**Czech University of Life Sciences in Prague**

**Faculty of Environmental Sciences**

# Metal uptake by vegetation in Staré Ransko deposit

**Bachelor thesis**

**Author: Barbora Hrůšová**

**Supervisor: doc. Mgr. Martina Vítková, Ph.D.**

**2024**

**CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE**
Faculty of Environmental Sciences

# BACHELOR THESIS TOPIC

| | |
|---|---|
| Author of thesis: | Barbora Hrůšová |
| Study programme: | Environmental Engineering |
| Thesis supervisor: | doc. Mgr. Martina Vítková, Ph.D. |
| Supervising department: | Department of Environmental Geosciences |
| Language of a thesis: | English |

Thesis title: **Metal uptake by vegetation in Staré Ransko deposit**

Objectives of thesis: The main objective of the thesis is to investigate the risk metal contents in vegetation over the Ransko massif and assess the bioavailability of these metals from soils in the surveyed area.

Methodology: The thesis will include theoretical and practical parts. The literature review will be focused on selected metal(loid) characteristics in the environment and their plant uptake. Materials and methods will include the site description, sample collection and preparation, and analytical methods. The results will be presented in graphical form and properly described in the text. Discussion will include results interpretation and discussion with relevant literature. Conclusions will be clear and concise.

The proposed extent of the thesis: 30 pages

Keywords: old mine, spruce uptake, cadmium, nickel, copper, metal transport

Recommended information sources:


1. CORREA-BETANZO J., ALLEN-VERCOE E., MCDONALD J., SCHROETER K., CORREDIG M., PALIYATH G., 2014. Stability and biological activity of wild blueberry (Vaccinium angustifolium) polyphenols during simulated in vitro gastrointestinal digestion. Food Chemistry 165, 522-531.
2. KUANG L., WANG Z., ZHANG J., LI H., XU G., LI J., 2022. Factor analysis and cluster analysis of mineral elements contents in different blueberry cultivars. Journal of Food Composition and Analysis 109, 104507.
3. ORTIZ-DELVASTO N., GARCIA-IBAÑEZ P., OLMOS-RUIZ R., BÁRZANA G., CARVAJAL M., 2023. Substrate composition affects growth and physiological parameters of blueberry. Scientia Horticulturae 308, 111528.
4. REIMANN C., KOLLER F., KASHULINA G., NISKAVAARA H., ENGLMAIER P., 2001. Influence of extreme pollution on the inorganic chemical composition of some plants. Environmental Pollution 115, 239-252.

5. SCHRENK D., BIGNAMI M., BODIN L., CHIPMAN J.K., DEL MAZO J., GRASL-KRAUPP B., HOGSTRAND C., HOOGENBOOM L.R., LEBLANC J.-C., NEBBIA C.S., NTZANI E., PETERSEN A., SAND S., SCHWERDTLE T., VLEMINCKX C., WALLACE H., GUÉRIN T., MASSANYI P., VAN LOVEREN H., BAERT K., GERGELOVA P., NIELSEN E., 2020. Update of the risk assessment of nickel in food and drinking water. EFSA Journal 18, 6268.
6. YANG H., WU Y., DUAN Y., ZHANG C., HUANG Z., WU W., LYU L., LI W., 2022. Metabolomics combined with physiological and transcriptomic analyses reveal regulatory features associated with blueberry growth in different soilless substrates. Scientia Horticulturae 302, 111145.
7. ZHAO R., LI J., MA Y., LV Y., 2020. A field study of vertical mobility and relative bioavailability of Cu and Ni in calcareous soil. Environmental Pollutants and Bioavailability 32, 121-130.

Expected date of thesis defence: 2023/24 SS - FES

Electronically approved: 27. 3. 2024
**prof. RNDr. Vladislav Chrastný, Ph.D.**
Head of department

Electronically approved: 2. 4. 2024
**prof. RNDr. Michael Komárek, Ph.D.**
Dean

Author’s statement

I hereby declare that I have independently elaborated the bachelor/final thesis with the topic of: The uptake of minerals by blueberries in Staré Ransko deposit and that I have cited all of the information sources that I used in the thesis as listed at the end of the thesis in the list of used information sources.

In Prague 28.3.2024 ________________________________

Acknowledgment

I would like to thank to my supervisor doc. Mgr. Martina Vítková, Ph.D. with guiding me through this bachelor thesis. I would also like to thank the people who were in a field with me and taught me so many things. This includes Dr. Solveig Pospiech, Lucija Dujmovic and Dr. Pertti Sarala. Also, big thanks to the Czech Geological Survey and people from there such as Mgr. Vojtěch Wertich, Ph.D. and RNDr. Petr Rambousek. Lastly, I would like to thank to RNDr. Karel Malý without whom I would not be able to attend the SEMACRET project of the EU horizon program that was funded under grant agreement number 101057741 and forward to write the thesis.

## Abstract

The purpose of this study was the detection of risk metal(loid)s such as As, Cd, Cu, Ni, Pb, Zn and the assessment of contamination level in the surveyed area of Staré Ransko deposit. Considering its rich mining history, Staré Ransko may represent potential hazard for its inhabitants. Therefore, we investigated these risk metals and the uptake by Norway spruce using sampling bark. Each sample was always taken from trees within the same species, of approximately the same age and from various distances from the deposit (i.e. contamination source). Three representative bark samples were collected, 1) close to the old mine, 2) in a waterbody area known as “mineralization Jezírka”, and 3) a control sample outside the studied area (i.e. off-site control sample). We found traces of many risk metal(loid)s differed due to their soil background, location and vegetation cover. The highest concentrations of metal(loid)s contamination was detected at the waterbody location (also known as “mineralization Jezírka”) and the least risk metal(loid) contamination was confirmed for the off-site (control) sample. Our results showed that the old mines can be a source of contamination of the studied area and potential mobility of several risk elements, depending on the site characteristics and its distance from ore deposit.

**Keywords:** Old mine, spruce uptake, cadmium, nickel, copper, metal transport

## Abstrakt

Účelem této studie byla detekce rizikových kovů a metaloidů jako je As, Cd, Cu, Ni, Pb, Zn a posouzení úrovně kontaminace ve zkoumané oblasti ložiska Staré Ransko. Staré Ransko může vzhledem ke své bohaté hornické historii představovat pro své obyvatele potenciální nebezpečí. Proto jsme tyto rizikové kovy zkoumali odběrem kůry ze smrku ztepilého. Každý vzorek byl vždy odebrán ze stromů stejného druhu, přibližně stejného stáří a z různých vzdáleností od ložiska (tj. zdroje kontaminace). Byly odebrány tři reprezentativní vzorky kůry, 1) v blízkosti starého dolu, 2) v oblasti vodní plochy známé jako „mineralizační Jezírka" a 3) kontrolní vzorek mimo studovanou oblast (tj. kontrolní vzorek mimo lokalitu). Zjistili jsme, že stopy mnoha rizikových kovů se lišily v důsledku půdního pozadí, polohy a vegetačního typu. Nejvyšší koncentrace kontaminace kovy a metaloidy byly zjištěny v lokalitě vodního toku (též „mineralizační Jezírka") a nejméně rizikové kontaminace kovy a metaloidy bylo potvrzeno u vnějšího (kontrolního) vzorku. Naše výsledky ukázaly, že staré doly mohou být zdrojem kontaminace na studovaném území a potenciální mobility několika rizikových prvků v závislosti na charakteristice lokality a její vzdálenosti od rudného ložiska.

**Klíčová slova:** Starý důl, příjem prvků smrkem, kadmium, nikl, měď, transport kovů

# Table of Contents

# 1. Introduction

The bachelor thesis deals with metals and metalloids in the soil and their transfer to blueberries (*Vaccinium myrtillus L.*). This issue is relevant both from theoretical and practical points of view, because of the high demand of blueberries and their frequent consumption. One of the many significant aspects about blueberries and their vitamin and mineral content is the disease prevention. As the products on market have been easier to reach, blueberries have received much attention by becoming one of the five healthy fruits by the Food Agriculture Organization due to their health benefit effects and the content of antioxidants and anticancer properties. Also with their popularity, blueberries are among the most commercialized fruits in the international market (Kuang et al. 2022). Moreover, blueberries are used for research very often, either regarding health benefit for humans, bioavailability of specific elements, or risk assessment in drinking water and food (Kalt 2019). For purpose of this thesis, blueberries were chosen for their ability to uptake various elements from soil. They are known to be good filters. Blueberries especially in old mining areas, can thus be a source of metal(loids) in the human food chain. Our sampling area is located around Staré Ransko where the Czech Government mined iron ore dating from 1393 till 1989. We selected four different areas for blueberries collection. Each area differs in the distance of old ore deposits. The target risk metals and metalloids that we were looking for were nickel (Ni), cobalt (Co), arsenic (As) and copper (Cu). Various risk elements can occur in the old environmental burdens after ore mining (Musilová 2022). Such research can help determine the food safety and assess potential risk for inhabitants living around Staré Ransko or similar areas.

## 1.1 Background

Blueberries are widely spread and widely distributed group of persistent flowering plants, with more than 200 species. They belong to the *Vaccinium* genus. They are evergreen, deciduous woody plants with different sizes. From small shrubs that range from 60 cm to lowbush and even highbush trees up to 4 meters. Blueberry includes both consumable fruit and officinal fruit-bearing shrubs that belong to the *Ericaceae* family (Yang et al. 2023).

Blueberries have many health benefits for humans or even animals. They are rich in vitamins (C and K), flavonoids, phenolic acids, bioactive compounds, and polyphenols (Yang et al. 2022). They are filled with antioxidants, which help with cellular and molecular damage. Polyphenols such as anthocyanins have many benefits, anthocyanins have the capacity to modulate second messenger systems and gene expression, prevent chronic degenerative diseases through their antioxidant function, or can alter enzyme activities, inhibit growth and proliferation of cancer cells. They also can prevent obesity, type II. diabetes and reduce the indices of metabolic syndrome because of enzymes called a-amulase and a-glucosidase (Correa- Betanzo et al. 2014). Blueberry plant is composed of smooth elliptical leaves that are arranged alternately along the dotted stems, twigs that remain green during winter (enabling the plant to photosynthesize even during winter) (Reimann et al. 2001), a small urn-shaped flower and a berry with many seeds with deep indigo to black color when full-blown. Anthocyanin is responsible for the color and represents one of the most important contents in blueberry that determines nutraceutical quality and human benefits. This pigment analysis is very important for determining bioactive compounds composition (Ortiz- Delvasto et al. 2023). At the beginning, elements are in the soil, during plant growing they are taken up via roots and then transported to the leaves. Elements that can be taken up by blueberries and most often analyzed are: iron (Fe), calcium (Ca), copper (Cu), magnesium (Mg), potassium (K), zinc (Zn), aluminum (Al) and many others.

