CHAPTER IV:
RESULTS
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A. ACUTE TOXIC EFFECTS ON GROWTH AND SURVIVAL

I. Copper

1. Effects on shoot height

Changes in the shoot height (SH) in control and Cu exposed plants on 3, 6, 9, 12 and 15 day of exposure are shown in Table 1.

On day 3, increase in SH was found to be higher in control when compared to those in the Cu exposed plants. The control plants showed an increase in SH by 2.4 cm during this period. On the contrary, SH increment ranged from 0.4 - 2.3 cm in plants exposed to 0.25 - 14.25 mg L\(^{-1}\) Cu. On the other hand, plants exposed to 25.45 mg L\(^{-1}\) Cu did not show any growth during this period. One way ANOVA revealed that the differences in SH was significantly different (F=9.648, p<0.001). Multiple comparisons using Tukey test showed that SH increase in plants exposed to 8.14 and 14.25 mg L\(^{-1}\) was significantly lower than that of the control and that of 0.25 mg L\(^{-1}\) Cu exposed plants as well (p<0.05). The test also revealed that the reduction at 25.45 mg L\(^{-1}\) was statistically significant from those of the control (p<0.01) and those of 0.25 - 2.54 mg L\(^{-1}\) Cu exposed plants (p<0.05).

On day 6, increase in SH was observed in control and in plants exposed to Cu concentrations of 0.25 - 14.25 mg L\(^{-1}\), with highest increment of 5.3 cm in control and lowest of 0.5 cm in 14.25 mg L\(^{-1}\). However, SH in 25.45 mg L\(^{-1}\) Cu exposed plants showed a reduction of 0.03 cm due to the appearance of stem tissue necrosis on day 6. It may be noted here that in this 15 day experiment, necrosis of stem tissue could be visually detected from day 3 onwards in plants exposed to Cu concentrations of 2.54 - 25.45 mg L\(^{-1}\). However, at 2.54 - 4.58 mg L\(^{-1}\), necrotic damage was minimal so that positive increments of growth could be observed. On the
other hand, necrosis resulted in partial collapse of stem tissue to reduce SH in plants exposed to 25.45 mg L\(^{-1}\). The differences in SH on day 6 were significant as revealed by one way ANOVA (F=12.447, p<0.001). The increase in SH in control was significantly higher than those in 4.58, 8.14, 14.25 and 25.45 mg L\(^{-1}\) (p<0.05) as shown by multiple comparisons with Tukey test. The test also revealed that the reduction of SH in 25.45 mg L\(^{-1}\) was significant from those of control (p<0.001) and 0.25 - 2.54 mg L\(^{-1}\) Cu treatment (p<0.05).

On day 9, the highest SH increment of 10.8 cm was observed at 0.25 mg L\(^{-1}\) of Cu followed by control with 10.5 cm and the lowest increment of 0.8 cm occurred in 14.25 mg L\(^{-1}\). Between day 6 and 9, most of the plants exposed to 25.45 mg L\(^{-1}\) Cu which underwent necrosis of stem tissue died. One way ANOVA revealed that the differences in SH were significant (F=13.492, p<0.001). Multiple comparisons with Tukey test revealed that the increases in SH in control and 0.25 - 2.54 mg L\(^{-1}\) Cu treatment were significantly higher than those in 8.14 and 14.25 mg L\(^{-1}\) (p<0.001).

On day 12, highest increment in SH was shown in 0.25 mg L\(^{-1}\) Cu (18.1 cm) followed by 2.54 mg L\(^{-1}\) (17.9 cm) and control (17.1 cm). The lowest increment was in 14.25 mg L\(^{-1}\) (0.9 cm). One way ANOVA revealed that the differences in SH on day 12 were statistically significant (F=16.518, p<0.001). Multiple comparisons with Tukey test showed that the increases in SH in control and 0.25 - 2.54 mg L\(^{-1}\) Cu were significantly higher than those in 4.58 - 25.45 mg L\(^{-1}\) Cu (p<0.05).

On day 15 of exposure, increment in SH in control, 0.25 and 2.54 mg L\(^{-1}\) Cu were 19.7, 19.7 and 17.9 cm, respectively (Table 1), while those exposed to 4.58 showed a lower increment of 9.6 cm. Plants exposed to 8.14 and 14.25 mg L\(^{-1}\) had reductions in SH of 0.5 cm and 0.01 cm, respectively, while the few surviving plants in 25.45 mg L\(^{-1}\) Cu did not record any growth. One
way ANOVA revealed that the differences in SH were significant (F=15.538, p<0.001). Multiple comparisons with Tukey test showed that change in SH at 8.14 mg L\(^{-1}\) was significantly lower than that of control and 0.25 - 4.58 mg L\(^{-1}\) Cu (p<0.001). The test further revealed that the changes of SH in 14.51 and 25.45 mg L\(^{-1}\) Cu were significantly lower than those of control and 0.25 - 4.58 mg L\(^{-1}\) Cu (p<0.001).

Overall changes in SH at the end of 15 day were analyzed using one way ANOVA and it revealed that the differences in SH were significant (F=29.875, p<0.001). Multiple comparisons with Tukey test showed that the increase in SH at 8.14 mg L\(^{-1}\) was significantly lower than those of control (p<0.001) and 0.25 - 4.58 mg L\(^{-1}\) (p<0.05). The test also revealed that the increase in SH at 4.58 mg L\(^{-1}\) was significantly lower than those of control and 0.25 - 2.54 mg L\(^{-1}\) of Cu (p<0.001) but higher than that of 8.14 mg L\(^{-1}\) (p<0.05). On the other hand the reductions in SH at 14.51 and 25.45 mg L\(^{-1}\) were significantly different from those of control (p<0.001) and Cu concentrations of 0.25, 2.54 and 4.58 mg L\(^{-1}\) (p<0.05). However, the differences among the control, 0.25 mg L\(^{-1}\) and 2.54 mg L\(^{-1}\) Cu were not significant (p>0.05).

2. Effects on number of new nodes

Changes in the number of new nodes (NN) in control and Cu exposed plants on 3, 6, 9, 12 and 15 day of exposure are shown in Table 2. NN continued to increase in the control plants till the end of the experiment. NN in plants exposed to 0.25 - 4.58 mg L\(^{-1}\) also increased till the end of the experiment but at rates lower than that of the control.

On day 3 of exposure, the highest mean NN of 1.1 was observed in control, which was followed by that in 0.25 mg L\(^{-1}\) (0.7) and 2.54 mg L\(^{-1}\) Cu (0.6). The lowest increment of 0.1 occurred in 14.25 mg L\(^{-1}\) Cu exposed plants. But the plants exposed to 25.45 mg L\(^{-1}\) did not produce any NN and most of them died after day 6. One way ANOVA revealed that the
differences in NN on day 3 were significantly different (F=4.588, p=0.001) among the control and Cu-exposed plants. Multiple comparisons using Tukey test showed that the increases in NN in 8.14 and 14.25 mg L\(^{-1}\) were significantly lower than those in control (p<0.05).

On day 6, control plants had the highest NN of 2.4 and lowest of 0.1 at 14.25 mg L\(^{-1}\). On the contrary, a few of the plants exposed to 2.54 mg L\(^{-1}\) showed necrotic symptoms in stem tissues resulting in NN (0.8) which was lower than those of 4.58 mg L\(^{-1}\) (1.1). The differences in NN were significant as revealed by one way ANOVA (F=7.155, p<0.001). Multiple comparisons with Tukey test showed that the increases in NN in control and 0.25 mg L\(^{-1}\) Cu were significantly higher than that in 8.14 - 25.45 mg L\(^{-1}\) Cu (p<0.05). And again the increase of NN in control was higher than that of 2.54 mg L\(^{-1}\) (p=0.026).

On day 9, the highest NN of 3.4 was observed in plants exposed to 0.25 mg L\(^{-1}\), followed by that of 3.1 in control, and 2.6 in 2.54 mg L\(^{-1}\) Cu (Table 2). One way ANOVA revealed that the differences in NN were statistically significant (F=11.370, p<0.001). Multiple comparisons with Tukey test revealed that the differences in NN among the control and 0.25 - 4.58 mg L\(^{-1}\) Cu exposed plants were not statistically significant. Again, NN in control and 0.25 - 2.54 mg L\(^{-1}\) of Cu treatment were significantly higher than those in 8.14 - 25.45 mg L\(^{-1}\) (p<0.05).

Similar trends in the appearance of NN were observed in control and 0.25 mg L\(^{-1}\) Cu exposed plants on day 12 and 15 with control having the highest NN of 4.3 and 4.9, respectively, followed by that of 4.3 and 4.6 in 0.25 mg L\(^{-1}\) Cu exposed plants. However, plants exposed to 8.14 mg L\(^{-1}\) showed reduction of NN by 0.11 and those exposed to 14.25 mg L\(^{-1}\) showed changes in NN from 0.7 to 0.4 due to stem tissue necrosis. The differences in NN were significant as revealed by one way ANOVA on day 12 (F=14.257, p<0.001) and on day 15 (F=19.151, p<0.001). Multiple comparisons with Tukey test revealed that the changes in NN in
8.14 and 14.25 mg L\(^{-1}\) Cu-exposed plants on day 12 were significantly lower than those in the control and 0.25 - 4.58 mg L\(^{-1}\) Cu. The test further revealed that the reductions in 8.14 and 14.25 mg L\(^{-1}\) Cu on day 15 were significantly different from that in the control and in Cu concentrations of 0.25 - 4.58 mg L\(^{-1}\) (p<0.001).

Overall changes in the appearance of NN at the end of 15 day were found to be significant as revealed by one way ANOVA (F=34.986, p<0.001). Multiple comparisons with Tukey test showed that the NN reductions in 8.14 - 25.45 mg L\(^{-1}\) were significantly lower than those in the control and Cu concentrations of 0.25 - 4.58 mg L\(^{-1}\) (p<0.001). The test also revealed that the increase in NN in 4.58 mg L\(^{-1}\) was significantly lower than those of the control (p=0.011). However, the differences among the control, 0.25 mg L\(^{-1}\) and 2.54 mg L\(^{-1}\) Cu were not significant (p>0.05).
Table 1: Changes in shoot height (SH) in control and Cu exposed *I. aquatica* during a 15 day experiment.

<table>
<thead>
<tr>
<th>Cu Conc. (mg L⁻¹)</th>
<th>Change in SH (cm)</th>
<th>Day3</th>
<th>Day6</th>
<th>Day9</th>
<th>Day12</th>
<th>Day15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>2.4±0.3</td>
<td>5.3±0.7</td>
<td>10.5±1.9</td>
<td>17.1±3.1</td>
<td>19.7±3</td>
</tr>
<tr>
<td>0.25</td>
<td></td>
<td>2.3±0.6</td>
<td>5.1±1</td>
<td>10.8±2.3</td>
<td>18.1±3.6</td>
<td>19.7±3.7</td>
</tr>
<tr>
<td>2.54</td>
<td></td>
<td>1.7±0.4</td>
<td>2.8±1.2</td>
<td>10±1.5</td>
<td>17.9±2.5</td>
<td>17.9±3.5</td>
</tr>
<tr>
<td>4.58</td>
<td></td>
<td>1.3±0.2</td>
<td>2.5±0.5</td>
<td>5.7±2</td>
<td>8±3.1</td>
<td>9.6±3.3</td>
</tr>
<tr>
<td>8.14</td>
<td></td>
<td>0.8±0.3</td>
<td>0.9±0.3</td>
<td>1.1±0.4</td>
<td>1.2±0.4</td>
<td>-0.5±0.4</td>
</tr>
<tr>
<td>14.25</td>
<td></td>
<td>0.4±0.2</td>
<td>0.5±0.2</td>
<td>0.8±0.3</td>
<td>0.9±0.6</td>
<td>0.01±1.2</td>
</tr>
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<td>25.45</td>
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<td>-0.03±0.03</td>
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</tr>
</tbody>
</table>

* Significant difference from corresponding value in control at p<0.05. Values are mean ± S.E.; n=9.

Table 2: Changes in number of new nodes (NN) in control and Cu exposed *I. aquatica* during a 15 day experiment.

<table>
<thead>
<tr>
<th>Cu Conc. (mg L⁻¹)</th>
<th>Change in NN</th>
<th>Day3</th>
<th>Day6</th>
<th>Day9</th>
<th>Day12</th>
<th>Day15</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td></td>
<td>1.1±0.3</td>
<td>2.4±0.4</td>
<td>3.1±0.5</td>
<td>4.3±0.6</td>
<td>4.9±0.5</td>
</tr>
<tr>
<td>0.25</td>
<td></td>
<td>0.7±0.2</td>
<td>2±0.5</td>
<td>3.4±0.6</td>
<td>4.3±0.8</td>
<td>4.6±0.9</td>
</tr>
<tr>
<td>2.54</td>
<td></td>
<td>0.6±0.2</td>
<td>0.8±0.8</td>
<td>2.6±0.7</td>
<td>3.7±0.8</td>
<td>4±0.7</td>
</tr>
<tr>
<td>4.58</td>
<td></td>
<td>0.4±0.2</td>
<td>1.1±0.4</td>
<td>2.2±0.5</td>
<td>3.1±0.5</td>
<td>3.6±0.6</td>
</tr>
<tr>
<td>8.14</td>
<td></td>
<td>0.2±0.2</td>
<td>0.2±0.2</td>
<td>0.2±0.2</td>
<td>0.4±0.2</td>
<td>-0.11±0.2</td>
</tr>
<tr>
<td>14.25</td>
<td></td>
<td>0.1±0.1</td>
<td>0.1±0.1</td>
<td>0.6±0.3</td>
<td>0.7±0.4</td>
<td>0.4±0.5</td>
</tr>
<tr>
<td>25.45</td>
<td></td>
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<td>0±0</td>
<td>0±0</td>
</tr>
</tbody>
</table>

* Significant difference from corresponding value in control at p<0.05. Values are mean ± S.E.; n=9.
3. Effects on number of new leaf

The number of new leaf produced in control and plants exposed to different concentrations of Cu on 3, 6, 9, 12 and 15 day of exposure are shown in Table 3. New leaves (NL) kept on appearing till the end of the experiment in control and plants exposed to the lower concentrations of Cu, i.e., 0.25 - 4.58 mg L\(^{-1}\). However, no NL appeared in plants exposed to the higher concentrations of Cu, i.e., 8.14 - 25.45 mg L\(^{-1}\) from day 3 onwards.

Appearance of NL was found to be the highest in control with 0.4 and 1.3 and the lowest in 4.58 mg L\(^{-1}\) with NL of 0.2 and 0.6 on day 3 and 6 of the exposure. One way ANOVA revealed that reduction of NL was significant on day 3 (F=2.905, p=0.015) and day 6 (F=12.265, p<0.001). No appearance of NL could be observed in plants exposed to 8.14 - 25.45 mg L\(^{-1}\) Cu from day 3 onwards. However, there was no significant difference in NL among plants in the control and those exposed to 0.25 - 4.58 mg L\(^{-1}\) Cu on day 3.

On day 6, the highest NL (1.3) was in the control, although the differences among the control and plants exposed to 0.25 - 2.54 mg L\(^{-1}\) were not statistically significant as revealed by multiple comparisons with Tukey test. The test also showed that NL in 4.58 mg L\(^{-1}\) was significantly lower than that of the control (p=0.024) (Table 3).

On day 9, the highest NL of 2.3 was observed in 0.25 mg L\(^{-1}\) followed by NL of 1.9 in control and 2.54 mg L\(^{-1}\) Cu. One way ANOVA revealed that the differences among the control and plants exposed to 0.25 - 2.54 mg L\(^{-1}\) were not statistically significant. However, NL in control and 0.25 - 2.54 mg L\(^{-1}\) were significantly higher than those in 8.14 - 25.45 mg L\(^{-1}\), while those in 0.25 mg L\(^{-1}\) was also significantly higher than that in 4.58 mg L\(^{-1}\) treatment (p=0.008).

The differences in the number of NL were statistically significant on day 12 (F=27.693, p<0.001) as shown by one way ANOVA. The highest number of NL on day 12 was found in
control, followed by that in 0.25 and 2.54 mg L\textsuperscript{-1}, although the differences among these groups were not statistically significant (Table 3). NL in control and 0.25 mg L\textsuperscript{-1} were significantly higher than that in 4.58 mg L\textsuperscript{-1}.

On day 15, the differences among the number of NL were statistically significant as shown by one-way ANOVA (F=27.435, p<0.001). Multiple comparisons using Tukey test revealed that the number of NL in control and 0.25 mg L\textsuperscript{-1} Cu were significantly higher than that in 4.58 mg L\textsuperscript{-1}. The differences among NL in control, 0.25 and 2.54 mg L\textsuperscript{-1} were not significant.

Overall appearance of NL at the end of 15 day exposure was statistically significant as revealed by one way ANOVA (F=41.589, p<0.001). Multiple comparisons with Tukey test showed that the appearance of NL in control and in 0.25 mg L\textsuperscript{-1} Cu was significantly higher (p=0.001) than that in 4.58 - 25.45 mg L\textsuperscript{-1} Cu. Further, the differences among NL among control, 0.25 and 2.54 mg L\textsuperscript{-1} were not significant.

4. Effects on plant mortality

The number of dead plants (DP) in Cu exposed \textit{I. aquatica} on 3, 6, 9, 12 and 15 day of exposure are shown in Table 4. On day 3 and 6 there was no DP in control as well as Cu exposed plants.

On day 9, DP was found to be 0.7 and 0.2 in plants exposed to 25.45 and 14.25 mg L\textsuperscript{-1} Cu, respectively. One way ANOVA showed that the occurrence of DP on day 9 was significant (F=11.250, p<0.001). Multiple comparisons with Tukey test revealed that the occurrence of DP in 25.45 mg L\textsuperscript{-1} was significantly higher than that in control and in 0.25 - 8.14 mg L\textsuperscript{-1} (p<0.001) as well as 14.25 mg L\textsuperscript{-1} Cu exposed plants (p=0.002). The test further revealed that DP in 14.25 mg L\textsuperscript{-1} was not significantly different from that in control and in Cu concentrations of 0.25 - 8.14 mg L\textsuperscript{-1} (p=0.370).
On day 12, plants exposed to 8.14 mg L\(^{-1}\) had DP of 0.3, and those exposed to 14.25 and 25.45 mg L\(^{-1}\) had DP of 0.4 and 0.8, respectively. One way ANOVA revealed that the differences in the occurrence of DP was significant (F=10.321, p<0.001) on day 12. Multiple comparisons using Tukey test showed that the occurrence of DP in 14.25 and 25.45 mg L\(^{-1}\) Cu-exposed plants was significantly different from those in control and in 0.25 - 4.58 mg L\(^{-1}\) Cu-exposed plants (p<0.05). However, the occurrence of DP in 8.14 mg L\(^{-1}\) was not significantly different from that in control (p=0.191) and in Cu concentrations of 0.25 - 14.25 mg L\(^{-1}\) (p>0.05) but was significantly lower than that in plants exposed to 25.45 mg L\(^{-1}\) (p=0.027).

On day 15, occurrence of DP was highest in 25.45 mg L\(^{-1}\) (0.8), followed by 14.25 mg L\(^{-1}\) (0.6), and then by 8.14 mg L\(^{-1}\) (0.3). The differences in the occurrence of DP were significant as revealed by one way ANOVA (F=11.410, p<0.001). Multiple comparisons using Tukey test revealed that the occurrence of DP in 14.25 and 25.45 mg L\(^{-1}\) was significantly different from that in control and in Cu concentrations of 0.25 - 4.58 mg L\(^{-1}\) (p<0.05). The test further revealed that the occurrence of DP in 8.14 mg L\(^{-1}\) was significantly lower than that in 25.45 mg L\(^{-1}\) (p=0.027) but not than that in control (p=0.191) and Cu concentrations of 0.25 - 4.58 and 14.25 mg L\(^{-1}\) (p>0.05).

Statistical analysis using one way ANOVA revealed that the differences in overall occurrence of DP at the end of 15 day were significant (F=19.867, p<0.001). Multiple comparisons using Tukey test revealed that the occurrence of DP in 25.45 mg L\(^{-1}\) was significantly higher than those in control (p<0.001) and in the other Cu concentrations of 0.25 - 14.25 mg L\(^{-1}\) (p<0.05). Further, DP in plants exposed to 14.25 mg L\(^{-1}\) was significantly higher than that in control and in Cu concentrations of 0.25 - 4.58 mg L\(^{-1}\) (p<0.001) as revealed by Tukey test.
Table 3: Number of new leaf (NL) in control and Cu exposed *I. aquatica* during a 15 day experiment.

<table>
<thead>
<tr>
<th>Cu Conc. (mg L$^{-1}$)</th>
<th>Appearance of NL</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day3</td>
<td>Day6</td>
<td>Day9</td>
<td>Day12</td>
<td>Day15</td>
</tr>
<tr>
<td>Control</td>
<td>0.4±0.2</td>
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<td>1.9±0.3</td>
<td>3.6±0.4</td>
<td>3.6±0.4</td>
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<td>0.25</td>
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<td>2.54</td>
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</tr>
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<td>4.58</td>
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<td>0.6±0.2$^*$</td>
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</tr>
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</table>

* Significant difference from corresponding value in control at p<0.05. Values are mean ± S.E.; n=9.

Table 4: Number of dead plants (DP) in control and Cu exposed *I. aquatica* during a 15 day experiment.

