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Accessing the Phytoremediation Potential of *Cicerarietinum* for Aspirin

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Abstract

Presence of xenobiotics such as pharmaceutical products, in the environment is raising serious concerns towards environment. With the increasing dependence on them, they have now become the focus of interest. Pharmaceuticals belong to therapeutic groups such as analgesics, antibiotics, contraceptives, anti-inflammatory, etc. Analgesics, one such class of pharmaceuticals, are consumed in high quantities either as prescribed ones or sold over-the-counter. Their market share is growing every year by 9.5%. Aspirin is one such commonly used analgesic. It can enter in the environment via several ways, either from manufacturer's plant or human excreta or as a veterinary medicine or as effluents from hospitals, clinics and so on. Owing to their chemical properties, their persistence in the environment has allowed their dispersal in water, soil and air and therefore may have ecotoxicological effects on non-target organisms. These pharmaceutical products are now being classified as emerging organic contaminants. Their effect on environment is not yet completely understood hence calls for the need to find ways to reduce their effects. Conventional treatment methods employed for their removal includes pump-and-treat systems, incineration, excavation, etc. but owing to their high cost, there is a necessity for other alternatives. Phytoremediation is one such alternative which offers several advantages over the conventional ones. It is the synergistic use of plants and its associated bacteria for the degradation of contaminants. In this study, the phytoremediation potential of *Cicerarietinum* (black chick pea) for aspirin under hydroponic conditions was evaluated. Various studies have reported favorable results for the phytoremediation of metals thus, indicating the need to

access the phytoremediation potential for aspirin. The results were highly promising indicating the possible usage of *Cicerarietinum*for other pharmaceuticals as well.

Keywords: Xenobiotics; Pharmaceutical products; Aspirin; Phytoremediation; Cicerarietinum

1. Introduction

In recent years, presence of various xenobiotic compounds in the environment such as pesticides, polyaromatic hydrocarbons (PAH), and pharmaceutical productsrepresents significant threat for environment thus, necessitating the studies for their further investigation. In 1977, pharmaceutical products were first identified as potential environmental contaminants [Hignite and Azarnoff, 1977]. Pharmaceutical drugs are defined as "chemicals used for diagnosis, treatment (cure/mitigation), alteration or prevention of disease, health condition or structure/function of the human body" [Daughton and Ternes, 1999]. The pharmaceuticals are divided into different therapeutic groups depending upon their intended use. Various groups such asanalgesics, antibiotics, antiepileptics, beta-blockers, lipid regulators, etc. have been detected in wastewater, sewage, groundwater, drinking water [Terneset al, 2002; Halling-Sørensenet al, 1998] in various quantities ranging from ng/kg to g/kg [Daughton and Ternes, 1999]. Analgesics are consumed in large quantities globally. A study reported the presence of various painkillers in surface and waste water such as acetylsalicylic acid (ASA), diclofenac, ibuprofen and some of the degraded compounds of ibuprofen, which were more toxic than their parent compounds [Buseret al, 1999]. Hence,pharmaceuticals along with their synthetic precursors and conversionproducts are extensively discharged into the environment. The pharmaceuticals can enter the environment by different routes including via their manufacturer, disposal of unused drugs and those which has expired, as bodily waste, veterinary use, etc. [K¨ummerer, 2010].Pharmaceuticals are designed to interact with specific drug targets at low concentrations and accordingly, acting upon the function of the living organism [Carlson et al, 2009]. Some of the drugs are excreted in free form, others get metabolized to various degrees and few also get converted to their more soluble forms via conjugate formation [Daughton and Ternes, 1999]. All these products eventually add to the existing list of thousands of pharmaceuticals which are added to the environment. Drug targets of these compounds are conserved across various organisms [Gunnarssonet al, 2008]. Owing to the intrinsic properties of these drugs such as lipophilic and low-biodegradability nature, they pose a potential for bioaccumulation and persistence in the environment [Christensen, 1998]. Therefore, it is essential to investigate the potential adverse effects of these products on non-target organism. Various articles have been published regarding the fate and effects of pharmaceutical products in the environment [Henschelet al, 1997; Halling-Sørensenet al, 1998]. Analgesics when used in veterinary medicines can be used for administration in ill-cows. When these animals die, their carcasses are consumed by scavengers such as vultures due to which tens of millions of vultures have been killed

