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Research Paper

Exploring Phytoremediation Potential for Estrogen Hormone

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ABSTRACT

In the present scenario, polluted environment of aquatic and soil systems is of concern for every scientist. Increasing pollutants in the form of tablets, complex organic compounds, and heavy metals released mainly from industries is becoming a big problem. Different types of pollutants are introduced in the environment. Those include organic and inorganic compounds from many sites like mining, smelting, electroplating, poultry, hospitals and farming. Many studies have suggested the ability of the plants to remove the contaminants like metals, pesticides, oils, and explosive chemicals. It is a cost-effective method. Many types of potential remediation techniques are present, from which, phytoremediation, being one of the reliable new technology can aid in the reduction of pollution in a way that is eco-friendly and cheap. Estrogen is becoming a major pollutant and it becomes the need of the hour to control its concentration in the environment and so more research on its remediation potential is necessary. Natural biological pathways as well as biochemical pathways in the plants are suggested for remediation for which some plants are studied of having high capacity to tolerate the contaminants. In this study, seeds of *Vigna radiata* were used to evaluate its remediation potential for estrogen. From the results, it has been concluded that 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 shoots was more than that of the roots in case of *Vigna radiata*. The percentage remediation of pollutant of estrogen was seen 0.08 ppm of estrogen respectively. On an average, around 76% of the pollutant is remediated. Hence, *Vigna radiata* show great potential in remediating estrogen.

Keywords: Phytoremediation, Estrogen, *Vigna radiata*, Flavonoids, Alkaloids, Tannins.

INTRODUCTION

Sex hormones like estrogen play a major role in developing female reproductive system and regulating secondary sex characteristics. In human body cholesterol is the source of this hormone which is secreted by the body in the endocrine system. Estrogen enters into the blood stream by binding to the macromolecular cell receptors present in the plasma membrane or inside the target glands and starts circulating inside soon after its formation. [1] The occurrence of natural estrogen hormones in minute concentrations in the environment have been examined by many scientists. [2] Steroidal estrogens from human and animal sources are constantly excreted into the environment. For example, a pregnant woman may excrete 10 mmol/day of the steroidal estrogens, estradiol and estrone and chicken manure can contain over 1 mmol/g of estrogen. [3] Other animals such as cows, swine, horses and goats also excrete large amounts of estrogen into the environment which also acts as a pollutant to the environment. Estrogen exists in three forms namely Estradiol (also β-estradiol or 17-β-estradiol), Estradiol is produced by the ovaries, placenta, adrenal cortex and testis whereas

Estriol and Estrone are produced by the placenta and other endocrine organs. Estradiol is secreted in a large proportion compared to the other two forms and is much stronger and prevalent with estrone being the weakest among the three. [4] Hormones are crucial for the proper functioning of the body. However, anything in more than the critical concentration becomes toxic to the body and when it is found outside the body, in the environment then it is treated as a pollutant, the removal of which becomes important. Estrogen is given to cattle to increase the milk production, to poultry birds to enhance the egg laying capacity and the quality of meat. There is also increasing use of birth control pills, which leads to increase of estrogen in the environment and becoming a pollutant. Estrogen from mammals (Estradiol, Estriol, Estrone) occurs very often in the environment and is found in oceans, rivers, lakes etc. Estrogen is excreted by humans, cows, poultry, horses etc. among which a pregnant lady excrete1000 times more estrogen compared to a non-pregnant female. [5] Sewage and effluents from human beings and mammals, respectively goes into the rivers and oceans through which estrogen enters into the ground water, water run-off and gets absorbed by the soil. The estrogen as a pollutant gets absorbed and accumulated in plant parts such as plant seeds, flowers, pollen, roots, shoots, oil, kernels and bulbs. [6]

The maximum acceptable concentration of estradiol in soil is 150 ng/kg. Though the soil treated with manureis reported to have up to 650 ng/kg estrogen. [7] Estrogen is associated with positive and negative changes in physiology of the body depending on the concentration being higher or lower than the required and accordingly responds to different stimulus from the environment. [8] It also affects health of fish, domestic animals. The elevated concentrations of estrogen effects male fish e.g. affect reproductive fitness, lower sperm count and alter other reproductive characteristics. It also causes reproductive alteration in domestic animals. The effect of estrogen can also cause loss in vision in domestic animals. The high levels of estrogen also play a role in determining the root and shoot development, process of germination and flowering in plants. Mammalian estrogen also influences shoot and root growth, pollination, corticosteroid resulting in biochemical changes in vascular pants. Therefore, estrogen pollution is becoming a vital environmental concern and deleterious effects on human, animal and plant growth and also development at significant level.

