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Bacterial Remediation of Yamuna River Water and its Impact on Seed Germination in Vitro

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Abstract—*In the growing awareness of relationships between human health and water pollution, it is essential to undertake regular monitoring and surveillance of important aquatic ecosystems. The present study deals with the assessment of physico-chemical parameters of untreated and bacterial consortium treated water and its effect on the growth of Cajanus cajan and Vigna radiata in vitro. The bacterial consortium treated water increased the germination percentage, shoot length, root length, shoot biomass and root biomass while in untreated water a significant decrease of growth was noticed. Among the different concentrations (25 %, 50 %, 75 % & 100 %) of Yamuna river water, 25 % concentration of untreated water & 100 % concentration of bacterial consortium treated water sample showed stimulatory effect on the seed germination & other growth parameters of Cajanus cajan and Vigna radiata over control. The observation revealed that the inoculation of bacterial consortium in water may release the nutrients through biodegradation of the organic/inorganic matter of water sources, which promote the plant growth.*

Keywords: *Bacterial consortium; Cajanus cajan; Germination percentage; Seedling growth; Vigna radiata; Yamuna River water*

1. INTRODUCTION

The increasing global population is widening the gap between the supply and demand for water and is reaching alarming level that in some parts of the world it is posing a threat to human existence. The quality of wastewater effluents is responsible for the degradation of receiving water bodies, such as lakes, rivers, streams. The rapid industrialization is accompanied by both direct and indirect adverse effect on environment. The rate of contamination of natural water bodies increases with increased industrialization [1]. Wastewater may be treated by physiochemical or biological methods, biological treatment is preferred over physicochemical as the former is cost effective, efficient and environmentally friendly [2, 3]. According to World health organization (WHO), the mortality rate of water associated diseases exceeds five million people annually with microbial intestinal infections accounting for more than 50 % [4]. River Yamuna is one of the most polluted rivers of India. Pollution levels in the Yamuna River water have raised, biological oxygen demand load has increased by 2.5 times between 1980 and 2000; from 117 tons per day in 1980 to 276 tons per day in 2005. The Yamuna River has been reduced to a small stream, draining industrial effluents, sewage, dirt and other toxic substances. The concept of Effective Microorganisms was developed by Japanese horticulturist Teuro higa from the University of Ryukyus in Japan. He reported in the 1970s that a combination of approximately 80 different microorganisms is capable of positively influencing decomposing organic matter such that it reverts into a life promoting process [5]. 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 [6-8]. The objective of this study was to isolate and identify bacteria from Yamuna River water and evaluate their remediation potential for use in germination and seedling growth of *Cajanus cajan* and *Vigna radiata* in vitro.

2. EXPERIMENTAL PROTOCOL

Sample Collection

The water samples were collected in pre-sterilized BOD bottles, from the river Yamuna situated in the city of Taj - Agra, India. The wastewater sample was then preserved at low temperature (4^0C) before commencement of the experiment.

Isolation and Identification of Bacterial Isolates

The bacterial isolates present in the Yamuna River water were isolated by Serial dilution (Pour-Plate) technique [9]. For the selected isolation of 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. After the incubation period the plates were observed for growth on the media [10]. When the colonies appear on agar plates, each one was sub cultured on a new agar plate until pure strains were obtained. The isolated bacterial cultures were identified on the basis of their morphological, physiological and biochemical characteristics features by Bergey's Manual of Systematic Bacteriology [11].

These cultures were further cross examined by BD-BBL Crystal Identification Autoreader (Becton Dickinson and Company, USA) for the identification surety. [12].

Bacterial Consortium preparation

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. Cell suspension was prepared using sterile distilled water and adjusted to 0.5 OD using UV-visible spectrophotometer [13]. 5% of the above stock solution 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.

Waste Water Characterization

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 [14].The efficiency of the bacterial consortium was evaluated with respect to changes in physico-chemical parameters of the wastewater samples after treatment by following the standard method [14]. For bacterial consortium treatment, 5% of EM solution containing bacterial consortium was inoculated into the water samples and incubated in an incubator. After incubation, the samples were analyzed for different physico-chemical parameters.

Impact of treated and untreated Yamuna water sample on seed germination (*Cajanus cajan, Vigna radiata*) in vitro

In *in vitro* studies the seeds of *Cajanus cajan* and *Vigna radiata* were collected from Dayalbagh Agriculture Farm, Dayalbagh, Agra, (India) and petriplates of 180 cm were used in this experiment. The seeds were sterilized with 1% $HgCl_2$ solution for two minutes and washed with sterilized distilled water 2-3 times to remove the contamination of seed coat prior to germination study. Twenty seeds were placed at an equi-distance in sterilized petriplates lined with Whatman 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. Each treatment including the control was run in triplicates. Plates were kept in an incubator and maintained under standard aseptically physiological conditions at 25-27˚C temperature, 16 hours light and 8 hours dark cycle. After alternate days the fixed amount of different concentration of untreated and bacterial consortium treated water samples was poured into plates upto7 days.

