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Phytoremediation potential of *Cicer arietinum* for Tetracycline

Manvi Makhijani[1], Sonal Gahlawat[1], Kirti Chauhan[1], Shubha Valsangkar[1] and Pammi Gauba[2]

*[1,2] Department of Biotechnology,
Jaypee Institute of Information Technology
A-10, Sec-62, Noida, U.P., India*

Abstract

Medicines play a cardinal role in the treatment, cure and prevention of disease in humans and animals. However, they may have unintended effects on the environment. These pharmaceuticals have been released into the environment for years, but the estimation of their quantities has been taken up recently by the researchers. It has been suggested that breakdown products and the combination of different biologically active compounds may have effects on the environment that are uncalled for. Pharmaceuticals can cast effects on bacteria and animals even in low concentrations. After being released into the environment, pharmaceuticals could be transported and distributed to air, water or soil. A lot of factors contribute in their distribution, out of which the characteristics of the receiving environment and chemical properties of the compound are the important ones. Sorption behaviour of the substance in the soils, sediment-water systems and treatment plants determines the transportation of the pharmaceutical between different environmental media. Conventional methods used for the removal of these pollutants are costly, thus generating need for a gainful method. Phytoremediation is a lucrative technology that uses plants to reduce, remove, immobilize or degrade environmental toxins. This method could be used to clean up sites with low to moderate levels of contamination, and is a passive technique. In the present study, *Cicer arietinum* (black chick pea) plants were grown in vitro, with calculated amount of tetracycline. *Cicer arietinum* is recognized for its ability to phytoremediate metal contaminants from the soil, therefore this study investigates its ability to phytoremediate tetracycline. The phytoremediation rate shown by *Cicer arietinum* seeds is promising thus, indicating their further exploitation for the removal of a potent pollutant from the environment.

Keywords- Pharmaceuticals and personal care products; Phytoremediation; Tetracycline; Cicer arietinum.

1. Introduction

Various pollutants are released into the environment every year, among which pharmaceuticals are becoming a cause of concern. There are many sources of these pharmaceuticals, such as 1. A surplus of human medicines, like antibiotics, statins, analgesics or cytotoxins used in cancer treatment, are produced and used in range of thousands of tons per year. After administration, they are absorbed and metabolized, after which some amount of chemicals may be excreted into the sewage system [Herklotz et al, 2009]. Metabolism of these drugs introduces hydrophilic functionalities into the pharmaceutical molecules that facilitate excretion in urine or feces [Katzung, 2001]. These compounds after entering into the wastewater treatment facilities are incompletely removed and can end up in the sewage sludge [Herklotz et al, 2009]. Treated sewage sludge can be classified as a biosolid, that can be applied to land as a rich source of nutrients for plants. This land application of biosolids further raises concerns about the environmental release of pharmaceuticals, which may be present in the biosolid [Deblonde and Hartemann, 2012]. Antibiotics form third largest group among all the pharmaceuticals in the human medicine. Mostly prescribed antibiotics are tetracyclines, β-lactams and macrolides [Thiele-Bruhn, 2003]; 2. Large amounts of veterinary medicines, like antibacterials, antifungals, and parasiticides, may also contribute to the stress on the environment by being released from aquaculture and agriculture. They are secreted to soils or surface waters by pasture animals. They can also indirectly enter the environment through the application of slurry and manure as fertilizers. Antibiotics for the treatment of fish or shrimp in aquaculture directly find their way into surface waters [Boxall, 2004]. In this category, 70% of the medicines are antibiotic agents. Tetracyclines, sulfonamides, aminoglycosides, β-lactams and macrolides are majorly used in veterinary medicines [Thiele-Bruhn, 2003]; 3. During the production of pharmaceuticals, the residues could be released into the surface waters [Boxall, 2004]; 4. Disposal of unused medicines and containers make up other minor routes of entry into the environment [Herklotz et al, 2009].

It has been suggested that pharmaceuticals can affect wildlife by causing feminization of male fish and reducing appetite of fishes. Steroids from contraceptives are strongly suspected to affect the fertility and development of fish, reptiles and aquatic invertebrates [Boxall, 2004]. Release of antibiotics may lead to the development of antibiotic resistance in bacteria [Herklotz et al, 2009; Onesios et al, 2009; Kummerer, 2008]. More subtle effects include impact on oocytes and testicular maturation, effects on insect physiology, inhibition or stimulation of growth in aquatic plant and algae species [Deblonde and Hartemann, 2012]. Studies have shown that the concentration of medicines in surface waters are below the levels that might be threatening to humans, but at the same time they have warned that uptake of these compounds from soils into crops and biomagnification through the food chain are yet to be quantified and thus can't be ruled out completely [Boxall, 2004; Onesios et al, 2009].

