TECHNISCHE UNIVERSITÄT WIEN

MASTER'S THESIS

Total reflection X-ray fluorescence analysis of trace elements in black teas and herbal infusions

Carried out at

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Abstract

The aim of this study was to determine elemental composition including trace element content in the various herbal solutions and teas, which could be used for medical treatment purposes, by the mean of Total Reflection X-ray Fluorescence Analysis (TXRF).

TXRF is an Energy Dispersive X-ray Fluorescence method with a special geometry, which is known and characterized by its easy sample preparation, especially for solutions, simple quantification and increased elemental measurement sensitivity, compared to conventional XRF.

Atomika 8030C spectrometer equipped with a Mo-W X-ray tube was used, where only Mo Kα energy was used for excitation. The measurements were conducted under normal conditions, Helium flow was not activated.

As there are not many research papers on trace element composition in herbal tea, there is also quite little information on how samples should be prepared. The methodology of sample preparation was developed and the data of 29 samples were collected.

After the measurements, 13 default elements and four occasional elements were detected. Different data evaluations, including statistical analysis, were used based on differences between measured samples.

Based on comparison between all samples for each separate element, the differences between black teas and herbal infusions were detected. The outcome that it is possible by means of spectrometric analysis to differentiate between teas and herbal infusions was supported by the PCA analysis. It was, however, impossible to distinguish between separate herbal samples. The fingerprint of the particular herbal sample was not identified, due to the lack of knowledge of origin of the plants, precise preparation procedure before selling, and other factors that could influence the results. After detailed analyses it was proved, that there are no traces of any kind of heavy metals in the samples (even in a wildly gathered peppermint).

The work performed in scope of this thesis was presented to the scientific community in three posters (at the European Conference on X-Ray Spectrometry 2016, the 66th Yearly Meeting of the Austrian Physical Society and at the 26th Seminar Activation Analysis and Gamma Spectroscopy) and a talk (at the 66th Yearly Meeting of the Austrian Physical Society).

Kurzfassung

Das Ziel dieser Studie war die Bestimmung der elementaren Zusammensetzung (und insbesondere auch des Gehalts an Spurenelemente) von verschiedenen Schwarz- und Kräutertees, welche zur medizinische Behandlung angesetzt werden, mittels Totalreflexion-Röntgenfluoreszenzanalyse (TXRF).

Die TXRF ist eine energiedispersive Röntgenfluoreszenzmethode welche sich vorallen durch ihre einfache Probenvorbereitung, speziell für Lösungen, durch einfache Quantifizierungen und verglichen mit Röntgenfluoreszenzanalyse (XRF) durch höhere Messempfindlichkeit auszeichnet.

Die Analysen wurden mit einem Atomika 8030C Spektrometer durchgeführt, das mit einer Mo-W Röntgenröhre ausgerüstet war. Dabei wurde jedoch lediglich die Mo Strahlung zur Anregung verwendet. Die Messungen wurden unter Standardbedingungen durchgeführt; Heliumspüllung war nicht aktiviert.

Da es nur wenig Forschung über die Zusammensetzung von Spurenelementen in Kräutertees gibt, sind auch nur wenige Informationen über Proben-Vorbereitungen vorhanden. Daher wurde eine Methode zur Probenvorbereitung entwickelt und Daten von 29 Proben gesammelt.

Nach den Messungen wurden 13 Default-Elemente und vier gelegentliche Elemente festgestellt. Verschiedene Datenauswertungen, einschließlich statistischer Analysen, wurden auf der Grundlage von Unterschieden zwischen gemessenen Proben verwendet.

Basierend auf dem Vergleich zwischen allen Proben für jedes einzelne Element wurden die Unterschiede zwischen schwarzen Tees und Kräuterinfusionen nachgewiesen. Das Ergebnis, dass es möglich ist, mittels spektrometrischer Analyse zwischen Tees und Kräuterinfusionen zu unterscheiden, wurde durch die PCA-Analyse unterstützt. Es war jedoch unmöglich, zwischen verschiedenen pflanzlichen Proben zu unterscheiden. Der Fingerabdruck der jeweiligen pflanzlichen Probe wurde aufgrund des fehlenden Wissens der Herkunft der Pflanzen, des präzisen Vorbereitungsverfahrens vor dem Verkauf und anderer Faktoren, die die Ergebnisse beeinflussen könnten, nicht identifiziert. Nach detaillierten Analysen wurde bewiesen, dass es in den Proben keine Spuren irgendwelcher Schwermetalle gibt (auch in einer wild gesammelten Pfefferminze).

Die Messungen, die im Rahmen dieser Abschlussarbeit verrichtet wurde, sowie die deraus gewonnenen Ergebnisse wurden der wissenschaftlichen Gemeinschaft mittels drei Posterpräsentationen (bei der „European Conference on X-Ray Spectrometry 2016“, während der 66. Jahrestagung der Österreichischen Physikalischen Gesellschaft und auf dem 26. Seminar Aktivierungsanalyse und Gammaspektroskopie) sowie im Zuge eines Vortrags (auf der 66. Jahrestagung der Österreichischen Physikalischen Gesellschaft) präsentiert.

Acknowledgements

At this point, I would like to mention all the people, who were involved in this Master's thesis. I wish to express my very great appreciation to:

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1. Introduction

The human health has been and stays one of the most important issues for the whole humanity. With the technical and therefore overall knowledge progress many questions arise: What is healthy food? How will it influence human body? What should / should not be included in daily diet? What does my food consist of? A lot of scientists all over the world are trying to answer those questions, to get better understanding of influence of the diet on human body.

Tea and all kinds of herbal solutions, after water, are the most common beverages in the world. [1] Therefore it is crucial to know the composition of such a widely used drink. There are only few research papers on teas (different sorts of tea from the *Camellia sinensis* plant) and even less information about other different herbs. Therefore, the purpose of this master's thesis was to select tea and herbal samples for establishing their elemental composition. For this purpose, the TXRF analysis was selected. The methodology of the sample preparation had to be also established.

1.1. Specific research questions

The field of research on herbal infusions is rather new and at the moment just slightly explored, therefore, during this master's work several particular questions of interest were identified to frame the research objectives:

1. What is the general elemental composition of the herbal solution and tea?
2. Is there a difference between elemental composition in teas and herbal solutions?
3. Is it possible to determine a fingerprint of the particular herb?
4. Is it possible to determine differences between plant families using elemental composition?
5. Is there any significant difference between different parts of the herbs (leaves, fruits, flowers and bark)?
6. Is there any influence on the elemental composition of the herbal infusion when using packaged tea instead of the loose leaves?
7. Are there any harmful elements in significant amounts in teas and herbal solutions?

These questions were considered and answered during this Master's work research.

1.2. Diploma thesis structure

Total X-ray Fluorescence analysis is a method, where the sample is examined by means of radiation. The sample is exposed to X-rays and is emitting X-rays back, which are then detected. For that reason, chapter 2 describes X-ray properties as well as XRF in general terms and particularly TXRF method.

To show scientific relevance of the chosen topic for this Master's thesis, chapter 3 is dedicated to the medical applications of tea and herbal infusions. It also concentrates on existing quality control methods and analysis of elemental composition of the plants. Chapter 4 shows the literature research, which was necessary for the following work to get orientation in the described research field. The literature research is finished with the conclusion and the next section (4.2.) describes the sample selection procedure.

After the theoretical part, in chapters 5 and 6 the practical part of the Master's work is shown. Chapter 5 deals with measurement preparation, describing used instrumentation and procedure parameters. It also uncovers the established sample preparation procedure. Chapter 6 is fully concentrated on the presentation of the results. It also contains description of the data processing and the evaluation of the method.

In chapter 7, the research questions from the paragraph 1.1. are listed again and elaborately answered, followed by conclusions and short summary.

Chapter 8 shows table of figures and tables, followed by the list of all references. In the appendix posters (as the first publications of the work) are to be found as well as documentation relevant to the research.

2. Properties of X-ray

In the year 1895, a new kind of radiation was discovered. This discovery was made by Wilhelm Conrad Roentgen (1845-1923). He was working with positively and negatively loaded electrodes. Both were inserted in a glass container (vacuum was created inside it) and shielded with heavy black paper. When high voltage was applied, all above mentioned resulted in a green colored fluorescent beam. This kind of radiation was not known before and was therefore called X-rays. For this finding Roentgen received the first Nobel Prize in physics in 1901.

2.1. X-rays

Further research caused a dispute whether X-rays are waves or corpuscular. Scatter- and ionization experiments led to controversial results. Today, it is known that X-rays originate in the atomic shell and are part of the electromagnetic spectrum, such as visible light or radio waves. Figure 1 shows comparison of wavelength, frequency and energy for the whole electromagnetic spectrum.

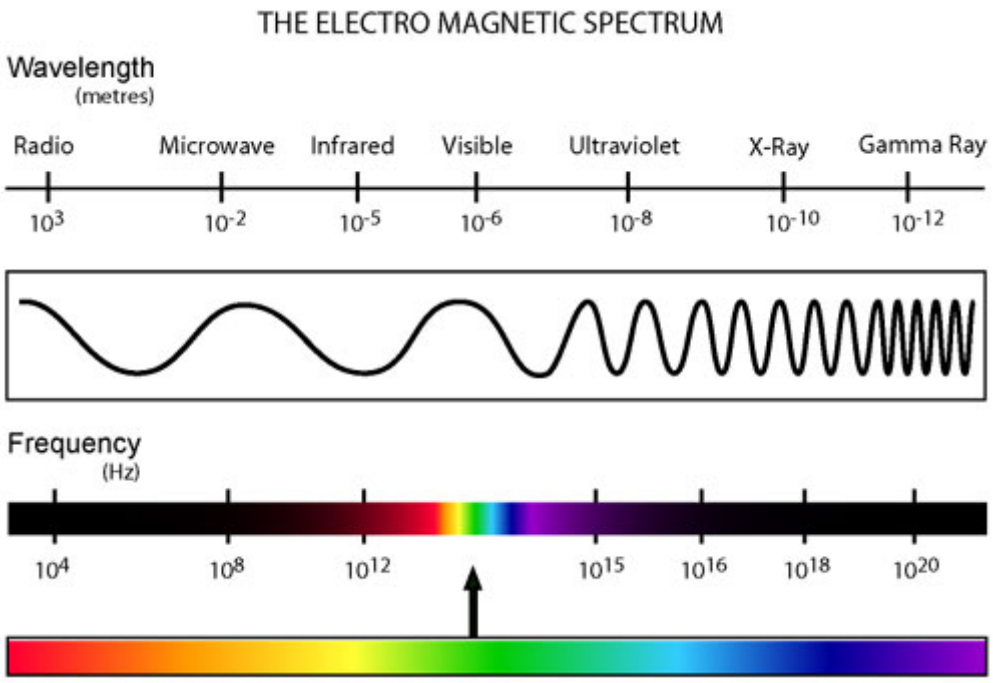

Figure 1 The electromagnetic spectrum. [2]

The energy E of X-rays reaches from 0.1 keV up to more than 100 keV. The energy of an electromagnetic wave is linked with its wavelength λ:

$$E = h\nu = \frac{h \cdot c_0}{\lambda} \quad \text{and} \quad c_0 = \nu \cdot \lambda \tag{2.1}$$

- h is Planck's constant ($h = 6.626 \cdot 10^{-34} J \cdot s = 4.135 \cdot 10^{-15} eV \cdot s$)
- ν is the frequency

- c_0 is the speed of light in vacuum ($3 \cdot 10^8 m/s$)

When all the constants are put into equation 2.1, this expression simplifies to:

$$E[keV] = \frac{1.239}{\lambda[nm]} \quad (2.2)$$

To get a deeper understanding of X-rays, it is necessary to be acquainted with Bohr's atomic model and the Pauli Exclusion Principle.

2.1.1. The Bohr model of atom

The Bohr's model or rather the Rutherford-Bohr model was first presented in 1913. Niels Bohr described atom as a solar system, where positively charged nucleus (as sun) is encircled by electrons on a designated orbits (as planets). The significant difference is however in attraction force: in solar system it is gravity, in atom rather electrostatic forces.

Bohr's model of atom uses following postulates:

1. An atom is made up of three particles: electrons, protons and neutrons (n). Electrons have a negative charge and protons have a positive charge, whereas neutrons have no charge. They are neutral. Due to the presence of equal number of negative electrons and positive protons, the atom as a whole is electrically neutral. The protons and n are located in a small nucleus at the center of the atom, which makes the nucleus positively charged, due to the presence of protons.

2. Electrons can rotate over the nucleus in orbits or shells. The energy levels / shells / orbits are denoted in two ways (Figure 2): either by the numbers 1, 2, 3, 4, 5 and 6 or by letters K, L, M, N, O and P. The energy levels are counted from center outwards.

3. The electrons can only stay in a stable mode, without radiating, in certain "stationary orbits" at a certain discrete set of distances from the nucleus. These orbits are associated with definite energies and are called energy shells or energy levels. During rotation within energy levels, the electron's acceleration does not result in radiation and energy loss as required by classical electromagnetic theory.

4. Electrons can only gain and lose energy by moving from one allowed orbit to another, absorbing or emitting electromagnetic radiation with a frequency υ determined by the energy difference of the levels according to the Planck relation:

$$\Delta E = E_2 - E_1 = h\upsilon \quad (2.3)$$

where h is Planck's constant ($h = 6.626 \times 10^{-34} J^{-s}$) and υ is the frequency of radiation absorbed or emitted, and E_1/E_2 are the energies of different orbits. The frequency of the radiation emitted at an orbit of period T is calculated same as in classical mechanics:

$$\upsilon = \frac{1}{T} \quad (2.4)$$

Bohr could determine the energy spacing between levels and come to an exactly correct quantum rule: the angular momentum *L* is restricted to those orbits where it is an integer multiple:

$$L = n \cdot \frac{h}{2 \cdot \pi} = n \cdot \hbar \qquad (2.5)$$

where n is a simple integer. The stationary states or allowed energy levels are only those where n = 1, 2, 3… This is called the Bohr quantum condition with principal quantum number n; and $\hbar = \frac{h}{2\pi}$. The lowest possible value of n is 1; this gives a smallest possible orbital radius of 0.0529 nm known as the Bohr radius. Once an electron is in this lowest orbit, it cannot circle any closer to the nucleus.

Each shell can contain only a fixed number of electrons: the general formula is that the n^{th} shell can in principle hold up to $2n^2$ electrons. Since electrons are electrically attracted to the nucleus, an atom's electrons (in ground state) will generally occupy outer shells only if the more inner shells have already been completely filled by other electrons.

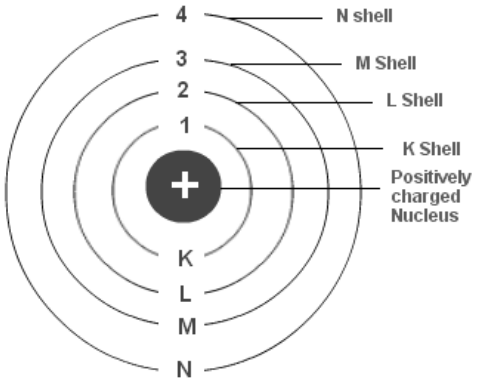

Figure 2 Energy levels around nucleus. [3]

The Pauli Exclusion Principle

There are no two electrons, which would have identical quantum numbers. This general principle is called Pauli Exclusion Principal and applies to electrons and to other particles of half-integer spin (fermions).

The nature of the Pauli Exclusion Principle can be illustrated by supposing that electrons 1 and 2 are in states "a" and "b" respectively. The wave function for the two-electron system would be

$$\psi = \psi_1(a)\psi_2(b) \qquad (2.6)$$

Where ψ_1 is a probability amplitude that electron 1 is in state "a" and ψ2 is a probability amplitude that electron 2 is in state "b", but this wave function is unacceptable because the electrons are identical and indistinguishable. To account for this, the linear combination of the two possibilities should be used, since the determination of which electron is in which

state is not possible to ascertain. The wave function for the state in which both, “a” and “b” states, are occupied by the electrons can be written as:

$$\psi = \psi_1(a)\psi_2(b) - \psi_1(b)\psi_2(a) \quad (2.7)$$

Where ψ is the probability amplitude that both states “a” and “b” are occupied by electrons 1 and 2 in either order. Thereto “-” is applied for fermions (half-integer spins) and “+” would be applied for bosons (integer spin). The minus sign in the relationship forces the wave function to vanish identically if both states are “a” or “b”, implying that it is impossible for both electrons to occupy the same state. [4]

It is therefore evident that different shells would be able to accept different amount of electrons. The outside shells can host more electrons through the building of subshells.

The K-shell configuration corresponds to the noble gas Helium. L-electrons have the principal quantum number n = 2. Its orbital angular momentum can have the values of l = 0 or l = 1. This includes (2l + 1) setting possibilities. To the state l = 0 belongs the magnetic quantum number m_l = 0, to the state l = 1 belong values m_l = 0 and m_l = ±1. All together this gives us four possible orbital angular momentum states. If we add two possible spin orientations, we get eight potential electron states for a principal quantum number n = 2. It means that maximally occupied L-shell will have $n_{MAX}(L) = 8 = 2 \cdot n^2$ electron places. If the K-shell is also completely filled, so we get in total 10 potential electron places. Exactly this constellation has the noble gas Neon. [5]

Shell	**Orbital angular momentum, l/m**					**Spin, s**	**n_{max}**
1 - K	0/0					±1/2	2
2 - L	0/0	1/(0, ±1)				±1/2	8
3 - M	0/0	1/(0, ±1)	2/(0, ±1, ±2)			±1/2	16
4 - N	0/0	1/(0, ±1)	2/(0, ±1, ±2)	3/(0, ±1, ±2, ±3)		±1/2	32
5 - O	0/0	1/(0, ±1)	2/(0, ±1, ±2)	3/(0, ±1, ±2, ±3)	4/(0, ±1, ±2, ±3, ±4)	±1/2	50

Table 1 Notations of the energy levels and the maximum occupation number according to the Pauli Exclusion Principle. [5]

2.1.2. Interaction between X-radiation and matter

By encountering with matter, the particular X-ray beam is changing its intensity (sometimes also its energy) due to interaction with it. This attenuation results from interactions of individual photons in the beam with atoms in the absorber [6]. There are four possible interaction mechanisms: photoelectric effect, Compton scattering, coherent scattering and pair production.

However, coherent scattering is not a major interaction process encountered in a field like, for example, radiography at the energies normally used. This form of photon interaction occurs when the energy of the X-ray or gamma photon is small in relation to the ionization energy of the atom. It therefore occurs with low energy radiation. The photon does not have

enough energy to liberate the electron from its bound state (i.e. the photon energy is well below the binding energy of the electron) so no energy transfer occurs. The only change is a change of direction (scatter) of the photon, hence 'unmodified' scatter [7].

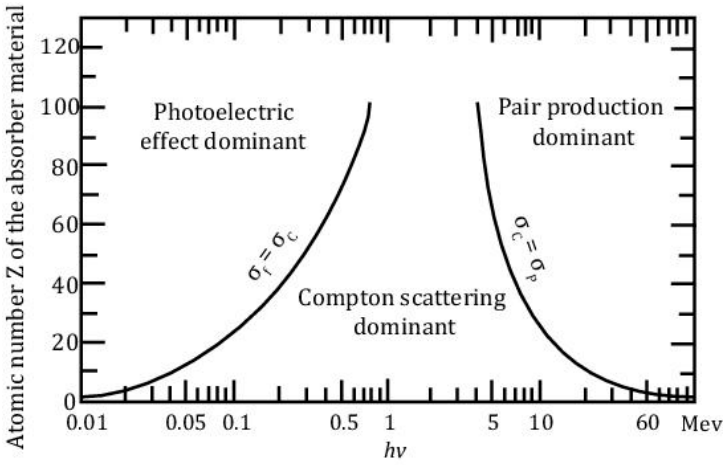

Figure 3 The interaction modes of X-rays with matter. [8]

Photoelectric effect

Photoelectric effect or photoelectric absorption (PEA) is a form of interaction between X-ray or gamma photon and matter. This is a dominant low energy photon process when the photon gets completely absorbed by an electron of an atom resulting in escape of the electron from the atom. Simply said, the electron is removed from its shell with a kinetic energy equal to the difference of energies: $E_{Photon} - E_{Binding}$. After the so-called photoelectron is emitted, an electron from a higher shell takes its place (Figure 4). To compensate the occurred difference in energy between the two shells, a characteristic X-radiation can be emitted. This secondary emission is also called X-ray fluorescence.

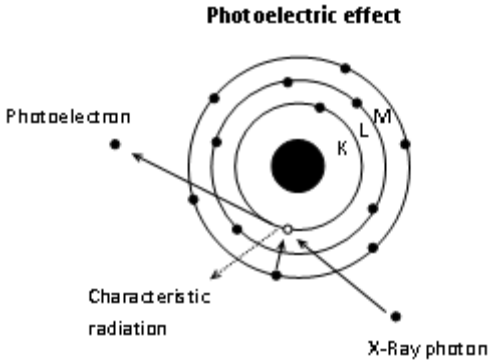

Figure 4 The photoelectric effect.

It is also possible, that the electron from a higher energy level, which is quickly filling in the hole after photoelectron ejection, instead of emitting a photon loses its energy to another electron from a higher energy level. In this case, there is no radiation but an electron emitted. The escaped electron or auger electron (Figure 5) has energy equal to the energy lost by the electron, which made the downward transition minus the binding energy of the electron that is ejected from the atom.

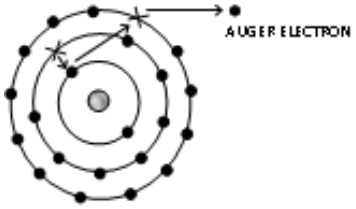

Figure 5 The Auger effect.

Compton scattering (X-ray Scatter)

The collision of a photon with a loosely bound outer electron of an atom or molecule or even with a free electron can lead to a change of direction and a loss of energy of the photon. This process is called *inelastic scattering* or *Compton scattering* (Figure 6). A strict relationship exists between energy loss and angle of deflection. [9]

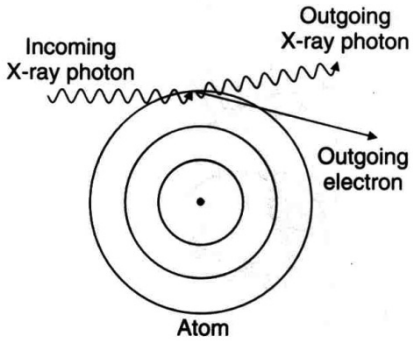

Figure 6 Compton scattering. [10]

The photons can be deflected in all directions. The loss of *energy* a photon suffers in Compton scattering results from the conservation of total energy and total momentum at the collision of the photon and the electron. A photon with the initial energy *E* keeps the part *E´* when it is deflected by an angle *ψ*, while the electron takes off the residual part of energy $dE = E - E'$. The fraction E'/E can be calculated according to

$$E' = \frac{E}{\left(1 + \frac{E}{E_0} \cdot (1 - \cos\psi)\right)} \qquad (2.8)$$

where E_0 is the rest energy of an electron which amounts to 511 keV.

Pair production

In a pair-production interaction, the photon interacts with the nucleus in such a manner that its energy is converted into matter. The interaction produces a pair of particles, an electron and a positively charged positron. These two particles have the same mass, each equivalent to rest mass energy of 511 keV. Pair production is a photon-matter interaction that is not

encountered in X-ray spectroscopy procedures because it can occur only with photons with energies in excess of 1022 keV, therefore it will not be considered here further.

To summarize, the interaction between radiation and matter and can be described partly by the wave picture and partly by the corpuscle picture. If an X-ray beam passes through matter, it will lose intensity due to different effects. The number of photons N_0 hitting upon a homogeneous sheet or layer of density ρ and thickness d is reduced to a fraction N being transmitted while the difference, $\Delta N = N_0 - N$, has been lost. The Lambert–Beer law controls the attenuation of intensity:

$$\frac{\Delta N}{N} = -\left(\frac{\mu}{\rho}\right) \cdot \rho \cdot \Delta d \qquad (2.9)$$

where μ is the linear attenuation coefficient, and $\left(\frac{\mu}{\rho}\right)$ is called the mass attenuation coefficient. The intensity exponentially depends on the thickness of the layer:

$$I(d) = I(0) \cdot e^{\left(\left(\frac{\mu}{\rho}\right)\cdot\rho\cdot d\right)} \qquad (2.10)$$

2.1.3. Radiation types

X-rays are basically produced by high-energy electrons bombarding a target (atom). When bombarding electrons penetrate into the target, some electrons travel close to the nucleus due to the attraction of its positive charge and are subsequently influenced by its electric field. [11] The course of these electrons will be deflected, and a portion or all of their kinetic energy will be lost. The principle of the conservation of energy states that in producing the X-ray photon, the electron has lost some of its kinetic energy (E_K):

$$E_K = E_K^I - E_{photon} \qquad (2.11)$$

where E_K – final kinetic energy, E_K^I – initial kinetic energy of the electron and E_{photon} – energy of X-ray photon.

The radiation can be divided into two types:

- Bremsstrahlung
- Characteristic X-rays

2.1.4. Bremsstrahlung

Bremsstrahlung or Braking radiation (as translated from German) generation occurs when an electron passes near the nucleus and it is slowed down and its path is deflected. Energy lost is emitted as a BremsstrahlungX-ray photon.

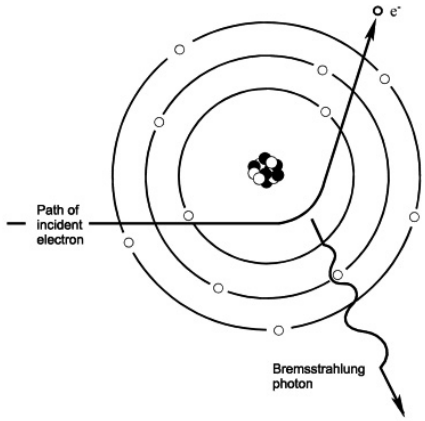

Figure 7 Bremsstrahlung or Braking radiation. [12]

Approximately 80% of the population of X-rays within the X-ray beam consists of X-rays generated in this way. Accelerated charges are releasing their electromagnetic radiation, and when the energy of bombarding electrons is high enough, the emitted energy will be the X-ray radiation. Braking radiation is characterized by a continuous distribution, which becomes more intense and shifts toward higher frequencies when the energy of the bombarding electrons is increased.

2.1.5. Characteristic Radiation

Characteristic radiation is described by photoelectric effect and was mentioned in the chapter *2.1.2 Interaction between X-radiation and matter*. It is, however, important to mention, that electrons can also be ejected by other electrons and not only electromagnetic waves. Essentially, characteristic radiation is a monochromatic radiation that is produced when an electron is ejected from an atom and another takes its place by jumping from another shell; the energy of the emitted photon is the difference between that of the two shell positions [13] (equation 2.10).

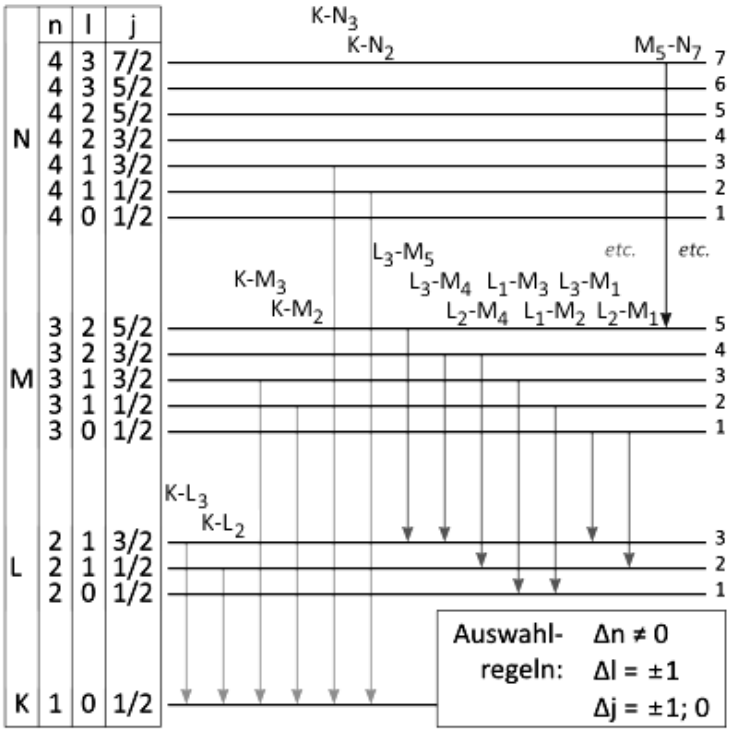

Figure 8 Energy levels transition. [14]

Figure 8 shows possible transitions of electrons in atom. Each of these transitions (K-L2, K-M3, etc.) results in a line on the spectrum. Most of the atoms have several shells and some sub shells, which results into the whole bunch of lines. The only two elements with a single energy line are Hydrogen and Helium.

There are some rules that need to be complied with in order to make the electron transition possible:

- $\Delta n \neq 0$
- $\Delta l = \pm 1$
- $\Delta j \in \{-1; 0; 1\}$

As it was mentioned in the paragraph 2.1.4, the Bremsstrahlungis represented by a continuous spectrum; in contrast, characteristic radiation is represented by a peak / line spectrum. Specific energy is needed to remove the electron from inner shell of the atom; those energies are individual for each element of periodic table; therefore, characteristic radiation can be used for spectroscopic analysis. It is possible to determine which elements are present in a sample by obtaining information about at which energies the characteristic radiation has appeared.

The relationship between the photon energy or energy peak and the element, which emits it, was discovered by Moseley in 1913. He found that the reciprocal wavelength and consequently the photon energy are dependent on the atomic number *Z* of the elements. [9]

$$E_{ij} = k_{ij} \cdot (Z - \sigma_i)^2 \qquad (2.12)$$

The k_{ij} and σ_i are constants for particular peaks or lines.

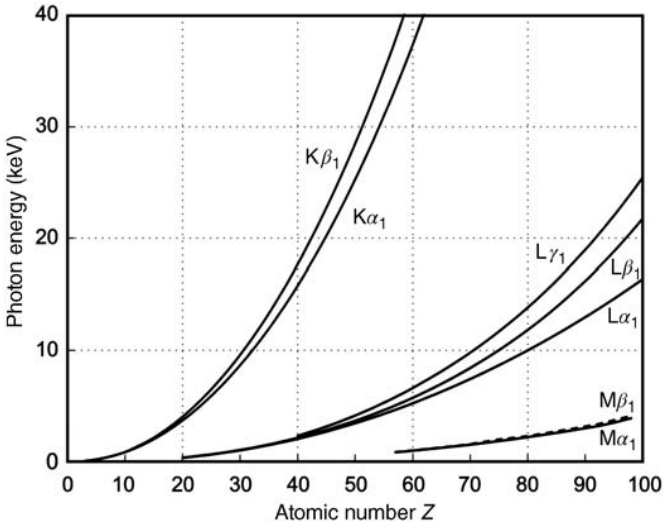

Figure 9 Photon energies depending on the atomic number Z, defined by Moseley law. [9]

In the Figure 9 the characteristic diagrams of photon energies are shown. Each of the lines represents a particular peak. Out of the Moseley law follows, that the atomic number of the elements can be correctly determined.

Out of the equation 2.12 follows that elements with different atomic number Z will have different energies for the same corresponding line. An example showing the spectrum with characteristic Ni peak (this sample is used as reference for Atomika 8030C spectrometer) can be seen in Figure 10. In the spectrum Si K_α → 1.740 keV from the sample carrier (quartz reflector), Ar K_α → 2.957 keV from air, Ni K_α → 7.472keV and Mo K_α → 17.443 keV can be seen. Characteristic spectrum was recorded with 100ng Ni sample (50kV, 37mA).

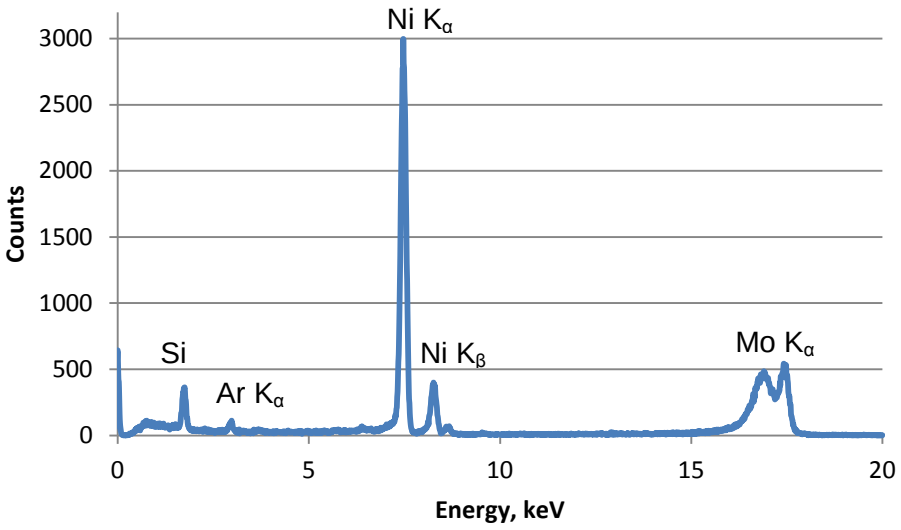

Figure 10 Characteristic spectrum with Ni K_α peak.

2.2. X-ray fluorescence analysis (XRF)

X-ray fluorescence analysis (XRF) is a non-destructive method used for determination of the multielement composition in a sample. This method provides qualitative and quantitative results, which is especially useful for materials analysis spectroscopy; it however, does not give any kind of information about the sample structure. The limits of detection are in the range of ppm (parts per million), but light elements (from Li till Na) are only detectable under specific conditions. This depends above other on the fluorescence yield, detector sensitivity, the measuring system, the vacuum and other.

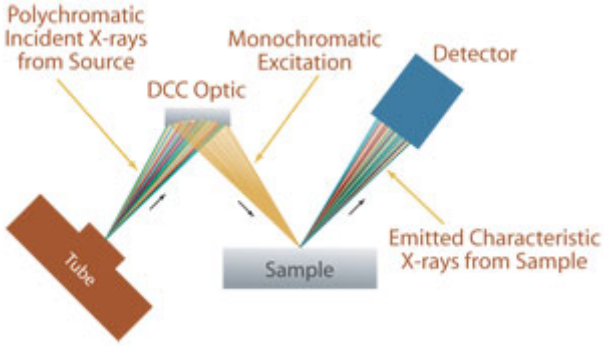

Figure 11 XRF system. [15]

The basic concept for all XRF spectrometers (Figure 11) is a source, a sample holding system, a sample, and a detection system. The source irradiates the sample and a detector measures the fluorescence radiation emitted from the sample. In most cases for XRF, the source is an X-ray tube. Alternatively, a radioactive source or a synchrotron can be used. Radioactive sources are complicated to handle and to store, so they are used quite rarely. The synchrotron is a very effective radiation tool, but it is complicated to get access to. The alternative radiation sources will not be discussed further in this work, as none of them was used for these measurements.

Based on the main measured parameter, there are two types of spectrometers that are used: wavelength dispersive spectrometer (WDS) and energy dispersive spectrometer (EDS).

Wavelength dispersive spectrometers

In wavelength dispersive X-ray fluorescence (WD XRF) spectrometers, all of the elements in the sample are excited simultaneously. The different energies of the characteristic radiation emitted from the sample are diffracted into different directions by an analyzing crystal or monochromator. By placing the detector at a certain angle, the intensity of X-rays with a certain wavelength can be measured. Sequential spectrometers use a moving detector on a goniometer to move it through an angular range to measure the intensities of many different wavelengths. In that case, the crystal is turned around an axis on top of its surface and vertical to the fluorescence beam, while the detector is turned simultaneously around this axis at a double angular speed.

Synchronal spectrometers are equipped with a set of fixed detection systems, where each system measures the radiation of a specific element. The principle advantages of WDXRF systems are high resolution (typically 5 – 20 eV) and minimal spectral overlaps. [16]

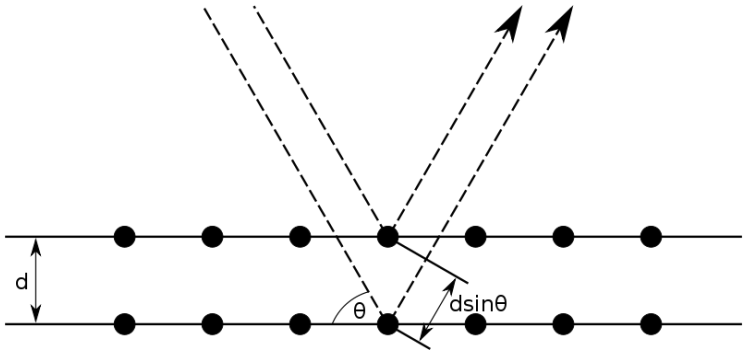

Figure 12 Bragg's diffraction and angle θ. [17]

Figure 12 shows a simplified picture of Bragg's diffraction and angle.

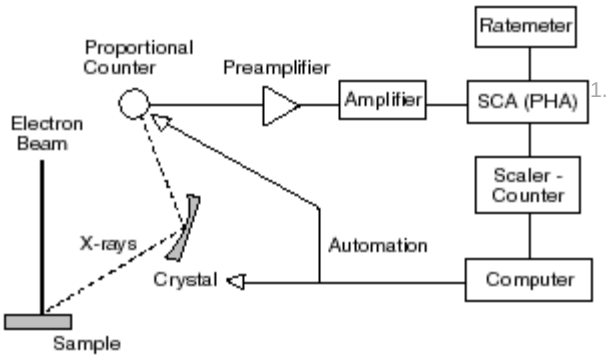

Figure 13 Wavelength-dispersive spectrometer (Goldstein et al. 1981) [18]

In figure 13 a rather simple and schematic version of WDS is shown. The main part is a positioning goniometer, that is, a mechanical device for angle measurement and for rotation of the object precisely about a fixed axis in space. It consists of two collimators, a Bragg crystal with a known interplanar spacing and an X-ray detector, mostly a gas-filled proportional detector. X-rays coming from the sample can be reflected from the crystal at a certain glancing angle and detected at twice this angle if their wavelength meets Bragg's law:

$$2d \sin\theta = n\lambda \qquad (2.13)$$

Energy dispersive spectrometers

In Energy Dispersive X-ray Fluorescence (EDXRF) spectrometers, all of the elements in the sample are excited simultaneously, and an energy dispersive detector in combination with a multi-channel analyzer is used to *simultaneously* collect the fluorescence radiation emitted from the sample and then separate the different energies of the characteristic radiation from each of the different sample elements. Resolution of EDXRF systems depends upon the detector, and typically ranges from 130 eV to 600 eV. [30]

The principal advantages of EDXRF systems are their simplicity, fast operation, lack of moving parts, and high source efficiency. The comparison of the WDXRF and EDXRF is provided below [19]:

WDXRF	EDXRF
+ high energy resolution	+ simply built
+ less spectral overlaps	+ fast operational
+ better peak to noise reduction	+ lack of any moving part in construction
+ high source efficiency	+ wide area of fluorescence radiation
	+ none or little sample preparation
	+ lower operating costs
- small area of fluorescence radiation (concentrated beam)	- poor spectral resolution
- time consuming (sequential analysis)	- peak overlaps
- complex mechanical design	- weak detection of light elements
- high operating costs	- complex cooling system (with liquid nitrogen)
- complicated sample preparation	- significant background noise

Because of the simplicity, speed, and convenience of operation the EDXRF is adapted for nearly all total reflection X-ray fluorescence (TXRF) instruments.

2.2.1. TXRF (Total reflection analysis and instrumentation)

The Total Reflection X-ray fluorescence analysis is an energy dispersive X-ray fluorescence method taking advantage of the reflectivity of the X-ray beam under small incident angles.

The main difference between common EDXRF spectrometer and TXRF spectrometer lies in the position of the radiation source and the detector. For TXRF spectrometer, the detector is placed over the sample and the radiation tube with the help of the monochromator is producing the X-ray beam that is on a glancing angle (near to critical angle) towards the sample carrier.

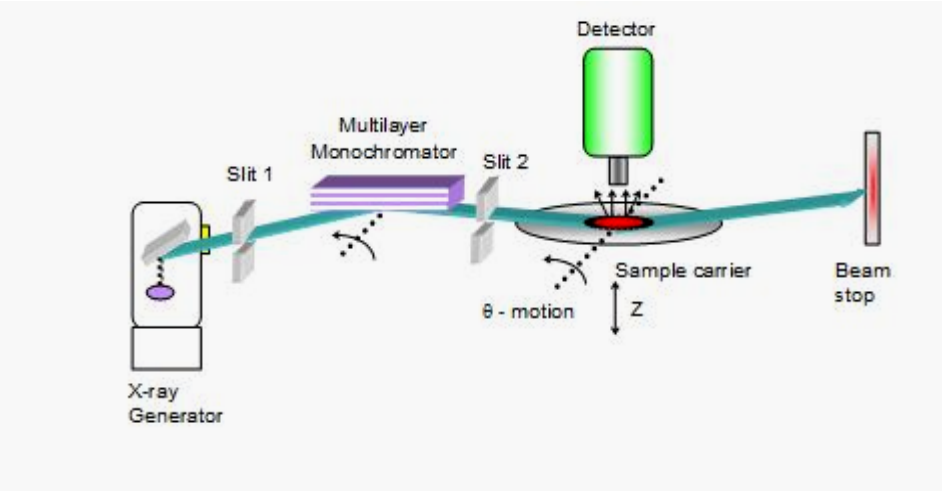

Figure 14 Total reflection X-ray fluorescence spectrometer [20]

The schematic reproduction of the setup of the TXRF system is shown in figure 14. The main feature can be also deduced from the same figure: the absorption of X-rays by the sample at present angle is minimal.

In general, TXRF is a variation of the XRF analysis with a special geometry. A TXRF setup holds three major advantages:

1. The primary beam hits the reflector at a very shallow angle against the sample, reflects from the sample carrier and the reflected beam passes through the sample again. This leads, thereby, to a double excitation and, therefore, to double the intensities. The *reflectivity R* increases close to 100% below the critical angle θ_C.
2. Due to the very shallow angle of incoming and reflected beam, the detector can be placed very close to the sample carrier (just a few mm away), whereby the absorption in the path between sample and detector decreases and the solid angle, seen by the detector, increases.
3. Since the beam experiences total external reflection, it penetrates the reflector only a few nanometers. Therefore, the reflector material will not be scattered as much, which results in very low background radiation.

Principle of total reflection

In a homogeneous medium, the X-ray beam behaves just like a light beam and follows a straight path on which the photons travel.

The difference between visible light beam and X-ray beam can be explained through refraction index. The index of refraction, as stated in Britannica vocabulary, is a measure of the bending of a light beam when passing from one medium into another. [21] The refractive index for light in the air equals 1,0003 and for vacuum it is 1. For X-ray the situation is a little different, the refractive index of the X-ray beam is marginally smaller than 1.0. It means that the velocity of X-rays in materials is greater than the velocity in air / empty space. For visible light beam true is the exact opposite.

The figure 15 graphically represents refraction, critical angle and total internal reflection of the incident ray.

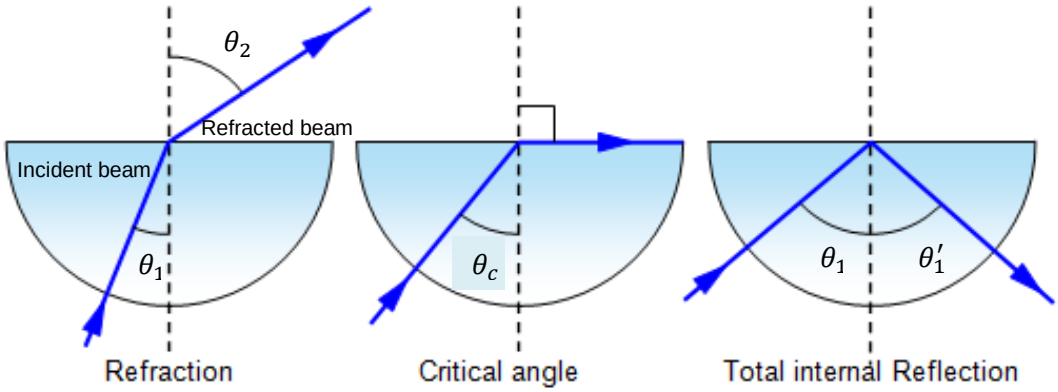

Figure 15 Different beam behaviours dependent on the incident angle (modified) [22]

Several known optic rules that are also valid for X-ray beam:

- The incident, reflected and refracted beams should exist in the same plane: the plane should be normal to the boundary plane.
- The glancing angles of the incident and the reflected beam are equal.

$$\theta_1 = \theta_1' \qquad (2.14)$$

- The glancing angles of the incident and the refracted beam follow Snell's law:

$$n_1 \cdot \sin\theta_1 = n_2 \cdot \sin\theta_2 \qquad (2.15)$$

where n is a refractive index, essentially it is a dimensionless number that describes how light propagates through medium (Formula 2.16):

$$n = \frac{c}{v} \qquad (2.16)$$

c – is speed of light in vacuum, v – is speed of light in medium. The refractive index determines how much light is bent, or refracted. Light propagation in absorbing materials is described by a complex-valued refractive index. The imaginary part then handles the attenuation, real part accounts for refraction. [23]

As mentioned above, the *R (reflectivity)* increases close to 100% below the critical angle θ_C. The relation between reflectivity and glancing angle for Mo–Kα is shown in the Figure 16. There are three elements calculated in this Figure: Si, Ni and Pt. X-ray were produced by Mo-K_α.

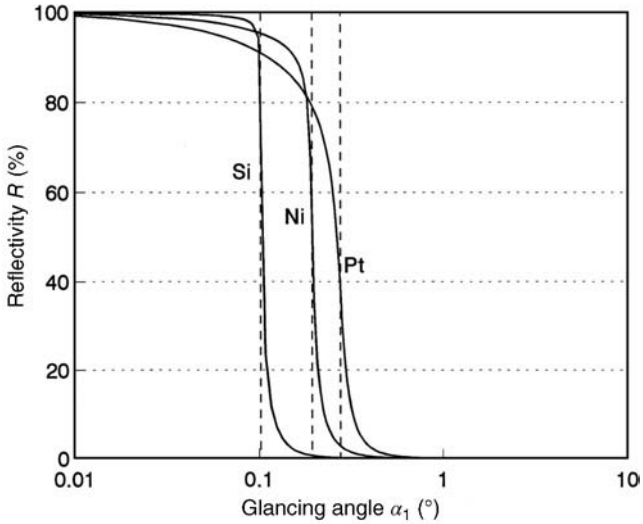

Figure 16 Dependence of the reflectivity on the glancing angle. [9]

If the glancing angle is lower than 1° the reflectivity for Mo-Kα is on the extreme low level, therefore it can be neglected. On the other hand, at the critical angle, the reflectivity tends to 100%. At this point, the reflectivity is dependent on the absorption β. [9] This means, that even by total reflection of the X-ray, a small quantity of the beam will get absorbed. This fact should be taken in the account for refraction index calculations:

$$n = 1 - \delta - i\beta \qquad (2.17)$$

The reflectivity is also element dependent. There are less absorbing materials, like Si, and for those elements, the switch to glancing angle is more explicit [9]. That is the reason why quartz glass (Silicon dioxide – SiO_2) is likely to be chosen as a sample carrier.

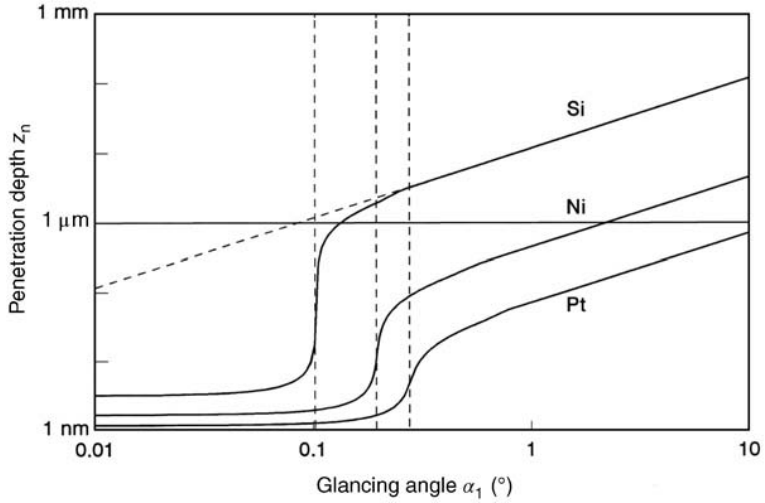

Figure 17 The penetration depth depends on the material and below the critical angle is just few nm. The radiation penetrates deeper into the medium, though through heavy elements it can penetrate less than the light elements. [9]

In the Figure 17 is shown the penetration depth that depends on the material and below the critical angle is just few nm. The radiation penetrates deeper into the medium, though through heavy elements it can penetrate less than the light elements.

