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Uptake of Cadmium by the Green Alga *Scenedesmus quadricauda* in the Presence of Selenium Nanoparticles

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Abstract: The observed nanoparticles consisted of elemental selenium as determined by X-ray spectroscopy (XRD) analysis indicated corresponding three intense peaks in the whole spectrum of 2Ɵ values ranging from 20 to 70. The green alga *Scenedesmus quadricauda* was incubated with cadmium, selenium and Selenium nanoparticles (Se-NPs) singly and in combination. In all treatments Selenium (Se) has counteract the toxicity of cadmium. The uptake of Cd^{2+} was reduced by selenium nannoparticales (0.02, 0.04mM). The growth rate was inhibited by the treatment of *S. quadricauda* with Cd^{2+} alone, however, in all selenium or nanoparticles containing culture *Scenedesmus* exhibited higher rates of growth. The photosynthetic pigments (Chl. *a*) as well as other metabolites of *S. quadricauda* cells seem to be the determinant in this work. Se-NPs variably abolished the effects of the Cd toxicity. The maximal positive effect of Se-NPs was found at a concentration of 0.04 while with lower concentration (0.01) it was negligible. The study provides a new insight into the impact of Selenium nanoparticles on *S. quadricauda*, especially with regard to its detoxicification of Cd^{2+}, bioaccumulation and bioremediation potential.

Keyboard: *Scenedesmus quadricauda*, nanoparticles, Selenium, Cadmium.

1 Introduction

Heavy metals, such as lead, copper, cadmium, zinc, and nickel, are among the most common pollutants found in both industrial and urban effluents [1]. Heavy metals affect all biological organisms, especially those in the aquatic ecosystem, in many important ways. Cadmium (Cd^{2+}) is one of the heavy metals most frequently implicated in environmental contamination. This metal is utilized in the manufacture of various products, such as batteries, chipsets, pigments, televisions, and semiconductors [2]. Cd^{2+} can attach to sulfated groups, as well as metalloproteins and metalloenzymes, thereby neutralizing their functions [3].

However, Cd^{2+} has no nutritional value for algae [4]. Algae comprise an essential component of aquatic ecosystems. In particular, green unicellular freshwater algae with high metabolism rates play the basic role in the primary production [5]. Important factors such as the effects of possible adaptation/acclimation to natural conditions could have considerable implications on metal toxicity data [6]. In this concept, Piotrowska-Niczyporuk et al. [7] suggested that phytohormones and polyamine play an important role in algal adaptation ability to metal contamination of the aquatic environment by inhibiting heavy metal biosorption and stimulating antioxidant enzyme activities. On the other hand, aquatic plants have evolved enzymatic (superoxide dismutase, catalase, ascorbate peroxidase) and non-enzymatic (ascorbate, glutathione) antioxidant mechanisms to prevent the oxidative stress caused by metals [8,9]. Algae are important bioremediation agents, and are already being used by many wastewater facilities. The potential for algae in wastewater remediation is however much wider in scope than its current role [10,11].

On the other hand, selenium is an essential trace mineral that functions as an antioxidant and promotes a healthy immune system. Selenium is required in remarkably small amounts, with recommended daily amounts measured in millionths of a gram (micrograms). Selenium is also toxic in larger amounts. Selenium has

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strong anti-cancer effects and is known to help detoxify the body and remove heavy metals including Mercury. In the early 1970's it was discovered that Selenium is incorporated into proteins to produce selenoproteins, important enzymes that are antioxidants (they destroy free radicals and prevent cellular damage). Selenoproteins boost the immune system and help regulate thyroid function.

