Chapter V

Evaluation of the Biological Treatment On Plant Growth and Yield
Increasing population is placing increasingly greater demand for the resources to meet their requirements. This leads to industrialization and consequent pressure on the existing natural resources. Increasing urban population and the consequent industrialization draw heavy quantity of water and provide a large quantity of wastewater or municipal effluent. The problems are further aggravating for disposal of these effluents. In the race towards industrialization, a number of industrial estates were established throughout the sub-continent. Textile industry in particular has been placed in the category of most polluting industries. However, the Indian Textile Industry is one of the largest in the world with a massive raw material and textile manufacturing base. Our economy is largely dependent on the textile manufacturing and trade in addition to other major industries. About 27% of the foreign exchange earnings are on account of export of textiles and clothing alone. The textiles and clothing sector contributes about 14% to the industrial production and 3% to the Gross Domestic Product (GDP) of the country. Owing to its huge economic contribution to the country, textile industry needs proper attention on its environmental concern. The improper and indiscriminate disposal of textile effluents in natural waters and land is posing serious problems.

One of the important measures is using these industrial effluents in tree plantations to control land degradation and improve environmental conditions. Uneven distribution of rainfall, long dry spells, soil water stress and nutrient deficiency constitutes the major constraint in the establishment of planted tree seedlings in dry areas, where better-quality water is becoming an increasingly scarce resource. Both the need to conserve water and to safely and economically dispose wastewater, make the use of municipal effluent in tree
plantation a very feasible option (Singh and Bhati 2005). In many parts of the world, municipal wastewater is used for the irrigation of various crops including agronomic, horticultural and tree crops (Mathur and Sharma 1984; Stewart et al., 1986; Urie 1986). Trees and shrubs are a better alternative than agricultural crops because of high growth rates and potential to produce high biomass on annual basis.

The textile effluent contains organic and inorganic chemical species that have adverse effect on growth of all plants and animals, because textile effluent used for irrigation contains heavy metals (Ni, Cd, Cr, Pb, Hg etc.), which accumulate in various parts of plants that result in various clinical problems in animals as well as human beings including hepatic and renal system damages, mental retardation and degradation of basal ganglia of brain and liver (Misra and Dinesh 1991). However, the adverse effects of textile effluents on plants depend on the type of species, types and concentrations of toxic materials in the effluent. After sequential approach to the problem, the toxicity of the biologically treated effluent using a novel bacterial strain JMC-UBL-27 isolated (As described in Chapter IV). Thus, the present study was aimed to assess whether the treated effluent could safely be used to irrigate crop plants. In addition, to assess up to what extent heavy metals present in irrigating water caused changes in plant growth at the germination and seedling stages, and to draw the relationships between modality of treatment and growth parameters of the plants two species of plant was chosen such as Brassica nigra and Cyamopsis tetragonolobus.
However, the individual steps involved are as follows.

- A lab scale study on the effect of raw effluent, chemically treated effluent and biologically treated effluent on the germination of *Brassica nigra* (mustard) and its growth characteristics.

- A field study on the effect of raw effluent, chemically treated effluent and biologically treated effluent on the morphometric and biochemical parameters in *Cyamopsis tetragonolobus* (Cluster Bean). In this experiment, the plants were treated according to the following regime.

  1. Well water (Control: Group I - WW)
  2. Raw effluent (before any treatment: Group II - RE)
  3. Sludge part of the chemical treatment (Group III - CTS)
  4. Water part of the chemical treatment (Group IV – CTW)
  5. Biologically treated effluent in total (Group V - BT)
Materials and Methods

Effect of effluents on the Growth *Brassica nigra* - (Mustard seeds)

To compare the efficiency of the biological treatment using the chosen isolate with the raw and chemically treated effluent, the effluents were simultaneously used to analyze the effect on the seed germination in mustard. The seeds of mustard (*Brassica nigra*) were sown uniformly in four groups of plastic trays, each one for the control (water alone), raw effluent, chemically treated effluent and biologically treated effluent. Effluent treatment was carried on for four days till the seeds germinated where in 20 ml of the respective effluents were used per tray to maintain appropriate moisture. Appearance of the shoot out of the seed for a length of about 0.5 cm was considered as ‘germinated’. The effect of treated and untreated effluents on germination was observed after 4 days. The plant was collected after 12 days for recording the morphological and biochemical parameters. All experiments were carried out in triplicates.