Both Cu and Ni are essential elements for plant growth. They can both move vertically in the soil profile, but their mobility is different. Copper is generally more sorbed in the soil than Nickel. There are studies showing higher Cu and Ni mobility in alkaline soils rather than in acidic soils (Zhao 2020). Also, the vertical mobility of these elements can change over time. Blueberry's composition differs from other fruits such as an apple. Blueberries' analyses showed lower elemental composition, in this sense, it shows preferences for $NH_4^+$(ammonium) over $NO_3^-$ (nitrate). There was a case, where mixed N sources showed higher growth then when there was a single N source since the induction of the expression of the related gene increased plant's root uptake and transport of ammonium and nitrate. Other analyses showed that increased ammonia could cause higher nutrient uptake simply by the decrease of soil pH required for blueberry growth and production (Ortiz- Delvasto et al. 2023).

## 1.2 Objectives

The main objective of the thesis is to investigate the risk metal contents in blueberries over the Ransko massif and assess the bioavailability of these metals from soils in the surveyed area. The deeper goal of this research is to evaluate the potential health risks associated with blueberries grown in old mine along with monitoring the risk elements behavior.

# 2. Review of literature

## 2.1 Metals in the Environment

### 2.1.1 Metals and Metalloids

Heavy metals are considered to be elements with atomic mass over 20 and specific gravity (density) above $5g/cm^3$. The word “heavy” is used for both metals and metalloids, that can be toxic to plants and to animals even at very low concentrations (Rascio & Navari-Izzo 2010). Metals are known to be good conductors of heat and electricity. They are malleable, meaning that they can be hammered into sheets, and ductile, which basically means “drawn into wire”. Most metals are solids when it comes to room temperature with silvery shine characteristics (except for mercury which is liquid) according to Vedantu for Metals, Non-metals, and Metalloids. Metals and metalloids are naturally occurring in the earth’s crust. Their concentration depends on their location. However, Earth’s crust is not the only place where we can find them. They also occur in foods, because they are absorbed by plants or animals, which then could get on our plate. We can call this process; “bioaccumulation”. There is a difference when it comes to accumulation of metals and heavy metals. Heavy metals bioaccumulate more easily, meaning they can reach higher concentration at which they are more toxic. Heavy metal toxicity has been proven as major threat, and it is associated with several health risks. Even though these metals do not have any biological role, they remain harmful for the human body. They could act as “pseudo” elements, but they could also interfere with metabolic processes. Pseudo-elements refer to elements that are not naturally occurring in the environment but are artificially created (Jaishankar et al. 2014). These elements have a short half-life and are highly unstable, making them hard to study. Heavy metal poisoning is when microscopic molecules of metals accumulate within your body. Toxic metals prevent your organs from functioning by binding on them. Accumulation of metals in body can cause many health problems. It can also result in excessive damage due to oxidative stress induced by free radical formation. Toxicity of metals depends on various factors, such as: absorbed dose, route of exposure, duration of exposure (Heavy metal poisoning 2022).

### 2.1.2 Natural Enrichment

Natural enrichment of metals in the environment refers to processes by which metals are naturally distributed in the environment. This is closely linked with geological and geochemical processes. Processes that are worth mentioning includes weathering of rocks, which is breaking down rocks. This releases metals that were resent in the rock to the soil. This process can occur through physical, chemical, or biological processes such as time, rain, wind, or temperature.

Biochemical processes are processes influenced by microorganisms or plants. Sedimentation is well known natural process in which rock is formed from small pieces of sand, stone, etc. that have been left by water, ice, or wind (Cambridge dictionary). Other processes involved in natural enrichment of metals are various geological processes such as volcanic activity or tectonic movements by which metals can be released into the environment (Garrett 2000). Hydrothermal processes can also be associated with volcanic activity. By this process hot water circulates through Earth's crust carrying metals along. Oxidation-reduction reactions transfers metals through the changes in redox conditions. Changes of redox influences solubility and mobility of metals, it can also lead to precipitation or dissolution of metals-bearing phases (Orellana 2011). All of these processes are dependent on time, climate, topography or even human activities.

### 2.1.3 Soil contamination

Soil contamination is defined as: "The occurrence of pollutants, that could be either anthropogenic or natural, in soil above a certain level, which causes either loss or deterioration of soil's function" (ESDAC 1970). Soil contamination is a Global Threat in almost all regions like Europe, Eurasia, Asia, and North Africa as indicated by the Food and Agricultural Organization of the United Nations (FAO). FAO states that degradation already affects one third of the world's soil (Iberdrola 2021). Changes in land use, such as increased urban-industrial expansion, infrastructure development, and tourism over the last few decades has resulted in soil contamination. Industrialization, urbanization, and use of agro-chemicals appear to be the three main causes of metal deposition in soils. According to Robinson (2023), soil degradation is one of the 15 biggest environmental problems of 2023, along with global warming from fossil fuels, food waste, biodiversity loss, plastic pollution, and many others. Within the major cause for soil degradation could be included erosion, increased salt content, loss of organic carbon, acidification, and chemical pollution. These causes could be either anthropogenic or natural. Actions like mining, urbanization, farming, transportation, industry could be included in anthropogenic activities. Soil contamination can cause harmful effects to people and to animals. Contaminants from soil can either be ingested through food that grew in the soil, absorbed through skin or by breathing dust. Mining practices are one of the main sources of environmental pollution by metals and metalloids. According to a global inventory by Nriagu and Pacyna (1988), in the late 1980's, about 356–857 × $10^6$ kg Pb, and 557–1360 × $10^6$ kg Zn were released into the environment annually through mining and smelting activities. More recently, Pirrone et al. (2010) estimated that ore mining and processing is responsible for 13% of global Hg (mercury) emissions.

## 2.2 Characteristic and mobility of selected metals

Lead (Pb) and Zinc (Zn) are examples of metals with different toxicity levels. Lead is a dense risk metal with relatively low melting point. It is used in various man-made products such as paint, pottery coatings, or dishware. Poising by Pb can cause serious damage to organs like brain, kidney, or blood cells. It represents non-essential elements that can be toxic even at low concentrations. On the other hand, Zn is considered as trace mineral which is used as an essential nutrient. It is one of the major aspects used for creating DNA or growing cells. Similarly to Pb, Zn can also cause some undesirable effects to human body when exceeding the daily dose, such as diarrhea, headache, nausea, or vomiting (Elmayel 2020).

For metal speciation is important to understand their reactivity, toxicity, and environmental impact (Templementon 2015). Mobility is another aspect important for metal characterization and refers to the ability of these metals to move within different environment such as soil, water, or air (Elmayel 2020). Mobility can be influenced by several factors. Since the metals exist in various chemical forms in the environment their mobility depends on pH, organic matter content, adsorption properties as well as on solubility. Solubility is defined as the ability of solute (solid, liquid, or gas) to dissolve into solvent and form solution. Mobility is generally strongly affected by soil's pH, the more alkaline soil, the more immobilized metals. The actual bioavailability is related to human exposure, water transport, and remediation strategies. Taking into consideration all these factors which effect the mobility, Zn is considered to be more mobile than Pb. Therefore, all the environmental factors need to be considered for proper assessment of potential metal mobility and toxicity (Li 2022)

## 2.3 Plant uptake of risk metals

### 2.3.1 Potential toxicity

Some risk metals (Cr, Ni, Co, Cu) at low concentrations play an essential role in the growth and development of plants but at concentrations higher than certain threshold levels cause toxicity to the plants. The metal toxicity depends upon number of factors like plant species, form in which metal is present in the soil, soil type, pH of the soil. Among all risk metals, only few are essential for the survival of both plant and animal and that too are required in very low amounts. Exposure greater than their permissible limits, leads to cellular and subcellular damage (damage to membranes, mitochondria, chloroplast) due to altered plant metabolism (Mahey 2020).

2.3.2 Principle of element uptake

Nutritional physiology of plant includes uptake, utilization, storage, internal transport, and recycling of mineral nutrients (Smethurst 2004). All of these aspects are very important for plant growth and crop yield. When we start at the beginning, we start underground with the plant roots. The essential organ for uptake of nutrients is represented by “root hairs”. When elements enter the root hairs, they continue to the center of the root, which is called the stele. From stele, nutrients are transported by xylem and phloem. Xylem moves mineral ions along with water and phloem is responsible for the movement of sugars, proteins, and other organic molecules in plants. The rate of nutrient uptake is independent of the water uptake, but concentrations of nutrients at root surfaces depend on soil water content.

There are three main ways for plants uptake of nutrients through the root (Fig. 1):

1) Simple diffusion: nonpolar molecules, such as O2, CO2, and NH3 moves passively through the cell lipid bilayer membrane without transport proteins.
2) Facilitated diffusion: solutes rapid movement by transport proteins.
3) Active transport: cells uptake requires energy, usually ATP, to move the molecules through the membrane.

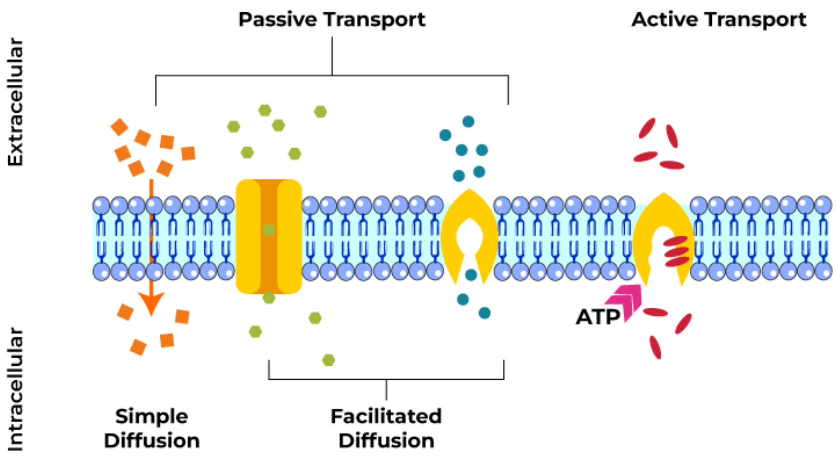

Figure 1. Characteristics of facilitated diffusion (GeeksforGeeks)

As mentioned above, plants uptake essential elements either from the soil through their roots or they can uptake those elements from the air through their leaves. Getting essential elements from the air is called photosynthesis. During photosynthesis, plants receive $CO_2$ (carbon dioxide) from the atmosphere to produce sugars and carbohydrates. Elements, water, air, and light are important factors for plant growth and reproduction.