Nanoparticles have been found in a varying range of applications in the past decade. They are more penetrable, easy vectors and are less toxic in nature [12]. They have unique optical, electrical and magnetic properties. The release of nanoparticles into the environment can be dangerous and toxic too in some cases and the study of the toxicity is known as nanotoxicology [13]. The inherent antimicrobial action of nano-selenium has not been studied before in a commercial scale. According to Eszenyi et al. [12], selenium nanoparticles are very good antioxidant and less toxic than other selenium forms. They do not trigger liver injury and less accumulated in the body than other forms. The production of nanoparticles organically using *Lactobacillus* sp. has been employed before. The biological preparation of nanoparticles is employed to get the nanoparticles of the desired size (100- 500nm). The effect of selenium salts on the growth of marine algae has been studied before and the study showed positive deteriorative effects on its growth [14]. Dash et al. [13] proved that selenium nanoparticles have been found to be less toxic than selenium salts in the environment. Thus, the aimed of this work was to abolish the toxicity of Cd^{2+} on the growth and metabolic activities of the green alga *Scenedesmus* by selenium or its nanoparticles.

2 Materials and Methods

2.1 Biosynthesis of selenium nanoparticles

Biosynthesis of selenium nanoparticles were carried out as described by [15]. Briefly, *Bacillus* sp. was grown in nutrient broth (NB) to an OD 600 nm of 1.0 at 30ºC. One ml of bacterial culture was transferred into fresh NB amended with 2 mM of sodium selenite (Na_2SeO_3). The bacterial culture flasks were incubated at 30ºC with shaking (150 rpm) for 48 hr. Bacterial cells containing red selenium particles were collected by centrifuge at 10,000 rpm and sonicated for 10 min. The released Se-NPs in supernatant that produced extracellulary were centrifuged at 25,000x g for 15 min and washed three times with distilled water. The obtained Se-NPs were used for further study.

2.2 Characterization of selenium nanoparticles

Ultraviolet-visible Spectroscopy (UV-Vis) was performed in a Perkin-Elmer Lambda Spectrophotometer. The studies of size, morphology and composition of the nanoparticles were performed by means of transmission electron microscopy (TEM) operated at 120 KV accelerating voltage (JTEM-1230, Japan, JEOL) with selected area electron diffraction (SAED). Samples for TEM studies were prepared by placing drops of the silver nanoparticles solutions on carbon-coated TEM grids. The obtained images were processed using the software ImageJ. ImageJ developed at the National Institutes of Health (NIH), USA is a Java-based public domain image processing and analysis program.

2.3 Algal strain and growth conditions

The green alga *Scenedesmus quadricauda* (Turpin) Br. was isolated from the River Nile at Assiut, Egypt and identified according to [16]. The culture was standardized at an optical density at 680nm of 0.1and inoculated in sterilized Bold's basal medium [17]. The media were contained different treatments of Cd (0.1), Se (0.04), Se-NPs (0.04), Cd (0.1)+ Se (0.01), Cd (0.1)+ Se (0.02), Cd (0.1)+ Se (0.04), Cd (0.1)+ Se-NPs (0.01), Cd (0.1)+ Se-NPs (0.02) and Cd (0.1)+ Se-NPs (0.04 ppm), prepared using analytical grade cadmium chloride, selenium dioxide and Se-NPs. The cultures were illuminated continuously with fluorescent tubes at a light intensity of 48 $\mu mol.m^{-2}s^{-1}$ for 6 days and they were incubated at a temperature of 25ºC. The cultures were harvested towards the end of the exponential phase. Three replicates were set up for each treatment. The growth of cultures was monitored by determination of the dry weight. The pigment content (Chl. *a*) was estimated spectrophotometrically according to Metzner et al. [18]. The specific growth rate (μ) calculated as chlorophyll *a* was determined using the following formula:

$\mu\ (h^{-1}) = (LnN_2\text{-}LnN_1)/\ (t_2\text{-}t_1)$, where N_2 and N_1 represent the chlorophyll *a* concentration at times t_1 (day 0) and t_2 (day 6), respectively.

2.4 Estimation of proteins and free amino acids

Using the method described by Lowry et al. [19], soluble, insoluble and total proteins were colorimetrically measured at 750nm. Free amino acids were estimated according to Lee and Takahashi [20].

2.5 Estimation of carbohydrates

All carbohydrates, including polysaccharides were spectrophotometrically determined according to Badour [21] using the anthrone sulfuric acid method.

2.6 Estimation of total lipids

The total lipids were determined by the sulfophosphovanilin method [22].

2.7 Determination of Cd uptake by S. quadricauda

The uptake of Cd^{2+} metal in (mg/g dry wt.) was detected.

