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COMPARATIVE ANALYSIS OF BIOREMEDIATION POTENTIAL OF NATIVE AND MARINE BACTERIA BY GERMINATION STUDY

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ABSTRACT

The effect of the various effluents on the germination and growth of *Pisum sativum* was studied in this piece of research work. The efluents used includes untreated or raw effluent as well as treated effluents. Three portions of the effluents were treated in three different kinds, first in which native bacteria isolated from the effluent itself was used for carrying out treatment, effluent in which marine bacteria isolated from a beach area was used for bioremediation and effluent in which marine bacteria isolated from a site at which the effluent is being discharged and proximal to an industry was used for treatment were used. Maximum percentage of germination was seen in effluent treated with native bacterium at a concentration of 10% (72% germination). At 50%, it gave 63% germination. The percentage germination seen in effluent treated with Tb (bacterium isolated from ocean, near the industrial plant) also gave 62% at 10% effluent concentration. Characters like number of leaves produced as well as the width of the leaves formed were measured to see whether there are any differences in these growth parameters on using the different kind of effluents for irrigation. Control as well as plantlets at irrigation with 10% of treated effluents using native as well as marine bacterium isolated from Veli cost showed maximum number of leaves(4). In the case of leaf width also, the maximum values were observed in control, irrigated with distilled water. This was followed by 10% effluent treated with bacterium isolated from Veli and then by native bacteria and bacteria isolated from Sanghumukham.

KEYWORDS: Bioremediation, effluent, native bacteria, germination, growth parameters.

INTRODUCTION

Industrialization has become an important factor in the development of a country's economy, through the establishment of plants and factories. But, industrial effluents lead to pollution which affects every environmental segment like surface water and soil and ground water on leaching. But some industries release their effluent without proper treatment, directly and indiscriminately to environment. This affects the nature of water quality and soil quality of the environment. Water pollution is the most important harmful effects of the environment. Industrial effluent is the mixture of harmful toxic metals and toxic elements which affect microbial diversity of the marine water and other freshwater resources. The area with polluted soil due to the presence of effluent will produce harmful effects on the environment, human health, agricultural production, animal husbandry etc.

Industries should treat the effluent prior to the release of this and then only plants, animals and humans can survive (Fric *et al.*, 1999). If the effluent is properly treated, organisms will survive and the environment will be available in a better condition for the organisms to survive. There can be some microorganisms living in the effluent which is capable of using the ingredients of the effluent. Their very presence shows that they are capable of using the potential toxic substances in it, and thus remediating it. Similarly, there may be so many potent microorganisms isolated from other unique ecosystems which may be capable of remediating the effluent. Several studies have shown that marine bacteria are candidate organisms which can be used for bioremediation activities because their metabolic pathways are developed to resist a variety of harsh conditions.

This is realized and as described in the previous chapters, the bioremediation potential of both native bacteria which are the original inhabitants of the effluent and that of bacteria isolated from marine environment is compared. In this chapter, the result of the bioremediation potential is checked by conducting a germination and growth study using garden pea seeds.

REVIEW OF LITERATURE

Sahare, (2014) suggested that pollution causes problems in the surrounding environment. Also, there are several reports which suggest that industrial effluents cause a significant damage to the germination and growth pattern of the plants. Jolly *et al*., (2012) found out that the growth pattern and yield of crop wheat is affected by industry effluent. In that study, in order to identify the differential effect of the treated and untreated effluents on the seed germination and growth of plant, wheat was selected. The study found out that the seed germination rate was better in treated effluent than in untreated effluent.

Abegunrin *et al*., (2015) reported that if the waste water is used for irrigation of crops continuously for a long period of time, it will increase the level of water repellency, adversely affect soil properties and make the soil to lose its fertility. A similar report was made by Chonker *et al.*, (2000) and found out that the high concentration of physico-chemical parameters in industrial effluent adversely affect the soil, water and plant growth. But at the same time some of the effluents have a plant growth promoting activity and hence can be used after proper dilution of the effluent. Kalaiselvi *et al*., (2010) also reported that the use of industrial effluent produce the irrigation purpose for crop plants is twofold. Dulaimi *et al*., (2012) reported the influence of industrial effluent on seed and seedling quality characters irrespective of crops.

