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Effect of Heavy Metals on the *Desmodesmus quadricauda* Isolated from the River Nile, Egypt

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Abstract

The wastewater of Iron and Steel Factories contains high level of metallic ions, especially Fe which represents the main component of the waste followed by Zn, Cu, Pb, Ni, Hg, Co, Cd, Mn and Cr. Bioassay experiment were achieved to determine the effect of industrial waste of Iron and Steel Factories at Helwan on growth, chlorophyll *a* and photosynthetic activity of one alga (*Desmodesmus quadricauda*) which represent one of the most dominant phytoplankton in the Nile River. The growth rate (counting and chlorophyll *a*) was more sensitive than photosynthetic activity. A synergistic effect was found between the trace metals of the waste. The toxicity of the waste decreased gradually by time. The EC_{50} of the growth rate values indicated that, the inhibitory effect of waste on growth of the alga showed slight decrease during the four days incubation, the phenomenon was enhanced on the 5th day when the EC_{50} reached to 46.84. The EC_{50} values of waste inhibited chlorophyll *a* showed a significant increase till the 3rd day when the EC_{50} reached to 47.79. The EC_{50} values had considerably decreased to 32.78 on the 4th day then abruptly increase to 47.32 on the 5th day. Industrial waste showed a significantly reduced the photosynthetic activity at 48% and 60% concentration. The maximum decreased observed on the 4th day and represented by 62.92% and 79.02% below their control values, respectively.

Keywords: *Desmodesmus quadricauda*; River Nile; Egypt; Heavy metals; Growth rate; Chlorophyll *a*; EC_{50}; Synergetic effect; Photosynthetic activity

Introduction

The accumulation of the heavy metals in aquatic organisms leads to danger effect on the aquatic food chain and endanger human health too. Heavy metals are considered the important reasons of the pollution in soil and aquatic ecosystem. The heavy metals cannot be biodegraded by micro-organisms into non-toxic or to volatile compounds as organic pollutants. Due to this reasons, these pollutants will eventually accumulate in the food chain.

Industrial wastes, agricultural run-off, municipal waste and accidental spillage are the main sources of the aquatic pollution. As well as the appreciable amounts of pollutants to air come from human activity as factories and automobile exhausts may enter the aquatic water bodies through rainfall or dry fall-out. The Nile receives increasing amounts of waste discharges, from several sources, as the rivers travels northward. River pollution problems are primarily due to human activities as domestic and industrial waste discharge as well as River transportation and agricultural return flows.

In the recent decades, the toxicity tests have been developed to expect the probable effect of industrial effluents on the aquatic ecosystem by using algae, crustaceans, mollusks and fishes [1,2]. The toxicity of heavy metals to phytoplankton was reviewed by Ref [3]. The toxicity of Copper and Cadmium was investigated on the alginate-immobilized algae. Ref [4] investigated the use of alginate-immobilized algae for testing the toxicity of cadmium and copper. The industrial wastes discharged on the banks of Periyar River in India were tested by using algal assays [5]. The effects of Zinc smelter waste to some diatoms were investigated by Rao and Mohanchand [6]. The effects of mercury on the cell population, chlorophyll *a* and photosynthesis of *Dunaliella minuta* was investigated by Gotsis [7]. The level of heavy metals in water and sediment was done by Sabri et al. [8] at River Tigris at Samarra impoundment (Iraq). Mingazzini [9] evaluated the different methods for quantitative toxicity of the pollutants on the River Po, Italy, by using *Selenastrum capricornatum*. The mutual effect of some heavy metals as Cadmium, Zinc and Copper on *Phaeosactylum trinutum* Bhlin was done by Wang et al. [10]. The metals chronic contamination the structure of phytoplankton of the Sado River (Prtugal) was reported by Monteiro et al. [11]. Lustigman et al. [12,13] studied the consequences of pH and Nickel on the growth of *Chlorella vulgaris*. As well as, they reported the effect of cobalt on the growth of *Chlamydomonas reinhardtii*. Abalde et al. [14] studied different characteristics of copper toxicity to *Dunaliella tertiolecta*.

Javed and Mahmood [15] discussed the metal toxicity of the plankton in the river Ravi from Shahdera to Bloki (India). They found that, metal toxicity showed significant difference due to variable discharges of un-treated industrial and sewage wastes into the river through different tributaries. The gathering of zinc in plankton was dependent completely and considerably on water temperature. However, amplify in water hardness drastically increased the accumulation of iron, manganese and nickel in plankton. Liu et al. [16], discussed the heavy metals and other pollutants enter the water through different ways are, algae as primary producers are the first affected creatures in the food chain and consequently affect the other trophic levels. Arunakumara and Xuecheng [17] studied the toxicity of lead and cadmium on the molecular genetic, physiological and morphological properties of the unicellular cyanobacterium, *Synechocystis* sp.

