

Full Length Research Paper

Environmental impact on morphological and anatomical structure of Tansy

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Accepted 19 April, 2010

Morphological and anatomical structure of Tansy (*Tanacetum vulgare* L.) from two specific locations in one town, depending on environmental conditions, were carried out: anthropogenic Ada Huja (polluted zone) and non anthropogenic Topcider park (unpolluted). Study included the differences in the structure of leaves, stem and roots. The plants from both sites showed visible morphological and anatomical changes in leaves. However, some reduction in these features were observed in leaves, which were collected from the anthropogenic Ada Huja site. Significant reduction in thickness of Tansy leaves from Ada Huja was recorded. Also, thickness reduction of leaf mesophyll, palisade parenchyma and upper and lower epidermis in plants from anthropogenic Ada Huja site was noticed. Similarly, reduction in the anatomical structures of leaves from polluted areas was also observed. Chlorophyll content in leaves from polluted and non polluted Topcider park, which is control site in this case, was measured too. Due to unfavourable environmental conditions, leaves of Ada Huja plants had lower chlorophyll *a* and *b* content. All recorded differences in morphological and anatomical structure and chlorophyll content of the Tansy were caused by differences of cumulative environmental conditions with dominant effects of the contamination degree of the location, soil type and microclimate.

Key words: *Tanacetum vulgare*, environmental conditions, anatomical structure, morphological structure, pollution degree.

INTRODUCTION

Different environmental conditions are always reflected to the plants. Intensive industrial production is usually connected with the emission of various pollutants to the environment (Kalandadze, 2003; Uaboi-Egbenni et al., 2009). It has been observed that plants even grow in urban areas affected greatly by varieties of pollutants (oxides of nitrogen and sulphur, hydrocarbon, ozone, particulate matters, hydrogen fluoride, peroxyacyl nitrates, etc). The car pollutants have long term effects on plants by influencing CO₂ contents, light intensity, temperature and precipitation. Plants need special protection because they are not only a source of food but are also helpful in cleaning the environment. Some of the authors (Bhatti and Iqbal MZ, 1988; Darley et al., 1963; Godzik and Halbwachs, 1986; Gupta and Ghose, 1988; Inamdar and

Chaudhari, 1984; Iqbal, 1985; Krause and Dohinger, 1987; Karenlampi, 1986; Nivova et al., 1983) have reported the effects of air pollution on the morphology and anatomy of different plants species.

Some pollutants even include heavy metals (Ciećko et al., 2005; Kabata-Pendias and Pendias, 1999). High heavy metal concentrations in the soil exerts negative effects on the plants (Gambus and Gorlach, 1992; Obata and Umebayashi, 1997). Plants usually adapt to high pollutant concentrations and unfavourable environmental conditions (Davis and Beckett, 1978; Wyszowski and Wyszowska, 2003), which is likely to result in different morphology and anatomy.

The anthropogenic influence is manifested in the creation of embankments and landfills, disposal of construction materials and land abuse. Therefore, physio-chemical characteristics of urban soil were significantly changed. Urban soil characterized by large fluctuations of physio-chemical properties in a short distances.

Urban plants, due to the absence of competition in the

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Table 1. Environmental features of Ada Huja and Topčider sites locations.

Environmental characteristic	Location	
	Ada Huja	Topčider
Tmean (°C)	15.1	13.6
Tmax (°C)	38.3	34.4
Tmin (°C)	-9.3	-12.3
Precipitation (mm/m2)	971.1	1005.4
Altitude (m a.s.l.)	75	140
Coord. N	44.823186	44.760630
Coord. E	20.525637	20.446234
Type of soil	Sand + building waste	Humus + sand

changed and extremely conditions, often represent the first pioneer species on anthropogenically modified habitats. Specific morpho-anatomical and physiological-biochemical characteristics are the result of plants adaptation on environmental conditions (Kovacic and Nikolic, 2005; Pandey et al., 2006). Stress conditions can disturb the formation of sclerenchymatic fibres in leaves of Poaceae (Gielwanowska et al., 2005).

This work was designed to investigate the effects of environmental conditions, dominantly presented by industrial pollution and consequently, soil type and microclimat, on morphology and anatomy of Tansy plant, growing near and far away from industrial zone. Tansy (*Tanacetum vulgare* L.) is a common and widespread plant. It can be cultivated and also grows spontaneously. Tansy has a wide distribution, which indicates a high ecologically plasticity and adaptability to different environmental conditions.