Following approximations can be applied to the reflectivity:

$$a_1 \ll a_{crit}: z_n \approx \frac{\lambda}{4\pi}\frac{1}{\sqrt{2\delta}} \qquad (2.18)$$

$$a_1 = a_{crit}: z_n \approx \frac{\lambda}{4\pi}\frac{1}{\sqrt{\beta}}$$

$$a_1 \gg a_{crit}: z_n \approx \frac{\lambda}{4\pi}\frac{a_1}{\beta}$$

In different cases, penetration depth α is dependent on one of two coefficients: β or δ. β is inclined in a ratio to the mass-absorption coefficient of the matter and δ is a determinative number, which is based on atomic constant value of the matter. [9]

When the beam is totally reflected on a very smooth surface, the interference zone appears. It results from interference between incident (incoming) and reflected beam. In the Figure 18, this zone is presented as a red-yellow triangle.

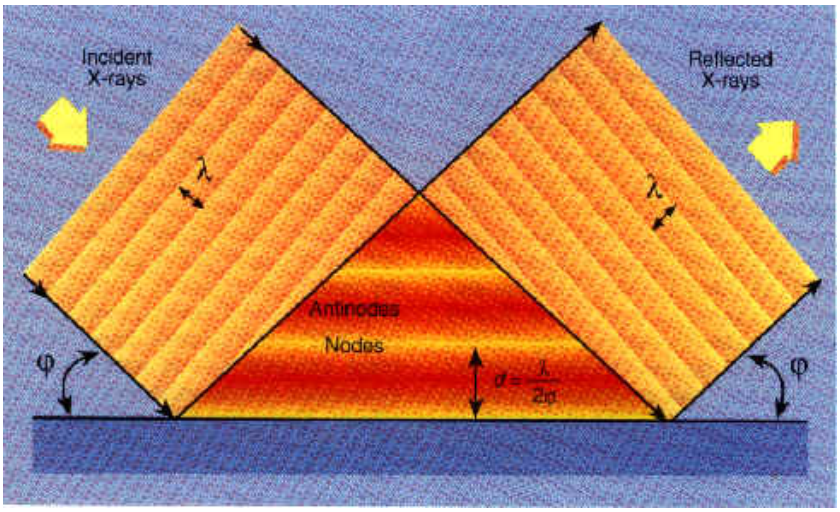

Figure 18 Interference of the incoming and the reflected X-ray. [24]

In this case, one will get at nearly four times of the original intensity. This feature helps TXRF method and in particular its special application - the Grazing Incidence X-ray Fluorescence Analysis (GIXRF) to operate with units of measurement as small as ppb (parts per billion).

2.2.2. TXRF instrumentation

Each (T)XRF construction is based on three core components: X-ray source, sample (sample holder), detection system. Furthermore, filters and monochromator are used.

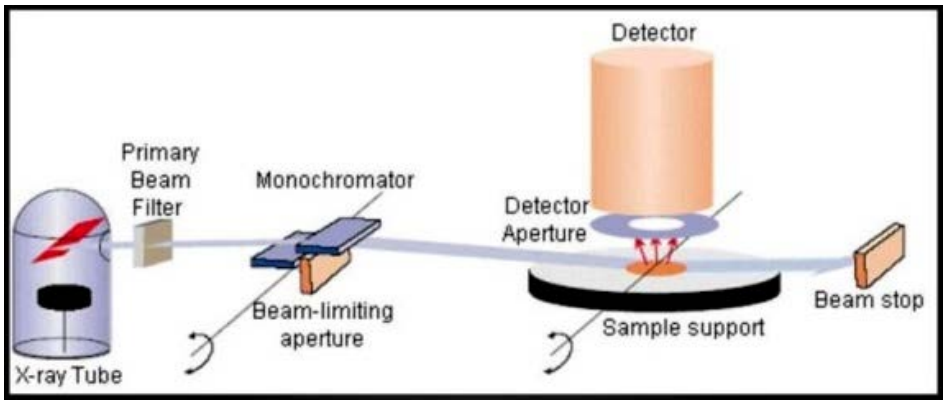

Figure 19 Basic design of the TXRF instrument. [25]

In the basic construction (shown in the Figure 19) of the conventional TXRF machine the polychromatic beam is produced by an X-ray tube, and then it goes through the primary filter to be collimated. Afterwards the beam is deflected by a monochromator, which alters the primary spectrum and allows selecting a specific X-ray energy for sample excitation. For some types of TXRF analysis, a simple quartz-glass block will be sufficient, as it will just cut off the high-energy part of the primary beam under the grazing incidence. However, for surface and thin layer analysis, the real monochromator is needed. For the measurements described in this thesis, a double multilayer monochromator with a fixed exit was used. After passing the monochromator, the monochromatic primary beam hits the sample carrier under

an angle smaller than the critical angle. The sample carrier is loaded with a small amount of a sample material: only 10 µl droplets.

The primary beam is reflected by the sample carrier and the sample is excited a second time. The X-ray fluorescence intensity from the sample can then be recorded with an energy-dispersive detector. The detector can be either a liquid nitrogen cooled solid-state (lithium-drifted silicon (Si(Li)) or a high-purity germanium (HPGe) detector (for high energy X-rays and gamma radiation), or a silicon-drift detector (SDD). For this particular work, the Si(Li) detector with 80 mm^2 active detection area was used.

The recording detector is mounted perpendicular to the carrier plane to obtain spectra with a minimum scattered background. In order to secure the detection of the fluorescence radiation within a large solid angle, the distance from detector to the sample can be reduced to less than 3 mm. A multichannel analyzer, leading to an energy-dispersive spectrum, then registers this intensity. Measurements can be carried out in ambient air, in helium or even in vacuum. Helium and vacuum are used to suppress or reduce the absorption (and the Ar peak in the spectrum) from the ambient air.

2.2.2.1. X-ray Tube

As mentioned above, to produce the X-ray beam an X-ray tube, radioactive source or a synchrotron is needed. In this study a Mo-W mixed alloy anode X-ray tube (2000W) was used.

A strip-like primary beam is needed for excitation, showing a high intensity in a certain spectral region. Therefore, a high-power X-ray source is mostly chosen for TXRF.

There are some different X-ray tubes: portable tubes, rotating anode tubes, X-ray tube with liquid anode, high-power tubes, and low-power tubes. All of them have some kind of anode and cathode. The cathode and anode are housed inside a glass “tube” and contained within vacuum [26].

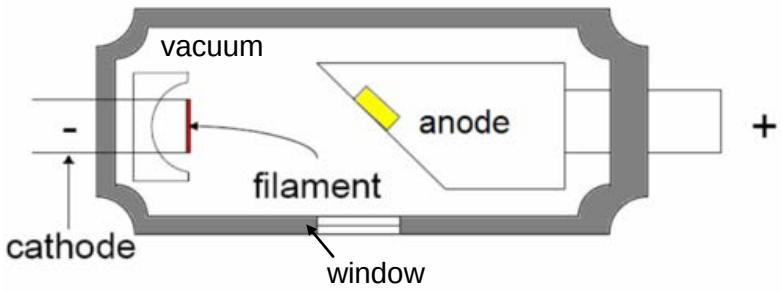

Figure 20 The X-ray tube [26, modified]

The filament is a very tightly wound coil of wire. When an electric current is passed through the filament, it heats up and emits electrons. When high acceleration voltage between cathode and anode is applied, the electrons get accelerated in vacuum and meet with the anode material with the $E_{kin} = e * U$. X-ray tubes produce about 99% heat and 1% radiation (characteristic and Bremsstrahlung). The radiation beam can leave the X-ray tube through the beryllium window and be used for the experiment.

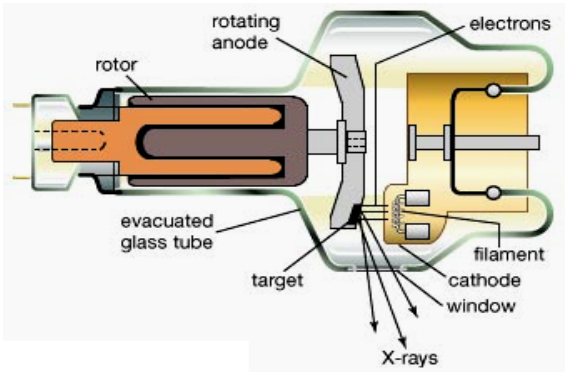

Figure 21 X-ray tube with rotating anode. [26]

Both the Bremsstrahlung and the characteristic radiation are produced by the anode; therefore there is a wide range of energies available for the measurement. Figure 22 shows a characteristic spectrum of a Mo X-ray tube. The two massive peaks can be undoubtedly seen: Mo - Kα and Mo - Kβ. The acceleration voltage was 45 kV, so the spectrum ended at 45keV. On the spectrum it can also be seen the *Bremsstrahlung*with the maximum at around 12 keV.

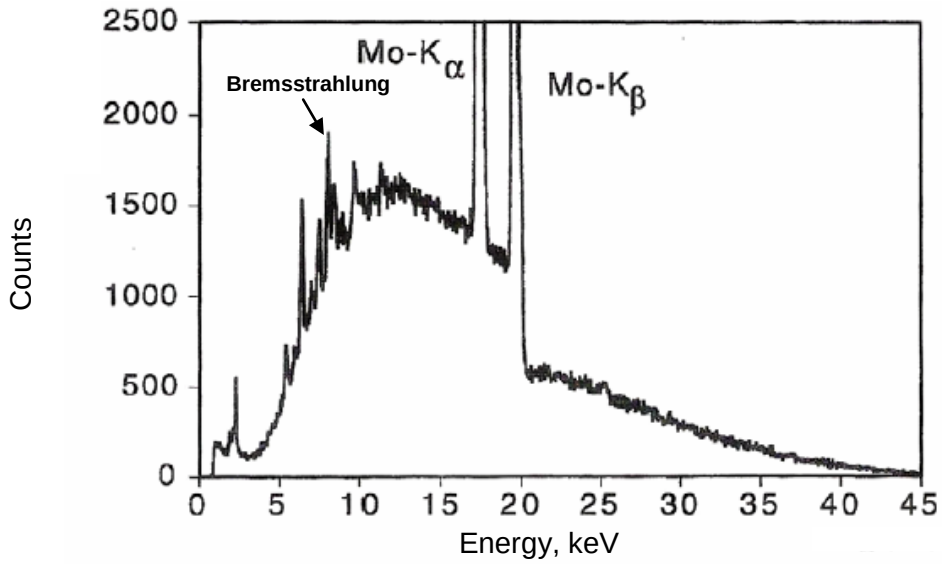

Figure 22 Characteristic spectrum for Mo X-Ray tube. [27]

A spectrum can display an intensity-distribution as a function of channel number, energy, wavelength or frequency. The spectral distribution depends on: take-off angle, applied high voltage and current.

2.2.2.2. Spectral modification

Spectral modification optics can include filter, secondary target, polarizer, high energy cut-off, monochromator, and focusing optics. Entire optics are meant to create a monochromatic beam with marginally possible background noise.

In this master's thesis, a multilayer monochromator with large bandwidth (100 eV) was used to extract the particular energy (for example, K-alpha energy of the X-Ray tube) from the characteristic spectrum of the Mo X-Ray tube. This step results in a small rectangular beam

(around 50 micrometer to 10 millimeter) with particular (only one) energy. For the sample, it means irradiation with very high intensities.

2.2.2.3. Sample

The sample preparation is considered being rather easy for the TXRF analysis. It depends, however, on the experiment design and requirements. Samples in a liquid form are perfect to analyze. Therefore, all the samples are preferably translated into liquid / suspension / solution. For the dissociation, triple distilled water or acids can be used. More about the sample preparation performed for this study can be found in the Chapter 5.3.

2.2.2.4. Detector

The X-Rays are detected by transforming the energy of the photons into electrical pulses available for further processing. For energy dispersive detectors, the incoming (fluorescence and scattering radiation) photon is absorbed in the active volume of the detector and its energy is proportional to the energy of the generated pulse. Generally, there are two distinct types of ED silicon detectors: Si(Li) and drift detectors.

- Si(Li) detectors (Solid State Detector - SSD)

In the EDS system the X-ray photons emitted from the sample are directly collected by a semiconductor detector. The schematic representation of it is shown in Figure 23. This special detector allows determining different energies of the entering photons and not only counts the individual photons. The semiconductor detector crystal has a P-I-N structure, where P stands for positive dopants such as the boron impurities, and N stands, respectively, for negative dopants in the Silicon or Germanium crystal. Boron is one of the most commonly appearing additives in the detector crystals and it influences sensitivity and conductivity of the whole detector. To compensate the drop of resistivity and raise of conductivity, the lithium impurities are added. Lithium is usually diffused into the crystal (it is shown in the name of the detector – Si(Li)) and is sensitive to the electric field, change of which allows it to “move” across the detector. And finally I is the intrinsic region and is susceptible to ionizing radiation. This region is also called the active volume, where electric field is applied due to reverse biasing. Due to relatively small band gap of the semiconductor the detector must be cooled. Cooling will prevent thermal generation of charge carriers in the intrinsic area and is usually done with liquid nitrogen (LN2). When absorbed in this region, the photon will produce photoelectron, Compton- or Auger electron. The number of electron hole pairs is proportional to the incoming photon. All the detected pulses are processed in an electronic measuring chain and finally sorted by a multichannel analyzer. The particular X-ray spectrum represents a content of this counter storage and can be observed already during the measurement. Afterward it can be processed directly by particular software on a dedicated computer.

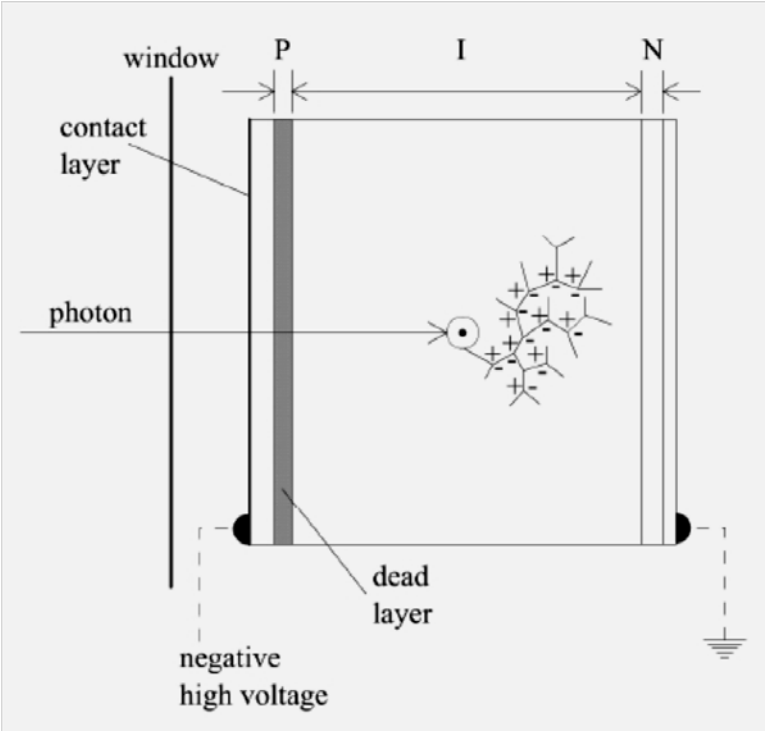

Figure 23 Semiconductor detector crystal for Si(Li) detector. [28]

It is necessary to protect crystal against moisture and other possible contaminations, which would otherwise all gather on the cooler detector surface. The contamination of the detector (also ice layer) would drastically reduce its resolution and sensitivity. The protection is usually done by implementing a thin Be (or other element with low Z number) window, which is around 10 to 100 µm thick. This measure is necessary for measurements in vacuum.

- Silicon drift detector (SDD)

These detectors are used mostly when high resolution and count rate are needed.

Same as semiconductors, drift detectors also must be cooled during the work. Usually a thermoelectric cooler is used; the detector chip is mounted directly on top of it (Figure 24).

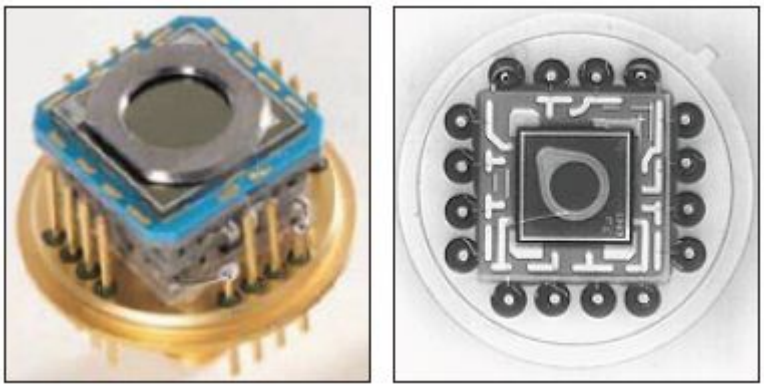

Figure 24 Commonly used SDD with usual mounting system. [29]

Nowadays, drift detectors have extreme small anode in relation to the rather big active area. On the backside there is a number of p – type rings and on the front a thin homogeneous layer (usually gold is used). The gold layer works as a cathode while switched to a negative

voltage and the rings on the other side also have negative voltage, which is increasing towards the outside. [9, 29] The schematic representation of SDD is shown in the Figure 26. The path of electrons is also shown; its trajectory towards the anode is possible due to the voltage difference in the rings.

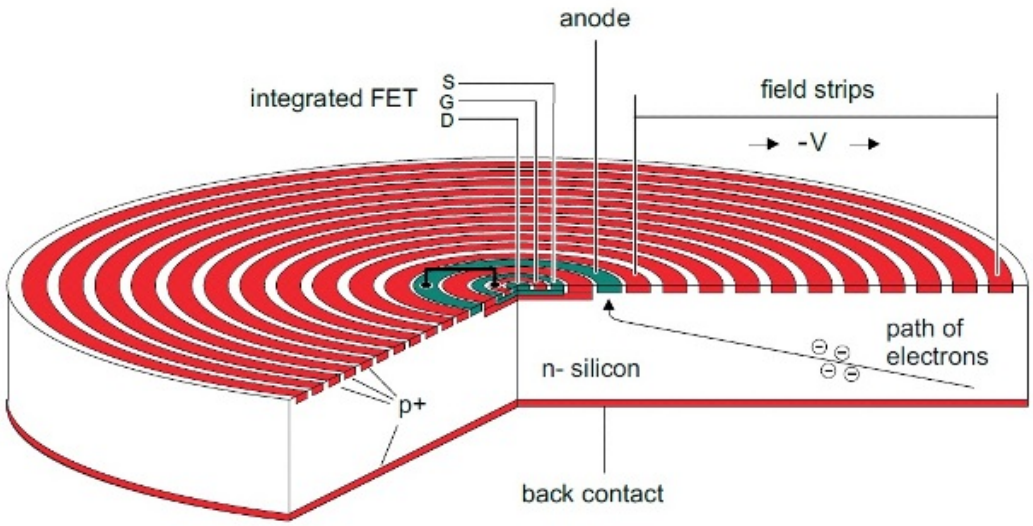

Figure 25 SDD. [29]

2.2.2.5. Artifacts

During the measurement, the detector collects and distinguishes at the same time different characteristic energies radiated from the sample. The whole detection system is commonly connected with the computer with analytical software, which is processing the collected data into a spectrum. In perfect case, each peak is representing separate element, contained in the sample. In reality, one needs to control each peak and consider whether it is not an artifact. Artifacts can generally occur during X-ray detection and processing afterwards [30]. Most common artifacts are the incident radiation peak from the source tube, escape peaks and sum peaks, due to elastic and inelastic scattering.

- Incident radiation peak

This artifact can originate from the source tube. In this master's work, this artifact resulted into a broad molybdenum peak, because the Mo Kα radiation was used.

- Escape peaks

Escape peak is a problem of rather strong signals recorded by the detector. It means that lower intensities of particular characteristic radiation are likely not to cause a visible escape peak. The situation is different for higher intensities. When such a photon is hitting the detector, there is a chance that the incident energy is high enough to produce a photoelectron within the detector. Consequently, the excited atom of the sensor material can produce an X-ray photon, which will be normally reabsorbed and detected as all the others, although, if the photoelectron is created near the edge of the detector it can escape the detector and the energy registered will be the energy of the original characteristic photon minus the energy of the Kα photon of Silicon. Position of the escape peak will be dependent on the position of the original peak it was created from. [9, 30]

- Sum peaks

This artifact happens due to pulse processor resolution limits. When two characteristic X-rays are arriving with too short time difference (shorter than a minimum, which is possible to record by a processor), the signals are just piling up and result in a single plotted sum peak in the spectrum instead of two separate peaks. [9, 30]

2.2.3. Qualitative and Quantitative analysis

As a result of the measurement, a spectrum will be generated with all the system measurable elements in it that the sample consists of. The position of the peaks is nothing more than energy being proportional to the element order number Z (order in the periodic table of the elements). Intensity – the height of the peaks – is dependent on the amount of the element in the sample (mass of the sample). It means one can examine the sample qualitatively (which elements can be obtained from the examined sample) and quantitatively (how much of each element is there exactly in the sample).

The qualitative analysis

It is the "easier" part of the analysis, because every element from the periodic system of elements (except for Hydrogen and Helium [31]) emits its individual characteristic X-ray fluorescence radiation. These characteristic energies are then mostly (as mentioned above, there is still a necessity for control in case of the artifacts) evaluated and interpreted by the processing software. Such a simple method is, therefore, very beneficial for the fast elemental analysis, when it is important to see the whole elemental composition of the unknown sample without destroying it [9].

The quantitative analysis

The more "complicated" part of the TXRF analysis. The intensity of the characteristic fluorescence radiation I can be presented with the following formula:

$$I\left(E_{K_{\alpha,i}}\right) = \int_{E_{Edge,i}}^{E_{max}} \int_{0}^{d} I_0(E) \cdot e^{-\mu(E)\cdot\frac{1}{sin\varphi_1}} \cdot c_i \cdot \tau_i(E) \cdot \frac{x}{sin\varphi_1} \cdot \left(1 - \frac{1}{S_{K,i}}\right) \cdot \omega_{K,i} \cdot p_{K_\alpha,i} \cdot \frac{1}{4 \cdot \pi} \cdot e^{-\mu(E_{K_{\alpha,i}})\cdot\frac{x}{sin\varphi_2}} \cdot f \cdot \epsilon\left(E_{K_\alpha,i}\right) \cdot dE \cdot d\Omega_1 \cdot d\Omega_2 \cdot dx \quad (2.19)$$

Where:

i	index for the characteristic line of the corresponding element
$E_{K\alpha,i}$	energy of the Kα beam from the corresponding element
$E_{Edge,i}$	absorption edge energy
d(x)	sample thickness
$\boldsymbol{I_0(E) \cdot d(E)}$	spectral distribution
τi(E)	photoelectric mass absorption coefficient of the K-shell
ωK,i	fluorescence yield for the K-shell
$\boldsymbol{p_{K_\alpha,i}}$	emission probability for Kα
ci	concentration of the respective element
$\boldsymbol{d\Omega_1, d\Omega_2}$	geometrical parameter

ϕ	angle at which radiation falls on the sample
$\boldsymbol{\mu(E)}$	attenuation coefficient
$\boldsymbol{f}$	factor to cover the absorption between sample and detector
$\boldsymbol{\epsilon}$	detector efficiency

The formula is integrated through the sample thickness and excitation radiation. [32]

It is possible to combine all the geometrical parameters in one geometric factor G:

$$G = \frac{d\Omega_1 \cdot d\Omega_2}{4 \cdot \pi \cdot sin\varphi_1} \cdot f \tag{2.20}$$

Q is a sum of the fundamental parameters which are dependent only on the basic physical properties:

$$Q_{f,i}(E) = \tau_i(E) \cdot (1 - \frac{1}{S_{K,i}}) \cdot \omega_{K,i} \cdot p_{K,i} \tag{2.21}$$

There are several special cases to consider for calculation of the intensity of the characteristic fluorescence radiation. Those would be: monochromatic radiation, endless thick and endless thin samples.

For this diploma thesis, the monochromatic Mo–Kα radiation was used. It is impossible to create a completely monochromatic radiation, but the multilayer monochromator can reduce the bandwidth to a small beam, which could be considered monochromatic. For the assumed monochromatic ray, the integral over the energy is not necessary and therefore it is convenient to try to create a monochromatic beam by any means when the quantitative analysis should be carried out.

In this project, the infinite thin sample or the thin layer approximation was used. It means, that the sample is assumed to be so thin, that there is no absorption of the X-ray radiation by the sample at all. In this case, the intensity can be calculated as follows:

$$I\left(E_{K_{\alpha,i}}\right) = G \cdot \epsilon(E_{K_{\alpha,i}}) \cdot I_0(E) \cdot Q_{f,i}(E) \cdot c_i \tag{2.22}$$

Out of the equation 2.23 it is evident that concentration of an element *i* and its intensity is directly proportional to the element *i* sensitivity S_i:

$$c_i = \frac{I_i}{S_i} \tag{2.23}$$

The sensitivity depends only on the fundamental parameters and the measurement parameters. If the experiment setting and environment are constant and an internal standard with known concentration ($c_{stand.}$) is added, then the concentration of the unknown element *i* can be calculated as:

$$c_i = \frac{S_{\text{stand.}}}{S_i} \cdot \frac{\mathrm{I}_i}{\mathrm{I}_{\text{stand.}}} \cdot \mathrm{c}_{\text{stand.}} \tag{2.24}$$

Abovementioned proves the TXRF having a rather simple quantification procedure, which makes this method quite popular for different research fields, particularly for trace elements analysis.

Figure 26 shows a typical calibration curve. This particular plot was done for Sulfur with four different concentrations (represented by black dots). The concentrations are aligned in almost a straight line. On the x-axis is: $\frac{I_{S\,K_\alpha}}{I_{Ti\,K_\alpha}} \cdot c_{Ti}$ - number of counts for Sulfur divided by number of counts for internal standard Titanium multiplied with concentration of the standard.

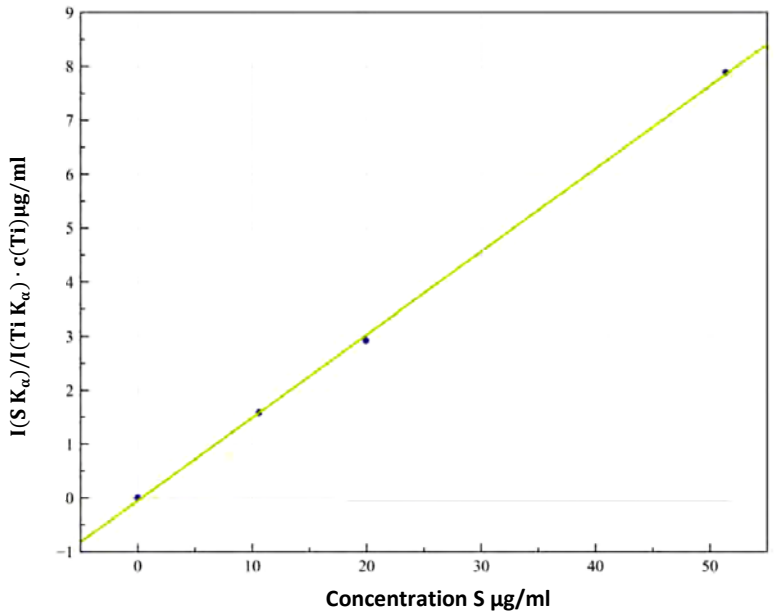

Figure 26 Calibration curve for Sulfur. [33, modified]

The sensitivity parameter $\frac{S_{stand.}}{S_i}$ (equation 2.24) is calculated through the calibration curve. Figure 27 shows a relative sensitivity curve over atomic number of the elements. In the graph are present Fluorine (F), Sodium (Na), Magnesium (Mg), Aluminium (Al), Phosphorous (P) and Sulfur (S). It is evidential, that with the higher atomic number, the relative sensitivity of an element is drastically increasing.

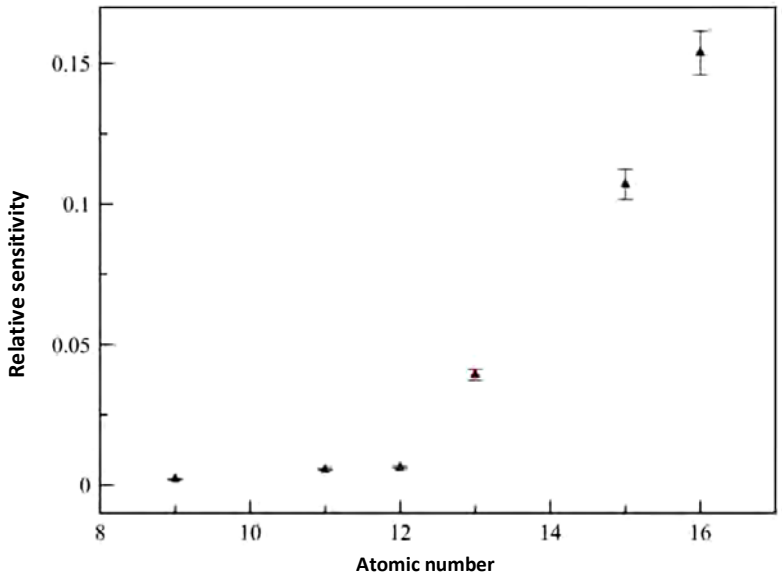

Figure 27 Relative sensitivity of the calibrated elements. [88, modified]

For this Master's thesis, there was no additional calibration necessary as the Atomika 8030C was already calibrated.

2.2.4. Fields of application

TXRF has a wide range of applications. This method has become increasingly popular in micro and trace elemental analysis [25]. In the list below are presented the most known application of TXRF spectroscopy [23]:

- ***Industrial/Technical applications***: Si-wafer surfaces, GaAs-wafer surfaces; implanted ions depth and profile variations; thin films single layers, multilayers; crude oil, fuel oil, grease; chemicals (acids, bases, salts, solvents); fusion/fission research (transmutational elements in Al + Cu, Iodine in water)
- ***Mineralogy***: ores, rocks, minerals, rare earth elements, etc.
- ***Fine Arts / Archeological***: pigments, paintings, varnish; bronzes, pottery, jewelry; textile fibers, glass.
- ***Forensic***: Dollar bills, gunshot residue, drugs, tapes, sperm, finger prints.
- ***Environmental applications***: water (rain, river, sea, drinking water, waste water…); aerosols, airborne particles, dust, fly ash; soil [36] sediments, sewage sludge; plant material such as algae, hay, leaves, lichen, moss, needles, roots, wood; coal, peat.
- ***Foodstuff***: fish, flour, fruits, crab, mussel, mushrooms, nuts, vegetables [35], wine, tea [36, 37]; cognac.
- ***Medicine / Biology / Pharmacology***: body fluids, for example: blood [38, 39] and blood serum, urine, amniotic fluid; tissue like kidney, liver [40], lung, nails, stomach, colon and hair [39].
- ***Various***: enzymes, polysaccharides, glucose, proteins, cosmetics, bio-films.

3. Medical applications of tea and herbal infusions.

Let food be thy medicine and medicine be thy food.

Hippocrates, father of medicine, 431 B.C.

For thousands of years, herbal infusions have been consumed for medicinal reasons. China is deemed to be the country of origin. The first historical records appear in the earliest Chinese Pharmacopeia, which is attributed to the Chinese emperor Shennong (around 3,000 B.C.). In the 1st century A.D., the Greek physician and pharmacologist Dioscorides describes more than 600 medicinal plants, which may be used for preparation of infusions in his book "De Materia Medica" (literally medicines). In the course of the past centuries, herbs and fruits have been adopted in sometimes even unusual manner to get relieve for certain symptoms of different illnesses. The consumption of herbal and fruit infusions – pure or mixed – just for enjoying the taste has become very popular in the recent history. Resulting from an increased health awareness of the consumers, drinking herbal and fruit infusions started to boom in the 1980's. [41]

3.1. History of usage and current situation

The first written evidence of using medical plants for preparation of drugs is approximately 5000 years old and was found on a Sumerian clay slab from Nippur. The writing contained 12 recipes for drug preparation using over almost 250 various plants (poppy, henbane, mandrake, etc.).

The most known medical writer of the ancient history, Dioscorides, in his work "De Materica Medica", describes 944 drugs, 657 of them have a plant origin. Dioscorides describes the outward appearance, locality, mode of collection, making of the medicinal preparations, and the therapeutic effect of the plants. The plants having mild effect are dominant, but there are also references to those containing alkaloids or other matter with strong effect (fragrant hellebore, false hellebore, poppy, buttercup, jimson weed, henbane, deadly nightshade). [42] The most appreciated domestic plants with mild effect were willow, chamomile, garlic, onion, common mallow, ivy, nettle, sage, common centaury, coriander, parsley, sea onion. They were used to cure wounds, stings, burns, and ulcers; for cleansing and rinsing the eyes, ears, nose, and mouth; to relieve headache and stomach ache; some were used as diuretics, some for gynecological purposes, and as antipyretics. [42]

The end of 19th and early 20th centuries were marked with the development of technologies allowing sophisticated chemical production of antibiotics and other synthetic medicines. Therefore, the usage of raw herbal material for preparation of medicines was left with less or no interest and was endangered to be eliminated. However, at the beginning of the 21st century a significant increase in herbal medicine production occurred. For example, in China the total value of herbal medicine manufactured in 1995 reached approximately 2.5 billion US$ and in 2015 sales totaled in around 14 billion US$ [43]. This substantial change can be related to the increased attention towards so-called healthy lifestyle. The problems like

obesity and depression can be eliminated with engaging a healthier lifestyle, eliminating bad habits and on-time medical controls; even a longevity can be achieved by sport activities and diet change - these are the most popular and promulgated ideas on TV, Internet and Radio for the last decade. The market is filled with production that is labeled BIO or Organic. It can be expected that due to these tendencies, more consumers are directed towards herbal medicines.

To the 31st of December 2013 there were 1319 traditional herbal medicines registered and 622 herbal medicines authorized in European Union. The European Medicines Agency [44] provides the list of 184 herbal medicines (in form of leaves, roots, seeds, flowers, oils, etc.) for human use.

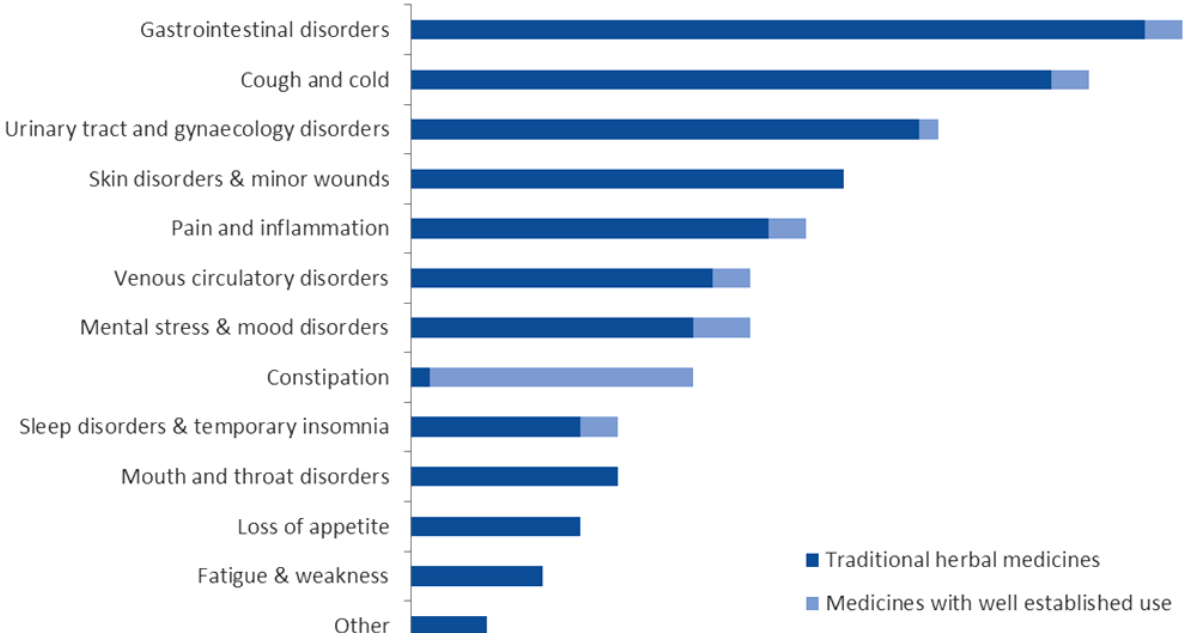

Figure 28 The use of herbal medicines for the time period of 2004 to 2014. [44]

The Figure 28 shows a statistical graph representing use of the herbal medicines separated in the fields of application over the time period from 2004 till 2014 in the European Union. The data was presented by the European Medicines Agency [45]. It becomes indisputable that herbal medicines are increasingly in demand and in some fields of application are even more favored than medicines with well-established use.

3.2. Medical properties of plants

As already mentioned above, herbal medicines are used since ancient time and over the years, even having ups and downs, they do not lose their popularity. The wisdom of the ages in usage of herbal medicines is corroborated by present-day knowledge. Plants display broad spectrum of pharmacological activities, including antimicrobial, anti-inflammatory, adaptogenic, stimulatory and sedative action [43, 46]. They are used as cholagogic, hypotensive, capillary-enforcing, antiulcer, anticholinesterase inhibitors, anticancer, spasmolytic, analgesic, and analeptic medications [46]. Herbs and phytopreparations are effective in therapy and prevention of various human diseases, e.g. cardiovascular and nervous diseases, gastrointestinal disorders and dermatological conditions, and even

malignancies [43, 46]. They are declared to serve as a treatment and prevention of diseases, health promotion and for enhancement of the span and quality of life [43].

The use of herbal medicine is associated with a number of advantages, as opposed to synthetic drugs. Due to complex composition herbs are characterized by broad range of activities, softer effect and less aggressive side effects. Therefore, plant medications can be administered over longer period of time, which is especially beneficial in therapy of chronic conditions in order to achieve a successful treatment results and overall health enhancement. Furthermore, the advantages of rather low price and wide availability should be mentioned, including the possibility to get most medicinal herbs without prescription.

Herbal material has complex chemical composition, and the medical properties of plants result from the sum of those physiologically active substances (PAS). Out of rather large list of possible PAS in the plants, several groups are of particular interest due to their therapeutic effects. Among them are alkaloids, terpenoids (triterpene and steroid saponins), phenolic compounds, glycosides (cardiac glycosides), and polysaccharides. For example, phenolic compounds are present in medical plants in different concentrations and for some plants are defining the mode of therapeutic action. Even for a plant effects of which are related to other types of PAS, phenolic compounds determine the specific properties that differ from those of synthetic drugs with the same therapeutic effects [46]. The methods of extraction of specific PAS (vitamins) or the sum of several PAS are studied intensively. There are papers reporting studies for vitamin extraction, Omega 3 and 6 essential fatty acids, flavonoids and many other physiologically active substances of organic nature. [46, 47]

It becomes evident, that there is a wealth of experience and expertise in organic compounds of herbal material, including array of established methods of qualitative and quantitative analysis. However, at the same time, the mineral composition of plant is downplayed or overlooked.

3.3. Quality control methods (according to the World Health Organization guidelines)

The World Health Organization (WHO) has established an international document, where recommended quality control methods for herbal materials are compiled. [48] The document does not perform the imperative function but provides rather an informative suggestion. The manual describes a series of tests for assessing the quality of medicinal plant material. The tests are designed primarily for use in national drug quality control laboratories in developing countries, and complement those described in the international pharmacopoeia. Following tests are included:

- Thin-layer chromatography
- Determination of extractable matter
- Determination of volatile oils
- Determination of bitterness value
- Determination of tannins
- Determination of swelling index
- Determination of foaming index
- Determination of haemolytic activity

The methods listed above refer solely to the analysis of organic compounds (either non-specified or particular group of active substances) and are obligatory according to the pharmacopoeia. The mineral composition (intrinsic micro- and microelements of the plant) or mineral contaminants are assessed in following tests:

- Determination of ash ("physiological ash", sum of mineral components of the plant itself; "non-physiological" ash, which is the residue of the extraneous matter, e.g. sand and soil, adhering to the plant surface)
- Determination of arsenic and toxic metals
- Radioactive contamination

The determination of ash test is an obligatorily performed method, which is not showing the whole range of elements though. The other two tests are also directed towards few particular metals. Also all three verification methods are so-called *exclusion tests*; they are focused on what should not be found in the herbal material.

It can be observed that the routine tests for thorough and complete element composition are not being performed. The lack of systematic approach and controlling systems for determination of "obligatory" elemental composition of a particular plant or the mixture of plants can be noted.

The properties evaluation of the herbal medicines should cover at least aspects such as selection and handling of raw material, safety, efficacy and stability of the finished product. The mentioned processes include a wide range of diverse scientific investigations. Most commonly included are physical, chemical and biological evaluations that are employing various analytical methods and tools. [49] Physical evaluation includes visually identifying the plant; microscopic evaluation – detailed identification and crude test for impurities; biological evaluation involves tests on living animals; chemical evaluation covers screening; isolation; identification and purification of the chemical components. The chemical screening or tests may include color reaction verification, which helps to determine the identity of the drug substance and possible adulteration. In the cases when previous techniques are not applicable the chromatographic tests are performed [49].

3.4. Analysis of elemental composition of plants

There is number of compelling arguments in favour of detailed examination of mineral composition of plant material and herbal medications:

1. Mineral metabolism in plants is inextricably connected with production and/or accumulation of organic substances.

The PAS metabolism includes primary precursors, assimilability, active transport, activation or inhibition of enzymes catalyzing biosynthesis, and compartmentalization. Therefore, underlying mechanisms of these molecular regulations are of considerable interest. Various metabolic stages are needed for PAS isolation and accumulation and they become possible due to compartmentalization. Metals, which are playing the role of cofactors or activators of enzymes and catalyzing various stages of natural compound metabolism, can modulate these processes.

Co, Zn, Fe, Cu, Mn, and Cr are substantially present in a plant and play an important role as cofactors or activators of enzymes for accumulation of PAS of various types (alkaloids, phenolic compounds, terpenoids, etc.). [46]

2. Macro- and microelements of plants as contribution to dietary uptake.

Most of the necessary macro- and microelements that are needed for normal functioning of the human organism are obtained through the everyday diet. Therefore, it is highly important to know and understand which elements and in what amounts are daily consumed.

For example, the study conducted in the United Kingdom determined that black tea drinking is a major source of dietary Mn and intakes commonly exceed proposed adequate values of 1.8–2.3mg Mn/day and, on occasion, even surpass upper limits of 10–11 mg/day. [50] One Latvian study of some indigenous berries showed that even though some of them had relatively low concentrations of inorganic elements, the consumption of these kinds of fruits may serve as a good source for some elements, since they contain some essential elements in higher quantity, which amounts to 15% or higher rate of the Recommended Dietary Allowances (RDA) or Dietary Reference Intake (DRI). For instance, blueberry pro 100g had higher amount of molybdenum – around 37.4% of the daily need and bilberry pro 100g had manganese at around 94.1 % of the daily need.[51] The USA study on mineral analysis of commercially available teas determined the low levels of Na. Therefore, those herbal infusions can be labeled as ‘‘Very Low Sodium’’ (35 mg Na or less per 236 ml) and can be included in a low-Na diet. R.N. Gallaher et al suggest it would be useful to include the more popular varieties in the national food consumption surveys to determine levels at which commercial herbal infusions are being consumed. [52]

3. Mineral compounds can contribute to pharmacological properties of the plants and produced herbal medications.

There are several studies devoted to one particular element in a single plant or common dietary product. Several investigations were done to prove the medical properties of a plant and its feature to accumulate useful elements. The paper from New York Cancer Institute states that there is evidence of sulfur (S-methylcysteine), which can be found in garlic, being protective against cancer. Scientists also claim, that selenium (Se-methylselenocysteine), which is biochemically similar to sulfur, could be more efficient for the same cause. In the presented study, usual domestic garlic was compared to the selenium enriched one. The results showed a better performance of selenium enriched garlic compared to other samples, which allows suggesting a higher potential of it in cancer protection. [53] The research on Se in plants was also done by John W. Finley in his paper: “Reduction of Cancer Risk by Consumption of Selenium-Enriched Plants: Enrichment of Broccoli with Selenium Increases the Anticarcinogenic Properties of Broccoli”. Se from high-Se broccoli decreased incidence of aberrant crypts in rats with chemically induced colon cancer by more than 50%, compared with controls. Results suggested that development of methods to increase the natural accumulation of Se in broccoli may greatly enhance its health promoting properties. [54] Silicon concentration and its application for bone disorders treatment is mentioned and investigated in a paper of M. H. Fernandes et al. They demonstrate that Equisetum arvense or common horsetail (quantity of Si in the plant is up to 25% dry weight) can negatively modulate osteoclastogenesis, in other words common horsetail can be used as a treatment for bone disorders characterized by a hyperactivation of osteoclasts. [55]

Those are just some of the published papers that are dealing with minerals in plants. There is an on-going research on data for specific elements in selected plants and other natural sources: “Chemical and medico biological properties of chaga” by M. Ya. Shashkina, P. N. Shashkin and A. V. Sergeev [56]; “Nutritional Value of Edible Seaweeds” by P. MacArtain, C.I.R. Gill, M. Brooks, R. Campbell, and I.R. Rowland [57] and many others.

4. Safety reasons, including heavy metals, radioactive contamination should be controlled.

A belief that herbal medicines are automatically safe is widespread, but is not necessarily true. There are several factors that need to be considered while using herbal medicines: the combination with other medications (herbal or other origin), variability of plant species, growing conditions, soil contaminations, biologically active constituents [43]. This can result in contamination and falsification of herbal material. They also can contain toxic compounds. There are, unfortunately, no normalized control procedures for every herbal component that can be used for drug production. There is also very little information on elemental composition of particular herbs. Several identified problems are correlated to the quality of herbal medicines. Some of them are listed below:

Herbal medicines often consist of many components, and not a single element.

- Plant materials are chemically and naturally variable, which does not allow an easy normalized control procedure.
- The operational unit of the herb is often unknown. It is not clear what is supposed to be inside the herb that would provide a desired result.
- There is a difference in raw material according to source and original quality.
- Herbal quality can also be affected by the methods of harvesting, drying, storage, transportation, and processing. [58]

The World Health Organization (WHO) states, that it is currently unable to recommend limits for contaminants and residues in herbal materials and medicines, because they are too diverse and there is a lack of international consensus (WHO guidelines for assessing quality of herbal medicines with reference to contaminants and residues, World Health Organization, Geneva, 2007.).

One of the commonly possible risks that are associated with plants is the contamination with heavy metals. The heavy metals represent the biggest danger for human health that can come from plants. This contamination mostly appears due to environmental pollution (motor vehicles, power plants, factories, etc.). As already mentioned above, the World Health Organization states, that it is currently unable to recommend limits for contaminants and residues in herbal materials and medicines.

Specifically, should be mentioned existing and proposed acceptable intake levels for heavy metals, e.g. Hg, Pb and As:

	USEPA	USFDA	JECFA	CAL Prop.65	British Pharmacopeia	WHO Monographs on selected medical plants	*ATSDR*	Committee on Dietary Allowances, Food and Nutrition
Arsenic (As)	0.0003 mg/kg bw-day		0.015 mg/kg bw-day	No Significant Risk Level 0.010 mg/day	5 ppm			
Cadmium (Cd)	0.001 mg/kg bw-day		0.007 mg/kg bw-day			0.3 ppm		
Chromium (Cr)	0.003 mg/kg bw-day	Reference Daily intake 0.12 mg/day		No Significant Risk Level $1x10^{-6}$ mg/day			0.2 mg/day	0.05-0.2 mg/day
Lead (Pb)			PTWI 0.025 mg/kg bw	Acceptable Intake Level 0.0005 mg/day		10 ppm		
Mercury (Hg)	Inorganic – $3.0 x 10^{-4}$ mg/mg Methyl-mercury – 1.0 x 10-10 mg/kg		PTWI 0.0033 mg/kg – bw for methyl-mercury	Acceptable Intake Level 0.0003 mg/day				

Table 2 Data for daily intake or Reference dose. [59]

The data that served as a basis for derivation of acceptable daily intake or Reference dose for some particular elements (Cd, Cr, Pb and Hg) is presented in the Table 2. The abbreviation “bw” stands for body weight.