The cultures were centrifuged at the end of the experiment at 5000 rpm for 20 min. The supernatants were discarded and the residual algal pellets were washed with sterilized distilled water and then the algal biomasses were transferred to known weight. The supernatants were used for the determination of the residual metal ions contents in mg/l. The initial metal ions contents in mg/l were determined in control without algal cell. Supernatants were treated with a mixture of acids such as HNO_3 and HCl in the ratio of 2: 1. The samples were estimated using Inductively Coupled Plasma Emission Spectrometer (ICAP 6200). Metal uptake in (mg/g dry wt.) was calculated according to the equation of Volesky and May-Phillips [23]:

Metal uptake (mg/g) $= V\,(C_i - C_f) / W$

Where, C_i is the initial metal concentration (mg/l); C_f is the final metal concentration (mg/l); V is the volume of the reaction (l); W is the total biomass (g).

3 Results and Discussion

In this work, a study on extracellular biosynthesis of SeNPs by the culture of *Bacillus* sp. was described. Visual observation of the bacterial culture incubation with sodium selenite (Na_2SeO_3) at room temperature in the dark showed a color change from light yellow to red color whereas no color change could be demonstrated in autoclaved bacterial cell or media with $NaSeO_3$ alone (Fig. 1).

Fig. 1. Digital photograph of conical flasks containing the bacteria *Bacillus* sp. supernatant reacted with aqueous solution of 2 mM sodium selenite (Na_2SeO_3). It is observed that the color of the solution turned from colorless/or light yellow to red after 24 h (conical 3), at 30ºC, indicating the formation of Se-NPs. Blank control, (conical 2) containing supernatant only, and (conical 1) containing Na_2SeO_3 only.

Thus, Se (IV) was reduced by a biological rather than a chemical process because no Se (IV) reduction was observed in the broth (Fig. 1) without the addition of bacterial cells. These colour changes were consistent with other studies on Se-NPs synthesis or stabilized by enzymes/proteins or any other stabilizer [15]. In addition, the characteristic red color of the solution was due to the excitation of the surface plasmon vibrations of the Se-NPs and provided a convenient spectroscopic signature of their formation [24]. UV–Vis absorption spectroscopy was also used to confirm the biosynthesis of Se-NPs, a well-defined absorption peak maxima at 285 nm, located between 200 and 300 nm (Fig. 2), was due to the formation of selenium nanoparticles from Se(IV) [25,26].

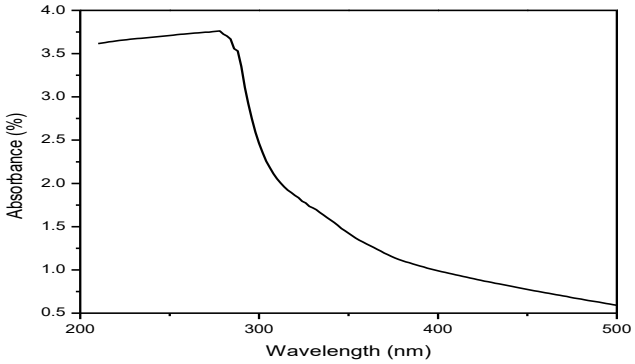

Fig. 2. UV- absorption spectrum of Se-NPs exhibited a well-defined absorption peak maxima at 285nm of such band is assigned to surface plasmon resonance of the selenium particles.