Tansy is principally used as antihypertensive remedy, antispasmodic, antihelminthic and carminative, stimulant to abdominal viscera, tonic and emmenagogue, anti-diabetic, diuretic and antihypertensive. Its extract has been reported to exhibit antitumor (Konopa et al., 1967), anti-inflammatory (Williams et al., 1999), antioxidant (Bandoniene et al., 2000; Mantle et al., 2000), antimicrobial activity (Holetz et al., 2002), antimalarial effect (Jansen, 2006) and vasorelaxing effect (Lahlou et al., 2008). It has also been reported to have an effect on experimental gastric ulcer on various infectious diseases (Tournier et al., 1999; Holetz et al., 2002). Essential oil production in Tansy is an indicator of plant adaptation on habitat conditions (Stevovic et al., 2009). Ecological role of essential oils is reflected in the interaction of plants with environmental factors. Essential oil helps plants easily adapt to the environmental stress conditions: drought, high temperature, intense radiation and heavy metal contents (Abu-Darwish and Abu-Dieyeh, 2009).

The main object of this study is to explore the morphological and anatomical features of Tansy plants as adaptability indicator. The goal was to prove the statement that the plants answer on environmental stress, from different anthropogenic and non anthropogenic site

locations, by changing morphology and anatomy of leaves, stem and roots.

MATERIALS AND METHODS

Environmental characteristics of the Ada Huja and Topčider localities

The climatological features of the sites included mean annual precipitation (mm/m²), mean annual temperature (Tmean, °C), minimum annual temperature (Tmin, °C) and maximum annual temperature (Tmax, °C). The data covered 2007 – 2009 and came from the Serbian Meteorological and Hydrological Service. The altitudes (m a.s.l.) and geographical coordinates of the localities were taken from 1: 100 000 topographic maps. For statistical analysis, the geographical coordinates were converted from degrees, minutes and seconds to a decimal system of north and east coordinates. Each site was also characterized by the data on the soil type and contamination degree. The environmental characteristics of the investigated localities are shown in Table 1.

Plant material

Fresh whole Tansy plants were collected from two specific ecological habitats: anthropogenic Ada Huja and non anthropogenic Topčider. One of them is anthropogenic Ada Huja (industrial zone), but the other one is non anthropogenic Topčider park (green area). Tansy was growing on damp hill and building waste materials on Ada Huja and next to the Topčider River in Topčider Park. Plant materials were harvested in three different places on each locality. The sample size was 20 plants per each place. Fresh mass of one plant was approximately 300 g. Total sample size was 180 per the site locality. Plants were three times harvested during the one flowering season at the end of August during the blossom stage of development. The plant reaches a height of 150 - 200 cm at that time. Fresh plant material was air dried between the sheets of porous paper, for about a month. All subsequent investigations were made with this prepared material marked as "air-dry matter". After "air-dry matter" leaves, stem and roots were separated from whole plants.

Anatomy study

Different parts of plant (leaf, stem, and root) were fixed in FAA (formalin-acetic acid-ethanol 10:5:85), dehydrated in a graded ethanol series and embedded in paraffin wax at 58°C. Sections (8 µm thick) were stained with haematoxylin. These sections were

Table 2. List of Tansy leaf characters used in this study. Tansy originated from polluted Ada Huja and unpolluted Topčider zones.

Character (thickness μm)	Ada Huja			Topčider		
	Mean \pm SE	min	max	Mean \pm SE	min	max
Leaf	393.37 \pm 41.10 a	351.20	483.50	542.22 \pm 56.40 a	501.60	650.85
Mesophyll	186.73 \pm 20.15 b	126.00	258.30	268.60 \pm 29.30 b	173.25	346.50
Upper palisade parenchyma	70.25 \pm 8.60 c	44.10	119.70	96.89 \pm 10.10 c	38.50	157.5
Lower palisade parenchyma	52.11 \pm 6.12 d	31.50	94.50	74.97 \pm 8.60 d	31.50	126.00
Spongy parenchyma	58.41 \pm 6.11 d	28.35	110.25	60.75 \pm 7.20 d	15.75	100.80
Upper epidermis	13.17 \pm 1.80 e	9.45	37.80	21.21 \pm 2.90 e	5.30	18.90
Lower epidermis	12.70 \pm 1.60 e	5.45	44.10	19.80 \pm 2.10 e	7.30	18.90

Values in each column marked by different letters are significantly different at 0.05 using the LSD test. SE = Standard error; min = minimum; max = maximum.

examined under the microscope for quantitative measurements of cuticle, epidermal layer, mesophyll and parenchyma cells of leaf with ocular scale. Well haematoxylin staining sections were photographed with an Olympus BX51 from permanent slides. All measurements and observations were made three times.