<table>
<thead>
<tr>
<th>Cu Conc. (mg L$^{-1}$)</th>
<th>Change in DP</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day3</td>
<td>Day6</td>
<td>Day9</td>
<td>Day12</td>
<td>Day15</td>
</tr>
<tr>
<td>Control</td>
<td>0±0</td>
<td>0±0</td>
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<tr>
<td>0.25</td>
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</table>

* Significant difference from corresponding value in control at p<0.05. Values are mean ± S.E.; n=9.
II. Nickel

1. Effects on shoot height

The changes in SH in control and plants exposed to different concentrations of Ni on 3, 6, 9, 12 and 15 day of exposure are shown in Table 5. Increase in SH was observed in control and in plants exposed to Ni concentrations of 0.22 - 7.14 mg L\(^{-1}\) till the end of the experiment. But those exposed to higher Ni concentrations of 12.51 and 22.33 mg L\(^{-1}\) showed reduction in SH on day 15.

On day 3, 6 and 9, SH increased in all the Ni treatments and control plants. Increase in SH was found to be highest (4.5 cm) in 7.14 mg L\(^{-1}\), followed by 3.8 cm in 0.22 mg L\(^{-1}\), and then by 3.7 cm in 4.02 mg L\(^{-1}\) on day 3. One way ANOVA revealed that the differences in the increase in SH were significant (F=4.366, p=0.002). Multiple comparisons with Tukey test showed that the increase in SH at 7.14 mg L\(^{-1}\) was significantly higher than those of 12.51 and 22.33 mg L\(^{-1}\) (p<0.05) but not from control (p=0.942). The test also revealed that the increase in SH at 12.51 mg L\(^{-1}\) was significantly lower than that in 0.22 and 4.02 mg L\(^{-1}\) of Ni (p<0.05) but not from control (p=0.076).

On day 6, maximum increment of 12.7 cm in SH was found in control and lowest of 1.1 and 2 cm in 12.51 and 22.33 mg L\(^{-1}\) respectively. The differences in the increase in SH on day 6 were significant as revealed by one way ANOVA (F=6.198, p<0.001). Multiple comparisons using Tukey test showed that the increase in SH in 22.33 mg L\(^{-1}\) Ni-exposed plants was significantly lower than that in control (p=0.003) and 0.22 mg L\(^{-1}\) (p=0.008). The test further revealed that the increase in SH in 12.51 mg L\(^{-1}\) was significantly lower than that in control (p=0.001) and also than that in 0.22 and 4.02 mg L\(^{-1}\) (p<0.05).

On day 9, highest increment in SH was 19.1 cm in control plants, followed by 18.3 cm in plants exposed to 0.22 mg L\(^{-1}\), and 13.6 cm in 2.23 mg L\(^{-1}\). One way ANOVA showed that the
differences in the increase in SH were significant (F=7.915, p<0.001). Multiple comparisons with Tukey test revealed that the increase in SH in control and in 0.22 mg L\(^{-1}\) were significantly higher than those in 12.51 (p<0.001) and 22.33 mg L\(^{-1}\) (p=0.001). The test further revealed that the increase in SH in 2.23 mg L\(^{-1}\) was significantly higher than that in 12.51 mg L\(^{-1}\) (p=0.015) but not that in the control (p=0.690).

On day 12, necrosis occurred in 22.33 mg L\(^{-1}\) Ni but since necrotic damage was minimal, positive increments of growth in SH were observed. However, there was no further growth, and SH changed from 3.4 to 2.1 cm on day 12 and from 2.1 to 1.3 cm on day 15 in 22.33 mg L\(^{-1}\) Ni exposed plants. Plants exposed to 12.51 mg L\(^{-1}\) showed reduction from 3 to 0.1 cm on day 15 of exposure. One way ANOVA revealed that the differences in SH were significant on day 12 (F=10.232, p<0.001) and on day 15 (F=16.858, p<0.001) of exposure. Multiple comparisons using Tukey test on day 12 showed that the changes in SH in 12.51 and 22.33 mg L\(^{-1}\) Ni were significantly lower than that in control (p<0.001) and that in Ni concentrations of 0.22 - 4.02 mg L\(^{-1}\) (p<0.05). Tukey test on day 15 revealed that the increase in SH at 7.14 mg L\(^{-1}\) was significantly lower than that in control (p=0.009) and the reduction at 12.51 and 22.33 mg L\(^{-1}\) were significant when compared to control (p<0.001) as well as Ni concentrations of 0.22 - 7.14 mg L\(^{-1}\) (p<0.05).

Overall changes in SH at the end of 15 day was statistically significant as revealed by one way ANOVA (F=20.195, p<0.001). Multiple comparisons with Tukey test showed that the increase in SH at 4.02 and 7.14 mg L\(^{-1}\) was significantly lower than that in the control (p<0.05) and the reduction at 12.51 and 22.33 mg L\(^{-1}\) was also significant from those in the control and in 0.22 - 7.14 mg L\(^{-1}\) Ni exposed plants (p<0.001). The test also revealed that the differences among the control, 0.22 and 2.23 mg L\(^{-1}\) of Ni were not significant (p>0.05).
2. Effects on number of new nodes

The changes in number of new nodes (NN) in control and plants exposed to different concentrations of Ni on 3, 6, 9, 12 and 15 day of exposure are shown in Table 6. NN in control and lower concentrations of Ni of 0.22 - 4.02 mg L\(^{-1}\) increased till the end of the experiment. Plants exposed to 2.23 mg L\(^{-1}\) showed a lower increment in NN till day 9 of the experiment. NN was reduced in 12.51 mg L\(^{-1}\) on day 15 and in 22.33 mg L\(^{-1}\) of Ni treatments from 12 day onwards.

On day 3, lowest NN of 0.2 was observed in both 12.51 and 22.33 mg L\(^{-1}\) Ni exposed plants, followed by plants in 2.23 mg L\(^{-1}\) with 0.3 increments in NN. One way ANOVA showed that the differences in NN were significant (F=8.720, p<0.001). Multiple comparisons using Tukey test revealed that the increase in NN in 2.23, 12.51 and 22.33 mg L\(^{-1}\) was significantly lower than that in control (p<0.05).

On day 6, highest NN of 5 was observed in control and lowest of 0.3 in 12.51 mg L\(^{-1}\) of Ni treatment. One way ANOVA revealed that the differences in NN on day 6 were significant (F=8.720, p<0.05). Multiple comparisons using Tukey test showed that the increase in NN in control was significantly higher than that in 2.23, 12.51 and 22.33 mg L\(^{-1}\) (p<0.05). The test further revealed that the increase in NN in 0.22, 4.02 and 7.14 mg L\(^{-1}\) was significantly higher than that in 12.51 mg L\(^{-1}\) of Ni exposed plants (p<0.05).

On day 9, lowest NN of 0.5 was observed in 12.51 mg L\(^{-1}\) followed by 1 in 22.33 mg L\(^{-1}\) Ni. Plants exposed to 2.23 and 7.14 mg L\(^{-1}\) showed NN in the range of 2.7 - 3.2. The differences in the increase in NN were significant as revealed by one way ANOVA (F=9.023, p<0.001). Multiple comparisons with Tukey test showed that the increase in NN in plants exposed to 2.23, 7.14, 12.51 and 22.33 mg L\(^{-1}\) was significantly lower than that in the control (p<0.05). The test
further revealed that the increase in NN in 0.22 and 4.02 mg L\(^{-1}\) was significantly higher than that in 12.51 mg L\(^{-1}\) (p<0.5) and those exposed to 22.33 mg L\(^{-1}\) was also significantly lower than that in 4.02 mg L\(^{-1}\) (p=0.038).

On day 12, maximum NN of 7.3 was found in control followed by that of 4.7 in plants in 4.02 mg L\(^{-1}\) treatment, while the lowest NN of 0.7 was observed in 12.51 mg L\(^{-1}\). Plants exposed to 22.33 mg L\(^{-1}\) Ni showed reduction in NN. One way ANOVA showed that the differences in NN on day 12 were significant (F=9.204, p<0.001). The reduction in NN in 22.33 mg L\(^{-1}\) was significantly different from that in control (p<0.001) and in Ni concentrations of 0.22 - 7.14 mg L\(^{-1}\) (p<0.05) as revealed by multiple comparisons with Tukey test. The test also revealed that the increase in NN in 12.51 mg L\(^{-1}\) was significantly lower than that in control (p<0.001) and in 4.02 mg L\(^{-1}\) Ni treatment (p=0.043).

On day 15, NN reductions of 1.3 and 2.7 in plants exposed to 12.51 and 22.33 mg L\(^{-1}\) Ni was observed. Highest NN of 8.2 was observed in control. One way ANOVA revealed that the differences in NN were significant (F=15.671, p<0.001). Multiple comparisons with Tukey test showed that the reduction in NN in 12.51 and 22.33 mg L\(^{-1}\) was significant from that in the control (p<0.001) and in Ni concentrations of 0.22 - 7.14 mg L\(^{-1}\) (p<0.05). The test also showed that the increase in control was significantly higher than that in 7.14 mg L\(^{-1}\) (p=0.024).

Overall changes in NN at the end of 15 day was significant as revealed by one way ANOVA (F=29.590, p<0.001). Multiple comparisons using Tukey test showed that the increase in NN in control was higher than that in 0.22 - 7.14 mg L\(^{-1}\) Ni treated plants (p<0.05) as well as in 12.51 and 22.33 mg L\(^{-1}\) (p<0.001). The test further revealed that the reduction in 12.51 and 22.33 mg L\(^{-1}\) was also significantly different from plants treated with the other Ni concentrations of 0.22 - 7.14 mg L\(^{-1}\) (p<0.05).
Table 5: Changes in shoot height (SH) in control and Ni exposed *I. aquatica* during a 15 day experiment.

<table>
<thead>
<tr>
<th>Ni Conc. (mg L⁻¹)</th>
<th>Day3</th>
<th>Day6</th>
<th>Day9</th>
<th>Day12</th>
<th>Day15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.5±0.8</td>
<td>12.7±2</td>
<td>19.1±1.5</td>
<td>28.2±2.4</td>
<td>31.2±2.2</td>
</tr>
<tr>
<td>0.22</td>
<td>3.8±0.7</td>
<td>11.8±2.8</td>
<td>18.3±4.7</td>
<td>21.2±5.8</td>
<td>22.6±5.7</td>
</tr>
<tr>
<td>2.23</td>
<td>2.1±0.6</td>
<td>8.5±1.3</td>
<td>13.6±1.9</td>
<td>21.2±5.8</td>
<td>22.6±5.7</td>
</tr>
<tr>
<td>4.02</td>
<td>3.7±1.1</td>
<td>9.1±2.8</td>
<td>11.9±3.1</td>
<td>16.4±3.2</td>
<td>18.8±2.5</td>
</tr>
<tr>
<td>7.14</td>
<td>4.5±0.8</td>
<td>8.2±0.7</td>
<td>11.9±1.6</td>
<td>14.4±2.6</td>
<td>15.3±2.5</td>
</tr>
<tr>
<td>12.51</td>
<td>0.5±0.3</td>
<td>1.1±0.4</td>
<td>2.1±0.8</td>
<td>3±1.4</td>
<td>-0.1±0.9</td>
</tr>
<tr>
<td>22.33</td>
<td>1.3±0.5</td>
<td>2±0.8</td>
<td>3.4±1.2</td>
<td>2.1±1.6</td>
<td>-1.3±1.1</td>
</tr>
</tbody>
</table>

* Significant difference from corresponding value in control at p<0.05.
Values are mean ± S.E.; n=6.

Table 6: Changes in number of new nodes (NN) in control and Ni exposed *I. aquatica* during a 15 day experiment.

<table>
<thead>
<tr>
<th>Ni Conc. (mg L⁻¹)</th>
<th>Day3</th>
<th>Day6</th>
<th>Day9</th>
<th>Day12</th>
<th>Day15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.2±0.7</td>
<td>5±1</td>
<td>6.3±1</td>
<td>7.3±1</td>
<td>8.2±1</td>
</tr>
<tr>
<td>0.22</td>
<td>1±0.4</td>
<td>2.8±0.8</td>
<td>3.8±1</td>
<td>4.3±1.2</td>
<td>4.3±1.2</td>
</tr>
<tr>
<td>2.23</td>
<td>0.3±0.2</td>
<td>1.7±0.4</td>
<td>2.7±0.7</td>
<td>3.7±0.8</td>
<td>4.2±1</td>
</tr>
<tr>
<td>4.02</td>
<td>1.2±0.3</td>
<td>3.2±0.8</td>
<td>4±0.5</td>
<td>4.7±0.4</td>
<td>5.5±0.4</td>
</tr>
<tr>
<td>7.14</td>
<td>1.2±0.3</td>
<td>2.8±0.2</td>
<td>3.2±0.3</td>
<td>3.5±0.4</td>
<td>3.5±0.4</td>
</tr>
<tr>
<td>12.51</td>
<td>0.2±0.2</td>
<td>0.3±0.2</td>
<td>0.5±0.3</td>
<td>0.7±0.5</td>
<td>-1.3±1.2</td>
</tr>
<tr>
<td>22.33</td>
<td>0.2±0.2</td>
<td>0.5±0.3</td>
<td>1±0.4</td>
<td>-0.8±1.4</td>
<td>-2.7±1</td>
</tr>
</tbody>
</table>

* Significant difference from corresponding value in control at p<0.05.
Values are mean ± S.E.; n=6.
3. Effects on number of new leaf

The appearance of NL in control and in plants exposed to different concentrations of Ni on 3, 6, 9, 12 and 15 day of exposure are shown in Table 7. NL in control and in Ni concentrations of 0.22 - 12.51 mg L\(^{-1}\) continued to increase till the end of the experiment.

On day 3, appearance of NL in control and in all the Ni treatment plants was at the range of 1.5 - 0.3 with maximum NL (1.5) in 0.22 mg L\(^{-1}\) treatment plants and lowest of 0.3 in 12.51 mg L\(^{-1}\). However, the difference in the NL was not significant as revealed by one way ANOVA (F=1.581, p=0.182).

On day 6 and 9, appearance of NL was found to be highest in control with NL of 3 and 4.2 and lowest of 0.8 and 1.2 in 12.51 mg L\(^{-1}\). And those exposed to 22.33 mg L\(^{-1}\) of Ni showed NL of 1 and 1.3 on day 6 and 9 of exposure. One way ANOVA revealed that the differences in the appearance of NL were significant on day 6 (F=5.726, p<0.001) and on day 9 (F=6.5508, p<0.001). Multiple comparisons with Tukey test showed that the appearance of NL in 12.51 - 22.33 mg L\(^{-1}\) Ni-exposed plants was significantly lower than those in control, and in 0.22 and 7.14 mg L\(^{-1}\) of Ni treatment (p<0.05) on both day 6 and 9 of exposure.

On day 12, highest NL of 4.8 was in control, while lowest of 1.2 was in 12.51 mg L\(^{-1}\) Ni treatment. The differences in the appearance of NL were significant as revealed by one way ANOVA (F=8.769, p<0.001). Multiple comparisons with Tukey test revealed that the appearance of NL in 12.51 mg L\(^{-1}\) was significantly lower than that in control (p<0.001) and in the Ni concentrations of 0.22 - 7.14 mg L\(^{-1}\) (p<0.05). The test further revealed that the appearance of NL at 22.33 mg L\(^{-1}\) was lower than that in control (p<0.001) and in 0.22 - 2.23 and 7.14 mg L\(^{-1}\) of Ni treatment (p<0.05).
On day 15, highest NL of 5.7 was observed in control, while lowest NL of 1.3 was in 12.51 mg L\(^{-1}\). One way ANOVA revealed that differences in the appearance of NL were significant (F=9.138, p<0.001). Multiple comparisons with Tukey test showed that the appearance of NL in 12.51 and 22.33 mg L\(^{-1}\) was significantly lower than that in control (p<0.001) and in the remaining Ni concentrations of 0.22 - 7.14 mg L\(^{-1}\) (p<0.05).

Overall differences in the appearance of NL at the end of 15 day were significant as revealed by one way ANOVA (F=15.191, p<0.001). The appearance of NL in plants treated with 12.51 and 22.33 mg L\(^{-1}\) Ni was significantly lower than that in control (p<0.001) and in Ni treatments of 0.22 - 7.14 mg L\(^{-1}\) (p<0.05) as revealed by multiple comparisons using Tukey test. The test also revealed that the appearance of NL in 2.23 mg L\(^{-1}\) was significantly lower than that in the control (p=0.039).

4. Effects on plant mortality

The changes in the occurrence of DP in plants exposed to different concentrations of Ni on day 3, 6, 9, 12 and 15 day of exposure are shown in Table 8. Control and plants exposed to Ni concentrations of 0.22 - 7.14 mg L\(^{-1}\) showed no DP till the end of the experiment.

On day 9 and 12, occurrence of DP of 0.2 was observed in 12.51 mg L\(^{-1}\) Ni treated plants. However, no significant difference in the occurrence of DP was observed on day 9 and 12 (F=1.000, p=0.441) as revealed by one way ANOVA.

On day 15, plants exposed to 22.33 mg L\(^{-1}\) started having DP of 0.5 which was higher than that (0.2) in 12.51 mg L\(^{-1}\). One way ANOVA revealed that differences in the occurrence of DP on day 15 were statistically significant (F=3.214, p=0.013). Post-hoc Tukey test revealed that DP in 22.33 mg L\(^{-1}\) was significantly different from that in control and in 0.22 - 7.14 mg L\(^{-1}\) Ni treatments (p=0.029) but not from that in 12.51 mg L\(^{-1}\) Ni exposed plants (p=0.303). The test
also revealed that the occurrence of DP in 12.51 mg L$^{-1}$ was not significant from that in control (p=0.918) and in Ni concentrations of 0.22 - 7.14 mg L$^{-1}$ (p=0.918).

Overall changes in the occurrence of DP at the end of 15 day were statistically significant as revealed by one way ANOVA (F=2.685, p=0.016). Multiple comparisons with Tukey test showed that the occurrence of DP in 12.51 and 22.33 mg L$^{-1}$ was significantly different from that in control and in Ni concentrations of 0.22 - 7.14 mg L$^{-1}$ (p=0.018).
Table 7: Number of new leaf (NL) in control and Ni exposed *I. aquatica* during a 15 day experiment.

<table>
<thead>
<tr>
<th>Ni Conc. (mg L⁻¹)</th>
<th>Appearance of NL</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day3</td>
<td>Day6</td>
<td>Day9</td>
<td>Day12</td>
<td>Day15</td>
</tr>
<tr>
<td>Control</td>
<td>1.2±0.3</td>
<td>3±0.4</td>
<td>4.2±0.3</td>
<td>4.8±0.5</td>
<td>5.7±0.6</td>
</tr>
<tr>
<td>0.22</td>
<td>1.5±0.2</td>
<td>3±0.4</td>
<td>3.5±0.6</td>
<td>4±0.7</td>
<td>4.5±0.8</td>
</tr>
<tr>
<td>2.23</td>
<td>0.7±0.2</td>
<td>1.8±0.4</td>
<td>2.5±0.6</td>
<td>3.7±0.4</td>
<td>4.3±0.5</td>
</tr>
<tr>
<td>4.02</td>
<td>0.8±0.4</td>
<td>2.2±0.6</td>
<td>2.8±0.5</td>
<td>3.5±0.3</td>
<td>4.7±0.5</td>
</tr>
<tr>
<td>7.14</td>
<td>1.2±0.3</td>
<td>3±0.3</td>
<td>3.3±0.3</td>
<td>3.7±0.3</td>
<td>4.2±0.4</td>
</tr>
<tr>
<td>12.51</td>
<td>0.3±0.2</td>
<td>0.8±0.3⁺</td>
<td>1.2±0.4⁺</td>
<td>1.2±0.4⁺</td>
<td>1.3±0.5⁺</td>
</tr>
<tr>
<td>22.33</td>
<td>0.8±0.4</td>
<td>1±0.4⁺</td>
<td>1.3±0.3⁺</td>
<td>1.5±0.4⁺</td>
<td>1.5±0.4⁺</td>
</tr>
</tbody>
</table>

* Significant difference from corresponding value in control at *p*<0.05. Values are mean ± S.E.; n=6.

Table 8: Number of dead plants (DP) in control and Ni exposed *I. aquatica* during a 15 day experiment.

<table>
<thead>
<tr>
<th>Ni Conc. (mg L⁻¹)</th>
<th>Change in DP</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day3</td>
<td>Day6</td>
<td>Day9</td>
<td>Day12</td>
<td>Day15</td>
</tr>
<tr>
<td>Control</td>
<td>0±0</td>
<td>0±0</td>
<td>0±0</td>
<td>0±0</td>
<td>0±0</td>
</tr>
<tr>
<td>0.22</td>
<td>0±0</td>
<td>0±0</td>
<td>0±0</td>
<td>0±0</td>
<td>0±0</td>
</tr>
<tr>
<td>2.23</td>
<td>0±0</td>
<td>0±0</td>
<td>0±0</td>
<td>0±0</td>
<td>0±0</td>
</tr>
<tr>
<td>4.02</td>
<td>0±0</td>
<td>0±0</td>
<td>0±0</td>
<td>0±0</td>
<td>0±0</td>
</tr>
<tr>
<td>7.14</td>
<td>0±0</td>
<td>0±0</td>
<td>0±0</td>
<td>0±0</td>
<td>0±0</td>
</tr>
<tr>
<td>12.51</td>
<td>0±0</td>
<td>0±0</td>
<td>0.2±0.2</td>
<td>0.2±0.2</td>
<td>0.2±0.2</td>
</tr>
<tr>
<td>22.33</td>
<td>0±0</td>
<td>0±0</td>
<td>0±0</td>
<td>0±0</td>
<td>0.5±0.2⁺</td>
</tr>
</tbody>
</table>

* Significant difference from corresponding value in control at *p*<0.05. Values are mean ± S.E.; n=6.
B. CHRONIC TOXIC EFFECTS ON GROWTH

I. Copper

1. Effects on shoot height

Changes in the shoot height of control and plants exposed to different concentrations (0.025 - 4.58 mg L\(^{-1}\)) of Cu on 3, 6, 9, 12, 15, 18, 21, 24, 27 and 30 day of exposure are shown in Fig 3. In this 30 day experiment control plants and those exposed to 0.025 - 0.13 mg L\(^{-1}\) of Cu continued to increase their SH throughout the experiment while those exposed to 0.25 - 4.58 mg L\(^{-1}\) showed reduction in SH due to stem tissue necrosis.

