Chapter-4

Determination of growth parameters of E. crassipes, V. spiralis, L. minor grown in industrial effluent
Abstract
The effect of organic and inorganic pollutants of effluent on the growth parameters like leaf number/plant, leaf area/plant, and chlorophyll content of the phytoremediators has been studied. It has been observed that with the increase in phytoremediation period, there is increase in leaf number and leaf area but a negative effect on chlorophyll a, chlorophyll b and total chlorophyll. The maximum change in growth parameters was observed in P20 treatment. Among three phytoremediators E. crassipes exhibited more growth than V. spiralis and L. minor.
4.1 Introduction
Phytoremediation with aquatic plants is an aesthetically pleasant, ecofriendly, cost effective, solar driven, passive technique that is useful for cleaning up environmental pollutants with low to moderate levels of contaminants\(^1\). The aquatic plants covering the wetland areas play an important role in sequestering large quantities of nutrients\(^2,4\) and metals\(^5-8\) from the environment by storing them in their roots and shoots. The plants have ability to absorb these pollutants from wastewater due to their sophisticated metabolism and detoxification mechanism. The wetland plants take up the heavy metals from the environment but tend mainly to accumulate them in the below ground tissues\(^9,10\). However the capacity to accumulate heavy metals in the above ground plant tissues represents the suitability of plants for phytoextraction\(^11\). It has been observed that accumulation of organic and inorganic pollutants result in increase in leaf number and leaf area per plant. The heavy metals accumulation is responsible for decrease in total chlorophyll and negatively affects the Chl a/Chl b ratio\(^12,13\). Extent of increase and decrease in growth parameters was in proportion to the concentration of the effluent and duration of exposure.

4.2 Experimental
4.2.1 Equipments
1. Microprocessor UV visible Spectrophotometer (Model No. 1371EI)
2. Cetrifugate
3. Mortar and pestle
4.2.2 Reagent
Acetone (80%) 
Analytical grade acetone was diluted to 80 percent acetone

4.2.3 Plant analysis
4.2.3a Leaf Number
The leaf number per plant was counted on each observation date.
4.2.3b Leaf area/plant
Average leaf area/leaf was calculated by drawing the area of 10 leaves representing different size on graph paper. An average leaf area per plant was calculated by multiplying the number of leaves/plant with average leaf area/Leaf.

4.2.3c Determination of Chlorophyll

Procedure
500 mg of finely cut and well-mixed sample of plant was weighed into a clean mortar and pestle. Ground the tissue to a fine pulp with the addition of 20 ml of 80% acetone. Centrifugated for 5 minutes and supernatant was transferred to a conical flask. This procedure was repeated until the residue was colourless. The mortar and pestle was washed thoroughly with 80 % acetone and clear washings were collected in the volumetric flask. The volume was made to 50 ml with 80 % acetone. The absorbance of the solution was read at 645 and 663 nm against the 80% acetone (solvent)\textsuperscript{14,15}.

Calculation
The amount of chlorophyll present in the extract (mg chlorophyll / gm tissue) was calculated using the following equations

\[
\text{mg Chlorophyll a/g tissue} = 12.7 \times (A_{663}) - 2.69 \times (A_{645}) \times \frac{V}{W} \\
\text{mg Chlorophyll b/g tissue} = 22.9 \times (A_{645}) - 4.68 \times (A_{663}) \times \frac{V}{W} \\
\text{mg total Chlorophyll} = \text{Chlorophyll a} + \text{Chlorophyll b}
\]

Where A = Absorbance at specific wave lengths
V = Final volume of chlorophyll extract in 80% acetone
W = Fresh weight of the tissue extracted.

4.3 Results
4.3.1 Initial growth parameters of E. crassipes, V. spiralis and L. minor
Initial growth parameters like leaf number per plant, leaf area per plant, chlorophyll a, chlorophyll b and total chlorophyll of \textit{E. crassipes}, \textit{V. spiralis} and \textit{L. minor} were recorded and represented in table 4.1. The data was shown in table 4.1. It was found that leaf number per plant was more in \textit{Vallisneria} (12.75). \textit{Eichhornia} has more leaf
area per plant (135.27 cm²). Total chlorophyll content is more in *Vallisneria* followed by *Lemna* and *Eichornia*. Conc. of chlorophyll a is more in *E.crassipes* and *V. spiralis* as compared to chlorophyll b but in *L. minor* the conc. of chlorophyll b is more in comparison to chlorophyll a.

