Full Length Research

Metabolic responses of Psidium guajava L. trees irrigated with polluted water

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Accepted 8 January, 2016; Published 22 January, 2016

The main objective of the present study was to clarify the main possible effects of waste water from El-Wafaeyya drain (polluted site) on some primary and secondary metabolites, some nutrient and heavy metals, photosynthetic pigments as well as fruit quality of seedy Psidium guajava trees irrigated with polluted water exhibited high accumulation of Fe, Mn, Zn, Cu, Pb, Co and Cd compared to that irrigated with control water. On the other hand, total photosynthetic pigments were significantly inferior in P. guajava leaves irrigated with polluted water compared to that of the control. The higher content of total protein together with total sugars was found in leaves of control site. High content of proline, free amino acids, antioxidants, total phenolics, total flavonoids and tannins were recorded in tree leaves treated with polluted water. The reverse was true for total alkaloids and saponins content. Regarding fruits, the negative effect was concentrated on the economic yield and number of fruits rather than fruit weight and size. Similarly, irrigation with polluted water caused lower water content, total proteins, total carbohydrates and vitamin A and C but higher dietary fibers.

Key words: Water pollution, guava, secondary metabolites, heavy metals, fruit quality.

INTRODUCTION

Chemical industries and agricultural organic wastes have created severe problems, as they release thousands of chemicals and materials to the environment (Abd El-Naim et al., 1997, Hinrichsen and Tacio, 2002). Pollution of the aquatic environments is a serious and considered as increasing problem (Sasaki et al., 1997) and has deleterious effects on terrestrial plants irrigated from contaminated water sources (McGlashan and Hughes, 2001).

Municipal wastewater is typically used for the irrigation of crops and fruit trees, mainly in per-urban ecosystems and is known to contribute significantly to the heavy metals content (Chen et al., 2005; Singh et al., 2004). Excessive accumulation of heavy metals in agricultural soils through wastewater irrigation significantly affects food quality and safety (Muchuweti et al., 2006). Heavy metals may impair plant physiology by reducing growth and nutrient uptake, interfering with photosynthetic processes and inhibiting fundamental enzymatic reactions if accumulated at high concentrations (Duruibe et al., 2007; El-Gammal, 2012; Malizia et al., 2012). Egenda et al. (2015) reported that the plants could be labeled as accumulators of pollution and there is a very strong relationship between soil and plants.

In Egypt, more than 95% of Egyptian water resources come from River Nile whose quality, however, dramatically worsened in the past few years because of pollution due to growing industrialization (Barakat, 2004; Mohamed et al., 2010). Seedy Psidium guajava L. (Guava, Myrtaceae) are widely planted especially in Beheira, El-Sharkia, around Alexandria and newly reclaimed lands (Samson, 1980). Trees growing near industrial factories and different contamination sources were subjected to heavy metals pollution either from air, irrigation water and/or soils (Haggag and El-Kobbia, 1981) which might affect the human health especially when these metals were found in high levels in the edible parts of the fruit (Sadek and El-Darier, 1992).

The present work was a trial to elucidate the main probable effects of agricultural waste water from El-Wafaeyya drain (polluted site) and Ferhaash canal (control) on some primary and secondary metabolites,
some nutrients and heavy metals, pigment content as well as fruit quality of *P. guajava* L.

**MATERIALS AND METHODS**

**Sampling and analysis of water and soil**

Ten samples of each water and soil (underneath the trees) were collected during summer 2015 from the control (Herhaash canal) and polluted (Wafaeeya drain) sites and the water samples were kept in sterilized plastic bottles in a refrigerator at 5°C until used. Water samples were analyzed for some of their physical and chemical characteristics as well as oxygen forms. Similarly, soil samples were also analyzed for some of their physical and chemical characteristics and the concentrations of trace elements were determined by Atomic Absorption Spectrophotometer (Perkin Elmer modelc., Norwalk, CT, USA). All the above mentioned analyses were according procedures outlined by Allen et al. (1984).

