

## AIR POLLUTION TOLERANCE INDEX OF *Plantago major* IN STEELWORKS AREA OF ZENICA, BOSNIA AND HERZEGOVINA

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### Abstract

Atmospheric pollution is among the largest anthropogenic impacts on the ecosystem. In numerous studies it was observed that plants, especially those that grow in urban areas, are heavily influenced by different pollutants and their survival is correlated with structural and metabolic adaptation to stressful environmental conditions. Primary objective of this study was to determine the index of tolerance to air pollution (APTI) of plantain (*Plantago major*), on two locations in Zenica. The results indicated that index of tolerance to air pollution of *P. major*, APTI, is higher in individuals sampled from the contaminated site, than those in the control area.

Key words: *air pollution, plantain, APTI*

Research article

### Introduction

Plants take an active part in the circulation of nutrients and gases such as carbon dioxide, oxygen and also provide an enormous leaf surface for the absorption and accumulation of air pollutants resulting in reduction of the pollution level in the environment (Escobedo et al., 2008). Atmospheric pollution is among the largest anthropogenic impacts on the ecosystem (Hijano et al., 2005). In numerous studies it was observed that the plants, especially those that grow in urban areas, are heavily influenced by different pollutants and their survival is

correlated with structural and metabolic adaptation to stressful environmental conditions (Gostin, 2009). Sensitivity and responses of plants to air pollution are very variable. Plant species that are more sensitive are used as biological indicators of air pollution. Responses of the plants to air pollution on physiological and biochemical levels can be understood by analysing the specific factors that determine the resistance and sensitivity. Urban areas can be contaminated by many pollutants such as SO<sub>2</sub>, CO, NO and heavy metals and the plants that grow there are exposed not just to one pollutant but a variety of pollutants including their complex interactions (Agarwal, 1985; Tiwari et

al., 1993). The main objective of this study was to determine the air pollution tolerance index (APTI) of *Plantago major*, collected from two sites in the Zenica area in order to determine the tolerance of this species to the existing atmospheric conditions.

## Materials and methods

### Study area

Sampling of plant material was performed in October 2015 in two localities in the area of Zenica: Tetovo and Smetovi. Tetovo is located at latitude 44°13'51.18" and longitude 17°53'19.71" and Smetovi at latitude 44°14'41.77" and longitude 17°58'42.05". Tetovo is located near steelworks ArcelorMittal, the source of air pollution. Plants collected at Smetovi locality were used as control samples.

The samples were collected in three biological replicates (fully developed leaves of *P. major*) and transferred into the Laboratory of Plant Physiology, Faculty of Science, University of Sarajevo. The samples were stored in a refrigerator at +4°C for the purpose of further analysis.

### Relative Water Content (RWC)

Relative water content was analysed according to Liu and Ding (2008). Five leaf samples of each individual per investigated site were selected for analysis. Fresh weight was obtained by weighing the fresh material. The leaves were then immersed in water overnight, for the purpose of determination of turgid mass. The leaves were then dried in an oven at 70°C overnight and reweighed to obtain the dry weight. After determining the parameters of fresh, dry and turgid weight of the leaves, relative water content was calculated using the formula:

$$RWC (\%) = \frac{FW - DW}{TW - DW} \times 100$$

where FW is fresh weight, DW is dry weight and TW is turgid weight.

### Leaf extract pH

Leaf extract pH was determined according to Singh and Rao (1983). Fresh plant material was homogenized in a mortar with the addition of 10 ml distilled water. The content of the mortar was then filtered and pH of the leaf extract determined using pH meter (872 pHlab Metrohm, Swissmade) after calibration with buffer solutions at pH 4 and pH 7.

### Total chlorophyll and carotenoid content

Extraction of pigments was carried out from fresh plant material (0.25 g), by maceration in 80% acetone (v/v). Following the centrifugation of the macerate (Biofuge A centrifuge manufacturer Heraeus sepatech) at 1000 rpm, for 15 minutes, the supernatant was collected for further analysis.