Field Trial: Effect of Treated and Untreated Effluent on *Cyamopsis Tetragonolobus* (Cluster Bean)

The seedlings of cluster bean were sown uniformly in a 100 sq. ft of a wet ground. The seeds were allowed to germinate and grow. After 25 days the plant was raised to a maximum height of 22cm. The plant was treated with 6 different groups of water / effluents such as (1) water as control, (2) raw effluent (untreated textile effluent),
(3) sludge of the chemically treated effluent, (4) supernatant of the chemically treated effluent and (5) biologically treated effluent separately. The treatment was carried out once in every 4 days for about one month in which the respective groups received 2 litres of water/effluents/treated effluents / sludges as appropriate. After the treatment period for about one month, the plant samples were collected according to the groups of treatment and their morphometric and biochemical parameters were recorded as given in the Table.

5.1

**Chlorophyll Estimation**

Chlorophyll 'a' and 'b' contents and total chlorophyll were determined according to the method of Arnon (1949). Fresh leaves (0.1 g) were ground in 80% acetone and centrifuged at 10,000 rpm for 5 min. Absorbance of the supernatant was read at 645, and 663 nm using a spectrophotometer (Schimadzu, UV-VIS- 1800, Japan).

\[
\begin{align*}
\text{Chl.}a \text{ (mg g}^{-1}\text{f.wt)} &= [12.7(\text{OD 663}) - 2.69(\text{OD 645})] \\
& \times V/1,000 \times W \\
\text{Chl.}b \text{ (mg g}^{-1}\text{f.wt)} &= [22.9(\text{OD 645}) - 4.68(\text{OD 663})] \\
& \times V/1,000 \times W \\
V &= \text{volume of the extract (ml)} \\
W &= \text{weight of the fresh leaf tissue (g)}
\end{align*}
\]
Determination of Proteins, Carbohydrates and Lipids

Proteins were extracted using the modified procedure of Ganjewala and Luthra (2007) and estimated using the Bradford method (1976). The total sugars and reducing sugars were determined according to Yemm and Willis (1954) and Nelson (1944), respectively.

Statistical Analysis

The data recorded from both these experiments were subjected to two way analysis of variance (ANOVA) using MS Excel (Office 2007).
Results

Effect of Effluents on the Germination of Mustard Seeds

There was a remarkable performance in the germination percentage of mustard seeds under biologically treated effluent to about 83.66 ± 0.5 % when compared to that of the water (Control Group) which demonstrated a germination of 91.66 ± 2.51 % on day 4. In contrast to this, the germination % was significantly too low in the other cases with the raw effluent (36.33 ± 1.52 %) and chemically treated effluents (29±3 %) (Figure 5.1, 5.20; Table 5.2).

Effect of Effluents on the Total Height of the Plant, Shoot Length and Root Length

The effluents had marked effect on the growth of the plant, shoot length and root length in comparison to the control when measured on day 12. The height of the plant was significantly higher in the biologically treated effluent group (11.23 ± 0.37 cm) than the other two effluents (5.9 ± 0.25 cm and 4.93 ± 0.2 cm in Raw effluent and Chemically treated effluents respectively). Similarly, shoot length and root length also showed better results than that of the raw effluent and the chemically treated effluent. However, the parameters in the control group were the best with a total height of 13.3 ± 0.5 cm (Table 5.3; Fig. 5.2, 5.21).
Effect of Effluents on Wet and Dry Weight of Plant

The wet weight and dry weight of the plant was analyzed and found that the biologically treated group was better than that of the chemically treated and raw effluent group of plants. In control, the wet and dry weights were 2.1 ± 0.15 gm and 0.28 ± 0.032 gm in contrast to the biologically treated effluent group of plants that were 1.52 ± 0.04 and 0.19 ± 0.02 gm respectively. On the whole, the biologically treated effluent group of plants demonstrated better results than the raw and chemically treated effluents in comparison with control-group of plants (Figure 5.3; Table 5.4)

Effect of Effluents on the Number of Leaves

Numbers of leaves were almost equal in control and biological treatment group with 3.6 ± 0.57 and 3.3 ± 0.57 respectively, where as that of the raw and chemically treated plant was lower. However, there was no difference in the number of leaves between the raw (2 ± 0.0) and chemically treated group of plants (1.6 ± 0.57) (Figure 5.4; Table 5.5).