### 2.3.3 Bioavailability of metals

Soil supplies the plant with macronutrients, such as: N, P, K, Ca, Mg and S, and micronutrients such as Zn, Cu and Cl in smaller amounts. The bioavailability of metals in soil refers to the proportion of the metal that is potentially accessible for uptake by plants. Their availability depends on many factors, such as chemical form of the metal, environmental conditions, or soil properties. Each metal is different, for example Co is more sensitive to phosphate precipitation than other elements. The bioavailability and mobility of metals in contaminated soil can be affected by many factors, such as physiochemical properties like soil pH, organic matter, water content, cation exchange capacity, soil texture, and soil microbiota (Martorell el al. 2011). The uptake of metal concentration into the plant can happen in two ways; active (metabolic) process or passive (diffusion or mass flow) process. The difference between these two processes lays in the ion concentration. For active process low ion concentration dominates, while for passive process it is the high ion concentration. Elements that can be taken up via active uptake are Cu and Zn and for passive uptake preferred elements are Cd, Cr, Pb or Ni (Degryse et al. 2006). Plants also have the ability to modify bioavailability of risk metals. This modification occurs in the rhizosphere by changing physicochemical properties and their biological composition (Chen et al. 2013). For example, vegetables are considered as the major source of human exposure to risk metal poisoning, and it contributes to 90% of total metal intake, while the rest 10% occurs through inhalation of dust or dermal contact (Martorell et al. 2011). Risk elements, such as metals have the ability to move from contaminated soil and bioaccumulate in vegetables and further cause health risk. However, risk metal poisoning has reduced over the last 20 years, because of the awareness and measures our society took.

### 2.3.4 Hyperaccumulators

We define “hyperaccumulators” as plants with an ability to grow on metalliferous soils and to accumulate high amounts of risk metals in the aerial organs without any phytotoxic effect (Fig. 2). Plants that have this kind of ability belong to distant families (Rascio& Navari-

Izzo 2010). These plants are all over the world, ranging from Latin America to Europe. Good examples of these plants are blueberries (focus of this thesis), sunflower (*Helianthus annuus*), tape grass (*Vallisneria americana*), duckweed (*Lemna minor*) and many others. We distinguish three main hallmarks of hyperaccumulators from non-hyperaccumulating taxa; a strongly enhanced rate of metal uptake, a faster root-to-shoot translocation, and a greater ability to detoxify and sequester risk metals in leaves. The main difference between hyperaccumulators and non-hyperaccumulators are in the genes. These differences include sequestration in cell walls, more specifically transmembrane transporters such as ZIP (zipper interacting protein), HMA (high microbial abundance), MATE (multidrug and toxic compound extrusion), and MTP (microsomal triglyceride transfer protein) families.

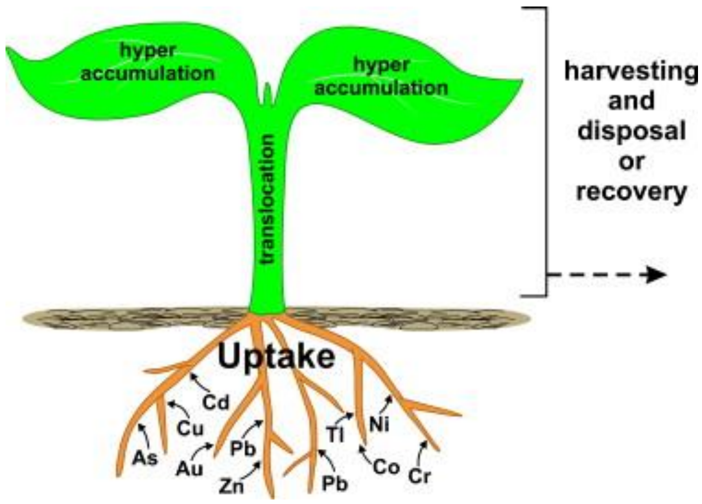


Figure 2. Phytoremediation and phytomining of risk metals rich soils by using plants which hyperaccumulate these metals in above-ground organs (Rascio et al. 2011)

Studies have shown that the reason behind the process of "hyperaccumulation" is simply a defense mechanism of the plants. For plant to survive high amounts of metal concentration, it needs to translocate contaminant above ground where it does not cause that much of damage to the plant. This process is called detoxification. This occurs in places such as epidermis, trichomes or cuticle. All this occurs in the plant's leave, which is interesting, because photosynthesis is also happens in the leaves. Hyperaccumulation was used by humans to cleanse contaminated soil in the past (Rascio & Navari-Izzo 2010). Soil washing is also technique used by humans to wash the contaminants out of the soil. Soil washing physically separates soil from its contaminants (DeVroom 2023). Unfortunately, with removing the metal contaminants with plants can also cause removing the essential nutrients from the soil. Hyperaccumulators play an important role in phytoremediation or phytomining, which are processes of using plants to reduce toxic metal concentrations in the soil. This is crucial for environmental cleanups, especially in areas that are affected by previous mining activities or industrial pollution. Studies for hyperaccumulators are significant not only for the environment but also for ecological management. The potential of hyperaccumulators tolerance of high metal concentrations offers valuable insight into the plant's survival strategies (Sharma 2021).

## 2.4 Blueberry

### 2.4.1 Growth Conditions

Blueberry is a native plant to North America that has expended over thousands of years. They can be found in various regions all over the world, from North America all the way to Oceania (Fig. 3). Only exception is Antarctica and Greenland. The best condition for their growth is acidic rather than alkaline soil. Acidic soils with pH ranging from 4.5 to 5.0. If the soil is not acidic enough, there are many methods for lowering the soil pH to the optimal level. Adding peat moss, adding vinegar to the water and many other methods. Blueberry's requirement does not end with only pH. For their thrive, they also need well- drained soil with high organic matter content and appreciate consentient moisture. Enough water is also very important. It is said that blueberries need, during growing season, at least 2.55 cm of water per week. When the plant starts to riper, it needs as much as 10.15 cm of water per week. Planting of blueberries should be done in the spring with spacing around 1.2 m to 2 m from each plant. Water and soil are as important as sun. Blueberry should not be placed in a shade, but in the place where the sun shines according to Arbor Day Foundation.

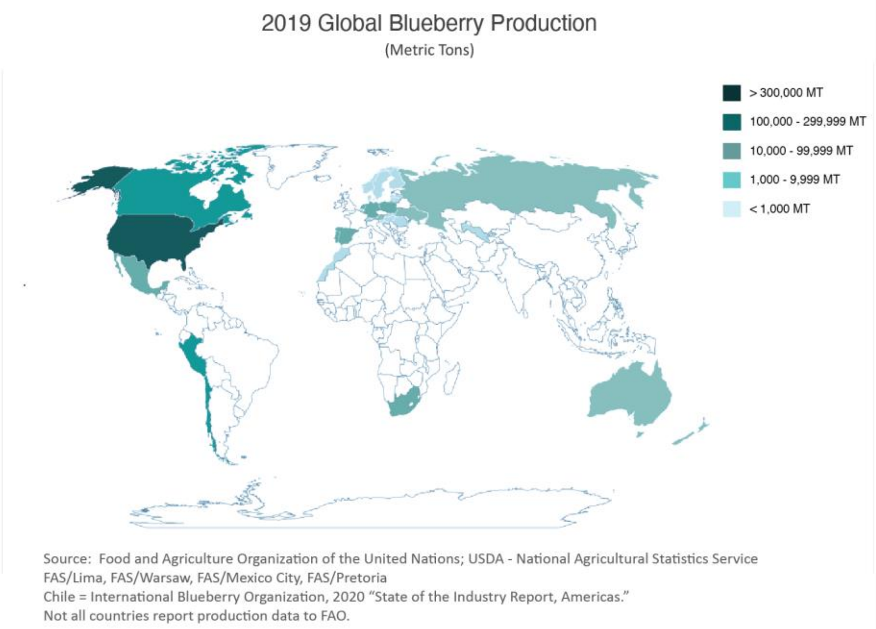


Figure. 3 Global blueberry production in 2019 (Protzman 2021)

### 2.4.2 Metal contents in different blueberry cultivars

There have been many studies about metal contents in different species of blueberries. Difference that has been attributed have shown variability of blueberry's genetic differences. Along with gene differentiation, environmental factors such as growing condition and soil composition are good examples of factors that influence cultivars. When we get back to metals, Cd-related stress is worth mentioning. Cadmium stress evokes alternations in various morphological, physiological, biochemical, and molecular processes of plants (Genter 1996). Not only the pH level, but also the Cd can influence the accumulation and uptake of minerals by blueberries. There have been many analyses conducted about soil profiles and heavy metal content in blueberry fruits to indicate levels of metal pollution. It was found that blueberries carry only a small amount of metal contaminants, which gave them the green light, making them relatively safe and healthy food choice for humans (Yang et al. 2023).

### 2.4.3 Risks associated with blueberries from mining sites

Blueberries that grow on sites or near the sites where mining activities took place can pose several health risks. These risks could either occur from soil contamination or water contamination (Fig.4). Manea et al. (2020) performed a study on the health risk assessment of dietary risk metals intake from fruits and vegetables collected from old mining areas in Carpathians mountains. This study mainly focused on Fe, Mn, Cu, Ni, Pb and Cd. For estimating the health risks associated with these metals, they used total hazard quotients (THQ) to estimate the noncarcinogenic health risks associated with metal contaminated vegetables and fruits and TTHQ (total target hazard quotients) to calculate the overall potential health risk. This study emphasized the need for future investigation but overall, the findings suggested various degrees of health risk associated with the consumption of metal-contaminated vegetal food from different mining areas, with Pb and Cd being the major contributors to potential health concerns (Manea et al. 2020).

The risks associated with blueberries from mining sites could include:

1) Risk metal(loid) uptake: Blueberries are known for being good hyperaccumulators. If they grow on site close to the old mines, there is a chance that they did uptake the harmful elements from the soil, which could lead to ingesting hazardous elements. These elements could include Pb, As, Cd.