		Arsenic (As)	Lead (Pb)	Cadmium (Cd)	Chromium (Cr)	Mercury (Hg)	Copper (Cu)	Total toxic metals as lead
For herbal medicines								
Canada	raw herbal materials	5 ppm	10 ppm	0.3 ppm	2 ppm	0.2 ppm		
	?nished herbal products	0.01 mg/ day	0.02 mg/ day	0.006 mg/ day	0.02mg/ day	0.02 mg/ day		
China	herbal materials	2 ppm	10 ppm	1 ppm		0.5 ppm		20 ppm
Malaysia	?nished herbal products	5 mg/kg	10 mg/ kg			0.5 mg/kg		
Republic of Korea	herbal materials							30 ppm
Singapore	?nished herbal products	5 ppm	20 ppm			0.5 ppm	150 ppm	
Thailand	herbal material, ?nished herbal products	4 ppm	10 ppm	0.3 ppm				
WHO recommendations (2)			10 mg/kg	0.3 mg/kg				
For other herbal products								
National Sanitation Foundation draft proposal (Raw Dietary supplement)[a]		5 ppm	10 ppm	0.3 ppm	2 ppm			
National Sanitation Foundation draft proposal (Finished Dietary Supplement)[a]		0.01 mg/ day	0.02 mg/ day	0.006 mg/ day	0.02 mg/ day	0.02 mg/ day		

Table 3 Example of national limits for As and toxic metals in herbal medicines and products per day. [48]

Some national standards and limits for raw herbal material and finished herbal products are shown in the Table 3. For example, Canada has added to list of controlled elements As and Singapore – Cu.

Notable are also the differences in choice of elements requiring a control and also variety in limits.

3.5. On teas (beverage) and herbal extractions

1. *Camellia sinensis*

All the mentioned points above, such as quality control, safety reassurance, medical properties and analysis of elemental composition of herbs, are important at least because of the increasing use of the herbal infusions or teas. Next to water, tea is the most widely consumed beverage in the world. In the European Union, all the tea is imported and in 2014, a total of 242.687 t of tea were imported into the EU [41]; in 2015 Americans consumed over 80 billion servings of tea, which is more than 13.6 billion liters.

The most popular is still tea, made out of the leaves of *Camellia sinensis* – the evergreen tea plant. From the same plant at least five different types of the tea can be obtained: Black, Green, Oolong, Dark and White teas. The difference comes from the various degrees of processing and the level of oxidization of the leaves.

The history of tea counts nearly 5,000 years. It is considered that the first tea was made by accident. The legend says that in 2737 BC some tea leaves were by chance blown into the pot of boiling water at the house of Chinese Emperor Shennong.

In Europe and American colonies tea has become popular around 1600. In 1840 the *Afternoon Tea* was created by the Duchess of Bedford. She began regularly taking tea with a light snack around 4:00 p.m. In 1904 tea bags and iced tea were invented, both in U.S.

Leading tea-producing countries include Argentina, China, India, Indonesia, Japan, Kenya, Malawi, Sri Lanka, Tanzania and Taiwan. [1, 41]

2. Herbal infusions

The *Camellia sinensis* tea is often used in a composition with some other leaves, flowers, fruits or other parts of different plants. It is mostly done to modify the taste of a beverage. The taste is also one of the reasons why there is a big market for herbal and fruit teas. There are up to 300 different plants and 400 parts of plants that could be used for making herbal and fruit beverages [41]. Almost all imaginable parts of herb can be used: leaves, for example, peppermint or salvia; fruits and berries – black currant and apple; flowers, like chamomile and lavender; roots as for ginseng or ginger tea; last but not least, seeds can also be used to make infusions (fennel and fenugreek seeds). In different countries (continents) different herbal teas will be considered traditional, the reason for that is mostly geographical distribution of plants. Consumption of such a beverage is often associated with its taste and is not only driven by any specific medical reason, although possible or expected effects are usually known to the consumer.

3. Officinal formulations

According to the European Pharmacopoeia [60] there are two formulations which can be considered matching tea. They differ only by the technology of preparation.

- Infusions with hot water

The description is clear from the name: the chosen herb is brewed with freshly boiled water. Usually it is steeped in the water for quite some time: 5 minutes (some bagged teas) to over

30 minutes (herb mixtures from wild gathering). The brewing time depends on the type of a plant and on the solubility of the elements in the tea (aroma particles and other). Then the herbal material is removed and the infusion is ready.

- Decoctions

Decoction is a method of extraction of crude and dissolved chemicals from herbal material by boiling it. This method is usually used for rather “hard” plant material such as roots or bark. The raw material is boiled for up to two hours and then strained.

Both of the methods are used for medical purposes, but the infusion preparation is much more common in an average household. Therefore, for the presented research the infusion method was chosen.

4. Advantages of the tea-like extractions.

Probably each household uses herbal infusions in the everyday life: for its taste or for its remedial qualities. There are undoubtable advantages of the tea-like extractions:

- Rather easy and fast preparation;
- Water as an extracting agent (no need for ethanol, methanol, and oils);
- Wide range of extractable substances;
- Widely available;
- No prescription needed;
- Mostly has a low cost.

3.6. Conclusion and discussion

It is visible that more detailed investigation of plants for use as herbal medicines is needed. The advantages of tea-like extractions make them very attractive for consumer.

The most challenging and at the same time the most promising would be a multi-elemental analysis. This analysis is gaining popularity as it can refer to the biggest advantage of the herbs – diversity in its composition. It is expected to achieve a potentiating effect due to the presence of multiple active compounds [43].

To achieve a user-like level (the way the average consumer is preparing the beverage) it is necessary to establish controlling system for herbal decoctions and infusions, as those are the most common ways medicinal herbs are used by an average consumer.

4. Tea analysis with TXRF

During the last decade, TXRF gained a lot of popularity as an established technique for multi-element determination of trace elements in various foods. It was also largely employed for the analysis of the beverages.

In this master's thesis, the TXRF method was used to identify elemental composition including trace element content in the various tea and herbal infusions. To get an idea where to start the research and what is already done, a literature analysis of already existing studies that have something in common with the planned measurements was performed.

4.1. Analysis of existing studies

Between 1970 and 1985, TXRF papers appeared only sporadically. However, in the years after 1986, their number grew explosively from some 3 to about 125 papers per year with large fluctuations. [9] Nowadays, there is very high number of publications about trace element analysis with total X-ray fluorescence spectroscopy. The topics of publications are also highly diverse: research on blood [38], human liver [40], human hair [39], and soil [36]. There are also some research papers about food and different beverages. In this chapter, the most relevant to a present study, papers and publications on trace element analysis of food and beverages will be discussed.

4.1.1. "Total Reflection X-ray Fluorescence Analysis of Foodstuff"

PhD thesis by Rogerta Dalipi from University of Brescia. [61]

In her study, R. Dalipi combined TXRF data with a chemometric approach to differentiate the honey and tea samples according to their botanical origin. She also demonstrated the suitability of this method for food traceability and quality control. The suspension of vegetal samples in de-ionized water, followed by TXRF analysis, demonstrated to be accurate and reliable for multi-elemental determination of this kind of samples.

R. Dalipi stated that elemental composition is characteristic to the type of tea and is principally attributed to the way the teas are processed, as well as geographical origin of tea plants (soil composition, climate, local environmental conditions and agricultural practices). TXRF was used to determine the solubility of a range of elements in a set of seven black, ten green and one oolong tea, one ginseng and nine mixed herbal teas. The analysis was done for made teas (tea leaves were milled into a fine powder by the author), herbs and their infusions. To control the results, the certified reference material "apple leaves" 1515 SRM was used. For infusion preparation 200mg of the sample were used and 50mL of ultrapure de-ionized water. The beaker was heated until boiling and then left for 5 minutes (extraction time) under agitation. The exact amount of tea sample, which was diluted with 1mg/L of Ga, is not known. Ga was used as an internal standard.

The obtained results were then treated with principal component analysis (PCA), to determine two groups: mixed herbs and teas. PCA was performed using covariance matrix on the complete raw data set (concentration values). The separation of two groups was successful for tea and herbs infusions and there was no clear separation for leaves. There is no detailed information about what kinds of herbal teas were used and which herbs, roots, seeds or flowers they contained. There is also no information whether the mixed samples

contained the same plant families or if they were different, which gives us no information for evaluation of the herbs infusion measurement.

4.1.2. "Multielement analysis of Chinese tea (*Camellia sinensis*) by total-reflection X-ray fluorescence"

M. Xie, A. von Bohlen, R. Klockenkämper, X. Jian, K. Günther. [62]

In this study, TXRF was also used for tea examination. The authors chose 39 tea samples, all of them of different quality and kind. Tea samples were produced in different regions of China. As reference material, the certified GBW 08505: tea was used. The points of interest of this study were: concentrations of elements in the tea and their solubility in infusions as well as the influence of the origin, type and quality of the samples on the results.

In this work, M. Xie et al. measured digested tea leaves and solutions. For solutions, following parameters were used: 1g of the respective tea leaves and 50 ml boiling distilled water. Extraction time was taken to be 5 minutes, after that all solutions were filtered by means of a folded filter paper – type 3427 ½, followed by cooling. Next step was to acidify the samples with HNO_3 and add Gallium (Ga) as an internal standard (IS).

The settings of all measurements were the same: Mo X-Ray tube (50 kV and 10-38 mA); 200 s live time; ambient air.

The authors managed to determine 15 various elements. When compared to the results of this diploma thesis, it will be noticed that there are differences between detected elements. In above-mentioned study Pb and Se were detected and Br was completely absent, and in the current thesis Br was one of the "standard" elements, detected in entire sample set. There are two main differences between this particular research and the one by M. Xie, A. von Bohlen, R. Klockenkämper, X. Jian,and K. Günther: the tea sample preparation procedure and the type of the samples. As a part of conclusion, the authors are mentioning that the solubility of elements from a tea sample is influenced by the conditions under which the tea infusion is prepared (e.g. extraction time and temperature); therefore, it is difficult to compare these results to those obtained in the different studies.

4.1.3. "Application of trace element and stable isotope signatures to determine the provenance of tea (*Camellia sinensis*) samples"

Tamara S. Pilgrim, R. John Watling, Kliti Grice. [63]

This work is not related to TXRF analysis, but is also devoted to the analyses of the tea samples (*Camellia sinensis*). Tamara S. Pilgrim et al. were using complimentary organic and inorganic isotope techniques. That means the distribution of stable organic and inorganic isotopes was identified. The measurements were performed by the means of micromass Isoprime isotope ratio mass spectrometer and for solutions with Solution analysis using inductively coupled plasma mass spectrometry (ICP-MS). After data acquisition the linear discriminant analysis (LDA) of the isotope ratios and mineral concentrations was performed. LDA is a supervised statistical analysis, which is usually used for dimensionality reduction purposes [64], in order to be able to recognize patterns or clusters in the data. Supervised means one needs to predetermine the entity group before using LDA.

Authors had 83 samples with established origin, harvest time, quality and type. Additionally they had 20 more samples purchased from diverse China stores. These samples were used

as unknown (as if the origin were unknown). The liquid sample (tea solutions) preparation was rather complicated. Tea leaves were acidified four times and then heated; each treatment had different temperature and was performed during various periods of time.

As a result of the study some sort of the tea data base was created, which is determined to show the real origin of the beverage. The LDA allowed a 97.6 % correct classification.

This study was not designed for elemental composition of tea samples as such. However, it is indisputable that principal component analysis again proved to be a relevant tool in sample coupling.

4.1.4. Conclusion of analysis of existing studies

There are several studies that have tea samples as their main object of interest; however, most of them are dealing with the tea plant – *Camellia sinensis* and with the determination of the origin of the samples for future quality control. The presented master's thesis is unique in the way that it is dealing with various popular herbal infusions, which a typical consumer would use. This approach gives us a possibility to develop a method to control herbal tea production and to improve understanding of healing qualities of commonly used herbal infusions. The focus of the study was put on the elemental analysis and on the differentiation between types / families of herbs and not on the origin of the plants.

4.2. Sample selection

For this study, 16 different tea and herb types were selected. In total, there were 29 different samples.

The selection was made subjectively, based on own perception of popularity. The priority however was directed towards herbal teas, which are used as alternative / home medicine. Most of the samples were bought in Austrian pharmacies. All selected tea types are listed in the Table 4 with the origin description and Latin names.

It was interesting to compare same types of tea from different manufacturers and see if any differences can be found between loose herbal teas and the pre-packaged ones. Also for two samples (Oolong and Pu-erh tea) it was chosen to determine different steeping. The same leaves were brewed several times and tea from each brewing time was taken as a separate sample. This was done only for two black tea samples, as in the instructions for those teas were explicitly mentioned that they can be steeped several times (Oolong 3 to 4 times; Pu-erh tea up to 12 times).

Nr.	Sample name	Tea	Latin Name	Manufacturer	Description on the package
1	Anis	Anise	*Pimpinella anisum*	“Kräuter Kottas”, Wien 1010, Freyung 7	
2	Baldrian#1	Valerian	*Valeriana officinalis*	„Graben Apotheke“, ‚Zum schwarzen Bären‘ Mag. Pharm. M. Derflinger OHG 1010 Wien; geprüfte Apothekenqualität nach dem Arzneibuch	Calming
3	Baldrian#2	– – –	*Valeriana officinalis*	“Apotheke im Stadion Center”, Baldrianwurzel, valid till 08.2016	By state of erousal, sleeping disorder, stress related gastrointestinal pain
4	BioDarjeeling	Bio Darjeeling	*Camellia sinensis*	SPAR PREMIUM, aus kontrolliert biologischem Anbau. Region Darjeeling, Norden Indiens.	
5	Brennnessel	Urtica (Nettles)	*Urtica dioica*	“SonnentoR” from controlled organic cultivation.	Mild tea fits for using in spring for disease treatment.
6	EZA Darjeeling	Darjeeling	*Camellia sinensis*	Weltladen 1010 Wien, certified organic production.	
7	Fenchel#1	Fennel	*Foeniculum vulgare*	“Riga Pharmaceutic Fabric”, Duntes iela 16/22, Riga, Latvia	
8	Fenchel#2	– – –	*Foeniculum vulgare*	“Kräuter Kottas”, Wien 1010, Freyung 7/ 31.07.2015	By flatulence, spasmodic gastrointestinal disorder
9	FixFenchelBeutel	Fennel Package	*Foeniculum vulgare*	“Teekanne”, hergestellt in Österreich/ 06.2018	
10	Kaesepappel	Common Mallow	*Malva sylvestris*	„Kaiser Josef-Apotheke“, Alserstr.51, 1080 Wien	By catarrhs of the upper airways and by indigestion
11	Kalmus	Calamus	*Acorus calamus*	„Graben Apotheke“, ‚Zum schwarzen Bären‘ Mag. Pharm. M. Derflinger OG 1010 Wien/ 31.7.14	
12	Kamille	Chamomile	*Chamomilla vulgaris*	„Graben Apotheke“, ‚Zum schwarzen Bären‘ Mag. Pharm. M. Derflinger OHG 1010 Wien	Anti-inflammatory, against stomach troubles
13	KamilleBeutel	Chamomile Package	*Chamomilla vulgaris*	„Alnatura“, aus biologischer Landwirtschaft; Herkunft: Ägypten, Osteuropa	
14	Lapacho	Lapacho	*Tabebuia impetiginosa*	Kräuterhaus Mag. Kottas; Wien 1010, Freyung 7; in Arzneibuchqualität	
15	Lavendel	Lavender	*Lavandula angustifolia*	Kräuterhaus Mag. Kottas; Wien 1010, Freyung 7; in Arzneibuchqualität /12.08.2015	By state of erousal, sleeping disorder, indigestion
16	Minze Oma	Peppermint	*Mentha piperita*	Picked by hand in Latvia, garden and outer forest.	
17	Minze#2	– – –	*Mentha piperita*	Kräuterhaus Mag. Kottas; Wien 1010, Freyung 7; in Arzneibuchqualität	By gastrointestinal and bile disorder
18	Pfefferminze Beutel	Peppermint Package	*Mentha piperita*	„Alnatura“, aus biologischer Landwirtschaft; Herkunft: Ägypten, Osteuropa	
19-20	Oolong#1-#2	Oolong	*Camellia sinensis*	Brought as a present from China.	
21-25	Pu-erh#1-#5	Pu-erh	*Camellia sinensis*	Brought as a present from China Pu’er region, Yunnan, China.	
26	Salbei	Sage	*Salvia officinalis*	Austrian pharmacy’s self production.	
27	SalbeiBeutel	Sage Package	*Salvia officinalis*	“Teekanne”, hergestellt in Österreich/ 07.2017	
28	Schafgarbenkraut	Yarrow	*Achillea millefolium*	„Graben Apotheke“, ‚Zum schwarzen Bären‘ Mag. Pharm. M. Derflinger OG 1010 Wien	By gastrointestinal and bile disorder
29	Thymian	Thyme	*Thymus vulgaris*	„Alnatura“, aus biologischer Landwirtschaft; Herkunft: Ägypten, Marokko, Osteuropa, Türkei	

Table 4 Selected tea types with Latin names, origin and description.

In the table 5, all the measured samples are list with additional information about which part of the particular plant was used.

Nr.	Sample name	Tea	Part of the plant
1	Anis	Anise	Fruits
2	Baldrian#1	Valerian	Roots
3	Baldrian#2	– – –	Roots
4	BioDarjeeling	Bio Darjeeling	Leaves
5	Brennnessel	Urtica (Nettles)	Leaves
6	EZA Darjeeling	Darjeeling	Leaves
7	Fenchel#1	Fennel	Fruits
8	Fenchel#2	– – –	Fruits
9	FixFenchelBeutel	Fennel Package	Fruits
10	Kaesepappel	Common Mallow	Whole herb
11	Kalmus	Calamus	Rhizome
12	Kamille	Chamomile	Flowers
13	KamilleBeutel	Chamomile Package	Flowers
14	Lapacho	Lapacho	Bark
15	Lavendel	Lavender	Flowers
16	Minze Oma	Peppermint	Leaves
17	Minze#2	– – –	Leaves
18	Pfefferminze Beutel	Peppermint Package	Leaves
19-20	Oolong#1-#2	Oolong	Leaves
21-25	Pu-erh#1-#5	Pu-erh	Leaves
26	Salbei	Sage	Leaves
27	SalbeiBeutel	Sage Package	Leaves
28	Schafgarbenkraut	Yarrow	Whole plant
29	Thymian	Thyme	Fruits

Table 5 Samples with used herb parts.

5. Preparations for measurements

The preparation for the measurements is an essential part of the working process. Instrumentation, correct settings and adjustments, sample preparation, cleaning procedures – all these aspects are covered in the present chapter.

5.1. Measurement instrumentation

Figure 29 ED TXRF Atomika 8030C.

The measurements for this study were performed with a commercial TXRF system – the Atomika 8030C. The used machine is an energy dispersive total X-ray fluorescence (ED TXRF) spectrometer. The equipment is situated in the X-ray laboratory of radiation physics working group in Atominstitut in Vienna.

5.1.1. Atomika 8030C

As the specific tool for multielement trace and thin layer analysis, the Atomika TXRF 8030C provides simultaneous and fast determination of all elements within the range from sodium to uranium. Sophisticated measurement instrumentation provides detection limits down to the ppb level. These features make the TXRF 8030C a valuable analytic tool for a wide range of applications: contamination in water, dust or sediments; quantitative screening in the chemical industry; toxic elements in tissues and biological fluids; radioactive elements; process chemicals in the semiconductor industry.

The 8030C is designed for sample carriers with 3 cm diameter and about 3 mm height. Usually quartz reflectors are used, as their cleaning procedure is rather simple and they show no contamination. The automated loading procedure allows measurements of maximum nine cassettes with 18 reflectors each. Spectrometer has a 2.5 kW high power X-ray tube with Molybdenum-Tungsten (Mo/W) mix-anode.

After passing through the monochromator, the primary X-ray beam is hitting the sample under an angle below the critical angle. For the analysis of the characteristic fluorescence radiation a 80 mm2 Si(Li) detector is used. The detector is positioned over the center of the reflector. This position is fixed and cannot be changed. The measurement and evaluation software are installed on the computer, which belongs to the TXRF system. The evaluation system provides, among others, data- and result-files. The data files can be processed and depicted using common spreadsheet software, such as MS Excel. The result files are giving the total overview over used settings, including dead time of the measurement, and the peak combined intensities for each found element (Appendix 2).

The following terms should be introduced and clarified:

Live time is the active measurement time. This entire time a radiation signal is obtained and registered from the sample.

Dead time is the time the detector is busy with the processing of the signal and unable to record a new signal.

Real time is the time, which passes during the measurement. It is the sum of live and dead time.

For the energy calibration and to control the sensitivity of the detector, a 100ng Ni sample is used. It is located in fixed position through all the measurements and is launched for control after the period of time, which has to be preprogrammed.

The sample carriers are cleaned in the clean-bench and measured as blanks before using for experimental measurements. Combined with regular control measurements it assures a reliable and reproducible measurement conditions.

5.2. Sample carrier cleaning procedure

Sample carrier cleaning is an essential part of each measurement. The reproduceability and trustworthiness of the experiment depends on the clean carrier or reflector.

For that reason, all reflectors have been treated with a standard procedure after each use and examined with Atomika 8030C total reflection spectrometer for 1000 seconds measurement time each. For this project, quartz reflectors were used.

Cleaning procedure:

1. To remove big particles (visible dirt), reflectors were rubbed with clean wipe and a droplet of concentrated cleaning soap (Decon90).
2. Then rinsed with tridistilled water.
3. Rinsed with acetone.
4. After that, all reflectors were inserted into PTFE holder and rinsed with tridistilled (TriDis) water and with acetone.
5. Then the holder with sample carriers was inserted into a beaker with 300 ml TriDis water and 20 ml cleaning soap Decon90 and heated at the temperature of 75° C for 20 to 30 minutes.
6. After that, the holder with reflectors in it was rinsed with TriDis water and rinsed with acetone.
7. Next, all reflectors were carefully inserted in a beaker filled with nitric acid (HNO_3) and gently heated up to 70°C in an ultrasonic bath for 15 to 20 minutes.
8. Then they were rinsed with TriDis water and dried with acetone, to remove traces of TriDis Water droplets.

Steps 5 and 7 of the cleaning procedure are to be performed in the special laboratory with a fume hood (Figure 30) suitable for acids and using personal protection clothing (smock, goggles, and hand gloves). The room is equipped with a special laboratory extractor hood for acids and glass doors to provide maximum security, even while working with acid.

Figure 30 The room with a fume hood in it.

After the above-mentioned procedure, all cleaned reflectors were inserted into the Atomika 8030C TXRF machine and measured for 1000 seconds live time each. In Figure 31, a spectrum of a typical clean reflector is shown. There are three visible peaks, Si–Kα, Ar–Kα, Mo–Kα.

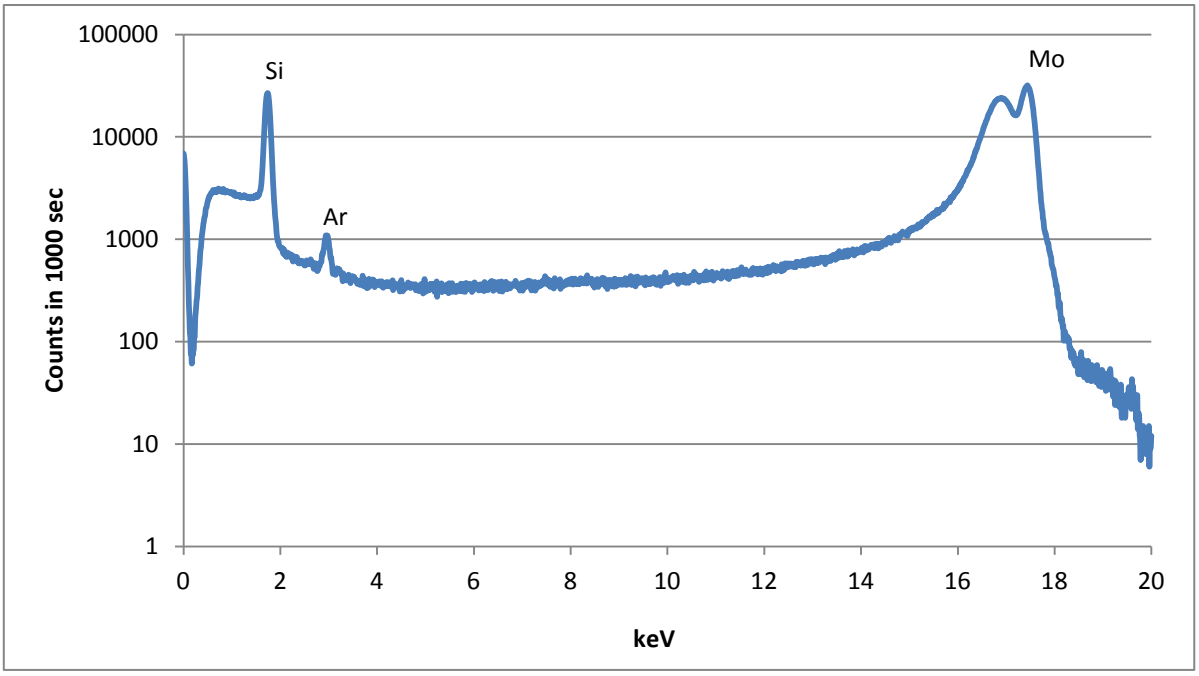

Figure 31 Spectrum of a typical clean reflector.

Silicon (Si) peak appears due to sample carrier (quartz = SiO_2) itself; Argon (Ar) peak is due to air presence during the measurement. In a vacuum, Ar would not be visible. The incident radiation (Molybdenum (Mo)) peaks are elastic and inelastic scattering from the exciting Mo radiation of the X-ray tube. In between those peaks there is no other contamination can be seen, so it can be concluded that the presented reflector is free of any contamination and can be used for sample measurements. To compare with Figure 31, Figure 32 displays a "dirty" reflector, which needs to be cleaned and measured again before using.

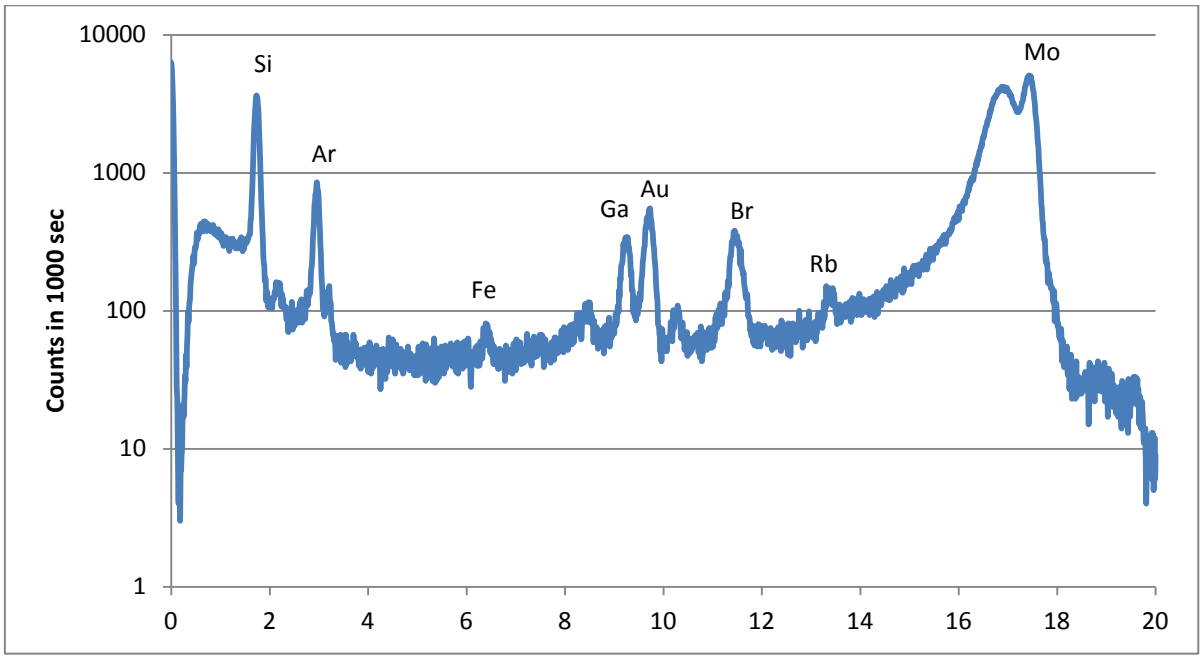

Figure 32 Spectrum of a contaminated reflector.

After sample carriers cleaning was performed, the beaker glass needed to be cleaned as well to make sure that there is no contamination possible during tea making process.

The beakers were rubbed with a clean pad and a drop of Decon90, then rinsed with tridistilled (TriDis) water and then dried with acetone. Approximately 100 ml to 150 ml of TriDis water was added into each beaker with around 10 ml of Decon90, and heated up for about 30 minutes (boiling of the cleansing solution should be avoided). Then beakers were again rinsed with TriDis water and acetone, and left to dry.

After all above-mentioned procedures, to reassure that all beakers are not exposed to any unwanted contamination, around 150 ml of TriDis water was boiled in each glass and then 10 µl of this boiled water from each glass were pipetted on a clean quartz reflector.

The measurement of boiled TriDis water out of the particular beaker was running for 1000 seconds to be maximally close to the original experiment, where tea sample will be also measured for 1000 seconds. Figure 33 is representative for a typical spectrum for a clean beaker. The clean beaker spectrum is comparable to the clean reflector spectrum (Figure 31).

When all the cleaning and control steps where done successfully, the sample measurement could be started. Also, all the equipment, used for the sample preparation, was reused. Complete cleaning circle should have been repeated each time after preparing each sample.

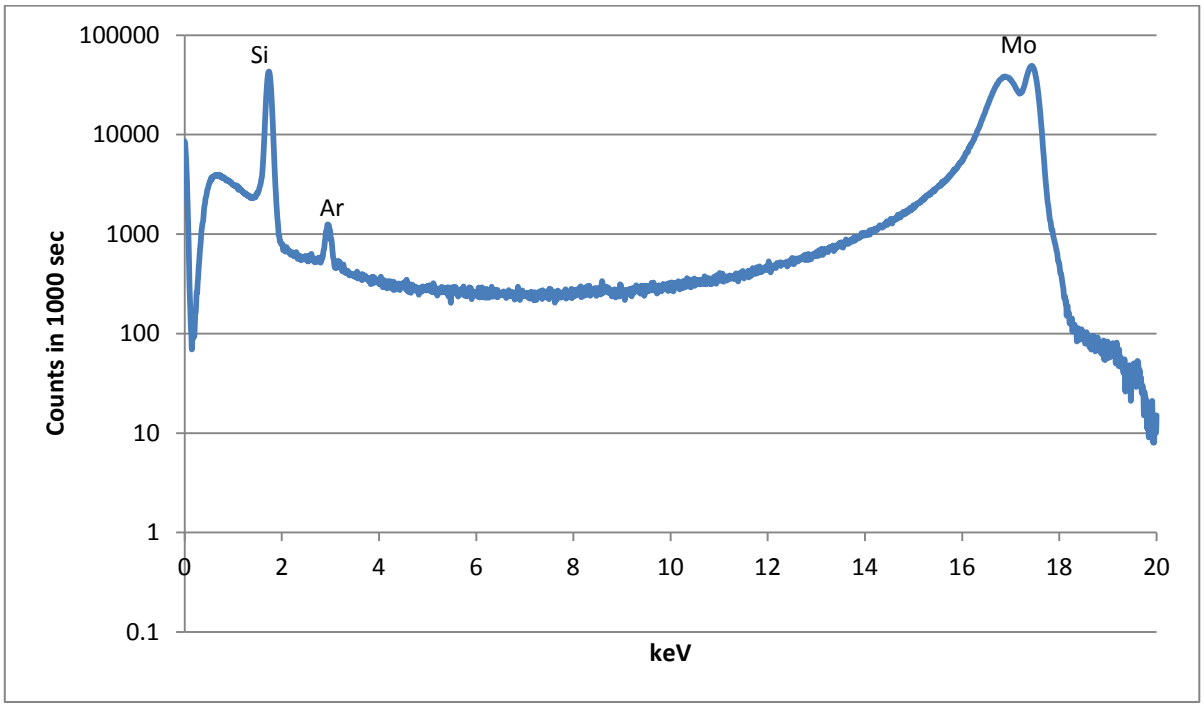

Figure 33 Spectrum of a not contaminated TriDis measurement.

5.3. Sample preparation

There is no normalized system for preparing tea samples; therefore, the protocol had to be established.

As the main interest of the master's thesis is based on herbs that could be used for treatment of particular symptomatic and mostly in-home conditions, it was found convenient to use just herbal infusions without any particular preparation except the one given by the manufacturer; meaning that herbs were not digested or processed.

To prepare tea / herbal infusion, 1g of the sample (leaves, bark, flowers or seeds) was steeped with 50 ml of tridistilled water; then brewed for 10 minutes. After an extraction time, the infusion was carefully refilled in a sterile beaker to remove most of the solid leftovers and left for the next 5 minutes to let the smallest particles settle. Next step was to refill the sample in the next clean beaker using one-way pipette, to provide clean sample without any visible particles. Approximately 500 mg of pure sample were spiked with 50 mg Ga used as a standard element. The solution was then homogenized by shaking. The used instrument for shaking is shown in the Figure 34.

Figure 34 "PHOENIX" instrument RA-VA10

The intension was to achieve the "home made" feeling, as the infusion would be done by an average consumer. As priory stated, tea preparation procedure was unchanged for each sample. However, there were some exceptions made.

The most notable change in the preparation procedure was done for the Pu-erh tea sample. After reviewing package instructions and existing on-line tutorials, it was decided to make five steeping of the same sample leaves. It occurred to be possible because of the quality of the presented sample. The description of each pu-erh tea states that a good quality tea can be brewed up to 12 times. The correct brewing also suggests that the first steeping would be only used for tea cups warming purposes and not for drinking. The samples were prepared as recommended by the manufacturer: the whole package (Figure 35), ~ 4.96g of pressed tea leaves, was steeped with ~85g of TriDis water with extraction time of only 30 seconds for each of five steeping.

Figure 35 Single portion of the packaged Pu-erh tea.

The packaged tea samples had also a difference in the amount of herbs and accordingly used water, as they were packaged with mass ranging from 1.5g to 2.25g. The amount of TriDis water was ~150g, as it was suggested on a tea box to use a standard cup for tea drinking.

For the measurements, clean reflectors were siliconized with 10µl of Silicone solution SERVA (particularly for siliconizing glass and metal) in isopropanol (Cat.No. 35130.03) each and dried on a hot plate in the flow hood. The siliconizing procedure is necessary in order to prevent the sample drop from spreading across the reflector. As the X-ray beam is pointed towards the center of reflector, it is convenient to concentrate the measured sample in the middle. After siliconizing, 10µl of the tested sample were pipetted in the middle of reflector and also dried on a hot plate in the flow hood. Constantly at least five carriers with the same sample were measured. All the reflectors were then inserted in a holder cassette of Atomika 8030C.

5.4. Validation with standard reference material NIST1640

Before starting the sample measurements, the calibration of Atomika 8030C had to be checked. For this purpose international standard reference material NIST1640 was measured (the data sheet attached in Appendix 2).

Element	Expected value	Corrected calculated value ± Standard deviation (STDEV)
K-K	944 ± 27	988.6 ± 75.2
Ca-K	7045 ± 89	8009.9 ± 286.51
Cr-K	38.6 ± 1.6	42.7 ± 1.55
Mn-K	121.5 ± 1.1	136.7 ± 3.16
Fe-K	34.3 ± 1.6	47.5 ± 3.78
Co-K	20.28 ± 0.31	23.4 ± 1.35
Ni-K	27.4 ± 0.8	35 ± 1.34
Cu-K	85.2 ± 1.2	98.2 ± 0.78
Zn-K	53.2 ± 1.1	74.6 ± 1.74
As-K	26.67 ± 0.41	30.9 ± 2.5
Se-K	21.96 ± 0.51	21.9 ± 2.18
Sr-K	124.2 ± 0.7	116.3 ± 6.21
Ba-L	148 ± 2.2	166.8 ± 25.66
Pb-L	27.89 ± 0.14	30.1 ± 2.73

Table 6 Comparison between expected and obtained reference material data

In the Table 6, a comparison between expected value of an international reference material and the measured value is shown. Corrected calculated value includes a concentration correction as the NIST1640 was spiked with 1ppm single Ga as reference material and was not measured purely. The proportions of NIST and Ga were about 9 to 1. Next to the corrected measured value, the corrected sample standard deviation (STDEV) was calculated. For statistical purposes, each measurement was repeated for five times. Out of those five measurements, with the integrated Word Excel function, the STDEVs were calculated for all the samples. STDEVs were calculated for each element in every sample with an exception when, by at least one measurement out of five, the element was not detected.

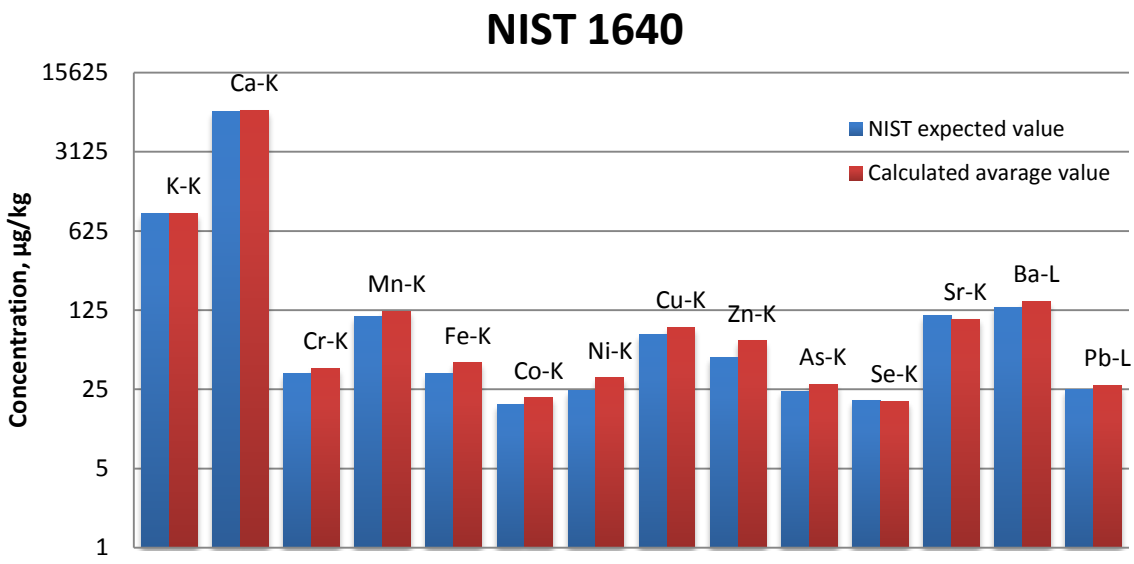

Figure 36 Graphical comparison between expected and obtained reference material data

From the Figure 36 it is possible to see, that expected and average values are quite comparable. The only noticeable difference is at Zn and Fe, 29% and 28% respectively. This could however been due to a possible contamination and is still considered as not significant.

From this measurement the conclusion was made, that ATOMIKA 8030C has a valid calibration and the planned experiment could be performed.

All measurements were performed with following pre settings:

Measurement Live Time	**1000 seconds**
Excitation	Mo-K
Filter	Zr_20 (thin)
Tube current	37 mA
Tube voltage	50 kV
System resolution	90-100 eV

Individual for each sample and measurement were number of counts per second and average dead-time. It was found convenient to keep dead-time at the level near 50%, however in most of the case when dead time was between 30% and 40% it was also tolerated, as the tube current was still 37 mA.

The percentage value of the dead time (D) can be calculated with following formula:

$$D = \frac{(n_{in} - n_{out})}{n_{in}} \cdot 100\% \qquad (2.25)$$

Where n_{out} is the output count rate (processed count rate) and n_{in} is the input count rate. During a percentage D of the whole measurement time, the detector system will be “dead”, and “live” for the remaining percentage (100 - D).

6. Results

In this chapter, data results both from black tea and the herbal infusions, as well as data processing will be presented and a method evaluation will be given.

6.1. Data Results

Data results are separated in four sub points: black tea, herbal infusions, separate elements and principal component analysis. Sections were separated in a way to provide the clearest understanding of the results. The discussion will follow in the chapter 7 Conclusion. Each section will thoroughly display the acquired and calculated results.

6.1.1. Black tea

During this master's thesis three different sorts of black tea were measured: Darjeeling, Oolong and Pu-erh tea. In total, it resulted in nine different samples: Darjeeling and BIO Darjeeling (from two different manufacturers), Oolong#1 and Oolong#2, Pu-erh#1 – #5. In case of Oolong and Pu-erh tea, the same sample and the same leaves were used several times. Due to manufacturer's suggestion and available preparation procedure, it was decided to have two steeping of chosen Oolong tea and five steeping of Pu-erh tea.

BIO Darjeeling

For the measuring of the most samples a normalized procedure should be been created in order to be able to compare the results. In case of the BIO Darjeeling the established normalized procedure was used.

After preparing the sample (details described in the chapter 5.3), it was measured at least five times to reassure accuracy.

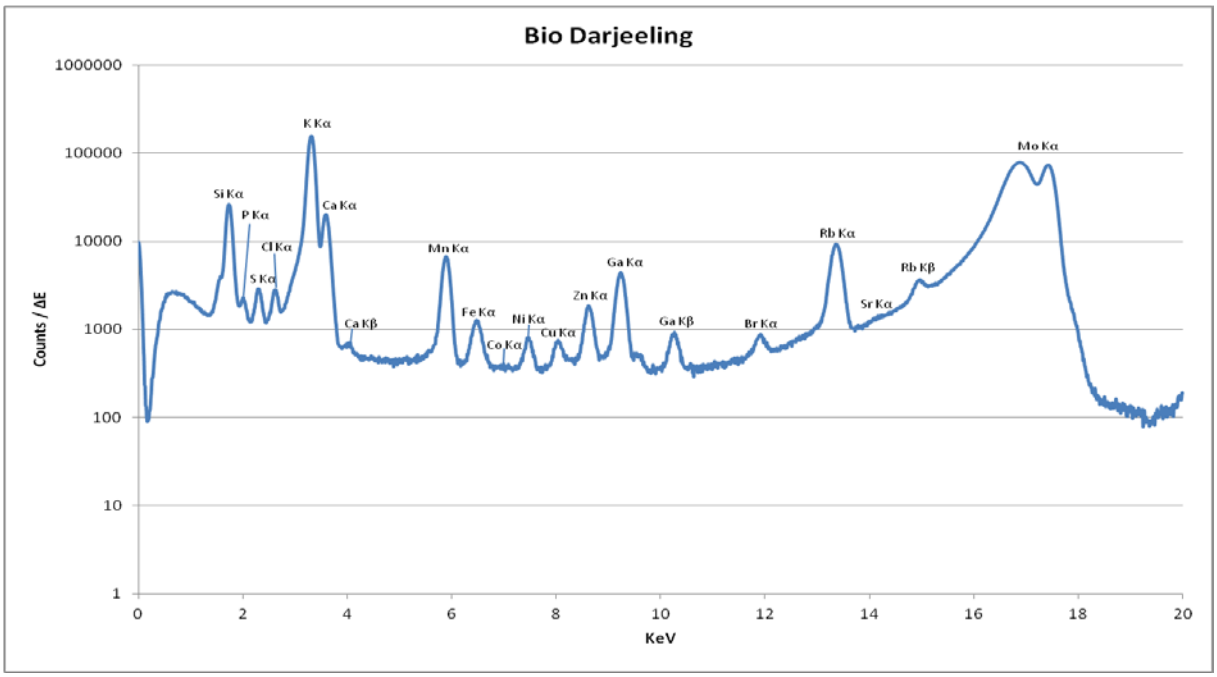

Figure 37 Characteristic spectrum of BIO Darjeeling sample.

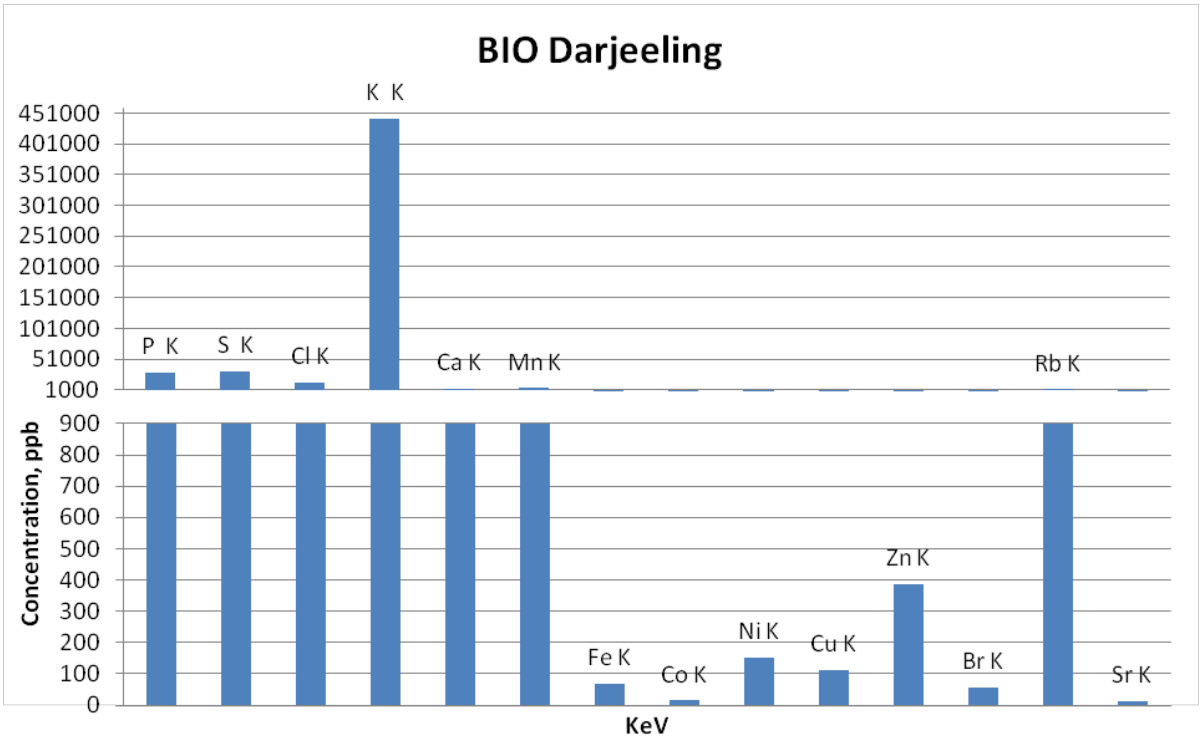

Figure 38 Column diagram showing the concentration of found elements in the sample – BIO Darjeeling.

The Figure 37 shows rather qualitative analysis of the sample; it means the elements contained in the sample are identified. On the other hand, Figure 38 better demonstrates the amount of the found elements. The exact numbers are listed in the table 6. All average values are normalized over the amount of internal standard Ga.

In this sample, the biggest values have Potassium, Sulfur and Phosphorus; the least amount has Strontium und Cobalt.

Darjeeling

Second Darjeeling tea sample was bought in a different place as the BIO Darjeeling. It was interesting to compare same tea, but from different production origin and manufacture. This sample also underwent the normalized sample preparation procedure.

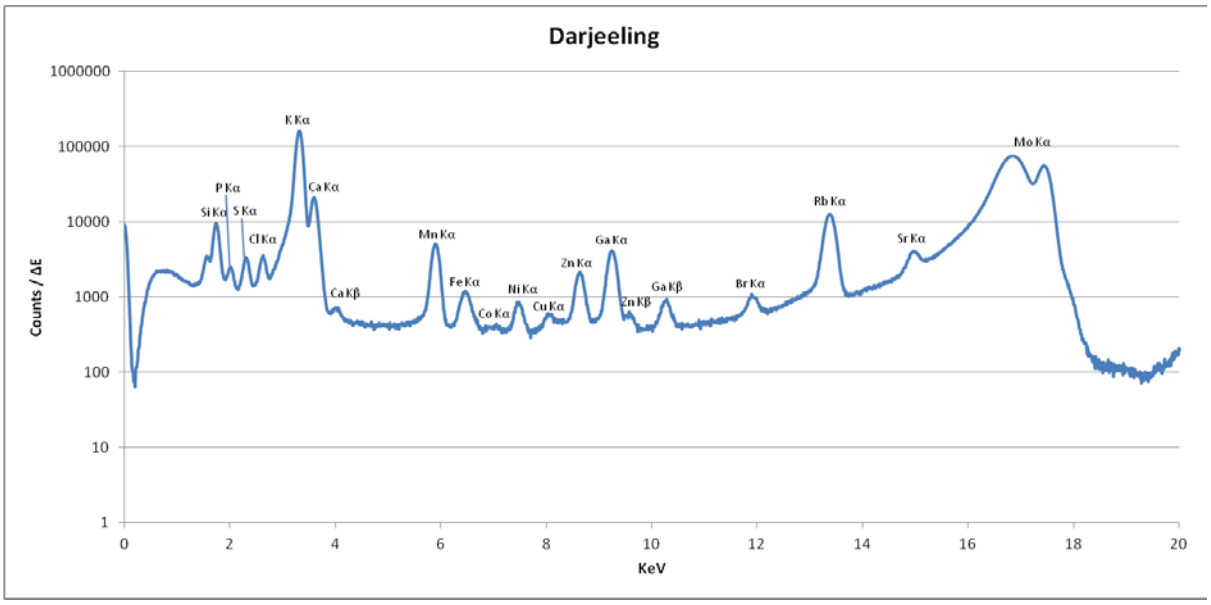

Figure 39 Characteristic spectrum of Darjeeling sample.