Effect of Effluents on Chlorophyll Content in Mustard Plant

Chlorophyll contents in terms of mg g⁻¹ f. wt (Chl a, Chl b and Chl T) were higher in the case of the control group (0.64 ± 0.02; 0.64 ± 0.15; 0.88 ± 0.01) of plants than the other treatment groups. However, the biologically treated effluent group of plants demonstrated
slightly higher levels (0.53 ± 0.01; 0.53 ± 0.007; 0.53 ± 0.007) than that of the chemical and raw effluent treated group of plants (Figure 5.5; Table 5.6).

**Effect of Effluents on the Total Carbohydrate, Reducing Sugar and Protein Content of the Plant Leaf**

The total carbohydrate content of 100 mg of plant material revealed about 1300 ± 72 µg in the control group where as it was significantly lower in the raw effluent (1000 ± 85 µg) and chemically treated group of plant (1100 ± 93µg). However, the biologically treated group of plants revealed higher levels (1200 ± 120 µg) than that of the chemically treated group of plants (Figure 5.6). The total reducing sugar content (µg /100mg) was highest in the control group of plants (590 ± 19 µg) than the others. However, biologically treated effluent group of plants revealed higher reducing sugar level (580 ± 48µg) than the chemically treated (570 ± 12µg) and raw effluent treated group of plants (550 ± 21 µg) (Figure 5.7). The content of protein in the leaves of the plant was higher in control (2000 ± 142 µg) than the other group of treatments. Chemical and biologically treated group of plants showed equal amounts of protein content. However, the protein level was least among the groups in raw effluent (1600 ± 74 µg) (Figure5.8).
Morphometric Analysis of the *Cyamopsis Tetragonolobus* (Cluster Bean)

In this field experiment, five different treatment groups were tested (Fig. 5.22, 5.23). They are Control: Group I – WW (Well water); Group II – RE (Raw effluent); Group III – CTS (Sludge part of the chemical treatment); Group IV – CTW (Water part of the chemical treatment) and Group V – BT (Biologically treated effluent in total). Morphometric parameters such as total height, shoot length, root length, wet-weight of the plant, no. of leaves in a plant, no. of nodes in a plant, no. of pods per plant, length of pods, weight of the pod, no. of seeds per pod, yield per plant and gross yield of treatment groups, were recorded after 60 days. All the data have been tabulated and presented (Tables 5.7 to 5.14; Figures 5.9 to 5.16).

In general the Group I-WW revealed best growth and yield characteristics as expected. The Group IV-CTW and Group V-BT comparatively had similar impact in all the parameters tested. However, when comparing supernatant of the chemically treated effluent (Group IV) and biologically treated effluents (Group V), the latter showed better growth characteristics on the plant though not significantly different on most of the parameters such as height of the plant (Table 5.7; Figure 5.9), wet-weight of the plant (Table 5.8; Figure 5.10), No. of leaves, nodes and seeds (Table 5.9; Figure 5.11), weight of pods (Table 5.12; Figure 5.14) and gross yield (Table 5.14; Figure 5.16). Likewise, the sludge of the chemically treated effluent and the raw effluent had adverse effect on the growth of plants. Among this two, the former demonstrated the most toxic nature on plants. Raw effluent had poor growth supporting properties when analyzed. There was
significant difference observed when analyzing the no. of pods per plant and yield per plant between various treatments (Table 5.9, 5.13; Figure. 5.11, 5.15, 5.24).

