EFFECT OF WASTEWATER AND GREY WATER REUSE ON THE GERMINATION AND EARLY GROWTH OF BARLEY AND ONIONS

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ABSTRACT
This paper evaluates the effect of wastewater reuse on the germination and growth at early stages of barley and onions. Treated wastewater, partially treated industrial wastewater, grey water, and car wash water, in addition to tap water as control were used to test their effect on germination percentage and germination speed of barley and onions and on the early growth stages of barley. Results have shown that germination percentage and germination speed of onions and barley were significantly reduced when treated wastewater and car wash water were used. All water qualities produced higher shoot lengths and lower root lengths of barley compared to tap water, with car wash water producing the highest shoot to root length ratio followed by treated wastewater. The shoot dry weight to root dry weight of barley of all irrigation water types used was not significantly different. The recommendation that resulted from this research is that these types of wastewaters should be avoided during the early growth phases of barley and onions.

Keywords: Barley, grey water, industrial wastewater, onions, seed germination, wastewater reuse.

1. Introduction

Jordan is one of the four poorest countries in the world with respect to water resources on a per capita basis. Available water resources are projected to decline to only 91 cubic meter per capita per year for all uses by 2025 (Ammary, 2007). Therefore a water management plan, in which non-conventional water resources use is a main component, should be adopted. Wastewater reuse has the lowest marginal cost in Jordan, and therefore, will continue to be a main component in Jordan's water budget. In addition grey water has been and is being considered for reuse in a number of rural areas in Jordan (Halalsheh et al., 2008; Jamrah and Ayyash 2008; Al-Hamaiedeh and Bino, 2010; Al-Mashaqbeh et al., 2012). In this paper, Grey water which accounts for 50-80% of household wastewater generation rate (Friedler and Hadari, 2006) refers to domestic wastewater that comes out from the shower, laundry facilities, washbasins, and the kitchen.

Several crops are sensitive while others are less or not sensitive for the use of wastewater as an irrigation water (Tadros et al., 2012). Many researchers have evaluated the effect of irrigation water quality on plant growth based on their effects on seed germination and early plant growth (Crowe et al., 2002; Casa et al., 2003; Liu et al., 2007; Tadros et al., 2012; Malaviya et al., 2012). Germination percentage and Germination Rate Index (GRI) as a measure of germination speed have been used to evaluate germinability (Tadros et al., 2012). A number of parameters have been used to evaluate the success of plantations based on seedling evaluations. These include, shoot or root height (Kaya et al., 2006), shoot length to root length ratio (Tadros et al., 2012), diameter, dry mass, or the ratio of shoot dry mass to root dry mass (Bernier et al., 1995; Tadros et al., 2012). An imbalance in shoot to root ratio can cause transplanting shock (Bernier et al. 1995). The root mass is correlated with the ability of a plant to absorb water, while the shoot mass is correlated with the amount of water a plant loses through transpiration (Wilson, 1988). Therefore, the shoot to root ratio has been used as a measure to evaluate the drought avoidance potential, with seedlings having a low shoot to root ratio values, have a higher drought avoidance potential (Bernier et al., 1995). For the same seeds, however, an equilibrium between the amount of shoot to the amount of root (shoot: root ratio) must be present (Wilson, 1988).

The present study aims at studying the effect of four different wastewaters, namely treated domestic wastewater (TWW), partially treated industrial wastewater (IW), partially treated grey water (GW), untreated car wash wastewater (CWW), and tap water (TW) as control, on the germinability of barley and onions seeds, and on the early growth stages of barley. The effect of these types of wastewaters on the growth of these plants is lacking.

2. Materials and methods

The effect of wastewater and grey water reuse on germination and early growth stages of barley and onions was studied using treated domestic wastewater (TWW), partially treated industrial wastewater (IW), partially treated grey water (GW), untreated car wash wastewater (CWW), and tap water (TW) as control. The quality of the four different types of wastewater and tap water (TW) used as control are shown in Table 1.

All tests were conducted according to Standard Methods for the Analysis of Water and Wastewater (APHA, 2000). In Table 1, emerging pollutants were not measured as it is believed that the different types of wastewaters (namely TWW, GW, CWW, and TW) used in this study should have (if any) an insignificant concentration of these types of pollutants. IW, in principle, could have such pollutants, but in this case, the type of IW used is partially treated blue water from a denim laundry facility. The possibility of this water having such pollutants at significant levels is very low.

