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Studies on Phycoremediation Of Chlorpyrifos And Heavy Metal Chromium Using Algae

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Abstract
The discharge of pesticides and heavy metals into aquatic ecosystem has become a matter of concern and these pollutants are introduced into the aquatic system as a result of industrial operations. The conventional methods in removal of these components has proven expensive and difficult. The increase in bio-magnification of these pollutants have affected the ecosystem. Due to the awareness of its ecological effects of the pollutants, years of research is being done to find solution for this. Recent studies on the use of bio-sorbents for heavy metal removal have revealed immense potential of algae for bio-sorption. In this study a new and cost-effective method was studied involving the removal of pesticides and heavy metal from aquatic system. The freshwater algae were collected and screened for bio-adsorption and biodegradation of the pollutants. Stress tolerance of the algae against pollutants was studied, later the pollutants were given as feed to the algae and their growth was studied. The fresh water algae have greater potential in biodegradation and bio-adsorption of the pollutants, further studies can be done to maximize the efficiency of the algae and reduce the pollutants from aquatic habitat.

Keywords
biodegradation; bio-adsorption; heavy metals; microalgae; bioremediation; adsorption

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INTRODUCTION
Insecticide is a chemical substance or mixture of substances used to kill an insect. Toxicity is the degree to which a substance can damage an organism. Toxicity can be referred to the effect on a whole organism, such as an insect, pest, birds, soil and so on. The use of insecticide in agriculture can leave chemical residue on food commodities. Toxicity of a pesticide is its capacity or ability to cause an illness. The toxicity of a particular pesticide is determined by subjecting the test macro

and microalgae to varying dosages of the active ingredients. The active ingredient is the chemical component in the pesticide product that controls the pest. The two types of toxicity are acute and chronic. Acute toxicity of a pesticide refers to the chemical's ability to cause injury to a person or animal from a single exposure, generally of short duration. The chronic toxicity of a pesticide is determined by subjecting test animal to long-term exposure to the active ingredient. All the organophosphate insecticides act on the nervous system of the parasites as inhibitor of acetyl cholinesterase. Its role is to terminate the transmissions of nervous signals. Its exposure has also been linked with persistent developmental disorders and autoimmune disorders. About 30% of agricultural produce is lost due to pests. Hence, the use of pesticides has become indispensable in agriculture. The abusive use of pesticides for pest control has been widely used in agriculture. However, the indiscriminate use of pesticides has inflicted serious harm and problems to humans as well as to the biodiversity (Gavrilescu, 2005; Hussain*et al*., 2009).

Metals are elements that occur naturally in rocks in relatively low concentrations. They have useful properties and are important components in our daily life. Metals and metalloids comprise about 75% of the known elements. Only H, B, C, N, P, O, S, halogens, and noble gases are not included in this category. Based on chemical and physical properties (the chemical approach), metals have been classified as light, heavy, and metalloids (semi-metals). The term heavy metal was widely used and refers to metals and metalloids with an atomic density greater than 5 g cm3. Sometimes the term toxic heavy metals are used to emphasize the impact of these elements on the environment and more specifically on their effect on the biota (the biological approach). Since heavy metals exert toxic effects on living organisms, they are termed toxic heavy metals. Some of the heavy metals, such as copper, nickel, and zinc, are, at very low concentrations, essential for life (also termed microelements or trace elements) because they play important roles in metabolic processes taking place in living cells (Gadd*,* 1993). However, elevated levels of these metal ions are toxic to most prokaryotic and eukaryotic organisms. Other heavy metals such as cadmium, lead, and mercury are nonessential and are known to cause severe damage in organisms even at very low concentrations. Metal speciation (chemical forms) is determining metals solubility, mobility, availability, and toxicity. It is generally accepted that for most metals the free ion is the species' most toxic to aquatic life (Sunda & Guillard, 1976; Anderson & Morel, 1978). Some organic forms such as methyl-mercury are taken up very efficiently by living organisms. It is more toxic than other mercury species (George, 1991).