**Table 4.1: Initial growth parameters of *E. crassipes*, *V. spiralis* and *L. minor***

<table>
<thead>
<tr>
<th>Growth parameters</th>
<th><em>E.crassipes</em></th>
<th><em>V.spiralis</em></th>
<th><em>L.minor</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf number/plant</td>
<td>6.50</td>
<td>12.75</td>
<td>2.5</td>
</tr>
<tr>
<td>Leaf area/plant</td>
<td>135.27 cm²</td>
<td>123.15 cm²</td>
<td>50.4 mm²</td>
</tr>
<tr>
<td>Chlorophyll a</td>
<td>0.75</td>
<td>0.89</td>
<td>0.43</td>
</tr>
<tr>
<td>Chlorophyll b</td>
<td>0.46</td>
<td>0.68</td>
<td>0.66</td>
</tr>
<tr>
<td>Total chlorophyll</td>
<td>1.21</td>
<td>1.57</td>
<td>1.09</td>
</tr>
</tbody>
</table>

4.3.2 Effect of different concentrations of pulp and paper mill effluent on the growth parameters of *Eichhornia crassipes*,

The growth parameters of *Eichornia crassipes*, like leaf number, leaf area, chlorophyll a, chlorophyll b and total chlorophyll were analyzed in 0% conc., 20% conc. and 40% conc. of pulp and paper mill effluent for a period of 50 days. The data is represented in table 4.2 and fig 4.1 a to 4.1e. The data showed that maximum increase in leaf number /plant and leaf area /plant and and maximum decrease in chlorophyll content was observed in P20 treatment among 20% and 40% of nonphytoremediated and 20% and 40% phytoremediated treatments.

Per plant number of leaves and leaf area increased up to 76.9% and 64.3% in P20 treatment and 61.5 and 46.2% in P40 treatment under a phytoremediation period of 50 days. Chlorophyll a, chlorophyll b and total chlorophyll were decreased by 34.7%, 34.8% and 34.7% in P20 treatment and 46.7, 45.7 and 46.3% in P40 treatment respectively for pulp and paper mill effluent after 50 days of phytoremediation.
Table 4.2: Effect of pulp and paper mill effluent on the growth parameters of *E.crassipes*

<table>
<thead>
<tr>
<th>Days</th>
<th>10 Days</th>
<th>20 Days</th>
<th>30 Days</th>
<th>40 Days</th>
<th>50 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameters</td>
<td>P0</td>
<td>P20</td>
<td>P40</td>
<td>P0</td>
<td>P20</td>
</tr>
<tr>
<td>Leaf No./Plant</td>
<td>6.50</td>
<td>8.75</td>
<td>7.50</td>
<td>6.25</td>
<td>9.50</td>
</tr>
<tr>
<td>Leaf area(cm²/plant)</td>
<td>132.67</td>
<td>168.27</td>
<td>149.12</td>
<td>120.53</td>
<td>196.51</td>
</tr>
<tr>
<td>Chlorophyll a</td>
<td>0.57</td>
<td>0.67</td>
<td>0.59</td>
<td>0.49</td>
<td>0.62</td>
</tr>
<tr>
<td>Chlorophyll b</td>
<td>0.39</td>
<td>0.43</td>
<td>0.40</td>
<td>0.31</td>
<td>0.40</td>
</tr>
<tr>
<td>Total chlorophyll</td>
<td>0.96</td>
<td>1.10</td>
<td>0.99</td>
<td>0.80</td>
<td>1.02</td>
</tr>
</tbody>
</table>
Fig. 4.1a: Change in leaf no./plant of *E.crassipes* by different concentrations of effluent

Fig. 4.1b: Change in leaf area/plant of *E.crassipes* by different concentrations of effluent
Fig. 4.1c: Change in chlorophyll a of *E. crassipes* by different concentrations of effluent.

