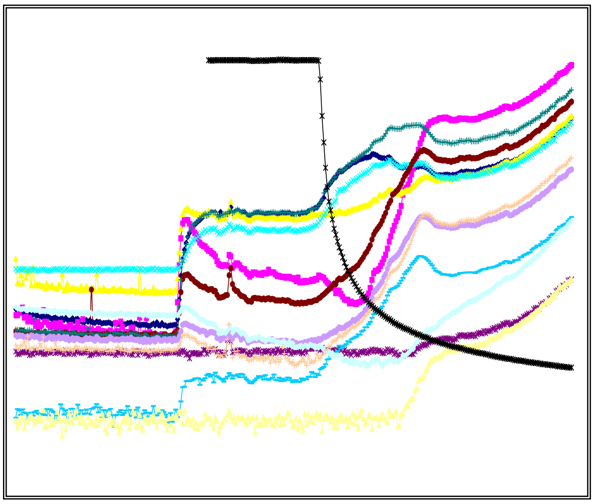

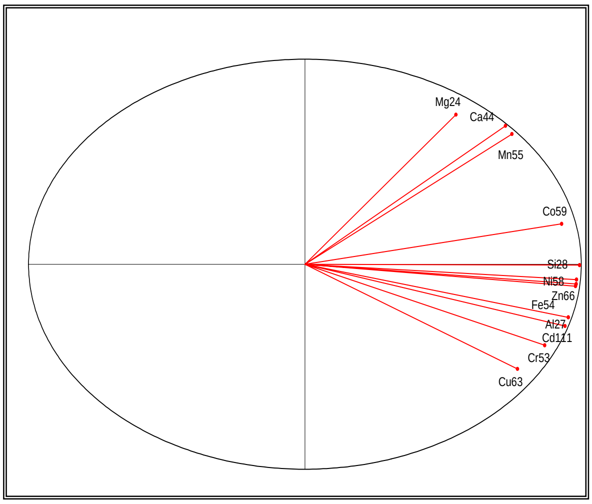

Continuous leaching-ICP-MS for the study of metal speciation in sediments

Master's Thesis in the 'Applied Environmental Measurement Techniques'

GEORGE NABIN BAROI

Department of Civil and Environmental Engineering
Division of Water Environment Technology
CHALMERS UNIVERSITY OF TECHNOLOGY
Göteborg, Sweden 2006
Master's Thesis 2006:34

MASTER’S THESIS 2006:34

Continuous leaching-ICP-MS for the study of metal speciation in sediments

Master’s Thesis in the *‘Applied Environmental Measurement Techniques’*

GEORGE NABIN BAROI

Department of Civil and Environmental Engineering
Division of Water Environment Technology
CHALMERS UNIVERSITY OF TECHNOLOGY

Göteborg, Sweden 2006

Continuous leaching- ICP-MS for the study of metal speciation in sediments

Master's Thesis in the *'Applied Environmental Measurement Techniques'*

GEORGE NABIN BAROI

Master's Thesis 2006:34

Department of Civil and Environmental Engineering
Division of *Water Environment Technology*
Chalmers University of Technology
SE-412 96 Göteborg
Sweden
Telephone: + 46 (0)31-772 1000

Department of Civil and Environmental Engineering
Göteborg, Sweden 2006

Continuous leaching- ICP-MS for the study of metal speciation in sediments

Master's Thesis in the *'Applied Environmental Measurement Techniques'*
GEORGE NABIN BAROI
Department of Civil and Environmental Engineering
Division of *Water Environment Technology*
Chalmers University of Technology

ABSTRACT

The advancement of multi-element analysis technique and the high sensitivity of ICP-MS opens the possibility of speciation analysis. Due to its high sensitive detector, ICP-MS is used in on-line speciation measurements since it has the potentiality to generate real time data and can be connected to separation techniques. Here ICP-MS is used for online monitoring of soluble metals associated with their adsorption to sediments. The use of multivariate statistical analysis helps in the analysis of element distribution and the relation among trace elements and major elements. Since speciation analysis with so called sequential extraction techniques produce discrepant results, the proposed method may provide a better alternative to study the speciation f metals in sediments.

Three different acid (HNO_3) concentrations are used for the leaching of twelve trace and major elements. Ca and Mg are the first major elements leached from the sediments with a maximum concentration in the aqueous phase at pH pH 2.3±0.38 and 2.4±0.43 respectively. Fe/Mn is maximum at pH 2.2±0.26 and 2.2±0.2 respectively. The results also show an overlap of Mn between Ca and Fe based phases. Ni, Co and Zn are found to be associated with Fe/Mn phase and Cd, Cr, Cu is found to be less associated with Ca phase. Significant readsorption and redistribution happens for Ca, Mg, Mn, Fe, Ni, Zn, Co, Cu and Al at different phases and it is quite clear at 1% HNO_3.

Key wards: Leaching, speciation, association, sorption, phase, pH, ICP-MS

ACKNOWLEDGEMENT

I would like to express gratitude to my honourable supervisor Sebastien Rauch for his continuous guidance, support, scientific advice and insights. I am very happy to work with him and grateful for his intensive care on my study and active co-operation in experiments.

I am also grateful to my examiner professor Greg Morrison for providing me a space with computer facilities in the WET department.

I must give thanks to Jesper and Mona for continuous assistance and encouragement. A friendly environment helps me not to scare in the laboratory. It is a very big achievement in my life.

I want to remember my parents, classmates and friends for their inspiration and suggestions to finish this task.

Göteborg
April 2006

George Nabin Baroi

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

1 INTRODUCTION

Sediment is an important sink (Singh et al., 2005; Förstner, 1992; Nürnberg, 1984; González et al., 2000) and carrier (Pardo et al., 1990) of trace elements in the aquatic environment. Trace elements in sediments are considered serious important environmental pollutants that can results from anthropogenic activities (Birch et al., 1996), although they can also be released from natural sources such as weathering of soils and volcanic eruption (Singh et al., 2005). The mobilization of these metals can occur in ecosystems due to changes of environmental conditions such as pH, redox potential, organic and inorganic complexion, microbial activities (Förstner, 1992; Roychoudhury, 2005) and physical process related to strong currents or dredging (González et al., 2000). Since trace elements are not bio-degradable, they might accumulate in plants or organisms (Nürnberg, 1984) and deteriorate their lives. Thus measurement of trace elements is important for assessment of the environmental impact of polluted sediment.

Several studies referring to the determination of total metal concentration (metals associated with suspended and bottom sediments) (Birch et al., 1996; Tessier et al., 1979) infers that all forms of metals present in the sediment have equal environmental effects. But biological and toxicological studies confirm only certain environmentally available forms that are harmful for living organisms need to be determined (Kot et al., 2000). Therefore, the mobility, bioavailability and subsequent toxicity of trace elements become important aspect in the research area (Singh et al., 2005; Förstner, 1992; Nürnberg, 1984; González et al., 2000). The bioavailability of toxic metal species is a complex phenomenon which depends on the chemical kinetics of the species and the interaction of species at different phases (Morrison et al., 1989). To describe the distribution and transformation of metal species a new approach termed ‘speciation’ have been taken under consideration where individual chemical and physical forms of species is identified. But due to some complexity of heterogeneous systems (Förstner, 1992; Rauch et al., 2000), speciation is restricted to transformation and/or distribution or identification of chemical species or only analytical activity of species. Therefore, typical metal species are determined operationally defined procedure (Förstner, 1992).

The possible forms of trace elements dissolved in water are hydrated ions, inorganic and organic complexes and metal species associated with heterogeneous colloidal dispersions where as particulate forms of metal species adsorbed with mineral matrix or clay particles (Morrison et al., 1989). In the speciation measurement, only some of these forms are considered for sediment quality criteria susceptible to environmental conditions. Therefore, several procedures have been developed for trace element extraction from sediments and soil (Tessier et al., 1979; Kersten1986; Hirner, 1990; Gatehousea 1977). Tessier et al.(1979) developed a sequential extraction procedure that consists of five geochemical phases. This analytical procedure found difficulties on selectivity (Kheboian and Bauer, 1987), recovery, readsorption and redistribution (Gómez-Ariza et al., 1999; Nirel and Morel, 1990). Later Communities Bureau of Reference (BCR) have prescribed a three steps protocol that subsets some drawbacks of different procedures and standardize the methodology. This extraction technique seems very straighter forward where samples are digested in each phase. However, it is a great effort to make all scientific works into one platform but still it remains some doubts. Some studies still do not support this new procedure for difficulties on reproducibility (Sahuquillo et al., 1999) and strict use of reagents in each phase which raises questions about the selectivity of phases (Marin et al., 1997; Beauchemin et al., 2002; Pardo et al., 1998). Therefore, a new method needs to be developed to distinguish phases responsible for releasing the most mobile fractions. Further an observation should be made to know how element redistribution happens. Although some studies measured element redistribution in different phases by measuring the loss of elements in each phase (Gómez-Ariza et al., 1999) but it is a tedious process. A pictorial clear observation needs to understand this phenomenon where phase transformation can be observed.