**Sampling of plant materials**

Five *P. guajava* trees were selected in each site where they are homogenous, more or less at the same age and received irrigation water from the two abovementioned sites since 5 years. Ten samples from leaves and fruits (summer 2015) were collected from the trees at the two study sites. The samples were washed thoroughly with running tap water then distilled water to remove dust particles from leaf surfaces. All samples were dried in an oven at 70°C till constant weight then powdered in an electrical grinder.

**Determination of some nutrient elements and heavy metals**

The concentrations of some nutrient elements and heavy metals in leaves and fruits of *P. guajava* were determined by Atomic Absorption Spectrophotometers and the heavy metal pollution index (HPI) was calculated using the equation given by Usero et al. (1997) to compare the total heavy metal load at different sampling locations;

$$HPI = \left( \frac{C_{f_1} \times C_{f_2} \times C_{f_3} \times C_{f_4}}{n} \right)^{1/n}$$

Where “Cf” is the concentration of “n” heavy metals in vegetative samples.

**Determination of photosynthetic pigments as well some primary and secondary metabolites**

Chlorophyll a, chlorophyll b and carotenoids (mg g⁻¹ fresh wt. of leaves) were determined spectrophotometrically according to Metzner et al. (1965). Total proteins, total soluble sugars, proline and total free amino acids were determined by method described by Lowry et al. (1951); Yem and Willis (1954); Bates et al. (1973); Moore and Stein (1984) respectively. Leaves plant extract was tested for the scavenging effect on DPPH radical according to the method described by Peiyuan et al. (2011). As well, total phenolic and flavonoids were determined using the Folin-Ciocalteu reagent and a colorimetric assay according to the methods of Singleton et al. (1999) and Kim et al. (2003) respectively. As well, total alkaloids, saponins and tannins were calculated as described by Kam et al. (1999), Yoko et al. (2001) and Sultana et al. (2012) respectively. All the aforementioned determinations were carried out in plant leaves.

Ten composite fruit samples were collected from the trees of the two sites and thoroughly washed with distilled water. The yield parameters [number of fruits tree⁻¹, fruit weight (g), fruit size (cm³) and fruit yield tree⁻¹ (Kg)] were evaluated. Thereafter, the samples were then sliced to small pieces and oven dried at 75°C for 72 h. The dried samples were then powdered and stored in plastic polythene bags ready for digestion (Lokeshwari and Chandrappa, 2006). The fruits were analyzed for their content of dietary fibers and both vitamin A and C (McCleary et al., 2010 and Brody, 1994 respectively).

**Protein profile**

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was performed to distinguish and fragment total soluble protein for *P. guajava* leaves attained irrigation water from control and polluted source according to the method of Laemmli (1970).

**Estimation of genomic template stability (GTS, %)**

Each separate bands effect observed in the protein profiles (disappearance of bands, appearance of new bands and variation in band intensities in comparison with control profiles) was considered in order to assess any DNA damage and the template genomic stability (percent) was calculate for each experimental group of fish with the chosen primer. Genomic template stability (GTS, %) was calculated as follows:

$$GTS \% = \left(1 - \frac{a}{n}\right) \times 100$$

Where “a” is the number of RAPD polymorphic profiles detected in each sample treated and “n” is the number of total bands in the control (Tice et al., 2000).

**Statistical analysis of the data**

Data were fed to the computer and analyzed using SPSS software package version 20.0. Comparison between the
two sites in the present study completed using independent t-test. Significance of the obtained results was judged at the 5% level. The procedures were according to Kotz et al. (2006).

RESULTS AND DISCUSSION

Water and soil characteristics

The variation in pH, EC, TDS, turbidity, salinity as well as different oxygen forms (DO, BOD and COD) in water samples collected from Ferhaash (control site) and El-Wafaeyya drain (polluted site) during summer of 2015 are listed in Table 1. The differentiation (D) between the two study sites with respect water analysis was calculated by the following equation:

\[ D = 1 - \frac{\text{Control}}{\text{Polluted}} \times 100 \]