**Biochemical Parameters**

Total carbohydrates, total reducing sugar, total protein and chlorophyll contents were analyzed for various treatments. The results are presented in the tables and graphs (Table 5.10; Figures 5.12, 5.17 to 5.19). There were insignificant differences among the biochemical parameters. However, the values of biological treatment were closer to that of the control than the values of the chemically treated effluent (supernatant and sludge) to the control.
<table>
<thead>
<tr>
<th>Morphometric characters</th>
<th>Biochemical parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean height of the plant</td>
<td>Total carbohydrate</td>
</tr>
<tr>
<td>Mean shoot length</td>
<td>Reducing sugar</td>
</tr>
<tr>
<td>Mean root length</td>
<td>Protein content</td>
</tr>
<tr>
<td>Mean weight of the plant</td>
<td>Chlorophyll content</td>
</tr>
<tr>
<td>Mean dry weight</td>
<td></td>
</tr>
<tr>
<td>No. of leaves (mean)</td>
<td></td>
</tr>
<tr>
<td>No. of nodes (mean)</td>
<td></td>
</tr>
<tr>
<td>Mean yield of the treatment group</td>
<td></td>
</tr>
<tr>
<td>(in numbers)</td>
<td></td>
</tr>
<tr>
<td>Mean yield of the treatment group</td>
<td></td>
</tr>
<tr>
<td>(in Kg)</td>
<td></td>
</tr>
<tr>
<td>Mean Length of the pod</td>
<td></td>
</tr>
<tr>
<td>Mean no. of seeds per pod</td>
<td></td>
</tr>
</tbody>
</table>

*Table 5.1 showing list of morphometric characters and biochemical parameters studied.*
### Table 5.2: Brassica nigra: Percentage of Germination among the treatments on day 4.

<table>
<thead>
<tr>
<th>Treatment Groups/Parameter</th>
<th>Control</th>
<th>Raw Effluent</th>
<th>CT-Sludge</th>
<th>BT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage of Germination</td>
<td>91.66±2.51&lt;sup&gt;a&lt;/sup&gt;</td>
<td>36.33±1.52&lt;sup&gt;b&lt;/sup&gt;</td>
<td>29±3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>83.66±5.03&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*Values followed by same letters in a column are not significantly different (p ≤0.05) (n=3, mean ± SD)*

### Table 5.3. Mean values of total height; root length and shoot length Brassica nigra on day 12

<table>
<thead>
<tr>
<th>Treatment Groups/Parameters</th>
<th>Control</th>
<th>Raw Effluent</th>
<th>CT-Sludge</th>
<th>BT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Height in cm</td>
<td>13.3±0.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.9±0.25&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.93±0.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>11.23±0.37&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Root Length in cm</td>
<td>1.98±0.37&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.47±0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.37±0.26&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.71±0.25&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Shoot Length in cm</td>
<td>11±0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.15±0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.33±0.23&lt;sup&gt;c&lt;/sup&gt;</td>
<td>9.1±0.2&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*Values followed by same letters in a column are not significantly different (p ≤0.05) (n=3, mean ± SD)*
Values followed by same letters in a column are not significantly different ($p \leq 0.05$) 
($n=3$, mean ± SD)

**Table 5.4: Brassica nigra: Wet and Dry weight of the Plant under Different Treatments**

<table>
<thead>
<tr>
<th>Treatment Groups/Parameters</th>
<th>Control</th>
<th>Raw Effluent</th>
<th>CT-Sludge</th>
<th>BT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wet Wt in gm</td>
<td>2.1±0.15(^a)</td>
<td>0.92±0.035(^b)</td>
<td>0.78±0.056(^c)</td>
<td>1.52±0.04(^e)</td>
</tr>
<tr>
<td>Dry Wt in gm</td>
<td>0.28±0.32(^a)</td>
<td>0.13±0.015(^b)</td>
<td>0.09±0.005(^c)</td>
<td>0.19±0.02(^d)</td>
</tr>
</tbody>
</table>

Values followed by same letters in a column are not significantly different ($p \leq 0.05$) 
($n=3$, mean ± SD)

**Table 5.5: No. of Leaves per plant among all the treatments in Brassica nigra**

<table>
<thead>
<tr>
<th>Treatment Groups/Parameters</th>
<th>Control</th>
<th>Raw Effluent</th>
<th>CT-Sludge</th>
<th>BT</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of leaves</td>
<td>3.6±0.57(^a)</td>
<td>2(^b)</td>
<td>1.6±0.57(^b)</td>
<td>3.3±0.57(^a)</td>
</tr>
</tbody>
</table>
Values followed by same letters in a column are not significantly different (p ≤ 0.05) (n=3, mean ± SD)

