CHARLES UNIVERSITY IN PRAGUE

Faculty of Pharmacy in Hradec Králové

Department of Analytical Chemistry

Comparison of manual and dynamic extractions of selected transition metals from solid samples

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ABSTRACT

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Title of thesis: Comparison of manual and dynamic extractions of selected transition metals from solid samples

The analysis of trace metals in soil is a subject of study in many laboratories. Due to large industrial areas soil can be highly contaminated. This work focused on two easy methods – manual extraction and single flow extraction with the same extractant – acetic acid. Two methods, two concentrations of acetic acid (0.11 M and 0.43 M), and two samples were used to evaluate, which conditions were more effective. As a detection device inductively coupled plasma - optical emission spectrometry was selected and two different wavelengths were used to prevent interferences.

Manual extraction was more time-consuming and less reproducible than the dynamic extraction. Higher concentration of acetic acid extracted a larger amount of metals and the extraction showed higher reproducibility of the results. In both soils the contaminations with Cu outreached the permitted level for this metal (according to the requirement of the government of the Balearic Islands) and are potentially contaminated.

ABSTRAKT

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Název diplomové práce: Porovnání manuální a dynamické extrakce vybraných přechodných kovů z pevných vzorků

Analýza stopových kovů v půdě je předmětem studií mnoha laboratoří. Díky rozsáhlým průmyslovým zónám může být půda vysoce kontaminována. Tato práce se soustředila na dvě jednoduché metody - manuální extrakci a průtokovou extrakci se stejným extraktantem – kyselinou octovou. Dvě metody, dvě koncentrace a dva vzorky byly použity k vyhodnocení nejvýhodnějších podmínek. Jako detekční zařízení byla vybrána optická emisní spektrometrie s indukčně vázanou plazmou a dvě vlnové délky k zabránění interferencí.

Manuální extrakce byla více časově náročná a hůře opakovatelná než ta dynamická. Vyšší koncentrace kyseliny octové (0,43 M) extrahovala vyšší množství kovů a extrakce vykazovala vyšší opakovatelnost výsledků. U obou půd kontaminace mědí přesáhla maximální povolené množství tohoto kovu (podle požadavků vlády Baleárských ostrovů) a jsou potenciálně kontaminované.

PROHLÁŠENÍ / STATEMENT

„Prohlašuji, že tato práce je mým původnim autorským dílem. Veškerá literatura a další zdroje, z nichž jsem při zpracováni čerpala, jsou uvedeny v seznamu použité literatury a v práci řádně citovány. Práce nebyla využita k získani jiného nebo stejného titulu.“

"I declare this thesis is my original work of authorship. All literature and other sources I used for writing the thesis are listed in the bibliography and properly cited in the work. The work has not been used in order to obtain other or equal degree."

Hradec Králove, 15. 5. 2015 _____

Vendula Kucharčíková

ACKNOWLEDGEMENT

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1 List of abbreviations

AA   agriculture areas

BCR   Community Bureau of Reference

IA   industrial areas

ICP-OAS   Inductively Coupled Plasma - Optical Emission Spectrometry

LA   living areas

MTP   maximal threshold permitted

PN   pneumatic nebulizer

USN   ultrasonic nebulizer

2 Introduction

Soil samples can be contaminated with heavy metals from natural as well as by antropogenic sources. The metal cations are generally bound to the solid material being the mineral fraction and organic matter.

By leaching, these metals can penetrate into the groundwater, contaminate growing plants, and present a risk for humans. This is because heavy metals can have a significant influence on the human health. Some of them cumulates in the body and causes acute or chronic poisoning. It is therefore necessary to assess the level of contamination of toxic metals in the environment.

In this work, two simplest methods for soil analysis – manual extraction and single flow extraction with one extractant – were tested and compared. Two different soils were used as model samples and two extractant concentrations were studied. Acetic acid was used as extractant. Concentrations in the extract were measured by ICP-OES technique and the results were compared and discussed.

3 Aim and description of the work

The objective of this thesis were:

- Extraction of the selected heavy metals cadmium, chromium, copper, lead, zinc, and nickel from the soil samples using acetic acid as extractant.

- Measurement of their concentration by the analysis by inductively coupled plasma - optical emission spectrometry.

- Comparison of two different methods of extraction and two different concentrations of extractant.

- Evaluation of the results and discussion about the suitability of both methods and the appropriate concentration of the extractant in respect of efficiency and reproducibility.

4   Theory

4.1   Metals

Metals represent 3/4 of the natural 98 elements of the periodic table. With the exception of mercury they are solid at room temperature, most have a high melting point, shiny, opaque, malleable, and have a great electrical and thermal conductivity. According to their density, metals are divided into two groups – light and heavy. 5000 kg/m^3 is considered as a borderline[1].

4.1.1   Properties of the metals of interest

The type of chemical bonds and crystal structure define the typical properties of metals. The number of valence electrons is not enough for the formation of covalent bonds[1]. Each element is special, so it is better to describe their properties separately. This work is focused on chrome, lead, copper, zinc, cadmium, and nickel.

Chromium (Cr, from Greek world *chroma* – color – according to the colors of its compounds) was discovered in 1797. The main use of this metal is the production of nonferrous alloys. The most stable oxidation state of chromium is +III and this ion is the basis of many coordination complexes [2]. It is an essential micronutrient that is necessary for sugar metabolism [3]. However, the oxidation state +VI has very strong oxidative effects [2] and is a well-known carcinogen.

Lead (Pb) is the only element in this work that belongs to the p-block of the periodic table. Unlike most metals lead has low melting point (327.5 °C). Ancient Egyptians used lead as a roof cover. It was also used in Europe until this century then the acid rain made its use impractical. It is used in the production of batteries, petrol, alloys etc. Nowadays the most important source of environmental contamination is mining and processing of ores. The oxidation state +II is more typical than +IV. Lead

pipes for water can be dangerous if the water inside contains free oxygen. This leads to the production of lead hydroxide that is soluble in water and contaminates water, which is responsible for health problems. In HF, HCl, and H_2SO_4 lead produces a protective layer, however if the atmospheric oxygen is presented, lead will react with some organic acids (such as acetic acid) [4] [5].

Copper (Cu) is known for thousands of years. Unlike the alkali metals, copper has a higher ionization energy and smaller ionic radius. This is why copper has a higher melting point, density, and hardness. Nowadays it is used for the production of electrical cables, water pipes, and alloys. This metal is stable in dry air but dissolves in hot H_2SO_4 and in concentrated as well as diluted HNO_3. It easily reacts with halogens and sulphur. Typical oxidation states are +I and +II [2].

Zinc (Zn) is a silverish metal with bluish gloss and is known for thousands years. Unlike most metals zinc has low melting point (419.5°C) and boiling point (907°C). Zinc is a good reducing agent. In contact with acids hydrogen is released. Zinc in oxidation state +II forms frequently complexes [2].

Cadmium (Cd) was discovered in 1817. It belongs to the same elemental group 12 as zinc, so that is has similar characteristics. It has a lower melting point (320.8°C) as well as a lower boiling point (765°C) than Zn [2]. Cadmium is used as a stabilizer in plastics and for the production of color pigments [4]. Cadmium also reacts with acids and products hydrogen. Its typical oxidation state is +II [2].

Nickel (Ni) was discovered in 18th century but objects from nickel were found in thousands years ago. Nickel can be found in nature in its pure metallic form or as form of ores. It is a silvery metal with good ductility and elongation and ferromagnetic properties. Its reactivity is low due to a low reduction potential, but at higher temperature nickel is covered with a thin layer of oxide. It is used for the production of alloys, coins, cutlery, batteries etc. Nickel in oxidation state +II frequently creates complexes [2].

4.1.2 Harmful effects of metals on human health and environment

As mentioned above chromium in oxidation state +VI have very strong oxidative effects. 2% of Cr^{3+} is absorbed in digestive tract. Respiration is also an easy way of entry. This can be more dangerous because the metal remains in the lungs for a long time and increases the risk of lung cancer and bare bronchial asthma, causes nasal irritation and hypersensitive reactions in lungs. Genotoxicity, immunosuppression, and carcinogenicity are also proven effects of Cr^{6+} [4,3]. Chromium in soil is mostly present in +III form and the absorption by plants is low so there is not a significant health risk for humans.

Toxic effects of lead are known for centuries and are well identified. It is more dangerous for children, because their digestive tract is more permeable for this metal. Also evolving brain is the most sensitive part for toxic effects. Lead accumulates in the body (90% of accumulated lead is stored in bones) for many years and can cause problems after reaching the toxic level – symptoms are not specific but include usually nausea, constipation, insomnia, and gray lines on gums. According to many studies, the poisoning is the worst for the hematopoietic system, nerve system, digestive tract and kidneys [4]. The concentration of lead in soil determines its concentration in groundwater. Lead bioavailability depends also on properties of the soils. Analysis on earthworms showed that the main factors are the pH value (a lower pH increases the bioavailability), cation-exchange capacity, and the presence of amorphous iron and aluminium oxides [6]. When a soil contains Fe and Mn, Pb lead undergoes chemical transformation and stays bonded in the soil, so the composition of soil has also an influence on lead bioavailability.

Copper is essential for the human organism but also can be toxic. Exposure to vapors is common for people working in metallic industry and causes "metal fever" but chronical damage has never been proven. $CuSO_4$ was used for its emetogenic effects. It was also used for suicide – low concentrations damage the gastrointestinal tract, higher concentrations cause icterus and kidney damage. Production of copper is growing in recent years. Copper concentrates in the mineral fraction of soil. If the soil contains manganese oxides or organic compounds, the bioaccessibility of copper is higher [4].

Zinc is an important essential micronutrient - more than 300 human enzymes contain zinc. Normal daily intake is necessary for growth and reproduction [7]. During the smelting of ores vapors often escape into the atmosphere. Inhalation causes "metal fever" (like copper). It means that after working day, laborers have headache, sore throat, cough etc. All of them disappeared after a chelation therapy. Chronical poisoning has not been described yet. Zinc plays probably some role in carcinogenesis because people with malign tumors have a lower Zn concentration in blood [4]. Most cases of acute toxicity are caused by food poisoning also because of storage of water in galvanized containers, which causes digestive problems [8].

Cadmium is not essential for human metabolism and has a damaging effect on human health. There are 2 ways of exposure: inhalation or digestion. From inhaled Cd, 10-50% are absorbed in lung depending on the particle size [9]. The main source of intoxication is contaminated food and water. Symptoms of acute poisoning are nausea, vomitus, headache, excessive saliva production. At higher concentration, Cd causes water loss (thanks to diarrhea), which results in kidney, lung, and heart failure. In some studies it was found out that exposure to cadmium can cause hypertension while the mechanism has not yet been explained. Also teratogenicity and carcinogenicity of Cd were proven. [4] The typical cure for Cd overdose is a chelation therapy. It showed good therapeutic effects in humans and animals [9].

Nickel is present in the human body as a part of enzymes and has a meaning during a blood production. Nickel belongs to the main causes of contact dermatitis as an allergic reaction. A risk factor is working with nickel in factories, wearing watches (made of nickel), contact with nickel coins, dishes etc. This problem can be treated with oral application of disulfiram or a diet with low content of nickel. Acute toxicity of Ni is relatively low but chronic exposure to vapors containing nickel causes lung cancer. A significant risk represents carbonyl nickel. Acute symptoms are non-specific but after 12-120 hours lung damage can occur and cause death [4].

4.2   Soil

Soil is a complex heterogenic material including a mineral fraction, organic matter, gas, and water. Soil can be defined as a natural mixture formed from surface weathering of the earth's crust and organic residues under the influence of soil-forming factors. It is a substrate for plants, an environment for many microorganisms and a water reservoir. Soils are classified among others according to the grain size. Their size has an effect on technological properties, coherence and adherence [10].

4.2.1   Soil properties

The extractable fraction of heavy metals from soil is influenced by the soil pH and by the presence of other chemical compounds or ions. The mobility of heavy metal ions increases with lower pH. Soil has some buffer capacity (aluminium ions, CO_2, carbonates, cation exchange reaction). Soil usually has a pH within the range of 4.0 - 8.5 – depending on the region. Oxides of Fe, Mn, and Al can precipitate and adsorb other ions so that these become non-extractable. There are many mechanisms that have an effect on the final extracted concentrations of metals (pH, organic components, adsorption, precipitation…) [11].

4.2.2   Soil sampling

Soil sampling can be a tricky task. If it is done wrongly, results are misrepresented. It is not just a random process but a well-considered part of the analytical procedure. The sample must be representative for the soil of interest and the area of sampling because soil is a non-homogenous mixture of fine particles, stones, air, rotten as well as fresh organic material.

It is necessary to use some statistic facts. Arithmetic mean and standard deviation are the best known. Sampling and also following analysis are expensive, so it is important to find out the lowest amount of samples that provide sufficient confidence [12].

The method of sampling depends on the type of soil, the analyte of interest, and the later performed analysis. For heavy metals, sampling from a depth between 30 and 60 cm is suitable. Usually the final sample consists of 30 separate samples that were taken in exact location according to a plan made in the beginning [13] . The next step is homogenization and drying. After this process all parts of the sample should have the same composition.

4.3 Bioaccessibility

There is a difference between bioavailability and bioaccessibility parameters. The International Organization for Standardization divides the term bioavailability into 3 following steps:

BIOACCESSIBILITY – the highest concentration of metal that is leachable. The maximum amount of metal which can be extracted.

ENVIRONMENTAL BIOAVAILABILITY – the part of fraction that is able to penetrate through a cell membrane. That means that not whole amount of metal can get to the organism.

TOXICOLOGICAL BIOAVAILABILITY – processes in organism such as metabolism, elimination, production of deposits. The part of fraction, which causes the toxic effect and is not otherwise deactivated.

It follows that in case of a zero bioaccessibility, the bioavailability is also zero. However, if the bioavailability is zero, bioaccessibility can be high – it does not depend on it. So it is important to distinguish these terms very carefully [14].

4.4 Methods

Speciation (= process of identification and quantification) of metals in a soil is very important. Soil can be a source of contamination for plants and animals. In 1979 Tessier et al. designed a five step extraction procedure shown in Table 1. The

exchangeable fraction is a fraction of metals that is adsorbed. Heavy metals can be associated with carbonates according to many works. This fraction is collected in step II. Step III focuses on metals that are bound to Fe-Mn oxides. Metals can be also bound to organic matter (for example living organisms). The last fraction contains metals that are hold in crystal structure of minerals presented in the soil [15].

In 1987, Community Bureau of Reference (BCR) established the program to harmonize the methodology of determining heavy metals in solid samples. It was an interlaboratory and international trials that should provide the proof that the tested procedure is well repeatable. Three methods of sequential extraction were tested and also one single extraction method. The main difference between the new and the old BCR scheme was the replacement of the first two steps in Tessier's model by only one step (nowadays the most common scheme has three steps). These trials showed to a need to create reference materials [16] [17]. Few years later Ure et al. tested prepared reference materials under different conditions to study the extraction conditions for achieving the best reproducibility [18].

In spite of the improvement of the BCR method and complete automation, it is still very time consuming. This work focuses on the single extraction with acetic acid. Acetic acid extracts the exchangeable fraction of trace metals and released metals after acidification, i.e. the fraction bounded as carbonates [17].

