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DEPARTMENT OF ENVIRONMENTAL GEOSCIENCES

Diploma Thesis

Optimization of the matrix separation methodology for Cd
isotope analysis of environmental samples

Author: Argyrios Mangolis

Supervisor: Ing. Hana Šillerová, Ph.D.

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Argyrios Mangolis

Land and Water Management

Thesis title

Optimization of the matrix separation methodology for Cd isotope analysis of environmental samples

Objectives of thesis

The main objective of the thesis is to optimize sample matrix separation methodology which must be performed prior Cd isotope analysis of environmental samples. The aim of the separation is to obtain a pure Cd fraction which contains more than 90% of Cd originally presented in a sample before the separation. All the other elements must be separated completely from the sample. The aim is to develop methodology which can be applied for different environmental samples and especially soil.

Methodology

The environmental samples (soil etc.) will be collected at 9 sampling sites in Ostrava region. The samples will be decomposed in a mixture of acids and analyzed for the metal concentration by ICP-MS. Depending on Cd concentration a specific portion of each decomposed sample will be taken on a chromatographic column equipped with a specific exchange resin. The separation efficiency must be over 90%. The separated pure Cd fraction will be analyzed for Cd isotope composition by TIMS spectrometer. Isotope data will be used for tracing the Cd sources and eventually for the pollution sources apportionment.

The proposed extent of the thesis

50-60

Keywords

cadmium, isotope fractionation, matrix separation, environmental pollution,

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Vossler, T., Cernlkovsky, L., Novak, J., et al., 2015. Atmosph. Pollution Res. 6, 454-463.
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The Diploma Thesis Supervisor

Ing. Hana Šillerová, Ph.D.

Supervising department

Department of Environmental Geosciences

Electronic approval: 12. 4. 2017

prof. RNDr. Michael Komárek, Ph.D.

Head of department

Electronic approval: 12. 4. 2017

prof. RNDr. Vladimír Bejček, CSc.

Dean

Prague on 13. 04. 2017

Declaration

I hereby declare that the work presented in this thesis is, to the best of my knowledge, original work, except as cited in the text. I have listed all data sources, literature and publications from which I acquired the necessary information for the completion of this study.

Prague ----- 18th of April 2017

Argyrios Mangolis

Abstrakt

Stopování zdrojů kovů v životním prostředí je důležitým klíčem k našemu chápání jejich biogeochemického cyklu a cest vedoucím k znečištění. Kadmium patří mezi nejvíce toxické a život ohrožující látky znečišťující životní prostředí, takže stopování jeho zdrojů je velmi důležité a užitečné. V této studii byly odebrány vzorky půdy v silně industrializovaných oblastech Ostravska, (Horní Slezsko, Česká republika). Cílem byla optimalizace separační metody, která má zajistit kvantitativního oddělení kadmia z půdních vzorků pro účely izotopové analýzy. Byla analyzována koncentrace vybraných kovů a polokovů, pro posouzení úrovně kontaminace na Ostravsku. Po separaci byla vyhodnocena její účinnost a vyhodnocena vhodnost této metody pro separaci Cd v případě vzorků půdy. Metoda byla prokázána jako vhodná a účinnost separace byla obvykle nad 95%.

Klíčová: Kadmium, izotopová frakcionace, separace matrice, znečištění životního prostředí

Abstract

Tracing the sources of metals in the environment is an important key in our understanding of their biogeochemical cycling and the pollution pathways. Cadmium species are among the most toxic and harmful environmental contaminants, so tracing its sources is a very important and useful tool. In this study, soil samples were collected from the heavily industrialized region of Ostrava, Upper Silesia, Czech Republic. Matrix separation methodology was optimized, in order to separate pure cadmium fraction from the soil samples for further isotopic analysis. Concentration of selected metal(loid)s was determined to evaluate the level of contamination in the Ostrava region. Finally matrix separation efficiency was determined to evaluate if it is a suitable method for Cd separation from environmental samples prior the isotopic analyses. The Cd separation was proved to be an efficient and suitable method for further isotopic analysis of soil samples, the separation efficiency was usually over 95%.

Keywords: Cadmium, isotope fractionation, matrix separation, environmental pollution

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1. Introduction

Soils may be contaminated by the accumulation of metals and metalloids through emissions from the rapidly expanding industrial areas, mine tailings, disposal of high metal wastes and paints, land application of fertilizers, animal manures, sewage sludge, pesticides, wastewater irrigation, coal combustion residues, spillage of petrochemicals, and atmospheric deposition (Khan et al., 2008). Soils are the major sink for metals released into the environment by aforementioned anthropogenic activities and unlike organic contaminants, which are oxidized to carbon oxide by microbial action, most metals do not undergo microbial or chemical degradation (Kirpichtchikova et al., 2006), and their total concentration in soils persists for a long time after their introduction. To identify the metal source metal isotope analysis has been recently successfully applied, based on the principal that the metal isotope signature in the environment corresponds to its source(s) (Wiederhold et al., 2015). Some useful applications of Cd isotopes have been identified in addition to those pertaining to extraterrestrial materials. For example, it has been found that industrially-processed cadmium is highly isotopically fractionated compared to “natural” terrestrial cadmium. (Schmitt et al., 2009). Under such circumstances, the mass dependent fractionation of Cd is an interesting new tracer for anthropogenic cadmium, and as such can be used to monitor heavy metal pollution and cycling of Cd in the environment (Cloquet et al., 2005). Therefore the main aim of this study is to evaluate the applicability of Cd isotopes for tracing Cd contamination in soil from Ostrava region (Czech Republic), a region that is considered to one the most polluted places in Europe. The purpose of this thesis is to develop an efficient methodology of matrix separation from the analyte (Cd) in order to obtain a pure Cd fraction for the isotope analysis selected from the most polluted areas in Ostrava region, and its implications for tracing the true sources of Cd. All these data could prove valuable and to provide insight into cadmium implications in order to develop innovative technics and methods appropriate for green and affordable remedial options.

Objective of thesis

The main objective of the thesis is to optimize sample matrix separation methodology which must be performed prior Cd isotope analysis of environmental samples. The aim of the separation is to obtain a pure Cd fraction which contains more than 90% of Cd originally presented in a sample before the separation. All the other elements must be separated completely from the sample. The aim is to develop methodology which can be applied for different environmental samples and especially soil.

Methodology

The environmental samples (soil etc.) will be collected at 9 sampling sites in Ostrava region. The samples will be decomposed in a mixture of acids and analyzed for the metal concentration by ICP-MS. Depending on Cd concentration a specific portion of each decomposed sample will be taken on a chromatographic columns equipped with a specific exchange resin. The separation efficiency must be over 90%. The separated pure Cd fraction will be analyzed for Cd isotope composition by TIMS spectrometer.

2.Literature Review

2.1 Industrial Pollution

Environment has been polluted by industry for many decades, industries have been discarding their waste products into the soil and water while emissions and discharges from all these processes are been spread out into the atmosphere potentially harming and contaminating. Disposal of industrial wastes into landfills is among the artificial aspects contributing towards soil pollution. Industrial wastes contain varied amounts of toxic materials and chemicals, which can accumulate in the top soil thereby depreciating the fertility and biological activity of the soil due to soil poisoning. It is also important to indicate that industrial waste and emissions are the largest contributors to the global pollution problem.

Mining and smelting often result into a widespread contamination of the surrounding environment by metals and metalloids. Metals and metalloids such as As, Cd, Cr, Cu, Hg, Ni, Pb etc. at high concentration are the reason for serious concern due to their accumulation in food chains. They can be released into the environment by industrial activity at a rate far higher than by natural processes. Another dangerous characteristic of these metals is their ability to bioaccumulate. This means an increase in the concentration in a biological organism over time, compared to their concentration in the environment. Trace elements cannot be degraded like organic pollutants and they may transform and become stable and persistent contaminants that accumulate in soil. (Han & Fengxiang X. 2007). Once they reach soil, are adsorbed by initial fast reactions (minutes, hours), followed by slow adsorption reactions (days, years) and are, therefore, redistributed into different chemical forms with varying bioavailability, mobility, and toxicity (Shiowatana et al., 2001). This distribution is believed to be controlled by reactions of heavy metals in soils such as mineral precipitation and dissolution, ion exchange, adsorption, and desorption, aqueous complexation, biological immobilization and mobilization, and plant uptake (Levy et al., 1992).

Because most of the practices to monitor effects of metallurgical industry use concentration data alone, evaluation of the range and source apportionment is often complicated. In this case, the study area is located in the Moravia-Silesian in the

Northeast Czech Republic. The area is considered to be one of the regions with the worst air quality in the EU (Horalek et al., 2007). Anthropogenic pollution in this area mainly results from the heavy steel and coal industries but also from the dense transport infrastructure in the region (Mikuska et al., 2015). Accordingly severe air pollution in Upper Silesia, especially in the vicinity of the city of Ostrava near the Czech-Polish border, is well known. The city of Ostrava is a highly industrial city with production of bituminous coal and metallurgical industry. The city itself has a long history of coal production, which began at the late 18th century, while today, in the 20th century the city highly focused on the development of mining, steel and other heavy industries. (Weissmannová et al., 2015). Black coal mines in the area originate from the 19th century, and three areas currently have a total of 8 active mines and 63 closed mines. The maximum coal production occurred in the 1980s, with a production of approximately $25 \cdot 10^6$ tons of coal (Novak et al., 2003). Additionally, several stationary industrial sources of pollutants (e.g. metals, particulate matter, nitrogen oxides, sulphur dioxide, and benzo(a)pyrene) exist directly in the city or in the close vicinity. That is why the region belongs to the most polluted ones in Europe and is contaminated not only by the metal and coal processing industries but also from the local combustion and traffic situation in the city of Ostrava.

