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Determination of risk elements in different species of edible flowers

Diploma Thesis

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Affidavit

I declare that I have elaborated my thesis "Determination of risk elements in different species of edible flowers" independently under the supervision of the supervisor of the thesis and with the use of literature and other information sources, which are quoted in the work and listed in the bibliography at the end of the thesis. Furthermore, as the author of this thesis, I declare that I have not infringed the rights of third parties in connection with its creation.

V Praze dne 18.4.2019 ______________________________

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Stanovení obsahu rizikových prvků v různých druzích jedlých květů

Souhrn

Jedlé květy se opět stávají populárními v západní společnosti, především díky jejich atraktivnímu vzhledu jako ozdobě, nebo ve formě dezertů. Četné vědecké studie zaměřují svou pozornost na nutriční a organoleptické vlastnosti jedlých květů, což naznačuje vysoký potenciál využití jedlých květin. Podle legislativy EU se jedlé květy řadí k novým potravinám, které podléhají stejným požadavkům na jakost jako ostatní potraviny (1,2,3).

Rizikové prvky uvolňované do životního prostředí přispívají k širokému spektru toxických účinků na živé organismy v potravinovém řetězci. Rostlinná akumulace kovů v nadzemních částech může vést ke zvýšení akumulace prvků v ornici v důsledku depozice listů nebo může vytvořit expoziční dráhu prvku do potravního řetězce. Evropská unie však dosud nestanovila maximální bezpečné limity pro některé rizikové prvky.

Cílem práce je shrnout současné poznatky o jedlých květech. Dále se zaměřit na rizikové prvky vyskytující se v životním prostředí, jejich toxicitu a akumulaci v rostlinách. Na závěr určit obsah rizikových prvků ve vybraných jedlých květech jejichž sběr proběhl v ČR a zhodnotit potenciální riziko jejich konzumace.

Experiment byl proveden pomocí ICP-OES pro stanovení obsahu prvků po předchozí extrakci vysušených homogenizovaných vzorků květů v kyselině dusičné. Výsledky podporují hypotézu, že jedlé květy nepředstavují riziko pro spotřebitele ve smyslu rizikových prvků.

Klíčová slova: rizikové prvky, jedlé květy, ICP-OES, toxicita, maximální bezpečné limity

Determination of risk elements in different species of edible flowers

Summary

Edible flowers are again becoming popular in the western society mainly thanks to their attractive appearance as a garnishing or in a form of desserts. Numerous scientific studies focus their attention to the nutritional and organoleptic properties of edible petals, which suggests a high potential of edible flower usage. In the EU, edible flowers belong to novel foods that are subject to the same quality requirements as other foods (1,2,3).

Risk elements released into the environment contribute to a wide range of toxic effects on living organisms in food chain. Plant accumulation of metals in epigeal tissues may lead to an increase of metal accumulation in topsoil, via leaf deposition, or may create an exposure pathway for metal introduction into the food chain, including the edible flowers. Nevertheless, the maximum upper safe limits for some of the risk elements are not yet established by the European Union.

The aim of this work is to summarize current knowledge about edible flowers. Furthermore, to focus on risk elements occurring in the environment, their toxicity and accumulation in plants. And finally determine the content of risk elements in selected edible flowers collected in the Czech Republic and evaluate the potential risk of their consumption.

The experiment was carried using the ICP-OES for determination of contents of elements after previous extraction of dried homogenized flower samples in nitric acid. The results support the hypothesis, that edible flowers do not pose a risk to the consumer in the term of risk elements.

Keywords: risk elements, edible flowers, ICP-OES, toxicity, maximum safe upper limits

Content

1 Introduction

The aim of this study is to present the last knowledge gathered about the food commodity of edible flowers, including nutritional values and the legislative requirements on quality. Secondly, the focus is on risk elements occurring in the environment, their toxicity and accumulation in plants.

By flower, it is meant "*the specialized part of an angiospermous plant that occurs singly or in clusters, possesses whorls of often colorful petals or sepals, and bears the reproductive structures (such as stamens or pistils) involved in the development of seeds and fruit : BLOSSOM"* (Merriam-Webster Dictionary).

Edible flowers have been known across the world for centuries and their tradition in European cuisine slowly decreased. Some were used as colorants, medicinal herbs or part of religious rituals. They were even ascribed supernatural powers. In ancient Rome, people believed they could gain the ability to speak with fairies after eating *Calendula officinalis*. We cannot exactly estimate when were flowers introduced to human diet, but we can assume it was long time ago regarding the references about edible flowers in numerous ancient texts such as Bible. The civilizations that mention consuming edible flowers are many: China, Egypt, Rome Empire, Greece, the Native-American civilizations etc. For instance a Sumerian herbal from 2500 BC mentions saffron crocus, planted for its colouring properties as an medicinal and food ingredient. Greek literature speaks also about other flowers like poppy, carnation, lotus or artichoke. (Newman & Kirker 2016)

Some of them are widely used in every household until now, such as cauliflower, artichoke, Brussel sprouts and broccoli, although they are commonly known as vegetable, it is their flowering part that is being consumed. Nowadays edible flowers celebrate a comeback in the western society mainly thanks to their attractive appearance as a garnishing or in a form of desserts. Nevertheless they have much more to offer than just pleasing our visual senses. Numerous scientific studies focus their attention to the nutritional and organoleptic properties of edible petals. For example Benvenuti et al. (2016) carried a study of antioxidant power and anthocyanin content of edible flowers, finding out that the antioxidant activity (levels of ascorbic acid) are equal and sometimes exceed the power of forest-fruit berries. This suggests a high potential of edible flower usage. Besides that, interest rises also thanks to the new processing technologies and storage possibilities. (Newman & Kirker 2016) Toxic compounds released into the environment contribute to a wide range of toxic effects on living organisms in food chain (Dembitsky & Řezanka 2003). Plant accumulation of metals in epigeal tissues may lead to an increase of metal accumulation in topsoil, via leaf deposition, or may create an exposure pathway for metal introduction into the food chain (Mertens et al. 2004).

2 The aim of this work and hypothesis

2.1 The aim of this work

The aim of the work was to summarize the current trends and knowledge about the use of edible flowers. Furthermore, to determine the content of risk elements in selected edible flowers collected in the Czech Republic and to assess the potential risk of their consumption.

Nowadays edible flowers celebrate a comeback in the western society mainly thanks to their attractive appearance as a garnishing or in a form of desserts. Nevertheless they have much more to offer than just pleasing our visual senses. Numerous scientific studies focus their attention to the nutritional and organoleptic properties of edible petals. For example Benvenuti et al. (2016) carried a study of antioxidant power and anthocyanin content of edible flowers, finding out that the antioxidant activity (levels of ascorbic acid) are equal and sometimes exceed the power of forest-fruit berries. This suggests a high potential of edible flower usage. Besides that, interest rises also thanks to the new processing technologies and storage possibilities. (Newman & Kirker 2016) Risk elements released into the environment contribute to a wide range of toxic effects on living organisms in food chain (Dembitsky & Řezanka 2003). Plant accumulation of toxic compounds in epigeal tissues may lead to an increase of risk elements accumulation in topsoil, via leaf deposition, or may create an exposure pathway for them introduction into the food chain (Mertens et al. 2004).

2.2 Hypothesis

Hypothesis 1: edible flowers do not pose a risk to the consumer in terms of risk elements.

3 Literature research

3.1 Nutritional composition of edible flowers

3.1.1 Main nutrients

Firstly, attention is put to the main nutritional characteristics of edible flowers. The contents of common nutritional components like proteins, lipids, saccharides and vitamins are similar to the contents of other plant organs, for example leaf vegetables. Many of the studied substances have chemoprotective or medicinal effect and they decrease the risk of various metabolic diseases. Especially beneficial for the human health are compounds with antioxidant effect, such as phenols, carotenoids and others. The plant parts composition of medicinals has been known for decades by contrast the knowledge of the compound composition of edible flowers of ornamentals and other plants is still being investigated. (Kopec 2004). The nutritional composition is significantly variable both at the different plant species and in the different plant components. (Rop et al. 2012)

In general, flower can be divided into three parts concerning its structure and nutritional value. The pollen, the nectar and the petals. Pollen is a rich source of proteins, amino acids, saccharides, saturated and unsaturated lipids, carotenoids, flavonoids etc. It is present in small amounts and it lacks taste.

Another part is the nectar, sweet liquid luring insects to feed on the flower. It is composed of a variety of saccharides (fructose, glucose, sucrose), amino acids (mainly prolin), proteins, lipids, organic acids, phenolic components, inorganic ions, alkaloids, terpenoids, etc. (Rop et al. 2012) (Mlcek & Rop 2011)

And finally the third are the petals and other flower parts. They are also source of above mentioned compounds and mainly not negligible series of vitamins. (Rop et al. 2012) The colours of the flowers are mainly given by the contents of carotenoids and flavonoids among other chemicals. (Friedman et al. 2010)

The main constituent of edible flowers is water varying between 70-95 %. The energetical value is quite low varying between 75-465 kJ /100 g of fresh weight depending on the content of carbohydrates. As to the content of macronutrients the levels of carbohydrates are varying between 10 and 90.2 g/100g dry weight among different species. A great variation occurs also in the content of proteins ranging from 2.0 to 52.3 g/100g dry weight. (*Begonia boliviensis* and *B. oleracea* var. *Italica,*) Lipid contents range between 1.3 to 6.1 g/100g dry weight. (Fernandes et al. 2017)

3.1.2 Minerals

Based on the study of Rop et al. (2012), we can say that edible flowers are great source of elements. Among tested flowers, the most abundant macroelement in plants was potassium (1,842.61 mg/kg to 3,964.84 mg/kg of FM) followed by phosphorus (202.11 mg/kg to 514.62 mg/kg of FM), sodium, magnesium and calcium. When comparing these values to the contents of some fruit and vegetables it has been found that the contents in edible flowers are very similar or even higher. Relatable results have been found in microelements (iron, manganese, copper, zinc, magnesium). (Rop et al. 2012) Minerals in general have an important role in human nutrition as they are essential to the metabolism.

3.2 Legislative of edible flowers and quality assessment

Following part of the research will focus on the legislative issues and the main criteria for quality assessment.

3.2.1 Food commodity determination

Edible flowers as we know them are not yet described as a single food commodity. Before introducing an edible flower product to the market it is obligatory to render to Czech Agricultural and Food Inspection Authority: 1) whether it is the type of flower intended for human consumption, 2) whether it is a novel food within the meaning of the new Regulation (EC) No 2015/2283 on novel foods and novel food ingredients and 3) whether it does not pose a health risk when consuming. (Státní zemědělská a potravinářská inspekce 2016)

According to the new Regulation (EC) Novel Food is defined as food that had not been consumed to a significant degree by humans in the EU before 15 May 1997, when the first Regulation on novel food came into force. 'Novel Food' can be newly developed, innovative food, food produced using new technologies and production processes, +as well as food which is or has been traditionally eaten outside of the EU. (Council of the European Union & European Parliament 2015)

3.2.2 Legislative requirements on quality

The quality requirements on edible flowers used for human nutrition are much higher than the ones determined for decoration.

According to the new Regulation (EC) No 2015/2283 the novel foods can not:

1. pose a health risk for consumers,
2. mislead the consumer,
3. be so different from foods and food ingredients to which they are intended to be replaced for, so that their normal consumption for consumers would be inappropriate considering the nutritional point. (Council of the European Union & European Parliament 2015)

Act No. 110/1997 Coll. prescribes that such foods may be marketed only as packaged (European Parliament 1997). Besides that, they should follow other legislative requirements regulating food quality and food safety, the same as other regulations related to foods. (Réblová 2014)

The health risk criteria and economical efficiency are very important. All the manipulation after the harvest has to be performed in the range of temperature within 4-7 C to retain their quality given by the species (2-7 days). (Kopec 2004) The flowers of ornamental plants are very delicate and sensitive material with tendency to microbial decay and mechanical damage. In order to prevent this, it is essential to ensure the right manipulation after the harvest. Flowers should be placed into perforated plastic bags or containers, which protect them against contamination, wilting and condensation of vapours. Harvested materials are rapidly cleaned, cooled and sometimes their shaped is modified to achieve better storage conditions. Subsequently they are stored at the temperature varying between 1 to 4 C for a period of 2 upto 14 days. (M. Kelley et al. 2003)

3.3 Quality evaluation

3.3.1 Sensoric quality

The main criteria evaluated by the consumers are the ones perceived by their senses: attractive appearance, color, size, taste, consistency, aroma, juiciness, surface of the petals. (Rop et al. 2012) In the study carried by M Kelley et al. (2001), consumers usually preferred most yellow and orange colors, than blue and combinations of other colors. For instance, the containers of edible flowers with more than one color of nasturtium and/or containers of nasturtiums with additional types of flowers were more favored. The diversity of the taste and the texture depends on the species, some flowers can be tender and crisp whereas others fragile or even silky. (Rop et al. 2011) Many of the sensoricaly active compounds present in edible flowers increase the efficiency of the nutrient digestion through psycho-physiological pathways. (Kopec 2004)

3.3.2 Economic quality features

Another criteria valued after the aesthetic appearance is their wholesomeness and suitability for the efficient economic use. (Herzog 1994) It is meant their capability to be grown on larger areas and the stability of their yields. Besides this, they should show at least a minimum resistance to diseases and pests (Neugebauerová & Vabkova 2009). It has showed that growing medicinal plants is very suitable addition to ecological farming regarding the minimum amount of pests and pathogens these plants suffer from. Not negligible is also the economical efficiency connected with their production. Relatively small surface can be used as a source of income and at the same time opportunity for further development even for small farms. The distinctively higher prices of the product are given by the above standard quality requirements resulting from high ratio of manual work and environmental value added of bioproducts which could not be reached by conventional agriculture. (Mitáček 2011) Medicinal plants are valued as an interesting element in the agricultural rotation enhancing the visual quality of the landscape therefore they contribute to increasing popularity of agrotourism on Czech farms. By their colorful diverse flowers they support natural pollinators' ecosystems. Their presence also positively affects other crops by allopathic influence of specific compounds, mainly silicas. (Mitáček 2011)

3.3.3 Some of the factors affecting the quality of edible flowers

Perhaps the most important, their medicinal soundness and nutritional characteristic were discussed by following authors. Anesini & Perez (1993) found that the values of antioxidant activity of edible flowers in cold storage did not change that much even after one week, thanks to the presence of gallic acid – one of the essential antioxidants of edible flowers.