Table 1: Five step fractionation procedure sequential extraction of heavy metals

Fraction	Extracted fraction	Extractants
I	exchangeable	1M $MgCl_2$, 1M NaOAc
II	bound to carbonates	1 M NaOAc + HOAc
III	bound to Fe-Mn oxides	0.3 M $Na_2S_2O_4$ + 0.175 M Na-citrate 0.04 M NH_2OH-HCl in 25% (v/v) HOAc
IV	bound to organic matter	0.02 M HNO_3 + 30% H_2O + 3.2 M NH_4OAc
V	residual	HF-$HClO_4$ mixture

4.5 Inductively Coupled Plasma - Optical Emission Spectrometry

Inductively Coupled Plasma - Optical Emission Spectrometry (ICP-OAS) is a technique and instrument for elementary analysis. It represents a big progress in metalanalysis. Nowadays this device is standard equipment for large laboratories. The very first ICP-OAS was presented in 1974.
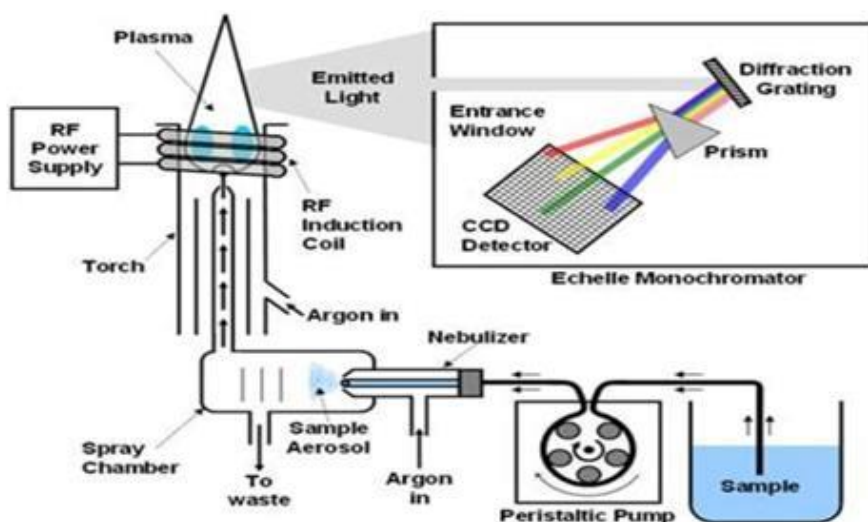

Figure 1: Scheme of an ICP-OAS instrument [19]

The illustration scheme of an ICP-OES is given in figure 1. It is possible to measure up to 70 elements in one injection or just one specific. Samples have to be in liquid or gaseous form, solid samples require prior liquid extraction or dissolution. In case of liquid samples, the sample is driven into a nebulizer to transform it by the help of argon gas into an aerosol and to carry it to the central channel of the plasma generation. The plasma torch is generated by induction heating of the argon flow, i.e. by variation of a high current. Due to the extremely high temperature – 10 000 K – the aerosol is rapidly vaporized and all compounds are broken down to their elements. The elements are excited and at energy decrease at leaving the plasma, they emit light on element specific wavelengths. With the help of mirrors, lenses, and a monochromator, the light is guided to a photodetector, which converts the light into an electric signal.

In this work, likewise a nebulizer was used as a sample introduction system (the system that introduces the sample into the plasma). There are many requirements for

this device such as the ability to use / nebulize very small volumes, low price, easy of use etc. The droplets formed by the nebulizer have to be very small – ca 8 μm – to reach the plasma, larger drops or particles are eliminated in the spray chamber seated between the nebulizer and the plasma.

There are two types – pneumatic nebulizers (PN) and ultrasonic nebulizers (USN). The PN is less efficient and only 5% at maximum of the sample volume reaches the plasma. Compared to the PN, an ultrasonic nebulizer is more efficient (10-20% of the sample reaches the detector). This nebulizer is not affected by the argon flow rate, which is in contract to the PN. Therefore, it is possible to extend the time of transporting the sample from nebulizer to plasma. The advantage is that the sensitivity is higher and the detection limit is lower. Disadvantages of the USN are a higher price, a longer timer of analysis, incompatibility with fluoric acid etc.

Torch – optic configuration – there are three types – axial, radial, and dual view (combination of previous two). Radial view plasma operates in vertical position and the analytical zone is observed from the side of plasma. Axial view done is the opposite – horizontal position and analyte is observed from the end, in which case the detection limit is lower. Experiments in this work used a dual view configuration.

The ICP generates photons, which pass through a monochromator. Because the light intensity is low, it is very important to multiply it. For this purpose, a photomultiplier tube is used.

Interferences are not so common compared to other techniques. But some of them are also presented, especially background interference. This type of interference is caused by multielement nature of plasma and the ability of ICP to excite almost every element. There are also some steps for prevention. In this work two different wavelength were selected for each metal. If one of them contained interferences, the second one would be used [20] [21].

5 Material

5.1 Sample

Two samples were used for this work. They were labeled as SN3-SN4 and SN6. Both were collected in Mallorca and were collected in the industrial areas.

5.2 Laboratory equipment

The following materials and instruments were used for this work:

5.2.1 Instruments

Magnetic stirrer: J.P.Selecta (Spain)

pH meter: Eutech instruments pc2700 (The Netherlands)

Centrifuge: EPPENDORF Centrifuge 5804R (Germany)

Peristaltic multi-syringe pump: CRISON Multi-Burette 4S (Spain)

ICP-AES: Perkin Elmer OPTIMA 5300 DV (Spain)

Water purifier: Millipore Milli-Q Gradient A10 (filter – 0,22 μm Millipak express) (Spain)

Microcolumn: Microcolumn is a container for solid samples. The biconical shape was chosen due to the option for bidirectional flow, which does not make hifg overpressure so easily. The composition is shown in picture 1 [22]. This one was built for a sample weight of 1g (maximum).

5.2.2 Consumables

Filters:

- Fluorophore Membrane Filter, PTFE, 0.45 μm – for the column (Spain)
- Fisher scientific – X200 syringe filter 25mm 0.45 μm, NYLON – syringe filter (Spain)

5.2.3 Software

Coco soft – created by David Cocovi (University of Balearic Islands)
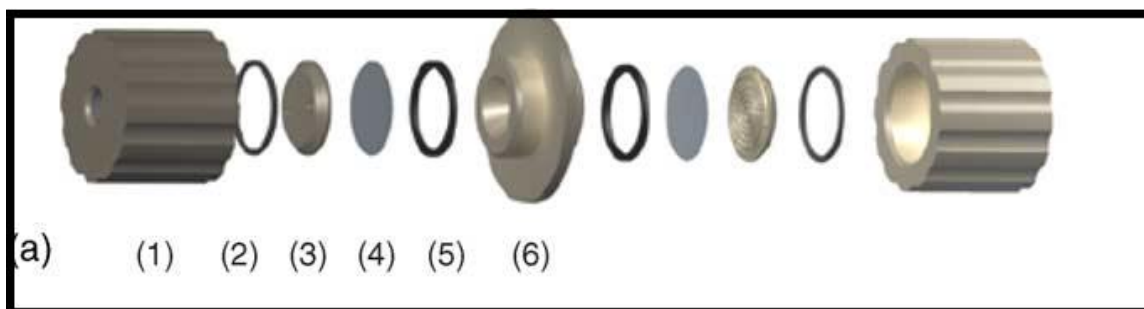
Figure 2: Scheme of microcolumn - (1) filter house, (2) o-ring, (3) filter support, (4) membrane filter, (5) silicone gasket, (6) sample container

5.3 Chemicals

5.3.1 List of chemicals
Acetic acid – **Glacial acetic acid (≥99.85%) by Sigma Aldrich** (Spain)

Nitric Acid - **FLUKA Nitric acid, =69.0%, TraceSELECT®, for trace analysis by Sigma Aldrich** (Spain)

Stock solution of metals – **Fluka multielement standard solution 5 for ICP, TraceCERT by Sigma Aldrich** (Spain)

5.3.2 Preparation of solutions

0.11M Acetic Acid

24.7 ml of glacial acetic acid was added to 500 ml of distilled water and then diluted to 1000 ml volume. This solution should be stored in a closed polyethylene container.

0.43M Acetic Acid

6.3 ml of glacial acetic acid was added to 500 ml of distilled water and then diluted to 1000 ml volume. This solution should be stored in a closed polyethylene container.

Nitric acid bath

1.4 l of nitric acid was added to 7 l of distilled water and mixed. Final concentration was 20% (v/v).

Carrier for ICP-AES

20 ml of nitric acid was added to 1000 ml volumetric flask and filled with water. Final concentration was 0.02% (v/v).

Calibration solutions for ICP-AES

Acid solution – 0.63 ml of acetic acid in case the extractant corresponds to 0.11 M acetic acid (2.462 ml for 0.43 M acetic acid) was added to 100 ml volumetric flask, 2 ml of nitric acid was also added and filled with water.

Preparation of calibration solutions – certain amount (according to chosen concentration) of stock solution of metals was added to the test tube and filled with the acid solution to 10 ml. This process was performed directly on the balance and the weights of the empty tube, tube with stock solution, and final weights were written down to calculate the real concentrations inside.

Table 2: Calibration solutions and their composition, which were used in these experiments. (Values refer to 100 ml of preparation)

Solution	Concentration (ppm)	Volume of stock solution (μl)	Volume of acetic acid(ml)
blank	0	0	10.00
std A	5	5	9.99
std B	10	10	9.99
std C	50	50	9.95
std D	100	100	9.90
std E	200	200	9.80
std F	500	500	9.50
std G	1000	1000	9.00

6   Methods

6.1   Manual extraction

The manual extraction is a method based on equilibrium conditions. First, the sample bottle is shaken for 2 min to homogenize the content. Then, 1 g of soil is given to a glass beaker of a volume of 100 ml and 40 ml of extractant was added. The beaker was attached to a laboratory stand with clamps and placed above but not onto a magnetic stirrer. The beaker should not be in direct contact with the magnetic stirrers to avoid unwanted increase of temperature in the reaction mixture (picture 2, 3). Inside the beaker, a stir bar was placed for continual stirring with the same speed (2715 rpm). The top of the beaker was covered with parafilm M to prevent evaporation of the acetic acid. The extraction was stopped exactly after 16 hours (overnight) and the temperature was measured. Six extractions were done in parallel [23].

Parafilm was taken off and the beaker and the content were transferred to a centrifuge tube. Same weights of centrifugation tubes were required. The sample was centrifuged for 15 min at 20°C at 3000 rpm. The supernatant was transferred to a plastic vessel, the solid part remained in centrifuge tube and was weighed to determine the remaining volume of sample material. To find out the real amount of extract, the vessel was weighed before and after supernatant transfer. For storage 800 μl of nitric acid were added and the closed vessel was put to a fridge at 4°C. It has been found that this is the optimal storage temperature [24].

There were 6 manual extractions performed at the same time – 4 with the soil and 2 without it (blanks). This is one way how to get the same conditions for one set of samples (time and room temperature are the same). Blank samples were treated like the real ones, stirred, centrifuged, acidified.

The manual procedure was done in total four times – with two different concentrations of extractants (acetic acid 0.11 M and 0.43 M) and with two different soil samples (soils taken at two different places in Mallorca).
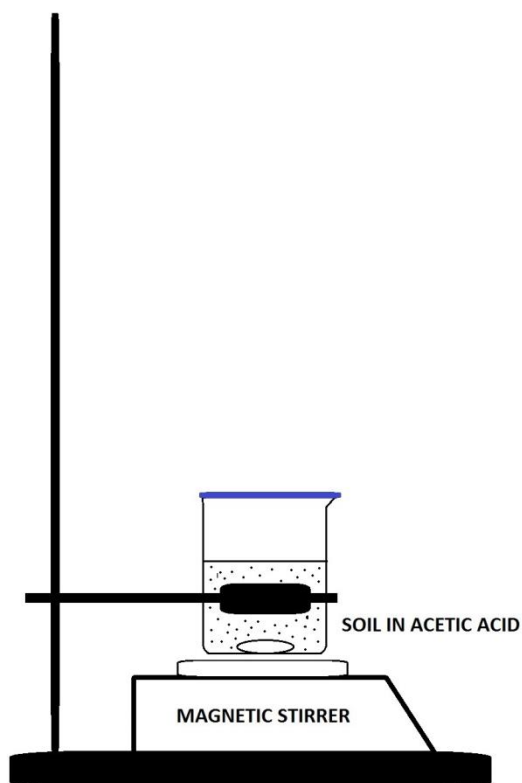
Figure 3: Apparatus used for manual extractions

6.2 Flow extraction

The main difference of dynamic extraction to the former one is that the method is based on non-equilibrium conditions.

The microcolumn was stored in a nitric bath to avoid contamination of metals and to be perfectly cleaned between two consecutive extractions. The dry column was put together with filters (0.45 μm and 0.5 g of soil material. Before, the sample bottle was shaken for 2 min to homogenize the content. Then microcolumn was closed and connected to the apparatus used for the dynamic extraction showing schematically in figure 4.

To the end of whole system another filter was added (Fig. 4). The acetic acid was aspirated into the syringe. The multisyringe pump was used as a single syringe pump. It was used because a single syringe pump was not available. On the top of the syringe

there was a valve. This valve allows changing connections. There are two possibilities. The first possibility is to connect pump with a reservoir of the extractant. In this position it is possible to aspirate the solution or return it back to the reservoir. The second position allows to release the extractant into the system [25].

Fractions were collected in plastics tubes, which were weighed before. 40 replicates were performed from each sample with an extraction volume of 5 ml each one., flow rate of 1.5 ml/min for soil SN6 and 1.2 ml/min for soils SN3 – SN4. In addition, two blank extractions that contain just extractant were done. The time to collect one fraction was 230 s. The fractions were collected manually and then tubes were weighed again to know the real amount of fractions.

The pH of the fractions was measured and then the same acidification process as above was performed i.e. addition of 100 µl of a nitric acid. The tubes were stored in a fridge (4°C) until the measurement.
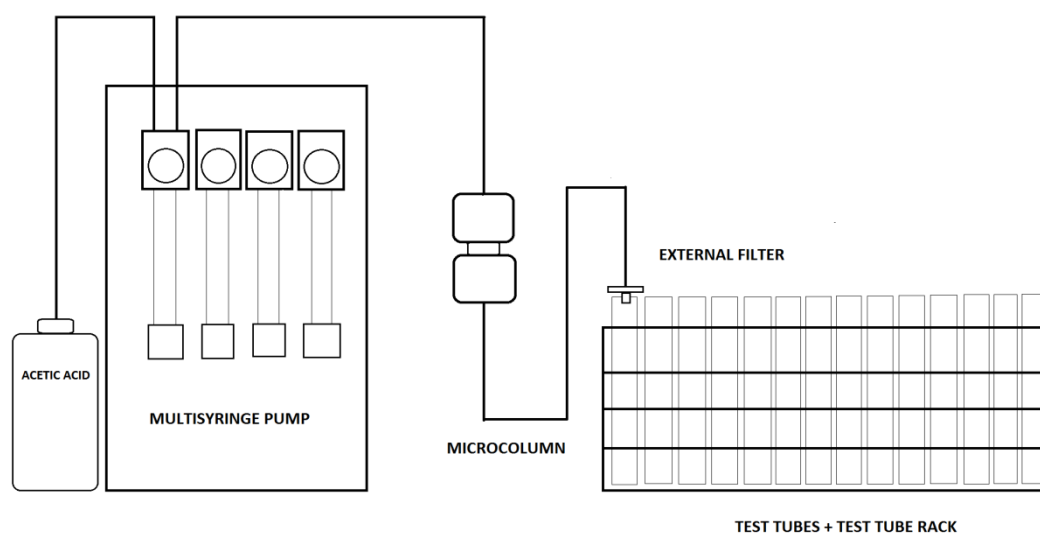
Figure 4: Apparatus used for flow extraction – The acetic acid was aspirated by multisyringe pump. Then the extractant got into the microcolumn and finally into the tube. Before entering the tubes it had to get through external filter.

