Investigating the individual and mixed effects of heavy metals (Copper and Cadmium) and linear alkyl benzene sulfonate (LAS) on growth and reproduction of Scenedesmus obliquus algae

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Abstract: In this study, we investigated the individual and mixed effects of heavy metals (Cu and Cd) and detergent (LAS) on growth and reproduction of Scenedesmus obliquus algae. We have conducted several tests to determine acute toxicity of pollutants in algae in individual and mixed manners through Selenastrum bottle test method. We used five test samples and a control sample and repeated the tests three times. Concentration ranges were determined by the logarithmic method and finally, the obtained results were calculated by probit analysis and the values of correlation coefficient, EC, and LC (10, 50, and 90) for pollutants were obtained in individual and mixed manners. The results obtained in tests of acute toxicity of algae and values of EC(10, 50 and 90) from the individual effects of heavy metals (Cadmium and Copper), LAS detergent and mixed effects of (Cd and LAS) mixture and (copper and LAS) mixture were, respectively, as the following: Cd(0.068, 0.127, and 0.237), Cu (0.53, 1.5, and 4.24), LAS (10.40, 21.53, and 130), LAS + Cd (0.013, 0.066, and 0.33), LAS + Cu (0.035, 0.21, and 1.32). The obtained allowed limits were 0.0127, 0.15, 2.153, 0.0066, 0.021 mg per liter, respectively, with correlation coefficients of 92, 98, 93, 90 and 95 percent, respectively. According to the non-parametric test of Kruskal-Wallis at 95% of confidence level, we can conclude that, there is no significant difference between copper and mixture of copper and LAS in terms of the effects on algae ($P < 0.05$). In addition, according to the non-parametric test of Kruskal-Wallis at 95% of confidence level, we can conclude that, there is significant difference between LAS and mixture of LAS and copper in in terms of the effects on algae ($P < 0.05$).

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1. Introduction

Algae have an important role in the food chain of the waters as well as protecting the invertebrates in the ecosystem. These invertebrates can be affected by algae in the following ways:

A. Through the adjustment in graze rates, the growth and reproduction rates of algae will be stimulated by increasing nutrients. Therefore, algae are supported in their final growth phase.
B. The increase in graze rate will reduce frequency of phytoplankton.

Therefore, one can say that, the effects of pollutants on aquatic communities (invertebrates) can increase or decrease the frequency of algae (Rouhi Baksh, 1998).

Chemical and physical differences in different species are the most important factor in determining the biological concentration of a pollutant. For example, one can mention the differences in the surface to volume ratio of the species so that, smaller species have the wider surface to absorb pollutants in comparison to larger species with the same weight (Steven & et al, 1995).

Heavy metals of cadmium and copper available in sediments, biologically, accumulate by algae. Through the observations, it is found that, algae, naturally, have more places to connect to the metals than dry sediments and are capable of intervention in collapsing heavy metals from sediments into the water. Algae play an important role in obtaining the heavy metals that are naturally found in sediments and are the representatives of the presence of these metals in invertebrates and finally in fish through the food chain (Alavi, 2003). The importance of algae is in biomass and biodegradation of pollutants which shows this procedure in the food chain as well as the way it protects the algae from other living species of aquatic ecosystems (Sharon & Belliger, 1976). Detergents are relatively capable of breaking plasma membrane proteins and causing the petroleum hydrocarbons and heavy metals enter into the cells. Detergents transform oil materials into small droplets; thus, make it easy for oil material to enter the body followed by reduced growth and reproduction. According to the results, one can say that, sometimes the amount of detergents in the ecosystem may be less than the Lc 50 obtained in the laboratory, but even this amount of concentration in ecosystems may increase the percentage of deaths if be mixed with other chemicals (Tehrani, 2000).

2. Material and Methods

In order to test the acute toxicity of heavy metals and detergents on green algae, we used Selenastrum Bottle Test standard method (Miller, 1978).

Before the cultivation of algae and adding certain amounts of the heavy metals, we must initially calculate the amount of algae required to be added to cultivation environment which is based on dry weight in milligrams per liter as follows.