Metal isotopes have been introduced as “fingerprints” of environmental pollution (Cicchella et al., 2008). In general, each metal source has a distinct or sometimes overlapping isotope ratio, but of course this is valid only for multi-isotope systems. The isotope composition of metals in the environment reflects the mixing of their sources, and source apportionment can be quantified in cases where all potential sources are characterized and have specific ratios. Isotopic measurement is superior to using concentrations alone in tracing pollutant sources because isotope ratios are significantly more sensitive tracers than elemental concentrations or ratios (Cheng et al., 2010). Metal isotope studies thus provide a convenient approach for studying and tracing the sources of pollution in different environmental compartments (Doucet et al., 2002). This approach has been widely used in the past for tracing Pb pollution and its sources, especially because the wide availability of analytical techniques necessary for such studies (for example a quadrupole ICP-MS) is usually sufficient. Besides Pb, other isotope systems, such as Cd, Cr and Zn have recently been proposed as tracers of environmental pollution and biogeochemical cycling in pioneer studies (Ettler,

2016). Potential use of Zn and Cd isotopes as tracers has been increasingly explored since Wombacher et al. (2004) and Cloquet et al. (2005) identified that high temperature processes (e.g., evaporation of Cd) cause isotopic fractionation.



Figure 1. Ostrava smog (www.radio.cz/en/section/curraffrs/environment-group-challenges-ministry-moves-to-tackleair-pollution-in-ostrava), František Tichý

2.2 Cadmium (Cd) properties and utilization

Cadmium is a lustrous, silver-white, ductile, very malleable metal, it is not an essential element for living organisms, because it is highly toxic for plants and animals. It is stable at the Earth's surface primarily in the Cd^{2+} Cd (II) oxidation state. Its surface has a bluish tinge and the metal is soft enough to be cut with a knife, but it tarnishes in air. It is soluble in acids but not in alkalis. Cadmium occurs in the earth's crust at an abundance of 0.1–0.5 ppm, namely it occurs at low concentrations in the lithosphere, averaging 0.2 mg/kg, 0.53 mg/kg in surface soils, and 0.66 mg/kg as dry weight in the plant foodstuffs. It is commonly associated with zinc, lead, and copper ores. It is also a natural constituent of ocean water with average levels between 5 and 110 ng/L with higher levels reported near coastal areas and in marine phosphates and phosphorites (Morrow et al., 2001). It is similar in many respects to zinc but it forms more complex compounds. Cadmium occurs in most terrestrial materials, it is a major constituent of the minerals greenockite and otavite, but commercially more important is its presence as a minor element, in sphalerite. It can also be found as an impurity in Pb ores, such

as galena (U.S. Geological Survey, 2013). The majority of Cd is used to produce the metallic Cd electrode plate found in nickel–cadmium (Ni–Cd) batteries, significant Cd is also used in pigments, as plastic stabilizers and coatings (US.Solar Society Institute). Cadmium has been also used particularly to electroplate steel, where a film of cadmium only 0.05 mm thick will provide complete protection against corrosion. Furthermore it has the ability to absorb neutrons, so it is used as a barrier to control nuclear fission in power plants.

The largest atmospheric sources of cadmium are, metal smelting, trash incineration, primarily the burning of nickel–cadmium batteries, which can be deposited as solid waste and fossil-fuel combustion such as coal or oil (Bhanarkar et al., 2005). Natural emissions to the environment can result from volcanic eruptions, forest fires, generation of sea salt aerosols, or other natural phenomena (Environment Protection Agency); (Shevchenko et al., 2003). Cadmium may also escape into the environment from iron and steel production facilities because it is used mainly for many industrial processes as mentioned above. The fate of cadmium in air is through transport and deposition, because particulate matter can travel long distances in the atmosphere and then deposit wet or dry onto surface soils and water, which can result in elevated cadmium levels even in remote locations (Shevchenko et al. 2003).

The main sources of cadmium include atmospheric deposition and direct application methods such as phosphate fertilizers which were used widely in the past decades and sewage sludge disposal. Some phosphate fertilizers could contain up to 300 mg Cd/kg (Alloway et al., 2012). The application of agricultural inputs such as fertilizers, pesticides, and biosolids (sewage sludge), the disposal of industrial wastes or the deposition of atmospheric contaminants increases the total concentration of Cd in soils, and the bioavailability of this Cd determines whether plant Cd uptake occurs to a significant degree (Wegglar et al., 2004). Cadmium is very biopersistent but has few toxicological properties and, once absorbed by an organism, remains resident for many years.

Cadmium mobility in soil depends on several factors including the pH of the soil and the availability of organic matter. Generally, cadmium will bind strongly to organic matter and this will, for the most part, immobilize cadmium (Autier et al., 2004) However, immobilized cadmium is available to plant life and can easily enter

the food supply. Low pH favors the accumulation of Cd in soil and tends to be more available at this pH (acidic). (Kirkham et al., 2006)

The International Agency for Research on Cancer (IARC) classifies Cadmium in Group 1. This means that Cd is **carcinogenic** to humans and the US Environmental Protection Agency (EPA) has determined that it is a probable human carcinogen by inhalation. Epidemiological data from occupational settings confirm lungs being the primary target organ while cadmium is not considered a carcinogen by ingestion. The critical health effect of ingested cadmium is on the kidneys where it damages the blood filtration system, which results in proteins being excreted in urine. The severity of the effect depends on duration and magnitude of exposure. Cadmium exposure in the workplace takes place during mining and work with cadmium containing ores. Additional occupational exposure may occur during manufacture of products containing cadmium such as paints and during work such as plating, soldering, and welding (National Institute of Occupational Safety and Health, 1990). Skeletal damage is another critical effect of long-term exposure to Cd at levels somewhat higher than those for which kidney problems occur. Cadmium is mainly stored in the liver, kidneys and bones. Excretion is slow, with a very long half-life (decades) in the human body. Cadmium concentrations in most tissues increase with age.

Below are mentioned several different ways of human exposure to cadmium:

- Food accounts for approximately 90% in the general, for non-smoking population.
- Less than 10% of the total exposures among general populations occur due to inhalation of low levels of cadmium in ambient air and through drinking water.
- Smoking is an important source of cadmium exposure by inhalation, both for smokers and, through second-hand smoke, for non-smokers.

The level of dietary exposure can exceed the guidelines set by the United Nations Food and Agriculture Organization (FAO) and the World Health Organization (WHO). According to available data, the average weekly intake of cadmium from food in most countries is within the range of 0.7–2.8 $\mu\text{g}/\text{kg}$ body weight per week, which is below the provisional tolerable weekly intake (PTWI) of 7 $\mu\text{g}/\text{kg}$ body weight per week. In

some groups, like smokers and some vulnerable groups like people with kidney problems, their intake can reach or be above the tolerable limit.

2.3 Biological cycling and isotope fractionation of metals in the environment

Many metals are involved in biological cycling. Identifying isotopic imprints of biogeochemical processes in environmental samples (“biosignatures”) has been a major motivation of metal isotope research from the beginning (Bigeleisen, et al., 1996). Most of the biological processes are governed by the same physical and chemical principles as abiotic processes. Although many biologically controlled processes can induce significant metal isotope fractionation, the effect is usually linked to one of the processes above like redox change, complexation, sorption or diffusion. Many biological processes are kinetically controlled for example bond-breaking in enzymatic reactions and may thus induce kinetic isotope effects (Von Blanckenburg et al., 2009). Whether such effects are preserved in signatures of natural samples depends, similarly as for abiotic processes, strongly on pool sizes and the fraction reacted during a transformation process (Wiederhold et al., 2015).

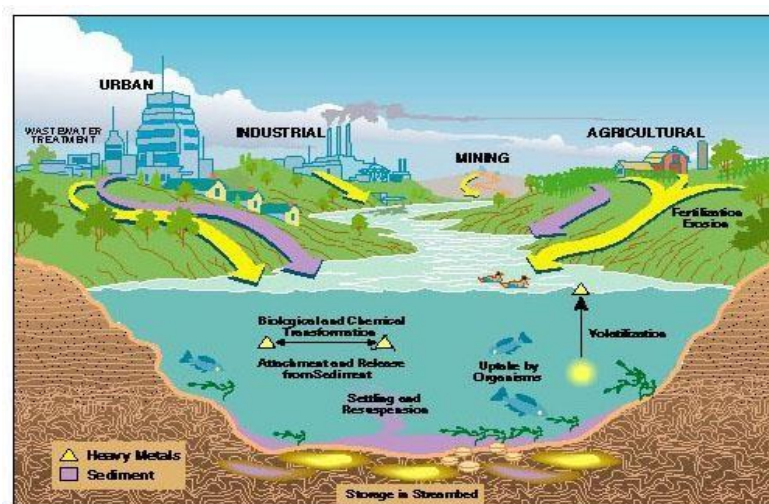


Figure 2. Heavy Metal Pathways (adapted from 1995 U.S. Geological Survey Circular)

Cadmium has eight stable isotopes with atomic masses of 106, 108, 110, 111, 112, 113, 114 and 116. These isotope abundances are 1.25%, 0.89%, 12.5%, 12.8%, 24.1%,

12.2%, 28.7%, and 7.49%, (Böhlke et al., 2005) respectively, which mean that this is the average amount of cadmium isotopes occurring naturally on Earth. The interest in the isotopic composition of cadmium (Cd) occurring in natural/geological materials has increased significantly in recent years. The Cd isotope abundance variations of δ ($^{114}\text{Cd}/^{110}\text{Cd}$) observed range from -13.4‰ up to 24.8‰ (Wombacher et al., 2003). In terrestrial samples the observed isotope abundance variation for δ ($^{114}\text{Cd}/^{110}\text{Cd}$) amounts from -3.6‰ to 3.4‰ . The determination of the δ values requires a standard material that can be used as a reference for all measurements. (Pritzkow et al., 2006). Ideally, an isotope reference material should be used that is well characterized in terms of its isotope abundance. The abundance of Cd is usually at a level measurable in ppb in rocks and meteorites. Cadmium has lower boiling point ($\sim 760^\circ\text{C}$) than Zn, and like Zn, Cd in ores likely evaporates during smelting, favoring escape of light Cd isotopes in the exhaust, as recently demonstrated (Cloquet et al., 2006). Cadmium behaves like Zn during electroplating, potentially resulting in a large pool of isotopically light Cd in the environment (Environmental Protection Agency, 2014).

Stable isotope fractionation causes a shift in the isotope ratio between reactant and product of a reaction, however this change is mostly very small, and the isotopic mass balance of the overall system remains unchanged. The biochemical cycling of metals in natural systems is often accompanied by stable isotope fractionation, which can now be measured due to recent analytical advances. More specifically stable isotope fractionation in natural samples can be divided into *kinetic* and *equilibrium* effects.

