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Review

Environmental impact quantification and correlation between site location and contents and structure of Tansy

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The aim of this work was to quantify the most significant impact from the polluted environment and to review the correlation between pollution indicators and the content and structures of *Tanacetum vulgare* L. (Tansy). Heavy metals as mercury, lead, cadmium, chromium and nickel are considered as pollution indicators. The micro and macro elements concentration of S, P, K, Ca, Mg, Na, Fe, Si, Al and Mn were tested too. The concentration of the essential oil was analyzed as indicator of Tansy environmental adaptability. Essential oils are practically agents which represents correlation between plant and environment. The type of Tansy was selected for laboratory research, because it belongs to most speeded urban flora and vegetation, in which the imperative to adapt is very strong. All those parameters was monitored on two different locations: industrial zone (anthropogenic) and park area (non anthropogenic) for comparison, as zero stage of the environment, before pollution. The differences were also recorded in the morphological and anatomical structure and chlorophyll *a* and *b* content of the Tansy and they were caused by differences of cumulative environmental conditions, with dominant effects of the contamination degree of the location, soil type and microclimate.

Key words: Anthropogenic factors, correlation, environmental impact, environmental zero stage, quantification.

INTRODUCTION

Tanacetum vulgare (Asteraceae/Compositae, syn. *Chrysanthemum vulgare* L. (common name Tansy) has a lot of very interesting and examined pharmacological aspects.

T. vulgare is a common and widespread plant. Tansy can be cultivated and it also grows spontaneously. Its wide distribution indicates a high ecologically plasticity and adaptability to different environmental conditions. This plant is often used as antihypertensive antispasmodic, antihelminthic and carminative agents, as stimulant to abdominal viscera, as a tonic and as antidiabetic and diuretic agents.

Extensive industrial production is usually connected with the emission of various pollutants to the environment (Kalandadze, 2003). As a consequence of the industrial

revolution, there is an enormous and increasing demand for heavy metals that leads to high anthropogenic emission of heavy metals into the biosphere (Ayres, 1992). Plants are largely immobile organisms and in the metal-contaminated environment it is usually the root of a plant that is the primary contact site with the metal ions. In order to survive, plants must have developed on one side, efficient and specific mechanisms by which heavy metals are taken up and transformed into a physiologically tolerable form, providing the essential elements for the plants' metabolic function (Stevovic et al., 2010a). On the other side, excess of these essential elements or those toxic heavy metal ions that do not play a role in metabolism, to which plants are exposed, have to be metabolically inactivated (Zeng, 1996).

Plant cells must have developed a mechanism by which the metal ion, entering the cytosol of the cell, thus preventing the metal from inactivating catalytically active or structural proteins to protect themselves from heavy metal poisoning (Zeng, 1996). Heavy metals interact with

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several functional groups of proteins, primarily SH-groups. As a result, protein conformation is changed, and many enzymes with SH-groups in their active centers lose their activities (Ivanov et al, 1998). This precludes, for instance, an inducible system. In the case of plants, one should expect a constitutive mechanism for the detoxification of heavy metal ions.

Some essential aromatic and medicinal plants are capable to accumulate heavy metals from contaminated soils (Abu-Darwish and Abu-Dieyeh, 2009; Stevovic et al., 2010a). Anthropogenic influence on the surroundings is reflected in the amount of macro- and microelements in the soil and plants. Due to the industrial contamination, the amount of macro- and microelements in soil affects the plants qualities (Stevovic et al., 2010b). Trace elements play an important role in the metabolism of the healthy and diseased plants. The presence of trace elements in sugarcane plants gives a clear picture of the distribution of these elements in the soil where plants absorb essential and necessary trace elements from the soil (Velmurugan et al., 2009). It gives an idea about the structure and composition of the soil, sugarcane plant and sugar products (Mohamed, 1999).

Specific morpho-anatomical and physiological-biochemical characteristics are the result of plants adaptation on environmental conditions (Stevovic et al., 2010c).

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 the plant to easily adopt to the environmental stress conditions: drought, high temperature, intense radiation and heavy metal contents (Abu-Darwish and Abu-Dieyeh, 2009). The quality of essential oil depends on the external environmental condition (Scheerer, 1984). Natural selection favors survival of the population with the composition of essential oil as a higher adaptive value.

The aim of this research was to define the correlation between the site location characteristics and heavy metal, micro-and macro elements and essential oil content, as well as morpho anatomical structure of Tansy.

