

CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE

FACULTY OF ENVIRONMENTAL SCIENCES

DEPARTMENT OF ENVIRONMENTAL GEOSCIENCES



**Cr(VI) reduction during adsorption onto selected soil
components using isotopic approach**

DIPLOMA THESIS

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DIPLOMA THESIS ASSIGNMENT

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Environmental Geosciences

Thesis title

Cr(VI) reduction during adsorption onto selected soil components using isotopic approach.

Objectives of thesis

Main aim of this diploma thesis is to investigate redox transitions associated with the adsorption of Cr(VI) on commonly occurring soil components including clay minerals and humic acids. Isotopic analyses will be performed to obtain a more detailed insight into redox processes.

Methodology

Dissolution of the solid samples of kaolinite, illite and humic acids after adsorption of Cr(VI).

Cr(VI) separation from the matrix using a modified anion exchange chromatography method prior to Cr isotopic analysis.

Cr isotopic measurements on a multicollector inductively coupled plasma mass spectrometer (MC ICP-MS, Neptune, Thermo, Germany).

The proposed extent of the thesis

60

Keywords

Cr(VI), adsorption, reduction, clay minerals, humic acids, isotopes

Recommended information sources

- Bullen, T.D., 2007. Chromium stable isotopes as a new tool for forensic hydrology at sites contaminated with anthropogenic chromium. In: Bullen, T.D., Wang, Y. (Eds.), *Water-Rock Interaction, Proceedings and Monographs in Engineering, Water and Earth Sciences*, pp. 699–702.
- Ellis, A.S., Johnson, T.M., Bullen, T.D., 2002. Chromium isotopes and the fate of hexavalent chromium in the environment. *Science* 295, 2060-2062.
- Ellis, A.S., Johnson, T.M., Bullen, T.D., 2004. Using chromium stable isotopes to quantify Cr(VI) reduction: lack of sorption effect. *Environmental Science & Technology* 38, 3604-3607.
- Šillerová, H., Chrastný, V., Čadková, E., Komárek, M., 2014. Isotope fractionation and spectroscopic analysis as an evidence of Cr(VI) reduction during biosorption. *Chemosphere* 95, 402-407.

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DECLARATION:

I hereby declare that I wrote this Diploma Thesis titled **“Cr(VI) reduction during adsorption onto selected soil components using isotopic approach”** under control and direction of my supervisor - Mgr. Veronika Veselská, Ph.D. I have listed all used literature references and information are cited at the end of the thesis.

In Prague, June 29th, 2020

BSc. Jahongir Rustamov

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ABSTRACT

Cr(VI) is classified as having harmful impacts on soil system, groundwater and plants in terms of high toxicity. Investigating and monitoring processes that assess Cr(VI) mobility, redox transformation, and speciation greatly helps to prevent the negative impact of Cr(VI) on soil, air, or atmosphere. Identifying the chromium species in soil and discovering effective ways to reduce their effect is still considered one of the difficult scientific tasks. It is due to the difficulty of the analytical procedure and the possibility of the transformation of chromium species at each level of the analytical processes.

This diploma thesis focuses on quantifying the extent of reduction Cr(VI) during the adsorption of Cr(VI) on two commonly occurring clay minerals, illite, kaolinite, and humic acid. For this reason, the isotope fractionation method was used as one of the effective methods for describing reduction-oxidation transitions associated with the adsorption of Cr(VI).

Work is aimed at the determination of how selected clay minerals and humic acids differ in their ability to reduce Cr(VI). Isotope analyses provide a suitable tool for the distinction between adsorption and reduction of Cr(VI). Cr isotope fractionation during Cr(VI) adsorption was determined using an inductive coupled plasma mass spectrometer (ICP - MS).

Keywords: *Cr(VI), adsorption, reduction, clay minerals, humic acids, Cr isotopic fractionation.*

ABSTRAKT

Cr (VI) je klasifikován jako látka, která má z hlediska vysoké toxicity škodlivé účinky na půdní systém, podzemní vodu a rostliny. Průzkum a monitorování procesů, které hodnotí mobilitu Cr (VI), redoxní transformaci a speciaci, výrazně pomáhá předcházet negativnímu dopadu Cr (VI) na půdu, vzduch nebo atmosféru. Jedním z obtížných vědeckých úkolů je identifikace druhů chrómu v půdě a objevování účinných způsobů, jak snížit jejich účinek. Je to kvůli obtížnosti analytického postupu a možnosti transformace druhů chrómu na každé úrovni analytických procesů.

Tato diplomová práce se zaměřuje na kvantifikaci rozsahu redukce Cr (VI) při adsorpci Cr (VI) na dva běžně se vyskytující jílové minerály, ilit, kaolinit a kyselinu huminovou. Z tohoto důvodu byla metoda izotopové frakcionace použita jako jedna z účinných metod pro popis redukčních oxidačních přechodů spojených s adsorpcí Cr (VI).

Diplomová práce je zaměřena na stanovení toho, jak se vybrané jílové minerály a huminové kyseliny liší ve své schopnosti redukovat Cr (VI). Izotopové analýzy poskytují vhodný nástroj pro rozlišení mezi adsorpcí a redukcí Cr (VI). Cr izotopová frakcionace během adsorpce Cr (VI) byla stanovena pomocí indukčního vázaného plazmového hmotnostního spektrometru.

Klíčová slova: Cr (VI), adsorpce, redukce, jílové minerály, huminové kyseliny, Cr izotopová frakcionace.

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1. INTRODUCTION

Understanding the behaviour of contaminants in soil and the aquatic environment leads to improve tracking, monitoring, and maintaining contaminants.

Cr(VI) is a well-known toxic element, classified as a contaminant in terms of high toxicity, which has many negative impacts on the environment that causes serious problems, such as skin irritation kidney and gastric damage, including lung cancer. Monitoring processes that assess Cr(VI) mobility and speciation, as well as maintaining redox transformation of Cr(VI) greatly helps to prevent the negative impact on soil, air, or atmosphere.

Chromium is a useful element in terms of usage in different industrial sectors, such as the production of different alloys, dyes, many other applications, and products. Cr(VI) widely releases into the environment from different large processing facilities: metallurgical, chemical-refractory industries, ore mining, and refining. (Gattulo *et al.*, 2018).

Reduction reactions of chromium occur during the adsorption processes. Isotopic analysis is becoming an important tool to track the reduction of toxic and soluble Cr VI. (Ellis *et al.*, 2004). Chromium isotopes analysis is becoming a high-tech method for monitoring Cr(VI) involved aqueous systems. Isotope fractionation research has been conducted to some Cr(VI) reductants: ferrous iron Fe(II)_{aq}, humic substances, or microbial strains. (Bauer *et al.*, 2018).

In environmental and paleo-environmental research, small ranges in Cr isotope ratios have been observed in terrestrial samples because of biological, mass-dependant fractionation and adsorption processes. Isotope fractionation methods have been used for a better understanding of the behaviour of Cr(VI) in hydrology, geology, geochemistry (Rodler *et al.*, 2015).

In chromium isotopes analysis $\delta^{53}\text{Cr}/^{52}\text{Cr}$ values illustrate isotope fractionation of chromium. It describes the importance, precisely constraining the $\delta^{53}\text{Cr}/^{52}\text{Cr}$ signature of a local Cr source which is crucial for quantification of the reduction kinetics of Cr(VI) in non-anthropogenic contaminated aquatic systems.(Farkaš *et al.*, 2013).

In this work, isotopic analyses were performed to investigate reduction-oxidation transitions associated with the adsorption processes of Cr(VI) on kaolinite, illite, and humic acids. Understanding the Cr(VI) reduction mechanisms is very important to be able to select suitable remediation technologies.

1. AIMS OF THE THESIS

The main aim of the diploma thesis is to determine the reduction of Cr(VI) during adsorption processes and to examine how the selected clay minerals and humic acid differ in their capability in Cr(VI) reduction.

Isotopic analyses were conducted to get more detailed insight into Cr(VI) redox processes during adsorption on several soil components kaolinite, illite, and humic acid.

2. LITERATURE REVIEW

3.1. General information about the chromium

Chromium (Cr), 24 atomic number, is transition silver-grey coloured, odourless metal, antiferromagnetic, quite inert to acids, , one of the most abundant elements on Earth (21st) with 122 ppm (0.0122 %), as well as soil's most frequently found metal pollutants. (Gallios and Vaclavikova, 2008).

Chromium takes an active part in several applications because of its unique characteristics such as corrosion resistance, hardness, and colour. Mainly used in the manufacturing of stainless steel. Iron-nickel-chrome alloys contain an incredible variety of the most important metals in modern technology. Various compounds of Cr(VI) are used in chrome-plating industries, automobiles, steel and alloys, leather tanning, and ammunition factories. Chromium is also widely used in chromium - based catalysis procedures. (Gattulo et al., 2018).

The United States and Russia are considered as major chromium-metal producing countries for electrolytic chromium metal whereas, France, Russia, China, and the United Kingdom are leader countries for aluminothermic chromium metal production.(National Research Council 1995)¹.

The understanding of the speciation of Cr in the solid phase and aqueous phase is crucial in developing approaches and designing a treatment scheme. Commonly, Cr⁶⁺ is available in the aqueous phase which is dissolved from the Cr-containing solid phases.

Following charts (see *figure 1*) show the worldwide chromium metal production.

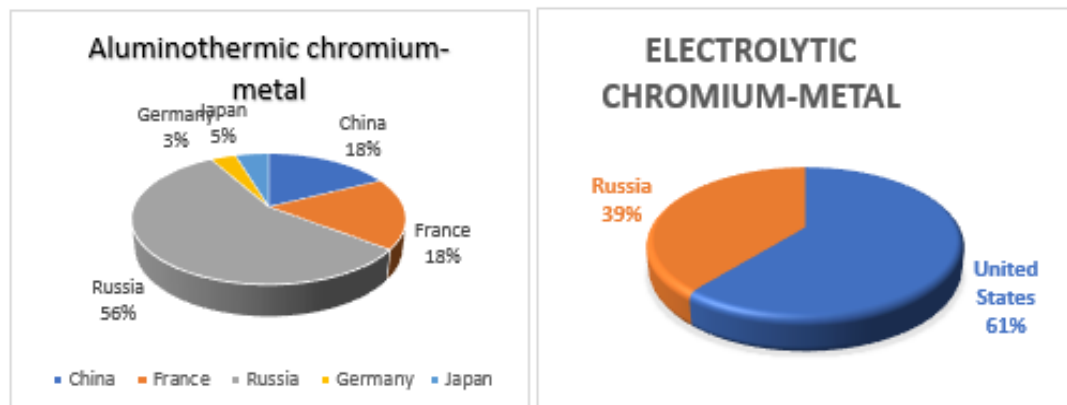


Figure 1: Global major producers - manufacturers of aluminothermic and electrolytic chromium-metal. (National Research Council 1995)¹.

According to the Toxics Release Inventory, chromium released from 3400 large processing facilities in the USA to the air was measured at above 18900 tons in 2008. Coal and oil combustion cause an estimated 1723 metric tons of Cr(VI) emitted to the atmosphere per year in the US. On the other hand, chromium-plating sources contribute 700 metric tons of Cr(VI) per year to the atmosphere. [Agency for Toxic Substances and Disease Registry (ATSDR 2010)².

Chromium sources are divided into 3 categories: *environmental, occupational, and non-occupational sources*:

Environmental sources: cement clouds of dust, topsoil, and rocks, airborne emissions from chemical plants and incineration facilities, effluents from chemical plants, contaminated landfills, asbestos lining erosion, road dust from catalytic converter erosion and asbestos brakes. (HazDat 2000).

Occupational sources: So-called industrial sources: anti-algae agents, production of high-fidelity magnetic audio tapes, copier servicing, plating, electroplating, antifreeze, leather tanning, tattooing, textile manufacturing, rinse waters, welding of steel and alloys, anodizing baths engaged in the manufacturing human activities, etc. (Owlad et al., 2009)

Non-occupational sources of chromium mainly considered as the result of two categories: smoking and diet, contaminated soil, wastewater, and the air. (Gattullo et al., 2018). The main sources of chromium are shown in *figure 2*.

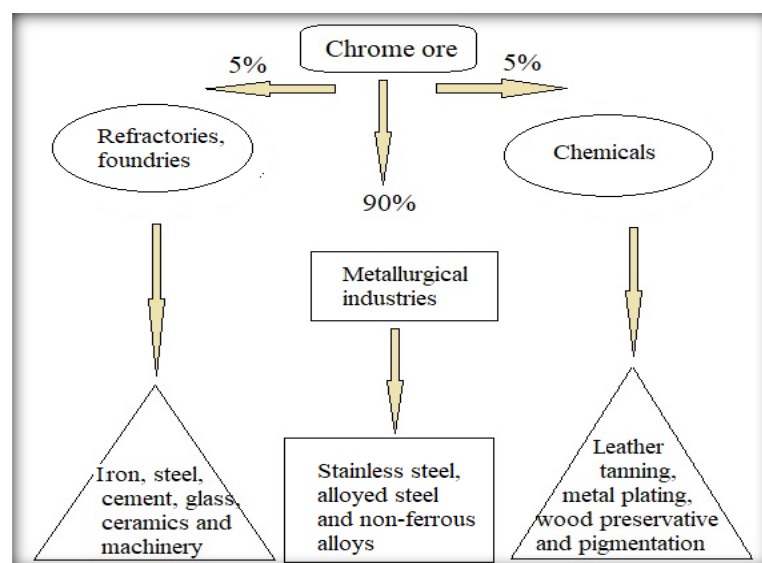


Figure 2: Chromium sources and usage in various industries (Dhal et al., 2013).

