

# Determination of trace elements in the Lavender inflorescence (*Lavandula angustifolia* Mill.) — Lavender oil system

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## Abstract

Bulgaria is a famous world producer of essential oils. Quality control of the lavender oils produced is an important analytical task in view of their wide use in perfumery and cosmetics, the food industry and aromatherapy. The present paper studies the relationship between the trace elements content in lavender inflorescence (*Lavandula angustifolia* Mill.) and in lavender oil derived from this inflorescence. Three model regions were investigated: two of them are situated far from industrial areas — the village of Zelenikovo and the town of Pavel Banya, and one region is located near the Plovdiv Non-Ferrous Metallurgical Plant. The content of the elements Cd, Cr, Cu, Fe, Mn and Pb in plants was determined after acid digestion by flame and electrothermal atomic absorption spectrometry (ETAAS). Lavender oils were analyzed directly by ETAAS. The results undoubtedly show that there is no strong correlation between the trace elements content in inflorescence and in essential oils. It might be assumed that the trace elements present and accumulated in the inflorescence do not pass in lavender oil during the production by steam distillation.

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## 1. Introduction

High quality lavender oil is produced through steam distillation of lavender inflorescence (*Lavandula angustifolia* Mill.). One kilogram of lavender oil is derived from about 120–150 kg of lavender inflorescence, depending on the climatic conditions. Essential lavender oils are very complex matrices consisting of various classes of organic compounds — terpenes, hydrocarbons, esters, alcohols, aldehydes, ketones, etc. [1,2]. Essential oils are widely used in perfumery and cosmetics and in the food industry [3–5]. Their therapeutic and bactericidal properties are well-known [5–9]. The wide use of essential oils requires strict control with respect to various undesirable impurities, such as artificially added substances [10,11], pesticide residues [12,13], toxic elements, etc.

No specific requirements and standards (Bulgarian or European) were found in literature regarding the trace metal content in essential oils. However, elements like Fe, Cr, Cu and Mn could most probably affect oil stability during storage due to

their catalytic properties, and the Cd and Pb content is subject to restrictions due to the high toxicity of these elements. In general, depending on the use of the oils, the standards for the respective product are observed — foodstuffs, cosmetic products, etc. There are only several papers related to the determination of trace elements in essential oils [14–16]. One of them is based on preliminary dry ashing of oil samples followed by ETAAS determination of trace elements [14]. Cd, Cu, Pb, Zn, Mn have been determined in citrus oils potentiometrically after hydrochloric acid extraction and the results have been compared with those obtained by ETAAS, after wet digestion of the sample [15,16]. Direct ETAAS has been used for the determination of As in rose and lavender oils and a very low content of As was found [17]. Our survey of literature showed that there is no systematic data on the levels of trace elements in essential lavender oil produced from different regions in Bulgaria. In this respect, no data has been published on the potentials of instrumental methods for the determination of trace elements in essential oils.

The aim of the present study was to perform an analysis of lavender inflorescence and lavender essential oils produced from these inflorescences in order to delineate the migration of

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Fig. 1. The map of Bulgaria and sampling areas.

trace elements (Cd, Cr, Cu, Fe, Mn and Pb) in the lavender inflorescence — essential oil system. As a first step, it was necessary to elaborate a set of methods for ETAAS determination of trace elements in lavender oil. As a second step, lavender inflorescences originating from three model regions and lavender oils produced from them were analyzed. Two of the regions are far from any industrial activities, in relatively unpolluted areas — the village of Zelenikovo (in the southern part of Sredna Gora mountain, one locality) and the town of Pavel Banya (in the northern part of Sredna Gora mountain, with four separate localities-Atyovo, Kara, Rosena and Chaliyata). One region is located very close to Plovdiv Non-Ferrous Metallurgical Plant (some 2 km away) (see Fig. 1). A sample of laboratory-produced lavender oil (Clavenger equipment) was also analysed in order to establish the impact of the processing equipment on the trace element content in the final product. Samples of lavender inflorescence were taken from a distillery immediately prior to oil production and they were acid-digested without any other preliminary treatment (without prior washing or air blowing). Lavender oils were analyzed directly by ETAAS.

