Journal of Fertilizers & Pesticides

Hussein et al., J Fertil Pestic 2016, 7:2
DOI: 10.4172/2471-2728.1000173

Research Article Open Access

Phycoremediation of Some Pesticides by Microchlorophyte Alga, *Chlorella* Sp.

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Abstract

Every year, pesticides are found in surface and ground waters in Egypt. Pesticides are uncommon usage and applied in high amounts in agricultural activities. The present study investigated the possible removal of some herbicides from water using the microalgae *Chlorella vulgaris* Microorganisms are capable of decomposing a range of organic pollutants and the main focus in previously published studies has been on bacteria and fungi. Microalgae are microorganisms that have different morphological, physiological, and genetic traits that confer the ability to produce different biologically active metabolites. Because of the high capacity of microalgae in biosorbing heavy metals, most of their studies concentrated on this advantage, but fewer studies reported the removal of organic pollutants such as pesticides. The experiments were conducted as the following; the first was long-term experiment (5 days) using growing cells, and the second was short-term experiment (60 min) using dead and living cells. In the long-term experiment, the presence of growing algae resulted in removal percentages of pesticides ranged from 87% to 96.5%, while in the short-term study, the presence of live algae cells led to removal percentages ranged from 86 to 89% and dead algae biomass achieved removal ranged from 96% to 99%. The main mechanism behind the removal of pesticides in the water phase is proposed to be biosorption onto the algal cells. This conclusion is based on the short duration required for removal to occur.

Keywords: Phycoremediation; *Chlorella vulgaris*; Pesticides; Bioremoval efficiency; Algae biomass; LC-MS/MS

Introduction

In the near future, water reuse will become especially important in densely populated arid areas where there is an increasing demand to supply water from limited supplies. Human well-being in a future world will depend more heavily upon this sustainable resource and the characterization of emerging contaminants will become important for ecological and human health risk assessments and commodity valuation of water resources [1,2]. Egypt is an agricultural country. Agricultural activities account for 28% of total national income, and nearly half of the country's work force is dependent on the agricultural subsector for its livelihood. An increase in environmental contamination by various chemicals such as OCPs and herbicides are anticipated along the Nile Delta, which is referred to as "Green Lungs of Egypt" [3]. Furthermore, the chemical industry in Egypt is, by far, the main source of hazardous waste release in developed regions. These industries have encountered frequent problems in disposing of the hazardous waste they generate. In addition to the foregoing pollution, water pollution is exacerbated by agricultural pesticides, raw sewage, and urban and industrial effluents [4]. Consequently, pesticides residues in water, plants and grasses may be ingested by herbivorous animals and eventually find their way into tissues [5]. Thus, the remediation of pesticides is very urgent, especially bioremediation by microalgae.

Chemical properties of the pesticide such as molecular weight, functional groups and toxicity affect the metabolic degradation of it [6]. Algae appear to be more able to metabolize organic compounds with low molecular weights than larger molecules [7-9].

Atrazine have effects on health that classified in three groups developmental reproductive and cancerous US Department of Health and Human Services (USDHHS). In developmental causes post implantation losses, decrease in fetal body weight in complete bone formation, neuro development effects, delayed puberty and impaired development of reproductive system. The effects harmful on reproductive system include pre-term delivery, miss carriage and various birth defects. The cancerous effects include Non-Hodgkin's lymphoma, prostrate, brain, testes, breast and ovarian cancer [10] Atrazine used widespread and toxicity necessitates search for remediation technology. Several methods are available for remove atrazine from contaminated soil, water and wastewater such as chemical treatment, incineration, adsorption, phytoremediation and biodegradation. Biodegradation of atrazine is a complex process depends on nature and amount of atrazine in soil or water. The biodegradation of atrazine in environment is limited by microorganism's available [11]. The major steps of atrazine degradation pathway are Hydrolysis, dealkylation, deamination and ring cleavage. Process dealkylation of amino groups to give 2–chloro 4–hydroxyl -6- amino– 1, 3, 5 triazine is unknown.

In hydrolysis, atrazine degradation occurs by hydrolytic pathway is consist of three enzymatic steps catalyzed AtzA, AtzB and AtzC that hydrolysis the bound between c-cl and then Ethyl and isopropyl groups catalyzed and in the end of this process producing of cyanuric acid that convert to ammonia and carbon dioxide by AtzD, AtzE and AtzF enzymes [12].

The main objective of the present study is to examine the possibilities for utilization of the microalgae *Chlorella vulgaris* to simultaneously remove a number of herbicides, pesticides and insecticides in concentrations representative of their residue values in monitoring reports in water.