In Czechia is quite common also the harvest of medicinals from free nature. The norms for ecological farming allow the certification of freely picked flowers as „bio-product" pressummed it has been confirmed the chemical cleaness of their original area. (Mitáček 2011)

When picking flowers in free nature, one should avoid picking at the areas close to dumps, factories, public communications (at least 100 m from a road) – the risk of pollutant contamination. In general we avoid places which could be chemically polluted and we never pick flowers on endangered list of species or growing in natural protected areas. (Lánská 2006)

The medicinal soundness (i.e. Absence of pathogens) of edible flowers depends on respected limits of the contents of toxic agents. Before consuming flowers picked up freely in nature it is always necessary to identify them. It is highly recommended not to consume edible flowers of ornamental plants from non-tested cultivars and/or florist's shops. They could contain potentially toxic compounds after the treatment by herbicides, fertilizers and other chemicals. A part from the toxic agents, even the flowers which may seem sound and clean can provoke allergic reactions to their non-defined components. Allergic response has often been caused by chrysanthemum flowers; manifested mainly as rash and eczema (Osimitz et al. 2006).

3.4 Accumulation of risk elements by plants

Before focusing on the determination of risk elements in edible flowers, the basic processes of elements accumulation by plants are being described.

Raskin et al. (1994) depicted the relationship of plants and some elements with metallic properties such as ductility, conductivity, density, stability as cations, ligand specificity, etc. and an atomic number >20. Some of them are essential to some life forms: vanadium, chromium, manganese, iron, cobalt, nickel, copper, zinc, molybdenum and some are required by plants: manganese, iron, copper, zinc, molybdenum and possibly nickel.

Sometimes risk elements are present in soil as natural components or as a result of human activities including metal-rich mine tailing, smelting, electroplating, gas exhausts, energy and fuel production, intensive agriculture, sludge dumping or downwash from power lines. (Castillo 2016) There are areas in which the levels of natural background concentrations exceed recommended safety levels given by regulatory agencies. However, some plants adapted to these environments and evidently some of them grow and prosper better on the soils with increased metal concentration. They are called metallophytes and they are used as mineral deposits indicators (Brooks 1982)

In general plants can be divided in **1/ metal excluders**, which prevent entering metal to their aerial parts, but not roots and **2/ metal non-excluders**, which actively accumulate metals in their above ground tissues. Metals non-excluders can be further divided in **a/ indicators** – their levels of metals in above ground tissues are equal to the levels in the soil, **b/ hyperaccumulators** – the levels of metal in their tissues can multiply exceed the concentration in the soil or in non-accumulating species. (Raskin et al. 1994)

According to one definition a plant containing > 0,1% of Ni, Co, Cu, Cr, Pb or 1% of Zn is considered hyperaccumulator. (Raskin et al. 1994) The largest family of hyperaccumulators in our climate (temperate) is *Brassicaceae*. (Baker et al. 1988) Interestingly, one of the extremely efficient hyperaccumulator is New Caledonian tree (*Pycnandra acuminata*), it has been reported the content of more than 11 % Ni in its latex tissue (dry weight). (Baker et al. 1988)

Mechanisms of metal accumulation

High amount of metals in soil are bound to organic matter (humus), inorganic (clay) or present in insoluble precipitates. In order to uptake metals, plants must first mobilize them in the soil. To do so, they utilize several pathways.

1/ metal-chelating molecules (phytosiderophores) can be secreted to the rhizosphere to chelate and solubilize soil-bound metal, for instance mugineic acid, avenic acid or nicotianamine. (Kinnersley 1993) Some metal-chelating proteins can also have a function as siderophores in plants.

2/ roots reducing soil-bound metal ions by specific plasma membrane bound metal reductases.

For instance pea plants reduce Fe(III) and Cu(II) (Welch et al. 1993)

3/ plant roots can solubilize heavy metals by acidifying their soil environment with protons extruded from the roots, lower pH solubilizes precipitates and releases metal ions

similar mechanism in dicotyledonous plants with Fe deficiency (Crowley et al. 1991)

All three mechanisms can be performed by mycorrhizal fungi or root-colonizing bacteria. (Raskin et al. 1994)

3.5 Risk elements

Human nutrition contains numerous metallic elements including sodium, potassium, iron, calcium, boron, magnesium, selenium, copper and zinc. Those stated above are essential to human organism and are present in trace quantities whereas other metals have no physiological function in human body and when consumed regularly they can cause serious damage to the whole metabolism. Most metals naturally occur in the earth's crust (background level) or in elevated levels due to anthropogenic activities such as agricultural and industrial processes, therefore they can pass to food-chain. They are present either as the pure metal or in a form of compounds composed of metallic and non-metallic element. Main concern considering the health risk rise these metals: mercury, lead, cadmium, tin, arsenic, other reports mention also chromium and uranium contaminating food and water. Nickel and beryllium are known for their negative effects on worker at the workplace. Those are mainly associated with inhalation of contaminated dust causing lung injuries. Normally they are not present in foodstuffs in toxic amounts. (Food Safety Authority of Ireland 2009)

By the term risk elements is meant a group of elements occurring in the environment which are associated with contamination and environmental risk, often wrongly referred to as heavy metals. The utilization of the term „heavy metal“ has been numerously questioned for its impreceness. It is based on the chemical density of the elements. Not all of them are categorized as metals plus there is lack of scientific evidence proving that all their compounds are highly toxic. (Pourret & Bollinger 2018)

The risk elements are represented by these: As, Be, Cd, Co, Cr, Cu, Hg, Mo, Ni, Pb, Ti, V, Zn. The group of so-called heavy metals, which have significant toxicological properties is included. Some of the risk elements are, however, essential for living organisms in trace concentrations (copper, zinc, chromium), but at higher concentrations they are toxic, others are toxic even at low concentrations (lead, mercury, cadmium). (Němeček et al. 2010)

Alloway (2010) distinguished three groups of risk elements depending on increasing concentration in the environment and living organisms.

1) Trace elements – they are present in living organisms or natural environment in very low concentrations, they are essential to the right functioning of metabolism. For humans for example zinc, chromium and iron.

2) 'Heavy metals' – those are the metals of specific atomic weight higher than 5 g/cm3 (cadmium, mercury and lead).

3) Toxic metals – their concentration is above the optimal level and they are harmful to the human organism and ecosystem.

Metals and other elements may be naturally occurring in food or can pass into the food-chain as a consequence of anthropogenic activities including industrial and agricultural processes. Particular focus is given to the elements of serious harmful effect on human organism: mercury, lead, cadmium, tin and arsenic. The toxicity of mercury and lead is multiplied as a result of their ability to accumulate in biological tissues, in a process commonly known as bioaccumulation. Due to their negative effects on health and the bioaccumulation properties of some of the elements, it is necessary to control their levels in the foodstuffs to ensure its food safety for consumption. (Food Safety Authority of Ireland 2009)

3.6 Legislative limits of risk elements in EU

3.6.1. EU Framework

The basic principles of EU legislation on contaminants in food are described in Regulation 315/93/EEC:

- *„Food containing a contaminant to an amount unacceptable from the public health viewpoint and in particular at a toxicological level, is not to be placed on the market*
- *Contaminant levels must be kept as low as can reasonably be achieved following recommended good working practices*
- *Maximum levels must be set for certain contaminants in order to protect public health."* (Council of the European Communities n.d.)

Maximum levels for mercury, lead, cadmium and tin in foodstuffs are set by European Commission Regulation No 1881/2006, the framework EU legislation which sets maximum levels for chemical contaminants in foodstuffs. This Regulation establishes maximum levels (MLs) for these metals in a wide range of foodstuffs including milk, meat, fish, cereals, vegetables, fruit and fruit juices, and also sets a maximum level for mercury in fish and fish products. (European Commission 2006)

3.6.2. Study of risk elements toxicity and reference values

The study will further summarize the main information concerning the risk elements introduction to food chain, toxic effects and reference values set by statutory organs or authorized institutions. In general, we distinguish acute toxicity, described as an immediate effect of one dose or short inhalation exposure and chronic toxicity resulting the effect of repeated doses or long continuous exposure. (Linhart 2014)

3.6.2.1. Lead

As a contaminant of the natural environment, lead occurs naturally or as a result of anthropogenic activities. The concentration of lead, Pb, in water, soil and air has decreased in last two decades in developed countries, thanks to a significant reduction of the sources resulting in its dispersion to environment. However, in some parts of the world its concentration in the environment is still increasing. (Baird & Cann 2012) EFSA (2010) stated that the main sources of lead in European diet are cereals, vegetables and tap water. Average daily intake through food for and adult ranges from 0.36 to 1.24, up to 2.43 μg/kg body weight (b.w.) per day in high consumers in Europe. Conclusions of this report is that current levels of exposure to lead pose a low to negligible health risk for adults nevertheless it poses a potential risk of neurodevelopmental effects in foetuses, infants and children.

Directly after consumption the highest levels of lead are present in human blood but eventually they stabilize. The surplus of the element enters the soft tissues mainly brain among other organs. Eventually lead is stored in bones. People with calcium deficiency are in higher risk, due to its competitive relationship with Calcium (Pb^{2+} x Ca^{2+}). A recommended prevention are dietary supplements of Ca. Increased income of lead can cause a bone impairment and dental cavities. High levels of inorganic lead leads to general poisoning of the metabolism. The toxicity of lead is proportionate to its content in soft tissues. It is stored in bones and can be remobilized. At the highest risk are fetuses and children under the age of 7. Numerous studies prove that children brain is suffering by 2-3 point IQ deficit for each 100 ppb in bloodstream. (Baird & Cann 2012).

High-level intakes of lead usually result in colic. The early symptoms are abdominal pain, constipation, nausea, vomiting and anorexia (ATSDR 2007). Children are in risk of developing toxic encephalopathy. However, regarding the lead bioaccumulation properties, chronic toxicity poses a much greater risk.

First studies of lead poisoning reported that individuals affected by occupational exposure suffered by increased mortality due to renal and cardiovascular disease (ATSDR 2007), which has been confirmed by current evidence. Moreover, another concern represent non-fatal effects of lead on human organism, such as neurotoxicity and cardiovascular effects. (EFSA 2010)

Commision Regulation (EC) No 1881/2006 on the Maximum Levels of Contaminants in Certain Foodstuffs provides the value of Maximum Levels for Brassica vegetables, leaf vegetables and cultivated fungi (27) 0.30 mg/kg of fresh weight. (European Commission 2006)

3.6.2.2. Cadmium

Cadmium, Cd belongs to the subgroup of transition metals. The only common ion of cadmium is the 2+ species.

Over 90 % of cadmium in the surface environment originates from anthropogenic activities including rock phosphate fertilizers, the ash from fossil-fuel combustion, waste from cement manufacture and metallurgical works, municipal refuse and sewage sludge, and atmospheric deposition. (Pan et al. 2009) Primarily, cadmium occurs together with zinc, therefore it is produced as a by-product of zinc smelting. Additional origins of the element entering the environment are coal burning and incineration of the waste material containing cadmium and the production of portable nicd batteries. (Baird & Cann 2012)

There are several bioavailable chemical forms of the element in fresh water systems (inorganic solid phases $CdCO^3$, $Cd(OH)^2$, CdS, the chelated and insoluble organic bound Cd, precipitated with hydrous oxides of Mn and Fe etc.) (Pan et al. 2009) The freshwater contamination leads to pollution of surrounding area by agricultural irrigation or floods which carry the sediments. (Pan et al. 2009)

First evidence about the harmful effects of cadmium in the environment were observed in Japan as consequence of the water contamination from a zinc-lead mine in 1960. Local people, exposed to the rice planted on contaminated fields and water from the area over 30 years, reported cases of a osteoporosis-like disease known as „ita-ita byo“. (Pan et al. 2009)

Acute intoxication by inhalation of cadmium causes respiratory systém impairment including shortness of breath, lung edema and destruction of mucous membranes. (Seidal et al. 1994)

Individuals suffering from longer-term exposure experienced kidney damage, due to high-level accumulation of Cd in these organs. (Barbier et al. 2005) Miller (1996) concluded cadmium acts as an endocrine disruptor with estrogenic properties which can potentially cause prostate cancer. (Tallaa et al. 2007) Heavy smokers intake of Cd can be around double the amount of the average intake in population. (Baird & Cann 2012). The IARC (International Agency for Research on Cancer) placed cadmium on a list of human carcinogen group I. However the current studies support the assumption that cadmium has carcinogenic effects only when inhaled. (Jin et al. 2003)

According to Regulation (EC) No 1881/2006 latest amended by Regulation (EC) No 629/2008 Cadmium maximum levels (MLs) in foodstuffs (mg/kg wet weight) of category 17.

Leaf vegetables, fresh herbs, celeriac and the following fungi: *Agaricus bisporus* (common mushroom), *Pleurotus ostreatus* (Oyster mushroom), *Lentinula edodes* (Shiitake mushroom) are set to 0.20. (EU Commission 2008)

3.6.2.3. Arsenic

Arsenic is a contaminant present in environment in a natural form but also due to anthropogenic activity. European population is exposed to its effects mainly through diet. The foodstuffs with the highest content of arsenic are as follows: cereals and products derived from them, foods intended for special dietary purposes, bottled water, coffee, beer, fish and vegetables. The major source of arsenic in diet is fish and seafood with over 90 %, yet most arsenic in seafood appears in less toxic form (arsenobetaine in fish and arsenosugars in others). (European Food Safety Authority 2009)

Arsenic was used as a main compound of war gases, Adamsite and Lewisite, in World War I. The main symptoms of the intoxication were respiratory irritation and extensive, slow-healing blisters. Malachowski (1990) states three typical signs of gas poisoning by arsenic: abdominal pain, hematuria and jaundice. (Malachowski 1990) The effects of acute toxicity appear in 30 minutes, the response may be slower when poisoned by food. The symptoms include gastrointestinal hypotension and tachycardia, multiorgan failure may follow. (Malachowski 1990)

With time, arsenic is accumulated in lungs and may lead to respiratory cancers. (Aposhian 1989) An Occupational Safety and Health Administration (OSHA) has connected arsenic to lung cancer. (National Institute for Occupational Safety and Health 1975)

Chronic exposure causes conjunctivitis and eczematoid or allergic dermatitis. Basal cell and squamous cell carcinomas can develop years after exposure. Another usual effect reported is alopecia. (Wagner et al. 1979) (Jackson & Grainge 1975) Lugo's et al. (1969) study confirmed arsenic's teratogenic effect.