## 2. Experimental

### 2.1. Instrumentation

Flame atomic absorption spectrometric measurements were carried out on a Perkin Elmer Zeeman 1100 B spectrometer with an air/acetylene flame. The instrumental parameters were optimized in order to obtain maximum signal-to-noise ratio. Electrothermal atomic absorption spectrometric measurements were carried out on a Perkin Elmer Zeeman 3030 spectrometer with an HGA-600 atomizer. The light sources used were hollow cathode lamps for Cr, Cu, Fe, Mn and Pb and an electrodeless discharge lamp for Cd (Perkin Elmer). The spectral band-pass was 0.7 nm. Pyrolytic graphite-coated graphite tubes coated with Pt as permanent modifier and tungsten impregnated tubes were used as atomizers. Permanent modification with Pt was achieved by triple injection of 40  $\mu\text{L}$   $(\text{NH}_4)_2\text{PtCl}_6$  modifier solution, 1000  $\mu\text{g L}^{-1}$  in 5% v/v HCl with 1000 °C ashing temperature and 2100 °C atomization temperature. Tungsten impregnation was

Table 1

The optimized HGA-600 temperature program for ETAAS measurements of oil samples

| Step | Temperature, °C   | Ramp, s | Hold, s | Ar flow, mL min <sup>-1</sup> |
|------|---|---------|---------|-------------------------------|
| 1    | 50  | 10      | 20      | 300                           |
| 2    | 150   | 10      | 20      | 300                           |
| 3    | 900 <sup>a</sup> , 1200 <sup>b</sup> , 1300 <sup>c</sup>  | 20      | 60      | 300                           |
| 4    | 1400 <sup>a</sup> , 2200 <sup>b</sup> , 2500 <sup>c</sup> | 0       | 3       | 0                             |

<sup>a</sup> Cd and Pb.

<sup>b</sup> Cu, Mn and Fe.

<sup>c</sup> Cr.

performed as follows: an uncoated graphite tube was left in 10% m/V  $\text{Na}_2\text{WO}_4$  solution for about 24 h. After drying at room temperature, the tube was heated according to the following temperature programme: 1. 100 °C for 30 s, 2. 1400 °C for 30 s, 3. 2650 °C for 10 s [18]. Sample solutions (20  $\mu\text{L}$ ) were introduced into the graphite atomizer using an AS-60 auto-sampler. Atomic absorption signals were recorded on an Anadex printer. Only peak areas were used for quantification. The optimal instrumental parameters for the ETAAS measurements were investigated and are presented in Table 1.

### 2.2. Reagents

All reagents were analytical reagent grade. The stock standard solutions of Cd, Cr, Cu, Fe, Mn and Pb 1000  $\mu\text{g mL}^{-1}$  were Titrisol, Merck in 2% v/v  $\text{HNO}_3$ . The working standard solutions were prepared daily by proper dilution with organic solvent (p.a. Merck). Nitric acid was additionally purified by sub-boiling distillation.

Stock standard solution of Pt 980  $\mu\text{g mL}^{-1}$  in 5% v/v HCl (Aldrich) was injected (40  $\mu\text{L}$ ) into the graphite furnace to obtain permanent modifier coatings. Doubly distilled water was used in all experiments.

### 2.3. Sample preparation

#### 2.3.1. Mineralization of the plant samples

The plant samples were taken from the distillation installations immediately before feeding the plant mass into the equipment and were subjected to mineralization without prior washing or air blowing.

A plant sample, ca. 0.5 g, was placed in a 25 mL beaker, 10 mL of concentrated nitric acid were added, the beaker was covered with a watch glass and left to stay for one night. Then the sample was heated (120 °C) on a sand bath, still covered with the watch glass, until the volume of the solution was reduced to 1–2 mL. After cooling down, 2 mL of  $\text{H}_2\text{O}_2$  were added and the sample was heated again for further 10 min. Finally, the sample solution was transferred to a 25 mL measuring flask and doubly distilled water was added up to the 25 mL mark.