MATERIALS AND METHODS

Site locations

Soil samples and fresh whole Tansy plants were collected from two specific ecological habitats: anthropogenic and non anthropogenic. One of them was Belgrade industrial zone but the other one was a park area on the periphery of the Belgrade town. Plant materials were harvested at the end of August in three different places on each locality.

Soil and plant sample collection for heavy metals analysis

The soils were sampled at 0 to 15 cm and 15 to 30 cm depth and then transferred into well labeled polyethylene bags for storage and

laboratory tests. Tansy plants found growing on the refuse dump soils were uprooted, labeled and taken to the laboratory for the analysis of their partitioned parts (leaf, stem and root).

Soil preparation and physicochemical analyses

In the laboratory, the soils were dried at ambient temperature (25°C), crushed in a porcelain mortar and sieved through a 2 mm (10 meshes) stainless sieve. Air dried samples were stored in polyethylene bags for subsequent analysis. The 2 mm fraction was used for the determination of selected soil physicochemical properties. Soil pH was measured using a pH meter (Thermo Orion 250, Orion Research, Inc., Boston, MA, USA) and H₂O according to Folsom et al. (1981). The soil/solution ratio was 1:2.

Preparation of plant material for heavy metal analysis

Plant material was milled in a domestic blender (Zepter MixSy, type VG-022-K) and after sieving (laboratory Erweka sieves); a sample with a mean particle diameter size of 0.7 mm was obtained. A prepared batch was kept in a hermetically sealed bag and stored at 8°C for 2 days before use, in order to avoid loss of volatile compounds.

Essential oil preparation for heavy metal analysis

Herb material (20 g) was submitted to hydrodistillation in a Clevengertype apparatus for 2 h according to Yugoslav Pharmacopoeia IV. The obtained oil was dried over anhydrous sodium sulphate, measured, poured in hermetically sealed dark-glass containers and stored in a freezer at -4°C until analyzed by atomic absorption spectrophotometer. The obtained oil was acidified with 1% (v/v) HNO₃ for analysis by flame AAS, Perkin/Elmer 4000.

Soil macro and micro-elements analysis

Soil was collected from 25 to 30 cm depth under the Tansy roots. The texture of soil was determined by the Bouyoucos hydrometer method. The total nitrogen (N) was measured using a semi Micro-Kjeldhal technique. Sodium (Na), potassium (K), magnesium (Mg), calcium (Ca), aluminum (Al), silicon (Si), iron (Fe) and manganese (Mn) were determined by flame atomic absorption spectrophotometry (AAPS) in the device manufacturing Perkin Elmer (USA) model 403 with deuterium background correction. Phosphorus (P) was determined spectrophotometrically in the form of blue molybdate-vanadium spectrophotometers produced by Carl Zeiss (Germany) model Specol 10 and total sulfur was determined in the form of barium sulphate. Aluminum and silicon was separated merging with sodium metaborate. Soil samples (about 1.5 kg) were dried; ground to fine powder and homogenized (the largest particle size was about 0.1 mm). The samples were collected from 10 different places (about 1.5 kg/each place) of both site localities.

Plant macro and micro-elements analysis

For macro and micro-elements analysis, plant material was air dried between the sheets of porous paper, for about a month. They were ground to fine powder using pestle and mortar. For the determination of total N, 150 mg of the plant powder was digested in concentrated H₂SO₄ following automated Kjeldahl method. The digested product was diluted to 100 ml and the resulting ammonium was then measured using FIA (flow injection analysis) method. Ca, Fe, K, Mg and P were determined by boiling 2.5 g of the powdered

Table 1. Heavy metals content in the soil samples (in mg/kg).

| Site location | Soil depth (cm) | Heavy metal (mg/kg) | | | | |
|-------------------|-----------------|---------------------|------|------|------|------|
| | | Hg | Pb | Cd | Cr | Ni |
| Anthropogenic | 0-15 | 13.1 | 19.0 | 13.4 | 14.0 | 20.0 |
| | 15-30 | 7.0 | 17.0 | 11.2 | 6.0 | 10.0 |
| Non anthropogenic | 0-15 | 0.9 | 6.0 | 2.1 | 4.6 | 4.3 |
| | 15-30 | 0.7 | 3.0 | 0.5 | 1.8 | 2.0 |

leaf material in concentrated HNO₃. Excess acid was decanted and the boiled sample was diluted to 25 ml. The elements were then determined using ICP OES (inductivity coupled plasma optical emission spectroscopy) method.