The JECFA Provisional Tolerable Daily Intake (PTDI) for inorganic arsenic is 0.002 mg/kg bodyweight, equivalent to 0.12 mg/day for a 60 kg adult. As has been stated before, most of the arsenic in the diet is from fish and most of the arsenic in fish is in the less toxic organic forms. (European Food Safety Authority 2009)

3.6.2.4. Beryllium

Beryllium is a metal found in the Earth's crust. Minerals rich in beryllium are processed for use in aerospace, weapons, nuclear, and electronics industries. Atmospheric deposition to surface waters and soil is mainly a result of industrial processing of beryllium and combustion of fossil fuels (especially coal). (Baird & Cann 2012)

Besides exposure in workplace, the most common way of intoxication is through diet in drinking water and foodstuff. (ATSDR 1993)

Exposure to beryllium and its compounds is much more risky via inhalation than by ingestion. Particularly affected are lungs, skin and eyes, eventually it can lead to death. (Okutani et al. 1993) Unfortunately, there is not enough scientific evidence on human intoxication through ingestion. The oral tolerable intake of 0.002 mg/kg body weight per day was derived.

International Agency for Research on Cancer (IARC) (1993) of the World Health Organization (WHO) data considers beryllium and beryllium compounds as carcinogenic to humans.

Acute toxicity of beryllium manifests as so called Acute beryllium disease including chemical pneumonitis. It may be fatal in 10 % of cases. (Scott 1989)

Chronic exposure to beryllium leads to development of Chronic beryllium disease also known as berylliosis. It is an inflammatory disease of lungs caused by inhalation of dust contaminated by soluble and insoluble beryllium particles. It is demonstrated by the formation of granulomas (pathological clusters of immune cells) with varying degrees of interstitial fibrosis and involves a beryllium-specific immune response. (Kriebel et al. 1988)

3.6.2.5. Vanadium

Vanadium widely occurs in the earth's crust. It is present in the natural form of around 70 minerals, nevertheless not as metallic vanadium. The most common oxidation states of vanadium are +3, +4, and +5. Vanadium is not among essential minerals for humans. Among foods examined for vanadium dill seeds and black pepper contained the most vanadium, 431 and 987 µg/kg, respectively (Myron et al. 1977). The intake of vanadium from normal food ranges between 10-20 µg/person/day or 0.2-0.3 µg/kg body weight/ day. This daily intake is at least three orders of magnitude below the lowest doses reported to cause adverse effects. Regarding the dietary supplements for athletes, the intake can be close to the doses causing adverse effects in rats and humans (daily doses up to 18 mg vanadium/person/day) Hence, prolonged ingestion of such supplements can lead to poisoning. A maximum intake level of 1.8 mg vanadium/day for adults is recommended by FNB and EGVM stated that there are insufficient data to establish a safe upper level (FNB 2001; EGVM 2003)

Scientific evidence confirms that oral ingestion can cause gastrointestinal distress in humans. The available data do not allow to define the highest level of oral intake which could be seen as tolerable. Therefore, nutritional requirements or intake recommendations have not been established. The SCF (1993) stated that evidence supporting the essentiality of vanadium has yet to be established. Addiotionally, there were reported adverse effects on kidneys, spleen, lungs, blood pressure, reproductive and developmental toxicity in rodents, caused by vanadium compounds.

Acute intoxication after oral ingestion varies among species. WHO (1988) found out, that vanadium is in general better tolerated by rodents than larger animals such as horse. Domingo et al. (1991) carried a study on chronic toxicity of orally administered vanadium in rats. Signs of intoxication included decreased weight gain, increased serum concentrations of urea and creatinine. Vanadium compounds had adverse effects on kidneys, spleen, lungs and blood pressure. In one case, effects showed even at 0.8 mg vanadium/kg body weight/day administered for 3 months (Domingo et al. 1985). A NOAEL cannot be derived from these studies.

3.6.2.6. Zinc

Zinc is a post-transition metal. It occurs as Zn2+ in all tissue and fluids in the body. Its total body content ranges between 2 and 4 g. It is present in food supplements.

For human is zinc essential for growth and development, testicular maturation, neurological function, wound healing and immunocompetence. It is part of over 300 zinc enzymes, for example superoxide dismutase, alkaline phosphatase and alcohol dehydrogenase. (Coleman 1992)

Acute toxicity in humans demonstrates mainly by gastrointestinal distress, including nausea, vomiting, loss of appetite, abdominal cramps, diarrhea, and headaches (Taper et al. 1980)

Chronic toxicity is connected with copper deficiency, the symptoms include among others decreased concentrations of plasma copper and decreased activity of the copper containing enzymes, superoxide dismutase and caeruloplasmin, altered lipoprotein metabolism and impaired immune function (Sandstead 1995) The adverse effects occur only after excessive consumption of dietary supplements rich in zinc (more than 150 mg/day) for longer-term.

An UL of 25 mg/day is recommended. (SCF 2003)

3.6.2.7. Chromium

There are two different forms of chromium occurring in the environment. First Chromium III naturally present as one of the essential nutrients of human diet. Its functions account glucose, protein, and fat metabolism. Second form, chromium VI, is occurring in environment rarely. Nevertheless it has raised a concern for public safety. It is solely produced by industrial processes and sometimes unfortunately contaminates drinking water. As documented in the movie Erin Brockovich, who was an american law-ecological activist working on the biggest case of refundation victims of industrial pollution by hexavalent chromium. The individuals were suffering from numerous adverse effects of chromium VI, including several kinds of cancers, skin edemas, pulmonary disease etc. (Soderbergh 2000)

EFSA sets the tolerable daily intake (TDI) of 0.3 milligrams per kilogram of body weight per day for chromium III. (EFSA 2014)

The Panel did not deliver a safe level ('TDI') for chromium VI, although EFSA (2014) considers an intake by drinking water of chromium VI to be in concern for infants.

Even though scientific data concluded that chromium (III) compounds can bind to DNA molecule and produce DNA-protein cross-links under certain circumstances, they differ from hexavalent chromium and they did not produce gene mutations. (IARC 1990)

The level given by legislation applies solely to trivalent chromium and not to chromium picolinate which is being excluded from the guidance due to the results of in vitro studies. The data which suggested it may damage DNA through a mechanism which remains unknown (EGVM 2002)

WHO considered that supplementation of chromium should not exceed 250 µg/day (WHO 1996)

3.6.2.8. Copper

The most abundant sources of copper in human diet are organ meats, seafood, nuts and seeds (Pennington et al. 1995). Maximum content of copper in drinking water is set to 2 mg/L by EU legislation (Council of the European Union 1998). Environmental pollution by copper is due to emissions from mines, smelters and foundries and burning coal. Food and Nutrition Board recommendation for adults is to consume a dietary intake of 0.9 mg copper/day. (FNB 2001)

Effective homeostatic controls are in place to reduce absorption and increase excretion, if excess copper is ingested. Nevertheless, there are documented cases of acute and chronic copper poisoning.

Acute toxicity in humans appears frequently and mainly results from contamination of food stuffs or beverages from copper containing vessels or dispensers. Among the symptoms are salivation, epigastric pain, nausea, vomiting and diarrhoea (Olivares & Uauy 1996). Copper ions show to be irritable for mucosal membranes and higher daily intakes in water have caused general gastric irritation (U.S. EPA 1987). Chuttani et al. (1965) reports a 48 cases of copper sulphate poisoning as a mean of suicide in India. WHO (1993) sets the fatal oral dose of copper salts about 200 mg/kg body weight .

As to chronic toxicity in humans, data showed that chronic doses of copper might lead to acute liver failure (O'Donohue et al. 1993).

An UL of 5 mg/day is derived. (EGVM 2003)

3.6.2.9. Nickel

Nickel is a metal naturally present in environment, it can enter the food-chain through environmental contamination resulting from anthropogenic activities. Although nickel is not essential to humans, it is present in a few dietary sources, among them most abundantly in cocoa (8.2-12 mg/kg), soya beans (4.7-5.9 mg/kg) or oatmeal. (Flyvholm et al. 1984) Consumption of such foodstuffs can result in exceeding of recommended tolerable daily intake. (SCF 1993)

Short-term exposure can lead to allergic reactions, both by touch and also from ingestion in food or water including development of eczemas and dermatitis in sensitized individuals. (EFSA 2015a)

Chronic toxicity after oral ingestion studied in rats showed to reduce their kidneys, spleen, lungs, and the myeloid system. Moreover, perinatal mortality was increased. (American Biogenics Corporation 1988) Dieter et al. (1988) discovered that nickel was primarily accumulated in kidney.

Based on the scientific opinion on the risks to human health from nickel in food and drinking water a safe level was set. Tolerable daily intake (TDI) of 2.8 micrograms per kilogram of body weight was established. Regarding the current chronic dietary exposure of public to nickel in food, EFSA considers nickel to be of concern. (EFSA 2015b)

3.6.2.10. Cobalt

Cobalt is a quite rare element present in air, surface water, leachate from hazardous waste sites, groundwater, soil, and sediment, it is essential to mammals in the form of vitamin B12. Daily dietary intake of cobalt for humans ranges between 5 and 50µg/day. Its sources in environment include natural ones including forest fires, wind-blown dust etc. And anthropogenic such as burning of fossil fuels, sewage sludge, phosphate fertilizers, etc. Occupational exposure is mainly due to inhalation of industrial dusts during processing of cobalt compounds for alloys and hard metals. The most common way of exposure for humans is through food. with estimated intake of 5– 40 µg/day through the diet. Most of the ingested cobalt is inorganic.

Morvai et al. (1993) tested rats exposed to cobalt chloride through diet for three weeks suffered from cardiac damage.

Cobalt allergy developing after exposure to cobalt and its compounds exhibits as dermatitis. Dermatitis can be a consequence of presence of cobalt in different mediums such as hair dyes, antiperspirants or fertilizers. (Nielsen et al. 2000)

In both human and animals, cobalt is excreted in urine. Curtis et al. (1976) studied metabolism of volunteers orally administering cobalt showing the elimination by urine covered 6-8 % of initial dose in the first week.

Based on the study on mice exposed to cobalt by inhalation, National Toxicology Program (1998) concluded there was clear connection to carcinogenicity.

There was not enough data to establish a tolerable intake for chronic ingestion. Regarding a short-term study in six human volunteers, ATSDR (2004) set an intermediate-term (15–364 days) minimal risk level of 50 μg/kg body weight per day.

3.7. The study of analyzed edible flowers

Within the framework of the work the elements of the following families were determined, therefore the research deals with the description of accumulation of risk elements in families.

3.7.1. Asteraceae

3.7.1.1. Accumulation

Plant species belonging to this family are used for remediation of heavy metals and radionuclides. (Gawronski et al. 2011) For example a sunflower is considered the best species in European conditions for phytoremediation, thanks to its sufficient uptake of heavy metals including antimony. (Tschan et al. 2008). The wide range of ornamental plants of Asteraceae producing high amounts of biomass can be utilized for phytoremediation of urban areas. As the species with higher biomass production are always prefered above the lower once in terms of phytoremediation. (Ma et al. 2001)

3.7.1.2. Bellis perennis

Common name known as daisy is a perennial plant that produces offsets. Originates from meadows of Europe and Turkey. It has oblanceous to oblique spathulate leaves. Daisy blooms from the end of the winter to the end of summer with individual white pinkish heads of 1-3 cm wide, on long flower stems. Lingual flowers are white and tubular are yellow. Of the Bellis perennis variety, many cultivars have been derived, they belong to the group of biennials cultivated in spring beds. (Brickell 2008) Daisy is especially popular among flower-eaters thanks to its unique appearance and wide occurrence. It grows closeby human households from spring to autumn. All the parts of the plant are edible, the taste of the flower slightly resembles nuts. The whole flowers with parts of straws are harvested and edible. It can be stored in the fridge for few days. Their taste is quite universal so it can be used in both in the sweet and at the salty cuisine. In order to not lose its visual attractiveness it is prefered to be used in raw state. (Vlková 2015)

Haselgrübler et al. (2018) identified and quantified numerous polyphenolic compounds in Bellis perennis such as apigenin glycosides, quercitrin and chlorogenic acid which are responsible for reduction of blood glucose levels. The conclusion of their study suggests application of *Bellis perennis* extracts in food supplements for prevention and support-treatment of Type 2 Diabetes Mellitus.

3.7.1.3. Centaurea cyanus - blue

The cornflower spread almost over the whole Europe slowly vanished within the seventies in the last century due to use of herbicides. (McVicar 2005)

Upright perennial with lanceolate leaves. The lower leaves have several pear-shaped lobes and are felted from below. Dark blue inflorescences with a diameter of 2.4-4 c, with violet-blue central flowers flourishing from the end of spring until mid-summer. Flower buds carry tubular lobed flowers, the outer ones are longer than others. It occurs in the regions of the temperate zone and in the north, especially in Europe and the Mediterranean. (Brickell 2008) The blue pigment responsible for its colorful petals, protocyanin, was first isolated by Bayer (1958). Anthocyanin with metals such as Fe, Mn and other element Ca to maintain its bright shade.