First, we placed a specific piece of 0.45 micron paper filter into a glass plate and heated it for two hours in 105°C of temperature. Then we took the plate out of the machine and placed into the desiccator to adjust the temperature. After elapsing the required of time, the paper filter was accurately and quickly removed with forceps and its weight was measured and recorded using a ten thousandth digital scale. Then, regarding the concentration of algae, we placed some of the mentioned algae from the net stock on the aforementioned filter and placed it on the vacuum pump to absorb water from cultivation medium. After this step, we, again, placed the paper filter in the plates and put it on the oven with 105 °C temperature for another two hours. After elapsing this time and adjusting the filter's temperature, we measured its weight which is the weight of dry algal cell plus 0.45 microns paper filter (Piri & Oradog, 1997).

Now, concerning the difference between primary and secondary weights, the amount of algae required to be added to the cultivation environment can be determined through a series of mathematical calculations. After determining the amount of algae, using calibrated and sterile pipette, we added the algae from major stock into the flasks in a special cultivation room equipped with UV light bulbs in sterile laminar box. In this experiment, five samples were used as test samples for each heavy metal and detergent, individually and mixed together, and a sample was used as control sample. The tests were conducted for three times and then the heavy metals and detergent solutions were added to the flasks, individually and mixed. The solutions were added with different concentrations which were calculated based on the logarithmic concentration series for each of the heavy metals. Before transferring flasks to the cultivation table, 2 ml of each sample was gathered an stabilized by 4% formaldehyde in order to count the algae. After algae cultivation, adding heavy metals, and putting air pipettes on the door of the flasks, they should be transferred to the cultivation table which equipped with fluorescent lamps. The proper experimental conditions for the growth of algae are 3500±3350 lux of illumination, 14 hours a day and 25 ± 2°C of temperature (Piri & Oradog, 1997).

Acute toxicity test will be conducted within 96 hours and after this time; the aeration will be stopped in order to recount algae.

Using probit analysis (Finny, 1971) the data were analyzed and Ec (10-50-90) or Lc were estimated. In order to calculate the regression line and correlation coefficients of the data, the Quatro Pro and Statgraphics software were used.

To realize the significant difference between the data and investigate this significant difference, the non-parametric Kruskal-Wallis test and Duncan Multiple Range Analysis, a = 0.05 were used.

3. Results

The overall results showed that, the effect of cadmium is more than copper and LAS. Comparing the EC50 of the three pollutants on the Scenedesmus algae showed that, Cd is 11.8 times more toxic than copper and is 169 times more toxic than LAS. Compared to other two pollutants, LAS, in low concentrations, increases the algae growth because, during its biodegradation by bacteria, the water phosphate will be increased resulting in increased algae density (Table 1).

According to the nonparametric Kruskal - Wallis test at 95% of confidence level, we can conclude that, there is statistically significant difference in terms of effects on algae between cadmium, copper and LAS ($P < 0.05$). However, in 99% level of confidence there is no statistically significant difference ($P > 0.01$). The Duncan Multiple Range Analysis (DunCan) shows no significant difference between copper, cadmium and LAS and it is due to the relatively high standard deviation of the Copper and LAS.

Concerning the parametric Kruskal - Wallis test at 95% of confidence level, we can conclude that, there is no significant difference between copper and copper-LAS mixture in terms of the effects on algae ($P < 0.01$).

Concerning the parametric Kruskal - Wallis test at 95% of confidence level, we can conclude that, there is significant difference between LAS and copper-LAS mixture in terms of the effects on algae ($P < 0.01$).

However, at 99% of confidence level there is no statistically significant difference between LAS and copper- LAS mixture ($P > 0.01$). Given the above results we can say that, there is no significant difference between cadmium and cadmium-LAS mixture in terms of the effects on the algae ($P < 0.05$). Moreover, one can conclude that, there is no statistically significant difference between LAS and LAS-Cadmium mixture ($P < 0.05$).