Table 5.6: Chlorophyll contents in Brassica nigra among all treatments

<table>
<thead>
<tr>
<th>Treatment Groups/Parameters</th>
<th>Control</th>
<th>Raw Effluent</th>
<th>CT-Sludge</th>
<th>BT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chl a</td>
<td>0.64±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.42±0.009&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.4±0.006&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.53±0.015&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Chl b</td>
<td>0.64±0.0157&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.2±0.003&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.162±0.007&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.53±0.007&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Chl Total</td>
<td>0.884±0.011&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.64±0.008&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.281±0.008&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.53±0.007&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values followed by same letters in a column are not significantly different (p ≤ 0.05) (n=3, mean ± SD)

Table 5.7: Chlorophyll contents in Brassica nigra among all treatments

<table>
<thead>
<tr>
<th>Treatment Groups/Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ht of plant</td>
<td>123±4.58&lt;sup&gt;a&lt;/sup&gt;</td>
<td>75.6±3.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>70.3±1.52&lt;sup&gt;b&lt;/sup&gt;</td>
<td>99.6±6.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>102.6±5.03&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mean Root Length</td>
<td>26±2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>19.3±1.52&lt;sup&gt;b&lt;/sup&gt;</td>
<td>18.6±1.52&lt;sup&gt;b&lt;/sup&gt;</td>
<td>21.6±1.52&lt;sup&gt;b&lt;/sup&gt;</td>
<td>21±2.64&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mean Shoot Length</td>
<td>97±2.64&lt;sup&gt;a&lt;/sup&gt;</td>
<td>56.3±1.52&lt;sup&gt;b&lt;/sup&gt;</td>
<td>51±2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>78±4.58&lt;sup&gt;d&lt;/sup&gt;</td>
<td>81.6±2.51&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values followed by same letters in a column are not significantly different (p ≤ 0.05) (n=3, mean ± SD)

Table 5.7: Mean height (in cm) of the root, shoot and the total plant in Cyamopsis tetragonolobus under different treatments.

Well water (Control: Group I - WW); Raw effluent (before any treatment: Group II - RE); Sludge part of the chemical treatment (Group III - CTS); Water part of the chemical treatment (Group IV – CTW); Biologically treated effluent in total (Group V - BT)
### Table 5.8: Wet Weight of the *Cyamopsis tetragonolobus* under different treatments.

<table>
<thead>
<tr>
<th>Treatment Groups/Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Wet Wt of Plant</td>
<td>260.3±5.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>130±5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>124.3±4.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>225±5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>227.6±2.51&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values followed by same letters in a column are not significantly different (*p* ≤0.05) *(n=3, mean ± SD)*

### Table 5.9: No. of Leaves and Nodes in *Cyamopsis tetragonolobus* under different treatments.

<table>
<thead>
<tr>
<th>Treatment Groups/Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of leaves</td>
<td>65.6±3.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>40±1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>39.3±3.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>58.6±1.52&lt;sup&gt;c&lt;/sup&gt;</td>
<td>60.3±1.52&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>No. of Nodes</td>
<td>34.3±2.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.6±1.52&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.6±2.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>29±2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>30.3±0.5&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>No. of Pods</td>
<td>49&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20&lt;sup&gt;b&lt;/sup&gt;</td>
<td>17&lt;sup&gt;c&lt;/sup&gt;</td>
<td>38.3&lt;sup&gt;d&lt;/sup&gt;</td>
<td>43.6&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>No. of Seeds</td>
<td>10.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>9.3&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values followed by same letters in a column are not significantly different (*p* ≤0.05) *(n=3, mean ± SD)*

Well water (Control: Group I - WW); Raw effluent (before any treatment: Group II - RE); Sludge part of the chemical treatment (Group III - CTS); Water part of the chemical treatment (Group IV – CTW); Biologically treated effluent in total (Group V - BT)
### Table 5.10: Chlorophyll contents in Cyamopsis tetragonolobus among all the treatment groups