Varma and Sharma, (2012) studied the use of textile effluent and diary effluent on seedling growth and plant growth of wheat. Sharmila *et al*., (2009) reported that bioremediation resulted in a significant improvement in the quality of the effluent and studied the effect of different concentrations of industrial effluent on germination of seeds. The suitability of use of pharmaceutical effluent in irrigation was also studied and it was identified that it is not good for the plants at high concentration, but at the same time, if there is a scarcity of water, then it can be properly diluted and used. Khan Jadoon *et al*., (2013) found that the germination of various vegetables decreased when it was irrigated with industrial effluent and affected the germination and reduced growth. Singh and Antil, (2012) showed that some effluents can be used as a good source of irrigation as it contained so many nutrients including Nitrogen, Phosphorus and Potassium. Some researchers (Naddafi *et al*., 2005; Parameswari and Udayasoorian 2013). have found out that, irrigation using industrial effluents increased the growth and productivity of plants and thus can be used as a cheap alternate for water. Hamidović *et al*., (2016) reported that the effluent after treatment with native bacteria has resulted in increased fertility of soil and hence increased plant growth.

MATERIALS AND METHODS

The experimental plant selected for conducting this study was the commonly cultivated vegetable, garden pea, *Pisum sativum*. This plant was selected because of the easy availability of its seeds and also due to the ease of growth.

Plate 1. Experimental plant

SELECTION OF EFFLUENT

Untreated Effluent

Effluent was collected from a nearby industry. The collection procedures were in according to the standard microbiological sampling techniques. The samples were maintained in cold chain and were refrigerated.

TREATED EFFLUENT

Using Native Bacteria

A portion of effluent was treated using the native bacteria which were enriched from the effluent itself. The process of effluent bioremediation explained in the earlier chapters. This treated effluent was used to irrigate the pea seeds as well as plants to find its effect on germination and growth.

USING MARINE BACTERIA

Another portion of the effluent was treated using marine bacteria, present in the Department Laboratory as part of another research work. Bacterial strains were isolated from two shores Veli and Sanghumukham. The sampling sites were selected on the basis of activity at the both shores. Veli is near to the industry selected for the study and Sanghumukham is a beach side with human interference. The activity will have some effect on the microbial diversity.

EFFLUENT DILUTION

Three dilutions of the untreated as well as treated effluent (native bacteria and two types of marine bacteria) were prepared (10%, 50% and 100%). Pure water was used as control.

GERMINATION STUDIES

GERMINATION STUDIES ON PETRIDISH

Germination studies were conducted using *Pisum sativum* seeds. The seeds selected for the study were of same size. Ten undamaged seeds were selected for each germination plate. Pre-sterilized petriplates were taken based on the number of effluents. The experiments were carried out in triplicate. Pure distilled water was taken as the control.

Measured quantities of effluent were added in each sterile petriplate with sterile cotton pads. Ten pea seeds were put in each petriplate. A total of thirteen petriplates were taken. One was marked control and the cotton pad was soaked with sterile distilled water. To the first three petriplates, 10%, 50% and 100% untreated effluent was added. To the second set of three petriplates, corresponding concentrations of effluent treated with native bacteria was added. To the third and fourth set of petriplates, the effluent treated with two strains of marine bacteria was added and kept for two days. The number of seeds germinated was counted (Rahman *et al*., 2002).

GROWTH MEASUREMENTS

After germination, the radicles as well as the plumule length of the seedlings were measured to find the effect of the effluents on the successful germination and growth of the seedlings. The length of root and shoot was measured with the help of a wetted twine (for flexibility) and a scale. From the values of length of radicle and plumule, the seedling length was found out. Finally Vigour Index was calculated. Vigour tests are calculated to find out how the seeds perform under the condition of stress. It is opposite of germination test. The findings of germination studies and calculated vigour index, it provide a complete performance profile for a range of field conditions and hence it helps to take decision on the conditions to which the seeds will be exposed to.