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Received November 29, 2016; **Accepted** December 04, 2016; **Published** December 15, 2016

Citation: Hussein MH, Abdullah AM, Eladal EG, El-Din NIB (2016) Phycoremediation of Some Pesticides by Microchlorophyte Alga, *Chlorella* Sp. J Fertil Pestic 7: 173. doi:10.4172/2471-2728.1000173

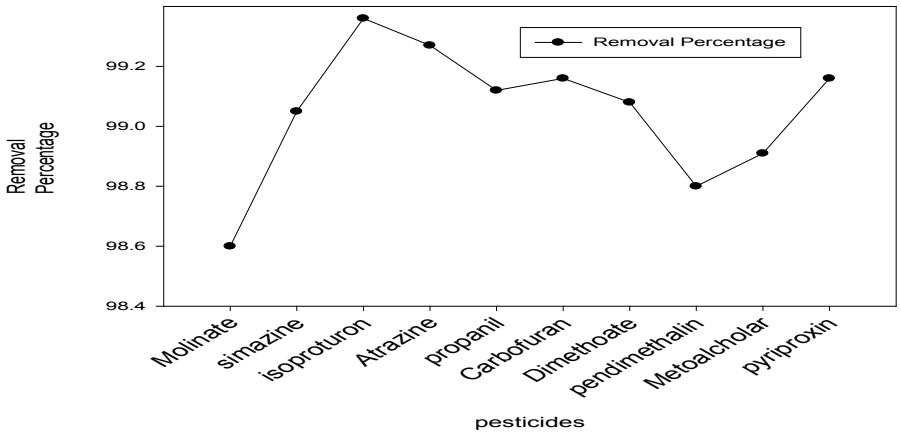

Figure 1: Short-term study; 2 µg/L pest. Mix STD, Lyophilized Chlorella 0.6 g dry weight per liter, Short term: 1 h.

Materials and Methods

Algal strain isolation, identification and culture conditions

Fresh water *Chlorella vulgaris* was isolated from water sample from river Nile. Culture purification was according to [13] and the alga was identified according to Ref. [14]. *Chlorella vulgaris* was grown in axenic cultures at 27 ± 2°C under continuous illumination 3600 lux in 500 Ml Erlenmeyer flasks, containing 200 mL BG11 medium [15] for 5 days incubation period in an Illuminated Memmert incubator.

Pesticides

Custom standard mixture (Atrazine, Molinate, Simazine, Isoproturon, Propanil, Carbofuran, Dimethoate, Pendimethalin, Metoalcholar, Pyriproxin) 0.1 mg/mL for each in methanol was purchased from Accustandard Inc., USA. The standard was obtained from The Reference Laboratory for Drinking Water, Cairo, Egypt. Standard solution containing the 10 micro contaminants in methanolic solution was added to each flask (final water or medium volume of 0.1 L) to obtain a final concentration of 2 µg L^{-1} and 10 µg L^{-1}. The concentration 10 µg L^{-1} was kept in high concentration level for further detection of pesticides in agricultural surface water following a runoff or spray drift events [16,17].

Short-term study

Lyophilized biomass was prepared by cultivating *Chlorella vulgaris* under certain conditions described in the previous section for Five days. Collecting the biomass using centrifugation (3000 g, 15 min, Bench-top - TD5B, Germany), after washing once with distilled water, the pellet was lyophilized in a freeze dryer for 24 h. Storing the lyophilized biomass in dark conditions at room temperature.

The lyophilized biomass was stored under dark conditions at room temperature whereas under similar growth conditions were used to produce the living biomass. After five days, an equal amount of live biomass was centrifuged. The dry biomass was crushed by a small mortar to a powder, ahead of the experiment. An initial concentration of 2.0 µ gL^{-1} and 10 µgL^{-1} (Figures 1 and 2) was obtained by adding the pesticide mix to sterile Milli Q water. The experiments included Lyophilized algal biomass, living algal biomass and a control without any biomass, with three replicates per experiment. The amount of biomass (lyophilized or live) added to each replicate corresponded to 0.6 g dry weight per liter (6×10^7 cells ml^{-1}). There were three replicates per treatment and the total volume of each replicate was 100 ml. The treatments were stirred on a shaker orbital at a speed of 380 rpm for 1 h at room temperature. After one hour, the biomass was removed from the aqueous phase by centrifugation (4000 g, 20 min, Bench-top - TD5B, Germany) and the samples were stored in the freezer at -20°C until analysis (Thermo Scientific™ MaxQ™ 4450 Benchtop Orbital Shakers, USA), the experiments were conducted. After one hour, by centrifugation (4000 g, 20 min, Bench-top - TD5B, Germany) the biomass was removed from the aqueous phase and the samples were kept in the freezer at -20°C until analysis. The pH values at the end of the experiment were measured and found to be 6.2 ± 0.06 in treatment with dead algae, 6.4 ± 0.2 in live algae and 6.4 ± 0.4 in the control, The pH was measured using a Hach® HQ40d pH meter.

