The Effects of Heavy Metals on Pollen Germination and Pollen Tube Length in the Tobacco Plant

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Abstract: This study was carried out to determine the effects of heavy metals (Ni, Fe, Pb, Co, Cd, Hg, Al, Zn and Cu) on pollen germination and pollen tube length in the tobacco plant (Nicotiana tabacum L.) cv. Karabağlar.

The results showed that enhanced concentrations of heavy metals, except Fe, decreased the pollen germination rates and the pollen tube lengths. With Fe concentrations, on the other hand, a positive relationship was determined between the pollen characteristics examined.

The most toxic effect on pollen germination was seen with the applications of Cu, Ni and Hg; on pollen tube length, on the other hand, a similar tendency was determined with the applications of Hg, Cd and Ni. The toxic effects of Co, Al and Fe were found to be low on both of the pollen characteristics. As a result, all the heavy metals examined prevented pollen germination and tube growth in the tobacco plant, but their toxicity levels varied.

Key Words: Pollen Germination, Pollen Tube Length, Heavy Metals, Tobacco

Ağır Metallerin Tütün Bitkisinde Polen Çınlanması ve Polen Tüpü Uzunluğuna Etkileri

Özet: Ağır metallerin (Ni, Fe, Pb, Co, Cd, Hg, Al, Zn ve Cu) tütün (Nicotiana tabacum L.) bitkisinde polen çinlanması ve polen tüp uzunluğu üzerine etkileri araştırılmıştır.

Araştırma bulgularına göre; Fe dışında diğer ağır metallerin artan konsantrasyonlarına bağlı olarak polen çinlanma oranı ve polen tüp uzunluğunda azalma görülmüştür. Fe konsantrasyonları ile incelenen polen özellikleri arasında önce pozitif sonra negatif bir ilişki saptanmıştır.

En yüksek toksik etki; polen çinlanmasında Cu, Ni ve Hg uygulamalarıyla, polen tüp uzunluğunda ise Hg, Cd ve Ni uygulamalarıyla görülmüştür. Co, Al ve Fe’nin toksik etkisi dereceleri ise her iki polen özelliği üzerine de düşük düzeyde bulunmuştur. Sonuç olarak, incelenen tüm ağır metaller tütündeki polen çinlanmasını ve tüp uzamasını engellemiş ancak toksite dereceleri oldukça geniş sınırlar arasında değişmiştir.

Anahtar Sözcüklер: Polen Çi nlanması, Polen Tüpü Uzunluğu, Ağır Metaller, Tütün

Introduction

Industrial chimney gases and exhausts from traffic cause air pollution in terms of heavy metals, which can also accumulate in soil, resulting in rapid uptake by plants. Rain, irrigation water rich in heavy metals, and agricultural chemicals are the most important sources of contamination. Moreover, chimney gases resulting from metallurgical procedures, dust and particles scattered around, and the wastes filtered from garbage mix in the deep and surface water and subsequently increase the heavy metal contents of soil (1). Presently, parallel to the rapid growth in industrialization, environmental pollution is also increasing, in which heavy metals like Ag, Au, Br, Cd, Cu, Fe, Hg, and Pb constitute a significant part. In the case of plants, the permeability of the cell membrane, the biochemical activities at the macromolecular level, and the regular growth and reproduction of cells are negatively affected. Pollutants of this kind at ppm levels usually cause anomalies in mitotic division, and chromosome aberrations (2,3). Many studies report that heavy metals such as Cd, Co, Fe, Pb, Zn, Cu and Hg at toxic levels
inhibit pollen germination, pollen tube growth and seed germination, causing ultra-structural changes. These studies also indicate that heavy metals applied to seeds at levels of 10-200 ppm inhibit the growth of plumula and radicles. Other negatively affected characteristics include germination percentage, germination index, root and shoot lengths and root and shoot dry matter rates (4,5). Some studies claim that heavy metals, particularly Pb and Cd, in the exhaust gases, engine oils and tires of vehicles in regions with heavy traffic (6,7) and acid rain (8) inhibit pollen germination and tube growth in plants, reaching toxic levels in the plant leaves. There are also findings indicating that heavy metals cause cytogenetic anomalies in plants, like the inhibition of mitosis division, decrease in the mitotic index (cell division frequency), and chromosomal anomalies (9). In two different studies on the effects of Al and Hg on meristematic root cells of Allium cepa L. and Allium sativum L., the results not only prove that these two heavy metals inhibit root growth, decrease the mitotic index and cause mitosis anomalies (10,11), but also show that these metals, even in minute amounts, damage the vital functions of plants. The effects of the main heavy metals on pollen germination and pollen tube length in the tobacco plant were investigated in this study as well.