The highest and the smallest values of this sample are the same as for BIO Darjeeling; they also have the same magnitude.

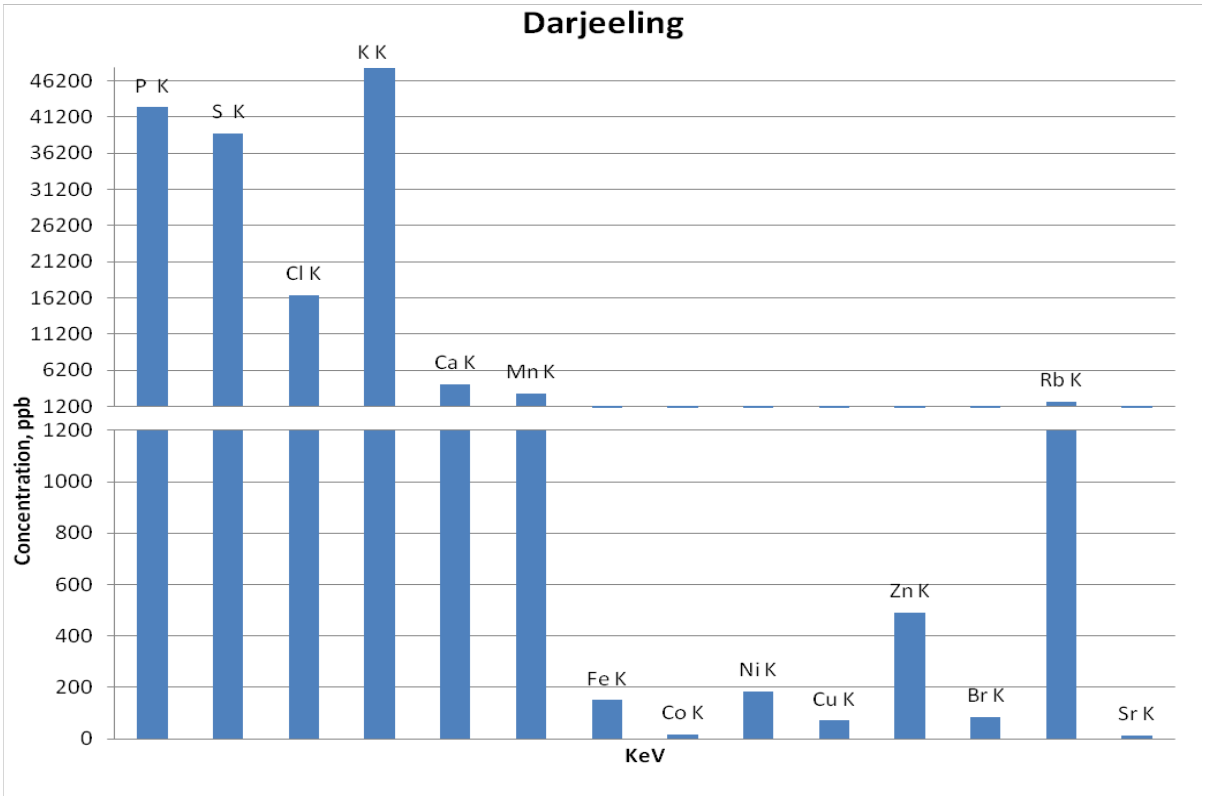

Figure 40 Column diagram showing the concentration of found elements in the sample – Darjeeling.

Element	Average Value Bio Darjeeling, ppb	STDEV Bio Darjeeling	Average Value Darjeeling, ppb	STDEV Darjeeling	Difference, %
P K	29523.9	2177.14	42519.5	1357.95	31%
S K	30882.5	1424.86	38871.8	652.2	21%
Cl K	12323.6	713.09	16512.9	597.3	25%
K K	442283	11027.46	477041.5	6393.99	7%
Ca K	3127.7	136.98	4267.8	134.12	27%
Mn K	3833.6	49.53	2969.8	20.09	23%
Fe K	66.7	23.91	150.6	10.23	56%
Co K	14.4		16.7	3.3	14%
Ni K	153.2	5.64	184.3	4.5	17%
Cu K	113.3	2.05	70.7	4.29	38%
Zn K	386.5	13.5	492.4	4	22%
Br K	57.7	2.13	83.5	2.46	31%
Rb K	1253.6	13.25	1823.9	16.97	31%
Sr K	12.7	2.59	13.5	4.02	5%

Table 7 Comparison of the average values between BIO Darjeeling and Darjeeling tea samples; and the percentage difference between them.

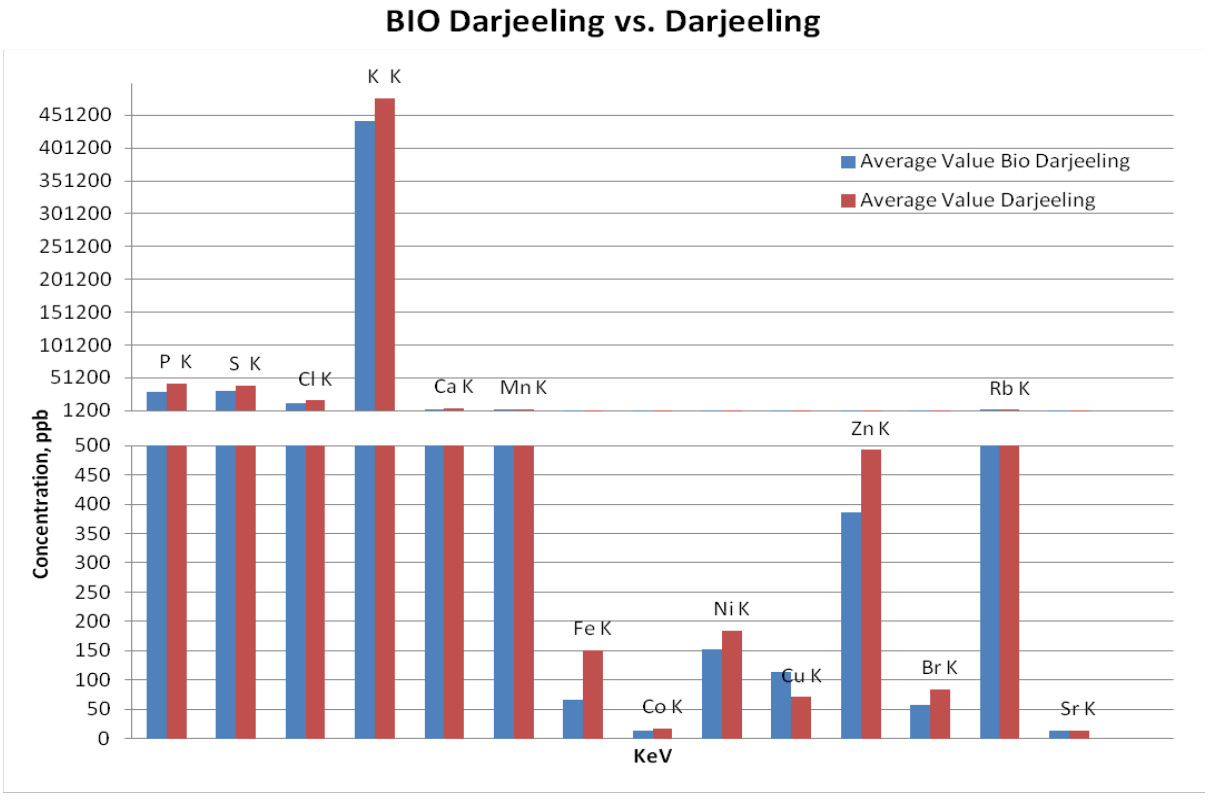

Figure 41 Comparison between average values of BIO Darjeeling and Darjeeling.

From the Figure 41 and the table 7 it is evident, that there are not that many differences between samples. The most notable fluctuation is for Iron – 56%. Iron as an element is generally a special case for this particular study and will be discussed in the chapter 6.2.

Oolong tea

By manufacturer of this tea was suggested that users can brew one portion of leaves for around four times. It was decided to make two steeping of the same leaves. The preparation procedure was not different from Darjeeling tea samples.

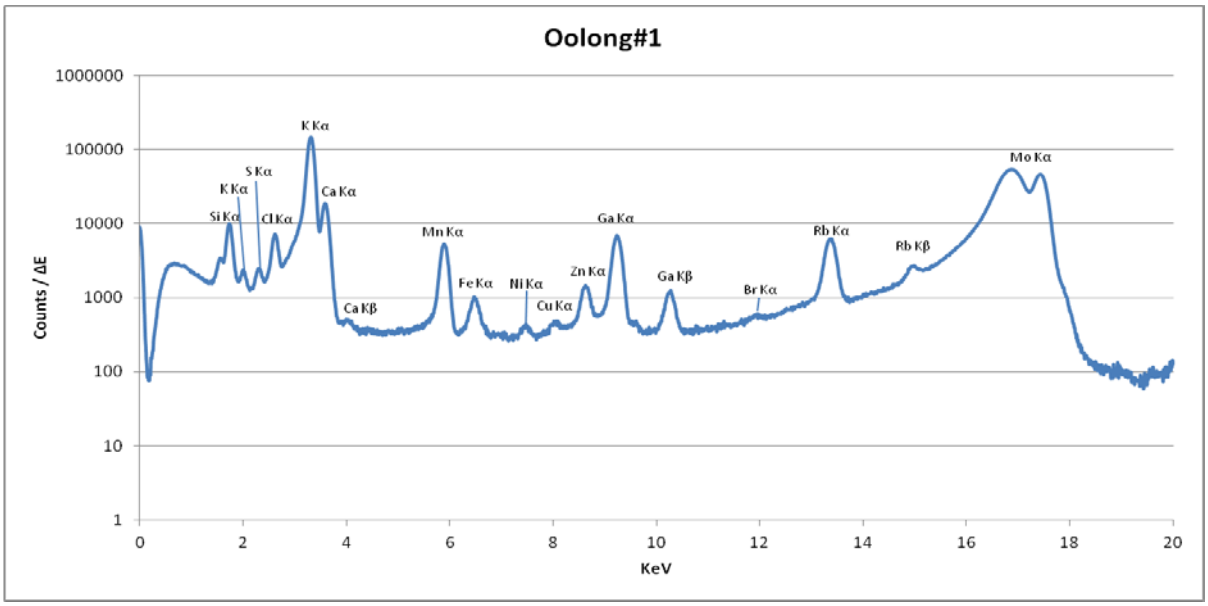

Figure 42 The characteristic spectrum of the Oolong#1 sample.

The Figure 42 represents the characteristic spectrum of the first steeping of the Oolong leaves. The smallest traced elements here were Bromine, Copper and Nickel. The spectrum of the first solution was chosen, because in the second water there were even less elements, which makes the spectrum less visual.

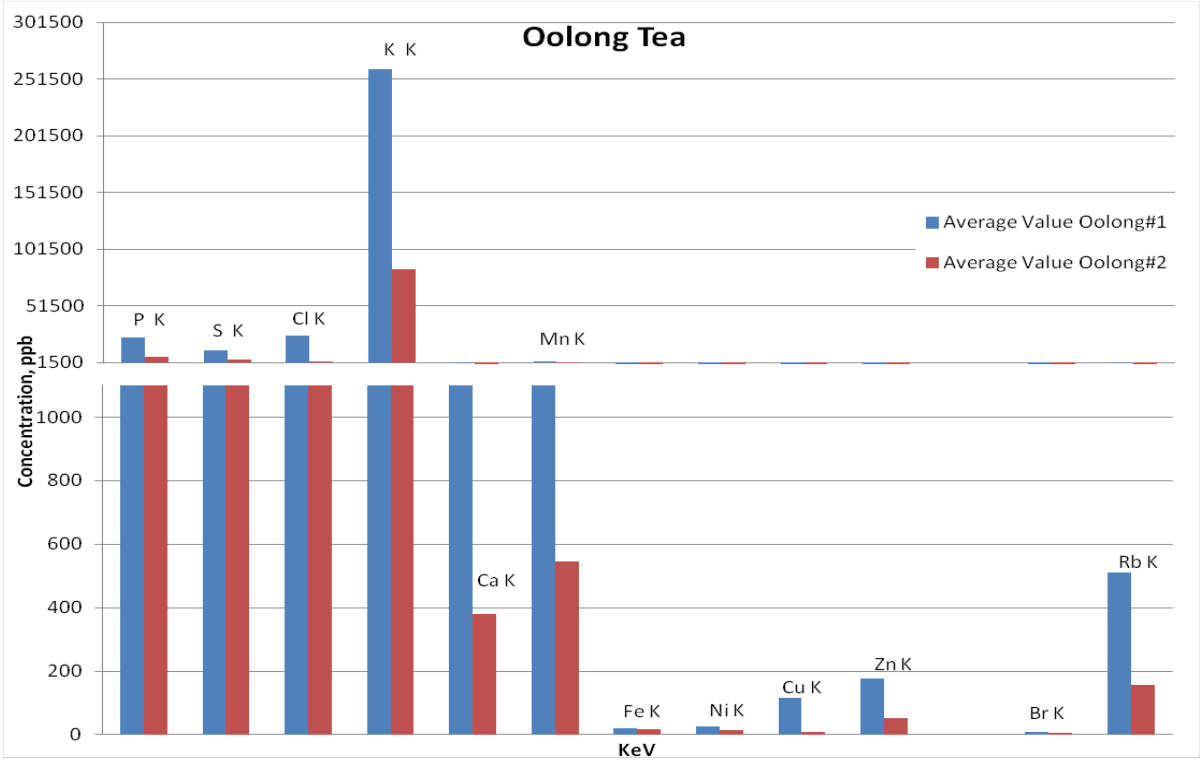

Figure 43 Comparison of the average values between samples Oolong#1 and Oolong#2.

Element	Average Value Oolong#1, ppb	STDEV Oolong#1	Average Value Oolong#2, ppb	STDEV Oolong#2	Difference, %
P K	23649.3	896.35	6359.7	760.44	73%
S K	12175	209.04	3943.3	432.93	68%
Cl K	24895.1	372.06	2892.2	585.56	88%
K K	260158	2349.08	83613.8	3120.53	68%
Ca K	1111.1	40.14	380.7	19.41	66%
Mn K	1849.3	10.37	545	9.17	71%
Fe K	19.1	5.93	18.2	5.86	5%
Ni K	26.6	1.72	15.4	3.56	42%
Cu K	115.1	207.56	9.5	1.51	92%
Zn K	178.0	8.24	51.7	4.07	71%
Br K	9. 4	1.21	6.3		33%
Rb K	509.4	2.27	157.1	2.64	69%

Table 8 Comparison of average values between Oolong#1 and Oolong#2 tea samples; and the percentage difference between them.

After comparing results from measurements of both samples (Figure 43 and table 8), the degradation of the trace elements in the subsequent brewing is recognizable. In average the reduction is in between of 65% and 85%. There are several exceptions though: iron amount is practically identical in both sample, it is also very low; copper is also a notable element as after first brewing it is practically completely extracted out of the leaves.

Pu-erh Tea

Pu-erh tea is a Chinese tea, grown and produced in the Pu-erh area of the Yunnan province. [65] This type of tea can be found as loose-leaf, but more often it is compressed in a disc. [66]

There is a special preparation technology (described in detail in a chapter 5.3), which among other suggests to pour out the first brewed water. It is usually used to warm up the tea cups, but it is not used for drinking. Based on this fact, it was decided to measure the first steeping and to compare it with the subsequent ones. All together one compressed portion of pu-erh leaves was brewed for five times.

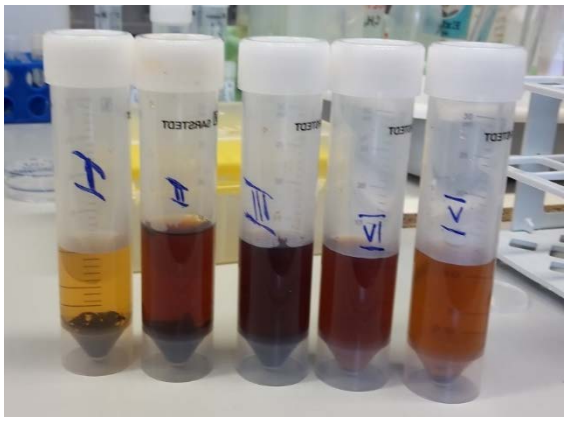

Figure 44 Color correlation of the five steeping of Pu-erh tea.

Figure 44 shows the beautiful color correlation between five different steeping of the pu-erh leaves. From the first to third extraction, the color is getting darker and towards fifth sample it is lightening again.

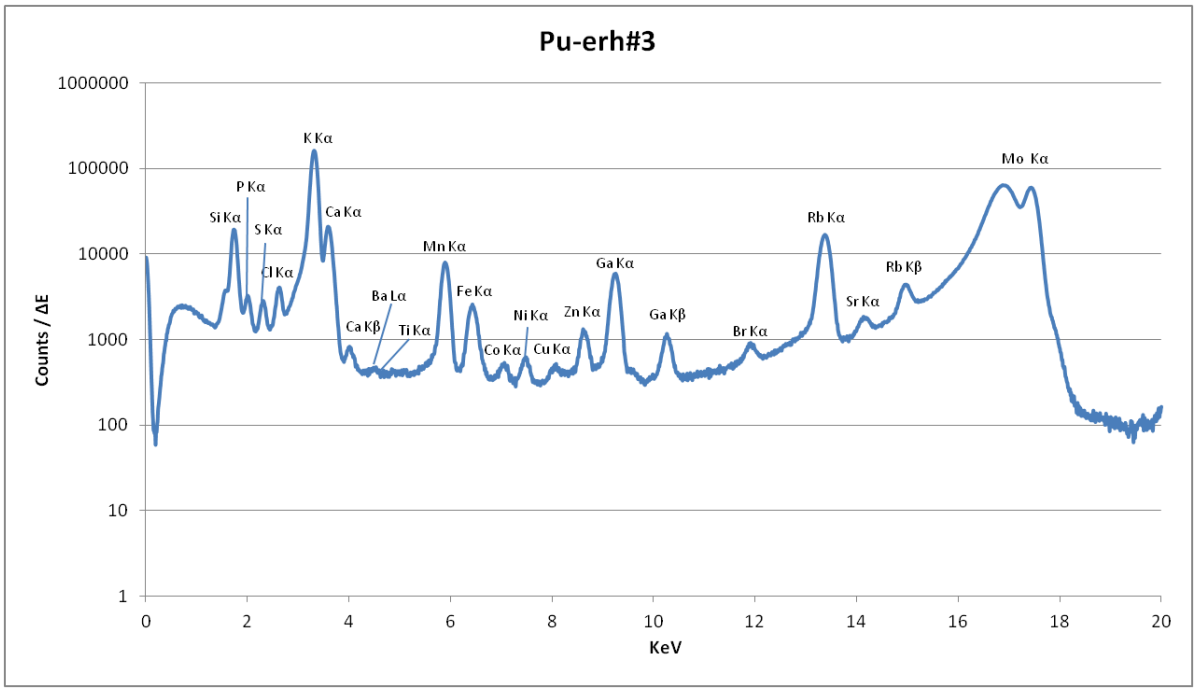

Figure 45 The characteristic spectrum of the Pu-erh#3 sample.

The characteristic spectrum of the Pu-erh tea sample is shown on Figure 45. The third steeping (with the darkest color) was chosen as an illustration, because it contains the most amounts of elements (detailed comparison in the table 9). Notable are Barium L alpha line (Ba Lα) and Titanium K alpha line (Ti Kα), which are rather rare elements in the tea samples. These elements are present in the very small amounts and are not clearly visible on a spectrum. It is possible, that these elements will be seen more pronounce with more sensible equipment. The largest met element is again the Potassium.

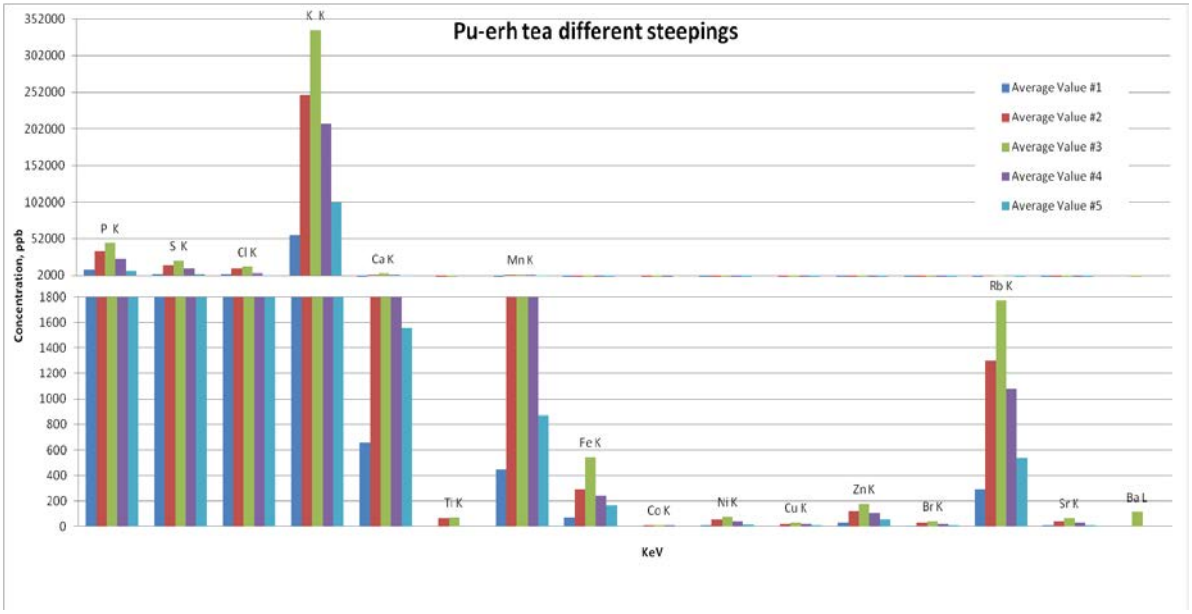

Figure 46 Comparison of the average values between samples Pu-erh#1 - #5.

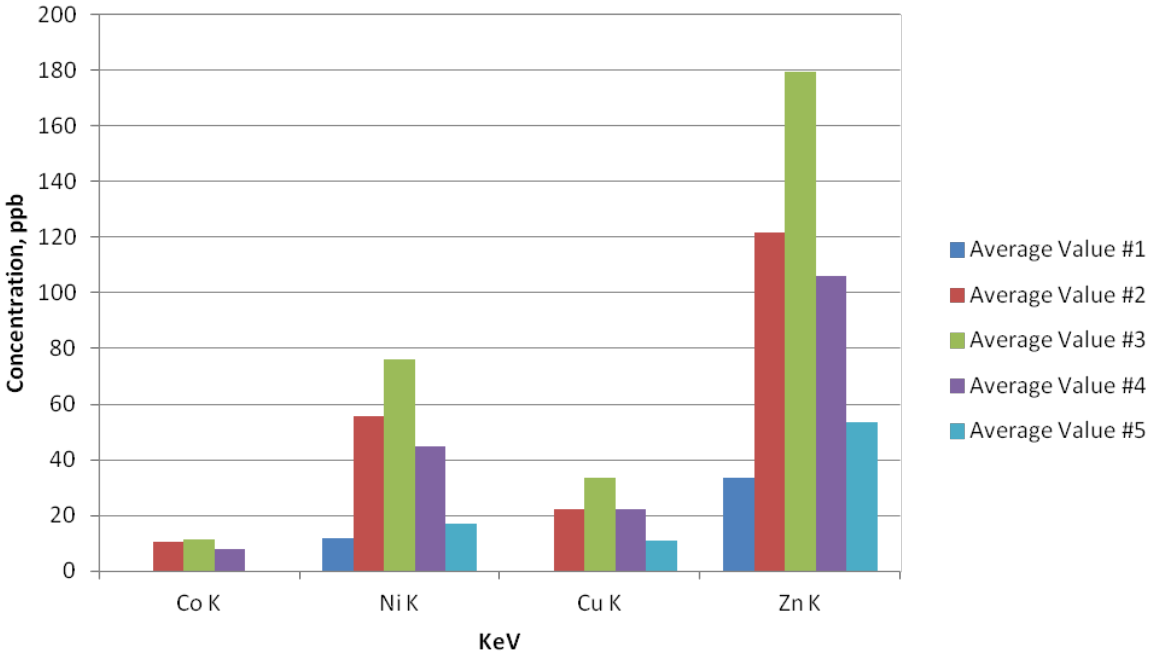

Figure 47 Close-up of the comparison of the average values between samples Pu-erh#1 - #5 for Cobalt, Nickel, Copper and Zinc.

The Figure 46 shows a bar diagram as comparison of average values, normalized over the internal standard Ga, between all five Pu-erh tea samples. The close-up of one section of the

diagram 46 is shown in the Figure 47. It displays elements with very small quantities, and is here for better demonstration of the distribution of those elements in all five samples. The distribution matches exactly the color distribution of the Pu-erh specimens, both for elements present only in small concentrations and for abundant elements.

Element	Average Value #1, ppb	STDEV #1	Average Value #2, ppb	STDEV #2	Average Value #3, ppb	STDEV #3
P K	8479.2	115.35	31791.8	608.69	41442	2670.11
S K	3701.2	287.87	14842	281.87	19780.4	877.44
Cl K	3287.4	256.06	10634.7	401.51	12488.1	1105.53
K K	51593.7	946	226356.3	2973.64	304982.9	11072.32
Ca K	598.2	12.37	2408.7	100.9	4037.9	75.95
Ti K			59		62.4	2.48
Mn K	404.2	9.97	1996.8	22.63	3073.7	78.55
Fe K	61.2	5.6	262.5	34.72	493.9	17.93
Co K	0		9.5		10.3	1.56
Ni K	10.9	2.82	50.6	2.81	68.7	4.92
Cu K	0	0.23	20.2	1.25	30.2	1.81
Zn K	30.7	3.98	110.6	2.84	162.4	7.3
Br K	6.7	1.67	31	1.57	40.5	3.05
Rb K	261.8	5.43	1181.8	21.87	1603.5	40.73
Sr K	7.7	1.16	39.7	2.39	56.9	1.64
Ba L					105.3	2.19

Element	Average Value #4, ppb	STDEV #4	Average Value #5, ppb	STDEV #5
P K	22065.8	1603.58	7386	1297.6
S K	10229.1	400.31	3529.2	165.99
Cl K	4675.1	854.44	1819.8	223.62
K K	190202.1	3007.77	92596.1	3195.17
Ca K	2462	71.43	1416.1	52.55
Ti K	46.8			
Mn K	1842.8	26.44	791.7	16.59
Fe K	219.3	19.05	150.6	45.56
Co K	7.3		0	
Ni K	40.8	3.42	15.4	1.32
Cu K	20.3	2.37	10.1	0.7
Zn K	96.5	5.39	48.6	1.53
Br K	20.3	1.52	8.2	0.81
Rb K	986.7	10.34	490.1	13.33
Sr K	28.2	1.89	10.1	1.28
Ba L				

Table 9 Comparison of average values between Pu-erh#1 to Pu-erh#5 tea samples

The table 9 shows collation of the normalized average values over all five samples. Notable is that all the highest amounts are in the third sample and that the data distribution matches the color distribution perfectly.

6.1.2. Herbal tea

All together there were 13 different sorts of herbs. Leaves, blossoms, seeds and bark were used to create herbal infusions, using established standard procedure. Among natural loose herbs, the packaged teas were also measured. The brewing process for the tea-bags was done as suggested on the package (as the quantity of the herb in the bag in each product is slightly different).

Anise

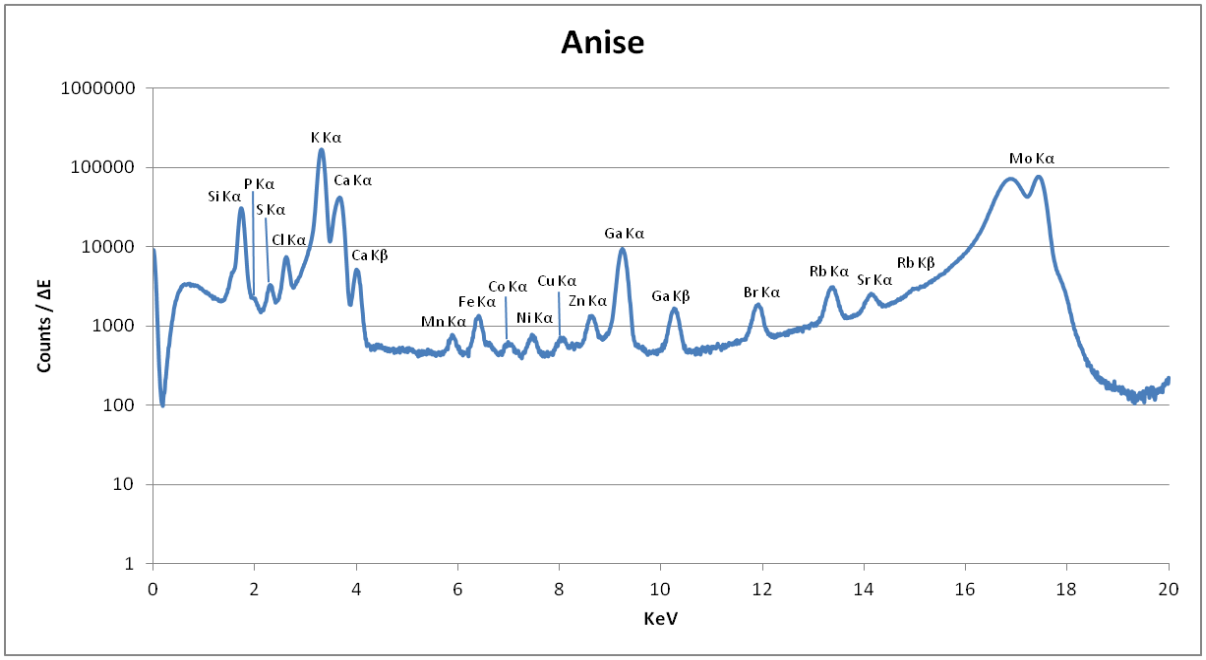

Figure 48 The characteristic spectrum of the Anise sample.

Figure 48 shows the characteristic spectrum of the anise infusion. In this sample the overall amount of elements is rather small (Table 10). The highest value has potassium followed by calcium.

Element	Average value Anise, ppb	STDEV Anise
P K	5150,4	287.24
S K	11516,5	536.24
Cl K	15464,5	608.69
K K	193231,5	4664.85
Ca K	29531,6	454.34
Mn K	78,7	4.3
Fe K	153	37.43
Co K	10,2	3.47
Ni K	47,3	2.17
Cu K	29,6	2.25
Zn K	93,6	2.87
Br K	83,4	3.51
Rb K	111,9	2.36
Sr K	51,6	1.54

Table 10 Average values of elements in Anis infusion sample normalized over the IS concentration.

Valerian

There are two different samples of Valerian: Valerian#1 and Valerian#2. Both obtained in different pharmacies in Vienna. It was interesting to compare same kind of herb from various manufacturers.

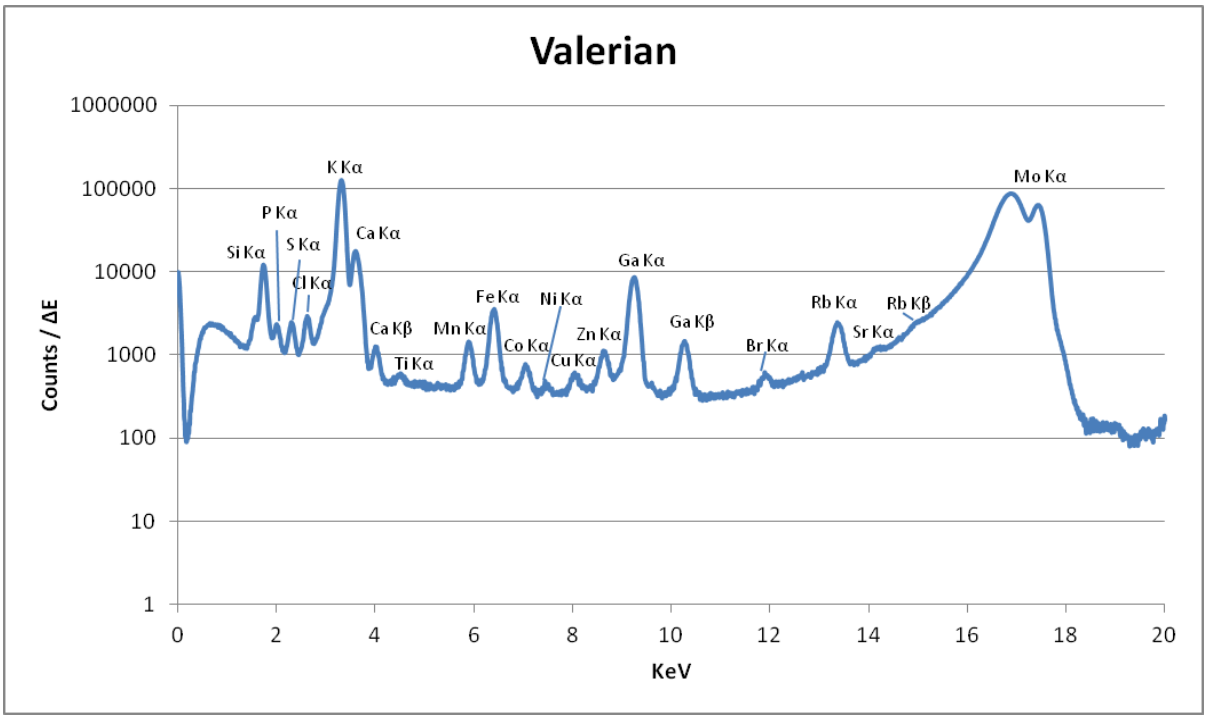

Figure 49 The characteristic spectrum of the Valerian#1 sample.

The characteristic spectrum of the Valerian#1 sample is shown in the Figure 49. The sample shows traces of Titanium. There are small amounts of Strontium and Nickel, as well as Titanium.

Element	Average Value Valerian #1	STDEV Valerian #1	Average Value Valerian #2	STDEV Valerian #2	Difference, %
P K	30538.2	2397.32	21273.1	875.02	30%
S K	16030.8	1254.67	14241.4	489.22	11%
Cl K	9306	679.08	7632.2	210.38	18%
K K	233974.3	7815.89	188376.9	2311.01	20%
Ca K	4701.7	178.64	6269.6	61.82	25%
Ti K	68.6	30.9	86.9	15.76	21%
Mn K	258.3	8.06	310.5	3.22	17%
Fe K	428.7	32.61	719.8	95.42	40%
Ni K	20.1	2.78	18.4		9%
Cu K	22.4	3.78	36.5	1.42	39%
Zn K	116.7	5.07	100.4	3.2	14%
Br K	10.4	0.85	14.6	1.57	29%
Rb K	92.4	3.53	134.9	1.45	32%
Sr K	15.2	1.99	10.2	1.64	33%

Table 11 Comparison between average values of elements in Valerian#1 and Valerian#2 infusion sample normalized over the IS concentration.

Table 11 shows numerical comparison between results of the two separate Valerian samples. Most of the elements have difference between them in the range between 20% and

40%. However Nickel, Sulfur and Zinc are more comparable. The bar diagram (Figure 50) shows that visually the elements with smaller amounts are perceived to have less difference even though numerically it appears significant (as an example Figure 50: Iron and Copper).

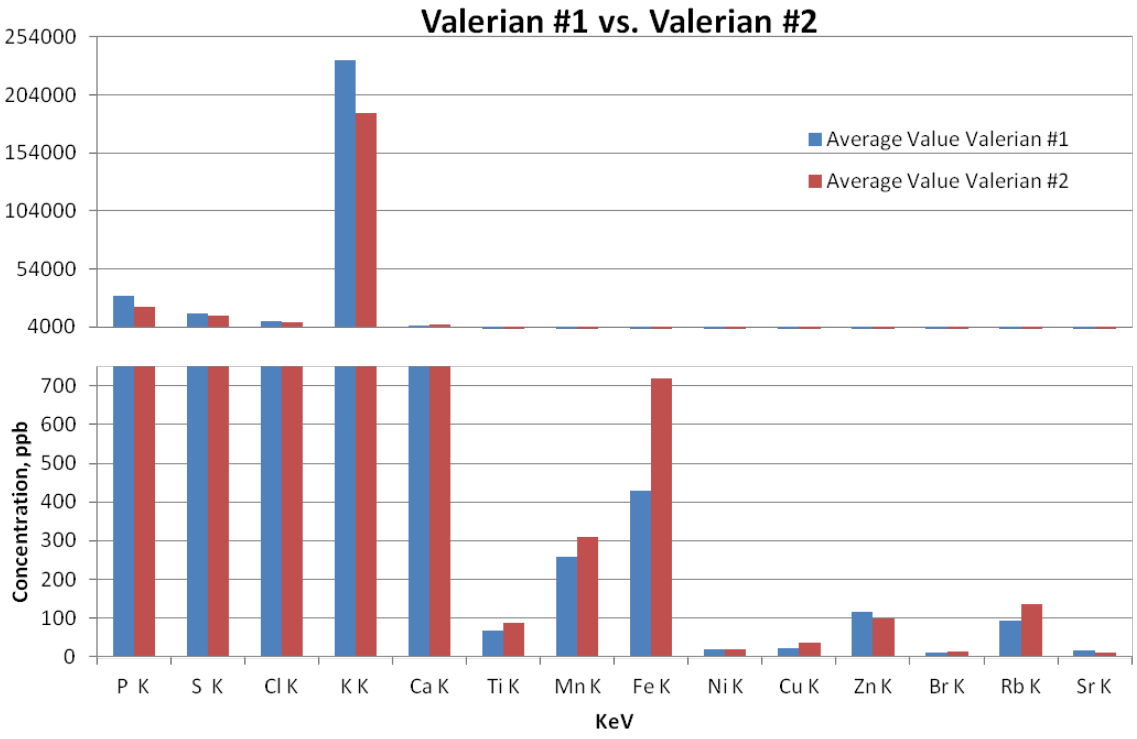

Figure 50 Comparison of the average values between samples Valerian#1 and Valerian#2.

Fennel

For this particular sort of herbs, three different samples were chosen: two samples of loose fruits (often mistakenly considered to be seeds) and one packaged tea from the local store.

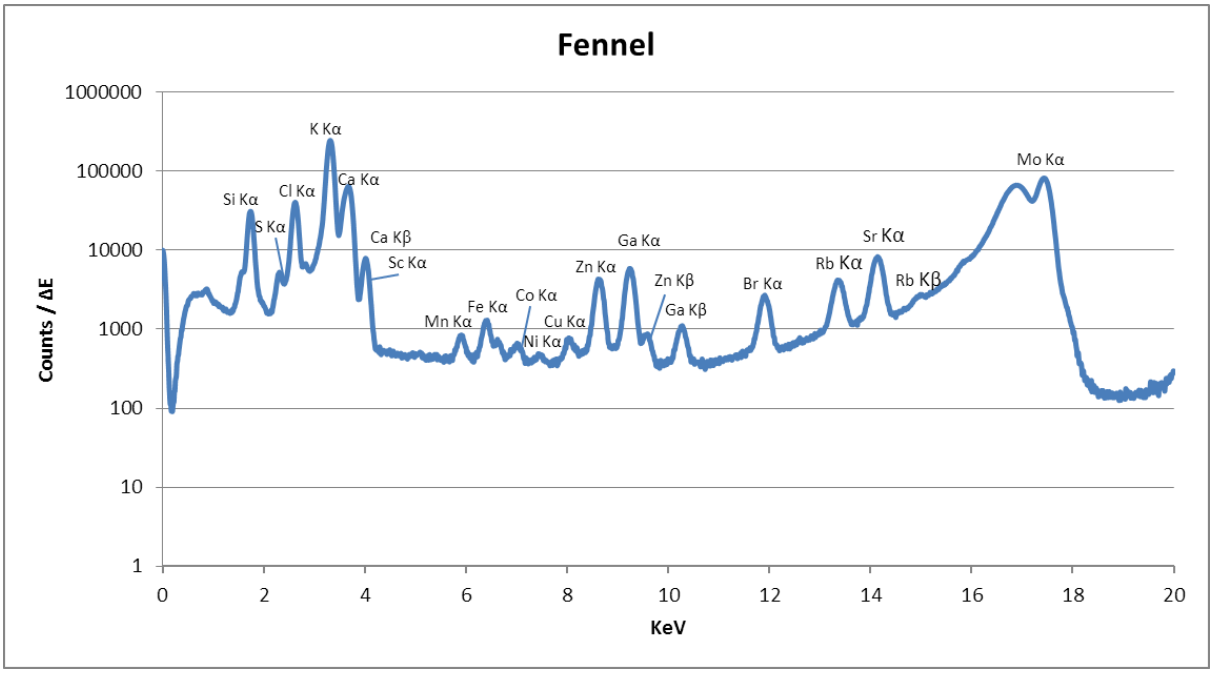

Figure 51 The characteristic spectrum of the Fennel#1 sample.

For the representation of the characteristic spectrum of the Fennel sample, sample Fennel#1 was chosen, because it has the most elements in it. There are traces of Cobalt, Scandium and Nickel. The highest value has Potassium followed by Chlorine and Calcium.

Element	Average Value Fennel#1, ppb	STDEV Fennel #1	Average Value Fennel#2, ppb	STDEV Fennel #2
P K	5215.8	767.61	4737.4	330.23
S K	34459.1	1862.13	5668.5	274
Cl K	189247.8	11765.67	11794.8	577.29
K K	499331.2	32288.69	102799.7	3657.29
Ca K	84025.3	2095.01	10226.8	207.6
Sc K	89.1	0.12		
Mn K	171.3	4.42	11.4	1.94
Fe K	293.8	87.47	32.4	19.01
Co K	31.9	5.21		
Ni K	19.5	4.4	4.7	0.88
Cu K	75.7	2.27	17.1	2.58
Zn K	796.4	8.48	18.3	6.53
Br K	271	5.15	30.3	1.27
Rb K	332	6.09	19.1	1.46
Sr K	671.8	4.74	19	1.32
Element	**Average Value Fennel Package, ppb**	**STDEV Fennel Package**	**Difference #1 to #2, %**	**Difference #1 vs. Package**
P K	7604.8	1040.15	9%	31%
S K	23440.7	677.83	84%	32%
Cl K	91123.4	3000.75	94%	52%
K K	317564.9	7099.47	79%	36%
Ca K	22879.8	277.61	88%	73%
Sc K				
Mn K	88.8		93%	48%
Fe K	214.4	5.64	89%	27%
Co K	10.1	26.69		68%
Ni K	27.5	1.92	76%	29%
Cu K	106.6	2.2	77%	29%
Zn K	200.4	3.14	98%	75%
Br K	54.8	4.05	89%	80%
Rb K	110.3	4.14	94%	67%
Sr K	225.4	2.44	97%	66%

Table 12 Comparison between average values of elements in Fennel#1, Fennel#2 and Fennel Package infusion samples normalized over the IS concentration.

From the Table 12 it can be seen that there is a great difference between both loose fruits samples (Fennel#1 and Fennel#2), except Phosphorus (only 9%), other elements varies from 75% to 98%.

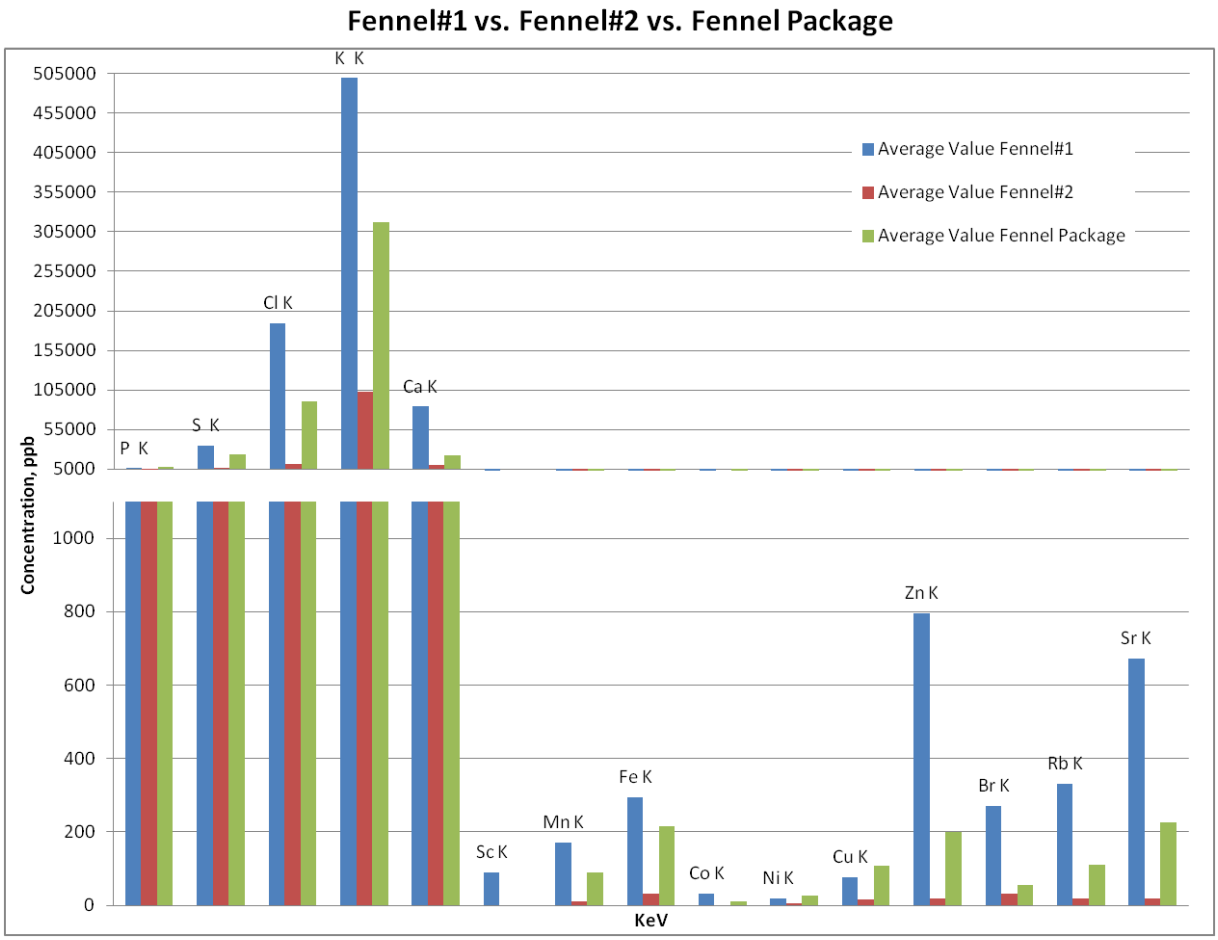

Figure 52 Comparison of the average values between samples Fennel#1, Fennel#2 and Fennel Package.

Figure 52 represents a bar diagram as a comparison between all three fennel samples. The packaged tea has overall a median value.