Kinetic isotope effects are caused by different reaction rates, of lighter and heavier isotopes and are only preserved in incomplete processes. Such processes include, for example, evaporation, diffusion or biological processes in which the enzymatically mediated breaking of bonds favours those bonds involving light isotopes due to their higher zero-point energies. The influence of kinetic effects on the sample isotopic composition depends strongly on the relative extent of reaction progress (Xiao et al., 2013). The largest effects are observed in remaining reactants which can be strongly enriched in heavy isotopes due to the continuous preferential removal of light isotopes, especially when reactions have proceeded far toward completion. Obviously, if reactants are completely transformed into products, the imprint of kinetic isotope

effects during the process is erased and isotope ratios of products are identical to initial reactants.

A Rayleigh fractionation model is often used to describe the evolution of isotope ratios during incomplete unidirectional reactions within closed systems. The Rayleigh equation applies to an open system from which material is removed continuously under condition of a constant fractionation factor. However, such processes can proceed under different boundary conditions, even when the fractionation factors are the same. One such system is the so-called "closed" system or 2-phase equilibrium model, where the material removed from one reservoir accumulates in a second reservoir in such a manner that isotopic equilibrium is maintained throughout the process (Carol Kendall and Eric A. Caldwell 1998). This concept does not only apply to kinetically controlled systems with negligible backwards reactions rates, but to all systems in which the product is physically removed or otherwise prevented from isotopically interacting with the reactant (Wienderhold et al., 2015).

Equilibrium isotope effects occur when two phases react with forward and backward reactions proceeding at equal rates. In this case, the relative isotopic abundance is controlled by energy differences in bonding environments of reaction partners which have reached isotopic equilibrium. However the time scales required to reach isotopic equilibrium can differ from those required to obtain concentration equilibrium (Meija et al., 2012). At isotopic equilibrium heavy isotopes are enriched in "stronger bonding environments" thermodynamically explained by lower zero point energies of bonds in molecules with heavy isotopes compared to bonds with light isotopes of the same element. (Xiao et al., 2013)

2.4 Natural Variations of isotopic abundances

The isotopes of any element participate in the same chemical reactions, rates of reaction and transport, however, they depend on nuclidic mass, and isotopic substitutions subtly affect the partitioning of energy within molecules. These deviations from perfect chemical equivalence are termed **isotope effects**. As a result of such effects, the natural abundances of the stable isotopes of practically all elements involved in low-temperature geochemical (< 200°C) and biological processes are not precisely constant. Stable isotope data are expressed as delta values (δ) by normalizing

isotope ratios in samples to the ratio of a standard material. Because the resulting values are usually very small, delta values are expressed in parts per thousand, by multiplying with 1000 and adding the per mil sign (‰). However, this factor should not be part of the delta value definition according to recent IUPAC recommendations (Ryu et al., 2014).

2.5 The delta notation

Because the interesting isotopic differences between natural samples usually occur at and beyond the third significant figure of the isotope ratio, it has become conventional to express isotopic abundances using a differential notation. To provide a concrete example, it is far easier to say and to remember, that the isotope ratios of samples A and B differ by one part per thousand. The notation that provides this advantage is indicated in general form below this means of describing isotopic abundances was first used by Urey (1948) in an address to the American Association for the Advancement of Science, and first formally defined by McKinney *et al.* (1950).

$$\delta^A_{XSTD} = \left(\frac{^A R_{\text{sample}}}{^A R_{\text{STD}}} \right) - 1$$

Equation 1. General form for calculation of delta notation

Where δ expresses the relative deviation from a standard composition expressed in parts per thousand (‰). R stands for a specific isotope ratio of the element studied, while the determination of the δ values requires a standard material that can be used as a reference for all measurements. Ideally, an isotope reference material should be used that is well characterized in terms of its isotope abundance (Pritzkow et al., 2006).

All Cd isotopic results are reported as notation as already suggested Wombacher and Rehkamper, 2004, which is the deviation of the Cd isotope composition of a sample relative to Cd standard.

$$\delta_{114/110} \text{ Cd} = \left[\frac{(114\text{Cd}/110\text{Cd})_{\text{sample}}}{(114\text{Cd}/110\text{Cd})_{\text{standard}}} - 1 \right] * 1000$$

Equation 2. Delta notation calculated for Cadmium Isotopes

The principle of mixing two pools or reservoirs is illustrated below (fig. 3.) visualizing the influence of pool sizes and isotope signatures on the resulting mixture. For example, if the geogenic background of a soil has an isotopic composition of +0.5 ‰ relative to the reference standard and the soil has been polluted by an anthropogenic source with a delta value of -1.5 ‰, the difference in delta value of -1 would indicate an anthropogenic contribution of 75% (Johnson et al., 2012). Within that range, differences as small as 0.00001(-) can provide information about the source of the cadmium and about processes in which the cadmium has participated. (Hayes et al., 2004)

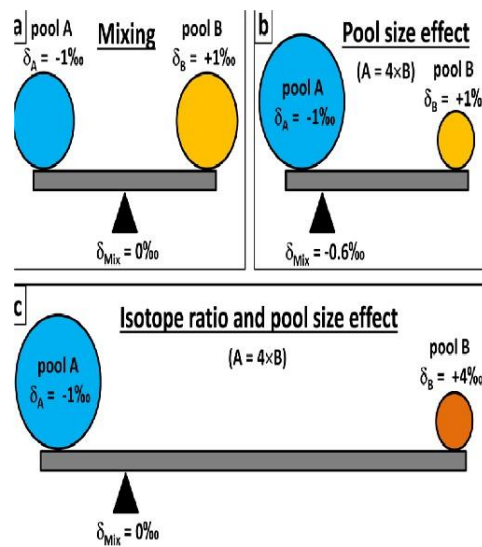


Figure 3. Schematic illustration of the principles of mixing models used for source tracing with metal stable isotope signatures. Panel a depicts the mass balance between two metal pools of opposite isotope signatures and equal size. The effect of different pool sizes (pool A = 4 × pool B) is shown in panel b, and the combined effect of different pool sizes and isotope signatures is illustrated in panel c. (Johnson et al., 2012)

2.6 Cadmium (Cd) isotope fractionation

The evaporation and condensation of refractory and moderately volatile elements in the solar system generally occurs at temperatures that are inferred to be too high to generate measurable isotope fractionations between equilibrated vapour and condensates. On the other hand, significant kinetic and equilibrium isotope fractionation has been observed for redox sensitive metals and for metals that are not redox sensitive in nature such as Cd and Zn. Because Cd in many forms is mobile in the environment identifying its sources is a crucial aspect for remedial intervention (Chrastny et al., 2015). Cadmium release to the environment is related with its use and the industrial processes of its transformation, because is used widely from recharging batteries to metal coatings in aerospace industry. As a consequence from all these processes, it is discharged into the environment and possibly poses risk for human and animal health because its dry and wet deposition, results in pollution of adjacent areas (U.S. Geological Survey, 2013). In addition to that many more human induced activities (smelting, refining, urban waste or burning of coal) may involve volatilization and that leads to mass dependent Cd isotope fractionation. (Cloquet et al., 2006). For all these reasons it is understandable how significant is to utilize cadmium stable isotopes in order to determine the true origins of contamination.

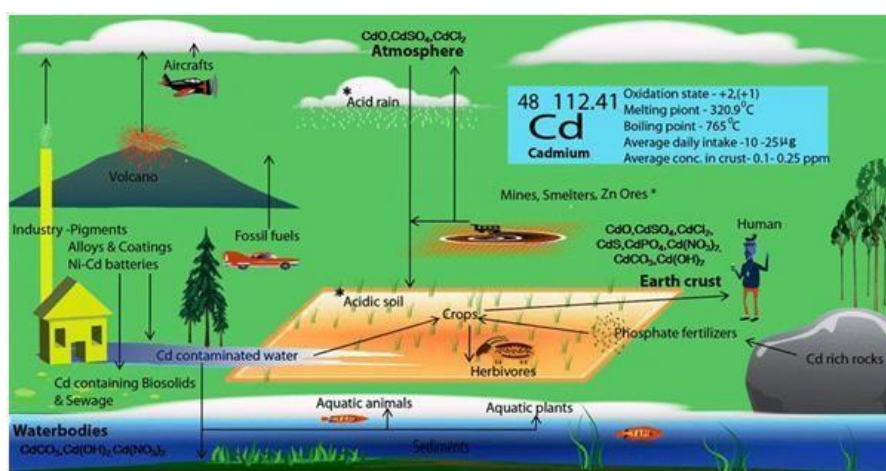


Figure 4. Biogeochemical Cycle of Cadmium (Image Source: by Abin Sebastian, Majeti Narasimha, Vara Prasad)

2.7 Cd isotope industrial fractionation

The principal of industrial fractionation is related with many and different processes occurring in the industry field. Smelters, pigments, plastic stabilizers, along with other human induced activities, burning of coal, urban waste, refining and metal coating, where is involved volatilization that leads to mass dependent metal isotope fractionation which is increased by other physical or chemical reactions. Metal isotope signatures can be used in different ways to deduce information about composition and history of environmental samples while the most important applications are **source** and **process** tracing.

Source Tracing is based on the mixing of reservoirs with different isotope signatures. If the isotopic compositions of the involved endmembers are known and sufficiently distinct, contributions of different source materials in a sample can be quantified by mixing calculations. This can be an alternative, cost-effective approach in order to identify pollutant sources by using bio-indicators, which are biological processes or species used to assess environmental quality. (Holt et al., 2011)

In addition to that, moss and lichens are the most commonly used bio-indicators of atmospheric pollution. They are well-suited to this role because they lack roots and are non-vascular, depending fully on the atmosphere for nutrients and water. Furthermore, they do not have a protective epidermis, so nutrients and pollutants are easily absorbed into a moss or lichen's tissue. The first work using moss as a bio-indicator of atmospheric heavy metals was done by Ruhling and Tyler (1968) in Sweden. The utilization of mosses as bio-indicators of atmospheric heavy metals, note that moss concentrations of cadmium and lead are more consistently correlated with measurements of atmospheric deposition than concentrations of other metals. The correspondence of instrumentally measured heavy metal concentrations and those in moss suggest that moss could be used to complement existing networks of air-quality monitors (Donovan et al., 2016).