There are many routes through which pharmaceuticals can enter the environment, but waste water treatment plants forms a major source. Municipal waste water treatment plants are designed to remove pharmaceuticals from the waste water, but their residues remain in the output of these treatment plants [Jelic et al, 2012]. Conventional waste water treatment plants generally employs two attenuation mechanisms: 1. Sorption to particles; 2. Biotransformation [Sedlak and Pinkston, 2003]. Some extensively use activated sludge processes [Jelic et al, 2012], reverse osmosis [Sedlak and Pinkston, 2003], advanced oxidative processes, activated carbon adsorption, membrane filtration and membrane bioreactors [Hoang et al, 2013]. Excavation, photolysis, incineration and pump-and-treat systems are some of the established methods of remediation [Pilon-Smits, 2005].

Due to various limitations of the methods mentioned above, there is a need to adopt a method which can give significant results and is cost-effective at the same time. In past decades, phytoremediation has been emerged as a promising technology and have been successfully applied on a pilot scale [Hoang et al, 2013]. It is a term used for a group of technologies that use plants to reduce, remove, immobilize or degrade environmental toxins. It uses plants to fasten up the degradation of organic contaminants, which are in concert with root rhizosphere microorganisms [Peer et al, 2006]. Plants can also be used to remove pollutants from municipal waste water, agricultural runoff drainage water, industrial waste water and landfill leachate. Air containing soot particles, dust, halogenated volatile hydrocarbons can also be filtered by plants [Pilon-Smits, 2005]. This method could be used to clean up sites with low to moderate levels of contamination, and is a passive technique [Ahalya and Ramachandra, 2006]. There are six aspects of phytoremediation: Phytoextraction, Phytodegradation, Phytovolatization, Phytostimulation, Rhizofiltration and Phytostabilization. These methods are not separate and can occur simultaneously to give fruitful results [Peer et al, 2006].

Plants required for phytoremediation have general properties such as: fast growth rate, high biomass yielding capacity, large and dense root systems, high levels of degrading enzymes, ability to accumulate large amounts of pollutants in harvestable tissues and are hardy, competitive and tolerant to pollution. Phytoremediation has gained popularity in past ten years, among government and industries due to advantages like, relatively low cost, easy implementation, ability to conjugate with other methods and *in situ* processing [Pilon-Smits, 2005].

2. Materials and Methods

2.1. Growing Cicer arietinum in presence of Tetracycline

Cicer arietinum (black chick pea) was grown *in vitro* in the presence of Hoagland Media (pH 6.5) [Blankendaal et al, 1972] and varying concentrations of tetracycline in the culture tubes. The total volume of media and tetracycline solution was kept at 30 ml and the working concentrations of tetracycline solution (1 mg/ml) was prepared as 0.5%, 1%, 2.5%, 5% and 7% by volume of total solution. Thus, these five concentrations were labeled as A, B, C, D and E respectively. We used two controls, first one being seeds and media and the second one was media and drug. The first control was maintained to rule out the amount of nutrients being taken up by the plants and second control was prepared in order to negate the effect of degradation of tetracycline in the solution. We had plants in triplicate to account for error. Since, plants were to be grown hydroponically, autoclaved (121 ºC, 20 minutes, 15 psi) sponge pieces were used to give support to the seeds in the culture tubes. For growing the plants, the *C. arietinum* seeds were surface sterilized by washing first with 70% ethanol followed by four-five washes with distilled water. Two seeds were added to each tube which were then covered with cotton plugs. All the tubes in which the plants were covered with aluminium foil and placed in the Plant Tissue Culture Room, wherein the temperature was maintained at 24.8 ºC. The sample from each tube was collected on particular days, aseptically. Aluminium foil was removed once the germination stage was over. The entire protocol was repeated with double (2X) and triple (3X) concentrations of tetracycline.

Table 1. Initial Concentrations of Tetracycline

Sample	1X (mg/mL)	2X (mg/mL)	3X (mg/mL)
A	0.005	0.01	0.015
B	0.01	0.02	0.03
C	0.025	0.05	0.075
D	0.05	0.10	0.15
E	0.075	0.15	0.225

2.2. Sampling

Samples were taken in 1.5 mL eppendorf tubes for all the three sets, on a regular basis to check the effect of phytoremediation.