Pesticide persistence in environment is caused by either their physiochemical properties or the lack of organisms able to degrade them. Light, heat or humidity could lead to loss of some pesticides by either volatilization or degradation. Contrastingly, degradation caused by organisms, could help decreasing considerably the pesticide persistence in environment. This information could be used to improve elimination of the undesirable effects of pollutants by using organisms; such an approach has been called bioremediation. The ability of organisms to bioremediate insecticides is mainly based on their biodegradation activity. Now the bioremediation is done with the help of plants, macro and micro algae too. Any factor which can alter growth and metabolism, would also affect biodegradation. Some bio degraders need other substrate to degrade pollutants, this phenomenon is called co-metabolism. When insecticide degradation occurs it usually involves more than one organism. It seems that the presence of different organisms is essential for an adequate biodegradation. Insecticide might undergo degradation reactions like de-chlorination, cleavage, oxidation, reduction by different organisms. Currently, organophosphorus pesticides form the major and most widely used group among the various groups of pesticides that are used worldwide and it accounts for more than 36% of the total world market (Ghosh *et al*., 2010). Organophosphates (OP) are used to control a variety of sucking, chewing and boring insects, spider mites, aphids, and pests that attack crops like cotton, sugarcane, peanuts, tobacco, vegetables, fruits and ornamentals.

A wide range of anthropogenic activities contribute to the discharge of heavy metals to the environment, for example, intensive agriculture, metallurgy, energy production, and microelectronic and sewage sludge. Heavy metals are stable and persistent environmental contaminants since they cannot be degraded or destroyed. Therefore, their toxicity poses major environmental and health problems and requires a constant search for efficient, cost-effective technologies for detoxification of metal-contaminated sites. The passive (not metabolically mediated) binding of metals by living or dead biomass is commonly referred to as bio sorption. This process can be used to remove and recover toxic heavy metals from metal bearing industrial wastewaters (Volesky, 1990). Interest has primarily focused on heavy metals due to their known toxicity as they are discharged in small quantities by numerous industrial activities into the environment, where they tend to accumulate, being concentrated throughout the food chain. This aspect coupled with their persistence results in a serious health hazard threatening water supplies and populations depending on them. Compared to conventional techniques such as precipitation or synthetic ion exchange resins, bio sorption offers the advantage of rendering it possible to achieve drinking water quality at low cost. This is the case because cheaply available biomass, such as waste products from other industries (e.g., food and fermentation industry) or abundant marine algae, may be used as effective bio sorbents. The information is mostly relevant to the description of metal accumulation by living plants or *to* the toxic effects of metals on their metabolism. Sometimes the levels of metals found in algal biomass served to detect the water contamination by metallic species (Vymazal*,* 1990).

Algae possess a high metal binding capacity (Ramelow et al., 1992; Holan and Volesky, 1994) whereby the cell wall plays an important role in metal binding *(*Crist et al., 1988*;* Kuyucak and Volesky, 1989). This is due to the presence of carboxyl and sulfate groups in algal cell wall polysaccharides which can act as binding sites for metals. The cell wall matrix of green algae contains complex hetero polysaccharides which also offer carboxyl and sulfate groups (Lee,1980). At low pH they will be protonated and thereby become less available from the binding of metals, which explains why the binding of many metals increases with increasing pH (Greene et al., 1987; Ramelow et al., 1992). The competition between protons and metal ions for the same carboxyl and sulfate binding sites has been described by a pH sensitive bio sorption isotherm model which allows the prediction of pH effects on metal binding and the amount of protons bound (Schiewer and Volesky, 1995).

