BIOLOGICAL REMOVAL OF HEAVY METAL POLLUTION FROM WASTEWATER BEFORE DISCHARGING IN MARINE ENVIRONMENT

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ABSTRACT: The pollution increases, industrialization and rapid economic development impose serve risks to availability and quality of water resources in many areas worldwide. Discharge of heavy metals from metal processing industries is known to have adverse effects on the environment.

Great focus is being put on the research into improving the quality of wastewater by using of microalgae. The basic aim of the present study was to evaluate the possibilities and the performance of *Chlorella salina* (green alga) for bio-removal of heavy metals from wastewater before discharging in marine environment by studying the effect of the treatment process on its growth and the ability to remove different heavy metals (Zn, Ni, Mn, Fe and Cr) as toxic pollutants in wastewater.

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INTRODUCTION

Marine pollution is a global problem in several senses. It defined as "The introduction by man, directly, or indirectly of substances or energy to the marine environment resulting in deleterious effects such as: hazards to human health, hindrance to marine activities, impairment of the quality of seawater for various uses and reduction of amenities. Some marine pollution problems are local, but many have international implications. Particularly if the effects of pollution on the living resources of the sea are considered, very few marine pollution problems can be considered matters of exclusively local interest.

The problems of aquatic pollution are likely to exacerbate and pose significant ecological risk/public health risk in the coming years, especially in developing countries. Coastal and marine pollution has already caused major changes in the structure and function of phytoplankton, zooplankton, benthic and fish communities over large areas including impacts on public health.

By far the greatest volume of waste discharged to the marine environment is sewage. Sewage effluent contains industrial waste, municipal wastes, animal remains and slaughterhouse wastes, water and wastes from domestic baths, utensils and washing machines, kitchen wastes, faecal matter and many others. Huge loads of such wastes are generated daily from highly populated cities and are finally washed out by the drainage systems which generally open into nearby rivers or aquatic systems. Sewage contains in itself a diverse array of polluting agents including pathogens, organic substances, heavy metals and trace elements and so on, which pose direct and indirect effects on ecosystems and organisms **(Islam, 2004)**. Many of the dangerous goods most commonly transported across the marine environment, They lead to release different chemical substances including toxic heavy metals **(IMO, 2015)**.

The industrial areas are generally highly populated or the industries are usually established near highly populated areas. Therefore, higher pollution load from industrial sources is generally accompanied by a higher risk of domestic and sewage pollution. **Robson and Neal (1997)** studied the water quality in term of pollution from industrial and domestic sources and reported higher pollution loads from domestic sources where the industrial pollution loads are also higher.

Heavy metals and trace elements are by-products of many industrial processes, and as such are discharged as wastes into the marine environment**.** They enter the marine environment through atmospheric and land-based effluent sources. The metals considered toxic and which are of concern have been restricted largely, but not exclusively, to the ten which appear to be most poisonous to marine life.

Wastewater arising from electroplating and metal cleaning industries often contains excessive amounts of heavy metals and causes serious water pollution problems (Robson and Neal, 1997).

In the aquatic environment, the little quantities of some heavy metals, such as: Cu, Zn, Fe, Mn and Ni are essential for biological systems functions, but their excessive concentration can be toxic to living organisms. Other heavy metals such as Cd, Hg and Pb are non-essential and therefore have toxic effects on living organisms,(Ahmed *et al.*, 2018).

The effects of marine pollution to coastal resources are extensive, impacting on the flora, fauna and entire ecology of the coastal environment. Marine pollutants tends to adversely alter or degrade the environment to extreme conditions (Elenwo and Akankali, 2015). Fish are of great economic importance, but are affected immensely by various chemicals including heavy metals directly or indirectly in different ways. Of particular interest is the impact of heavy metals pollution caused to fisheries and other commercial use of coastal and marine habitats. Most of the world’s important fisheries have now been damaged to varying extent (Windom,1992).Once in the system, metals concentrate in protein-rich tissues such as liver and muscle. High trace element burdens in marine mammals have been associated with a variety of responses. These include lymphocytic infiltration, lesions and fatty degeneration in bottlenose dolphins, and decreasing nutritional state and lung pathology (Siebert *et al*., 1999). Mercury shows an age-related accumulation and strong bio magnification in the food web (Nigro and Leonzio, 1996). Correlations have also been reported between age and cadmium levels in the kidneys of harbor porpoises from the east coast of Scotland (Falconer *et al*., 1983). Goldberg (1995) reviewed different sources of heavy metal inputs into the sea and their possible role in ecosystems, heavy metals interference are reported to cause an increase in the permeability of the cell membrane in phytoplankton and other marine algae, leading to the loss of intracellular constituents and, therefore, cellular integrity. Kayser (1976) reported change in cell shape of phytoplankton as a result of heavy metal incorporations and such changes in shape are much likely to be related to the loss of cellular integrity. Similarly, Davies (1978) reported production of very large cells of phytoplankton as an effect of copper and mercury and found that the size spectrum of cells was related to the mercury concentrations. They concluded that metals inhibit independent cell division in phytoplankton and, therefore, they grow big in size.