On day 3, the highest increase in SH of 1.3 cm was observed in 0.025 mg L\(^{-1}\) of Cu followed by that of 0.9 cm in the control. The lowest increase of 0.3 cm was observed in 2.54 mg L\(^{-1}\) Cu exposure. One way ANOVA revealed that the differences in the increase in SH among the different treatments on day 3 were statistically significant (F=6.172, p=0.001). Multiple comparisons with Tukey test showed that the increase in SH at 0.025 mg L\(^{-1}\) was significantly higher (p<0.05) than that in 0.13, 2.54 and 4.58 mg L\(^{-1}\) Cu exposed groups but not than that in control (p=0.425).

On day 6, the highest increment in SH was observed in 0.25 mg L\(^{-1}\) Cu (2.2 cm) and lowest in 4.58 mg L\(^{-1}\) Cu (0.8 cm). The difference in SH was statistically significant as revealed by one way ANOVA (F=2.901, p=0.032). Multiple comparisons with Tukey test showed that the increase in SH in 0.25 mg L\(^{-1}\) was significantly higher than that in 4.58 mg L\(^{-1}\) (p=0.048) but not than that in control (p=0.191).

From day 9 onwards the increase in SH in 0.13 mg L\(^{-1}\) was found to be the highest till the end of the experiment. SH increased to 3.9 cm in 0.13 mg L\(^{-1}\) followed by 3.6 cm in 0.25 mg L\(^{-1}\).
Cu with the lowest in control (1.8 cm) on day 9 of the exposure. However, one way ANOVA showed that the change in SH was not statistically significant (F=2.410, p=0.063).

On day 12, reduction in SH of 4.13 cm occurred in 4.58 mg L$^{-1}$ Cu while plants exposed to 0.13 mg L$^{-1}$ showed maximum increment with SH of 4.8 cm. The difference in SH was significant as revealed by one way ANOVA (F=2.568, p=0.05). Multiple comparisons with Tukey test revealed that the increase in SH in 0.13 mg L$^{-1}$ was significantly higher than that in 4.58 mg L$^{-1}$ but not from that in control (p=0.952).

SH at 4.58 mg L$^{-1}$ of Cu continued to reduce from day 12 of exposure till the end of the experiment by 3.7 cm on day 15 of the exposure. The differences in SH on day 15 was not significant as revealed by one way ANOVA (F=2.856, p=0.057).

On the other hand, plants exposed to 0.13 mg L$^{-1}$ showed an increment of 6.9 cm from 5.5 cm on day 18 of exposure. One way ANOVA revealed that the increase in SH was statistically significant (F=2.856, p=0.034). Multiple comparisons with Tukey test showed that the increase in SH in 0.13 mg L$^{-1}$ of Cu was significantly different from that in 4.58 mg L$^{-1}$ (p=0.015) but not from that in control (p=0.691).

On day 21 of exposure, plants treated with 2.54 mg L$^{-1}$ of Cu started showing necrosis in stem tissues with reduction of 2.3 cm in SH which continued till the end of the experiment. Plants in 4.58 mg L$^{-1}$ treatment had a reduction of 3.2 cm. One way ANOVA revealed that the difference in SH was not significant (F=2.053, p=0.103).

The reduction in SH in 4.58 mg L$^{-1}$ was 14.2 cm while those exposed to 0.13 mg L$^{-1}$ of Cu showed an increment of 7.9 cm on day 24. The difference in SH on day 24 was statistically significant as revealed by one way ANOVA (F=5.395, p=0.002). Multiple comparisons with
Tukey test showed that the reduction in SH in 4.58 mg L\(^{-1}\) was significantly different from that in control (p=0.014) and in 0.025 - 0.25 mg L\(^{-1}\) Cu exposed plants (p<0.05).

Plants exposed to 0.25 mg L\(^{-1}\) of Cu showed reduction of 3.2 cm in SH, while those exposed to 4.58 mg L\(^{-1}\) showed reduction of 14.6 cm on day 27. One way ANOVA showed that the differences in SH were significantly different (F=3.446, p=0.016). Multiple comparisons with Tukey test revealed that the reduction in SH in 4.58 mg L\(^{-1}\) was significantly different from that in 0.08 and 0.13 mg L\(^{-1}\) of Cu treatment (p<0.05). The test further revealed that the reduction in SH in 0.25 and 2.54 mg L\(^{-1}\) was not significantly different from that in control (p=0.951 and 0.936) and in the other Cu treatments (p>0.05).

On day 30, plants exposed to 0.13 mg L\(^{-1}\) of Cu showed highest SH increment of 8.4 cm followed by SH of 4.3 cm in 0.08 mg L\(^{-1}\), while those exposed to 4.58 mg L\(^{-1}\) Cu showed maximum reduction of 14.6 cm. One way ANOVA revealed that the differences in SH were statistically significant (F=3.219, p=0.021). The increase in SH in 0.13 mg L\(^{-1}\) was significantly higher than that in 4.58 mg L\(^{-1}\) (p=0.014) but not than that in control (p=0.960) and that in 0.025 - 0.08 mg L\(^{-1}\) and 0.25 - 2.54 mg L\(^{-1}\) Cu exposure.

Overall increase or decrease in SH in plants in control and different Cu exposure concentrations at the end of 30 days were statistically significant as revealed by one way ANOVA (F=15.586, p<0.001). Multiple comparisons with Tukey test showed that SH in plants exposed to 4.58 mg L\(^{-1}\) was significantly different from that in control (p<0.001) as well as from those in Cu concentrations of 0.025 - 2.54 mg L\(^{-1}\) (p<0.05). The test further revealed that SH in 2.54 mg L\(^{-1}\) was significantly different from that in 0.13 mg L\(^{-1}\) of Cu (p=0.006) but not from that in control (p=0.969).
2. Effects on number of new nodes

The changes in the appearance of NN in control and Cu (0.025 - 4.58 mg L\(^{-1}\)) exposed plants on 3, 6, 9, 12, 15, 18, 21, 24, 27 and 30 day of the exposure are shown in Fig 4. There were no new nodes in control and in all the exposed plants at different Cu concentrations on day 3 of the exposure.

On day 6, new nodes appeared in Cu treatments of 0.08, 0.25 and 2.54 mg L\(^{-1}\), while control plants and those exposed to 0.025, 0.13 and 4.58 mg L\(^{-1}\) produced no new nodes. One way ANOVA revealed that the differences in the appearance of NN on day 6 were significantly different (F=4.222, p=0.006). However multiple comparisons with Tukey test showed that the difference in NN on day 6 was not significantly different when compared with that in control and in the other Cu treatments. However, LSD test showed that the appearance of NN in 0.25 and 2.54 mg L\(^{-1}\) was significantly higher from that in control (p=0.006) and in plants exposed to 0.025, 0.13 and 4.58 mg L\(^{-1}\) Cu (p=0.006).

On day 9, control and 0.08 - 4.58 mg L\(^{-1}\) Cu exposed plants bore new nodes with the exception of plants exposed to 0.025 mg L\(^{-1}\) which bore new nodes from day 18 of the exposure. Among the plants which bore new nodes, 0.25 and 2.54 mg L\(^{-1}\) Cu treated plants showed highest NN of 2 and lowest of 0.7 in control. The differences in the appearance of NN on day 9 were statistically significant as revealed by one way ANOVA (F=7.185, p<0.001). Multiple comparisons with Tukey test revealed that the increase in NN in 0.25 and 2.54 mg L\(^{-1}\) treatments was significantly higher than those in control (p=0.028). Again the absence of NN in 0.025 mg L\(^{-1}\) was significantly different from those in 0.08, 0.25, 2.54 and 4.58 mg L\(^{-1}\) Cu (p<0.05) but not from that in control (p=0.584).
A reduction of 3.3 in NN was observed in 4.58 mg L\(^{-1}\) Cu on day 12 due to stem tissue necrosis, while all the other exposed plants in Cu concentrations of 0.08 - 2.54 mg L\(^{-1}\) continued to increase NN with highest NN of 2.3 in 2.54 mg L\(^{-1}\) Cu and lowest NN of 0.7 in control. The differences in the change in NN were statistically significant as revealed by one way ANOVA (F=2.738, p=0.040). Multiple comparisons with Tukey test showed that the reduction in NN in 4.58 mg L\(^{-1}\) Cu was significant from that in 2.54 mg L\(^{-1}\) Cu (p=0.039) but not from that in control (p=0.255).

Reduction in NN continued in 4.58 mg L\(^{-1}\), while it continued to increase in Cu concentrations of 0.08 - 2.54 mg L\(^{-1}\) with the highest NN increment of 3 in 2.54 mg L\(^{-1}\) Cu on day 15 of exposure. One way ANOVA revealed that the differences in the change in NN were significant (F=2.986, p=0.029). Multiple comparisons with Tukey test showed that the reduction in NN in 4.58 mg L\(^{-1}\) Cu was significant from that in 2.54 mg L\(^{-1}\) Cu (p=0.031).

On day 18, plants exposed to 0.025 mg L\(^{-1}\) Cu produced NN of 0.7 followed by control with NN of 1.3 and those exposed to 0.13 and 2.54 mg L\(^{-1}\) Cu showed higher NN of 3, while those exposed to 4.58 mg L\(^{-1}\) continued to show reduction in NN till the end of the experiment. The differences in the change of NN on day 18 were significant as revealed by one way ANOVA (F=2.990, p=0.028). The increase in NN in 0.13 and 2.54 mg L\(^{-1}\) Cu was significantly higher than that in 4.58 mg L\(^{-1}\) (p=0.035) but not than that in control (p=0.947) as revealed by multiple comparisons with Tukey test.

On day 21, plants exposed to 2.54 mg L\(^{-1}\) started showing reduction in NN due to stem tissues necrosis that continued till the end of the experiment. However, no significant differences in the appearance of NN were observed on day 21 as revealed by one way ANOVA (F=1.739, p=0.161).
On 24 day of exposure the extent of reduction in nodes became higher in plants exposed to 4.58 mg L\(^{-1}\) Cu with a reduction of 10.7, while those exposed to 0.13 mg L\(^{-1}\) Cu showed the highest NN of 3.3. One way ANOVA showed that the differences in the change in NN were significant (F=2.906, p=0.036). Multiple comparisons with Tukey test revealed that the reduction in NN in 4.58 mg L\(^{-1}\) Cu was significantly different from those in control (p=0.014) and in 0.025 - 0.25 mg L\(^{-1}\) Cu exposed plants (p<0.05).

On day 27 and 30, plants exposed to 0.25 mg L\(^{-1}\) also showed reduction of NN, while plants in control and those exposed to 0.025 - 0.13 mg L\(^{-1}\) Cu continued to show increase in NN with highest NN of 2.3 on day 27 and 2.7 on day 30 in 0.13 mg L\(^{-1}\) Cu exposed plants. One way ANOVA showed that the differences in the change of nodes were significant on day 27 (F=2.906, p=0.032) and on day 30 (F=3.129, p=0.024). Multiple comparisons with Tukey test revealed that the increase in NN in 0.13 mg L\(^{-1}\) was significantly higher than that in 4.58 mg L\(^{-1}\) (p<0.05) but not than that in control (p>0.05).

Overall changes in NN was analyzed using one way ANOVA and were found to be significant (F=12.496, p<0.001). Multiple comparisons with Tukey test revealed that the reduction in NN in 4.58 mg L\(^{-1}\) Cu was significant from that in control (p<0.001) and the other Cu concentrations of 0.025 - 2.54 mg L\(^{-1}\) (p<0.001).
Figure 3: Changes in shoot height (SH) in control and Cu exposed *I. aquatica* during 30 day exposure. [d=day], * Significant difference from corresponding value in control at p<0.05.

Figure 4: Changes in number of new nodes (NN) in control and Cu exposed *I. aquatica* during the 30 day exposure. * Significant difference from corresponding value in control at p<0.05.
3. Effects on number of new leaf

The number of new leaf (NL) produced in control and plants exposed to different concentrations of Cu (0.025 - 4.58 mg L\(^{-1}\)) on 3, 6, 9, 12, 15, 18, 21, 24, 27 and 30 day of the exposure are shown in Fig 5. On day 3 of the exposure no new leaf appeared on control plants and all the other Cu exposed plants.

On day 6 new leaves were produced in 0.025, 0.08, 0.13 and 0.25 mg L\(^{-1}\) Cu with the same NL of 0.3. On day 9, control and all the Cu exposed plants bore new leaves with maximum NL of 1 in 0.13 mg L\(^{-1}\) Cu and lowest of 0.3 in 0.025 and 4.58 mg L\(^{-1}\) Cu. However one way ANOVA revealed that the differences in the appearance of NL were not significant on day 6 (F=1.000, p=0.451) and on day 9 (F=0.872, p=0.532).

On day 12, 0.13 mg L\(^{-1}\) bore the highest NL which continued till the end of the experiment. Highest increment in NL of 1.7 in 0.13 mg L\(^{-1}\) Cu, and lowest NL of 0.3 in 0.025 mg L\(^{-1}\) Cu was observed. However on day 12, the differences in the production of NL were not significantly different as revealed by one way ANOVA (F=1.375, p=0.270).

On day 15, highest NL of 3 in 0.13 mg L\(^{-1}\) followed by NL of 1 in 0.08, 0.25 and 2.54 mg L\(^{-1}\) Cu and lowest being of 0.3 in 0.025 mg L\(^{-1}\) Cu was observed. One way ANOVA revealed that the differences in the appearance of NL on day 15 were statistically significant (F=8.056, p<0.001). Multiple comparisons with Tukey test showed that the increase in NL in 0.13 mg L\(^{-1}\) was significantly higher than that in control (p<0.001) and that in the other Cu exposed plants in 0.025 - 0.08 mg L\(^{-1}\) and 0.25 - 4.58 mg L\(^{-1}\) (p<0.05).

On day 18 and 21, plants exposed to 0.08 mg L\(^{-1}\) Cu showed further increase in NL from 1 to 1.3 and those exposed to 0.13 mg L\(^{-1}\) Cu had NL of 3.3. One way ANOVA showed that the differences in the appearance of NL were significant on day 18 and 21 (F=7.373, p<0.001).
Multiple comparisons with Tukey test revealed that the increase in NL in 0.13 mg L\(^{-1}\) Cu on day 18 and 21 was significantly higher than that in the control (p=0.001) and in the other Cu treatments (0.025 - 0.08 mg L\(^{-1}\) and 0.25 - 4.58 mg L\(^{-1}\) Cu) at p<0.05.

On day 24 and 27, NL in control plants increased from 0.7 to 1, with the highest NL of 3.3 found in 0.13 mg L\(^{-1}\) Cu. The differences in NL on day 24 and 27 were statistically significant as revealed by one way ANOVA (F=5.754, p=0.001). Multiple comparisons with Tukey test showed that the increase in NL in 0.13 mg L\(^{-1}\) Cu on day 24 and 27 was significantly higher than that in control (p<0.05) and in the other Cu treatments of 0.025 - 0.08 mg L\(^{-1}\) and 0.25 - 4.58 mg L\(^{-1}\) (p<0.05).

On day 30, further increase in NL was observed in control from 1 to 1.3 and also in 0.08 mg L\(^{-1}\) Cu from 1.3 to 1.7, although NL in other Cu treatments remained the same as that of day 27. One way ANOVA revealed that the differences in NL on day 30 were significant (F=5.467, p=0.002). Multiple comparisons with Tukey test showed that although control plants showed increase in NL, it was significantly lower than that in plants exposed to 0.13 mg L\(^{-1}\) Cu (p=0.027). Again the increase in NL in 0.13 mg L\(^{-1}\) Cu was significantly higher than that in plants exposed to Cu concentrations of 0.025, 0.25, 2.54 and 4.58 mg L\(^{-1}\) Cu (p<0.05) but not than those in 0.08 mg L\(^{-1}\) Cu (p=0.090).

Overall changes in NL at the end of 30 day were analyzed using one way ANOVA and were found to be significant (F=27.699, p<0.001). Multiple comparisons with Tukey test revealed that the increase in NL in 0.13 mg L\(^{-1}\) Cu was significantly higher than that in control (p<0.001) and than that in Cu exposed plants in 0.025, 0.08, 0.25, 2.54 and 4.58 mg L\(^{-1}\) (p<0.05). The test further revealed that the increase in 0.08 mg L\(^{-1}\) Cu was significantly higher than that in 0.025 and 4.58 mg L\(^{-1}\) Cu (p<0.05) but not than that in control (p=0.429).

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4. Effects on tiller number

The changes in tiller number (TN) in control and all the Cu (0.025 - 4.58 mg L⁻¹) exposed plants on 3, 6, 9, 12, 15, 18, 21, 24, 27 and 30 day of exposure are shown in Fig 6.

On day 3, control plants and all those exposed to Cu produced no new tiller with the exception of 0.25 mg L⁻¹ Cu treated plants which produced TN of 0.3. However, differences in the appearance of TN were not significant as revealed by one way ANOVA (F=2.000, p=0.111).

On day 6, TN of 0.3 was observed in 0.08, 0.13 and 4.58 mg L⁻¹ Cu exposed plants, although the differences in the production of TN were not significant as revealed by one way ANOVA (F=1.000, p=0.451).

On day 9, 12 and 15, appearance of new tiller was observed in all the Cu exposed plants with highest TN of 0.7 in 0.08, 0.13 and 2.54 mg L⁻¹ Cu. On day 15, TN increased from 0.3 to 0.7 in 0.025 mg L⁻¹ Cu. One way ANOVA revealed that the differences in the appearance of TN were not significant on day 9 and 12 (F=0.533, p=0.777) and on day 15 (F=0.578, p=0.744).

Control plants produced TN of 0.3 on day 18 and those exposed to 4.58 mg L⁻¹ Cu increased the TN production from 0.3 to 1. However, the differences remained not significant (F=0.278, p=0.941).

On day 21 and 24, plants exposed to 4.58 mg L⁻¹ Cu produced the highest TN of 1, while lowest TN of 0.3 was observed in control and in 0.25 mg L⁻¹ Cu exposed plants. However, one way ANOVA revealed that the differences in the appearance of TN were not significant on day 21 and 24 (F=0.278, p=0.941).
Further increase in TN was observed in 0.025 and 0.08 mg L\(^{-1}\) Cu from 0.7 to 1 on day 27 of the exposure but again the differences were not significant as revealed by one way ANOVA (F=0.515, p=0.790).

On day 30 of the exposure, plants exposed to 0.13 mg L\(^{-1}\) Cu increased the TN from 0.7 to 1, while in all other Cu exposed and control plants no further increase in TN was observed. One way ANOVA showed that the differences in the appearance of TN on day 30 were not significant (F=0.603, p=0.725).

However on analyzing the overall increased in TN at the end of 30 days the differences in the production of TN were found to be significant as revealed by one way ANOVA (F=2.607, p=0.018). Multiple comparisons with Tukey test showed that the appearance of TN in 0.08 and 4.58 mg L\(^{-1}\) Cu was significantly higher than that in control (p=0.043).
Figure 5: Increase in number of new leaf (NL) in control and Cu exposed *I. aquatica* during 30 day exposure. * Significant difference from corresponding value in control at p<0.05.

Figure 6: Increase in tiller number (TN) in control and Cu exposed *I. aquatica* during 30 day exposure.
5. Effects on width of leaf blade

The increase in the width of leaf blade (WLB) in control and plants exposed to different concentrations of Cu (0.025 - 4.58 mg L$^{-1}$) on 3, 6, 9, 12, 15, 18, 21, 24, 27 and 30 day of the exposure are shown in Fig 7.

Increase in the WLB of 0.03 cm was observed only in control plant on day 3 of the exposure.

On day 6 and 9, increase in WLB was also observed in 0.13, 0.25 and 2.54 mg L$^{-1}$ Cu treatment plants. However one way ANOVA revealed that the differences in the changes in WLB were not statistically significant on day 3 (F=2.000, p=0.111), day 6 (F=1.133, p=0.378) and day 9 (F=1.600, p=0.197).

On day 12 and 15, WLB further increased in plants exposed to 0.13 mg L$^{-1}$ Cu from 0.07 to 0.13 cm on day 12 and to 0.17 cm on day 15, while it remained constant in control and other Cu exposed plants. Plants exposed to 0.08 and 4.58 mg L$^{-1}$ Cu showed no increment in WLB upto 15 day of the exposure. One way ANOVA showed that the differences in the increase in WLB were significant on day 12 (F=3.200, p=0.022) and day 15 (F=3.538, p=0.014). Multiple comparisons with Tukey test revealed that the increase in WLB in 0.13 mg L$^{-1}$ Cu was significantly higher than that in 0.025, 0.08 and 4.58 mg L$^{-1}$ Cu but not than that of control (p>0.05) on both day 12 and 15.