Common Mallow

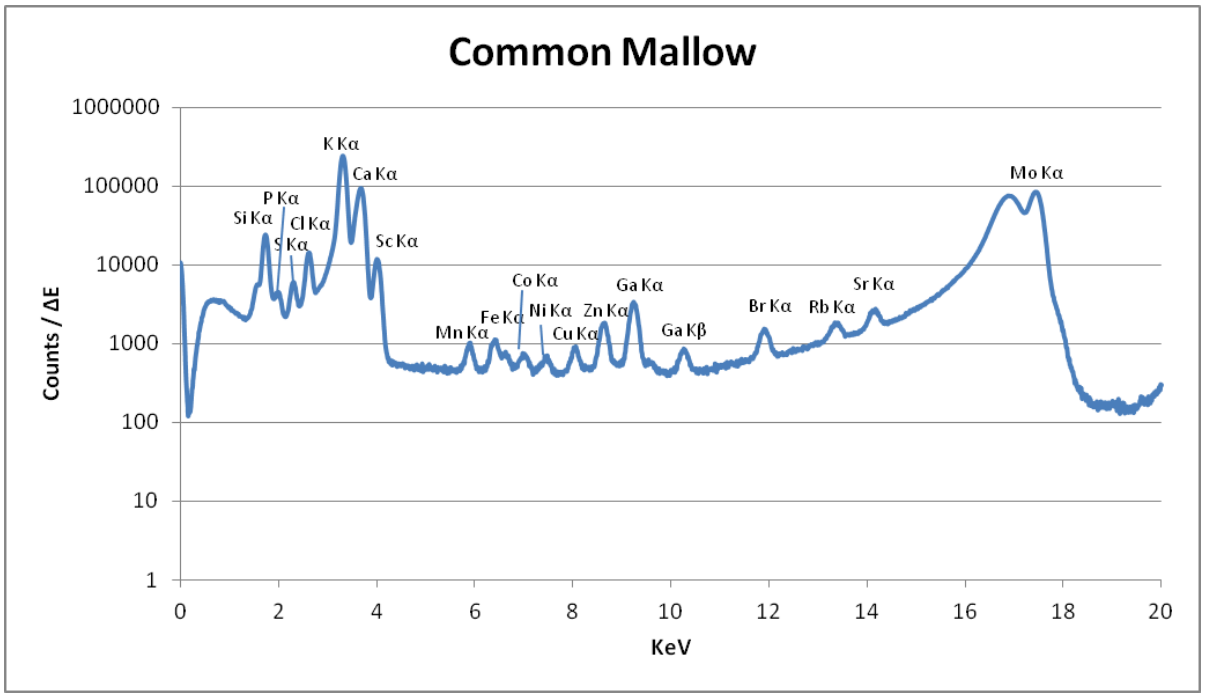

Figure 53 The characteristic spectrum of the Common Mallow sample.

Next sample was common mallow – interesting fact about this sample is, that in several sources (67, 68) there are listings of beneficial metals that are said to be found in the plant. Among them Iron, Zinc, Magnesium, Potassium and Selenium [67,68]. Magnesium can not be detected by means of Atomika 8030C, however Selenium can be detected and apart from all the other elements Selenium is the one that is not detected in measured sample. Magnesium is complicated to measure, because of the intense Si peak (it comes from the sample holder glass), which is overlapping with K line peak of Magnesium. The smallest value (see Table 13) has Cobalt, followed by Nickel (both less than 100 ppb).

Element	Average value, ppb	STDEV Common Mallow
P K	103173.3	9075.69
S K	90246.0	2504.51
Cl K	110958.7	2982.93
K K	910063.6	31688.24
Ca K	237379.2	4391.51
Mn K	422.5	36.24
Fe K	389.6	64.56
Co K	50.1	10.01
Ni K	97.9	10.25
Cu K	174.2	14.15
Zn K	528.0	34.67
Br K	200.0	6.13
Rb K	111.7	6.14
Sr K	191.0	4.96

Table 13 Average values of elements in Common Mallow infusion sample normalized over the IS concentration.

Calamus

Calamus is the only sample where the plants rhizome was used. The preparation of the infusion was done using already established procedure (for detail see chapter 5.3).

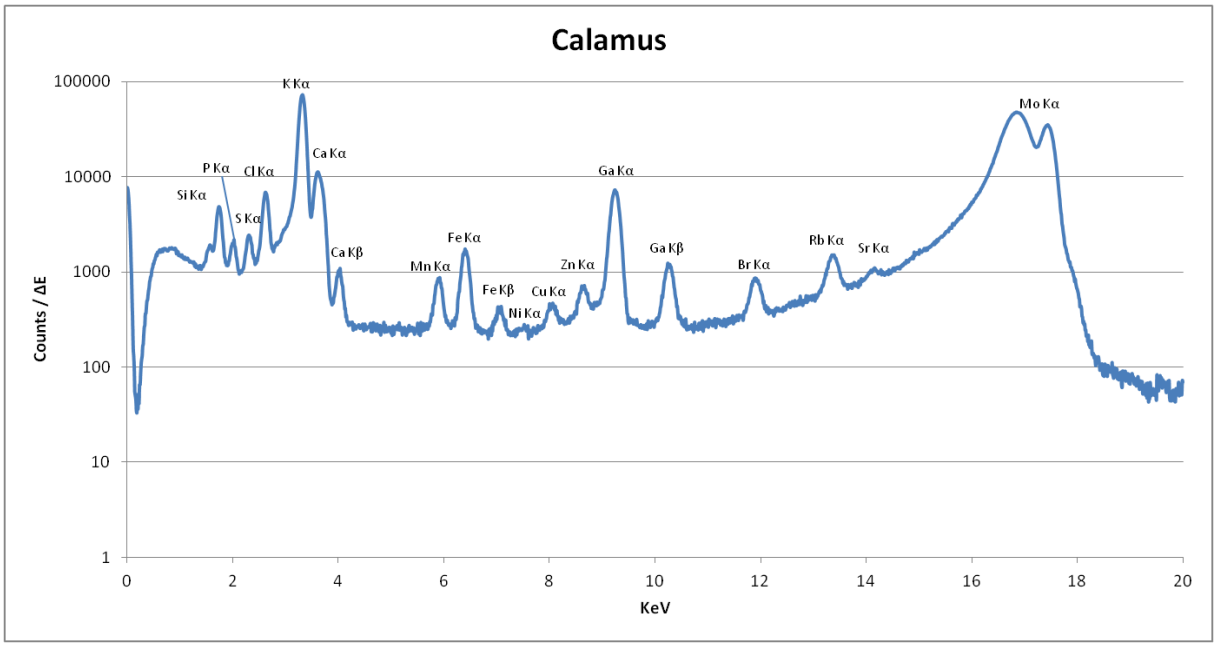

Figure 54 The characteristic spectrum of the Calamus sample.

The presented sample has rather small variety of elements. The lowest value has Nickel: it was detected not in all repeated measurements of one sample, suggesting the amount of the Nickel is very small. A little bit more of concentration has Strontium, which, however is to be found in all control measurements.

Element	Average Value, ppb	STDEV Calamus
P K	24205.8	5420.05
S K	16192.5	3467.61
Cl K	25350.8	4096.99
Ar K	703.0	11831.03
K K	122375.5	618.59
Ca K	6626.9	14.49
Mn K	210.1	41.3
Fe K	341.2	3.88
Ni K	7.7	2.81
Cu K	35.8	5.28
Zn K	46.4	1.47
Br K	52.6	2.82
Rb K	73.3	1.32
Sr K	15.6	5420.05

Table 14 Average values of elements in Calamus infusion sample normalized over the IS concentration.

The Table 14 shows the average value of elements from all five control-measurements of the Calamus infusion sample. The data was normalized over the internal standard element Gallium.

Chamomile

Chamomile tea is one of the all-time favorites among herbal teas. It is also one of the most famous. The Chamomile is known as the “all-rounder” [69] for its calming, anti-inflammatory and spasmolytic effects, which is especially beneficial against stomach troubles. There were two Chamomile samples measured: pure blossoms and one bio packaged tea.

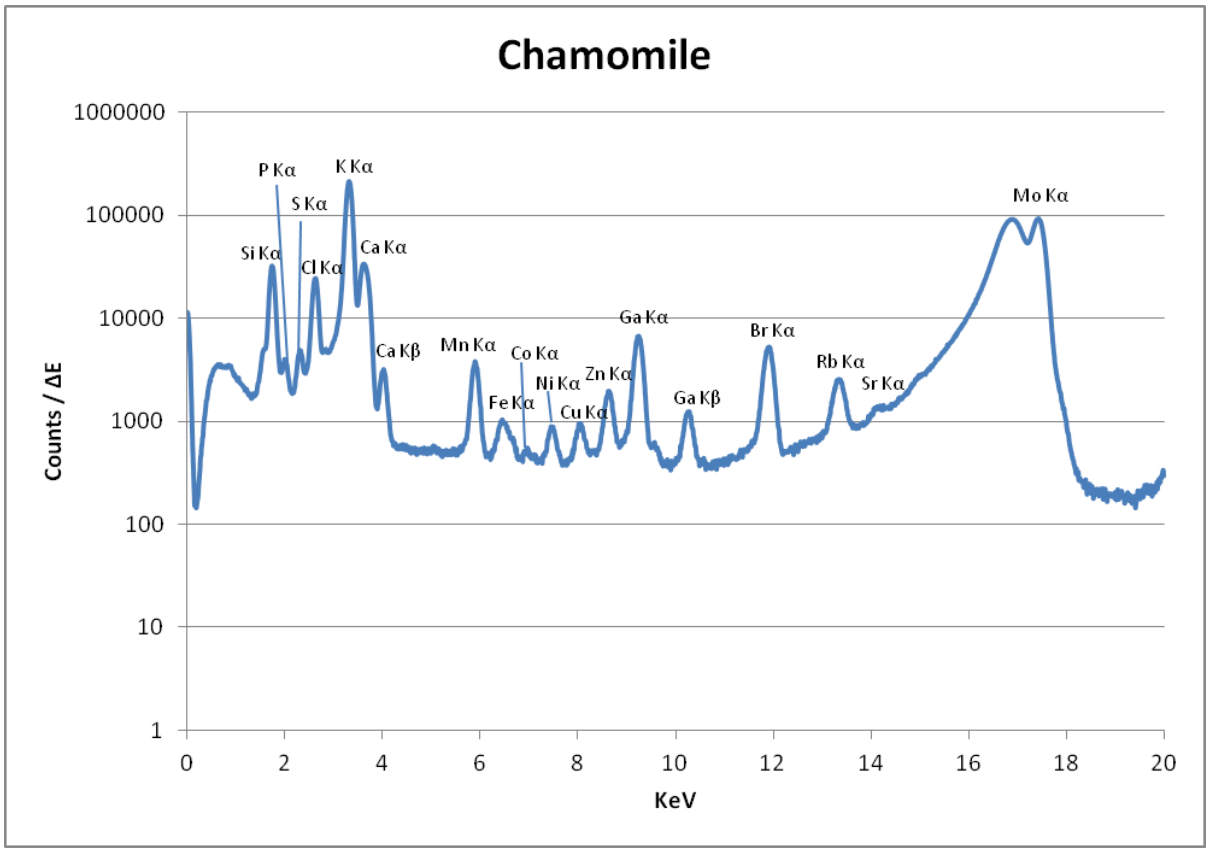

Figure 55 The characteristic spectrum of the Chamomile sample.

The characteristic spectrum of the Chamomile sample (Figure 55) shows traces of 14 elements. The biggest value has Potassium and the lowest value Cobalt in both samples.

Elements	Average Value Chamomile, ppb	STDEV Chamomile	Average Value Chamomile Package, ppb	STDEV Chamomile Package	Difference, %
P K	50212.5	2518.05	17137.9	1431.55	66%
S K	34075.6	1976.45	28465.6	1122.03	17%
Cl K	105746.9	8260.96	178702.5	17871.6	41%
K K	411916.6	19841.98	252164.2	2798.08	39%
Ca K	26803.5	948.68	38660.1	404.62	31%
Mn K	1300.2	36.29	292.7	7.73	78%
Fe K	73.2	21.85	95.8	22.99	24%
Co K	14.3	4.71	18.1	1.7	21%
Ni K	113.9	2.92	28.3	2.66	75%
Cu K	101.4	5.79	61.7	2.39	39%
Zn K	278.6	11.03	954.7	5.05	71%
Br K	557.1	20.17	325.4	5.8	42%
Rb K	128.6	4.13	48.5	2.4	62%
Sr K	21.2	1.13	391.9	2.05	95%

Table 15 Comparison between average values of elements in Chamomile and Chamomile Package infusion samples normalized over the IS concentration.

Table 15 and Figure 56 show comparison between packaged and loose herbal tea. The highest difference between both samples is seen in Strontium, it is 95%. Out of the bar diagram it is clear that it is impossible to conclude that one of the samples has higher / smaller amount of each present element. Even though the amount of elements is the same the value of them varies in samples from element to element. Out of 14 elements, five have higher value in packaged Chamomile sample.

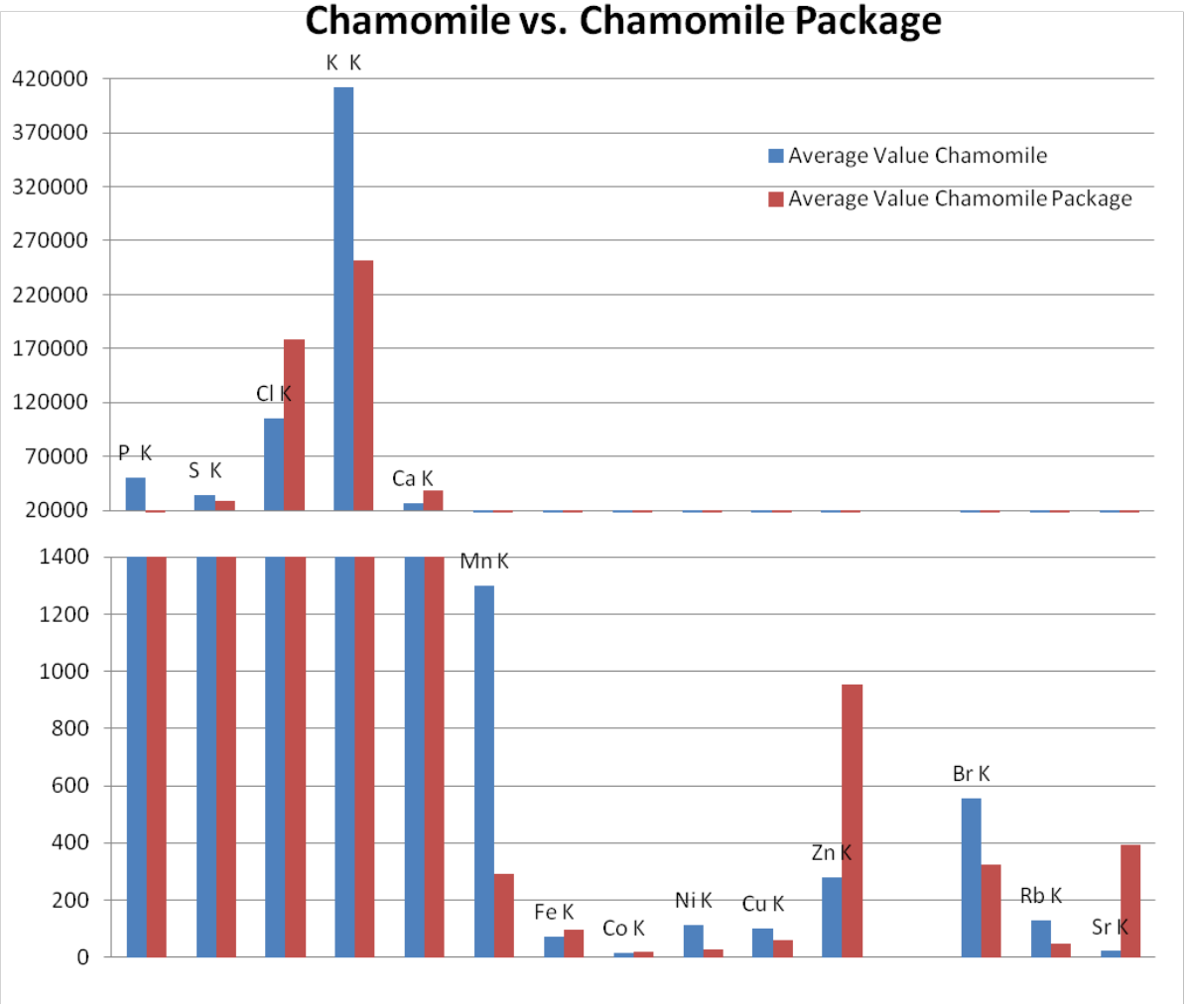

Figure 56 Comparison of the average values between Chamomile and Packaged Chamomile samples.

Lapacho

Lapacho is the only sample where the plants bark was used. The name of this herbal tea comes from the name of the tree, the inner bark of which is used, nowdays, as tea. [70] The established procedure of preparing the infusion was used here without any changes.

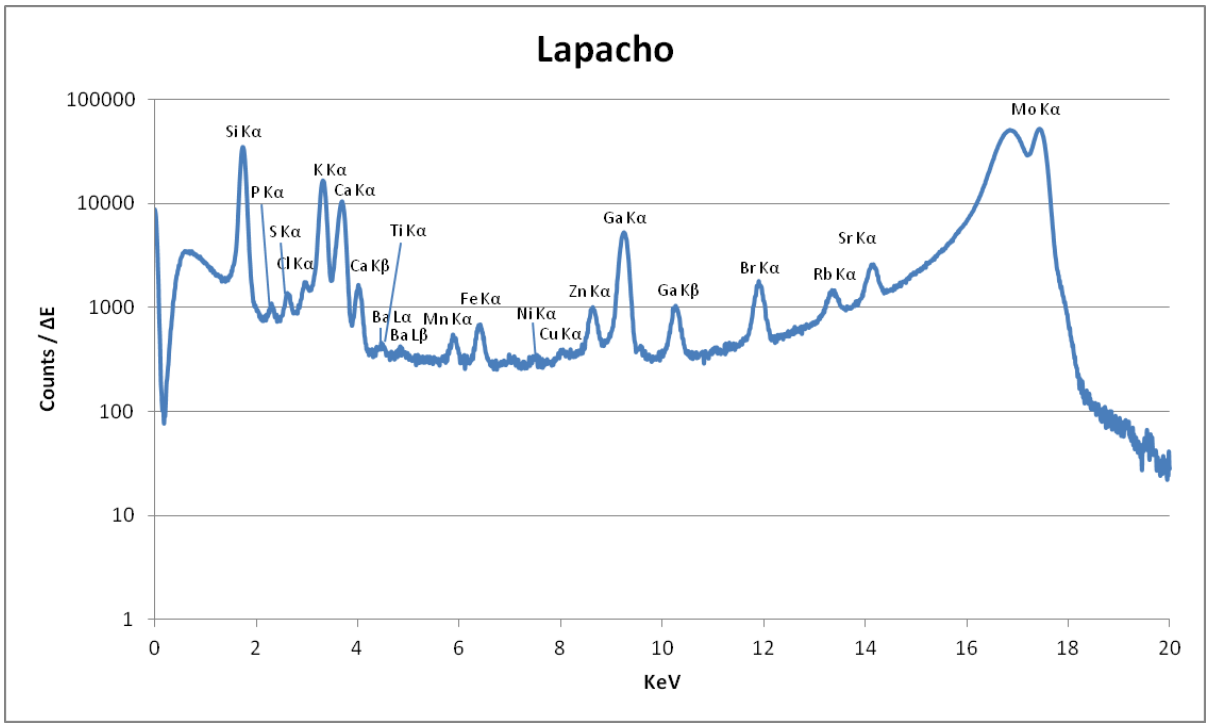

Figure 57 The characteristic spectrum of the Lapacho sample.

This sample has several interesting elements, which are rather uncommon to be found in tea: Barium L-line and Titanium K-line. The smallest value has Nickel and the highest (as it seems, in all teas and herbal infusions) has Potassium.

Element	Average Value, ppb	STDEV Lapacho
P K	1599.8	
S K	3437.7	361.69
Cl K	4717.7	1654.92
K K	35540.8	690.56
Ca K	15085.3	364.9
Ti K	46.9	
Mn K	100	4.57
Fe K	115.5	25.39
Ni K	9.6	2.53
Cu K	14.3	1.88
Zn K	116.2	19.49
Br K	172.7	6.86
Rb K	54.2	2.01
Sr K	141.7	2.86
Ba L	191.3	32.08

Table 16 Average values of elements in Lapacho infusion sample normalized over the IS concentration.

Out of the numerical representation of the elements (Table 16) it can be seen that overall values are rather small: except Phosphorus, Sulfur, Chlorine, Potassium and Calcium, all other ten elements are in the range from 9 to about 200 ppb.

Lavender

For Lavender tea, the blossoms of the *Lavandula angustifolia* (latin) were used. The infusion had light purple color and very intense scent. Lavender is more known for its essential oil, but it also can be used as tea.

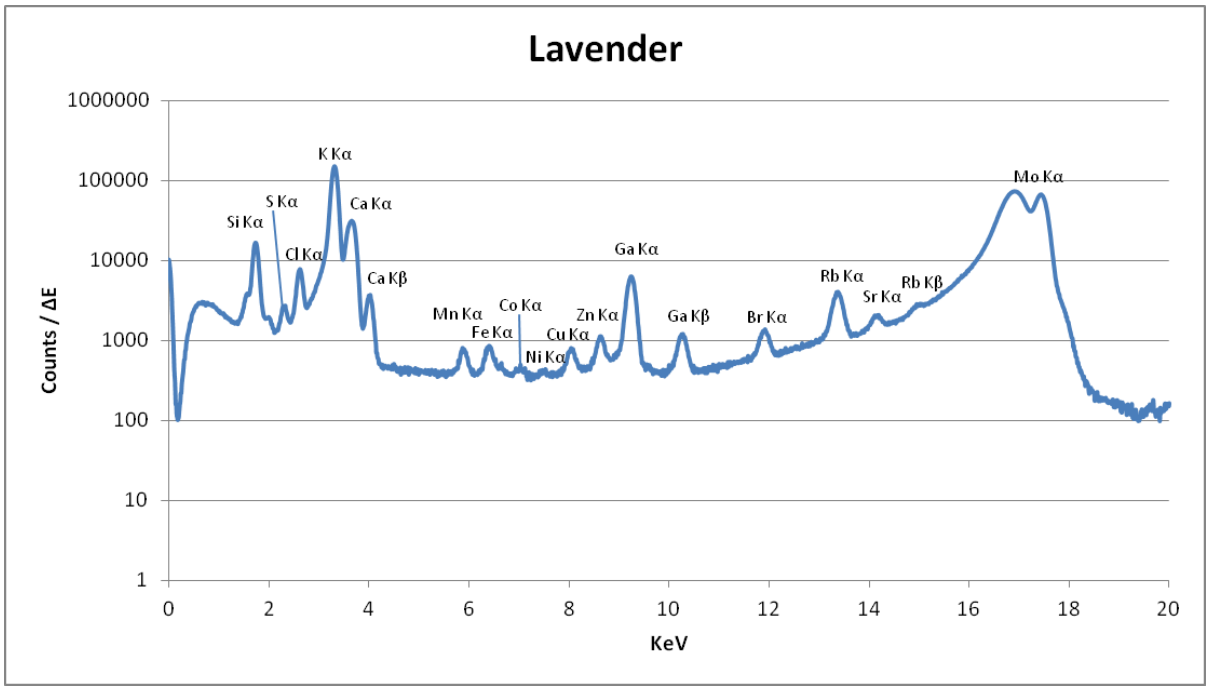

Figure 58 The characteristic spectrum of the Lavender sample.

The Figure 58 shows the characteristic spectrum of the sample. The average values of elements in Lavender infusion sample normalized over the IS concentration are shown in the Table 17. There one can see that the values of the most elements (except P, S, Cl, K and Ca) are quite low. However, the traces of Cobalt are present.

Element	Average Value, ppb	STDEV Lavender
P K	11207.7	1056.53
S K	16711.8	973.73
Cl K	30588.1	1433.12
K K	289898.9	6444.7
Ca K	34069	776.46
Mn K	175	8.47
Fe K	161	20.45
Co K	11.3	1.11
Ni K	13	2.24
Cu K	81.3	3.7
Zn K	117.6	2.72
Br K	85.7	3.01
Rb K	291.4	4.42
Sr K	59.3	1.78

Table 17 Average values of elements in Lavender infusion sample normalized over the IS concentration.

Peppermint

Peppermint is one of the most popular and beloved herbal beverages. It is known for its specific aroma and calming qualities.

For measurements were used two different samples of loose Peppermint leaves and one packaged tea. The loose leaves teas were brought from different countries: Minze#2 was obtained in Austria and MinzeOma was a gathering of wildly growing peppermint in Latvia.

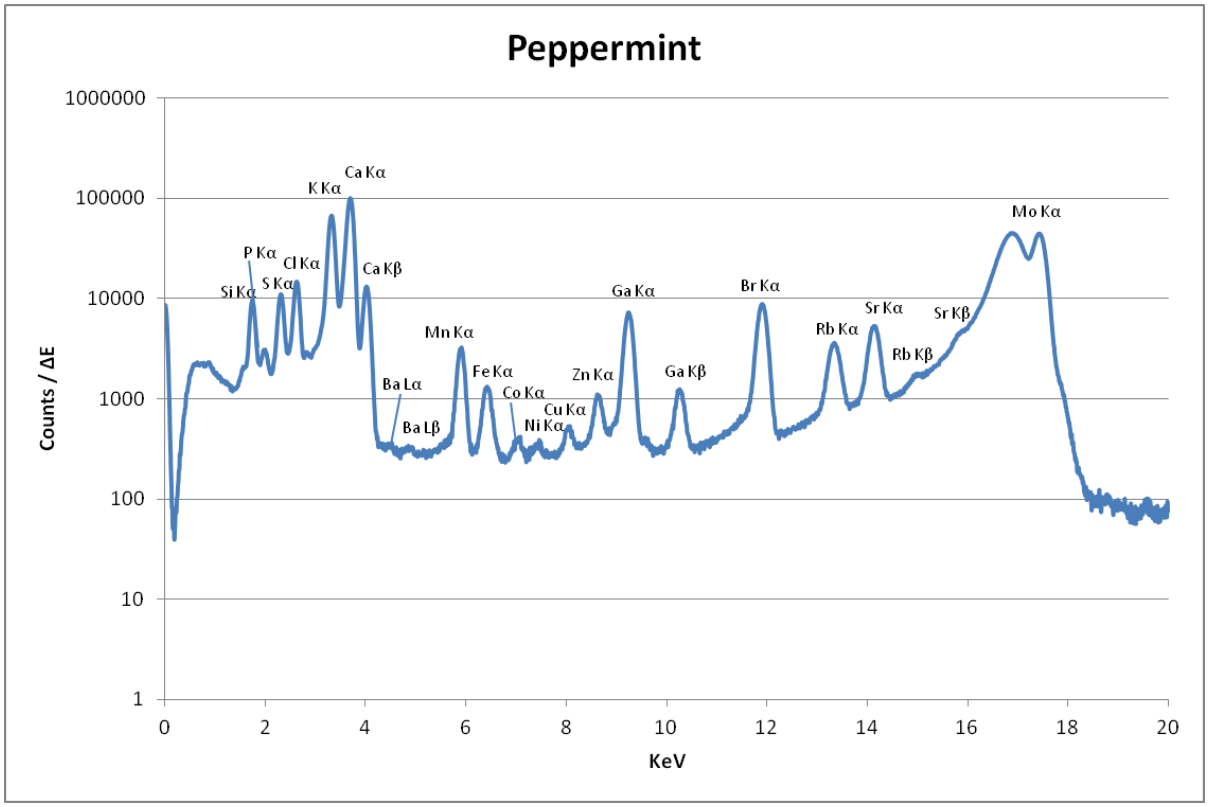

Figure 59 The characteristic spectrum of the Peppermint sample.

As representative sample (Figure 59) for the peppermint samples the MinzeOma sample was selected, as it has the highest values among all three samples. The Ba L-line is present in this sample and in the Peppermint Package sample (Table 18), it is, however, absent in Minze#2 sample.

Element	Average Value MinzeOma, ppb	STDEV MinzeOma	Average Value Minze#2, ppb	STDEV Minze#2
P K	20334.5	1559.48	92708.1	1013.26
S K	98599.9	5627.94	33072.5	934.39
Cl K	56301.8	2077.09	245397.7	10051.01
K K	112067.9	3477.81	722927.5	16871.25
Ca K	120312	3426.02	92708.1	1013.26
Mn K	1035	18.3	318.7	4.63
Fe K	244.8	7.68	72.7	10.41
Co K	9.2	1.05	35.4	9.23
Ni K	14.5	3.9	48.8	4.08
Cu K	45.5	6.93	90.4	7.22
Zn K	123.2	7.26	166.2	9.29
Br K	885.4	18.68	354.5	9.19
Rb K	172.9	3.19	80.4	3.75
Sr K	345.2	9.25	57.2	2.52
Ba L	150.2	27.8		

Element	Average Value Peppermint Package, ppb	STDEV Peppermint Package
P K	14684.8	1200,24
S K	59780.7	431,03
Cl K	125513.7	1450,9
K K	259058.9	2035,42
Ca K	50689.8	177,02
Mn K	263.2	3,92
Fe K	146.7	48,24
Co K	10.5	1,61
Ni K	15.6	3,06
Cu K	35.8	2,87
Zn K	105.1	5,15
Br K	171.5	3,7
Rb K	81.1	3,29
Sr K	388.5	4,19
Ba L	110.1	

Table 18 Comparison between average values of elements in Minze#2, MinzeOma and Peppermint Package infusion samples normalized over the IS concentration.

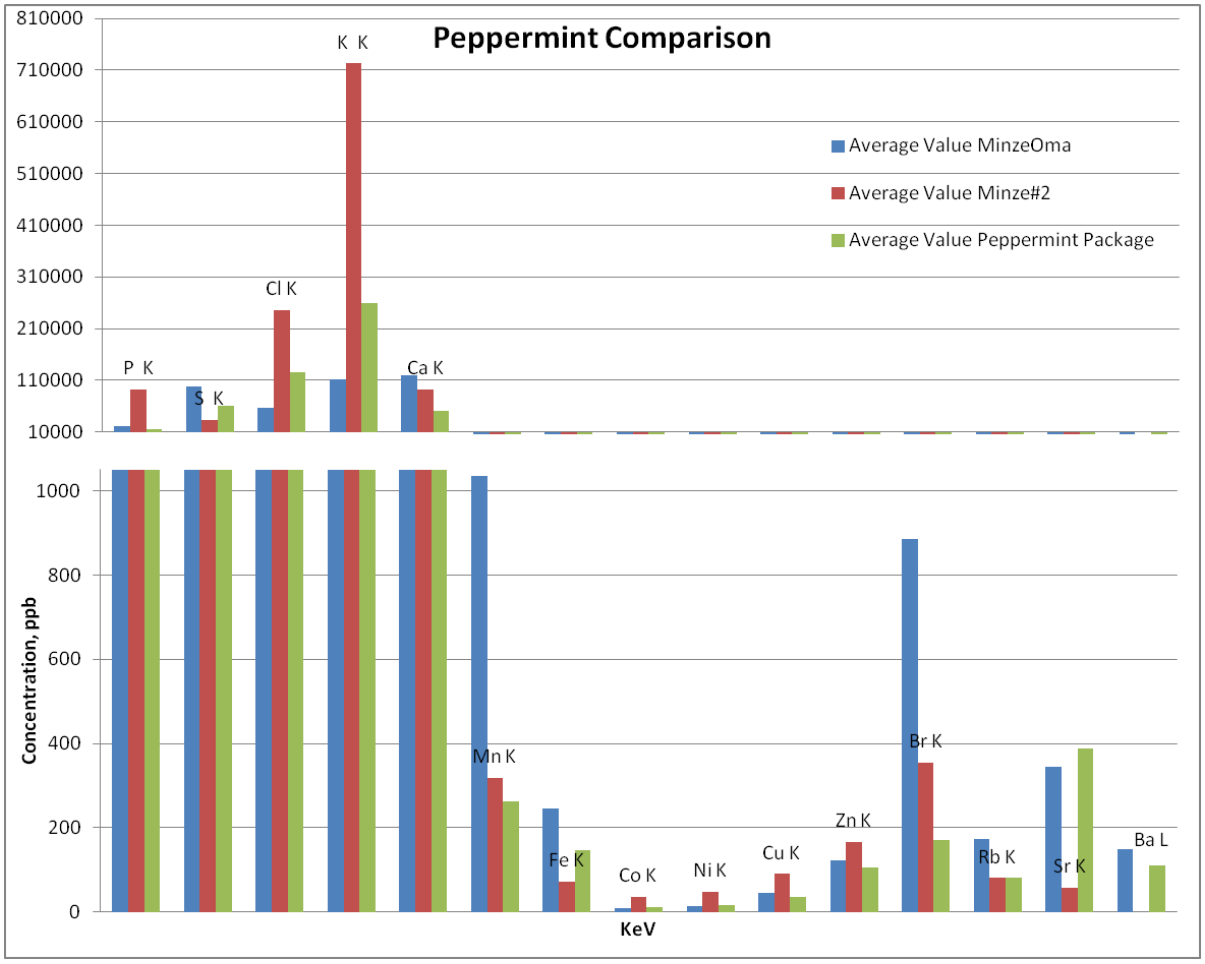

Figure 60 Comparison of the average values between MinzeOma, Minze#2 and Peppermint Package samples.

To make the results visual, the bar diagram (Figure 60) was created. It shows comparison of the average values between MinzeOma, Minze#2 and Peppermint Package samples. The most of Phosphorus, Chlorine, Potassium, Cobalt, Nickel, Copper and Zinc has Minze#2 sample. The only element where packaged tea has the at most value is Strontium.

Sage

For this type of herb were also taken two samples: loose leaves Sage and packaged tea Sage Package.

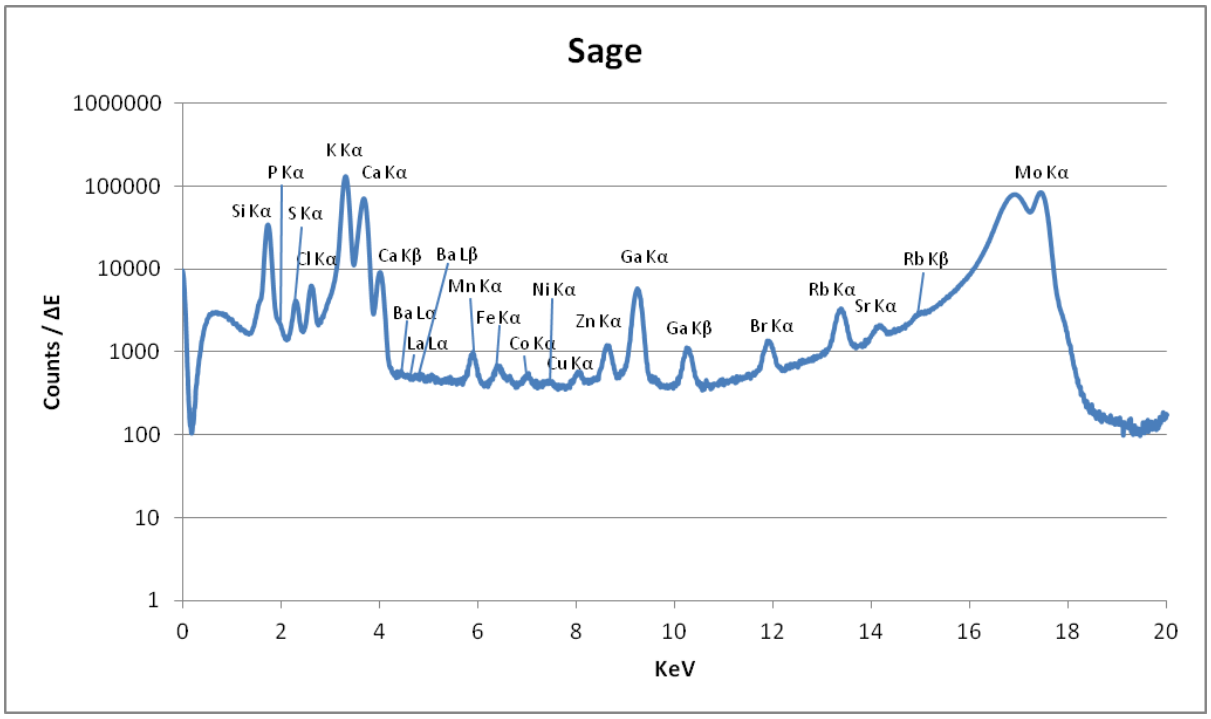

Figure 61 The characteristic spectrum of the Sage sample.

Characteristic spectrum of the Sage sample (Figure 61) shows all trace elements found in this infusion. Rather unexpected is detected Lanthanum L-line. The highest value has Potassium and the lowest value Nickel (Table 19).

Element	Average Value Sage, ppb	STDEV Sage	Average Value Sage Package, ppb	STDEV Sage Package
P K	7215	1118,57	9701.5	1077,9
S K	35812	1043,12	20340.5	706,41
Cl K	25859.3	1443,65	18306.8	2373,63
K K	278938.1	8752,88	213570.7	6688,76
Ca K	103330.6	1450,55	71547.4	917,97
Mn K	234.4	4,77	268.9	6,17
Fe K	64.3	11,02	231.1	37,36
Co K	13.3	2,34	9.4	1,51
Ni K	11	2,54	20.8	2,71
Cu K	36	2,14	19.5	4,22
Zn K	160.1	4,45	144	1,99
Br K	103.9	3,51	121.5	4,32
Rb K	249.9	9,81	151	5,24
Sr K	59.8	1,98	82.7	2,91
Ba L	142.8		144	
La L	102.7			

Table 19 Comparison between average values of elements in Sage and Sage Package infusion samples normalized over the IS concentration.

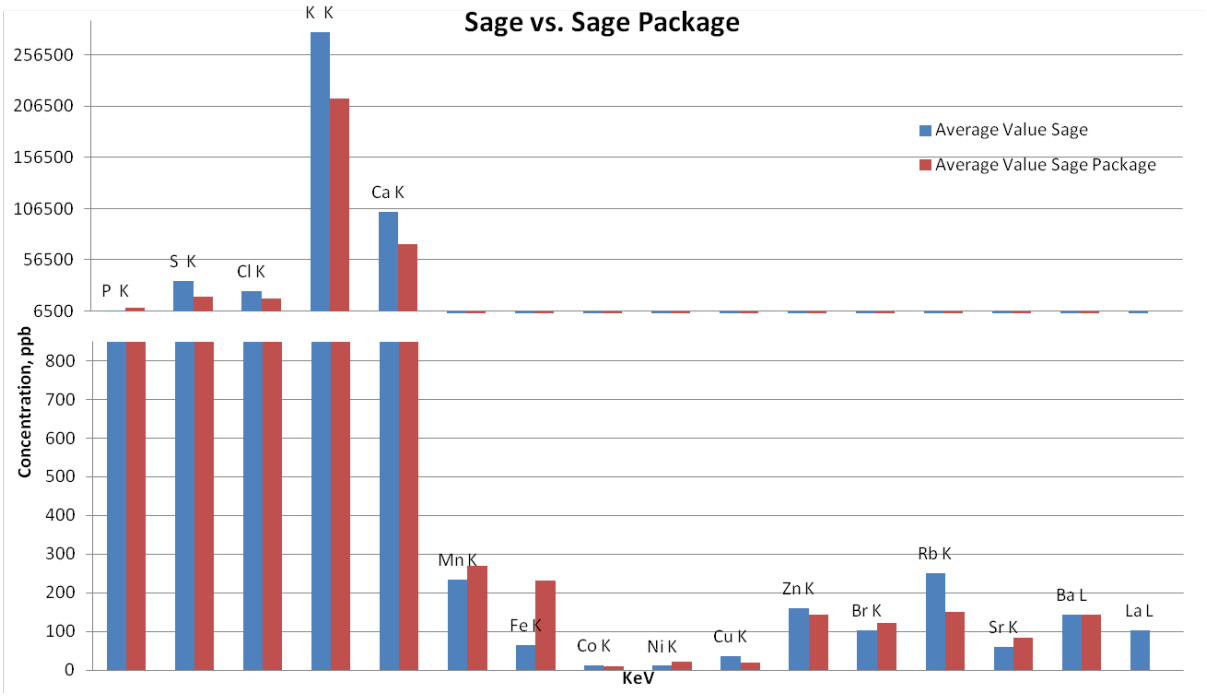

Figure 62 Comparison of the average values between Sage and Sage Package samples.

Figure 62 shows comparison of the average values between Sage and Sage Package samples. 16 elements were detected, out of those, 6 elements have higher values in Sage Package sample.

Yarrow

From this herb the leaves were used. The established preparation procedure was used.

The yarrow is rather uncommon in commercial tea production, especially a solo herb. However, it is freely obtainable in the pharmacies. For its antiseptic features and for its ability to stimulate the appetite, yarrow was used already 3000 years ago in Greece. [71, 72]

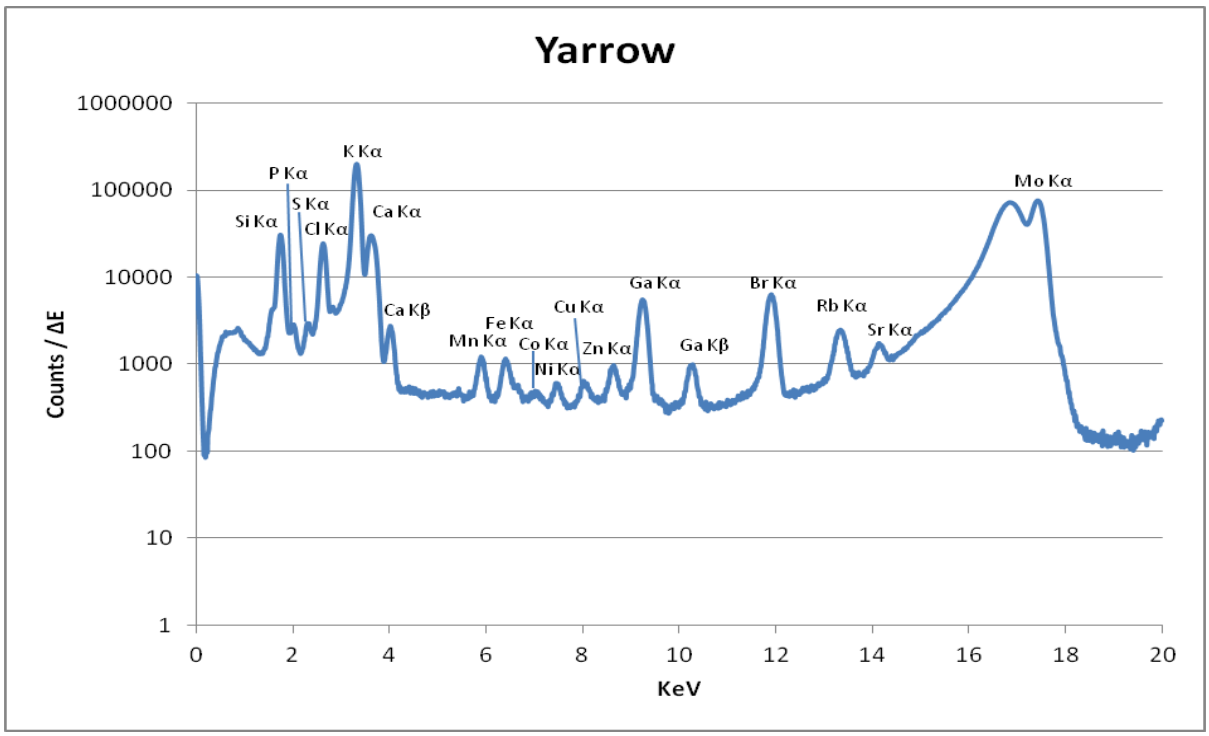

Figure 63 The characteristic spectrum of the Yarrow sample.

The characteristic spectrum of the Yarrow sample is shown in Figure 63. The highest value has again Potassium, the smallest value Cobalt. All together 14 elements were detected (average values are shown in the Table 20).

Element	Average Value, ppb	STDEV Yarrow
P K	38078.6	1281,82
S K	17454.4	591,53
Cl K	115227.7	1984,81
K K	417273.2	6007,55
Ca K	25764	127,4
Mn K	348	9,32
Fe K	164	64,39
Co K	13.4	3,23
Ni K	51.3	8,64
Cu K	63.1	3,14
Zn K	107.2	6,58
Br K	756.1	13,03
Rb K	129.8	3,3
Sr K	75.3	1,73

Table 20 Average values of elements in Yarrow infusion sample normalized over the IS concentration.

Thyme

For sample preparation Thyme was used in the “herba” form, which means the whole plant with dried leaves and flowering tops. Although during decoction preparation, it was noticed, that the seeds were also present.

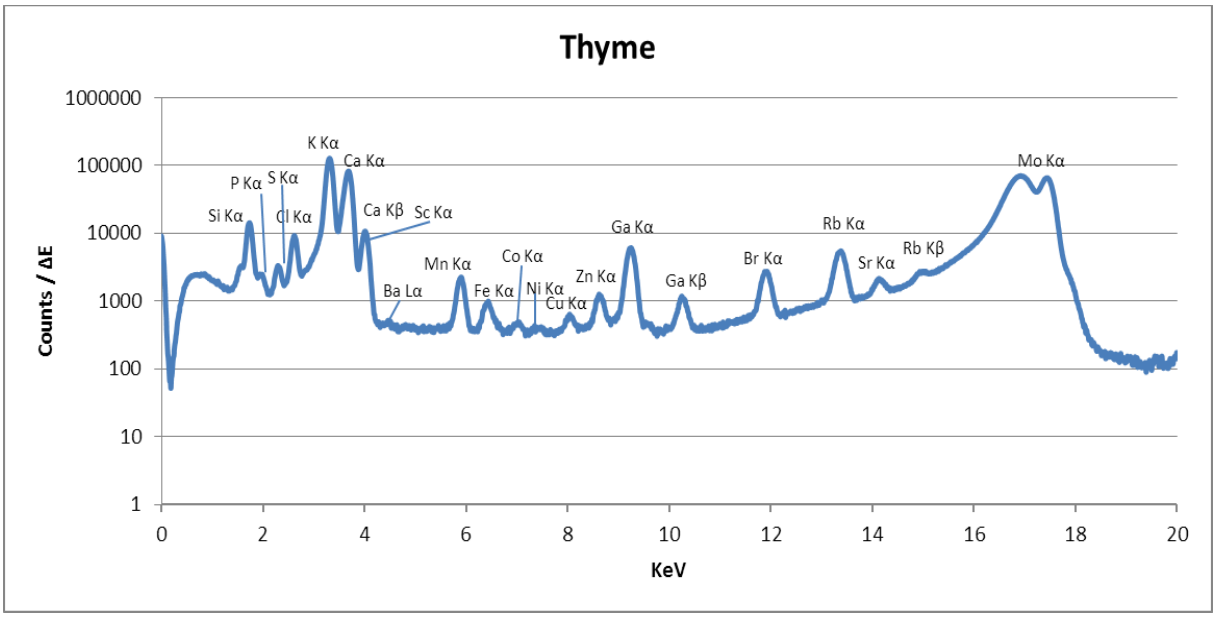

Figure 64 The characteristic spectrum of the Thyme sample.

The characteristic spectrum of the Thyme sample (Figure 64) shows that the smallest value out of all elements has Nickel, followed by Cobalt. The highest value, as for all tea samples, has Potassium followed by Calcium. All numerical values are shown in the Table 21. Interesting case is here for Sc Kα – line, which is detected by the equipment software, because it is completely buried under Ca Kβ – line. For this reason, it is hard to confirm the presence of Scandium in the sample.

Element	Average Value, ppb	STDEV Thyme
P K	14001.9	1850,79
S K	23421.4	571,05
Cl K	38380.9	1127,04
K K	248275.1	2607,85
Ca K	112100.9	1283,71
Sc K	149.2	34,5
Mn K	779	16,77
Fe K	221.3	34,64
Co K	15	2,65
Ni K	12.3	2,63
Cu K	56.6	3,33
Zn K	161.8	2,87
Br K	270.4	5,46
Rb K	449	9,49
Sr K	72.5	2,68
Ba L	184.9	18,39

Table 21 Average values of elements in Thyme infusion sample normalized over the IS concentration.

Nettles

Nettles (Urtica) is a stinging herb, but it is used to treat allergy symptomatic, generally for inflammation reduction, also nettles is an official source of the K vitamin and is used as haemostatic remedy during dysmenorrhea. [73] Leaves were used for preparing the infusion.