Vigour Index = V.I. = Germination percentage x total length of seedling (cm)

GROWTH STUDIES IN POT

Studies were also carried out in tins of uniform size with all the above mentioned effluents as well as with one control which was irrigated with plain water. Good soil from the same plot was taken and sieved in a 2 mm mesh. Five hundred grams of soil was taken in each tins. Three different concentrations (10%, 50% and 100%) of treated and untreated effluent were used for irrigation. Same quantities of control as well as various effluents were used for irrigation and it was done at an interval of 24 hours. The complete setup was placed for fifteen days. Parameters such as plant height, number of leaves formed and leaf width were measured.

RESULTS AND DISCUSSION

STUDIES ON PETRIPLATES

The effect of various concentrations effluent on the germination of pea seeds was studied and the results are represented here (Table: 1. and Figure: 1.). A set of control seeds were also maintained, irrigated with distilled water. It can be seen that the control plate showed 100% germination after 24 hours. In other petriplates and effluents both untreated and treated, the germination percentage decreased as the concentration of treated and untreated effluent increased. Even though it decreased, the germination percentage of the treated effluents at 10% and 50% were not too low. This shows that the bioremediation carried out was good with both native bacteria as well as marine bacterial isolates. Among the native and non-native bacteria, native bacteria were found to be better candidates. The positive control with distilled water showed a cent percent germination whereas, in all percentages of untreated effluent, the germination percentage was too low.

Table: 1. Germination Percentage.

Control (%)	Effluent Concentration		10%	50%	100%
100	Untreated		12	5	0
	Treated	Native Bacteria	72	63	38
		Tb	62	44	31
		Bb	58	45	29

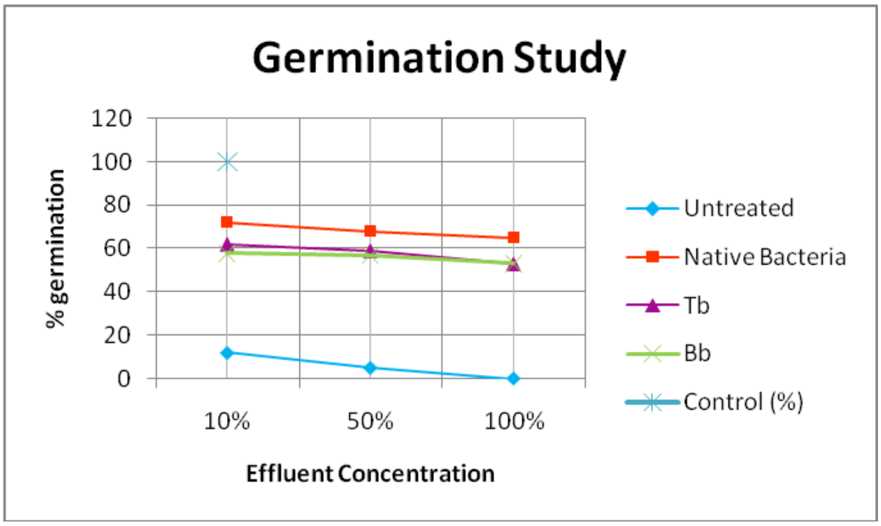


Figure: 1. Graph showing the percentage of germination

Rodger *et al*., (1957) reported that high osmotic pressures of the germination solution makes imbibitions more difficult and retard germination, while the ability of seeds to germinate under high osmotic pressure differs with variety as well as species. This may be the reason why in untreated effluent the percentage of germination was too low.

There are several studies which show that untreated effluent can in some cases increase the growth as well as germination rate of plants. Biradar *et al*., (1989) reported that industrial effluents at lower concentrations stimulated the physiologically inactive seeds to germinate. Similarly, Jerath and Sahai, (1982) reported the fertilizing effect of lower concentrations of the effluent and optimum conditions provided for seed germination. This may be due to the minerals present in these effluents which may act as micronutrients for the seeds. Rajannan, *et al*. (1998) also studied the effects of tannery effluent at different concentrations (25, 50, 75 and 100%) on seed germination of *Oryza sativa* and found that the germination was inhibited by 25 and 50% effluent and fully dormant by 75 and 100% effluent. The germination studies are shown in Plate: 1.