Quantities of chlorophyll a, chlorophyll b, total chlorophyll and carotenoid were determined by absorbance measurement at 663 nm (chlorophyll a), 646 nm (chlorophyll b) and 440 nm (carotenoids) using spectrophotometer (*Perkin-Elmer spectrophotometer Lambda 25*). Calculation of chlorophyll a and chlorophyll b and carotenoid contents was done according to the formulas by Porra et al. (1989) and Holm (1954):

$$\text{Chl a} = 12.25A_{663} - 2.55A_{646} (\mu\text{g/g})$$

$$\text{Chl b} = 20.31A_{646} - 4.91A_{663} (\mu\text{g/g})$$

$$\text{Chl a + b} = 17.76A_{646} + 7.34A_{663} (\mu\text{g/g})$$

$$\text{Car} = 4.69A_{440} - 0.267\text{Chl a + b} (\mu\text{g/g})$$

### Ascorbic acid (AA)

Extraction of ascorbic acid was carried out from 0.5 g of fresh material, in 5 ml of distilled water and 2-3 drops of glacial acetic acid (99.8%), obtained supernatant was used for analyses.

Titrimetric analysis of ascorbic acid was carried out according to the following procedure: a solution of 10 ml of the filtrate, 50 ml of distilled water and 0.5 ml of 1% starch was mixed and immediately titrated to endpoint with a standardized solution of iodine (0.002 mol L<sup>-1</sup>). Titration was repeated twice for samples from both sites. Results are expressed in mg/g (Tahirović et al., 2012).

#### *Air Pollution Tolerance Index (APTI)*

Air Pollution Tolerance Index (APTI) was determined by Singh and Rao (1983) using the formula:

$$APTI = \frac{A(T + P) + R}{10}$$

where A is ascorbic acid content (mg/g FW), T is total chlorophyll content (mg/g FW), P is pH of leaf extracts and R is relative water content (% of the leaves).

## **Results and discussion**

#### *Relative Water Content*

To calculate relative water content in *Plantago major*, values of fresh, turgid and dry mass of leaves were determined. The results (Table 1) indicate that individuals sampled at the site of Tetovo, show increase in relative water content in relation to the locality Smetovi, control site. Dhanam et al. (2014) obtained similar results by recording higher relative water content in leaves from polluted sites than in those at the control site.

These results suggest that relative water content plays an important role in maintaining homeostasis in plants that are under stress due to air pollution. Plant species with high relative water content are tolerant to pollutants. High relative water content in plant allows it to maintain physiological homeostasis when exposed to pollutants when transpiration rates are usually high. It also serves as an indicator of

plant resistance to drought conditions (Dhanam et al., 2014).

#### *Leaf extract pH*

Leaf extract pH is another necessary parameter in determination of APTI. Variation was observed in pH values of leaf extracts depending on sampling location. The pH of the extract of leaves (Table 1) sampled from the contaminated site (5.85) was lower than the values recorded at control sites (8.6).

Leaf extract pH has a very important role in determining the level of tolerance of plants to any kind of pollution. High pH can affect the efficiency of conversion of hexoses into ascorbic acid, while at low pH revealed good correlation with sensitivity to air pollution and with reduced photosynthetic activity in plants. Higher pH allows greater tolerance of plants to air pollution (Dhanam et al., 2014).

Photosynthetic activity was reported to be very dependent upon the pH of the leaf (Yan-ju and Hui, 2008) and photosynthesis is reduced in plants with low leaf pH values (Turk and Wirth, 1975). In a study on the individuals from two different sites it was found that plants stationed in the industrial contaminated area had an acidic pH value, while individuals in the control area showed neutral to slightly basic pH (Rai et al., 2013), which is in line with the results obtained on *P. major*.

#### *Content of photosynthetic pigments*

Analysis of the content of photosynthetic pigments included the determination of the concentrations of chlorophyll a, chlorophyll b, total chlorophyll content and carotenoids. Analysis showed higher content of chlorophyll a, chlorophyll b and carotenoids in individuals sampled from the contaminated area (Table 1).