2) Soil pollution: Contaminated soil can affect the blueberry plant itself. It could influence the yield, quality, nutrition composition etc.
3) Health concerns: Consuming contaminated blueberries can cause many health risks. These effects can be caused by long-term ingestion of toxic elements at increased levels.

Figure. 4 Illustration of old mining site (modified), (McNab 2018)

## 2.5 Norway spruce

Norway spruce shares some similarities with blueberries. These similarities include evergreen plant, prefer moist soil, economic importance, cultural significance (treetime.ca). Norway's spruce is used for paper production or lumber, this could be considered as economic significance (forestryandland.gov.scot). And as for cultural significance goes, this tree is very often used by many families around the world as a Christmas tree (treetime.ca). It also has a value for wildlife, being habitat to many species such as beetles, birds, or insects. Its cones serve as food for small mammals like a red squirrel.

*Picea abies* is a coniferous tree which can reach up to 40 meters and live up to 1 000 years. This trees' appearance can be seemed like triangular shape, wide at the bottom with narrowing to the top. Norway spruce is bearing cones along with needles. Cones are usually 9-17 cm long with red-brown color and diamond-shape, rounded scales. It's bark, when the tree is young, is a coppery grey-brown color with the appearance of smooth surface, but it is rough to the touch. Older tree's bark is darker then in younger ones, color ranging from purple to brown with cracks. Twigs have the same color, no matter the age of the tree, being orange-brown, grooved, and hairless (woodlandtrust.org.uk).

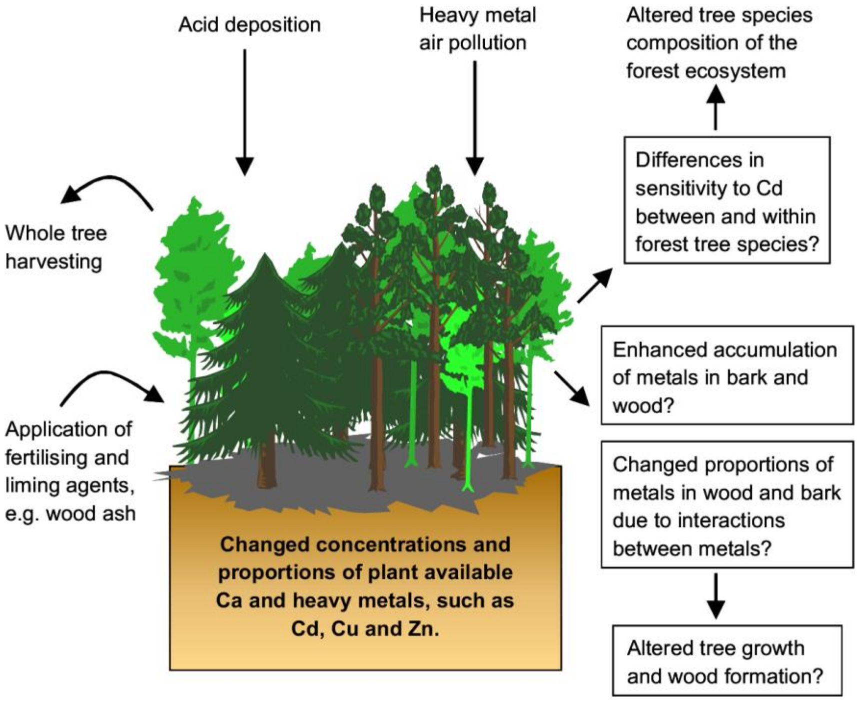


Figure 5. Human activities like logging, air pollution, and soil treatments affect metal levels in forest soils, impacting metal accumulation in tree wood and bark, altering tree growth, and changing tree species composition. (Osteras 2004)

Norway spruce can, as blueberry, uptake risk metals from soil through their roots. Roots are the major pathways of risk metals into the tree, but there are other ways of getting inside the tree, such as leaves or bark (Lepp 1975). The uptake of risk metals is dependent on factors such as soil pH, metal bioavailability, redox potential, organic matter content, or competing ions and cations binding capacity (Osteras 2004), (Fig.5). As I mentioned metal ions, their concentration increases in plants when there is an increase around them (Greger 1999). When absorbed, they then move to the outer layer of the roots, also known as the apoplast. This movement is done either by diffusion or mass flow (Marschner 1995). Apoplast is considered to play crucial role in nutrient uptake in many trees, is it the intercellular space outside of the plant's cell membrane (Fig.6). Apoplast serves as pathway for movement of water, minerals, or other nutrients (Farvadin 2020). Before entering the root cells, metal cations must pass the apoplast. Risk metals bound either to the cell wall or enter the cytoplasm (Pineros 1997).

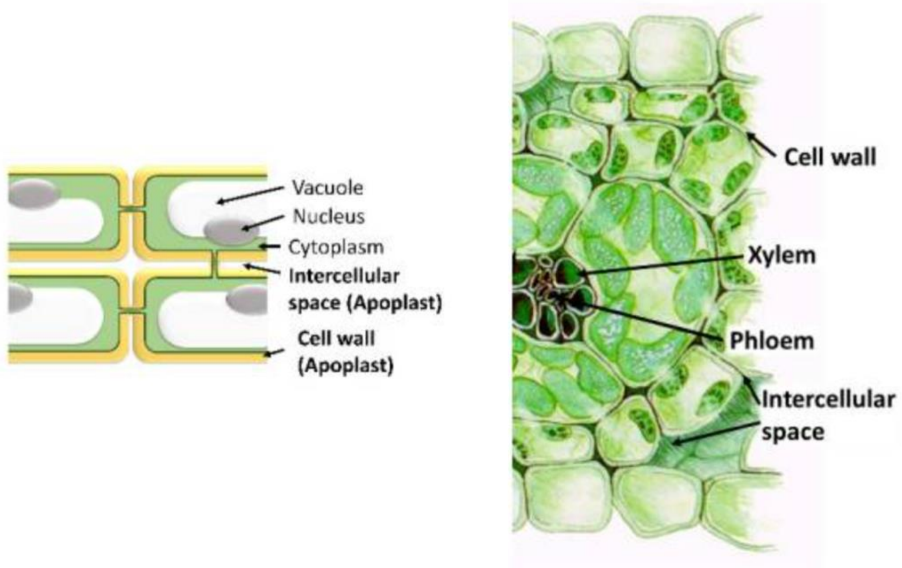


Figure 6. Spaces and structures that form the apoplast in plants (Farvardin 2020)

When the metal ions reach the xylem they are transported upward to the tree trunk. In higher plants such as our Norway spruce, roots are observed to contain higher levels of risk metals compared to other structures such as leaves, flowers, or fruits (Greger 1999). Although risk metals tend to accumulate in roots, as said above, due to binding to the cell walls, they are able to move upward and accumulate in wood or barks. The importance for risk metals in Norway spruce is connected with its economic importance. Risk metals might not harm the tree in noticeable way, but it could affect paper production (Osteras 2004).

# 3. Materials and Methods

## 3.1 Site description

The research was carried out in Staré Ransko National Natural Reserve. It is located in northeast of Vysočina Region. This little village has only 183 inhabitants (Wikipedia 2021). Sampling was not done in this village rather than around it. Staré Ransko is known for its natural resources since the late 17th century (Historie báňské činnosti v oblasti Starého Ranska, 2017). Natural resources that were mined in last century include elements such as Fe, Ni, Cu, Co, Zn. Mining was banned in 1989 (Mining.cz).


Figure 7.a) National Natural Reserve- Ancient ore mines, b) forest with Norway spruce, common spruce, grass, and blueberry (author: B. Hrůšová)

We sampled on many locations from which three sites were chosen for this thesis. Sample No. 345 was taken from the old mine; therefore, it can be considered “on-site” sample, denoted here as “old mine”. The area was very sunny with a lot of green cover.

Many blueberries with big leaves were observed along with mountain ash (*Sorbus*), Norway spruce and grass.

Sample No. 348 was not far away from sample No. 345, it was basically situated at the mineralization Jezírka and it was close to waterbody Ranská Jezírka. This sample had different cover then the first one. Although it was also located close to old mine, different one, the vegetation cover was quite different. It was not as sunny area as the location before because it was deeper in the woods. The ground was not as green, it was rather brown, covered by needles that fell from spruces, along with many horsetails and Lilly of the valley. Horsetails are considered as good indicators for gold mines (Brooks et al. 1981). As we literally dug deeper into the soil, we found Ni and Cu sulphides. Also, with taking samples from the soil, we discovered the subsoil to be troctolite, olivine and gabbro.

The last selected sample No. 372, which is considered to be "off-site" (i.e. control sample not affected by any mining activities). This sample was taken in different region, Pardubický Region. On this site, there was no biotite, only little of muscovite and mostly K-feldspar and quartz present. Vegetation on this location was not very rich. The only trees that were present were spruces along with peaty moss and sphagnum moss.

