was also measured using the standard curve of glucose based on micrograms on liter. Here, to draw a 500 mg l^{-1} glucose curve, different consistencies of 0.2, 0.4, 0.6, 0.8, 1, 3, 5 and 7 mg l^{-1} were prepared and 2 ml per each of those solutions were taken, while all were involved in the same process.

2.7 Catalase Enzyme Activity Measurement

Catalase enzyme activity rate measurement was performedwith the method proposed by Pereira (2002). Here, the catalase enzyme activity rate together with H_2O_2 reduction level was investigated by studying light wave changes at 240 nanometers for 1 minute. First, 0.15 gr alga was prepared and ground at 1.5 ml of 50 mill molar potassium phosphate buffer (7.5 pH) containing 1% of 1 millimolar polyvinyl pyrollidone (PVP). All of the extraction phases were done in the presence of ice and then, 3.5 ml H_2O_2 was combined with 50 ml aquapura before taking 70 microliter and adding 2.83 ml potassium phosphate buffer (without PVP and EDTA). And finally, 100 microliter of the resulting substance was added and centrifuged at 4 ºC, 2000 spin for 20 minutes.

Then, the clear solution formed was used to measure the catalase enzyme activity. The absorption rate was recorded immediately after 1 minute at 240 nanometers. The control solution was prepared here by adding 2.83 mm potassium phosphate buffer (without PVP and EDTA) to 70 microliter of 2% H2O2, and then adding 100 microliter potassium phosphate buffer containing PVP and EDTA (Pereira et al., 2002).

2.8 Dismutase Superoxide Enzyme Activity Measurement

Dismutase superoxide enzyme activity was measured based on a method proposed by Giannopolitis et al (1997). Here, 3 ml of reaction solution was containing 50 mm potassium phosphate buffer (pH 7.8), 13 mm of 75 μM nitroblute trazolium, 2 μM riboflavin, 0.1 EDTA and 100 microliter enzyme extraction. The reaction started by removing the aluminum foil and exposing the samples to 5000 LUX light for 15 minutes. Then, the samples absorption rate was recorded at 560 nanometers immediately. Two control samples were used here containing no enzyme extraction, one of which was shed by light for 15 minutes, while the other one received no light.An enzyme activity unit is composed of some enzyme which prevents 50% of NBT (reduced) at 560 nanometers (Giannopolitisetal., 1977).

2.9 Heavy Metals Measurement (Copper and Cadmium) Absorbed by Alga

Researchers at the present study measured the metals substances by solving the samples in acid kept in closed tubes, using microwave and atomic spectrophotometer.

To this end, 0.2 gr samples, completely dried, were taken, specific TFM tubes required were located on a flat area and the 0.2 gram samples were added to the tubes together with 6 ml of 65% HNO_3 and 2 ml of 30% H_2O_2. Then, closed the tubes and located them in microwaves adjusted to the following heat control program:

Step	Time	Temperature	Microwave power
1	10 minutes	200 °C	Up to 1000 Watt
2	10 minutes	200 °C	Up to 1000 Watt

After passing the required time, the samples were examined by an atomic spectrophotometry device to measure copper and cadmium levels.

2.10 Statistical Analysis

The present study was carried out as a completely accidental study with 4 repetitions. Treatments used at this study contained different levels of copper and cadmium. The data analysis variance and mean comparisons of the study treatments was conducted based on Duncan comparative test at 5% chance, data distribution was normal and the data analysis was performed using SPSS program. And finally, excel program was applied to design charts and graphs.

3. Results

There was a significant difference in the average growth of the samples after 14 days of incubation at different doses. In all treatments except 0.5 ppm cu and 2 ppm cu, a descending rate in growth was seen, while the highest growth rate was at 0.5 ppm cu treatment. As a result, diatoms growth was under the influence of copper and cadmium heavy metals in a way that the first one increased and the second one decreased their growth rate (Figure 4).