Increased use of metals and chemicals in process industries has resulted in generation of large quantities of effluent that contain high level of toxic heavy metals and their presence poses environmental–disposal problems due to their non-degradable and persistence nature. In addition, mining, mineral processing and extractive-metallurgical operations also generate toxic liquid wastes. Environmental engineers and scientists are faced with the challenging task to develop appropriate low-cost technologies for effluent treatment. Conventional methods for removing metals from aqueous solutions include chemical precipitation, chemical oxidation or reduction, ion exchange, filtration, electrochemical treatment, reverse osmosis, membrane technologies and evaporation recovery. These processes may be ineffective or extremely expensive especially when the metals in solution are in the range of 1–100 mg $^{-1}$. Another major disadvantage with conventional treatment technologies is the production of toxic chemical sludge and its disposal/treatment becomes a costly affair and is not eco-friendly. Therefore, removal of toxic heavy metals to an environmentally safe level in a cost effective and environment friendly manner assumes great importance. In the present study algae was used for degradation of pesticide (Chlorpyrifos) and biosorption of chromium.

MATERIALS AND METHODS

SAMPLE COLLECTION

Samples of fresh water algae *Spirullinaplantensis, Azolla* and *Spirogyra* were collected from ponds, lakes and damp soil places in and around Coimbatore, South India. Samples were collected in glass bottles and then transferred to BG11 media. The algae were washed twice with tap water and thereafter with double distilled water thoroughly to remove epiphytes and adhering dirt particles. Then the samples were subjected to sub-culturing, thus inoculating it into BG11 media. The samples were cultured at room temperature with the optimum pH of 7.5 with proper light intensity and aeration.

STUDIES ON PESTICIDE DEGRADATION BY ALGAE

SCREENING OF ALGAE FOR PESTICIDE

The algal sps were tested for their ability to grow on chlorpyriphos by inoculating to the medium containing chlorpyriphos (25, 50, 75, 100 ppm). The best tolerable sps were used for further analysis.

DEGRADATION OF PESTICIDE BY ALGAE

The medium containing chlorpyriphos (50 pm) was prepared and sterilized. The selected strains were inoculated and incubated at 30°C for 7 days. At regular intervals, samples were drawn and analysed. The biodegradation potential of the selected strains was evaluated by analysing the residual chlorpyriphos in the medium. one ml of the sample was transferred to centrifuge tubes and centrifuged at 10,000 RPM for 10 minutes. The supernatant was separated out and extracted with chloroform. Analiquote of test solution containing 1.2 to 18µg of chlorpyrifos was taken in a 25ml graduated tube and 1ml of Sodium hydroxide was added to it. The test solution was kept for 20 minutes at room temperature for complete hydrolysis. Then 1ml of Para aminbenzoic acid was added and shaken thoroughly and kept at 5ºC for 15 minutes for full colour development and wine red colour was obtained. The solution was then diluted to the mark with water and the absorbance was measured at 520 nm against a reagent blank (Khan *et al*., 2007).

SCREENING OF ALGAE FOR HEAVY METAL RESISTANCE

The culture medium was dispensed in test tubes and sterilized for 15 min at 121°C. To each of the labelled tubes, 500µg of the appropriate chromium metal salt solutions was added and incubated at 28°C for 7 days. The tubes were qualitatively measured for turbidity. (Konopka*et al*., 1999).

BIOSORPTION OF METALS BY ALGAE

Chromium metal salt (50mg/l) was added to the media with algal sps and incubated for 24 hrs. The culture supernatant was obtained by spinning down the culture at 10,000 rpm for 20 minutes. The degradation activity of the sps was determined by measuring the absorbance of the culture supernatant (2ml) at OD 542nm using UV spectrophotometer (Kalme*et al*., 2007)

RESULTS

COLLECTION AND IDENTIFICATION OF SAMPLE

In this study algal samples were collected from fresh water ponds of Coimbatore. The samples were identified by normal microscopic method.

Scientific classification

Kingdom : Plantae

Order : *Zygnematales*

Family *: Zygnemataceae*

Division *: Chlorophyta*

Class *: Zygnematophyceae*

Genus : *Spirogyra*

Fig 1: *Spirogyra*and *Spirulina*

Scientific Classification

Kingdom : Eubacteria

Order : *Spirulinales*

Family : *Spirulinaceae*
Domain : *Bacteria*
Genus : *Spirulina*
Phylum : *Cyanobacteria*