Long-term study

A final concentration of 2.0 µgL^{-1} and 10 µg L^{-1} was obtained by adding the pesticide mix to sterile BG11. The experiments consisted of one experiment with growing *Chlorella* and a control without any biomass. There were three replicates per experiment and the total volume of each replicate was 100 ml. In the treatment with *Chlorella*. and inoculum of 10% (v/v) of a five-day old culture was added which resulted in a starting density of 3×10^6 cells ml^{-1}.

The control treatment received an inoculum of 10% (v/v) of sterile BG11. The experiments were kept under the growing condition described above for 5 days. After the experiment the biomass was removed by centrifugation (4000 *g*, 20 min, Bench-top - TD5B, Germany) and samples of the aqueous phase were taken and stored in the freezer until analysis. After the experiment, the cell density was 3×10^7 cells ml^{-1} and the pH was measured to 7.6 ± 0.03 in the treatment with algae and 7.43 ± 0.03 in the control.

Chromatographic analyses

Samples (50 ml) from the aqueous solution were sent to The Reference Laboratory for Drinking Water, Cairo, Egypt for chromatographic analyses. Reference method EPA 536 (EPA 536, 2007), were used to conduct the pesticides analysis, which is based on a combination of liquid chromatography (LC) and mass spectroscopy (MS) specifically called LC-MS/MS (tandem-MS). Tandem-MS (Xevo-TQ-S, Waters Corporation, Milford, MA, USA) provides low detection limits and very high security, which means that more substances can be tracked at lower level.

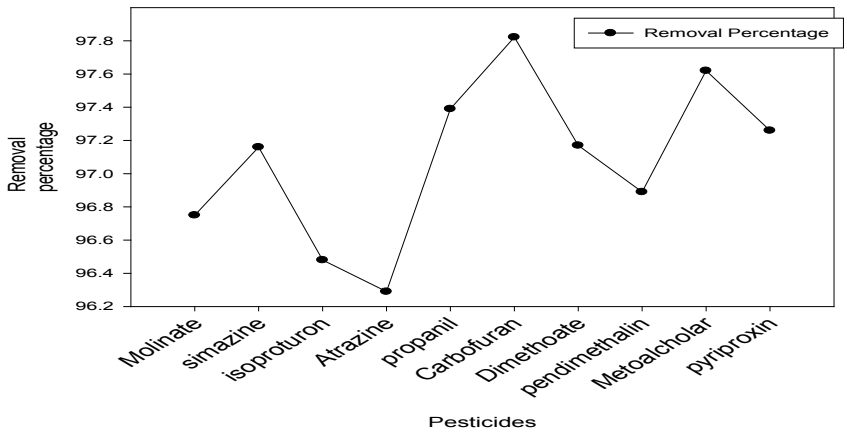

Figure 2: Short-term study; 10 µg/L pest. Mix STD, Lyophilized Chlorella 0.6 g dry weight per litre, Short term: 1 h.

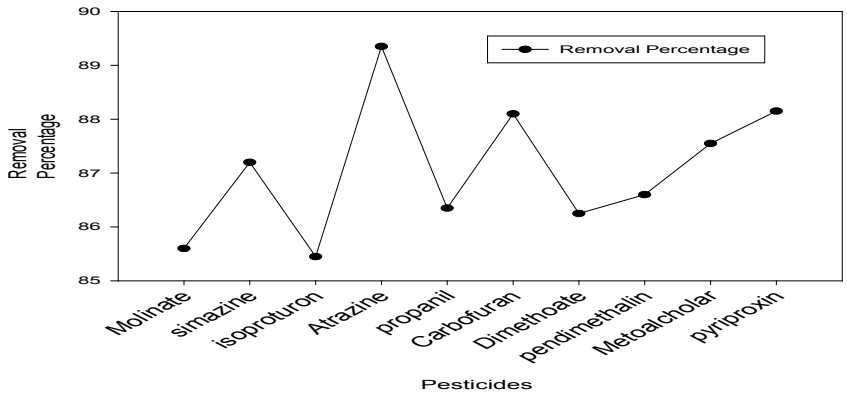

Figure 3: Short-term study; 2 µg/L pest. Mix STD, Live Chlorella 0.6 g dry weight per liter, Short term: 1 h.