Recent studies show that algae can accumulate heavy metal ions, consequently, it is possible to use algae to restore the heavy metal from

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contaminant water [18-20]. The bioassay tests concerning about the biosorption of the several metal ions were done by Mane and Bhosle [21]. They reported the effect of copper, iron, selenium, manganese and zinc on *Spirogyra* sp. and *Spirulina* sp. Małgorzata Rajfur [22] reviewed the sorption of heavy metals in different species of algae as results of pollution by wastewater. Kanchana et al. [23] studied the role of different algal species in the recovery and removal of heavy metals through biosorption process. Chaidir et al. [24] reported the capability of *Scenedesmus dimorphus* and some microalga species for bio-elimination of Cd^{+2} under different pH concentrations, which The pH is one of the most important controlling parameters in the adsorption process of heavy metal ions and algae tolerance. Çelekli et al. [25] reported that the survival of the green algae in the aquatic environment contaminated with heavy metals serve on its ability to generate and conveyance signals that accommodate the metabolism. Cheng et al. [26] investigated the effect the stress of cadmium ions on the growth, antioxidant system and physiological of *Chlorella vulgaris*.

Desmodesmus quadricauda is Chlorophyceae, have obtuse/truncated cell poles (differentiated by the presence or absence of spines respectively). The genus of *Desmodesmus* or *Scenedesmus* is colonial and non-motile. This species is a highly efficient photosynthetic organism [27]. The response of *Desmodesmus quadricauda* against the effect of the toxicity of some heavy metals will be study in this article.

Materials and Methods

Experimental bioassay

Bioassay experiment was done to verify the effect of the industrial waste of Iron and Steel Factories on growth, Chlorophyll *a* and Photosynthetic activity of the Nile green alga *Desmodesmus quadricauda* Trup de Brebission.

Test alga: The main characters of these algae can be summarized as follows:

Desmodesmus quadricauda Trup de Brebission has two to eight cells in a single series. Cells cylindrical-ovoid, rounded, apices of the outer cells with a long curved spine, inner cells without spines, or with a small papilla.

Isolation, purification and preparation of the bioassay test: *Desmodesmus quadricauda* Trup de Brebission was collected from the River Nile by using a fine mesh plankton net. The steps of isolation and purification approved by Pringshiem [28] were followed to obtain unialgal cultures. *D. quadricauda* was isolated from the Nile and maintained in a unialgal culture under optimum laboratory conditions.

Clonal culture of *Desmodesmus quadricauda* Trup de Brebission was drawn by micropipette isolation of a single cell from the water sample which was collected from the Nile - Egypt. The steps of isolation and purification approved by Pringshiem [28] were followed to obtain unialgal cultures. Cultures were grown under sterile conditions on conical flask with pasteurization media collected from Rod El-Farag (non polluted area). The sample was enriched with 0.1 g/l $NaNO_3$ and 0.02 g/l K_2HPO_4 [29]. This media considered as a control media. On the other hand, industrial waste samples collected from the discharged point of the of Iron and Steel Factories. These wastes pasteurized and kept in dark plastic bottle in refrigerator at 4°C.

Chemical analyses were carried out on the basic medium and industrial effluent waters. Analysis of Chemical Oxygen Demand (COD), Dissolved oxygen (DO), alkalinity, chlorinity Cl%, PO_4-P, total phosphorus, NH_4-N, NO_2-N, NO_3-N, total nitrogen, hardness (Ca and Mg), SO_4, SiO_3-Si were done according to APHA [30]. The Atomic Absorption Spectrophotometer (Model Perkin-Elmer 3010) equipped by Graphite Furnace (Model HG600) as well as Hydrite Unite (Model HG20) were used to analyze the heavy metals Fe, Cu, Pb, Co, Ni, Cd, Cr, Mn, Zn and Hg for both basic medium and waste water following the procedures of APHA [30]. The tested concentrations of the waste were chosen after several range finding tests with the selected alga. The waste concentrations ranged from; 0 (control), to those inhibit growth of the alga by more than 50% of the control. The selected waste concentrations were 12, 24, 36, 48 and 60%.