7 Conditions

7.1 Conditions for manual extraction

Sample weight

For manual extraction 1.0000g was tested [23].

Amount of extractant

40 ml of acetic acid were used to extract heavy metals from the soil [23].

Stirring time

Stirring for 16 hours [23].

pH

The value of pH was measured at the beginning of experiment (acetic acid alone) and at the end (acetic acid with soil) [26].

Centrifugation

3000 rpm, 15 min, 20°C – these conditions proved to be sufficient enough during optimization.

7.2 Conditions for dynamic extraction

Sample weight

For flow extraction 1 g of soil was tested as first. This amount caused so high back pressure that extensive leaking occurred. For this reason, the sample amount was decreased to 0.5 g.

Amount of extractant

5 ml of acetic acid each round, 40 replicates.

Flow rate

3 ml/min. [27] seemed to be too high (after few minutes the soil occurred in the tubes and nothing remained in the microcolumn), so the flow rate was lowered to 2.5 ml/min, 2 ml/min and finally to 1.5ml/min.

pH

The same reason as above. The pH value was measured in the acetic acid before the experiment and then after extraction before the acidification with nitric acid.

Filter

The biggest problem was the fact that almost the whole amount of soil got off the column during the extraction. For this reason, an external filter was added at the end of apparatus to prevent entering of soil to the tubes.

8 Results and discussion

The summary of results (concentration – weight of metal (mg) in 1 kg of soil) is shown in tables 1 and 2. For manual extraction, these numbers are average values from 4 measurements of manual extraction and 3 measurements in the case of flow extraction.

Graphs 1-24 show the amount of extracted metals in each replicate. Graphs 25-46 show the extraction kinetics.

Table 3: Summary of results for soil SN6

SN6	manual - 0.11 M (mg/kg)	manual - 0.43 M (mg/kg)	flow - 0.11 M (mg/kg)	flow - 0.43 M (mg/kg)
Cr	0.0000	2.6139	2.0040	0.2076
Pb	0.0000	52.9511	48.6217	98.6876
Cu	2.0505	27.2192	65.9906	55.6875
Zn	0.6127	0.4173	53.5221	72.4326
Cd	0.0000	0.4173	1.3133	0.0117
Ni	0.8512	13.8503	4.9185	3.9057

Table 4: Summary of results for soil SN3 – SN4

SN3 - SN4	manual - 0.11 M (mg/kg)	manual - 0.43 M (mg/kg)	flow - 0.11 M (mg/kg)	flow - 0.43 M (mg/kg)
Cr	0.0126	0.9176	0.0000	0.1010
Pb	0.0000	0.2602	0.0000	3.9690
Cu	11.5896	499.3104	197.0233	368.6460
Zn	2.3888	93.9093	36.5110	63.6542
Cd	0.0000	0.1480	0.0000	0.2106
Ni	0.1594	0.4233	0.0000	1.1757

8.1 Results of manual and dynamic extractions

The figures 5-48 clearly show that the reproducibility of extracted amount of metals is low. This can be caused by the small amount of soil used for the extractions or by inhomogeneity of the sample material. The samples were stored in 400 ml plastic bottles and gravity causes sedimentation. There was very important step to shake the bottle fist before the experiment.

While using the microcolumn, the soil got off and was held in the external filter. It could be caused by the fact that the size of filter was big, flow rate was too high or does not seal enough. This may be the reason why the results are not very reproducible. In each case soil remained in the column for different time.

Results were considered repeatable when the relative deviation was 10% or less.
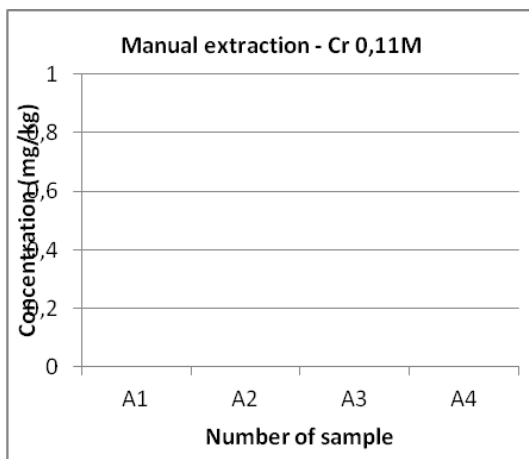
Figure 5: Manual extraction with 0.11 M acetic acid (soil SN6)

The presence of Cr was not proven. The concentration was under the detection limit in all repetitions.
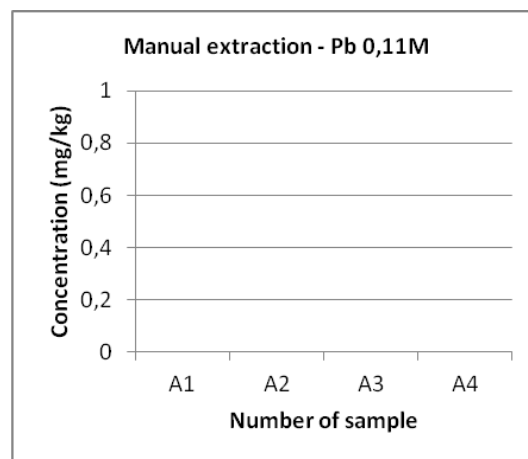
Figure 6: Manual extraction with 0.11 M acetic acid (soil SN6)

The presence of Pb was not proven. The concentration was under the detection limit in all repetitions.
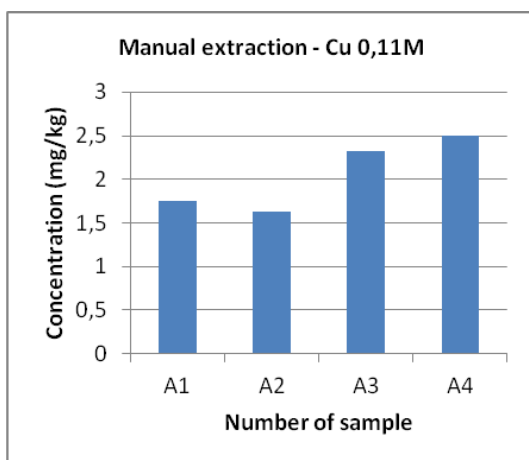
Figure 7: Manual extraction with 0.11 M acetic acid (soil SN6)

Low concentrations were detected in each repetition but the reproducibility is not sufficient.
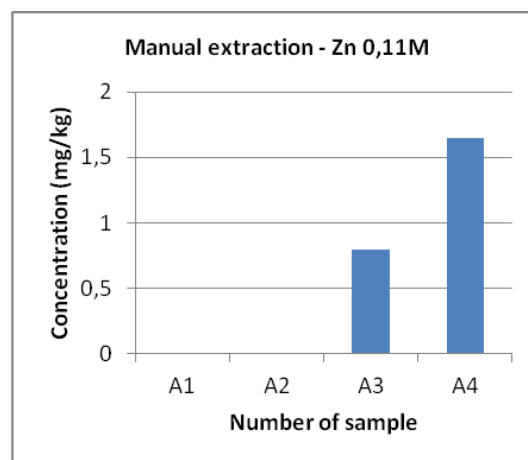
Figure 8: Manual extraction with 0.11 M acetic acid (soil SN6)

Low concentrations of Zn were detected in two repetitions but the reproducibility is not sufficient.
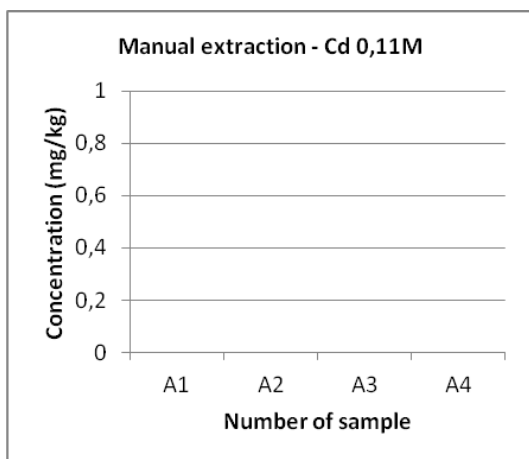
Figure 9: Manual extraction with 0.11 M acetic acid (soil SN6)
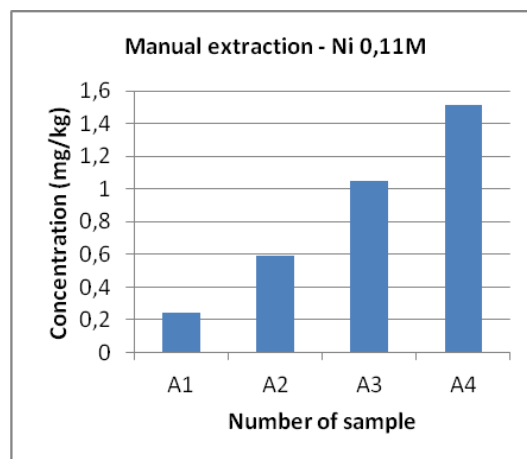
Figure 10: Manual extraction with 0.11 M acetic acid (soil SN6)

The presence of Cd was not proven. The concentration was under the detection limit in all repetitions.
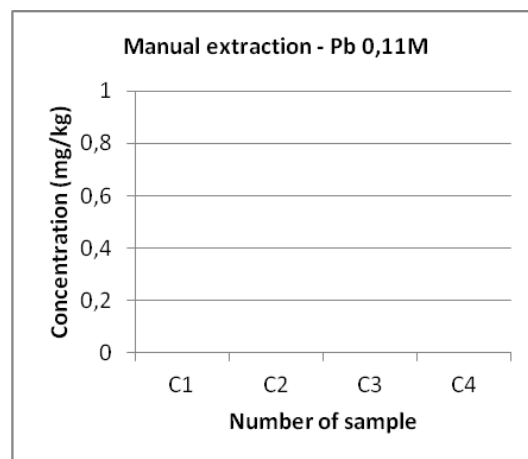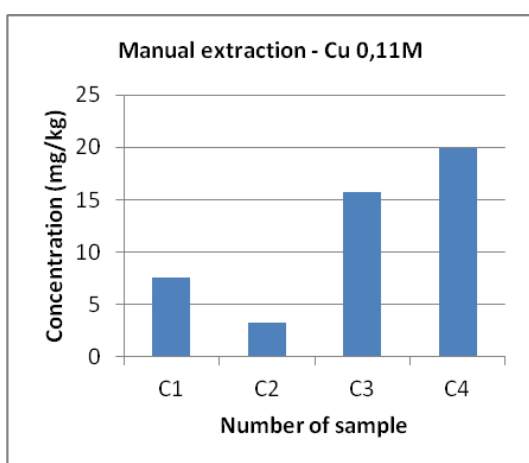
Low concentrations of Ni were proven in each replicate but the reproducibility is not sufficient.
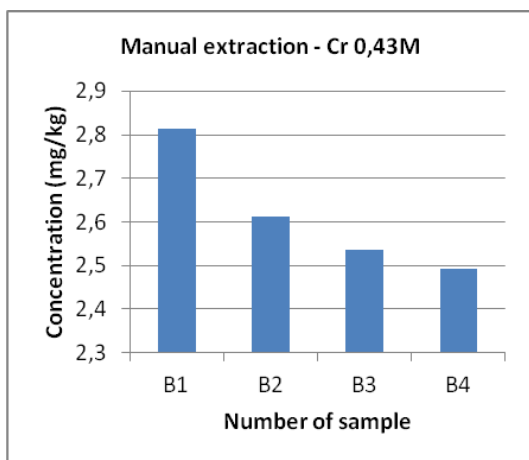
Figures 11: Manual extraction with 0.43 M acetic acid (soil SN6)

Figures 12: Manual extraction with 0.43 M acetic acid (soil SN6)

Low concentrations of Cr were proven in each replicate but the reproducibility is not sufficient.
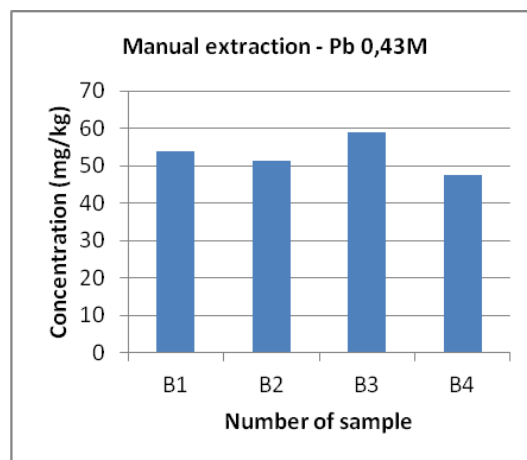
High concentrations of Pb were proven in each replicate with a good reproducibility. The concentration is higher than normal permitted level in soil.
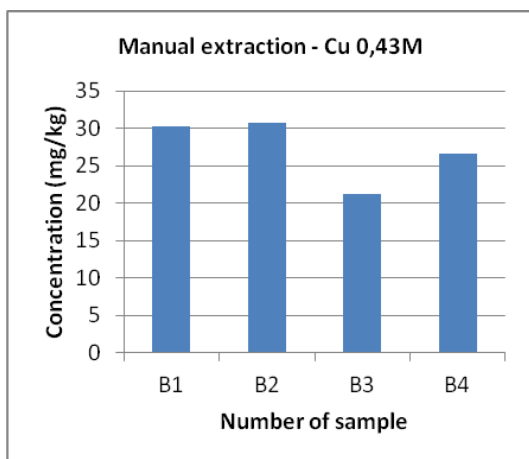
Figures 13: Manual extraction with 0.43 M acetic acid (soil SN6)

High concentrations of Cu were proven in each replicate, but with no significant reproducibility. The concentration is higher than normal permitted level in soil.
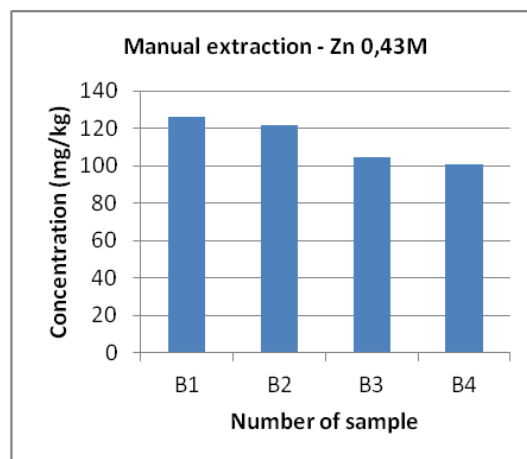
Figures 14: Manual extraction with 0.43 M acetic acid (soil SN6)