On day 18 of exposure, WLB increased to 0.025 cm in 0.08 mg L$^{-1}$ Cu treatment and to 0.27 cm in plants exposed to 0.13 mg L$^{-1}$ Cu. The differences in the change in WLB were significant as revealed by one way ANOVA (F=2.990, p=0.028). Multiple comparisons with Tukey test showed that WLB in 0.13 mg L$^{-1}$ Cu exposed plants was significantly higher than those in 0.025 and 4.58 mg L$^{-1}$ Cu (p=0.025) but not than the control (p=0.969).
On day 21, plants exposed to 0.08 mg L\textsuperscript{-1} Cu showed no further increment in WLB but those exposed to 0.13 mg L\textsuperscript{-1} Cu showed increment in WLB from 0.27 to 0.28 cm. One way ANOVA showed that the differences in the increase in WLB were significant (F=3.628, p=0.013). Multiple comparisons with Tukey test showed that the increase in WLB in 0.08 mg L\textsuperscript{-1} Cu treatment was significantly higher than that in 0.025, 0.08 and 4.58 mg L\textsuperscript{-1} Cu treatment plants (p<0.05) but not from that in control (p=0.082).

On day 24, plants exposed to 0.13 mg L\textsuperscript{-1} Cu showed increment in WLB from 0.28 to 0.3 cm on day 24 and from 0.3 to 0.33 cm on day 30. On day 30, control plants also showed increase in WLB from 0.07 to 0.1 cm. One way ANOVA revealed that the differences in the increase of WLB were significant on day 24 (F=3.824, p=0.010), on day 27 (F=3.824, p=0.010) and on day 30 (f=3.522, p=0.014). Multiple comparisons with Tukey test showed that the increase in WLB in 0.13 mg L\textsuperscript{-1} Cu was significantly higher than that in 0.025 and 4.58 mg L\textsuperscript{-1} Cu exposed plants (p<0.05) but not than that in control (p>0.05) on day 24, 27 and 30 of the exposure.

Overall changes in the WLB at the end of 30 day were found to be significant as revealed by one way ANOVA (F=21.034, p<0.001). Multiple comparisons with Tukey test showed that WLB in plants exposed to 0.025 and 4.58 mg L\textsuperscript{-1} Cu were significantly different from that in control (p=0.026) and in 0.13, 0.25 and 2.54 mg L\textsuperscript{-1} Cu exposed plants (p<0.05). The test further revealed that the increase in 0.13 mg L\textsuperscript{-1} Cu was significantly higher than that in control (p<0.001) and than that in 0.025 - 0.08 mg L\textsuperscript{-1} and 0.25 - 4.58 mg L\textsuperscript{-1} Cu exposed plants (p<0.001).
6. Effects on fresh weight

The changes in fresh weight (FW) in control and plants exposed to different concentrations of Cu (0.025 - 4.58 mg L\(^{-1}\)) at the end of 30 days are shown in Fig 8. At the end of 30 days, FW increased in control and in plants exposed to 0.08 and 0.13 mg L\(^{-1}\) Cu while those exposed to 0.025, 0.25 - 4.58 mg L\(^{-1}\) Cu showed reduction in FW from the initial FW values ranging from 1.231 - 0.133 g. Highest increase in FW of 0.12 g was observed in plants exposed to 0.13 mg L\(^{-1}\) Cu, followed by 0.023 g in plants exposed to 0.08 mg L\(^{-1}\) Cu, and then by control plants with 0.004 g. Maximum reduction of 1.231 g was observed in 4.58 mg L\(^{-1}\) Cu, followed by 0.246 g in 0.25 mg L\(^{-1}\) Cu exposed plants. One way ANOVA revealed that the differences in the change of FW were statistically significant at the end of 30 day (F=14.760, p<0.001). Multiple comparisons with Tukey test showed that the reduction in FW in 4.58 mg L\(^{-1}\) Cu was significantly different from that in control (p<0.001) and in plants exposed to the other Cu concentrations of 0.025-2.54 mg L\(^{-1}\) (p<0.001). Although highest increment in FW was observed in 0.13 mg L\(^{-1}\) Cu, it was not significantly different from that in control (p=0.993).
Figure 7: Increase in width of leaf blade (WLB) in control and Cu exposed *I. aquatica* during 30 day exposure.

Figure 8: Change in fresh weight (FW) in control and Cu exposed *I. aquatica* at the end of 30 day exposure. The values in x-axis are in mg L\(^{-1}\).
II. Nickel

1. Effects on Shoot height

The changes in SH in control and in plants exposed to different concentrations of Ni (0.02 - 7.14 mg L\(^{-1}\)) on day 3, 6, 9, 12, 15, 18, 21, 24, 27 and 30 day of the exposure are shown in Fig 9. SH in *I. aquatica* continued to increase in control plants till the end of the experiment, as well as in plants exposed to 0.02 - 4.02 mg L\(^{-1}\) Ni, but at rates lower than that in the control. However, SH remained the highest in control plants from 6 day onwards. SH in plants exposed to 7.14 mg L\(^{-1}\) Ni increased only up to day 12 of exposure which subsequently got reduced due to stem tissue necrosis and resultant collapse of cells.

On day 3, highest increase in SH of 2.85 cm was observed in control and in plants exposed to 0.02 mg L\(^{-1}\) Ni, followed by 2.8 cm in 4.02 mg L\(^{-1}\) Ni exposed plants, with the lowest of 0.4 cm in 0.22 mg L\(^{-1}\) Ni. One way ANOVA revealed that the differences in the increase in SH were statistically significant (F=5.362, p=0.005). Multiple comparisons with Tukey test showed that the increase in SH in 0.22 mg L\(^{-1}\) Ni was significantly lower than that in control (p=0.006) and that in 0.02 and 4.02 mg L\(^{-1}\) Ni exposed plants (p<0.05).

On day 6, highest increase in SH of 5.9 cm was in control plants, and lowest of 0.9 cm in 0.22 mg L\(^{-1}\) Ni exposed plants. The remaining Ni exposed plants increased SH at the range of 1.8 - 4.75 cm. One way ANOVA revealed that the differences in the increase of SH on day 6 were significantly different (F=9.155, p<0.001). Multiple comparisons with Tukey test showed that the increase in SH in control was significantly higher than that in 0.11, 0.22, 4.02 and 7.14 mg L\(^{-1}\) Ni treatment.

On day 9, plants exposed to 0.22 mg L\(^{-1}\) Ni had the highest SH increase from 0.9 to 10.9 cm, while the lowest increase of 3.15 cm was in 7.14 mg L\(^{-1}\) Ni exposed plants. Increase in SH
of 23.6 and 25.7 cm on day 9 and 12, respectively, was seen in control plants. One way ANOVA revealed that the differences in the increase in SH were statistically significant on day 9 (F=8.516, p=0.001), and day 12 (F=7.629, p=0.001). Multiple comparisons with Tukey test showed that the increase in SH in control was significantly higher from those in plants exposed to all the Ni exposure concentrations (p<0.05) on both day 9 and 12.

On day 15, reduction in SH of 1.6 cm was observed in 7.14 mg L\textsuperscript{-1} Ni; plants exposed to 0.02 - 4.02 mg L\textsuperscript{-1} Ni increased SH in the range of 10 - 14.45 cm; while control plants had an SH increase of 25.85 cm. One way ANOVA showed that the differences in SH on day 15 were significant (F=6.091, p=0.003). Multiple comparisons with Tukey test showed that the reduction in SH in 7.14 mg L\textsuperscript{-1} Ni was significantly different from those of the control (p=0.001) and of 4.02 mg L\textsuperscript{-1} Ni exposed plants (p=0.045). The test further revealed that increase in SH in 0.11 mg L\textsuperscript{-1} Ni was significantly lower than that of control plants (p=0.049).

A reduction in SH in plants exposed to 4.02 mg L\textsuperscript{-1} from 5.95 to 5 cm due to stem tissue necrosis was observed on day 18, although this reduction was not statistically significant. Highest SH increment of 26.2 cm was observed in control plants on day 18, while the lowest increment of 10.5 cm was in 0.11 mg L\textsuperscript{-1} Cu treated plants. The difference in the changes of SH was significant as revealed by one way ANOVA on day 18 (F=3.897, p=0.017), day 21 (F=4.001, p=0.015) and on day 24 (F=4.070, p=0.014). Multiple comparisons with Tukey test showed that the reduction in SH in 7.14 mg L\textsuperscript{-1} Ni was significant when compared to control (p=0.004) on day 18, 21 and 24, but not in comparison with 0.02 - 4.02 mg L\textsuperscript{-1} Ni exposed plants.

On day 27, plants exposed to Ni concentrations: 0.02 - 4.02 mg L\textsuperscript{-1} continued to increase SH at the range of 10.9 - 14.85 cm while those exposed to 7.14 mg L\textsuperscript{-1} Ni further reduced the
SH by 6.1 cm. The differences in the change of SH on day 27 was significant as revealed by one way ANOVA (F=6.247, p=0.002). Multiple comparisons with Tukey test showed that the reduction in SH in 7.14 mg L\(^{-1}\) was significant when compared to control (p=0.001) and to plants exposed to Ni concentrations of 0.22, 2.23 and 4.02 mg L\(^{-1}\) (p<0.05).

On day 30, plants exposed to 0.02 mg L\(^{-1}\) Ni further increased SH by 12.1 cm and those exposed to 7.14 mg L\(^{-1}\) Ni further reduced by 7.05 cm. One way ANOVA showed that the differences in the change of SH were significant (F=6.264, p=0.002). Multiple comparisons with Tukey test revealed that the reduction in SH in 7.14 mg L\(^{-1}\) Ni was significant when compared to control (p=0.001) and to plants treated with 0.02, 0.22, 2.23 and 4.02 mg L\(^{-1}\) Ni (p<0.05).

Overall changes in SH in control and plants exposed to different concentrations of Ni at the end of 30 day were statistically significant (F=27.672, p<0.001). Multiple comparisons with Tukey test revealed that the reduction in SH in 7.14 mg L\(^{-1}\) Ni was significant when compared to control (p<0.001) and to the other Ni exposed plants (p<0.001). The test further revealed that the increase in SH in control was significantly higher than that in plants exposed to 0.02 - 4.02 mg L\(^{-1}\) Ni exposed plants (p<0.001).

2. Effects on number of new nodes

The changes in NN in control and Ni (0.02 - 7.14 mg L\(^{-1}\)) exposed plants on day 3, 6, 9, 12, 15, 18, 21, 24, 27 and 30 day of the exposure are shown in Fig 10. NN continued to increase in control and plants exposed to Ni concentrations of 0.02 - 4.02 mg L\(^{-1}\) throughout the experiment. NN remained the highest in control plants from 9 day onwards. On the contrary, plants exposed to 7.14 mg L\(^{-1}\) Ni showed reduction in NN from day 15 onwards due to stem tissues necrosis.
On day 3, plants exposed to 0.02 mg L\(^{-1}\) Ni produced highest NN of 2 followed by control and 7.14 mg L\(^{-1}\) Ni exposed plants with 1.5 new nodes, while those exposed to 0.22 mg L\(^{-1}\) Ni produced no new nodes. One way ANOVA showed that the differences in the appearance of NN on day 3 were significant (F=5.250, p=0.005). Multiple comparisons with Tukey test showed that the increase in NN in 0.02 mg L\(^{-1}\) Ni was significantly higher than that in 0.11, and 2.23 mg L\(^{-1}\) Ni exposed plant (p<0.05), but not than that in control (p=0.903). The test also revealed that NN in 0.22 mg L\(^{-1}\) Ni was significantly different from that of the control (p=0.048) and from those of 0.02 and 7.14 mg L\(^{-1}\) Ni exposed plants (p<0.05).

On day 6, plants exposed to Ni concentrations of 0.02 - 4.02 mg L\(^{-1}\) continued to increase NN in the range of 3.5 - 0.5. However, no increase was recorded in 7.14 mg L\(^{-1}\) Ni exposed plants. One way ANOVA revealed that the differences in the appearance of NN were statistically significant (F=6.500, p=0.002). Multiple comparisons with Tukey test showed that the increase in NN in 0.02 mg L\(^{-1}\) Ni was significantly higher than that in 0.11, 0.22 and 2.23 mg L\(^{-1}\) Ni exposed plants (p<0.05) but not than that in control (p=0.980). The increase in NN in 0.22 mg L\(^{-1}\) Ni was significantly lower than that in control (p=0.016), and in 0.02 mg L\(^{-1}\) Ni exposed plants (p=0.004) as revealed by Tukey test.

On day 9, control plants produced the highest NN of 7, followed by 2.23 and 4.02 mg L\(^{-1}\) Ni with NN values of 3 and 3.5, while the lowest value of 2 was in 7.14 mg L\(^{-1}\) Ni exposed plants. One way ANOVA revealed that the differences in the increase in NN were significantly different (F=5.282, p=0.005). The increase in NN in control was significantly higher than that in 2.23, 4.02 and 7.14 mg L\(^{-1}\) Ni exposed plants (p<0.05) as revealed by multiple comparisons with Tukey test.
On day 12, plants exposed to 7.14 mg L$^{-1}$ Ni produced the lowest NN of 2 only. One way ANOVA revealed that the differences in the increase in NN were significant (F=4.128, p=0.014). Multiple comparisons with Tukey test showed that the increase in NN in 7.14 mg L$^{-1}$ Ni was significantly lower than that in the control (p=0.005).

On day 15, plants exposed to 7.14 mg L$^{-1}$ Ni started showing reduction in NN due to stem tissue necrosis and collapse of cells which continued till the end of the experiment. One way ANOVA revealed that the differences in the change in NN on day 15 was significantly different (F=6.810, p=0.002). Multiple comparisons with Tukey test showed that the reduction in NN was significantly different from that in the control (p<0.001) and from that in 0.02, 0.11, 0.22 and 4.02 mg L$^{-1}$ Ni exposed plants (p<0.05).

On day 18, plants exposed to 7.14 mg L$^{-1}$ Ni showed NN reduction of 1. One way ANOVA revealed that the differences in the changes in NN on day 18 were significantly different (F=6.370, p=0.002). Multiple comparisons with Tukey test showed that the reduction in NN in 7.14 mg L$^{-1}$ Ni was significantly lower than control (p=0.001) and from plants in 0.02, 0.22 and 4.02 mg L$^{-1}$ Ni treatment (p<0.05).

On day 21 and 24, NN in 4.02 mg L$^{-1}$ Ni reduced from 6 to 4 due to stem tissue necrosis and resultant collapse, although no further reduction was observed till the end of the experiment. One way ANOVA revealed that the differences in the appearance of NN were significant on day 21(F=3.786, p=0.019), and on day 24 (F=3.500, p=0.025). Multiple comparisons with Tukey test showed that the reduction in NN in 7.14 mg L$^{-1}$ Ni was significantly different from the control value (p<0.05) on day 21 and 24.

On day 27, further reductions in NN ranging from 1 to 2.5 in 7.14 mg L$^{-1}$ Ni was observed while plants exposed to 0.02 - 2.23 mg L$^{-1}$ Ni continued to increase their NN. One way
ANOVA revealed that the reduction in 7.14 mg L\(^{-1}\) Ni on day 27 was significantly different from that in the control (p<0.001) and from that in plants in 0.02 - 4.02 mg L\(^{-1}\) Ni (p<0.05).

On the last day of exposure i.e. 30 day, plants exposed to 0.22 and 4.02 mg L\(^{-1}\) Ni showed increase in NN from 5 to 5.5 and from 4.5 to 5 but were lower than that of control (7.5). Those exposed to 0.02, 0.11 and 2.23 mg L\(^{-1}\) Ni showed no further increase in NN. One way ANOVA revealed that the differences in the change in NN on day 30 were significantly different (F=4.723, p=0.008). Multiple comparisons with Tukey test showed that the reduction in NN was significantly different from that in the control (p=0.003) and from plants exposed to Ni concentrations of 0.22 and 4.02 mg L\(^{-1}\) (p<0.05).

Overall changes in NN at the end of 30 day were found to be statistically significant as revealed by one way ANOVA (F=24.881, p<0.001). Multiple comparisons with Tukey test showed that the increase in NN in control was significantly higher than that in all the plants exposed to 0.02 - 7.14 mg L\(^{-1}\) Ni (p<0.001). The test further revealed that the reduction in NN in 7.14 mg L\(^{-1}\) Ni treatment was significantly different from control (p<0.001) and from those in the other Ni treatments of 0.02 - 4.02 mg L\(^{-1}\) (p<0.001).
Figure 9: Changes in shoot height (SH) in control and Ni exposed *I. aquatica* during 30 day exposure. * Significant difference from corresponding value in control at p<0.05.

Figure 10: Changes in number of new nodes (NN) in control and Ni exposed *I. aquatica* during 30 day exposure. * Significant difference from corresponding value in control at p<0.05.
3. Effects on number of new leaf

The increase in NL in control and plants exposed to different concentrations of Ni (0.02 - 7.14 mg L⁻¹) on day 3, 6, 9, 12, 15, 18, 21, 24, 27 and 30 day of the exposure are shown in Fig 11. NL increased in all the Ni exposed plants till the end of the experiment but at rates lower than that in control.

On day 3, appearance of NL was observed in control and in plants exposed to all the Ni concentrations with the exception of 2.23 and 7.14 mg L⁻¹ Ni. The highest appearance of NL of 1 was observed in 0.02 mg L⁻¹ Ni treatment while control plants showed the appearance of NL of 0.5. However one way ANOVA revealed that the differences in the appearance of NL on day 3 were not significant (F=1.250, p=0.340).

On day 6, NL increased from 0.5 to 2 in control plants, while those exposed to 2.23 and 7.14 mg L⁻¹ Ni bore no NL. One way ANOVA revealed that the differences in the appearance of NL were significantly different (F=4.700, p=0.008). Multiple comparisons with Tukey test showed that the increase in NL in control was significantly higher than that of 2.23 and 7.14 mg L⁻¹ Ni treated plants (p<0.05).

On day 9, control plants and all the Ni exposed plants produced new leaves with highest NL of 5 in control, and lowest NL of 0.5 in 7.14 mg L⁻¹ Ni. The differences in the appearance of NL on day 9 were statistically significant as revealed by one way ANOVA (F=6.920, p=0.001). Multiple comparisons with Tukey test showed that the increase in NL in control was significantly higher than that in 2.23 and 7.14 mg L⁻¹ Ni exposed plants (p<0.05).

On day 12, all Ni exposed plants continued to produce NL but lower than the control with lowest NL of 1 in 7.14mg L⁻¹ Ni exposed plants, respectively. One way ANOVA showed that the differences in the increase of NL on day 12 were significant (F=5.037, p=0.006). Multiple
comparisons with Tukey test revealed that the increase in NL in control was significantly higher than that in 2.23 and 7.14 mg L\(^{-1}\) Ni treated plants (p<0.05).

On day 15, increase in NL was observed in control plants and those plants exposed to Ni concentrations of 0.02 - 4.02 mg L\(^{-1}\), while in those exposed to 7.14 mg L\(^{-1}\) Ni no further appearance of NL was observed till the end of the experiment. One way ANOVA revealed that the differences in the appearance of NL on day 15 were statistically significant (F=3.717, p=0.020). Multiple comparisons with Tukey test showed that the increase in NL in control was significantly higher than that in 7.14 mg L\(^{-1}\) Ni treatment (p=0.010).

On day 18, 21, 24 and 27, control plants had the highest NL of 6 followed by NL of 5 in 4.02 mg L\(^{-1}\), and lowest of 1 in 7.14 mg L\(^{-1}\) Ni exposed plants. One way ANOVA revealed that the differences in the appearance of NL was significantly different on day 18, 21, 24 and 27 (F=4.723, p=0.008). Multiple comparisons with Tukey test showed that the increase in 7.14 mg L\(^{-1}\) Ni was significantly lower than that in the control (p=0.005) and from that in 4.02 mg L\(^{-1}\) Ni exposed plants (p=0.026) on day 18, 21, 24 and 27 of exposure.

On day 30, control plants continued to have the highest NL of 6. Lowest NL of 1 was in 7.14 mg L\(^{-1}\) Ni exposed plants. One way ANOVA revealed that the differences in the appearance of NL on day 30 were statistically significant (F=6.784, p=0.002). Multiple comparisons with Tukey test showed that the increase in NL in 7.14 mg L\(^{-1}\) Ni was significantly lower than that in the control (p<0.05) and from 0.22 and 4.02 mg L\(^{-1}\) Ni treated plants (p<0.05). The test further revealed that the increase in 2.23 mg L\(^{-1}\) Ni was also significantly lower than that of control (p=0.028).

Overall differences in the increase in NL at the end of 30 day were statistically significant as revealed by one way ANOVA (F=20.336, p<0.001). Multiple comparisons with Tukey test
showed that the increase in NL in control was significantly higher than that in Ni exposed plants in concentrations of 0.02 - 2.23 mg L\(^{-1}\) and 7.14 mg L\(^{-1}\) Ni \((p<0.001)\), but not than that in 4.02 mg L\(^{-1}\) Ni \((p=0.186)\).

4. Effects on tiller number

The increase in TN in control and Ni \(0.02 - 7.14\) mg L\(^{-1}\) exposed plants on day 3, 6, 9, 12, 15, 18, 21, 24, 27 and 30 of exposure are shown in Fig 12. TN in control plants increased throughout the experiment. Plants exposed to Ni concentrations of 0.02 - 4.02 mg L\(^{-1}\) Ni also increased the TN till the end of the experiment but at rates lower than that of the control. Plants exposed to 7.14 mg L\(^{-1}\) Ni produced no new tiller during the experiment.

On day 3 and 6, maximum TN of 1.5 was observed in control plants followed by 0.5 TN in 0.02 mg L\(^{-1}\) Ni exposed plants while other Ni exposed plants produced no new tiller. One way ANOVA revealed that the differences in the appearance of TN were significant on day 3 and 6 \((F=13.500, p<0.001)\). Multiple comparisons with Tukey test showed that the increase in TN in control was significantly higher than that in plants exposed to Ni concentrations of 0.02 - 7.14 mg L\(^{-1}\) \((p<0.05)\) on day 3 and 6.