Inductively coupled plasma mass spectrometry (ICP-MS) is one of the most common spectroscopic methods for determination of metal concentrations in environmental samples (Sastre et al., 2002). Low detection limit, low sample consumption makes it useful for trace element analysis (Falciani et al., 2000). The advancement of multi-element analysis technique and high sensitivity of ICP-MS opens the possibility of speciation analysis (Rauch et al.,2000, Baca et al., 2003). Due to its high sensitive detector, currently ICP-MS is used in on-line speciation measurement (Beauchemin, 2002; Sanz-Medel and Blanco-González, 2001; Plantz et al., 1989, MacFarlane et al., 2005; Baca et al., 2003). Advance data management and presentation techniques help for real time data visualization.

This paper describes an analytical method that will help to reduce short coming involved in sequential extraction procedures by continuous leaching ICP-MS. This method is dynamic and automated to reduce manual operations. Since ICP-MS has the potentiality to generate real time data, this technique will give information instantly while reactions happen between analyte and reagent. A statistical analysis will be performed on the basis of this data set to find element distribution and the relation among trace elements and major elements.

2 AIMS AND OBJECTIVES

Since the extraction poses difficulties to provide meaningful description of redistribution, mobility, an effort has been made to depict those problems through a dynamic process with minimum laboratory facilities. Therefore, the objectives that are made to fulfil this effort are as follows:

- Develop simple procedure to measure leaching and better understand speciation in sediments.
- Provide a statistical analysis method to interpret the results
- Study kinetics of metal desorption

This study will not measure total concentration of each element and mobility and the pollution level in terms of concentration will not be discussed.

3 BACKGROUND

3.1 Important concepts

Leaching of trace metals in the environment depends on chemical equilibrium and kinetics which are function of pH, redox potential (Borg et al., 1996), ligands (Calace et al., 2004). To get better understand, therefore, some concepts are briefly described for further understanding of leaching and speciation.

3.1.1 Ligands

A functional group, atom or ion that combine with other atoms or ions, usually metals, by donating electrons through a coordinate covalent bond and the compound formed in this way is termed as complex. Simply, a ligand is any atom or group of atoms attached to the species of interest. For metal complex, metal ions are lewis acids which accept electron from electron donating Ligands that are lewis bases. For an example, in the following reaction of silver ions with cyanide ions, Ag^+ act as lewis acid where as cyanide act as a lewis bases and they from a complex ion silver cyanide.

$Ag^+ + 2\ CN^- \leftrightarrow Ag\ (CN)_2^-$

Generalized form of the above reaction can be written as:

$aMe^{n+} + bL^{m-} \leftrightarrow (Me_bL_a)^{n-m}$

Where 'Me' means 'Metal' and 'L' means 'Ligand' (Harris, 2002).

Water as a ligand:

Addition of water in to sediment might produce metal complex ($Me(OH_2)_{x-1}(OH)^{n-1}$). Where, Me^{n+} combine with hydroxide ions and form hydrate complex. In this combination proton for water molecules released in to water.

$Me^{n+} + H_2O \leftarrow\!\!\text{------}\!\!\rightarrow Me(OH)^{n-1} + H^+$

$Me^{n+} + OH^- \leftarrow\!\!\text{------}\!\!\rightarrow MeOH^{n-1}$

The above two reactions might be written in the following one form.

$Me\ (OH_2)_x^{n+} \leftarrow\!\!\text{------}\!\!\rightarrow Me(OH_2)_{x-1}(OH)^{n-1} + H^+$

Here $Me(OH_2)_{x-1}(OH)^{n-1}$ is hydrated metal complex where hydroxide is acting as a ligand. (Benjamin, 2002)

Organic compounds as ligands

Typical organic compounds with ligands such as humic substances (HS) can contain various moieties such as carboxyl, hydroxyl and amino groups that can complex metal cations in various ways. The solubility and mobility are strongly influenced by the presence of humic substances and other naturally occurring synthetic ligands.

3.1.2 Metal speciation

The term “speciation” used in different fields eg. toxicology, clinical chemistry, geochemistry, environmental chemistry for expressing the meaning of transformation of species, distribution of species and analytical activity for concentration determination. Even, in biology ‘speciation’ is used for evolution of species. Therefore, the meaning of speciation becomes more confusing in the technological advancement in analytical measurement. Here few terms related to speciation have been discussed on the basis of IUPAC with other studies for better understand.

‘Speciation’ refers to the fact that a metal ion (or a group of atoms) in solution can potentially exist in a variety of forms (i.e. as a free ion; as a number of solution complexes; and potentially in different oxidation states). Metal speciation is a process of identification and quantification of ***forms*** of a metal (Benjamin,2002) where each of that individual physico-chemical forms together makes the total concentration of that metal (Florence, 1981) . For example, $Fe(OH)_2$, Fe_2O_3, $Fe(OH)_3$, Fe_2^{++}, Fe_3^{++} are some of the forms of iron in aqueous solution either in ferrous (Fe (II)) sate or in ferric (Fe(III)) state. Among the two forms ferrous iron is soluble and toxic however, in this case it does not mean that higher concentration of Fe (III) has no environmental effects rather there is a potential risk for redox reaction that can generate free radicals like OH- which cause oxidation of tissue. Therefore, the identification and quantification of each and individual species are important.

Many reports have been published on speciation where speciation normally refers to the determination of ***species of metals and metalloids*** including organometallic compounds.(Das et al.,1995). According to IUPAC speciation means ‘distribution of an element amongst *defined chemical species* in a system’ (Templeton et al. 2000). The mobility of metal species from one environmental medium to other depends on their reactivity or kinetics which intern makes them-selves available in that medium. Therefore, in that sense speciation meaning is of ‘describing the distribution and transformation of metal species in various media’ (Calace et al., 2004).

Speciation is very significant for determination of trace and ultra-trace element concentration and there are difficulties involved in the choice of analytical techniques. Since the concentration of a trace element is low, its individual species might be at concentrations below the detection limit of sensitive analytical instruments. Although, it is now possible to determine various elements at the nanogram level in deferent samples, very little success has been achieved so far. Three types of speciation techniques can be defined i.e. chemical, functional and operational.

Chemical speciation

Chemical speciation refers to the determination of specific forms of an element defined as to isotopic composition (eg. ^{204}Pb, ^{206}Pb, ^{207}Pb,^{208}Pb), electronic or oxidation sate (eg. Fe(II), Fe (III)), and/or complex or molecular structure (organic or inorganic) in a given solution matrix (Templeton et al. 2000). Therefore, chemical speciation refers (a) functional group such as ‘plant-available species’, (b) operationally defined species and (c) as particular compounds or oxidation states of an element. (Davidson et al., 1994)

Functional speciation

Functional speciation refers to the analytical activities of identifying and/or measuring the one or more individual chemical species that play an important role in the environment (Templeton et al. 2000). Actually scientist wants to know this species through analytical chemistry. It is not an easy task to measure functional species. Since long time, a direct method of analysis has been performed to measure the species by tissue analysis. There is a scope for development of a new method of this analysis.

Operational speciation

In the operational speciation technique, a specific kind or set of metal species defined according to their physical (eg. size, solubility) and chemical (eg. bonding, reactivity) properties (Templeton et al. 2000) before extraction are quantified that means operational procedures for determining typical metal species in environmental samples (Calace et al., 2004). Such as BCR or ASTM three steps sequential extraction standardize technique is widely used as an operationally defined speciation technique (Morrison et al.,1989; Kebbekus et al., 1998; Harris, 2002) where, specific set of metals species such as carbonate, oxides, sulfide fractions are targeted for extraction. Again, metal extracted from soil with an acetate buffer' is an operational definition. Operational speciation interchangeable used as functional speciation.

3.1.3 Leaching

Leaching is an important process that frequently occurs in the natural systems. Metals can be trapped into pore water of sediment particles or adsorbed by organic cells, ligand complex or even on the surface of sediment particles or organic compounds [Calace et al., (2004), Usero J., et al.,(1998)]. If metals are released from one of the phases, it will be called leaching. The accumulated metals in the sediment may exist in different forms such as particulate, ionic, complex, salt which depends of the geochemical conditions in the system. Under prevailing conditions, metals with different solubility may come in to liquor solution. Metals that are trapped in pore water can be leached easily either mechanical process or chemical process. But metals that are absorbed by other species are not easily leached since metals are chemically bonded with that species.

Chemical aspect of leaching

The chemical process of leaching depends on several factors such as reaction time, pH, sorption, redox and complexation. The time of contact for reaction can be varied from a second to several days. Acid-Base or complexion reaction usually takes less time (few seconds) where as sorption takes through few hours to several days or even months.

pH strongly affects leaching, redox transformation, complexion and sorption process. As shown in figure 3.1a, the leaching of metal species increases at both higher and lower value of pH and the concentration in the solution is lower while pH is normal. This similar pattern can be observed in the sorption process where anion species are sorbed at lower value of pH and cation species are sorbed at higher value of pH (Figure 3.1b). Changes of proton in the solution influence oxidation and reduction reaction, while adding acid increase proton into solution that changes ion exchange and speciation chemistry. Higher and lower value of pH will increase solubility of metals without complexes, on the other hand the concentration of oxide metals complexes is low when pH is high (Figure 3.1c).