The bioassay experiment was done in 500 ml, clean dry Erlenmeyer flasks containing 200 ml sterilized medium. Treatments and control flasks were inoculated with 10,000 *D. quadricauda* cells ml^{-1} in a logarithmic growth phase. Cultures (experimental and control flasks) were maintained at in a local made incubator at 25 ± 1°C under 14 h light: 10 h dark (L: D) cycle and and light intensity of 4000 Lux. The duration of bioassay test was 5 days. Three replicate flasks were used for control and each concentration of the waste.

Determination of cell growth: Growth response of *D. quadricauda* was measured by various parameters as the following

Growth rate: Cell densities in control and treated culture were counted by haemocytometer. Eight counts were made for each flask. Daily estimation of growth rate of either control or treated alga was carried out. The growth rate was calculated according to Kratz and Mayers's equation [31]. The EC_{50} (the effective concentration of waste which causes 50% reduction in growth parameters compared to the control) was calculated by using Linear regression [32].

Time required for division (Td): Td was calculated for each tested concentration according to Guillard's equation [33].

Inhibitory%: Inhibitory effects in algal growth test can be calculated according to Blankley [34].

Measurement of pigments (Chlorophyll *a*)

Samples were filtered using Syringe Filtration System (Sartarious), connected with a plastic holder (25 mm), through glass microfiber filter (Whatmann GF/F). The filters containing the samples were extracted with 90% aqueous acetone and the tissue grinder (Tomas type) was used for complete extraction [30]. The extract was clarified by centrifugation at 2000 rpm using T24 centrifuge. The samples were measured by Turner III Filter Fluorometer. Standard Chlorophyll *a* (Sigma product) was used for calibration of the fluorometer. The corresponding phaeophytin values were also measured after acidification with 2 drops of 5% HCl and measured again. The concentration of Chlorophyll *a* and phaeophytin was calculated according APHA [30], and the results recorded µg/L.

Photosynthetic activities

Photosynthetic activities of control and treated culture were determined by applying C^{14} tracer technique [35]. This technique is simple but requires more precautions and expensive laboratory requirements Abou-Waly et al. [36]. Each vial was counted in a series for 3 times, each times was 3 minutes and the results were printed directly as Count Per Minute (CPM). The recommendation for the C^{14} methods about handling, preparation and cautions were applied according to APHA [30].

Results

Effect of industrial waste on growth, chlorophyll *a* and photosynthetic activity of *Desmodesmus quadricauda*

The basic medium used in this experiment was filtered and sterilized Nile water collected from Cairo Sector (Rod Elfrag area). The waste of Iron and Steel Factories was collected from the main drain of these factories which discharges in the Nile at the point of discharge of Iron and Steel Factories, Helwan. Physico-chemical characters of the medium and waste are given in Table 1 and Figure 1.

Effect of industrial waste on growth: The growth rate of the tested alga progressively decreased with increasing waste concentrations. Figure 2 demonstrates an oblivious decreased in cell count as an indicator for the growth rate. At 12% and 24% waste, the growth of the alga showed a significant decrease with time to the second day, and their inhibitory effect began to decrease from the third day to the end of the experiment when the percentage decrease in growth reached to 17.78% and 36.00% of the corresponding control.

With increasing of waste concentrations to 36% and 48%, its inhibitory effect had significantly increased. On the first day, their inhibitory effect was identical and reduced the growth of the alga by 59.38% of the control. At 48% waste, the inhibitory effect showed an increase with time and reduced the cell density by 65.08% of the control on the fourth day. On the fifth day, an obvious drop in the toxicity of these concentrations (36% and 48%) was observed and they reduced the cell density by 44.67% and 54.67% of the control.

At 60% waste, the growth of the green alga was gradually decreased, reached a maximum depletion of 80.32% of the control on the third day and slightly decreased to 76.65% 77.56% of the control on the 4th and 5th days, respectively. Table 2 showed the growth rate (K) of the control was gradually decreased with time, while at 12% waste, it showed a slight increase on the 2nd day and then began to decrease reaching the minimum of 0.74 on the 5th day. At 24% waste, the growth rate had slightly increased on the 2nd day (0.90) and started to decrease from the 3rd day to end of the experiment. At 36%, 48% and 60% waste, the growth rate of the tested alga had considerably increased with time, reaching the maximum of 0.76, 0.68 and 0.55 on the 4th day. Although its values were decreased on the 5th day.