Anatomy study

Different parts of the fresh 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.

Root, shoot and leaves sections were examined under the microscope for quantitative measurements of cuticle, epidermal layer, mesophyll and parenchima cells of the leaf with ocular scale. Well haematoxylin staining sections were photographed with an Olympus BX51 from permanent slides.

Chlorophyll content measurements

Chlorophyll content (Chl *a+b*) was determined according to Wintermans and Demots (1965) at the end of the experiment. Samples 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.

Plant preparation for essential oil content

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".

Methods for determination of the content and composition of essential oil

Essential oil content was determined by distillation with water steam according to pharmacopoeia (Radulovic et al., 2007). The composition of essential oil was determined by the method of gas chromatography (GC) and a combination of gas chromatography mass spectrometry (GC-MS). GC was performed on the instrument of production (Varian USA, model 3400), which was equipped with split/splitless injector (1:20) and the working temperature was 266°C. Column production (JW USA, type DB-5), had a length of 30 m, internal diameter of 0.25 mm and film thickness of 0.25 µm. The bearing gas was hydrogen with a flow rate of 1 ml/min at 210°C. The temperature of the column was liner programmed from 60 to 285°C rate of 4.3°C /min. Flame ionization detector was feed to 300°C. GC-MS was performed on the production of mass spectrometers (Finnigan Mat, Germany model 8230), with the ion

trap detector (ITD) (model of ITD-705, the same manufacturer). Hromatograf gas associated with the mass spectrometers, as well as working conditions was the same for GC, with the feed off. Output column was connected through the interface directly from the ITD at a temperature of 240°C. Particular essential oil components were identified by standards as well as by comparison of their relative retention times, retention indices and mass spectra with those of the WILEY 275 spectral library (2001).

RESULTS AND DISCUSSION

Heavy metals in the soil and in Tansy

The results thus showed typical mercury distribution with Hg levels decreasing with distance from the pollutant (Stevovic et al., 2010a). Mercury is generally of low mobility because of its high density, which explains the high concentrations in the soil samples. On the other hand, the data obtained also indicated that, the Hg concentration in Tansy roots, stems and leaves increased linearly as the soil Hg concentration (Table 2). The results thus, showed a typical distribution and accumulation of Hg through plant vascular elements from roots to leaves. Essential oil concentrations of Hg in the investigated locations were not detected (Table 3). The concentration of Pb in the surface soil samples decreased from 19 mg/kg (anthropogenic zone) to 6.0 mg/kg (non anthropogenic zone) (Table 1). Higher Pb level was detected in the plants harvested from the anthropogenic zone (4.8 mg/kg in leaves, 1.6 g/kg in stems and 2.0 mg/kg in roots), than in the plants from the non anthropogenic zone (0.7, 0.2 and 0.4 mg/kg), (Table 2). Essential oil of the Tansy plants from the anthropogenic zone consisted 10 times higher level of Pb (0.1 mg/kg) than in the non anthropogenic zone (0.01 mg/kg), (Table 3). Cadmium was detected in the soil, in the Tansy plant materials and in the essential oils on both investigated site locations. Soil samples from the anthropogenic zone (13.4 and 11.2 mg/kg; for two different depths, respectively) had higher level Cd than samples from the non anthropogenic zone (2.1 and 0.5 mg/kg) (Table 1). Tansy plants from the anthropogenic zone consisted from 2 times higher Cd concentration than plants from the non anthropogenic zone (Table 2). Cadmium, as well as Lead, distribution in Tansy decreased in the order: leaves > roots > stems. The same Cd trends persisted in the essential oils (Table

Table 2. Heavy metals content (mg/kg) in the different parts of Tansy plants.

| Site location | Tissue | Hg | Pb | Cd | Cr | Ni |
|-------------------|--------|------|-----|------|-----|------|
| Anthropogenic | Leaf | 0.2 | 4.8 | 0.7 | 0.3 | 1.1 |
| | Stem | 0.5 | 1.6 | 0.2 | 0.4 | 1.6 |
| | Root | 1.0 | 2.0 | 0.4 | 0.6 | 1.9 |
| Non anthropogenic | Leaf | 0.01 | 0.7 | 0.3 | 0.1 | 0.05 |
| | Stem | 0.02 | 0.2 | 0.08 | 0.2 | 0.1 |
| | Root | 0.04 | 0.4 | 0.2 | 0.4 | 0.3 |

Table 3. Heavy metals solubility (%) in sage leaf essential oil.

| Heavy metal | Essential oil | |
|-------------|---------------|-------------------|
| | Anthropogenic | Non anthropogenic |
| Hg | ND | ND |
| Pb | 0.1 | 0.01 |
| Cd | 0.02 | 0.01 |
| Cr | 0.04 | ND |
| Ni | 0.1 | ND |

ND = Not detected.