Chandawat et al. (2011) found that the chlorophyll content of all plant species tested varied in accordance with the level of pollution

**Table 1.** Air Pollution Tolerance Index (APTI) of *Plantago major* on two sites in Zenica

	<b>Tetovo</b>	<b>Smetovi</b>
Fresh weight (g)	0.72	0.47
Turgid weight (g)	0.82	0.54
Dry weight (g)	0.12	0.08
RWC (%)	85.32	82.96
Chlorophyll <i>a</i> (mg/g)	0.85	0.67
Chlorophyll <i>b</i> (mg/g)	0.22	0.21
Total chlorophyll (mg/g)	1.08	0.88
Carotenoids (mg/g)	0.28	0.25
pH	5.81	6.08
Ascorbic acid (mg/g)	1.23	0.70
APTI	9.37	8.78

in a given area, as well as in accordance with species tolerance and sensitivity to air pollution. Certain air pollutants reduce and others increase the content of total chlorophyll in the plant (Dhanam et al., 2014).

Tripathi and Gautam (2007) recorded large loss of chlorophyll in the leaves of plants that have been exposed to high levels of air pollution, which once again points to the role of chloroplasts in the plant and supports the argument that the chloroplast is a primary place of attack of air pollutants on the plant.

#### *Ascorbic acid*

The results (Table 1) show that the content of ascorbic acid is higher in individuals from contaminated sites (1.23 mg) compared to individuals in the control area (0.7 mg).

Ascorbic acid plays an important role in the synthesis of the cell wall in plants, photosynthetic carbon fixation and the cell division (Lakshmi et al., 2008). The increased value of ascorbic acid content may be caused by the defence mechanisms of the plant itself. The results obtained by Dhanam et al. (2014) concur with the research of Chandawat et al. (2011) and

Rai et al. (2013) who observed higher levels of ascorbic acid in the leaves of the most tolerant plants and those plants stationed near polluted areas (Dhanam et al., 2014). Tripathi and Gautam (2007) noted large variations in the levels of ascorbic acid among all samples regardless of the collection site.

Elevated levels of ascorbic acid in plants that were located in the area with high levels of air pollution can be caused by higher rate of production of reactive oxygen species during photooxidation (Tripathi and Gautam, 2007). Elevated levels of ascorbic acid recorded in *P. major* from Tetovo locality could be attributed to plant defence mechanisms in conditions of high air pollution.

#### *Air Pollution Tolerance Index (APTI)*

APTI is calculated on the basis of biochemical parameters that were analysed in the study. All these parameters play very important role in determining the resistance and sensitivity of plant species. In this study, APTI values of plants from contaminated sites were higher when compared to the control (Table 1). Air pollution in urban and industrial areas can be

adsorbed, absorbed, accumulated or integrated into the plant, and the toxin can cause a variety of injuries to the plants. The level of injury will be high for sensitive and low for tolerant plant species. Sensitive species are indicators of air pollution while tolerant ones could be used for reducing levels of air pollution (Subrahmanyum et al., 1985). Species with APTI lower than 10 are characterized as sensitive to air pollution and can be used for biomonitoring levels of air pollutants (Agrawal et al., 1991). Tolerant plant species accumulate pollutants, so planting polluted areas with these plants has great benefit. Based on the data given in Table 1., it is evident that APTI values (9.37 at contaminated site; 8,78 at control site) place *P. major* among sensitive plant species that may be used as appropriate indicators of air pollution for studied area.

## Conclusions

The results of four parameters studied within APTI support the hypothesis that *Plantago major* belongs to the species sensitive to air pollution, which is consistent with the classification of plants based on APTI (Singh and Rao, 1983). Air pollution is one of the greatest threats to the disruption of the ecological state of the environment. Due to the rise in industrialization, there is constant danger of deforestation caused by air pollution. These facts indicate the importance of determining Air Pollution Tolerance Index (APTI) for different plant species, as well as the use of obtained results in future planning and the establishment of control measures against air pollution.

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