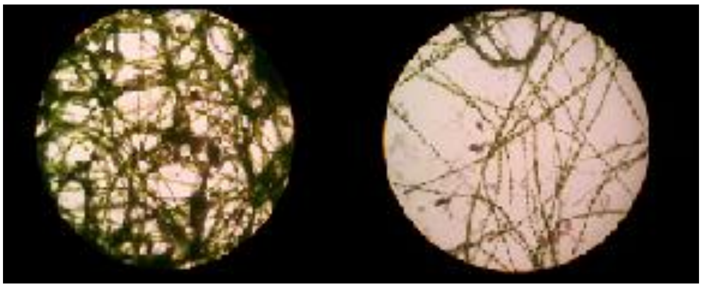


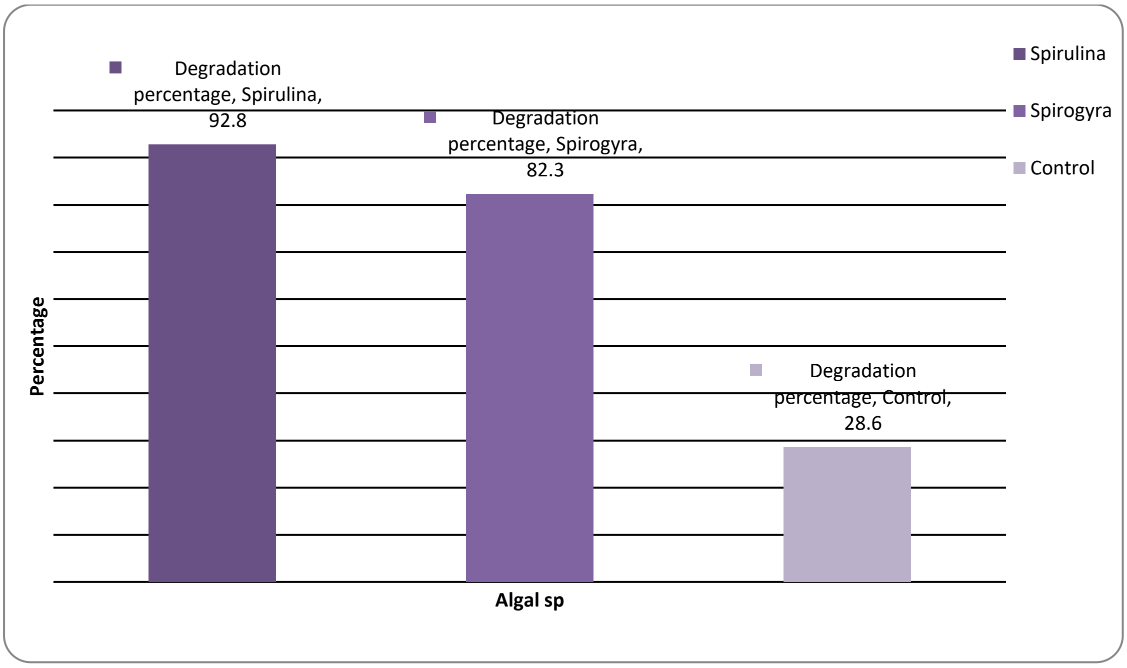
Fig 2: *Spirogyra*and *Spirulina*

*Spirogyra*is a member of the algae. These are simple plants ranging from single celled organisms to complex seaweeds. They contain chlorophyll and food through photosynthesis. *Spirogyra*can reproduce both sexually and asexually. *Spirulina* is microscopic blue green algae that exist as a single celled organism. It contains billions of years of evolutionary wisdom in its DNA and is an offspring of earths first photosynthetic life forms.