2.3.Estimating Concentration of Tetracycline

A standard graph of tetracycline was prepared initially [Sultan et al, 1988]. For estimating the concentration of tetracycline in the samples, 0.5 mL ferric ammonium sulphate solution (100mg ferric ammonium sulphate in 100mL 0.001M H_2SO_4) was added to the test tubes. To these, 0.5 mL sample was added and kept for incubation for 20 minutes at room temperature. After the incubation, 1.5 mL of 0.001M H_2SO_4 was added to the tubes. The O.D. of the solutions was recorded at 423nm using Shimazu UV-1800 Spectrometer. The concentration of tetracycline solution in the samples was then determined with the help of the standard graph.

Table 2. Preparation of solutions for taking O.D. of the samples

Tube Name	Ferric Ammonium Sulphate Solution (mL)	Sample (mL)	Sulphuric Acid (mL)
Seed + Media	0.5	0.5	1.5
Media + Drug	0.5	0.5	1.5
A	0.5	0.5	1.5
B	0.5	0.5	1.5
C	0.5	0.5	1.5
D	0.5	0.5	1.5
E	0.5	0.5	1.5

3. Results and Discussion

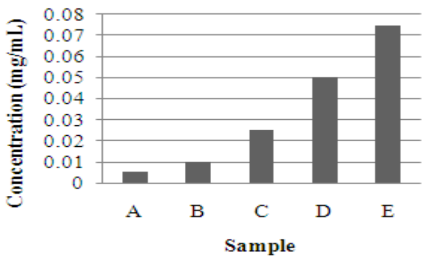
For 1X, plants grew very well exhibiting a fine developed root and shoot system by the 21st day thus, showing no phytotoxicity due to presence of tetracycline. Decrease in the concentration of tetracycline was observed for all the samples in a day wise analysis. While calculating the decrease in the concentration of tetracycline, the degradation of the drug was also estimated and taken into account. The overall decrease in the concentration of tetracycline has been reported here and it can be observed that it had an increasing trend from sample A (having least concentration of tetracycline) to sample E (having highest concentration of tetracycline) i.e. the decrease in the concentration was least in sample A and highest in sample E. With increase in time, the concentration of drug in the media decreased with time, thus indicating phytoremediation potential of *C. arietinum* plants.

On further increasing the concentration of tetracycline in set 2X, it was observed that the growth of plants was a little slow as compared to 1X and did not attain much height. This showed that at high concentration, tetracycline showed phytotoxic effects on the plants. However, a decrease in the concentration of tetracycline was observed on a day wise basis. The overall decrease in the concentration was lowest in sample A and highest in sample E. Thus, phytoremediation potential was maximum in case of the sample having highest concentration.

When compared to 1X and 2X, growth of plants was least in 3X set due the high concentration of tetracycline. Apart from this, trend similar to 1X and 2X in the overall decrease in the concentration of tetracycline was followed here as well.
Following table shows the initial and final concentrations of tetracycline in all the sets. From the table, it can be clearly seen that in each sample and set, there is a decrease in the final concentration from the initial concentration. Thus, indicating the phytoremediation of tetracycline by the plants.

Table 3. Initial and Final Concentrations of tetracycline in all sets

	1X		2X		3X	
Sample	Initial Concentration (mg/mL)	Final Concentration (mg/mL)	Initial Concentration (mg/mL)	Final Concentration (mg/mL)	Initial Concentration (mg/mL)	Final Concentration (mg/mL)
A	0.005	0.002	0.01	0.0007	0.015	0.0022
B	0.01	0.0023	0.02	0.0014	0.03	0.0045
C	0.025	0.01	0.05	0.0022	0.075	0.0081
D	0.05	0.01	0.10	0.0058	0.15	0.025
E	0.075	0.004	0.15	0.006	0.225	0.02



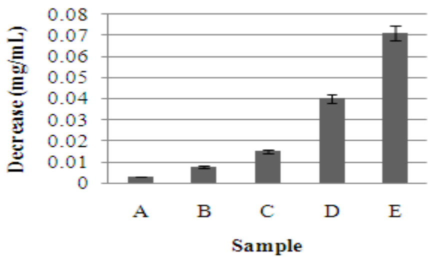
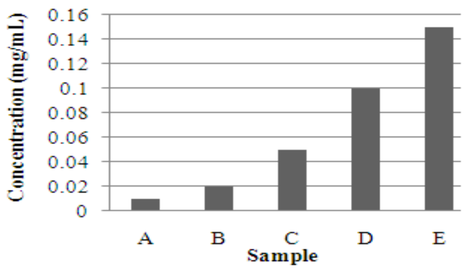