Materials and Methods

Nicotiana tabacum L. cv. Karabağlar was used as the research material in this study. Unbloomed flower buds of healthy plants were collected when the tips of their corollas had become pink, and pollen was examined under laboratory conditions (12).

Standard solutions of each heavy metal under consideration (Ni, Fe, Pb, Co, Cd, Hg, Al, Zn and Cu) were prepared with distilled water as follows: 30, 60, 90, 120 µM Hg as HgCl2; 50, 100, 150, 200 µM Al as AlCl3; 60, 120, 180, 240 µM Zn as ZnSO4; 70, 140, 210, 280 µM Pb as Pb(NO3)2; 90, 180, 270, 360 µM Cd as CdCl2; and 100, 300, 500, 700 µM Ni, Fe, Cu and Co as, subsequently, NiCl2, FeCl2, CuSO4 and CoCl2 (4,10,11,13-15).

In the experiment, a germination medium containing 100 ppm H3BO3 and 15% sucrose was used to provide the germination of pollens (16).

Blotting paper was placed into each sterile petri dish, and 2 slides were put on the blotting paper. Then 50 µl nutrient medium and 50 µl heavy metal solutions were dropped onto the slides. Further, pollen taken from flower anthers with a pin was carefully spread on this solution. The control treatment was solely 50 µl distilled water + 50 µl germination medium. The experiment was performed in randomized parcels with two replications. Subsequently, the petri lids were closed and incubated at 24°C for 24 hours.

Finally, the slides in the incubated petri dishes were examined and counted under a microscope in terms of germinated and ungerminated pollen in 4 different observation sections to determine the germination ratio of pollen in percentages. As for the average length of pollen tubes, the lengths of 10 germinated pollen tubes randomly chosen from each of the 4 different observation sections were measured and the averages were recorded. The results are given in microns (µ) and the concentrations given in the tables decreased at a rate of 1/2 due to the dilution of the medium.

Results and Discussion

The effects of zinc as ZnSO4, nickel as NiCl2, iron as FeCl2, copper as CuSO4, cobalt as CoCl2, cadmium as CdCl2, mercury as HgCl2, lead as Pb(NO3)2 and aluminum as AlCl3 on pollen germination and pollen tube lengths of the tobacco plant are given in Tables 1, 2 and 3.

As seen in the tables, while the germination rates of pollen decreased with the application of heavy metal solutions, the pollen tube lengths were negatively affected. Pollen germination was inhibited most by Cu, Ni and Hg, and least by Al, Fe and Co. The pollen tube length, on the other hand, was affected most by Hg, Cd and Ni and least by Fe, Al and Co. Heavy metal effects may be due to different tolerance levels of tobacco pollen. When the effects of heavy metals on pollen germination were examined, in the groups where Cu, Co and Al were applied, no statistically significant difference was found between the control and the lowest concentrations applied. Similar results in the case of pollen tube length were only seen on Co application. This may be due to the low toxicity of low doses of the heavy metals under consideration. When the pollen germination in the group where Fe had been applied was compared with the control, the results showed that pollen germination increased with the 100 µl dose and decreased with the 300, 500 and 700 µl doses. The results also revealed that
### Table 1. The effects of Ni, Fe, Cu and Co as NiCl₂, FeCl₂, CuSO₄ and CoCl₂ respectively on pollen germination and pollen tube length in the tobacco plant.