High concentrations of Zn were proven in each replicate, but with a good reproducibility. The concentration is higher than normal permitted level in soil.
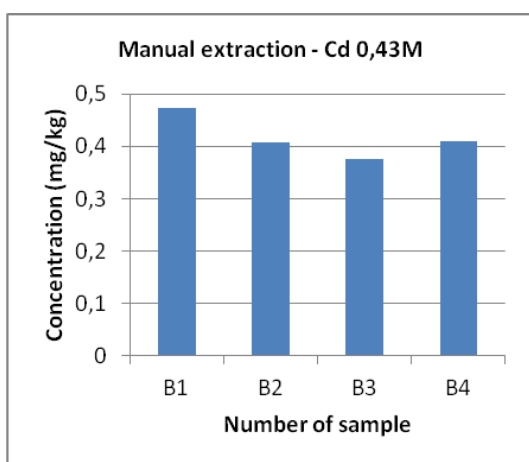
Figures 15: Manual extraction with 0.43 M acetic acid (soil SN6)

Normal concentrations of Cd were proven in each replicate with a good reproducibility. The concentration is higher than normal permitted level in soil.
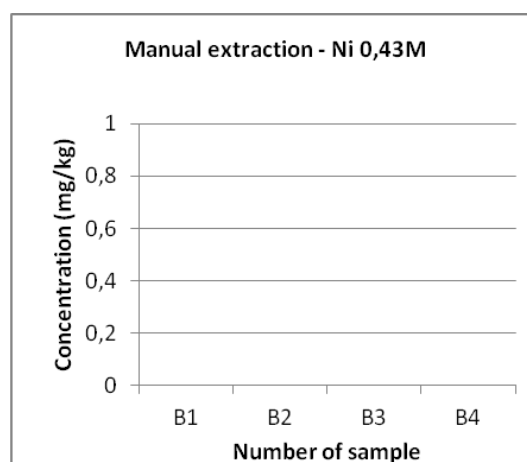
Figures 16: Manual extraction with 0.43 M acetic acid (soil SN6)

The presence of Ni was not proven. The concentration was under the detection limit in all repetitions
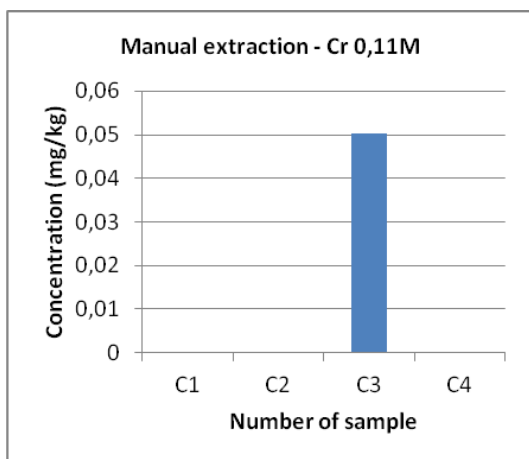
Figure 17: Manual extraction with 0.11M acetic acid (soil SN3-SN4)

Figure 18: Manual extraction with 0.11M acetic acid (soil SN3-SN4)

The concentration of Cr was in one case over the detection limit but the amount of the metal in the sample was very low.

The presence of Pb was not proven. The concentration was under the detection limit in all repetitions.

Figure 19: Manual extraction with 0.11M acetic acid (soil SN3-SN4)

Figure 20: Manual extraction with 0.11M acetic acid (soil SN3-SN4)

High concentrations of Cu were proven in each replicate, but with no significant reproducibility. This concentration was not higher than permitted, so it was considered as not contaminated.

Zn was detected only in 2 replicates and the concentrations were markedly lower than maximum permitted level.
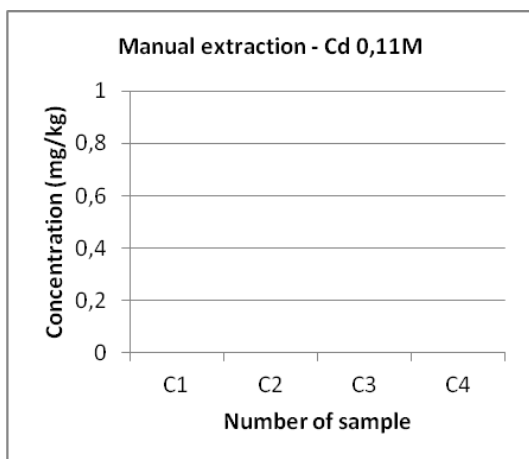
Figure 21: Manual extraction with 0.11M acetic acid (soil SN3-SN4)

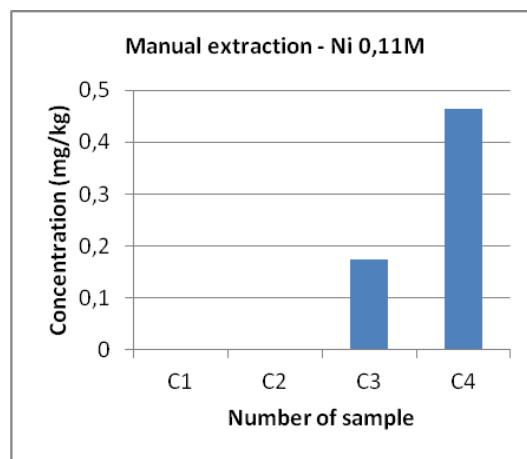The concentration of Cd was below the detection limit in all repetitions.

Figure 22: Manual extraction with 0.11M acetic acid (soil SN3-SN4)

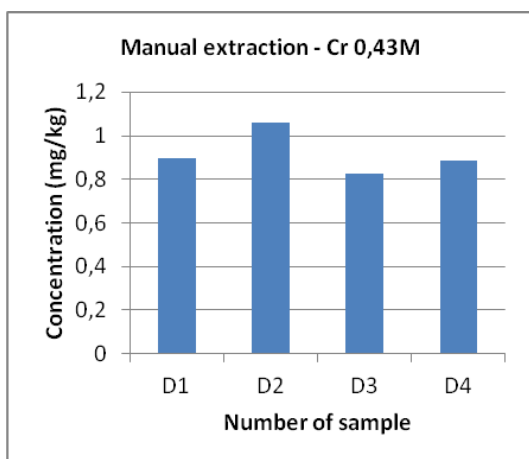Ni was detected only in two replicates but in low concentrations.

Figure 23: Manual extraction with 0.43M acetic acid (soil SN3-SN4)

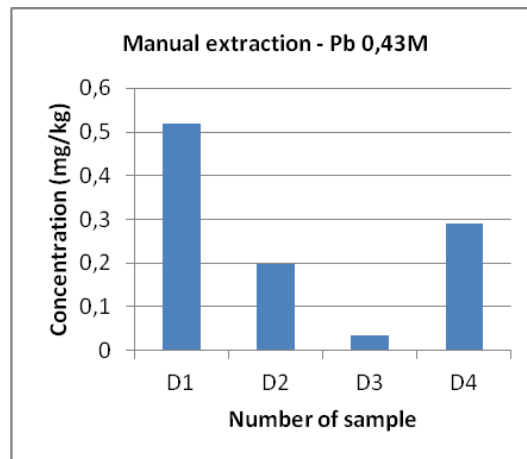Low concentrations of Cr were proven in each replicate with a good reproducibility.

Figure 24: Manual extraction with 0.43M acetic acid (soil SN3-SN4)

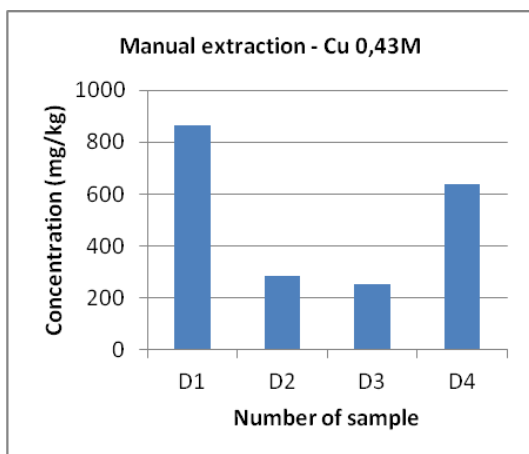Low concentrations of Cr were detected in each replicate but with no significant reproducibility.

Figure 24: Manual extraction with 0.43M acetic acid (soil SN3-SN4)

High concentrations of Cu were measured in each repetition nut with no significant reproducibility. These concentrations were higher than maximum permitted level so the soil was potentially contaminated.
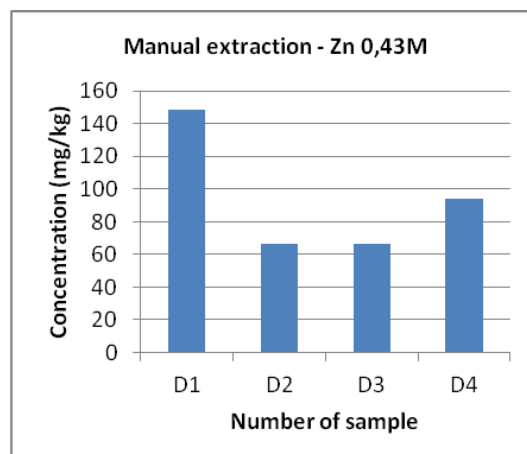

Figure 24: Manual extraction with 0.43M acetic acid (soil SN3-SN4)

High concentrations of Zn were measured in each repetition with no significant reproducibility. These concentrations were higher than maximum permitted level so the soil was potentially contaminated.
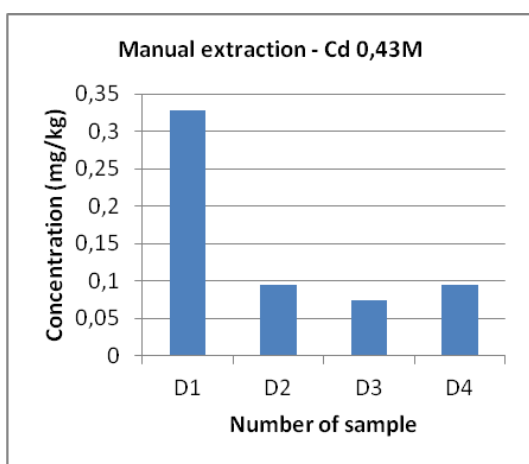

Figure 24: Manual extraction with 0.43M acetic acid (soil SN3-SN4)

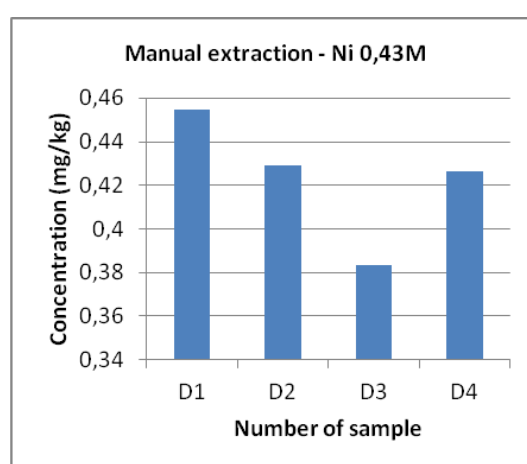Very low concentrations of Cd were detected with no significant reproducibility.

Figure 24: Manual extraction with 0.43M acetic acid (soil SN3-SN4)

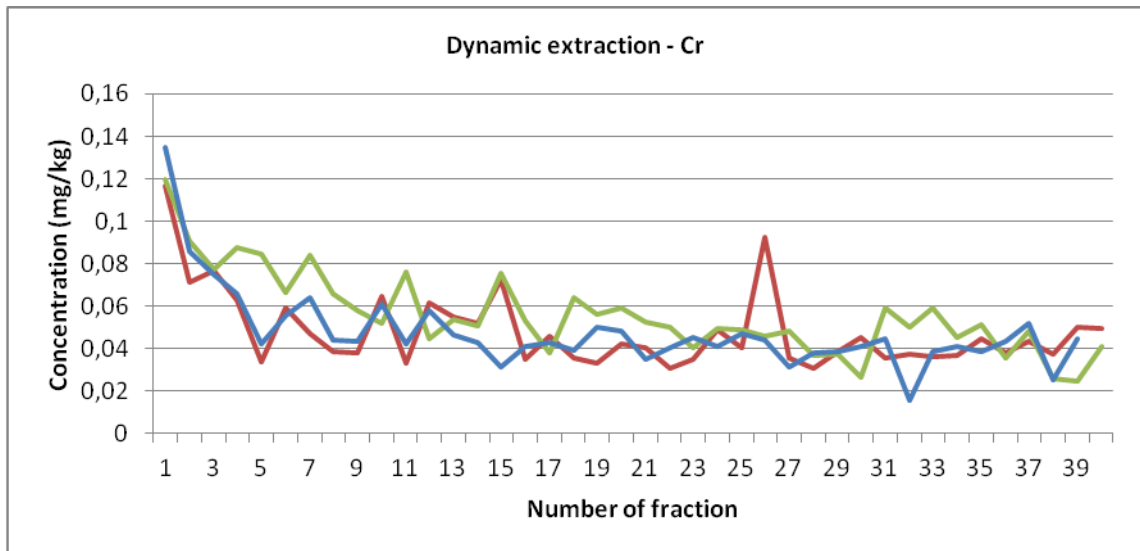Very low concentrations of Ni were detected with a good reproducibility.