GROWTH MEASUREMENTS

LENGTH OF RADICLE, PLUMULE AND SEEDLING

The lengths of plumule, radicle and the total seedling were measured for different treatments and are given as Table: 2 and Figure: 2. Growth in untreated effluent was shown to be minimum and good growth in control. All other treated effluents at all percentages showed almost similar growth pattern. This measurements also shows that the effluent was almost well remediated with the microorganisms used, making these organisms candidates for effluent remediation.

Table 2: The length of radicle, plumule and the seedling length.

Effluent		Con.	Radicle (cm)	Plumule (cm)	Total Seedling Length (cm)
Control			5.1	4.9	10
Untreated		10%	2.7	2.9	5.6
		50%	0.5	0	0.5
		100%	0	0	0
Treated	Native Bacteria	10%	4.0	4.1	8.1
		50%	3.8	3.9	7.7
		100%	3.7	3.7	7.4
	Tb	10%	4.1	4.3	8.4

		50%	3.9	4.1	8
		100%	3.7	4.0	7.7
	Bb	10%	4.2	3.9	8.1
		50%	4.1	3.7	7.8
		100%	4.0	3.6	7.6

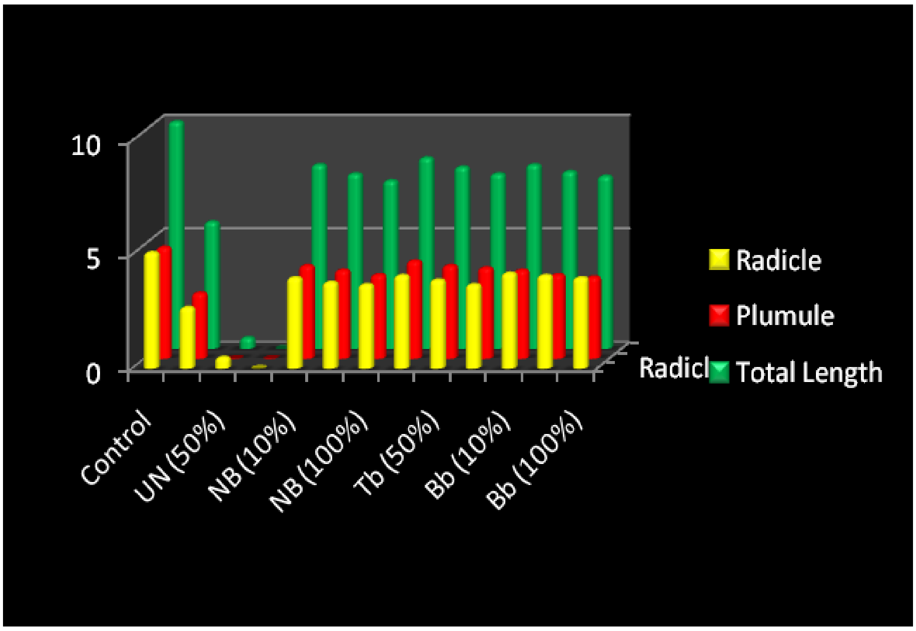


Figure: 2. Graph showing seedling characteristics.