Many flavonoid aglycones (quercetin, kaempferol, isorhamnetin, apigenin, luteolin, hispidulin) and their glycosides, phenolic acids including caffeic, chlorogenic, neochlorogenic and isochlorogenic acids have been isolated from the aerial parts of the Centaurea cyanus L. These compounds demonstrated antioxidant, anti-inflammatory, antibacterial, gastroprotective activity and cytotoxic effect, when tested *in vitro.* (Litvinenko & Bubenchikova 1988)

For its brightening character was used as an ingredient to French eye water „Eau de Casselunettes“ (McVicar 2002) The flowers are suitable as an edible decoration, they can be added to salads, on bread or desserts. Dried can be used as a part of spice mixture or tea herbal fusion. Their taste is neutral. (Beiser 2014)

3.7.2. Caryophyllaceae

3.7.2.1. Accumulation

Plants of the taxonomic family *Caryophyllaceae* are highly frequented on salt or metal polluted soil, including the species *Dianthus carthusianorum*. (Baranowska-Morek & Wierzbicka 2004) As the ornamental species of this family do not grow high biomass they are not used for phytoremediation, although they can indicate polluted sites and predict a well conditioned places for growth of tolerant species. (Gawronski et al. 2011)

3.7.2.2. Dianthus pink

Dianthus sp. Is originally a perennial native to Europe and Asia from which many varieties were cultivated. The leaves are linear to lanceolate, gray with waxy tinge. Flowers bloom all summer and are exceptionally durable after cut. Flowers are usually scented, reminiscent of cloves. They form end umbels, each flower has a short tubular base and five leaves. (Brickell 2008)

Dianthus sp. was considered so charming by the ancient greeks that they named them „the flower of flowers“. They were widely used during celebrations, as a symbol of engagement. For their clove-like taste they were also used as a spicy. The flowers can be also glazed and the petals added to soups, sauces, syrops, liquors and wines. It is recommended to separate the pungent calyx from petals. (Bremness 1994) They are suitable decoration for refreshing summer meals or sweet and savory dishes. Most preferably, they are used as ingredients of smooth creams and foams such as mayonnaise, whipped cream or butter. Heat treatment is not recommended. (Vlková 2015)

Phenols present in carnation (*Dianthus caryophyllus*) prove fungitoxic property towards fungi species of genus *Fusarium* sp. (Curir et al. 2003)

3.7.3. Hydrangaceae

3.7.3.1. Philadelphus coronarius

It is a wide upright deciduous shrub with ovate toothed leaves and bowl-shaped white flowers with prominent stamens. Short, top-of-the-line inflorescence blooms with 5-9 cups of simple, intensely fragrant, cream-colored white flowers. It is valued ornamental plant especially in the temperate zone. (Brickell 2008) Some of the species of Philadelphus L. have antibacterial, antiradical and immunomodulatory effects therefore they could be used for isolation of active substances (Valko et al. 2006) Valko et al. (2006) investigated the cytotoxic effect of water extracts from leaves and branches of *Philadelphus coronarius* L. On human skin carcinoma cell line with satisfying results suggesting the extracts isolated from the plant should be further investigated for usage after chronic treatment.

3.7.3.2. Accumulation

Philadelphus coronarius L. Planted in urban areas is valued for its ability to retente the contaminants of air-dust (Wang & Li 2006).

3.7.4. Rosaceae

Rosa sp. Is valued since ancient times as an extraordinary flower. It was introduced from Persia and inspired many artists, warriors and lovers. The rose extract was used as a medicine and food ingredient. The rose wine origins in Persia. Turkish honey was also made from rose water. Besides that, rose petals were used to produce jam, vinegar, pate or as a garnishing. (Bremness 1994)

3.7.4.1. Accumulation

Gawronski et al. (2011) described this family as possessing above average tolerance to pollution nevertheless not so high such as some of the families mentioned above. Often used in polluted urban areas, mainly species serving as ornamentals among them for example Rosa rugosa or Rosa rugotida cultivated on traffic dividing stripes. Species from this family are employed as biomonitoring indicators of pollution by risk elements (Calzoni et al. 2007) such as Cd, Pb, and Zn. (AKGÜÇ & Ozyigit 2008)

3.7.4.2. *Rosa* 'Gloria Dei' yellow and *Rosa* 'Gloria Dei' pink

Also known as *Rosa* Peace or 'Mme A. Meilland' is lushly growing, densely branched tea hybrid with shiny dark green leaves. It has flat and densely full fragrant flowers of yellow color with pink tinge. Inflorescence have a diameter of 15 cm and flourish from summer to autumn. (Brickell 2008)

Both in European traditional medicine (Newman & Kirker 2016) and in Indigenous traditional knowledge was rose considered highly effective remedy. Yi et al. (2007) carried a study on antioxidant properties of wild British Columbia roses, finding out that all extracts exhibited strong antioxidant activity, which also correlated with antimicrobial activity including antimicrobial activity against yeast and Gram-positive bacteria.

3.7.5. Lamiaceae

Plants belonging to family *Lamiaceae* are known for their antioxidant activity. Particularly sage is common traditional medicine with most of its antioxidants identified. Majority of its free radical-scavenging substances belongs to the group of phenolic compounds. (Pokorný 1991) The genus *Salvia* (*Lamiaceae*) contains almost 900 species worldwide. Many plants of *Salvia* genus are used as herbal tea and as food flavouring, in cosmetics, perfumery and the pharmaceutical industries. (Chalchat et al. 1998)

The name „Salvia“ origins from a latin word „salvere“ which means „to be in a good health, to heal, to take things easy“. This herb was highly valued through the history across the continents, for its medicinal value even considered holy by some cultures. In the 17th century, Chinese traders would exchange one box of dried salvia leaves for three boxes of their tea. According to the Roman rules, plant could not be harvested by iron tools, which would decrease its therapeutic properties. Both leaves and flowers are used in great variety of recipes. In small amount, flowers can be used in a salad, or for a tea infusion. The leaves are often added to food as a digestive agents especially to greasy, spicy meals which could be difficult to digest. Other culinary purposes include vinegar, butter or filling. (Bremness 1994) Sage grows abundantly in the Mediterranean on sunny limestone rocks. There are three subspecies in nature: officinalis from Syria to Crimea, minor in Yugoslavia and lavandulifolia in Spain.

It acts antiseptically, anti-inflammatory, antidiarrheal, decreases sweating and improves gallbladder function. It is used in the cosmetic industry, for the production of toothpastes, as a mouthwash while suffering from toothache, oral mucosal inflammation or angina pectoris. (Lánská 2006)

3.7.5.1. Accumulation

Moreno-Jiménez et al. (2011) argued that levels of transfer of trace elements to above-ground tissues of Rosmarinus officinalis was limited whereas Díez Lázaro et al. (2006) reported that Thymus mastichina and Lavandula stoechas highly accumulate different metals and metalloids such as Ni, Cr, Co, Mn, Zn and As in their tissues. The aromatic oil of flowers, stems and leaves of *Lamiaceae* family is used in food products, perfumes and cosmetics, ensuring their production is economically profitable. (García et al. 1998) Cala et al. (2005) comes to a conclusion that plants of this family could be used as alternative crop grown on soils with „heavy metal“ contamination and maybe even used for production of oil assuming its composition would not be altered. Angelova et al. (2006) carried a study on heavy metal accumulation of several *Lamiaceae* species (peppermint (*Mentha piperita* L.), sage (*Salvia officinalis* L.), and clary sage (*Salvia sclarea* L.)), cultivated at near proximity to areas of metal works polluted by heavy metals. Among the selected plants, clary sage had the highest and sage had the lowest concentrations of metals in its tissues. This suggests that accumulation properties vary even among the species of the same genus. The content of Pb, Zn, and Cd in the leaves from sage and peppermint exceeded the maximum regulatory permissible concentrations and could possess a risk if used as a food ingredient. The contents of Pb, Cu, and Cd in the oil of *Salvia sclarea* and *Mentha piperita* cultivated nearby contaminated areas were also higher than the accepted permissible concentrations, nevertheless they were suitable for usage in the perfumery, cosmetics, and tobacco industries. Clary sage proved to be a hyperaccumulator of metals and could be used for phytoremediation. (Angelova et al. 2006)

3.7.5.2. Salvia lavandulifolia

It is a perennial with a wooden base and mostly ground-long long felted leaves. In the middle of summer, it creates blue-violet flowers. (Brickell 2008) Its aroma is slightly balsamic, it is being used for teas. (Bremness 1994)

Tildesley et al. (2003) et al. (2003) conducted a study on memory enhancing effects of Spanish sage on young healthy adult volunteers, revealing its positive influence on cognitive functions, mainly recalling of the words. This suggests Spanish sage extract could be used in early stage treatment of Alzheimer disease.

The compositions of *S. lavandulaefolia* and *S. officinalis* are very much alike, although they vary in the amount of thujone, neurotoxic oil which can be harmful when consumed in higher doses. Spanish sage has much lower content. (Leung & Foster 1996) Therefore *S. lavandulaefolia* was considered more appropriate for therapeutic purposes (Mantle et al. 2000)

3.7.5.3. Salvia austriaca

Taxodione, an antibacterial, cytotoxic and antitumor compound, was extracted from *Salvia austriaca* roots. It has been proved that it demonstrates inhibiting activity towards the human acetylcholinesterase enzyme and should be further studied for pharmaceutical purposes. (Kuźma et al. 2012)

3.7.5.4. Salvia virgata

Salvia virgata is in Turkish called ‘‘yılancık”, it's mainly used as a treatment of wounds and diseases of skin. In the West Turkey, blood cancer is treated by the decoction made of above-ground tissues. (Baytop 2000) Tosun et al. (2009) concluded from his study on Antioxidant properties of several *Salvia* species that the genus *Salvia virgata* (among other species) has significant antioxidant and free radical-scavenging activity, therefore could be employed as an antioxidant.

3.7.6. Tropaeolaceae

3.7.6.1. Accumulation

Monk cress has been used in many studies investigating its accumulation properties of arsenic and phenyl arsenic compounds. Zacarías et al. (2011) tested the effect of metal-contaminated soil on feasibility of several plant species, among them Monks cress. Given the results of the study it has showed that Monks cress proved extraordinary ability to accumulate As with only slight decrease in production of its biomass compared to other species. Based on this study it could be used for phytoextraction purposes.

3.7.6.2. Tropaeolum majus

Tropaeolum majus is a lush annual plant with deciduous or drooping stem. Rounded up to the kidney leaves are rippled at the edges. They bloom in summer and autumn. The flowers are yellow, orange or red with a conspicuous spur. (Brickell 2008) Garden nasturtium (*Tropaeolum majus*) is considered one of the most popular sources of edible flowers (M. Kelley et al. 2002)

The buds' and leaves' aroma ressembles a garden cress. Young seeds have stronger aroma therefore they are sometimes used as a substitution of a horseradish in mayonnaise. Leaves are rich on content of vitamin C, thus it is often recommended to use the plant whilst in the cold. Monks cress can be used in salads, sauces and other recipes. (Bremness 1994)

Tropaeolum majus is widely spread traditional medicine in Brazil known as “capuchinha”, “chaguinha”, and “nastúrcio”. Its leaves are used as a remedy of a few diseases such as cardiovascular, infections of urinary tract, asthma and constipation. (Calil Brondani et al. 2016)

Results of scientific studies proved presence of many bioactive antioxidant substances including fatty acids (erucic, oleic, linoleic), flavonoids (quercetol 3-glucoside and kaempferol glucoside) isolated from the leaves and seeds (Medeiros 2000) and glucosinolates in leaves. (Griffiths et al. 2001) Pharmacological tests supply evidence about its beneficial properties for example both in vitro and in vivo antitumor activity (Pintão et al. 1995), antithrombotic activity (Medeiros et al. 2000) and antibacterial activity against infections (Goos et al. 2006).

UL - Tolerable upper intake levels for vitamins and minerals - EFSA
ATSDR - Agency for Toxic Substances and Disease Registry

4 Materials and methods

4.1 Standards and reagents

HNO3 (p.a., Lach-ner, CZ) deionized water of conductivity 18.2 M ohm (MILLI-Q Element, Millipore, France), 120 Nylon disk filters (Cronus, UK), ICP-OES (DUO i-Cap 7000, Thermo Scientific)

4.2 Samples preparation

Ten dried samples of flowers were kindly provided by Faculty of Horticulture (Mendel University, Czech Republic). They were harvested within a period of January and August 2016 at the Botanical garden of the Mendel University. The orchard is situated at the South-Moravia region at Lednice, Czech Republic. The average altitude is 173 m above sea level, mean annual precipitation 473 mm and mean annual temperature 9.8 C. (ČHMÚ 2018a, 2018b) The soil type of the area is classified as modal chernozem. (Česká geologická služba n.d.)Air-dried samples were sent on 11.9.2016 in polyethylene bags to the Czech University of Life Sciences in Prague. See a Table 1. for a precise date of collection and the amount of a sample received. They were further stored in laboratory of the Department of the Quality of Agricultural Products. The species of flowers are as follows daisy (*Bellis perennis*), pink (*Dianthus sp.*), sweet mock-orange (*Philadelphus coronarius*), peace rose in yellow and pink variety (*Rosa Gloria-dei*), spanish sage (*Salvia lavandulifolia*), Austrian sage (*Salvia austriaca*), wand sage (*Salvia virgata*), Indian cress (*Tropaeolum majus*) and cornflower (*Centaurea cyanus*) Latin, common and Czech names are listed in Table 2.. For the risk elements analysis, the dried samples were homogenized. The 9 of them was sieved through a 2mm stainless-steel sieve laboratory biomass grinder (MF 10 Basic, IKA, Germany).

In the case of *Centaurea cyanus* liquid nitrogen was used to freeze the sample and then it was grinded in a friction bowl with a beater to prevent losses due to a limited amount of the sample.

Collected edible flowers

Table 1. Collected edible flowers shows the plant species, genus, (variety), date of collection and amount of sample. Flowers were collected at Botanical garden of Mendel University at Lednice and sent to CULS on 11.9.2016.

	Genus	Species	Variety/comm nt	Collection	Weight (g)
1	*Bellis*	*perenis*		3/1/2016	2.67
2	*Dianthus*		pink	5/24/2016	2.02
3	*Philadelphus*	*coronarius*		6/14/2016	2.54
4	*Rosa*		´Gloria Dei´- llow	5/24/2016	2.62
5	*Rosa*		´Gloria Dei´- nk	5/24/2016	5.53
6	*Salvia*	*lavandulifolia*		6/7/2016	1.46
7	*Salvia*	*austriaca*		5/26/2016	0.58
8	*Salvia*	*virgata*		5/24/2016	1.7
9	*Tropaeolum*	*majus*		6/30/2016	1.76
10	*Centaurea*	*cyanus*	blue	6/8/2016	4.18

Vocabulary for analysed edible flowers

Table 2. Vocabulary of analysed edible flowers shows three different names for each species. Latin, English and Czech.