Figure 1a. *Initial concentration of tetracycline (1X)* **Figure 1b.** *Overall decrease in concentration of tetracycline (1X)*



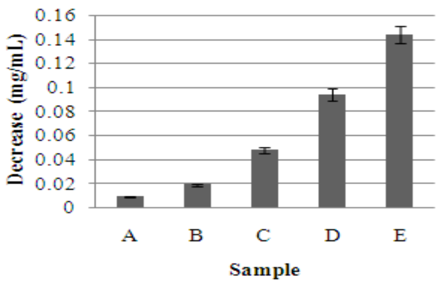
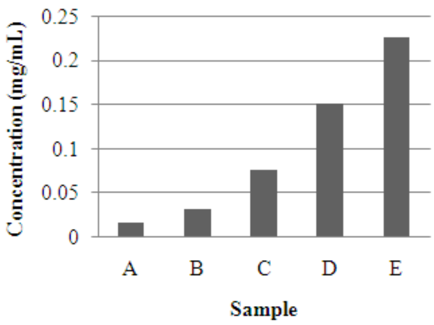


Figure 2a. *Initial concentration of tetracycline (2X)* **Figure 2b.** *Overall decrease in concentration of tetracycline (2X)*



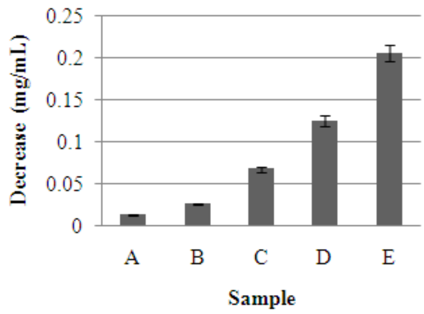


Figure 3a. *Initial concentration of tetracycline (3X)* **Figure 3b.** *Overall decrease*
in concentration of *tetracycline (3X)*

After analyzing the results of the sets individually, a comparative graph between the sets was plotted. The comparison showed that as the concentration of the set is increased, the phytoremediation potential increased.

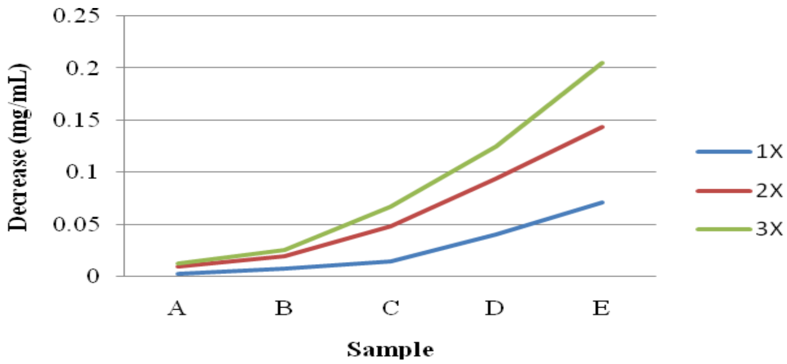


Figure 4. *Comparison of overall decrease in concentration between 1X, 2X and 3X*

4. Conclusion

To the best of our knowledge, this is the first work reporting the ability of *C. arietinum* to phytoremediate tetracycline. Various studies have demonstrated the ability of *C. arietinum* to phytoremediate metals, but this study shows the potential of *C. arietinum* to remove tetracycline from the environment. In this study, a direct relationship between the initial concentration and phytoremediation was observed. The overall decrease in the concentration of tetracycline in case of 2X was 2.5 times of 1X and for 3X, it was 3.5 times. In addition to this, the growth of plants was best in case of set 1 but the maximum rate of phytoremediation was made known by set 3. Phytoremediation is a cost-effective method and can be used to remove a variety of contaminants. The ability of *C. arietinum* to take up metals and now antibiotics, makes it a useful plant for clearing up industrial wastes. More horizons need to be explored, one of which may be the phytoremediation of hormones. Accumulation of the pharmaceutical products in the plant parts could also be done in order to get a clear insight of this technique.

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