At contaminated sites, commonly occurring metals are lead (Pb), chromium (Cr), zinc (Zn), cadmium (Cd), copper (Cu), mercury (Hg), arsenic (As) and nickel (Ni), and their total concentration in soils persists for a long time after their introduction and thus, biogeochemical activity of soil is affected including organisms living therein (Bryan and Langston 1992; and Sharifuzzaman, *et al*., 2015).

Removal of metals from waste water is achieved principally by the application of several processes such as adsorption, sedimentation, electrochemical processes, ion exchange, cementation, coagulation flocculation, filtration and membrane processes, chemical precipitation etc. Phyto-remediation has emerged as the most desirable technology which uses plants for removal of environmental pollutants or detoxification to make them harmless. There are several plants used in the phyto-remediation has a considerable capacity of metal absorption, its accumulation and reducing the time of decontamination of an ecosystem (**Soma Halder, 2014)**.

Biotechnology of microalgae has gained importance in recent years due to the development of new production and environmental technology. Because the growth of algae requires unexpensive substrates such as solar light and CO_2, microalgae can be used as cheap and effective biocatalyst to obtain high added-value compounds from simple metabolites to complex molecules, ie., proteins, vitamins, carotenoids, pigments or polysaccharides **(Carlos *et al*., 1997 and Liu *et al*., 2004).**
The study aims to measure the effect of biological treatment on heavy metals removal from wastewater using green algae (*Chlorella salina*).

MATERIALS AND METHODS

Biological material

The biological material chosen in this research was the axenic unicellular green alga *Chlorella salina* obtained from phycology laboratory, Botany Department, Faculty of Science, Alexandria University. Hover, a routine bacterial test was carried out every now and then by inoculating one drop of the culture at the beginning and at the end of the experiment into previously autoclaved 10 ml Vials containing a bacterial growth medium (F. medium). This medium contained 0.lg K_2HPO_4; 0.5 g peptone in 100 ml water and 0.5 g beef extract **(Stein, 1973)**. After incubation for 48 hours the turbidity was examined. Positive cultures were discarded. The basal medium for *Chlorella salina* was used in this work described by **Boussiba *et al*.,(1987)**. It consists of filtered seawater enriched by two solutions (a) and (b). Solution (a) consists of the following salts (g/L natural seawater):

Solution (a) **g /L**		Solution "b" (microelement) consists of (mg/L distilled water)	
Na_2EDTA	0.019	$ZnCl_2$	40.00
$KN0_3$	1.000	H_3BO_3	600.0
$FeCl_3.6H_2O$	0.0 14	$CuCl_2.H_2O$	40.00
KH_2PO_4	0,070	$MnCl_2$	400.0
		$CaCl_2$	1.500
		$(N H_4)_6 Mo_7O_{24}.4H_2O$	370.0

To prepare the enriched sea water medium one ml of solution (b) was added to one liter of solution (a) pH was then adjusted at 7.5.

Culture conditions

The axenic culture of *Chlorella salina* was grown in 50 ml of the basal medium. The flask inoculated with a known number of cells in 250 ml Erlenmeyer Pyrex-glass flasks under controlled laboratory conditions (temperature at 28°C ± 3°c and light at 80µmolmolm^{-2}S^{-1}) in a culturing chamber. This temperature was chosen depending on the results of **Ginzburg and Ginzburg,(1981)** who observed among others insignificant variation in the growth rate of *Chlorella* species in the range of temperature corresponding to 26°C.