<table>
<thead>
<tr>
<th>Tre. (µM)</th>
<th>Germ. (%)</th>
<th>Pollen tube length (µ)</th>
<th>Tre. (µM)</th>
<th>Germ. (%)</th>
<th>Pollen tube length (µ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cont.</td>
<td>90.60a</td>
<td>230.58a</td>
<td>Cont.</td>
<td>90.60b</td>
<td>230.58c</td>
</tr>
<tr>
<td>100</td>
<td>74.41b</td>
<td>162.41b</td>
<td>100</td>
<td>95.11a</td>
<td>290.03b</td>
</tr>
<tr>
<td>300</td>
<td>61.40c</td>
<td>122.56c</td>
<td>300</td>
<td>84.73b</td>
<td>303.11a</td>
</tr>
<tr>
<td>500</td>
<td>18.82d</td>
<td>48.03d</td>
<td>500</td>
<td>75.36c</td>
<td>235.41c</td>
</tr>
<tr>
<td>700</td>
<td>8.11d</td>
<td>21.00d</td>
<td>700</td>
<td>55.66d</td>
<td>166.46d</td>
</tr>
<tr>
<td>LSD (5%)</td>
<td>11.22</td>
<td>31.99</td>
<td>LSD (5%)</td>
<td>12.77</td>
<td>90.40</td>
</tr>
</tbody>
</table>

Tre: Treatment, Germ: Germination, Cont: Control

### Table 2. The effects of Cd, Hg, Pb and Al as CdCl₂, HgCl₂, Pb(NO₃)₂ and AlCl₂ respectively on pollen germination and pollen tube length in the tobacco plant.

<table>
<thead>
<tr>
<th>Tre. (µM)</th>
<th>Germ. (%)</th>
<th>Pollen tube length (µ)</th>
<th>Tre. (µM)</th>
<th>Germ. (%)</th>
<th>Pollen tube length (µ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cont.</td>
<td>90.60a</td>
<td>230.58a</td>
<td>Cont.</td>
<td>90.60a</td>
<td>230.58a</td>
</tr>
<tr>
<td>90</td>
<td>76.16b</td>
<td>160.61b</td>
<td>30</td>
<td>44.93b</td>
<td>115.40b</td>
</tr>
<tr>
<td>180</td>
<td>53.63c</td>
<td>80.83c</td>
<td>60</td>
<td>21.70c</td>
<td>116.90b</td>
</tr>
<tr>
<td>270</td>
<td>23.96d</td>
<td>28.83d</td>
<td>90</td>
<td>7.66d</td>
<td>32.03c</td>
</tr>
<tr>
<td>360</td>
<td>17.00d</td>
<td>17.33d</td>
<td>120</td>
<td>11.20d</td>
<td>8.03d</td>
</tr>
<tr>
<td>LSD (5%)</td>
<td>10.39</td>
<td>46.37</td>
<td>LSD (5%)</td>
<td>4.24</td>
<td>16.05</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tre. (µM)</th>
<th>Germ. (%)</th>
<th>Pollen tube length (µ)</th>
<th>Tre. (µM)</th>
<th>Germ. (%)</th>
<th>Pollen tube length (µ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cont.</td>
<td>90.60a</td>
<td>230.58a</td>
<td>Cont.</td>
<td>90.60a</td>
<td>230.58a</td>
</tr>
<tr>
<td>70</td>
<td>71.23b</td>
<td>197.06b</td>
<td>50</td>
<td>93.50a</td>
<td>189.83b</td>
</tr>
<tr>
<td>140</td>
<td>53.30c</td>
<td>98.76c</td>
<td>100</td>
<td>77.86b</td>
<td>171.03b</td>
</tr>
<tr>
<td>210</td>
<td>36.16d</td>
<td>108.06c</td>
<td>150</td>
<td>60.23c</td>
<td>126.43c</td>
</tr>
<tr>
<td>280</td>
<td>24.13d</td>
<td>36.63d</td>
<td>200</td>
<td>62.43c</td>
<td>106.11c</td>
</tr>
<tr>
<td>LSD (5%)</td>
<td>11.64</td>
<td>22.21</td>
<td>LSD (5%)</td>
<td>9.07</td>
<td>32.59</td>
</tr>
</tbody>
</table>