On day 9, besides control and 0.02 mg L\(^{-1}\) Ni exposed plants, those exposed to 0.11 and 2.23 mg L\(^{-1}\) Ni also produced TN of 0.5. However, on day 12, TN increased from 1.5 to 2 in control and from 0.5 to 1 in 2.23 mg L\(^{-1}\) Ni exposed plants. One way ANOVA revealed that the differences in the appearance of TN were significant on day 9 \((F=6.000, p=0.003)\) and on day 12 \((F=7.500, p=0.001)\). Multiple comparisons with Tukey test showed that the increase in TN in control plants on day 9 was significantly higher than that in 0.22, 4.02 and 7.14 mg L\(^{-1}\) Ni treatment \((p=0.004)\). However, increase in TN in control on day 12 was significantly higher
than that in 0.02 - 0.22 and 4.02 - 7.14 mg L\(^{-1}\) Ni exposed plants (p<0.05) but not than that in 2.23 mg L\(^{-1}\) Ni (p=0.184).

On day 15, plants exposed upto Ni concentration 2.23 mg L\(^{-1}\) produced new tiller but at rates lower than control plants, while those exposed to 4.02 and 7.14 mg L\(^{-1}\) Ni produced no new tiller on day 15, 18 and 21 of exposure. The differences in the appearance of TN were significant on day 15 and 18 (F=4.000, p=0.015) and also on day 21 (F=4.364, p=0.021) as revealed by one way ANOVA. Multiple comparisons with Tukey test showed that the absence of tiller in 4.02 and 7.14 mg L\(^{-1}\) Ni treated plants was significantly different from that in control on day 15, 18 and 21 (p<0.05).

On day 24, TN in control plants further increased to 2.5, followed by TN of 1.5 in plants exposed to 4.02 mg L\(^{-1}\) Ni. One way ANOVA revealed that the differences in the appearance of TN on day 24 were significantly different (F=4.538, p=0.009). Multiple comparisons with Tukey test showed that the increase in TN in control plants was significantly higher than those in 0.11, 0.22, 4.02 and 7.14 mg L\(^{-1}\) Ni exposed plants (p<0.05).

On day 27 and 30, further increase in TN was observed in control and Ni exposed plants at concentrations of 0.02 - 4.02 mg L\(^{-1}\). One way ANOVA revealed that the differences in appearance of TN were not significant on day 27 (F=2.810, p=0.052) and on day 30 (F=2.593, p=0.067).

Overall changes in the appearance of TN at the end of day 30 were statistically significant as revealed by one way ANOVA (F=28.419, p<0.001). Multiple comparisons with Tukey test showed that the increase in TN in control was significantly higher than that in all the Ni exposed plants (p<0.001).
Figure 11: Increase in number of new leaf (NL) in control and Ni exposed *I. aquatica* during 30 day exposure. * Significant difference from corresponding value in control at p<0.05.

Figure 12: Increase in tiller number (TN) in control and Ni exposed *I. aquatica* during 30 day exposure. * Significant difference from corresponding value in control at p<0.05.
5. Effects on width of leaf blade

The increase in WLB in control and in plants treated with different concentrations of Ni (0.02 - 7.14 mg L\(^{-1}\)) on day 3, 6, 9, 12, 15, 18, 21, 24, 27 and 30 day of exposure are shown in Fig 13. WLB remained unchanged in control and all the Ni exposed plants on day 3, 6 and 9 day of the exposure.

On day 12, highest increase in WLB was observed in 0.22 mg L\(^{-1}\), although it did not increase in those exposed to 7.14 mg L\(^{-1}\) Ni. One way ANOVA revealed that the differences in the change of WLB was significant (F=4.500, p=0.010). Multiple comparisons with Tukey test showed that the increase in 0.22 mg L\(^{-1}\) Ni treatment was significantly higher than that in 4.02 and 7.14 mg L\(^{-1}\) Ni (p<0.05) but not than that in control (p=0.527).

On day 15 also, those exposed to 0.22 mg L\(^{-1}\) Ni showed highest WLB of 0.125 cm. However, one way ANOVA revealed that the differences in WLB were not significant as revealed by one way ANOVA (F=2.167, p=0.109).

On day 18 and 21, plants exposed to 0.22 mg L\(^{-1}\) showed the highest WLB, while those in 2.23 mg L\(^{-1}\) Ni had the lowest WLB increment of 0.05 cm. One way ANOVA revealed that the differences in the changes in WLB were significant on day 18 and 21 (F=6.200, p=0.002). Multiple comparisons with Tukey test revealed that the increase in 0.22 mg L\(^{-1}\) Ni treatment was significantly higher than that in 2.23 mg L\(^{-1}\) Ni exposed plants (p=0.034) but not than that in control (p=0.989) on day 18 and 21.

On day 24, the highest increase in WLB of 0.21 cm was in control plants, followed by 0.18 cm in 0.22 mg L\(^{-1}\) Ni, and then by 0.13 cm in 0.11 mg L\(^{-1}\) Ni exposed plants. The differences in the increment of WLB on day 24 were significant as revealed by one way ANOVA (F=11.246, p<0.001). Multiple comparisons with Tukey test showed that the increase in WLB in control
plants was significantly higher than that in 0.02 and 2.23 - 7.14 mg L\(^{-1}\) Ni exposed plants (p<0.05).

On day 27 and 30, WLB remained highest in control, followed by that in 0.22 mg L\(^{-1}\). One way ANOVA revealed that the differences in the change in WLB were significant on day 27 and 30 (F=10.714, p<0.001). Multiple comparisons with Tukey test showed that the increase in WLB in 0.02, 2.23 - 7.14 mg L\(^{-1}\) Ni exposed plants was significantly lower than that in control (p<0.05) and 0.22 mg L\(^{-1}\) Ni exposed plants (p<0.05) on day 27 and 30 of exposure. The test further revealed that WLB in 7.14 mg L\(^{-1}\) Ni was significantly different from that in control (p<0.001) and from those in 0.11 - 0.22 mg L\(^{-1}\) Ni exposed plants (p<0.05).

Overall changes in WLB at the end of 30 days were found to be statistically significant as revealed by one way ANOVA (F=38.386, p<0.001). Multiple comparisons with Tukey test showed that increase in WLB in control and in 0.22 mg L\(^{-1}\) Ni exposed plants were not different from each other, but significantly higher than that in 0.02 - 0.11, and 2.23 - 7.14 mg L\(^{-1}\) Ni treated plants (p<0.05).

6. Effects on fresh weight

The changes in the fresh weight (FW) in control and plants exposed to different concentrations of Ni (0.02 - 7.14 mg L\(^{-1}\)) at the end of 30 days are shown in Fig 14. FW increased maximally in control plants followed by plants exposed to different concentrations of Ni (0.02-4.02 mg L\(^{-1}\)), but those exposed to 7.14 mg L\(^{-1}\) Ni treatment showed reduction in FW from their initial FW. One way ANOVA revealed that the changes in FW at the end of 30 day exposure were statistically significant (F=37.450, p<0.001). Multiple comparisons with Tukey test showed that the increase in FW in control was significantly higher than that in 0.11 and
4.02 - 7.14 mg L\(^{-1}\) Ni treatment, but not than those in 0.02, 0.22 and 2.23 mg L\(^{-1}\) Ni exposed plants.

![Graph](image)

Figure 13: Increase in width of leaf blade (WLB) in control and Ni exposed *I. aquatica* during the 30 day exposure. * Significant difference from corresponding value in control at p<0.05.

![Graph](image)

Figure 14: Change in fresh weight (FW) in control and Ni exposed *I. aquatica* at the end of 30 day exposure. The values in x-axis are in mg L\(^{-1}\).
C. CHRONIC TOXIC EFFECTS ON BIOCHEMICAL ACTIVITIES

I. Copper

1. Changes in Chlorophyll content

Changes in chlorophyll \( a \), chlorophyll \( b \) and total chlorophyll content of control and plants exposed to different concentrations of Cu (0.025 - 4.58 mg L\(^{-1}\)) at the end of 30 day exposure are shown in Figures 15, 16 and 17. Chlorophyll \( a \), chlorophyll \( b \) and total chlorophyll content decreased in all concentrations of Cu treatment. Highest chlorophyll \( a \) content of 0.46 mg g\(^{-1}\) was found in control plants whereas the lowest of 0.25 mg g\(^{-1}\) was found in 0.025 mg L\(^{-1}\) Cu. Similar results were also obtained in case of chlorophyll \( b \) and total chlorophyll content with control plants having the highest concentrations of 0.23 and 0.69 mg g\(^{-1}\), while the lowest concentrations of 0.17 and 0.42 mg g\(^{-1}\) were found in plants treated with 0.025 mg L\(^{-1}\) Cu. One way ANOVA revealed that the differences in chlorophyll content were not significant for chlorophyll \( a \) (F=0.462, p=0.832), chlorophyll \( b \) (F=0.169, p=0.984) and total chlorophyll (F=0.319, p=0.923) at the end of the 30 day experiment.

Chlorophyll ratio (Chlorophyll \( a/b \) ratio) of control and Cu exposed plants are shown in Fig 18. Chlorophyll \( a/b \) ratio decreased in all the Cu treated plants with the lowest ratio of 1.4 mg g\(^{-1}\) in 0.13 mg L\(^{-1}\) Cu followed by 1.5 in 0.025 mg L\(^{-1}\) Cu. Highest chlorophyll ratio was observed in control plants with a value of 2.01 followed by 1.9 at 4.58 mg L\(^{-1}\) Cu treatment. However, one way ANOVA revealed that the differences among the chlorophyll ratios were not statistically significant (F=2.323, p=0.054).
Figure 15: Chlorophyll $a$ content in control and Cu exposed $I. \ aquatica$ at the end of 30 day exposure.

Figure 16: Chlorophyll $b$ content in control and Cu exposed $I. \ aquatica$ at the end of 30 day exposure.
Figure 17: Total chlorophyll content in control and Cu exposed *I. aquatica* at the end of 30 day exposure.

Figure 18: Chlorophyll *a/b* ratio in control and Cu exposed *I. aquatica* at the end of 30 day exposure.
2. Changes in protein content

The changes in protein content in stem and leaf tissues of control and Cu (0.025 - 4.58 mg L\(^{-1}\)) treated \(I.\) \(aquatica\) plants at the end of 30 day exposure are shown in Fig 19 and 20. In plants exposed to 2.54 and 4.58 mg L\(^{-1}\), changes in protein content in stem and leaf tissues were observed only up to day 9 and 3 due to the appearance of necrosis thereafter. Since necrosis set in after 30 day of exposure in plants exposed to 0.25 mg L\(^{-1}\) Cu, protein content in both stem and leaf tissues could be observed up to 30 day of exposure. In case of stem tissue, plants exposed to Cu concentrations of 0.025 - 0.13 mg L\(^{-1}\) showed slightly higher protein concentrations in the range of 1.12 - 1.15 mg g\(^{-1}\) when compared to that in control (1.11 mg g\(^{-1}\)). Plants exposed to 0.25 - 4.58 mg L\(^{-1}\) had reductions in protein concentrations of 0.62 - 1.04 mg g\(^{-1}\) when compared to control. Increases in protein concentrations from that in control in plants exposed to Cu concentrations of 0.08 - 0.13 mg L\(^{-1}\) were 3.3% in 0.13 mg L\(^{-1}\) Cu exposed plants, followed by 2.46% in 0.08 mg L\(^{-1}\) Cu at the end of 30 day exposure. Plants exposed to 0.25, 2.54 and 4.58 mg L\(^{-1}\) Cu showed reduction of 6, 26.2 and 44.2% from that in control. However, the differences were not statistically significant as revealed by one way ANOVA (F=1.984, p=0.076).

Leaf tissues of plants exposed to 0.025 - 0.25 mg L\(^{-1}\) Cu showed increase in protein content of 1.64 - 2.04 mg g\(^{-1}\) from that in the control (1.63 mg g\(^{-1}\)). Leaf tissues of plants exposed to 2.54 and 4.58 mg L\(^{-1}\) Cu showed reduction in protein content (1.2 and 1.4 mg g\(^{-1}\)) as compared to control. At the end of 30 day highest protein content in the leaf tissues was observed in 0.13 mg L\(^{-1}\) Cu exposed plants (2.04 mg g\(^{-1}\)) while lowest (1.2 mg g\(^{-1}\)) was in 2.54 mg L\(^{-1}\). One way ANOVA revealed that the differences in protein content in the leaf tissues were significantly different (F=2.813, p=0.015). Multiple comparisons with LSD test showed that the increase in
protein content in plants exposed to 0.13 mg L\(^{-1}\) Cu was significantly higher than that in control (p=0.031) and in 0.025, 2.54 and 4.58 mg L\(^{-1}\) Cu exposed plants (p<0.05).

Figure 19: Protein content in stem tissues of control and Cu exposed \(I.\ aquatica\) at the end of 30 day exposure.

Figure 20: Protein content in leaf tissues of control and Cu exposed \(I.\ aquatica\) at the end of 30 day exposure. * Significant difference from corresponding value in control at p<0.05.
3. Changes in total carbohydrates content

The changes in the total carbohydrates concentrations in control and Cu (0.025 - 4.58 mg L$^{-1}$) exposed plants in the stem tissues of *I. aquatica* are shown in Fig 21. In this biochemical analysis, plants exposed to 2.54 and 4.58 mg L$^{-1}$ Cu showed the appearance of necrosis after day 18 and 9 of the exposure, so the changes in the carbohydrates content could be observed up to day 18 and 9 of exposure for both stem and leaf tissues. However, in plants exposed to 0.25 mg L$^{-1}$ Cu, necrosis set in after 30 day of exposure, so carbohydrates content in both stem and leaf tissues could be observed up to 30 day of exposure. Plants exposed to 0.08 and 0.13 mg L$^{-1}$ was found to have higher carbohydrates content (5.9 and 5.7 mg g$^{-1}$) than that in control (5.3 mg g$^{-1}$). But those exposed to 0.025, 0.25 - 4.58 mg L$^{-1}$ Cu showed reduction of 10.8 - 38% from that of control. On the contrary, those exposed to 0.08 and 0.13 mg L$^{-1}$ Cu showed increase of 10.8 and 7.2% as compared to control. The differences in the carbohydrates content were statistically significant as revealed by one way ANOVA (F=2.798, p=0.015). Multiple comparisons with LSD test revealed that the carbohydrates content in 2.54 and 4.58 mg L$^{-1}$ Cu were significantly lower than that in control (p<0.05) and that in 0.08 and 0.13 mg L$^{-1}$ Cu treatment plants (p<0.05).

The changes in the total carbohydrates content in the leaf tissues in control and plants exposed to different concentrations of Cu (0.025 - 4.58 mg L$^{-1}$) are shown in Fig 22. In case of leaf tissues plants exposed to 0.025 - 0.13 mg L$^{-1}$ Cu showed total carbohydrates content in the range of 3.12 to 3.74 mg g$^{-1}$ which is more or less similar to control plants (3.21 mg g$^{-1}$). But those exposed to 0.25 - 4.58 mg L$^{-1}$ had lower carbohydrates content (2.9 - 2.5 mg g$^{-1}$) than that in the control. The highest total carbohydrates content in the leaf tissues of *I. aquatica* plants at the end of 30 day was observed in 0.13 mg L$^{-1}$ Cu treated plants with an increase of 14.1%. The
lowest was in 4.58 mg L^{-1} with a reduction of 23% from that in control plants. In spite of reduction in carbohydrates content in 0.25 - 4.58 mg L^{-1} Cu, the differences were not statistically significant as revealed by one way ANOVA (F=1.258, p=0.285).

Figure 21: Total carbohydrates content in stem tissues of control and Cu exposed *I. aquatica* at the end of 30 day exposure. * Significant difference from corresponding value in control at p<0.05.

Figure 22: Total carbohydrates content in leaf tissues of control and Cu exposed *I. aquatica* at the end of 30 day exposure.
4. Changes in ascorbic acid content

The changes in ascorbic acid (AsA) content in the stem tissues in control and in plants exposed to different concentrations of Cu (0.025 - 4.58 mg L\(^{-1}\)) are shown in Fig 23. In this case necrosis set in plants exposed to 2.54 and 4.58 mg L\(^{-1}\) Cu after day 9 and 3 of exposure, so AsA content in both the stem and leaf tissues in these respective concentrations were taken only upto day 9 and 3 of exposure. However, in plants exposed to 0.25 mg L\(^{-1}\) Cu, necrosis set in after 30 day of exposure, enabling AsA analysis in both stem and leaf tissues up to 30 day of exposure. AsA content in stem tissues increased in all the Cu treated plants with highest increase of 0.0098 mg g\(^{-1}\) in 0.025 mg L\(^{-1}\) Cu exposed plants, and lowest increase of 0.0087 mg g\(^{-1}\) in 0.25 mg L\(^{-1}\) Cu exposed plants as compared to that in control plants (0.006 mg g\(^{-1}\)). However, the differences were not statistically significant as revealed by one way ANOVA (F=0.239, p=0.963).

The changes in AsA content in the leaf tissues in control and in plants exposed to different concentrations of Cu (0.025 - 4.58 mg L\(^{-1}\)) are shown in Fig 24. In case of leaf tissues also, Cu induced neither stimulatory nor inhibitory effects on AsA content of \textit{I. aquatica} plants as compared to control as shown in Fig 24. Unlike stem tissues, highest AsA content of 0.014 mg g\(^{-1}\) was observed in 4.58 mg L\(^{-1}\) and lowest of 0.006 mg g\(^{-1}\) in 0.13 mg L\(^{-1}\) Cu exposed plants. Control plants and those exposed to 0.025 - 0.08 and 0.25 - 2.54 mg L\(^{-1}\) Cu showed AsA content of 0.0098 - 0.012 mg g\(^{-1}\). One way ANOVA revealed that the differences in the AsA content were not statistically significant (F=0.941, p=0.481).
Figure 23: Ascorbic acid (AsA) content in stem tissues of control and Cu exposed *I. aquatica* at the end of 30 day exposure.

Figure 24: Ascorbic acid content (AsA) in leaf tissues of control and Cu exposed *I. aquatica* at the end of 30 day exposure.
II. Nickel

1. Changes in chlorophyll content

Changes in chlorophyll $a$, chlorophyll $b$ and total chlorophyll content in control and Ni exposed plants (0.02 - 7.14 mg L$^{-1}$) at the end of 30 day exposure are shown in Figures 25, 26 and 27. In the present study, Ni did not induce either stimulatory or inhibitory effect on chlorophyll $a$, chlorophyll $b$ and total chlorophyll content of *I. aquatica* plants. Since plants exposed to Ni concentration of 7.14 mg L$^{-1}$ bore no leaf on 30th day of exposure due to the occurrence of necrosis, chlorophyll content was analyzed upto 18 day. Plants exposed to 0.11 mg L$^{-1}$ showed reduction of 28.8, 23.32 and 26.9 percent in chlorophyll $a$, chlorophyll $b$ and total chlorophyll content, respectively, compared to the control plants. On the other hand, increase of 8.04, 3.3 and 6.5 per cent from that of the control was observed in chlorophyll $a$, chlorophyll $b$ and total chlorophyll content in plants exposed to Cu concentration of 2.23 mg L$^{-1}$. However one way ANOVA revealed that the differences in chlorophyll content were not significant for chlorophyll $a$ (F=0.472, p=0.824), chlorophyll $b$ (F=0.364, p=0.896) and total chlorophyll content (F=0.409, p=0.868).

Ni spiking increased the chlorophyll ratio (chlorophyll $a/b$ ratio) by 4.4, 4.5 and 11.7% in 0.22, 2.23 and 4.02 mg L$^{-1}$ from those of control (Fig 28). At the same time, reduction of 5.3, 13.2 and 6.8% also took place in 0.02, 0.11 and 7.14 mg L$^{-1}$ Ni treated plants. One way ANOVA revealed that the differences in chlorophyll $a/b$ ratio were not statistically significant (F=1.549, p=0.192).
Figure 25: Chlorophyll $a$ content in control and Ni exposed $I. aquatica$ at the end of 30 day exposure.

Figure 26: Chlorophyll $b$ content in control and Ni exposed $I. aquatica$ at the end of 30 day exposure.
Figure 27: Total chlorophyll content in control and Ni exposed *I. aquatica* at the end of 30 day exposure.

Figure 28: Chlorophyll *a/b* ratio in control and Ni exposed *I. aquatica* at the end of 30 day exposure.
2. Changes in protein content

The changes in the protein content in the stem tissues in control and Ni exposed plants at concentrations of 0.02 - 7.14 mg L\(^{-1}\) at the end of 30 day are shown in Fig 29. In this study Ni induced inhibitory effects on protein content of \(I.\ aquatica\) stem tissue. Plants exposed to Ni concentrations of 0.02 - 7.14 mg L\(^{-1}\) had lower protein concentrations ranging from 0.32 to 0.51 mg g\(^{-1}\) compared to 0.61 mg g\(^{-1}\) in control. Plants exposed to 4.02 mg L\(^{-1}\) Ni showed highest reduction of 46.9% followed by 46.3% reduction in 7.14 mg L\(^{-1}\) Ni, with lowest reduction of 16.5% in 0.11 mg L\(^{-1}\) Ni. The differences in the reduction in the protein content in the stem tissues were found to be statistically significant as revealed by one way ANOVA (\(F=4.599, p<0.001\)). Multiple comparisons with Tukey test revealed that the protein content at Ni concentrations 2.23, 4.02 and 7.14 mg L\(^{-1}\) was significantly reduced as compared to control (\(p<0.05\)).