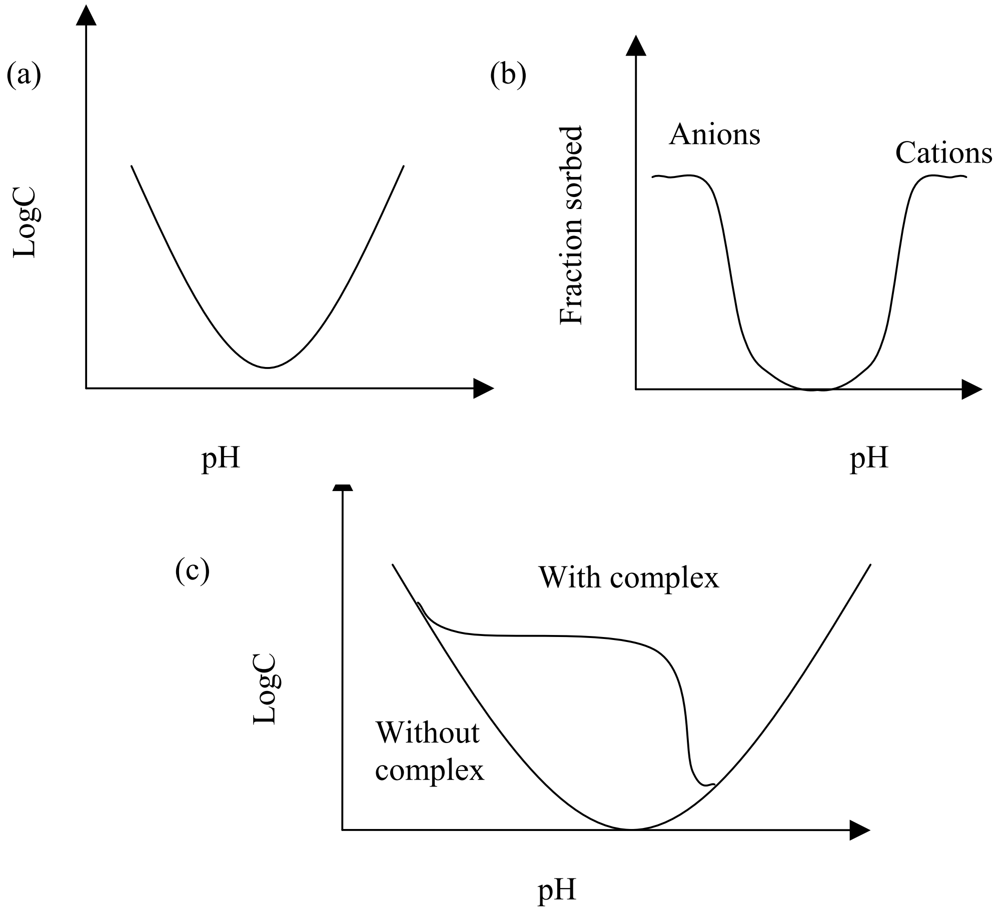

Figure 3.1 Effects of pH on the equilibrium of chemical kinetics a. speciation, b. sorption processes and c. redox transformation and complex formation.

(source: http://www.epa.gov/epaoswer/hazwaste/test/pdfs/batchel.pdf)

3.2 Extraction schemes and leaching

Determination of extractable trace metals in sediment and soil has long history. Since late 1970's various methods have been developed dealing with single and sequential extraction schemes such as Tessier et al. (1979) developed five steps sequential procedure, but none of them have been accepted due to the lack of uniformity in the determination and extraction procedures cause difficulty to compare results. The results obtained were mostly 'operational specific' that means the forms of metals are defined by the procedure used for determination. Therefore, BCR and other EU expertise organized a project to eliminate these problems through improvement and harmonization of measurement procedure and thereby developing an extraction protocol for sequential extraction of trace elements (Salomons, 1993 ; Ph.Quevauviller et al.,1993 ; Ure et al.,1993 ; Ph.Quevauviller, 1998). A three steps sequential extraction protocol is the consequence of this project. But still the BCR proposed procedure poses some difficulties such as systematic error, total experimental time and phase selections. Different methods have been developed to reduce those difficulties.

3.2.1 Different procedures used for leaching tests in environmental studies

There are two ways of determining sediment speciation, theoretically by thermodynamic analysis and the other is by experimental techniques. The thermodynamic techniques can only suggest the expected trace element species distribution, which is suitable only when the number of sample is less and thereby uncertainty involves due to changes of complex physio-chemical characteristics of metals. On the other hand, experimental techniques can perform operationally depending on the sample properties. Therefore, experimental analysis becomes more popular than theoretical analysis. Among other experimental analysis such as microwave digestion (Bettinelli, 1989) and direct instrumental techniques like X-Ray fluorescence (Battiston et al.,1993), sequential extraction procedure is one of the widely used experimental techniques where fractions are defined operationally. Several reports published with different operational or selective sequential extraction techniques such as Gatehouse et al.(1977), A.Tessier et al.(1979), Salomons and Förstner (1980), Kersten and Förstner (1986). Tessier's proposed method is the most widely used among them. This method has five consecutive sediment fractions: exchangeable, carbonate, reducible (Fe/Mn-Oxides), oxidable (organic and sulfide) and residual. Since this method defines each environmental phases, different laboratories at different places used this method for speciation measurement for various environmental samples. But, while comparing the results between two different laboratories, not only Tessier method but also all the methods show a significant variation in phases. Many studies report their variability in the sample pre-treatment (freezing-drying) (Kersten and Förstner, 1986), Oxic-anoxic sample (Wallmann et al.,1993), adsorption, readsorption, reproducibility (Nirel et al.,1990; Gómez-Ariza, 1999) etc. and therefore, questions have been raised on phase selectivity (Kheboian et al.,1987).

Under these circumstances, BCR proposed a three steps operational extraction protocol to reduce those problems and finally standardize this method using Standards, Measurements and Testing program (SM&T) providing certified reference materials (CRM) for quality control of this procedure.(Ph.Quevauviller et al.,1993, 1998). BCR proposed fractions involve only mobile fractions: exchangeable/carbonate, reducible (Fe/Mn-Oxides), oxidable (Organic and Sulfide). Strict quality control improves highest extraction of metals compare with other methods. Due to operational or selective chemical extraction procedure, still criticism has been raised for phase selectivity, readsorption and redistribution of elements and reproducibility.

Several studies try to improve these problems applying different techniques. Studies reported methods for *improvement of extraction time*: A continuous flow fractionation proposed by Spivakov et al. (2005), Tongtavee et al. (2005), Fedotov et al., 2005, *sample handling and pretreatment*: Sequential-injection analysis (SIA) proposed by Economou (*2005*), by MacFarlane et al.(2005), *Elemental association (bonding):* LA-ICP-MS proposed by Rauch et al. (2000).

3.3 Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

3.3.1 Principle of ICP-MS

Inductively Coupled Plasma Mass Spectrometry (ICP-MS 'ELAN 6000' PE Sciex) is a very sensitive and powerful analytical method used to analyze trace elements (Figure 3.2). The principle of this instrument is that ionized or excited atoms produced by the application of plasma to the sample or analyte, are separated and identified according to their mass and charge ratio (m/z). The whole ICP-MS system consist of five sections automated sample introduction, inductive coupled plasma, interface, mass spectrometry and finally data processing and display section.

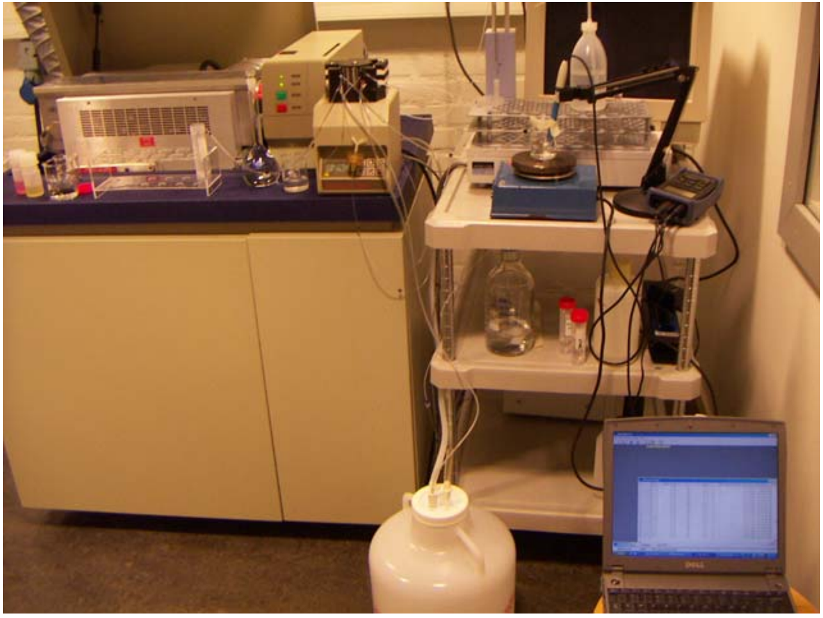

Figure 3.2 Picture of Chalmers ICP-MS Laboratory

Sample introduction:

The liquid sample, a common form of sample introduction, is forced into pneumatic nebulizer at a flow rate of 1mL min^{-1} by the peristaltic pump. Although other types of nebulizer can be used such as concentric, cross-flow, glass frit, hildebrand grid, V-groove or babington type. The cross-flow nebulizer in ELAN 6000 creates an aerosol, a fine suspension of liquid particle in a gas, from liquid sample and transfer into the mixing chamber, called Fazel-Scott Spray chamber, from which these fine aerosols are carried by a stream of Ar through a heated tube to evaporate and separate solvent through condensation. Finally the analyte reaches to the plasma as a dry aerosol through inner spray chamber. (Harris, 2002; Mendham et al., 2000; Ebdon et al.,1998; Rauch 2001)

Inductive Couple Plasma (ICP):

'Plasma is a partially ionized gas with sufficiently high temperature to atomized, ionized and excite most of the elements in the Periodic Table' (Ebdon et al.,1998). To produce plasma argon, helium or air is usually used. In this case argon gas is used since it produces relatively pure form of ions or excited atoms.