#### 2.3.2. Preparation of the lavender oil samples

The lavender oil samples were taken from the producer after the end of the distillation process. The samples were diluted

Table 2  
Elements content, ng g<sup>-1</sup> in lavender inflorescence and lavender oil (three parallel determinations)

| Regions                                       | Localities    |                    | Element content, mean±s (ng g <sup>-1</sup> ) |                      |                       |                     |                        |          |
|---|---------------|--------------------|---|----------------------|-----------------------|---------------------|------------------------|----------|
|   |               |                    | Cd  | Cr                   | Cu                    | Fe                  | Mn                     | Pb       |
| Pavel Banya                                   | Atyovo        | Inflorescence      | 13±1  | 90±10                | 7.2±1.1 <sup>a</sup>  | 114±12 <sup>a</sup> | 39±4 <sup>a</sup>      | 90±8     |
|   |               | Oil                | 0.82±0.07                                     | 1.8±0.2              | 5.9±0.4               | 170±12              | 394±14                 | 7.3±0.5  |
|   | Kara          | Inflorescence      | 50±5  | 220±20               | 10.9±1.2 <sup>a</sup> | 353±28 <sup>a</sup> | 163±11 <sup>a</sup>    | 670±5    |
|   |               | Oil                | 0.63±0.05                                     | 9.4±0.4              | 44.3±3                | 840±32              | 452±19                 | 25.3±1.5 |
|   | Rosena        | Inflorescence      | 14±2  | 150±18               | 8.3±1.1 <sup>a</sup>  | 256±23 <sup>a</sup> | 31±4 <sup>a</sup>      | 80±6     |
|   |               | Oil                | 0.57±0.04                                     | 3.1±0.2              | 10.8±0.7              | 190±11              | 21±2                   | 14.7±0.9 |
|   | Chaliyata     | Inflorescence      | 12±1  | 130±12               | 9.7±1.2 <sup>a</sup>  | 123±14 <sup>a</sup> | 43±4 <sup>a</sup>      | 270±15   |
|   |               | Oil                | 0.65±0.05                                     | 2.9±0.2              | 39±2                  | 210±14              | 104±9                  | 18.6±1.1 |
| Zelenikovo                                    | Inflorescence | 11±1               | 40±4  | 8.4±1.1 <sup>a</sup> | 66±9 <sup>a</sup>     | 39±4 <sup>a</sup>   | 770±70                 |          |
|   | Oil           | 0.71±0.05          | 1.6±0.1                                       | 11.9±0.8             | 100±9                 | 336±16              | 17.3±0.9               |          |
| Metallurgical Plant                           | Inflorescence | 27 <sup>a</sup> ±3 | 570±45  | 79±8 <sup>a</sup>    | 477±38 <sup>a</sup>   | 41±3 <sup>a</sup>   | 1406 <sup>a</sup> ±112 |          |
|   | Oil           | 0.72±0.05          | 0.04±0.01                                     | 8.4±1.1 <sup>a</sup> | 66±9 <sup>a</sup>     | 25±2                | 10.2±0.7               |          |
| Laboratory produced lavender oil <sup>b</sup> |               |                    | 0.23±0.05                                     | 0.8±0.1              | 11.4±0.9              | 13±2                | 5.3±0.7                | 7.8±0.7  |

<sup>a</sup> Results in µg g<sup>-1</sup>.

<sup>b</sup> Inflorescence from Zelenikovo.

directly in an autosampler cup placed on analytical balance, by dispensing such amounts of essential oil and organic solvent (1,4-dioxan) so as to obtain 1+1, 1+2 m/m dilution. Spiked samples were prepared by adding the proper volumes (10–30 µL) of aqueous standard solution. The cups were sealed with Parafilm and shaken to obtain homogeneous solutions; the diluted samples proved to be stable for at least 1 week at ambient temperature. The cups were opened just before making the measurements so as to avoid uncontrolled evaporation of organic solvent.