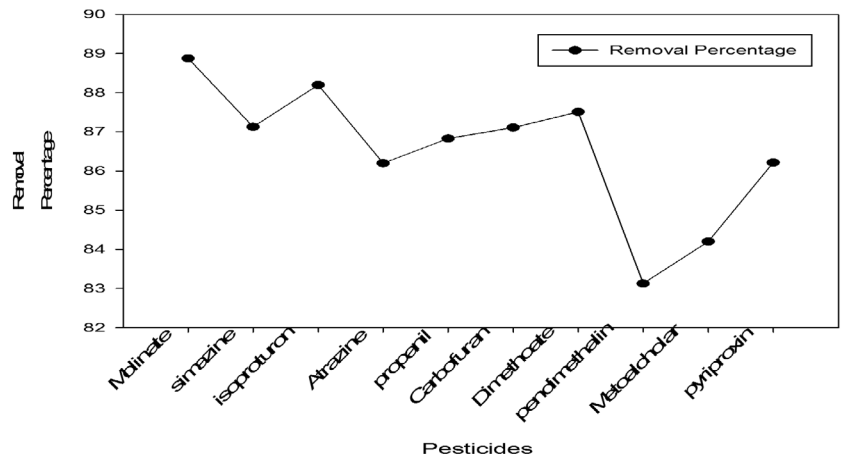

Figure 4: Short-term study; 10 µg/L pest. Mix STD, Live Chlorella 0.6 g dry weight per liter, Short term: 1 h.

Results

Biosorption/Short-term study

Both lyophilized and living biomasses of *C. vulgaris* achieved a good removal percentages for the two concentrations of pesticides 2 µg/L and 10 µg/L. In the short-term study, Figure 1 shows removal percentages ranged from 98.6 to 99% by lyophilized algae.

Figure 2 shows that: in the short – term study, the presence of lyophilized algae at the concentration of 10 mg/L led to removal percentages range 96% to 97.8 5%.

Figure 3 shows that: In the short-term study, the presence of live algae cells at the concentration of 2 µg/L led to removal percentages ranged from 86 to 89.6%.

Figure 4 show that: In the short-term study, the presence of live

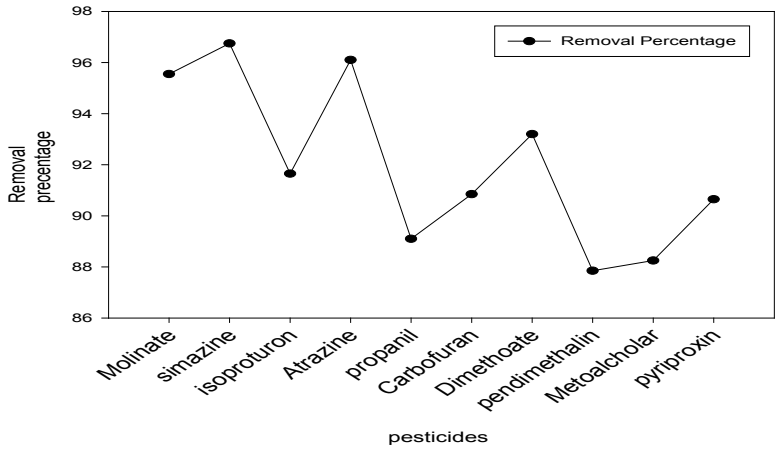

Figure 5: Long-term study; 2 µg/L pest. Mix STD, Chlorella an inoculum of 10% (v/v), 5 days starting density of 3 × 10^6 cells ml^{-1}.

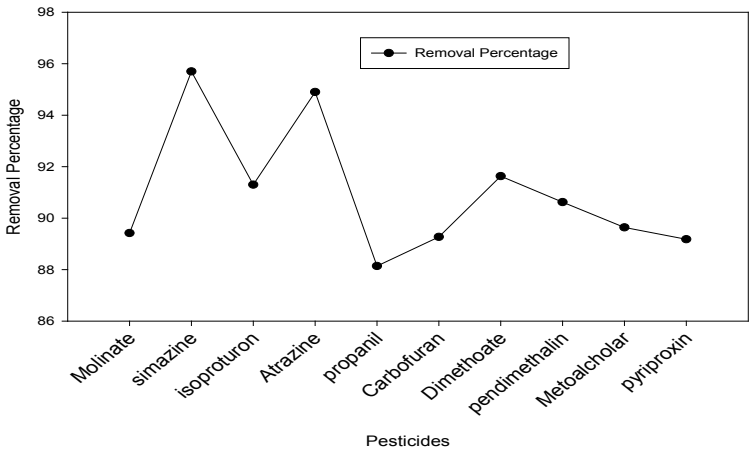

Figure 6: Long-term study; 10 µg/L pest. Mix STD, Chlorella an inoculum of 10% (v/v), 5 days starting density of 3 × 10^6 cells ml^{-1}.

algae cells at the concentration of 2 µg/L led to removal percentages ranged from 86 to 89%.