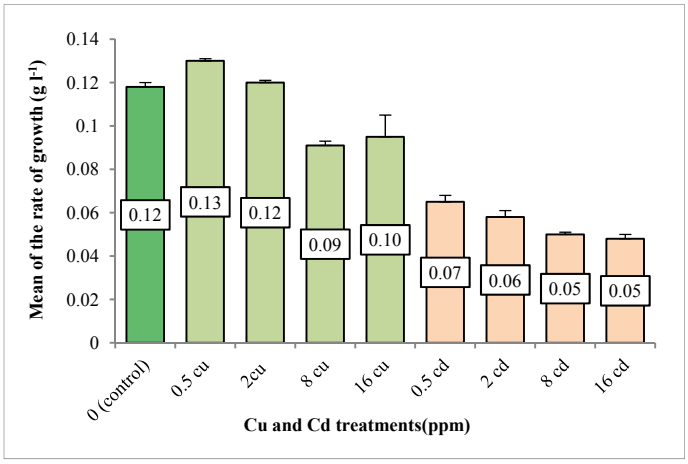

Figure 4. Alga growth after 14 days of different treatments by cadmium and copper

There was a significant difference in chlorophyll a consistencies after 14 days of incubation at different doses. Chlorophyll a measures compared with control, in all copper treatments had an ascending and in all cadmium treatments had a descending growth rate with the highest chlorophyll a concentration at 8 ppm Cu treatment (Figure 5).

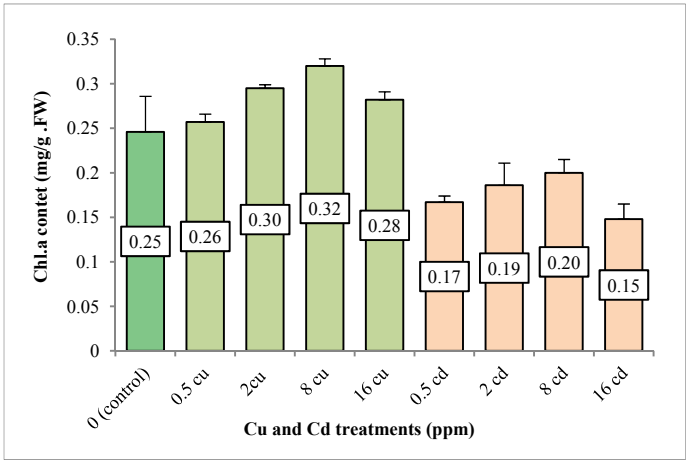

Figure 5. Chlorophyll a concentration in cadmium and copper treatments

There was a significant difference in carotenoid concentration measures after 14 days of incubation at different doses. The highest carotenoid concentration was at 8 ppm cu treatment (Figure 6).

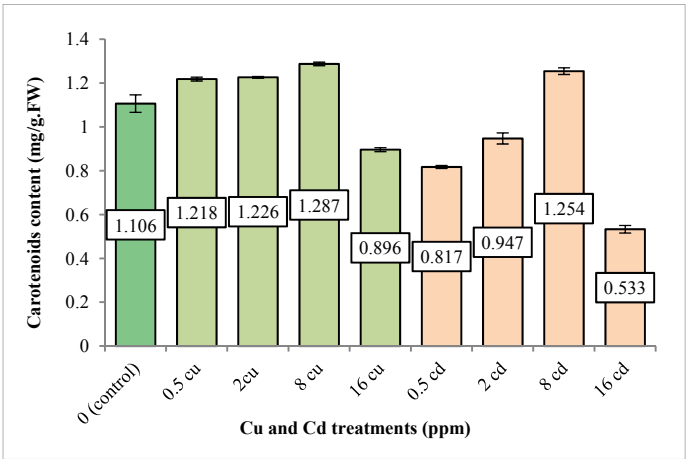

Figure 6. Carotenoid concentration in cadmium and copper treatments

There was a significant difference in reductant glucose concentration measures after 14 days of incubation at different doses. There was also an ascending growth rate in all copper treatments while compared to the control, and the highest soluble sugars concentration average was at 16 ppm cd treatment (Figure 7).