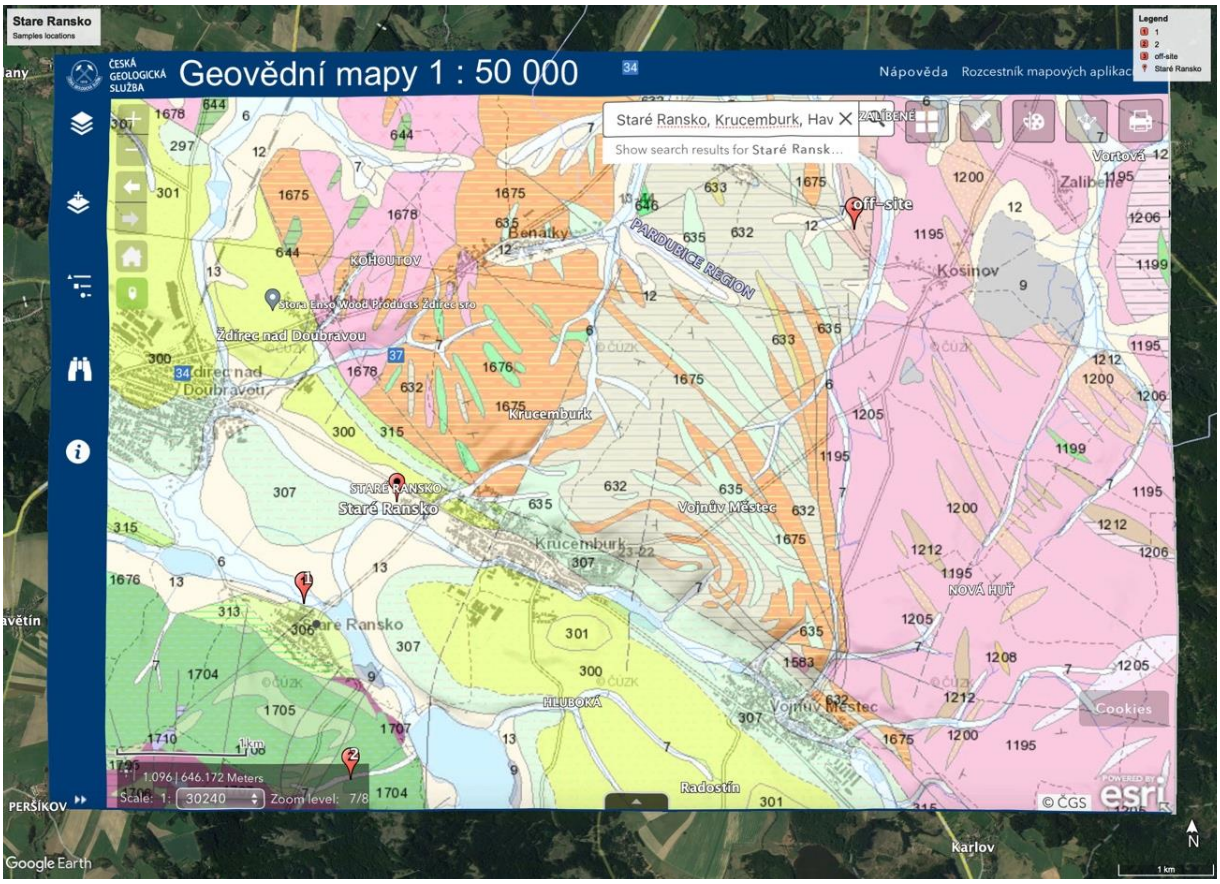


Figure 8. Geoscientific maps showing Staré Ransko area (Česká geologická služba)

Geology of old mine location shows a surface of sandstone calcareous clay. For location mineralization Jezírka, the surface is stony clay. The last sample from off-site location is on top of fine-grained biotic mostly pearl gneiss. All this information was gathered from Česká Geologická Služba, Geovědní mapy.

## 3.2 Bark sampling

We chose Norway spruce for bark sampling. Sampling was done always by the same people with the same procedure. From every location, we picked 3-5 trees within the sampled area. Those trees were usually surrounded the soil samples (that were taken as well but not studied in this thesis), therefore not very close to each other. The distance between those trees was approximately 10 meters. Radius from the soil samples didn't exceed more then 15 meters. We always tried to pick trees with the same age, condition, and species. For bark sampling we used specific tools such as draw knives, paper bag, pair of gloves. We collected 50 grams of bark from each sampled tree. Along with collection of bark, we collected also twigs and one cone. It is necessary to note that we also collected blueberries samples; however, due to the extreme delay and miscommunication with the MASA institute labs, we have not received the blueberries analyzed samples. Therefore, only the bark samples are presented and discussed in this thesis.

Figure 9. bark of Norway spruce, method of sampling (author: B. Hrůšová)

## 3.3 Sample preparations and instrumental analyses

Samples were carefully, with gloves, transferred from paper bag into mini plastic test bottles. Sealed to prevent any material contamination and marked for recognition. All provided materials were analyzed using coupled plasma mass spectrometry (ICP-MS) and inductively coupled plasma optical emission spectroscopy (ICP-OES), depending on the total concentrations level of each element. All the samples were prepared and analyzed in MASA Institute certified laboratory.

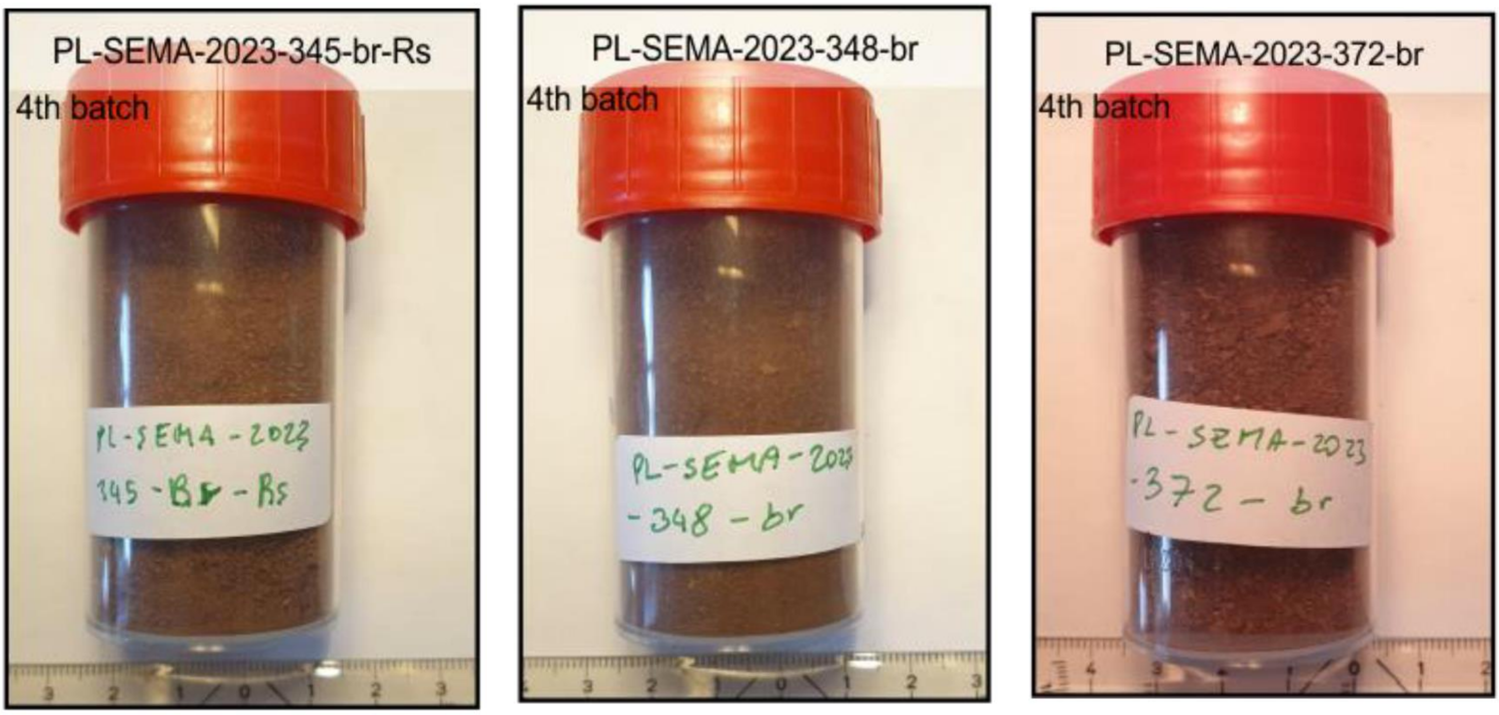


Figure 10. Sample analysis of 345, 348, 372 (MASA institute)

For full digestion, the homogenized sample material was placed in digestion vessels (Savilex cups) weighed in. To check the measurements, additional plant standards were weighed for quality control. Hydrofluoric acid (HF) and concentrated nitric acid ($HNO_3$) were used for the complete digestion of the plant material, hydrogen peroxide ($H_2O_2$) and hydrochloric acid (HCl) were also used. After the last smoke of $HNO_3$ and $H_2O_2$, a certain amount of HCl is added to the samples. The sample containers are closed tightly, and the solutions are heated. After cooling, the lids are removed to evaporate the sample acid mixtures. In a final step, the samples are placed in the measuring solution, then transferred, homogenized by heating with the lid closed, to a defined level volume diluted and can then be analyzed.

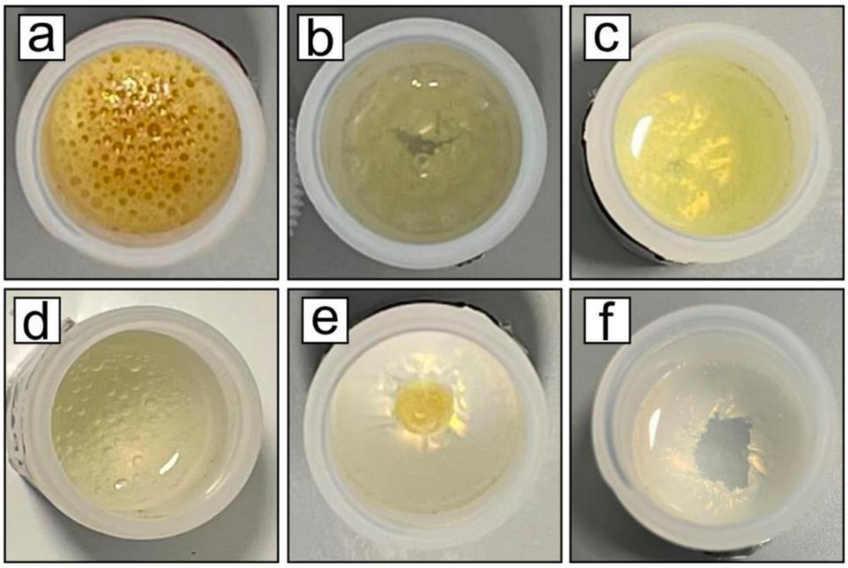


Figure 11. Digestion procedure of samples (MASA Institute)

Different steps of the digestion procedure: a) after adding $HNO_3$ and $H_2O_2$, b) after cold reaction, c) after again addition of $HNO_3$ and $H_2O_2$, d) after cold reaction, e) after fuming off the HCl, f) after homogenization in $HNO_3$ and $H_2O$.

External standard solutions with a known composition are used to control the analyses treated the same as the samples. The trace element analysis is carried out using ICP-MS (iCAP TQe from the company ThermoScientific) and the ICP-OES (iCAP Pro XP from ThermoScientific). To assess the quality. The external standards are also measured during the measurements in order to minimize the standard deviation and increase the accuracy of the analyzes to e (<2% RSD (2S)). The samples are used as quality control an internal standard was added (Be).