<table>
<thead>
<tr>
<th>Treatment Groups/Parameter</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chl a</td>
<td>0.86±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.7±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.66±0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.81±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.84±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Chl b</td>
<td>0.51±0.018&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.34±0.024&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.26±0.015&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.4±0.066&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.47±0.025&lt;sup&gt;d,a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Chl Total</td>
<td>1.38±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.96±0.015&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.89±0.036&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.2±0.047&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.27±0.03&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*Values followed by same letters in a column are not significantly different (p ≤0.05) (n=3, mean ± SD)*

### Table 5.11: Length of the Pods among all the treatment groups

<table>
<thead>
<tr>
<th>Treatment Groups/Parameter</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length of the pod in cm</td>
<td>16.3±0.258&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.1±0.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.9±0.15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.7±0.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>15.7±0.32&lt;sup&gt;d,a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*Values followed by same letters in a column are not significantly different (p ≤0.05) (n=3, mean ± SD)*

Well water (Control: Group I - WW); Raw effluent (before any treatment: Group II - RE); Sludge part of the chemical treatment (Group III - CTS); Water part of the chemical treatment (Group IV – CTW); Biologically treated effluent in total (Group V - BT)
<table>
<thead>
<tr>
<th>Treatment Groups/Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight of individual pod in gm</td>
<td>5.97±0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.04±0.14&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.73±0.19&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.15±0.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.58±0.24&lt;sup&gt;c,a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*Values followed by same letters in a column are not significantly different (p ≤ 0.05) (n=3, mean ± SD)*

**Table 5.12: Weight of individual pods in Cyamopsis tetragonolobus among all the treatments**

<table>
<thead>
<tr>
<th>Treatment Groups/Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wt of Pod/plant in gm</td>
<td>181.57±3.75&lt;sup&gt;a&lt;/sup&gt;</td>
<td>85.9±1.71&lt;sup&gt;b&lt;/sup&gt;</td>
<td>75.1±4.57&lt;sup&gt;c&lt;/sup&gt;</td>
<td>149.97±3.98&lt;sup&gt;d&lt;/sup&gt;</td>
<td>173.98±8.12&lt;sup&gt;e,a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*Values followed by same letters in a column are not significantly different (p ≤ 0.05) (n=3, mean ± SD)*

**Table 5.13 : Yield per plant (Cyamopsis tetragonolobus) among all the treatments**

Well water (Control: Group I - WW); Raw effluent (before any treatment: Group II - RE); Sludge part of the chemical treatment (Group III - CTS); Water part of the chemical treatment (Group IV - CTW); Biologically treated effluent in total (Group V - BT)
<table>
<thead>
<tr>
<th>Treatment Groups/Parameter</th>
<th>Group I (Total Yield in Kg)</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Yield in Kg</td>
<td>3.15±0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.84±0.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.64±0.07&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.76±0.07&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.92±0.09&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values followed by same letters in a column are not significantly different (p ≤0.05) (n=3, mean ± SD)

Table 5.14: Gross yield in *Cyamopsis tetragonolobus* treatment group

Well water (Control: Group I - WW); Raw effluent (before any treatment: Group II - RE); Sludge part of the chemical treatment (Group III - CTS); Water part of the chemical treatment (Group IV - CTW); Biologically treated effluent in total (Group V - BT).
**Fig. 5.1** Graph showing the percentage of germination in Brassica nigra among the different treatment groups.

**Fig. 5.2.** Mean values of total height; root length and shoot length in Brassica nigra on day 12.

**Fig. 5.3:** Graph showing wet and dry weight of Brassica nigra among the four treatment groups.

**Fig. 5.4:** Number of Leaves per plant among the four treatment groups in Brassica nigra.
**Fig. 5.5:** Chlorophyll contents in Brassica nigra among all the four treatment groups

**Fig 5.6:** Levels of total carbohydrates in the leaves of Brassica nigra among the four treatment groups

**Fig 5.7:** Levels of protein in the leaves of Brassica nigra among the four treatment groups.

**Fig 5.8:** Levels of reducing sugar in the leaves of Brassica nigra among the four treatment groups
Fig. 5.9: Mean height (in cm) of the root, shoot and the total plant in \textit{C. tetragonolobus} among the treatment groups.