Chlorophyll content measurements

Chlorophyll content (Chl *a+b*) was determined according to Wintermans and Demots (1965) at the end of the experiment. Samples (five discs each) were taken from fully expanded leaves of each treatment and were extracted with 95% ethanol in a water bath at 80°C. Full extraction of chlorophyll was achieved when the sample was discolored. The absorption of the extracts was measured at 665 and 649 nm with an LKB Ultraspec II spectrophotometer.

Statistics and repetition

Three measurement repetitions had been performed for each of the 180 samples. The results were analyzed using completely randomized design and tested according to least significant difference (LSD) test.

RESULTS

Environmental characteristics

Environmental characteristics of polluted Ada Huja and unpolluted Topčider site localities were investigated. Studied localities had similar general climate but different microclimate conditions. Moreover, differences in the composition of the soils were established. Soil samples from Ada Huja contained sand and building waste, while samples from Topčider had humus and sand (Table 1). However, Ada Huja had higher mean annual temperature and smaller precipitation than Topčider location. In addition, due to sandy soil and faster drainage in the underground, Ada Huja had dryer conditions than Topčider location.

Morphological characteristics

The morphological studied Tansy of both sites was shown that plants had similar height, shape, size and color of stem, root and heads. Purple brown stems were densely covered with dark green leaves. The leaf had lancet incisions. Yellow heads consists of a dense and numerous inflorescences.

However, Tansy plants showed visible morphological changes of leaves. Plants from Ada Huja had a thinner leaves than plants from Topčider, due to of the impact of industrial pollutants.

Anatomical characteristics

This study uses numerical methods to describe significance of anatomical features of Tansy collected from anthropogenic Ada Huja and non anthropogenic Topčider sites. Anatomy characters of leaves collected from Ada Huja and Topčider zones were shown in Table 2.

Tranversal section of the leaf

A transverse section taken from the middle part of the leaves was observed (Figures 1 and 2). After measuring the thickness of leaves differences were noticed. Leaves of Tansy from Ada Huja (393.37 μm) were thinner than leaves from Topčider (542.22 μm). Well developed cuticle on the surface of leaves originating from that site location was observed. Upper and lower epidermis consists of a single layer of rectangular or orbicular cells. Also, epidermal cells had a different shape and size. There were many multicellular trichomes on both epidermis. Stomata occur on both epidermal surfaces, level with neighboring cells. Also, stomata cavities were large in leaves of Tansy plants from both localities. Mesophyll consists of the palisad parenchyma and spongy parenchyma (Figure 1). Thickness of leaf mesophyll from

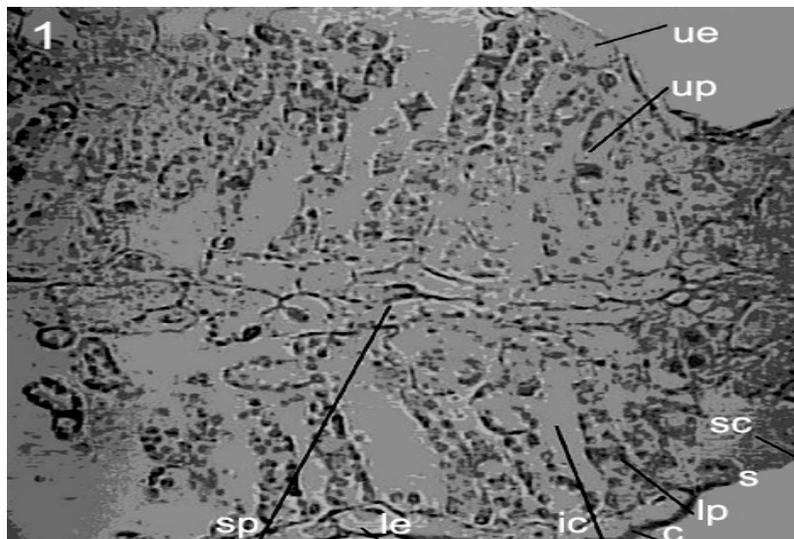


Figure 1. A transverse section of Tansy leaf from Ada Huja. C = Cuticle, ue = upper epidermis, le = lower epidermis, up = upper palisad parenchyma, lp = lower palisad parenchyma, sp = spongy parenchyma, ic = intercellular cavity, s = stomata, and sc = stomata cavity.