Process Tracing is based on the concept that a sample has been affected by a transformation process causing a shift in the isotope signature. An example is the

partial transformation of soluble and insoluble elemental species involving a separation of aqueous and solid phases (Schoenberg et al., 2008).

2.8 Column chromatography method

Column chromatography method is used for purification of an environmental sample, i.e. separation of Cd fraction and matrix. The matrix represents all the elements and substances presented in the environmental sample, except Cd. The resin used for the Cd separation on chromatography column is called AG1-X8, it is extensively purified to remove both organic and inorganic impurities. The resin is sized to consistently give narrow wet mesh ranges, which provide high resolution and excellent reproducibility. It is available as anion exchanger and it is primarily used for the separation of low molecular weight compounds such as inorganic ions, organic acids, nucleic acids, or carbohydrates. It also has a lower physical resistance to shrinking and swelling, so that it absorbs more water and swells to a larger wet diameter. Resin, AG 1-X8, works in chloride form and is used for sorption, exchange, and separation of low molecular weight inorganic anions, and in applications such as cyclic nucleotide assays and fractionation of organic acids.



Figure 5. AG1-X8 anion Exchange resin (100–200 mesh)

3.Methodology

3.1 Study area and Sampling sites

The study area is located in the Moravia Silesian Region in the Northeast Czech Republic. The city of Ostrava is known for its long mining and smelting history. The landscape forms a valley that is known as the Moravian Gate, which leads from the southwest to the northeast and into the Silesian region of Poland. Air typically flows through the valley, predominantly from the southwest. Anthropogenic pollution in this area mainly results from the heavy steel and coal industries but also from the dense transport infrastructure in the region (Mikuska et al., 2015).

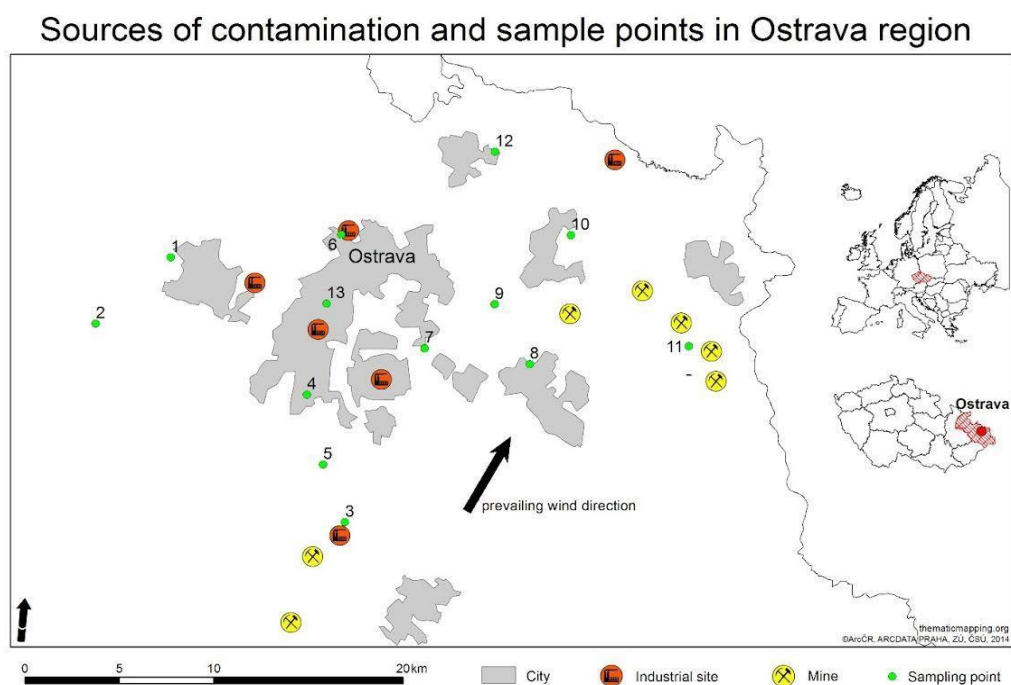


Figure 6. Sampling points and sources of contamination in Ostrava region (Francova et al., 2016)

3.2 Soil Sampling

Sampling was carried out in August 2016, and soil samples were collected in Ostrava region. For our study, soil samples came for the sampling sites 1, 6, 7, 9, 10 and 13. The sites were chosen according to the predominant wind direction (northeast) and positioned with respect to the main industries in the area. In the figure 6 we can observe all the sampling points, industrial sites and mines in the Ostrava region while in figure 7, we can see the sampling sites according to the city's infrastructure. Soil profiles were sampled in a 1 x 1-m-wide pit and sectioned into 3 or 4 parts according to the natural development and visible changes within the profile down to the mineral horizon. The top surface composed of fresh litter or grass cover was removed. The sampling points were chosen in order to include both polluted and non-polluted areas so as to achieve the optimum result for our analysis.

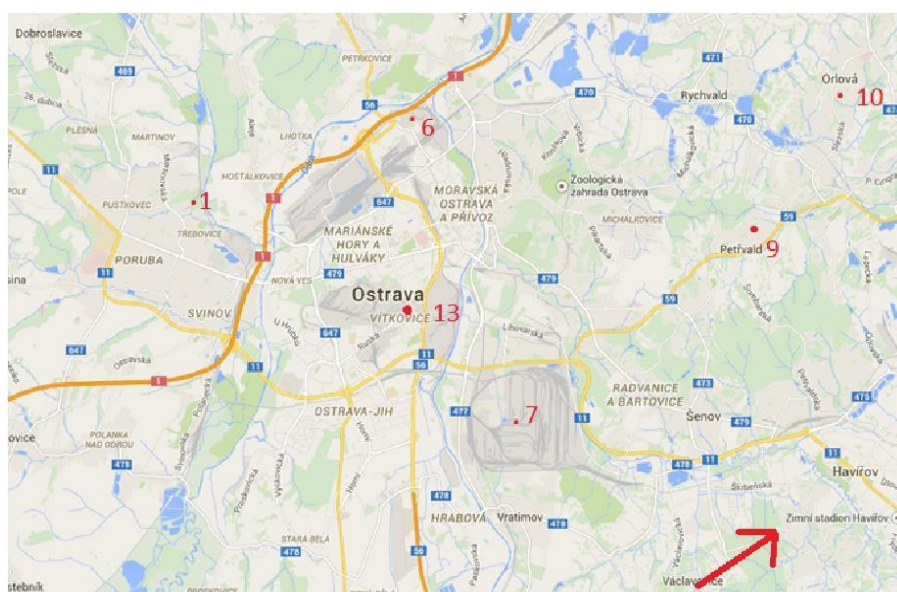


Figure 7. City of Ostrava and Sampling points and areas (googlemaps.com)

More specifically for our sampling points, site 1 was one of the less polluted sites, although there is a black coal, power plant nearby in Poruba Area. Main sources of pollution there also derive from the surrounding households and from local

combustion uses. Sampling site 6, is considered to be the most polluted, it is located inside the industrial area near a high energy coal plant and in the surrounding area (Privoz) there is also an old coal factory. Site 7 is considered to be in the main polluted area of the region mostly from lead while, one of the biggest metal smelters called Arcelor Mittal Ostrava a.s is located there. Sampling site 9 is positioned in a non-industrial area (Petrvald), although the prevailing northeast wind maybe has an important contribution towards pollution of the locale. Site 10 is located in the area called Orlova with 3 active mines around in the general surrounding. Lastly, is sampling site 13 which is located in the city centre of Ostrava, the area Hulvaky-Vitkovice. This site has 3 active industries around it (Vítkovice Power Engineering a.s., Evraz Vítkovice Steel a.s., Vítkovice Mechanika a.s.) and is considered polluted not only by industries but also by the dense traffic of the city.

3.3 Preparation and Decomposition of soil samples

Soil samples were air dried and homogenized (2 mm sieved). The fine fraction of soil was used for the analysis. For the soil decomposition a standard method **EPA-3051A** was used. This method is appropriate for soil, sediments, sludge and oils. Approximately 250 mg of soil was weighed (fig. 8.) into a special Teflon vessel and consequently 9ml of nitric acid (69%) HNO_3 , 3ml of hydrochloric acid HCl and finally 1ml of hydrofluoric acid HF were added.



Figure 8. Laboratory Scale



Figure 9. Acids used and Oven vessel

In the microwave (fig. 10), the samples undergo 3 different stages, firstly the temperature is rising up to 170° C, then remain in this temperature for 10 minutes and eventually the temperature drops to 70° C and remains until the end of the procedure.



Figure 10. Microwave oven used for Soil decomposition

Finally the samples are collected and transferred into savillex Teflon vessels and let to dry up onto a hot plate (fig. 11).

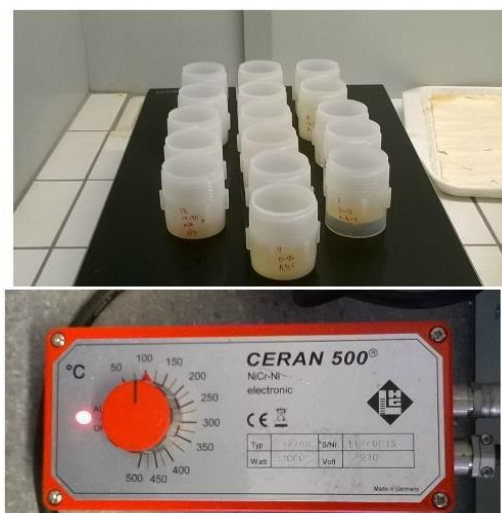


Figure 11. Hot-plate (Ceran 500)

Consequently, the addition of 20ml demineralized water, the samples are gathered, filtered with a syringe and transferred into different containers in order to store them for the following separation procedure.

3.4 Chemical Solutions

Chemicals used in this study were ultra-clean, ultra-pure acids for isotope analysis, concentration of impurities in these chemicals is <10 ppt. Ultra-pure water (electrical resistivity 18.2 M Ω .cm, miliQ, USA) was used to prepare all the solutions. The solutions had to be prepared with specific molarity and volume. The HNO₃-HBr mixtures that are required for the anion-exchange chemistry were prepared freshly on the day of use, because both acids slowly react with one another. To avoid interferences and matrix effects, chemical separation of Cd from the sample matrix is a prerequisite for precise isotope ratio measurements. Separation of Cd from the elements Pd, In, and Sn is particularly important, because these elements can generate direct isobaric interferences.