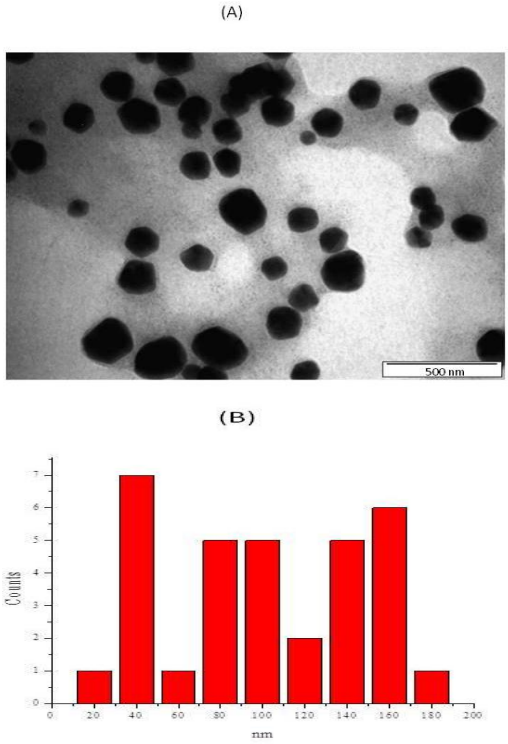

Fig. 3. (A) TEM image of the Se-NPs produced by the reaction of 2 mM sodium selenite (Na_2SeO_3) solution with bacteria *Bacillus* sp. supernatant incubated at 37ºC for 48h and (B) its particle size distributions.

Transmission electron microscopy (TEM) analysis demonstrated that, the size of nanoparticles ranged from 40nm to 168nm and spherical in shape (Fig. 3). Formation

Table 1. Effect of cadmium, selenium and Se-NPs singly and in combination on the growth parameters and some metabolites of *Scenedesmus quadricauda*

Parameters / Treatments		Chl. *a* (µgml^{-1})	D.W. (mgl^{-1})	µ(d^{-1})	Proteins			Free amino acids	Carbohydrates			Total lipids
					S.P.	Ins.P.	T.P.		S.C.	Ins.C.	T.C.	
					mg/g dry weight							
Control		5.19 ±0.02^{f}	1221 ±26^{b}	0.256	18.46 ±3.8^{a}	20.4 ±0.3^{a}	38.8 ±3.5^{ab}	41.2 ±6.0^{abc}	9.3 ±2.7^{a}	42.2 ±5.6^{a}	51.5 ±8.3^{a}	92.3 ±1.2^{d}
Cd (0.1)		0.32 ±0.04^{bc}	1120 ±15^{a}	0.167	18. 7 ±1.2^{a}	34.2 ±2.4^{de}	52.8 ±3.6^{de}	59.2 ±6.7^{cd}	11.9 ±1.0^{ab}	93.9 ±1.2^{c}	105.9 ±2.2^{d}	64.3 ±0.6^{bc}
Se (0.04)		2.76 ±0.1^{e}	1150 ±18^{a}	0.150	25.9 ±1.1^{b}	30.0 ±0.4c^{d}	55.9 ±1.5^{e}	62.3 ±4.3^{d}	20.5 ±0.4^{c}	63.1 ±0.7^{b}	83.6 ±1.1^{c}	99.6 ±2.7^{e}
Se-NPs (0.04)		6.20 ±0.18^{g}	1210 ±13^{b}	0.286	16.0 ±3.7^{a}	21.4 ±0.8^{a}	37.4 ±2.9^{a}	27.2 ±0.8^{a}	10.2 ±0.7^{ab}	50.0 ±0.9^{ab}	60.2 ±0.2^{ab}	64.8 ±1.9^{c}
Cd (0.1)+ Se (0.01)		0.27 ±0.04^{ab}	1125 ±15^{a}	0.013	14.1 ±0.7^{a}	24.0 ±0.9^{ab}	38.1 ±0.2^{a}	39.3 ±7.1^{ab}	13.2 ±0.4^{ab}	112.9 ±4.1^{d}	126.1 ±4.5^{e}	62.5 ±0.1^{bc}
Cd (0.1)+ Se (0.02)	(mM)	0.36 ±0.02^{bc}	1130 ±11^{a}	0.017	16.9 ±0.2^{a}	26.9 ±3.1^{bc}	43.9 ±2.9^{abc}	52.5 ±8.3^{bcd}	15.7 ±1.0^{bc}	133.1 ±6.9^{e}	148.8 ±5.9^{f}	60.9 ±0.5^{bc}
Cd (0.1)+ Se (0.04)		0.27 ±0.01^{ab}	1135 ±12^{a}	0.285	16.0 ±0.3^{a}	30.4 ±0.4^{cd}	46.4 ±0.1^{bcd}	50.5 ±5.2^{bcd}	9.4 ±1.1^{a}	104.8 ±0.7^{cd}	114.2 ±1.7^{de}	110.5 ±1.5^{f}
Cd (0.1)+ Se-NPs(0.01)		0.07 ±0.007^{a}	1138 ±13^{a}	0.003	13.9 ±0.7^{a}	33.3 ±0.0^{de}	47.3 ±0.7^{cd}	29.5 ±2.2^{a}	15.1 ±4.4^{abc}	50.2 ±6.8^{ab}	65.3 ±2.4^{ab}	46.5 ±0.8^{a}
Cd (0.1)+ Se-NPs(0.02)		0.53 ±0.03^{c}	1142 ±4.0^{a}	0.025	30.7 ±0.2^{b}	35.9 ±1.2^{e}	66.6 ±1.1^{f}	43.1 ±8.9^{abc}	13.1 ±0.3^{ab}	52.84 ±7.3^{ab}	65.9 ±7.0^{ab}	61.4 ±6.2^{bc}
Cd (0.1)+ Se-NPs(0.04)		1.29 ±0.005^{d}	1145 ±10^{a}	0.023	45.8 ±4.1^{c}	56.6 ±0.0^{f}	102.3 ±4.1^{g}	49.9 ±0.2^{bcd}	13.1 ±0.7^{ab}	59.4 ±4.8^{b}	72.5 ±5.5^{bc}	56.7 ±1.5^{b}