Table 1: Experimental results of testing heavy metals of Cd, Cu and LAS detergent, individually and mixed, on Scenedesmus obliquus algae

Type of pollutant	Concentrations in the Scenedesmus obliquus algae samples (Ppm)	Regression line equation in 96 hours, y = ax + b	The correlation coefficient (r)%	Lethal concentration in mg per liter in 96 hours		
				EC10	EC50	EC90
Cd	0.1-0.6	y= 5.802x+10.148	92	0.068	0.127	0.237
Cu	0.79-5	y= 2.8592x+4.4912	93	0.53	1.5	4.24
LAS	19.6-74	y= 2.3489x+1.3378	98	10.40	21.53	130
LAS + Cu	0.15-4.24 and 2.15-130	y= 1.6333x+6.0818	90	0.035	0.21	1.32
LAS + Cd	0.127-0.237 and 2.15-130	y= 1.8858x+7.2103	95	0.013	0.066	0.32

Figure 1: The effect of Cadmium on Scenedesmus obliquus

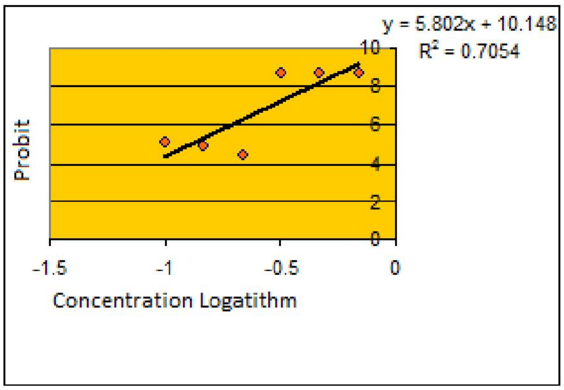


Figure 2: The effect of Copper on Scenedesmus obliquus

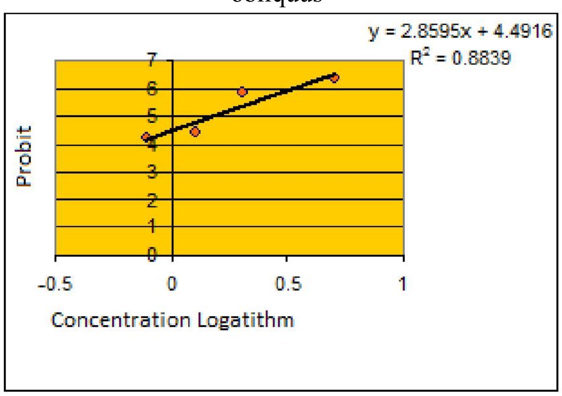


Figure 3: The effect of LAS on Scenedesmus obliquus

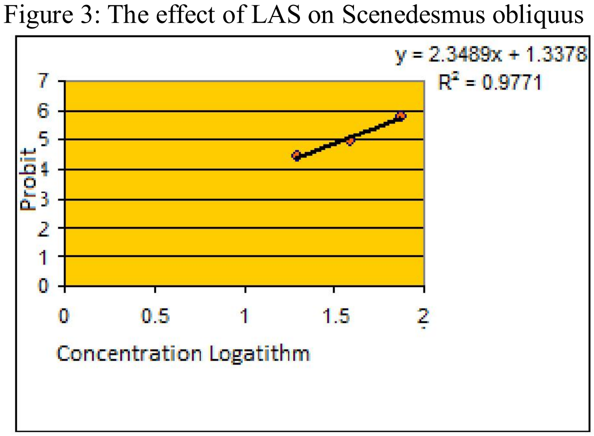


Figure 4: The effect of Cadmium and LAS on Scenedesmus obliquus

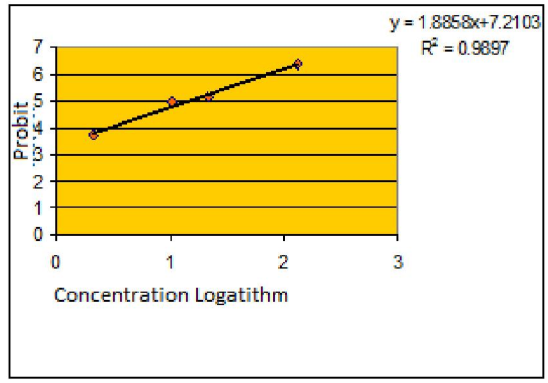
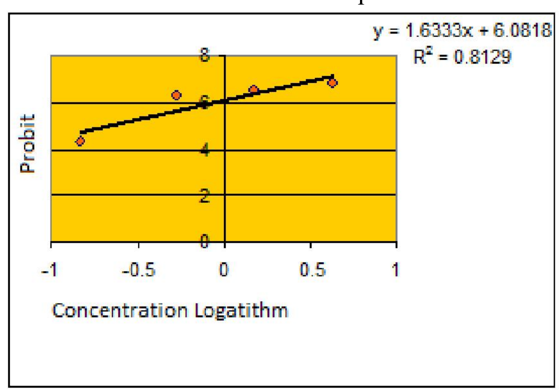