The EC_{50} values reported in Figure 3 indicated that, the inhibitory effect of waste on growth of the alga showed slight decrease during the four days incubation, the phenomenon was enhanced on the 5th day when the EC_{50} reached to 46.84.

Effect of industrial waste on chlorophyll *a* of the tested alga: The response of *D. quadricauda* to different waste concentration is shown in Figure 4. The results can be briefly explained as follows:

- The chlorophyll *a* content of the alga showed a gradual decrease with increasing of waste concentrations, although its values had considerably increased with time.
- At 12% waste, the chlorophyll *a* value was decreased. On the 1st day, the phenomenon was reduced with time and its inhibitory effect reduced to the minimum on the 4th and 5th days (19.34 and 20.47%).
- At 24% waste, the depilatory effect had remarkably increased during 2nd and 3rd days that reduced the chlorophyll *a* content by 42.96% and 42.39% of the corresponding control. On the 4th day the inhibitory effect of waste decreased to 38.69% and 38.22% for the 5th day.
- With increasing of waste to 36% and 48%, the inhibitory effect exhibited a significant increase to the 2nd day. This phenomenon began to decrease on the 3rd day to the end of experiment when the percentage decrease in chlorophyll *a* reached to 46.98% and 57.48% of the corresponding control.
- The highest inhibitory effect observed at 60% waste concentration. The chlorophyll *a* decreased gradually with increasing waste concentration reached to 62.47% of the corresponding control on the 5th day.
- The EC_{50} values of waste inhibited chlorophyll *a* Figure 3 showed a significant increase till the 3rd day when the EC_{50} reached to 47.79. The EC_{50} values had considerably decreased to 32.78 on the 4th day then abruptly increase to 47.32 on the 5th day.

Effect of industrial waste on photosynthetic activity of *Desmodesmus quadricauda*: C^{14} uptake reflects the physiological activity where the photosynthetic activity depends mainly upon the culture conditions and the growth phase of the culture.

Figure 5 shows that, effect of the Industrial waste water on the photosynthetic activity. This decreased considered less than their effect upon cell density and Chlorophyll *a* especially at low concentration of waste.

At 12% waste, the photosynthetic activity of the alga had gradually decreased till the 3rd day; a phenomenon was reversed from the 4th day to the end of experiment. With increasing waste concentration, the inhibitory effect was progressively increased reaching the maximum of 38% of the control on the 4th day. This phenomenon was completely reversed on the 5th day when the depilatory effect reached to the minimum of 7.56% of the corresponding control.

With regard to 36% waste, the inhibitory effect had significantly increased with time, reaching to 48.95% of the corresponding control on the 4th day. Its depilatory effect decreased on the 5th day to 34.51% of the control. Industrial waste showed a significantly reduced the photosynthetic activity at 48% and 60% concentration. The maximum decreased observed on the 4th day and represented by 62.92% and 79.02% below their control values, respectively.

On the 1st and 2nd days, their values were higher than the 50% of the control even the highest dose of waste. Figure 5 showed the EC_{50} values that, the inhibitory effect of waste on photosynthetic activity decreased gradually till to the minimum value of 45.16 on the 5th days.

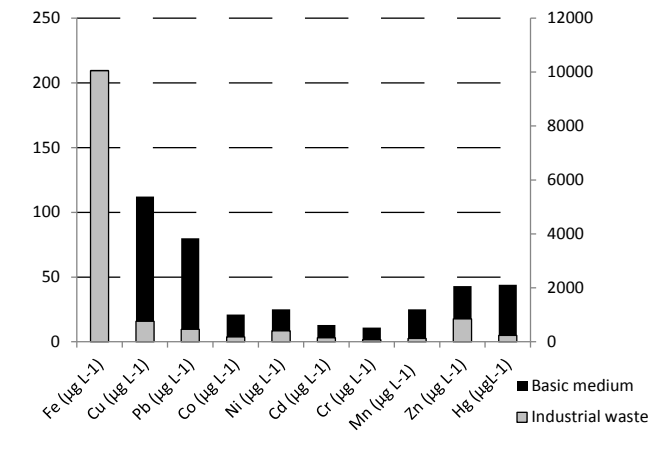

Figure 1: The metallic ions of basic media and industrial waste.