Tre: Treatment, Germ: Germination, Cont: Control
pollen tube length increased with the 100 and 300 µl doses and decreased with the 500 and 700 µl doses. This situation can be explained by the finding that Fe is a plant nutrient and subsequently has a very low toxicity level (17).

When the highest concentrations of the heavy metal solutions are considered in comparison with the control group, the reductions in pollen germination rate and pollen tube length are given in Table 4.

Tables 1, 2 and 3 prove that both pollen germination and pollen tube lengths were negatively affected by the application of heavy metals in increasing concentrations. Pollen germination was most negatively affected by Cu at a rate of 92%, but pollen tube length was affected at a lower level (77%). On the other hand, Ni and Hg affected both the pollen germination and pollen tube length at the highest level, showing a high toxicity for the tobacco plant. Among Cu, Zn and Fe, which are elements necessary for plant nutrition, Fe showed the lowest toxicity. It inhibited germination and pollen tube length at the rate of 35%, even at the highest dose (700 µl). The elements with the lowest negative effect on pollen germination and pollen tube length were Fe, Al and Cu. Fe in particular is very important due to its active role in photosynthesis. In a study on tobacco anther culture, the significance of Fe presence in the medium was noted for embryogenesis. It has also been indicated that, in a medium with no Fe, embryoids proceed no further than the globular stage (18). In another study on the bioconcentrations of heavy metals in the plant structure, it has been indicated that the uptake and transportation of the elements such as Mn, Fe, Zn, Cu and Mo (which act as micronutrients for plants) are at higher rates than those of other trace elements. It has also been claimed that Cd, Cu, Hg and Ni are more toxic than Pb and Zn for plants (19). Heavy metals do not only affect the electron transportation during respiration negatively (20), but also inhibit the plant growth indirectly by preventing enzyme activity partly or completely. The decline in enzyme activity and respiration also affect the pollen germination and pollen tube length negatively (17). In an experiment on *Lens esculenta* L., seed germination decreased noticeably with the applications of Cu, Cd, Hg and Zn, and amylase and peroxidase isoenzymes in the plumula, radicles and cotyledons increased during the germination, showing enzyme activation prevention by heavy metals (13). In another study on aromatic plants, the negative effect of heavy metals at 6-10 ppm Cd, 60-150 ppm Cu, 100-500 ppm Pb and 400-800 ppm Zn levels was claimed to be damaging on seed germination and root growth (14). The effects of Cd, Co, Cu, Fe, Hg, Mn, Zn and Al on the ultra-structure and pollen tube growth of *Lilium longiflorum* has also been studied and the highest rate of toxic effect was reported to be caused by Cd, Cu and Hg (15). Some other studies state that compounds with Hg among heavy metals prevent DNA replication and protein synthesis, causing mitotic anomalies, and that Cu has similar effects, causing chromosome anomalies (21).

In conclusion, the results proved that all heavy metals have negative effects on pollen characteristics; however, the damage to pollen varies with the dose. All plants show some tolerance against different pollutants. The area
where the research material of this study was collected is at risk from heavy gas and dust particle pollution caused by three thermal power plants. The importance of the subject will be realized better when the information that dust and gas particles reach a distance of 20 to 100 km and contain heavy metals at different rates is considered (22). In this study, the relations between pollen characteristics of the tobacco plant, which has economic importance, and heavy metal contamination were examined and some negative characteristics were described.

References

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