D.W= dry weight, µ= specific growth rate, S.P. = soluble proteins, Ins. P. = insoluble proteins, T.P. = total proteins, S.C. = soluble carbohydrates, Ins. C. = insoluble carbohydrates, T.C. = total carbohydrates. The data are given as averages of three replicates ± standard error. Values followed by the different letters are significantly different at $p < 0.05$.

of nanospheres through the reduction of Se (IV) by different bacterial strains has also been reported earlier [27,28,29]. Furthermore, the observed nanoparticles consisted of elemental selenium as determined by X-ray spectroscopy (XRD) analysis. These results indicated corresponding three intense peaks in the whole spectrum of 2Θ values ranging from 20 to 70 (Fig. 4). The diffraction peak at 2Θ value of 23.780, 29.797 and 43.878 can be indexed to the (100), (101) and (102) planes of the face-centered cubic (fcc) red elemental selenium, respectively [15].

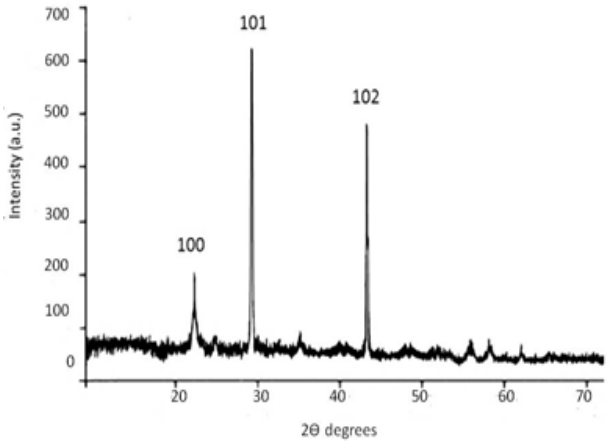

Fig. 4. Representative XRD pattern of Se-NPs synthesized by the reaction of 2 mM sodium selenite (Na_2SeO_3) solution with bacteria *Bacillus* sp. at pH 7.0.

Over the last few years, increasing human population and industrial development have led to an increase of contaminants in aquatic systems [30]. Accordingly, studies reporting the effects of heavy metals on aquatic organisms are currently attracting more attention, particularly those focused on industrial and urban pollution. Contamination of coastal waters with trace metals through sewage and other anthropogenic sources has become a severe problem [31].