The followings are some types of ionization processes that might occur in the ICP:

$M + A^+ \rightarrow M^+ + A^+ + e^-$ (ion – atom collision)

$M + A \rightarrow M^+ + A + e^-$ (atom – atom collision)

$M + e^- \rightarrow M^+ + 2\,e^-$ (electron impact)

$Ar^+ + X \rightarrow Ar + X^+$ (charge transfer)

$Ar^m + X \rightarrow Ar + X^+ + e^-$ (penning ionization†)

†This ionization could be happened while meta-stable atom collide with neutral atom and ionized the neutral atom by consequent de-excitation of the meta-stable atom

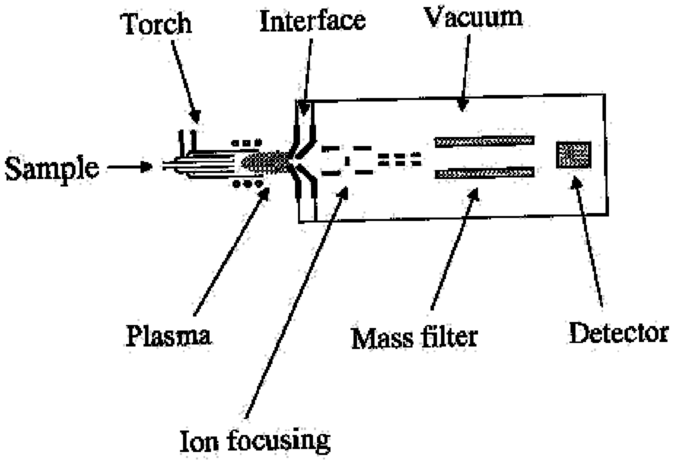

Figure 3.3 Cross section of ICP-MS reproduced from Rauch S (2001)

In ICP, the plasma torch comprises three concentric silica quartz tubes (Figure 3.3) each of which is open at the top. Two or three turns of a radio-frequency (RF) induction coil are wrapped around the top opening of this quartz tube. The sample in the form of aerosol carried out by argon is passed through the central tube. Auxiliary argon is passed at a rate ~1 L min^{-1} through the middle tube and plasma gas (argon gas) is passed at a rate 10-15 L min^{-1} through the second or middle tube maintains the plasma. The helical pattern of the plasma flow of plasma gas provides stability and helps to thermally isolate the outermost quartz tube. The ignition of plasma, done by a spark from Telsa coil probe, ionizes Ar gas. Thus, seeded free electrons are accelerated by the radio-frequency field and absorb enough energy form the coil to maintain constant higher plasma temperature (6000-10000K). Accelerated electrons, collide with atoms, transfer their energy to the entire gas. The outer tube passes coolant gas to protect quartz torch from over heating. These ions and atoms transfer into mass spectrometer through interface (Harris, 2002; Ebdon et al.,1998; Thompson et al., 1989).

Interface:

The interface plays the important role of transferring ions from the extremely hot plasma into the mass spectrometer. The mass spectrometer does not work at this high temperature, and requires high vacuum to avoid collisions between ions and background gas molecules which divert the ions from their trajectory in a magnetic filed. Therefore, an interface has been introduced between the ICP and the mass spectrometer. Temperature has been reduced by making a series of vacuum chambers held at consecutively lower pressure. Plasma and the mass spectrometer kept at a same horizontal axis to direct plasma onto water cooled nickel sampling cone with one millimeter diameter orifice. Behind sampling cone the vacuum pressure is approximately 10^{-3} bar. A fraction of the plasma gas together with analyte ions can pass though the orifice and a sudden expansion is formed due to this high vacuum pressure which might take a form of a cone called Mach disk and the region of that the expansion take place is called ‘zone of silence’, which is the representative of the ion species in the analyte.

Another water cooled cone called skimmer with smaller orifice size than sample cone is placed just behind the sample cone in such a way that a small portion of ‘zone of silence’ can pass through its orifice. The pressure in this chamber is approximately 10^{-7} bar. From the skimmer, the ions are passed as an ion beam to the mass spectrometer through a series of ion lenses. (Harris, 2002; Ebdon et al., 1998; Thompson M. et al., 1989)

Mass spectrometry (MS):

Once the ions are introduced in to the mass spectrometer, ions are separated according to their mass to charge ratio (m/z). When ions are singly charged then m/z is equivalent to mass of that atoms. Several mass spectrometers such as sector field, time-of-flight, ion trap mass and quadrupole used for mass analysis. In this study ICP-MS ELAN 6000 uses quadrupole mass filter. Quadrupole consists of four parallel metal rods where oppositely rods are connected with radiofrequency and direct current (DC) which creates an electric field depending on the m/z ratio. The electric field deflects ions and makes a complex trajectory. Only specified value of m/z, which can be fixed by rapidly varying of voltage of the electric filed, allows passing of ions through this quadrupole and transfers to the ion detector and other ions colloid with rods and they lost before reach the detector. (Harris, 2002; Ebdon et al., 1998; Siuzdak, 1996; Rauch, 2001; Thomas, 2004)

Data processing and display

The detector transfers atoms information to data reduction and system control. ELAN NT software systems can control hardware and methodology including quality control checks, graphical display with real time data. The ELAN 6000 can not only detect all elements in the periodic table but also their isotope and isobars. It can measure up to eight orders of magnitude. (http://web.uct.ac.za/depts/geolsci/facilities/icpms/lectures/lec1.html)

3.3.2 Interferences of ICP-MS

An effect that causes change the signal or spectrum while the concentration of the analyte remains same is called interferences. This effect can be due to spectral, chemical reaction or ionization of analyte. Generally elements of oxides, hydroxides and ions (Vaughan et al.,1986) cause spectral interferences and inductively coupled plasma can atomized completely of analyte oxides and hydroxides and therefore this effect is almost negligible in this case. Chemical interferences can be happened due to incomplete atomization of analyte and this caused by other components present in the sample. For example, SO_4^{2-} and PO_4^{3-} inhibit atomization of Ca^{2+}. Ionization interference is caused due to low ionization of analyte. Generally this type of interferences has been observed in the analysis of alkali earth metals since they have lowest ionization potentials. All the effects discussed above are under main two broad categories which are spectroscopic interferences and non-spectroscopic interferences (Evans et al., 1993).

Interferences can be due to background gas like Ar , for example, the plasma has background of argon oxide (ArO, mass 56) which cause interference for the most abundant ^{56}Fe isotope. Interference with the same mass is called spectral interferences which is also termed as *isobaric interference* (Rauch, 2001; Thopmson et al., 1989; Evans et al., 1993). In ICP-MS, this problem is suppressed by separating each species by its higher resolution (MacFarlane et al., 2005; Morita et al. 1989). Even isobaric interferences can be reduced by using alternative sample preparations methods (sample dissolution, precipitation and solvent extraction, online separation), alternative sample introduction method (desolvation, thermal vaporaizaiton, laser ablation, hydide/vapour generation), other methods (altenative gas and mixed gas plasmas, alternative plasma sources, multivariate correction methods) (Evans et al., 1993). Description of these methods are beyond the scope of this study.

Again, interferences can only happen due to matrix. Everything in the sample other than analyte is termed as matrix. A change (reduction or suppression and enhancement) in the signal caused by anything other than analyte is termed as matrix effects or non-spectral interferences. Matrix induced interferences can be as: ‘ionization interferences’ (due to high concentrations of matrix elements), ‘ion sampling interferences’ (a change in the ion population in the sample) and ‘mass-dependent signal drift’ (possibly due to deposition of salt on the ion optics) (Evans et al., 1993; Beauchemin et al., 1987; Thopmson et al., 1989). These effects can be reduced by sensitizing the spectrometer before analysis by nebulization of a solution containing responsible element, internal standardization (Thopmson et al., 1989; Rauch, 2001), isotopic dilution, separation methods and flow injection (Evans et al., 1993).