3). However, Cd and Pb were only found in the essential oil from the non anthropogenic zone in low concentration of 0.01 mg/kg, while the other heavy metals were not detected (Table 3). Chromium in the soil samples from the anthropogenic zone had higher values for the two depths (14.0 and 6.0 mg/kg, respectively) than the soil samples from the non anthropogenic zone (4.6 and 1.8 mg/kg), (Table 1). Accumulation of Cr in the Tansy plants was higher in the samples from anthropogenic zone than in the samples from the non anthropogenic zone (Table 2). However, chromium was not detected in the essential oils from the non anthropogenic zone (Table 3). Anthropogenic site location had significantly higher concentration of Ni (20.0 and 10.0 mg/kg) than the non anthropogenic zone (4.3 and 2.0 mg/kg) (Table 1). Nickel concentration amount was significantly higher in the anthropogenic zone plants, than the concentration in the samples from the non anthropogenic zone (Table 2). Nickel as well as Hg and Cr were not detected in essential oil of the non anthropogenic plants (Table 3).

The heavy metal content depends on the type of plant, environment in which the plant is grown, the level of industrial development of the region, air pollution, soil and climate conditions, etc., (Zenk, 1996). According to the WHO criteria, the results showed that the measured values were in the regular limits on the site locations, of the non anthropogenic zone. Distribution of the heavy metals among the plant organs was selective and depended on the part of the plant, surface characteristics of the plant organ and the element that was examined. Because of that, the root, stem and leaves were investigated. The Hg concentration in the root, stem and

leaves from the anthropogenic site were up to 20 times more than those in the plants at the non anthropogenic site (Table 2). Our results about Hg content are in agreement with results of Fernández-Martínez et al. (2005). Concentration of Pb in the anthropogenic soil was higher (19.0 and 17.0 mg/kg) than the non anthropogenic soil (6.0 and 3.0 mg/kg), (Table 1). The level of Pb in Tansy is not hazardous to human health. Concentration of Pb in the Tansy essential oil from the anthropogenic (0.1 mg/kg) had maximum permissible concentrations (Table 3). Tansy roots contained lower Cd amount than the leaves (Table 2). Also, according to Angelova et al. (2005), deposition of Cd in the leaf was higher than that in the root. The Pb and Cd distribution in Tansy decreased in the order: leaves > roots > stems as also noticed in *Salvia officinalis* (Blagojević et al., 2009). The essential Tansy oils from the anthropogenic (0.02 mg/kg) and non anthropogenic (0.01 mg/kg) zones showed permissible concentrations of Cd (maximum in the essential oil was 0.05 mg/kg, respectively). Roots of Tansy plants from the non anthropogenic zone consisted similar values of Cr as those of *Zea mays* and *Sorgum vulgare* plants (Oviasogie et al., 2009). The content of Ni in the Tansy leaves and essential oils from the anthropogenic site was much higher than the permissible concentrations. Also, the levels of Ni in all the specimens of *Thymus vulgaris* in Jordan (Abu-Darwish and Abu-Dieyeh, 2009) indicated higher concentrations than the average (0.1 to 0.5 mg kg⁻¹) reported by Kastori et al. (1997) for the same plant. This higher concentration of Ni may be due to the vicinity of the road and the intensity of the plant, which was progressively increased with locations full of vehicles and industrial activities in Amman or neighboring southern locations with cement factories in their surroundings (Jaradat et al., 1999). Our results about Ni and Pb contents in the soil and plants are in accordance to the research done by Loranger and Zayed (1994). These authors reported that, Ni was higher in industrial and residential areas, which may be due to their use as fuel additives just like Pb. Also, the concentration of Hg, Pb, Cd, Cr and Ni were higher in soil samples on 0 to 15 cm depth than on 15 to 30 cm depth on all the site locations. These results are in agreement with those of Oviasogie et al. (2009). Soil, plant and essential oil samples from the non anthropogenic area in Belgrade, consisted of

Table 4. Macro- and microelements content in soil samples from the anthropogenic and non anthropogenic site locations.