Phytoremediation

Phytoremediation is a process of removing contaminants from the environment with the help of plants. This method uses plant uptake mechanism. It has gathered a lot of attention as a solution for the problems related to contamination in soil and water. Different plant species have different mechanisms making them unique for different contaminants. [9]

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 it is always kept in mind that it should be able to tolerate the pollution level in soil, easy to grow, and fast growing. Thus, *Vigna Radiata* was chosen for the study as it has been found capable of tolerating the pollution level in the soil. *Vigna radiata* (mung bean) is a plant of legume family and is found in the Indian subcontinent. It is rich in proteins, minerals and also helps in maintaining the nitrogen balance of the soil. *Vigna radiata* has also been reported to have potential for heavy metals but not much work has been done regarding organic pollutants like estrogen. Some of the major advantages of using *Vigna radiata* are germination time is less, can grow in every season. [10]

Plants used in Phytoremediation

Selection of plants depends upon various factors such as root length, nature of toxic particles, composition of the soil potential of plants accumulation. The different plants can be used for phytoremediation are *Vigna radiata*, [10] alfalfa, [11] Arrowroot, Alpine Bluegrass, Blue-green Algae, Bermuda Grass, Duck Weed, Hybrid Poplar Tree, Rye Grass, Sudan Grass, Yellow or White Water Lilies. [12][13]

MATERIALS AND METHODS

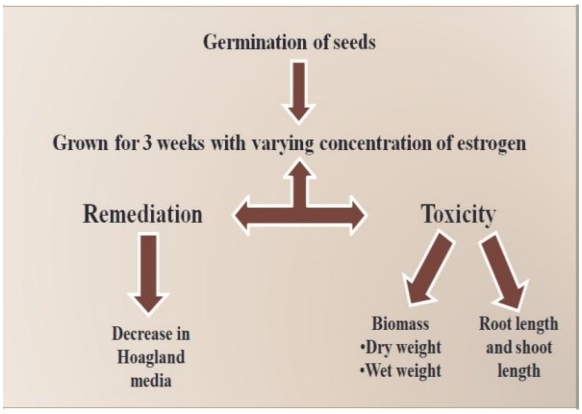


Figure 1: Work Flow

The Standard Curve

Standard graph of estrogen was plotted after taking the readings on spectrophotometer at 270 nm. Stock was made by dissolving 12Estrogen tablets (2mg each) into 48 ml of methanol. [14]

Germination of Seeds

Vigna radiata seeds need to be grown on sterile media. This requires prior seed sterilization to prevent the growth of microbial contaminants present on the seed surface. Therefore, seeds were sterilized using 70% ethanol and were washed with 0.1% of $HgCl_2$. [15] Germination of seeds was done with Hoagland media. The sterilized and germinated seeds were grown with three different concentrations of estrogen ranging from 0.02-0.1 ppm[Fig 2], 5-25 ppm[Fig 3]and 50-25ppm[Fig 4]for three weeks.