To study the effect of the quality of wastewater reused on germination (ISTA, 2003), twenty seeds of both barley and onions were placed on wet autoclaved filter paper in sterile petri dishes. The filter paper was made wet by placing 5 ml of the wastewater in concern on the filter paper. The petri dishes and the accompanying seeds were then maintained at 20 °C in darkness in an incubator for the whole germination experiment. The filter paper was kept wet by adding 2 ml of wastewater in concern to the filter paper when it gets dry. Four replicates for each type of water tested were performed. Complete germination was defined by radicle emergence (Crowe et al., 2002), and the time for such emergence was recorded. The
germination rate index (GRI) was used to indicate the speed of germination, as defined below (Tadros et al., 2012):

\[
\text{GRI} = \frac{\text{No. of germinated seeds}}{\text{Days of first count}} + \frac{\text{No. of germinated seeds}}{\text{Days of final count}}
\]

**Table 1: Quality of irrigation water used in the experiments (mg/l, except as noted).**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Tap Water (TW)</th>
<th>Grey Water (GW)</th>
<th>Treated Wastewater (TWW)</th>
<th>Industrial Wastewater (IW)</th>
<th>Car Wash Water (CWW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.9</td>
<td>8.0</td>
<td>7.7</td>
<td>7.1</td>
<td>7.6</td>
</tr>
<tr>
<td>EC (µS cm(^{-1}))</td>
<td>647</td>
<td>720</td>
<td>1240</td>
<td>4000</td>
<td>810</td>
</tr>
<tr>
<td>TDS</td>
<td>440</td>
<td>440</td>
<td>805</td>
<td>2500</td>
<td>510</td>
</tr>
<tr>
<td>BOD</td>
<td>-</td>
<td>34</td>
<td>25</td>
<td>53</td>
<td>120</td>
</tr>
<tr>
<td>COD</td>
<td>-</td>
<td>50</td>
<td>44</td>
<td>96</td>
<td>220</td>
</tr>
<tr>
<td>(\text{PO}_4^{3-})</td>
<td>-</td>
<td>2</td>
<td>17</td>
<td>3</td>
<td>1.3</td>
</tr>
<tr>
<td>(\text{NO}_3^-)</td>
<td>-</td>
<td>3</td>
<td>50</td>
<td>28</td>
<td>4.2</td>
</tr>
<tr>
<td>Ca</td>
<td>46</td>
<td>63</td>
<td>48</td>
<td>58</td>
<td>51</td>
</tr>
<tr>
<td>Mg</td>
<td>14</td>
<td>16</td>
<td>27</td>
<td>35</td>
<td>18</td>
</tr>
<tr>
<td>Na</td>
<td>75</td>
<td>80</td>
<td>142</td>
<td>786</td>
<td>83</td>
</tr>
<tr>
<td>Cl</td>
<td>128</td>
<td>95</td>
<td>183</td>
<td>980</td>
<td>140</td>
</tr>
<tr>
<td>Zn, Fe, Mn, B</td>
<td>&lt; 1</td>
<td>&lt; 1</td>
<td>&lt; 1</td>
<td>&lt; 1</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>Oil and Grease</td>
<td>-</td>
<td>4</td>
<td>&lt; 1</td>
<td>&lt; 1</td>
<td>7</td>
</tr>
<tr>
<td>MBAS*</td>
<td>-</td>
<td>6</td>
<td>0.5</td>
<td>0.8</td>
<td>14</td>
</tr>
</tbody>
</table>

* Methylene Blue Active Substances

**2.1 Statistical Analysis**

To evaluate the effect of the different water types on early stage growth of barley, shoot lengths, root lengths, shoot fresh weight, root fresh weight, shoot dry weight, and root dry weight were measured. From these measurements both the shoot length to root length ratio (SL/RL) and shoot fresh weight to root fresh weight ratio (SDW/RDW) were calculated. The differences between results of different waters were evaluated using Fisher’s Least Square Difference tests (LSD) at 0.05 probability level using SAS software (Tadros et al., 2012).

**3. Results and discussion**

**3.1 Effect on Onions seed germination**

Table 2 shows the effects of the different wastewater qualities on the germination of onions seeds. Table 2 shows that the different wastewater qualities produced different germination percentages of onions seeds after 13 days of incubation. TW and IW produced the highest germination percentages (89.7% after 13 days of incubation) and they were assigned the letter A to indicate that they have scored the highest germination percentage. Germination percentages when using TWW (76.7%) and CWW (82%) were the lowest. In Table 2, TWW was assigned the letter C, while CWW was assigned the letter BC. The meaning of these letters is as follows. To evaluate the differences between the germination percentages of the different water types used, the value of Fisher’s Least Square Difference (LSD) at 0.05 probability level was computed and found to be equal to (6.6). As the letter A was assigned to a germination percentage of 89.7% as shown above to TW and IW, a treatment that produced a germination percentage that is lower than 89.7% by LSD (6.6 in Table 2) would be assigned the letter B. A treatment that produced a germination percentage that is lower
than 89.7% by twice the value of LSD would be assigned the letter C, which is the case for TWW. CWW was assigned the letter BC because it produced a germination percentage that lies below B (by not more than LSD) and above C (by not more than LSD). TW and CWW have germination percentages (76.7% and 82%, respectively) that are lower than TW (89.7%) by more than LSD (6.6) showing that the difference is significant in comparison with TW. Although, IW and GW produced lower germination percentages than TW, the difference was non-significant according to Fisher’s least significant difference (LSD), except for day 10 for GW. The germination rate index (GRI) followed a similar trend to germination percentage, with TWW (9.75) and CWW (10.79) having the least values, while TW scored the highest value of 12.41. Again, the LSD for the GRI (1.11) after 13 days of incubation shows that the differences between TW and TWW and CWW are significant, while the difference with the IW and GW are non-significant.