The changes in the protein content in the leaf tissues in control and Ni (0.02 - 7.14 mg L\(^{-1}\)) exposed plants at the end of 30 day are shown in Fig 30. In case of leaf tissues also inhibitory effects of Ni on the content of protein was observed. In this study, control plants had the highest protein content (0.63 mg g\(^{-1}\)) as compared to all the Ni exposed plants (0.02 - 7.14 mg L\(^{-1}\)) which had protein content in the range of 0.31 - 0.54 mg g\(^{-1}\). Plants exposed to 7.14 mg L\(^{-1}\) of Ni showed highest reduction of 50.2% from control, while the lowest of 13.9% decrease from control was in 0.02 mg L\(^{-1}\) Ni exposure. One way ANOVA revealed that the differences in the protein content in the leaf tissues were statistically significant (\(F=3.729, p=0.002\)). Multiple comparisons with Tukey test showed that the protein content in 2.23-7.14 mg L\(^{-1}\) of Ni exposed plants was significantly reduced from those in the control plants (\(p<0.05\)).
Figure 29: Protein content in stem tissues of control and Ni exposed *I. aquatica* at the end of 30 day exposure. * Significant difference from corresponding value in control at p<0.05.

Figure 30: Protein content in leaf tissues of control and Ni exposed *I. aquatica* at the end of 30 day exposure. * Significant difference from corresponding value in control at p<0.05.
3. Changes in total carbohydrates content

The changes in the total carbohydrates content in the stem tissues of control and plants exposed to different concentrations of Ni (0.02 - 7.14 mg L\(^{-1}\)) are shown in Fig 31. Total carbohydrates content was reduced in all the Ni exposed plants. Control plants showed the highest carbohydrates content of 5 mg g\(^{-1}\) and those exposed to 0.02 - 2.23 mg L\(^{-1}\) had carbohydrates in the range of 4.3 - 4.7 mg g\(^{-1}\). Plants exposed to 4.02 - 7.14 mg L\(^{-1}\) Ni showed lower carbohydrates content of 4 and 3.8 mg g\(^{-1}\). The highest reduction of 24.4% was observed in 7.14 mg L\(^{-1}\) Ni and lowest of 5.4% was observed in 0.11 mg L\(^{-1}\) Ni exposed plants. However, the differences were not significant as revealed by one way ANOVA (F=1.172, p=0.327).

The changes in the total carbohydrates content in the leaf tissues in control and Ni (0.02 - 7.14 mg L\(^{-1}\)) exposed plants are shown in Fig 32. Plants exposed to 0.22 mg L\(^{-1}\) of Ni showed higher carbohydrates content of 4.5 mg g\(^{-1}\) when compared to the control (4.2 mg g\(^{-1}\)). Plants exposed to the other Ni treatments showed reduction in carbohydrates content in the range of 3.1 - 3.7 mg g\(^{-1}\) as compared to control. Highest reduction percent of 27.5% was observed in 4.02 mg L\(^{-1}\) of Ni and lowest of 10.1% in 0.11 mg L\(^{-1}\) of Ni exposed plants. One way ANOVA revealed that the differences in the carbohydrates content were statistically significant (F=2.227, p=0.048). Multiple comparisons with LSD test showed that carbohydrates content in 4.02 mg L\(^{-1}\) of Ni was significantly lower than that in control (p=0.040). The test further revealed that carbohydrates content in 0.22 mg L\(^{-1}\) of Ni was significantly higher than that in 2.23 - 7.14 mg L\(^{-1}\) of Ni (p<0.05) but not that in control (p=0.462).
Figure 31: Total carbohydrates content in stem tissues of control and Ni exposed *I. aquatica* at the end of 30 day exposure.

Figure 32: Total carbohydrates content in leaf tissues of control and Ni exposed *I. aquatica* at the end of 30 day exposure. * Significant difference from corresponding value in control at p<0.05.
4. Changes in ascorbic acid content

The changes in ascorbic acid (AsA) content in stem tissues in control and plants exposed to different concentrations of Ni (0.02 - 7.14 mg L\(^{-1}\)) are shown in Fig 33. Ni treatment induced stimulatory effects in the AsA content except in plants exposed to 0.11 mg L\(^{-1}\) (0.0043 mg g\(^{-1}\)) which had lower AsA content when compared to that in the control (0.0059 mg g\(^{-1}\)). Plants exposed to 7.14 mg L\(^{-1}\) of Ni had similar AsA content as that in control (0.0059 mg g\(^{-1}\)). The highest AsA content at the end of 30 day was observed in 4.02 mg L\(^{-1}\) (0.0073 mg g\(^{-1}\)) followed by 2.23 mg L\(^{-1}\) of Ni exposed plants (0.0072 mg g\(^{-1}\)). However, one way ANOVA revealed that the differences were not statistically significant (F=0.642, p=0.697).

The changes in AsA content in the leaf tissues in control and Ni (0.02 - 7.14 mg L\(^{-1}\)) exposed plants are shown in Fig 34. Lowest AsA content in leaf tissues was observed in 0.11 mg L\(^{-1}\) of Ni (0.0043 mg g\(^{-1}\)). Plants exposed to the other Ni treatments showed higher AsA content than that in the control (0.0049 mg g\(^{-1}\)) in the range of 0.005 to 0.0082 mg g\(^{-1}\). Highest AsA content in leaf tissues at the end of 30 day was observed in 4.02 mg L\(^{-1}\) of Ni with 0.0082 mg g\(^{-1}\) followed by 2.23 mg L\(^{-1}\) of Ni exposed plants with 0.0078 mg g\(^{-1}\). The differences were, however, not significant as revealed by one way ANOVA (F=0.704, p=0.648).
Figure 33: Ascorbic acid (AsA) content in stem tissues of control and Ni exposed *I. aquatica* at the end of 30 day exposure.

Figure 34: Ascorbic acid (AsA) content in leaf tissues of control and Ni exposed *I. aquatica* at the end of 30 day exposure.
D. CHRONIC TOXIC EFFECTS ON ENZYME ACTIVITIES

I. Copper

1. Catalase activities

The changes in catalase (CAT) activities in stem tissues of control and Cu (0.025 - 4.58 mg L\(^{-1}\)) exposed plants at the end of 30 day are shown in Fig 35. Plants exposed to 2.54 and 4.58 mg L\(^{-1}\) showed necrosis in the stem tissues due to which CAT activities was taken only upto day 9 and 3 of the experiment, respectively. Cu exposed plants in the other concentrations did not show necrosis till the end of 30 day. Cu treatment resulted in the reduction of CAT activities in the stem tissues with the exception of plants treated to 0.08 mg L\(^{-1}\) which showed a 23.2% increase in activity from that of control. The highest reduction in CAT activities was observed in plants exposed to 4.58 mg L\(^{-1}\) with a reduction percent of 60% from that in control. One way ANOVA revealed that the differences in the CAT activities were statistically significant (F=3.478, p=0.003). Multiple comparisons with LSD test showed that the increase in CAT activity at 0.08 mg L\(^{-1}\) Cu exposed plants was significantly higher than that of plants exposed to 0.025, 0.13, 2.54 and 4.58 mg L\(^{-1}\) (p<0.05) but not than that in control (p=0.174). The test also revealed that CAT activity in plants exposed to 0.13 and 4.58 mg L\(^{-1}\) was significantly lower than that in control (p<0.05).

The changes in CAT activities in leaf tissues in control and Cu (0.025 - 4.58 mg L\(^{-1}\)) exposed plants at the end of 30 days are shown in the Fig 36. In contrast to stem tissues CAT activities in leaf tissues were increased in Cu exposed plants in Cu concentrations of 0.13 - 4.58 mg L\(^{-1}\). But reduction in CAT activities was observed in those exposed to 0.025 and 0.08 mg L\(^{-1}\) of Cu. Maximum increase in CAT activities was observed in plants exposed to 2.54 mg L\(^{-1}\) with an increase percent of 56.7% from that of control. While those exposed to 0.025 and 0.08 mg L\(^{-1}\)
Cu showed reduction percent of 5.1 and 19.1% from control plants. One way ANOVA revealed that the differences in the CAT activities at the leaf tissues were statistically significant (F=3.297, p=0.004). Multiple comparisons with LSD test showed that the increase in CAT activities in 2.54 mg L$^{-1}$ of Cu was significantly higher than that in control (p=0.007) and Cu exposed plants in concentrations of 0.025 - 0.25 and 4.58 mg L$^{-1}$ (p≤0.05).
Figure 35: Catalase (CAT) activities in stem tissues of control and Cu exposed *I. aquatica* at the end of 30 day exposure. * Significant difference from corresponding value in control.

Figure 36: Catalase (CAT) activities in leaf tissues of control and Cu exposed *I. aquatica* at the end of 30 day exposure. * Significant difference from corresponding value in control.
2. Guaiacol Peroxidase activities

Guaiacol Peroxidase (GPOD) activities in stem tissues of control and Cu (0.025 - 4.58 mg L\(^{-1}\)) exposed plants at the end of 30 day are shown in Fig 37. Plants exposed to 2.54 and 4.58 mg L\(^{-1}\) showed necrosis in the stem tissues due to which GPOD activities was taken only upto day 9 and 3 of the experiment. Plants exposed to 2.54 mg L\(^{-1}\) did not show prominent necrosis till the end of 30 day. Treatment by higher concentrations of Cu (0.13 - 4.58 mg L\(^{-1}\)) reduced GPOD activities in stem tissues of *I. aquatica* plants. However, plants exposed to lower concentrations of Cu (0.025 - 0.08 mg L\(^{-1}\)) showed increased activities of GPOD. Increase of 47.5 and 24% was observed in GPOD activities in plants exposed to 0.025 and 0.08 mg L\(^{-1}\) Cu. However, those exposed to 0.13, 0.25, 2.54 and 4.58 mg L\(^{-1}\) Cu showed reduction of 18.3, 15.7, 21.7 and 8.3% in GPOD activities. One way ANOVA revealed that the differences in GPOD activities were significantly different (F=3.260, p=0.006). Multiple comparisons with Tukey test showed that the increase in GPOD activities in 0.025 mg L\(^{-1}\) Cu exposed plants was significantly higher than those in 0.13 - 2.54 mg L\(^{-1}\) Cu treatment at p<0.05.

The changes in GPOD activities in leaf tissues in control and Cu (0.025 - 4.58 mg L\(^{-1}\)) exposed plants at the end of 30 days are shown in Fig 38. In case of leaf tissue reduction in GPOD activities was observed in all Cu exposed plants. Highest reduction of 67.2% was observed in plants exposed to 2.54 mg L\(^{-1}\) Cu followed by 4.58 mg L\(^{-1}\) with 58.6% reduction. Lowest reduction of 12.4% was observed in plants exposed to 0.08 mg L\(^{-1}\), followed by 27.3% in 0.25 mg L\(^{-1}\) Cu exposed plants. One way ANOVA revealed that the reduction in GPOD activities was statistically significant (F=2.283, p=0.043). Multiple comparisons with LSD test showed that the increase in GPOD activities in control plants was significantly higher than 0.025, 0.13, 2.54 and 4.58 mg L\(^{-1}\) Cu treated plants (p<0.05). The test also revealed that
reduction in GPOD activities in 0.025 mg L$^{-1}$ was also significantly lower than that in 0.08 mg L$^{-1}$ Cu exposed plants (p=0.026).
Figure 37: Guaiacol Peroxidase (GPOD) activities in stem tissues of control and Cu exposed *I. aquatica* at the end of 30 day exposure.

Figure 38: Guaiacol Peroxidase (GPOD) activities in leaf tissues of control and Cu exposed *I. aquatica* at the end of 30 day exposure. * Significant difference from corresponding value in control at p<0.05.
II. Nickel

1. Catalase activities

CAT activities in stem tissues in control and Ni (0.02 - 7.14 mg L$^{-1}$) exposed plants at the end of 30 day are shown in Fig 39. In case of stem tissues CAT activity was stimulated under Ni treatment. Highest increase of 48.2% in CAT activity was observed in stem tissues in 0.22 mg L$^{-1}$ of Ni treatment as compared to control. Plants exposed to 0.02 mg L$^{-1}$ showed lowest increase of 25.3% from that in control. Plants exposed to 0.11, 2.23, 4.02 and 7.14 mg L$^{-1}$ showed increased CAT activity in the range of 39 to 40.3% from that in control. The increase in CAT activity was statistically significant as revealed by one way ANOVA (F=3.466, p=0.003). Multiple comparisons using LSD showed that CAT activities in control was significantly lower than those in 0.11 - 7.14 mg L$^{-1}$ Ni exposed plants (p<0.05).

The changes in CAT activities in leaf tissues of control and plants exposed to different concentrations of Ni (0.02 - 7.14 mg L$^{-1}$) are shown in Fig 40. In all the Ni treatment concentrations increase in CAT activities from that in control was observed with the exception of 0.22 mg L$^{-1}$. Highest increase of 58.7% was in 0.02 mg L$^{-1}$ followed by 36.9% and 32.3% in 4.02 and 0.11 mg L$^{-1}$ of Ni treatment, respectively. Plants exposed to 2.23 and 7.14 mg L$^{-1}$ showed increase of 13.2 and 12.6 % in CAT activities as compared to control plants. On the other hand, 0.22 mg L$^{-1}$ of Ni treated plants showed 23.4% reduction with respect to control plants. One way ANOVA revealed that the differences in the increase in CAT activities were statistically significant (F=3.351, p=0.004). Multiple comparisons with Tukey test showed that increase in CAT activity in 0.02 mg L$^{-1}$ was significantly higher than that in control (p=0.013) and in 0.22, 2.23 and 7.14 mg L$^{-1}$ Ni exposed plants(p<0.05).
Figure 39: Catalase (CAT) activities in stem tissues of control and Ni exposed *I. aquatica* at the end of 30 day exposure. * Significant difference from corresponding value in control at p<0.05.

![Catalase activities in stem tissues](image)

Figure 40: Catalase (CAT) activities in leaf tissues of control and Ni exposed *I. aquatica* at the end of 30 day exposure. * Significant difference from corresponding value in control at p<0.05.

![Catalase activities in leaf tissues](image)
2. Guaiacol Peroxidase activities

GPOD activities in stem tissues of control and Ni (0.02 - 7.14 mg L\(^{-1}\)) exposed plants at the end of 30 day are shown in Fig 41. In case of stem tissues, increase with respect to that in control of 20.8 and 11.9\% was observed in 0.02 and 0.11 mg L\(^{-1}\), while in the other Ni concentrations i.e. 0.22 - 7.14 mg L\(^{-1}\) GPOD activities were inhibited. The lowest reduction of 2.2\% was observed in GPOD activities in plants exposed to 4.02 mg L\(^{-1}\) as compared to that in control plants. Plants exposed to 0.22, 2.23 and 7.14 mg L\(^{-1}\) had GPOD reductions ranging from 18.05 to 30.4\% with respect to that in control stem tissue. The differences in the reduction of GPOD activities were significant as revealed by one way ANOVA (F=2.425, p=0.029). Tukey test showed that increase in GPOD activity in 0.02 mg L\(^{-1}\) was significant from that in 2.23 mg L\(^{-1}\) of Ni treatment (p=0.034) but not from that in control (p=0.756).

GPOD activity was inhibited in leaf tissues of *I. aquatica* under different concentrations of Ni (0.02 - 7.14 mg L\(^{-1}\)) treatment at the end of 30 day (Fig 42). Leaf tissues of plants exposed to 7.14 mg L\(^{-1}\) showed highest reduction of 62.47\% from that in control plants, while lowest reduction of 7.5\% was observed in 4.02 mg L\(^{-1}\) of Ni treatment. Plants exposed to 0.02 and 2.23 mg L\(^{-1}\) showed reduction of 55.6 and 51.8\%, while 0.11 and 0.22 mg L\(^{-1}\) Ni exposed plants had GPOD reductions of 28.2 and 32.2\% when compared to the control. One way ANOVA revealed that reduction in GPOD activities was significant (F=3.600, p=0.003). Multiple comparisons using Tukey test showed that reduction in GPOD activities in 0.02, 2.23 and 7.14 mg L\(^{-1}\) were statistically significant with respect to control (p<0.05).
Figure 41: Guaiacol Peroxidase (GPOD) activities in stem tissues of control and Ni exposed *I. aquatica* at the end of 30 day exposure.

* Significant difference from corresponding value in control at $p<0.05$.

Figure 42: Guaiacol Peroxidase (GPOD) activities in leaf tissues of control and Ni exposed *I. aquatica* at the end of 30 day exposure. * Significant difference from corresponding value in control at $p<0.05$. 

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E. METAL ACCUMULATION IN I. AQUATICA

I. Copper

1. Cu accumulation at 2.54 mg L\(^{-1}\) Cu exposure

Fig 43 shows the accumulation of Cu in different tissues of I. aquatica exposed to 2.54 mg L\(^{-1}\) Cu on day 5, 10 and 15 of exposure along with that in the control plants. The amount of metal accumulation in root, stem and leaf was found to be greater in Cu exposed plants than in those of control.

The concentration of Cu in root of plants exposed to 2.54 mg L\(^{-1}\) Cu was highest on day 15 of exposure (750.3 µg g\(^{-1}\)), followed by that on day 10 and day 5 (707.7 and 412.9 µg g\(^{-1}\), respectively). The lowest concentration was that in control plants (251.1 µg g\(^{-1}\)). One way ANOVA revealed that the difference in the amount of metal accumulation among root tissue in control and Cu treatment on different days was statistically significant (F=3.871, p=0.038). Multiple comparisons with LSD test showed that the amount of Cu accumulations in root of control was significantly lower than those of Cu-exposed plants on day 10 and 15 of exposure (p<0.05), but not than that on day 5.

The highest Cu accumulation in stem tissue was on day 10 (104.7 µg g\(^{-1}\)), followed by that on day 15 and day 5 (102.5 and 76.8 µg g\(^{-1}\), respectively), while the lowest was that in control (14.9 µg g\(^{-1}\)). One way ANOVA revealed that the difference in the amount of metal accumulation was statistically significant in stem (F=34.454, p<0.001). Multiple comparisons showed that the amount of Cu accumulation in control stem was significantly lower than those in Cu exposed plants on day 5, 10 and 15 (p<0.001) as revealed by Tukey test.

The highest Cu concentration in leaf tissue was found in plants on day 15 (47 µg g\(^{-1}\)), followed by that on day 10 and 5 (36.8 and 33.4 µg g\(^{-1}\), respectively), with the lowest
accumulation in control leaf (19.1 µg g$^-1$). One way ANOVA revealed that the difference in the amount of metal accumulation was statistically significant (F=5.368, p=0.014). Multiple comparisons with Tukey test revealed that Cu accumulation in leaf tissue in control plants was significantly lower than those in Cu-exposed plants on day 15 (p=0.009), but not on day 10 and 5.

When tissue-wise accumulation irrespective of days was compared, root was found to accumulate the highest concentration of copper followed by stem and leaf (Fig 4). Root accumulated 6.6 times higher Cu concentrations as compared to that in stem, and 16 times higher as compared to that in leaf at the end of 15 day of the experiment. One way ANOVA showed that the differences in the accumulation of Cu among the tissues were significant (F=36.645, p<0.001). Multiple comparisons with Tukey test showed that the amount of Cu accumulation in root of Cu exposed plants was significantly higher than those in stem and leaf tissue (p<0.001).
Figure 43: Cu accumulation in different tissues of control and 2.54 mg L$^{-1}$ Cu exposed *I. aquatica* on 5th, 10th and 15th day of the exposure.

Figure 44: Cu accumulation in different tissues of 2.54 mg L$^{-1}$ Cu exposed *I. aquatica* at the end of 15 day exposure.
2. Changes in Cu accumulation after 30 days

The changes in the amount of Cu accumulation in the whole plant bodies irrespective of root, stem and leaf tissues in control and different concentrations of Cu (0.13, 0.25 and 4.58 mg L\(^{-1}\)) at the end of 30 day are shown in Fig 45. The amount of Cu accumulation in the whole plant bodies was higher in all the Cu exposed plants as compared to control plants. Highest Cu accumulation of 2047.5 µg g\(^{-1}\) with increase of 98.6% was observed in 4.58 mg L\(^{-1}\) Cu exposed plants, followed by 151.8 µg g\(^{-1}\) Cu with increase of 80.8% in 0.25 mg L\(^{-1}\), and then by155.2 µg g\(^{-1}\) Cu with 81.3% increase in 0.13 mg L\(^{-1}\) Cu exposed plants, from that in control, which had 29.1 µg g\(^{-1}\) Cu accumulation at the end of 30 day exposure. One way ANOVA revealed that the differences in the accumulation of Cu were statistically significant (F=4.312, p=0.028). Multiple comparisons with LSD test showed that the Cu accumulation in the whole plant body in control was significantly lower than that in 4.58 mg L\(^{-1}\) of Cu exposed plants (p=0.010) but not than that in 0.13 and 0.25 mg L\(^{-1}\) Cu exposed plants (p>0.05). The test further revealed that the increase in Cu accumulation in 4.58 mg L\(^{-1}\) was significantly higher than that in 0.13 and 0.25 mg L\(^{-1}\) Cu exposed plants (p<0.05)

The changes in the amount of Cu accumulation in root, stem and leaf tissues of control and of plants exposed to different concentrations of Cu (0.13, 0.25 and 4.58 mg L\(^{-1}\)) at the end of 30 day are shown in Fig. 46. During this 30 day experiment necrosis set in plants exposed to 4.58 mg L\(^{-1}\) Cu on 3-15 day of exposure. Further, only stem and root tissues were analyzed for metal uptake in this Cu concentration, since leaves were totally absent.