4 THE PROPOSED METHOD

In the BCR extraction procedure, factions have been defied in two categories, one is 'pseudo-total' content and the other is 'residual' content. Pseudo-total content is considered as the most hazardous for environment since they contain the mobile fractions. BCR three stages procedure demonstrate this 'pseudo-total' content under tree groups such as carbonate and/or exchangeable fractions, Fe/Mn oxides fraction, and finally sulfide and organic fractions where all these phases are pH dependent. The most mobile like carbonate and/or exchangeable fractions are considered in the first phase and the less mobile like sulfide and organic fractions are considered in the third phase under low pH and Fe/Mn oxides are considered in between them. Therefore, an idea has been developed that can give a clear picture of toxic metal species and relation among them under various pH condition.

4.1 The idea

The dependence of metal leaching at different pH level is known from different technical information. Progressive acid flux into sediment can change pH level which intern causes metal mobilization from sediments (Thomas et al., 1994; Rauret et al., 1991). Exchangeable fractions (weakly bonded with solid surfaces), carbonate, hydrous iron and manganese oxides have higher solubility product constant and they are easily available at higher pH (Morrison, 1984). Under this consideration, a continuous acid flux at various concentrations (eg. 0.1%, 1%, 10% HNO_3) has been used to perform this study. Therefore, the most mobile fractions (carbonate and exchangeable fractions) are expected to be released at the fist phase. Continuous acid flow can decrease pH level at which the disintegration of binding of trace and major elements are expected since higher concentration of H^+ or lower value of pH reduces larger size of metal complex.

As discussed in the previous sections, BCR and all other sequential procedure are criticized due to readsorption or precipitation during extraction and strict reagent use in every step. To observe readsorption and/or precipitation, here, analytes are not completely be extracted from sample rather to observe the kinetics in a particular phase and during the phase change, a continuous acid flux is maintained.

Since the multi element capability of ICP-MS enables the analysis of both majors and trace elements, it is possible to study the binding in phases (as in sequential extraction) by analysis both trace elements and major elements present in the phases. Again, ICP-MS can produce real time data. To observe fractional distribution and readsorption in each phase, a continuous injection of solution in to ICP-MS has been made. The change of pH in the solution can be measured by pH meter with a certain interval of time and all data will automatically transfer to computer. Therefore a correlation among the metal species at certain pH can be derived statistically.

Due to strong digestion capacity, nitric acid is selected as reagent. Compare to other acids nitric acid has a high oxidizing capacity. However, very dilute nitric acid can dissolve almost all trace elements of concern because most of the nitrates are water soluble. Nitric acid also helps to reduce isobaric interferences while performing analysis through ICP-MS (Beauchemin, 2002).

4.2 Sampling

A laboratory preserved sample which has been collected by students in the AEMT programme of Chalmers is used in this experiment. The sediments were collected from lake Vällsjön in Göteborg. Vällsjön is a fairly shallow lake, situated lower down in the landscape. It was below the sea level (Sohlenius G. et al.,(2004)) during the last ice age and the surrounding soil is therefore thicker and richer. For several decades up until the 1970s the lake was contaminated with domestic waste water and run-off from landfill sites. Contamination is less now a day.

Sediments at different depths have been preserved in small plastic containers. In this study, sediments in each container have been mixed manually up to 10 minutes. These homogenized sediments again poured in to a separate plastic container and then preserved into refrigerator for further experiment. Since sediments at different level of depth are homogenized, it may lose its original state of equilibrium. It is assumed that metals in the sediment sample are at equilibrium state. Actually, the purpose of this homogeneity is get representative sample for analysis.

As the sediments are preserved into refrigerator for several months and the sediment containers are not properly poured, brownish precipitation (Fe-hydroxide) is observed on the container wall. This observation confirms that the sediment samples are oxidized prior experiment. The sediments are well mixed while taking samples from the container. In each experiment, 5gm of sample is taken for leaching analysis and mixed with MilliQ (0.25 µm) water to make the sample volume 80ml. The volume ‘80ml’ is considered arbitrarily to dilute samples properly in water and for better reactions of each sediment particles with acid. Although, BCR protocol suggests taking 1gm of sample but in this case, 5gm considered as well representative of the total sediment rather than one gram sample. Even, to avoid sample loss, sieving of sample is discarded. All experiments are performed with acid washed apparatus (beakers, tubes, filter system) at room

Figure 4.1 Picture of sample container showing brownish color on the surface of the container.

temperature (20±1^{0}C). Three samples have been prepared for analysis at different acid concentrations 0.1%, 10% and 1% (HNO_3) respectively.

4.3 Experimental setup

A simple experimental setup is designed for acid flux, analyte separation through filtration and finally injection in to ICP-MS (Figure 4.2). Tubing system is, therefore, arranged so that, analyte mixes with HNO_3 (reagent). To observe the flow and/or filter effect, internal solution (Rh solution 50 ppb) is used passing through filter media. Filter system is nothing but a plastic filter box (Figure 4.2). Inside the filter box cellulose filter with pore size 0.45µm is used for filtering. Two tubes, one carrying sample with analyte and the other carrying internal solution, meet just before the filter system. The folw is maintained by using a peristaltic pump roller linked with ICP-MS (Figure- 4.3). There are four pipes with diameter (0.03 inch innner daimeter) used to connect each other.

Figure 4.2 Picture of filter system used in the experiment

A glass beaker containing 5gm of sample is pored with MilliQ water to make the total volume 80ml. Magnetic strried machine is used for well mixing of reagent and sample. A pH meter (WTW Multiline P4, F/SET-3) with data logging is used for pH measurement. The pumping rate and the diameter of the tubes are kept same for all sample analysis so that the flow rate in each tube (1,2,4,5) (Figure-4.4),

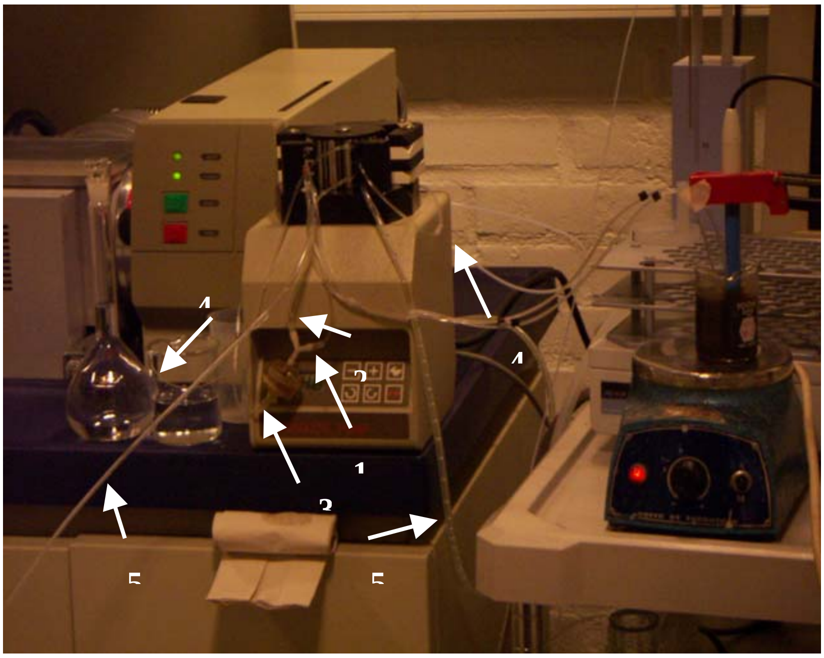

Figure 4.3 Picture of experimental setup

before and after the peristaltic pump remains same 0.5 ml/min and the flow rate after filtration becomes 1 ml/min (0.5 + 0.5 = 1ml/min) in tube 3, as tube carrying analyte (tube-1) solution has flow rate 0.5ml per minute and tube carrying internal solution (tube-2) has flow rate 0.5 ml/min. Agin tube passing reagents (HNO_3) (tube-4) has flow rate 0.5 ml/min, therefore, total vloume of solution in to the sample beaker remains same. Another pipe (tube-5) is used to pump out the soultion from ICP-MS to waste bottle.