Growth measurements

The growth of the tested organism was determined by cell count using the hemacytometer slide consists of 16 small squared areas, each of 0.0025mm^2 and 0.1mm depth. The total volume of the big square was 0.004 mm^3. By means of sterilized pipette a drop of homogenous algal suspension was transferred to the hemacytometer slide the cover was put and the cells were counted microscopically after setting of cells. At least 5 replicates were taken to get the mean number of cells per ml culture.

Wastewater treatment

Primary treatment carried out through the preliminary sieving step at station to get-rid of the large suspended solids. Wastewater sample was supplied from Wastewater Treatment Station at Abees district, Alexandria City, Egypt in plastic-bags.

Heavy metal analysis:

Analysis of Zn, Ni, Mn, Fe, Cr, Pb, Co, Cu and Hg were determined spectrophotometry in mg/l according to Standard Methods for the Examination of Water and Wastewater **(APHA, 1985)**.

The analysis were done for all heavy metals in central lab of faculty of science, Alex. University except Hg determined in laboratory of Ministry of agriculture in Cairo using the following instruments :

1- Perkin – Elmer atomic absorption spectrophotometer model 2380 equipped with air – acetylene burner and HGA- 400 graphite furnace.
2- Hollow cathode lamps for Zn, Ni, Mn, Fe and Cr.

For determine heavy metals after cultivating the alga *Chlorella salina* in wastewater, centrifugation of wastewater was done for 20 min. using angle rotator centrifuge. The supernatants were used for analyzing different heavy metals.

Statistical analysis:

Data obtained in the present investigation were statistically analyzed using the Least Significant Difference test (LSD) at 1% and 5% levels of probability **(Snedecor and Cochran, 1967)**.

CORRELATION

It is the relationship between two data sets, that are scaled to be independent of the unit of measurement. It is used to determine whether two data move together, that is whether large values of one set are associated with large values of the other (positive correlation), whether small values of one set are associated with large values of the other (negative correlation) or whether values of the two set are un related (**Dixon, 1988**).

RESULTS

In the light of the experimental results recorded in the tables and figures it is clear that number of cells increased gradually reached its maximum at the 12th day of culturing in case of normal state and wastewater medium but with different degrees. This revealed that, wastewater acted as inhibitory agent for the growth.

Table 1. Growth parameter measured by cell number of Chlorella salina cultured for 16 days at normal state and wastewater medium.

	Normal state	**Wastewater**
Time (Days)	**Cell No. ×10^6 cells/ml**	**Cell No. ×10^6 cells/ml**
0	**3.70 ±0.01**	**3.70 ±0.01**
2	**4.07 ±0.03**	**3.95 ±0.02**
4	**4.55 ±0.03**	**4.65 ±0.027**
6	**5.37 ±0.02**	**4.81 ±0.03**
8	**6.44 ±0.01**	**4.97 ±0.14**
10	**6.66 ±0.03**	**5.11 ±0.03**

12	**6.70 ±0.04**	**5.27 ±0.05**
14	**5.22 ±0.06**	**3.63 ±0.07**
16	**4.92 ±0.03**	**3.12 ± 0.05**

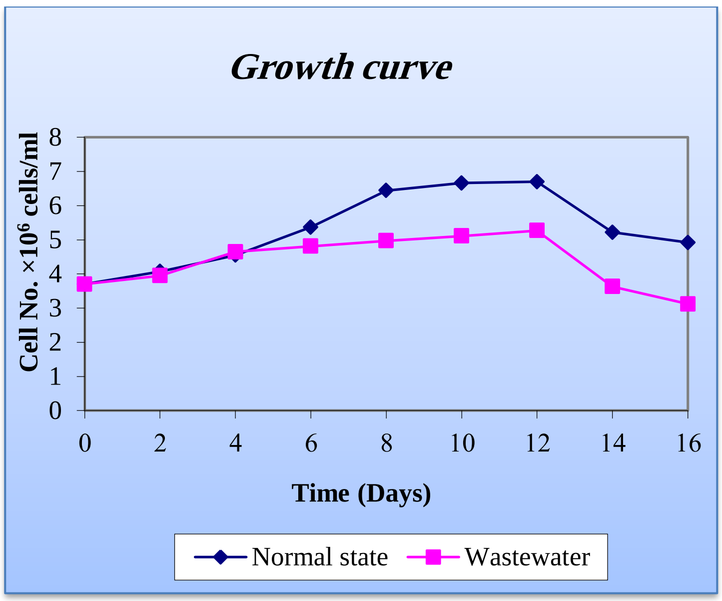

Fig1: Growth curve in cell number of *Chlorella salina* cultured for 16 days in both normal and wastewater media.

