



## Phytoremediational Honey Locust Trees' (*Gledischia triancanthos* L.) Efficacy in Reducing Air Pb and Cd Levels

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**ABSTRACT:** Because plant absorption of heavy metals is effective in decreasing environmental pollution, the present study examines the absorption of heavy metals by *Gledischia triancanthos* L. a species of trees planted in the green space along the Tehran- Karaj highway. This study was conducted in a split-plot design with three replications. The experimental treatments parameters consisted of the distances of the planted trees from the midline axis of the highway (0, 15, and 30 m) and three different sampling times (at the opening of the leaves, in late June, and before fall). The results showed that by the passage of time and especially before fall, the levels of lead increased significantly. The highest level of cadmium was observed in the trees' mid-growth period. For trees at a greater distance from the highway, the chlorophyll a levels were significantly higher, but chlorophyll b and total chlorophyll were not influenced by the distance from the highway. The results of the distance and time interactions have demonstrated that at the closest distance from the highway, the chlorophyll a levels significantly decreases over time. At distances of 15 and 30 m from the highway, the chlorophyll a levels increased and decreased, respectively, in the mid-growth and end-growth periods. At the closest distance from the highway, the levels of lead were significantly increased with the passage of time. By the time point before fall, the levels of lead were increased at 15 and 30 m from the highway. At the distance nearest to the highway, during the mid-growth period, the levels of cadmium reached their maximum values, and in the fall, were increased at 15 and 30 m from the highway.

**Keywords:** green space, heavy metals, air pollution, highway border, Honey Locust Trees'

### INTRODUCTION

One of the most significant principles in creating green spaces is to use trees and shrubs that are not merely resistant to environmental pollutants but also purify air, water, and soil. Plants play a crucial role in preventing the dispersion of noise and add to the esthetic value of urban and industrial areas.

Heavy metals may be absorbed by plants, particularly in agricultural soils, which then may be ingested by humans using the plants (Chehreghani *et al.*, 2009).

Therefore, information about the distribution of heavy metals such as lead, zinc, cadmium, and copper in urban environments assists in sustainable development and in creating a safer greener environment for urban dwellers. The forenamed metals are naturally present in the soil in small amounts. However, various urban, industrial, and agricultural activities such as mining,

waste management, the manufacture of aerosols, and the use of pesticides and fertilizers causes the levels of these metals to increase in the soil; to put it another way, the soil is threatened by these increased metal levels (Abdullahi *et al.*, 2009). Cadmium is one of the most important heavy metal pollutants in the environment, although it is not harmful to plant growth but is highly lethal to humans and animals (Salardini & Mojtahedi, 1988).

The idea of using plants to remove metals from contaminated soils was first developed by Utsunomyia and Chaney in 1991, the first "plant breeding" using zinc and cadmium was introduced by Baker (Ghosh & Singh, 2005). Amini (2011) concluded that species of cedar, ash, mulberry, and new leaves can be used as bio-tracers for airborne heavy metals in dry areas (2011).

By considering the growth behavior, absorption degree, and durability of two types of grass, *Trifolium repens* and *Lolium perenne*, in areas contaminated with heavy metals near lead, zinc, and cadmium mines, Bidar *et al.* (2007) showed that the metals were absorbed more by the roots than by the branches and that the absorption rate was the highest for cadmium, followed by zinc and lead. Through the changes in superoxide dismutase and malondialdehyde activity, the oxidative stresses of the plants were determined. The oxidative changes had a close relationship with the levels of the metals present, the plant tissue sampled, and the plant species. In comparison with *T. repens*, it seemed that *L. perenne* showed a larger response to oxidative stress from heavy metals (Bidar *et al.*, 2007).

Plants are used worldwide to mitigate soil contamination, which is considered to be one of the best ways of improving the environment. Despite the relevance of this technique, its use has been overlooked in Iran. The present study seeks to address the lack of systemic research on *Gledischia triancanthos* L.' resistance to adverse environmental conditions, including the presence of airborne heavy metals from vehicle emissions.