All Cu exposed plants showed higher accumulation of Cu than control plants in root, stem and leaf tissues. Among plant tissues, root accumulated maximally as compared to stem and leaf in control as well as Cu exposed plants. Highest Cu accumulation of 4474.7 µg g\(^{-1}\) Cu in
root tissue was observed in plants exposed to 4.58 mg L\(^{-1}\) Cu with increase of 98.4% from that in control plants (71.9 µg g\(^{-1}\)), while those exposed to 0.13 and 0.25 mg L\(^{-1}\) Cu accumulated 436 and 427.8 µg g\(^{-1}\) of Cu with increase of 83.5 and 83.2%, respectively. Lowest Cu accumulation in root tissues was observed in control plants.

In case of stem tissues, highest Cu accumulation of 1667.7 µg g\(^{-1}\) was observed in 4.58 mg L\(^{-1}\) Cu exposed plants with increase of 99.2% from that in control plants which had lowest Cu accumulation of 13.1 µg g\(^{-1}\). Plants exposed to 0.13 and 0.25 mg L\(^{-1}\) Cu accumulated 22.9 and 21.2 µg g\(^{-1}\) of Cu.

In case of leaf tissues, plants exposed to 0.13 and 0.25 mg L\(^{-1}\) Cu accumulated 6.7 and 6.5 µg g\(^{-1}\) of Cu with 66.1 and 65.3% increase, respectively, from that of control plants.

No statistical analysis could be performed for this experiment, since the tissues had to be analyzed as pooled samples because of scarcity of tissue material.
Figure 45: Cu accumulation in whole plant tissues of control and Cu exposed *I. aquatica* at the end of 30 day exposure. * Significant difference from corresponding value in control at p<0.05.

Figure 46: Cu accumulation in different tissues of control and Cu exposed *I. aquatica* at the end of 30 day exposure.
II. Nickel

1. Changes in Ni accumulation after 30 days

Fig 47 shows Ni uptake values in the whole plant bodies irrespective of root, stem and leaf tissues at different concentrations of Ni (0.11, 0.22 and 4.02 mg L\(^{-1}\)) along with control plants. Ni accumulation increased with increase in Ni concentration in this 30 day exposure. Highest Ni accumulation of 1111.4 µg g\(^{-1}\) was observed in plants exposed to 4.02 mg L\(^{-1}\) with 99.6% increase, followed by 42.5 µg g\(^{-1}\) accumulation in 0.22 mg L\(^{-1}\) Ni exposed plants with 89% increase, with respect to lowest Ni accumulation of 4.7 µg g\(^{-1}\) in control plants. The differences in Ni accumulation were statistically significant as revealed by one way ANOVA (F=3.716, p=0.042). Multiple comparisons using LSD test showed that Ni accumulation in 4.02 mg L\(^{-1}\) was significantly higher from those in control (p=0.016) and also from 0.11 and 0.22 mg L\(^{-1}\) Ni exposed plants (p<0.05).

Fig 48 shows accumulation of Ni in different tissues of plants in control and different concentrations of Ni (0.11, 0.22 and 4.02 mg L\(^{-1}\)) at the end of 30 day exposure. Ni exposed plants showed higher accumulation of Ni from that in control in root, stem and leaf tissues of plants.

Ni was maximally accumulated in root tissues of plants exposed to 4.02 mg L\(^{-1}\) with 2682.4 µg g\(^{-1}\) and increase of 99.95%, followed by that in 0.22 mg L\(^{-1}\) with 102.5 µg g\(^{-1}\) and 97.4% increase, and then by that in 0.11 mg L\(^{-1}\) with 96.3 µg g\(^{-1}\) and 97.2% increase, from those of control plants with lowest Ni accumulation of 2.6 µg g\(^{-1}\) in root tissues.

In stem tissue, highest Ni accumulation was observed in 4.02 mg L\(^{-1}\) with 131.8 µg g\(^{-1}\) and increase of 96.1%, followed by 0.11 and 0.22 mg L\(^{-1}\) of Ni exposed plants with 13.4 and 12.3
µg g\(^{-1}\) and increase of 61.5% and 58%, respectively, from that of control plants, which had lowest accumulation of 5.2 µg g\(^{-1}\) of Ni in its stem tissue.

In case of leaf tissue, maximum Ni accumulation of 519.9 µg g\(^{-1}\) was observed in 4.02 mg L\(^{-1}\) with 98.8% increase, followed by 0.22 and 0.11 mg L\(^{-1}\) of Ni exposed plants with 12.9 and 8.7 µg g\(^{-1}\) and 51.6 and 28.8% increase, respectively, from that in control plants with lowest Ni accumulation of 6.2 µg g\(^{-1}\).

No statistical analysis could be performed for this experiment, since the tissues had to be analyzed as pooled samples because of scarcity of tissue material.
Figure 47: Ni accumulation in whole plant tissues of control and Ni exposed *I. aquatica* at the end of 30 day exposure. * Significant difference from corresponding value in control at p<0.05.

Figure 48: Ni accumulation in different tissues of control and Ni exposed *I. aquatica* at the end of 30 day exposure.
F. STUDY ON THE INDUCTION OF NECROSIS BY COPPER AND NICKEL

I. Copper

1. Appearance and progress of necrosis

Table 9 shows the period of appearance of necrotic symptoms in *I. aquatica* plants exposed to graded Cu concentrations of 0.025 - 4.58 mg L\(^{-1}\), along with the percent of length of stems showing necrosis in plants exposed to 2.54 and 4.58 mg L\(^{-1}\) Cu during the 70 day exposure. Necrosis first appeared on the lower part of the stem which was in direct contact with the growth medium (i.e. Hoagland solution) containing different concentrations of Cu. Subsequently, necrosis gradually extended to the upper part of the stem.

In plants exposed to 0.025 and 0.08 mg L\(^{-1}\) Cu, no sign of necrosis could be visually detected during the exposure period. But those exposed to 0.13 - 4.58 mg L\(^{-1}\) Cu showed progressive, concentration-dependent appearance of necrosis (Early Necrosis: EN) followed by its spread in the stem tissue (Advanced Necrosis: AN). However, in case of leaf and root, appearance of necrosis was not as distinct as that observed in stem tissue, and instead of showing EN and AN, the roots and leaves appeared to wither and dry up rapidly with signs of shrinking of tissues.

During this exposure, the earliest appearance of EN and AN was detected in 4.58 mg L\(^{-1}\) Cu-exposed plants on day 3 - 4 and day 15, respectively. This was followed by 2.54 mg L\(^{-1}\) Cu with appearance of EN and AN on day 9 and 19, respectively (Fig 49). In plants exposed to 0.13 mg L\(^{-1}\), appearance of EN and AN was detected on day 33 - 58 and 49 - 67, respectively; and in case of plants exposed 0.25 mg L\(^{-1}\), on day 19 - 28 and 34 - 42, respectively (Table 9).

When necrotic length of plants exposed to 2.54 and 4.58 mg L\(^{-1}\) Cu at EN were compared, the percent length of stems with necrosis in 2.54 mg L\(^{-1}\) Cu-exposed plants at EN stage was
found to be 2.86 times higher than that in 4.58 mg L\(^{-1}\) Cu-exposed plants (Table 9). However in case of AN, a reverse result was found where the percent length of stems with necrosis in 4.58 mg L\(^{-1}\) Cu-exposed plants was found to be 2.05 times higher than that in 2.54 mg L\(^{-1}\) Cu-exposed plants.

Table 9: Appearance of early (EN) and advanced necrosis (AN) in the stem of *I. aquatica* on exposure to graded Cu concentrations and % necrotic length in stem tissues.

<table>
<thead>
<tr>
<th>Cu concentration (mg L(^{-1}))</th>
<th>Period of appearance of necrotic symptoms (day)</th>
<th>% length with necrosis (Mean±S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EN</td>
<td>AN</td>
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<td>0.03</td>
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<td>-</td>
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<tr>
<td>0.08</td>
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<td>-</td>
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<tr>
<td>0.13</td>
<td>33-58</td>
<td>49-67</td>
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<tr>
<td>0.25</td>
<td>19-28</td>
<td>34-42</td>
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<tr>
<td>2.54</td>
<td>9</td>
<td>19</td>
</tr>
<tr>
<td>4.58</td>
<td>3-4</td>
<td>15</td>
</tr>
</tbody>
</table>

-Necrotic symptoms not observed
NM: Not measured
Figure 49: (A) *I. aquatica* plants exposed to 2.54 mg L\(^{-1}\) Cu showing non-necrotic (NN), early necrotic (EN) and advanced necrotic (AN) tissues on 20 day; (B) *I. aquatica* plants exposed to 4.58 mg L\(^{-1}\) Cu showing non-necrotic (NN), early necrotic (EN) and advanced necrotic (AN) tissues on 15 day of exposure.
2. Effects on biochemical content

2.1. Protein content

Fig 50 shows the concentration of protein in control (CN) and also in NN, EN and AN tissues irrespective of concentration of Cu-exposure during the 70 day exposure along with Recovery (RC) tissues after 20 days of recovery treatment at the end of the exposure experiment. Comparisons of protein content in different tissues revealed that stem tissues in AN portion had the lowest protein concentration of only 0.55 mg g\(^{-1}\) followed by EN portion with 0.84 mg g\(^{-1}\) of protein. The highest protein concentration of 1.08 mg g\(^{-1}\) was observed in NN portion of the plants, which was even more than that in the control plants. Stem tissue of recovery plants (RC) after 20 days of rearing in HS without added Cu was found to have 0.94 mg g\(^{-1}\) of protein, which was close to that of the control (CN) value of 0.96 mg g\(^{-1}\). One way ANOVA revealed that the differences in the protein content among the different tissues were statistically significant (F=3.683, p=0.008). Multiple comparisons with Tukey test showed that the protein concentration in NN tissue was significantly higher than that in AN tissues (p=0.002) but not than that in control (p=0.618) and not than those of EN and RC (p>0.05). No significant difference was observed between the CN and RC stem tissues.

Fig 51 represents the protein concentrations in different Cu treatments irrespective of NN, EN and AN portions along with those in control and in recovery plants from 2.54 mg L\(^{-1}\) (RC1) and also from 4.58 mg L\(^{-1}\) Cu (RC2). It was observed that the 4.58 mg L\(^{-1}\) Cu exposed plants had the highest protein concentration of 1.01 mg g\(^{-1}\), while the 2.54 mg L\(^{-1}\) Cu-exposed plants had the lowest protein concentration of 0.73 mg g\(^{-1}\). This was followed by RC1 with 0.9 mg g\(^{-1}\) protein. Control plants and plants exposed to 0.13 - 0.25 mg L\(^{-1}\) and RC2 showed protein concentrations in the range of 0.93 - 0.96 mg g\(^{-1}\). However, one way ANOVA revealed
that the differences in the protein concentrations among these treatments were not statistically significant (F=0.686, p=0.661).

Figure 50: Protein content in early necrotic (EN), advanced necrotic (AN) and non-necrotic (NN) stem tissues of *I. aquatica* along with recovery (RC) and control (CN) tissues.

Figure 51: Protein content in the whole plant body of *I. aquatica* including necrotic tissues in different Cu concentrations along with the tissues of recovery plants exposed to 2.54 mg L\(^{-1}\) (RC1) and 4.58 mg L\(^{-1}\) (RC2) after rearing in HS without added Cu.
2.2. Total carbohydrates content

Fig 52 shows the concentration of total carbohydrates in different tissues (NN, EN and AN) irrespective of the concentration of Cu exposure along with CN during the 70 days exposure and also in RC tissues after 20 days of recovery treatment at the end of the exposure experiment. NN tissues had the highest total carbohydrates concentration of 5.13 mg g\(^{-1}\) while AN tissues had the lowest of 1.5 mg g\(^{-1}\) carbohydrates concentration, followed by RC tissues with 1.9 mg g\(^{-1}\). The carbohydrates concentration in EN was 3.3 mg g\(^{-1}\) which was close to that of the CN value of 3.5 mg g\(^{-1}\). One way ANOVA revealed that the differences in the carbohydrates concentration among the different tissues were statistically significant (F=4.622, p=0.002). Multiple comparisons with Tukey test showed that the total carbohydrates content in NN was significantly higher than that in AN tissues (p=0.001) and in RC stem tissues (p=0.023) but not than that in control (p=0.606). No significant difference was observed between the CN and RC stem tissues.

Fig 53 represents the total carbohydrates concentration in different Cu treatments irrespective of NN, EN and AN portion along with those in control and also in RC1 and RC2. It was observed that stem tissues of plants exposed to 4.58 mg L\(^{-1}\) had the highest carbohydrates concentration of 5.9 mg g\(^{-1}\) followed by 2.54 mg L\(^{-1}\) Cu treatment with 3.7 mg g\(^{-1}\) of carbohydrates, while RC1 had the lowest carbohydrates concentration of 1.8 mg g\(^{-1}\), and RC2 with 2 mg g\(^{-1}\) carbohydrates. Plants exposed to 0.13 and 0.25 mg L\(^{-1}\) Cu had carbohydrates concentration of 2.3 and 2.5 mg g\(^{-1}\). One way ANOVA revealed that the differences in the total carbohydrates concentration in different Cu concentrations and in RC1 and RC2 were statistically significant (F=2.267, p=0.046). Multiple comparisons with LSD test showed that the total carbohydrates concentration in 4.58 mg L\(^{-1}\) was significantly higher than that in 0.25
and 0.13 mg L\(^{-1}\) (p<0.05) and also that in RC1 and RC2 (p<0.05) but not than that in control (p=0.138).

Figure 52: Total carbohydrates content in early necrotic (EN), advanced necrotic (AN) and non-necrotic (NN) stem tissues of *I. aquatica* along with recovery (RC) and control (CN) tissues.

Figure 53: Total carbohydrates content in the whole plant body of *I. aquatica* including the necrotic tissues in different Cu concentrations along with the tissues of recovery plants exposed to 2.54 mg L\(^{-1}\) (RC1) and 4.58 mg L\(^{-1}\) (RC2) after rearing in HS without added Cu.
2.3. Ascorbic acid content

Fig 54 represents the AsA concentrations in NN, EN and AN tissues irrespective of the concentration of Cu exposure along with CN during the 70 days exposure and in RC tissues after 20 days of recovery treatment at the end of the exposure experiment. It was observed that AN tissues had the highest AsA concentration of 0.0048 mg g\(^{-1}\) followed by EN tissues with 0.0046 mg g\(^{-1}\) of AsA. While the lowest AsA concentration of 0.0017 mg g\(^{-1}\) was observed in RC tissues, that of control and NN tissues had AsA values of 0.0025 and 0.004 mg g\(^{-1}\), respectively. However, the differences in AsA concentration were not statistically significant as revealed by one way ANOVA (F=1.338, p=0.264).

During this 70 day experiment for observing AsA concentration, there were no signs of either EN or AN in plants exposed to 0.25 mg L\(^{-1}\) Cu. Therefore, comparison of AsA concentration was done among 0.13, 2.54 and 4.58 mg L\(^{-1}\) of Cu exposed plants along with control and RC1 and RC2 as shown in Fig 55. Plants exposed to 4.58 mg L\(^{-1}\) Cu had the highest AsA content of 0.0053 mg g\(^{-1}\) followed by 2.54 mg L\(^{-1}\) Cu exposed plants with AsA content of 0.0044 mg g\(^{-1}\), while RC2 tissues had the lowest AsA concentration of 0.0014 mg g\(^{-1}\). Plants exposed to 0.13 mg L\(^{-1}\) of Cu, control plants and RC1 had AsA in the range of 0.0014 to 0.0025 mg g\(^{-1}\). The differences were not statistically significant as revealed by one way ANOVA (F=0.962, p=0.450).
Figure 54: Ascorbic acid (AsA) content in early necrotic (EN), advanced necrotic (AN) and non-necrotic (NN) stem tissues of *I. aquatica* along with recovery (RC) and control (CN) tissues.

![Ascorbic acid content in stem tissues](image1)

Figure 55: Ascorbic acid (AsA) content in the whole plant body of *I. aquatica* including the necrotic tissues in different Cu concentrations along with the tissues of recovery plants exposed to 2.54 mg L$^{-1}$ (RC1) and 4.58 mg L$^{-1}$ (RC2) after rearing in HS without added Cu.

![Ascorbic acid content in whole plant body](image2)
3 Effects on enzyme activities

3.1. Catalase activities

The alterations in CAT activities in NN, EN and AN stem tissues of *I. aquatica* along with those in CN and RC tissues are shown in Fig 56. CAT activities were found to increase in all tissues including RC stem tissues when compared with that in control tissues. The highest percent increase of 68.27% was observed in EN tissues, followed by 62.03 and 57.28% in NN and AN tissues, while the lowest percent increase of 48.82% was observed in RC tissues. One way ANOVA showed that the differences in CAT activities were statistically significant (F=2.759, p=0.034). Multiple comparisons with Tukey test revealed that the increase in CAT activity in EN tissues were significantly higher than that in CN (p=0.024), although the other differences were not significant.

Fig 57 shows the increase in CAT activities in different concentrations of Cu along with RC1 and RC2 tissues of *I. aquatica*. When comparing the concentration wise percent increase in CAT activities from that of control, the highest increase of 78.45% was observed in 4.58 mg L\(^{-1}\) Cu exposed plants, followed by 74.8% in 2.54 mg L\(^{-1}\), 52.34% in RC2, 47.22% in 0.13 mg L\(^{-1}\), and the lowest percent increase of 47.22% in RC1 plants. The differences in the CAT activities were statistically significant as revealed by one way ANOVA (F=7.070, p<0.001). Multiple comparisons with Tukey test revealed that the increase in CAT activities in 4.58 and 2.54 mg L\(^{-1}\) Cu exposed plants was significantly higher than those in tissues of 0.13 mg L\(^{-1}\) Cu treated plants (p<0.05) and also from those of control and RC1 (p<0.05).
Figure 56: Catalase (CAT) activities in early necrotic (EN), advanced necrotic (AN) and non-necrotic (NN) stem tissues of *I. aquatica* along with recovery (RC) and control (CN) tissues.

* Significant difference from corresponding value in control at p<0.05.

Figure 57: Catalase (CAT) activities in the whole plant body of *I. aquatica* including the necrotic tissues in different Cu concentrations along with the tissues of recovery plants exposed to 2.54 mg L\(^{-1}\) (RC1) and to 4.58 mg L\(^{-1}\) (RC2) after rearing in HS without added Cu.

* Significant difference from corresponding value in control at p<0.05.
3.2. Guaiacol peroxidase activities

Fig 58 represents the comparison of GPOD activities in NN, EN and AN tissues along with CN and RC tissues. The highest GPOD activity of 0.18 µmol min⁻¹ mg⁻¹, which represented a percent increase of 11.64% from that in control was observed in EN tissues. On the contrary, NN and AN tissues showed GPOD activities of 0.14 and 0.11 µmol min⁻¹ mg⁻¹, which represented reduction of 11.07 and 29.15%, respectively, from that in control. GPOD activity of 0.15 µmol min⁻¹ mg⁻¹ was observed in RC tissues, which was equivalent to 0.9 % reduction from the GPOD activity of 0.16 µmol min⁻¹ mg⁻¹ in control. However, neither the increment nor the reductions in GPOD activities were statistically significant as revealed by one way ANOVA (F=1.085, p=0.376).

Fig 59 shows the reduction in GPOD activities in 0.13 - 4.58 mg L⁻¹ Cu treated plants along with RC1 and RC2. Since during this experiment, plants exposed to 0.25 mg L⁻¹ Cu showed no sign of necrosis, GPOD activities were observed in the remaining Cu concentrations of 0.13, 2.54 and 4.58 mg L⁻¹ Cu, where necrosis was observed. The lowest GPOD activity of 0.12 µmol min⁻¹ mg⁻¹ was observed in RC2, followed by 0.13 µmol min⁻¹ mg⁻¹ in 0.13 mg L⁻¹ Cu exposed plants, and 0.14 - 0.17 µmol min⁻¹ mg⁻¹ in 2.54 and 4.58 mg L⁻¹ Cu treated plants and RC2 plants. All these activities were lower than that in control (0.19 µmol min⁻¹ mg⁻¹). However, one way ANOVA revealed that the changes in GPOD activities were not statistically significant (F=1.081, p=0.384).
Figure 58: Guaiacol Peroxidase (GPOD) activities in early necrotic (EN), advanced necrotic (AN) and non-necrotic (NN) stem tissues of *I. aquatica* along with recovery (RC) and control (CN) tissues.

Figure 59: Guaiacol Peroxidase (GPOD) activities in the whole plant body of *I. aquatica* including the necrotic tissues in different Cu concentrations along with the tissues of recovery plants exposed to 2.54 mg L⁻¹ (RC1) and 4.58 mg L⁻¹ (RC2) after rearing in HS without added Cu.
4. Cu accumulation in different necrotic tissues and in different Cu concentrations

Fig 60 represents the comparison of Cu accumulation among NN, EN and AN tissues along with CN and RC tissues. The highest Cu accumulation of 993.85 µg g⁻¹ was observed in AN tissues followed by 139.1 µg g⁻¹ in EN tissues, 27.7 and 18 µg g⁻¹ in NN and RC tissues, respectively, with the lowest accumulation in CN tissues with 14.9 µg g⁻¹. One way ANOVA revealed that the differences in the Cu accumulation among different tissues were statistically significant (F=11.384, p<0.001). Multiple comparisons with LSD test showed that the amount of Cu accumulated in AN tissues was significantly higher than that of RC, NN, EN and CN (p<0.05). The test further revealed that the Cu accumulation in RC, NN and CN was significantly lower than that of EN (p<0.05).