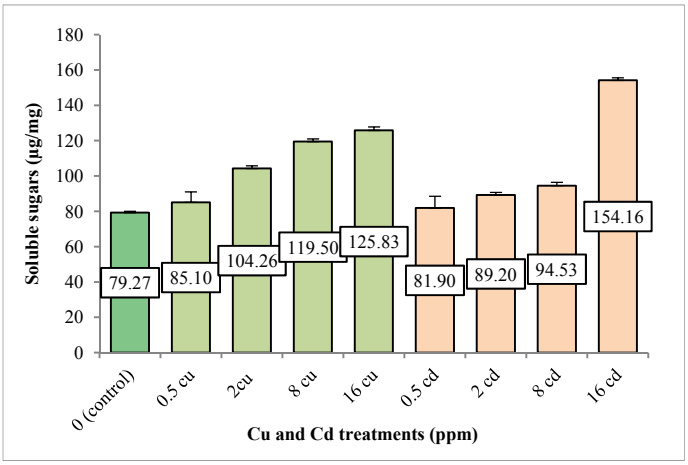

Figure 7. Soluble sugars in cadmium and copper treatments

There was a significant difference in catalase enzyme activity measurement results after 14 days of incubation at different doses. There was also an ascending growth rate in enzyme activities at all treatments while compared to the control, and the highest catalase enzyme activity average after 14 incubation days was at 16 ppm Cd treatment (Figure 8).

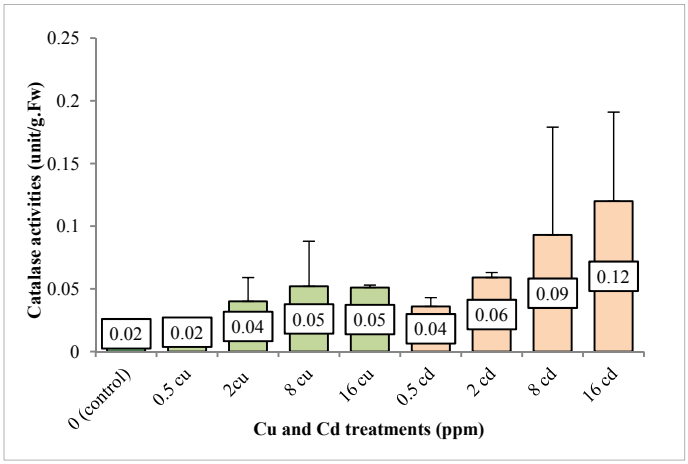

Figure 8. Catalase enzyme activity in different cadmium and copper treatments

There was a significant difference in superoxide dismutase enzyme activity measures after 14 days of incubation at different doses. There was also an ascending growth rate in enzyme activities at all treatments**,** and the highest superoxide dismutase enzyme activity average was at 16 ppm cd treatment (Figure 9).

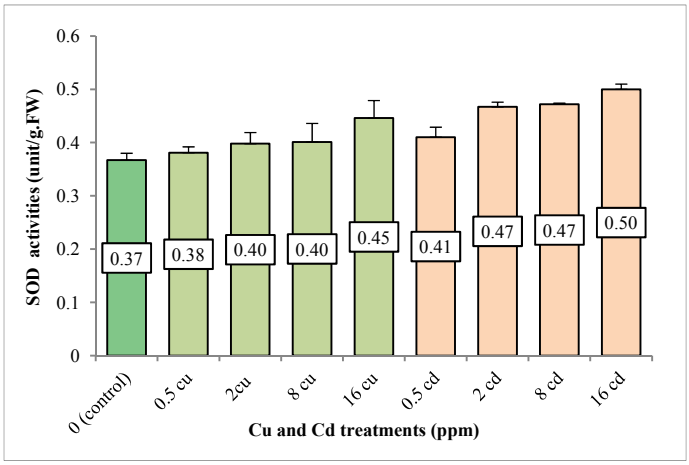

Figure 9. SOD enzyme activity in different cadmium and copper treatments

There was a significant difference in Cu concentration measures (mg l^{-1}) after 14 days of incubation at different doses. The highest Cu concentration measure was at 16 ppm treatment (Figure 10).