The calculated values of D were -10.88, 60.4, 86.3, 69.32, 83.76, -21.3, 72.62 and 52.65 for pH, EC, TDS, turbidity, salinity, DO, BOD and COD respectively. All the evaluated parameters indicated that they are significantly dominant in the water of the polluted site compared to the control except for pH and DO which attained the negative sign. The World Health Organization (WHO) and European Union (EU) recommended limits as 5-6 and 6-8 for pH respectively. The two organizations recommended a value of 250 (μS/cm) for conductivity. At high pH, metals tend to form insoluble metal mineral phosphates and carbonates, whereas at low pH they tend to be found as free ionic species or as soluble organometals and are more bioavailable (Sandrin and Hoffman, 2007). The measured conductivity values of the soil samples (601 and 1516 μS/cm for the control and polluted sites respectively) testify the presence of trace metal ions ionizable materials in the polluted water.

Generally, Table 2 showed the values of some physical and chemical properties of the soil collected from the orchards in the two study sites. It was noticeable that the difference in soil texture between the two sites was not significant. Similar to Table 1 the D values were also calculated to distinct between the two study sites. The calculated values of D were -10.88, 60.4, 86.3, 69.32, 83.76, -21.3, 72.62 and 52.65 for pH, EC, TDS, turbidity, salinity, DO, BOD and COD respectively. All the estimated parameters denoted that they are significantly (as evaluated by t-test) dominant in the polluted site compared to the control except for OM and P which attained the negative sign.

Accumulation of heavy metals

The accumulation of heavy metals in soils is of increasing concern to researchers min the agricultural practices because the metals are biomagnified by plants. Accumulation of heavy and trace metals in plants occur by various sources but soil is considered the major one. Consumption of vegetables and fruits containing heavy metals is one of the main ways in which these elements enter the human body (Egbenda et al., 2015). In the present study, concentrations of some nutrient elements and heavy metals in P. guajava leaves exhibited a significant variation between the control and polluted sites (Figure 1). In addition, heavy metal pollution index (HPI) was calculated. The data indicated that the leaves of the study species related to the orchards in the polluted site attained high concentrations of Fe, Mn, Zn, Cu, Pb, Co and Cd compared to that irrigated with control water. It worth note that Mg was the only element which achieved a reverse trend and the Co was not detected in the control leaves. Subsequently, the heavy metals pollution index (HPI) in polluted leaves was about 2 folds that displayed by control leaves. The concentration of heavy metals was considered to be higher in leaves of the trees irrigated with polluted water compared with the control. This could be due to the dominant of metals in polluted water and subsequent uptake by P. guajava trees growing there in the polluted site. The responses of plants to high level of heavy metal content of irrigation water differ with species. For example, some plant species can be injured and their quality may be change by the increased heavy metal content in their environment. Others, called indicators can tolerate heavy metals (Pugh et al., 2002). Also, some plants have the capability to safely accumulate of heavy metals in different ways (Kim et al., 2003). Our results were consistent with those obtained by several authors (Sharma et al., 2006, 2007; Marshall et al., 2007) who reported that crops and vegetables grown in soils contaminated with heavy metals demonstrated greater accumulation of heavy metals than those grown in uncontaminated soil.

Photosynthetic pigments

Data of the current study indicated that the total photosynthetic pigments were significantly minor in P. guajava leaves irrigated with polluted water compared to that of the control (Figure 2). The value in polluted leaves was reduced to about one-third that occurred in the control. The relative values for the chlorophyll fractions (Chl a, Chl b and carotenoids) were 2.6, 4.3 and 4.46 respectively. This may be due to the negative effects of heavy metals on the content of chlorophyll and photosynthesis yield. Exposed to Cr, the content of chlorophyll of P. guajava leaves rose (Yang et al., 1999). The same results were observed for guava where the content of chlorophyll decreased and the chlorophyll a/b ratio was kept at 2 under Cr stress (10-40 mg/L) (Yang et al., 2001). Sun and Wang (1985), however, reported that the chlorophyll a/b ratio of macrophytes decreased as the
Table 1. Some physical characteristics and oxygen forms of surface water sampled from the control site (Ferhaash canal), as well as polluted site (El-Wafaeyya drain) during summer of 2015.