The detected average values of some heavy metals of the wastewater before treatment were recorded in table (2) .These heavy metals were Zn, Ni, Mn, Fe, Cr, Pb, Co, Cu and Hg.

The studied wastewater was analyzed for its heavy metals content before and after treatment with the tested algal species cleared that, some elements were found at high concentration and even exceeded the accepted level of concentration, others are found at low concentration which could be considered as safe. The results of the analyses showed also that, other elements were still absent or found at very low concentration, the highest concentration was found in case of Ni and the lowest one was Mn .

Table 2. Heavy metals concentration (mg/l) in wastewater sample .

Heavy metal	Concentration (mg/l)
Zn	**0.090**
Ni	**0.159**
Mn	**0.027**
Fe	**0.117**
Cr	**0.033**
Pb	-----
Co	------
Cu	----
Hg	----

Table 3. Concentrations (mg/l) and removal percentage of heavy metals Zn, Ni, Mn, Fe and Cr from the wastewater by *Chlorella salina* after cultured for 16 days.

Heavy metal	Concentration in waste water (mg/l)	Concentration after treatment (mg/l)	% of removal
Zn	0.090	0.041	54.44
Ni	0.159	0.00	100.00
Mn	0.027	0.003	88.89
Fe	0.117	0.00	100.00
Cr	0.033	0.00	100.00

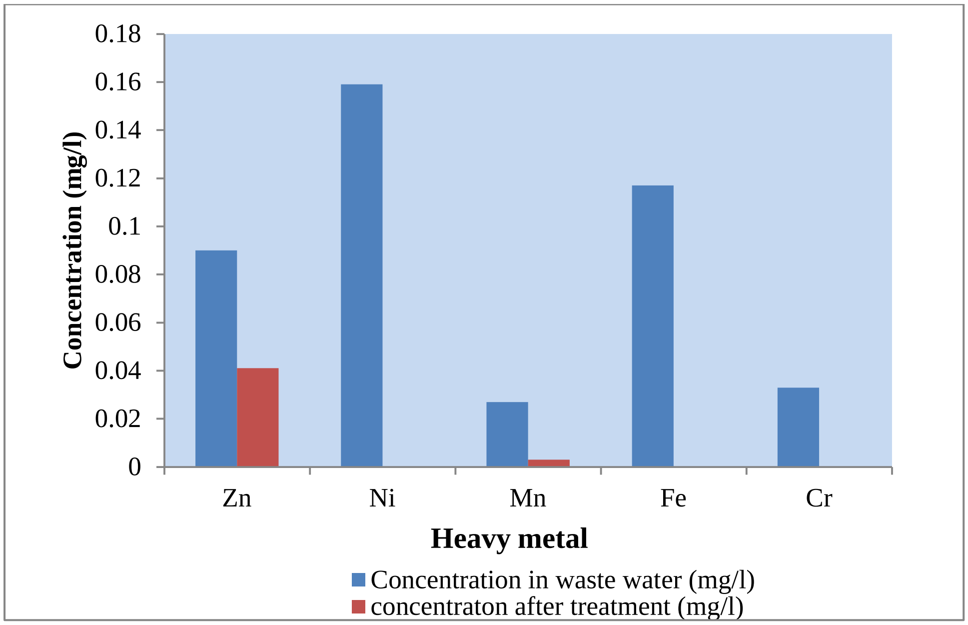

Fig 2 :Concentrations (mg/l) of heavy metals Zn, Ni, Mn, Fe and Cr in wastewater

before and after treatment by *Chlorella salina* culturing for 16 days.

Data obtained in table (3) and recorded in figure (2) cleared that, the bio-removal efficiency of Chlorella salina in different heavy metal uptake which differed according to the type of metal.