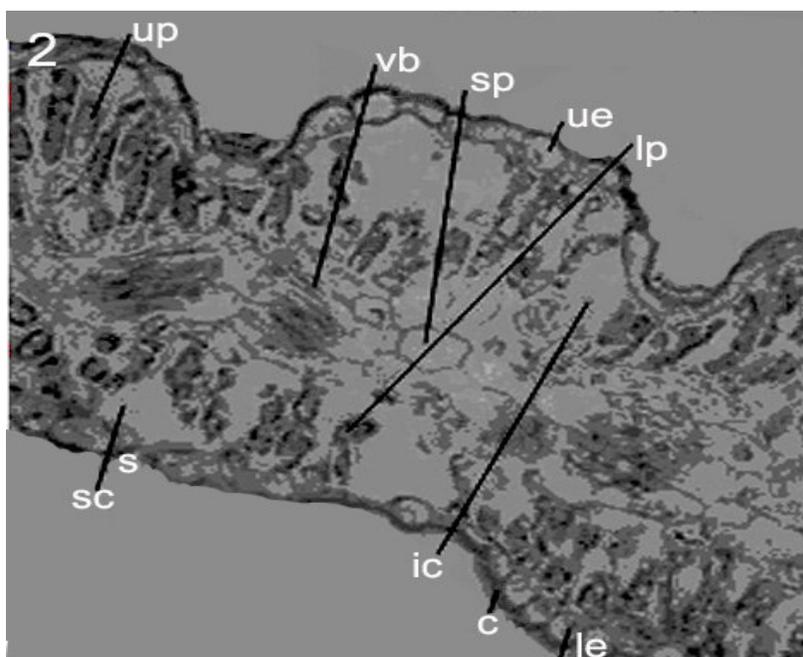


Figure 2. A transverse section of Tansy leaf from Topcider. C = Cuticle, ue = upper epidermis, le = lower epidermis, up = upper palisad parenchyma, lp = lower palisad parenchyma, sp = spongy parenchyma, ic = intercellular cavity, s = stomata, sc = stomata cavity, and vb = vascular bundle.

from Ada Huja (186.73 μm) was significantly thinner than mesophyll of Topcider (258.30 μm) plants (Table 2).

Leaf mesophyll from Ada Huja consists of 1 or 2 layers of elongated palisade cells. Palisad cell had many chloroplasts and large intercellular cavities. Upper (70.25

μm) and lower (52.11 μm) palisade parenchyma from Ada Huja were thinner than in Topcider plants (119.7 and 94.5 μm).

Solitary vascular bundles are surrounded by parenchymatous and orbicular cells. Also, leaves from Ada

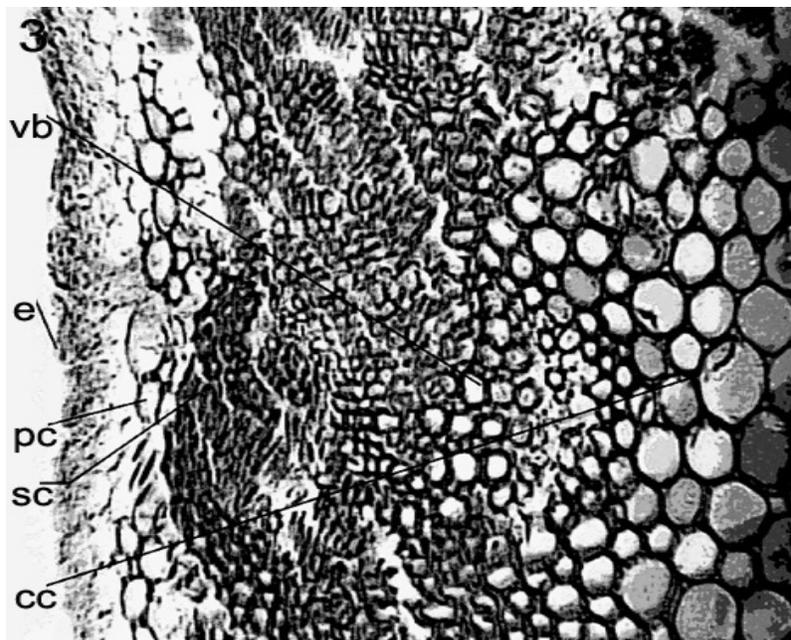


Figure 3. A transverse section of Tansy stem from Ada Huja. e –epidermis, pc – primary cortex, cc – central cylinder, sc – sclerenchymatic cells, and vb – vascular bundle.