Fig 61 shows the Cu accumulation in 0.13, 0.25, 2.54 and 4.58 mg L⁻¹ Cu irrespective of NN, EN and AN portion along with RC1, RC2 and CN tissues. The highest Cu accumulation of 869.3 µg g⁻¹ was observed in 4.58 mg L⁻¹ Cu followed by 2.54 mg L⁻¹ Cu exposed plants with 509.3 µg g⁻¹. Lowest Cu accumulation of 7.03 µg g⁻¹ was observed in RC1 followed by RC2 with 7.4 µg g⁻¹. Control plants accumulated 14.9 µg g⁻¹ of Cu and 0.13 and 0.25 mg L⁻¹ Cu exposed plants accumulated 58.8 and 51.3 µg g⁻¹ of Cu, respectively. One way ANOVA revealed that the differences in the amount of Cu accumulation among different treatments were statistically significant (F=4.679, p=0.002). Multiple comparisons with LSD test showed that the amount of Cu accumulation in 2.54 and 4.58 mg L⁻¹ was significantly higher than those in CN, 0.13 and 0.25 mg L⁻¹ of Cu treatment, RC1, and RC2 (p<0.05) but not between them (p=0.777).

When HS solution containing 4.58, 2.54 and 0.25 mg L⁻¹ of Cu were analyzed for Cu concentration during the occurrence of EN and AN in plant samples, it was found that HS
medium treated with 4.58 and 2.54 mg L\textsuperscript{-1} of Cu contained 3.046 and 2.42 ppm Cu, respectively, during EN stages. And those exposed to 0.25 mg L\textsuperscript{-1} contained 0.64 ppm Cu content during the AN stage.
Figure 60: Cu accumulations in early necrotic (EN), advanced necrotic (AN) and non- necrotic (NN) stem tissues of *I. aquatica* along with recovery (RC) and control (CN) tissues.

* Significant difference from corresponding value in control at p<0.05.

Figure 61: Cu accumulation in the whole plant body of *I. aquatica* including the necrotic tissues in different Cu concentrations along with the tissues of recovery plants exposed to 2.54 mg L\(^{-1}\) (RC1) and 4.58 mg L\(^{-1}\) (RC2) after rearing in HS without added Cu. * Significant difference from corresponding value in control at p<0.05.
5. Recovery of plants exposed to 2.54 (RC1) and 4.58 (RC2) mg L\(^{-1}\) Cu after rearing in HS without added Cu.

Table 10 shows the increase in SH, NN, NL and number of new roots (NR) from initial day of recovery treatment in RC1 and RC2 to 15 and 20 day of the treatment. It was observed that SH, NN, NL and NR in RC1 and RC2 increased with the increase in the recovery period (Fig 62 and 63). Increase in SH in RC1 was 9.7 and 16.1 cm on 15 and 20 day, respectively. That of RC2 was 5 and 7 cm on 15 and 20 day, respectively. In the case of NN also gradual increase of 3 to 7.5 in RC1 and of 1.3 to 1.7 in RC2 on 15 and 20 day of recovery treatment was observed.

There was no leaf and root in the RC1 and RC2 plants at the beginning of the recovery period. However, NL of 1.5 and 4 in RC1, and 2 and 4 in RC2, was recorded on 15 and 20 day of recovery experiment, respectively. NR also increased to 7 and 9 in RC1, and to 10.3 in RC2 on 15 and 20 day of recovery treatment, respectively.

Table 10: Increase in shoot height (SH), number of new nodes (NN), number of new leaf (NL) and number of new root (NR) in recovery \(I.\ aquatica\) exposed to Cu concentrations, 2.54 (RC1) and 4.58 mg L\(^{-1}\) (RC2) after rearing in HS without added Cu.

<table>
<thead>
<tr>
<th>Recovery plant from Cu conc. (mg L(^{-1}))</th>
<th>Increase in SH (cm), NN and NL (Mean ±S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SH</td>
</tr>
<tr>
<td>2.54 mg L(^{-1})</td>
<td></td>
</tr>
<tr>
<td>15 day</td>
<td>9.7±2.7</td>
</tr>
<tr>
<td>20 day</td>
<td>16.1±3.25</td>
</tr>
<tr>
<td>4.58 mg L(^{-1})</td>
<td></td>
</tr>
<tr>
<td>15 day</td>
<td>5±3.1</td>
</tr>
<tr>
<td>20 day</td>
<td>7±4.5</td>
</tr>
</tbody>
</table>

Values are mean ±S.E.
Figure 62: (A) *I. aquatica* plant after one day of recovery from 2.54 mg L\(^{-1}\) Cu exposure; (B) *I. aquatica* plant after one day of recovery from 4.58 mg L\(^{-1}\) Cu exposure.

Figure 63: (A) *I. aquatica* plant after 20 day of recovery from 2.54 mg L\(^{-1}\) Cu exposure; (B) *I. aquatica* plant after 20 day of recovery from 4.58 mg L\(^{-1}\) Cu exposure.
II. Nickel

In case of Ni, necrosis occurred in 7.14 mg L\(^{-1}\) of Ni and very rarely in 4.02 mg L\(^{-1}\) Ni exposed plants. Even in plants exposed to 7.14 mg L\(^{-1}\) Ni, the appearance of necrosis was uncertain and irregular, because of which only protein and carbohydrates analysis, Ni accumulation and SEM studies were done. In the case of 7.14 mg L\(^{-1}\) of Ni, signs of EN appeared during 9 - 11 days and that of AN during 22 - 30 day of exposure. The RC plants in this case failed to recover. Appearance of EN and AN in plant exposed to 4.02 mg L\(^{-1}\) of Ni could not be observed satisfactorily because of the uncertainty in occurrence of necrosis.

1. Effects on protein and carbohydrates content and Ni accumulation in different tissues

Table 11 shows the comparisons of protein, total carbohydrates and Ni accumulation in NN, EN and AN tissues of 7.14 mg L\(^{-1}\) Ni exposed plants along with control. Protein concentration in CN was found to be highest with 0.72 mg g\(^{-1}\) followed by NN tissues with 0.4 mg g\(^{-1}\). The lowest protein concentration was observed in AN tissues with 0.25 mg g\(^{-1}\) followed by EN with 0.26 mg g\(^{-1}\). One way ANOVA revealed that the differences in protein concentration among the tissues were significantly different (F=5.834, p=0.008). Multiple comparisons with Tukey test showed that the protein concentration in CN was significantly higher than those in EN and AN (p<0.05) but not than that in NN (p=0.123).

In case of total carbohydrates, CN had the highest concentration with 2.1 mg g\(^{-1}\) followed by NN tissues with 1.12 mg g\(^{-1}\) while AN had the lowest carbohydrates concentration of 0.8 mg g\(^{-1}\) followed by EN tissues with 1.04 mg g\(^{-1}\) of carbohydrates. One way ANOVA revealed that the differences in carbohydrates content among the tissues were statistically significant (F=3.577, p=0.039). Multiple comparisons with Tukey test showed that the carbohydrates
content in CN was significantly higher than that in AN tissues \((p=0.027)\) but not than those in NN and EN tissues \((p>0.05)\).

The amount of Ni accumulation was found to be lowest in CN stem tissues with 5.2 \(\mu g\ \text{g}^{-1}\). In NN and EN stem tissues, Ni accumulation was found to be 702.3 and 769 \(\mu g\ \text{g}^{-1}\), respectively. The highest Ni concentration was observed in AN tissues with 4503.4 \(\mu g\ \text{g}^{-1}\). Pooled samples were taken for analyzing the Ni accumulation in CN and different tissues (NN, EN and AN) of 7.14 mg L\(^{-1}\) Ni exposed plants. Hence, no statistical analyses could be performed.

When 50% HS solution containing 7.14 mg L\(^{-1}\) Ni was analyzed for Ni concentration during the occurrence of AN in plant samples, it was found that the solution contained 4.54 ppm of Ni.

Table 11: Reduction in protein and total carbohydrates content and increase in Ni accumulation in non-necrotic (NN), early necrosis (EN) and advanced necrosis (AN) tissues of 7.14 mg L\(^{-1}\) Ni exposed \textit{I. aquatica} along with control (CN).

<table>
<thead>
<tr>
<th>Ni concentration (mg L(^{-1}))</th>
<th>Protein content (mg g(^{-1}))</th>
<th>Total carbohydrates content (mg g(^{-1}))</th>
<th>Ni accumulation ((\mu g\ \text{g}^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>CN 0.72 ±0.1</td>
<td>2.1±0.9</td>
<td>5.2</td>
<td></td>
</tr>
<tr>
<td>7.14 NN 0.4±0.13</td>
<td>1.12±0.22</td>
<td>702.3</td>
<td></td>
</tr>
<tr>
<td>EN 0.26±0.12*</td>
<td>1.04±0.3</td>
<td>769</td>
<td></td>
</tr>
<tr>
<td>AN 0.25±0.11*</td>
<td>0.8±0.2*</td>
<td>4503.4</td>
<td></td>
</tr>
</tbody>
</table>

* Significant difference from corresponding value in control at \(p<0.05\). Values are mean ±S.E.
G. ULTRASRUCTURAL CHANGES IN *I. AQUATICA* STEM REVEALED BY SCANNING ELECTRON MICROSCOPY

1. Copper

1. SEM studies at low Cu exposure

Scanning Electron Microscopic (SEM) images of stem tissues of control and Cu exposed plants (0.025 mg L\(^{-1}\)) at the end of 30 days exposure are shown in Figures 64 and 65. SEM study of stem tissues was done on plants exposed to 0.025 mg L\(^{-1}\) of Cu and was compared with that of control plants. Plants exposed to 0.025 mg L\(^{-1}\) of Cu showed regular arrangements of cortical tissues (CT) and normal structure of xylem and phloem elements as that of control. However, coarsened appearance of the epidermal cells (EC) was observed in the Cu exposed plants whereas control plants showed a regular and uniform appearance of EC.
Figure 64 (A-B): Scanning electron micrographs of *I. aquatica* stem tissues of control plants at the end of 30 day exposure. (CT-Cortical tissues; EC-Epidermal cells; PE-Phloem element; XE-Xylem element).

Figure 65 (A-B): Scanning electron micrographs of *I. aquatica* stem tissues exposed to 0.025 mg L\(^{-1}\) Cu at the end of 30 day exposure. (CT-Cortical tissues; EC-Epidermal cells; PE-Phloem element; XE-Xylem element).
2. SEM studies on Cu-induced necrotic tissues

Fig 66 shows the scanning electron micrograph (SEM) of control plant during the 70 day exposure. The transverse section of stem samples under control condition consisted of monolayer and rectangular shaped epidermal cells (EC) as shown in Fig 66 (A). Cortical tissues (CT) were regularly arranged in control samples. Fig 66 (B) showed the arrangement of vascular bundles (VB) tissues in the control sample with normal orientation of hexagonal/pentagonal shaped xylem element (XE) and phloem element (PE).

Fig 67 shows the stem section of NN tissues of 0.13 mg L$^{-1}$ Cu treated *I. aquatica*. No discernible alteration in the anatomical structures was observed. Fig 67 (A) reveal the regular arrangements and normal orientation of CT. XE and PE structures are normally arranged (Fig 67B).

Fig 68 shows the stem section of EN tissues of 0.13 mg L$^{-1}$ Cu treated *I. aquatica*. Transverse view of stem section showed slight distortion in the EC but no particular alterations were observed in CT as shown in Fig 68 (A). Intercellular air spaces (AS) became smaller in EN stem tissues and that of vascular tissues showed subnormal appearance as observed in Fig 68 (B).

Fig 69 shows the stem section of AN tissues of 0.13 mg L$^{-1}$ Cu treated *I. aquatica*. Transverse view of stem section shows slight distortion in epidermal cells (Fig 69A). Fig 69 (B) shows the gradual degeneration of PE along with the associated surrounding tissues but no particular alteration was observed in XE.
Figure 66 (A-B): Scanning electron micrographs of *I. aquatica* stem tissues in control plants during the 70 day exposure. (CT-Cortical cells; EC-Epidermal cells; PE-Phloem element; XE-Xylem element).

Figure 67 (A-B): Scanning electron micrographs of non-necrotic (NN) stem tissues of *I. aquatica* in 0.13 mg L\(^{-1}\) Cu treatment. (CT-Cortical tissues; EC-Epidermal cells; PE-Phloem element; XE-Xylem element).
Figure 68 (A-B): Scanning electron micrographs of early necrotic (EN) stem tissues of *I. aquatica* in 0.13 mg L\(^{-1}\) Cu treatment. (AS- Intercellular air spaces; CT-Cortical tissues; EC-Epidermal cells; VB- Vascular bundle).

Figure 69 (A-B): Scanning electron micrographs of advanced necrotic (AN) stem tissues of *I. aquatica* in 0.13 mg L\(^{-1}\) Cu treatment. (CT-Cortical tissues; EC-Epidermal cells; PE-Phloem element; XE-Xylem element).
Fig 70 shows the NN stem tissues of 0.25 mg L$^{-1}$ Cu treated *I. aquatica*. CT becomes slightly enlarged but no distinct change in outer epidermal cells was observed (Fig 70A). No severe change in PE was observed in NN tissues, although slight thickening of the vascular tissues could be observed (Fig 70B).

Fig 71 shows the EN stem tissues of 0.25 mg L$^{-1}$ Cu treated *I. aquatica*. Distortion of monolayer epidermal cells was observed and CT layer became irregular and thickened (Fig 71A). Serious impairment in the normal appearance of vascular tissues along with the surrounding tissues was observed (Fig 71B).

Fig 72 shows the AN stem tissues of 0.25 mg L$^{-1}$ Cu treated *I. aquatica*. Severe distortions in the outer epidermal cells were observed along with reduction in diameter of CT and the layer (CT) also became irregular and thickened (Fig 72A). Fig 72 (B) shows the alterations in vascular tissues where PE is totally degraded along with the associated surrounding tissues.

Fig 73 shows the NN stem tissues of 2.54 mg L$^{-1}$ Cu treated *I. aquatica*. No specific alteration was observed in the CT structure and also in the layer (Fig 73A). In this NN tissue, PE became less distinct in structure and also degenerated. However, XE tissues remained intact with slight irregularity in shape (Fig 73B).

Fig 74 represents the EN stem tissues of 2.54 mg L$^{-1}$ Cu treated *I. aquatica*. Fig 74 (A) shows the slightly deformed EC when view transversely and tightly arranged CT layer. The structural appearance of vascular tissues was seriously altered. XE became smaller and was covered with cellular debris and that of PE was mostly degraded and becomes smaller in size (Fig 74B).

Fig 75 represents the AN stem tissues of 2.54 mg L$^{-1}$ Cu treated *I. aquatica*. CT were thickened and become smaller besides this some layer of the tissues was blocked (Fig 75A).
Fig 75 (B) shows the serious impairment in the arrangement of vascular tissues where both XE and PE were covered with cellular debris. Tissues surrounding the VB were also greatly degraded.

Fig 76 shows the necrotic stem tissues of 4.58 mg L$^{-1}$ Cu treated *I. aquatica*. Fig 76 (A) shows the EN stem tissues of 4.58 mg L$^{-1}$ Cu treated *I. aquatica*. It was observed that the diameter of PE became smaller and that of XE became irregular. Greater destruction was observed in the tissues surrounding the VB and also in the CT. Cellular debris was observed all over the CT layer. Fig 76 (B) represents the AN tissues where outer epidermal cells lost their normal rectangular shape. CT also became thickened and smaller and some of them were also blocked. Vascular tissues were greatly reduced where diameter of the XE and PE became smaller and were also blocked. Surrounding tissues of VB and pith layer (PH) changed their normal orientation.
Figure 70 (A-B): Scanning electron micrographs of non-necrotic (NN) stem tissues of *I. aquatica* in 0.25 mg L$^{-1}$ Cu treatment. (CT-Cortical tissues; EC-Epidermal cells; PE-Phloem element; XE-Xylem element).

Figure 71 (A-B): Scanning electron micrographs of early necrotic (EN) stem tissues of *I. aquatica* in 0.25 mg L$^{-1}$ Cu treatment. (CT-Cortical tissues; EC-Epidermal cells; PE-Phloem element; XE-Xylem element).
Figure 72 (A-B): Scanning electron micrographs of advanced necrotic (AN) stem tissues of *I. aquatica* in 0.25 mg L\(^{-1}\) Cu treatment. (CT-Cortical tissues; EC-Epidermal cells; PE-Phloem element; XE-Xylem element).

Figure 73 (A-B): Scanning electron micrographs of non-necrotic (NN) stem tissues of *I. aquatica* in 2.54 mg L\(^{-1}\) Cu treatment. (CT-Cortical tissues; PE-Phloem element; XE-Xylem element).
Figure 74 (A-B): Scanning electron micrographs of early necrotic (EN) stem tissues of *Ipomoea aquatica* in 2.54 mg L$^{-1}$ Cu treatment. (CT-Cortical tissues; EC-Epidermal cells; PE-Phloem element; XE-Xylem element).

Figure 75 (A-B): Scanning electron micrographs of advanced necrotic (AN) stem tissues of *Ipomoea aquatica* in 2.54 mg L$^{-1}$ Cu treatment. (CT-Cortical tissues; EC-Epidermal cells; PE-Phloem element; PH-Pith layer; XE-Xylem element).
Figure 76: (A) Scanning electron micrographs of early necrotic (EN) and (B) advanced necrotic (AN) stem tissues of I. aquatica in 4.58 mg L$^{-1}$ Cu treatment. (AS- Intercellular air spaces; CT-Cortical tissues; EC-Epidermal cells; PE-Phloem element; PH- Pith layer; VB-Vascular bundle; XE-Xylem element).
Fig 77 shows the EN stem tissues of 8.14 mg L\(^{-1}\) Cu treated \textit{I. aquatica}. No specific alteration was observed in the epidermal cells but CT layer became irregular losing its normal appearance (Fig 77A). Fig 77 (B) shows the mass degradation of CT and deformed vascular tissues. XE and PE were poorly developed and their structures were also thickened.

Fig 78 shows the AN stem tissues of 8.14 mg L\(^{-1}\) Cu treated \textit{I. aquatica}. EC lose its normal appearance and that of CT layer were irregularly arranged (Fig 78A). Fig 78 (B) shows the alteration in the VB and its surrounding tissues. XE showed irregularity in its structure and that of PE showed poor development and loss of its normal pentagonal/hexagonal structure.

II. Nickel

1. SEM studies at low Ni exposure

SEM images of Ni exposed plants at concentrations 0.02 and 0.22 mg L\(^{-1}\) at the end of 30 days are shown in Figures 79 and 80. Ni treatment on \textit{I. aquatica} stem exhibited subnormal appearance of epidermis, cortical cells and vascular bundle elements (Xylem and phloem). SEM images of transverse sections of stem samples under control and 0.02 mg L\(^{-1}\) of Ni treatment show uniform appearance of CT, normal structures of XE and, PE with regularly arranged EC. Stem tissues of plants treated with 0.22 mg L\(^{-1}\) of Ni showed impairment in the arrangement of EC and irregularity of CT but XE arrangements were similar to those of control.
Figure 77 (A-B): Scanning electron micrographs of early necrotic (EN) stem tissues of *I. aquatica* in 8.14 mg L\(^{-1}\) Cu treatment. (CT-Cortical tissues; EC-Epidermal cells; VB-Vascular bundle).

Figure 78 (A-B): Scanning electron micrographs of advanced necrotic (AN) stem tissues of *I. aquatica* in 8.14 mg L\(^{-1}\) Cu treatment. (CT-Cortical tissues; EC-Epidermal cells; PE-Phloem element; XE-Xylem element).
Figure 79 (A-B): Scanning electron micrographs of *I. aquatica* stem exposed to 0.02 mg L\(^{-1}\) Ni at the end of 30 day exposure. (CT-Cortical tissues; EC-Epidermal cells; PE-Phloem element; VB- Vascular bundle; XE-Xylem element).

Figure 80 (A-B): Scanning electron micrographs of *I. aquatica* stem exposed to 0.22 mg L\(^{-1}\) of Ni at the end of 30 day exposure. (CT-Cortical tissues; EC-Epidermal cells; PE-Phloem element; VB- Vascular bundle; XE-Xylem element).
2. SEM studies on Ni-induced necrotic tissues

Fig 81 shows the AN stem tissues of 4.02 mg L\(^{-1}\) of Ni treated \(I.\ aquatica\). The orientation and arrangement of CT were altered. Deposition of cellular debris was observed all over the CT layer as shown in Fig 81 (A). Irregular shaped XE and PE were observed along with thickening in phloem tissues as shown in Fig 81 (B).

Fig 82 (A) shows the EN tissues of 7.14 mg L\(^{-1}\) Ni exposed plants. No severe impairment in the shape and arrangement of vascular tissues were observed. CT were tightly arranged losing their normal and regular appearance. EC also remained intact in this tissue.

Fig 82 (B-C) shows the AN tissues of 7.14 mg L\(^{-1}\) of Ni treated \(I.\ aquatica\). Complete distortion in EC and CT were observed in stem tissues (Fig 82B). Serious impairment of the normal orientation of almost all the cells was observed in AN tissues along with complete distortion of epidermal and cortical cells with slight thickening in phloem tissues (Fig 82C). In addition, AN tissues showed cellular debris all over their surface thus making the appearance of stem section coarse and rough.
Figure 81 (A-B): Scanning electron micrographs of advanced necrotic (AN) stem tissues of *I. aquatica* in 4.02 mg L\(^{-1}\) Ni treatment. (CT-Cortical tissues; PE-Phloem element; Xylem element).

Figure 82: (A) Scanning electron micrographs of early necrotic (EN) stem tissues of *I. aquatica* in 7.14 mg L\(^{-1}\) Ni treatment; (B-C) Scanning electron micrographs of advanced necrotic (AN) stem tissues of *I. aquatica* in 7.14 mg L\(^{-1}\) Ni treatment. (CT-Cortical tissues; EC-Epidermal cells; VB-Vascular bundle).