2.2. The behaviour of Cr(VI) in soils

Chromium occurs in a wide range of valence forms in soils. Cr^{2+} , Cr^{3+} , Cr^{6+} are the most common oxidation states. Cr(VI) is present as chromate CrO_4^{2-} and dichromate $\text{Cr}_2\text{O}_7^{2-}$ in soil and groundwater. Cr(VI) remains stable in shallow aquifers, topsoil (under oxidizing conditions). (Dayan et al., 2001).

Chromium (6+) is stable in sandy soils with low concentrated organic matter. This form is readily mobilized by groundwater and sediments based on redox potential and pH environment. Cr(VI) species easily go into through cell membranes in plants. Cr(VI) absorbs into membranes more than Cr(III) species. (Mohan et al, 2006).

Table 1 displays the total concentration of Cr in the soils, compared with other metals.

Metal	Concentration range mg/kg	Regulatory limit mg/kg
Arsenic	0.1-102	20
Cadmium	0.1-345	100
Chromium	0.005-3950	100
Copper	0.03-1550	600
Mercury	0.001-1800	270
Lead	1-6900	600

Table 1. Heavy metals prevailing in soils (Ahemad, 2015)

Chromium chemically changes in soil as well as in water, and atmosphere. Chromium forms complex compounds with metals. For example, crocoite (PbCrO_4), bentonite $\text{Ca}_6(\text{Cr}, \text{Al})_2(\text{SO}_4)_3$ and tarapacaite (K_2CrO_4), vauquelinite ($\text{CuPb}_2\text{CrO}_4\text{PO}_4\text{OH}$). Increasing the hexavalent Cr(VI) amount in the soil is almost the result of human activities (Oliveira, 2012).

The concentration and binding mechanisms of chromium increase proportionally with the depth of all soil profiles. Almost in all surface horizons of soils Cr appears as lithogenic origin. Cr^{6+} accumulates in deeper soil horizons, for example in planosol and luvisol soils. The distribution of Cr alters according to soil pH, redox potential, and soil moisture. (Hernandez et al., 2003).

Chromium easily binds with the functional groups on the surface of humic acids to form complex compounds. The toxicity of chromium and bioavailability are not only depending on their chemical forms. Mobility is depending on the structure of the compounds, soil components, microorganisms, and soil pH etc. (Kotás, et al.,2000).

Chromium and its compounds are highly influencing the microbial - physiological activity of the soil which causes a decrease of overall biomass. Cr(VI) reduces the seed germination and fertility, decreases nutrient uptake and development of plants etc. Cr(VI) enters cells as dichromate/chromate $\text{Cr}_2\text{O}_7^{2-}$, CrO_4^{2-} ions, then by metabolic reduction. It changes to unstable Cr^{5+} , Cr^{4+} forms, and finally reduces into stable form Cr^{3+} . (Ahemad, 2015).

Soils have 3 different electric (redox) potential states (Eh), which describes a system's overall reducing or oxidizing capacity. It is a dominating factor to identify the biochemical behaviour of chromium in soil.

anoxic: $\text{Eh} < +100 \text{ mV}$.

suboxic: $+350 \text{ mV} < \text{Eh} < +100 \text{ mV}$.

oxic: $\text{Eh} > +350 \text{ mV}$.

Chromium species (3+ and 6+) are sensitive to redox potential. Cr(VI) has strong oxidizing potential Eh (+1.38 V) in acidic solution. (Shahid et al., 2017).

Cr(VI) species dominates in oxygen-rich environments at the range of neutral to alkaline pH. (Ball et al., 2004).

Influence on humans will be more threatening where agricultural products are growing in an area that has been overly contaminated with chromium. Using the phosphorus (phosphate) fertilizers is one of the main reasons for increasing the amount of chromium in soils. The content of chromium varies widely in soils, on average about 250 mg.kg^{-1} . (Bencko et al., 1995).

According to the US Environmental Protection Agency (USEPA-2008), the safe level of Cr(VI) should be no more than 5 ppm in uncontaminated areas. (Zhang *et al.*, 2019). It is harmful that when the concentration of Cr is even as low as 0.05 ppm (5 $\mu\text{g/kg}$) in soil. (Ahemad, 2015).

The allowable level of total Cr in agricultural soils in the Czech Republic is 100-200, mg kg⁻¹ (Shahid et al., 2017).

World Health Organization (WHO) states that the maximum safe limit for chromium is 0.05 mg l⁻¹ (Owlad et al., 2009).

2.3. Remediation of Cr(VI) in soils.

Knowing the source of chromium gives the information to choose correct chromium removal techniques and treatment methods. Industrial wastes contain a large amount of chromium, including other heavy metal pollutants. They are not easily detoxified or removed without advanced techniques (Owlad et al., 2009).

One of the remediation methods for Cr(VI) is reducing Cr(VI) into its Cr(III) form. Therefore, determining the total quantity of Cr in the soil system and its oxidation changes is vital during the remediation processes. The reduction of Cr(VI) is a key step in the remediation process of Cr contaminated soils (Pettine, 2000).

There are many methods being implemented for the Cr(VI) remediation processes. For example, zero-valent iron (ZVI) anode electrolysis in a separated electrode system, many microorganisms such as *Shewanella. sp*, *Bacillus. sp*, *Pseudomonas. Sp*, *Escherichia coli* (Zhang et al., 2019), and biochar have been used in order to describe the remediation of Cr(VI) (Zhang et al., 2013).

The main idea behind zero-valent iron (ZVI) anode electrolysis method is that oxidation of iron electrodes produces ferrous ions, which is considered a reducing agent in the phase. During this process chrome-iron, stable precipitate (Fe₁₅Cr₅(OH)₆₀) is formed (Sarahney et al., 2012).

Permeable zero-valent iron (Fe⁰) reactive barriers (PRB) and in situ redox manipulation (ISRM) zones are effectively being used in the remediation of Cr-contaminated aquifers. The degree of the reduction of Cr(VI) can be calibrated by observing the changes of the ⁵³Cr/⁵²Cr ratio using the isotopic fractionation factor for specific reactions (Basu and Johnson, 2012).

It is important to determine the potential for exposure to Cr(VI) at hazardous waste sites on a case by case basis. Studying the negative effects of Cr(VI) in soils leads to find new, modern remediation and localization techniques. Manufacturing new

remediation techniques help to protect soil and water resources from further degradation according to the level of pollution. For instance, agricultural biomass and pyrite are used as reactive agents during Cr(VI) removal in aqueous solutions (Kantar et al, 2015).

Nowadays soil remediation and soil recovery from chromium is in the spotlight, because of its persistent nature, toxicity and biodegradability that causing environmental issues. Cr(VI) which are released into the environment cannot be biologically decomposed or easily detoxified. It can be stored in natural aquatic bodies, in the human body, and in the soil (Hwang et al., 1994).

Bioremediation in situ, such as phytoremediation, exploiting the metal-accumulating plants in soils is considered economical and ecologically accessible methods. But these processes are slow and time-consuming that require to enhance plant biomass (Ahemad, 2015).

Bioremediation is a cost-effective, eco-friendly, sustainable contaminant detoxification. During remediation, it is important to monitor the level of toxicity and effectiveness of Cr(VI) reduction to optimize remediation conditions. Biomass application in Cr(VI) rich soils improves the reduction rate of Cr(VI). During the reduction process OH^- ions releases and produced less mobile - $\text{Cr}(\text{OH})_3$ Bioremediation processes of Cr(VI) using biochar and chromium reduction is illustrated in *figure 3* (Zhang, Bolan et al. 2013).

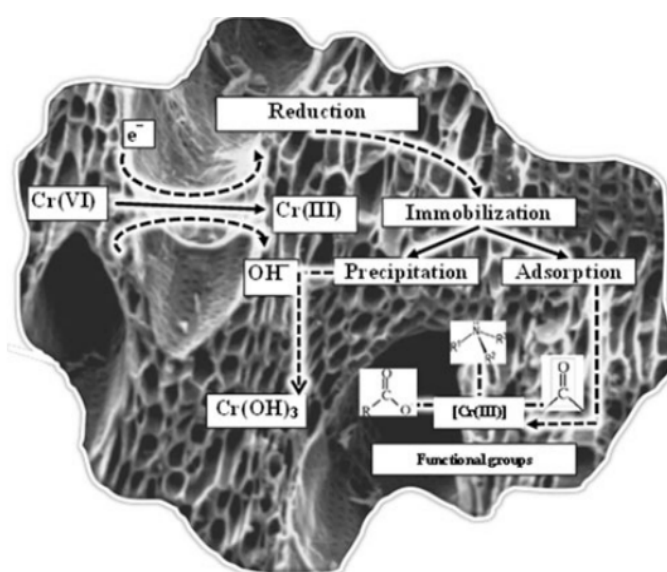
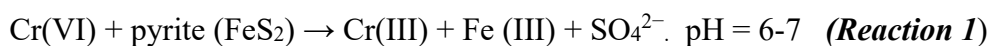


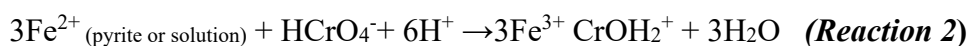
Figure 3. Chromium reduction and immobilization in biochar carbon-amended soils (Zhang, Bolan et al. 2013).

Biochar is the final product from the agricultural waste biomass residues during pyrolysis. Biochar has a high capacity to adsorb Cr(VI). Several physicochemical reactions occur on the surface: a) cation exchange with Ca^{2+} , Mg^{2+} , and other cations resulting in complex compounds with humic matter and mineral oxides within biochar; b) surface complexation with different functional groups, mineral hydroxides c) physical adsorption and surface precipitation that stabilizes Cr^{6+} . Generally, biochar increases soil pH, decreases the leaching of Cr(VI) through redox reactions. In remediation procedures application of biochar provides a new solution to the soil contamination problems (Zhang et al., 2013).

Cr(VI) removal by pyrite occurs together with the oxidation of Fe(II) to Fe(III) and S_2^{2-} into SO_4^{2-} at the pyrite surface. due to the reduction of Cr(VI) to Cr(III). While pyrite surface is negatively charged, therefore reduction of $(\text{CrO}_4)^{2-}$ leads to precipitation of $\text{Cr}(\text{OH})_3$, $\text{Fe}(\text{OH})_3$ and $\text{Fe}^{3+}-\text{Cr}^{3+}$ (oxy) hydroxides ($\text{Cr}_x\text{Fe}_{(1-x)}\text{OOH}$) under alkaline conditions (see *reaction 1*) (Kantar et al., 2015).



Reduction of Cr(VI) to Cr(III) by pyrite-solution interface, under oxygen-free conditions (*see reaction 2*) (Kim et al., 2002).



Reduction of Cr(VI) by SO_2 is a common remediation method used in treatment from industrial wastewaters. The *reaction 2* is very rapid at pH 4. As pH increases e.g. pH 7, the reduction rate decreases. The effect of pH has been explained with distribution of species formed when SO_2 dissolves in water. SO_2 produces sulfurous acid (H_2SO_3), which dissociates into HSO_3^- and SO_3^{2-} ions (*see reactions 10,11.*) (Ahn M., 2004).

Remediation of Cr(VI) is accomplished by adsorption, reduction, membrane filtration, extraction, ion exchange methods, phytoremediation. etc. Diffusion, adsorption-desorption, precipitation-dissolution, oxidation, and complexation are chemical and physical processes that control the transportation rate and mobility of chromium in soil (Zhang et al., 2019).

Adsorption methods - using a variety of natural and synthetic Cr VI sorbents that are used during the remediation processes, such as, clay minerals, industrial wastes

activated carbons, biological materials, etc. Adsorption processes depend on some parameters: surface area of sorbents, pH, and temperature (Owlad et al., 2009).

Membrane filtration methods used mainly chromium treatment from wastewaters. Several types of membranes such as inorganic, polymeric, and liquid membrane filtration have a high capability of removing Cr(VI). Membrane separation of Cr(VI) suitable technique that may be used even at room temperature (Pugazhenthii et al., 2005).

Extraction methods- Concentration of chromium can be determined using a sequential extraction method. This technique has been recommended by (BCR) Community Bureau of Reference. However, knowing the total concentration of the Cr(VI) cannot provide significant information about Cr forms in soils, their mobility, bioavailability, or potential risks to the environment (Wali et al., 2014).

Phytoremediation is an economical and ecologically effective method in situ bioremediation approaches to clean chromium polluted soils. But this method is slow and time-consuming (Ahemad, 2015).

Ion Exchange methods: In recent years, this method is popular among treatment processes. Synthetic Ambersep 132 and Dowex 2-X4 (strong alkaline anion resin (OH⁻ - type)) ion exchange resins were used to separate Cr⁶⁺ from aqueous solution. Ion exchange method contains four steps:

- 1) capturing the chromic acid.
- 2) converting chromic acid into to sodium chromate form.
- 3) converting the sodium chromate back into chromic acid using cationic (H-type) ion exchange resins
- 4) regeneration of disturbed resin by hydrochloric acid.