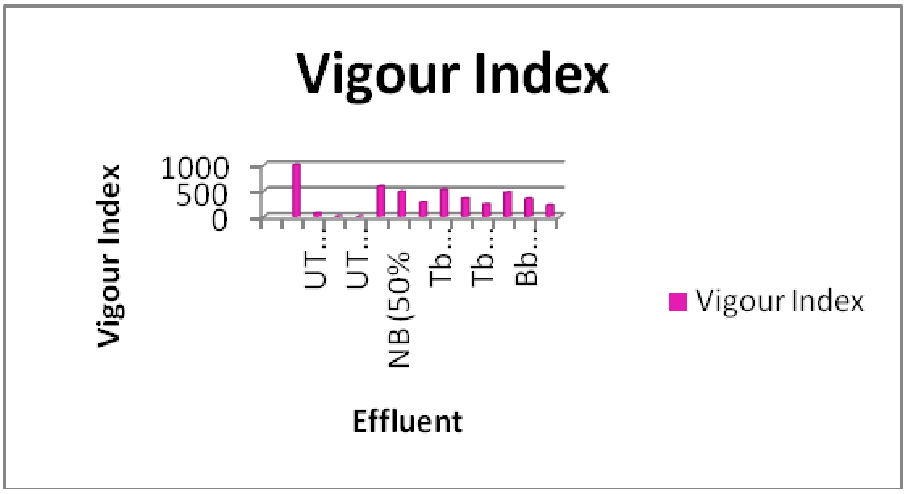
The length of the radicle, plumule as well as the total seedling also was greatest in control, irrigated with distilled water. Untreated effluent irrigation resulted in very poor growth of the seedlings where as 10% concentration of effluents showed fair growth in length of plumule, radicle as well as total seedling length. Sharma, *et al*. (2002) studied the effect of fertilizer factory effluents (0, 1, 2, 5, 10, 25, 50 and 100%) on seed germination of tomato cultivars PED, Pusa Ruby and Rupal-I. He found that the percentage germination as well as growth decreased step by step with rising concentration of effluents. It has been reported that the effluents have an inhibitory effect on seed germination and growth performance of wheat cultivars, pulses like green gram, (El-Nashar, 1998) red gram, and other crops like onion and tomato.

VIGOUR INDEX

Vigour Index of the seedlings was calculated and the result is shown in Table: 3. and Figure: 3.

Table 3: Table showing vigour index.

Effluent	Germination (%)	Seedling Length (cm)	Vigour Index
Control	100	10	1000
UT (10%)	12	5.6	67.2
UN (50%)	5	0.5	2.5
UT (100%)	0	0	0
NB (10%)	72	8.1	583.2
NB (50%	63	7.7	485.1
NB (100%)	38	7.4	281.2
Tb (10%)	62	8.4	520.8
Tb (50%)	44	8	352
Tb (100%)	31	7.7	238.7
Bb (10%)	58	8.1	469.8
Bb (50%)	45	7.8	351
Bb (100%)	29	7.6	220.4



The vigour index also shows that the control ie., the seeds in distilled water showed high vigour index. Reports show that it is difficult to get 100% vigour. The vigour index is used to describe the performance of seeds when sown in the field, (Perry, 1984).

GROWTH STUDIES IN SOIL

PLANT HEIGHT

The length of the plant in soil on irrigation with various effluents studied was measured to understand the effect of the effluents. The observed results are as shown in Table: 4.

Table: 4. Influence on plant height.

Effluent		**Con.**	**Plant Height (cm)**
Control			28.0
Untreated		10%	10.0
		50%	7.1
		100%	4.5
Treated	Native Bacteria	10%	21.3
		50%	18.7
		100%	14.0
	Tb	10%	23.0
		50%	15.0
		100%	7.0
	Bb	10%	18.5
		50%	9.8
		100%	9.4

The values show that the maximum length is attained by control plant. Bioremediated effluents treated using marine bacterial strains from Veli also showed good growth at 10% effluent concentration. The effluent treated with native bacteria also came close to these values.

LEAF NUMBER AND LEAF WIDTH

Characters like number of leaves produced as well as the width of the leaves formed were measured to see whether there are any differences in these growth parameters on using the different kind of effluents for irrigation. The results of the observations are given in Table: 4. and Figure: 4.

Table: 5. Leaf number and Leaf width in various treatments.

Effluent		**Con.**	**Leaf No.**	**Leaf Width (cm)**
Control			4	6.5
Untreated		10%	2	3.0
		50%	2	2.5
		100%	1	2.1
Treated	Native Bacteria	10%	4	5.8
		50%	3	3.5
		100%	3	2.9
	Tb	10%	4	6.3
		50%	3	3.7
		100%	2	4
	Bb	10%	3	5.8
		50%	2	3.5
		100%	3	2.9