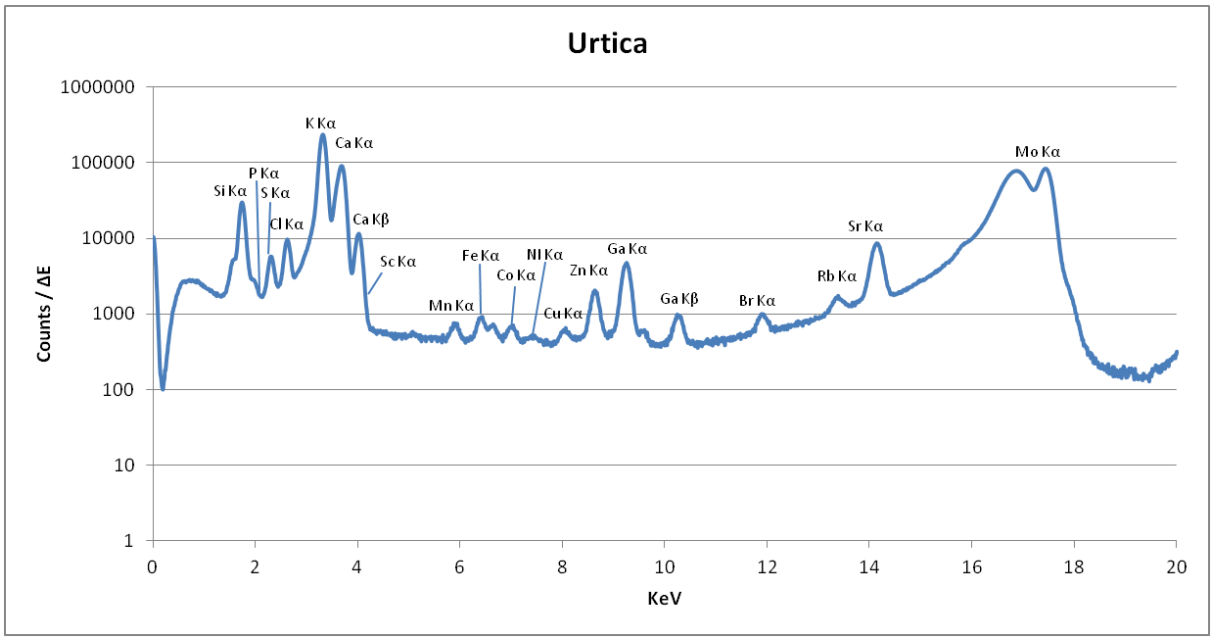

Figure 65 The characteristic spectrum of the Nettles sample.

The characteristic spectrum of the Nettles sample is shown in the Figure 65. The analyzing software of the Atomika 8030C has detected Scandium. It is hard to evaluate this element by hand, because Sc peak is overlapping with Ca Kβ – line peak. One more interesting peak is seen between Iron Kα and Cobalt Kα peaks. It is a sum peak of two times Potassium (as it has the biggest value).

Element	Average Value, ppb	STDEV Nettles
P K	13819.2	1573,53
S K	68721.9	7056,66
Cl K	53839.9	3979,3
K K	643430.2	52675,46
Ca K	160317	1883,64
Sc K	215.8	49,67
Mn K	161.4	6,44
Fe K	190	33,09
Co K	27.1	7,27
Ni K	22.7	6,91
Cu K	61.8	6,79
Zn K	418.2	22,83
Br K	71.7	4,14
Rb K	75.1	8,18
Sr K	863.8	24,39

Table 22 Average values of elements in Nettles infusion sample normalized over the IS concentration.

All average values of elements in Nettles infusion sample normalized over the international standard concentration are shown in the Table 22.

6.1.3. Default Elements and Occasional Elements

During measurements from all the samples, there were identified 13 “default” elements and 4 “occasional” elements. To get an overview and the general idea how different are the samples, it was decided to compare all tea solutions for each element separately. The elements will be displayed in conformity with their energies.

1. Phosphorus

The first element to be reviewed is Phosphorus. Out of Figure 66 it can be seen, that all samples have rather different amount of Phosphorus. It is highly variable in between all teas.

However, the highest value has Common Mallow, followed by Minze#2. Interesting enough is that other peppermint samples are way lower and are less comparable to the Minze#2 sample.

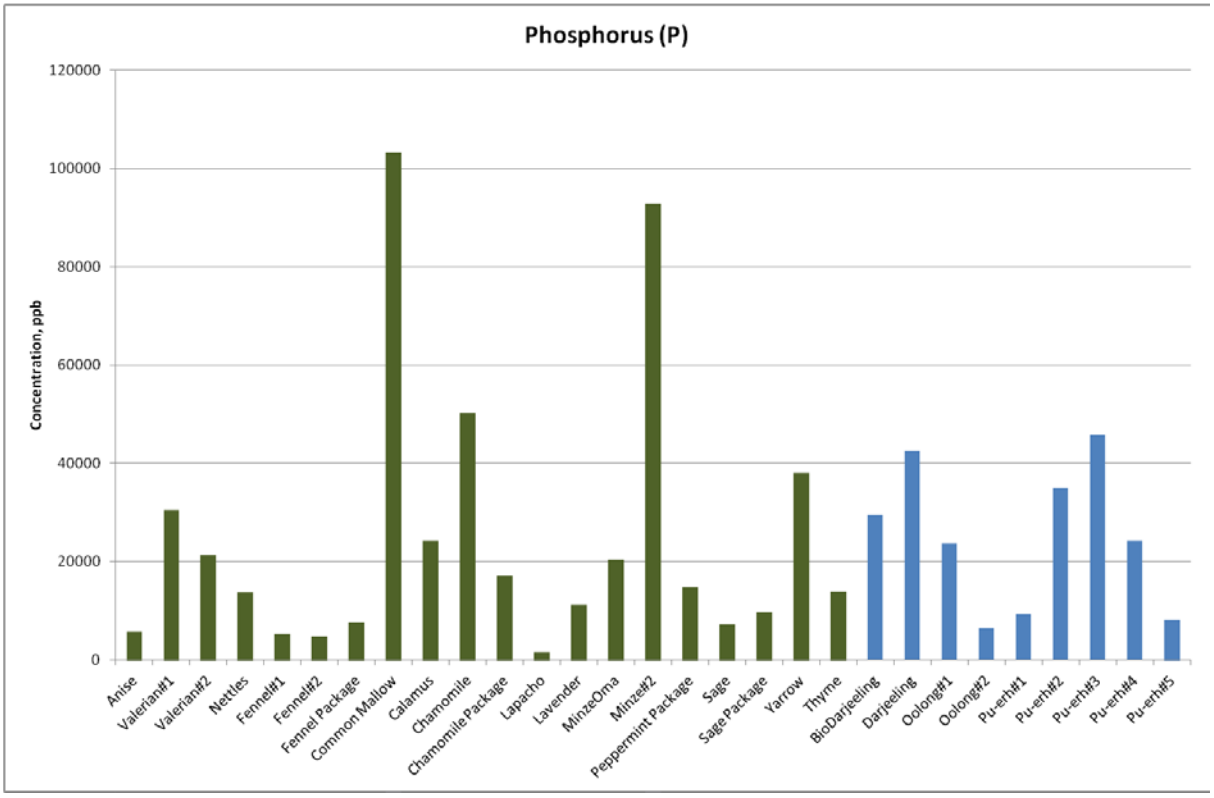

Figure 66 Comparison between all samples for Phosphorus.

The daily intake recommendation for Phosphorus for an adult person is 700mg, and upper limit is 4g daily until 70 years old and 3g after 70. [74] In case of the Common Mallow tea, one would get around 103.2 mg per liter.

2. Sulfur

Figure 67 shows comparison between all samples for Sulfur. Again, there is rather no distinct pattern how the values are distributed (Figure 67). There are four samples that have notably higher values of sulfur: MinzeOma, Common Mallow, Nettles, and Peppermint Package.

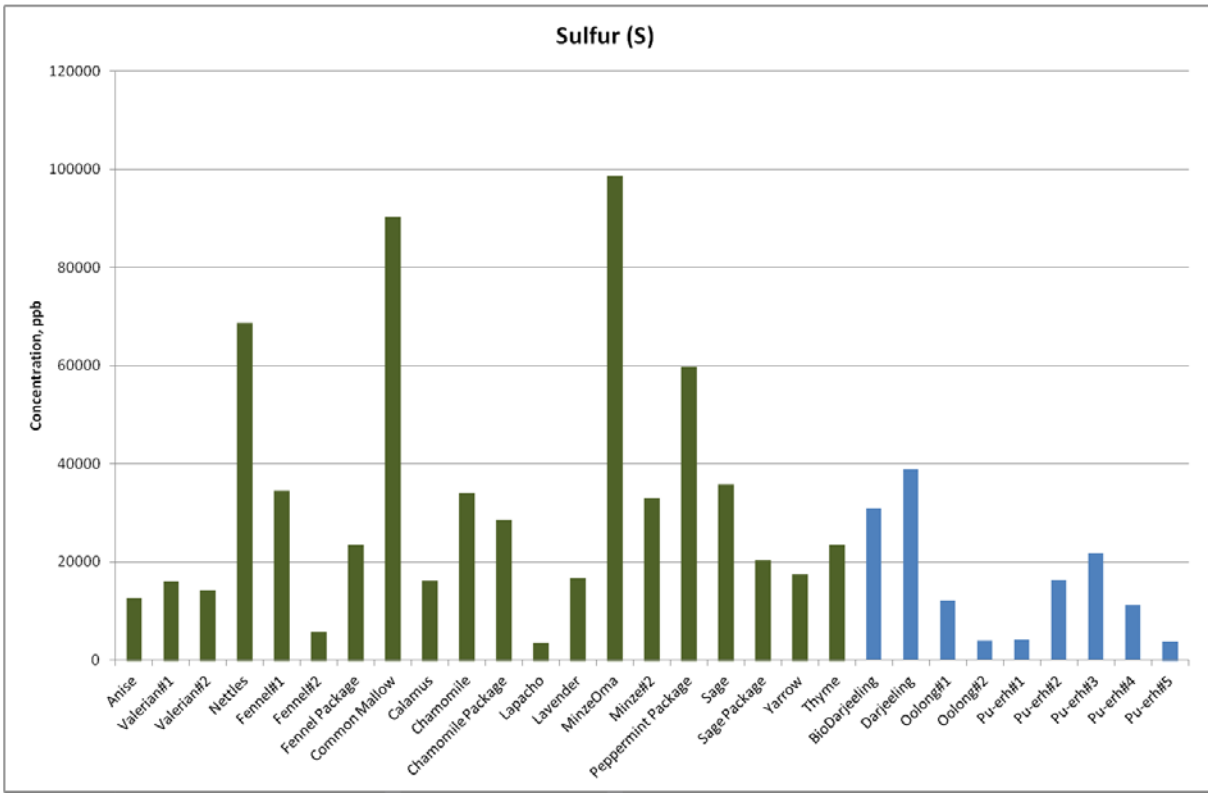

Figure 67 Comparison between all samples for Sulfur.

The Institute of Medicine from the National Academy of Sciences provides a list of Recommended Dietary Allowances (RDAs) for particular nutrients [74]. However, there is no official RDAs for Sulfur. That means the daily intake suggestion should be based on the health condition / age of the particular individual.

3. Chlorine

The comparison bar diagram for chlorine is shown in the Figure 68. Out of all herbal infusions the smallest amounts of chlorine is observable in the Lapacho, Valerian#1 and Valerian#2 samples, as well as in Fennel#2 sample. It is also evident, that in overall black teas (regardless of the type of the tea) have smaller Chlorine values in comparison with herbal infusions.

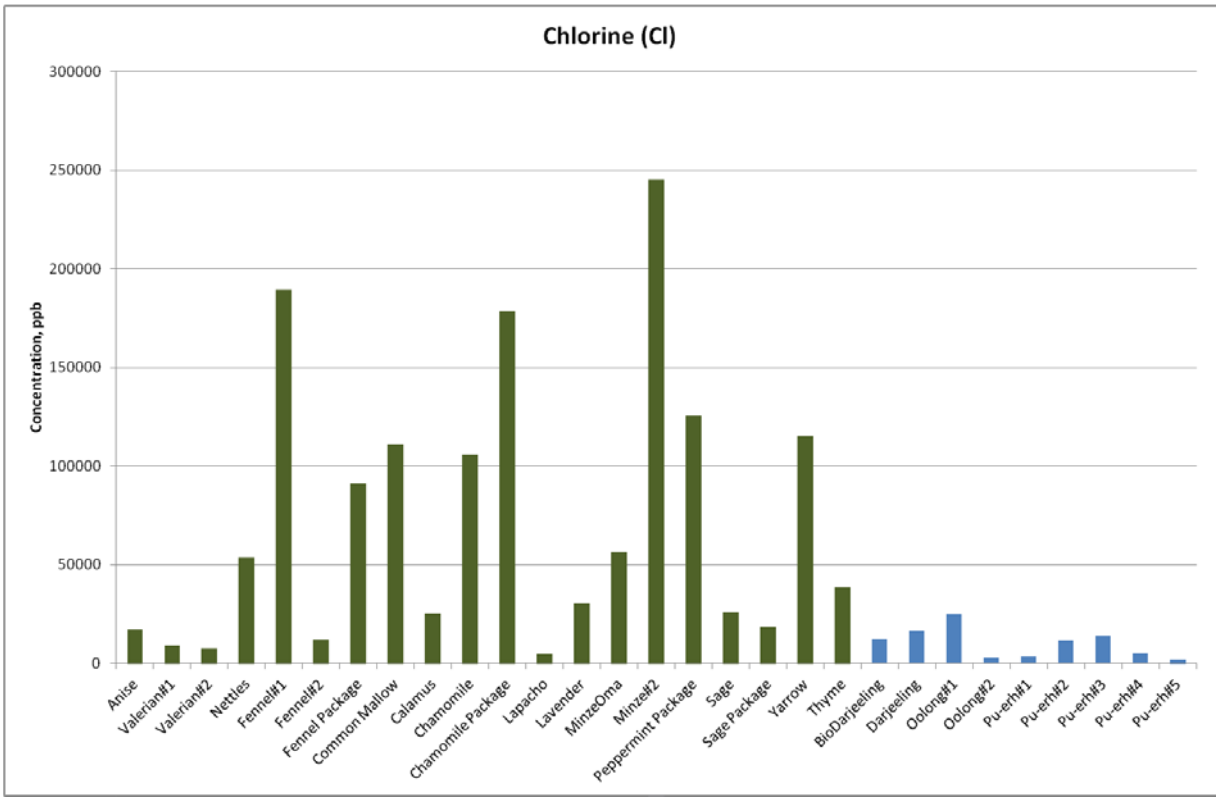

Figure 68 Comparison between all samples for Chlorine.

The daily intake norm of Chlorine is not suggested by RDAs, however, other sources suggest 3400mg in chloride form. [75]

4. Potassium

This element had the highest value for each of the samples. Most of it is, however, in Common Mallow, minze#2 and Nettles.

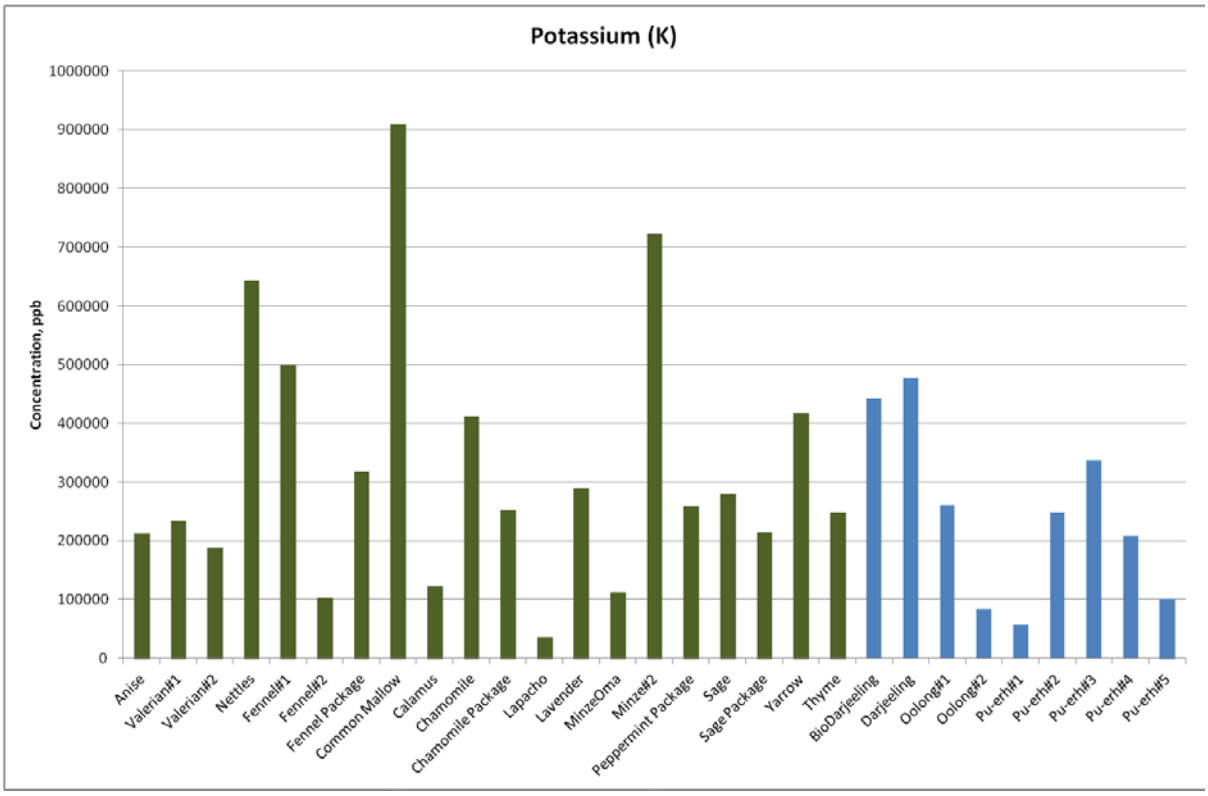

Figure 69 Comparison between all samples for Potassium.

There are no established RDAs or any upper limit for Potassium consumption. However, one of the sources mentions that overdoses (more than 3.5 g per day) can cause indigestions, intestinal problems or even heart problems. [75]

5. Calcium

Calcium is a mineral necessary for life. It is an essential building material for bones and teeth, but it is also important for muscle, hormones, nerve function and blood building.

Interesting result is observable in the Figure 70, where it is clearly visible that all teas, originating from *Camellia sinensis* plant, lack calcium trace almost complete, compared to the higher values in the most herbs. Common Mallow, Nettles, MinzeOma and Thyme are the leaders on Ca amount among the herbal samples.

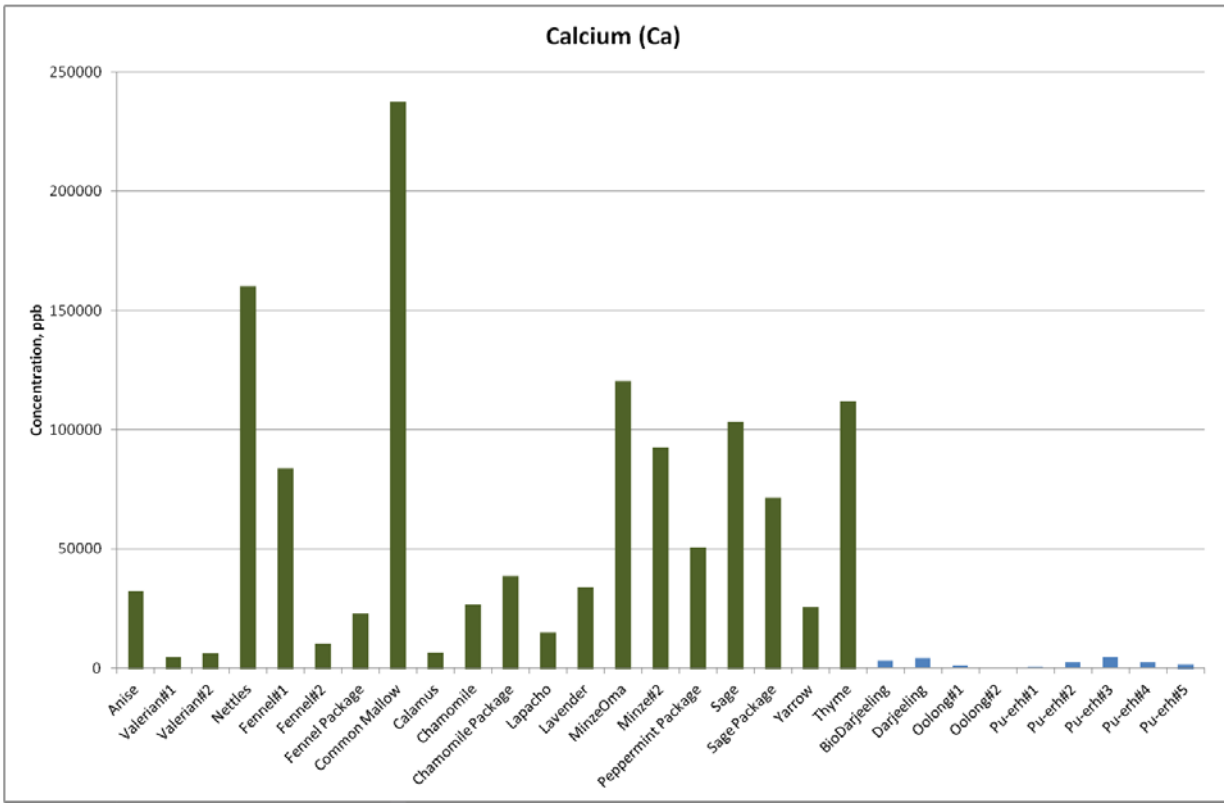

Figure 70 Comparison between all samples for Calcium.

There are clearly defined RDAs and upper limits for Ca [74]. It is 1000 mg per day for an adult up to 50 years and upper limit is as high as 2500 mg a day. By performing simple calculations, one can find out how to reach the daily dose of Ca by drinking herbal solution:

Recommended daily intake of Ca: 1000 mg
Standard teacup – 250 ml
Value of the Ca in Common Mallow sample is 240000 ppb
It makes → 240 mg per liter or 60 mg per cup
In this case, daily intake norm will be reached with *17 teacups a day*

6. Scandium

Scandium (Sc) is one of the occasional elements, which were found in tea and herbal infusions. In total, it was possible to detect Scandium in two samples: Nettles and Thyme (Figure 71). In case of Sc, it was necessary to rely on the analysing software, because Ca Kβ line has usually higher intensity and is overlapping with the Sc Kα line.

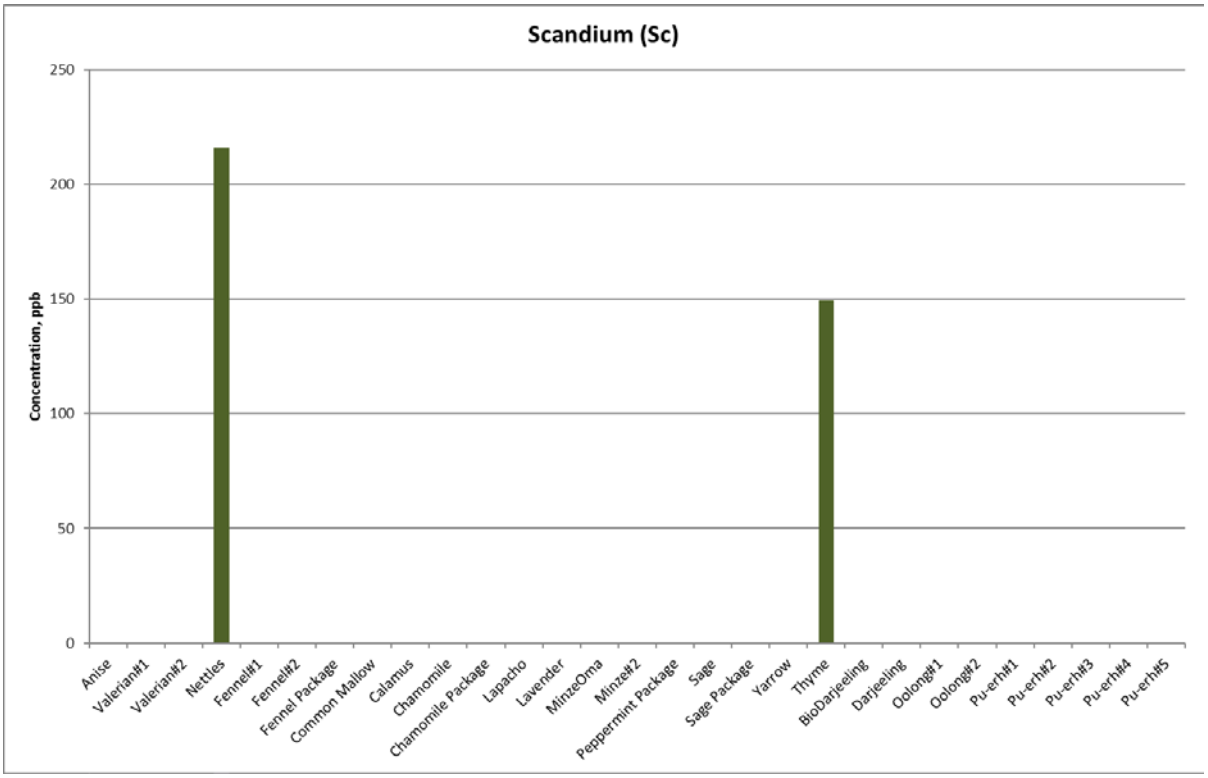

Figure 71 Comparison between all samples for Scandium.

The Scandium is not an element one expects to have in daily consumed food. There are also no known RDAs for it. However, there is a report from John Emsley, stating that the upper limit for the Sc could be around 0.0001 mg [76]. However, in his report, J.Emsley is referring to *Camellia sinensis* plants, so to black teas.

7. Titanium

This element appears in only five of the samples and is therefore also occasional. Out of black teas, only Pu-erh tea has it present. It is mostly seen in the sample Pu-erh#3 (Figure 72), the third brewing of the leaves with the highest values among all Pu-erh samples. In herbal infusions, Titanium found in both Valerian#1 and Valerian#2 samples, and Lapacho.

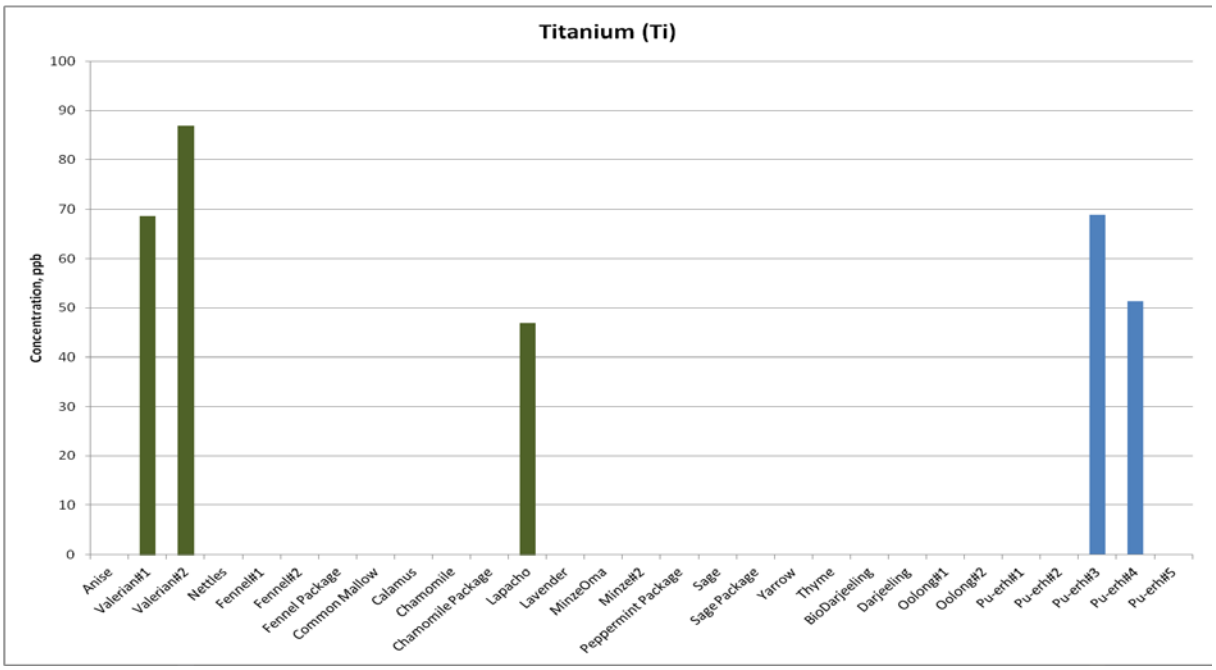

Figure 72 Comparison between all samples for Titanium.

It is convenient to suggest, that Ti enters in the plant through the soil, as it is mostly found in stones / ground / soil.

8. Manganese

Manganese is known to be a very important mineral for healthy human organism, as well as for most living organisms. It is used for maintaining the correct and healthy bone structure, is responsible for healthy functioning of the brain and whole nervous system. [77]

Figure 73 shows the bar comparison diagram between all the samples. Interestingly enough, black teas are shown to have more Manganese than any of the herbal teas. Out of herbal infusions, the highest values are in Chamomile and MinzeOma samples. The least amount of Mn is in Fennel samples.

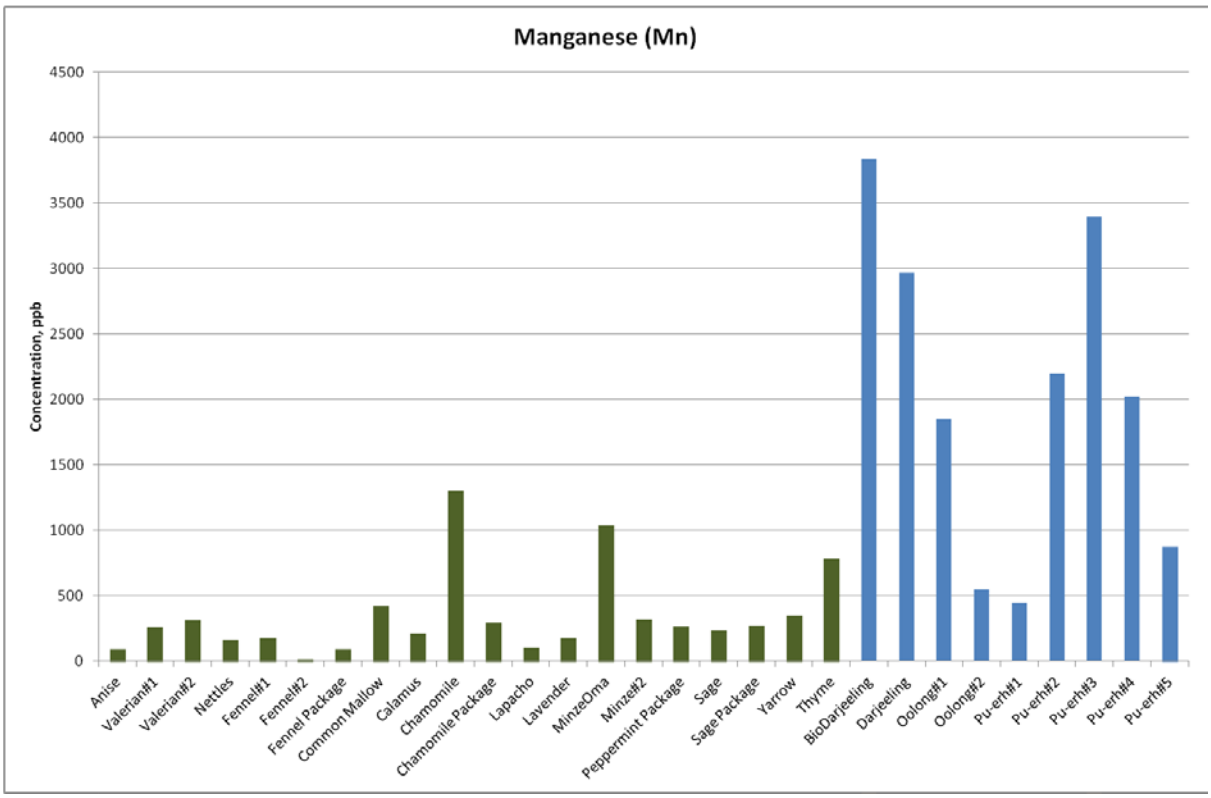

Figure 73 Comparison between all samples for Manganese.

There are no RDA's established for Manganese, although there is an adequate intake suggestion and upper limit for it. An adequate suggestion ranges between 1.8 and 2.6 mg per day. [74] It is crucial not to underestimate the importance of the upper limit (11 mg for an adult), because an extensive consumption of Manganese can cause liver troubles, anemia due to malabsorbtion of Fe and even symptoms similar to Parkinson disease [77].

9. Iron

In the human body, Iron is contained in the red blood cells and is essential for living. It is used as the electron transfer system in the body and for the cellular respiration.

Figure 74 shows the comparison over element Iron for all the samples. The values are quite different and there is no pattern of distribution.

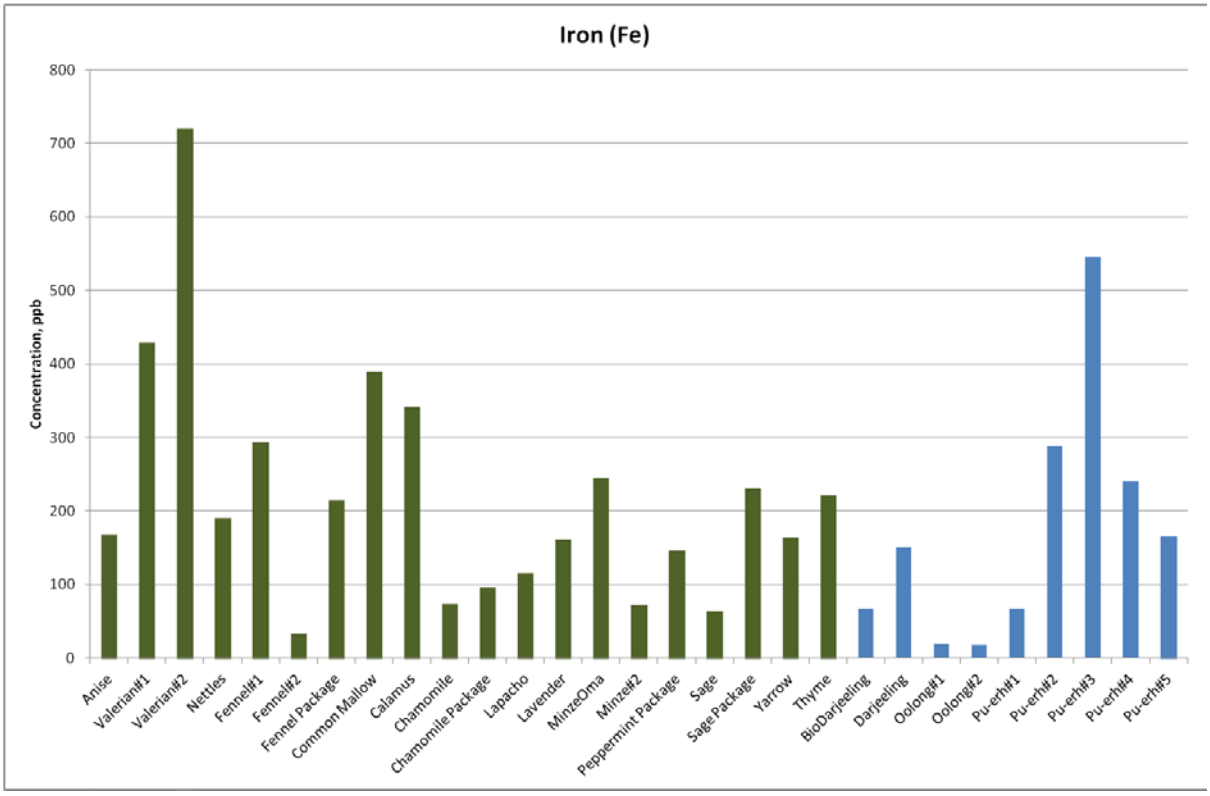

Figure 74 Comparison between all samples for Iron.

Figure 75 shows comparison between five statistical measurements of one sample – Anise. The values of Iron in identical sample vary from 94.2 to 278.4, which is factor three higher. By such high dispersion of the experimental data it is impossible to relay on the average value.

The reason for such great scatter in Anise sample is not entirely clear. It is, however, evidential, that not all the measured samples had similar problem. Figure 76 shows another five statistical measurements of the Peppermint sample (MinzeOma). The scatter in this case is rather irrelevant and average value is assumed representable.

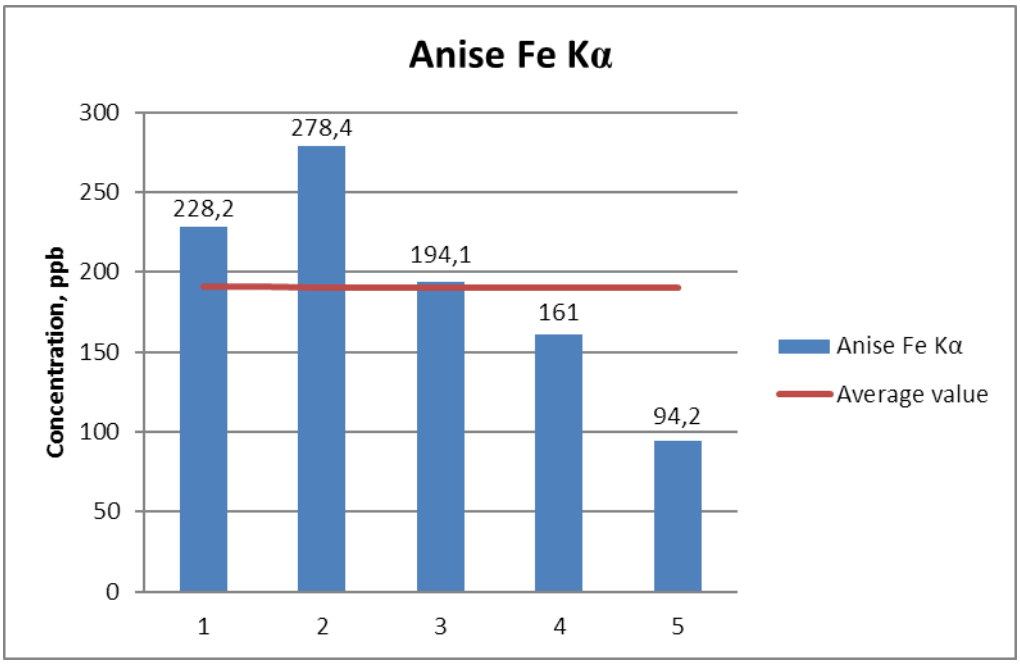

Figure 75 Comparison between five measurements of one and the same Anise sample.

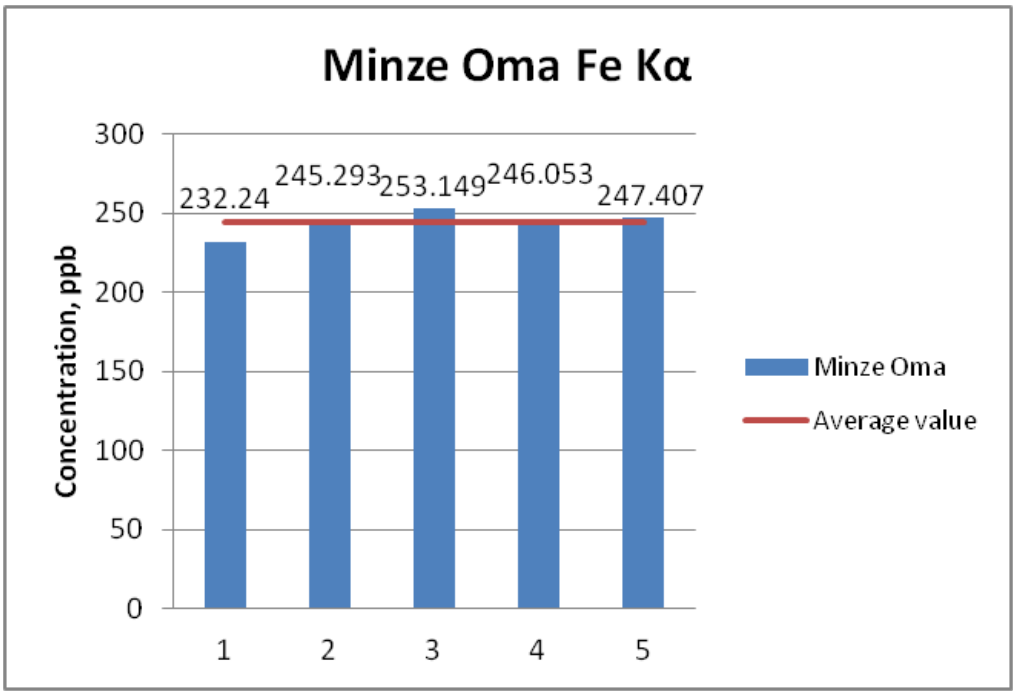

Figure 76 Comparison between five measurements of one and the same Minze Oma sample.

10. Cobalt

Cobalt is one of the occasional elements; however, it is present in most of the samples.

The element cobalt is usually associated with vitamin B12, as it represents an essential part of the structure of the vitamin. B12 is needed for healthy red blood cells, and, in case of deficiency, this might result in anemia. [78]

In the Figure 77, the comparison between all samples containing Cobalt is presented. Only two types of black tea (3 samples in total) show some traces of Co; out of herbal teas, the Common mallow and the Minze#2 are having the highest value. In general, the values of all samples are in a very low range (just up to 50 ppb).

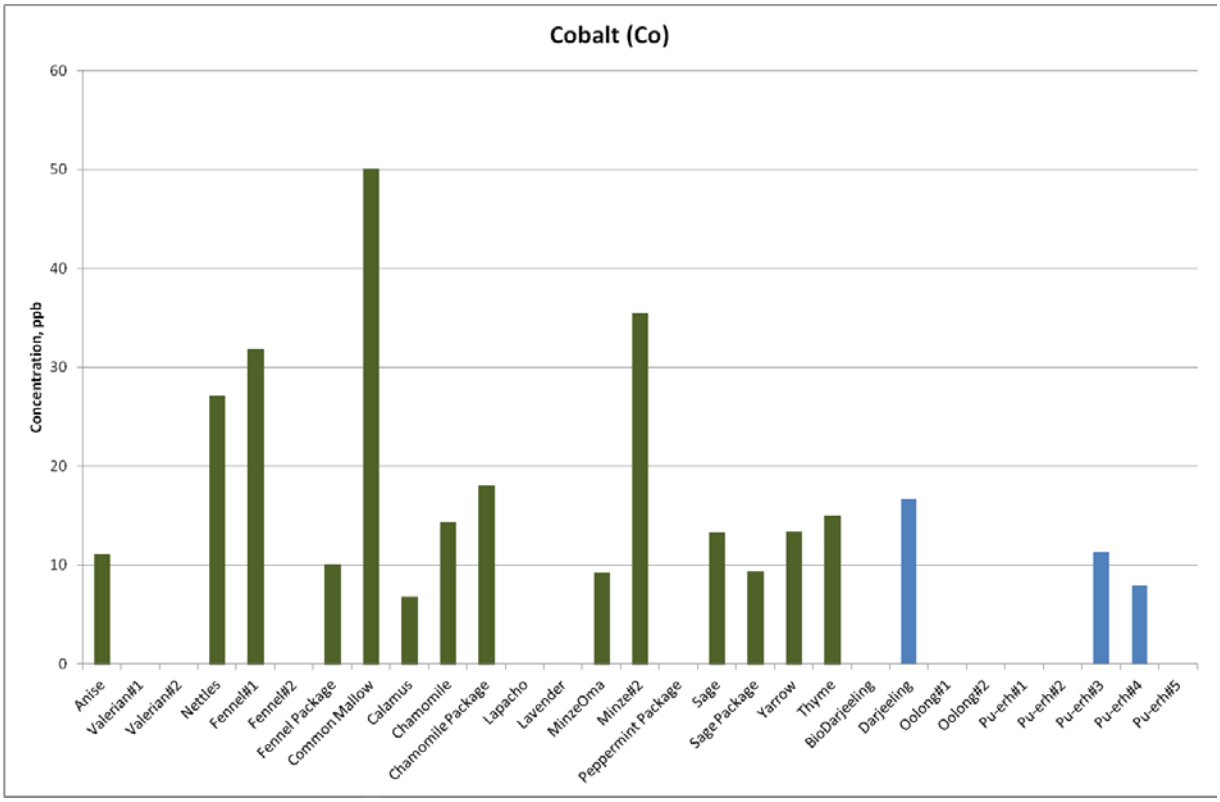

Figure 77 Comparison between all samples for Cobalt.

The RDA for vitamin B-12 is 2.4 µg per day; the upper limit of intake is not established at the moment. [74]

11. Nickel

Nickel is a metal, for which the essentiality for human health is not determined [79]. However, it is impossible to avoid it complete. Ni is naturally present in the soil (especially in volcanic dust), it is used in the industry for battery production, jewelry, metal alloys for coins, stainless steels, and even color ceramics. [80] There is no established upper consumption limit for Nickel, but the overconsumption can lead to allergies (contact dermatitis), adverse respiratory effects, and there are also suspicions for carcinogenesis of Ni. [79, 80]

The highest values of Ni (Figure 78) were found in Darjeeling tea samples, followed by Chamomile and Common Mallow.

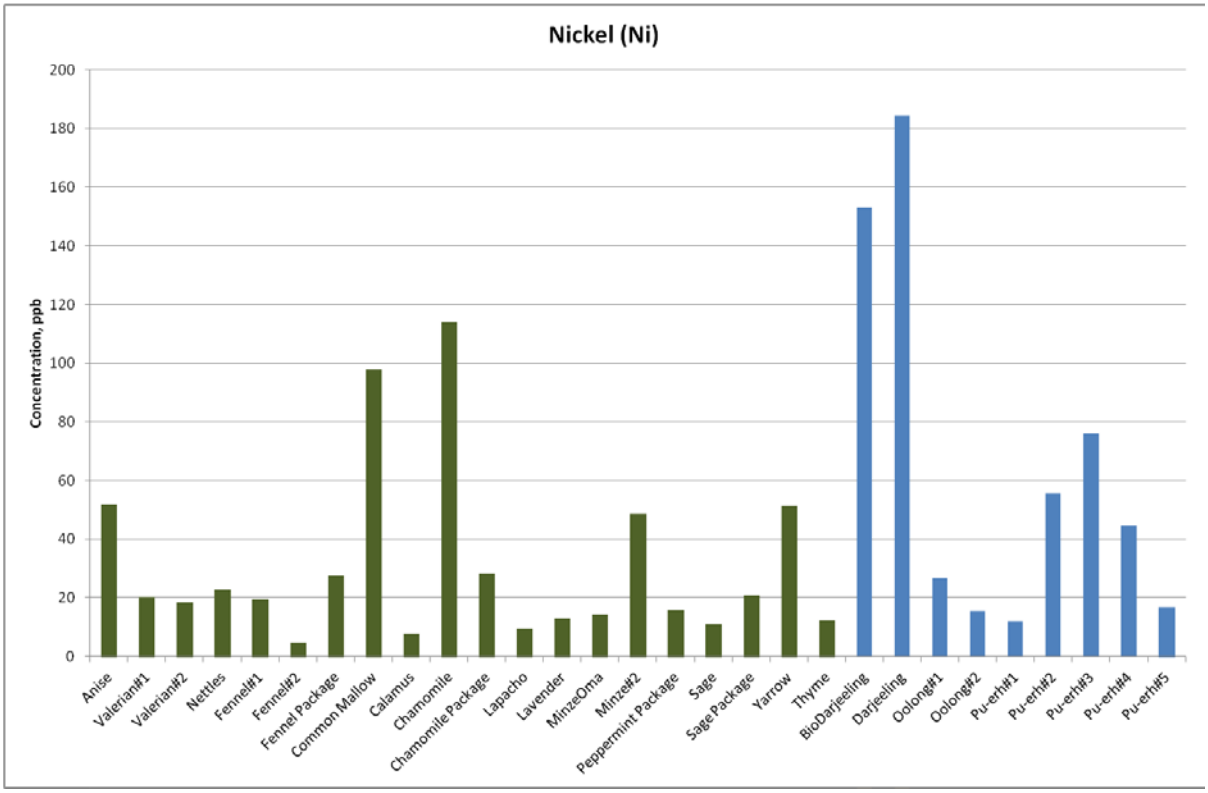

Figure 78 Comparison between all samples for Nickel.

12. Copper

Copper is a vital component for bone strength, blood cells maturation (red and white), iron transport, cholesterol and glucose metabolism, heart muscle contraction and brain development. [81]

Cu was detected in all the samples, except Pu-erh#1 (Figure 79). Already in the next brewing (Pu-erh#2 sample) of Pu-erh sample, Cu is observable. The highest value has Common Mallow sample, followed by BioDarjeeling and Oolong#1.

