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# Influence of Bacterial Consortium Treated Yamuna Water on Seedling Growth of Vigna Radiata L. In Vitro

KEYWORDS: Bacterial consortium, Vigna radiata, Yamuna River water

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**ABSTRACT** *In the present research work, we attempted two investigational systems: one is treatment of the Yamuna River waste water by bacterial consortium and the other is impact of treated and untreated water on seedling growth of Vigna radiata in vitro. The bacterial consortium treated water sample showed a sharp reduction in BOD i.e. 59% and 72% in COD. The study of seed germination in vitro was carried out at 25, 50, 75 and100% concentrations of treated and untreated water. In case of seed germination among the different concentrations of Yamuna water, 25% concentration of untreated water and 100% concentration of bacterial consortium treated water showed stimulatory effect on the seed germination and other growth parameters of Vigna radiata over control. The result of the study indicates that Effective bacterial consortium helps in the reduction of water impurities to a extent and can be effectively used for irrigation.*

## INTRODUCTION

Water is one of the most important natural resources essential for all forms of life. This resource is being contaminated everyday by various anthropogenic activities, such as rapid growth of population, urbanization and industrialization that ultimately make the environment polluted [1]. In Agra (city of Taj) the Yamuna River has always been the most important fresh water resources. However the Yamuna water is used in every sector of development like agriculture, industry, transportation, aquaculture, public water supply etc. Huge load of wastes from industries, domestic sewage and agriculture practices find their way into the Yamuna, resulting in large scale deterioration of the water quality and affect the physico-chemical parameters of water. The status of the Yamuna river water is very much useful as it determine the physiological life cycle of plants, animals and human kingdom. Recently biological waste treatment is emerging as a natural environment friendly, permanent and greater public acceptance as less expensive and minimal site distribution technique of waste elimination [2, 3, 4].

The concept of effective microorganisms (EM) was developed by Professor Teruo Higa, University of the Ryukyus, Okinawa, Japan [5, 6]. EM consists of mixed cultures of beneficial and naturally occurring microorganisms that can be applied as inoculants to increase the microbial diversity of soils and plant. Research has shown that the inoculation of EM cultures can improve soil quality, soil health, and the growth, yield, and quality of crops. Microbes are reported to take effective part in bioremediation of wastes [7, 8].They can neutralize the organic contaminants by oxidizing them to carbon dioxide. However, in biological treatment, the microorganisms degrade the organic pollutants using them as a carbon source to produce metabolic energy to survive.

The effects of various industrial effluents, sludge materials and metal elements on seed germination, growth and yield of crop plants have captivated the attention of many workers [9, 10, 11].Hence an attempt has been made to study the effect of the Yamuna river water (both treated and untreated) on germination and seedling growth of Vigna radiata in vitro.

## MATERIAL AND METHODS

### Sample collection and Bacterial Isolation

Water samples were collected in sterilized BOD bottles, from the river Yamuna situated in the city of Taj - Agra, India. The water samples were analyzed to determine their physical, biological and chemical characteristics. For the selected isolation bacteria Nutrient agar media were used. Nutrient Agar medium was prepared by mixing Peptone-5 g, Beef extract-3 g, Sodium chloride-5 g, Agar-20 g, pH-7 in 1000 ml distilled water. The Yamuna water sample directly streaked on Nutrient media and incubated at 37°C for 24 h. After the incubation period the plates were observed for growth on the media [12].

### Physico-chemical analysis of Yamuna waste water

The analysis of initial physico-chemical parameters such as DO, BOD, COD, TDS, TSS, TS, acidity, alkalinity and hardness of collected Yamuna water samples were carried out by standard methods of APHA [13].

### Preparation of inoculums

Bacterial cultures (Rhodopseudomonas palustris, Rhodobacter spheroides, Escherichia coli, Bacillus subtilis, B. cereus, B. thuringiensis, B. fusiformis, Lactobacillus sp) were inoculated individually in pre-sterilized 100ml Nutrient broth. The flask was kept in a shaker at 120 rpm for 16 to 18 h at 30°C. The culture broth was centrifuged at 10000 rpm for 20 min. Bacterial cell suspension was prepared using sterile distilled water and adjusted to 0.5 OD using UV-visible spectrophotometer. 5% of the above bacterial consortium of Effective Microorganisms was added in Jaggary Solution (Jaggary-100g, Yeast extract-10g, Distilled water- 1000ml pH-6.5. Dissolve all the ingredients in distilled water and autoclaved at 121˚C at 15 lbs for 15 min.).The inoculated Jaggary medium was incubated at 37˚C temperature for 5 days.