in Asia [Vollmer and Gee, 2010]. Moreover, studies have reported that analgesics have the ability to inhibit the growth of aquatic plants [Pomatiet al, 2004]. As reported, salicylates have a damaging effect and can cause gastrointestinal hemorrhage [Scott, 1966].A number of initial reports have advocated that low concentrations of human and veterinary pharmaceuticals can adversely affect a variety of organisms such as fish, snails, etc. still, a lot more research is required to evaluate the threats posed by pharmaceuticals on the environment. Presence of these pharmaceuticals will always be detected at various levels as long we continue to use them even if it's meant to be in very low concentrations.

Acetylsalicylic acid (ASA) or aspirin,was introduced in 1890s [Dreser, 1899]. It is the most widely used drug worldwide [Vane and Botting, 2003]. It is used for treatment of mild to moderate pain and exhibits other properties such as anti-inflammatory, anti-pyretic and anti-platelet [Awtry and Loscalzo, 2000].ASA is hydrophilic in nature and thus, is rapidly and completely absorbed in human body. Acetylsalicylic acidis hydrolyzed in the body to salicylic acid [Zwieneret al, 2002]. ASA is found to be almost fully metabolized to salicylic acid leaving less than 1% which is excreted as parent compound [Schowanek and Webb, 2005]. ASA has been found to act through inhibition of biosynthesis of cyclic prostanoids i.e. thromboxane A2, prostacyclin and other prostaglandins [Awtryand Loscalzo, 2000]. It has been reported that acetylsalicylic acid is readily biodegradable in nature [Jjemba, 2008] yet the compound is still detected in the environment indicating its persistence [Nakadaet al, 2006]. Various methods have been established for the removal of pharmaceuticals from the environment such as incineration, pump-and-treat systems, incineration, etc. [Smits, 2005]

Phytoremediation is a group of technologies which uses the ability of plants for the removal of contaminants from the environment. The plants in association with their rhizospheric bacteria can contain, sequester, remove, degrade or modify the contaminants from various sites including soils, groundwater, sediments, surface water, etc. (Tsao, 2003). Contaminants can include petroleum hydrocarbons, pesticides, metals, explosives, pharmaceutical products.
The aim of this study was to evaluate the potential of *Cicerarietinum* to tolerate and remove ASA from a contaminated substrate using in-vitro culture. Studies have reported promising results for the phytoremediation of many metals by *Cicerarietinum*thus, signifying its importance for the removal of other contaminants also such as pharmaceuticals products.

2. Materials and Methods

2.1 Plant Growth

Seeds of *Cicerarietinum* were grown hydroponically in culture tubes in the presence of Hoagland media [Blankendaalet al, 1972] (pH 6.5) and varying concentration of aspirin. The seeds were grown in broth using autoclaved sponge (121°C, 15psi, 20 minutes) as the support. There were 5 different concentrations of aspirin. The

concentrations were made according to the following percentage w.r.t the final volume of the media being used in the culture tube i.e.0.5%, 1%, 2.5%, 5% and 7%. Triplicates for each concentration were used to account for error due to variability. Controls were also made to check if the drug is getting degraded without any seed in the normal environmental conditions also. For this, aspirin was added to Hoagland's medium without any seeds. Another control was made to rule out any variation due to the absorption of nutrients from the media by the seeds. This involved the germination of seeds in the presence of media without any drug concentration. The seeds were surface sterilized with ethanol for 5 minutes and then subsequently washed with distilled water four-five times. Each culture tube had 2 seeds. All the growth experiment was done in a plant tissue culture room which had appropriate conditions for their growth i.e. 24.8°C. The culture tubes were covered with aluminum foil during germination days, after which germinated seeds were placed under white light for further growth. The whole experiment was subsequently repeated by increasing the concentration of aspirin in the media i.e. 2X and later on 3X. The initial concentrations in for all the 3 sets are provided given below.

2.2 Sampling:

A 1-mL micropipette was used to withdraw samples from the culture tubeson a regular basis to check the effect of phytoremediation. The samples were collected in autoclaved 1.5 mL eppendorf tubes and were stored at 4°C to inhibit further drug degradation for later use.