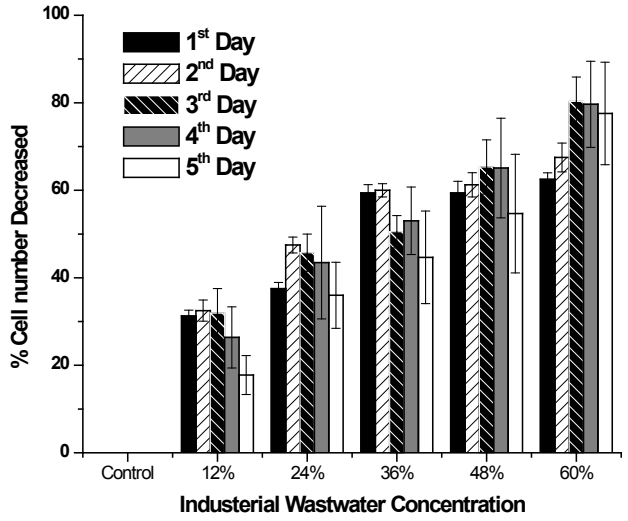

Figure 2: Percentage decrease in Cell population as a result of Industrial Wastewater.

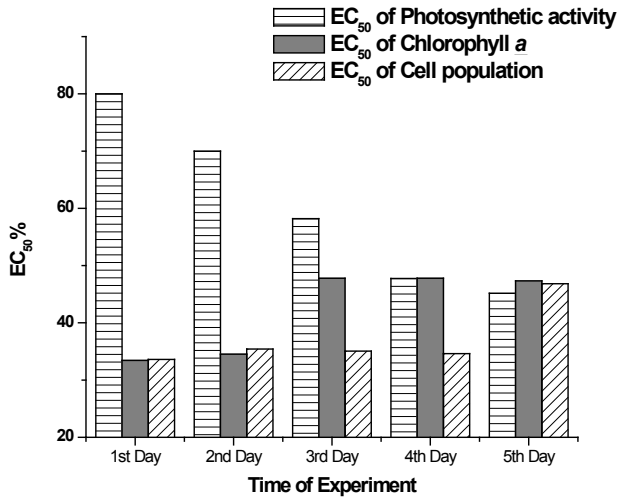

Figure 3: The effect of Industrial wastes on the EC50.

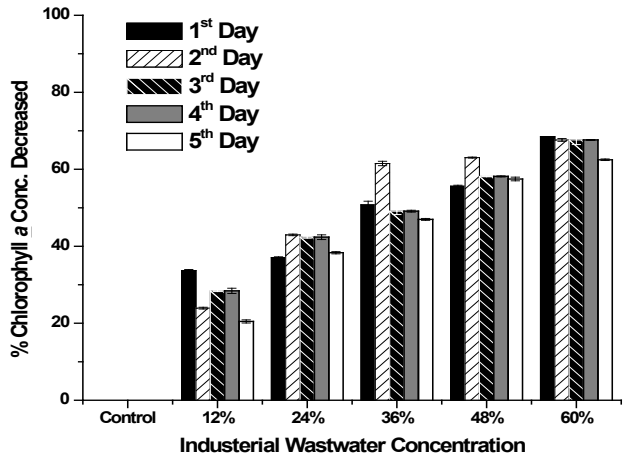

Figure 4: Percentage decrease in Chlorophyll a content as a result of Industrial Wastewater.

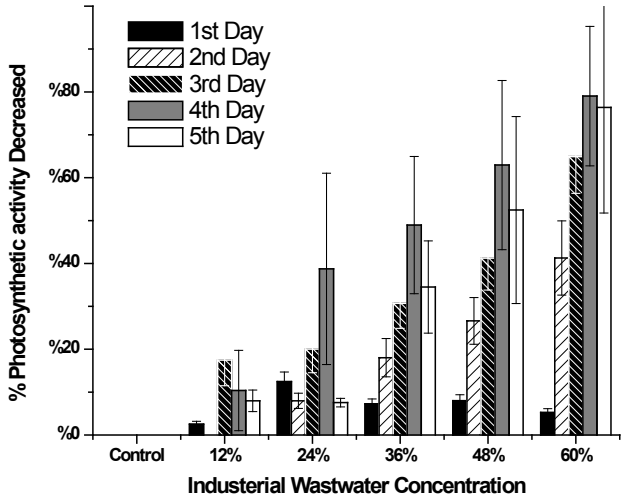

Figure 5: Shows that, effect of the Industrial waste water on the photosynthetic activity.

Discussion

The wastewater of Iron and Steel Factories contains high level of metallic ions, especially Fe which represents the main component of the waste followed by Zn, Cu, Pb, Ni, Hg, Co, Cd, Mn and Cr. Low doses of some of these metals are known to be essential for growth and metabolic activities of phytoplankton species, while their high concentrations cause severe drop in growth and metabolism of these organisms [37].