Effect of treated and untreated Yamuna water sample on seed germination (Vigna radiata) in vitro.

The seeds of Vigna radiata were surface sterilized with 1% HgCl2 solution for two minutes and washed with sterilized distilled water 2-3 times. Twenty seeds were placed at an equi-distance on the periphery of each pre- sterilized petri-plates lined with Whatman's Filter paper No. 41. Known volume of different concentration (25%, 75%, 50% and 100%) of untreated and bacterial consortium treated waste water samples were poured into different petri-plates. Control was also run simultaneously using sterilized distilled water. Each treatment including the control was run in triplicates.

The plates were kept in diffused light at 25-27˚C in BOD incubator. Germinated sedds were recorded at fix time inter-

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val. Seed germination in each treatment was counted on 7th day for the caclutation of germination percentage, root and shoot length were measured by meter scale and root and shoot biomass by dry weight method.

## RESULTS AND DISCUSSION

### Physico-chemical characteristics of untreated and treated Yamuna waste water sample

In present study for remediation purpose eight indigenous bacterial strains were isolated from Yamuna water and were tentatively identified as Rhodopseudomonas palustris, Rhodobacter spheroides, Escherichia coli, Bacillus subtilis, B. cereus, B. thuringiensis, B. fusiformis, Lactobacillus sp by Bergey's Manual of Systematic Bacteriology [14].

The physico-chemical characteristics of Yamuna waste water before and after treatment are presented in Table I. All the physico-chemical parameters except DO showed maximum values in untreated Yamuna water sample but the bacterial consortium treated showed much reduction in the physical and chemical parameters to the way of improvement. These findings can serve as an important contribution towards an economic and simplified the biological methods for the waste water treatment using bacterial consortium.

The present findings are supported with the observations of Joshi and Sharma [15] they performed bioremediation of sewage through bacterial inoculation under in vitro and reported a reduction in BOD and COD after 24, 48 and 72 h. Maximum reduction (79.45% in BOD and 72.25% in COD) was observed after inoculation period of 72 h with bacterial cultures.

Ayyasamy [16] supported the present findings, they used different combinations of bacterial and fungal cultures and observed that consortium of five bacterial strains (Alcaligenes sp, Corynebacterium sp, Micococcus sp, Bacillus sp, Pseudomonas sp.) showed the maximum percentage reduction in BOD, COD and other parameters of effluent in comparison to single and dual bacterial cultures. Kumar and Bhoopathi [17] also supported the present result and reported that the consortium of different bacterial species (Pseudomonas sp, Cellulomonas sp, Alcaligenes sp) showed the significant reduction in different parameters of sago factory industry.

**TABLE-1 PHYSICO-CHEMICAL CHARACTERISTICS OF YAMUNA WATER BEFORE AND AFTER TREATMENT WITH BACTERIAL CONSORTIUM.**

| Physico-chemical parameters | Untreated* | Treated* | Percentage reduction (%) |
|---|---|---|---|
| Colour | Brown | Colourless | - |
| Odour | Foul smell | Odourless | - |
| pH | 8.9 | 7.3 | - |
| Acidity (mg $L^{-1}$ ) | 395 | 102.5 | 74 |
| Alkalinity (mg $L^{-1}$ ) | 895 | 95 | 89 |
| Hardness (mg $L^{-1}$ ) | 986 | 542 | 45 |
| TDS (mg $L^{-1}$ ) | 43.5 | 6.3 | 86 |
| TSS (mg $L^{-1}$ ) | 26.7 | 5.2 | 81 |
| TS (mg $L^{-1}$ ) | 16.7 | 1.1 | 93 |
| DO (mg $L^{-1}$ ) | 8.8 | 86 | 90 |
| BOD (mg $L^{-1}$ ) | 21.7 | 8.9 | 59 |
| COD (mg $L^{-1}$ ) | 100 | 28 | 72 |

*mean from three replicates.