Huja had 2 or 3 layers of isodiametric spongy parenchymatic cells with lower intercellular cavities.

Thickness of upper and lower palisade parenchyma from Topcider was 1.3 times greater than palisade parenchyma of plants from Ada Huja. However, spongy parenchyma of Tansy leaves from Topcider had similar thickness, as well as spongy tissue from Ada Huja (Figure 2). Vascular bundles were well developed in leaves from both site localities.

Transversal section of the stem

A transverse section taken from the middle part of the stem of plants from both polluted (Figure 3) and unpolluted (Figure 4) sites showed round shape. There are no specific anatomy changes between samples of stem from both localities. Stem consists of epidermis, cortex and central cylinder.

Stem cortex consists of 4 or 5 layers of usually oval parenchyma cells in plants from both localities. Parenchyma consists of cells (occupy the most of stem) had a different shape, size and thin cell walls and chloroplasts. There is a sclerenchymatic sheath between these bundles. Vascular bundles are of different sizes. Pith cells are large and cylindrical of Tansy from both localities (Figures 3 and 4).

Transversal section of the root

A transverse section taken from the middle part of the

root of plants from polluted Ada Huja (Figure 5) and unpolluted Topcider (Figure 6) sites was observed. There are no specific anatomy changes between samples of root from both localities. Epidermis on the root surface in plants from both site localities was noticed. Primary cortex obtained between epidermis and central cylinder. Primary cortex was more developed than central cylinder. Between phloem and xylema was conducting parenchyma. Cells of primary cortex had thin cell walls. Vascular bundles with radial shape were closed.

In light of the mentioned differences in leaf anatomy, leaf chlorophyll content in Tansy was measured (Table 3).

Chlorophyll content

Leaves from polluted Ada Huja had significantly lower chlorophyll content than control Topcider leaves. Relation of total chlorophyll amount in Topcider (62.2 mg/100 g) and Ada Huja (36.7 mg/100 g) leaf samples were 1.69 (Table 3). However, relation between chlorophyll a and b content in samples from both site locations was 1.5 (Ada Huja, Chl a = 21.9; Chl b = 14.8; and Topcider Chl a = 37.3; Chl b = 24.9).

DISCUSSION

Ada Huja and Topcider site localities have similar general climate, but different type of soil, contamination degree and different microclimate (Table 1). Since plants originating

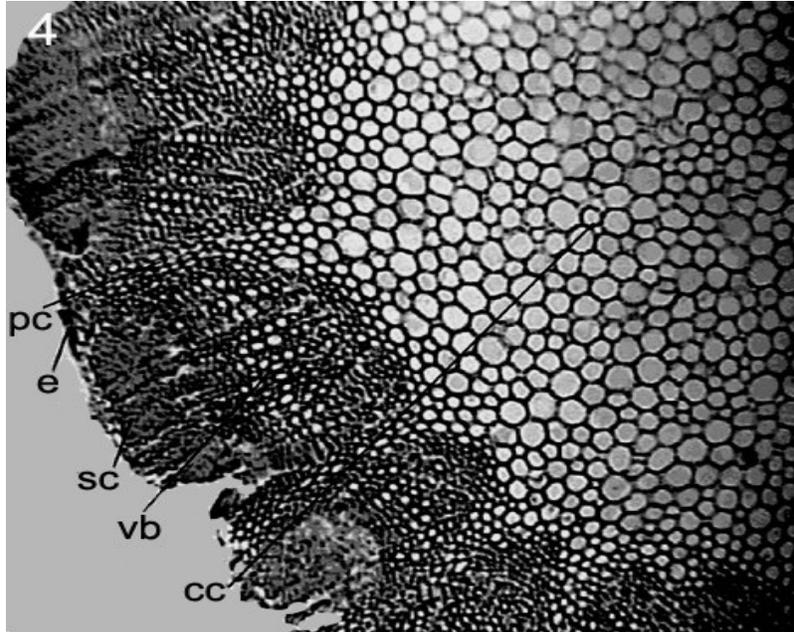


Figure 4. A transverse section of Tansy stem from Topcider. e = Epidermis, pc = primary cortex, cc = central cylinder, sc = sclerenchymatic cells, and vb = vascular bundle.