There is no available data about the combined effects of different metallic ions on growth and productivity of phytoplankton species. Toxicity testing with unicellular algae is increasingly being used to assess the impact of potential pollutants on aquatic ecosystems. This is largely due to their high sensitivity and reproducibility under laboratory conditions, along with their high ecological significance as the foundation of most aquatic food chains [38].

The present data indicated that, the amplitude of growth inhibition of the tested alga *Desmodesmus quadricauda* under the influence of the waste depends on its concentration and exposure time. The highest toxicity of the waste was found on the 1st day when the EC_{50} values were 33.6% and 33.53% for growth and chlorophyll *a*, respectively. The inhibitory effect of the waste had decreased with time reaching to the least level on the 5th day when its EC_{50} values were 46.84% and 47.32%, for the two parameters respectively. On the 1st and 2nd days, the photosynthetic activity of the tested alga was still higher than 50% of the control even at the highest waste concentration; its value had decreased on the 5th day (EC_{50}, 45.16%). This means that, the growth and biomass of the alga were more sensitive to the waste compared to the photosynthetic activity.

By calculating the concentration of each metal in the EC_{50} of the waste at the end of experiment and comparing these concentrations with the corresponding EC_{50} of each metal on phytoplankton species (from the available literatures), one can reach to specific information about synergistic and /or antagonistic interaction of the metals. The calculated concentrations of different metals in EC_{50} of the waste are in Table 3.

Concerning Iron, the data reported by Ibrahim [39] and Shabana

Parameters	Basic medium	Industrial waste
Air Temperature (°C)	22	24
Water Temperature (°C)	20	21
pH	7.4	7.2
Conductivity (m mohs Cm^{-1})	0.50	1.25
COD (mg L^{-1})	9.4	1.2
Dissolved Oxygen (mg L^{-1})	7.35	4.2
Alkalinity Carbonate (mg L^{-1})	45	32
Bicarbonate (mg L^{-1})	175	125
Chlorosity (Cl%)	0.090	0.560
Orthophosphate (µg L^{-1})	42.05	62.65
Total Phosphorus (µg L^{-1})	85.54	220.43
NH_4-N (µg L^{-1})	28.34	170.69
NO_2-N (µg L^{-1})	15.36	185.47
NO_3-N (µg L^{-1})	25.40	151.90
Total nitrogen (mg L^{-1})	1.30	2.45
Calcium (mg L^{-1})	95.53	169.55
Magnessium (mg L^{-1})	135.4	600.35
Silicate (mg L^{-1})	4.59	5.67
Sulphate (mg L^{-1})	52.36	134.65

Table 1: Effect of Industrial Waste on Growth.

			Control	12%	24%	36%	48%	60%
1st Day	Cell Population	K	1.27	0.89	0.80	0.37	0.37	0.29
		TD	0.55	0.78	0.87	1.90	1.90	2.43
	Chlorophyll a	K	1.07	0.66	0.61	0.36	0.26	0.00
		TD	0.28	0.46	0.50	0.83	1.16	0.00
2nd Day	Cell Population	K	1.09	0.90	0.77	0.63	0.62	0.53
		TD	0.64	0.78	0.91	1.10	1.13	1.32
	Chlorophyll a	K	0.96	0.82	0.68	0.48	0.46	0.39
		TD	0.32	0.37	0.45	0.63	0.66	0.77
3rd Day	Cell Population	K	1.01	0.88	0.81	0.78	0.66	0.47
		TD	0.69	0.79	0.86	0.90	1.06	1.48
	Chlorophyll a	K	0.91	0.80	0.73	0.69	0.62	0.54
		TD	0.33	0.38	0.42	0.44	0.49	0.57
4th Day	Cell Population	K	0.95	0.87	0.80	0.76	0.68	0.55
		TD	0.74	0.80	0.87	0.92	1.02	1.27
	Chlorophyll a	K	0.91	0.86	0.82	0.73	0.71	0.65
		TD	0.33	0.35	0.37	0.42	0.43	0.47
5th Day	Cell Population	K	0.78	0.74	0.69	0.66	0.62	0.48
		TD	0.89	0.94	1.01	1.05	1.12	1.44
	Chlorophyll a	K	0.78	0.73	0.68	0.65	0.61	0.58
		TD	0.39	0.41	0.45	0.47	0.50	0.52

Table 2: Effect of Industrial wastewater on Growth rate (K) and time of duplication of the Cell population and chlorophyll a of *Desmodesmus quadricauda*.