| Site location | Element (%) | | | | | | | | | |
|-------------------|-------------|------|------|------|------|------|------|-------|-------|------|
| | S | P | K | Ca | Mg | Na | Fe | Si | Al | Mn |
| Anthropogenic | 0.03 | 0.01 | 0.26 | 6.35 | 0.20 | 3.50 | 1.02 | 74.80 | 11.80 | 0.05 |
| Non anthropogenic | 0.06 | 0.06 | 0.25 | 1.00 | 1.00 | 3.04 | 0.51 | 28.31 | 6.54 | 0.06 |

Table 5. Macro- and microelements content in shoot and root of Tansy from the anthropogenic and non anthropogenic site locations.

| Site location | Tissue | Element (%) | | | | | | | | | |
|-------------------|--------|-------------|------|------|------|------|------|------|------|------|-------|
| | | S | P | K | Ca | Mg | Na | Fe | Si | Al | Mn |
| Anthropogenic | Shoot | 0.11 | 0.12 | 4.43 | 1.70 | 0.20 | 0.01 | 0.02 | 0.58 | 0.20 | 0.015 |
| | Root | 0.20 | 0.06 | 2.25 | 3.33 | 0.11 | 0.02 | 0.05 | 1.00 | 0.40 | 0.007 |
| Non anthropogenic | Shoot | 0.22 | 0.78 | 4.40 | 0.90 | 1.00 | 0.01 | 0.01 | 0.38 | 0.10 | 0.02 |
| | Root | 0.40 | 0.37 | 2.21 | 2.00 | 0.58 | 0.02 | 0.02 | 0.80 | 0.20 | 0.01 |

lower concentration of the investigated heavy metals.

Micro-and macro elements

Soil samples collected from the anthropogenic zone had pH 8.2. However, pH value measured on the non anthropogenic zone was 7.2. The contents of macro- and microelements in the soil and Tansy plants were expressed in percentage of dry weight (Stevović et al., 2010b). Concentration of total sulfur in the soil sample from the non anthropogenic zone (0.06%) was two times higher than that of the anthropogenic (0.03%) site location (Table 4). The concentration of phosphorus (0.01%) was poor in the anthropogenic zone, while the concentration in the non anthropogenic zone soil was high (0.06%) (Table 4). Also, soil samples from the non anthropogenic zone had 6 times more P than samples from the anthropogenic zone. The soil from the anthropogenic zone was mostly composed of sand, while soil from the non anthropogenic zone was a mixture of humus and sand. Soil samples from the non anthropogenic zone consisted more levels of S and P than soil samples from the anthropogenic zone (Table 4). Soils on both site locations contained similar amount of potassium (0.26 and 0.25%) and sodium (3.50 and 3.04%) (Table 4). Therefore, soil samples from the anthropogenic zone had six times more of Ca, two times more of iron, silicon and aluminum than the soil samples from the non anthropogenic zone. However, soil samples from the non anthropogenic zone had five times more of magnesium than samples from the anthropogenic zone (Table 4). Also, mineral elements contents were measured in Tansy plants from the anthropogenic-contaminated industrial zone and non anthropogenic area. Concentrations of sulfur in shoots (0.22%) and roots (0.40%) of the plants from the non anthropogenic zone was two times higher than the concentration in plants from the anthropogenic (0.11 and 0.20%) site locations

(Table 5). Also, P amount in shoots (0.78%) and roots (0.37%) of the plants from the non anthropogenic zone were 6 times more than that in Tansy from the anthropogenic zone (0.12 and 0.06%), (Table 5). However, Tansy plants from the anthropogenic and non anthropogenic zone had similar amounts of K in the shoots and roots, as well as Na and Mn. Nevertheless, the shoots and roots of Tansy plants from the non anthropogenic zone had five times Mg than that in the shoot and roots from the anthropogenic zone. Plant samples from the anthropogenic site consisted two times Ca, Fe, Si and Al than those of samples from the non anthropogenic site location (Table 5). Tansy plants adapted to the high Ca concentration in the anthropogenic soil. The Tansy plants absorbed more Ca, Fe, Si and Al due to higher concentrations of the minerals in the anthropogenic soil. Concentrations of macro- and microelements in the soil were reflected to from the concentration in the Tansy plants. Also, plants from the anthropogenic zone adapted to the soil with a lot of sand and high concentrations of Ca, Fe, Si and Al.