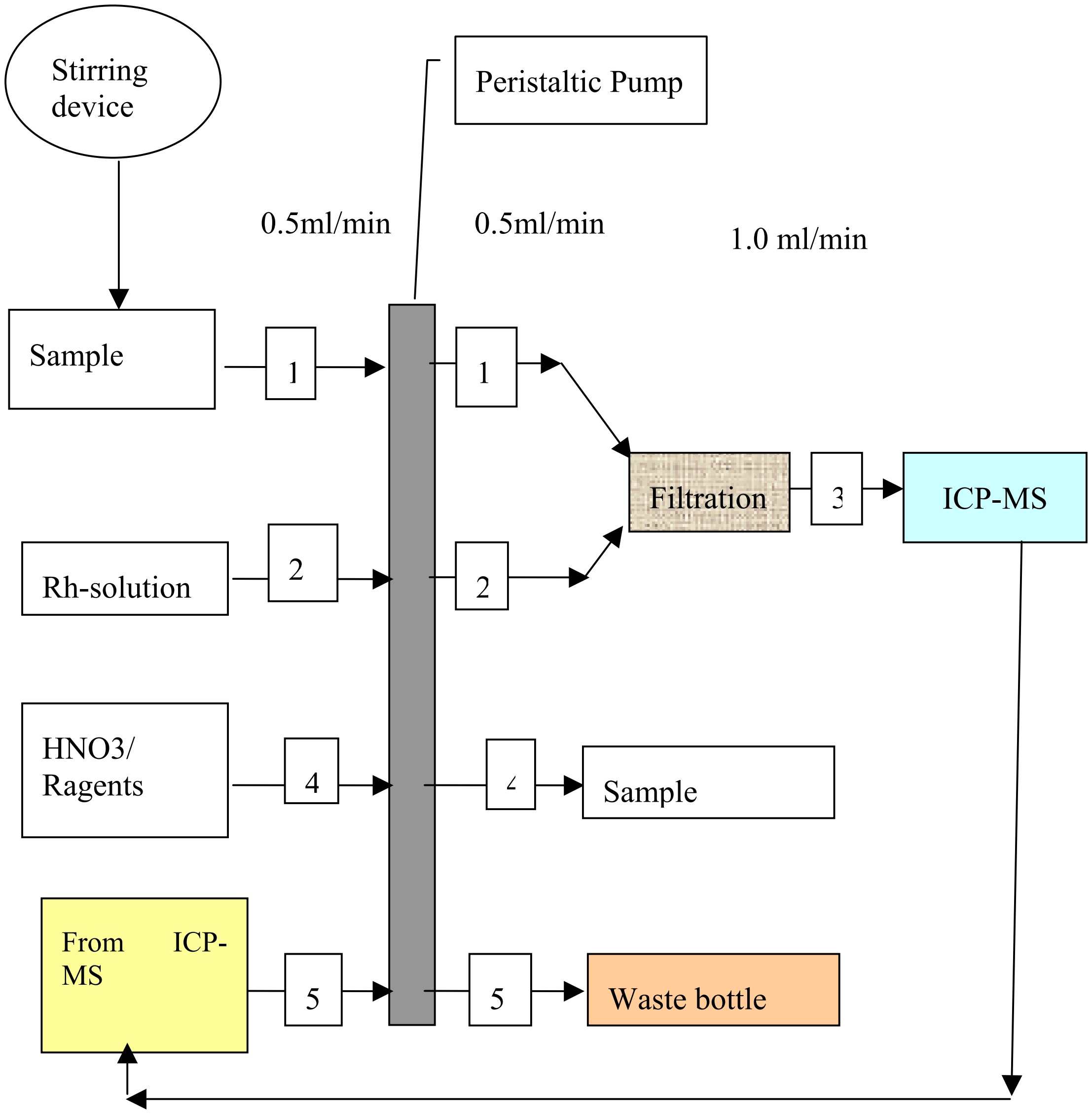

Figure 4.4 Schematic digram of the experimental setup

The ICP-MS is very sensitive and need special precautions before run the sample. Higher values of plasma R.F power can result large pick of signal comparatively with less nebulizer flow rate. There is also a close relationship with sample depth and nebulizer gas flow rate since optimum nebulizer gas flow rate which is a function of nuclide mass. Therefore, higher R.F power and higher nebulizer flow rate is considered to shift the zone of higher metal ions density towards the tip of the sampling cone (Vandecasteele et al.,1993). Table-4.1 shows the parameters considered for ICP-MS analysis.

Table 4.1 ICP-MS parameters for analyzing elements

RF power	1200 W
Plasma gas	Ar , 16 L min^{-1}
Auxiliary gas	Ar, 0.9L min^{-1}
Nebulizer gas	Ar, 0.86 L min^{-1}
Analytes	Ca44, Mg24, Mn55, Fe54, Fe57, Ni58, Ni60, Zn66, Cd111, Cr53, Co59, Cu63, Cu65 Al27, Si28, S3, Rh103
Data acquisition	Peak Hopping
Dwell time	10 Mili second
Sweeps per reading	20
Run time	≈ 1 hour

5 RESULTS AND DISCUSSION

The result and discussion are made on the basis of three samples as mentioned in the previous section. The number of samples is relatively less and no reference samples have been analyzed to validate this method. However, the results of these three samples seem quite interesting to support this method. Few problems that could not be overcame for the ICP-MS analysis such as back pressure which was happened due to filter clogging while sample was passing through the filter. Therefore, the total time used for plotting figures may not be same for all three samples. Although, the results used for analysis are consistent and no clogging effects have been observed. Seventeen elements (Ca44, Mg24, Mn55, Fe54, Fe57, Ni58, Ni60, Zn66, Cd111, Cr53, Co59, Cu63, Cu65 Al27, Si28, S3, Rh103) are analyzed by ICP-MS but only twelve (Ca44, Mg24, Mn55, Fe54, Ni58, Zn66, Cd111, Cr53, Co59, Cu63, Al27, Si28) of them are discussed.

5.1 Sensitivity analysis

To check the sensitivity of ICP-MS, MilliQ water is injected into ICP-MS through the filter media (Figure-5.1). After certain interval of time, standard solution (200 ppb with 23 elements) is added, for a few seconds, through reagent tube. Except Si all elements are very sensitive to ICP-MS and probably Si is not present with in the 23 elements in the standard solution. Sensitivity for each element is calculated from the resulted peak signal for concentration calculation of each element for a certain time for one ppb (Appedix-I, *table*-I.1).

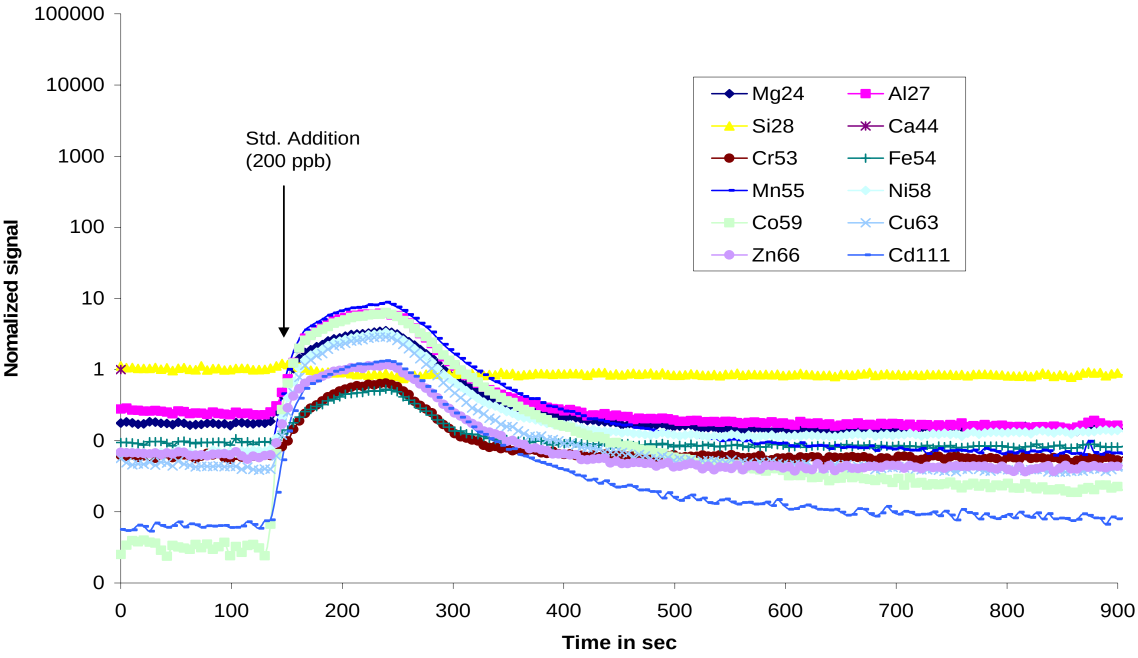

Figure 5.1 ICP-MS measurement of analyte for sensitivity analysis using standard solution

5.2 Phase analysis at different acid concentration

The phase analysis is performed by producing few graphs (see Appendix-II) with data from ICP-MS. Normalized data on the primary Y-axis are plotted in log scale where as pH on secondary Y-axis and time on the X-axis are plotted in linear scale. More than one graph has been produced for one sample on the basis of phase differences like Ca-phase, Fe/Mn-phase and so on.

5.2.1 Leaching at 0.1% HNO_3

The phases are identified while plotting the normalized data produced from ICP-MS for elements leaching from sample-1 at 0.1% HNO_3. Figure-II.1 in Appendix-II shows that the parallel release of Ca and Mg produces peak at pH 2.6, which means Ca and Mg are in the same phase. Although, Mn starts leaching with Ca and Mg but it produces peak at lower pH= 2.4 with Fe (Appendix-II Figure-II.2). It can be explained that Mn have fractions of both Ca-phase and Fe-phase. The maximum release of Ca, Mg happen at pH 2.6 but according to BCR and Teisser et al procedure this value is fur below since both methods expect that Ca and Fe/Mn fractions should be leached at pH>5.0. The result of Mn also conflict with those two methods. However, the difference is not as large as Ca fractions. Identical release of Si, Ni,Co and Zn with Fe can be explained as Fe oxides fraction. The distinctive leaching behaviour has been observed for Al. During the sample analysis, the leaching of Al declines and even it continues in the acidic condition. After all, Al starts leaching at a very low pH where Al produces maximum peak with Cd which means they can be occurred from the same source. The declination of Cu-release in the acidic conditions can also be observed at low pH (Appendix-II Figure-II.3). Leaching of Cr in 0.1% HNO_3 condition is very low and it is very hard to identify the peak and even it is not possible to get idea about the relation of Cr with Ni.

