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PHYTOREMEDIATION OF PHARMACEUTICAL PRODUCTS

SWARNA SHIKHA, PAMMI GAUBA*

Department of Biotechnology, Jaypee Institute of Information Technology, Sector-62, Noida -201 307, Uttar Pradesh, India. Email: pammigauba@hotmail.com

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ABSTRACT

Objective: This study focuses on the application of phytoremediation technology in remediation of some pharmaceutical products such as antibiotics (ciprofloxacin) and hormones (progesterone and estrogen).

Methods: *Cicer arietinum* is a well-studied and documented plant for phytoremediation of heavy metals, in specific. Here, we tried to focus this potential of this plant for the pharmaceutical products by an ultraviolet-visible spectrophotometric method in Hoagland–Arnon solution.

Results: The results imply that *C. arietinum* can be used for phytoremediation of pharmaceutical products with an average of 60% of the pollutant being remediated.

Conclusion: The percentage remediation of pollutants is 60, 64.66, and 63.3 of ciprofloxacin, progesterone, and estrogen, respectively, by *C. arietinum.*

Keywords: Antibiotics, Hormones, Cicer arietinum, Ultraviolet spectrophometer, Pharmaceutical products.

INTRODUCTION

In phytoremediation, we use plants and their associated microorganisms to stabilize or reduce contamination in an environment in different forms [1]. As it is a natural process, it can be effective on contaminants over large cleanup areas and at shallow depths [2]. Plants are selected on various factors such as ability to extract or degrade the contaminants of concern, adaptation to local climates, high biomass, deep root structure, compatibility with soils, growth rate, easy for planting and maintenance, and ability to take up a large amount of water through their roots [3]. Phytoremediation involves mitigating the pollutant concentrations in contaminated soils with plants that are able to degrade or eliminate pesticides, solvents metals, explosives, crude oil, and various other contaminants from the soil that contain them [4].

Ciprofloxacin is an antimicrobial agent belonging to the fluoroquinolone group [5]. It is commercially available for the treatment of a broad range of infections against Gram-negative and Gram-positive bacteria by inhibition of DNA gyrase [6]. Small amounts of pharmaceuticals present in everyday food may generate strains that are resistant microorganisms in human and animal organisms [7]. That is why pharmaceutical products have now become harmful in the environment [8]. Due to their lipophilic nature, they are able to permeate biomembranes and prevent their inactivation before the therapeutic effect [9]. Therefore, drugs have the properties to accumulate in organisms and cause changes in ecosystems [10].

MATERIALS AND METHODS

Materials

Pharmaceutical grade antibiotics ciprofloxacin (100 mg) and steroid hormones progesterone - provera tablets (5 mg), estrogen - estradiol (2 mg) were used, which are widely being used in India. Plants were grown in Hoagland–Arnon solution [11] *in vitro*.

Plant selection

This is a very important step while planning a phytoremediation experiment as correct choice of plant is very important to achieve significant remediation. While choosing a plant things to keep in mind that it should be able to tolerate the pollution level in soil, easy to grow, and fast growing [12]. Thus, *Cicer arietinum* was chosen for the study as it has been found capable of tolerating the pollution level in the soil.

Methods

From the working stock solutions, appropriate dilutions of antibiotic and hormones were made with Hoagland–Arnon solution. The wavelength of solutions was taken in a range of 200-500 nm and recorded the ultraviolet (UV) spectrum of antibiotic and hormones using Arnon–Hoagland solution in the reference cell as a blank. Absorbance maxima (λ_{max}) for ciprofloxacin, progesterone, and estrogen were found to be 316, 410, and 410 nm, respectively.

RESULTS AND DISCUSSION

Regarding analytical methodology, a study was undertaken by Amjad *et al.* [13] on residual antibiotics in tissues that showed good spectrophotometric results in comparison to those found with high-performance liquid chromatography (HPLC). Moreover, Dey *et al.* [14] estimated flucloxacillin by UV-visible (VIS) spectrophotometric method. In 2003, Mendez *et al.* [15] analyzed the validation of HPLC and UV spectrophotometric methods for the determination of meropenem and found that there is no significant difference between the results obtained by these two methods. It was also found that UV-spectrophotometric method was simple, accurate, precise, and economical [16].

Keeping the aforementioned background in mind, the present study has been undertaken to determine the phytoremediation potential of *C. arietinum* against pharmaceutical products by UV-VIS spectrophotometric method.

1. Percentage remediation of progesterone, ciprofloxacin, and estrogen using *C. arietinum* during germination period of 7 days.