The only disadvantages of ion-exchange methods are that resins are not available for major trace metals, also it is time-consuming process with high operational cost (Lin and Kiang 2003).

Several kinds of research and studies have been tested in recent decades, mainly based on adsorption, reduction, precipitation, extraction and ion exchange, surface

complexation processes. Cr(VI) adsorption increases proportionally within increasing the concentration of Al and Fe oxides in the soils (Dhal et al., 2013).

Fractionation techniques have been proposed during the oxidation of Cr(III) to Cr(VI). This process also indicates the mobility and bioavailability of chromium. Cr separation from a sample matrix is a very important step in the determination of Cr isotope ratios. The behaviour and chemical characteristics of Cr³⁺ and Cr⁶⁺ species are very different. This problem can be solved by the converting chromium ions into one oxidation state by reduction during the ion exchange separation process (Pontér et al., 2016).

2.4. Characteristics of selected Cr(VI) reductants in soils

Cr(VI) can be reduced into Cr(III) by organic soil components, clays, Fe (II)-ferrous minerals. They are the main reducing agents in the soil. Cr(VI) redox processes during adsorption in the soil are controlled mainly by the presence of Fe-(oxy) hydroxides, Mn-(oxy) hydroxides and soil organic matter. The reduced Cr is rapidly adsorbed in an acidic pH environment and removed from the solution. In nature, the rate of reduction is slow (Kwak et al., 2018).

2.4.1. Clay minerals

Clay minerals together with humic acids are very important to soil components in terms of chromium reduction processes. A variety of low-cost adsorbents such as agricultural, industrial wastes, natural and synthetic clay minerals have been used to remove chromium, from soils. Clay minerals are being used over the past decades as effective adsorbents due to their strong ion exchange and complex formation capabilities (Bekkouche et al., 2012).

Clay minerals are defined as hydrous aluminosilicates minerals that contain colloid fractions; particle size is < 2 µm. (Ajouyed, et al., 2011). Clay minerals are textures that mix with a small amount of water resulting in soft - elastic mineral substance. Clay minerals have been widely used in a range of environmental applications as “natural hunter” where they catch cations and anions which are toxic to humans and wildlife. Clay minerals have high cation exchange capacity, catalytic properties, swelling capacity, high specific surface area, and consequently strong adsorption capacity (Ajouyed, et al., 2011).

Surfaces of clay minerals are electrostatically negatively charged. Surface layers are neutralized by interlayer metal cations: such as K^+ , Na^+ , NH_4^+ , Mg^{2+} , Ca^{2+} or hydroxide octahedral groups $Al(OH)_3$, $Mg(OH)_2$. The buffer capacity of clays and the initial soil pH play an important role in terms of mobility of Cr(VI). Observations have been attributed that Cr^{6+} reduces into Cr^{3+} in acidic environment and Cr^{6+} adsorbs in larger amount than Cr^{3+} onto clay minerals (Lukman et al., 2013).

The structure of clays is mainly tetrahedral and octahedral. The structural formula of tetrahedral clays is T_2O_5 , where T represents metals: Si^{4+} , Al^{3+} , Mg^{2+} and less frequently Fe^{3+} cations. There is a composite layer which is formed by linking tetrahedral and octahedral structures known as 1:1 structure. The layout of oxygen and silicon atoms located within polyhedrons. (see figure 4). In such structures, OH groups engage the upper part of plane layers. When the octahedral layer is surrounded by two tetrahedral layers is known as 2:1 structure. If the 2:1 or 1:1 layers have the electrostatically negative charge the layers are neutralized by interlayer cations: such as K^+ , Na^+ , NH_4^+ , Mg^{2+} , Ca^{2+} , or hydroxide octahedral groups $Al(OH)_3$, $Mg(OH)_2$. (this structure coincides with Illite).

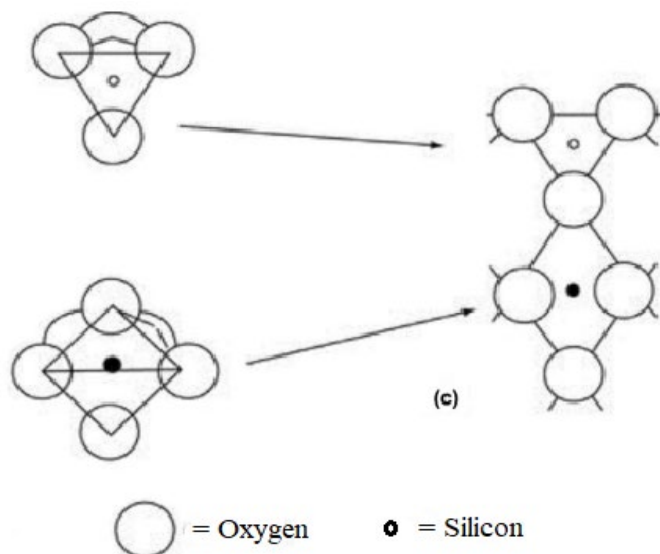


Figure 4. Tetrahedrally co-ordinated (a), octahedrally co-ordinated (b); cation polyhedrons (c) linked octahedral and tetrahedral polyhedrons (Huggett, 2004).

The size of clays depends on the type of interlayer cations. X-ray diffraction spectroscopy analysis is used to identify the structures of clay minerals. Water in the interlayer site weakly binds with outer surface clay particles depending on their size and charge.

This following *table 2* shows the cation exchange capacity (CEC) of selected clay minerals.

Kaolinite	3-18(*meq/100g)
Illite	10-40 (meq/100g)

Table 2. Cation exchange capacities (CEC) of selected clay minerals.

**milliequivalents/gram* (Huggett, 2004).

Kaolinite – single-layered (1:1, Tetrahedron – Octahedron) clay mineral with approximate structure $Al_2O_3 \cdot 2SiO_2 \cdot 2H_2O$ (TO). One layer of silica (SiO_4), linked with the layer of gibbsite ($Al_2O_3 \cdot 3H_2O$). There are not any cation or water molecules between layers. The distance between the two layers is 7 Å. Kaolinite is used as a raw material in producing cement – structural clay products and manufacturing ceramics (Ayadi et al., 2013).

Illite (micaceous mineral) 2:1 layered clay, (TOT) Tetrahedron – Octahedron - Tetrahedron is a phyllosilicate or aluminosilicate mineral. Approximate chemical composition - $(R^+(Al_{2-x}Mg,Fe(II),Fe(III))_xSi_{4-y}Al_yO_{10}(OH)_2)$. The interlayer distance is 10 Å. (1 angstrom is 0.1nanometre).

The approximate chemical formula slightly differs from muscovite (K, H_3O^+) $(Al, Mg, Fe)_2(Si, Al)_4O_{10}[(OH)_2,(H_2O)]$. less octahedral Al, less interlayer K and more Si, Mg, and H_2O in structure (Huggett, 2004).

The structure of phyllosilicate – kaolinite and mica- phyllosilicate – illite is shown in *figure 5*. Illite has cations between edge of interlayers where interlayers within kaolinite are linked by (H_3O^+ or hydrogen bonds).

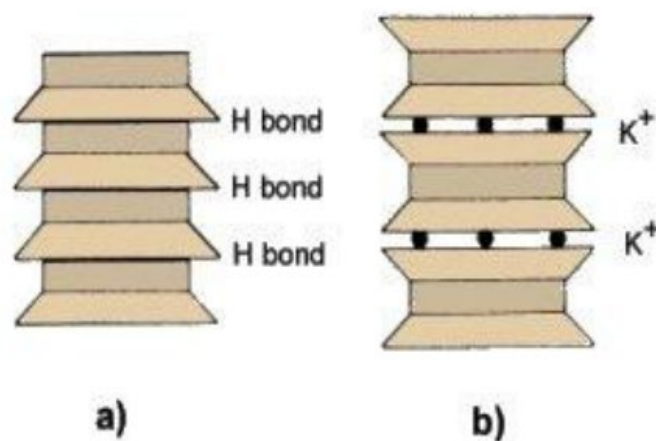


Figure 5. Structure of a) kaolinite b) Illite. Source: (Ayadi et al., 2013).

Kaolinite mineral has been studied for treatment with groundwater and wastewater. The origin of kaolinite mineral is a result of extremely weathering of metamorphosed sedimentary rocks with a low SiO₂ amount. It mainly occurs in iron-poor acid rocks, in a stable moist, and warm environment at low pH (Huggett, 2004).

2.4.2. Organic soil components

Laboratory studies have confirmed that plants and soil organic matter highly impact on mobility and transport of chromium in soils. Soil horizons with high soil organic components are associated with lower total chromate (CrO₄⁻) ratios than the horizons with lower organic content. The addition of organic matter has a significant influence on chromium reduction because Cr(VI) reduction rates are enhanced by the soil organic matter content (Kitchen et al., 2012).

The organic soil components occupy 5% of the total soil volume (see *figure 6*).

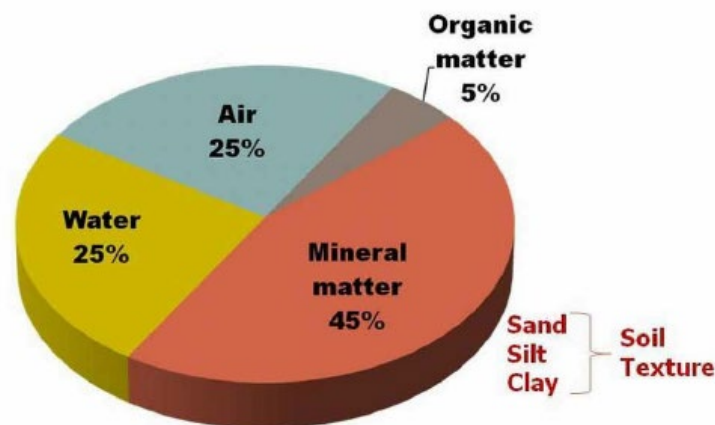


Figure 6. Soil components. source (Toor et al., 2009).

Organic matter is the result of the residues of plants and animals. Organic matter also contains live biomass: micro-organisms; partially decomposed materials; and non-living materials. Organic soil components balance the soil pH by binding or releasing cations. The decomposition process involves microbial activity that produces gas, water, and new bacterial cells, as well as volatile acids. (Toor and Shober, 2009).

Soil organic matter contains mostly 50% carbon, 40% oxygen, 5% hydrogen, 4% nitrogen, and 1% sulfur. Most importantly organic soil components bind Cr(VI) prevents releasing it as constituents from soils. Soil organic matter provides many important benefits. It helps to maintain the structure of the soil, pH, soil fertility, and plant nutrient uptake (Toor and Shober, 2009).

Heavy elements intend to accumulate in surface organic horizons. Accumulation consequently affects the biological activity thanks to their strong affinity with soil organic components. It leads to delay of decomposition of soil organic matter (Hernandez et al., 2003).

Organic soil components can be found in various decomposition stages. The decomposition process involves microbial activity that produces gas, water, and new bacterial cells, as well as volatile acids. Organic soil humus is the final product of the microbial process. It is a complex mixture of various organic compounds (Al-Khafaji Andersland, O. B., 1992). Complex structure of molecular fulvic and humic acids are shown in following *figure 7*.

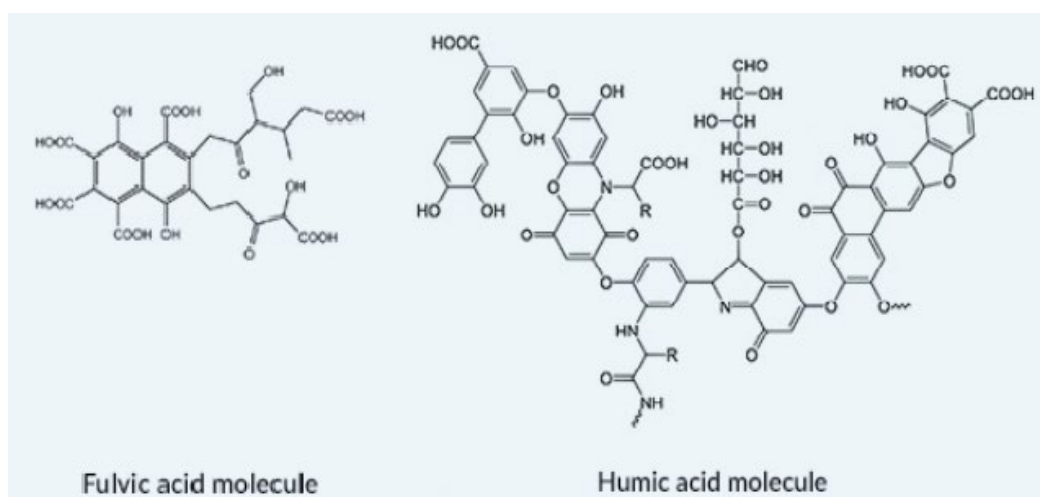


Figure 7. Molecular structure of fulvic and humic acids (Al-Khafaji Andersland, O. B., 1992).