Latin	**English (common name)**	**Czech**
Bellis perennis	daisy	sedmikráska
Dianthus (odr-pink)	pink	hvozdík
Philadelphus coronarius	sweet mock-orange	pustoryl věncový, pravý jasmín
Rosa (Gloria -dei žl.)	rose 'peace', glory to god, Mme A. Meillande	-//-
Rosa (Gloria dei. růž.)		
Salvia lavandulifolia	Spanish sage	šalvěj levandulolistá
Salvia austriaca	Austrian sage	šalvěj rakouská
Salvia virgata	wand/southern-meadow sage	šalvěj prutnatá
Tropaeolum majus	Garden nasturtium, indian cress, monks cress	lichořeřišnice větší, řicha, kapucínka
Centaurea cyanus	Cornflower, Bachelor's button, basket flower	chrpa modrá

4.3 Extraction according to ICP-Forest

Homogenized flowers were weighed to 0.5 g and transferred to Teflon vessels (Savillex, USA) with 10 ml of 65% HNO3 (p.a., Lach-ner, CZ). They were kept overnight in a laboratory temperature, closed but not sealed. Then the Teflon vessel was sealed and the mixture was heated at 120°C on hot plate for 2 hours. The digested solution was then quantitatively transferred to the 50 ml volumetric flask and filled up to the mark with deionized water (conductivity 18.2 MΩ) The solution was filtered through 120 Nylon disk filters (Cronus, UK) prior analysis. The samples were diluted 10 times (5 ml/50 ml) and 10 ml of each diluted solution was transferred to test tubes in rack. The contents of selected elements (Cd, Pb, As, Be, Mo, Va, Zn, Cr, Cu, Ni, Co) were determined by means of ICP-OES (DUO i-Cap 7000, Thermo Scientific) under standard analytical conditions. Quality of digestion and analysis were controlled by using blanks and the standard reference materials (NIST SRM 1575a Pine Needles and NCS DC 73351 Tea).

4.4 Statistical analysis

Firstly the statistical analysis focuses on individual elements. Means and standard deviations are set for each genus with more than one sample. The concentration of selected elements were tested using an Analysis of Variance test (ANOVA) at a probability level of 5%. For a statistical analysis a freely available software R-studio was used.

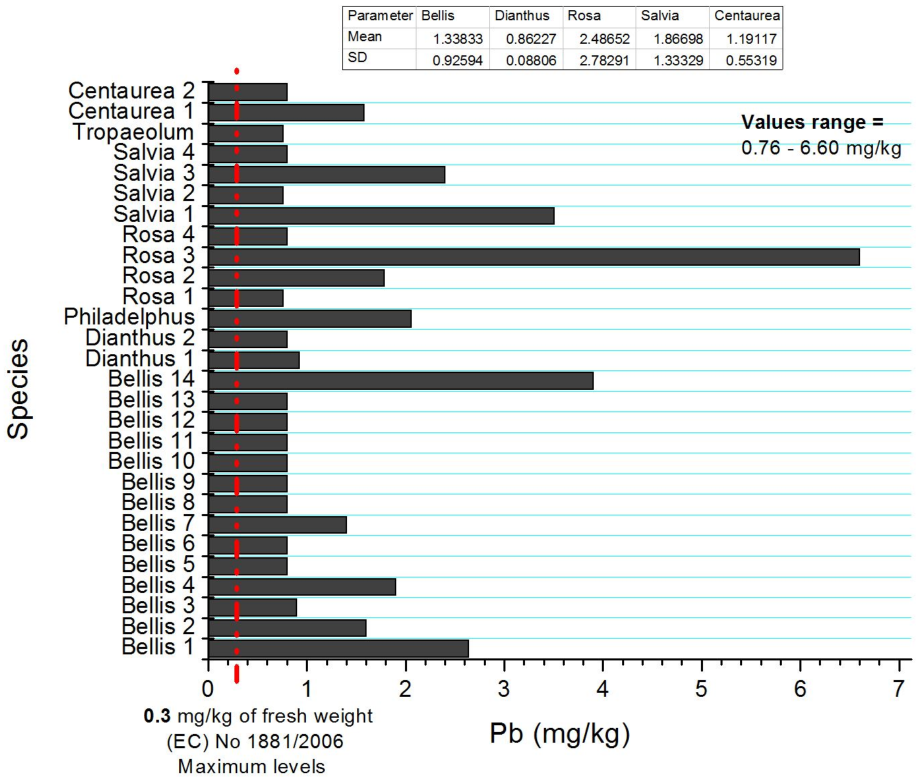
5 Results

The contents of risk elements in edible flowers were evaluated individually according to elements. Means and standard deviations were established for the species with more than one sample. In case of *Philadelphus* sp. and *Tropaeolum* sp. was not possible to determine mean and standard deviation. For the concentrations below the detection limit, a constant of a half of the detection limit was introduced. From the available data, only three flowers were chosen for the ANOVA (*Bellis* sp., *Rosa* sp. and *Salvia* sp.). Because only these flowers were measured in more than two samples, the minimum number of samples required in each population to be compared. A p value of less than 0.05 was considered to indicate statistical significance. The Shapiro-Wilk test, the As, Be, Co and Pb indicated to not have a normal distribution of the dataset. Thus As, Be, Co and Pb were excluded from the further assumption assessment and the ANOVA test. The concentration of elements in flowers have a degree of dependence since one element in plants could favor the presence of another element(s), in complex uptake and metabolic pathways inside the plants.

Another factor to consider is the influence of error in the measurement, which can be errors of the methodology, human, equipment, environmental interferences, cross-contamination, etc.

The contents of lead in analyzed edible flowers

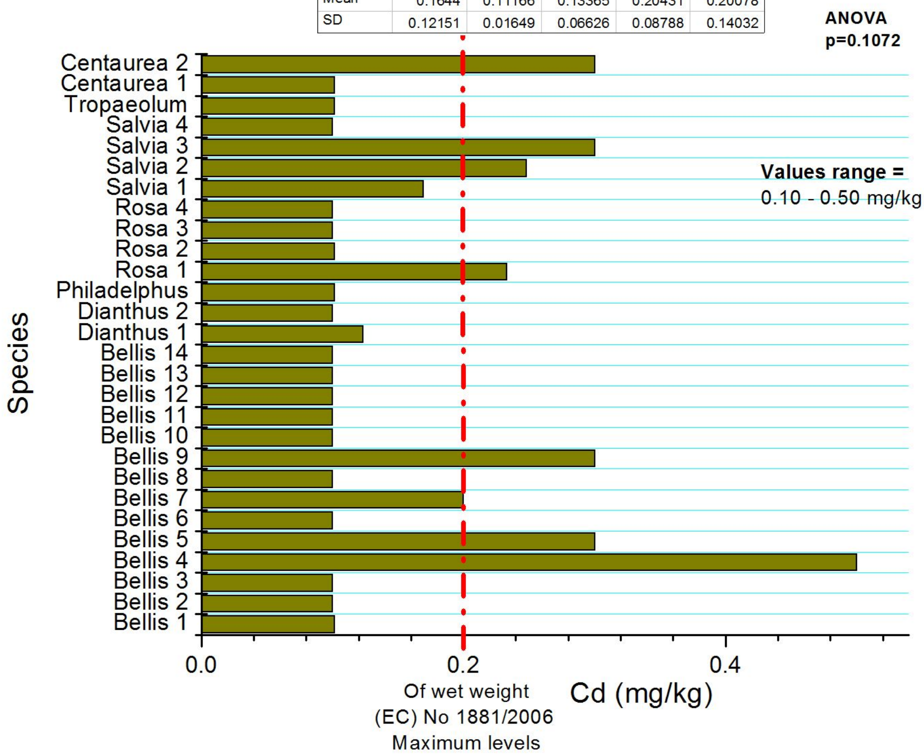
Figure 1. The contents of lead in analyzed edible flowers showing the (EC) No 1881/2006 Maximum Levels in certain foodstuffs 0.3 mg/kg of fresh weight, mean and standard deviation values for *Bellis*, *Dianthus*, *Rosa*, *Salvia* and *Centaurea*. The common mean for all samples is 1.54 mg/kg and standard deviation 1.31 mg/kg. Concentrations below the detection limit were substituted by ½ of detection limit. In case of lead the ½ DL is 0.76. Values are within range of 0.76 - 3.51 mg/kg.



The contents of cadmium in analyzed edible flowers

Figure 2. The contents of cadmium in analyzed edible flowers showing the (EC) No 1881/2006 Maximum Levels of Cadmium in certain foodstuffs 0.2 mg/kg of wet weight, mean and standard deviation values for *Bellis*, *Dianthus*, *Rosa*, *Salvia* and *Centaurea*. The common mean for all samples is 0.16 mg/kg and standard deviation 0.1 mg/kg. Concentrations below the detection limit were substituted by ½ of detection limit. In case of cadmium the ½ DL is 0.10. Values are within range of 0.10 - 0.50 mg/kg. Cadmium p-value in the ANOVA test is (p=0.1072), which is higher than the critical value (p=0.05), thus the null hypothesis is accepted. There is no significant difference in the concentrations of Cd in the three compared flowers (*Salvia*, *Rosa* and *Bellis*).

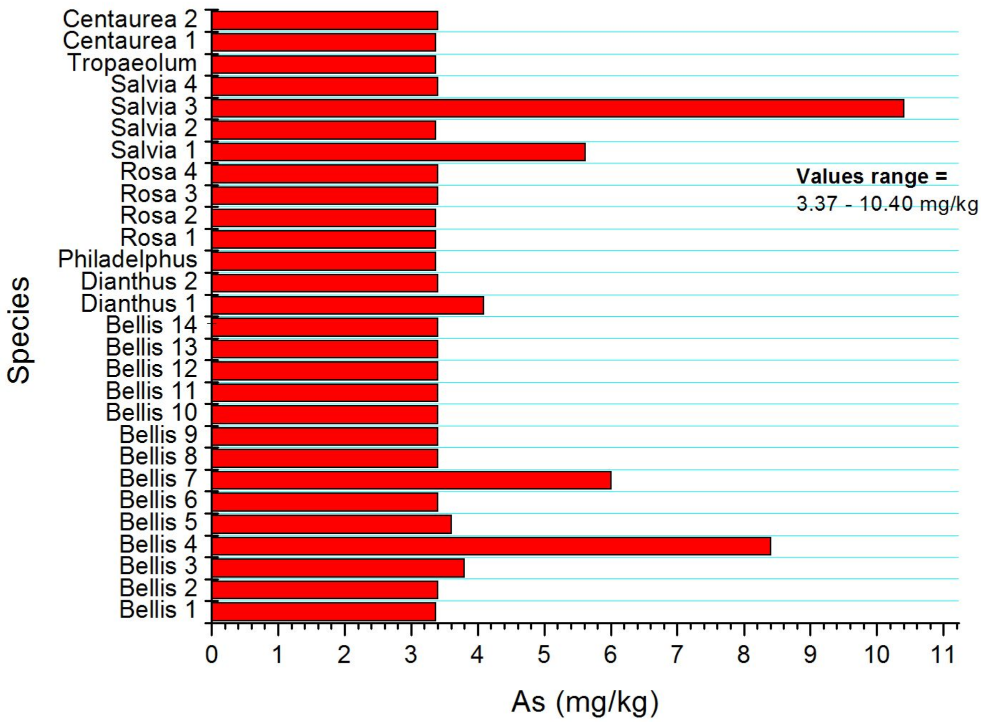
Parameter	Bellis	Dianthus	Rosa	Salvia	Centaurea
Mean	0.1644	0.11166	0.13365	0.20431	0.20078
SD	0.12151	0.01649	0.06626	0.08788	0.14032



The contents of arsenic in analyzed edible flowers

Figure 3. The contents of arsenic in analyzed edible flowers showing the mean and standard deviation values for *Bellis*, *Dianthus*, *Rosa*, *Salvia* and *Centaurea*. The common mean for all samples is 4.04 mg/kg and standard deviation 1.67 mg/kg. Concentrations below the detection limit were substituted by ½ of detection limit. In case of arsenic the ½ DL is 3.37. Values are within range of 0.37 - 10.40 mg/kg. (*Salvia officinalis*)

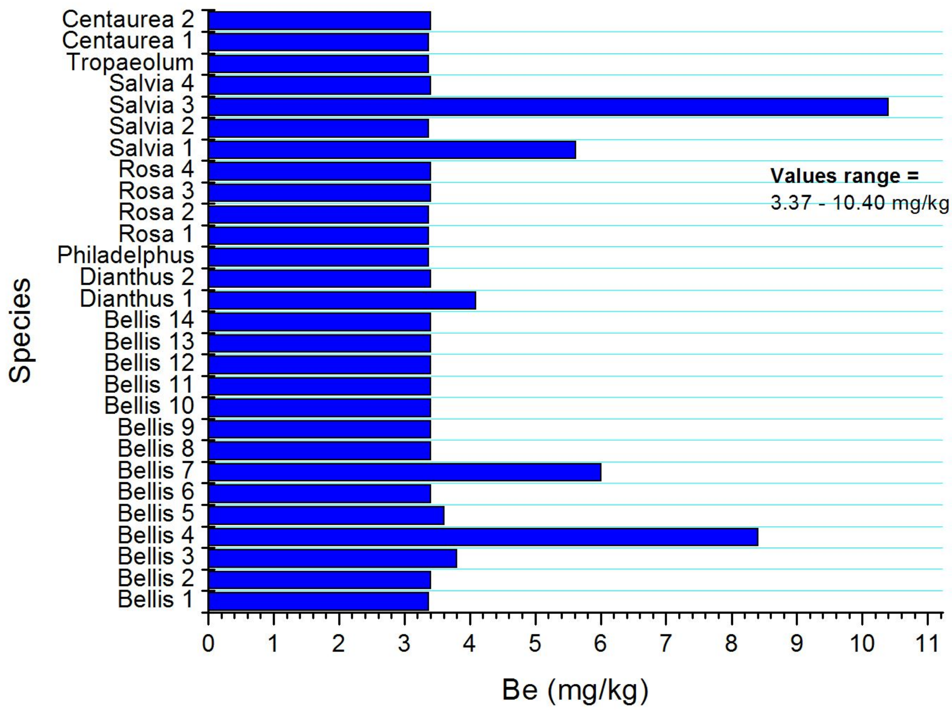
Parameter	Bellis	Dianthus	Rosa	Salvia	Centaurea
Mean	3.98348	3.74531	3.38437	5.69583	3.38437
SD	1.44634	0.48834	0.01804	3.30767	0.0221



The contents of beryllium in analyzed edible flowers

Figure 4. The contents of beryllium in analyzed edible flowers showing the mean and standard deviation values for *Bellis*, *Dianthus*, *Rosa*, *Salvia* and *Centaurea*. The common mean for all samples is 4.04 mg/kg and standard deviation 1.67 mg/kg. Concentrations below the detection limit were substituted by ½ of detection limit. In case of beryllium the ½ DL is 3.37. Values are within range of 0.37 - 10.40 mg/kg.