3. RESULTS AND DISCUSSION

Physico-chemical analysis of water

The waste water samples were collected from three different sites of Yamuna River of Agra, city of Taj. From the collected samples, total 15 bacterial strains were obtained. The bacterial isolates having highest prevalence i.e. 100% throughout were selected and identified on the basis of their morphological, physiological and biochemical characteristics. After biochemical test for confirmation of the identified bacteria, a new technique of BD-BBL Crystal Mind Auto reader was also used for rapid identification of bacteria. It showed results between 95 to 99% purity of identified bacteria. The bacteria were tentatively identified as: *Rhodopseudomonas palustris, Rhodobacter spheroides, Escherichia coli, Bacillus subtilis, Bacillus cereus, B. thuringiensis, B. fusiformis, Lactobacillus* sp. The physico-chemical characteristics of Yamuna waste water before and after treatment are presented in Table 1 and Fig.1. 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. Saini [15] reported that the physico-chemical parameters of untreated domestic waste water were beyond the tolerance limit but after treatment with bacterial consortium all the parameters were sharply reduced, below the tolerance limit. 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	Site 1 (Kailash Temple)		Site 2 (Poiyah Ghat)		Site 3 (Hathi Ghat)	
	Untreated	Treated	Untreated	Treated	Untreated	Treated
pH						
ACIDITY	8.9	7.3	9.6	7.8	7.9	7.1
ALKALINITY	395	102.5	498.7	79.3	234	97.2
HARDNESS	895	95	1197	124	459	56.8
TS	986	542	1834	524	654	243
TDS	43.5	6.3	56.8	7.0	29.3	8.3
TSS	26.7	5.2	32.9	4.9	20.2	6.1
DO	16.7	1.1	23.9	2.1	9.1	2.2
BOD	8.8	86	3.8	62.4	5.6	78.9
COD	21.7	8.9	29.9	3.3	10.3	0.8
	100	28	152.9	24.3	84.9	23.9

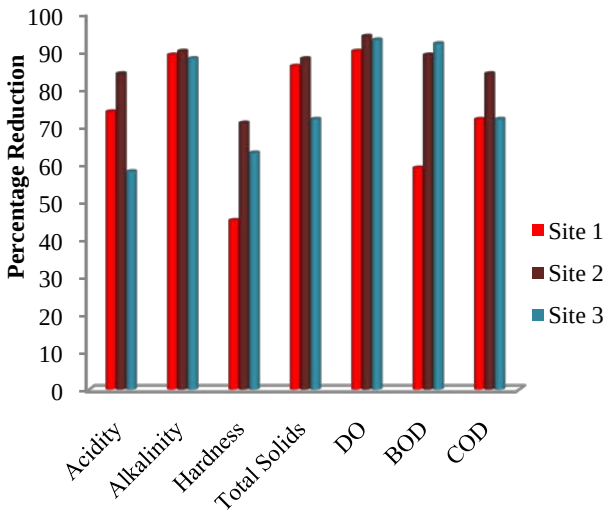

Fig. 1: Percentage reduction in Physico-chemical parameters of waste water

Impact of treated and untreated Yamuna water sample on seed germination (*Cajanus cajan, Vigna radiata*) in vitro

In case of seed germination, the effect of untreated and treated water on *Cajanus cajan* and *Vigna radiata* is presented in Table 2 and 3, Fig. 2 and 3. In *in vitro* study on *Cajanus cajan* and *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 *Cajanus cajan* and *Vigna radiata*. These findings are similar with the report of Mythili and Karthikeyan [13] reported that the higher concentrations (80 and 100%) of tannery effluent suppressed the germination of blackgram and sunflower.

Table 2: Effect of untreated and bacterial consortium treated water sample on the growth of *Vigna radiata* L. *in vitro*

Concentration	Shoot length (cm)	Root Length (cm)	Shoot Biomass (gm)	Root Biomass (gm)
	Mean ± S.D	Mean ± S.D		
CONTROL			0.31	0.04
UNTREATED	7.6 ± 2.0	4.7 ± 0.4		
100%			0.98	0.28
75%	10.7 ± 0.7	7.8 ± 0.2	1.12	0.31
50%	13.4 ± 0.4	9.2 ± 0.2	1.42	0.39
25%	13.3 ± 0.5	9.9 ± 0.3	1.52	0.42
TREATED	13.8 ± 0.2	10 ± 0.1		
100%			3.89	2.98
75%	15.8 ± 0.7	12.4 ± 0.3	2.97	2.12
50%	15.6 ± 0.2	11.7 ± 0.1	2.89	1.97
25%	14.8 ± 0.2	11.6 ± 0.2	2.79	1.89
	14.2 ± 0.1	11.2 ± 0.4		

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 It may be due to the effect of higher amount of total solids and heavy metals stress on the seed germination process in untreated effluent. Paliwal [18] and Singh [19] observed that high concentrations of waste water caused the reduction of seedling growth due to the body system, resulting in ultimate damage to the seed. The results are in accordance with the report given by Verma [20] that the higher concentration of the waste water contains more pollution load, which causes deleterious effect on crop production.