Figure 5: The effect of Copper and LAS on Scenedesmus obliquus



4. Discussions

The results indicate that, cadmium is more toxic than green algae. The 0.127 mg per liter of concentration of this metal reduces the Scenedesmus Obliquus algae population by 50% and its allowed limit is 0.0127 mg per limit. Furthermore, the effective concentration of this metal is 0.1 to 0.6 mg per liter. Studies of Reddy et al. (1997) show that, this algae cat resist up to 6 mg per liter without any lethal effect. However, above 2 mg per liter of concentration, the alga faces considerable reduction in growth rate (Figure 1).

Rand (1995), in his studies on green algae, found the minimum and maximum toxicity of copper 1.99 and 7.11, respectively, which are relatively in agreement with our results. However, the results of lower concentrations have higher effect and they are also lower than the lethal limit reported by Rand.

According to the algal cell counts and quantitative study of the effects of copper on this alga, it was found that after 96 hours of cultivation, the percentage of algal density of Scenedesmus Obliquus are 80.7, 52.8, and 18.5 mg per liter compared to control as 0.79, 1.26, and 2 mg per liter. With increasing the copper concentration, algal density is reduced so that, at 5 mg per liter of concentration, the density is equal to 4.7 percent (Figure 2).

The results indicate that, the 21.53 mg per liter of concentration of LAS reduces the Scenedesmus Obliquus algae population by 50% and its allowed limit is 2.153 mg per limit. Furthermore, the effective concentration of this pollutant is 19.6 to 74 mg per liter. Chattopadhyay & Konar (1991) showed that, the activity of organisms and algae may reduce the toxicity of detergent in the water. Therefore, we conclude that, algae by decomposing detergents in lower concentrations of detergents, increase the phosphate levels and consequently they grow. However, in higher concentrations, the algae cells will be affected by detergents and will not be able to grow. In addition, due to the reduction of oxygen, these materials cannot properly decompose (Figure 3).

The results of the effect of LAS + copper showed that, the 0.21 mg per liter of concentration reduces the algae population by 50% and the allowed limit of this mixture is 0.021 mg per liter. As results indicate, the EC50 effective concentration of LAS + Cu mixture is 7.1 times lesser than the Copper alone. This means that, when LAS is added to Copper, the toxicity of the Copper is increased so that, if 1.5 mg of Copper causes 50% of mortality in algae, after adding LAS, more than 90% of algae will be eliminated. The EC90 of Copper for the algae in mixture is 1.32 mg which is more than the EC50 of the Copper alone. When the Copper is mixed with LAS, its permeability is increased and is more toxic in lower concentration (Figure 5).

Okwuosa & Tomoregie (1995) stated that, the population of planktons which were exposed to chemical substances and LAS were significant reduced. They added that, the mixture of pollutants may affect the feeding, reproduction, and general activities of the planktons. The results of this research have shown that, the toxicity of LAS-Copper mixtures is 7 times more than the copper alone indicating the catalyzer role of the LAS. Increasing the concentration of pollutant mixture significantly reduces the density of algae.

In addition, the results of the effect of LAS + Cadmium showed that, 0.066 mg per liter of concentration reduces the population of this alga by 50% and its allowed limit is 0.006 mg per liter. The results also showed that, the toxicity of LAS + Cadmium mixture is two times more than the Cadmium alone (Figure 4).

Counting the algae and quantitative analysis of this mixture showed that, after 4 days of cultivation, the percentage of algae density, compared to the control group, for the following concentration of Cadmium + LAS mixture: (0.0127 and 2.15), (0.068 and 10.40), (0.127 and 21.53), and (0.237 and 130) mg per liter are 89.4, 52.8, 44.99, and 8.3 percent, respectively. Therefore, by increasing the concentration of this mixture, the algal density is decreased.

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