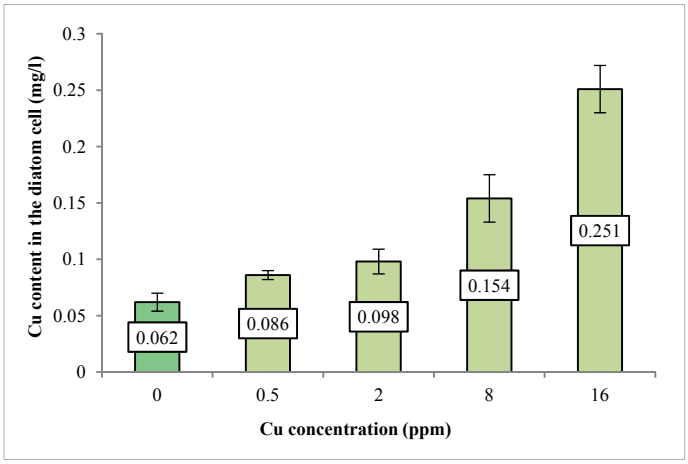

Figure 10. Copper absorption rate by diatom in different treatments

There was a significant difference in Cd concentration measures (mgl^{-1}) after 14 incubation days at different doses. The highest Cd concentration measure average after 14 incubation days was 16 ppm at treatment 8 (Figure 11).

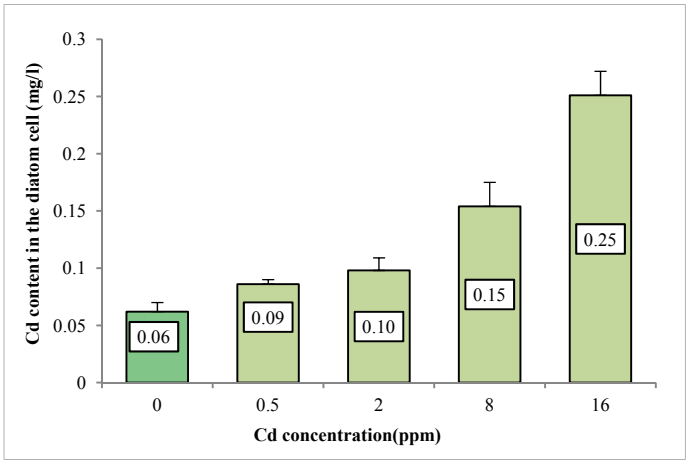

Figure 11. Cadmium absorption rate by diatom in different treatments

4. Discussion

As stated before, Chun's cultivation medium is suitable for algae growth and helps the diatoms to have an acceptable growth rate after 14 days. Copper is a very nutritious substance and an inseparable member of the enzymes being responsible for electron movements speeding up chloroplast reactions in plants (Geatke & Chow, 2003). It should be mentioned that a little zinc, copper, iron, etc. was used at Chun's medium to investigate their effects. As the results indicated, the highest growth rate was recorded at the copper's 0.5 ppm treatment, indicating that copper can improve the algae growth to some extent (2 ppm) (Figure 4). Although, higher than normal consistencies of copper may cause damage on plants tissues (Hall, 2002), like what happened at the present study. High concentration of copper at the plants leaves also may cause changes at processes such as photosynthesis, breathing, enzyme activities, integrity of DNA, etc. that finally lead to some growth issues (Schutzendubel & Polle, 2002; Posmyk et al., 2009).

Regarding cadmium that is not a necessary element for plants growth, a descending growth rate was recorded at all treatments, especially at the 16 ppm one. In the same line with many other studies conducted such as Larbi et al. (1997), Sandalio (2001), or Costa and Spitz (1997) who reported growth failure as one of the most immediate effects of cadmium on plants, the present study also brought about the same results once again.