### 3. Results and discussion

#### 3.1. ETAAS determination of the elements in lavender inflorescence and lavender oil

ETAAS determination of trace analytes in plant samples conventionally consists of acid digestion of the plant samples followed by ETAAS measurement. In the present study, mixture of HNO<sub>3</sub> and H<sub>2</sub>O<sub>2</sub> was used for the digestion of lavender inflorescence and almost complete mineralization of the plant matrix was achieved, thus avoiding filtration of the sample solution before instrumental measurement. Palladium (2.5 µg) was used as modifier for thermal stabilization of Cd in ETAAS determinations. Lead was measured in the absence of modifier since it is well known that the HNO<sub>3</sub> and H<sub>2</sub>O<sub>2</sub> serves as an internal modifier and allows 600 °C pyrolysis temperatures for lead, which are high enough for achieving reliable ETAAS measurements of this element. The accuracy of the analytical procedure used for lavender inflorescence analysis is confirmed by the analysis of CRMs (NIST 1571, orchard leaves, and NIST 1570, spinach). Very good compliance to the certified values (all within the 95% confidence interval) was established.

It was possible to analyze lavender oil by ETAAS after wet acid digestion of the samples. Lavender oil digestion is a long and tedious procedure, even using a MW oven. Sample amounts around 0.3 g could be mineralized completely by relatively high

amounts of HNO<sub>3</sub> and H<sub>2</sub>O<sub>2</sub>, which entails high blank values. Another approach for oil analysis used in the present work is direct ETAAS after dissolving the samples in a suitable organic solvent. The organic solvent used — 1,4-dioxane, ensures complete dissolution of the oil samples and a possibility to use aqueous standard solutions for calibration purposes [19]. Optimal instrumental parameters for ETAAS determination of trace elements in lavender oil dissolved in 1,4-dioxane were achieved on the basis of pretreatment and atomization curves constructed for each element; these are summarized in Table 1. Pyrolytic coated graphite tubes and W-impregnated graphite tubes were tested as atomizers. It was found that the W-impregnated tube is the most suitable atomizer for the determination of Fe and Pb (it was not necessary to use an additional modifier for Pb determination). The experiments showed that the use of Pt as permanent modifier ensures 900 °C loss-free pretreatment temperature for Cd and permits ETAAS determination of this element in lavender oil. The matrix interferences were evaluated through the ratio of the slopes of the calibration curves generated in the presence of lavender oil diluted in 1,4-dioxane and in the presence of aqueous standard also diluted in 1,4-dioxane. It was found that Cr, Cu, Fe, Mn and Pb could be determined in 1+2 m/m diluted lavender oil samples, and that 1+3 m/m lavender oil dilution is required for Cd determination. By using these dilution factors, the standard calibration curve generated with aqueous standards dissolved in 1,4-dioxane could be used for quantification of the trace elements.

#### 3.2. Analysis of the lavender inflorescence

The Cu, Mn and Fe contents of all lavender inflorescence samples, as well as the Cd and Pb contents of lavender inflorescence from the vicinity of the Plovdiv Non-Ferrous Metallurgical Plant, were determined by means of FAAS. The Cd and Pb contents of the samples from the regions of Pavel Banya (localities Atyovo, Kara, Rosena, Chaliyata) and Zelenikovo were determined by ETAAS. The Cr content in the all plant

samples was determined by ETAAS. The results from the analyses of plant samples are shown in Table 2. It can be seen that Fe and Mn are present at higher and significantly varying concentrations in the lavender inflorescence from unpolluted areas and, for the samples from the various regions, ranged from 66 to 352  $\mu\text{g g}^{-1}$  for Fe, and from 31 to 163  $\mu\text{g g}^{-1}$  for Mn. Copper is the element present at almost equal concentrations in all the samples from the various localities, with an average content of 8.9  $\mu\text{g g}^{-1}$ . The Cd, Cr and Pb content is much lower — with average contents of 0.018  $\mu\text{g g}^{-1}$  for Cd, 0.11  $\mu\text{g g}^{-1}$  for Cr and 0.44  $\mu\text{g g}^{-1}$  for Pb, for the samples from the unpolluted areas. Zelenikovo is the region with the lowest degree of pollution, far from industrial areas and busy highways, thus showing the lowest levels for all studied elements. It is worth mentioning that the four localities near Pavel Banya are very closely situated; however, almost twofold differences were observed in the contents of some elements, with the highest concentrations being measured in the Kara locality. This is possibly due to specific climatic factors in these four micro-regions. The trace element content of lavender inflorescence is influenced by the trace element content in the soils on which lavender was grown. Most probably, however, air pollution (aerosol depositions) and the climatic conditions are also very important factors influencing the trace element content of lavender inflorescence.