Humic acid (HA) is a dominant component among organic substances in soils, in sediments and aquatic media. Humic acid binds clays and metal oxides. Understanding the mechanisms of humic acid during adsorption on mineral surfaces is important. Ligand - cation exchange, electrostatic attraction, hydrogen bonding, and hydrophobic interaction is the key adsorption mechanisms to observe characteristics of humic acids (Chen et al., 2017).

There are several functional groups in humic acid (HA): carboxyl (COO^-), aromatic units; phenolic hydroxyls ($\text{C}_6\text{H}_5\text{O}^-$), amino (NH_2^-), hydroxyl (OH^-), etc. Functional groups react with both metal ions and clay mineral surfaces (Hongxia et al., 2016). Redox properties of humic acids are considered crucial for Cr(VI) reduction (Chen et al., 2017).

The main differences between humic and fulvic acids are the content of carbon and oxygen atoms, molecular weight, and their colour. Humic acids dark coloured, large molecules (size ranges 10000 to 100000) with many functional groups on edge-surface. Fulvic acids golden coloured, size is smaller than humic acids. Humic acids in the soil bind nutrients for better uptake into plants. They produce chelates with chromium and prevents from entering the membrane. Fulvic acids are responsible for nutrients transportation between soil and plant membranes. Fulvic acids are known as effective chelating compound thanks to its small molecular size and surface charges (Theologou; et al., 2013).

Humic acids are soluble in alkaline environment but not soluble in an acidic environment. ($\text{pH} < 7$). Fulvic acids are soluble in water. The degree (rate) of Cr(VI) removal is higher in the presence of humic acids compared to fulvic acids during the Cr(VI) removal processes from the soil solution (Mak et al., 2011).

Fulvic and humic acids altogether with reactive compounds like esters, carboxylic acids, carbonyl hydroxylates acids and phenols originated from microbial fermentation can reduce Cr(VI) into Cr(III) under pH-dependent reduction conditions (Theologou; et al., 2013).

2.5. Transformation Cr(VI) to Cr(III).

Cr(III) and Cr(VI) have significant differences and different chemical characteristics: toxicity, adsorption, bioavailability in soils, as well as translocation to cell-membrane and aerial parts of the plants. Environmental Protection Agency (EPA) regulates the features and allowable limits of chromium (Shahid et al., 2017).

Maintenance the ratio of two chromium forms is considerable due to regular constant transformations in ecosystems (Eksperiandova et al., 2002).

Cr(III) compounds are not hazardous, less mobile. (10-100 times less toxic than Cr(VI)) Cr(III) is stable and remains its form under a reducing environment. Oxidation of Cr(III) can occur with the presence of oxygen, chlorine, or ozone. Chromite ore (FeCr_2O_4), (MgCr_2O_4) contains a significant amount of Cr(III). (About 55% Cr_2O_3) (Meija et al., 2016).

Interactions between chromium oxidants/reductants and chemical equilibria of the aqueous phase are important. The reduction of Cr(VI) is generally carried out in the presence of sulfide, sulfur, magnesium, ferrous ions. The reduction can be enhanced by humic substances (M. Economou-Eliopoulos et al., 2014).

Cr₂O₃ is low soluble and less reactive. Cr(VI) toxicity and its carcinogenic effects have long been known with negative environmental impacts (Meija et al., 2016).

Cr(VI) can be reduced to its Cr(III). Few methods have been developed to enlighten the nature of Cr transformation in soil and wastewater (Bartlett et al, 1991).

Oxidation–reduction processes depend on the concentration of oxygen, reducing agents, sulfides, silicates, Fe, Mn ions, ligands, and type of catalysts. Ph is an influential key factor in reduction rate in the subsoil. In low pH, acidic conditions increase the release of Fe (II) from soil minerals to react with the Cr(VI) in the aqueous environment. The reduction of Cr(VI) increases also according to the content of organic matter. Reducing Cr(VI) to Cr(III) is an easier process than oxidizing Cr(III) to Cr(VI) in soils (Dhal et al, 2013).

Flow chart of oxidation (by MnO₂) and reduction sites (by Fe(II), S²⁻) of Cr(VI) and Cr(III) is illustrated in *figure 8*.

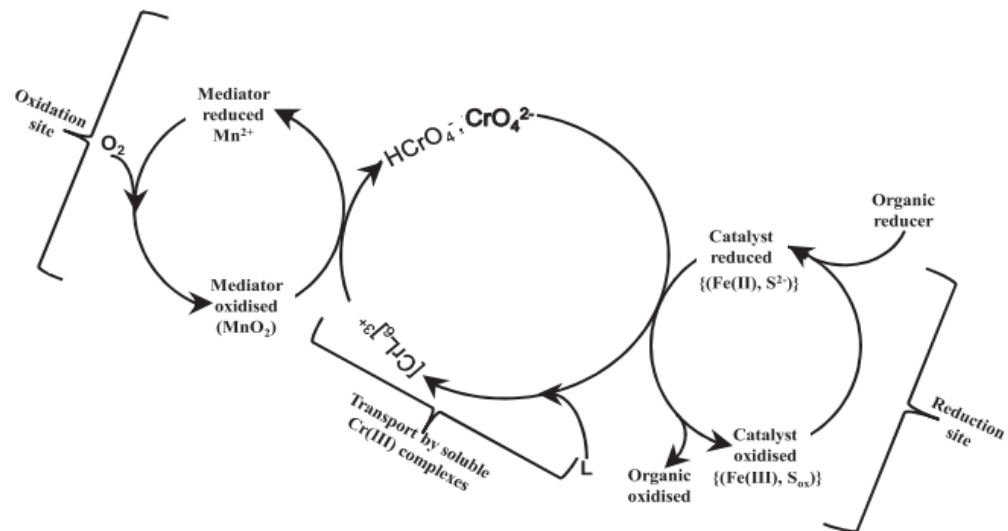


Figure 8. Oxidation/reduction of chromium in soil (Dhal et al., 2013).

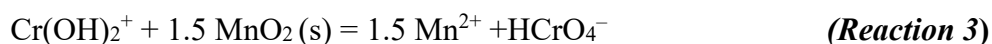
Chromium changes its form quite quickly in a low pH, acidic environment (reduction of Cr(VI) increases by decreasing pH, increasing temperature). (Desai et al., 2014). For example, Sulphite ion (SO_3^{2-}), is the main reductant for Cr(VI) at low pH (< 5.0). Ferrous iron Fe (II) is primarily reductant at pH =6.5-7.5 (Lin, 2002).

Cr(VI) in the soil categorized into two types of fractions: exchangeable fraction and non-exchangeable fraction.

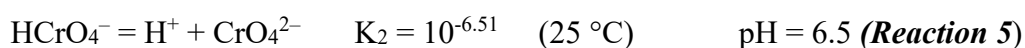
Exchangeable fraction - the fraction of chromium can be removed easily from the soil.

Non-exchangeable fraction – is the fraction where Cr(VI) is very strongly adsorbed or precipitated. Extraction methods are used to separated Cr(VI) from the fraction.

The parameters that control the oxidation rate of Cr(III): concentration of chromium (III), pH, initial available β -MnO₂ surface area. Chromium (III) oxidized to chromium (VI) by MnO₂ proportionally to a reduced amount of manganese. (oxidation rate increases by decreasing pH) serves as the electron acceptor, which is shown in *reaction 3* (Fendorf et al.,1992).



The proportion of Cr⁶⁺ ions (HCrO_4^- and H_2CrO_4) is generally anionic and mobile in a strongly acidic environment in moist soils. The following reactions occur in the aquatic medium as a function of pH and chromium total concentration. Distribution of these species according to pH is described by the following *reactions 4, 5*.

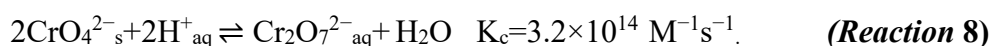


CrO_4^{2-} are soluble and predominant species at low pH. Dissociation of (CrO_4^{2-}) resulting HCrO_4^- at pH between 0.8 and 6.5, and CrO_4^{2-} at pH above 6.5 has been investigated as a function of pH (Rai et al., 1989).

According to further studies when pH is low and the total concentration of Cr(VI) is greater than 10 mM, HCrO_4^- dimerizes and results dichromate $(\text{Cr}_2\text{O}_7)^{2-}$ $K=10^{-1.54}$



The direction of *reaction 8* is favored according to pH conditions:



a) in alkaline solution, $\text{pH} > 7$ the reverse reaction is favored

b) in acidic solution, $\text{pH} < 7$ the forward reaction is favored (Ahn M. 2004).

Following *figure 9* shows the forms of Cr(VI) and Cr(III) in the aqueous systems depending on pH.

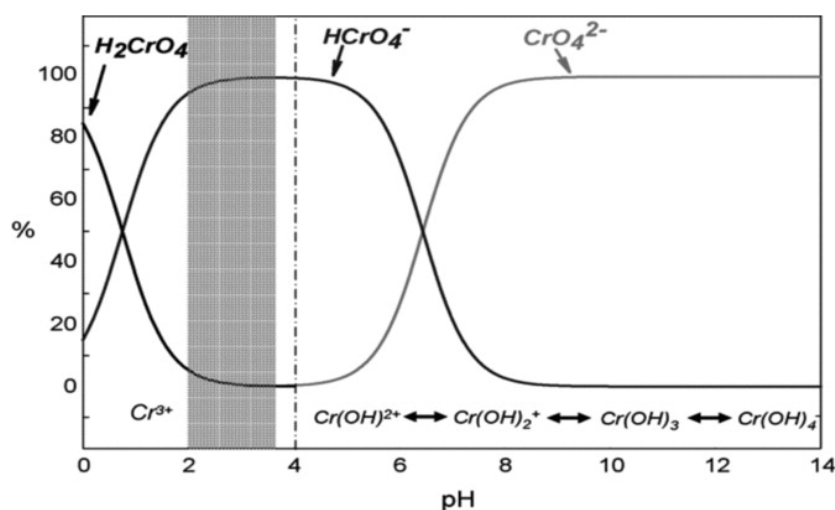


Figure 9: Species distribution diagrams of Cr(III) and Cr(VI) in an aqueous system source: (Rakhunde et al., 2012).

Cr(VI) mainly exists as chromic acid salts; (H_2CrO_4) – at $\text{pH} < 1$, hydrogen chromate ions (HCrO_4^-) at $\text{pH} 1-6.5$, chromate ions (CrO_4^{2-}) at above $\text{pH} 6.5$. As pH decreases Cr(III) forms become predominant. Dichromate ion $\text{Cr}_2\text{O}_7^{2-}$ produced when chromium concentration exceeds 1 g/L.

Figure 10 elaborates that ionic species in aqueous Cr(III) hydrolysis resulting hydroxide species $[\text{Cr}(\text{OH})_2^+, \text{Cr}(\text{OH})_2^{2+}, \text{Cr}(\text{OH})_3$ and $\text{Cr}(\text{OH})_4^-]$ at $\text{pH} < 3,5$. And $\text{Cr}(\text{OH})_3$ exists as a solid amorphous precipitate (Mohan and Pittman, 2006).

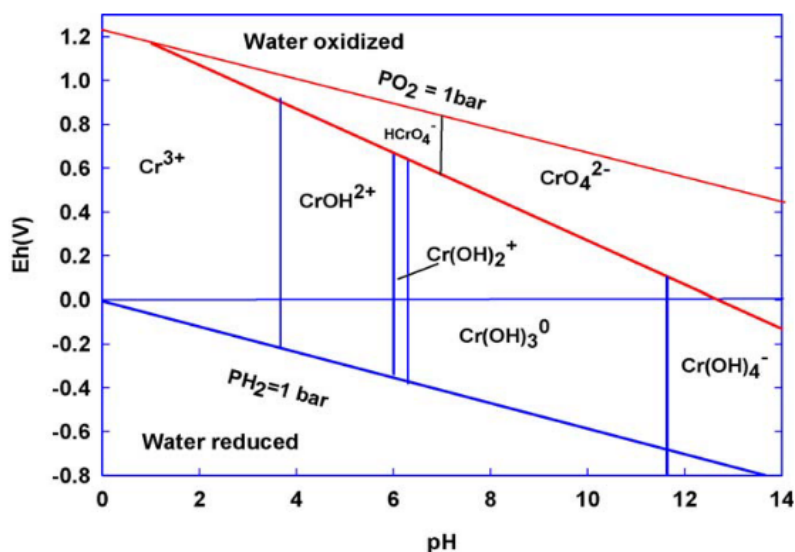
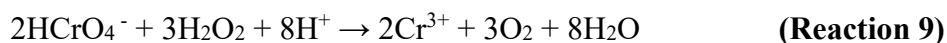


Figure 10. Eh–pH diagram for chromium. (Zink et al., 2010).

Hydrogen peroxide H_2O_2 also influences chromium reduction processes depending on the pH of the aqueous system. H_2O_2 produces hydroperoxyl radical (HO_2^*).

In case of $\text{Fe}(\text{II})$ and S^{4-} as reductants this active radical (HO_2^*) reacts with iron and SO_3^{2-} , as a result, active radical either promotes the production of Cr(III) (at high pH) or restricts the process (at low pH) These reactions rapidly proceed under acidic conditions. At low pH, under the alkaline environment, the reaction slows down because of precipitation of $\text{Fe}(\text{OH})_3$, the concentration of Fe^{3+} species becomes lower in the solution. The kinetic data of H_2O_2 involving redox processes evaluate the role of these two reductants in the natural aquatic systems (Assaf, Lin, 2002).