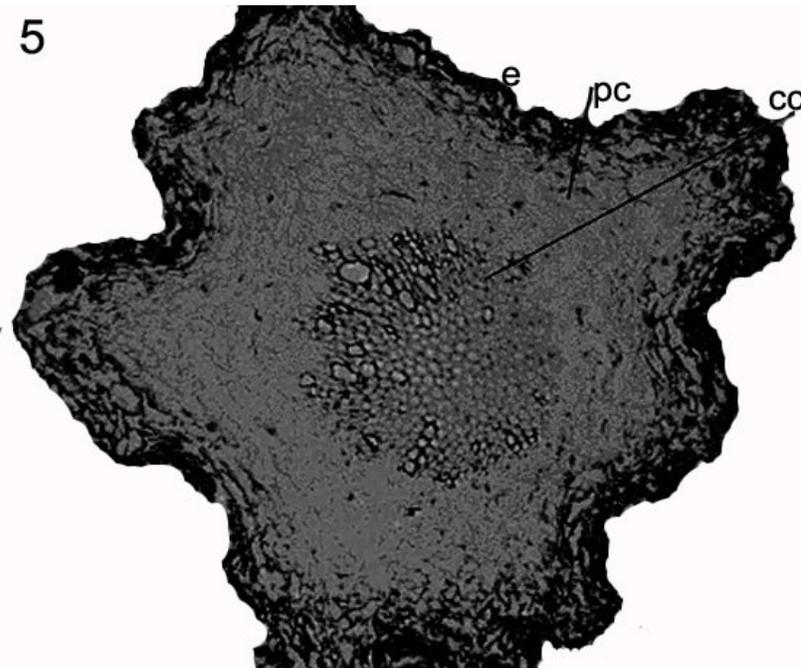


Figure 5. A transverse section of Tansy root from Ada Huja. e = Epidermis, pc = primary cortex, and cc = central cylinder.

from Ada Huja and Topcider grow under different micro-climate and similar general climate, influence of pollution and type of soil on the morphological and anatomical features, was dominant.

The morphological aspects of Tansy of both sites have shown that plants have slightly different, but similar height, shape, size and color of leaves, stem, roots and heads.

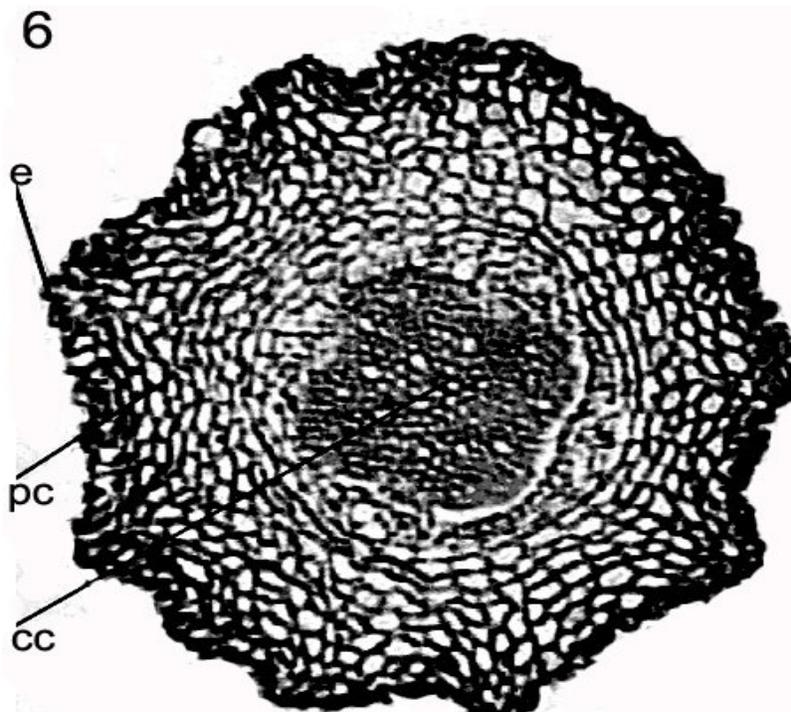


Figure 6. A transverse section of Tansy root from Topcider. e = Epidermis, pc = primary cortex, and cc = central cylinder.