# 4. Results and discussion

## 4.1 Arsenic

Arsenic became number one carcinogen by the International Agency of Research on Cancer (IARC) and its exposure through soil or water became serious concern for human health. Exposure of As is not usually direct, instead it accumulates in edible parts of plants and is afterwards consumed by human. It is not essential element for plant growth, although it serves as a key component in many processes (Farooq et al. 2016). These processes include cell division, function of cell walls, extension, and synthesis. Since the bioaccumulation of As occurs mainly in its roots, there is little of translocation above ground (Perez et al. 2012). The sampled location “waterbody” showed the highest concentration of As, followed by location “old mine” with very little difference as showed in Fig 12. Off-site (control) showed the lowest concentrations of As, as expected. What was not expected was the amount of As being quite close to old mine sampled location (concentration range between 0.10 and 0.11mg/kg).

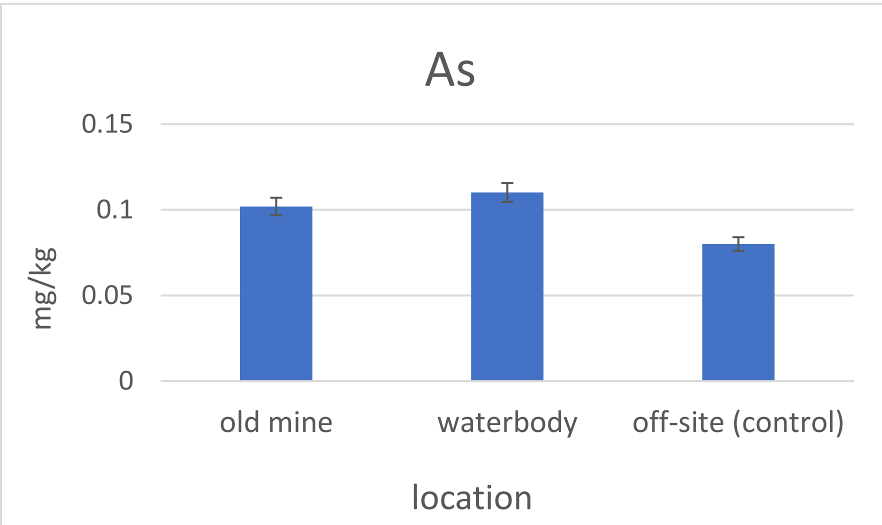


Fig.12 Arsenic concentration

## 4.2 Cadmium

Cadmium ranks as the third most significant environmental contaminant after Hg and Pb. What makes Cd different from other risk metals is its ability to effect not only humans but also animals even at plant tissue concentrations that are typically non-toxic. The World Health Organization (WHO) has set a maximum acceptable level of 0.3 mg/kg for root vegetable.

Higher plants have the ability to absorb Cd from both soil and water through roots. The accumulation of Cd can be effected by plant species, genotype, environmental conditions or the presence of other minerals and nutrients. Studies showed thad Cd content in plants was especially higher in soils with pH 4.0 compared to soils with pH 5.0, along with Cd levels higher in sandy soils rather then in clay soils (Eriksson 1989). For the sampled locations, Cd showed the highest concentrations on location "old mine" (0.87 mg/kg). This location was also on sandstone surface. The waterbody location, which showed the second highest concentrations of Cd had a geology of stony clay surface, supporting study mentioned above. Last sample taken from off-site location showed lowest concentrations of Cd, its geology being gneiss (Fig.13).

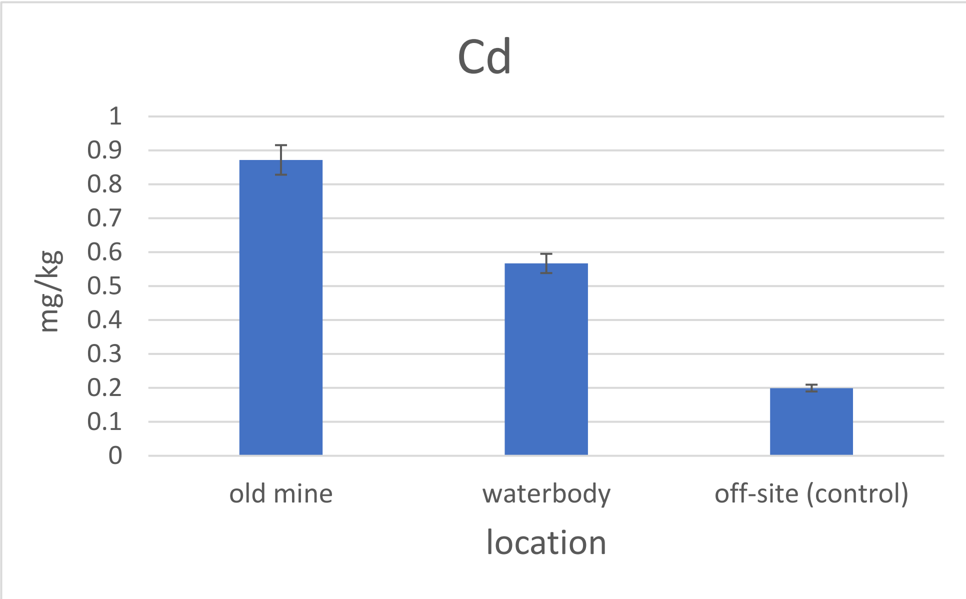


Fig. 13 Cadmium concentrations

## 4.3 Copper

Copper is an essential element with the ability of transition. This ability helps the element to cycle around between the oxidized Cu(II) and reduced Cu(I) which then makes it possible to be involved in biological processes such as photosynthesis, respiration, or cell wall metabolism (Burkhead et al. 2009). Copper also has a dual nature to plants: essential at an optimum level at the same time, toxic at high levels. Copper being highly toxic, root sequestration regulates mobility of Cu to upper parts of the plant. To prevent toxicity, excess Cu in plant above ground is chelated and sequestered efficiently (Lange et al. 2016). Copper showed highest concentrations in the waterbody sample on stoney clay surface (5.48 mg/kg;

Fig.14). Second highest concentrations of Cu is on old mine area with sandstone geological background. High concentration of Cu can be explained by study conducted by Cornwall (1956), who showed Cu occurance on wide variety of environments, which also includes clastic sedimentary rocks or oxidized zones of sulfide deposits. The last sample from off-site area showed again the lowest concentrations of Cu (Fig. 14).

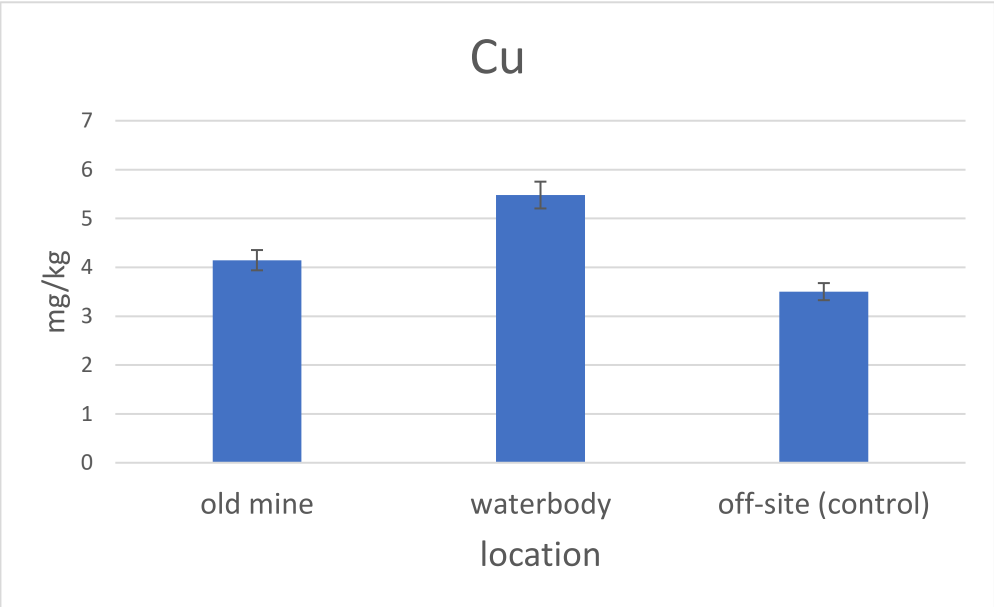


Fig. 14 Copper concentrations

## 4.4 Nickel

Nickel is an essenatial element with good electric conducitivity of and it is also only one of four elements that are ferromagnetic at or near room temperature. It is the fifth most common element on earth, its occurrence indicate more towards below the crust rather then inside of it (Pedersen 2016). Trace amount of this element are naturally occuring in many plants, vegetables and many more foods. Due to very low requirements, Ni deficiency is rare and often misdiagnosed because it does not show any symptoms on plants (Buechel 2023). However, in higher amounts it can accumulate in soil resulting in toxicity. This toxicity in plants leads to inhibiting seed germination, retarding growth, or disruption of metabolic processess (Ahmad 2011).

Nickel concentrations were detected the highest in spruce bark near the old mine (6.76 mg/kg), this can be explained by the iron mines and accompained risk elements in Staré Ransko. Nickel is always found in the combination with iron (Geology page-Nickel, 2014).

Since the location “waterbody” is also close to the old mine, there is consideralby high concentrations of Ni compared to off-site (control) location (Fig.15).