Earlier studies have been reported of the stoichiometry of the reduction reaction of Cr(VI) with H_2O_2 shown in two following reactions 9.10:



An excess of H_2O_2 will favor both reactions. Therefore, at least 10 times excess amount of H_2O_2 is required for effective reduction of Cr(VI) in aqueous solutions (Van Niekerk et al., 2007).

Oxidation/reduction reactions of Cr^{3+} and Cr^{6+} can occur thermodynamically, simultaneously. This dynamic reaction depends on so pH, Eh, hydrolysis, precipitation, cation exchange capacity (CAC), microbial – biological conditions, and concentration of metals (Shahid et al., 2017).

2.5.1. Biological reduction in situ.

Microorganisms, such as bacteria, algae, yeast, and fungi can reduce chromium (Hawley et al., 2004). It was also stated that bacteria increase the reduction of hexavalent into trivalent chromium in aerobic and anaerobic conditions (Cheung et al., 2007).

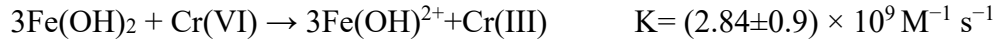
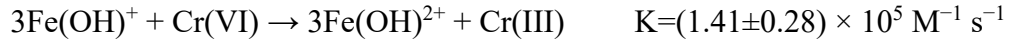
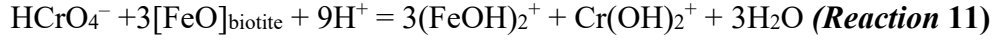
Recent studies suggest that Cr(VI) reduction can be enhanced using biomaterials: for example, fungal mycelia involves adsorption-linked reduction, including sorption of the hexavalent chromium. Another method of Cr(VI) reduction involves natural organic matter, which also associated with microbial activity (Park et al., 2007).

2.5.2. Chemical reduction in situ.

This method is performed by direct application of a chemical reducing reagents in liquid forms. The main purpose is to create a reactive zone in the aquifer. Alternatively, a solid chemical reducing agent medium is placed in the contaminated area to form an impermeable reactive barrier. Chemical reducing agents including FeO, iron-based and sulfur-based agents (Theologou; et al., 2013).

It is difficult to predict the ratio and amount of chromium reduction in soil. Several researchers have been investigating chromium (VI) reduction using organic compounds to its insoluble Cr(III). Enough source of electrons is available to reduce chromium (VI) to chromium (III) in organic material present in clays. The other source of reduction of chromium (VI) is Fe (II) ions which occur in hematite and biotite. Past studies related to mineral phases revealed that aquatic chromium (VI) components can be reduced also the presence of the small amount of Fe (II) in hematite and biotite, resulting in (Fe, Cr) (OH)₃ precipitate.

The following *reaction 11* describes chromate reduction through iron ions produced by the dissolution of iron species of hematite and biotite in an acidic environment (Rai et al., 1989).



Cr(VI) reduction occurs by oxidation of Fe^{2+} . However, rapid oxidation of Fe(II) by dissolved oxygen terminates Cr(VI) reduction. (see Table 3. a)

(Kim et al., 2002), (Theologou et al., 2013).

Here are in table 3 shown some chromium reduction reactions:

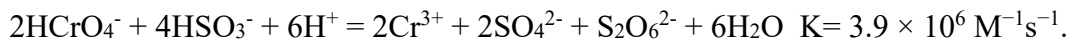
- a. $2(\text{CrO}_4)^{2-} + 2\text{Fe}^{2+} + \text{H}_2\text{O} + 4\text{H}^+ \rightarrow \text{Fe}(\text{OH})_3 + \text{Cr}_2\text{O}_3$
- b. $(\text{Cr}_2\text{O}_7)^{2-} + 6\text{Fe}^{2+} + 14\text{H}^+ \rightarrow 2\text{Cr}^{3+} + 6\text{Fe}^{3+} + 7\text{H}_2\text{O} \quad (\text{pH} < 7)$
- c. $(\text{CrO}_4)^{2-} + 3\text{Fe}^{2+} + 4\text{H}_2\text{O} \rightarrow \text{Cr}^{3+} + 3\text{Fe}^{3+} + 8\text{OH}^- \quad (\text{pH} > 7)$
- d. $3(\text{CrO}_4)^{2-} + 2\text{FeS} + 9\text{H}_2\text{O} \rightarrow 4[\text{Cr}_{0.75}, \text{Fe}_{0.25}] (\text{OH})_3 + \text{S}_2\text{O}_3^{2-} + 6\text{OH}^-$
- e. $2(\text{CrO}_4)^{2-} + 3\text{CaS}_4 + 10\text{OH}^+ \rightarrow \text{Cr}(\text{OH})_3 + 12\text{S}^0 + 3\text{Ca} + 2\text{H}_2\text{O}$
- f. $8(\text{CrO}_4)^{2-} + 3\text{H}_2\text{S} + 10\text{H}^+ + 4\text{H}_2\text{O} \rightarrow 8\text{Cr}(\text{OH})_3 + 3\text{SO}_4^{2-}$
- g. $4(\text{CrO}_4)^{2-}_{\text{aq}} + 6\text{NaHSO}_3_{\text{aq}} + 3\text{H}_2\text{SO}_4_{\text{aq}} + 8\text{H}^+_{\text{aq}} \rightarrow 2\text{Cr}_2(\text{SO}_4)_3_{\text{aq}} + 3\text{Na}_2\text{SO}_4^{2-} + 10\text{H}_2\text{O}$
- h. $2(\text{CrO}_4)^{2-}_{\text{aq}} + 3\text{SO}_2_{\text{aq}} + 4\text{H}^+_{\text{aq}} \rightarrow \text{Cr}_2(\text{SO}_4)_3_{\text{aq}} + 2\text{H}_2\text{O}$

Table 3. Hexavalent chromium involving chemical reduction reactions.

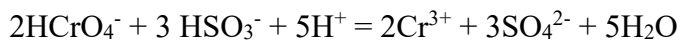
Source: (Theologou; et al., 2013).

Likewise, (see table 3, g) sulphite SO_3^{2-} reduces Cr(VI) at a strongly acidic environment (pH<5).

Following reactions 12, 13. describe the reduction mechanisms of Cr(VI) by SO_2 .



(Reaction 12)



(Reaction 13)

The reduction rate and directions of reactions by SO₂ reductant are related to the mechanisms of reaction based on the presence of reactants' amount:

- a) Cr(VI) reduction is favoured according to the *reaction 12* in the excess of SO₂;
- b) Cr(VI) reduction is favoured according to the *reaction 13* in the excess of (CrO₄)²⁻

However, SO₃²⁻ would not further reduce Cr(VI) once the concentration of Cr³⁺ exceeds 10⁻⁴ M. It is because aquatic S⁴⁺ becomes atmospheric equilibrium with atmospheric SO₂. (LaCount et al., 1987), (Ahn M, 2004).

2.6. Cr isotopes

Chromium forms over 20 different isotopes. Four of them are considered stable while nineteen are radioactive, and many have half-lives shorter than 24 hours. The ones listed below are the most stable atomic weight 50, 52, 53, and 54.

⁵²Cr-the most occurring in nature with an abundance of 83,8 %, followed by ⁵³Cr isotope with 9.5% abundance (*see table 4*).

Element	Cr 50 (amu*)	Cr 52 (amu)	Cr 53 (amu)	Cr 54 (amu)
Chromium	4.345%	83.789%	9.501%	2.365%

amu* - atom mass unit.

Table 4. *The percentage abundance of stable isotopes of chromium* (Moos and Boyle, 2019).

CHROMIUM-50 - (⁵⁰Cr) (1.8 x 10¹⁷ half-life years) is used for the calibration of neutrino detectors in solar neutrino observatories; synchrotron calibration and synthetic ruby production, detection of strong neutron fields, production of Cr⁵¹ and positron-emitting radionuclide (radioisotope) of manganese (Mn⁵¹)³.

CHROMIUM-52 - (⁵²Cr) is used for synthetic ruby production; research of doped semiconductors; photo absorption cross-section measurements, production of Fe⁵² radionuclides (radioisotopes) and Mn⁵¹ positron-emitting radionuclides (radioisotopes)³.

CHROMIUM-53 - (⁵³Cr) is used for the development of Nuclear Magnetic Resonance (NMR) spectroscopy in quality control and research for determining the content and purity of a Cr compounds as well as its molecular structure. This process

is based on radio signal processing devices; investigations of irregularities in structures of crystals, glass and ceramic materials through nuclear gamma resonance (NGR) and electron paramagnetic resonance (EPR), studies of metals and alloys through nuclear magnetic resonance (NMR) for production of magnetic semiconductors³.

CHROMIUM-54 - (⁵⁴Cr) used for studies that deal with metabolism and diabetes issues, likely to other chromium isotopes. This isotope is mainly used to produce synthetic rubies. All isotopes are available in their metal and chemical oxide forms³.

In recent years Cr stable isotopes have been widely used in environmental applications. Many scientists have demonstrated the monitoring of Cr-contaminated soil and water. Stable isotope ratios are useful to indicate sources and Cr reduction reactions. Recent studies using stable Cr isotopes for tracking redox processes in surficial environments were focused on the treatment of groundwaters, rivers, or seawaters. For that reason, the interpretation of Cr isotope data is important, and it is needed to find quantitative methods for chromium reduction processes. (Novak et al., 2014).

The chromium isotope system has been used to track the reduction of toxic and soluble Cr(VI) in groundwater. Cr isotopes are used to track paleoenvironmental changes. Isotopic analysis is an effective method to track Cr(VI) release in aqueous systems. The fractionation degree differs according to the type of reactive material. Shifts in the Cr ratio determines to find suitable removal mechanism of Cr(VI) (Jamieson-Hanes et al., 2015).

Isotope fractionation associated with Cr(VI) reduction tends to be larger than those accompanying Cr(III) oxidation on mineral surfaces. The reduction of dissolved Cr(VI) is a relatively fast kinetic process in terms of heavier isotopes of Cr in the aqueous form, compare to oxidation of Cr(III) which is a slow process (Novak et al., 2018).

Several studies have observed that identifying the small changes in the ⁵³Cr/⁵²Cr ratio could describe Cr sources in natural/anthropogenic sources and would be useful in tracking the chromium involved chemical processes. $\delta^{53}\text{Cr}/^{52}\text{Cr}$ ratios usually defined as relative deviations from the standard (Zhang et al., 2019).

The lighter isotopes have low dissociation energy. General feature in many oxidation–reduction reactions is that the lighter isotopes are more reactive, therefore, can react more actively than heavier isotopes. So, during the reduction, light isotopes will be preferentially reduced. As a result, the products of reduction will have lower $\delta^{53}\text{Cr}$ values, and the remaining reactants have higher $\delta^{53}\text{Cr}$ values (Zhang et al., 2019).

The lighter isotopes are preferentially reduced during Cr(VI) reduction, resulting in an enrichment of ^{53}Cr in the remaining Cr(VI) pools. The enrichment or depletion of ^{53}Cr is measured in aqueous solutions as the change in the ratio of $^{53}\text{Cr}/^{52}\text{Cr}$. It is expressed as $\delta^{53}\text{Cr}$ in units per mil (‰) relative to a standard (M. Economou-Eliopoulos et al., 2014).

3. MATERIAL AND METHODS

3.1. Description of materials and chemicals

Technical grade humic acids (nano powder size <50 nm) were purchased from Sigma Aldrich (Germany). The natural clay minerals: kaolinite (KGa-2) and illite (IMt-2) were bought from the Clay Minerals Repository (Clay Minerals Society).

All the reagents used for batch adsorption experiments and Cr(VI) separation were in analytical grade and have been purchased from Sigma Aldrich.

3.2. Adsorption experiment prior to isotopic analyses

Quantitative reduction capacities of clay minerals and humic acids have been tested during the Cr(VI) adsorption batch laboratory experiments. The adsorption experiments were conducted in duplicate samples for 24 hours aimed at assessing the main Cr(VI) reductants, under 101,325 kPa, at 25⁰C, prior to isotopic measurements.

All clay minerals and humic acids in mass 20g/l were separately added to the electrolyte solution (0.01M 250 ml KNO₃). Suspensions were shook using an orbital shaker for 24 hours (*see figure 11*). Initial Cr(VI) concentration was 10⁻⁴ M and pH = 4, 6.5 and 9. For individual experiments pH was adjusted using NaOH or HNO₃ (both 10⁻² M).

2x10 ml aliquot of the solution was removed from each batch before adding a solid phase to measure an initial Cr concentration. Samples are added in the initial solution. Also, 2x10 mL aliquot of the slurry was removed after adsorption, centrifuged (3000 rpm, 10 min), and filtered (0.2 μm, acetate cellulose) to analyse supernatants using MC ICP-MS. The rest of the slurry was subsequently filtered using a vacuum pump (filter DIA 90 mm; 84 g/m²) and the solid phase was frozen. Freeze-drying (24 hours). Freeze-dried samples were dissolved for isotopic measurements.