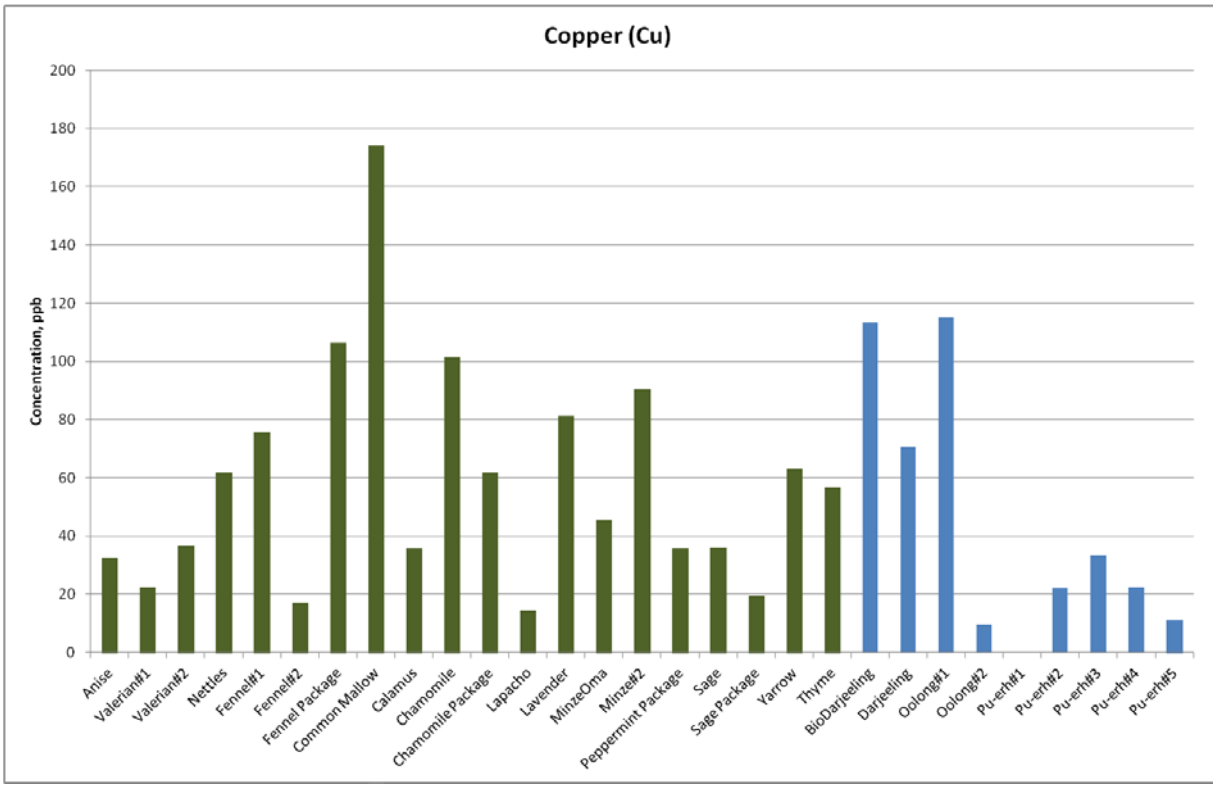

Figure 79 Comparison between all samples for Copper.

The RDA for Cu is 900µg for an adult person with upper limit up to 10000µg. [74] It will be however, complicated to achieve recommended daily amount by means of tea consumption, as the values of copper in tea are too small: from below detection to 175 ppb.

13. Zinc

Figure 80 presents a comparison between all samples for Zinc. The highest value of this element has Chamomile Package tea sample and the second highest value has Fennel#1. Interestingly, the lowest value has sample Fennel#2. The difference between values in the same herb could be explained by the difference of soil and environment both plants were growing in.

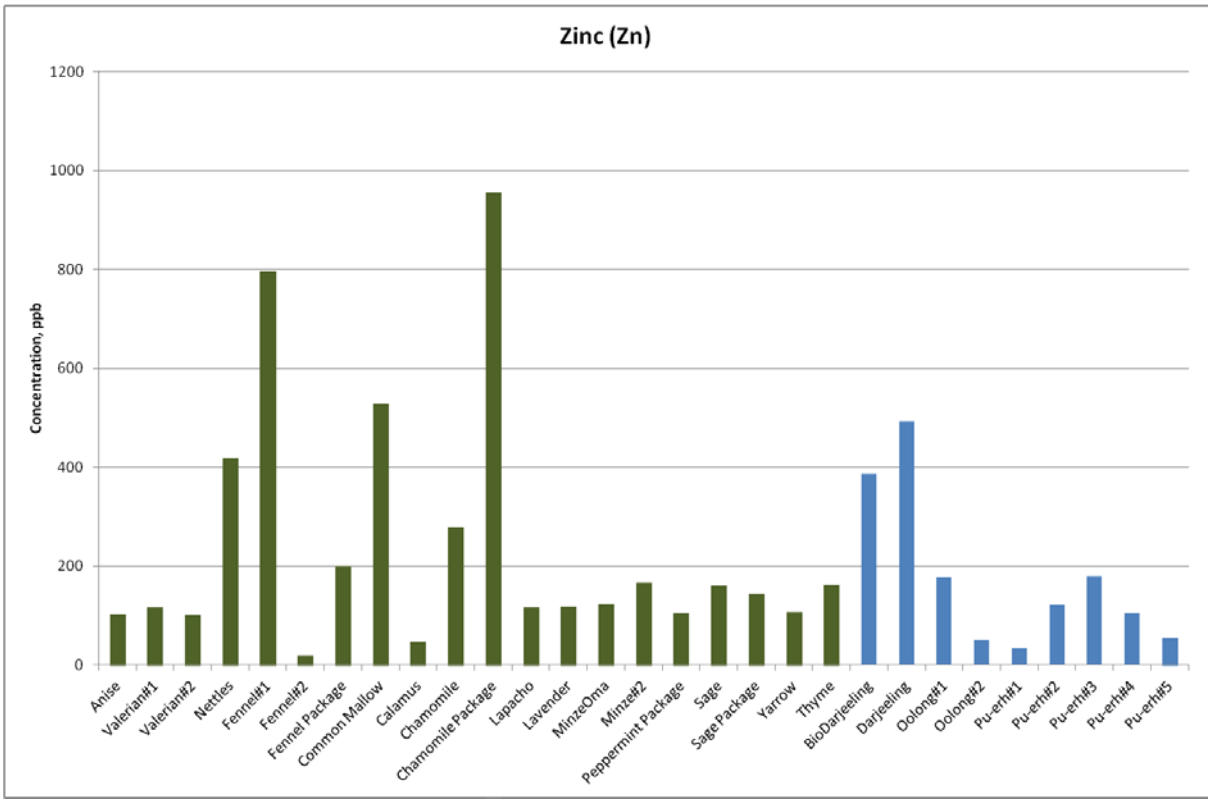

Figure 80 Comparison between all samples for Zinc.

RDA for Zinc is established: 11mg for males daily, 8mg for females, 11mg and 12mg for pregnant and lactating women. [74]

There are suppositions that Common Mallow leaves due to their values of iron, zinc and most vitamins could be recommended to be included into diet for pregnant and lactating women.

Recommended daily intake of Zn: 11 mg
Standard teacup – 250 ml
Value of the Zn in Common Mallow sample is 528 ppb
It makes → 0.528 mg per litter or 0.132 mg per cup
In this case, daily intake norm will be reached with *83.3 teacups a day*

It is evident that such an amount is impossible to consume through drinking tea alone.

14. Bromine

Comparison between all measured samples for Bromine is presented in Figure 81. In this bar diagram the separation of two groups (black tea and herbal infusions) can be observed: there are distinctly lower values in Darjeeling, Oolong and Pu-erh tea samples than in the herbal samples. The lowest value of the herbal samples has Valerian#1 and Valerian#2.

Bromine is found in sea water and Earth crust, the concentrations of it will vary in different places. For example, sample MinzeOma is a gathering of wildly grown peppermint in Latvia. This country has a long sea coast; which could lead to higher amounts of Br in soil. However, that is a hypothesis.

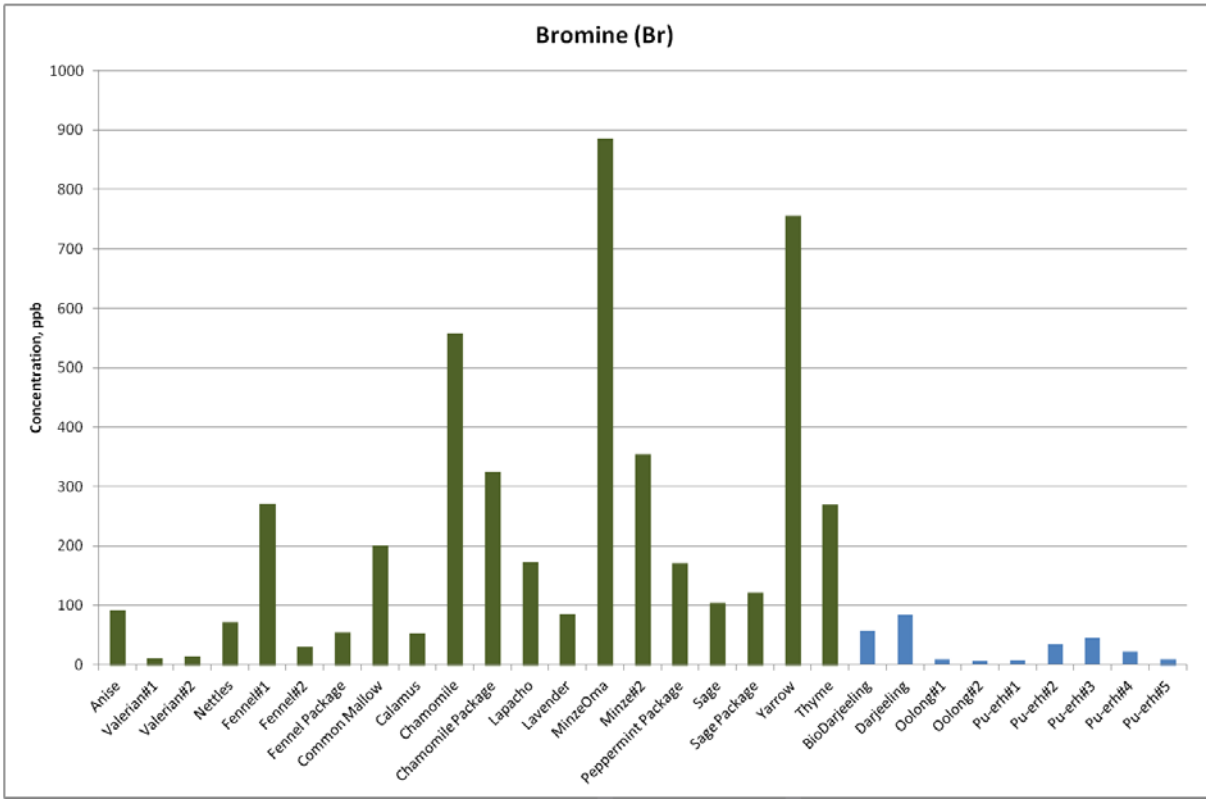

Figure 81 Comparison between all samples for Bromine.

There are no RDA's or any upper limits for Bromine consumption. [74] Br is used for water purification, as fire retardants, in some prescription drugs (although, these medications are minimized in production, at least in USA). If one is exposed to Br source for a long period of time, it could lead to cognitive failures, allergic reactions (cough, troubled breathing, headaches, and irritated mucous membranes), and in pregnant women might even increase risk of preterm birth. [82, 83]

15. Rubidium

Rubidium is an alkali metal, which is considered slightly radioactive and is mildly toxic when ingested. [84, 85]

The values of Rubidium in tea and herbal solutions are not too high; however, it is evident, that black teas contain considerably higher amounts of Rb than herbs (Figure 82).

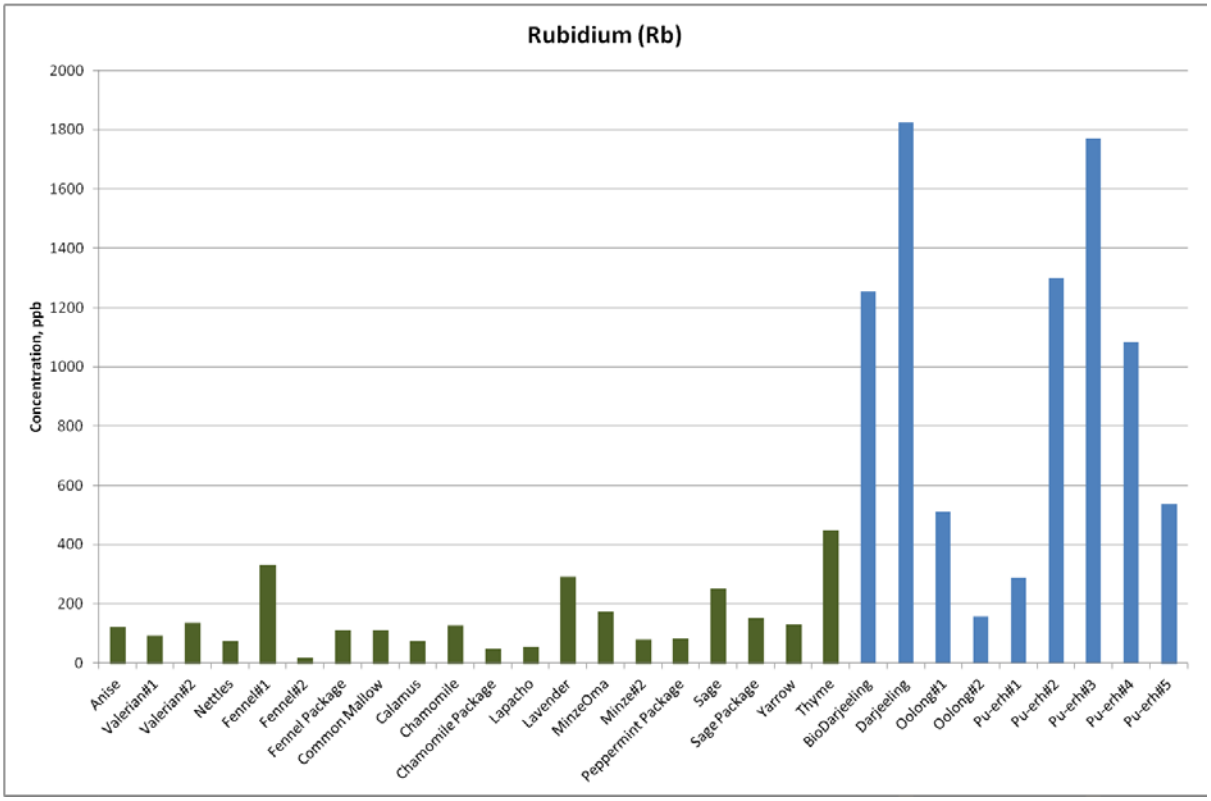

Figure 82 Comparison between all samples for Rubidium.

16. Strontium

Strontium was present in all measured samples, except Oolong tea. This element is present almost everywhere in small amounts (in rocks, soil, dust, coal, oil, surface and underground water, air, plants, and animals).

Highest values are found in Nettles and Fennel#1. In most other samples the values vary between below detection range and 200 ppb (Figure 83).

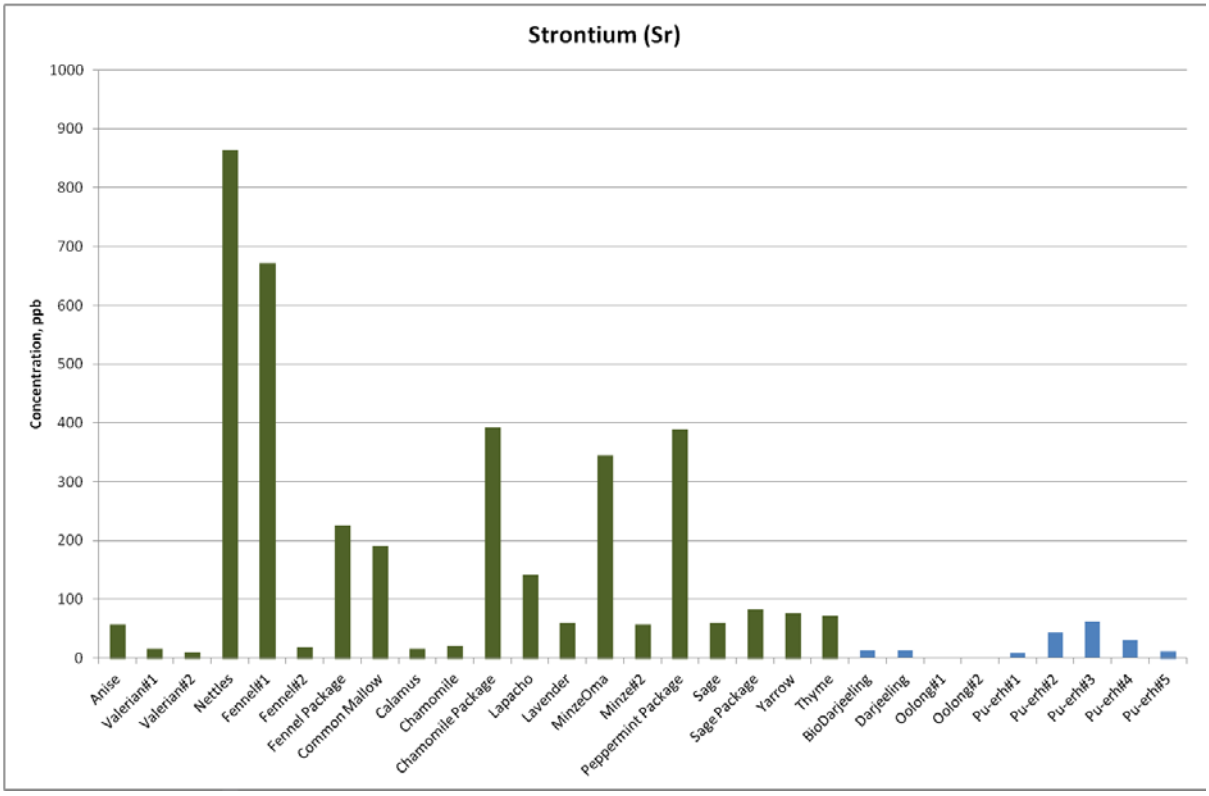

Figure 83 Comparison between all samples for Strontium.

There are two types of strontium: stable and radioactive. Consumption of stable Sr with everyday dietary products (including tea), will bring no harm. Some animal studies showed possibility of lethal cases, when enormous amount of Sr has been consumed. This is rather not applicable to an everyday human life. [86]

17. Barium

Barium is the last “occasional” element. It was detected in only five samples: 4 herbal infusions (Calamus, Lapacho, MinzeOma, Thyme) and one black tea (Pu-erh#3). Figure 84 shows the comparison between all five samples over Ba.

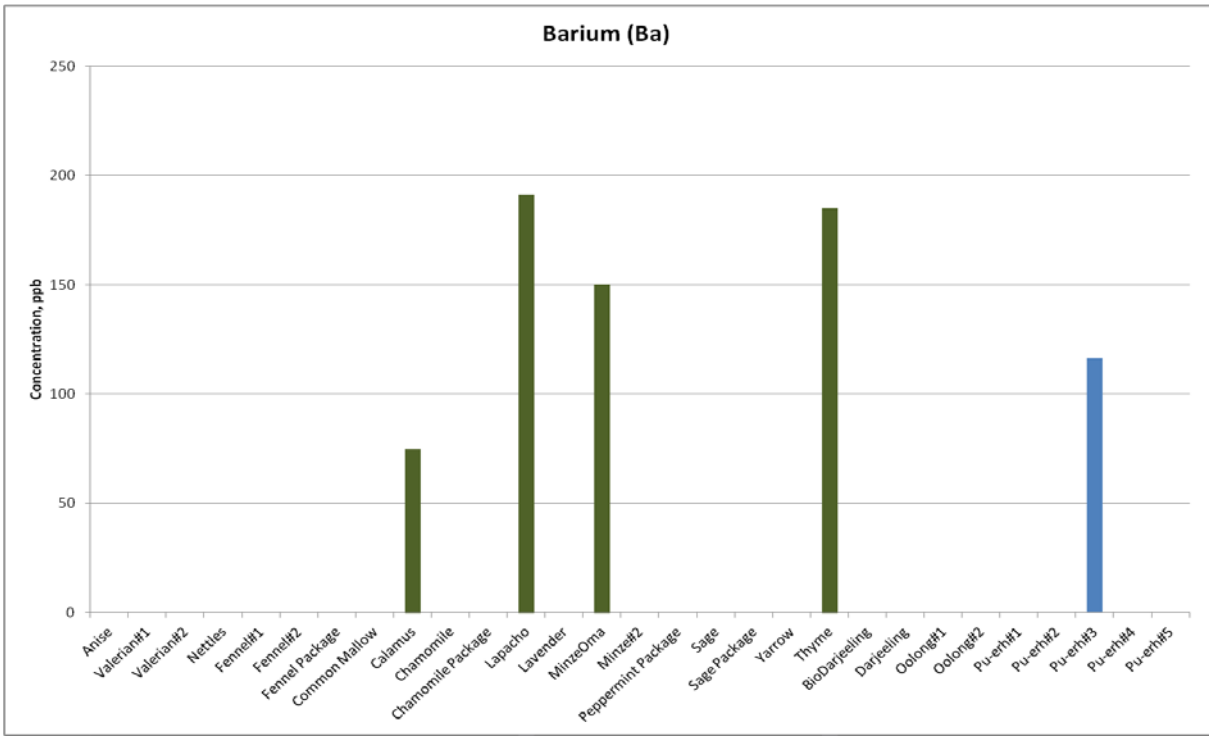

Figure 84 Comparison between all samples for Barium.

There are no upper limits or RDA for Ba consumption. The effect on human health is mostly dependent on the compound it is forming. There are very different possibilities: water soluble or not, can be crystals or powders. There are also several Ba compounds that are not usually found in nature and are used mostly in different manufactures. Therefore, the effects that Barium-compounds can cause in human body will be directly dependent on whether and how well they are soluble in water / stomach fluids. Even if the Ba-compounds that were consumed are dissolvable, a rather high dosage should be consumed to cause changes in heart rhythm or paralysis, and such “lighter” effects such as vomiting, abdominal cramps, diarrhea, difficulties in breathing, increased or decreased blood pressure, numbness around the face, and muscle weakness are also rarely found.

6.1.4. Principal Component Analysis (PCA)

Principal component analysis (PCA) is a method of multivariate statistics. This method is usually used for data with high amounts of dimensions. The analysis serves to structure, simplify and illustrate the extensive data sets by approximating a large number of statistical variables by a smaller number of possible linear combinations (the so-called main components). [87]

The PCA was used on all the tea and herbal infusion samples together. All analyses were performed by Dipl.-Ing. Dr.techn. Johannes Sterba with the R software for statistical computing.

The weak point of PCA analysis is working with data that have missing points. Therefore, before using the data set, it must be “cleaned” accordingly, so that only elements without missing values will be used.

```
formula<-paste("~",names(tee)[2])
for(i in names(tee)[-2]) {
  if(sum(is.na(tee[,i]))<1) {
    formula<-paste(formula,"+",i)
  }
}

formulaMin<-as.formula(formula)
```

Figure 85 Sample of the PCA code.

Figure 85 shows sample of the used PCA code.

Several separate evaluations of the data set were done:

1. All teas with normalized data
2. Only herbal teas with normalized data
3. Three herbal families
4. Loose herbal material against packaged tea

All teas with normalized data

The values of single elements have a high scatter: from less than a 100 ppb (as for Cu, Ni, Co…) up to over 100000 ppb (as for K, Ca, etc.). For this reason, using PCA, it is necessary to normalize the data, otherwise the elements with highest values (for example: K, S, Ca) would dominate the analysis. It will be done automatically: the average value will be taken as 0 and the standard deviation as 1. That would assure that more precise data has higher influence on the outcome.

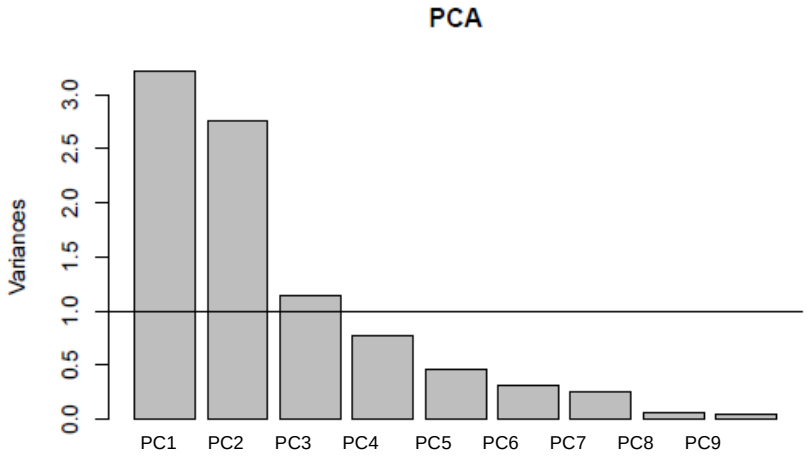

Figure 86 PCA for all teas with normalized data. Distribution of the main components.

Distribution of the main components (meaningful are the components over the 1.0 line) is shown in the Figure 86. The content of each component (its values) is shown below, in the Figure 87.

```
##            PC1          PC2          PC3         PC4         PC5
## S   0.44679496  0.002156695  0.2817318486 -0.41300417  0.30258836
## Cl  0.40901287 -0.150737287 -0.3380105464  0.38136727 -0.26738382
## K   0.48423848  0.141757927  0.0065389677  0.01432150 -0.53950450
## Ca  0.45414944 -0.172256410  0.2664306974 -0.35732236  0.10339065
## Mn -0.04644761  0.576145872 -0.0005322161 -0.11113370  0.09916795
## Fe  0.02339783  0.069592695  0.8028187723  0.55605132 -0.10018265
## Ni  0.18086671  0.507627135 -0.1427865280 -0.09500451 -0.27514708
## Zn  0.38872680  0.120008638 -0.2552939143  0.47447200  0.65312668
## Rb -0.07627580  0.564474489  0.0725721826  0.02022054  0.11419381
##            PC6         PC7         PC8         PC9
## S  -0.41011994 -0.38194953  0.03157961 -0.37918800
## Cl -0.62438520  0.03091123  0.14703271  0.25762281
## K   0.27268667  0.28119051 -0.28014479 -0.47137840
## Ca  0.22000441  0.39593832  0.12645462  0.57754821
## Mn -0.30640373  0.12260016 -0.66660791  0.30072104
## Fe -0.02262329 -0.13965313 -0.01880265  0.10243744
## Ni  0.29361351 -0.58938568  0.29049045  0.29413108
## Zn  0.32369892 -0.01392409 -0.08801027 -0.06996854
## Rb -0.18384736  0.48499551  0.58827042 -0.20433343
```

Figure 87 The loading of each component.

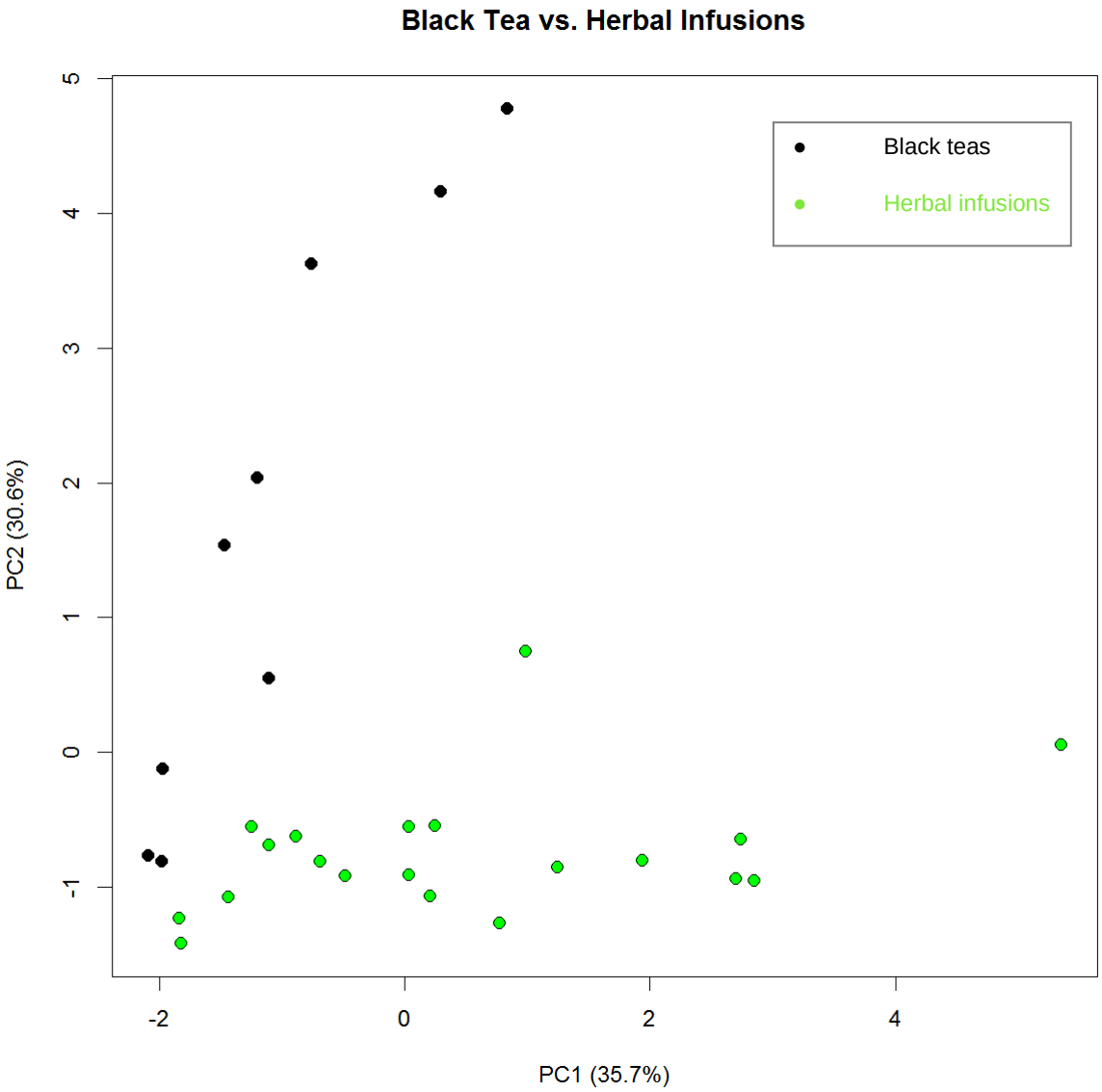

Figure 88 Graphical representation for PC1 and PC2, for all teas.

Figure 88 shows two main components of the PC analysis for all teas using normalized data. PC1 and PC2 are the most meaningful components, therefore, they were chosen for graphical representation. The distinct separation of the herbal and black teas is seen.

This result means that in case there is an unknown sample, after preparing it, according to the established method, and measuring it with TXRF spectrometer, one can define with great probability whether the sample is a black tea (*Camellia sinensis*) or some kind of herbal tea.

Only herbal teas with normalized data

Second PCA was done for all herbal infusions separately. It was done to try to reveal a fingerprint of the herbal tea, to distinguish between different herbs.

Figure 89 shows distribution of the main components of PCA for all herbal infusions with normalized data.

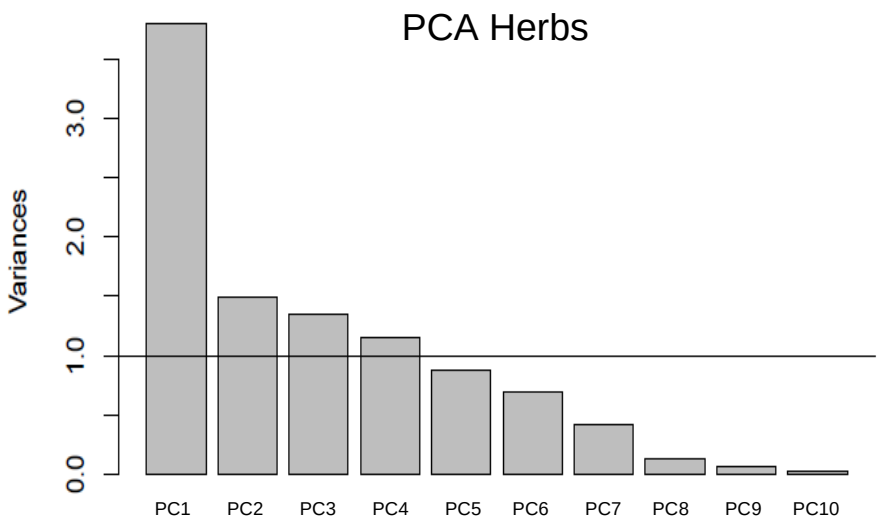

Figure 89 PCA for herbal infusions with normalized data. Distribution of the main components.

The most meaningful are first four components, for more precise plot: PC1 against PC2 were chosen (Figure 89). Figure 90 presents the element distribution over all components. The principal component analysis was done ignoring the “occasional” elements; it was done because of the features of the PCA. As soon as there is a sample, which does not have one of the “default” elements, the whole sample will be ignored by the program and data will be lost. For this measurement, main components (PC1, PC2, etc.) included following elements: P, S, Cl, K, Mn, Fe, Ni, Zn, Rb.

```
##           PC1        PC2         PC3         PC4         PC5         PC6
## P   0.40973525  0.1210008 -0.37193492  0.123696025 -0.10431856  0.22759341
## S   0.34887311 -0.3598592  0.06006111  0.129084332  0.47349096 -0.35528896
## Cl  0.33990340  0.3725857  0.27201452 -0.214208219 -0.17359025 -0.09827614
## K   0.45236639  0.1162451  0.06787769  0.214865174 -0.07202891  0.37438657
## Ca  0.38187914 -0.3612262  0.21652492  0.218361110  0.28954053  0.19889462
## Mn  0.19094127 -0.4186670 -0.33280795 -0.540960297 -0.12046593 -0.33851332
## Fe -0.05196053 -0.2685062 -0.21445885  0.653537061 -0.54246747 -0.28683645
## Ni  0.36706855  0.1202260 -0.40982155 -0.205667833 -0.21406433  0.02890568
## Zn  0.26377025  0.1522858  0.52837769  0.005311959 -0.30909215 -0.48729746
## Rb -0.03050739 -0.5366748  0.35872601 -0.257345181 -0.44229402  0.44432832
##           PC7        PC8         PC9        PC10
## P  -0.30817880  0.5694940 -0.27553087 -0.32548969
## S  -0.16153436 -0.3668039 -0.24089021 -0.39928475
## Cl -0.60763771 -0.2633395 -0.10919920  0.36807739
## K   0.09566940 -0.2758082  0.66691142 -0.23607239
## Ca  0.16006810  0.2905223 -0.04967701  0.62708353
## Mn -0.11783194  0.1731089  0.45054953  0.09439772
## Fe -0.15312876 -0.1470995  0.01832976  0.17358712
## Ni  0.54235378 -0.3668342 -0.37768778  0.15010451
## Zn  0.37132644  0.3354893 -0.00996208 -0.21092815
## Rb -0.07463734 -0.1121829 -0.24660367 -0.21004235
```

Figure 90 The loading of each component.

The plot of the two main components of all herbal solutions (Figure 91) shows all herbs, independent of the origin, type or part of the plant. Unfortunately, it is impossible to distinguish any kind of grouping. Even the same sorts of herbs are not being together (for example: Fennel, Peppermint, etc.).

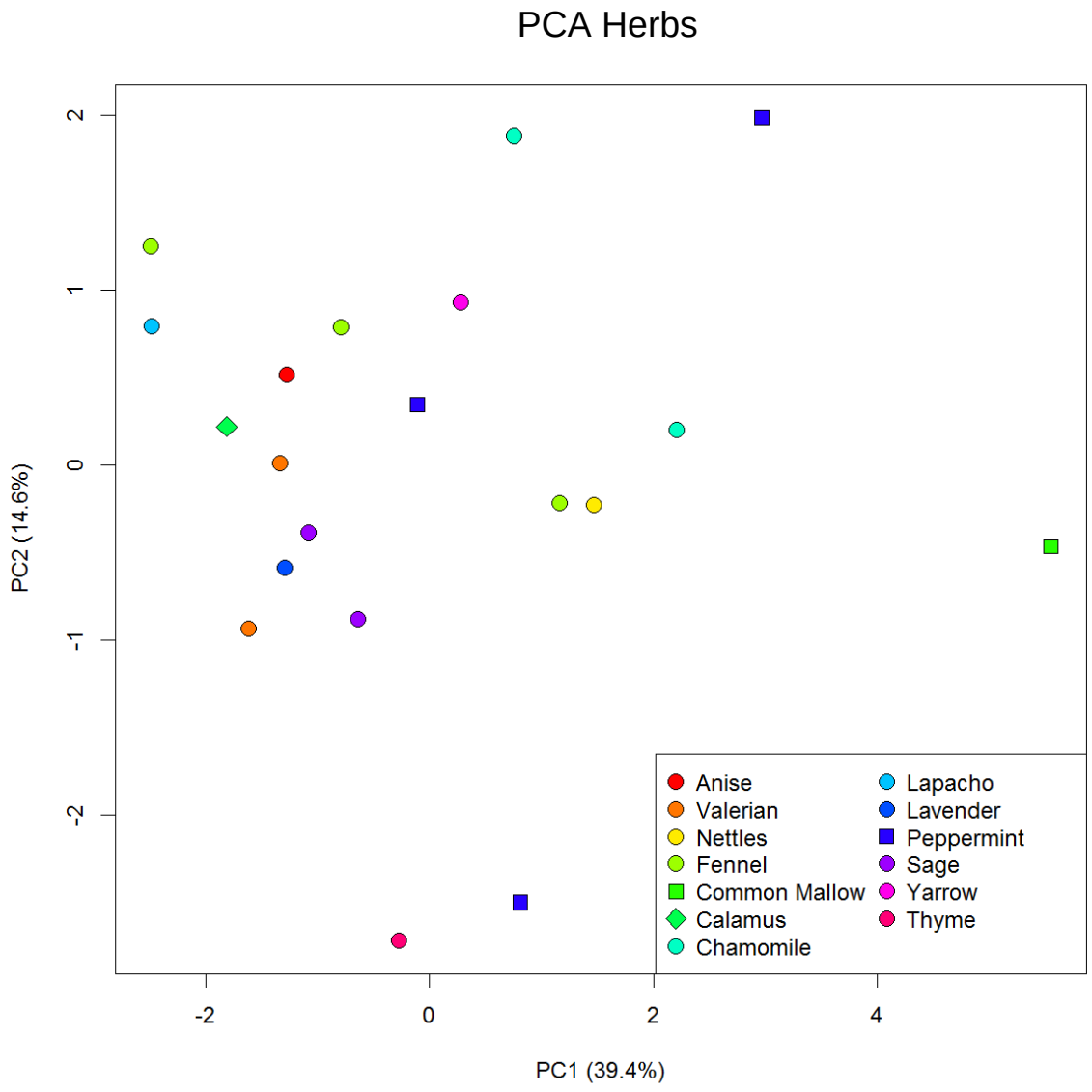

Figure 91 Graphical representation for PC1 and PC2, for all herbal infusions.

Herbal families

One of the hypotheses was that the plants belonging to the same family will have similar elemental composition, intrinsic to this family and thereby can be distinguished.

Three families were defined (the chosen three families combine most of the samples):

- *Apiaceae*: includes Anise and Fennel
- *Asteraceae*: includes Chamomile and Yarrow
- *Lamiaceae*: includes Lavender, Peppermint, Sage and Thyme.

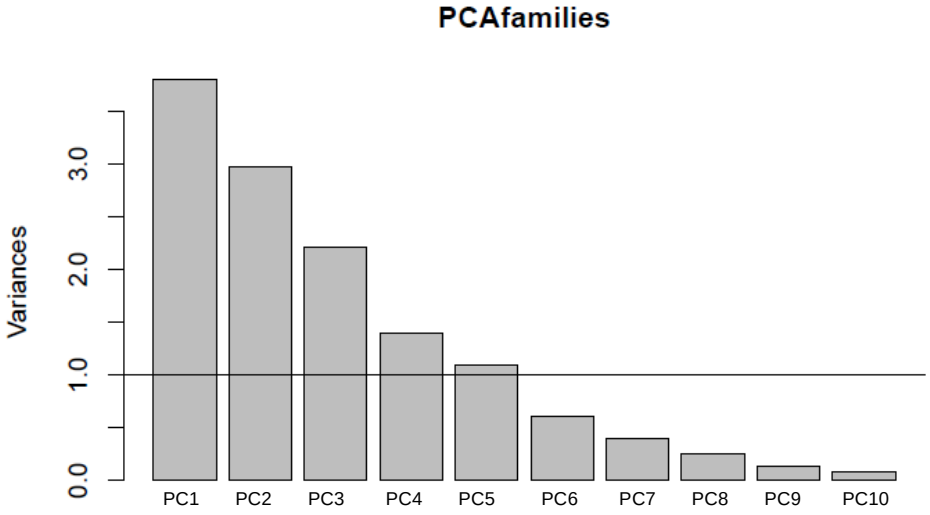

Figure 92 PCA for herbal families with normalized data. Distribution of the main components.

Figure 92 demonstrates distribution of the main components after PCA analysis for herbal families. The content of each PC is shown in the Figure 93. Used elements were: P, S, Cl, K, Ca, Mn, Fe, Ni, Cu, Zn, Br, Rb and Sr.

```
##            PC1         PC2         PC3          PC4         PC5
## P   0.42014516  0.20423364 -0.13336990  0.067485843 -0.34525367
## S   0.15639866 -0.38275965 -0.26047371 -0.355391027 -0.17743986
## Cl  0.41646297 -0.02674641  0.33318267 -0.119150194 -0.22348021
## K   0.40026792  0.11002397  0.21680636  0.317291331 -0.25014187
## Ca  0.07903014 -0.41169465 -0.18911000  0.231736474 -0.46659559
## Mn  0.26334886 -0.14304141 -0.46812474 -0.017850743  0.29934942
## Fe -0.02935011 -0.43375802  0.08076370  0.208938710  0.27362937
## Ni  0.33293028  0.26977463 -0.15776837 -0.005277513  0.41252854
## Cu  0.36808161  0.06275477  0.14557711  0.281006920  0.30316670
## Zn  0.17456875 -0.18962928  0.44582532 -0.170524536  0.23948204
## Br  0.31720090 -0.17456618 -0.33219652 -0.231075398  0.12419843
## Rb -0.04333005 -0.33134168 -0.04485408  0.640892698  0.12010703
## Sr  0.09801574 -0.40978927  0.37109824 -0.290492420  0.07289474
##            PC6           PC7         PC8         PC9        PC10
## P   0.05976923 -0.1087962524  0.04046918 -0.24877861  0.2963683
## S   0.15293666  0.4349439314 -0.18745764  0.14939936 -0.2232165
## Cl  0.01252123 -0.0493661016  0.03597651  0.04652722  0.3686072
## K   0.12653248 -0.1333367969 -0.14300516  0.30264263 -0.1718107
## Ca -0.26685183 -0.0456355055 -0.20639007 -0.30865194 -0.3225527
## Mn -0.29869568  0.1372383326 -0.10826034 -0.09483417  0.5365961
## Fe  0.57614000 -0.3324965429 -0.20555001 -0.39653732  0.1206333
## Ni -0.06282109 -0.2011979771 -0.58475475  0.16940373 -0.2953351
## Cu  0.20333700  0.6284876366  0.27593819 -0.24308069 -0.2219600
## Zn -0.58083214 -0.1487209611  0.07962783 -0.32603778 -0.1945898
## Br  0.12822304 -0.4294331569  0.61145370  0.16400025 -0.2672695
## Rb -0.24212985 -0.0004555182  0.15617581  0.45273501  0.1172565
## Sr  0.08232892  0.0727476797 -0.14907052  0.36684976  0.1757434
```

Figure 93 The loading of each component.

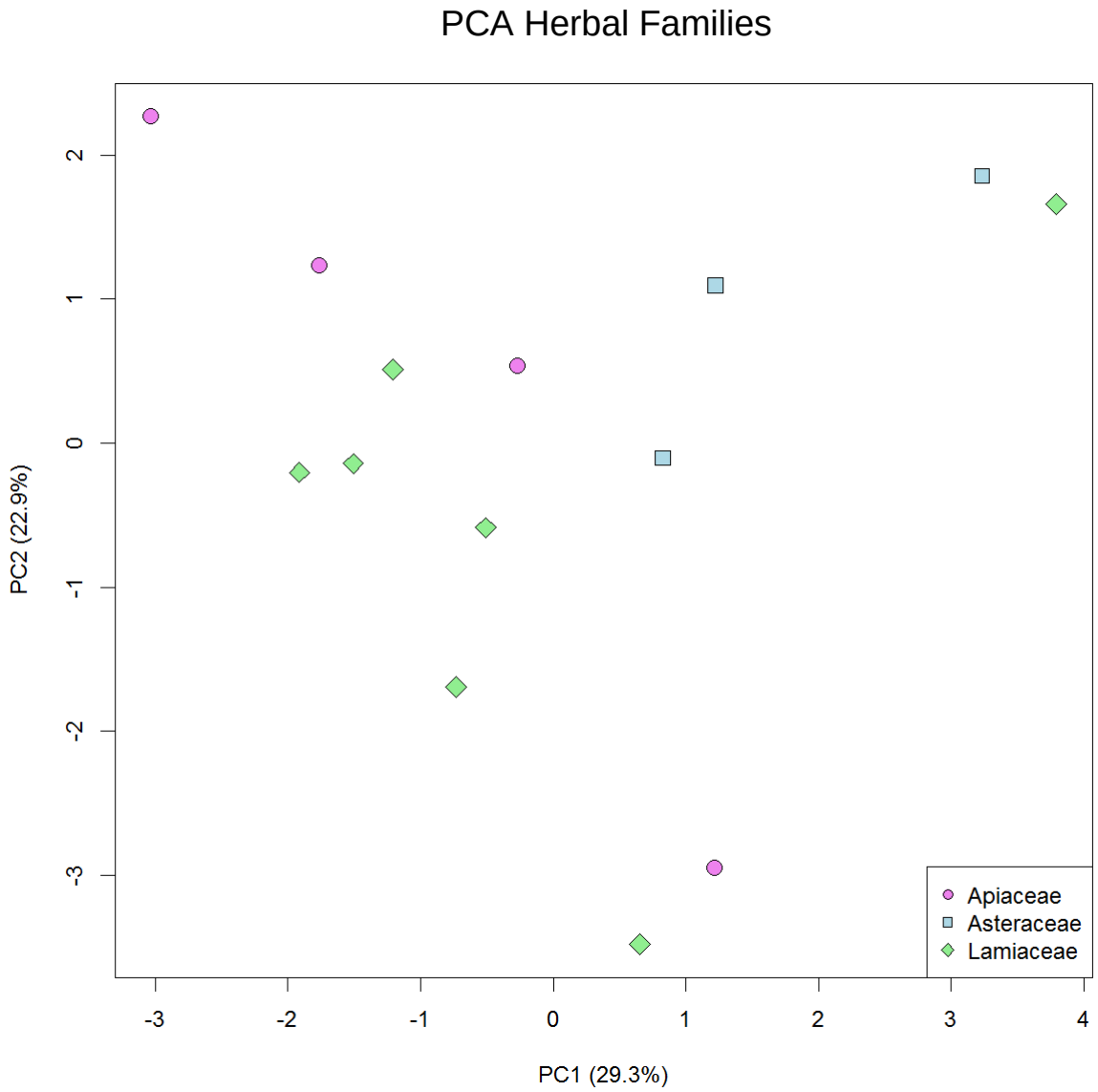

Figure 94 Graphical representation for PC1 and PC2, for herbal families.

The graphic image of the PCA for herbal families is shown in the Figure 94. Each group is represented by an individual number of samples and is coded in a different color for higher clarity. It is evident, that there are no indisputable group separations. The reason for this outcome will be discussed more thoroughly in the chapter 7 of this Master's thesis.

Loose herbal material against packaged tea

The fourth and the last PCA was held towards comparison between loose herbal material and packaged tea. There was a suspicion, that industrial packaging may cause contamination with one or several identical elements and that it would be possible to determine them.

Distribution of the main components is shown in the Figure 95. Four out of ten components are over the meaning line, however, PC1, PC2 and PC3 are considered to be more illustrative.