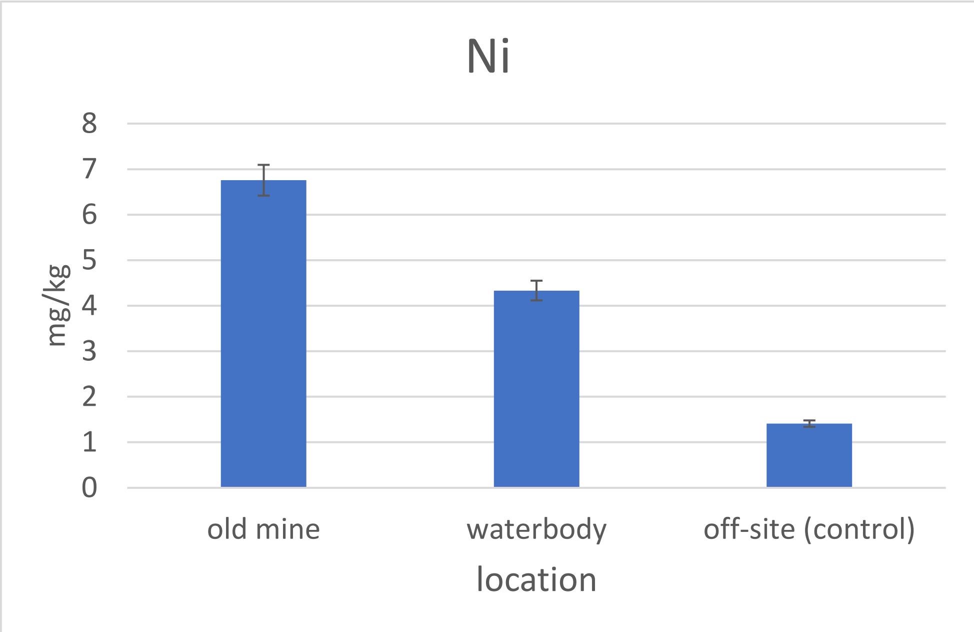


Fig. 15 Nickel concentrations

## 4.5 Lead

Lead is a highly toxic metal which can be found in various enviroments and tends to persist in soil for extended periods not only due to its resistance to degradation. Its toxic effects on plants can impact the plants growth, development, or physiological processes. Lead, as most of mentioned risk metals, gets absorbed by plant’s roots and then follows the water stream upwards. However, the highest concentrations of Pb still occurs in the roots, especially when the plant is young and the roots have thin cell walls. This action results of decreased uptake of Pb to above ground parts of the plant, which could seemed like an advatage. It can be considered as an advatage for food chain, along with using these plants for remedation techniques. Therefore, plants together with Pb can be very often used to assess environmental quality (Pourrut et al. 2011). Lead had highest concentrations in waterbody location (3.84 mg/kg). This area is also reffered as “mineralization Jezírka”, which would explain the obtained results. Since Pb is found mostly on sedimentary, igneous or metamorphic rocks (Ghazi et al. 1964). Not far behind was location ,,old mine”, which is also located close to location waterbody. As expected, the lowest concentration of Pb was off-site (control) area (Fig.16).

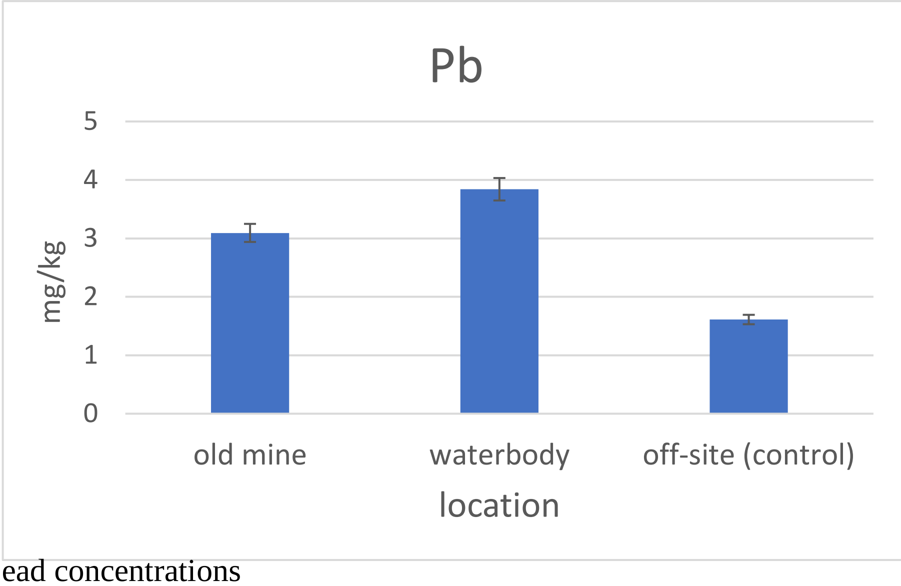


Fig. 16 Lead concentrations

## 4.6 Zinc

Zinc is essential micronutrient for both plants and animals (Gupta et al. 2016). It is also a trace element, which means that body requires only small amount of it, however it is necessary for many chemical reactions. It plays crucial role in the creation of DNA, growing of cells, healing damaged tissue, and supporting healthy immune system. Not all of Zn in soil can be taken up by plant's root. The specific form of Zn in which it is occuring in soil also plays a crucial role for availability and uptake. Zinc exists in soil in insoluble, exchangeable or water-soluble form. Most of the time, Zn occurs in insoluble form which makes it impossible for plant uptake, fraction is then exchangeable and can be afterwards taken up by roots through process like ion exchange (Barber 1995). Zinc is very mobile in wet environment, which explains the highest concentrations obtained on "waterbody" area (164.95 mg/kg). Since the area of "old mine" and "off-site" area had similar geology, and were dry rather than wet, the concentrations of Zn were very close to each other (Fig.17).

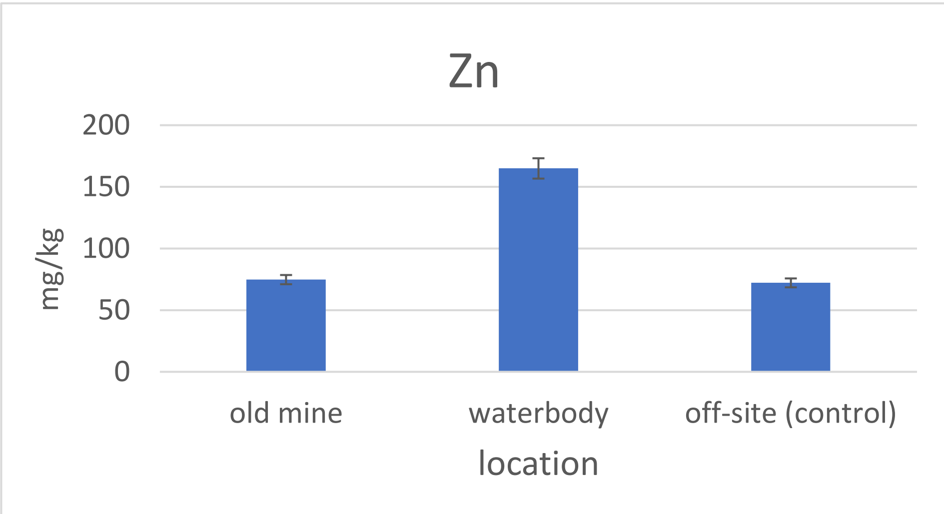


Fig.17 Zinc concentrations

# 5. General discussion

Even though the area of Staré Ransko is known for its history of iron mining in the last century, there were many risk metals located within the area of old mines or around it. First (old mine) and second (waterbody) chosen sites showed some similarities; however the area of waterbody seemed to have more contaminants either within the soil or in chosen samples such as bark from Norway spruce. The area of waterbody had the highest concentration for following risk metal(loid)s: As, Cu, Pb and Zn. For these risk metal(loid)s, the area of old mine had the second highest concentrations. As for risk metals like Cd and Ni, the area old mine had the highest concentrations.

Arsenic was surprisingly high for every location with difference only of 0.01 mg/kg between the old mine and waterbody, and 0.02 mg/kg between old mine and off-site. Study made by Garcia-Sanchez (2003) in Spain highlighted geology being similar to location Staré Ransko. More precisely, basement rocks in Spain were: sandstones, clays, limestones, mudstones, sands, gravels etc. Some of these basement's rocks are the same as in Staré Ransko area. This study came to conclusion that "As distribution in soil without mining influence is mainly determined by the nature of the soil parent rock" (Garcia-Sanchez 2003), which would also explain high concentrations in off-site area. Comparing the results of As to Cd, the concentrations between each location differ a lot. Cadmium was found in very high concentrations for the area of old mine. This could be explained by samples taken only 50 meters far from the old mine since Cd is mainly produced as byproduct of mining, smelting of ores (Wikipedia). This also explains why the off-site area has the lowest concentration of Cd. Copper had the highest concentrations in waterbody, but it did not differ much from other chosen locations. Since Cu has low mobility, meaning it does not leach through the soil profile as it was conducted in study made by Cornell University on Copper, the vegetation cover plays an important role when it comes to Cu. All of the sampled locations had very similar vegetation cover which would explain the obtained results. Nickel is a transition metal that mainly occurs with iron. This would explain the highest concentrations obtained in "old mine", followed by mineralization Jezírka location and lastly the off-site (control) location. Even though Pb has low solubility, the concentrations of Pb in sampled locations are quite high, even in off-site location. It is believed that small amounts of Pb can be absorbed by plants due to its strong binding with organic or colloidal materials (Pourrut et al. 2011). As of lead's natural levels remain below 50 mg/kg compared to 3.09 mg/kg (highest obtained concentration), this study would be confirmed. All results were usually in low concentrations of mg/kg; however, Zn is an exception. Zinc had the highest concentrations of all the risk metals. Waterbody area had 165 mg/kg and off-site area has concentration of 72.1 mg/kg. This can be explained by mobility of Zn. This element's mobility, especially in wet environment makes it easier for plants to uptake it from soil (Moreno-Lora 2020). Zinc is also an essential element, which would explain its occurrence in high amounts in all of sampled locations.

The last chosen location, the area off-site (control) area always showed the lowest concentrations for every evaluated risk metal. First two areas were very close to each other, therefore the concentration did not vary enormously. Off-site area was always with the lowest concentrations with a big difference for every risk metal except Zn and Cu. The biggest difference between the results were in risk metals like Ni and Cd.

## 6. Conclusion

We investigated risk metal concentrations in Norway spruce in Staré Ransko area. We sampled in three locations, based on their distances from the old iron mines. Two locations were located on site (directly on the old mines) and one location was considered to be off-site (control), far away from the old mines. We found traces of many risk metals: As, Cd, Cu, Ni, Pb, Zn. The concentrations of these risk metals differed due to their soil background, location and vegetation cover. The most contaminated location was waterbody area, also known as "mineralization Jezírka" and as we expected, location with the least risk metal contamination was confirmed to be off-site (control).

# 7. Bibliography


Elmayel I., Esbri J.M., Efren G.-O., Garcia-Noguero E.-M., Elouear Z., Jalel B., Farieri A., Roqueni N., Cienfuegos P, Higueras P., 2020, Evolution of the speciation and mobility of Pb, Zn and Cd in relation to transport processes in a mining environment, *International Journal of Environmental Research and Public Health.*

Templeton D. M., 2015, Speciation in metal toxicity and metal-based therapeutics, *Toxics.*

Rashid A., Schutte B. J., Ulery A., Deyholos M. K., Sanogo S., Lehnhoff E. A., Beck L., 2023, Heavy metal contamination in agricultural soil: environmental pollutants affecting crop health, Agronomy.