In remediation of ciprofloxacin with gram, it was observed that there was an increase in percentage remediation with an increase in ciprofloxacin concentration up to 20 ppm. Afterward, there was a decrease in percentage remediation at 25 ppm, which is caused by toxicity. In the case of progesterone and estrogen, remediation was found maximum with lower concentration and vice versa, i.e., at 50 ppm remediation percentage was 64.66 and 63.33 for progesterone

and estrogen, respectively, while at 500 ppm remediation percentage was 46.12 and 32.99 for progesterone and estrogen, respectively (Tables 1-3 and Fig. 1).

Table 1: Per	rcentage ren	nediation of	progesterone
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Sample	Initial progesterone concentration	Progesterone concentration after germination	Amount remediated	% Remediation
A	50	17.668	31.653	64.66
В	100	42.38	61.769	57.62
С	200	86.794	113.206	56.6
D	500	269.391	230.609	46.12

Table 2: Percentage remediation of ciprofloxacin

Sample	Initial ciprofloxacin concentration (ppm)	Ciprofloxacin concentration (ppm) after germination	Amount remediated	% Remediation
А	5	3.955	1.045	20.9
В	10	7.475	2.525	25.25
С	15	10.91	4.09	27.27
D	20	14.53	5.47	27.35
E	25	18.482	6.518	26.07

Table 3: Percentage remediation of estrogen

Sample	Initial estrogen concentration (ppm)	Estrogen concentration (ppm) after germination	Amount remediated	% Remediation
A	50	18.347	31.653	63.3
В	100	38.231	61.769	38.231
С	200	79.476	120.524	39.738
D	500	164.997	335.003	32.999

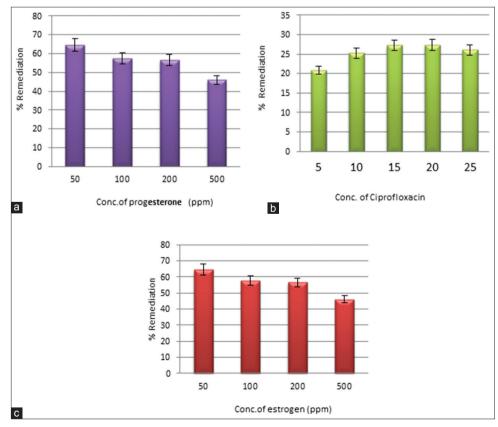


Fig. 1: Percentage remediation of (a) progesterone (b) ciprofloxacin (c) estrogen using Cicer arietinum

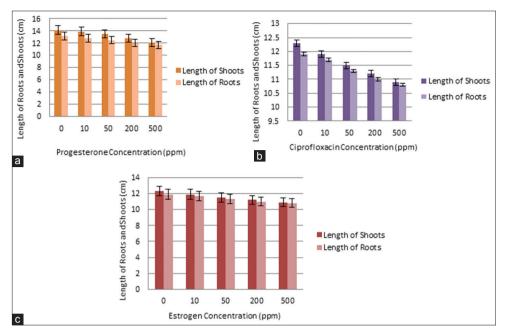


Fig. 2: Effect of (a) progesterone (b) ciprofloxacin (c) Estrogen on root and shoot length of Cicer arietinum

Table 4: A comparative study showing remediation potential of
germinating Cicer arietinum

Serial number	Contaminant	% Remediation
1	Antibiotic - ciprofloxacin	60
2	Hormone - estrogen	63.3
3	Hormone - progesterone	64.66

2. Effect of progesterone, ciprofloxacin, estrogen on root, and shoot length of *C. arietinum*.

It was observed that the decrease in the length of roots was more than that of the shoots in case of gram seed. There was greater than 50% decrease in root and shoot length in ciprofloxacin whereas in the case of hormones, it was observed that there was very minimal toxicity seen in lengths of roots and shoots of gram (Table 4 and Fig. 2).

CONCLUSION

A decrease in the lengths of roots and shoots of the germinating seeds was observed which indicates the remediating properties of the germinated plants. It was observed that the decrease in the length of roots was more than that of the shoots in case of gram seed. The percentage remediation of pollutants is 60, 64.66, and 63.3 of ciprofloxacin, progesterone, and estrogen, respectively. On an average, around 60% of the pollutant is remediated. Hence, gram seeds show great potential in remediating progesterone.

On the other hand, in the case of hormones, it was observed that there was very minimal toxicity seen in lengths of roots and shoots of gram. The results of present study indicate that *C. arietinum* accumulates high amounts of antibiotics and hormones in roots than shoots. It is a fast growing plant and has the ability to tolerate high concentrations of pollutant. Thus, it can be used for phytoremediation of pharmaceutical products.

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