The site locations had similar general climate but different microclimate conditions. Moreover, differences in the composition of the soils were established. Soil samples from the anthropogenic zone were mainly made of sand and building waste, while samples from the non anthropogenic site location had humus and sand. However, the anthropogenic zone had higher mean annual temperature and smaller precipitation than the non anthropogenic location. In addition, due to sandy soil and faster drainage in the underground, the anthropogenic zone soil was dryer than the soil in the non anthropogenic area. The macro- and microelement content depends mainly on the type of plant, environment (the level of industrial development of the region, air pollution, and soil and climate conditions) (Voutsas et al., 1996; Ngole and Ekosse, 2009). Plants growing in a polluted environment can accumulate macro- and microelements at high concentrations, causing a serious risk to human health when

plant based foodstuffs are consumed (Kabata-Pendis and Kabata-Pendis, 1984; Hovmand et al., 1983; Pais, 1997). Macro- and microelements uptake by roots depends on both the soil and plant factors (source and chemical form of elements in soil, pH, organic matter, plant species, plant age etc.). Element mobility and plant availability are very important when assessing the effect of soil contamination on plant minerals uptake and related phytotoxic effects (Mench et al., 1994). Interaction between minerals occurring at the root surface and within the plant can affect uptake, as well as translocation and toxicity (Luo and Rimmer, 1995; Ande and Onajobi, 2009). Adequate pH is necessary to assure that soil minerals are "plant available". Plants generally cannot take up the minerals they need when soil pH is at extremes. Higher concentrations of Ca, Si and Al in the investigated soil were in agreement with the pH higher value in the soil samples, which were also found in similar locations (Kato et al., 1997). However, soil on the non anthropogenic location had higher amount of S and P which caused better organic quality of humus soil on this site. The largest Ca, Fe, Si and Al levels in the soil samples from the anthropogenic site was measured. A higher value of these minerals in the anthropogenic soil due to this site location was dumped garbage and building materials. The results of this experiment are in agreement with the expected results. The nutritional and chemical value of Tansy plants were investigated as well as that of palm (Ezieshi and Olomu, 2007), *Amaranthus hybridus* (Akubugwo et al., 2007) using standard analytical methods. Sulfur was the most important element in the Tansy plants from the anthropogenic and non-anthropogenic localities. The concentration of Ca in anthropogenic Tansy plants was greater than that in the non anthropogenic plants. Similar results was reported by Kumar et al. (1999) and Rajakumar and Narayanaswamy (2004). Low level of Na and high level of K in Tansy from both site localities are necessary for good plant quality and excellent diuretic properties (Thangavelu et al., 2003). Amount of Mg in the soil and Tansy from the anthropogenic zone was very high. Kumar et al. (1999) and Rao (2000) showed that high Mg level gives plants a better taste, color and smell. It is of very great importance for Tansy plants from non the anthropogenic site and these are used in medicine and nutrition. Magnesium acts as cofactor in sugar synthesis when it is available in greater amount in the metabolic environment (Rajakumar and Narayanaswamy, 2004). The high concentration of iron in the Tansy plants from the anthropogenic site enables easier survival. Tansy is often present around the roads, dump, civil engineering sites, even on the heavy contaminated habitats. Fe amount in the Tansy plants, measured and presented in this paper are in correlation with the results of Kumar et al. (1999) and Rajakumar and Narayanaswamy (2004). Iron is essential for chlorophyll and protein formation, photosynthesis, electron transfer, oxidation and reduction of nitrates and sulphates and

other enzyme activities (Mohamed, 1999). Deficiency of iron causes internal chlorosis in newly emerging young leaves due to reduced chlorophyll synthesis, resulting in poor growth as well as loss in yield and sucrose content (Singh et al., 1992; Pais, 1997; Singh et al., 2000). Mg content in the non anthropogenic Tansy plants was high, which is in close agreement with the results obtained by Kulikova et al. (2005).

High manganese status for availability combined with high organic matter was observed to improve soil quality (Kulikova et al., 2005). Also, distribution of the macro- and microelements among the plant organs (shoot and root) depends on mobility of the examined minerals. P, K, Mg and Mn are mobile elements, therefore, these are more in the Tansy leaves. However, amounts of S, Ca, Na Fe, Si and Al were higher in Tansy roots, because these minerals were immobile. Results about mobility of macro- and microelements in Tansy are in accordance with results on agricultural plants (Vukadinovic and Loncaric, 1997). An ecological aspect of the role of macro- and microelements as well as the essential oils is reflected in the interaction of plants with the environmental factor. In a variety of stressful conditions: unfavorable geological, pedological, climatic, or biotic conditions, the number of plants rich in essential oils and poor in macro- and micro elements is increased. The Tansy plant like cane plant (Kumar et al., 1999) takes up its need of trace elements from the surrounding soil. Tansy plants survive in the urban conditions by finding ways of adapting to the environmental conditions.