<table>
<thead>
<tr>
<th>Location</th>
<th>Parameter</th>
<th>pH</th>
<th>EC (µS/cm)</th>
<th>TDS (mg/l)</th>
<th>Turbidity (NTU)</th>
<th>Salinity (mg/l)</th>
<th>DO (mg l⁻¹)</th>
<th>BOD (mg l⁻¹)</th>
<th>COD (mg l⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control canal</td>
<td></td>
<td>7.26 ± 0.01</td>
<td>601.0 ± 1.0</td>
<td>101.67 ± 1.53</td>
<td>13.66 ± 0.16</td>
<td>121.0 ± 1.0</td>
<td>7.30 ± 0.23</td>
<td>5.26 ± 0.20</td>
<td>12.71 ± 0.41</td>
</tr>
<tr>
<td>Polluted drain</td>
<td></td>
<td>6.67 ± 0.18</td>
<td>1516.7 ± 104.1</td>
<td>741.7 ± 37.1</td>
<td>44.53 ± 6.20</td>
<td>745.3 ± 53.3</td>
<td>3.42 ± 0.50</td>
<td>19.21 ± 1.51</td>
<td>26.84 ± 1.61</td>
</tr>
</tbody>
</table>

P: \( p \) value as evaluated by Student t-test. *: Statistically significant at \( p \leq 0.05 \).

TDS: Total dissolved salts, EC: Electrical conductivity, DO: Dissolved oxygen, BOD: Biochemical oxygen demand, COD: Chemical oxygen demand.

Table 2. Variation in some physical and chemical characteristics of soils sampled from the control site (Ferhaash canal), as well as polluted site (El-Wafaeyya drain) during summer of 2015.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Location</th>
<th>Control orchard</th>
<th>Polluted orchard</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>EC (ds/m)</td>
<td></td>
<td>35.11 ± 0.29</td>
<td>244.4 ± 13.0</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>pH</td>
<td></td>
<td>2.27 ± 0.03</td>
<td>6.81 ± 0.07</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Ca (meq/kg)</td>
<td></td>
<td>36.11 ± 0.34</td>
<td>129.8 ± 10.6</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Cl (meq/kg)</td>
<td></td>
<td>16.83 ± 0.54</td>
<td>106.1 ± 7.2</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>OM (%)</td>
<td></td>
<td>18.60 ± 0.87</td>
<td>8.18 ± 0.96</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

Clay (%)

| Clay (%)          |                  | 43.37 ± 2.11    | 42.95 ± 0.53     | 0.757 |
| Sand (%)          |                  | 28.83 ± 2.25    | 28.62 ± 0.71     | 0.883 |
| Silt (%)          |                  | 27.80 ± 0.34    | 28.43 ± 1.23     | 0.440 |

Textural grade

<table>
<thead>
<tr>
<th>Available nutrients (µg g⁻¹)</th>
<th>Nitrogen (N)</th>
<th>Phosphorus (P)</th>
<th>Potassium (K)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control orchard</td>
<td>11.68 ± 0.70</td>
<td>21.62 ± 1.01</td>
<td>57.46 ± 0.81</td>
</tr>
<tr>
<td>Polluted orchard</td>
<td>54.79 ± 6.63</td>
<td>7.99 ± 3.59</td>
<td>106.63 ± 15.75</td>
</tr>
</tbody>
</table>

P: \( p \) value as evaluated by Student t-test. *: Statistically significant at \( p \leq 0.05 \).

Some primary and secondary metabolites

The higher content of total protein together with total sugars was found in leaves of the control site. On the other hand, high leaf content of proline, free amino acids and antioxidants were recorded in leaves treated with polluted water (Figure 3). Total phenolics, flavonoids and tannins contents were significantly accumulated in leaves irrigated with polluted water. The reverse was true for total alkaloids and saponins content (Figure 4). Hong et al. (1991) and Li et al. (1992) reported that the dissolubility of proteins increased under Cd stress, which might be a detoxifying mechanism. On the other hand, the results of Qin et al. (1994) showed that the dissolubility of proteins of \( P. guajava \) decreased with an increase in Cd concentration higher than 0.1 mg/L. The polypeptide composition of \( P. guajava \) was degraded under Cd and Zn treatment, and the effect of Cd was more significant than that of Zn (Chen et al., 1999). Proline is an important substance for infiltrative calibration; its accumulation was adapted to the environmental stress (Tang, 1984). Under a lower stress with Cd the contents of proline increased little in \( P. guajava \) seedlings (Qin et al., 1994).
Figure 1. Variation in the concentrations (ppm) of some elements and heavy metals pollution index (HPI) in leaves of *Psidium guajava* grown in the control orchard (Ferhaash canal), as well as polluted orchards (El-Wafaeyya drain) during summer of 2015.