Li Q., Wang Y., Li Y., Li L., Tang M., Hu W., Chen L., Ai S., 2022, Speciation of heavy metals in soils and their immobilization at micro-scale interfaces among diverse soil components, *Elsevier.*

Musilova J., Frankova H., Lidikova J., Chlpik J., Vollmannova A., Arvay J., Harangozo L., Toth T., 2022, Impact of old environmental burden in the Spis region (Slovakia) on soil and home-grown vegetable contamination, and health effects of heavy metals, *Scientific Reports.*

Manea D. N., Ienciu A. A., Stef R., Smuleac I. L., Gergen I. I., Nica D. V., 2020, Health risk assessment of dietary heavy metals intake from fruits and vegetables grown in selected old mining areas- A case study: The banat area of Southern Carphathians, *Int. J. Environ. Res. Public Health.*

Acosta J.A., Faz A., Martinez-Martinez S., Arocena J.M., 2011, Enrichment of metals in soils subjected to different land uses in a typical Mediterranean environment (Murcia City, southeast Spain), *Elsevier,* 405-414.

Ettler V., 2016, Soil contamination near non-smelter ferrous metal smelters: A review, *Elsevier,* 56-74.

Jaishankar M., Tseten T., Anbalagan N., Mathew B. B., Beeregowda K. N., 2014, Toxicity, mechanism and health effects of some heavy metals, *Interdiscip Toxicol,* 60-72.

Protzman E., 2021, Blueberries around the globe-past, present and future, *International agricultural trade report.*

Drozdz P., Seziene V., Pyrzynska K., 2018, Mineral composition of wild and cultivated blueberries, *Biol Trace Elem Res*, 173-177.

Zeiner M., Cindric I. J., 2018, Harmful elements (Al, Cd, Cr, Ni, Pb) in wild berries and fruits collected in Croatia, *Toxics.*

Chen S., Zhuang Q., Chu X., Ju Z., Dong T., Ma Y., 2021, Transcriptomics of different tissues of blueberry and diversity analysis of rhizosphere fungi under cadmium stress, *BMC Plant Biology.*

Perez R., Laca A., Laca A., Diaz M., 2022, Environmental behavior of blueberry production at small scale in Northern Spain and improvement opportunities, *Elsevier.*

Kim R.-Y., Yoon J.-K., Kim T.-S., Yang J. A., Owens G., Kim K.-R., 2015, Bioavailability of heavy metals in soils: definitions and practical implementation- critical review, *Environmental Geochemistry and Health*, 1041-1061.

Olaniran A. O., Balgobind A., Pillay B., 2013, Bioavailability of heavy metals in soil: impact on microbial biodegradation of organic compounds and possible improvement strategies, *International Journal of Molecular Sciences*, 10197-10228.

Rascio N., Navari-Izzo F., 2011, Heavy metal hyperaccumulating plants: How and why do they do it? And what makes them so interesting? *Elsevier*, 169-181.

Khan A., S. Khan S, Khan A., Qamar Z., Waqas M., 2015, The uptake and bioaccumulation of heavy metals by food plants, their effects on plants nutrients, and associated health risk: a review, *Environmental Science and Pollution Research*, 13772-13799.

Kalt W., Cassidy A., Howard L. R., Krikorian R., Stull A. P., Tremblay F., Zamora-Ros R., 2020, Recent research on the health benefits of blueberries and their anthocyanins, *Advances in Nutrition*, 224-236.

Loredo-Portales R., Bustamante-Arce J., Gonzalez-Villa H. N., Moreno-Rodriguez V., Del Rio-Salas R., Molina-Freaner F., Gonzales-Mendez B., Archundia-Peralta D., 2020, Mobility and accessibility of Zn, Pb, and As in abandoned mine tailings of northwestern Mexico, *Environmental science and pollution research international*, 26605-26620.

Duan Y., Tarafdar A., Chaurasia D., Singh A., Chaturvedi Bhargava P., Yang J., Li Z., Ni X., Tian Y., Li H., Kumar Awasthi M., 2022, Blueberry fruit valorization and valuable constituents: a review, *International Journal of Food Microbiology*

Reimann C., Koller F., Kashulina G., Niskavaara H., Englmaier P., 2001. Influence of extreme pollution on the inorganic chemical composition of some plants. *Environmental Pollution* 115, 239-252.

Kuanga L., Wanga Z., Zhanga J., Lia H., Xua G., Li J., 2014. Factor analysis and cluster analysis of mineral elements contents in different blueberry cultivars. *Journal of Food Composition and Analysis*, 1-8.

Ortiz-Delvasto N., Garcia-Ibanez P., Olmos-Ruiz R., Barzana G., Carvajal M., 2023. Substrate composition affects growth and physiological parameters of blueberry. *Scientia Horticulturae*, 1-8.

Yang H., Wu Y., Duan Y., Zhang Ch., Huang Z., Wu W., Lyu L., Li W., 2020. Metabolomics combined with physiological and transcriptomic analyses reveal regulatory features associated with blueberry growth in different soilless substrates. *Scientia Horticulturae*, 1-14.

Correa-Betanzo J., Allen-Vercoe E., McDonald J., Schroeter K., Corredig M., Paliyath G., 2014. Stability and biological activity of wild blueberry (Vaccinium angustifolium) polyphenols during simulated in vitro gastrointestinal digestion. *Food Chemistry*, 522-531.

Zhao R., Li J., Ma Y., Lv Y., 2020. A field study of vertical mobility and relative bioavailability of Cu and Ni in calcareous soil. *Environmental Pollutants and Bioavailability*, 121-130.

Schrenk D., Bignami M., Bodin L., Chipman JK., Del Mazo J., Grasl-Kraupp B., Hogstrand C., Hoogenboom LR., Leblanc J-C., Nebbia CS., Ntzani E., Petersen A., Sand S., Schwerdtle T., Vleminckx C., Wallace H., Guerin T., Massanyi P., Van Loveren H., Baert K., Gergelova P., Nielsen E., 2020. Update of the risk assessment of nickel in food and drinking water. *EFSA Journal*, 1-101.

Raji Z., Karim A., Karam A., Khalloufi S., 2023, A review on the heavy metal adsorption capacity of dietary fibers derived from agro-based wastes: Opportunities and challenges for practical applications in the food industry, *Trends in food science & technology*, 74-91.

Wood E., Hein S., Mesnage R., Fernandes F., Abhayarante N., Xu Y., Zhang Z., Bell L., Williams C., Rodriguez-Mateos A., 2023, Wild blueberry (poly)phenols can improve vascular function and cognitive performance in healthy older individuals: a double-blind randomized controlled trial, *The American journal of clinical nutrition*, 1306-1319.

Iskandar I., Suryaningtyas D. T., Baskoro D. P. T., Budi S. W., Gozali I., Saridi S., Masyhuri M., Dultz S., 2022, The regulatory role of mine soil properties in the growth of revegetation plants in the post-mine landscape of East Kalimantan, *Ecological Indicators.*

Farooq A. M., Islam F., Ali B., Najeeb U., Mao B., Gill A. R., Yan G., Siddique K. H. M., Zhou W., 2016, Arsenic toxicity in plants: Cellular and molecular mechanisms of its transport and metabolism, *Evnironmental and Experimental Botany*, 42-52.

Čabalová I., Bélik M., Kučerová V., Jurczyková T., Hiziroglu S., 2021, Chemical and morphological composition of Norway spruce wood (Picea abies, L.) in the dependence of its storage, *Polymers*.

Ejidike I. P., Onianwa P. C., 2015, Assessment of trace metals concentration in tree barks as indicator of atmospheric pollution within Ibadan City, South-West Nigeria, *Journal of Analytical Methods in Chemistry.*

Eriksson J. E., 1989, The influence of pH, soil type and time on adsorption and uptake by plants of Cd added to the soil, *Water, Air and Soil pollution,* 317-335.

Vogelweith F., Thiéry D., 2018, An assessment of the non-target effects of copper on the leaf arthopod community in a vineyard, *Biological Control*, 94-100.

Shabbir Z., Sardar A., Shabbir A., Abbas G., Shamshad S., Khalid S., Natasha, Murtaza G., Dumat C., Shadid M., 2020, Copper uptake, essentiality, toxicity, detoxification and risk assessment in soil-plant environment, *Chemosphere.*

Ahmad M. S. A., Ashraf M., 2011, Essential roles and hazardous effects of nickel in plants, *Reviews of environmental contamination and toxicology*.

Gupta N., Ram H., Kumar B., 2016, Mechanism of zinc absorption in plants: uptake, transport, translocation and accumulation, *Reviews in Environmental science and Bio/Technology*, 89-109.

Osteras A. H., 2004, Interaction between calcium and heavy metals in Norway spruce.

Garcia-Sanchez A., Alvarez-Ayuso E., 2003, Arsenic in soils and waters and its relation to geology and mining activities (Salamanca Province, Spain), *Journal of Geochemical and Exploration*, 69-79.

Echevarria G., Massoura S. T., Sterckeman T., Becquer T., Schwartz C., Morel J. L., 2006, Assessment and control of the bioavailability of nickel in soils, *Environmental toxicology and chemistry*.

Brooks R. R., Holzbecher J., Ryan D. E., 1981, Horsetails (equisetum) as indirect indicators of gold mineralization, *Journal of Geochemical Exploration*, 21-26.

Ghazi A. M., Millette J. R., 1964, Lead deposits, *Environmental forensics*.

Moreno-Lora A., Delgado A., 2020, Factors determining Zn availability and uptake by plants in soils developed under Mediterranean climate, *Geoderma*.

Farvardin A., Gonzalez- Hernandez A. I., Llorens E., Garcia-Agustin P., Scalschi L., Vicedo B., 2020, The Apoplast: A key player in plant survival, *Antioxidants*.

Pineros M., Tester M., 1997, Calcium channels in higher plant cells: selectivity, regulation and pharmacology, *Journal of experimental botany*.

Jaishankar M., Tseten T., Anbalagan N., Mathew B. B., Beeregowda K. N., 2014, Toxicity, mechanism and health effects of some heavy metals, *Interdisciplinary toxicology*, 60-72.

Greger M., 1999, Metal Availability and Bioconcentration in plants, *Heavy metal stress in plants*, 1-27.