## MATERIALS AND METHODS

### A. Experimental parameters

This study was conducted between winter 2013 and winter 2014 at 40-hectare sites: in a botanical garden at the University College of Agriculture and Natural Resources at Tehran University, which is a park at the university, and in a green space in Dowlat Abad, Karaj, adjacent to Martyr Kalantari Terminal. The parameters in this experiment consisted of the distance of the *Gledischia triancanthos* L. trees' to the middle axis of the highway (0, 15, and 30 m) and the sampling times for the trees (opening of the leaves, late June, and before fall).

### B. Sampling Methods

Leaves, approximately in the amount of 10 grams, from *Gledischia triancanthos* L. were randomly selected for measurement. The sampling process was performed at three points in time: at the sprouting time, late June, and before fall. At each time point, the samples were collected from trees at three distances from the highway: 0, 15, and 30 m.

The sampling was taken from the presence of leaves at three levels of height; top, middle, and bottom on each tree, and also on four different directions at the crown of the plant, so that each sample contained leaves from each height and direction for a definite tree. After mixing the samples of every tree, the measurements were performed.

### C. Measurement Types

For each sample, the lead and cadmium levels were measured. The chlorophyll and carotenoid levels as well as the wet and dry leaf weights were measured.

### D. Measurement of Lead and Cadmium

To measure the lead and cadmium levels, 0.25 g of dried leaves were put into a 100 ml beaker, and 4 ml of sulfuric acid was added. After several shakings, the contents were transferred to the Digesdhal machine for extraction. After the digestion of the plant sample, the resulting solution was transferred to an atomic absorption set to measure the lead and cadmium levels (Hakimi, 2014).

### E. Measurement of Chlorophyll and Carotenoids levels

To measure the chlorophyll and carotenoid levels in the samples, 0.5g of wet leaves from each sample was crushed using liquid nitrogen. Then, 80% acetone kept at 0°C was added to extract the chlorophyll and carotenoid. The samples were centrifuged for 15 min, and the supernatant was used for the subsequent measurements. Absorption was determined by a spectrophotometer using a wavelength of 663.2nm and 646.2nm for chlorophyll and 470 nm for carotenoids. The chlorophyll levels were calculated according to the wet weight in mg and the formula provided in Lichtenthaler (1987). The carotenoid levels were calculated on the basis of mg/ml and related formulas as described previously (Hill *et al.*, 1986).

### F. Dry to Wet Leaf Weight Measurement

To measure the dry to wet leaf weight, 2g of leaf tissue from each tree was weighed out separately. Each leaf tissue sample was placed into an oven at 60°C for 72 h, so that each sample reached a constant weight following water loss. Thereafter, the weight of the dry samples was recorded and calculated on the basis of the formula provided by Otsubo and Iwaya-Inole (2000).

### G. Statistical Population

The existing *Gledischia triancanthos* L. were located in a 40-hectare area in the fields of the College of Agriculture and Natural Resources, Karaj. The data was initially recorded in Excel and transferred to SPSS software for statistical analysis using ANOVA.

## RESULTS

The results showed that the levels of the photosynthetic pigments chlorophyll and carotenoids significantly decreased before fall compared to when the leaves opened. The dry to wet weight significantly increased over time as well. The lead levels increased at the mid and late growth time points. Over time, the levels of lead significantly increased, while the highest level of cadmium occurred in the middle of the growing period (Tables 1 and 2).

**Table 1: ANOVA for Factors Affected by Time and Distance to the Highway.**

| Studying Features |    |               |               |                   |            |                   |          |         |
|-------------------|----|---------------|---------------|-------------------|------------|-------------------|----------|---------|
| Change sources    | df | chlorophyll a | chlorophyll b | Chlorophyll (a+b) | carotenoid | dry to wet weight | Pb       | Cd      |
| Rep               | 2  | 1.039**       | 1.19**        | 2.31**            | .07ns      | .00003ns          | 1.488ns  | .23ns   |
| Dis.              | 2  | 2.093**       | .53ns         | 20.84**           | 3.09**     | .00052**          | 37.569** | 1.114** |
| Error             | 4  | .278          | .36           | .45               | .52        | .00006            | .780     | .028    |
| Time              | 2  | 202.113**     | 130.47**      | 211.41**          | 54.68**    | .01758**          | 629.460* | 1.632** |
| Time*Dis.         | 4  | 3.41ns        | 1.112*        | 3.87**            | 2.37**     | .00016*           | 567.362* | 2.979** |
| Rep.*Time         | 4  | .36ns         | .47           | .36ns             | .10ns      | 0.00004 ns        | .552ns   | .084*   |
| Error             | 8  | 8.121         | .27           | .38               | .31        | .00004            | 1.163    | .024    |
| CV                |    | 6.2           | 11.5          | 5.1               | 10.9       | 1.1               | 6.2      | 19.1    |