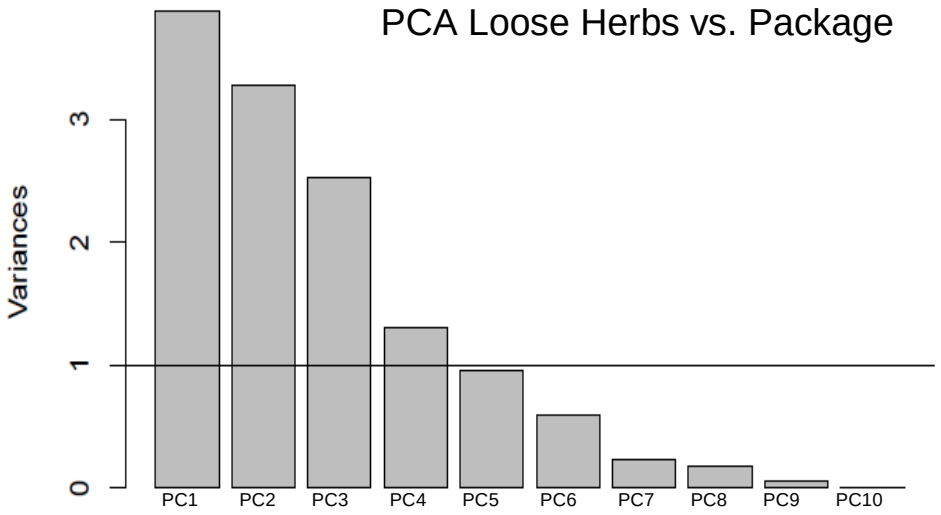

Figure 95 PCA for loose herbal material and packaged teas with normalized data. Distribution of the main components.

The loading of each separate PC is demonstrated in the Figure 96. It consists out of 13 commonly met elements in all the measured teas.

```
##          PC1         PC2         PC3         PC4         PC5         PC6
## P  0.37988579 -0.26564196 -0.08690628 -0.32118002 -0.191524923  0.04658273
## S  0.17563083  0.35547271 -0.34691391 -0.01757362 -0.296863425  0.20200425
## Cl 0.37501184 -0.02558415  0.34741427 -0.08667264 -0.369033292  0.03518793
## K  0.37215948 -0.13698442  0.27914795 -0.37068802  0.122851905  0.04943503
## Ca 0.14642646  0.35068256 -0.15822265 -0.54766340 -0.009794142 -0.25035369
## Mn 0.30610771  0.02880723 -0.43641313  0.29610817  0.145740172 -0.14443178
## Fe 0.04355655  0.43759845  0.10994064  0.08380932  0.247261467  0.54510651
## Ni 0.35464437 -0.27089580 -0.11670901  0.27477074  0.327356013 -0.09215673
## Cu 0.38582325 -0.12490814  0.18337218  0.17233169  0.246830630  0.37878417
## Zn 0.16075859  0.15613551  0.40610726  0.36190485 -0.138597855 -0.56281759
## Br 0.33284310  0.19522221 -0.36117688  0.17371741 -0.187829797 -0.09397805
## Rb 0.09590837  0.37625088  0.10080724 -0.19816645  0.604663313 -0.29287743
## Sr 0.09058560  0.41870323  0.30515523  0.22661330 -0.230745214  0.09654369
##           PC7         PC8         PC9         PC10
## P  -0.272980681  0.007691937 -0.10812251  0.04188097
## S   0.424178241  0.133447424  0.39580081  0.07016783
## Cl  0.009431448  0.187212028  0.03336341 -0.25777817
## K  -0.011434070  0.215391389 -0.09916908  0.45287974
## Ca -0.077819337 -0.283775441  0.33467707 -0.16617836
## Mn -0.054493930  0.168009057  0.04281255  0.56236290
## Fe -0.620610640 -0.036739805  0.14930668  0.03095294
## Ni -0.101961614  0.351412137  0.34638234 -0.51715962
## Cu  0.434972094 -0.586363448  0.02133383 -0.00418960
## Zn -0.183242779 -0.298391916  0.27350649  0.18940442
## Br -0.168931814 -0.254878811 -0.60633833 -0.24574167
## Rb  0.244438169  0.155310354 -0.27105307 -0.11258771
## Sr  0.172304635  0.380986396 -0.21957203 -0.02316958
```

Figure 96 The loading of each component.

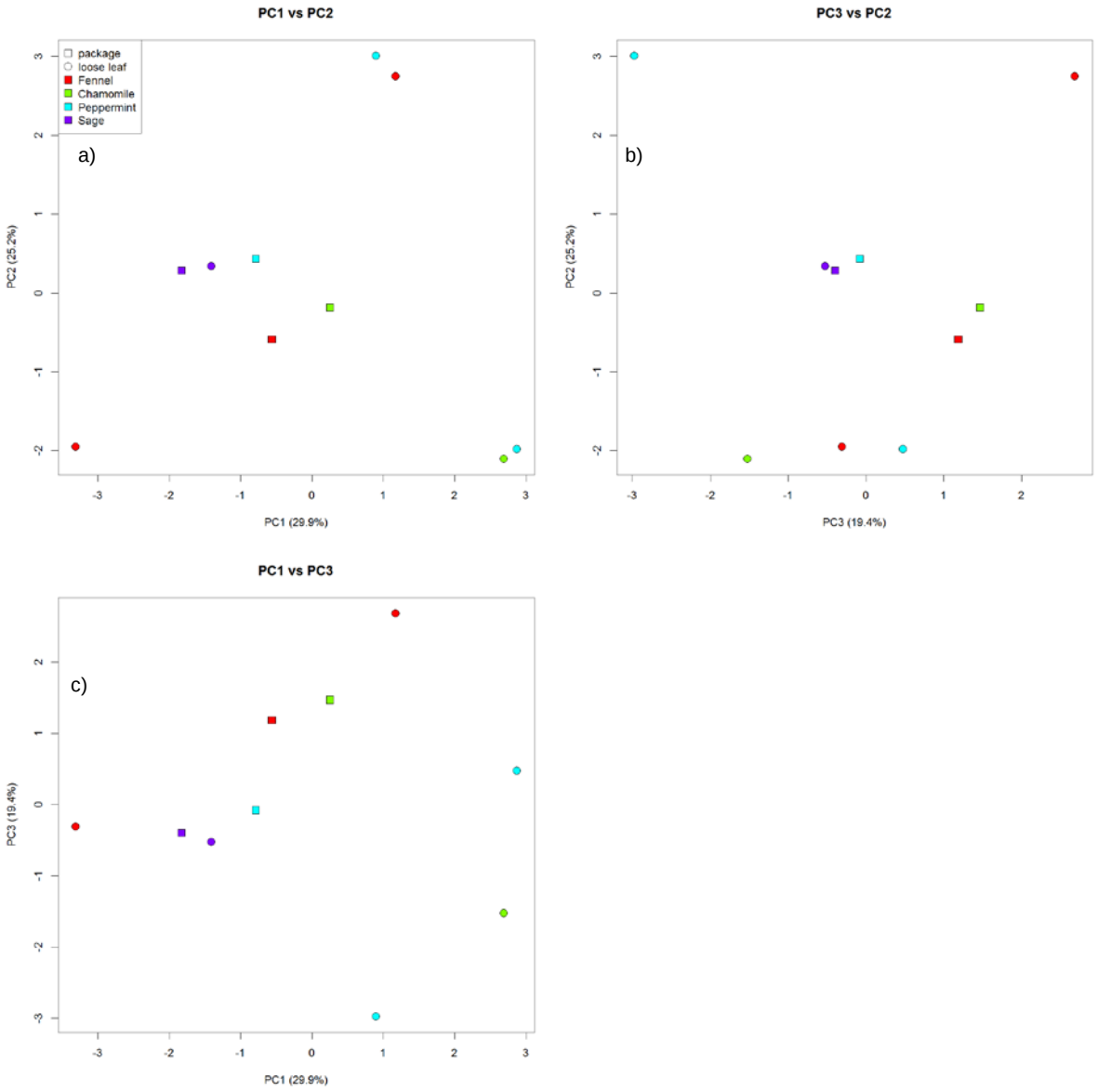

Figure 97 Graphical representation of main components (PC1, PC2 and PC3) for loose herbal material and packaged tea.

For the demonstration of the results of this analysis, it was decided to take three most meaningful principal components. Figure 97 a) shows correlation between PC1 and PC2; b) demonstrates PC2 and PC3; and graph c) PC1 over PC3. In all three graphs, loose herbal material samples have little in common, they are scattered over the whole plot. They are also rather scattered away from the corresponding packaged teas (exceptions are Sage and Sage Package samples). It is still however possible to suggest, that some similarities between packaged teas is notable. Packaged tea samples are rather situated in the center of the plot, in all three graphs.

6.2. Data processing

Data processing is an essential part of the measurement. It is responsible for the results interpretation, for its trustworthiness and repeatability. It is also usually a rather complex process which is developed for particular type of equipment or data set.

For TXRF a big part of the data processing is happening automatically with a help of software specially developed for particular spectrometer. The software is producing several types of files: .det, .lab, .spe, and .res. Out of these four different files, the .res or results file is directly usable (Attachment 3). After a conversion, from a .det file it is possible to get a .dat file which provides a set of numbers, allowing creating a spectrum of the measured sample. Other files are for internal use of the software.

The software itself has a function to mark and name the characteristic element peaks (Figure 98).

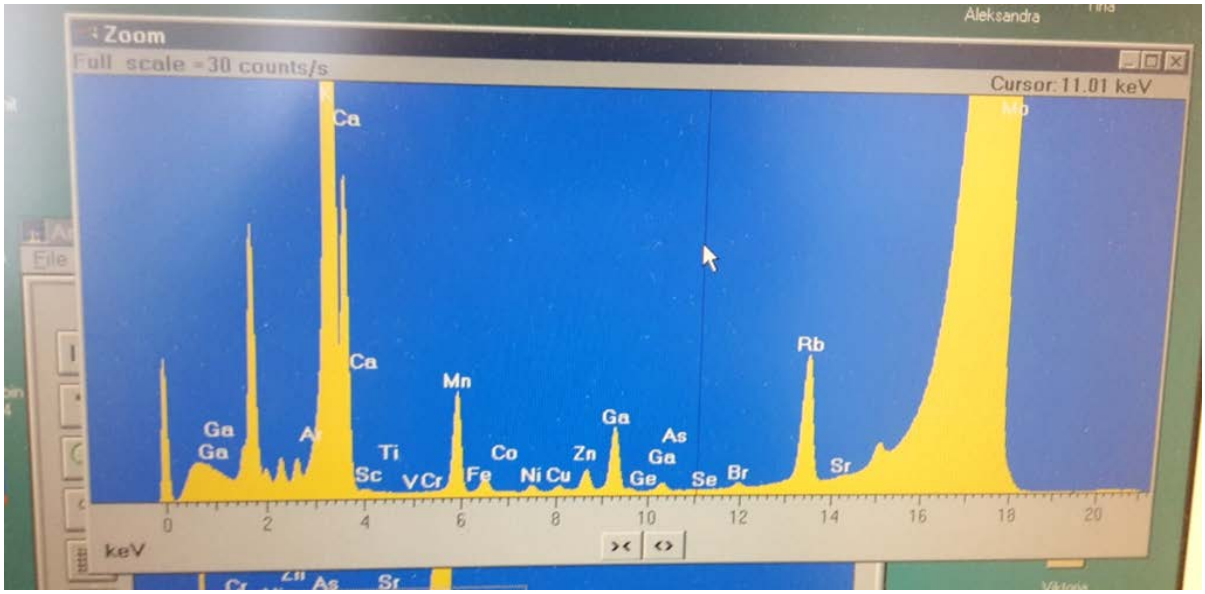

Figure 98 Spectrum generated by the software of Atomika 8030.

All peaks are predefined by user, meaning one can choose which elements are to be controlled and marked. When requested, element characteristic energy will be marked regardless whether there is a distinct peak present or no.

Evidently, the numerical results of such spectrum are recorded in corresponding .res file (Appendix 3). The first column contains elements which were predefined for control and type of the energy line (K-, L-, M- line). In the next column, the concentration value with calculated uncertainty (sigma) is shown. Concentration is calculated out of the raw data for each element. At first raw data is divided by the calibration factor of the particular element, and then divided by the reference count rate for 1 ng Nickel; and in the end, it is multiplied by the number of the atoms in one ng Ni (a constant value of $2040 \cdot 10^{10}$). Also, an important value is in the last column: Fit index. It shows how precise the calculated concentration (fitted) corresponds with initial one. [88]

Next step, after the data was analyzed by respective software, all converted .dat files were transferred into Excel program for establishing the characteristic spectra (examples are seen in chapters 6.1.1. and 6.1.2.).

6.3. Method evaluation

The presented method is rather outstanding for the measured type of samples. Liquid specimens are ideal for the total reflection X-ray spectrometer. Simple measurement preparation and fast obtaining of the end results, makes this method unbeatable for express whole spectrum evaluation. High resolution allows detection of the trace elements up to few ppb.

It is, however, necessary to stay critical, especially evaluating the trace elements. The little amounts of the sample and of the elements in it can lead to the misinterpretation and loss of the data. As mentioned in the chapter 2.2.2.5, there are several possible artifacts which can also lead to false results. It is necessary to check each recorded spectrum manually and determine whether escape peaks or sum peaks appeared. It is also important to evaluate overlapping peaks. It is not uncommon when software is detecting an element that is overlapped and completely invisible under another element with higher concentration.

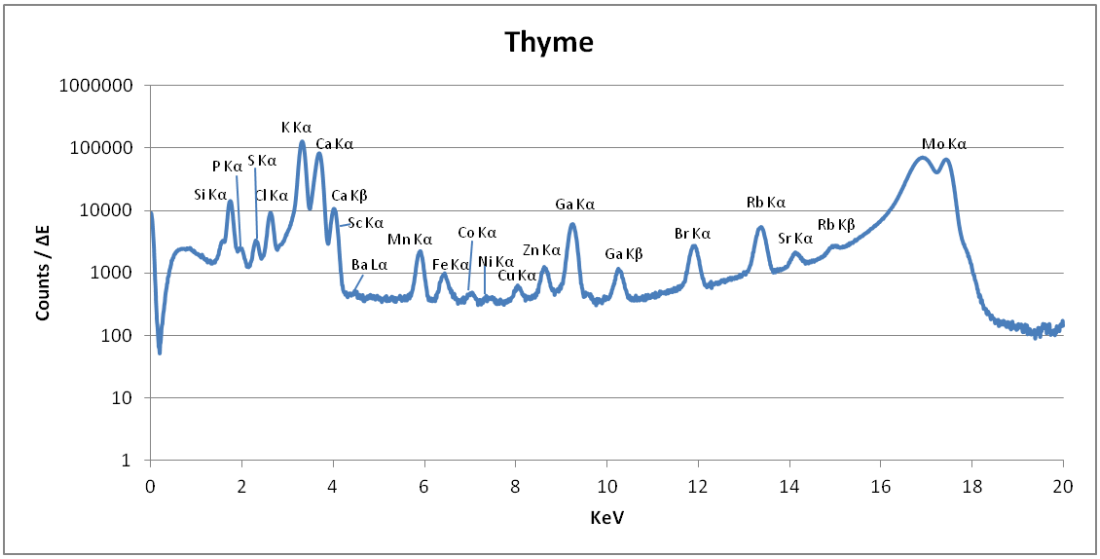

Figure 99 Typical Thyme sample spectrum

The Figure 99 shows a typical Thyme extraction sample, where the overlapping peak is observable. Element Sc Kα is completely covered under the higher signal of the intense Ca Kβ peak. The energy of the Sc Kα line is 4.09 KeV and for the Ca Kβ it is 4.012 KeV. As the peaks are not separated and represented as straight lines, but rather as a Gaussian distribution with ±σ, the Ca Kβ easily covered the Sc Kα.

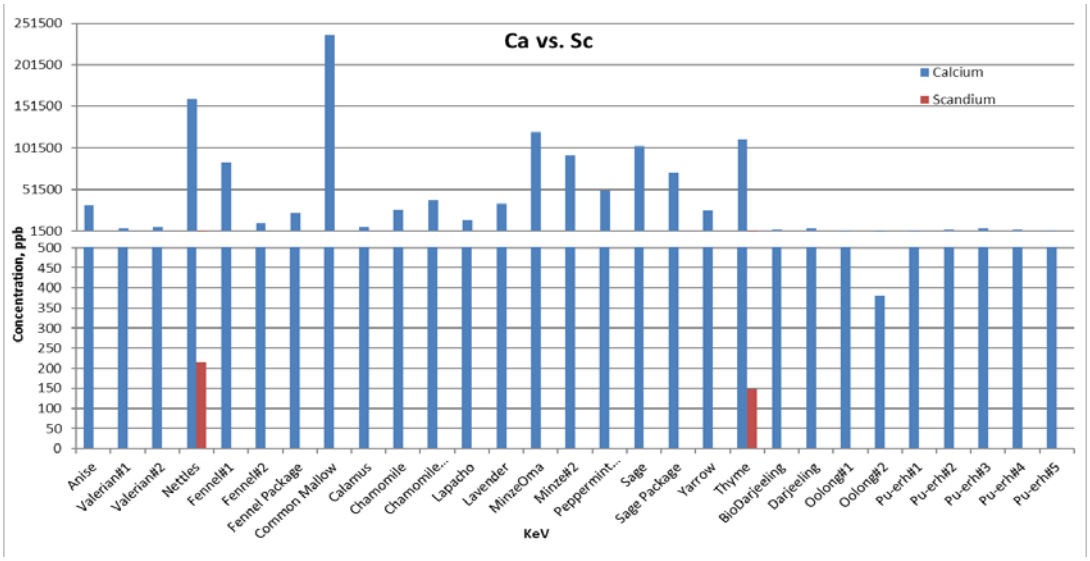

Figure 100 Overview of all samples Ca and Sc overlapping peaks.

Out of all the measured samples, Sc was detected in two: Nettles and Thyme (Figure 100). In both cases, amount of Ca was very high (over 100000 ppb). The Kβ lines of Ca will be at least a half of the Kα lines; by such high concentration of Ca at Kα lines, the Sc Kα will be completely hidden under the Kβ lines of Calcium. This leads to a conclusion, that Sc cannot be correctly determined in the presence of a high Ca content. The analyzing software has detected Sc in five samples all together, but after detailed analyzing, the Kβ line was visible in only Nettles and Thyme samples.

The frequency of appearance of the sum peaks is related to the spectral resolution of the device (Atomika 8030C has spectral resolution < 150 eV at the 5.89 keV for Mn); it varies from 125 eV to 150 eV by diverse equipment. The main component that defines the spectral resolution of the device is the process time while detection. The detected peak will be narrower and, therefore, the resolution will be better, when time that pulse processor has spent for measuring the upcoming X-ray impulse is longer. One still must take into account, that higher processing time will lead to higher dead time (time, when detector is not recording a signal). [89]

Other point in method evaluation concerns the selected samples.

The chosen method provided the whole element spectrum, and without additional information (about origin of the elements found in the samples), it was impossible to establish a fingerprint of the particular plant. Further note are presented in the chapter 7 Conclusion.

The sample selection, more particularly its origin, soil composition, preparation way – all together should be taken into account in order to achieve certain goals and tasks.

7. Conclusion

The purpose of this Master's thesis was to select tea and herb samples for establishing their elemental composition. Also in the beginning of this Master's thesis, seven specific research questions were defined. After finishing the experimental part of the work and presenting all the outcomes in the chapter 6, all opened questions can be answered.

Measurements of 29 different samples lead to the conclusion, that there are 13 default elements, which are found in all measured samples. Those elements are: Phosphorus, Sulfur, Chlorine, Potassium, Calcium, Manganese, Iron, Nickel, Copper, Zinc, Bromine, Rubidium, and Strontium. There are also four occasional elements, which could be found only in few samples. Those elements are: Cobalt, Titanium, Barium, and Scandium. To realize the differences between the samples, column diagrams for each element (default or occasional) were constructed. The elements were displayed in conformity with their energies.

Such a comparison gave a first realization in the question if it is possible to distinguish teas and herbal infusions. Elements like Ca, Sr, Br, and Cl are dominant primarily in the herbal infusions, whereas Rb and Mn are substantially more present in the black teas. The outcome that it is possible by means of spectrometric analysis to differentiate between teas and herbal infusions was supported by the PCA analysis. For this purpose, all samples with normalized data were analyzed. A rather clear division of the two types of beverages is notable in Figure 101, where black dots are black teas and green dots are herbal infusions.

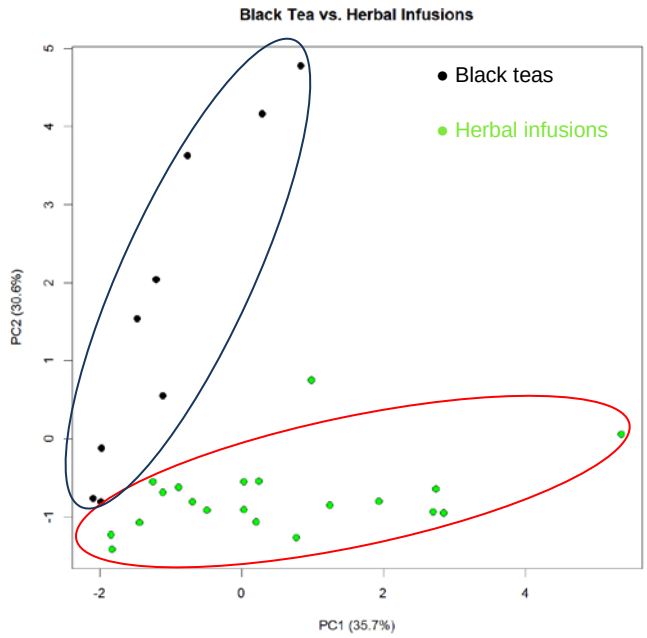

Figure 101 Two separated groups: black teas and herbal infusions.

The next step of the study was to try to identify the fingerprint of each herbal infusion. It proved to be impossible with the limited knowledge of origin of the plants including soil samples, precise preparation procedure before selling, and other factors that could influence the results. As proof for the prior statement, there are several column diagrams (Figures 41,

50, 52, 56, 60, and 62) comparing same sorts of tea / infusion. For example, there are three peppermint samples: loose leaves bought in Austrian pharmacy, loose leaves gathered as wildly growing plant in Latvia and one packaged tea claiming to consist of plants gathered at organic farms originating from Egypt and Eastern Europe. Figure 60, 81 and Table 18 thoroughly demonstrate how different the samples are. There is no trend, which could backup these differences. As already mentioned, such a scatter of the experimental data could occur out of lack of information. Such facts as exact origin, soil constitution, and way of packaging (especially for tea in bags) can give a necessary insight into establishing the plants' fingerprint. One more way to try to establish the fingerprint features database could be by using more sensitive equipment.

Next idea was to determine if it is possible to distinguish between different plant families. All herbs can be divided into several families, they all share morphological (vegetative and generative organs) similarities. Therefore, the objective was to check if they also share a similar elemental composition. It was not clearly recognizable from the bar diagrams of the elements (chapter 6.1.3. Default and Occasional Elements). For some elements, few families were rather comparable, and for other elements, the data were rather random. Figure 102 shows an example of Ni in all the samples, where the infusions prepared using the plants – members of Lamiaceae family are marked in red. The results here are rather inconclusive; therefore, a PCA was performed for three families (which include most of the herbal samples) with normalized data. Figure 94 represents obtained results. There is no visible consistent pattern. As possibility for obtaining better results, more sensitive spectroscopic equipment could be used.

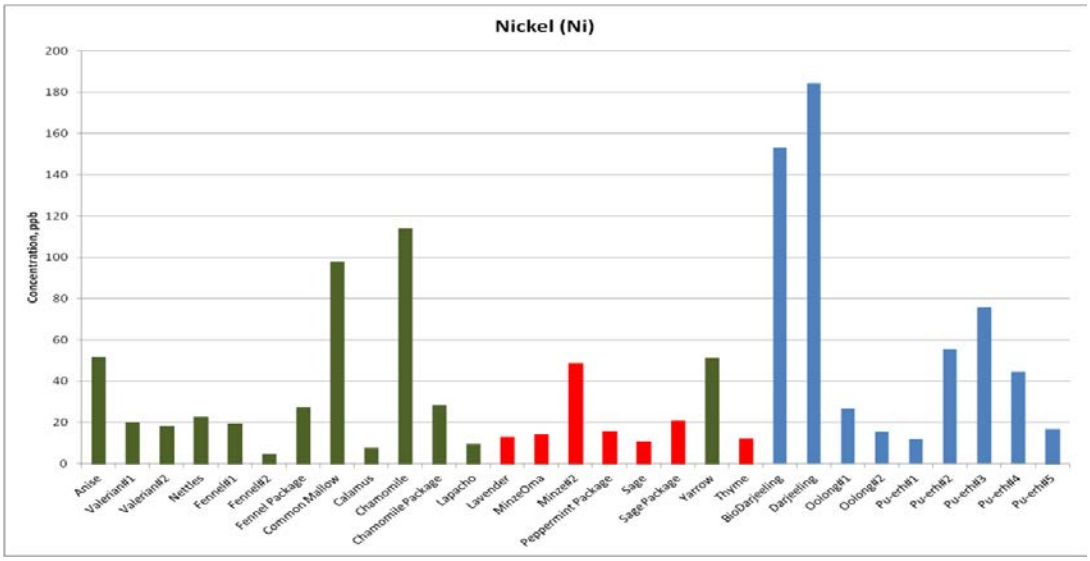

Figure 102 Ni in all samples.

Out of all 20 herbal samples, 16 were loose herbal material and 4 were made in the form of the tea bags. The exact procedure of particular tea bags production is not entirely known, for example, which bleacher was used. The goal here was to see if there is any likeness between packaged teas and to compare them to the same sort of loose herbal material. Figure 94 demonstrates PCA for this comparison. All the three sets look rather random, although a tendency of packaged teas to hold more together, independently of the type of herbs, is observable. On this point more thorough research could be applicable. It would be beneficial to look for lighter elements, such as Fluoride (F), as there are several articles mentioning F presence in some tea sorts and packages [90, 91].

After all measurements, a typical spectrum was created for each tea / herbal infusion sample. All the peaks were analyzed and controlled. It can be concluded, that no heavy metals were found in any of the samples, there is also no evidence for any toxic elements in any worrying dosage.

During the measurements of Pu-erh tea, five steeping of the tea were measured as separate samples. In the all preparation manuals, available on paper or in internet, there was a suggestion / a description to pour out the first steeping. It is basically thrown away or used to warm up the cups. It was interesting to take a look if this particular sample has anything special in it to justify the throwing out. With the obtained results (for more details see: chapter 6.1.1. paragraph Pu-erh tea), there is no confirmation of any harmful element to be present in the first steeping or any following for that matter. On the contrary, the first brewing of Pu-erh tea had the least amounts of elements in it, compared to the following.

For better understanding of the elemental composition of teas and herbal beverages, further extensive study could be reasonable. More diverse types of herbs could be controlled. The point of interest would be also to compare different parts of one plant in between (roots, leaves, blossoms and/or fruits).

8. References

8.1. Figures

8.2. Tables

8.3. Literature

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9. Appendix

1. Used abbreviations

FDA	food and drug association (US-united states)
JECFA	joined FAO/WHO expert committee on food additives
USEPA	US environmental protection agency
FAO	food and agriculture organization
WHO	world health organization
ATSDR	agency for toxic substances and disease registry
LDA	linear discriminant analysis
PCA	principal component analysis
RDA	recommended dietary allowance
XRF	X-ray fluorescence
TXRF	total reflection X-ray fluorescence
ED XRF	energy dispersive X-ray fluorescence
WD XRF	wavelength dispersive X-ray fluorescence
PTWI	provisional maximum tolerable weekly intake
mg/kg bw-day	milligram per kilogram of body weight per day

2. Certificate of Analysis NIST 1640

National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 1640

Trace Elements in Natural Water

This Standard Reference Material (SRM) is intended primarily for use in evaluating methods used in the determination of trace elements in fresh water. SRM 1640 is composed of natural fresh water collected from Clear Creek, CO, which has been filtered and stabilized with nitric acid at a concentration of 0.5 mol/L. A unit of SRM 1640 consists of approximately 250 mL of solution in a polyethylene bottle, which is sealed in an aluminized plastic bag.

Certified Values and Uncertainties: The certified values expressed as mass fractions and their expanded uncertainties are listed in Table 1 for 17 elements in SRM 1640. The certified values are equally weighted means of the results of two or more independent analytical methods or a single primary method. Each expanded uncertainty is based on a 95 % confidence interval for the mean, and includes an allowance for differences between the analytical methods used and an allowance for solution stability [1].

Reference Values and Uncertainties: The reference values expressed as mass fractions and their expanded uncertainties are provided in Table 2 for an additional ten elements. The reference values are means from a single method or two or more equally weighted means of results of independent analytical methods for which there is insufficient information to meet NIST certification criteria. Each expanded uncertainty is based on a 95 % confidence interval for the mean and includes an allowance for differences between the analytical method used and an allowance for solution stability but may not include all sources of uncertainty [1].

Information Value: The upper limit information value for thallium, expressed as a mass fraction in Table 3, is an estimate based on the instrumental limit of detection and measurements from a single unit of SRM 1640.

The analytical methods used for the characterization of this SRM are given in Table 4. All values are reported as mass fractions [2].

NOTICE AND WARNINGS TO USERS

Expiration of Certification: The certification of SRM 1640 is valid, within the measurement uncertainty specified, until **01 June 2010**, provided the SRM is handled in accordance with instructions given in this certificate (see "Instructions for Use"). This certification is nullified if the SRM is damaged, contaminated, or otherwise modified.

Maintenance of SRM Certification: NIST will monitor this SRM over the period of its certification. If substantive technical changes occur that affect the certification before the expiration of this certificate, NIST will notify the purchaser. Registration (see attached sheet) will facilitate notification.

Coordination of the NIST technical measurements was under the direction of J.R. Moody of the NIST Analytical Chemistry Division.

Statistical analysis of the experimental data was performed by W.F. Guthrie of the NIST Statistical Engineering Division.

Stephen A. Wise, Chief
Analytical Chemistry Division

Gaithersburg, MD 20899
Certificate Issue Date: 06 February 2008
See Certificate Revision History on Last Page

Robert L. Watters, Jr., Chief
Measurement Services Division

The overall coordination of measurements performed by the U.S. Geological Survey National Water Quality Laboratory, Arvada, CO, and by laboratories that participate in the Standard Reference Water Program was under the direction of K. Long.

Support aspects involved in the issuance of this SRM were coordinated through the NIST Measurement Services Division.

INSTRUCTIONS FOR USE

The SRM should be shaken before use because of potential water condensation. Samples should be analyzed at a room temperature of 22 °C ± 5 °C. To prevent possible contamination of the SRM, pipettes should not be inserted into the bottle. After use, the bottle should be recapped tightly and returned to the aluminized bag, which should be folded and sealed with sealing tape. This precaution will protect the SRM from possible environmental contamination and long-term evaporation.

The mass fractions given in Tables 1 and 2 are expressed as microgram per kilogram or milligram per kilogram. These values can be converted to mass concentrations with units of nanograms per cubic centimeter or micrograms per cubic centimeter, respectively, by multiplying by the density. The density of SRM 1640 at 22 °C was measured to be 1.0015 g/cm^3 ± 0.0005 g/cm^3 (identical to grams per milliliter).

Recognizing contamination at the microgram per kilogram level can be a serious problem, labware should be scrupulously cleaned and only high purity reagents employed. Sampling and manipulations, such as evaporations, should be done in a clean environment, such as a Class-100 clean hood.

Table 1. Certified Mass Fractions

Element	µg/kg	Element	µg/kg
Aluminum	52.0 ± 1.5	Iron	34.3 ± 1.6
Antimony	13.79 ± 0.42	Lead	27.89 ± 0.14
Arsenic	26.67 ± 0.41	Manganese	121.5 ± 1.1
Barium	148.0 ± 2.2	Molybdenum	46.75 ± 0.26
Beryllium	34.94 ± 0.41	Selenium	21.96 ± 0.51
Boron	301.1 ± 6.1	Silver	7.62 ± 0.25
Cadmium	22.79 ± 0.96	Strontium	124.2 ± 0.7
Chromium	38.6 ± 1.6	Vanadium	12.99 ± 0.37
Cobalt	20.28 ± 0.31		

Table 2. Reference Mass Fractions

Element	µg/kg	Element	mg/kg
Copper	85.2 ± 1.2	Calcium	7.045 ± 0.089
Lithium	50.7 ± 1.4	Magnesium	5.819 ± 0.056
Nickel	27.4 ± 0.8	Silicon	4.73 ± 0.12
Potassium	994 ± 27	Sodium	29.35 ± 0.31
Rubidium	2.00 ± 0.02		
Zinc	53.2 ± 1.1		

Table 3. Information Mass Fraction

Thallium	<0.1 µg/kg

Source and Preparation of Material:[1] A sample of about 3500 L of natural (fresh) water was obtained by the USGS at Clear Creek, CO. It was filtered through a 0.1 µm ultra filter and acidified with nitric acid. Analysis of the water by inductively coupled plasma mass spectrometry (ICPMS), before and after the stabilization process, showed that arsenic, beryllium, cobalt, selenium, and zinc were decreased in concentration during the stabilization process. These elements were adjusted to their original concentration levels by the addition of salts of the decreased elements. The stabilized solution was then pumped through an ultra filter, past a UV light source (for sterilization purposes), and then to a bottling station. At the bottling station, the bottles were rinsed with the sample and then filled.

Table 4. Methods Used for the Analysis of SRM 1640

Elements	Methods
Aluminum	DCP, ETAAS, ICP-AES, ICPMS
Antimony	ETAAS, Hyd-AAS, ICP-AES, ICPMS
Arsenic	ETAAS, Hyd-AAS, ICP-AES, ICPMS
Barium	DCP, ETAAS, ICP-AES, ICPMS, **ID-ICPMS**
Beryllium	ETAAS, ICP-AES, ICPMS
Boron	COLOR, ICP-AES, ICPMS, ID-TIMS
Cadmium	ETAAS, FAAS, IC, ICP-AES, ICPMS, ID-ICPMS
Calcium	DCP, FAAS, ICP-AES, ICPMS
Chromium	ETAAS, FAAS, IC, ICP-AES, ICPMS
Cobalt	ETAAS, ICP-AES, ICPMS
Copper	ETAAS, FAAS, IC, ICP-AES, ICPMS, ID-ICPMS
Iron	ETAAS, FAAS, ICP-AES, ICPMS, ID-TIMS
Lead	ETAAS, FAAS, IC, ICP-AES, ICPMS, **ID-ICPMS**
Lithium	ETAAS, FAAS, ICP-AES, ICPMS
Magnesium	DCP, FAAS, ICP-AES, ICPMS
Manganese	DCP, ETAAS, FAAS, ICP-AES, ICPMS
Molybdenum	ETAAS, ICP-AES, ICPMS, **ID-ICPMS**
Nickel	ETAAS, FAAS, ICP-AES, ICPMS, ID-ICPMS
Potassium	ETAAS, FAAS, FES, ICP-AES, ICPMS
Rubidium	ID-TIMS
Selenium	EAAS, Hyd-AAS, ICP-AES, ICPMS
Silicon	COLOR, ICP-AES, ICPMS
Silver	ETAAS, FAAS, ICP-AES, ICPMS, ID-ICPMS
Sodium	DCP, FAAS, FES, ICP-AES, ICPMS
Strontium	DCP, ETAAS, ICP-AES, ICPMS, **ID-ICPMS**
Thallium	ICPMS
Vanadium	ETAAS, ICP-AES, ICPMS
Zinc	FAAS, IC, ICP-AES, ICPMS, ID-ICPMS

[1] Certain commercial equipment, instrumentation, or materials are identified in this certificate to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the NIST, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

Methods given in bold indicate that a single NIST primary method was used for certification.

Methods

COLOR	Colorimetry
DCP	Direct current plasma atomic emission spectrometry
ETAAS	Heated graphite atomizer (electrothermal) atomic absorption spectrometry
FAAS	Flame atomic absorption spectrometry
FES	Flame emission spectrometry
Hyd-AAS	Hydride generation-atomic absorption spectrometry
IC	Ion chromatography
ICP-AES	Inductively coupled plasma-atomic emission spectrometry
ICPMS	Inductively coupled plasma mass spectrometry
ID-ICPMS	Isotope dilution-inductively coupled plasma mass spectrometry
ID-TIMS	Isotope dilution-thermal ionization mass spectrometry

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M. Morita; Regional Environmental Division of the National Institute for Environmental Studies, Japan Environmental Agency, Tsukuba, Japan

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[2] Taylor, B.N.; *Guide for the Use of the International System of Units (SI)*; NIST Special Publication 811, U.S. Government Printing Office: Washington, DC (1995).

Certificate Revision History: 06 February 2008 (Editorial change to correct the SRM number in Expiration section); 28 January 2008 (Update of expiration date and editorial changes); 20 January 2006 (This revision reflects an extension of the certification period); 17 March 2004 (This technical revision reports a change in the expiration date); 23 January 1998 (Revision reports the addition of an information value for thallium; 02 October 1997 (Original certificate date).

Users of this SRM should ensure that the certificate in their possession is current. This can be accomplished by contacting the SRM Program at: telephone (301) 975-6776; fax (301) 926-4751; e-mail srminfo@nist.gov; or via the Internet at http://www.nist.gov/srm.

3. The prior result sheet (acquired by the ATOMIKA 8030C software)

Sample: Kamille #1 Ga 51 cas 2/17
System ID: 943/4-543-1-2/01001 Listed at 10:29 on Monday, February 01, 2016

A T O M I K A T X R F 8 0 3 0 C

Filename: c:\txrfdata\ac1\j18\302.spe atomika, Job number 18
Measurement: 1000 seconds Date: 28.01.2016 Time: 12:05
Excitation: Mo-K (thin) Filter: Zr20
Tube current: 37 mA System resolution: 94 eV
Element list: DEFMO.SQS Avg DT: 40.2 % , 14211 cps

Element & line	Conc. ± sigma [ppb]	RSD [%]	Peak area ± sigma [counts/sec]	Fit index
Si K	********** ± ********	0.721	397.69 ± 0.93	4.9
P K	46936.781 ± 933.193	1.988	28.22 ± 0.53	5.8
S K	32833.167 ± 521.802	1.589	37.80 ± 0.54	14.1
Cl K	97634.381 ± 760.703	0.779	279.74 ± 1.05	42.0
Ar K	< 308.148		0.74 ± 0.76	15.5
K K	387478.228 ± 2669.478	0.689	2891.99 ± 2.79	9.3
Ca K	25580.036 ± 206.440	0.807	272.72 ± 1.18	3.4
Sc K	< 53.301		0.35 ± 0.41	0.9
Ti K	< 33.925		0.13 ± 0.33	0.1
V K	< 15.567		0.12 ± 0.28	0.2
Cr K	< 11.621		0.11 ± 0.28	0.9
Mn K	1250.166 ± 14.828	1.186	52.27 ± 0.51	5.7
Fe K	53.007 ± 6.587	12.427	2.77 ± 0.34	6.2
Co K	10.929 ± 4.604	42.128	0.70 ± 0.29	3.9
Ni K	113.393 ± 4.599	4.055	8.72 ± 0.35	0.3
Cu K	96.230 ± 4.161	4.324	8.72 ± 0.37	0.5
Zn K	259.875 ± 5.063	1.948	27.29 ± 0.50	0.6
Ga K ref.	1058.000 ± 7.217	0.682	127.01 ± 0.87	0.6
Ge K	Not detected			
As K	Not detected			
Se K	Not detected			
Br K	529.963 ± 6.093	1.150	103.00 ± 0.95	1.0
Rb K	122.797 ± 3.664	2.984	30.13 ± 0.88	2.2
Sr K	19.932 ± 2.603	13.061	5.52 ± 0.72	14.6
Ba L	< 76.313		0.41 ± 0.84	1.0
La L	< 109.126		1.29 ± 0.74	1.0
Pb L	< 11.850		1.02 ± 1.02	2.8
Pb M	Not detected			

Figure 103 The view of a result file directly after measurement.

4. Poster

Total X-ray reflection spectrometry of black tea and herbal infusions

A. Winkler, M. Rauwolf, A. Turyanskaya, J. Sterba, P. Wobrauschek and C. Streli

Atominstitut, TU Wien, Stadionallee 2, 1020 Vienna, Austria

1. Introduction

World Health Organization states [1], that it is currently unable to recommend limits for contaminants and residues for herbal materials and medicines. The reason for that is that there is not enough research on the elemental composition in raw and processed herbal materials. Challenging and at the same time the most promising research is expected to be a multielemental diagnostic as this is the biggest advantage of the herbal medicines over single-component drugs. It is expected to achieve a potentiating effect due to the presence of multiple active compounds [2]. TXRF is a universal and economic multielement method suitable for ultra micro- and trace analyses. It has a wide range of field of application and has recently become increasingly popular in micro and trace elemental analysis [3].

2. Aim

The aim of this study was to determine elemental composition including trace element content in the various tea and herbal infusions, among them those which are used for medical treatment purposes, by the mean of Total Reflection X-ray Fluorescence Analysis (TXRF).

3. Samples

- 3 tea (*Camellia sinensis*) sorts and 13 herbal-tea sorts where chosen:
  - Darjeeling, Oolong, Pu-erh
  - Anise, Valerian, Fennel, Common Mallow, Calamus, Chamomile, Lapacho, Lavender, Peppermint, Sage, Yarrow, Thymus, Urtica (Nettles)
- Resulting in total 29 different samples.

4. Method

Fig. 1: Total Reflection X-ray Spectrometer Atomika 8030C.

- Tea samples preparation was identical for all teas and herbal infusions, except pu-erh tea, in order to be able to make a comparison between them.
- Each sample was weighted: 1g of the sample (leaves, bark, flowers or seeds) was steeped with 50 ml of tridistilled water; then brewed for 10 minutes.
- Pu-erh tea sample was steeped several times and brewed for only 30 seconds each. It was done due to specific preparation rules for this tea.
- After infusion was ready 1ppm Ga as Internal Standard was added.

Setup properties:

- Measurement Live Time:	1000 seconds
- Excitation:	Mo-Ka
- Filter:	Zr_2O (thin)
- Tube current:	37 mA
- Tube voltage:	50 kV

5. Results

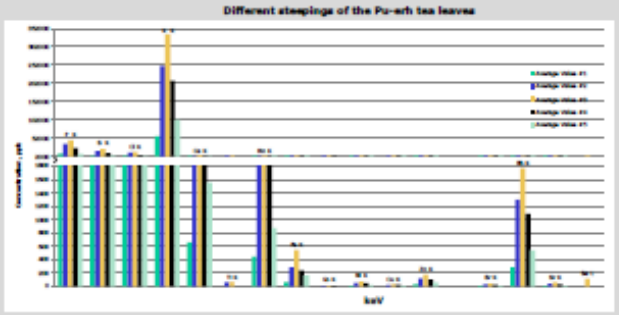

Fig. 2: Energy (keV) over concentration (ppb) distribution for five steepings of the same leaves for Pu-erh tea.

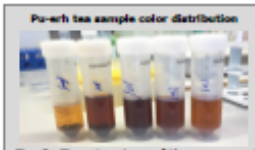

Fig. 3: Five steepings of the one sample of Pu-erh tea – color distribution.

For some elements, the difference in obtained results between samples was drastical (Ni and Mn in Fig.5). It was also possible to see the possible clusters as in Fig.6. All sorts of black tea showed nearly no or very little amount of Ca.

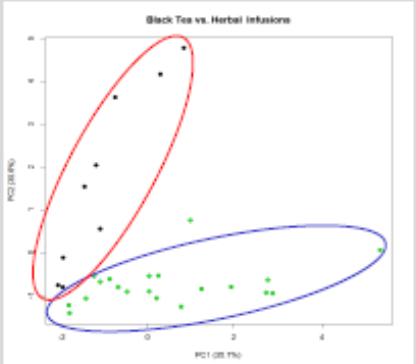

Fig. 4: Principal component analysis of the tea samples. Black tea (black dots) versus herbal infusions (green dots). PC1 include: S, Cl, K, Ca, Zn and PC2 mostly include: Mn, Ni, Rb, Ca.

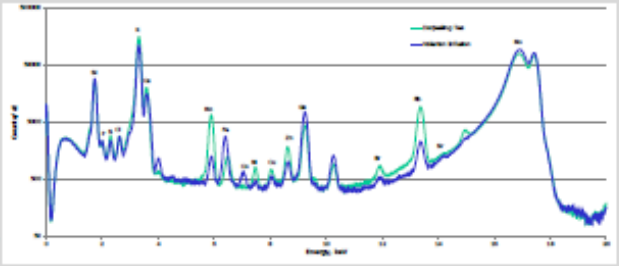

Fig. 5: -> Spectra of typical black tea (Darjeeling sample) and typical herbal infusion (Valerian sample). Both were normalized to Internal Standard.

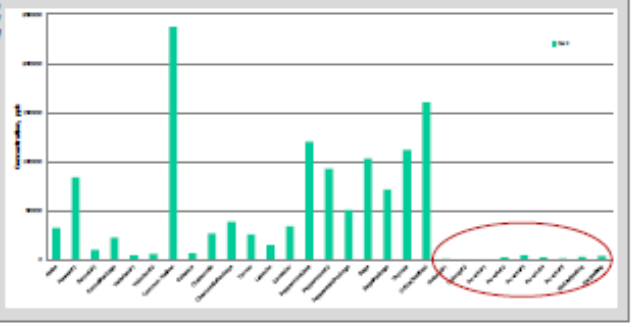

Fig. 6: -> Comparison of Ca concentrations for all samples.

Conclusions

– Differences between black teas and herbal infusions were identified. The origin of the sample was not taken into account, just elemental composition. Figure 4 shows results of the principal component statistical analysis, where two respective clusters can be clearly seen.
– Pu-erh tea showed to be an interesting case, as the elemental amount (independently of the element) correlates with the color of the steeping.
– The common elements for herbal infusions and teas were distinguished. In total 13 "default" (P, S, Cl, K, Ca, Mn, Fe, Ni, Cu, Zn, Br, Rb, Sr) and 5 "occasional" elements (Co, Tl, Sc, Ba) were found.

Outlook

More detailed research including soil and origin parameter could lead to establishment of an elemental fingerprint for particular herbal material.

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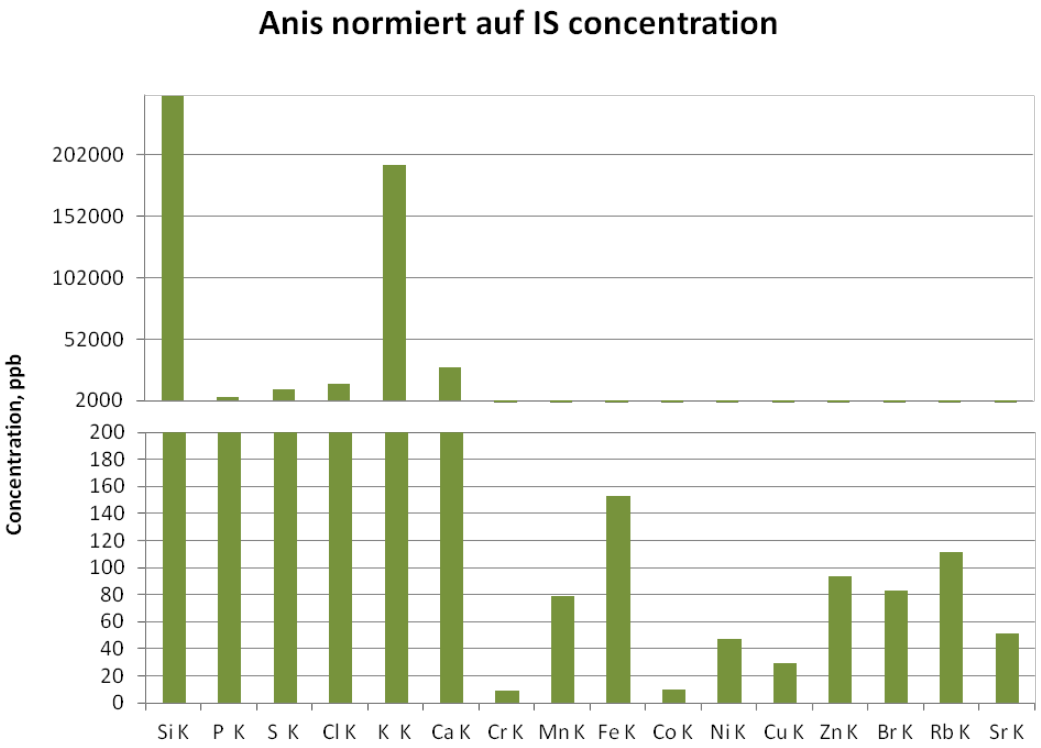
5. Anis


Anis normiert auf IS concentration
Concentration, ppb
202000
152000
102000
52000
2000
200
180
160
140
120
100
80
60
40
20
0
Si K
P K
S K
Cl K
K K
Ca K
Cr K
Mn K
Fe K
Co K
Ni K
Cu K
Zn K
Br K
Rb K
Sr K