Figure 2: Plants grown with 0.02-0.01 ppm estrogen

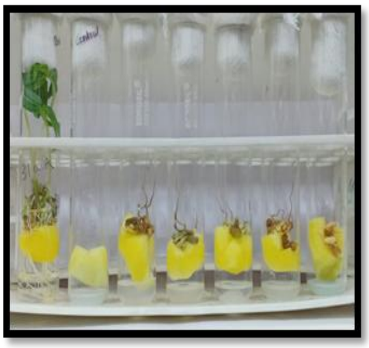
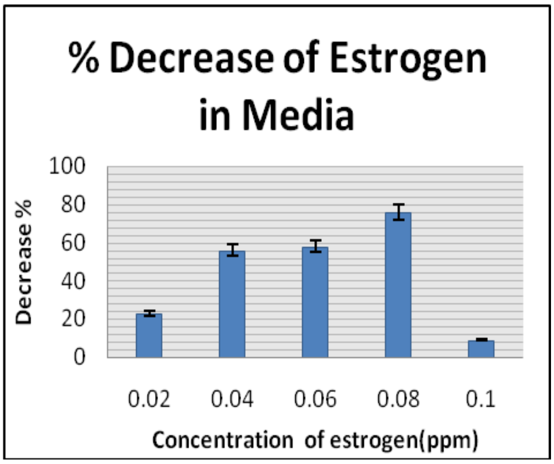


Figure 3: Plants grown with 5-25 ppm estrogen

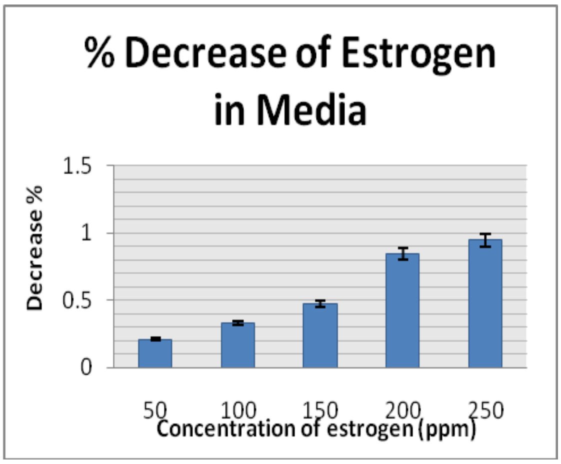
Figure 4: Plants grown with 50-250 ppm estrogen

RESULTS

Remediation



Graph 1: % decrease of estrogen in media at 0.02-0.1 ppm



Graph 2: % decrease of estrogen in media at 50-250 ppm

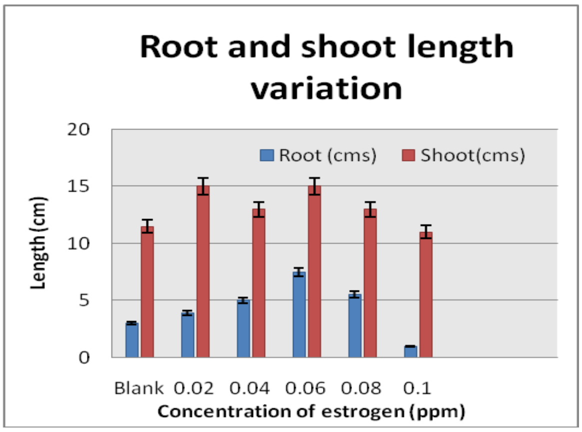
Remediation was analysed as a percentage and decrease of estrogen in Hoagland media It was observed that maximum remediation was seen at 76% ats 0.08 ppm [Graph 1& 2].

TOXICITY OF ESTROGEN IN PLANTS

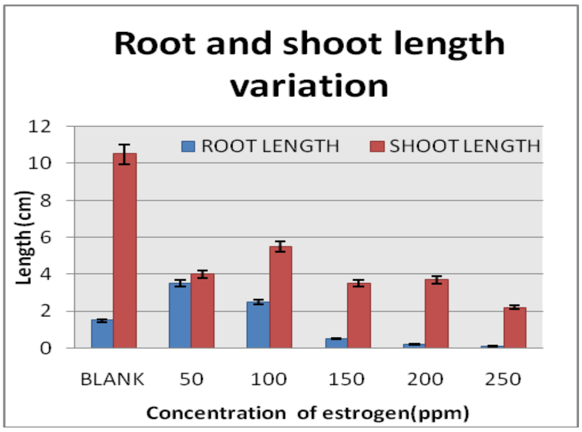
Toxicity was analyzed by three different parameters namely root and shoot length, biomass of plant, presence of chlorophyll content and phytochemical tests.