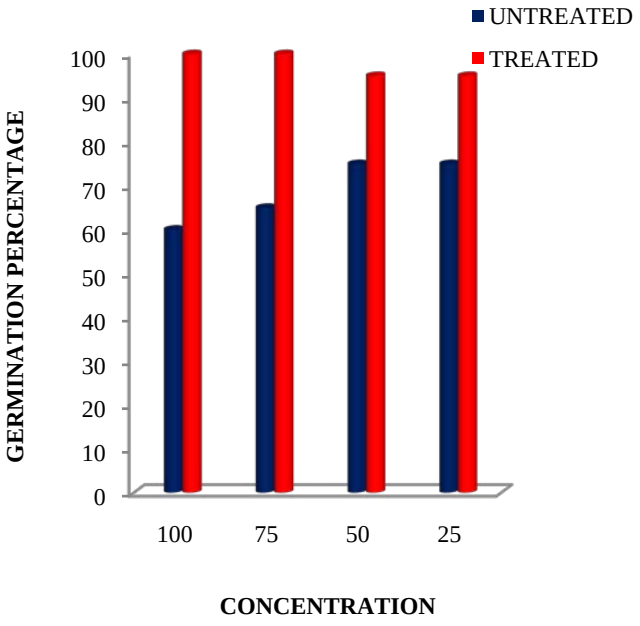

Fig. 2: Effect of untreated and bacterial consortium treated Yamuna water sample on Germination Percentage of *Vigna radiate* L. *in vitro*

Table 3: Effect of untreated and bacterial consortium treated Yamuna water sample on the growth of *Cajanus cajan* L. *in vitro*

Concentration	Shoot length (cm)	Root Length (cm)	Shoot Biomass (gm)	Root Biomass (gm)
	Mean ± S.D	Mean ± S.D		
CONTROL	8.5 ± 0.5	6.6 ± 0.4	1.49	1.23
UNTREATED				
100%	12.0 ± 0.3	8.5 ± 0.3	1.64	1.21
75%	13.1 ± 0.2	7.8 ± 0.2	1.73	1.24
50%	14.0 ± 0.1	6.6 ± 0.4	1.73	1.35
25%	15.0 ± 0.1	5.2 ± 0.1	1.82	1.38
TREATED				
100%	17.5 ± 0.3	13.5 ± 0.3	2.83	2.41
75%	16.7 ± 0.2	12.5 ± 0.2	2.41	2.11
50%	16.2 ± 0.3	11.7 ± 0.2	2.33	1.98
25%	15.7 ± 0.1	10.6 ± 0.1	2.11	1.74

mean±standard deviation from three replicates

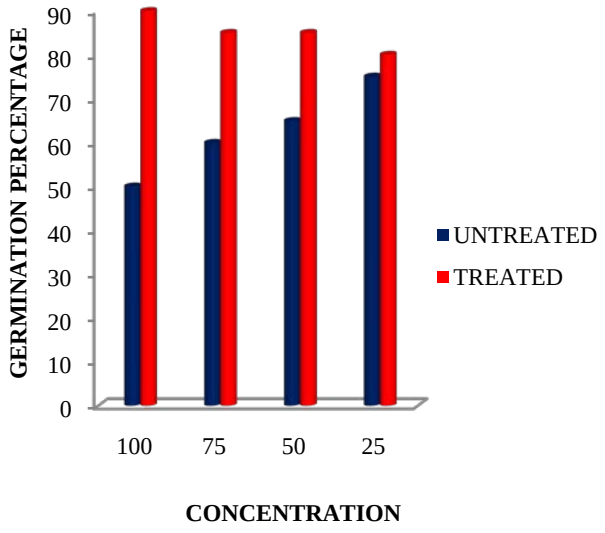

Fig. 3: Effect of untreated and bacterial consortium treated Yamuna water sample on Germination Percentage of *Cajanus cajan* L. *in vitro*

4. CONCLUSION

Perceiving bacteria as dangerous is now turning towards greater awareness of the microbial world as a fundamental element of life. EM is easy and convenient for use, safe and not harmful, low cost and economically effective and this has increases the effectiveness of application of this technology. 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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