Long-term study

For the long-term study, it was observed that the removal of pesticides had been achieved with high percentage, by the growing microalgae as illustrated in Figures 5 and 6.

Discussion

Pesticides are used worldwide. The general population can be exposed to low concentrations of agricultural pesticides through contamination of air, water, food supplies [18] and also through household use [19]. Due to the application of pesticides in agriculture or for the purposes of protection of public health such as malaria prevention, high exposures are linked to these compounds. Pesticides 'contamination of water has been well documented worldwide to be considered as a potential risk for the ecosystem. Pesticide residues are commonly existing in the aquatic environment as a result of surface runoff, leaching from surface pesticides' applications, careless disposal of empty containers, and through industry and domestic sewage [20,21].

Consequently, developing efficient treatment systems is necessary for remedying these pesticides in polluted water bodies or catching them in wastewater treatment before they pollute the environment. Ranged from 87% to 96.5%.

Short-term study

Both lyophilized and living biomasses of *C. vulgaris* achieved a good removal percentages for the two concentrations of pesticides 2 µg/L and 10 µg/L, In the short-term study, the presence of live algae cells led to removal percentages ranged from 86 to 89% and dead algae biomass achieved removal ranged from 96 to 99%.

Because of the short period (60 min) needed for the removal of pesticides, it implies that biosorption as the proposed mechanism. Since, there is insufficient time for active uptake or mentalization processes to occur.

Long-term study

At the end of the five-day experiment, the removal percentages ranged from 87 to 96.5% for all the pesticides were used. The removal percentages were near that was achieved by the living Chlorella in short-term experiment, which suggest that the pesticides removal in the long-term experiment is the same as in the short-term experiment, namely biosorption. However, in the long-term treatment there was

sufficient time for some mentalizations processes to happen by the algae. The algae may either have biosorbed, metabolized or facilitated the degradation of pesticides, or it can be due to a combination of those.

The recovery percentages of the control in the long-term experiment is the same as in the short-term experiment equal to 99%. These results demonstrate that the pesticides under study are very stable to hydro- and photolysis in aqueous media through all the experiments conducted.

This data indicates that it is a complex system where many effects take place simultaneously. Even though the herbicide concentration is not a significant factor by itself, it interacts with the other variables. The result of most practical interest is the high removal when algae is used, which indicates that the combination of an adsorption mechanism by the biological activity of algae to degrade herbicides, achieves more than 90% removal after 5 days of treatment.

Application of microalgae for treatment

In the present work it was proved that it was possible to remove not only the heavy metals as the previous studies achieved, but also pesticides from water by short time remediation with algal cells. The easily produced species *Chlorella vulgaris* is a promising organism to work with for the removal of heavy metals and pesticides from polluted water bodies.

This offers an idea to make a filter of dead algae biomass to be used for removal of pesticides from polluted water. As stated previously, using dead biomass instead of live has the advantages that the product will be stable and no risk for damaging the cells is expected. Dead biomass has also the ability to be recycled [22]. The biosorbed pollutants could be washed away from the algal biomass [23] and processed in a safe way [24] and thereafter the biomass filter itself could be reused.

Another idea is to use live *Chlorella vulgaris* in remediation systems, providing metabolization in addition to biosorption. *C. vulgaris* can grow in autotrophic, mixotrophic and heterotrophic modes [25] which gives the algae competitive advantages over bacteria and fungi in treatment of organic pollutants in certain environments, which acts as great advantage of using living cells [26].

Conclusion

In this study, pesticides removal by microalgae *Chlorella vulgaris* was investigated. Two main experiments were conducted: short-term for dead and living *Chlorella vulgaris*, to examine their ability for removal, and long-term treatment by growing *Chlorella vulgaris*. The results showed that the lypholized biomass achieved removal percentages reached up to 99% of pesticides and higher than living *Chlorella vulgaris* at the short-term experiments. On the other hand, long-term experiments proved the ability of growing *Chlorella vulgaris* for the removal of pesticides, which ranged from 87 to 96.5%. These results confirm that it is possible to remove more than 90% of these herbicides.

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