According to Issa et al. [32], green algae represent the dominant group in the total phytoplankton of the River Nile and *Scenedesmus quadricauda* was isolated and purified in some polluted waters. *S. quadricauda* showed a high efficiency of cadmium uptake in the presence of the high concentration of selenium. However, the percentage of Cd^{2+} uptake decreases by the treatment of algae with of selenium nannoparticales (0.02, 0.04mM) (Fig. 5). Selenium is known to help detoxify the body and remove heavy metals including Mercury. The highest growth rate was shown in *Scenedesmus* cultures treated with Cd and Se-NPs, followed by Cd and Se, and the lowest one was recorded in cultures containing Cd alone (Fig. 6). The effect of selenium salts on the growth of marine algae has been studied before and the study showed positive deteriorative effects on its growth [14]. Accordingly, the photosynthetic chlorophyll *a* and dry weight were almost the same trend (Table 1). The decrease of macroalgae growth rates [31], increased activities of glutathione reductase [33], changes in photosynthetic pigments [30, 34] were recorded by algae treated with Cd.

Concerning, the carbohydrate contents in all Cd treated culture were significantly enhanced than control cultures (without Cd or Se). In Se nanoparticles, however the carbohydrates were no obvious trend. From the data shown

in table (1) the total protein, lipids contents as well as free amino acids were increased in *Scenedesmus* cultured with Se or Cd and Se-NPs in comparison to other treatments. Dash *et al.* [13] proved that selenium nanoparticles have been found to be less toxic than selenium salts in the environment. Fawzy and Issa [35] reported that, increase of proteins and carbohydrates in cyanobacterial species appeared to be related to the pollution stress caused by heavy metals.

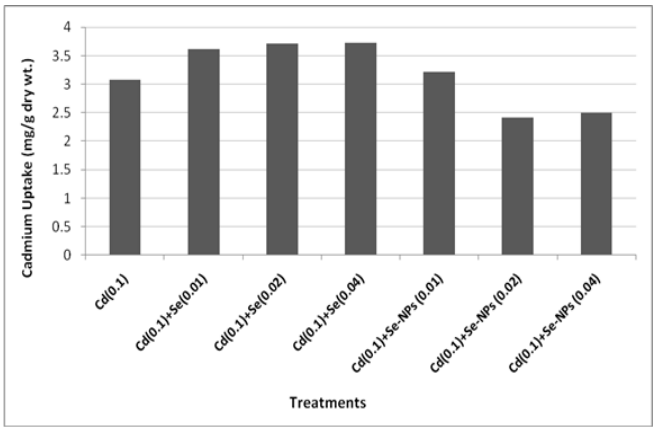

Fig. 5. Cadmium uptake by *Scenedesmus quadricauda* at different concentrations of selenium and Se-NPs.

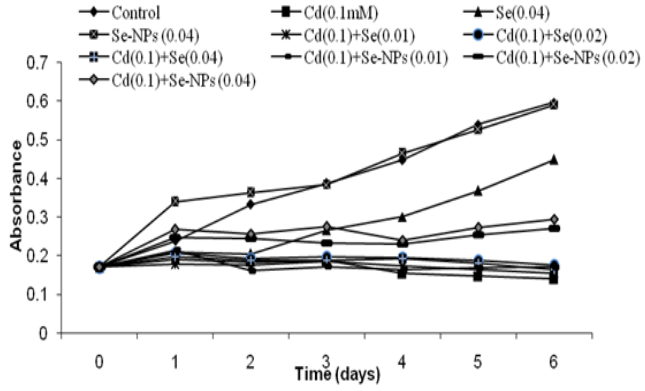

Fig. 6. Growth curve of *Scenedesmus quadricauda* under cadmium, selenium and Se-NPs singly and in combination as measured as optical density.

In conclusion, algae constitute an important component of our environment and ecosystem as a primary producer, contributing to nearly 40% of the global productivity of biomass. Observed toxic attributes of Cd on growth of aquatic photosynthetic algae are, therefore, matter of serious concern. On the other hand, Se nanoparticles can be employed for well-being by regulating algal growth toxicity by Cd in the water bodies. *Scenedesmus* has worldwide distribution and thrive in shallow ponds, lakes, water channels and reservoirs. Dense and prolific growth of these algae often interferes with fishing, irrigation, recreation, municipal water supply and other utilization of the water bodies. Selenium is known to help detoxify the algal growth and remove heavy metals especially cadmium.

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