DISCUSSION

Heavy metals are non-degradable elements naturally occurring in coastal seas. They are not particularly toxic as the condensed free elements but they are dangerous to living organisms in the form of cations with capacity to bind with short carbon chains. In this form, they bio-accumulate in marine organisms and concentrate year after year.

The principal mechanism of metallic cation sequestration involves the formation of complexes between a metal ion and functional groups on the surface or inside the porous structure of the biological material. Different types of organisms may be used for bio-monitoring, such as marine algae **(Besada *et al.*, 2009).** Metals are taken up by algae through adsorption over the cell surface very quickly which is called as physical adsorption and then these ions transported slowly into the cytoplasm through the process called chemisorptions (**Soma Halder,2014)**.

The blue green algae, *Phormidium sp*. Can successfully hyper-accumulate heavy metals like Cadmium, Zinc, lead, Nickel and Copper **(Wang *et al.*, 1995).**The algae has the advantages of low cost raw materials, more adsorbing capacity of heavy metals, no secondary pollution, moreover waste water cleaning capacity removing nitrate , ammonia and phosphate **(Noaman *et al*., 2004)**.

Wastewater can be used as a substrate for microalgal growth after a stabilization treatment by aeration or anaerobic digestion has been applied. This makes it possible to obtain a final effluent of an acceptable quality to be used for a various purposes **(Canizares *et al.*, 1995)**.

There are several reports that different species of several fresh water microalgae like *Chlorella sp., Anabaena sp., Westiellopsis sp., Stigeoclonium sp., Synecococcus sp.* etc. have high tolerant capacity for various heavy metals **(Dwivedi, 2012).**

The obtained results revealed that highest removal efficiency of nickel, iron and chromium by *Chlorella salina* recorded 100% removal efficiency for all theses elements. In view of the results obtained here , total bio-removal of Ni and Cr by *Chlorella salina* , this result goes in harmony with those obtained by **(David, 1981)** who found that the extracellular products produced by the blue-green algae may be non-specific and form complexes with other divalent cations as well result in decreasing the toxicity. These results go in harmony with those obtained by **(Christine *et al*., 2004)**. Also, **Rehan *et al*., (2004)** found that**,** *Chlorella sp*. could efficiently reduce 78% to 94% of nickel from the medium within 7-28 days when cultured under laboratory condition**.**

The results showed that, uptake of Fe was higher than Mn, result goes coincident with those obtained by (**Allan, 1997**), who found that, Fe is essential as constituents of the catalytic sites of several enzymes. Also, **(Hauck *et al*., 2003)** found that, Fe is essential for chlorophyll biosynthesis.

The results showed bio-removal efficiency of Mn by *Chlorella salina* reached (88.89%), this result goes coincident with those obtained by **(Ansari, 2004)** who found that many enzymes in algae and plants have been shown to be activated by manganese, also **(Mane *et al*., 2011)** found that, up take of Mn by *Spirogyra sp* reached to 99%.

The results revealed that algal tissue of the tested organism accumulate low level of Zn, this result goes coincident with those obtained by **Hammouda *et al*., 1995**). Zinc is an essential source for living things, it can help enzyme to work properly, it becomes an agent in transferring hydrogen in plant's photosynthesis, (**Tjahjono *et al* ., 2017).**

CONCLUSION

If adequate attention is given to the regulation of industrial discharges into the coastal zone, it is extremely unlikely that metals in the ocean will pose any significant threat to either the marine environment or to human health.

By far the greatest volume of waste discharged to the marine environment is sewage which contains in itself a diverse array of polluting agents including pathogens, organic substances, heavy metals and trace elements and so on, which pose direct and indirect effects on ecosystems and organisms.

Removal of metals from wastewater is achieved principally by the application of several processes such as adsorption, sedimentation, electrochemical processes, ion exchange, coagulation, filtration and membrane processes, chemical precipitation etc. Phyto-remediation has emerged as the most desirable technology which uses plants including algae for removal of environmental pollutants or detoxification to make them harmless.

The study aims to measure the effect of biological treatment on removal of heavy metals from wastewater using green algae (*Chlorella salina*). The obtained results revealed that highest removal efficiency of nickel, iron and chromium by *Chlorella salina* recorded 100% removal for all theses elements. Bio-removal efficiency of manganese and zinc reached 88.89% and 54.44% respectively. The efficiency of removal of heavy metal pollution differed according to the type of element.

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