Figure 2. Variation in the mean concentration of different pigment fractions, Chl. "a", Chl. "b", carotenoids and total pigments (mg g f.wt.⁻¹), in leaves of *Psidium guajava* grown in the control orchard (Ferhaash canal), as well as polluted orchards (El-Wafaeyya drain) during summer of 2015.
Fruit quality and yield

The fate of heavy metals in polluted water is a subject of study because of the direct potential toxicity to biota and the indirect threat to human health via the contamination of groundwater and accumulation in food crops (Martinez and Motto, 2000). Regarding fruits, irrigation with polluted water in the present study led to lower production and number of fruits per tree, but higher fruit weight and fruit size. Data indicating the negative effect of water pollution mainly concentrated on the economic yield and number of fruits rather than fruit weight and size. Similarly, irrigation with polluted water caused lower water content, total proteins, total carbohydrates and vitamin A and C but higher dietary fibers (Table 3a). The reason for this result could be attributed to the increase in the soluble

![Figure 3. Variation in some primary metabolites (mg g⁻¹) and antioxidant activity (%) in leaves of Psidium guajava grown in the control orchard (Ferhaash canal), as well as polluted orchards (El-Wafaeyya drain) during summer of 2015.](image1)

![Figure 4. Variation in some secondary metabolites (mg g⁻¹) in leaves of Psidium guajava grown in the control orchard (Ferhaash canal), as well as polluted orchards (El-Wafaeyya drain) during summer of 2015.](image2)
solids contents (Abd Allah et al. 1992). These data are in accordance with suggestion of Adams et al. (1994 and 1995), who reported that dry matter (DM) accumulation of plants decreased with increased salinity, but DM of fruits increased. Increasing salinity reduces DM production and increased the proportion of total DW in the fruits at the expense of the upper shoot.

Table 3b demonstrated a comparison between the concentrations of some nutrient elements and heavy metals in guava fruits collected from trees irrigated with polluted and control water. Generally, the data showed that the fruits related to the orchards in the polluted site attained high concentrations of all elements except Mg. Explicitly, Cr, Cd and Ni were accumulated in polluted fruits while completely absent in the control. Several studies have indicated that crops grown on water contaminated with heavy metals have higher concentrations of heavy metals than those grown on uncontaminated soil (Nabulo, 2006). Importantly, reduction percentages of about 83 and 80% in vitamin C and A respectively was exhibited by fruits harvested from P. guajava trees affected by irrigation with polluted water.

### Table 3a. Variation in some biochemical characteristics (a) and yield parameters (b) of Psidium guajava fruits grown on the control site (Ferhaash canal) as well as polluted site (El-Wafaeyya drain) during summer of 2015.

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Location</th>
<th>Control orchard</th>
<th>Polluted orchard</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Biochemical characteristic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water content (%)</td>
<td></td>
<td>80.22</td>
<td>63.02</td>
<td>0.002*</td>
</tr>
<tr>
<td>Total proteins (mg g⁻¹)</td>
<td></td>
<td>5.07</td>
<td>1.52</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Total carbohydrates (mg g⁻¹)</td>
<td></td>
<td>11.26</td>
<td>2.71</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Total dietary fibers (mg g⁻¹)</td>
<td></td>
<td>47.24</td>
<td>69.29</td>
<td>0.001</td>
</tr>
<tr>
<td>Vitamin C (mg g⁻¹)</td>
<td></td>
<td>1.28</td>
<td>0.21</td>
<td>0.002*</td>
</tr>
<tr>
<td>Vitamin A (mg g⁻¹)</td>
<td></td>
<td>0.061</td>
<td>0.012</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>b. Yield parameter</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of fruits tree⁻¹</td>
<td></td>
<td>97</td>
<td>58</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Fruit weight (g)</td>
<td></td>
<td>97.84</td>
<td>109.43</td>
<td>0.001</td>
</tr>
<tr>
<td>Fruit size (cm³)</td>
<td></td>
<td>73.28</td>
<td>87.87</td>
<td>0.015*</td>
</tr>
<tr>
<td>Fruit yield tree⁻¹ (Kg)</td>
<td></td>
<td>9.49</td>
<td>6.34</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