### Effect of bacterial consortium treated Yamuna waste water sample on seedling growth of Vigna radiata

In case of seed germination, the effect of untreated and treated water on Vigna radiata is presented in Table II and III respectively. In in vitro study on Vigna radiata, the growth parameters were observed after 7 days. In untreated water sample, the lower concentration (25%) proved to be very efficient in germination percentage and seedling growth, shoot and root biomass of Vigna radiata.

Paliwal and Singh [18,19] observed that high concentrations of waste water caused the reduction of seedling growth due to the presence of high amount of complex organic and inorganic matter and excess of various forms of cations and anions which on coming in contact with germinating seed may enter the body system, resulting in ultimate damage to the seed . The results are in accordance with the report given by Verma et al [20] that the higher concentration of the waste water contains more pollution load, which causes deleterious effect on crop production. The results are also similar with the findings of Francosis and Mass [21] who reported that adverse effect of 100% concentration of untreated water on plant growth may be due to the influence of ions in waste water in large quantity which makes them toxic to the seed embryo.

Our results are in concordance to that of Kannabiran and Pragasam [22]. They showed that the negative impact of higher concentration of waste water on seed germination of Vigna radiata might be due to the higher contents of dissolved solids, which prevent the germination by contributing the salinity of the solute absorbed by seeds before germination.

The higher concentration of bacterial consortium treated waste water showed more stimulatory effect on the germination percentage, seedling growth, shoot and root biomass of Vigna radiata in comparison to control and untreated water samples. Similar findings were reported by Kumar and Bhoopathi [17] that the bacterial consortium (Alcaligenes sp Bacillus sp and Pseudomonas sp) treated sago factory effluent better response in seed germination and seedling growth of Vigna radiata and Vigna munga in comparison to untreated and control. Our results are in concordance to that of Saini et al [23] they, reported the similar results that the maximum growth was found at 100% and minimum at 25% concentration over control. At 50% and 75% concentrations all the data increased with increasing the concentration of treated domestic waste water sample.

**TABLE-2 EFFECT OF UNTREATED AND BACTERIAL CONSORTIUM TREATED YAMUNA WATER ON SEEDLING GROWTH OF VIGNA RADIATA**

| Concentration | Germination percentage | Shoot length* (cm) | Root Length* (cm) | Shoot Biomass (g/plate) | Root Biomass (g/plate) |
|---|---|---|---|---|---|
| Control | 50% | M ± S.D* | M ± S.D* | 1.73 | 0.20 |
| Untreated | 30% | 10.6 ± 0.7 | 6.7 ± 0.9 | 1.05 | 0.11 |
| 100% | 45% | 10.0 ± 1.0 | 4.8 ± 1.4 | 1.57 | 0.16 |
| 75% | 50% | 12.1 ± 0.5 | 8.2 ± 0.3 | 1.20 | 0.19 |
| 50% | 55% | 12.4 ± 0.2 | 8.9 ± 0.4 | 2.03 | 0.24 |
| 25% | 100% | 12.9 ± 0.1 | 9.1 ± 1.5 | 3.64 | 1.08 |
| Treated | 100% | 17.4 ± 0.1 | 12.2 ± | 3.33 | 0.98 |
| 100% | 95% | 16.8 ± 0.1 | 0.4 | 3.30 | 0.50 |
| 75% | 95% | 16.1 ± 0.6 | 11.0 ± | 2.33 | 0.35 |
| 50% | | 15.4 ± 0.6 | 0.5 | | |
| 25% | | | 9.5 ± 0.2 | | |
| | | | 9.3 ± 1.1 | | |

*mean± standard deviation from three replicates.

## CONCLUSION

Perceiving bacteria as dangerous is now turning towards greater awareness of the microbial world as a fundamental element of life. The results of present study indicate that EM technology helps in the reduction of water impurities. Moreover, the regular monitoring of water pollution level of river basin, appropriate purification treatment and community participation in water resources management will certainly help managers in taking informed decisions for water resources sustainability and management.

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