Parameter	Bellis	Dianthus	Rosa	Salvia	Centaurea
Mean	3.98348	3.74531	3.38437	5.69583	3.38437
SD	1.44634	0.48834	0.01804	3.30767	0.0221

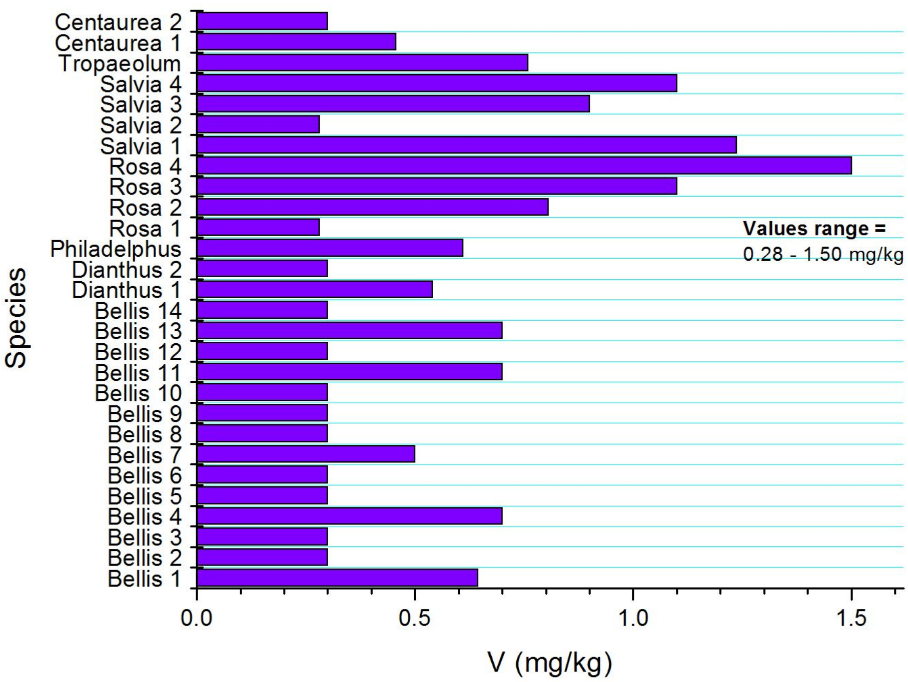


The contents of vanadium in analyzed edible flowers

Figure 5. The contents of vanadium in analyzed edible flowers showing the mean and standard deviation values for *Bellis*, *Dianthus*, *Rosa*, *Salvia* and *Centaurea*. The common mean for all samples is 0.58 mg/kg and standard deviation 1.31 mg/kg. Concentrations below the detection limit were substituted by ½ of detection limit. In case of vanadium the ½ DL is 0.28. Values are within range of 0.28 - 1.50 mg/kg. (*Rosa* Gloria 'Dei' orange). Vanadium p-value in the ANOVA test is (p=0.3171), which is higher than the critical value (p=0.05), thus the null hypothesis is accepted. There is no significant difference in the concentrations of V between *Salvia*, *Rosa* and *Bellis*.

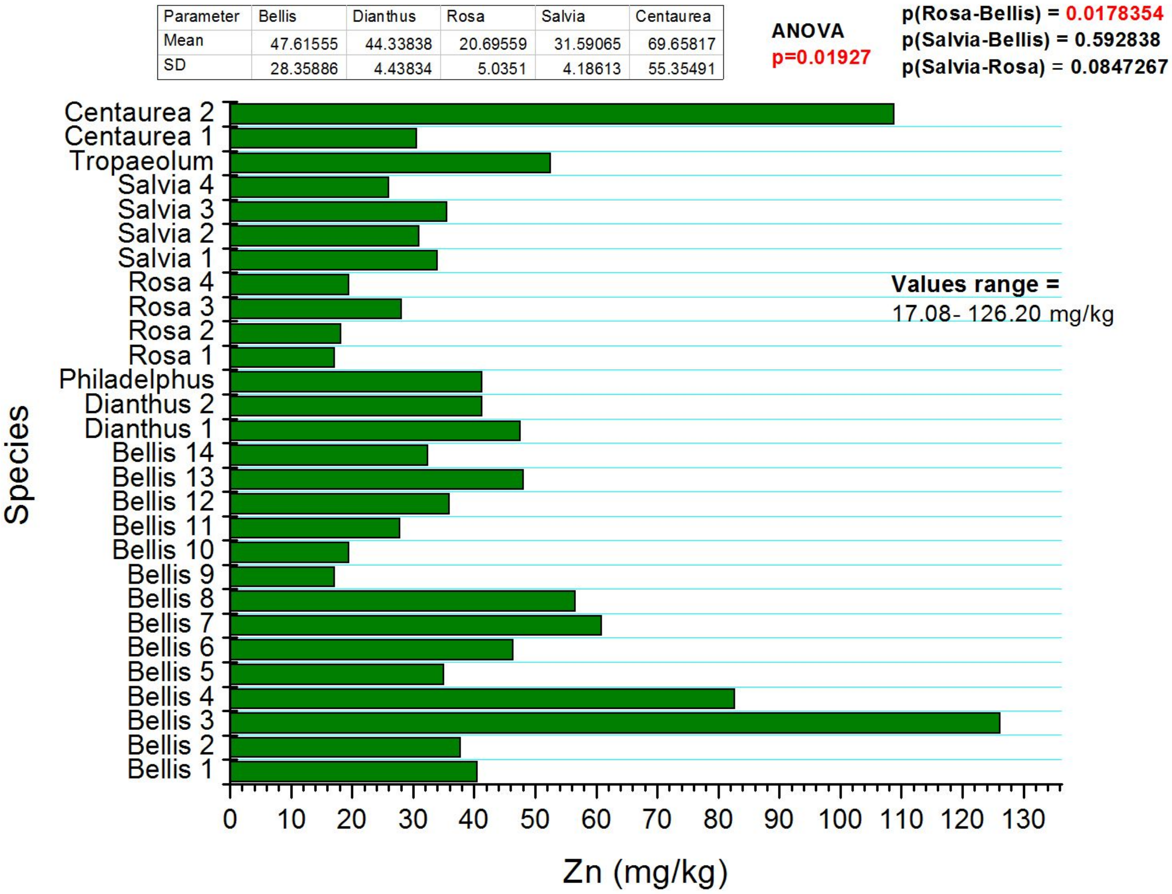
Parameter	Bellis	Dianthus	Rosa	Salvia	Centaurea
Mean	0.42459	0.41978	0.9215	0.87926	0.3781
SD	0.18	0.16939	0.51342	0.42237	0.11045

ANOVA
p=0.3171



The contents of zinc in analyzed edible flowers

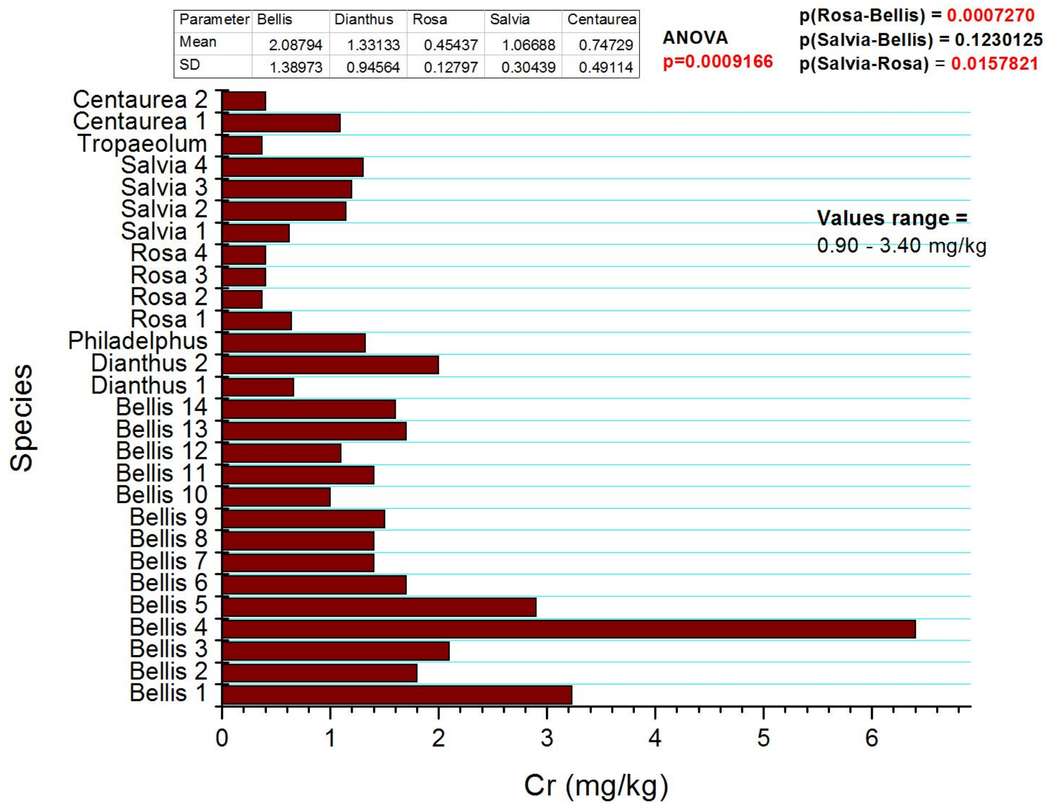
Figure 6. The contents of zinc in analyzed edible flowers showing the mean and standard deviation values for *Bellis*, *Dianthus*, *Rosa*, *Salvia* and *Centaurea*. The common mean for all samples is 42.76 mg/kg and standard deviation 25.81 mg/kg. Concentrations below the detection limit were substituted by ½ of detection limit. In case of zinc the ½ DL is 3.00. Values are within range of 17.10 - 126.20 mg/kg. Zinc p-value in the ANOVA test is (p=0.0.01927), it is lower than the critical value (p=0.05), thus the null hypothesis is rejected. There is significant difference in the concentrations of Zn between *Salvia*, *Rosa* and *Bellis*. The comparison between each flower showed that the significant difference of Zn content was: p(Rosa-Bellis) = 0.0178354.



Parameter	Bellis	Dianthus	Rosa	Salvia	Centaurea
Mean	47.61555	44.33838	20.69559	31.59065	69.65817
SD	28.35886	4.43834	5.0351	4.18613	55.35491

The contents of chromium in analyzed edible flowers

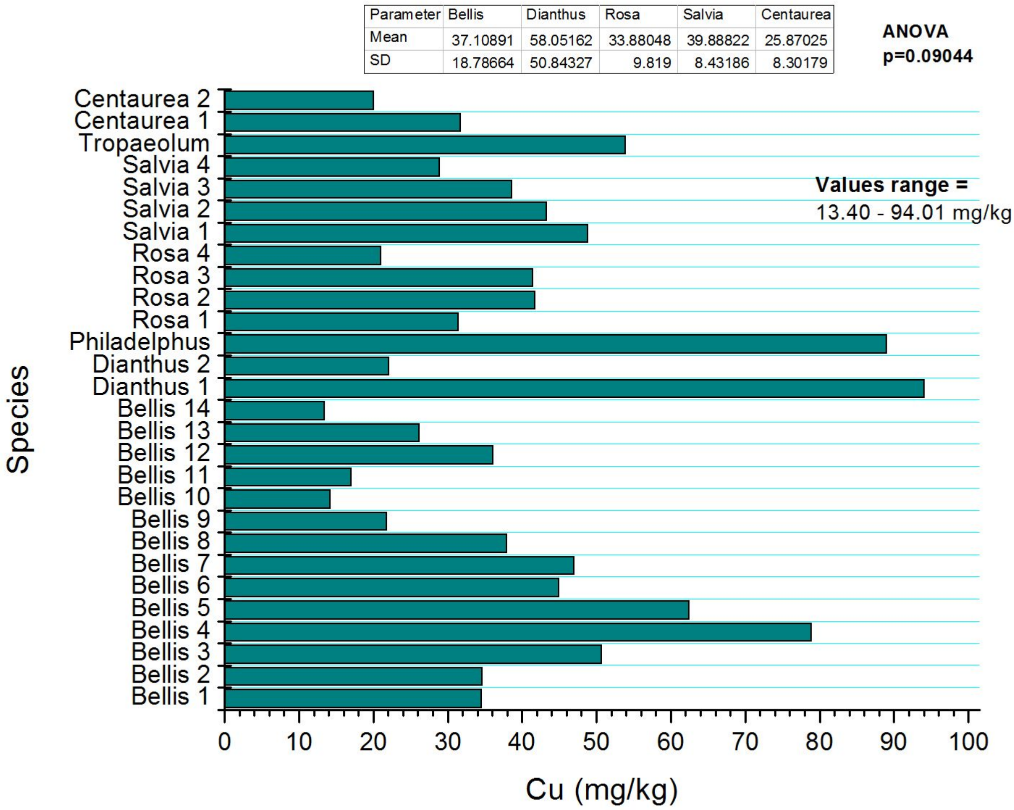
Figure 7. The contents of chromium in analyzed edible flowers showing the mean and standard deviation values for *Bellis*, *Dianthus*, *Rosa*, *Salvia* and *Centaurea*. The common mean for all samples is 1.47 mg/kg and standard deviation 1.20 mg/kg. Concentrations below the detection limit were substituted by ½ of detection limit. In case of chromium the ½ DL is 0.37. Values are within range of 0.37 - 6.40 mg/kg. Chromium p-value in the ANOVA test is (p=0.0009166), it is lower than the critical value (p=0.05), thus the null hypothesis is rejected. There is a significant difference in the concentrations of Cr between *Salvia*, *Rosa* and *Bellis*. The comparison between each flower showed that the significant difference of Cr content was for: Rosa-Bellis p = 0.000727 and Salvia-Rosa p = 0.01578.