Figure 25: Dynamic extraction with 0.11 M acetic acid (soil SN6)

Concentrations of Cr in all replicates are low. These measurements were performed with a good reproducibility. Tube number 26 could be little contaminated according to higher peak and also higher peak in following figures.
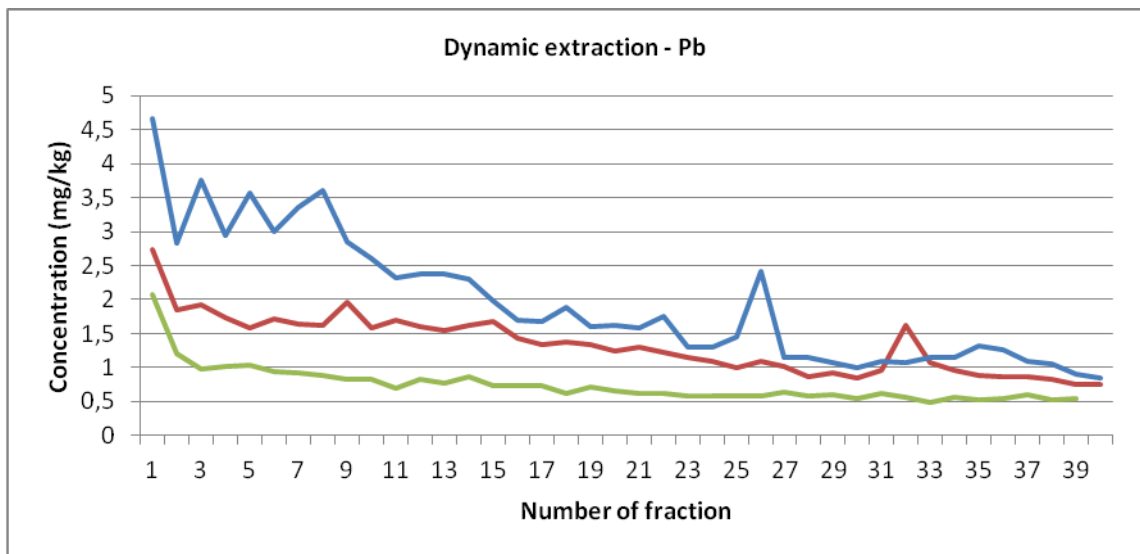

Figure 26: Dynamic extraction with 0.11 M acetic acid (soil SN6)

High concentrations of Pb were detected with no significant reproducibility.
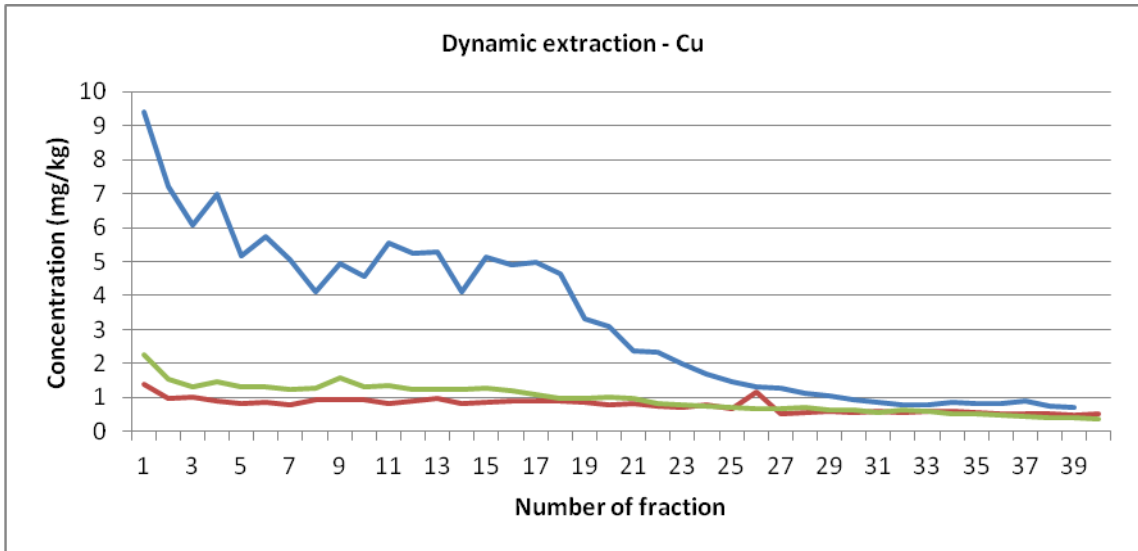
Figure 27: Dynamic extraction with 0.11 M acetic acid (soil SN6)

High concentration of Cu was detected only in one replicate. In the others there were much lower values measured so the reproducibility is also very low.
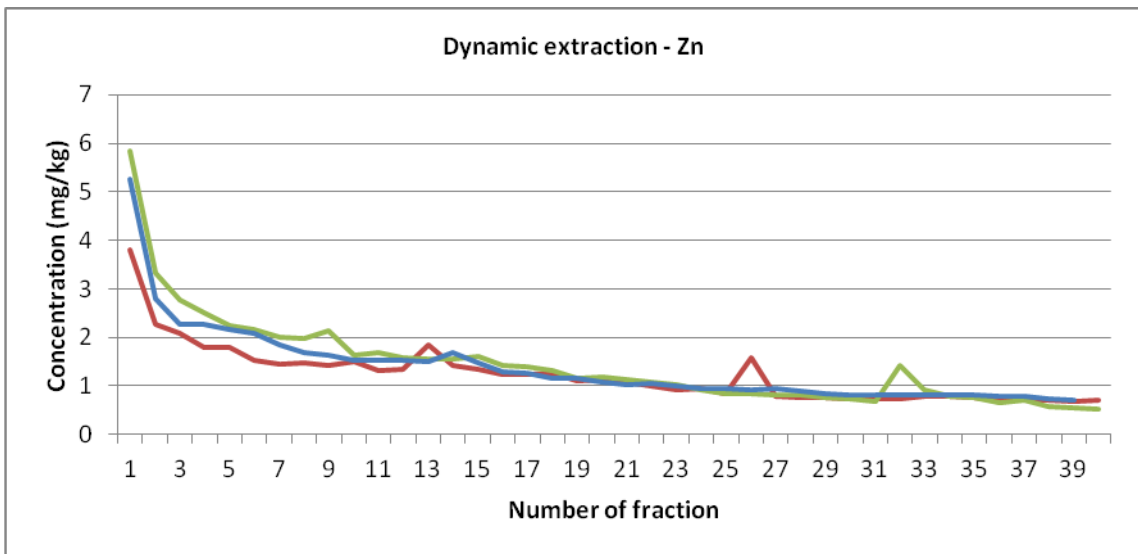

Figure 28: Dynamic extraction with 0.11 M acetic acid (soil SN6)

High concentrations of Zn were detected but it did not reach the permitted limit for Zn in soil.

Figure 29: Dynamic extraction with 0.11 M acetic acid (soil SN6)

Low concentrations of Cd were detected in all replicates with a good reproducibility. This concentration is on the border of permitted concentration.
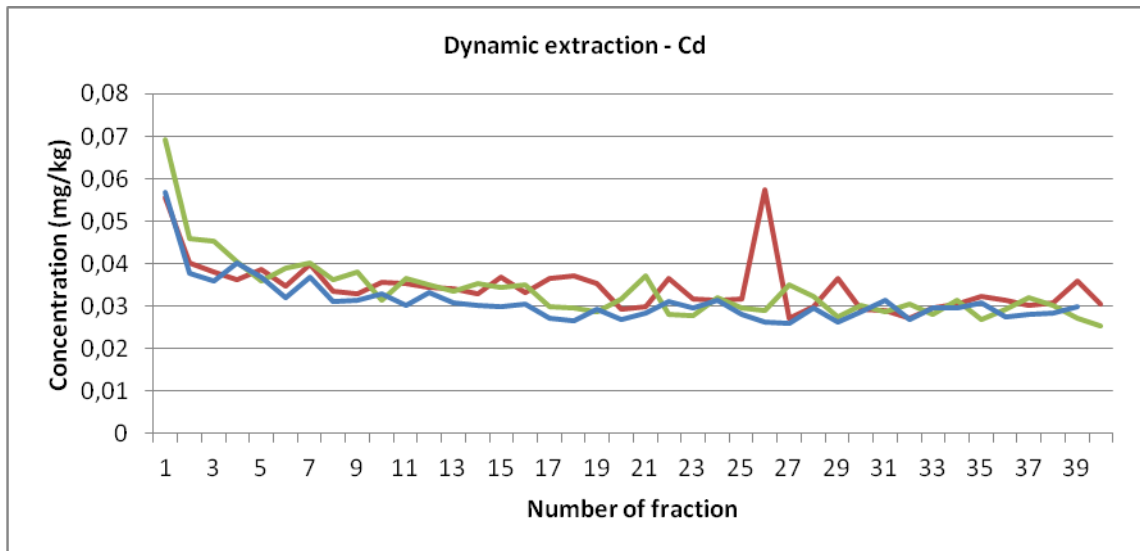

Figure 30: Dynamic extraction with 0.11 M acetic acid (soil SN6)

Low concentrations of Ni were detected in all replicates with a good reproducibility.

Figure 31: Dynamic extraction with 0.43 M acetic acid (soil SN6)

Very low concentrations of Cr were detected.

Figure 32: Dynamic extraction with 0.43 M acetic acid (soil SN6)

High concentrations of Pb were measured in all replicates. Tube number 14 of one replicate was probably contaminated because high concentrations of all metals were detected (it is obvious from the following figures).
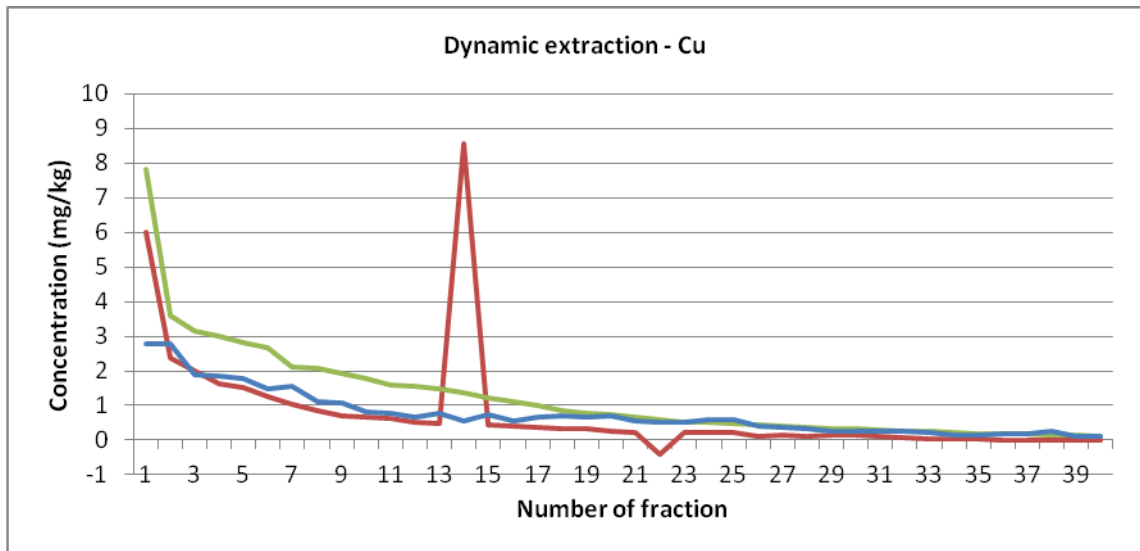
Figure 33: Dynamic extraction with 0.43 M acetic acid (soil SN6)

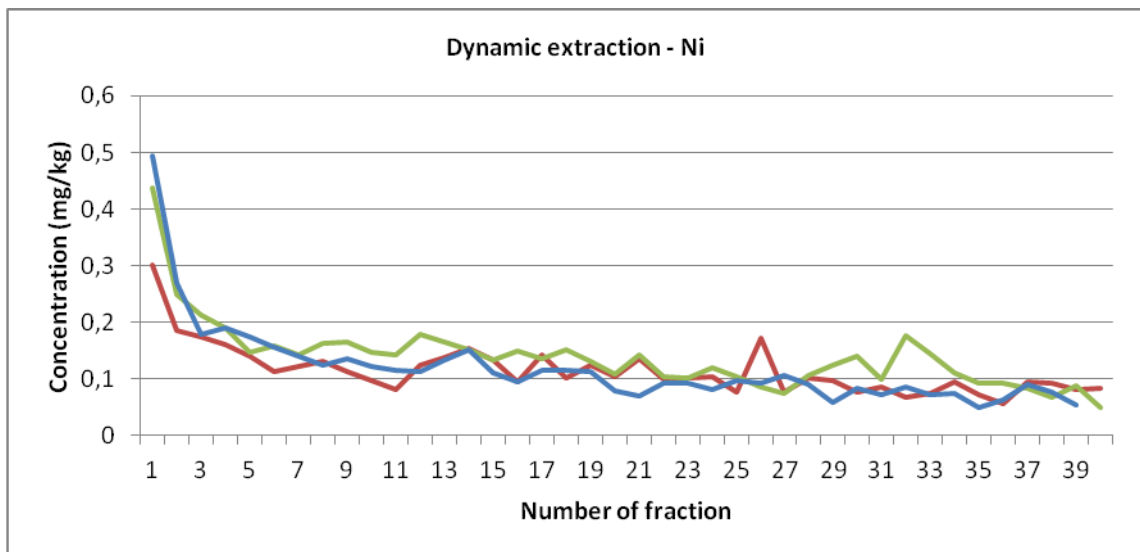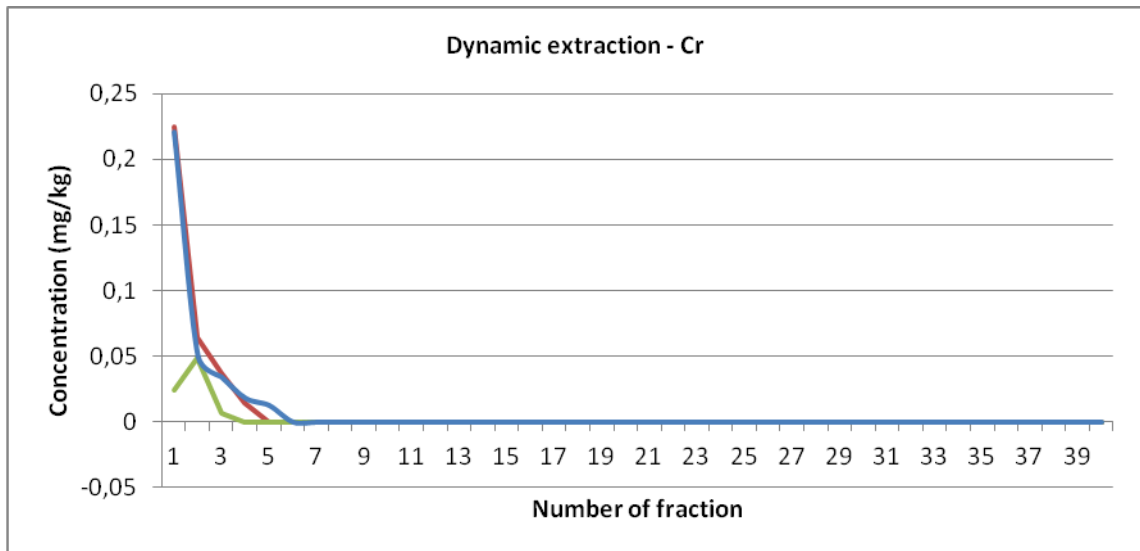High concentrations of Cu were detected with low reproducibility. These concentrations were on the border of permitted limit in soil.
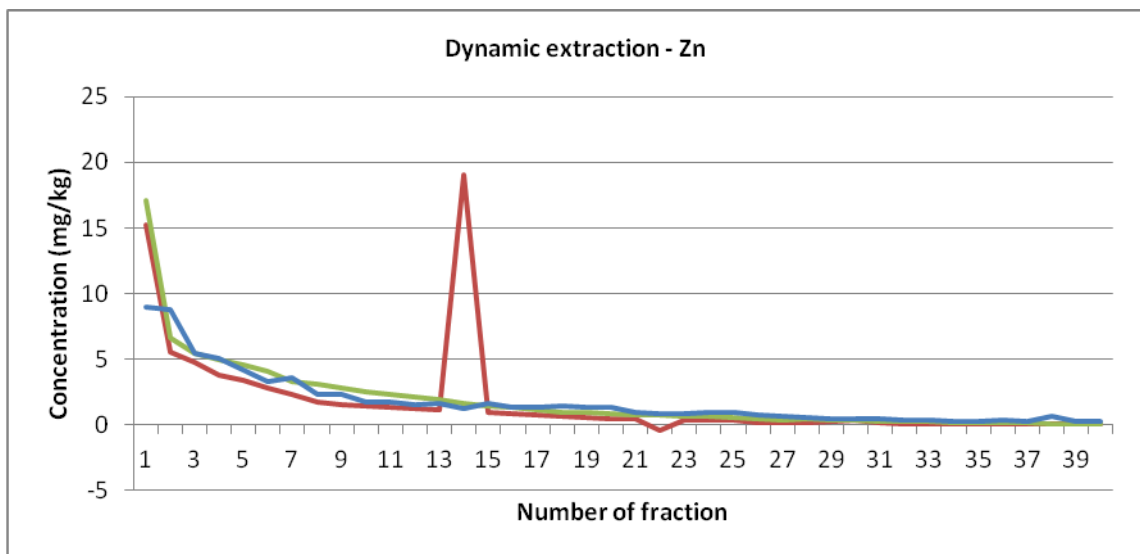
Figure 34: Dynamic extraction with 0.43 M acetic acid (soil SN6)