2.3 Estimation of concentration of Aspirin:

A standard graph was prepared for estimating the aspirin concentration in the samples. For this, a stock solution of aspirin with concentration (0.8 mg/mL or 5.8mM/L) was made along with 0.025M ferric chloride ($FeCl_3$). Different dilutions were prepared and their absorbance was taken at 530nm using Shimadzu UV 1800 Spectrophotometer.The experimental samples of respective days drawn in eppendorf tubes were prepared for spectrophotometry estimation. The absorbance recorded was further used for the calculating their concentration with the help of standard graph.

3. Results and Discussions

*C.arietinum*showed a remarkable tolerance to ASA. These plants exhibited minimum or no phytotoxic effects resulting from the exposure of ASA for 21 days. Table 1 shows the initial and final day concentrations of ASA in all the 3 set i.e. 1X, 2X and 3X. The final day concentrations represented the concentration in the media on 21^{st} day after maximum phytoremediation done by the plant. These final concentrations are very less when compared to the initial ones. Hence, it is evident that the drug has been phytoremediated by the plant in in-vitro. Figure 1 shows the comparison between initial (i.e. 3^{rd} day) and final (i.e. 21^{st} day) concentrations of ASA for 1X set. The graph shows that the presence of ASA had no inhibitory effect on the growth of plant. Instead, it is portraying the ability of the plants to phytoremediate the drug in high concentration. As a result of which, the concentration of ASA in the media kept

on decreasing with increase in time i.e. showing an inverse relation between the two. The growth of plants was impressive with fully developed root and shoot system. In the same way, when the concentration of ASA was further amplified in 2X set, the results were in sync with the initial ones. The proper growth of the plants indicated no phytotoxic effect due to ASA exposure. The plants growth was almost similar in set 1X and 2X. Moreover, the rate of phytoremediation showed a similar trend in 2X set i.e. the ASA concentration kept on reducing with time.

On further increasing the ASA concentration in set 3X, it was observed that the rate of phytoremediation of the drug increased with respect to the two previous sets i.e. 1X and 2X. This signifies that the rate of phytoremediation increases with increase in drug concentration. But, at this high concentration of ASA, the growth of the plant was not similar to that observed in 1X and 2X sets. The height of the plant was comparatively smaller. Even though the growth of the plants in 3X set was less as compared to the initial sets, this did not hamper the phytoremediation potential of the plants to phytoremediate the drug. While calculating the decrease in concentration of ASA, degradation of drug was estimated and taken into account.

Table 1: Initial and Final Concentration of ASA in 1X, 2X and 3X

	1X Concentration (mM/L)		2X Concentration (mM/L)		3X Concentration (mM/L)	
	Initial	Final	Initial	Final	Initial	Final
A	0.03	0.0015	0.06	0.0030	0.09	0.0088
B	0.06	0.0088	0.12	0.0040	0.18	0.0059
C	0.15	0.0039	0.30	0.0070	0.45	0.0093
D	0.30	0.0088	0.60	0.0320	0.90	0.0156
E	0.44	0.0147	0.87	0.0670	1.305	0.0191

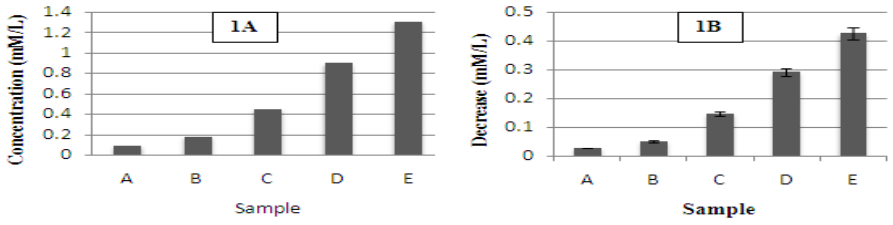

Figure 1: *(A) Initial concentration of ASA (1X). (B) Overall decrease in ASA concentration on 21st day (1X)*