**Table 2: Comparison of Averaged Time Effects on Study Indicators.**

| Studying Features           |                      |                      |                          |                   |                   |            |           |
|-----------------------------|----------------------|----------------------|--------------------------|-------------------|-------------------|------------|-----------|
| Time                        | chlorophyll a (mg/g) | chlorophyll b (mg/g) | chlorophyll (a+b) (mg/g) | carotenoid(µg/ml) | dry to wet weight | Pb (mg/kg) | Cd(mg/kg) |
| start of the growing season | 12.90a               | 3.75b                | 16.65a                   | 6.59a             | .58b              | 7.70c      | .49b      |
| end of the growing season   | 5.65b                | 6.70a                | 14.35b                   | 6.84a             | .64a              | 20.69b     | 1.27a     |
| onset of leaf fall          | 3.99c                | 1.21c                | 5.21c                    | 2.45b             | .66a              | 23.32a     | .71b      |

The results showed that at increased distances from the highway, the chlorophyll a levels significantly increased, but the chlorophyll b and total chlorophyll levels were unaffected. In addition, the carotenoid levels in the leaves significantly increased at distances of 15 and 30 m from the highway. However, the dry to

wet weight was unaffected by distance. At 30 m from the highway, the lead levels were significantly greater than those at closer distances. The cadmium levels of the nearest and the longest distance from the highway samples significantly increased (Table 3).

**Table 3: Comparison of the Average of Study Variance Factors Affected by the Distance to the Highway.**

| Studying Features |                      |                      |                          |                   |                   |            |            |
|-------------------|----------------------|----------------------|--------------------------|-------------------|-------------------|------------|------------|
| Distance          | chlorophyll a (mg/g) | chlorophyll b (mg/g) | chlorophyll (a+b) (mg/g) | carotenoid(µg/ml) | dry to wet weight | Pb (mg/kg) | Cd (mg/kg) |
| D1                | 6.98b                | 4.60a                | 11.58 a                  | 4.70c             | 0.62 a            | 16.88b     | 1.17a      |
| D2                | 7.66a                | 4.29 a               | 11.95a                   | 5.31b             | 0.63a             | 15.40 b    | 0.46 c     |
| D3                | 7.91a                | 4.77a                | 12.68a                   | 5.87a             | 0.63a             | 19.44 a    | 0.80 a     |

**Table 4: Interaction of Time and Distance from the Highway on Study Indices.**

| Studying Features |      |                      |                      |                          |                   |                   |            |            |
|-------------------|------|----------------------|----------------------|--------------------------|-------------------|-------------------|------------|------------|
| Distance          | Time | chlorophyll a (mg/g) | chlorophyll b (mg/g) | chlorophyll (a+b) (mg/g) | carotenoid(µg/ml) | dry to wet weight | Pb (mg/kg) | Cd (mg/kg) |
| D1                | T1   | 11.02b               | 3.29c                | 14.31b                   | 4.87c             | .57e              | 6.45g      | 0.26e      |
|                   | T2   | 6.15c                | 9.05a                | 15.20b                   | 7.13ab            | 0.62d             | 35.85b     | 2.95a      |
|                   | T3   | 3.76e                | 1.47d                | 5.23d                    | 2.10d             | .66ab             | 8.32f      | .29e       |
| D2                | T1   | 13.62a               | 3.95c                | 17.57a                   | 7.09ab            | 0.58e             | 7.54fg     | 0.32de     |
|                   | T2   | 5.19d                | 7.83b                | 13.02c                   | 6.48b             | 0.65bc            | 14.58d     | 0.24e      |
|                   | T3   | 4.16e                | 1.10d                | 5.25d                    | 2.36d             | 0.67a             | 24.08c     | 0.82bc     |
| D3                | T1   | 14.06a               | 4.02c                | 18.08a                   | 7.80a             | 0.58e             | 9.11f      | 0.74bc     |
|                   | T2   | 5.60cd               | 9.22a                | 14.82b                   | 6.92ab            | 0.64c             | 11.62e     | 0.63cd     |
|                   | T3   | 4.06e                | 1.07d                | 5.13d                    | 2.90d             | 0.67a             | 37.57a     | 1.03b      |