Fig. 4.1d: Change in chlorophyll b of *E. crassipes* by different concentrations of effluent.
4.3.3 Effect of pulp and paper mill effluent on the growth parameters of *Vallisneria spiralis*

The effect of varied concentrations of pulp and paper mill effluent on the growth characteristics of *Vallisneria spiralis* are represented in table 4.3 and fig.4.2a to 4.2e respectively. It has been observed that there is maximum increase in all the selected growth parameters in P20 treatment as compared to P40 treatment. Increased duration of phytoremediation caused corresponding increase in growth parameters of the *Vallisneria* as compared to the grown in P0 concentration.

In P20 treatment of pulp and paper mill effluent per plant number of leaves and leaf area were increased by 43.1 and 37.3% and in P40 treatment the increase was 31.4 and 17.1 % respectively after a phytoremediation period of 50 days.

Chlorophyll a, chlorophyll b and total chlorophyll were decreased up to 25.8, 36.7 and 30.5 percent in P20 treatment and 33.7, 48.5 and 40.1 percent in P40 treatment respectively.
Table 4.3: Effect of pulp and paper mill effluent on the growth parameters of *V. spiralis*

<table>
<thead>
<tr>
<th>Days</th>
<th>10 Days</th>
<th></th>
<th>20 Days</th>
<th></th>
<th>30 Days</th>
<th></th>
<th>40 Days</th>
<th></th>
<th>50 Days</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P0</td>
<td>P20</td>
<td>P40</td>
<td>P0</td>
<td>P20</td>
<td>P40</td>
<td>P0</td>
<td>P20</td>
<td>P40</td>
<td>P0</td>
</tr>
<tr>
<td>Leaf No./Plant</td>
<td>12.75</td>
<td>15.50</td>
<td>14.25</td>
<td>11.25</td>
<td>16.75</td>
<td>15.25</td>
<td>10.25</td>
<td>17.50</td>
<td>16.25</td>
<td>10.0</td>
</tr>
<tr>
<td>Leaf area(cm²/plant)</td>
<td>111.23</td>
<td>135.38</td>
<td>128.51</td>
<td>100.15</td>
<td>152.09</td>
<td>135.71</td>
<td>96.45</td>
<td>161.64</td>
<td>139.51</td>
<td>87.33</td>
</tr>
<tr>
<td>Chlorophyll a</td>
<td>0.75</td>
<td>0.95</td>
<td>0.83</td>
<td>0.68</td>
<td>0.87</td>
<td>0.78</td>
<td>0.62</td>
<td>0.81</td>
<td>0.73</td>
<td>0.57</td>
</tr>
<tr>
<td>Chlorophyll b</td>
<td>0.50</td>
<td>0.64</td>
<td>0.60</td>
<td>0.47</td>
<td>0.59</td>
<td>0.52</td>
<td>0.40</td>
<td>0.56</td>
<td>0.49</td>
<td>0.35</td>
</tr>
<tr>
<td>Total chlorophyll</td>
<td>1.28</td>
<td>1.59</td>
<td>1.43</td>
<td>1.15</td>
<td>1.45</td>
<td>1.30</td>
<td>1.02</td>
<td>1.37</td>
<td>1.22</td>
<td>0.92</td>
</tr>
</tbody>
</table>

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Fig. 4.2a Change in leaf no./plant of V.spiralis by different concentrations of effluent

Fig. 4.2b: Change in leaf area/plant of V.spiralis by different concentrations of effluent
Fig. 4.2c: Change in chlorophyll a of *V. spiralis* by different concentrations of effluent

![Graph showing change in chlorophyll a](image)

Fig. 4.2d: Change in chlorophyll b of *V. spiralis* by different concentrations of effluent

![Graph showing change in chlorophyll b](image)
4.3.4 Effect of pulp and paper mill effluent on the growth parameters of *L. minor*

The growth parameters of *Lemna minor* like leaf number, leaf area, total chlorophyll and total biomass were analyzed in 0% conc., 20% conc. and 40% conc. of pulp and paper mill effluent for a period of 50 days. The data is represented in table 4.4 and fig 4.3a to 4.3e. The data showed that maximum increase in growth parameters was observed in P20 treatment among P0, P20 and P40 treatments of pulp and paper mill effluent. Increased duration of phytoremediation caused corresponding increase in the all selected growth parameters.