3.5 Matrix Separation

In order to achieve the separation the resin (AG 1-X8) must be cleaned and activated. The resin is stored in water, while the amount added into the columns must be at least 1.5ml. Consequent is the cleaning where ultra-pure water is added, two times into the columns until it passes through, then 2% nitric acid in two doses and in the end ultrapure water again. Afterwards, the cleaning of the resin continues by adding 8M

HCl in the column and wait until it passes through, subsequently 0.5M HCl is added. Finally, follows the activation of the resin, for that a solution of 3M HCl is added into the columns.

Eluent	Volume (ml)	Eluted
2 mL Biorad AG 1-X8 anion exchange resin (100–200 mesh)		
2 M HNO ₃ (resin cleaning)	10	
8 M HCl (wash, conversion to Cl ₋ form)	20	
0.5 M HCl (wash, conversion to Cl ₋ form)	10	
3 M HCl (resin conditioning)	10	
3 M HCl (sample solution)	10-12	matrix
0.5 M HCl	30	matrix
1 M HCl	10	matrix
2 M HCl	10	
8 M HCl	12	Ag
0.5 M HNO ₃ -0.1 M HBr	2,5	
0.5 M HNO ₃ -0.1 M HBr	10	Zn
2 M HNO ₃	2.5	
2 M HNO ₃	8	Cd

Table 1. Table with the chemicals and volumes. Elution sequence of the anion-exchange and the resin chemistry for the separation of Cd.

The methodology was adapted from Wombacher et al. (2003). A two-stage column chemistry procedure was used in this study for the separation of Cd from geological matrices. In the first stage, anion-exchange chromatography serves to separate Cd from most matrix elements. The procedure largely follows published Cd separation protocols (Rosman and De Laeter, 1974; Loss et al., 1990), but an HNO₃-HBr mixture

was used for the elution of Zn (Strelow, 1978). In our method we did not use the second step as in Wombacher because eventually tin (Sn) was not a problem for interferences. Next step is to add the decomposed samples in the columns in order to obtain the pure Cd fraction. In this case we analysed 3-6 soil samples and we mixed them all together in order to get the preferable amount of Cd because as discussed the quantities in the samples were small. After the chemical separation the Cd fraction was dried down and added concentrated HNO_3 to remove all Cl from the sample solutions.

There were some critical points in the procedure of the column separation that needed attention. In the evaporated samples were added a 3M HCl solution and the samples were divided it into two or three duplicates. The samples are loaded into the columns and the following step is the part of the separation. The steps of the separation include 3 doses of 0.5M HCl solution, one dose of 1M HCl and after that, 2M HCl solution, followed by an 8M HCl solution. It is very important for the acids to be added slowly so we do not stir the resin. Finally a freshly made HNO_3 -HBr mixture which is prepared that day is added, in small doses so the reaction time lasts longer, Once this procedure is finished the matrixes are collected and analysed in order to identify all the elements in the samples apart from the cadmium which remained in the column. The final step of the separation is to collect the pure Cd fraction. To do that, a 2M HNO_3 solution is added into the columns in 4 doses in order for the reaction to last longer and the final product which passes through the column is the pure cadmium fraction.

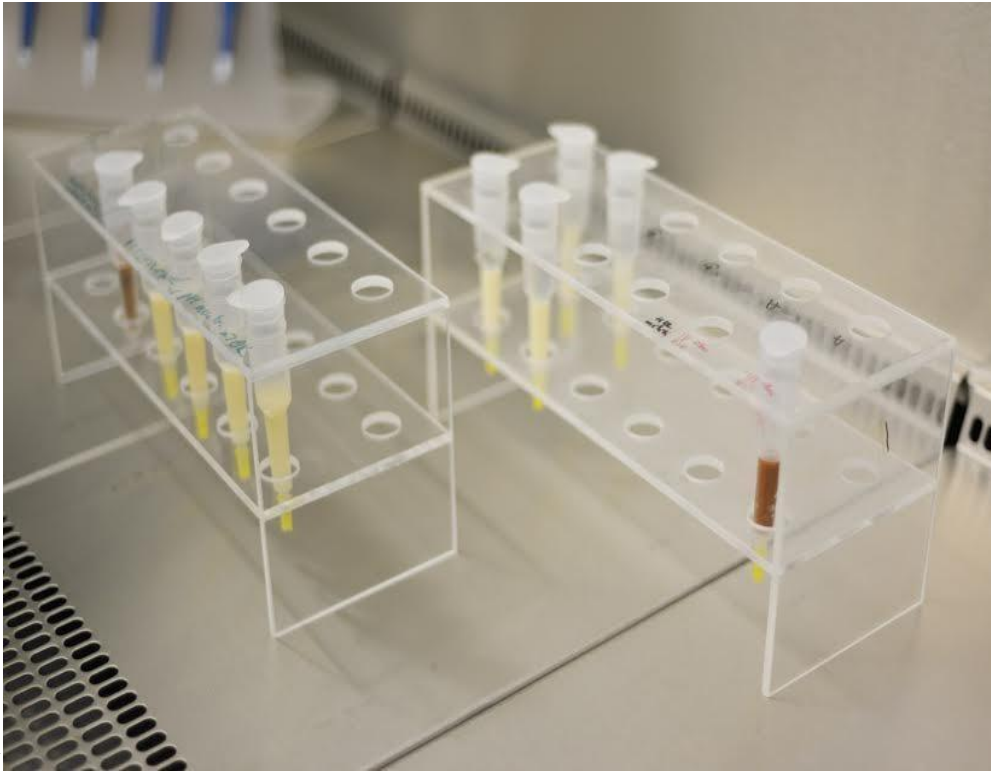


Figure 12. Column Separation

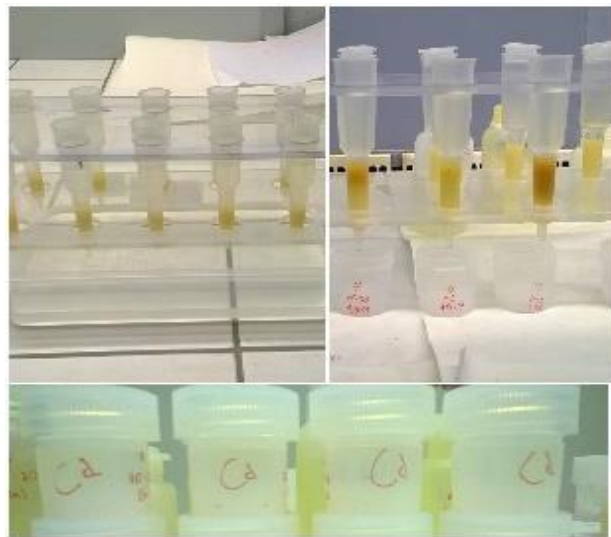


Figure 13. Column Separation-Cadmium Fractions

3.6 Matrix Separation Efficiency

Matrix separation efficiency was calculated for cadmium as well as some of the other selected elements (Ca, Na, Al, K, Fe, Zn) presented in high concentration in the environmental samples. The aim of this procedure was to identify the actual efficiency in percentage (%) of the column separation because in order to be successful, the remaining Cd fraction must be over 95% while the removal of the selected elements, must be over 90%. Some elements can cause interferences or can complicate Cd ionization during the isotope analysis so the percentage must be near 100% which means to complete separation of these elements from the matrixes.

3.7 Aliquot calculations

For the Cd isotope analysis 1 μg of Cd in each sample is required. To calculate the actual amounts of Cd in the soil samples recalculation from $\mu\text{g/L}$ to $\mu\text{g}/20\text{ ml}$ must be carry out. Most samples contained very low concentration of Cd, so replicates needed to be decomposed in order to have at least 1 μg of Cd in each sample. After the digestion of the soil samples we added 20ml demineralized water, while after the column separation we evaporated the samples and added 5 ml 2% HNO_3 . So the exact amount of concentration in our samples was in $\mu\text{g/L}$ and we were able to calculate the exact quantities in μg or mg/kg contained in the samples regarding what we needed for analysis. Below there is an example of our calculation with the replicates of site 1 and depth (0-10cm):

Sampling Site	Cd_B (µg/L)	Cd_A µg
1 (0-2cm)		
A	29.8	0.596
B	34.4	0.688
C	36.3	0.726

Table 2. Recalculation of Cadmium quantities

$$\text{Cd}_A = (\text{Cd}_B * 20 \text{ ml}) / 1000\text{ml} = (29.811 * 20) / 1000 = 0.5962 \text{ µg}$$

Equation 3. Example of Aliquot Calculation

The same procedure was used for all the replicates of cadmium and all the different selected elements existing in our soil samples.

4.Results

4.1 Concentration of Cd and other selected elements in the soil samples

The concentrations of selected metals and metalloids were determined using an inductively couple plasma mass spectrometry ICP-MS (iCAP Q, Thermo Fisher, Germany) (fig. 13).

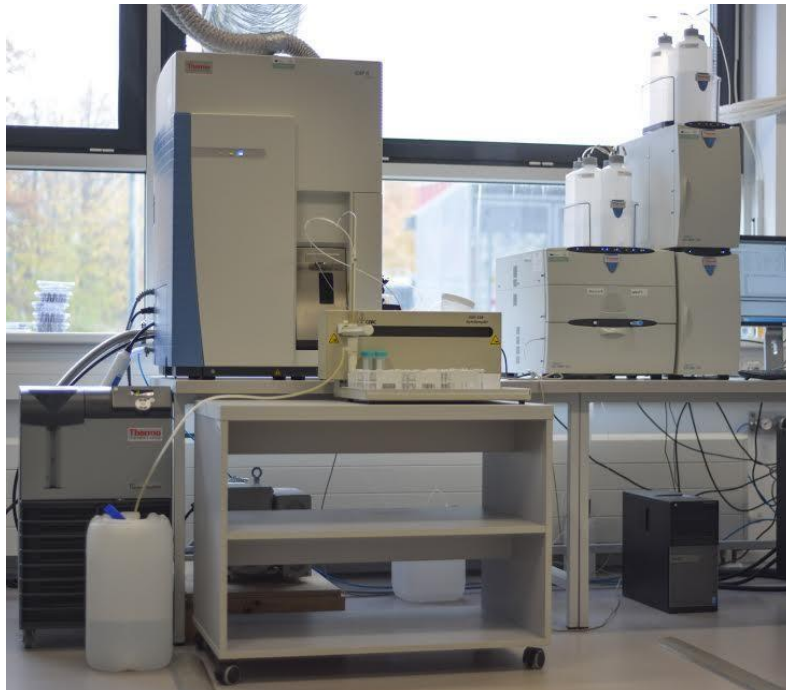


Figure 13. ICP-MS

The collected matrixes and cadmium fractions after the separation were analysed in order to determine concentration of Cd, Mg, Ca, Cr, Mn, Fe, Co, Ni, Zn, Cu, As, Pb, Na, Al, K. Below, presented in the Table 3, there are the average concentration of cadmium in *mg/kg* along with the standard deviation that obtained from the 6 different contaminated sites in Ostrava region.