Root and Shoot Length

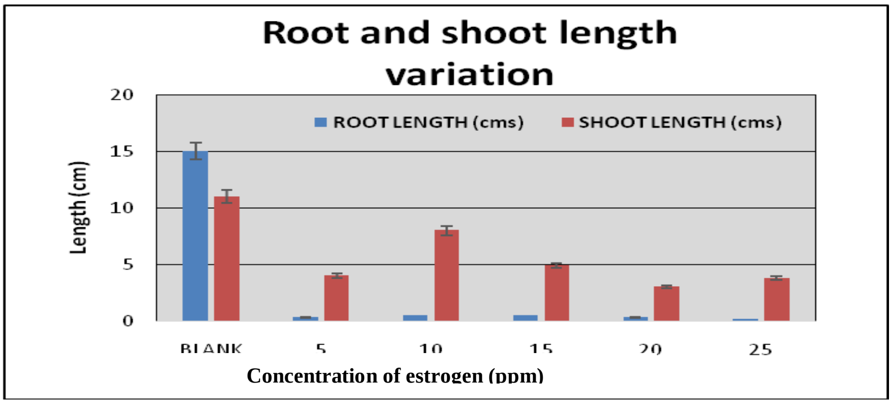
Varying concentrations of estrogen affects the root and shoot length of *Vigna radiata*. It was observed that at high concentrations of estrogen, there was decrease in the growth, whereas, in low concentrations of estrogen, the growth was comparatively increased.[Graph 3,4 & 5].



Graph 3: Root and shoot length at 0.02-0.1 ppm



Graph 4: Root and shoot length at 50-250 ppm



Graph 5: Root and shoot length at 5-25 ppm

LENGTH AT DIFFERENT CONCENTRATION OF ESTROGEN AFTER 3 WEEK

Figure 5: Length of root and shoot at 0.01-0.2 ppm

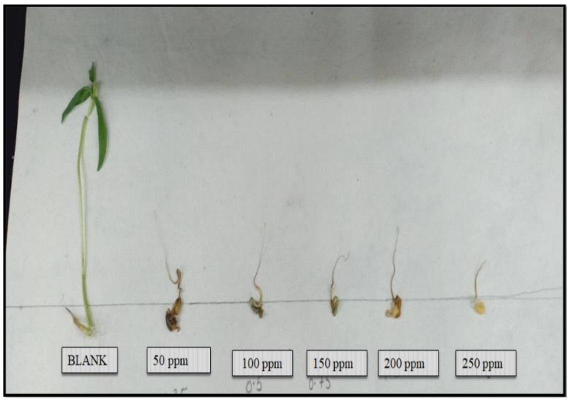


Figure 6: Length of root and shoot at 50-250 ppm

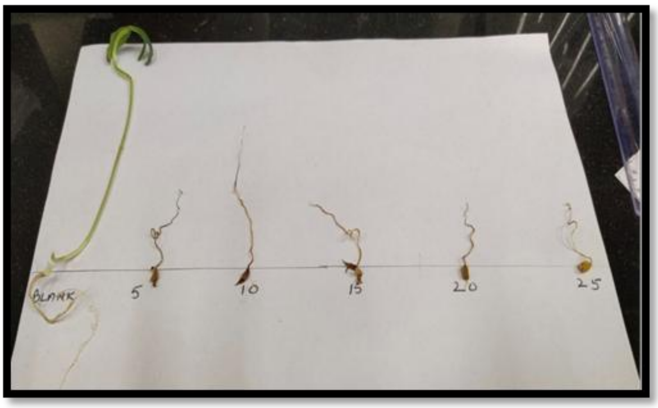
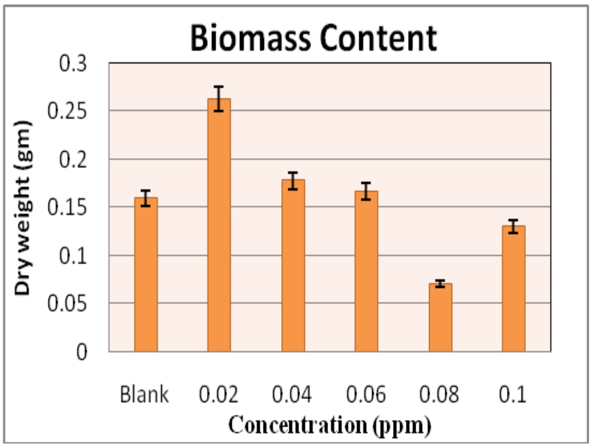