P-value as evaluated by Student t-test.

Protein electrophoresis

The distribution of protein bands in SDS gel of P. guajava (Plate 1) were studied to distinguish between control and polluted orchard. Generally, polluted sample reflected superiority in number and activities of protein pattern comparing with the control one with ten and seven protein bands respectively. Two common protein bands were monitored between the two samples with 98, 37 KDa respectively.

Five specific bands characterize control sample with 157, 135, 78, 73 and 43 KDa. On the other hand, polluted sample showed eight specific bands with 173, 140, 122, 54, 33, 24, 22 and 14 KDa. These results indicated that genomic template stability was significantly affected at polluted water. The general tendency of genomic template stability (GTS%) of polluted orchard was 41%. The experimental procedure adopted, and the successive precision the damage induced by polluted stress on the genome of proteins. The simultaneous use of qualitative and quantitative analyses of electrophoretic patterns makes the observations more reliable as regards the various genotoxic effects that these substances can be induced. RAPD profile analysis in conjunction with the evaluation of GTS % would prove a powerful eco-toxicalogical tool. Thus, DNA polymorphisms detected using RAPD analysis could be used as an investigation tool for environmental toxicology and as a useful biomarker assay that can be used as an early warning system (Becker et al. (2003); Etinosa et al. (2013); Fischerová et al. (2005)).

Conclusion

The present study indicated that P. guajava may consider as a bioindicator as they are very sensitive to water and soil pollutants. Such pollutants caused damage of their leaves, impair photosynthetic apparatus, enhance the accumulation of heavy metals and some secondary metabolites as well as alteration of protein pattern in their leaves and fruits. Awareness must be taking for the application of P. guajava fruits collected from polluted locations for human uses as they are unsafe, dangerous
Table 3b. Variation in the concentrations (ppm) of some heavy metals and heavy metal pollution index (HPI) of *Psidium guajava* fruits grown in the control site (Ferhaash canal), as well as polluted site (El-Wafaeyya drain) during summer of 2015.

<table>
<thead>
<tr>
<th>Element</th>
<th>Location</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control orchard</td>
<td>Polluted orchard</td>
</tr>
<tr>
<td>Mg</td>
<td>117.42</td>
<td>48.85</td>
</tr>
<tr>
<td>Mn</td>
<td>8.64</td>
<td>38.83</td>
</tr>
<tr>
<td>Cr</td>
<td>ND</td>
<td>11.88</td>
</tr>
<tr>
<td>Fe</td>
<td>12.88</td>
<td>227.78</td>
</tr>
<tr>
<td>Zn</td>
<td>0.78</td>
<td>27.49</td>
</tr>
<tr>
<td>Cd</td>
<td>ND</td>
<td>0.09</td>
</tr>
<tr>
<td>Cu</td>
<td>2.19</td>
<td>22.85</td>
</tr>
<tr>
<td>Ni</td>
<td>ND</td>
<td>.22</td>
</tr>
<tr>
<td>Pd</td>
<td>0.46</td>
<td>14.53</td>
</tr>
<tr>
<td>Co</td>
<td>ND</td>
<td>0.88</td>
</tr>
<tr>
<td>HPI</td>
<td>3.21</td>
<td>9.85</td>
</tr>
</tbody>
</table>

P-value as evaluated by Student t-test.

Plate 1. Analysis of protein fingerprinting patterns extracted from leaves of *Psidium guajava* grown in the control site (Ferhaash canal), as well as polluted site (El-Wafaeyya drain) during summer of 2015.

and risky.

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