Element	Growth	Chlorophyll a	Photosynthetic activity
Fe	4707.42	4756.61	4539.48
Zn	398.14	402.22	383.86
Cu	356.20	359.86	343.44
Pb	217.81	220.04	209.99
Ni	190.64	192.59	183.80
Hg	112.42	113.57	108.38
Co	84.31	85.18	81.29
Cd	70.26	70.98	67.74
Mn	58.55	59.15	56.45
Cr	37.00	37.38	35.68

Table 3: Concentrations of metals in 120 h. EC_{50} of the waste (ppb).

et al. [40], who investigated the single effect of iron the growth of some of Nile phytoplankton. Ibrahim [39] found that, low concentration of Fe^{+3} (1800 ppb) stimulated chlorophyll synthesis of *Coelastrum microporum, Desmodesmus opoliensis, Oscillatoria amphibia* and *Microcystis flos-aquae*, while high dose of 10.000 ppb caused rigorous drop in chlorophyll. Also Shabana et al. [40] recorded that, the lower (10^{-8} M) and higher concentrations (10^{-4} M) of Fe^{+3} inhibited growth of *Desmodesmus dimorphous*. Both Ibrahim [39] and Shabana et al. [40] did not mentioned EC_{50}.

For Zinc, Ali [41] reported that, the EC_{50} of Zn as a single effect on growth of *Desmodesmus obliqquus* was 1120 ppb. This revealed that, the level of Zn in the EC_{50} in the present bioassay, of the waste was much lower than its single effect. On the other hand, Rao and Mohanchand [6], they studied the combined effect of Zn of Smelter Factory on some marine diatoms and found the EC_{50} of the waste was 143.69 ppb and this value was much lower than the EC_{50} recorded in the present study.

For Copper, Ali [41] recorded that, the experimental EC_{50} of Cu was 980 ppb which inhibit 50% of the growth of *Desmodesmus obliqquus*. This value was higher than our EC_{50} value (356.2 ppb). Nyholm mentioned that, the experimental value of EC_{50} of Cu was 750 ppb on *Selenastrum capricornatum*. This means that, Cu with synergistic effect with other metals.

The EC_{50} of Pb of our study being 217.81, 220.04 and 209.99 µg L^{-1} for growth, chlorophyll *a* and photosynthetic activity, respectively. Monahan [42] recorded that, 500 µg L^{-1} Pb reduced the growth of *Selenastrum capricornatum, Chlorella pyrenoidosa, C. vulgaris* and *C. ellipsoidea* by 50%. Our EC_{50} was lower than the EC_{50} recorded by Monahan [42]. In this context, Our EC_{50} value was lesser than the value EC_{50} recorded by Monahan [42] and this may be due to the synergistic effect of Pb with other metals in the mixture.

The EC_{50} values of Ni in the present experiment were 190.64 ppb for growth. Drastic effect on *Desmodesmus obliquus* occurred at 2000 µg L^{-1} [41]. She recorded that, the experimental EC_{50} of Ni was 1120 µg L^{-1}. The EC_{50} recorded by Ali [41] are considerably higher than the results reported here and this reflect the energetic effect of Ni.

Mercury is the most toxic heavy metal. The present data agrees partially with Ibrahim [39], who stated that, by increasing Hg concentrations caused chlorophyll degradation of the Nile phytoplankter. He reported that, the activation of chlorophyll continued until 1.8 µg L^{-1} in case of *Coelastrum microporum, Oscillatoria amphibia* and *Microcystis flos-aquae*, while in case of *Desmodesmus opoliensis* until 10 µg L^{-1}. He did not mentioned anything about EC_{50}. Nasr [43] reported that, the values of EC_{50} of Hg were 234 and 252 µg L^{-1} of growth and chlorophyll *a* of *Cyclotella comta*. The result of EC_{50} of our work (113.57 ppb) was much lower than the value of EC_{50} recorded by Nasr [43]. By comparing the present data about the effect of Hg with Nasr [43]. We can concluded that, the extreme effect of mercury appeared obviously due to the synergistic effect with other metals in the waste.