5.2.2 Leaching at 1% HNO_3

Leaching of all elements from sample-2 at 1% HNO_3 happens at the same time and the identification of final peaks of individual elements is very difficult since the sharp decline of pH influences the leaching. Nevertheless, different phases can be analyzed on the basis of their primary resurgence and corresponding decline. Ca and Mg show almost same pattern of peak (Appendix-II Figure-II.4). The identical release and decline pattern of Mn with Ca and Mg which is similar to the leaching at 0.1% acid conditions. In the oxide phase (Fe/Mn fractions), Ni, Co and Zn are equally reactive with Fe (Appendix-II Figure-II.5). But Si, Al, Cr, Cu, Cd are shows distinctive pattern (Appendix-II Figure-II.6).

To find out any effects of sulfur fractions, sulfur leaching is analyzed at the same time with other elements. The sulfur content in the sediment is higher compare to other elements (Appendix-II Figure-II.7) and it starts leaching at pH 2.0. Since acid treated as reducible agent, BCR and Teisser et al. method only consider sulfur and organic fractions in the oxidizing phase at pH 2.0. Therefore, leaching of sulfur can be due to oxic condition of sediment. Leaching pattern of sulfur with Al is similar which gives an idea of Al-S complex formation. However, there could be sulfur complex with other trace elements.

5.2.3 Leaching at 10% HNO_3

Due to clogging problem in the filter systems, the total time for this sample (sample-3) experiment is relatively shorter than the analysis performed for other two samples. Here, only first peak is identified and discussed. The leaching behaviour at 10% HNO_3 is comparable with 1% HNO_3. In this case, sharp dropping of pH does not affect leaching and therefore, different phases are well identified. However, it seems that all elements start to leach below pH 2.0. The releasing pattern (Appendix-II Figure-II.8) of Mg, Ca and Mn at 10% of HNO_3 from this sample is same as sample-1. Ni, Co, Zn fractions are released with Fe (Appendix-II Figure-II.9). Although Cu and Al show same leaching behaviour both in the sample and acidic condition (Appendix-II Figure-II.10), Al starts earlier leaching than Cu which means the bonding of Cu is comparatively stronger. Again, Cu starts to leach with Cd, Cr, Si. Due to less analysis time of this sample, as mentioned earlier, the peaks are not identified to define their actual phase.

Theoretically, fractions that release with Ca/Mg fractions are termed as acid soluble or exchangeable fractions and are dangerous for environment when their concentrations become higher. From the above discussions Ca and Mg are identified in the same phase and have higher mobility (Tokunaga et al., 2005) where the distribution of Mn might be in both carbonate and oxide phases which supports by many studies (Hjorth, 2004). In contrast with both BCR and Teisser et al. procedure, comparatively higher release of Ca, Mg and Mn happens at pH 2.3±0.38, 2.4±0.43 and 2.2±0.26 respectively. Again, theoretically metals bonded with iron and manganese oxides are said to be reducible fractions. In all three samples, trace elements Ni, Co and Zn are released with Fe/Mn oxides at pH 2.2±0.2. The same results can be found in different studies such as Thomas et al.(1994), Tessier (1979), Usero et al.(1998).

Higher concentration of sulfur in sample-3 is not expected in the reducible stage. However, Teisser (1979) found low level of sulfur in the in the reducible stage. Furthermore, amorphous metal sulfides can be found in the reducible. Distribution of Cd, Cr, Al, Cu and Si can be in the oxides and sulfide phases. However, a low level of Cd, Cr and Cu are released in the exchangeable or carbonate phase which satisfy Teisser et al.,(1979) results.

5.3 Statistical analysis

Several studies have been carried out under BCR and Teisser methods but very few studies addressed the correlation of fractional distribution into sediments. Complete correlation of metal distribution has not been found yet due to differential binding behaviour at different phases. An effort has been made to perform analysis of bindings of trace metals with major elements through statistical analysis.

5.3.1 Correlation matrix analysis

Correlation analysis is a useful statistical tool for speciation analysis. It represents the strength of relationship between two variables. Since the objective of this study is to identify the association of elements, a symmetric correlation matrix has been calculated using XLSTAT statistical tools for better interpret the relations among elements. The ICP-MS normalized data for a particular sample, in this case sample 1, 2 and 3, has been correlated using ‘Pearson correlation coefficient’ at 95% significance level. The coefficient value ranges from -1 to 1, and it measures the degree of linear correlation between two variables. Pearson correlation coefficient gives an idea of how much of the variability of a variable (element) is explained by the other variable.

The bold values in the correlation matrix (Table-5.1) mean stronger correlation among trace (Mn, Cr, Ni, Zn, Co, Cd, Al, Cu) and major elements (Ca, Mg, Fe, Si) at 0.1% HNO_3. Mg has the strongest correlation with Ca which is followed by Mn. Again Ca has significant correlation with Mn followed by Mg. The association of Ca with Mn and Mg are greater or equal to 0.92 at 95% confidence level. Further, Mn also has stronger relationship with Co and Si than Fe. Again, a significant correlation has been observed between Fe and Al, Si, Ni, Co, Zn and Cd which are greater or equal to 0.94. Si shows the highest association with Cd followed by Ni, Zn, Fe, Al and Co. Except Ca, Mn, Mg, Co and Cu, all other elements have significant correlation with Al. The trace element Cr has greater affinity to Cu followed by Cd and Al. Again, Cu only has greater correlation, more than 0.9, with Cr. However, it also has sufficient correlation with Al (0.84).

The correlation factors for sample-2 at 10% HNO_3 are slightly higher than the previous sample (Table-5.2). Here also, the bold values in correlation matrix show strong association among variables. Under strong acid condition no significant changes in relation of Ca with Mn and Mg are observed except certain changes in coefficient values. Mg shows significant correlation, greater than 0.9, with Mn along with Ca. Similarly, Mn has important correlation with Co and Si along with Ca and Mg which may be an impression of oxide fractions. At this acid concentration Cu, Co and Cr shows a significant change of their relation with Al and Fe which was not clear in the sample-1 at 0.1% HNO_3 acid concentration. Al shows a significant relation with Cu and Co, coefficient greater than 0.9, with all other elements except Ca, Mg and Mn. However, Cu shows higher association with Cd rather than Al and Cr. Fe shows significant correlation with Cr along with other elements like Ni, Zn, Al, Cd, Co and Si. The relations of Si with all other elements are quite strong except Ca, Mg, Cu. This relation is possibly the formation of silicate matrix.

At 1% HNO_3 in sample-3, the correlation coefficient among trace and major elements are comparatively higher and shows above 0.9 at 95% confidence level the table (Table-5.3). This might give an impression of about the greater variability of elements and defining relationships among elements are quite impossible with these data.

Table 5.1 Correlation matrix (Pearson) for sample-1 at 95% confidence level

Variables	**Mg24**	**Al27**	**Si28**	**Ca44**	**Cr53**	**Fe54**	**Mn55**	**Ni58**	**Co59**	**Cu63**	**Zn66**	**Cd111**
Mg24	1											
Al27	0.340	1										
Si28	0.537	**0.946**	1									
Ca44	**0.920**	0.509	0.723	1								
Cr53	0.264	**0.909**	0.868	0.393	1							
Fe54	0.404	**0.967**	**0.967**	0.617	0.851	1						
Mn55	0.814	0.534	0.746	**0.971**	0.388	0.673	1					
Ni58	0.427	**0.962**	**0.972**	0.642	0.842	**0.999**	0.697	1				
Co59	0.534	0.831	**0.915**	0.774	0.666	**0.938**	0.851	**0.948**	1			
Cu63	0.157	0.837	0.775	0.258	**0.950**	0.751	0.244	0.737	0.520	1		
Zn66	0.422	**0.969**	**0.971**	0.628	0.851	**0.998**	0.676	**0.998**	**0.935**	0.751	1	
Cd111	0.327	**0.993**	**0.937**	0.482	**0.932**	**0.946**	0.497	**0.939**	0.788	0.883	**0.948**	1

Table 5.2 Correlation matrix (Pearson) for sample-2 at 95% confidence level

Variables	**Mg24**	**Al27**	**Si28**	**Ca44**	**Cr53**	**Fe54**	**Mn55**	**Ni58**	**Co59**	**Cu63**	**Zn66**	**Cd111**
Mg24	1											
Al27	0.518	1										
Si28	0.733	**0.957**	1									
Ca44	**0.964**	0.679	0.856	1								
Cr53	0.709	**0.910**	**0.950**	0.803	1							
Fe54	0.571	**0.990**	**0.974**	0.736	**0.915**	1						
Mn55	**0.901**	0.777	**0.917**	**0.982**	0.847	0.832	1					
Ni58	0.589	**0.988**	**0.978**	0.751	**0.918**	**0.999**	0.844	1				
Co59	0.696	**0.941**	**0.980**	0.847	**0.906**	**0.975**	**0.925**	**0.979**	1			
Cu63	0.332	**0.909**	0.828	0.461	0.823	0.876	0.551	0.869	0.765	1		
Zn66	0.589	**0.989**	**0.978**	0.748	**0.920**	**0.999**	0.839	**0.999**	**0.976**	0.879	1	
Cd111	0.503	**0.998**	**0.950**	0.660	**0.910**	**0.982**	0.755	**0.980**	**0.924**	**0.929**	**0.982**	1