High concentrations of Zn were detected with a good reproducibility. These concentrations did not reach the maximal permitted level.
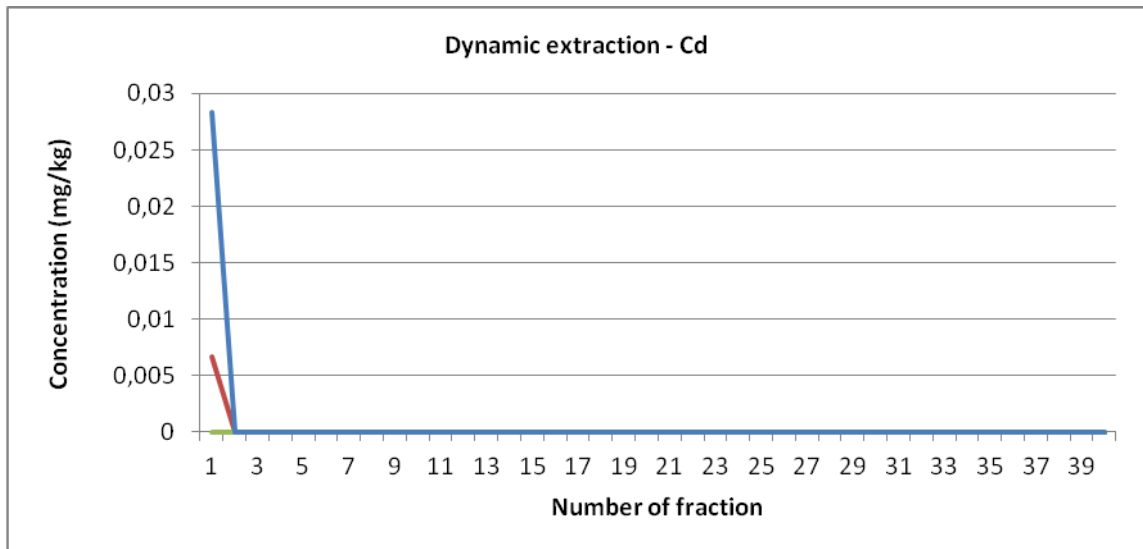
Figure 35: Dynamic extraction with 0.43 M acetic acid (soil SN6)

Very low concentrations were detected in two replicates. Results were reproducible.
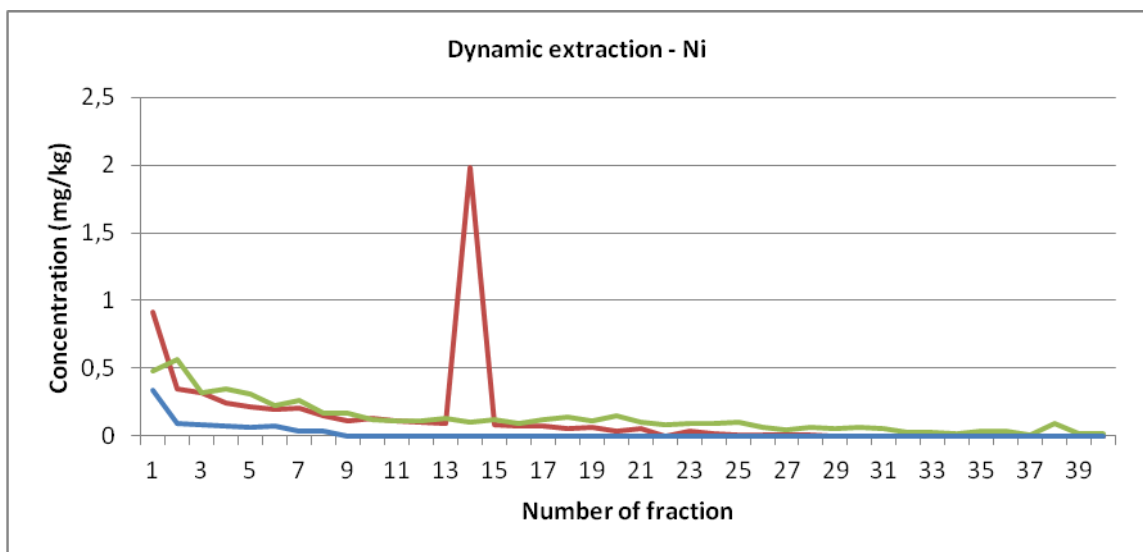
Figure 36: Dynamic extraction with 0.43 M acetic acid (soil SN6)
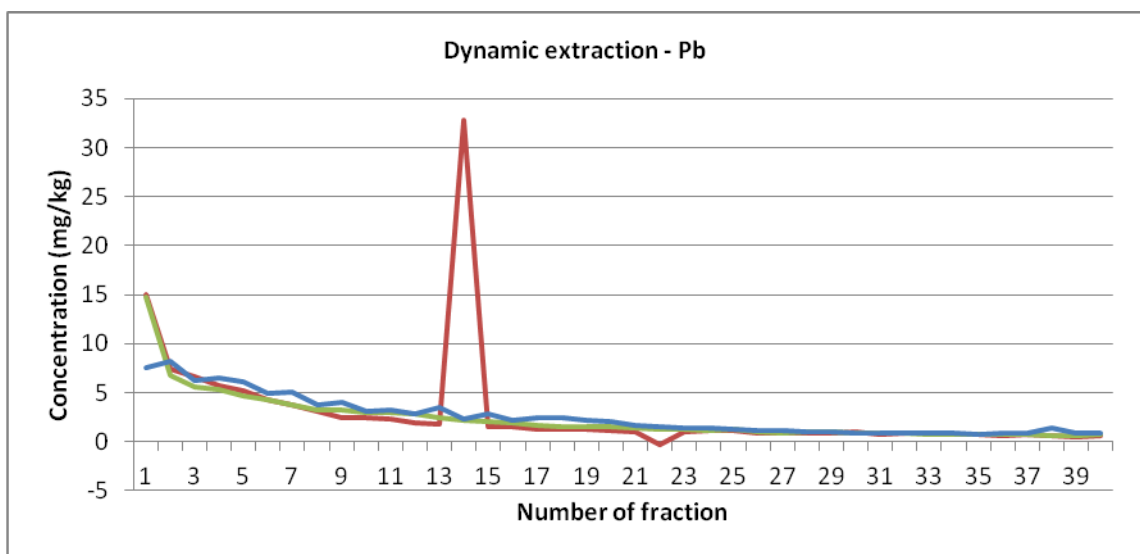
Low concentrations of Ni were detected. The reprodubility was affected mostly by the high peak in tube 14 (the same as above).
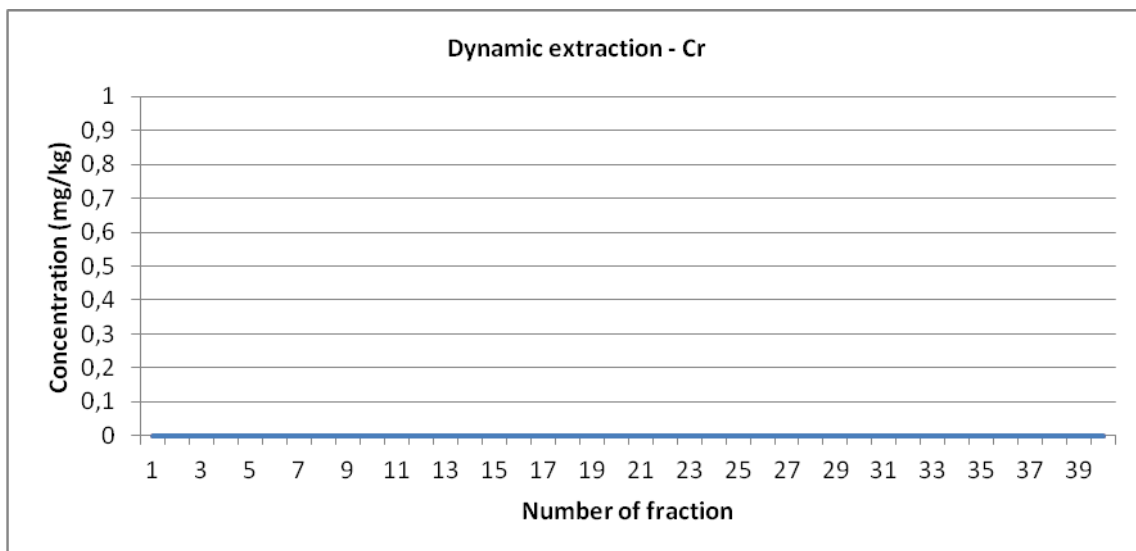
Figure 37: Dynamic extraction with 0.11 M acetic acid (soil SN3-SN4)

The presence of Cr was not proven. The concentration was under the detection limit in all repetitions.
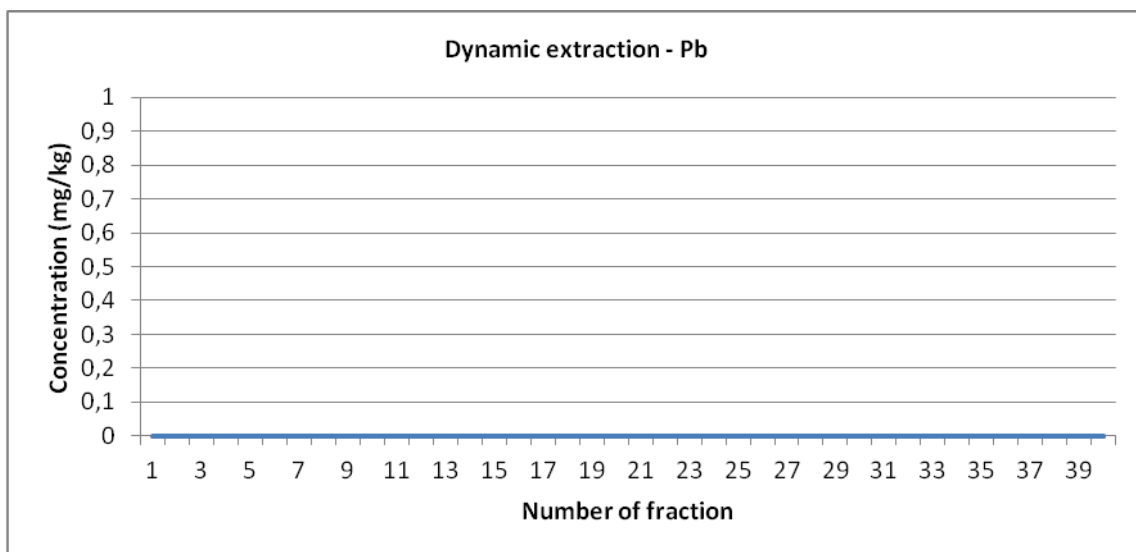
Figure 38: Dynamic extraction with 0.11 M acetic acid (soil SN3-SN4)

The presence of Pb was not proven. The concentration was under the detection limit in all repetitions.

Figure 39: Dynamic extraction with 0.11 M acetic acid (soil SN3-SN4)

High concentrations of metals were detected with no significant reproducibility. The extraction process was different this time because extracted maximum was not measured in the first tube.
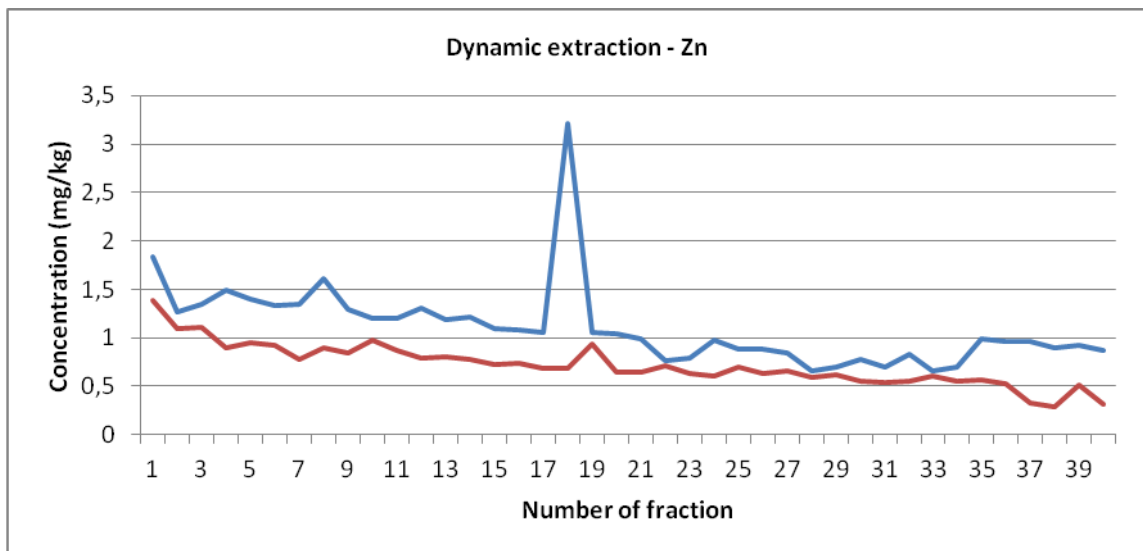
Figure 40: Dynamic extraction with 0.11 M acetic acid (soil SN3-SN4)

Low concentrations of Zn were extracted. The tube number 18 was probably contaminated because the extracted amount of Zn is even higher than in the firs fraction.
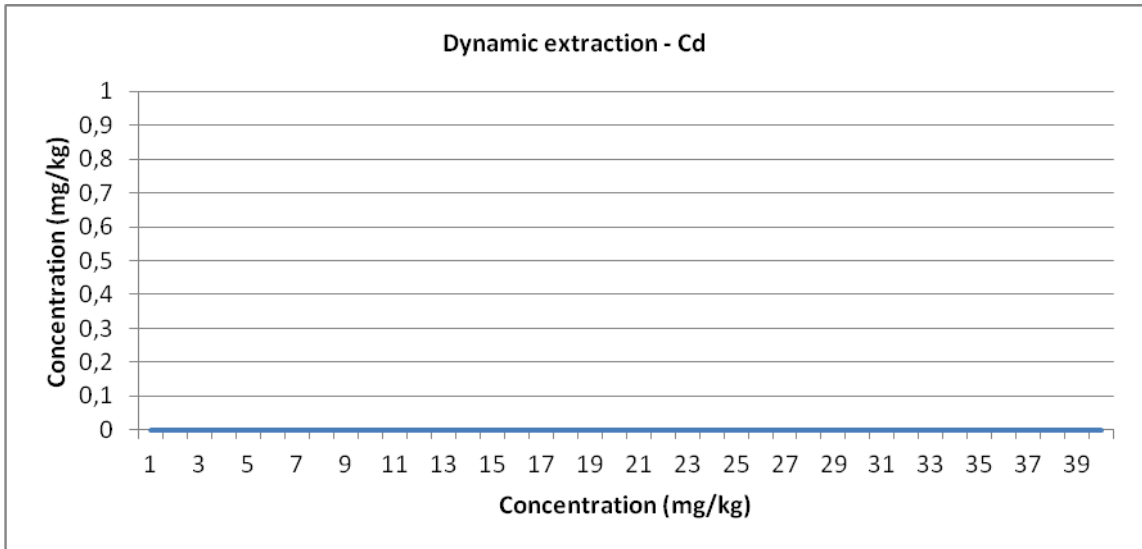
Figure 41: Dynamic extraction with 0.11 M acetic acid (soil SN3-SN4)