Fig. 5.10: Wet weight of the \textit{Cyamopsis tetragonolobus} under different treatment groups.

Fig. 5.11: No. of leaves and nodes in \textit{Cyamopsis tetragonolobus} under different treatment groups.

Fig. 5.12: Chlorophyll contents in \textit{C. tetragonolobus} among the different treatment groups.
Fig. 5.13: Length of the pods among the different treatment groups.

Fig. 5.14: Weight of individual pods in C. tetragonolobus among different treatment groups.

Fig. 5.15: Yield per plant in C. tetragonolobus among different treatment groups.

Fig. 5.16: Gross yield (in Kg) in C. tetragonolobus among different treatment groups.
Fig 5.17: Total carbohydrates in the leaves of *C. tetragonolobus* in five treatment groups.

Fig 5.18: Concentration of proteins in the leaves of *C. tetragonolobus* in five treatment groups.

Fig 5.19: Concentration of reducing sugar in the leaves of *C. tetragonolobus* in five treatment groups.
Discussion

There has been a strong global awakening during the last few decades regarding the proper management of existing natural resources. Among them, irrigation water is one which becoming costlier due to increasing demand of human population. Simultaneously the demand for food is also increasing, which has brought more and more land under cultivation and focused the attention on fertilizer and irrigation water. With these certain limitations, one has to turn to non-conventional recourses to meet the irrigation water demand. Among others, one of the most important irrigation as well as nutrient resources is industrial waste water, which consists of about 95% water and the rest as organic and inorganic nutrients. Since, its disposal is a big problem in urban areas, applying the textile waste water to agricultural field instead of disposing off in lakes and rivers can make crops grow better due to presence of various nutrients like N, P, Ca, Mg etc. (Kannan et al., 2005 and Khan et al., 2003). There can be both beneficial and damaging effects of irrigation with waste water on various crops including vegetables (Ramana et al., 2002;) especially the textile industry effluent water that contains much of undesirable qualities like opacity due to suspended particles, alkaline pH and toxic synthetic chemicals such as the dyes. The need is to assess waste water quality and plant species requirements before using treated waste water for crops production (Jothimani et al., 2002).

It is very important to know whether biodegradation of a dye leads to detoxification of the dye or not. This can be done by performing phytotoxicity and microbial toxicity tests of the original dye and its biodegradation products. In phytotoxicity studies, the seeds of
model plants can be treated with a particular concentration of the original dye and also with its biodegradation products. The effect of the treatment on percent germination and length of plumule and radicle can be evaluated and the results compared with those of the control (without treatment with dye and its biodegradation products). Differences

For sulfonated azo dyes, both aromatic sulfonic and azo groups and their metabolic intermediates (sulfonated and unsulfonated aromatic amines) represent important groups of environmental pollutants having toxic nature (Gottlieb et al., 2003; Junnarkar et al., 2006; Chen 2002). Improper disposal of dyeing effluents containing reactive azo dyes causes serious environmental and health hazards, they are being disposed off in water bodies and this water is being used for an agriculture purpose. Use of untreated and treated dyeing effluents containing water for the agriculture purpose has direct impact on the fertility of soil (Kalyani et al., 2009). Therefore, it is of concern to assess the phytotoxicity of the textile dye effluent before and after degradation by any mode of treatment. Seed germination and plant growth bioassays are the most common techniques used to evaluate the phytotoxicity (Saratale et al., 2010). Though, chemical treatment is being currently carried out in the industries that are under pressure to reduce pollutant-load, the water that is drained into the streams is not significantly improved in its qualities. Biological treatment is not only eco-friendly but also economically friendly. However, there is a need for a careful examination of presence of any undesirable toxic metabolites. Since, water is a commodity of high demand in the country, it is imperative to carryout toxicity tests atleast for the plants as it may be pumped for irrigation purposes. (Jadhav et al., 2010; Kalyani et al., 2008; Saratale et al., 2009). Therefore, any efficient treatment methodology
needs to be evaluated for its phytotoxicity. Likewise, on an expedition to find a feasible biological treatment of textile effluent water, two important edible crop plants (Brassica nigra and Cyamopsis tetragonolobus. L) have been tested for phytotoxicity of the biologically treated effluents in comparison to the chemical treatment.