In general, two phases: supernatant and solid phase are separated after the adsorption processes. Supernatant has been kept for the chromium separation from matrix elements. The solid phase was dissolved in a pressure digestion system prior to further isotopic analysis.



Figure 11. Suspensions are shaking using an orbital shaker (model GFL 3005).

3.3. Total dissolution of solid phases after adsorption prior to isotopic measurements

Total dissolution of samples after adsorption experiment was carried out in a pressure-digestion system (Anton-Paar, Austria) using method EPA 3051A (USEPA, 2007). Solid samples are changed into liquid phase using acid digestion techniques, to calculate absolute concentrations of metal elements in samples. EPA 3051A method involves several stages. The main step is releasing the metal ions from the solid phase transferring to the acid solution via the extraction process. This is an important and necessary step for the determination of content of chromium by MC ICP-MS. Thus, this method can ionize most elements including elements with a high ionization potential (i.e. elements that require high energy to remove an electron from an atom) (Vanhaecke et Degryse 2012).

The USEPA 3051A method is a modified method of 3051, which uses a combination of hydrochloric acid (HCl) and nitric acid (HNO₃) to boost digestion of chromium and iron contained soils samples. This method conducted in a closed microwave system provides higher temperature and pressure which provides efficient, safer, and faster digestion. However, 3051A method is not an absolute effective method, because it cannot recover 100 % of the metals in the soil sample.

The amount of 0.250 g of freeze-dried solid samples were added to Teflon vessels. Teflon pressure vessels containing a combination of nitric acid, hydrochloric, and hydrofluoric acids (9 ml of 65-69 % HNO₃, 3 ml of 32-35 % HCl, 1 ml of 48% HF). Solid samples were dissolved and subsequently filtered using a 0.45µm acetate cellulose membrane. After the filtration, samples heated and dried at 100°C in Savillex digestion Teflon vessels.(see figure 12).

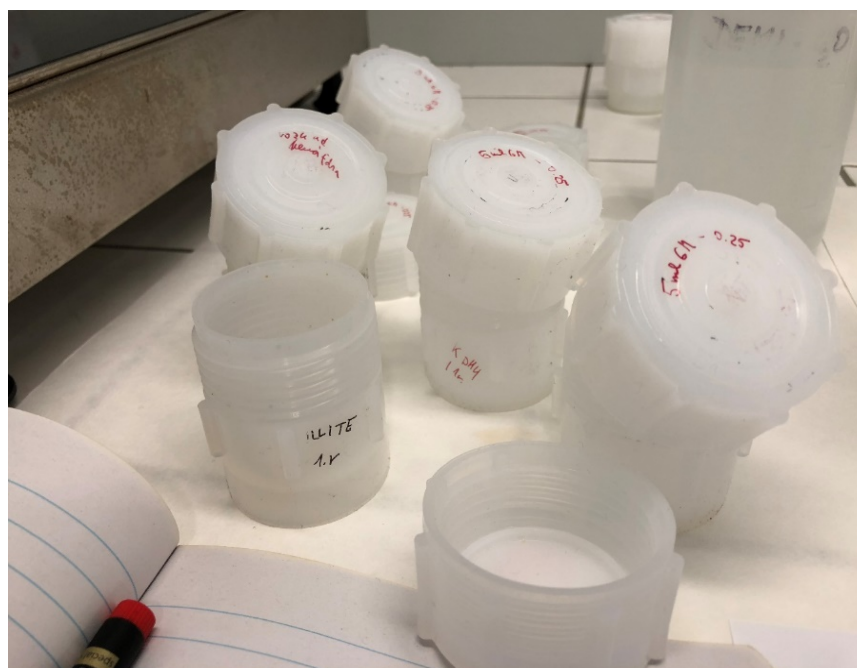


Figure 12. Savillex digestion Teflons. (photo by J. Rustamov).

Both supernatants and solid phases of humic acids were heated in a mixture of HNO₃ and H₂O₂ solutions (proportion is 1.5:1). This process was repeated many times until organic matter fully dissolved. Then samples were dissolved in 2 ml of 2% HNO₃ solution in Teflon vessels, then duplicated.

Duplicated samples were diluted and analyzed using ICP-MS (Thermo Fisher, iCAP Q) (see figure 13).



Figure 13. Decomposition of humic acids with 2% nitric acid. $pH = 4$ (left), $pH = 6.5 - 9$ (right).

It is considered that the two-step modified anion-exchange method has better separation performance for silicate materials. The separation procedure generally consists of two steps:

- a. *Fe removal.*
- b. *Cr separation.*

3.4. Fe separation from the matrix prior Cr(VI) separation

Modified anion exchange chromatography method (Bullen 2007) was used to separate Cr and Fe from matrix elements. All separation experiments were taken place in *Thermo - Scientific Biological Safety Cabinets*.

AG1-X8 100:200 mesh - chloride form– is an anion exchange resin AER (Bio-Rad, USA) used as the removal agent for all cations in samples. The separation process started off with filling the chromatography columns with resin up to the line. (ratio of anion resin with distilled water is 1:2).

Fe separation from sample matrix was performed in first place prior to Cr(VI) separation in the case of dissolved solid samples including supernatants of humic acids.

1st step. **Resin washing.** Resin are taken volume of 2 ml added to separation chromatography columns, washed 4 times with 5 ml, (20 ml) deionized water, so resin should not contain ions or impurities.

2nd step. **Initialization** (activation) the resin solution. Initialization performed using 6M concentrated hydrochloric acid HCl (4 times \times 5 ml of 6 M HCl). AE resin were activated by 6M HCl and the then washed 3 times with deionized H₂O to gain neutral pH in the columns.

3rd step. **Sample loading.** Samples were evaporated from 2% HNO₃ to volume 0.3-0.4 ml (approximately one small drop). While accurately and carefully adding the sample to the activated resin solution in the separation columns, it is important that resin should not disturbed or shook. The combination of 0.5 ml of 6 M HCl and then 20 μ l of H₂O₂ was applied to the initialized resin in chromatography columns. The resin caught only Fe cations where all other cations except Fe were transferred with 5 ml of 6M HCl into Nalgene. (see figure 14).

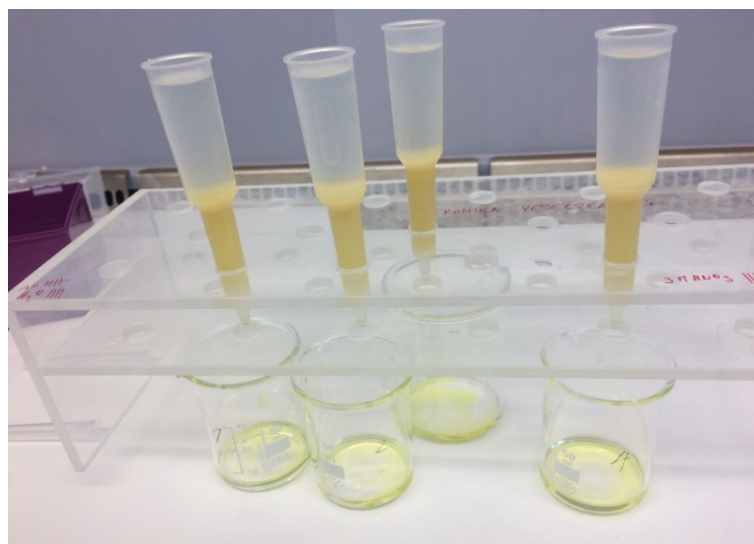


Figure 14. Releasing the Fe ions from the columns. (photo by J. Rustamov).

All Fe ions are captured by the column was released with 10 mL of 0.1 M HCl (see figure 15).

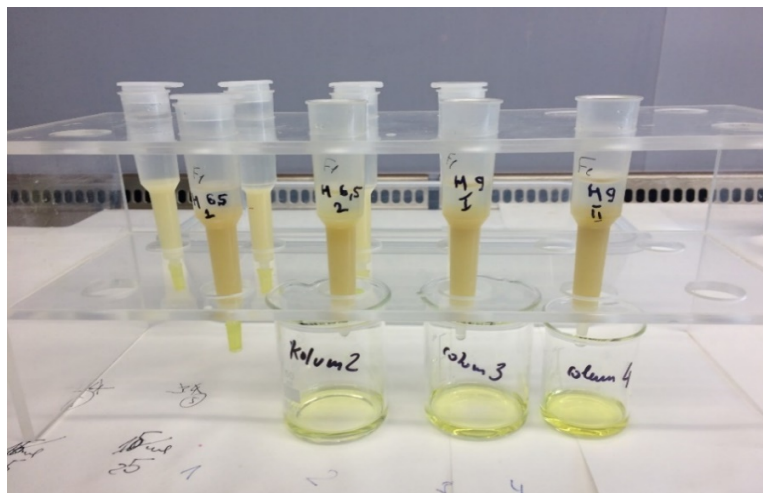


Figure 15. Fe ions released using 0.1M, 10 ml of HCl solution.

(photo by J. Rustamov).

Samples including all elements except of Fe in 5 mL of 6 M HCl were evaporated near dryness then diluted with deionized H₂O to 1 mL. This volume was ready for Cr(VI) separation. Iron ions are removed, and other matrix elements including Cr(III) are collected.

3.5. Cr(VI) separation from matrix prior isotopic analyses.

The samples then were subjected to modified anion exchange chromatography (Bullen, 2007; Šillerová et al., 2014) to separate Cr from the matrix components before the isotopic analysis.

10 mL reservoir gravity chromatography columns were filled with 2 mL AG1-X8 resin. Resin washed with 20 mL of deionized water and initialized by 15 ml of 6 M HCl. For Cr(VI) separation, environment within resin should be neutral. For this reason, resin was washed with deionized water to bring neutral pH (pH of effluent was controlled with litmus paper). In acidic environment, Cr(VI) is reducible to Cr(III), and it would be immediately eluted from the column. Cr(III) has low affinity for the resin in 6 mol L⁻¹ HCl solution.

Supernatants after adsorption experiment could have been directly loaded to the column in calculated volumes (for 2 µg of total Cr yield). However, samples after Fe separation contain Cr in form of Cr(III), which must be further transformed to

Cr(VI) prior Cr separation. The oxidation process of Cr(III) occurs with hydrogen peroxide (H_2O_2) and in an alkaline environment. Calculated volumes of Cr(III) in the matrix solution samples were treated with is oxidized to Cr(VI) using 30 μL of NH_4OH and 50 μl of H_2O_2 solution and then loaded into the AG1-X8 columns.

As a next step, *matrix elements are removed* using 20 ml of 0.1 M HCl solution, (4x5 ml of 0.1M HCl solution). Cr(VI) is retained on the AG1 resin. For discharging Cr(VI) from the columns, we had to change pH again, this time to strongly acidic. Finally, Cr(VI) is reduced to Cr(III) and collected using a mixture of ($\text{HNO}_3 + \text{H}_2\text{O}_2$) were added to the columns with 6 M HCl solution in ratio solution 1 mL of 6 M HCl + 50 μL H_2O_2 and subsequently 4 mL of 6 M HCl, applied twice. Cr separation efficiency was quantified by MC ICP-MS, Neptune, Thermo.

To release Cr(VI) from the column, the quantitative reduction to Cr(III)) was performed using H_2O_2 under strongly acidic condition (6 M HCl). Cr(VI) is to be separated after the matrix elements released by combination of ($\text{HNO}_3 + \text{H}_2\text{O}_2$) (*see figure 16*).

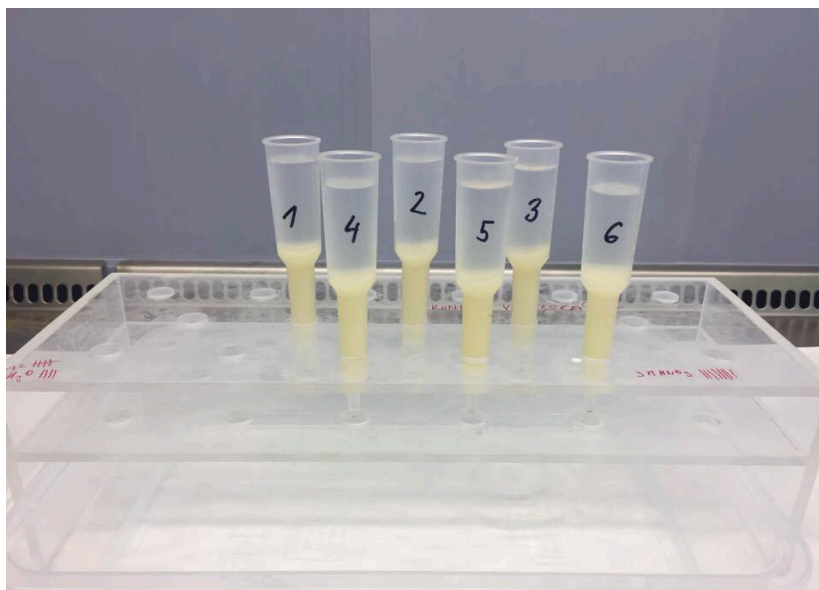


Figure 16. Chromium separation from the supernatant after removing all matrix elements. (Photo by J. Rustamov).

Samples were evaporated to near dryness to remove chlorides and nitrates (see figure 17).