The presence of Cd was not proven. The concentration was under the detection limit in all repetitions.
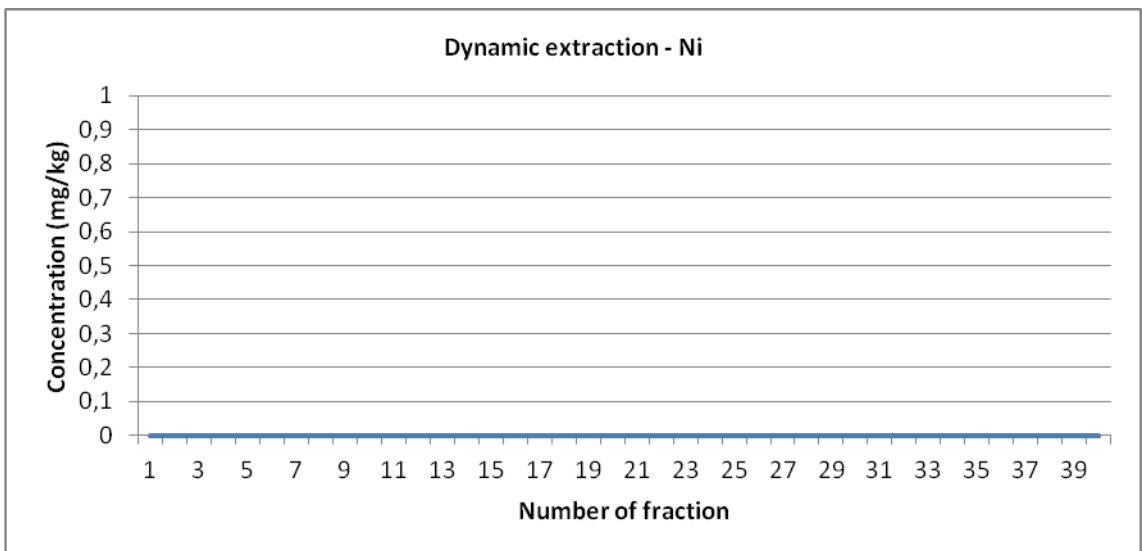
Figure 42: Dynamic extraction with 0.11 M acetic acid (soil SN3-SN4)

The presence of Ni was not proven. The concentration was under the detection limit in all repetitions.
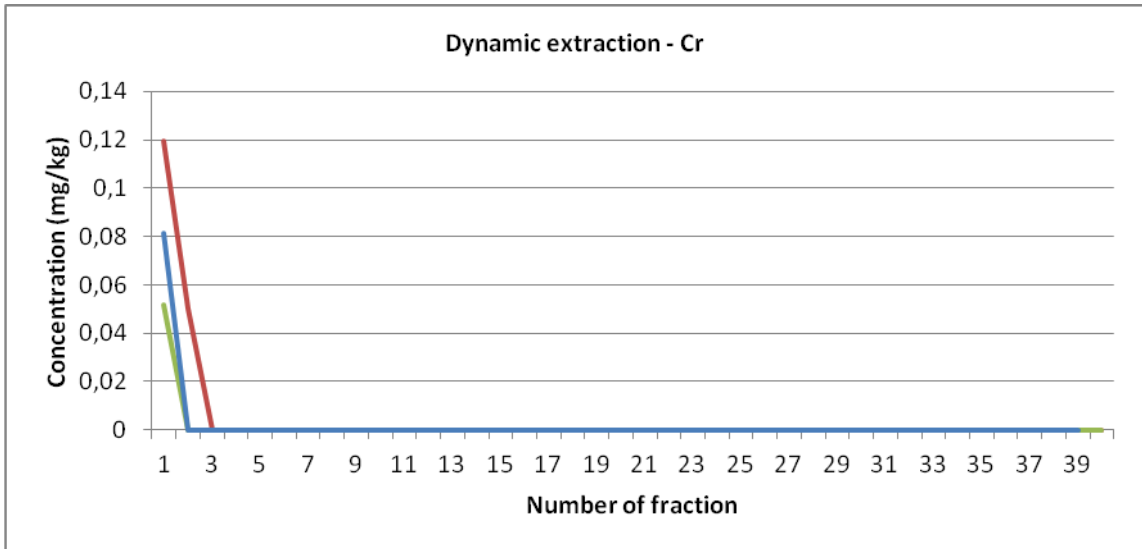
Figure 43: Dynamic extraction with 0.43 M acetic acid (soil SN3-SN4)

Very low concentrations of Cr were detected.
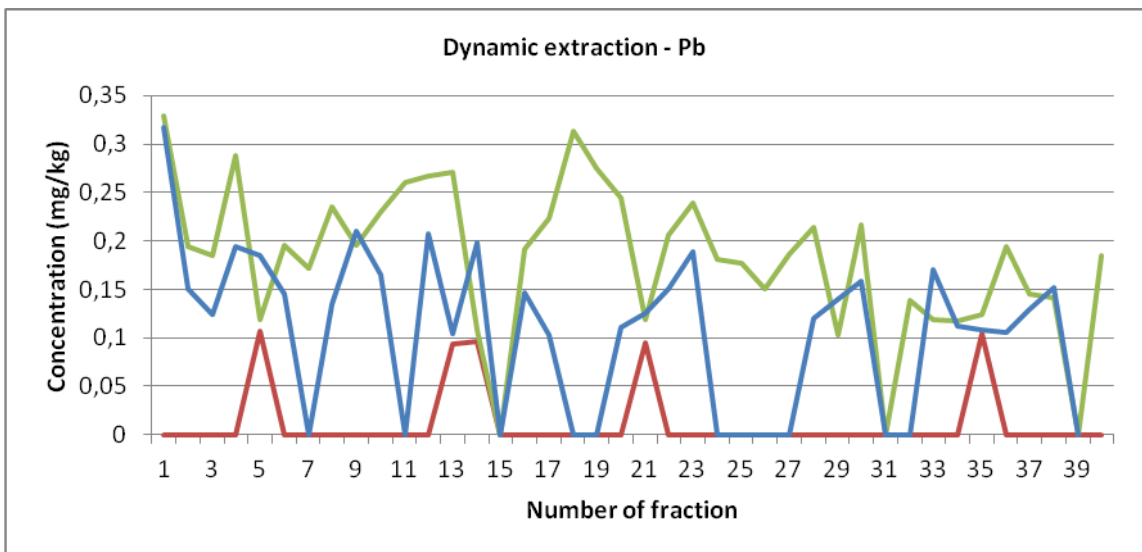
Figure 44: Dynamic extraction with 0.43 M acetic acid (soil SN3-SN4)

Low concentrations of Pb were detected with no significant reproducibility. The curve shaves was not very reproducible.
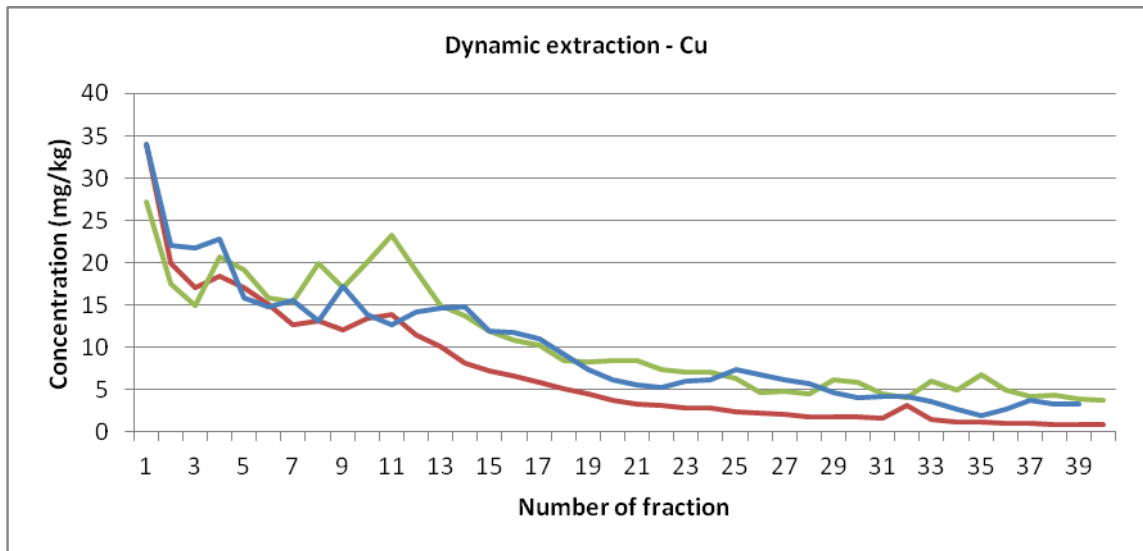
Figure 45: Dynamic extraction with 0.43 M acetic acid (soil SN3-SN4)

Very high concentrations of Cu were detected with low reproducibility. The extracted amount was higher than maximum permitted level so the soil was potentially contaminated.
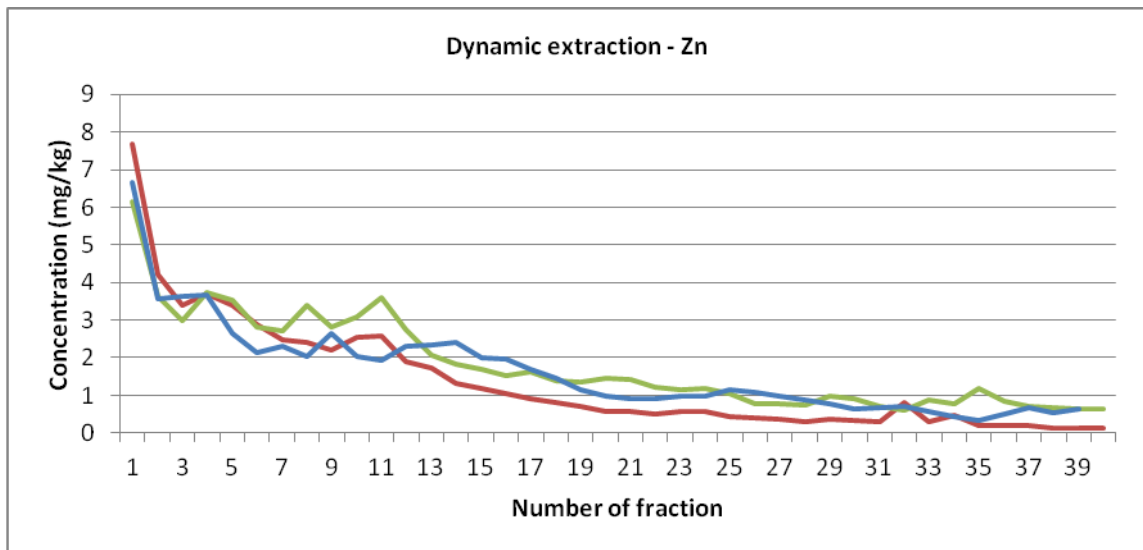
Figure 46: Dynamic extraction with 0.43 M acetic acid (soil SN3-SN4)

High concentrations of Zn were detected with low reproducibility. The concentrations did not reach the maximum permitted level.
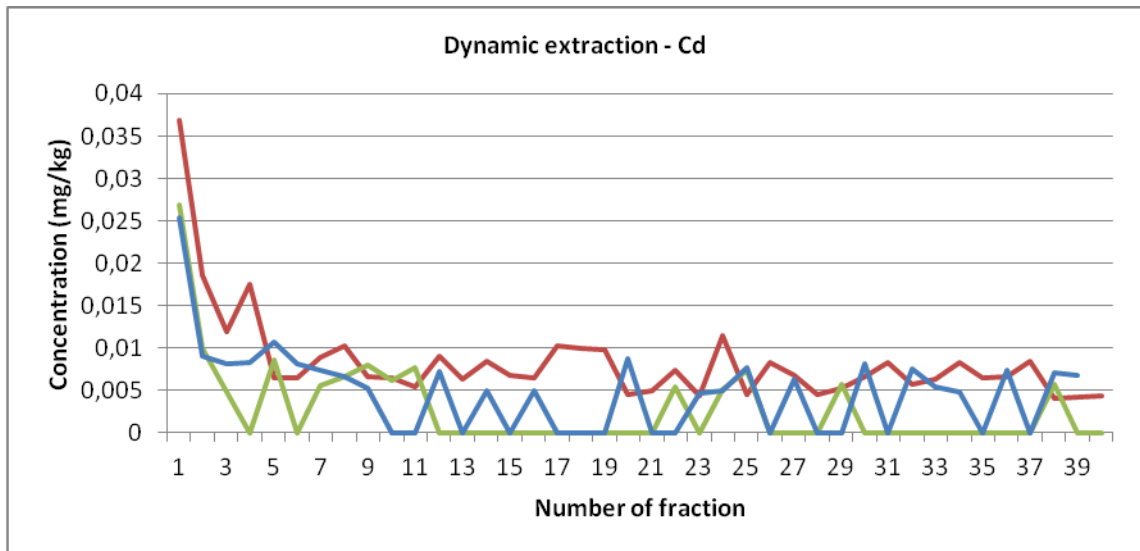
Figure 47: Dynamic extraction with 0.43 M acetic acid (soil SN3-SN4)