Table 3. Chlorophyll content in leaf samples from polluted (Ada Huja) and unpolluted (Topcider) site localities.

Chlorophyll (mg/100 g)	Ada Huja	Topcider
a	21.9 ± 2.6 a	37.3 ± 4.2 a
b	14.8 ± 1.8 b	24.9 ± 3.1 b
Total	36.7	62.2

Values in each column marked by different letters are significantly different at 0.05 using the LSD test.

However, morphology study of Tansy is mainly focused on detailed leaf features. Leaves of Tansy from Ada Huja (393.37 μm) are significantly thinner than leaves from Topcider (542.22 μm).

However, reduction in leaf area growing in the vicinity of heavy pollutants was also observed in many plants (Sodnik et al., 1987; Bhatti and Iqbal, 1988; Gupta and Ghose, 1988). Also, some hidden injury or physiological disturbance might have occurred which caused reduction in morphological and anatomical characters of other plant species (Gielwanowska et al., 2005; Makbul et al., 2006).

Anatomical studies of Tansy are mainly focused on leaf, stem and root features. Well developed cuticle on leaves from Ada Huja is an effective peripheral protection and adaptation to environmental conditions: increased insolation and temperature and life in the open habitat. Results about well developed Tansy cuticle in polluted

area are in accordance with results Godzik and Halbwacks (1986).

Leaf anatomy of Tansy also showed reduction mesophyll, palisade parenchyma and upper and lower epidermis in polluted area as compared to leaves collected from non polluted area. Significant reduction in thickness of leaves Tansy plants from Ada Huja are adapted to the continuous effect of different pollutants (heavy metals, building materials, oxides of nitrogen and sulphur, etc) which are released into the environment.

Also, other authors in the previous years also showed significant reduction in different leaf variables in the polluted environment in comparison with clean atmosphere (Ninova et al., 1983; Sodnik et al., 1987; Jahan and Zafar, 1992). Changes in shape and structure of thin walled mesophyll cells have been widely reported. Mesophyll cells are thin walled and are in direct contact with the environment through stomates (Karenlami, 1986; Szabo et al., 2006).

The Tansy palisade parenchyma cells become flattened due to continuous exposure to pollutants. Similarly, Iqbal (1985) and Jahan and Zafar (1992) have shown significant reduction in leaf palisade and spongy parenchyma in polluted population. Due to unfavourable environmental conditions, leaves of Ada Huja plants are poorer in chlorophyll *a* and *b* content. Results about Tansy chlorophyll amount are in accordance with results on other species (Krause and Dorchinger, 1987; Gielwanowska et al., 2005; Kofidis et al., 2008).

Plants growing in extreme environmental conditions have anatomically and morphologically different chloroplasts than plants growing in environmentally friendly conditions. Chlorophyll in leaf mesophyll had irregular shape, with pockets or invaginations inside the organelles. Both of them make the surfaces of chloroplasts larger, and result in an increase in the amount of substances exchanged between the chloroplasts and cytoplasm (Gielwanowska et al., 2005).

Also, the chlorophyll fluorescence parameters of coriander plants can provide a tool for early diagnosis of the use of growth retardant even before any signs of growth retardation are visible in the plants (Kofidis et al., 2008).

Conclusion

This study concentrated on the influence of different environmental conditions on Tansy morphology and anatomy. Environmental condition differences on two contrary different sites in one town, were dominantly reflected by the difference in the contamination degree, type of soil and microclimate. The leaves of Tansy growing in polluted habitat are thinner, contain less palisade parenchyma and chlorophyll *a* and *b* than leaves from unpolluted site. Undoubtedly, the anatomical and ultrastructural changes in the leaf structure were noticed, measured and described, taking into consideration all stress factors.

The possible positive human influence on the environment could be to decrease the pollutions and to improve soil quality by horticultural activities that is, designing parks in urban environment. The plants suffer in industrial zones and it is reflected in their morphology and anatomy too. The present study provides a good basis for further research on impact of the environment to morphological and anatomical structure of the plants.

ACKNOWLEDGMENT

This work was supported by the Ministry of Science and Environmental Protection of Serbia, grants No. 18031 and No. 143026.

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