Sampling sites	Cd Average concentration(mg/kg)	STDEV(+/-)
1 (0-2cm)	2.69	0.24
1 (2-10cm)	0.57	0.17
1 (10-20cm)	3.56	0.64
1 (20-Xcm)	3.70	0.37
6 (0-16cm)	6.04	0.21
6 (16-Xcm)	2.43	0.16
7 (0-15cm)	1.46	0.09
7 (15-30cm)	1.26	0.04
7 (30-x cm)	3.60	0.49
9 (0-15 cm)	8.00	0.75
9 (15-35cm)	0.57	0.12
9 (35-x cm)	2.94	0.04
10 (0-2cm)	0.88	0.03
10 (2-Xcm)	0.38	0.31
13 (0-10cm)	2.03	0.15
13 (10-20 cm)	2.06	0.15
13 (20-x cm)	3.79	1.01

Table 3. Average Cd concentration (mg/kg) and standard deviation in the studied sites

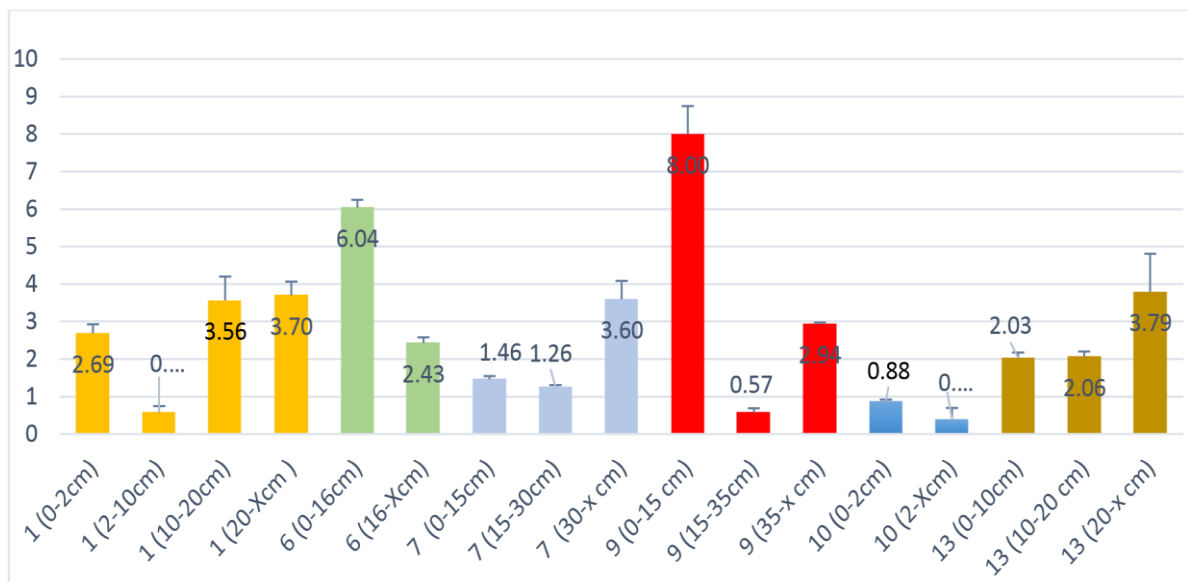


Figure 15. Average concentration and Standard deviation of cadmium (mg/kg) in the studied site

The average concentration of cadmium in all samples was determined and presented above in the diagram (fig 14). Cd concentration in the soil samples ranges between 0.38 and 8.0 mg/kg. Significant differences in Cd concentration existed across the 6 different sampling points. Site 9 and site 6, exhibit the highest Cd concentration, both in the upper soil layer. Site 6 is located in the industrial area of the city and the highest value is 6.04 mg/kg observed in the topsoil (0-16 cm) while in the deeper layer (16-x cm) the value is 2.43 mg/kg. However the highest concentration of cadmium was determined in site 9. This area, do not have any major industries around it, however the prevailing northeast wind probably influence the deposition of the emissions from the industrial and other adjacent areas of the region. In the top soil the amount of cadmium is 8.00 mg/kg while in the other layers is 0.57 mg/kg (15-35cm) and 2.94 mg/kg, (35-Xcm) pointing out the industrial origin of the contamination.

The two sites that are considered the most polluted of the region site 7 (near Arcelor Mittal) and site 13 (the city centre) exhibit similar trend in the amounts of cadmium. Higher concentrations appear in the deeper layers and lower in the topsoil, 1.46 (0-15cm), 1.26 (15-30cm), 3.6 (30-x cm) mg/kg (site 7) and 2.03 (0-10 cm), 2.06 (10-20 cm), 3.79 (20-x cm) mg/kg (site 13). Samples collected from the less polluted site 1, also presented high values of cadmium in the surface layer as well as the deeper layers, possible result from the coal factory nearby and the combustion of households in the surrounding. The lowest values of cadmium are in site 10, with 0.88 mg/kg (0-2cm) in the top soil and 0.38 mg/kg (2-x cm) in the deeper layer. This site is nearby three active coal mines which may be responsible for these quantities, while the northeast wind may also be responsible for the amounts that are found there. According to Francova et al (2017), the analysis of other environmental samples (lichens, snow, PM₁₀) show some similarities in the Cd concentration at the sites 1, 6, 7, 13. These high concentrations are a clear evidence of the recent contamination in Ostrava and its surroundings.

The amount of cadmium in the soil samples analysed was the lowest of all the elements analysed in all the samples. We could say that the highest values that been observed for cadmium, also match with the highest values that observed for the other special

elements. Sites 6, 7, 9 and 13 exhibit elevated values not only for cadmium but also for the remaining selected elements (Fe, Al, K, Na, Ca, and Zn). Exception is only the site 1 in which cadmium also exhibits high values, although is the site with the lowest quantities of the other elements in the topsoil, while the deeper layers present increased quantities of the selected elements.

Figure 16 summarizes the amount of g/L of the elements analyzed, apart from cadmium in the soil samples, in the different sampling sites and soil profiles.

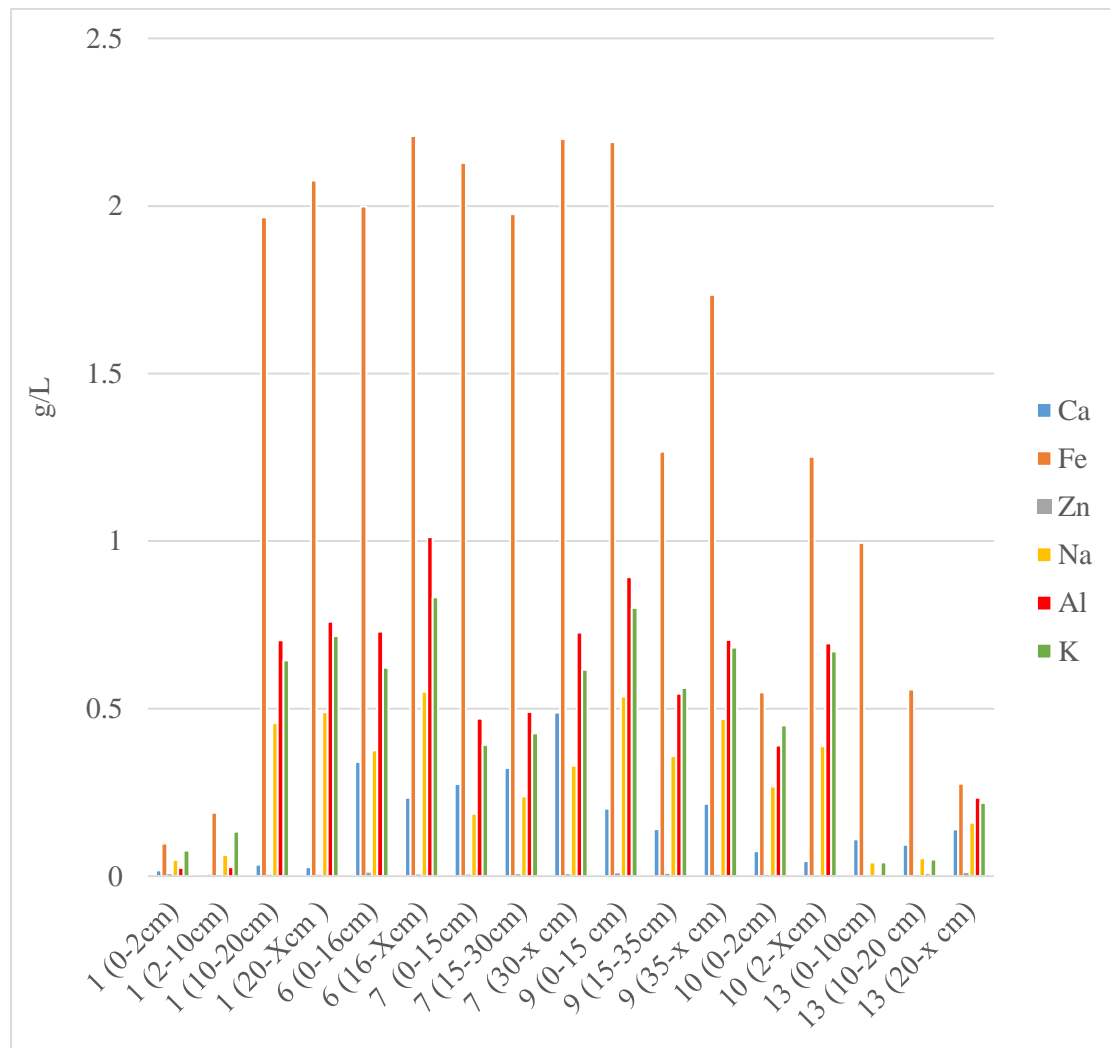


Figure 16. Average concentration (g/L) of selected Elements in different sites

As it can be observed the highest concentration in the samples is the iron (Fe), aluminium (Al) and potassium (K). The average concentrations in the soil samples follow this order: Fe > Al > K > Na > Ca > Zn > Cd. The most elevated quantities of iron, aluminium and potassium are observed in site 6, in the deeper soil layer (16-x cm).