Table 2: Germination percentage (%) and germination rate index (GRI) of onions under different water types

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Germination percentage (%)</th>
<th>GRI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 5</td>
<td>Day 6</td>
</tr>
<tr>
<td>Tap water (TW)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>Industrial water (IW)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>Grey water (GW)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>Treated waste water (TWW)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>Car wash water (CWW)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>AB</td>
</tr>
<tr>
<td>LSD</td>
<td>4.42</td>
<td>9.28</td>
</tr>
</tbody>
</table>

A: The water produced the highest score
AB: The water produced a lower score than score A, but the difference was non-significant according to LSD at 0.05 probability level. At the same time, the water produced a higher score than score B, but the difference was non-significant according to LSD at 0.05 probability level.
B: The water produced a lower score than score A and the difference was significant.
BC: The water produced a lower score than score B and a higher score than C, but the difference was non-significant.
C: The water produced a lower score than score B and the difference was significant.
D: The water produced a lower score than score C and the difference was significant.
S: At least one water produced a lower score than tap water in which the difference was significant
NS: All water types used produced differences from tap water that are non-significant.

In Table 2 (and all subsequent tables and figures), the water types that score the highest germination percentages are assigned the letter A. The letter B is assigned to water types that produced lower germination percentages that are significantly different than those assigned the letter A according to LSD at 0.05 probability level. The letter AB is assigned to water types that produced a lower score than score A, but the difference was non-significant according to LSD at 0.05 probability level. At the same time, the water produced a higher score than score B, but the difference was non-significant according to LSD at 0.05 probability level. In other words, water types that produced a germination percentage that cannot be considered either A or B are assigned the letter AB. Similarly BC is assigned to the water that scored a
germination percentage that is lower than score B and higher than score C, but the difference was non-significant to be included in either B or C. Similarly, the letter C is assigned to the water that produced a lower score than score B and the difference was significant, and so on.

Although the differences between TWW and CWW on one side and TW on the other are significant according to LSD at 0.05 probability level, the difference is not high for all practical purposes. IW and GW produced almost similar results to TW both for the germination percentage and the GRI.

3.2 Effect on barley seed germination

Table 3 shows the effects of wastewater reuse of different water qualities on the germination of barley seeds. Table 3 shows that germination percentage was affected by the quality of water used. The least germination percentage after 10 days of incubation occurred when using CWW with a percentage of about 64%, compared to 96%, 93%, 90%, and 88% for TW, GW, IW, and TWW, respectively. The germination rate index (GRI) followed the same trend as germination percentage, with the CWW having the least value of 18.21 compared to 27.77, 26.91, 25.74, and 24.95 for TW, GW, IW, TWW, respectively. The Fisher's least significant difference (LSD) at 0.05 probability level after 10 days of incubation (5.89 for germination percentage and 1.74 for GRI) shows that the differences between TW and all other wastewaters used, except GW, were significant. This is true for germination percentage and for the GRI.

**Table 3**: Germination percentage (%) and germination rate index (GRI) of barley under different water types

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Germination percentage (%)</th>
<th>GRI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 3</td>
<td>Day 4</td>
</tr>
<tr>
<td>Tap water (TW)</td>
<td>89.33</td>
<td>92</td>
</tr>
<tr>
<td>Industrial water (IW)</td>
<td>81.33</td>
<td>86.66</td>
</tr>
<tr>
<td>Grey water (GW)</td>
<td>86.67</td>
<td>90.33</td>
</tr>
<tr>
<td>Treated waste water (TWW)</td>
<td>77.33</td>
<td>83.33</td>
</tr>
<tr>
<td>Car wash water (CWW)</td>
<td>57.00</td>
<td>61.00</td>
</tr>
</tbody>
</table>

A: The water produced the highest score

AB: The water produced a lower score than score A, and a higher score than B, but the difference was non-significant according to LSD at 0.05 probability level.