Table 1: Growth of algal sps in media enriched with chlorpyrifos

Sps	25ppm	50ppm	75ppm	100ppm
Spirulina	+	+	+	_
Spirogyra	+	+	_	_
Azolla	+	-	-	-

Fig 1: Percentage degradation of chlorpyrifos



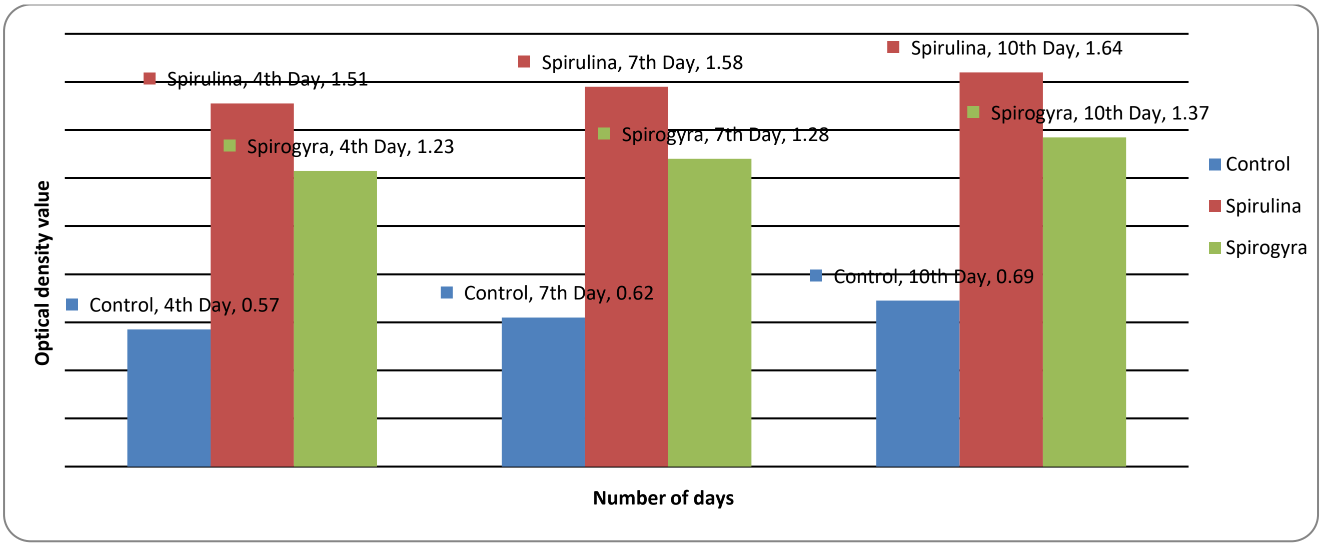


Fig 2: Turbidity Measurement of algal sps in medium inoculated with metal salt

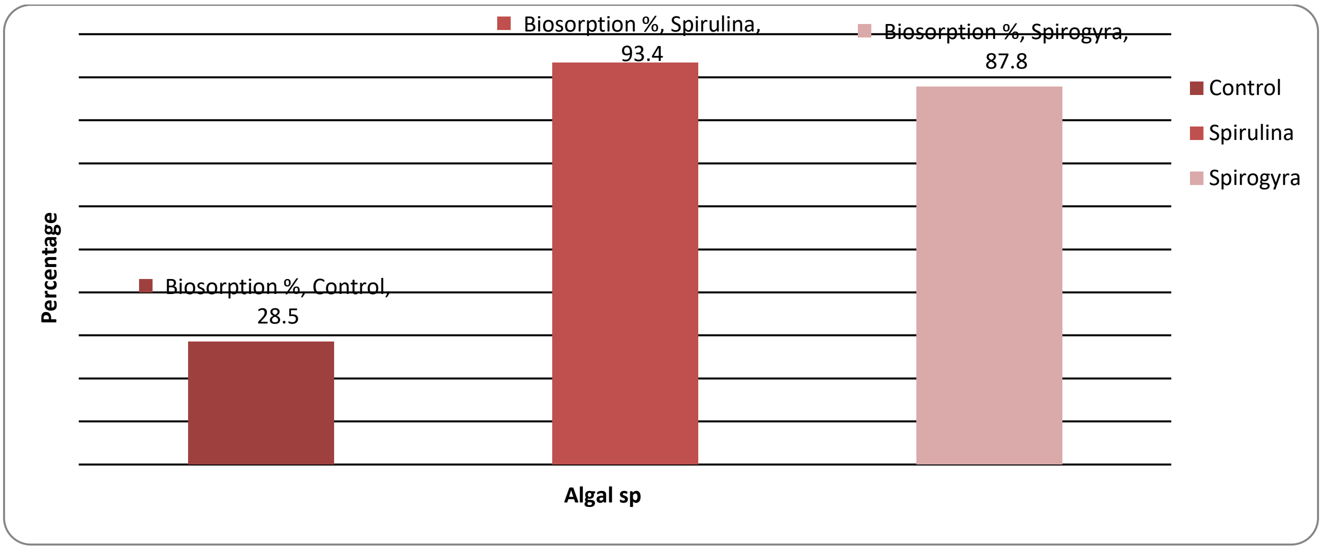


Fig 3: Biosorption % of chromium by algal sps

SCREENING OF ALGAE FOR PESTICIDE DEGRADATION

The present study showed the screening of the algae sps for the capability of degrading the organophosphosphate pesticide, Chlorpyrifos. The study included the primary screening of the organisms for the biodegradation initially in the liquid media with the pesticide and then utilization of the potential organisms to carry out biodegradation. It was observed from the results that *Spirulina* were capable of tolerating the pesticide up to a concentration of 75ppm, *Spirogyra* for 50 ppm and *Azolla* for 25 ppm. So, the first 2 sps were used for the biodegradation studies.

DEGRADATION OF PESTICIDE BY ALGAE

The results indicated that there was a significant difference between the chlorpyrifos estimated in the sample before and after degradation. The degradation percentage of *Spirulina* was significant that exhibited a degradation of 92.8 % in comparison to 28.6 % degradation of the control sample.

SCREENING OF ALGAL SPS FOR HEAVY METAL RESISTANCE

Spirulina showed higher resistance to heavy metal chromium than *Spirogyra* and control.

BIOSORPTION POTENTIAL OF ALGAE

The organism was inoculated, incubated and grown adequately in the presence of heavy metal and the absorbance was measured. *Spirulina*and *Spirogyra* showed 93.4 % and 87.8% biosorption of the heavy metal.

The results revealed that *Spirulina* and *Spirogyra* showed more efficiency for the phycoremediation of toxicants chlorpyrifos& chromium. Comparatively *Spirulina* exhibited more phycoremediation efficiency than *Spirogyra*. Control showed the least efficiency in both the studies.

SUMMARY & CONCLUSION