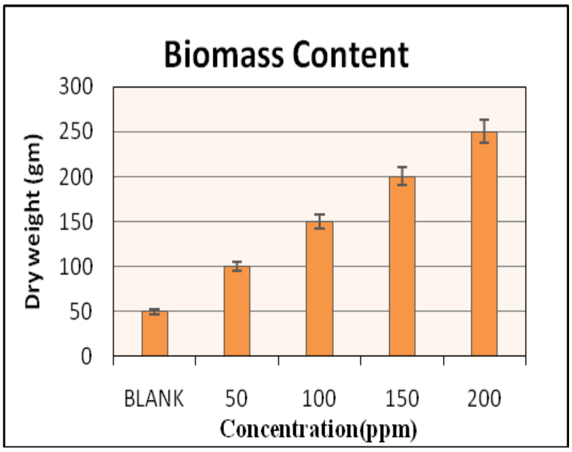
Figure 7: Length of root and shoot at 5-25 ppm

Biomass

Biomass of plants was measured by taking dry and wet weight of the plants. Graphs were plotted for different concentration of estrogen and dry weight [Graph 6&7].



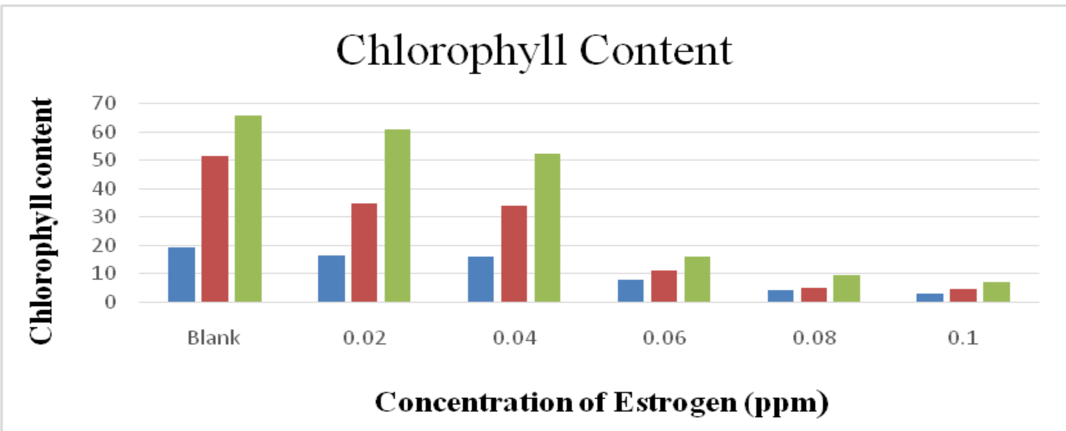
Graph 6: Biomass content of plants at 0.02-0.1 ppm



Graph 7: Biomass content of plants at 50-250 ppm

Chlorophyll Content

In this test, 1g of fresh plant samples with 80% acetone was taken and was transferred in the eppendorf then it was centrifuged at 5000 rpm for 5 minutes. Then supernatant was collected in another eppendorf and the pellet was washed with acetone until it decolourized. After that, supernatant and pellet were mixed in a test tube and OD was taken at 643 and 663 nm[Graph 8]. [16]



Graph 8: Chlorophyll Content of plants.