B: The water produced a lower score than score A and the difference was significant.

BC: The water produced a lower score than score B and a higher score than score C but the difference was non-significant.

C: The water produced a lower score than score B and the difference was significant.

D: The water produced a lower score than score C and the difference was significant.

S: At least one water type produced a lower score than tap water in which the difference was significant.

Barley irrigated with CWW lost about one third of the germination percentage as compared to seeds irrigated with TW. CWW has higher oil content and higher detergent content (measured using Methylene Blue Active Substances (MBAS)) compared to all other waters tested, especially TW. The water that has the
second oil content and detergent content is GW. However this water produced almost similar germination percentage to TW. The type of oil in both of these waters is different. The presence of used oil in CWW could be the reason for the reduction of germination percentage and germination velocity when used in the irrigation of barley seeds.

It should also be noted that although IW produced lower germination percentage than TW, it produced similar results as TWW and a slightly lower results than GW, despite the higher salinity of such water (2500 mg l\(^{-1}\)) as compared with 440 and 805 for GW and TWW, respectively. According to Pang et al. (2010), high salinity water should not be applied before or during germination, as crops are most sensitive to salinity in their early growth stages. This has not been observed here with barley seeds, similar to results observed by Tadros et al., (2012) for Leucaena Leucocephala seeds. This could be due to the presence of higher organic matter in IW (Uzair et al., 2009; Tadros et al., 2012), as shown in Table 1.

### 3.3 Effect on Barley early growth stages

To evaluate the effect of the different water qualities used on early growth stages of barley, the shoot lengths, root lengths, shoot length to root length ratio, and the shoot dry weight to root dry weight have been measured and calculated. Figure 1 shows that the different wastewater qualities produced different root and shoot lengths.

![Figure 1. Shoot lengths and root lengths of barley under different water types. For the root lengths, the letter (B) means that the value is significantly lower than (A) and significantly higher than (C) according to Fisher’s least square difference test (LSD), and so on. For the shoot lengths, the different treatments that have the same value (A) are not significantly different, while they are higher than the treatment that has the letter (B) according to LSD.](image)

TW scored the lowest shoot length (assigned the letter B as compared to A for all other treatments) and the highest root length (assigned the letter A, while other treatments were assigned the letters B, C, D, and E). In contrast, CWW scored the highest shoot length and the lowest root length (the letter E). The LSD shows that the differences in shoot lengths and root lengths of all the different wastewaters tested compared to TW were significant. All the different wastewaters used scored higher shoot lengths and lower root lengths than TW. This has also produced significant differences in the shoot length to root length (SL/RL) ratios.
between TW and TWW and CWW experiments, as shown in Figure 2. Figure 2 also shows that the differences in shoot dry weight to root dry weight (SDW/RDW) of all water types used were non-significant.

Although the effects of using the different types of water mentioned above on barley is lacking in the literature, the differences in shoot length to root length ratios produced by the different wastewater types and GW compared to TW could be explained by the differences in water quality of these water types. GW and CWW have high concentrations of organics, oil and grease, and detergents (measured as MBAS). Similarly, TWW has high concentrations of organics, nitrogen, and salinity, while IW has high salinity value. Each of these different water characteristics has affected the balance between the shoot and root of barley. It seems that the effects of the high concentrations of used oil and grease and detergent in the CWW has affected barley the most.

Figure 2: Shoot length to root length (SL/RL) ratio and shoot dry weight to root dry weight (SDW/RDW) ratio of barley under different water types. For SL/RL, the letter (B) means that the value is significantly lower than (A) and significantly higher than (C) according to Fisher's least square difference test (LSD). The letter (BC) means that the value is not significantly lower than B and is not significantly higher than C; it is in between according to LSD. For SDW/RDW, the different treatments have the same value (A) which means they are not significantly different according to LSD.

4. Conclusions

The following conclusions were drawn after the use of grey water, treated wastewater, industrial wastewater, and car wash water as irrigation water on the seeds of barley and onion, and after comparing the results with those obtained after the use of tap water for the same purpose. All differences were evaluated using Fisher's Least Square Difference test at 0.05 probability level using SAS software.

- The use of the above types of waters as compared to tap water has reduced the germination percentage and germination speed of barley and onion seeds, with barley affected more than onion, especially when car wash water was used.

- The root length of barley was affected negatively by the use of these water types as compared to tap water. Car wash water produced the highest shoot to root ratio of all water types used, while tap water produced the lowest value.
The use of these types of water has changed the balance between shoot length and root length ratio of barley. The shoot to root ratio is a characteristic of each plant and should be constant for the same crop. This ratio has been changed for all types of water used as compared to tap water.

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