From the results, it was obvious that the raw effluent and sludge (recovered from the chemical treatment) reduced the seed germination and early growth of the plants. Further, biologically treated textile effluent did not show any inhibitory effect on seed germination. These findings are in accordance with the others in similar studies. Mohammad and Khan (1985) found that industrial effluent reduced the germination percentage of kidney bean (Phaseolus aureus) and ladyfinger (Abelmoschus esculentus). While working with Cicer arietinum, Dayama (1987) reported that even highly diluted industrial effluent (5% of industrial effluent) adversely reduced the seed germination. Sesamum indicum (Neelam and Sahai 1988) Holchus lanatus (Bradshaw and McNeilly 1981) Agrostis stolonifera (Amzallag 1999). The reduction in germination percentage of Turnip in untreated textile effluent might have been due to presence of high concentration of Ni$^{2+}$, Cd$^{2+}$, and Pb$^{2+}$, and other toxic organic compounds that cause a range of cellular toxicities (Kadar and Kastori 2003). Although osmotic potential of the effluent was not recorded, it is also possible that the presence of high amount of salts and organic compounds in untreated textile effluent reduces the availability of water thereby resulting in reduced germination. This aspect is further supported by the fact that presence of high salts in water or soil reduces the germination and early growth of plants by salt-induced osmotic stress that varies from species to species (Ashraf 2004). Few years back, Ramana et al. (2002) found
that the osmotic potential of the distillery effluent is higher at higher concentrations, which
retards germination of different vegetable crops. Slight reduction in growth of the three
vegetable crops due to 100% treated textile effluent might have been due to the still
presence of some heavy metals. This argument has been further supported by findings of
Srivastava and Sahai (1987) who reported that irrigation with distillery effluent (distillery
effluent have a very low amount of toxic material) reduced the growth *Cicer aritinum.*
Overall, this inhibition in growth and germination may have been due to presence of heavy
metals such as Ni\(^{2+}\) and Pb\(^{2+}\) that cause toxicity at cellular as well as at the whole plant level
(Kadar and Kastori 2003). Furthermore, presence of heavy metals in the growth medium
also causes reduction in uptake of other essential nutrients thereby resulting in reduced
growth. For example, while working with tomato seedlings Palacios *et al.* (1998) concluded
that presence of elevated concentration of Ni in rooting medium may cause disturbances
and imbalances of different essential mineral elements.

Photosynthetic pigments such as chlorophyll ‘\(a\), ‘\(b\)’ and total chlorophyll were
decreased in the raw effluent and the chemical-sludge-treated group of plants. A decrease
in chlorophyll content may either be due to inhibition of chlorophyll synthesis or its
destruction or replacement of Mg ions (Barcelo and Gunse *et al.*, 1985; Chandra *et al*.,
2009). Sahai *et al.* (1983) reported similar observation when *Phaseolus radiatious* was
treated with distillery effluent. The more adverse effect of raw textile effluent and
chemically treated effluent on photosynthetic pigments in *Brassica nigra* and *Cyamopsis
tetragonologus* than that of the biologically treated effluent can also be explained that
presence of heavy metals like Ni and Pb damage the photosynthetic apparatus which
possesses both chlorophyll a and b (Seregin and Kozhevnikova 2006). Similar results were found by Krupa et al. (1993) and Sheoran et al. (1990) who observed a reduction in growth and chlorophyll concentration of bean and pigeon pea, respectively.

Consequently plants of the two vegetables irrigated with biologically treated effluent have demonstrated better growth than those of untreated (raw effluent) and chemically treated effluent. The results of the present study can be explained in view of the arguments of different scientists (Palacios et al., 1998; Kadar and Kastori 2003; Seregin and Kozhevnikova 2006) that lead, cadmium and nickel are highly toxic though at relatively low concentration. They interfere in enzyme action by replacing metals ions from metalo-enzymes and inhibit different physiological processes of plants (Agarwal 1999). Textile effluents contain various acids, alkalis, salts, or metal ions as impurities (Kaushik and Malik 2009). Based on the above results, treatment of wastewater can be considered as an effective method, which will help in reusing the effluent from industry for irrigation purpose.