Heavy metals increase the possibility of the tissues death by increasing lipid peroxidation and producing other

active oxygen species. Photosynthesis also is being affected by cadmium, knowing that effective enzymes on CO_2 stabilization are highly under the influence of cadmium (Sandalio, 2001; Larsson et al., 1998). Extra cadmium not only prevents rubisco enzyme activity that plays a key role in Calvin Cycle (Chaffei et al., 2003), but also causes disorder at breathing, providing and absorption of nourishing elements, nitrogen and sulfate metabolism (Balestrasse et al., 2001; Gussarsson et al., 1996; Haag-kever et al., 1999; Lee & Leustek, 1999). Together with appearing disorders at physiological processes, an increase in cadmium levels at different parts of plants may have negative effects on their growth. While a cell receives excessive amounts of heavy metals, different kinds of reactions are provoked against the stress caused by cadmium. To name a few, we can refer to an increase in proteins with small molecular mass or synthesis of peptides (Nakagamy & Hirt, 2004; Wang & Peverly, 1999).

The cells disorders affected on the plant growth may be caused by such different reasons as losing water, elasticity of the cells' walls (Costa & Spitz, 1997), losing necessary ingredients such as K, Ca, Mg and Fe (Gogorcena et al., 2002; Gussarsson et al., 1996), problems brought about by disorders at photosynthesis processes, breathing and nitrogen metabolism as a result of poisonous cadmium consistencies (Balestrasse et al., 2001; Haag-Kever et al.,1999; Larsson et al., 1998).

Most researchers believe that losing chlorophyll while experiencing a heavy metal stress such as the stress caused by cadmium is the main reason of disorders appeared in chlorophyll synthesis in plants, the thing that was proved by Mike et al. (1992) for wheat. The reason is that the plants start absorbing cadmium instead of iron, place it instead of Mg in chlorophyll molecules that prevent chlorophyll synthesis (Polle, 2001). In stressful conditions also the chlorophyll molecule death is certain (Wo'jcik et al., 2006).

Cadmium stress also decreases carotenoids numbers. It is caused by non photochemical suppression of carotenoid, chlorophylls, and finally led to disorders in their structure. Carotenoids may also play an antitoxin role for chlorophylls and decrease the poisonous effects of free radicals, as an example, reacting with chlorophylls to prevent forming free oxygen radicals. It finally leads to their death when acting as a defensive system to protect against oxidative stress imposed on the process (Sanita di Toppi & Gabbrielli, 1999). Losing carotenoid is actually a strategy applied by plants to resist against the oxidative stress imposed by cadmium. At the present study also the samples inseminated by cadmium 16 ppm had which the highest rate of carotenoid loses while compared with the other consistencies of copper and cadmium.

Carotenoid and green pigments decrease during the process as well as antioxidant enzymes increase caused by adding heavy metals consistencies proves the relationship between free radicals production and heavy metals increase. As a result, while a non-enzymatic defensive system becomes weak, the enzymatic system is being activated and helps the plants by opposing free radicals. It also worth mentioning that as proposed by Nyitrai et al. (2003), feeding lighter consistencies, and a combination, of cadmium, massicot, nickel and DCMU to plants or spraying the mixture on their leaves incorporates to improve chlorophyll synthesis process.

A descending rate for green pigments and chlorophyll a production in cadmium treatment, and an ascending rate of that in the copper treatment were seen at the present study, proving an increase in oxidative stress.

To protect themselves against oxidative damages, plants are equipped with a wiper system designed to operate against free radicals. The system contains antioxidant enzymes such as catalase, peroxide, dismutase peroxide and nonenzymic defensive systems like ascorbate, glutathione (Mittler et al., 2004). As presented in Figures 8 and 9, catalase and dismutase superoxide antioxidant enzymic activity increases while being influenced by cadmium and copper stress. Although it should be mentioned that an ascending rate was more apparent in cadmium than copper stress, while the highest point in both enzymes recorded measures was at cadmium 16 ppm treatment.