The experimental EC_{50} of Cd recorded by [41] was 950 µg. L^{-1} for *Desmodesmus obliquus* and this value was much higher than the result of our work (70.26 ppb). She reported that, Cd even at low concentration (50 µg.L^{-1}) inhibited the growth of *S. obliqquus.* In this respect, Wong et al. [44] reported the acute toxicity of Cd to *Ankistrodesmus falcatus* at 500 µg. L^{-1} and mainly inhibited photosynthetic activity by 50% and this level was considerably higher than our result (67.74 ppb). In the present bioassay, the minimum value of EC_{50} of Cd may be attributed to the synergistic effect of Cd with other metals in the waste.

Ali [41] using *Desmodesmus obliqquus*, reported EC_{50} value for chromium (710 ppb for growth). this level was considerably higher than the results reported here (37 ppb for growth of *S. quadricauda*) and this may be due to the same reason reported previously.

About Manganese, Shabana et al. [40] reported that, the physiological activity of *Desmodesmus dimorphous* was increased by lower concentration of Mn^{+2} (10^{-8} M). However, any rise in Mn^{+2} concentration induced a decrease in physiological activity. They did not reported any thing about EC_{50}.

The present data revealed that, the decrease of toxicity by time leads us to conclusion that, the tested alga develop increased resistance to the effect of these waste with increasing exposure time [7]. Also, this may be attributed to, the capability of the tested alga to absorb or adsorb these metals either passively or through the active site of the cells [45]. The effect of the effluents upon the tested alga were statistically significant at $P<0.05\%$. The differences in EC_{50} between the present study and those previously mentioned may be to species differences as well as the physiological responses are quite different [32]. Joy [5] mentioned that, the response to a particular industrial waste is dependent upon species and time of exposure as well as concentration. He reported the effluent inhibit the growth of *Nitzschia palea* where EC_{50} 74% of the waste while the growth of *Oocystis pusilla* was stimulated 50% at 21% of the effluent concentration. The combined effect of multimetalic ions are very complicated because it is related not only to the composition and proportion of mixture but also to the organisms that the trace elements acts upon. Consequently, it is preferable to act like this bioassay on the community not on single species. This may be due to the biochemical and physiological characteristics of different organisms are quite different. The manners in which certain metal some metals effect on organism cannot be used to described the effect of another organism [10].

In the present bioassay, the inhibition in population growth may be attributing to the toxicity of heavy metals which appears to exert their major effect by interfering with the activity of enzymes situated on cell membrane [46]. Also, Cu at high concentration might prevent the production of methionine which appears necessary for cell division as well as this metal inhibit cell division by binding reactive thiols which is important during mitosis division [46]. The presence of high concentration of Cd, Cu and Hg in the industrial wastewater showed a decrease in cell division and this may be due to the binding of metals to sulphydryl groups which are important in regulating cell division [37]. Visviki and Rachlin [47] observed the reduction in growth of *Dunaliella minuta* cells exposed to mixture of Cd and Cu. They concluded this reduction to the binding of these metals to sulfhydryl groups.

The present data revealed that, the low concentrations of the waste caused a slight to moderate effect on the tested alga and this may be attributed to an interference with the site of enzymes leads to increased the time required for division (Td). This phenomenon agrees with Gotsis [48] who reported the toxicity of heavy metals (e.g., Hg and Cu) upon the growth of micro-organisms through the acting on the site of Sulfur, since the biological cycle of Hg and Cu is fundamentally similar to Sulfur.

The chlorophyll *a* content is a good indicator of algal growth. In the present data, the growth rate of cell population almost parallels those for chlorophyll *a* concentration. C^{14} uptake reflects the physiological activity. The present findings revealed that, the cell population and chlorophyll *a* concentration approximately to the same extent whereas the photosynthetic rate was inhibited to significantly lesser degree. This

may suggest that, the effect of waste is primarily on cell division rather than cellular photosynthesis. The present findings agrees with Gotsis [7,48,49] who recorded the high concentration of Hg and Cu inhibited both cell population and chlorophyll *a* of *Dunaliella minuta* to some extent but photosynthetic rate was inhibited to a significantly lesser degree. Rai et al. [3] claimed that, Fe, Hg, Cu, Zn, Pb and Cd in high concentrations were toxic for photosynthetic activity through the affect on the degradation of chlorophyll *a* and plasma membrane.

It is obvious that, from the previous extrapolation from such laboratory experiments to field situations in attempts to predict the effects of metals upon natural phytoplankton communities is almost impossible. Several physico-chemical and biological factors limit the applicability of results obtained in laboratory experiments to field situation. In any case, bioassay tests using laboratory culture provide a cheap, sensitive, repeatable and convenient method for studying the effects of industrial wastewater discharged into the Nile on the metabolic processes of phytoplankton.

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