Per plant number of leaves and leaf area increased up to 50.8 and 15.5 percent in P20 treatment and 44 and 11.1 percent in P40 treatment under a phytoremediation period of 50 days. Chlorophyll a, chlorophyll b and total chlorophyll were decreased by 24.9, 8.5 and 15.3 percent in P20 treatment and 37.2, 16.6 and 23.8 percent in P40 treatment respectively for pulp and paper mill effluent.
Table 4.4: Effect of pulp and paper mill effluent on the growth parameters of *L. minor*

<table>
<thead>
<tr>
<th>Parameters</th>
<th>10 Days</th>
<th>20 Days</th>
<th>30 Days</th>
<th>40 Days</th>
<th>50 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P0</td>
<td>P20</td>
<td>P40</td>
<td>P0</td>
<td>P20</td>
</tr>
<tr>
<td>Leaf No./Plant</td>
<td>2.5</td>
<td>3.0</td>
<td>2.77</td>
<td>3.25</td>
<td>3.50</td>
</tr>
<tr>
<td>Leaf area(cm²/plant)</td>
<td>50</td>
<td>54.2</td>
<td>53.9</td>
<td>52.2</td>
<td>57.3</td>
</tr>
<tr>
<td>Chlorophyll a</td>
<td>0.392</td>
<td>0.478</td>
<td>0.413</td>
<td>0.323</td>
<td>0.429</td>
</tr>
<tr>
<td>Chlorophyll b</td>
<td>0.614</td>
<td>0.746</td>
<td>0.696</td>
<td>0.588</td>
<td>0.702</td>
</tr>
<tr>
<td>Total chlorophyll</td>
<td>1.006</td>
<td>1.224</td>
<td>1.109</td>
<td>0.911</td>
<td>1.131</td>
</tr>
</tbody>
</table>
Fig. 4.3a: Change in leaf no./plant of *L. minor* by different concentrations of effluent

Fig. 4.3b: Change in leaf area/plant of *L. minor* by different concentrations of effluent
Fig. 4.3c: Change in chlorophyll a of *L. minor* by different concentrations of effluent

Fig. 4.3d: Change in chlorophyll b of *L. minor* by different concentrations of effluent
4.4 Discussion

It has been observed that there is gradual increase in plant growth parameters like Leaf number per plant, leaf area per plant and decrease in chlorophyll a, chlorophyll b and total chlorophyll content with the increase in phytoremediation period. The maximum increase in growth parameters was observed in phytoremediators grown in 20 % concentration followed by 40 % concentration.

The experiment was done for assessing the effect of pulp and paper mill effluent including domestic waste on the growth characteristics of *E.crassipes, V.spiralis* and *L. minor*. The maximum growth was recorded in case of *Eichhornia* in comparison to *Vallisneria* and *Lemna* grown in wastewater for 50 days.

The toxicity of pulp and paper mill effluent on chlorophyll content was studied at different intervals by Srivastava and Pandey\textsuperscript{16}. They observed that the total chlorophyll content decreased with the increase in dilutions for 7, 14 and 21 days of exposure to
varied concentrations of the effluent in case of *Eichhornia crassipes*, *Pistia stratiotes* and *Hydrilla verticillata*. The extent of reduction is proportional to the duration of exposure and concentration of the effluent. The maximum inhibition in chlorophyll content was 57.72 and 28.5 percent in 100 % concentration at 168 hour of exposure in *Eichhornia crassipes* and *Spirodela polyrrhiza* respectively. The results of the present study regarding the reduction in chlorophyll content were comparable to the results of other workers\textsuperscript{17-18}. 
References