Extremely high contents of some elements such as Cd and Pb were found in lavender inflorescence from the region near Plovdiv Non-Ferrous Metallurgical Plant. The Fe and Mn in lavender inflorescence from this highly polluted region were very close to these levels in lavender inflorescence from the unpolluted regions. However, considering that this is a non-ferrous metallurgical plant, it might be expected that the levels of Cd, Cr, Cu and Pb would be hundred to thousand fold higher than the average levels of these metals in lavender inflorescence from the unpolluted regions. Such a great difference was not observed for the Cd, Cr, Cu and Pb content in the respective soils, which undoubtedly confirms the supposition that the level of trace elements in lavender inflorescence reflects mainly aerosol deposition and the degree of air pollution near the metallurgical plant.

### 3.3. Analysis of the lavender oil samples

The results from the analyses of lavender oils are also shown in Table 2. When comparing the results for lavender inflorescence and lavender oils, it can be seen that the content of the elements in oils is considerably lower than the element content in inflorescence. The toxic elements Cd and Pb are present at  $\text{ng g}^{-1}$  levels for all studied lavender oil samples. Furthermore, lavender oil produced from lavender inflorescence with extremely high content of toxic Cd and Pb, grown near the Non-Ferrous Metallurgical Plant, shows Cd and Pb contents close to the average values for these elements in other samples. The highest variability and highest concentrations were observed for the Fe and Mn contents in the various lavender oil samples. As a next step, multivariate correlation analysis was carried out and it showed that, for all the studied elements, the correlation

Table 3  
Multiple regression results

| Element | R (coefficient of multiple correlation) | Adjusted R |
|---------|---|------------|
| Cd      | 0.21                                    | -0.17      |
| Cr      | 0.59                                    | 0.19       |
| Cu      | 0.13                                    | -0.23      |
| Fe      | 0.34                                    | -0.11      |
| Mn      | 0.59                                    | 0.18       |
| Pb      | 0.41                                    | -0.24      |

coefficients for the trace element content of lavender inflorescence and lavender oils produced from that inflorescence are lower than 0.6, and for toxic elements like Cd and Pd, even lower than 0.5 (see Table 3). Taking into account that 1 kg of lavender oil is produced from 120–150 kg of lavender inflorescence, it can be concluded that the majority of the chemical species of Cd, Cr, Cu, Fe, Mn and Pb are aqueous soluble and have been transferred chiefly in the aqueous phase during distillation. Therefore, it can be concluded that high quality lavender oil can be produced from lavender inflorescence from relatively polluted regions. This conclusion is very important for the agricultural practice in Bulgaria. The analysis of the sample of laboratory produced lavender oil in Clevenger equipment shows lower values for Fe and Mn compared to the lavender oil samples produced in the distillery. Clearly, the Fe and Mn contents in lavender oils are affected mainly by the quality of the processing equipment. As a future step, in order to present the real distribution of trace elements between the aqueous phase and oil phase, thorough examination of each stage of the essential oil production process is required.

## 4. Conclusions

Essential oils are used in perfumery, cosmetics, the food industry, medicine and aromatherapy. Therefore, the control on their quality is very important. The present research was provoked by the lack of standards (Bulgarian and European) regulating the trace element contents in oils. The set of methods that has been elaborated for direct electrothermal atomic absorption determination of Cd, Cr, Cu, Fe, Mn and Pb was applied to lavender oils from six regions. The element content of the oils was considerably lower than the element content of the lavender inflorescence used for the production of the oils. No strong correlation was found between the trace element content in lavender inflorescence and in lavender oils (Table 3). Furthermore, high quality lavender oil could be produced from lavender plants grown in a relatively polluted region. The Fe and Mn content of lavender oil depends mainly on the quality of the processing equipment. However, for a fuller clarification of these issues, thorough research of each of the stages in the production process is necessary.

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