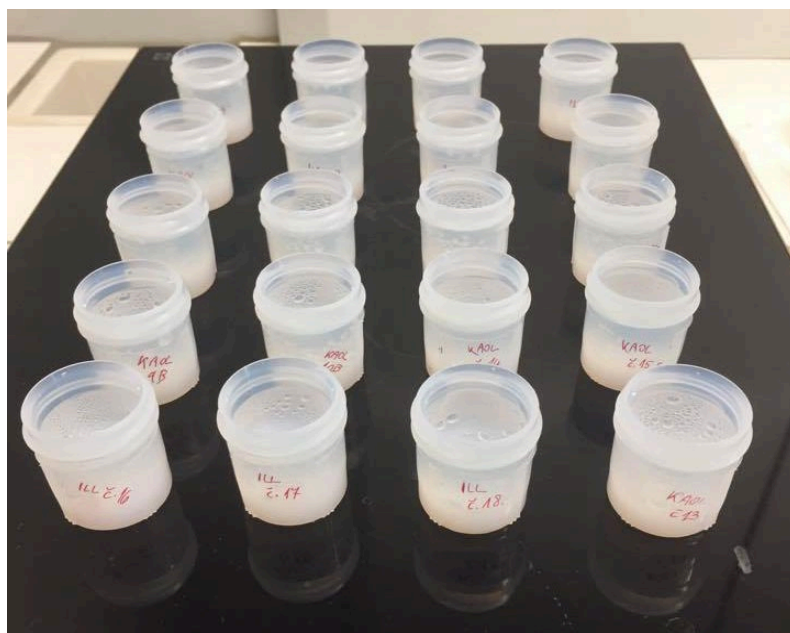


Figure 17. Samples are being evaporated after dissolution in strong acid, ready for further Cr isotope analysis (photo by J. Rustamov).

To eliminate the organic residues originating from the column resin bed, 50 μL H_2O_2 was added together with concentrated HNO_3 on a hot plate. Finally, samples were heated to near dryness and diluted with 2% HNO_3 to 5 ml prior isotopic analysis (see figure 18).

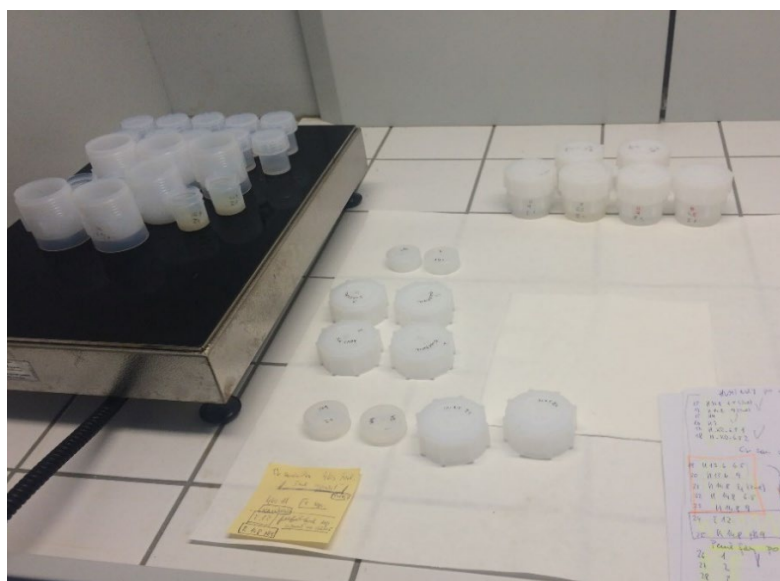


Figure 18. Samples are being evaporated, ready for Cr isotope analysis (photo by J. Rustamov).

The analytical steps of batch adsorption and Cr separation experiments prior to MC ICP MS isotopic analysis illustrated in (see figure 19).

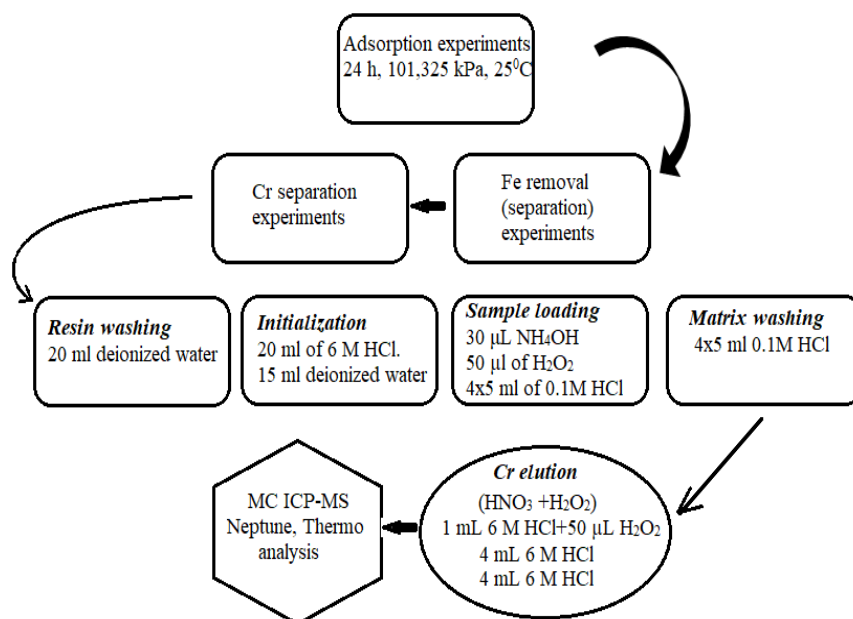


Figure 19. Flow chart of the Cr separation by modified anion exchange chromatography method.

The separation of Cr from a sample matrix is a very important step in the determination of Cr isotope ratios. Cr is separated providing a recovery of >95% from the matrix using columns of anion exchange resins.

3.6. Cr isotopic analysis

Cr isotopic measurements were performed using multi-collector inductively coupled plasma mass spectrometer (MC ICP-MS, Neptune, Thermo). The spectrometer is equipped with nine Faraday cups. Samples were introduced into the plasma, transferred to CETAC ARIDUS II™ Desolvating Nebulizer System (USA) which is specifically designed for ICP mass spectrometry. Additionally, a high degree of sample purification is required to avoid potential matrix effects in precisely determining Cr isotope ratios in samples using MC ICP-MS (Moos and Boyle, 2019).

Mass spectrometry can eliminate these direct interferences in extremely high resolution (>30000). The separation procedure must be applied as chromium samples must be free of titanium, vanadium, and iron (^{50}Ti , ^{50}V , and ^{54}Fe) to avoid isobaric interferences and matrix effects (Halicz et al., 2008).

The main difficulty of isotope analysis is “mass fractionation” (mass bias). Isotopic fractionation is mass dependent. Mass bias alters isotope ratio shift between the introduced sample and the measured ratios. *The double-spike technique* is used for mass bias correction, which enables accurate and precise correction of isotope fractionations during isotope measurements. Double spike technique allows precise correction of the laboratory-induced and instrumental Cr isotopes fractionation processes. This method is suitable for elements which has four stable isotopes (Coath, Elliott and Hin, 2017).

Corrections for mass bias were performed using a double-spike method involving spiking of sample with solution enriched in two isotopes (^{50}Cr and ^{54}Cr). The results were related to the Cr standard NIST SRM 979 and reported as δ -values for each sample were calculated using the following (*see equation 1*).

$$\delta^{53}\text{Cr} = \left(\frac{\left(\frac{\text{Cr}^{53}}{\text{Cr}^{52}}\right)_{\text{sample}}}{\left(\frac{\text{Cr}^{53}}{\text{Cr}^{52}}\right)_{\text{SRM}}} - 1 \right) \times 1000 \quad \text{Equation 1}$$

where, *SRM* represents the Cr isotopic reference material, SRM 979 National Institute of Standards and Technology (NIST) in the form of a hydrated chromium (III) nitrate, $\text{Cr}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$. ^{53}Cr , ^{52}Cr represents two different isotopes of interest, $(^{53}\text{Cr}/^{52}\text{Cr})_{\text{sample}}$ is the measured ratio of sample solution.

The resulting ($\delta^{53}\text{Cr}$) isotopic signature values are usually small, delta values are multiplied by 1000 and reported as adding per mil sign (‰), relative deviations in parts per million (ppm).

The δ -value refers to a ratio of a heavier isotope composition, a positive δ - value indicates to an enrichment in the heavier isotope compared to the standard, where negative value corresponds depletion. The $\delta^{53}\text{Cr}$ value of standard NIST 979 doped with a double spike has been monitored over the course of our study. The average value was $\delta^{53}\text{Cr} = -0.001 \pm 0.054$ (2SD) ($n=12$). The isotopic composition of all samples was corrected to this value.

The isotope composition of Cr may change during oxidation-reduction reactions. Cr isotope ratios can be an indicator of such reactions.

The isotopic fractionation is described by the fractionation factor (*see equation 2*).

$$\alpha_{\text{prod-react}} = \frac{\left(\frac{\text{Cr}^{53}}{\text{Cr}^{52}}\right)_{\text{product}}}{\left(\frac{\text{Cr}^{53}}{\text{Cr}^{52}}\right)_{\text{reactant}}} \dots \dots \text{Equation 2}$$

where, $^{53}\text{Cr}/^{52}\text{Cr}$ – ratio of reduced Cr at a given time (product) and in the reactant pool. Values for alpha α fractionation factor tends to be very close to 1.

Fractionation factor is also expressed as *enrichment factor*, ε : (see equation 3) (Ellis et al 2002).

$$\varepsilon = (\alpha - 1) \times 1000\text{‰} \quad \text{Equation 3}$$

Different reduction mechanisms and environments of Cr(VI) changes the $^{53}\text{Cr}/^{52}\text{Cr}$ ratio. Previously, Cr isotope fractionation during Cr(VI) reduction was examined under different temperatures. The fractionation factor gives quantitative insight into the reduction procedure according to the mechanism and kinetics of reactions (Basu and Johnson, 2012).

The relative isotopic difference Δ , between two substances in a closed system is calculated after adsorption according to equation 4:

$$\Delta^{53/52}\text{Cr}_{(lq1-lq2)} = \delta^{53}\text{Cr}_{lq1} - \delta^{53}\text{Cr}_{lq2} \quad \text{Equation 4}$$

where, $lq1$ – initial $\text{K}_2\text{Cr}_2\text{O}_7$ solution (reactant) (sample 1), $lq2$ – supernatant (product) (sample 2).

3.7. Mass balance

To verify and evaluate the isotopic and concentration data, the Cr concentrations and $\delta^{53}\text{Cr}$ values were determined in all “inputs” and “outputs” of the experiment. According to the mass conservation law the inputs must equal the outputs. The mass balance equation 5 applied:

$$m_1\delta_1^{53}\text{Cr} + m_2\delta_2^{53}\text{Cr} = m_3\delta_3^{53}\text{Cr} + m_4\delta_4^{53}\text{Cr} \quad \text{Equation 5}$$

Inputs: $m_1\delta_1^{53}\text{Cr} + m_2\delta_2^{53}\text{Cr}$ - (m_1 - initial mass (mg) of total Cr and Cr isotopic fractionation ratio (‰) - $\delta_1^{53}\text{Cr}$ in $\text{K}_2\text{Cr}_2\text{O}_7$ solution, m_2 – the intrinsic Cr mass (mg) in the solid sample and Cr isotopic fractionation ratio (‰) - $\delta_2^{53}\text{Cr}$ in intrinsic Cr.)

Outputs: $m_3\delta_3^{53}\text{Cr} + m_4\delta_4^{53}\text{Cr}$ - (m_3 – the total mass (mg) of Cr in the supernatants after adsorption and $\delta_3^{53}\text{Cr}$ - Cr isotopic fractionation ratio (‰) of supernatant, m_4 is the mass (mg) of total Cr (mg) adsorbed on the solid sample. $\delta_4^{53}\text{Cr}$ – fractionation ratio (‰) of Cr in solid phase after adsorption).

4. RESULTS AND DISCUSSION

The results of this work demonstrate that Cr isotopic fractionation occurred during reduction Cr(VI) by selected soil components. In general, intrinsic Cr plays an important role in Cr(VI) reduction within adsorption processes. The presence of intrinsic Cr influences the evaluation of isotopic data. Cr isotopic composition of the supernatants differs in terms of Cr isotopic signature.

4.1. The ability of clay minerals to reduce Cr(VI)

Clay minerals support reduction of Cr(VI) depending on Cr(VI) concentration and content of inorganic impurities. It has been confirmed by X-ray absorption spectroscopy (XAS) (data is not shown here), that Cr is originally present as an impurity in the clay minerals before the adsorption of Cr(VI). The presence of intrinsic Cr within individual soil clays represents real soil conditions. Isotopic data of kaolinite and illite is aggravated by the presence of intrinsic Cr.

Table 5 shows Cr isotope data of the initial Cr(VI) solution, intrinsic Cr, and supernatants after adsorption. Two isotopically different sources of Cr (solid phase and initial Cr(VI) solution) were mixed during adsorption of Cr(VI) onto clay minerals. Two different $\delta^{53}\text{Cr}$ isotope signatures of the intrinsic Cr were defined in Kaolinite ($0.193\pm 0.040\%$) and in Illite ($-0.317\pm 0.016\%$) compare to isotopic signature of initial Cr(VI) solution ($-0.475\pm 0.02\%$).