The results show that chlorophyll a levels significantly decreased with the passage of time and with the interaction between distance and time at 0 m from the highway. On the other hand, at 15 and 30 m, the chlorophyll a levels increased in the middle and late growth periods compared to the early period of the growth season (Table 4).

With the passage of time, the chlorophyll b levels significantly increased at 15 and 30 m. The highest total chlorophyll level was observed at 15 and 30 m from the highway. The total chlorophyll levels significantly increased at these distances by June but decreased at the same distances before fall. The carotenoid levels increased at the 0 m location over time but decreased at the 15 and 30 m prior to fall. The dry to wet weight significantly increased over time for all of the distances from the highway.

The lead levels significantly increased over time for the trees nearest to the highway. At the 15 and 30 m distances, the lead levels significantly increased as fall approached. The cadmium levels increased in trees at 15 and 30 m toward the fall, and reached a maximum level at 0 m during the middle of the growth period (Table 4).

#### DISCUSSION AND CONCLUSION

In a study on *Lonicera japonica*, Liu *et al.* (2009) determined that the species showed high cadmium tolerance and accumulation capabilities. Therefore, *L. japonica* is potentially effective in removing cadmium pollution.

In a study by Hajrasuliha *et al.* (2005) that was conducted in late June through the September of 2005 at Islamic Azad University, it was shown that acacia leaves could be used to bio-monitor air pollution in areas that had been contaminated by heavy metals such as lead, cadmium, zinc, manganese, copper, and iron. Acacia, when used as a bio-monitor, could also separate contaminated from non-contaminated sites (Hajrasuliha *et al.*, 2005).

Through the use of acacia species as bio-monitors, Amini *et al.* (2007) concluded that industrial and high-traffic areas of Isfahan, Iran were the main sources of the presence of heavy metals in air pollution and that acacia can be used as a safe bio-tracer for analyzing air and soil pollution in those areas (Amin *et al.*, 2007). In the present study, the cadmium levels significantly decreased at the nearest distance to the highway; however, because of the wind, the lead levels were the highest at a distance of 30 m.

Khajei *et al.* (2011) determined that for ash trees, the ability of the leaves to absorb cadmium in January and August was greater than the leaves, roots, and stems during the fall. In addition, the absorption of cadmium was found to be higher in ash leaves and in acacia stems. Therefore, by excluding the ash leaves from the environment, we can remove the pollutant. In the

present study, the cadmium levels increased during the mid-term growth of the plant and were the highest at the distance nearest the highway. However, the lead levels reached its highest amount at a distance of 30 m from the highway at the end of the growing period. Azimpour *et al.* (2012) showed that the sampling position influences the absorption levels of nickel in the leaves of the ash tree in May and October.

The process of changing the nickel levels in ash and leaf umbrella increases in contaminated areas. By and large, the results of this research consider the ash tree to be a repellent species. Similarly, Khajei (2009) showed that the leaf absorption levels of cadmium in the ash tree during January are higher than those prior to the falling of the leaves or in the roots of the acacia. In our study, we also observed that the cadmium levels in the mid-term growth period significantly increased.

In another experiment, it was observed that the chlorophyll levels in alfalfa decreased when the lead and zinc levels increased. Moreover, alfalfa was more effective than the pea and vetch plants in its ability to produce chlorophyll in the presence of accumulated lead and zinc (Nezami *et al.*, 2011). In a study on the effects of cadmium on rapeseed photosynthesis, cadmium was concluded to reduce turgor pressure and intracellular space. Eventually, photosynthesis decreases because of the enzymatic destruction of chlorophyll (Iranshahi *et al.*, 2009). The results showed that as the cadmium and lead levels increased, the photosynthetic pigment levels significantly decreased.

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