Very low concentrations of Cd were measured with low reproducibility. The values were low but also maximum permitted level was.
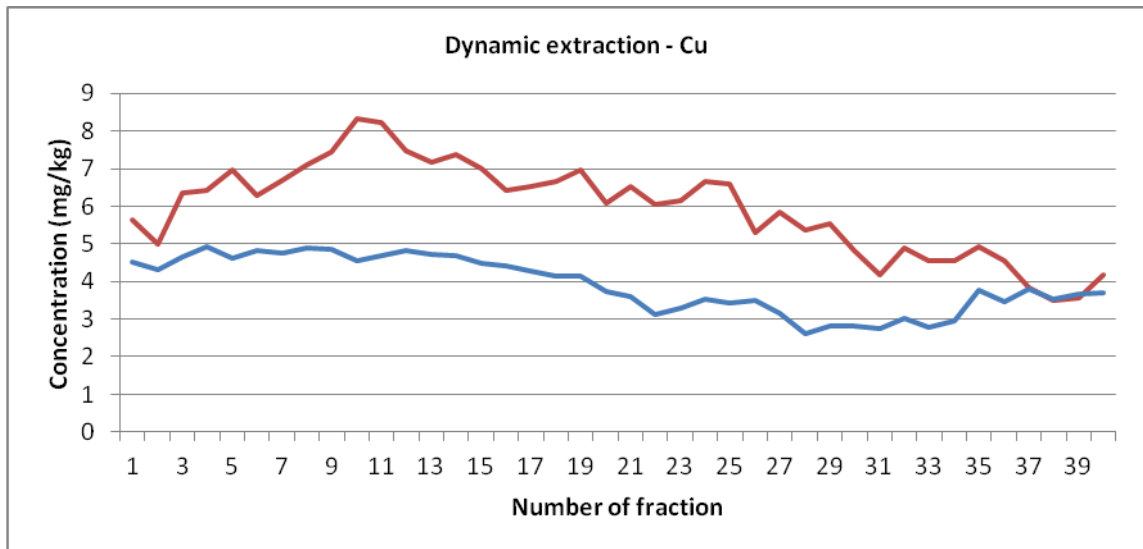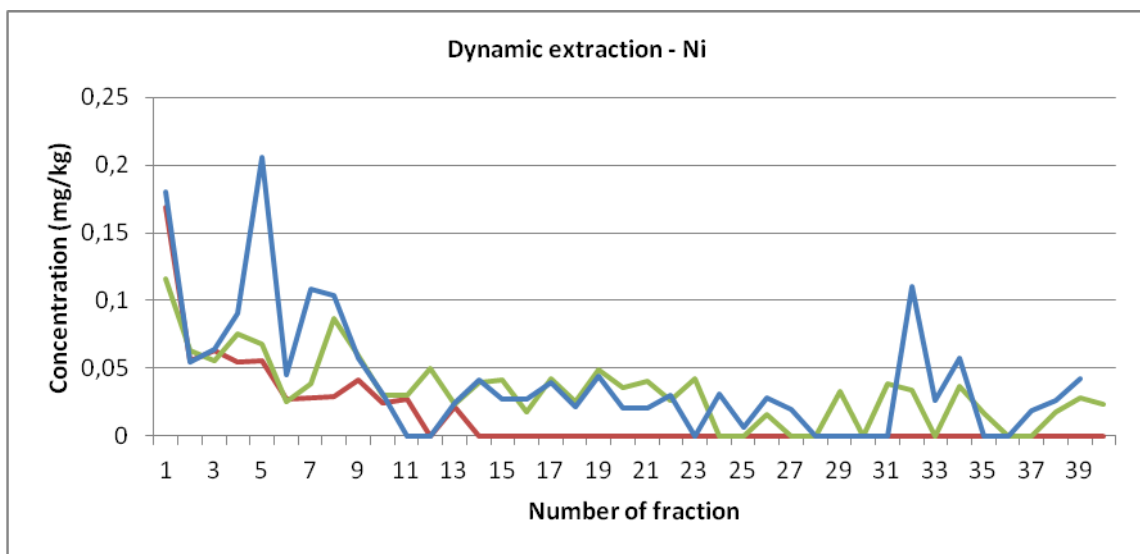

Figure 48: Dynamic extraction with 0.43 M acetic acid (soil SN3-SN4)

Low   concentrations   of   Ni   were   detected   with   low   reproducibility.

Table 5: pH before and after manual extraction – soil SN6

MANUAL	Number of sample	pH before extraction	pH after extraction
0.11M SN6	A1	2.75	7.50
	A2	2.75	7.20
	A3	2.75	7.24
	A4	2.75	7.15
0.43M SN6	B1	2.45	4.90
	B2	2.45	4.75
	B3	2.45	4.83
	B4	2.45	4.84

Table 6: pH before and after manual extraction – soil SN3 – SN4

MANUAL	Number of sample	pH before extraction	pH after extraction
0.11M SN3-SN4	C1	2.75	6.61
	C2	2.75	7.31
	C3	2.75	6.41
	C4	2.75	6.31
0.43M SN3-SN4	D1	2.46	4.34
	D2	2.46	4.38
	D3	2.46	4.33
	D4	2.46	4.36

Table 7: pH before and after dynamic extraction – soil SN3 – SN4

DYNAMIC	Number of sample	pH before extraction	pH after extraction
0.11 M SN3-SN4	E1	2.75	4.68
	E10	2.75	4.20
	E20	2.75	3.78
	E30	2.75	3.66
	E40	2.75	3.36
0.11 M SN3-SN4	F1	2.75	4.84
	F10	2.75	4.90
	F20	2.75	3.76
	F30	2.75	3.59
	F40	2.75	3.64
0.43 M SN3-SN4	A1	2.46	4.57
	A10	2.46	1.30
	A20	2.46	2.70
	A30	2.46	2.68
	A40	2.46	2.61
0.43 M SN3-SN4	B1	2.46	4.27
	B10	2.46	1.30
	B20	2.46	2.94
	B30	2.46	2.69
	B40	2.46	2.59
0.43 M SN3-SN4	C1	2.46	11.15
	C10	2.46	1.30
	C20	2.46	2.95
	C30	2.46	2.75
	C40	2.46	2.64

Table 7: pH before and after dynamic extraction – soil SN3 – SN4

Table 8: pH before and after dynamic extraction- soil SN6

DYNAMIC	Number of sample	pH before extraction	pH after extraction
0.11 M	A1	2.75	4.28
SN6	A10	2.75	3.95
	A20	2.75	3.75
	A30	2.75	3.58
	A40	2.75	3.51
0.11 M	B1	2.75	4.58
SN6	B10	2.75	4.20
	B20	2.75	3.72
	B30	2.75	3.54
	B40	2.75	3.35
0.11 M	C1	2.75	4.89
SN6	C10	2.75	4.70
	C20	2.75	3.71
	C30	2.75	3.54
	C40	2.75	3.37
0.43 M	X1	2.46	4.42
SN6	X10	2.46	3.29
	X20	2.46	3.10
	X30	2.46	3.30
	X40	2.46	2.84
0.43 M	Y1	2.46	3.78
SN6	Y10	2.46	3.50
	Y20	2.46	3.18
	Y30	2.46	2.92
	Y40	2.46	2.91
0.43 M	Z1	2.46	4.35
SN6	Z10	2.46	3.49
	Z20	2.46	3.11
	Z30	2.46	2.98
	Z40	2.46	2.98

8.2 Comparison of manual and dynamic extractions

8.2.1 Manual extraction – lower and higher pH of extractants

In the first moment it is noticeable that extractions with 0.11 M acetic acid (repetition A, C) show higher variability than the rest. In one repetition there was a zero amount of one metal and in another one this metal was detected. This observation indicates a low reproducibility and reliability. The concentrations of the metals of interest are also significantly lower than in case of 0.43 M acetic acid. This is probably caused by the buffering capacity of the soil. Basic oxides buffer the acidity of acetic acid and the metals that have the ability to be extracted after acidification could not be released. From the table 3 it is obvious that the final pH after extraction is neutral – the acetic acid was neutralized by basic substances (for example basic oxides in the soil sample).

The use of 0.43 M acetic acid seems more effective. In the series D repetitions D2 and D3 show clearly a lower content of all metals (lower than in repetition D1 and D4). This can be caused by an inhomogenous sample or inefficient stirring.

The higher concentration of acetic acid turned to be more effective. Extracted amount of metals was significantly higher. In case of 0.11 M acetic acid some metals (for example Pb in the soil SN3-SN4) did not even reach the detection limit to be detected. According to result obtained from the extraction with 0.43 M acetic acid it was clear that these metals are presented.

8.2.2 Dynamic extraction - lower and higher pH of extractants

It is logical to assume that the amount of extracted soil decreases with the number of fraction. However, according to the figures showing the extraction kinetics (Fig. 25-48), this assumption seems unjustified. When the soil contains around 1 mg/kg

of the metal (for example Ni), it is clear that the kinetic curve is not perfectly descending. These differences were not observed when the content is very high.

In the analysis of soil SN3-SN4 0.11 M tube number 18 appears to be contaminated and was considered as an outlayer and was therefore not taken into account. In case of soil SN6 there was no significant improvement while using higher concentration of acetic acid. On the contrary in case of the second soil there were almost double extracted amounts of metals. So it was also recommended to use 0.43 M acetic acid.

8.3 Manual and dynamic extraction

It is clear that the manual extraction with 0.11 M acetic acid is not effective because in some cases (for example for Pb and Zn in the soil SN6) the extracted amount did not even reach the detection limit. The concentrations were insufficient for quantification. On the other hand, a dynamic extraction with 0.11 M acetic acid shows better results. Much higher amounts of all metals are extracted.

Both manual and dynamic extractions with 0.43 M acetic detected the presence of metals. In case of the soils SN3-SN6, manual extraction shows even higher concentrations than the dynamic extraction. This can be caused for example by contamination of the extractant or laboratory glass or by an insufficient amount of fractions analyzed during dynamic extraction.

The operation of manual extraction was definitely simpler (equipment and process). Only one concentration one obtained and no information about the extraction kinetic was obtained. The sample did not completely dissolve.

8.4 Comparison of the used soil samples

There was not a significant difference between the results obtained for thee two tested soils. Soil SN6 reached the toxic level of Cu and Pb, soil SN3-SN4 was contaminated with Pb. The main difference is the content of individual metals that is shown in tables 1 and 2.

Table 9: Permitted levels of selected metals in Mallorca (taken from the requirements of the government of Balearic Islands) – Max normal - High threshold of what is considered normal background level, MTP – Maximal threshold permitted, LA – living areas, IA – industrial areas, AA – agriculture areas. When a measured concentration of the metal is lower than MAX normal, soil is not contaminated. When the concentration is between MAX normal and MTP, the soil is potentially contaminated and more studies should be done. If the concentration is higher than MTP, the soil is contaminated and other risk studies are required.

Metal	background level (mg/kg)	MAX normal (mg/kg)	MTP – LA (mg/kg)	MTP – IA (mg/kg)	MTP – AA (mg/kg)
Cd	0.46	0.7	2.5	70.0	3.0
Cr	35.0	59.0	200.0	700.0	140.0
Cu	18.5	32.0	125.0	1000.0	250.0
Ni	22.4	36.0	50.0	800.0	85.0
Pb	22.0	33.0	150.0	1000.0	250.0
Zn	49.4	90.0	250.0	3000.0	300.0

Both soils belonged to the category of the industrial areas. After comparison the results with Table 2 and 3 it was evident that the tested soil samples were not highly contaminated. The level of Cd is within the normal range with an exception of the flow extraction of the soil SN6 with 0.11 M acetic acid. Neither Cr nor Ni did exceed the normal level.

The soil SN6 contained possible contamination of Pb and Cu. Its levels outreached the maximum threshold of what is considered normal background level. The second soil sample (SN3-SN4) contained a risk concentration of Zn and Cu and was possibly contaminated. For this reason both soils should be studied in detail.

9 Conclusions

In conclusion it was found that both tested methods for soil analysis gave better results when using the higher concentration of the tested extractant acetic acid. The manual extraction method was more time-consuming and less reproducible in the obtained results. The pH value of the extractant after manual extraction with lower concentration of acetic acid (0.11 M) was neutral. The buffering capacity of the soil was high enough to completely neutralize the acid so the acid soluble fraction could not be extracted. The dynamic extraction with 0.43 M acetic acid showed to be the best method and achieved the highest reproducibility and highest amount of extracted metals. Both soil materials contained a higher amount of Cu than what is permitted by local legislation. The soil material SN3-SN4 showed furthermore a significant contamination with Pb.

10 Souhrn

Přítomnost toxických kovů v půdě může mít vážné důsledky. Mohou se dostat do lidského organismu a způsobit vážné zdravotní důsledky. Analýza půdy je značně složitý proces a existuje spousta možností k jejímu provedení. Tato práce je soustředěna na manuální a průtokovou extrakci s jedním extraktantem. Jako detekční zařízení byla zvolena optická emisní spektrometrie s indukčně vázanou plazmou. Měření probíhalo ve 2 vlnových délkách pro každý prvek pro případ, že by se v jedné vlnové délce vyskytly interference. Pro chrom byla využita vlnová délka 205,26 nm, pro olovo 220,35 nm, pro měď 324,75 nm, pro zinek 206,20 nm, pro kadmium 214,44 nm a pro nikl 231,60nm. Druhé vlnové délky obsahovaly více interferencí. Kalibrační roztoky byly připraveny pro každou koncentraci kyseliny octové zvlášť.

Cílem této práce bylo porovnat manuální a průtokovou extrakci vybraných kovů – Cd, Cr, Cu, Ni, Pb a Zn. Byli použity dvě koncentrace (0,11 M a 0,43 M) kyseliny octové a dva vzorky půd. Před odměřením půdy bylo nutno vzorek půdy pořádně protřepat, aby byl odebraný vzorek homogenní. První byla vyzkoušena manuální extrakce. 1g půdy s 40 ml kyseliny octové byl promícháván v kádince na magnetické míchačce po dobu 16 hodin. Poté byly vzorky zcentrifugovány a okyseleny kyselinou dusičnou na její 0,2% roztok. Poté byly měřeny pomocí ICP-OAS a výsledky graficky vyhodnoceny.

Druhá metoda byla průtoková extrakce s kyselinou octovou. Aparatura se skládala ze zásobníku s kyselinou, injekční pumpy, mikrokolony se vzorkem (0,5 g), externího filtru a sběrných zkumavek. Externí filtr byl přidán z důvodu, že vzorek v průběhu extrakce unikal mimo kolonu a pronikal i do sběrných zkumavek. Průtoková rychlost byla stanovena na 1,5 µl/min. Jako řídící software byl použit Cocosoft, což je program , který vytvořil David Cocovi z chemické laboratoře Universitat de les Illes Balears. Bylo sbíráno 40 frakcí po 5 ml, které byly následně okyseleny kyselinou dusičnou na její 0,2% roztok, změřeny pomocí ICP-OAS a výsledky graficky vyhodnoceny. Na začátku (kyselina octová) i na konci (ještě před okyselením kyselinou dusičnou) bylo měřeno pH extrahované tekutiny.

Manuální extrakce byla více časově náročná. Výsledky s nižší koncentrací kyseliny octové byly neuspokojivé. Extrahované množství bylo velmi nízké a opakovatelnost velice malá. Po 16 hodinách bylo změřeno pH, které bylo neutrální. Zde pufrovací kapacita půdy plně pokryla kyselost použité kyseliny octové a nemohlo tedy dojít k extrahování frakce kovů uvolnitelné v kyselém prostředí. Při použití 0,43M kyseliny octové došlo ke zvýšení množství extrahovaných kovů i opakovatelnosti měření. Nicméně průtoková extrakce byla značně výhodnější. Nezabrala tolik času (tři hodiny oproti šestnácti) a výsledky byly také více opakovatelné. Ukázalo se, že 40 frakcí je málo. Poslední zkumavka obsahovala ještě dostatečné množství extrahovaného kovu, tudíž extrakce neproběhla úplně do konce.

Výsledky extrakce byly porovnány s požadavky Španělského ministerstva zemědělství, které jsou uvedeny v tabulce č. 9. Množství mědi v obou vzorcích už přesáhlo maximální povolenou hranici. V půdě označené SN3-SN4 bylo ještě nadměrné množství olova.

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