PHYTOCHEMICAL TESTS

The sample is the complex and requires systematic analysis to ensure that all the constituents are identified properly. This can be done either by inorganic quantitative and organic qualitative tests

Standard methods are applied to identify the presence of phytochemicals in the aqueous extracts, such as alkaloids, tannins and flavonoids. Chemical tests performed indicate the presence of a particular phytochemical through a change in the color of the sample, which appears after the addition of certain reagents. [17]

Alkaloids

Marquis reagent was prepared from formaldehyde (40%) and concentrated sulfuric acid, which is a spot test for identifying small amounts of certain alkaloids and the presence, is indicated by a certain color change. In this test, 4 µl concentrated sulfuric acid (H_2SO_4) was added to plant extract. To this solution few drops of 40% formaldehyde were added, upon which the color changes to dark orange and purple thus indicating the presence of the alkaloids [Fig 9]. [18]

Flavonoids

20% sodium hydroxide (NaOH) was added to 2 µl of the plant extract, which gives the solution an intense yellow color. To this, 70% dilute hydrochloric acid (HCL) was added after which the yellow color disappears. Formation and then the disappearance of the yellow color confirmed the presence of flavonoids [Fig 10]. [18]

Tannins

10% ferric chloride ($FeCl_3$) was added to 2 µl of the plant extract which gave brownish blue or black color which indicated the presence of tannins [Fig 11]. [18]

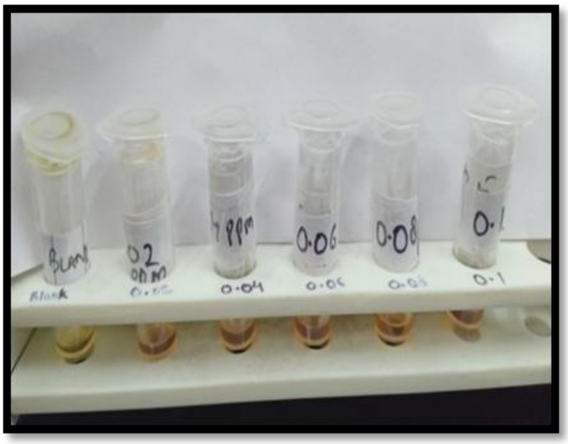


Figure 8: Test for presence of Alkaloids

Figure9: Test for presence of Flavonoids

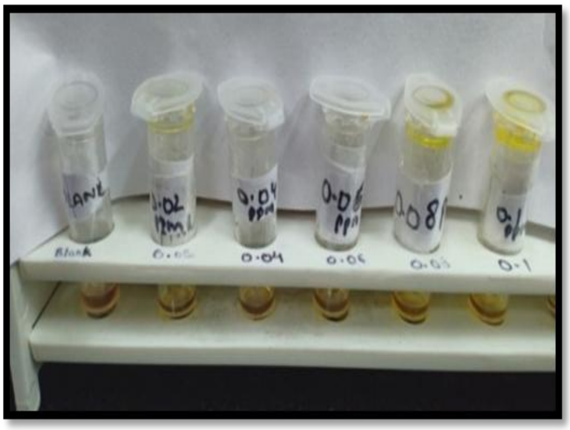


Figure 10: Test for presence of Tannins

Table 1: Qualitative Test

S. No	Concentration of estrogen (ppm)	Flavonoid	Alkaloid	Tannins
1	BLANK	+	+	+
2	0.02	+	+	+
3	0.04	+	+	+
4	0.06	-	+	+
5	0.08	-	++	++
6	0.1	+	+++	+++

CONCLUSION

From the results, it has been concluded that 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 shoots was more than that of the roots in case of *Vigna radiata*. The percentage remediation of pollutant of estrogen was seen 0.08 ppm respectively. On an average, around 76% of the pollutant is remediated. Hence, *Vigna radiata* show great potential in remediating estrogen. Toxicity was measured using different parameters such as root and shoot length, chlorophyll content and biomass. Phytochemical tests were also done to see the presence of flavonoids, alkaloids and tannins. On the other hand, in the case of hormones, it was observed that there was very high toxicity seen 15-20 ppm. The results of present study indicate that the plant can accumulate estrogen. Moreover, it is a fast-growing plant and has the ability to tolerate high concentrations of pollutant. Since estrogen is becoming a major pollutant, it becomes the need of the hour to control its concentration in the environment and so more research is necessary. Thus, it can be used for phytoremediation.

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