According to Francova et al., where Pb concentrations were calculated, the highest concentrations were observed at site 13 in lichens and snow, at site 7 in winter for PM₁₀ while site 6 presents low concentrations of Pb below 10 µg/L unlike our results where elevated values for our selected elements are observed in that site.

5. Discussion

5.1 Matrix separation efficiency

There were some critical points in the procedure of the column separation that needed attention so as to be efficient. In the evaporated samples were added a 3M HCl solution and the samples were divided it into two or three duplicates. Also in the steps of the separation it was very important to add the needed solutions very slowly so as do not stir the resin in the column. The HNO₃-HBr mixture was prepared daily and added in small doses, very slowly into the columns to achieve the maximum result, while in the final step of the separation where Cd fraction is collected, the 2M HNO₃ solution is added into the columns in 4 doses in order for the reaction to last longer. The efficiency of the matrix separation procedure is summarized in the tables below. Tables shows aliquots (in µg) of the selected elements in the decomposed samples **before (Table 4)** the procedure of matrix separation and **after (Table 5)**.

All sites and elements (μg)	Cd	Na	Al	K	Ca	Fe	Zn
1 (0-2cm)	2.01	2912	1518	4585	1027	5863	375.5
1 (2-10cm)	0.97	3811	1632	7976	403	11330	146.8
1 (10-20cm)	2.98	54809	84457	77282	4124	235918	504.1
1 (20-Xcm)	3.21	58723	91159	85989	3275	249082	471.2
6 (0-16cm)	3.56	30057	58383	49749	27300	159887	868.2
6 (16-Xcm)	2.63	66091	121389	99817	28054	265045	621.2
7 (0-15cm)	2.52	11200	28190	23479	16505	127720	314.7
7 (15-30cm)	2.49	14300	29408	25561	19397	118562	327.3
7 (30-x cm)	2.11	26376	58204	49321	39088	176006	444.2
9 (0-15 cm)	6.41	53638	89280	80106	20157	219082	870.1
9 (15-35cm)	3.07	35861	54475	56191	14049	126736	633.3
9 (35-x cm)	2.53	56324	84669	81833	25886	208153	525.7
10 (0-2cm)	2.51	21377	31195	36000	5937	43904	265.7
10 (2-Xcm)	2.83	54345	97194	93947	6308	175159	282.8
13 (0-10cm)	1.03	1664	161	1647	4401	39787	17.3
13 (10-20 cm)	1.55	4290	632	3979	7490	44569	181.8
13 (20-x cm)	2.86	9597	14030	13151	8399	16583	582.8

Table 4. Concentration (μg) of special Elements before column separation

All sites and elements(μg)	Cd	Na	Al	K	Ca	Fe	Zn
1 (0-2cm)	1.75	0.60	0.08	0.50	0.64	0.26	0.16
1 (2-10cm)	0.89	0.55	0.00	0.43	0.82	0.17	0.17
1 (10-20cm)	2.58	0.53	0.21	2.34	0.72	0.53	0.37
1 (20-Xcm)	3.04	0.23	0.09	0.38	0.83	0.14	0.58
6 (0-16cm)	3.40	0.14	0.07	0.02	0.70	0.17	0.31
6 (16-Xcm)	2.81	0.16	0.12	0.00	0.30	0.13	0.54
7 (0-15cm)	2.12	0.27	0.01	0.00	0.09	0.14	0.27
7 (15-30cm)	1.64	0.10	0.00	0.16	1.03	0.74	0.34
7 (30-x cm)	2.06	0.06	0.11	0.01	0.11	0.26	0.12
9 (0-15 cm)	5.26	0.10	0.06	0.03	0.19	0.14	0.27
9 (15-35cm)	2.39	0.09	0.05	0.06	0.34	0.09	0.19
9 (35-x cm)	1.98	0.32	0.05	0.74	0.43	0.26	0.29
10 (0-2cm)	1.49	0.09	0.00	0.18	0.66	0.45	0.25
10 (2-Xcm)	2.74	0.11	0.05	0.08	0.16	0.07	0.25
13 (0-10cm)	0.89	0.15	0.00	0.21	0.99	0.18	0.52
13 (10-20 cm)	1.50	0.14	0.00	0.18	0.81	0.19	0.55
13 (20-x cm)	2.40	0.07	0.07	0.11	0.27	0.20	0.43

Table 5. Concentration (μg) of special Elements after column separation

In Table 6, the actual efficiency (%) of the separation method for each sample is summarized. If the efficiency is lower than 95% this means that there is a higher probability of Cd isotope fractionation during the separation process. This fractionation could influence the Cd isotope analysis. Below are presented the average concentrations of the samples before and after the column separation. The matrix efficiency for the cadmium fraction was above 95% in 5 of the total 17 samples that analyzed indicating a higher possibility of Cd isotope fractionation in the columns. Concerning the removal of the other elements we can mention that was efficient, as the percent was below 10 % indicating that the method used was totally successful since the range of removal fluctuates from 0-3 %.

SITES & DEPTHS	Cd	Na	Al	K	Ca	Fe	Zn
Matrix Efficiency (%)							
1 (0-2cm)	87.1	0.0	0.0	0.0	0.1	0.0	0.0
1 (2-10cm)	91.7	0.0	0.0	0.0	0.2	0.0	0.1
1 (10-20cm)	86.7	0.0	0.0	0.0	0.0	0.0	0.1
1 (20-Xcm)	94.5	0.0	0.0	0.0	0.0	0.0	0.1
6 (0-16cm)	95.5	0.0	0.0	0.0	0.0	0.0	0.0
6 (16-Xcm)	106.6	0.0	0.0	0.0	0.0	0.0	0.1
7 (0-15cm)	84.1	0.0	0.0	0.0	0.0	0.0	0.1
7 (15-30cm)	65.9	0.0	0.0	0.0	0.0	0.0	0.1
7 (30-x cm)	97.8	0.0	0.0	0.0	0.0	0.0	0.0
9 (0-15 cm)	82.1	0.0	0.0	0.0	0.0	0.0	0.0
9 (15-35cm)	77.6	0.0	0.0	0.0	0.0	0.0	0.0
9 (35-x cm)	78.3	0.0	0.0	0.0	0.0	0.0	0.1
10 (0-2cm)	59.5	0.0	0.0	0.0	0.0	0.0	0.1
10 (2-Xcm)	96.6	0.0	0.0	0.0	0.0	0.0	0.1
13 (0-10cm)	86.2	0.0	0.0	0.0	0.0	0.0	3.0
13 (10-20 cm)	97.2	0.0	0.0	0.0	0.0	0.0	0.3
13 (20-x cm)	84.0	0.0	0.0	0.0	0.0	0.0	0.1

Table 6. Efficiency of matrix separation for Cd and Selected Elements

6. Conclusions

The main objective of this study was to optimize the matrix separation methodology prior to Cd isotope analysis in the soil samples, collected at several contaminated sites in Ostrava region. We present an efficient high purity chemical separation procedure for Cd separation from rich inorganic matrices. Although the separation is more complicated due to the fact that Cd concentration is very low in comparison with the matrix in the soil samples, the efficiency of Cd separation was over 90% in most of the samples and matrix separation was over 99% in all samples. It was necessary to divide one sample in 2 or 3 separation columns in order not to exceed the ion exchange capacity of the resin. In conclusion, the method is suitable for Cd separation from soil samples for further isotopic analysis. Moreover traceable amounts of Cd were determined in the soil samples from Ostrava region related to the adjacent industrial areas and the active mines that exist there.

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Websites

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Appendix

Sites	(mg)	Cd	Mg	Ca	Cr	Mn	Fe
1 (0-2cm)							
A	246,4	30	1628	28578	309	23446	89539
B	251	34	295	14534	529	51123	82292
C	251	36	251	8228	355	49723	121333
1 (2-10cm)							
A	252,1	34	461	16766	197	50196	52996
B	245,2	6	163	1589	317	7307	137644
C	251,2	9	299	1806	911	7025	375870
1 (10-20cm)							
A	255,7	55	190	520	857	9698	445984
B	249,7	40	120	320	933	4804	344540
C	257,6	41	49	-90	637	5090	275207

A	259,2	5	205961	63985	9730	68717	3832955
B	253,4	5	277335	65282	7595	80335	3227929
C	258,7	4	208957	76110	8317	77843	3669265
1 (20-Xcm)							
A	243,4	9	121	1966	896	5914	377698
B	258,4	5	3263	956	694	6729	305956
C	257,8	5	9	452	837	7063	363116
A	243,1	40	268535	60805	8323	61718	3562010
B	260	52	272427	53652	9345	68928	3906044
C	263,3	50	304932	45896	9573	66799	3939263
6 (0-16cm)							
A	250,2	13	3682	85443	2616	12528	836815
B	242,9	10	39867	86453	2546	11940	718042
A	258,7	80	303331	580740	8745	92617	3304716
B	254,5	75	264487	612362	7989	94058	3134756
6 (16-Xcm)							
A	252,6	5	73281	46665	2143	7959	744725
B	242	5	86839	50211	2330	7891	765523
A	258	33	227554	297907	7783	55946	3118467
B	253	30	330013	388750	6903	52771	2839132
C	242	27	343514	362198	6400	52954	2645032
D	251	32	399630	256990	7688	57579	3139394
7 (0-15cm)							
A	248,9	19	37878	100470	3571	17156	1398950
B	243	17	91020	116567	3215	16757	1317724
A	254,5	90	302359	608225	7750	99639	3669318
7 (15-30cm)							
A	250,8	16	91764	148892	3409	18709	1175046
B	249,5	15	32594	95393	2923	16134	1092476
A	250,5	93	341646	725586	11326	113187	3660558
7 (30-x cm)							
A	242,9	8	51834	84076	12191	28282	1091968
B	243,3	8	20744	57227	3486	10362	972004
A	248,4	49	175231	1176827	11947	107734	3531689
B	252,3	41	187757	636288	8637	63964	3204616
9 (0-15 cm)							
A	252,1	3	16	285	237	5081	87421
B	253	12	709	11510	1126	7786	326969
A	254,6	112	206264	361773	10819	85217	3665739
B	259,5	95	184888	320222	8805	74762	3599487
C	249	98	196553	314041	9020	77109	3274489
9 (15-35cm)							
A	254,7	7	1010	26955	169	12405	54352