Morphological and anatomical investigation

The morphological studies of the Tansy on both sites shown that, the plants had similar height, shape, size and color of stem, root and heads. However, Tansy plants showed visible morphological changes of the leaves. Plants from the anthropogenic zone had thinner leaves (393.37 μm) than the plants from the non anthropogenic (542.22 μm) site location, due to the impact of the industrial pollutants. The upper and lower epidermis consisted 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 epidermises. The stomata occur on both epidermal surfaces level with the neighboring cells. Also, stomata cavities were large in the leaves of the Tansy plants from both localities. Mesophyll consisted of the palisad parenchyma and spongy parenchyma. Thickness of the leaf mesophyll from the anthropogenic (186.73 μm) plants was significantly thinner than that of the mesophyll of the non anthropogenic (258.30 μm) plants. Leaf mesophyll from the anthropogenic zone consisted 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 the anthropogenic

zone were thinner than that in the non anthropogenic plants (119.7 and 94.5 μm). Solitary vascular bundles are surrounded by parenchymatous and orbicular cells. Also, leaves from anthropogenic zone had 2 or 3 layers isodiametric spongy parenchymatic cells with lower intercellular cavities. Thickness of the upper and lower palisade parenchyma from the non anthropogenic zone was 1.3 times greater than that of the palisade parenchyma of the plants from the anthropogenic area. However, the spongy parenchyma of the Tansy leaves from the non anthropogenic site had similar thickness, as well as the spongy tissue from the anthropogenic zone. Vascular bundles were well developed in the leaves from both site localities. Stem consisted of the epidermis, cortex and central cylinder. Stem cortex consisted of 4 or 5 layers of the usually oval parenchyma cells in the plants from both localities. The parenchyma had cells (occupy the most of stem) which had a different shape, size and thin cell walls and chloroplasts. There was a sclerenchymatic sheath between these bundles. Vascular bundles are of different sizes. Pith cells of the Tansy from both localities were large and cylindrical. Epidermis on the root surface in the plants from both site localities was noticed. Primary cortex was obtained between the epidermis and central cylinder. The primary cortex was more developed than the central cylinder. Between phloem and xylem was the conducting parenchyma. Cells of the primary cortex had thin cell walls. Vascular bundles with radial shape were closed. In light of the mentioned differences in the leaf anatomy, leaf chlorophyll content in Tansy was measured. Leaves from the polluted zone had significantly lower chlorophyll content than the control leaves. Relation to the total chlorophyll amount in the non anthropogenic (62.2 mg/100 g) and anthropogenic zones (36.7 mg/100 g) leaf samples was 1.69. However, the relation between chlorophyll *a* and *b* contents in samples from both site locations was 1.5 (anthropogenic zone, Chl *a*= 21.9; Chl *b*= 14.8; and non anthropogenic zone Chl *a*= 37.3; Chl *b*= 24.9). Anatomical studies of Tansy are mainly focused on leaf, stem and root features. Well developed cuticle on leaves from the anthropogenic zone is an effective peripheral protection and adaptation to the environmental conditions: increased insolation and temperature, and life in the open habitat. The results about well developed Tansy cuticle in the polluted area are in accordance with the results of Godzik and Halbwachs (1986). Leaf anatomy of Tansy also showed reduction in mesophyll, palisade parenchyma and upper and lower epidermis in the polluted area when compared with leaves collected from the non polluted area. Significant reduction in thickness of leaves of the Tansy plants from the anthropogenic zone was an adaptation to the continuously effect of different pollutants (heavy metals, building materials, oxides of nitrogen and sulphur, etc.) which were released into the environment. Also, other authors in previous years also showed significant reduction in the 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 the shape and structure of the 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 becomes flattened due to continuous exposure to pollutants. Similarly, Iqbal (1985) and Jahan and Zafar (1992) showed significant reduction in leaf palisade and spongy parenchyma in polluted population. Due to inconvenient environmental conditions, the leaves of the anthropogenic plants were poorer in chlorophyll *a* and *b* contents. The results about Tansy chlorophyll amount are in accordance with the results on other species (Krause and Dochinger, 1987; Gielwanowska et al., 2005; Kofidis et al., 2008).