The contents of copper in analyzed edible flowers

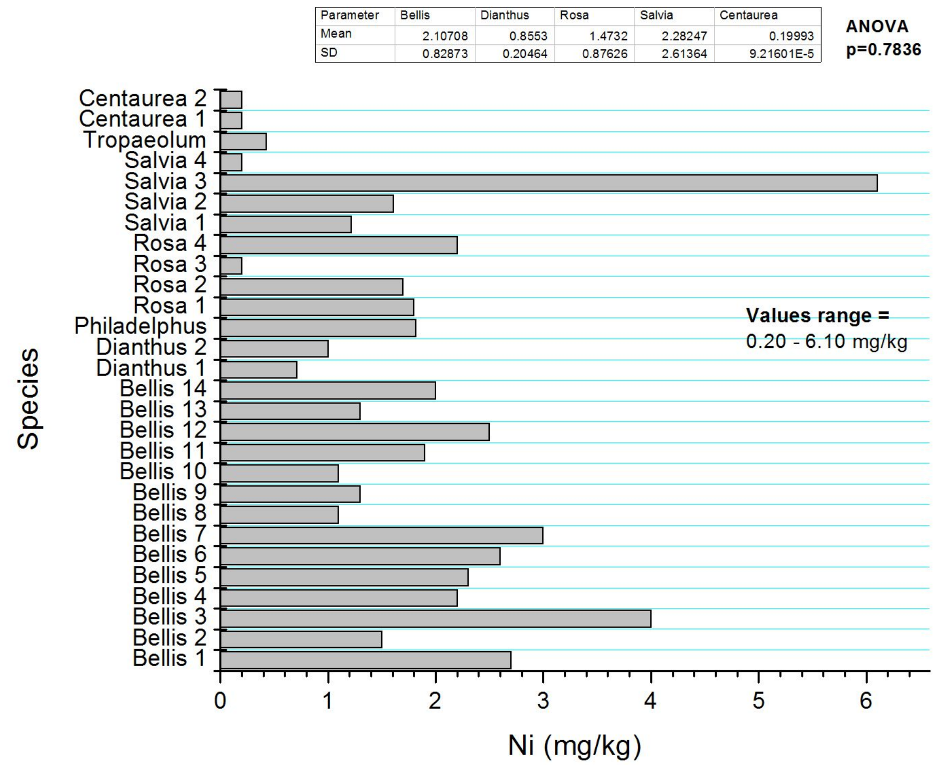
Figure 8. The contents of copper in analyzed edible flowers showing the mean and standard deviation values for *Bellis*, *Dianthus*, *Rosa*, *Salvia* and *Centaurea*. The common mean for all samples is 40.19 mg/kg and standard deviation 20.77 mg/kg. Concentrations below the detection limit were substituted by ½ of detection limit. In case of copper the ½ DL is 2.98. Values are within range of 0.76 - 3.51 mg/kg.

Parameter	Bellis	Dianthus	Rosa	Salvia	Centaurea
Mean	37.10891	58.05162	33.88048	39.88822	25.87025
SD	18.78664	50.84327	9.819	8.43186	8.30179



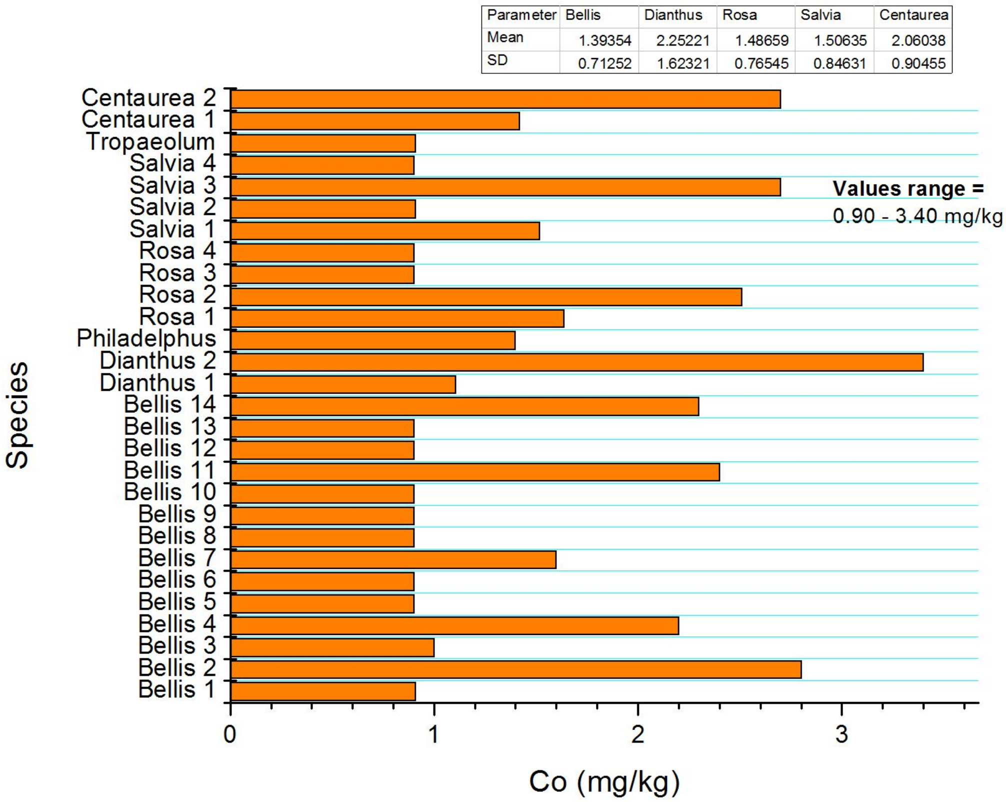
The contents of nickel in analyzed edible flowers

Figure 9. The contents of nickel in analyzed edible flowers showing the mean and standard deviation values for *Bellis*, *Dianthus*, *Rosa*, *Salvia* and *Centaurea*. The common mean for all samples is 1.75 mg/kg and standard deviation 1.26 mg/kg. Concentrations below the detection limit were substituted by ½ of detection limit. In case of nickel the ½ DL is 0.20. Values are within range of 0.20 - 6.10 mg/kg. (*Salvia officinalis*)



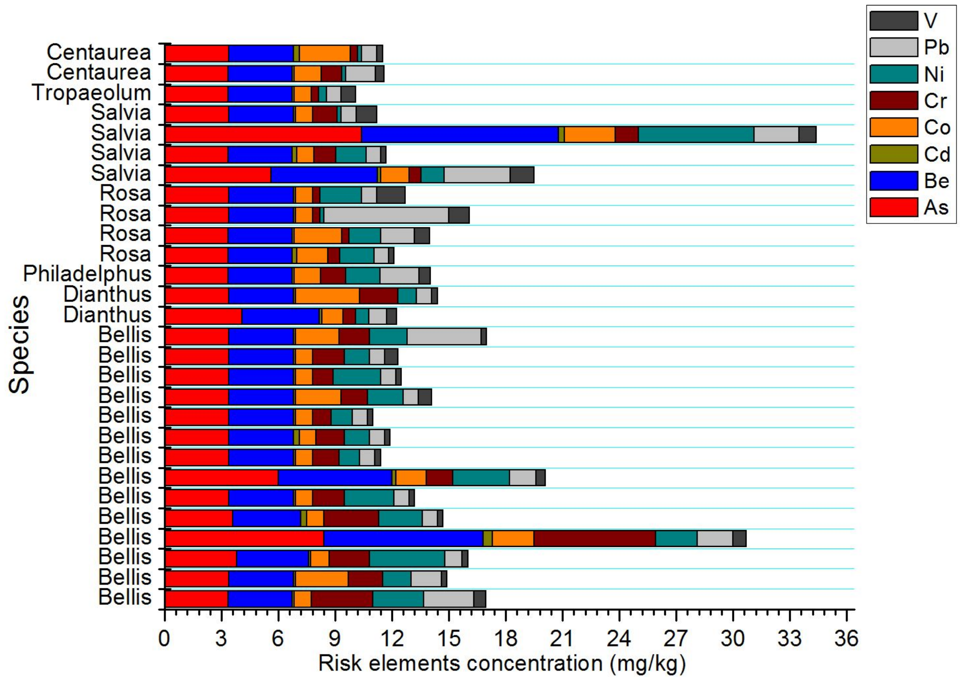
The contents of cobalt in edible flowers

Figure 10. The contents of cobalt in edible flowers showing the mean and standard deviation values for *Bellis*, *Dianthus*, *Rosa*, *Salvia* and *Centaurea*. The common mean for all samples is 1.51 mg/kg and standard deviation 0.77 mg/kg. Concentrations below the detection limit were substituted by ½ of detection limit. In case of cobalt the ½ DL is 0.91. Values are within range of 0.91 - 2.80 mg/kg.



Comparison of risk elements contents in different species of edible flowers

Figure 11. Showing the comparison of contents of risk elements in different species of edible flowers



6 Discussion

The discussion will try to describe the graphs from the part results connecting them with previous literature research and author assumptions. The average serving size portion has been estimated using the portion size recommended by FreshEdible Flowers^{TM} and further used as a constant value 14 g.

6.1 Lead Contents in analyzed samples

For lead (Figure 1.), as one of the heavy metals, are established Maximum Levels by Commision Regulation (EC) No 1881/2006 on the Maximum Levels of Contaminants in Certain Foodstuffs (27) 0.30 mg/kg of fresh weight. The maximum value measured 6.60 mg/kg belongs to one of the varieties of *Rosa* Gloria 'Dei'. Akguc et al. (2008) stated that members from family *Rosaceae* are used for a phytoremediation of sites polluted by Pb, this could explain the high contents of this family. Kabata-Pendias (2011) stated that usually the plant materials contain between 5-10 mg/kg of Pb, meaning the maximum value is within the standard whereas the common mean seems to be lower. It has to be said, that the MLs in foodstuffs are established for fresh weight samples and this research works with dried plants. We can assume that the concentration of the elements has risen significantly. The common mean 1.54 mg/kg and standard deviation 1.31 mg/kg show that the plants in general do not exceed the MLs, although the analysis was carried with dried samples. In the case of Özcan (2004), the Pb contents were within range 0.49 - 8.36 mg/kg. The highest value he measured belongs to *Rosemary*. Nevertheless among his samples was nearly a half below the detection limit suggesting that the contents are in general very low. Results of Grzeszczuk et al. (2018) seem to be similar, varying from 0.20 - 4.31 mg/kg. Additional studies should be carried in case of Pb to assess the comparison between fresh and dry weight of samples.

6.2 Cadmium contents in analyzed samples

The maximum value of Figure 2. showing contents of cadmium belongs to *Bellis perennis* 0.5 mg/kg. For example Gawronski et al. (2011) is describing the high accumulation property of family *Asteraceae*, especially towards heavy metals. Comparing it with the Maximum Levels given by EC in the amended EC Regulation 1881/2006 which sets the maximum level for fresh herbs on 0.20 mg/kg of wet weight, measured value exceeds the limit. We could again argue, that in the experiment were analyzed dried samples, in which the content per kg were of course more concentrated than in those of fresh weight. Furthermore, majority of the flowers had their content of cadmium below the detection limit and also below the maximum levels with a mean 0.16 mg/kg and standard deviation 0.10 mg/kg. Comparing the results with other studies, our results are slightly above the range of Özcan (2004), who measured the concentrations of Cd in plants in a range 0.01 - 0.14 mg/kg.

6.3 Arsenic contents in analyzed samples

Arsenic results are shown on Figure 3. most of the values were below detection limit, giving the common mean of 4.04 mg/kg and standard deviation 1.67 m/kg, suggesting that edible flowers content of arsenic is in general very low. The maximum value measured was represented by *Salvia officinalis* (10.40 mg/kg). Despite the study of Angelova et al. (2006) which indicates that *Salvia officinalis* has the lowest ability to accumulate metals among the plants of the same genus analyzed in the research. Zhu et al. (2013) analysed arsenic content in different herbal flowers with results for *Rosa rugosa* 0.37 mg/kg and *Lavandula angustifolia* 0.31 mg/kg. Comparing them with means for *Rosaceae* 3.38 mg/kg and *Lamiaceae* 5.70 mg/kg, both means in the study are significantly higher. Taking into account the recommended serving size by BrightFresh Edible Flowers of 14 g this gives us approximately 0.15 mg of As consumed in *Salvia officinalis*, regarding the JEFCA PTDI 0.12 mg per day for 60 kg adult, would not be suitable to be used as a food ingredient.

6.4 Beryllium contents in analyzed samples

There is not many studies regarding the contents of beryllium in plants. Griffiths (et al. 1977) indicated that contents of plant samples are generally below 1 mg/kg dry weight, exceptions make are some plants that uptake beryllium from soil.

The Figure 4. shows contents of beryllium in studied plants. The maximum value belongs to *Salvia officinalis* 10.40 mg/kg and *Bellis perennis* 8.40 mg/kg. This gives us 0.15 mg Be per serving for Salvia and 0.12 mg Be for *Bellis*.

The oral tolerable intake of 0.002 mg/kg body weight per day estimated by Bowen (1979), converted to 0.12 mg Be for 60 kg adult. From the numbers above, we can say that the maximum value would not be suitable for human consumption and the value belonging to *Bellis perennis* would. The mean 4.04 mg/kg and standard deviation 1.67 mg/kg prove that edible flowers in general contain tolerable amounts of this element and should not cause adverse effects in humans. Nevertheless the detection limit for beryllium is nearly 6 mg and the values in statistics represent half of the detection limit, we can only guess how high these numbers could be.

6.5 Vanadium contents in analyzed samples

The maximum value of Figure 5. represents 1.5 mg/kg and belongs to *Rosa* Gloria 'dei' (yellow variety). The mean value is 0.58 mg/kg and standard deviation is 0.34 mg/kg. For instance Özcan (2004) value for sage is 5.08 whereas our value 0.90 mg/kg is significantly lower. Regarding the means of family *Lamiaceae* in the two studies, for Özcan (2004) it is 8.74 mg/kg and our mean for *Lamiaceae* is 0.88 mg/kg, we could say ours is ten times less. More similar were results of Antal et al. (2009) who found the highest amount of V in flowering aerial parts, with an average of 0.76 mg/kg. particularly high was Wild Thyme, suggesting it may be interesting supplement in diabetes mellitus type II considering the antidiabetic effect of V. A maximum intake level recommended by FNB (2001) and EGVM (2003) is 1.8 mg vanadium/day for adults. It is clear that edible flowers are sound in terms of content of vanadium.