In the present study algae sps *Spirulinaplatensis* and *Spirogyra* were used for the biodegradation of pesticide chlorpyrifos & biosorption of heavy metal chromium. Both the sps showed more efficiency for the bioremediation of toxicants than control. *Spirulina* showed good degradation effect. The algae *Spirullinaplantensis* and *Spirogyra* were studied for biosorption of heavy metals of chromium at various concentrations. *Spirullinaplantensis* showed more tolerance to the heavy metal chromium &biosorption than *Spirogyra sps*. Organophosphate pesticides are the most commonly used pesticides worldwide. The continuous use of pesticide results in serious effects to environment including biomagnification and eutrophication problems. The chemical remediation of the pesticides would only increase the environmental pollution. The best method of eliminating the pesticide is the bioremediation of the pesticide.

Heavy metals are the most hazardous pollutants as they are non-biodegradable and undergo bio-accumulation in the food chain. This situation is alarming in the developing world. Chromium (Cr) compounds are highly toxic to plants and are detrimental to their growth and development. Contamination of agricultural soil by heavy metals has become a critical environmental concern due to their potential adverse ecological effects. Such toxic elements are considered as soil pollutants due to their widespread occurrence and causing acute and chronic toxic effects on all trophic levels due to biomagnification problems.

A dramatic increase in environmental contamination including soil, water and air due to anthropogenic activities have been reported in the last few decades. Phycoremediation is a novel technique that uses algae to clean up toxicants from the environment. In the present study an attempt was made to degrade the pesticide using the algal sps. As chromium compounds were used in dyes and paints and the tanning of leather, these compounds are often found in soil and groundwater at abandoned industrial sites, now needing environmental cleanup and remediation.

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