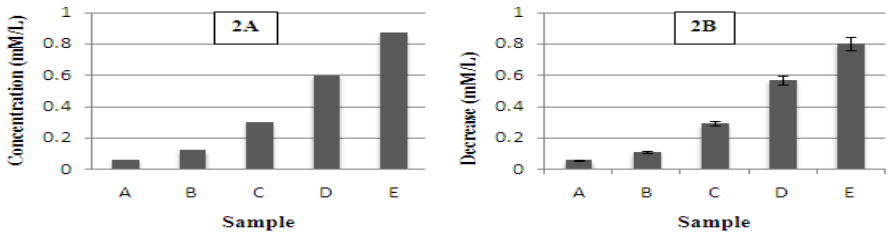

Figure 2: *(A) Initial concentration of ASA (2X). (B) Overall decrease in ASA concentration on 21st day (2X)*

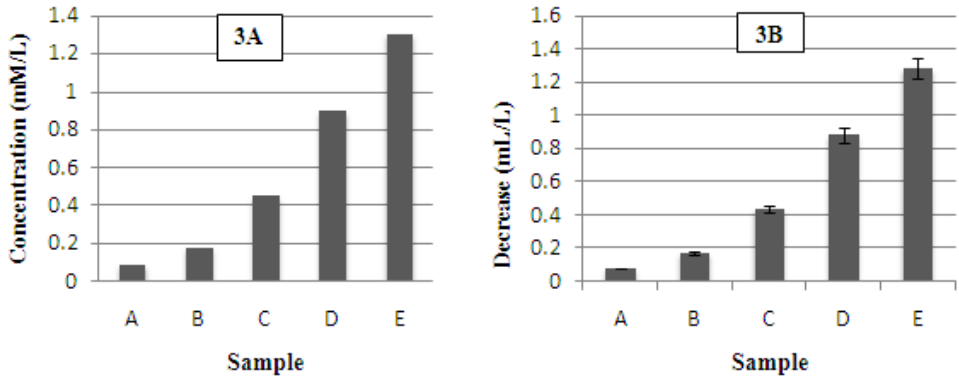

Figure 3: *(A) Initial concentration of ASA (3X). (B) Overall decrease in ASA concentration on 21st day (3X)*

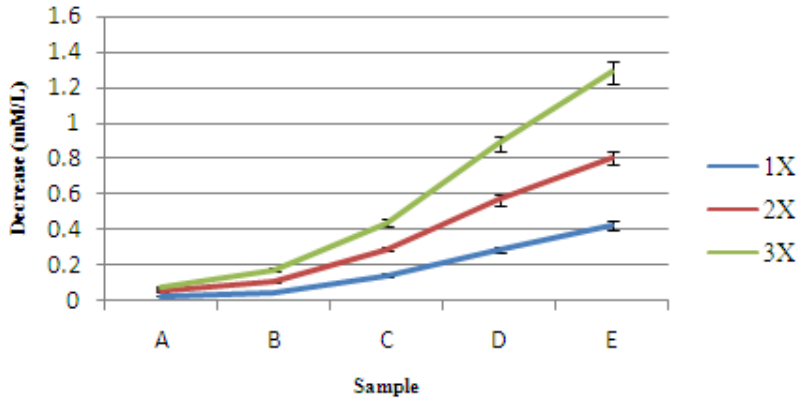

Figure 4: *Comparison between the overall decrease in concentration of ASA of 1X, 2X and 3X set*

On comparing sets 1X, 2X and 3X in terms of the overall decrease in ASA concentration, it can be concluded that the rate of phytoremediation was maximum in 3X set where the ASA concentration was the highest. Also, in each of the set, the maximum overall decrease in ASA concentration was maximum in sample E (having the highest concentration of ASA) and in sample A (having the lowest concentration of ASA), this overall decrease was the least.

4. Conclusion

The phytoremediation potential *C. arietinum* has been reported for metals but for pharmaceuticals, this is the first study which is exploiting its advantage for other environmental contaminants as well. In the present research work, the results obtained for phytoremediation of ASA are promising. A direct relationship between the initial concentration and phytoremediation rate has been observed i.e. as the concentration of ASA is increased, the rate of phytoremediation also increases. Even though the

growth of plant was less in 3X set as compared to set 1X and 2X, yet it is surprising that the rate of phytoremediation was maximum in 3X set. The overall decrease of ASA in set 2X was almost 2 times as compared to 1X while for the case of set 3X, it was 3 times. This finding unbolts interesting viewpoints for the utilization of *C. arietinum* for phytoremediation of other pharmaceutical products such as hormones, antibiotics, contraceptives, etc.

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