Samples	Initial pH	$\delta^{53}\text{Cr}_{\text{init}}\pm 2\text{SD}$	$\delta^{53}\text{Cr}_{\text{intri}}\pm 2\text{SD}$	$\delta^{53}\text{Cr}_{\text{eq}}\pm 2\text{SD}$	$\Delta^{53/52}\text{Cr}_{(\text{lq1-lq2})} \%$
Kaolinite	4	-0.475 ± 0.020	0.193 ± 0.040	-0.217 ± 0.081	-0.258
	6.5	-0.475 ± 0.020	0.193 ± 0.040	-0.209 ± 0.056	-0.266
	9	-0.475 ± 0.020	0.193 ± 0.040	-0.346 ± 0.044	-0.129
Illite	4	-0.475 ± 0.020	-0.317 ± 0.016	-0.398 ± 0.004	-0.077
	6.5	-0.475 ± 0.020	-0.317 ± 0.016	-0.431 ± 0.027	-0.044
	9	-0.475 ± 0.020	-0.317 ± 0.016	-0.632 ± 0.075	-0.157
Humic acids	4	-0.475 ± 0.020	-0.524 ± 0.059	0.039 ± 0.066	-0.514
	6.5	-0.475 ± 0.020	-0.524 ± 0.059	-0.185 ± 0.007	-0.29
	9	-0.475 ± 0.020	-0.524 ± 0.059	-0.210 ± 0.038	-0.265

Table 5. Cr isotopic data for the clay minerals and humic acid. $\delta^{53}\text{Cr}_{\text{init}}$ - initial $\text{K}_2\text{Cr}_2\text{O}_7$ solution, $\delta^{53}\text{Cr}_{\text{intr}}$ - intrinsic Cr in the samples, $\delta^{53}\text{Cr}_{\text{eq}}$ - supernatants after adsorption. $\Delta^{53/52}\text{Cr}_{\text{lq1-lq2}}$ = relative isotopic difference (%).

Speciation of intrinsic Cr in the samples have been confirmed XAS (Veselská et al., 2019). Intrinsic Cr was in Cr(III) form and was not desorbed from clay minerals in the blank experiment

Earlier studies show that clay minerals show highest Cr(VI) adsorption capacity at pH = 4, like lignite coal and bituminous coal were used as adsorbents (Kannan et al., 1991). This observation was attributed to reduction which occurs in acidic solutions of Cr⁶⁺ to Cr³⁺ and adsorption of Cr⁶⁺ was much larger than that of Cr³⁺ as investigated by (Huang et al., 1977). Adsorption of Cr(VI) onto the clay minerals can exceed up to 99%. where there is least (0.18%) desorption from clays (Lukman et al., 2013).

Cr(III) could be partially dissolved during Cr(VI) adsorption experiments. Clay minerals are main components of soils, which means that the effects of these minerals must be accurately described to determine the reduction and fractionation of Cr of during adsorption. Typically, clay minerals contain Al and Fe(II) oxides in their structure. Al and Fe(II). oxides reduce Cr(VI) once Cr(VI) is absorbed onto the clay surfaces. However, some dissolved Cr species can accumulate in the aqueous phases and may subsequently serve as catalysts (Deng et al., 2003).

The isotopic signature of this intrinsic Cr (δ^{53}) could influence the final isotopic composition in the supernatants after adsorption.

The reduction of Cr(VI) into Cr(III) caused Cr isotopic fractionation for both clay minerals. The changes in δ^{53} Cr vales were low (in case of kaolinite) and minimal change is obtained in case of illite. The isotopic heavier Cr(VI) fraction related to the initial Cr(VI) fraction in the K₂Cr₂O₇ solution was remaining in supernatants after adsorption while confirming Cr(VI) reduction.

There are significant δ^{53} Cr changes observed at pH 4 and 6.5 ($\delta^{53}\text{Cr}_{\text{eq(pH4)}} = -0.217 \pm 0.081\text{‰}$ and $\delta^{53}\text{Cr}_{\text{eq(pH6.5)}} = -0.209 \pm 0.056\text{‰}$) in presence of kaolinite. $\delta^{53}\text{Cr}_{\text{eq}}$ values were close to the initial K₂Cr₂O₇ solution ($\delta^{53}\text{Cr}_{\text{init}}$) value in presence of illite, at initial pH 4 and 6.5, ($\delta^{53}\text{Cr}_{\text{eq(pH4)}} = -0.398 \pm 0.004\text{‰}$, $\delta^{53}\text{Cr}_{\text{eq(pH6.5)}} = -0.431 \pm 0.027\text{‰}$). In case of illite that results show substantially less variation in Cr isotopic composition.

Isotopic $\delta^{53}\text{Cr}_{\text{eq}}$ values could be influenced by the dissolved intrinsic Cr(III). In the case of kaolinite ($\delta^{53}\text{Cr} = 0.193 \pm 0.040\text{‰}$) would be “positive”, shifting the $\delta^{53}\text{Cr}_{\text{eq}}$

towards positive values. In case of illite, ($\delta^{53}\text{Cr}=-0.317\pm 0.016\text{‰}$) would be “negative”, shifting the $\delta^{53}\text{Cr}_{\text{eq}}$ back to more negative values

The redox-independent Cr isotopic fractionation was confirmed as well in earlier studies (Saad et al. (2017)). To evaluate isotopic data more accurately the effect of the dissolved Cr(III) is considered which is initially present in clay minerals. The partial adsorption of Cr(VI) onto kaolinite and illite, was proven to have a minor effect on Cr isotopic fractionation (Ellis et al., 2004).

We can predict that both the inputs and outputs were well defined by the Cr concentration and isotope composition. In general, the good results between inputs and outputs obtained for both kaolinite and illite is accompanied by some deviations, especially in the case of kaolinite at pH 4 and illite at pH 4 and 6.5. These deviations may be caused both by the adsorption of Cr(VI) from the solution and the desorption of Cr(III) from the clay surfaces.

The isotopic mass balance of the system remains unchanged. In a similar mass range, the extent of natural isotopic variations is linked to the geochemical behaviour of the respective elements. This concept is visualized as linear scale and corrected for the mass difference effect. Elements with multiple oxidation states, presence in different aggregation states with existing variable bonding partners, shows larger isotopic variations than elements with one oxidation state which binds to the same bonding partners (Wiederhold, 2015).

4.2. Dominant role of humic acids in Cr(VI) reduction

Obtained data (*see table 7*) shows that the most significant change in the Cr isotopic composition was at pH 4 ($\delta^{53}\text{Cr}_{\text{eq}}=0.039\pm 0.0066\text{‰}$) with the maximal relative isotopic difference $\Delta=0.5\text{‰}$. Stable isotope fractionation causes a shift in the isotope ratio between reactant and product. The $^{53}\text{Cr}/^{52}\text{Cr}$ shift may reach up to 4‰ depending on the reduction agents (Basu et al., 2014; Ellis et al., 2002; Šillerová et al., 2014).

All the solutions after adsorption showed enrichment in the heavy isotope relative to the initial solution. The reduction of Cr(VI) by humic acids is accompanied by an enrichment in heavy Cr isotopes in the supernatant after the adsorption compare to the initial Cr(VI) solution ($\delta^{53}\text{Cr}_{\text{init}}=-0.475\pm 0.020\text{‰}$ (2SD, n=3)).

Generally, humic acids were shown to have good the reduction efficiency depending on their functional groups e.g. phenols and thiols and have been previously described as Cr(VI) reducing agents. Not many functional groups complexed with Cr(VI) have been detected on the surface of humic acids. Humic acids adsorb Cr(VI) at $\text{pH} < 4$ due to inorganic impurities (Al, Si, Fe, Ca), but soils with rich organic matter and clay minerals will be more efficient at retention of Cr(III) as a product of Cr(VI) reduction. (Scaglia, Tambone and Adani, 2013).

It was stated that the Cr(VI) reduction is slower at higher pH values near to neutral pH. Lower $\delta^{53}\text{Cr}$ values of humic acids corresponds the *lower* Cr adsorption efficiency. The reduction of Cr(VI) depends on the experimental conditions, not to mention particle size of sample, initial Cr(VI) concentration. (Wanner and Sonnenthal, 213).

Some other publications related to isotope fractionation have been conducted with Cr(VI) reductants; humic acids (with relative isotopic differences $\delta^{53}\text{Cr} = 3.1\text{‰}$) (Kitchen et al., 2012), microbial strains ($\delta^{53}\text{Cr} = 4.1\text{‰}$) (Sikora et al., 2008), ($\delta^{53}\text{Cr} = -4.2\text{‰} - 2.11\text{‰}$) in case of reductant aqueous $\text{Fe(II)}_{\text{aq}}$ (Basu and Johnson, 2012). and adsorbed Fe(II) or organic content onto the surface of clays attributes an effort on part of Cr(VI) reduction (Kwak et al., 2018).

The impact of isotopic signature on $\delta^{53}\text{Cr}$ values of dissolved Cr(VI) is dependent on the mass of exchangeable Cr(III) (Wang, Johnson and Ellis, 2015). As a result of Cr(VI) reduction in the water sheds, $\delta^{53}\text{Cr}$ values are positively fractionated in industrially contaminated waters in Czech Republic (Novak et al., 2014).

While humic acids are well soluble over a wide pH range (Melo et al., 2016), it is impossible to quantitatively separate the solid and liquid phase.

Mass flows cannot be quantified well as dissolved humic acids interfere in mass balance (see table 6).

Samples	pH	Initial solution		Intrinsic Cr		Treated solution		Treated solid phase		Mass balance	
		$\delta_1^{53}\text{Cr}$	m_1 (mg)	$\delta_2^{53}\text{Cr}$	m_2 (mg)	$\delta_3^{53}\text{Cr}$	m_3 (mg)	$\delta_4^{53}\text{Cr}$	m_4 (mg)	Inputs	outputs
Kaolinite	4	-0.475	1.44	0.193	0.19	-0.217	1.00	-1.349	0.29	-0.649	-0.612
	6.5	-0.475	1.44	0.193	0.19	-0.209	0.90	-0.466	0.47	-0.649	-0.409
	9	-0.475	1.44	0.193	0.19	-0.346	1.24	-0.324	0.41	-0.649	-0.561
Illite	4	-0.475	1.44	-0.317	0.20	-0.398	1.19	-0.658	0.17	-0.748	-0.587
	6.5	-0.475	1.44	-0.317	0.20	-0.431	1.22	-0.197	0.32	-0.748	-0.589
	9	-0.475	1.44	-0.317	0.20	-0.632	0.99	-0.225	0.33	-0.748	-0.696
Humic acids	4	-0.475	1.442	-0.524	0.12	0.039	0.35	0.006	0.40	-0.747	-0.016
	6.5	-0.475	1.442	-0.524	0.12	-0.185	0.54	-0.254	0.65	-0.747	-0.266
	9	-0.475	1.442	-0.524	0.12	-0.210	0.60	-0.588	0.25	-0.747	-0.274

Table 6: Data used for the mass balance calculation and ($\delta^{53}\text{Cr}$ values); the Cr isotopic fractionation ratio (‰). The mass balance of Cr (m values) in the initial $\text{K}_2\text{Cr}_2\text{O}_7$ solution, solid phases before adsorption and supernatant and solid phase after adsorption.

The obtained data confirms isotope fractionation is mass dependent. Mass balance applied to isotopic data to verify the isotopic fractionation of Cr during adsorption.

The small amount of Cr(VI) adsorption does not affect the results of $\delta^{53}\text{Cr}$ data considering the dissolved Cr(VI) remains in the supernatant after adsorption. The fraction of adsorbed Cr(VI) does not change during the process. Cr(III) is bound to the solid phase. (Basu and Johnson, 2012). However, inputs and outputs are affected by analytical procedure, but differences between them is smaller than the theoretical error.

5. CONCLUSIONS

The results of this work demonstrated that Cr isotopic fractionation occurred during the adsorption on selected soil clay minerals e.g. kaolinite, illite and humic acids and well reflects associated with reduction of Cr(VI). The obtained results are significant for identifying possible reduction mechanisms. The evaluation of obtained data reinforces the use of Cr isotopes to detect and quantify Cr(VI) reduction in clay minerals and humic acids.

Our work is based on a multidisciplinary approach on investigating the adsorption and reduction of Cr(VI) by soil components and humic acid under different pH (strongly, alkaline, neutral, and strongly acidic) conditions. Based on the results from isotopic analyses, we evaluated how the soil components influence Cr(VI) reduction in soils. Isotopic fractionation analysis confirms that the reduction of Cr(VI) is a fundamental key of Cr(VI) adsorption.

The obtained results proved that the chromium isotopic fractionation decreased in the order: humic acids - kaolinite – illite.

To conclude, isotopic approach has helped to obtain a more comprehensive information about the complex processes of adsorption/reduction of Cr(VI) in soils. The studied work and obtained results can help further to contribute to interpret Cr isotope data being collected in contaminated soils and more generally improve our knowledge of Cr isotope fractionation in a variety of geochemical applications to develop capable remediation methods.

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