Increased consistencies of copper in cells leads to H_2O_2 radical hydroxyl production by stepped up reaction with a metal named Haber-Weiss reaction (Palma et al., 1987). Protective mechanisms formed to react against active oxygen types also might, because of high consistencies of heavy metals, increase activities of antioxidant enzymes such as dismutase superoxide and peroxidase (Bueno & Piqueras, 2002), although effective elimination of superoxide and hydrogen peroxide needs an effective activity of a lot of antioxidant containing enzymes. Here, superoxide immediately turns into H_2O_2 under the effect of SOD (Bowler et al., 1992), and then H_2O_2 is being broken into H_2O and H by catalase (Noctor & Foyer, 1998).

Plants have a limited protective power while heavy metals stress increases. A lot of research has proved that high consistencies of heavy metals commonly decreases antioxidant enzyme activities (Polle & Schutzendubel, 2002). Autoxidation and Fenton also seriously weaken enzymatic defensive systems. For example, catalase enzyme activity is being stopped immediately by O_2 superoxide radicals, or hydroxide radicals stop dismutase superoxide

enzyme -2n-Cu activity (Casano et al., 1997). As a result, enzyme activity at lower concentrations of heavy metals increases, while it starts to decrease slowly at higher concentrations (based on the plant type) (Cao et al., 2004). It should also be mentioned that a lengthy influence period of heavy metals at first increases enzyme activities, especially peroxides, while after sometimes decreases those activities (Qadir et al., 2004). An increase in heavy metals, especially higher concentrations, stop catalase enzyme activity (Posmyk et al., 2009, Choudhary et al., 2007). Descending dismutase superoxide antioxidant enzyme activity rate at higher concentrations of heavy metals such as cadmium is possibly a result of its deactivation by excessive active oxygen production or disorders caused by synthesizing heavy metals instead of dismutase superoxide (dismutase superoxide).

As presented at Figure 4, reductant sugar measures increases while being stressed by cadmium and copper heavy metals, especially at 16 ppm treatment. The reason might be descending breathing rate and ascending insoluble sugars catalyzer enzyme activities, such as anvertase and sucrose synthesis, which decreases glucose use and increases its production (Shah & Dubey, 1998; Verma & Dubey, 2001).

Soluble sugars kinds are increased as a result of the imposed stress by heavy metals. Cadmium and copper concentration increased sugars in this alga.

Different conclusions are arrived about the potential effects of stress on glucose concentration in plants. Some researchers believed that glucose increases under stress (Jones & Turner, 1980), while the others have opposite views, saying that it decreases (Hanson & Hitz, 1982) or remains changeless (Morgan, 1992). Results of the present study, in the same line with the results presented by Alaoui et al. (2004) regarding copper treatment, also proved that reductant glucose increases under the toxicity influence of copper and cadmium. Some studies also conducted investigating sea diatom growth (*Amphora coffeaeformis*) under the influence of cadmium and copper. These heavy metals influence on the mentioned diatom's parameters of biochemical combination and its growth was measured, while the second parameter was measured by the diatom's chlorophyll content and proved its growth. Results then indicated that heavy metals significantly decreased carbohydrate, protein, amino acid and lipid rates (Anantharaj et al., 2011).

As presented in Figures 10 and 11, while cadmium and copper concentrations were increased in diatom cultivation medium, the alga absorbed more proportions of the heavy metals. As a result, diatom has a high capacity in metal absorption and duplication, the characteristics that increases this microscopic biomes capability in decontaminating wastewater. These diatoms should be identified to solve the problems caused regarding metal poisoning in the water ecosystem contaminations. As a result, complementary investigations should be carried out in industrial scale to decontaminate ecosystems from heavy metals.

5. Conclusion

Due to industrialization and addition of industrial waste water in lakes and rivers, the use of biological methods to clean water has been of interest to researchers. This study has been indicated effects of *Nitzchia* diatom on heavy metal extraction from water. *Nitzchia* is being in city's mere be able to stay in metallic stress and storage a lot of cu and cd ingredient. Therefore, it suggested that *Nitzchia* is a prepare candidate to purgation and cleaning up of industrial swages beside other systems.

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