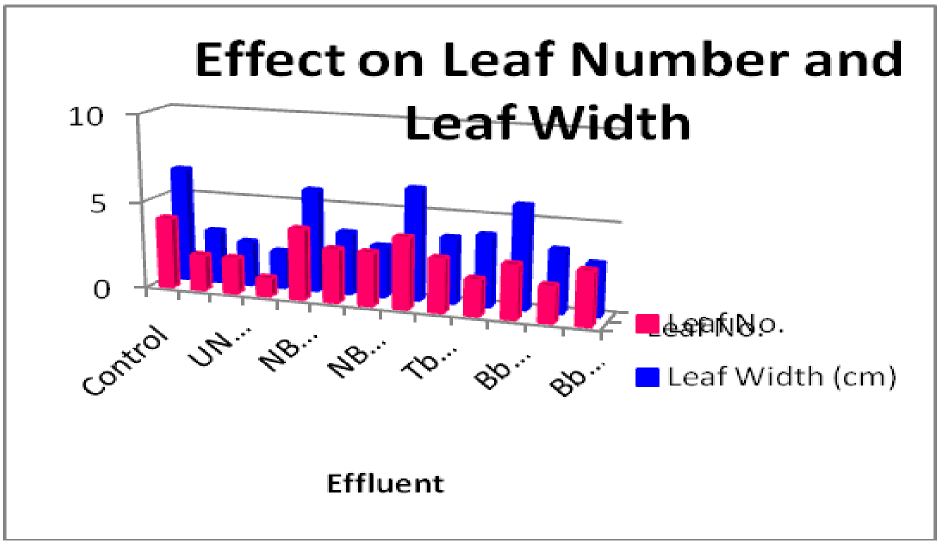


Figure 5: Graph showing effect of effluent on leaf number and width.

Control as well as platlets at irrigation with 10% of treated effluents using native as well as marine bacterium isolated from Veli cost showed maximum number of leaves (4). In the case of leaf width also, the maximum values were observed in control, irrigated with distilled water. This was followed by 10% effluent treated with bacterium isolated from Veli and then by native bacteria and bacteria isolated from Sanghumukham.

CONCLUSION

The effect of the various effluents on the germination and growth of *Pisum sativum* was studied in this chapter. The efluents used includes untreated or raw effluent as well as treated effluents. Three portions of the effluents were treated in three different kinds, that in which native bacteria isolated from the effluent itself was used for carrying out treatment, effluent in which marine bacteria isolated from a beach area was used for bioremediation and effluent in which marine bacteria isolated from a site at which the effluent is being discharged and proximal to an industry was used for treatment were used.

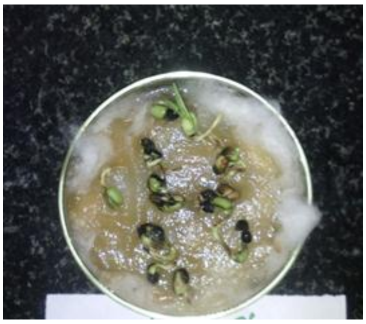
Maximum percentage of germination was seen in effluent treated with native bacterium at a concentration of 10% (72% germination). At 50%, it gave 63% germination. The percentage germination seen in effluent treated with Tb (bacterium isolated from ocean, near the industrial plant) also gave 62% at 10% effluent concentration. The reason for this can be explained. Native bacteria are the residents of the effluents and hence have the capacity to use the materials in the effluent as nutrients, either as carbon or as energy source. It is understood that microbes in stressed environment are adapted to these environments and develop diverse metabolic pathways, (Butler and Mason 1997; Ellis, 2000). As these capacities of microbes usually reside in plasmids, they can be transferred to others by horizontal gene transfer, (Abraham *et al*. 2002; Arunprasad and Bhaskara Rao 2010). Similar results were reflected in the values of vigour index as well as pot studies. Thus it was found out that bioremediation substantially improves the quality of effluent and the usability for irrigation.

Control

Untreated effluent (100%)

Native bacteria (10%)

Tb (10%)



Bb(10%)

Bb (50%)

Tb (100%)

Bb (100%)

Plate: 1. Germination studies of *Pisum sativum* using various effluents

Control

Untreated Effluent (100%)

10%Tb

Tb (50%)

Tb (100%)

Bb (10%)

50%Bb

100%Bb

Plate: 2. Growth Studies of Pisum sativum in pots using different effluents

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