B	253,4	9	345	10709	310	12628	129669
C	253,1	6	357	7868	118	7996	39227
A	255,6	63	220206	265454	8280	86691	2931004
B	252	69	126576	391466	8528	91212	3182545
9 (35-x cm)							
A	249,5	3	533	7280	116	8005	56201
B	246,8	5	407	6904	201	13631	93486
C	249,7	7	666	11948	841	11029	378039
A	257,2	38	192365	428209	7500	78182	3312004
B	256,5	37	165744	436975	7315	63460	3158789
C	249,4	37	119888	402989	7640	65688	3409130
10 (0-2cm)							
A	261,4	12	313	15450	155	13073	48426
B	259	11	286	4625	113	10140	32229
C	257,3	11	317	6828	218	11687	67219
A	261	91	122920	269922	5823	91363	2047313
10 (2-Xcm)							
A	245	1	178	924	154	2636	43372
B	241	5	75	1849	728	4664	294120
C	260	2	293	1135	87	4590	40852
A	247,5	46	120925	66243	7295	39225	2708281
B	250	38	99266	121846	7174	48553	2868693
C	243,2	39	55670	98324	6621	40838	2526274
D	244	10	5580	25082	1405	9084	276383
13 (0-10cm)							
A	252,4	27	98393	93179	5662	21762	1020897
B	256,3	25	113530	126876	5301	20255	968451
13 (10-20cm)							
A	243,3	23	142518	146537	5108	19533	1017341
B	252,3	26	169305	208734	6545	27744	1155103
A	252,3	0	-70	-355	-1	1	220
B	255,4	28	686	19232	159	17770	55798
13 (20-x cm)							
A	256,1	47	12651	136484	2813	40494	276383
B	260,2	37	10239	93140	2692	25809	276383
C	241,5	59	14816	190347	3831	46264	276383

Sites	(mg)	Ni	Cu	Zn	As	Co
1 (0-2cm)						
A	246,4	615	1174	6152	26	182
B	251	270	323	9395	36	78
C	251	247	338	3228	51	79
1 (2-10cm)						
A	252,1	218	280	3136	22	72
B	245,2	392	228	2102	28	135
C	251,2	401	253	2105	54	136
1 (10-20cm)						
A	255,7	459	224	1831	73	217
B	249,7	407	185	1623	57	122
C	257,6	341	167	1599	67	133
A	259,2	3543	2086	7537	1595	1529
B	253,4	2990	1667	5489	1334	1625
C	258,7	3300	1770	7124	1435	1580
1 (20-Xcm)						
A	243,4	387	253	2088	128	119
B	258,4	433	219	1814	69	172
C	257,8	440	217	1777	83	173
A	243,1	3376	1861	5181	1142	1561
B	260	3800	2075	6314	1252	1805
C	263,3	3810	2182	6386	1287	1767
6 (0-16cm)						
A	250,2	627	500	792	191	198
B	242,9	516	400	1072	172	165
A	258,7	3592	4022	21094	1825	1389
B	254,5	3617	3920	20454	1429	1449
6 (16-Xcm)						
A	252,6	586	311	1699	154	175
B	242	586	327	1694	156	170
A	258	3736	3057	8095	1226	1228
B	253	3368	2962	6104	1034	1185
C	242	3271	2477	5739	935	1167
D	251	3769	3039	7730	1188	1320
7 (0-15cm)						
A	248,9	673	587	1002	236	221
B	243	605	517	1087	209	197

A	254,5	2832	3485	13647	1192	1085
7 (15-30cm)						
A	250,8	606	546	1141	222	207
B	249,5	595	569	1157	210	226
A	250,5	3346	4252	14069	1321	1337
7 (30-x cm)						
A	242,9	555	350	1480	214	198
B	243,3	558	378	1489	204	190
A	248,4	3026	2787	10377	1538	1410
B	252,3	2813	2634	8862	1406	1112
9 (0-15 cm)						
A	252,1	304	142	1289	20	131
B	253	388	313	3782	64	111
A	254,6	3464	3516	14847	1852	1339
B	259,5	2964	3552	11187	1953	1168
C	249	2956	3007	12398	1748	1177
9 (15-35cm)						
A	254,7	587	366	3093	9	180
B	253,4	633	407	2136	27	195
C	253,1	447	288	1711	10	144
A	255,6	3729	2705	9625	1334	1325
B	252	3687	2823	15102	1444	1390
9 (35-x cm)						
A	249,5	466	244	1683	8	165
B	246,8	565	286	1937	65	213
C	249,7	602	360	2418	122	221
A	257,2	3361	2086	6965	1738	1458
B	256,5	3243	2116	6493	1655	1185
C	249,4	3317	2233	6791	1878	1341
10 (0-2cm)						
A	261,4	244	217	1859	27	109
B	259	238	142	1563	12	87
C	257,3	228	188	1723	18	97
A	261	1966	1850	8142	1553	773
10 (2-Xcm)						
A	245	171	94	441	22	60
B	241	297	194	758	121	106
C	260	272	143	696	12	103
A	247,5	2388	1571	3743	1437	839
B	250	2523	1485	3739	1429	959
C	243,2	2212	1358	3193	1240	825
D	244	472		1570	274	184
13 (0-10cm)						

A	252,4	672	899	368	371	183
B	256,3	608	819	496	350	172
13 (10-20 cm)						
A	243,3	652	844	581	443	190
B	252,3	775	976	379	415	219
A	252,3	0	1	0	0	0
B	255,4	743	1223	8128	15	182
13 (20-x cm)						
A	256,1	1044	42	9728	762	393
B	260,2	937	22	7857	662	326
C	241,5	1396	50	11557	1041	517

Sites	(mg)	Ag	Pb	Na	Al	K
1 (0-2cm)						

A	246,4	4	1207	99566	38414	119253
B	251	3	631	21409	18709	53080
C	251	4	682	24617	18783	56902
1 (2-10cm)						
A	252,1	3	376	22799	27488	53066
B	245,2	3	489	80526	24195	163960
C	251,2	9	731	87224	29916	181769
1 (10-20cm)						
A	255,7	5	356	74349	17579	164257
B	249,7	7	247	51555	35656	123157
C	257,6	5	344	50106	12656	138347
A	259,2		2878	866023	1345770	1134058
B	253,4		2641	836812	1379237	1133297
C	258,7		2692	861604	1431955	1170963
1 (20-Xcm)						
A	243,4	8	787	95828	21950	200323
B	258,4	5	261	90648	52793	203937
C	257,8	6	312	88983	25012	212511
A	243,1		1756	854197	1503437	1214446
B	260		2020	921245	1495650	1247297
C	263,3		2075	885256	1459129	1220960
6 (0-16cm)						
A	250,2	11	1420	15025	5026	34840
B	242,9	9	1101	18650	3852	34964
A	258,7		5266	705752	1488323	1234168
B	254,5		4669	763412	1421933	1183469
6 (16-Xcm)						
A	252,6	12	626	12331	7801	36280
B	242	8	630	6552	8456	39069
A	258		2692	866345	1495964	1231505
B	253		2467	769554	1455034	1177647
C	242		2372	781550	1535818	1217030
D	251		2659	868233	1566400	1289327
7 (0-15cm)						
A	248,9	40	2632	26611	3105	37469
B	243	15	1678	10441	4303	21161
A	254,5		5368	522933	1402110	1115299
7 (15-30cm)						
A	250,8	16	1492	74745	4889	30486
B	249,5	15	1445	52252	6584	38032
A	250,5		5994	587988	1458907	1209528
7 (30-x cm)						
A	242,9	16	666	47289	9041	32227

B	243,3	17	657	79451	8542	27677
A	248,4		2461	595127	1465932	1217566
B	252,3		2280	596953	1426688	1188583
9 (0-15 cm)						
A	252,1	4	73	58849	12657	133823
B	253	11	911	70437	42701	188476
A	254,6		5626	926717	1555080	1306741
B	259,5		4352	790165	1388304	1151349
C	249		5966	835742	1465251	1224913
9 (15-35cm)						
A	254,7	2	493	100787	32726	222557
B	253,4	3	630	99497	24122	219595
C	253,1	2	407	76051	22885	165030
A	255,6		4154	723496	1288359	1072988
B	252		4403	793231	1355642	1129384
9 (35-x cm)						
A	249,5	2	380	117140	25501	183550
B	246,8	2	664	107382	28264	222317
C	249,7	8	1007	132387	31668	225868
A	257,2		4457	818800	1344365	1120036
B	256,5		5961	790942	1325139	1103724
C	249,4		4501	849526	1478507	1236143
10 (0-2cm)						
A	261,4	3	624	111140	30577	228783
B	259	6	52	91685	15639	153344
C	257,3	5	242	102728	24252	168415
A	261		5520	763313	1489275	1249441
10 (2-Xcm)						
A	245	2	65	58660	27349	143336
B	241	8	439	94195	19469	255302
C	260	3	63	99963	18531	200700
A	247,5		3161	735173	1504884	1274514
B	250		2748	852068	1579627	1334010
C	243,2		2575	733824	1473530	1270310
D	244		650	143355	236323	219177
13 (0-10cm)						
A	252,4	17	2519	16080	5525	42650
B	256,3	15	2379	67116	2502	39710
13 (10-20 cm)						
A	243,3	15	2314	86412	4302	34119
B	252,3	16	2512	22437	4322	47602
A	252,3	0	1	-208	-340	190

B	255,4	5	1129	105641	22995	117018
13 (20-x cm)						
A	256,1		2934	161398	250766	219177
B	260,2		2448	151559	207000	219177
C	241,5		3390	166888	243730	219177