6.6 Zinc contents in analyzed samples

Zinc contents are shown in Figure 6. The maximum for zinc content is 126.20 mg/kg. The estimated mean 42.76 mg/kg and standard deviation 25.81 mg/kg. An UL of 25 mg/day is recommended (SFC 2002). All the measured contents are within the range of a safe upper limit. Zinc contents, as one of the microelements, are of course higher in comparison with other elements, nevertheless the calculated mean exceeds the results of other studies on its content. For example Grzeszczuk et al. (2018) with the mean 26.07 mg/kg (d.m.).

For the Zn, there is a statistically significant difference when comparing its concentration between Rosa and Bellis. In the comparison of Salvia-Bellis and Salvia-Rosa, it indicates no statistically significant difference in the Zn concentration.

6.7 Chromium contents in analyzed samples

Chromium is accumulated in higher amounts in the roots than aerial parts. Interestingly, the lowest amounts are always found in the vegetative and reproductive organs. The distribution in plants is stable and does not depend on soil properties and the element concentration. (Adki et al. 2012) Lago-Vila et al. (2015) investigated contents of minerals in two plant species. The contents analysed in two plants *Festuca* and *Juncus* varied between 0.73-82.84 mg/kg with the highest amount for one *Festuca* plant growing on contaminated soil, they indicate it could be considered a hyperaccumulator meeting the criteria of Cr content in shoots higher >50 mg/kg. Regarding the results of chromium contents in analyzed samples in Figure 2. the mean of the contents is 1.47 mg/kg, the standard deviation 1.20 mg/kg, therefore none of edible flowers is a hyperaccumulator. The maximum for *Bellis perennis* 6.4 mg/kg. Nevertheless all these values should be below the detection limit. Using the previous serving size of 14 g, this gives us about 0.09 mg of Cr consumed. EFSA (2014) sets the tolerable daily intake (TDI) of 0.3 milligrams per kilogram of body weight per day for chromium III. Calculated value for 60 kg adult is 18 mg per day. The TDI for total chromium was not established. Regarding the above stated results, the flowers do not pose a risk to consumer in terms of content of chromium.

In the case of Cr, it is indicated that there is a statistically significant difference when comparing the concentrations between *Rosa* and *Bellis*, and when comparing *Salvia* and *Rosa*. Whilst when comparing the Cr concentrations between *Salvia* and *Bellis*, it does not indicate a statistically significant difference.

6.8 Copper contents in analyzed samples

Copper's maximum contents presented in Figure 6. are those of *Dianthus* 94 mg/kg and Philadelphus 89 mg/kg. An UL of 5 mg/day was established by EGVM (2003). Therefore approximately 1.3 mg of Cu would be consumed in case of *Dianthus*, that being said, all the samples analyzed are within the recommended upper safe level. The mean content of Cu detected in the plants is 40.19 mg/kg and standard deviation 20.77 mg/kg. Given the mean value

8.01 mg/kg from Grzeszczuk et al. (2018) we could assume that plants chosen for the purpose of this study are very rich on copper. The range of concentrations of Cu 7.26 -10.23 measured for *Hibiscus* by Malik et al. (2013) is much lower compared with results of our study

6.9 Nickel contents in analyzed samples

Salvia officinalis is the most abundant on nickel content 6.10 mg/kg exceeding the mean of content in analyzed plants over almost 4 units (mean 1.75 mg/kg and standard deviation 1.26 mg/kg). 0.09 mg/14 g Tolerable daily intake (TDI) of 2.80 micrograms per kilogram of body weight was established. Conversion of TDI for 60 kg adult gives us 0.17 mg. All the plants are suitable for human consumption regarding the content of nickel. Nickel mean content at the study of Grzeszczuk et al. (2018) was 2.30 mg/kg, for example *Dianthus* with the content of 0.80 mg/kg compared with our obtained mean for nickel 0.85 mg/kg are results nearly the same.

6.10 Cobalt contents in analyzed samples

Grzeszczuk et al. (2018) measured content of Co in plants in range 0.07 - 1.62 mg/kg, results of this study are varying between 0.90 - 3.40 mg/kg, interestingly the maximum values are both for plants of *Dianthus* sp. alluding it has higher accumulating capacity for Co. Calculating the content for the highest value with the stated serving size 14 grams, it makes 0.05 mg of cobalt.

ATSDR (2004) set an intermediate-term (15–364 days) minimal risk level of 50 µg/kg (0.05 mg/kg of body weight per day deriving a limit 3 mg per day for 60 kg adult). It can be said that even the maximum value measured stays within the recommended maximum intake. The mean of the values is 1.51 mg/kg and standard deviation 0.77 mg/kg.

6.11.

Stacked bar chart on Figure 11. is showing the comparison of element content in different edible species excluding the two most abundant elements, copper and zinc. In this way is better visible the ratio of elements in the samples. The best accumulator of the elements is *Salvia officinalis*, followed by flower of genus *Bellis* sp. Interestingly, the ratio of Be and As seems to be very consistent, suggesting there could be a correlation between these two elements.

7 Conclusion

We can see that edible flowers deserve more attention not only in a matter of consumption but also the legislative description of it as a food commodity. As the research has demonstrated there are blanks in the norms dealing with its legislative requirements on quality.

The same can be said about the risk elements for which, in many cases, the safe upper limits have not been yet established by EU. It is clear that the risk elements can cause adverse effects in humans. What remains unclear are the synergic effects of number of elements on human organism.

This paper contains brief summarisation of some of the stated issues connected with edible flowers and the contents of risk elements with an objective to investigate the hypothesis that "Edible flowers do not pose a risk to human in terms of content of risk elements". Our observation declare that in general they do not pose a risk, nevertheless few maximums exceeded the reference values. In those cases a potential health risk could occur, namely to sensitized consumers, pregnant women and children.

More research should have been done to better lay down the legislative requirements on quality and ensure that human safety is secured.

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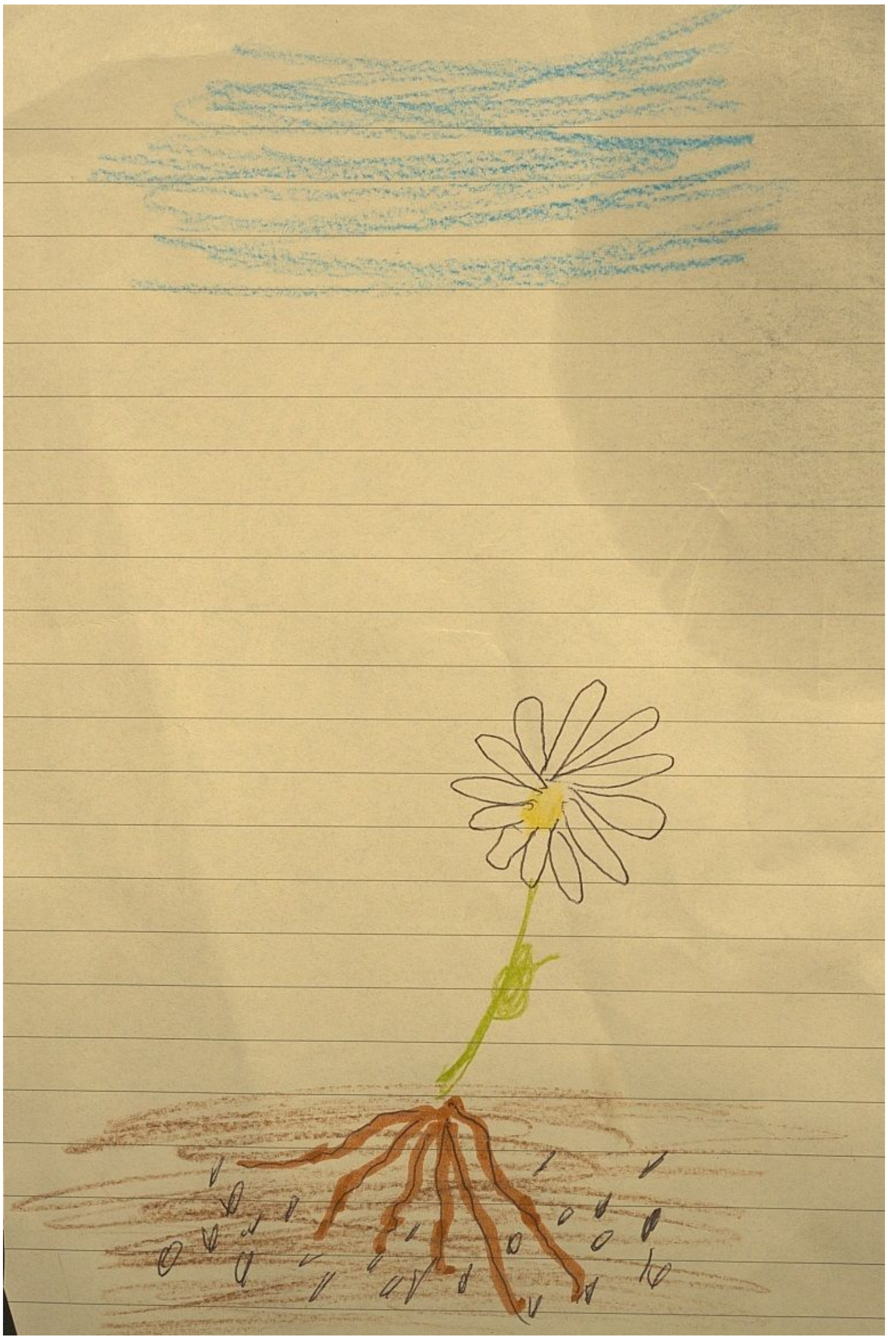
Appendix 2 - Acronyms and abbreviations

ATSDR - Agency for Toxic Substances and Disease Registry
EC - European Commision
EFSA - European Food Safety Authority
EGVM - Expert Group on Vitamins and Minerals
FNB - Food and Nutrition Board
IARC - International Agency for Research on Cancer
ML - Maximum Level
NOAEL – No Observed Adverse Effect Level
LOAEL – Lowest Observed Adverse Effect Level
OSHA - Occupational Safety and Health Administration
PTDI - Provisional Tolerable Daily Intake
PTWI - Provisional Tolerable Weekly Intake
SCF- Scientific Committee for Food
TDI - Tolerable Daily Intake
TWI - Tolerable Weekly Intake
UL – Tolerable Upper Intake Level: a highest level of nutrient intake daily that will not pose a risk of adverse effects for most people in the population
WHO - World Health Organization

Appendix 3 - Illustrations

Bellis perennis



Picture 1. *Bellis perennis* accumulating risk elements from metal contaminated soil by Veronika Miškovská 2019.

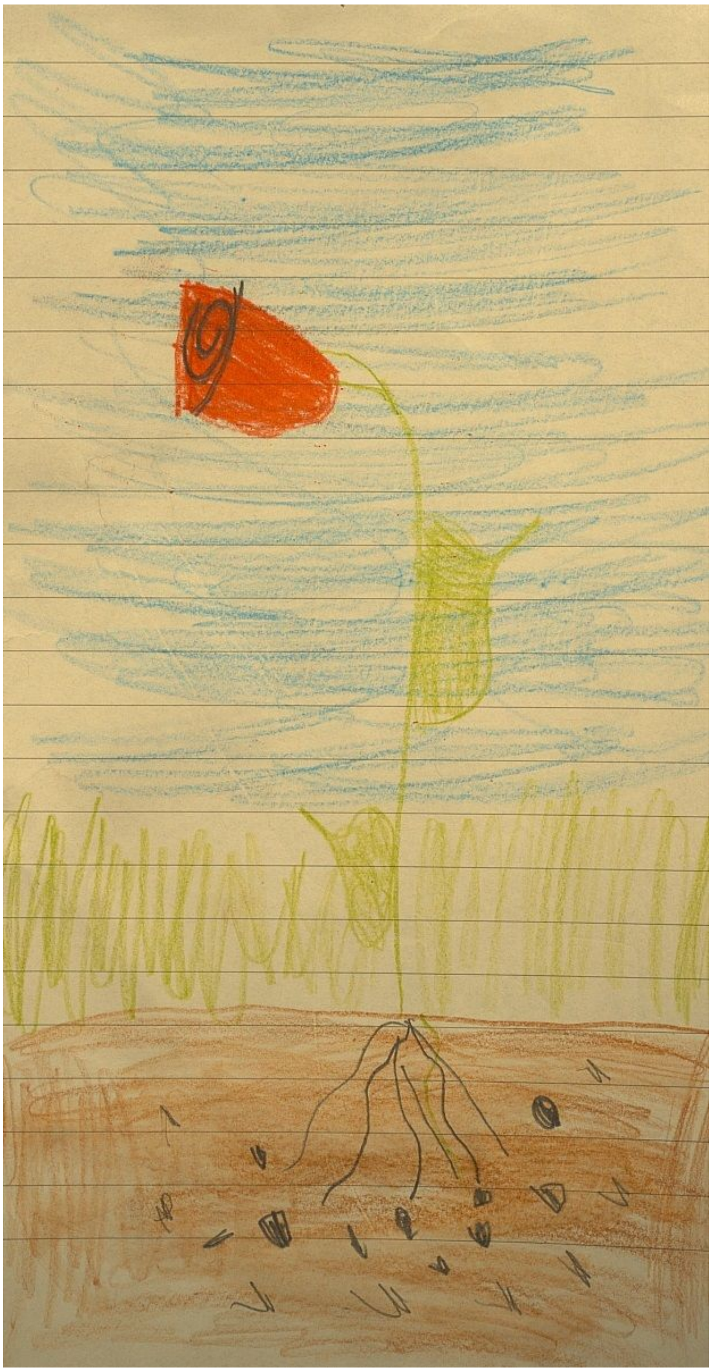
Centaurea cyanus

Picture 2. *Centaurea cyanus* accumulating risk elements from metal contaminated soil by Veronika Miškovská 2019.

Tropaeolum majus

Picture 3. *Tropaeolum majus* accumulating risk elements from metal contaminated soil by Veronika Miškovská 2019.

Rosa Gloria 'Dei'



Picture 4. *Rosa* Gloria 'Dei' accumulating risk elements from metal contaminated soil by Veronika Miškovská 2019.

BrightFresh EDIBLE FLOWERS

Picture 5. BrightFresh EDIBLE FLOWERS showing the recommended portion size of 14 grams, used for the conversion in part Results.