Environmental adaptability indicator

The concentration of the essential oil is analyzed as indicator of Tansy environmental adaptability. 22 different major and minor constituents from Tansy was separated and identified. Only six components (artemisia ketone, *trans*-chrysanthenyl acetate, *trans*-carveol acetate, linalool oxide acetate, piperitone and β -thujone), of about 14 identified, were detected in the plants from both sites. The total composition of the essential oil in the plants from the anthropogenic site was 80.7%, while in the non anthropogenic plants it was 75.5%, related to total amount. Essential oil of *T. vulgare* plants from the anthropogenic and non anthropogenic zones had different content of eight characteristic compounds. Specific Tansy essential oil compounds from the anthropogenic zone were: germacrene-D, α -cadinol, longiborneol, pinocarvone, santolina alcohol, spathulenol, α -thujone and undecane. However, the compounds characteristic to Tansy grown on the non anthropogenic site location were: α -phelandrene, β -phelandrene, *trans*-chrysanthenol, *p*-cresol acetate, *p*-cumene, linalool oxide, *cis*-pinochamphone and sabinene hydrate. *Trans*-chrysanthenyl acetate and *trans*-carveol-acetate (more than 50 %) were the main compounds in the plants from both site localities.

Tansy plants exposed to the anthropogenic influences had higher amount of the essential oil than the plants from the non anthropogenic zone (Stevović et al., 2009). The results presented in this paper clearly showed that, the *trans*-chrysanthenyl acetate was the most important component of the Tansy essential oil and that it was in correlation with the results of Popov et al. (2001). Toxic β -thujone was found in the essential oil of the Tansy plants grown on the polluted site (8.3%), while in the essential oil of plants from unpolluted site the concentration was much less (1.3%). Those results were expected. It was in accordance with the difference between

contamination of the industrial and green area zone (Vaverkova et al., 2008). Extremely high content of toxic thujone is very dangerous for human health (Gallino, 1988). Tansy plants growth on anthropogenic and non anthropogenic site locations belong to the *trans*-chrysanthenyl acetate chemotype, which is one of four different chemotypes of this species: thujone, *trans*-chrysanthenyl acetate, chrysanthenyl and camphoric (Popov et al., 2001) in Serbia. Toxic β -thujone was found in the essential oil of Tansy plants grown on the polluted site (8.3%), while in the essential oil of the plants from the unpolluted site, the concentration was much less (1.3%). The site location from where the plants are harvested for medical purposes is very important. *Trans*-chrysanthenol was found in the essential oil from the non anthropogenic zone in very small concentration (1.4%). Camphor was not found in the Tansy essential oil on both investigated habitats in the territory of Belgrade.

The close correlation between the level of the environmental pollution and pollutant contents in plant and soil was found. Essential oil amount is an environmental adaptability indicator and differences in morpho-anatomical structure of Tansy are a response on pollution.

Conclusion

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. Heavy metals were present in the soil and Tansy medicinal plant at different concentrations, which, in some cases, exceeded the permissible levels. This could be attributed to contamination from traffic and industrial activities.

Macro- and microelements were present in the soil and Tansy medicinal plant at different concentrations, which depended on the pollution degree. Moreover, high concentration of Ca, Fe, Si and Al in the industrial zone soil was closely related with the influence of anthropogenic factors. Tansy grown on the non anthropogenic site had a high nutritional value than the plants from the anthropogenic location. The results obtained and presented in this paper showed extreme sustainability and usefulness of Tansy plants, in spite of the negative anthropogenic environmental conditions. Further studies on macro- and micro elements contents in the wide spread plants are recommended.

The leaves of Tansy grown in the anthropogenic zone were thinner; contained less palisade parenchyma and chlorophyll *a* and *b* than the leaves from the non anthropogenic site. The possible positive human influence on the environment could be to decrease the pollutions and to improve soil quality by horticultural activities; designing parks in urban environment. The research presented in this paper provides a good basis for further research on impact of the environment to content and morphological and anatomical structure of the plants.

It was demonstrated too that, the specific essential oils compounds were adequate to represent the *T. vulgare* environmental adaptability. The total amount of essential oil in the Tansy plant found from both locations proved a higher degree of representative compounds in the plants from the anthropogenic area. It could serve as an indicator of the adaptability of this species on anthropogenic and anthropogenic environmental conditions, as well as its usability. Further experiments are required to isolate and to identify all other active principles also responsible for Tansy ecological plasticity.

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