Table 5.3 Correlation matrix (Pearson) for sample-3 at 95% confidence level

Variables	**Mg24**	**Al27**	**Si28**	**Ca44**	**Cr53**	**Fe54**	**Mn55**	**Ni58**	**Co59**	**Cu63**	**Zn66**	**Cd111**
Mg24	1											
Al27	0.945	1										
Si28	0.962	0.995	1									
Ca44	0.990	0.952	0.967	1								
Cr53	0.952	0.990	0.997	0.951	1							
Fe54	0.954	0.995	0.996	0.967	0.989	1						
Mn55	0.970	0.947	0.960	0.994	0.941	0.967	1					
Ni58	0.954	0.994	0.996	0.967	0.988	1.000	0.968	1				
Co59	0.955	0.984	0.988	0.975	0.976	0.996	0.980	0.997	1			
Cu63	0.948	0.981	0.993	0.946	0.996	0.982	0.934	0.980	0.969	1		
Zn66	0.956	0.995	0.999	0.963	0.995	0.998	0.958	0.997	0.990	0.991	1	
Cd111	0.953	0.992	0.997	0.953	0.998	0.990	0.944	0.989	0.978	0.997	0.996	1

5.3.2 Principal component analysis (PCA)

Multivariate statistical techniques have been used in several studies for pattern and relation identification among various data sets (Filgueiras et al., 2004; Tanaka et al., 2003; Pardo, 2004; Kartal, 2006; Relic, 2005). Principal component analysis (PCA) is one of the most popular multivariate techniques where data sets are reduced in to small number of variables. In this study XLSTAT statistical tools are used for PCA where the factors (F1, F2 etc), which are calculated from a correlation matrix, are plotted on factor axes in the vector space. Although all the variables are dimensionless, covariance matrix can be used instead of correlation matrix. In this study, to reduce effort, correlation matrix has been used. As XLSTAT can help to calculate correlation matrix rather than covariance matrix, it is ignored.

In the PCA, the most variable data sets are placed in the first factor and then second factor and so forth. Using correlation matrix eigen values and eigen vector matrix are computed which are used to calculate corresponding variability of the factor to reduce dimensions or factors without loosing much information. A cumulative variance gives better idea of important factors since first factor poses the highest variability.

Factor loading has been calculated from correlations between the variables and the factor axes. Highest value of factor loading for a variable (elements) represents grater contribution in a particular factor. The PCA plot of factor loading generate cluster of points and the lines represent corresponding vector line. Close cluster demonstrate greater degree of association of variables.

Results of Principal component analysis (PCA):

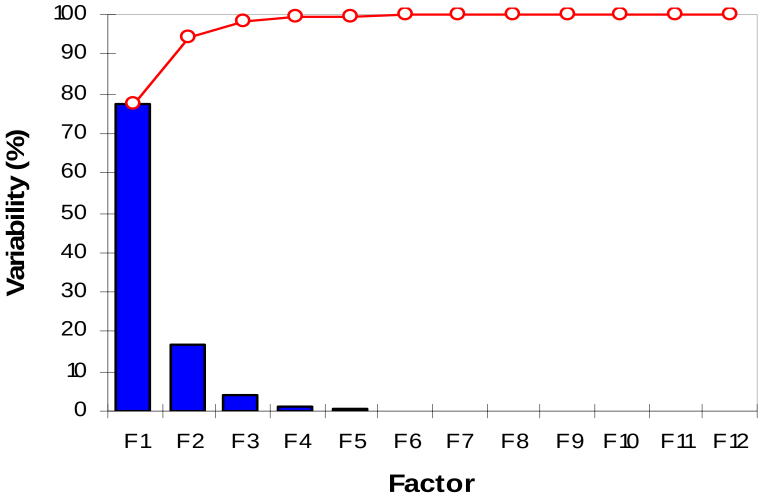

Figure 5.2 Screen plot for sample-1 at 0.1% HNO3

Using XLSTAT software, pearson correlation matrix (12 X 12) are calculated for PC analysis for each sample. Screen plot (Figure 5.2) shows that the factor F1 poses highest percentage of variability (77%) followed by factor F2. The cumulative of these two factors comprise 94% of the total variability. In factor F1, elements like Si, Ni, Zn, Fe, Al, Co and Cd are more dominating than the other elements like Ca, Cr, Mn and Cu. Again, Ca, Mn and Mg have positive loading and Cu and Cu have negative loading in factor F2. The loading of Mg in factor F2 is comparatively higher than the loading in factor F1. Since F1 and F2 together poses the maximum variability, a PCA graph is plotted in Appendix III Figure III.1 with these two factors (F1, F2) for cluster or association analysis of all variables. Ca and Mn are more likely rather than Mg. Among other elements Zn, Ni, Si forms a cluster with Fe, which is close to a cluster of Al and Cd. Variables like Cr, Cu and Co are unlikely to make up any close relationship. Since Factor F1 shows higher variability, the clusters are not well distinguishable.

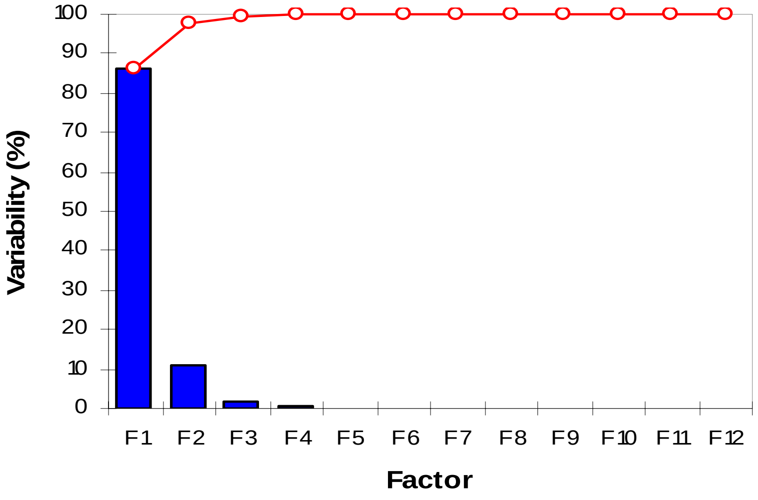

Figure 5.3 Screen plot for sample-2 at 10% HNO3

Strong acid concentration (10%) in sample-2 influences the variability of element kinetics since the variability of factor F1 is almost 9% higher than the factor F1 in sample-1. It is expected that the clusters of variables may not be well distinguishable as like as sample2.

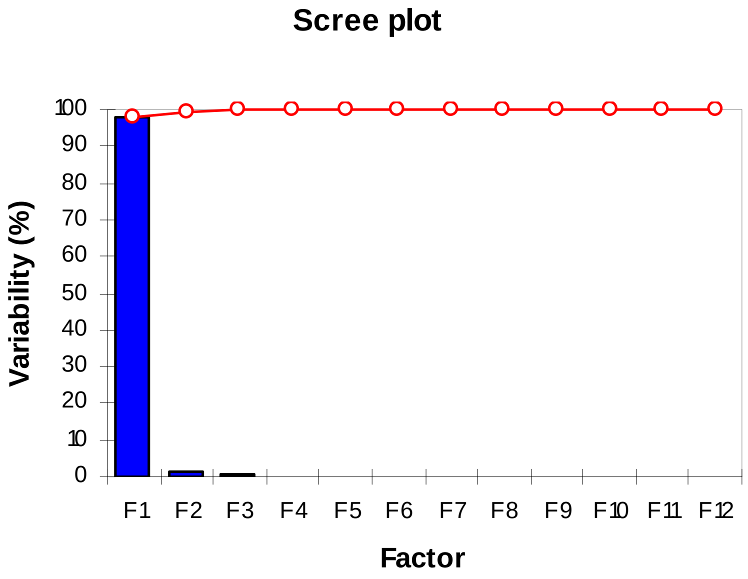

Figure 5.4 Screen plot for sample-3 at 1% HNO3

The screen plot of sample-2 (Figure 5.3) shows cumulative variability of all factors. Factor F1 alone contributes 86% where the individual contribution of variables Si Ni, Zn, Co, Fe, Al, Co, Cr is almost 9%. Ca, Mn, Mg and Cu are less dominant in F1 and Ca, Cu and Mg shows higher loading in F1 compare to F2. Further, the factor F2 is highly loaded by Mg but this loading is comparatively less than in factor F1. Except Mg, Ca, Si, Cr, Mn and Co all other variables have negative loading in F2 where Cu poses the highest negative loading. The PCA plot of factor F1 and F2 (Appendix III Figure III.2) shows similar grouping as discussed for sample-1 except Si and Cr. Variables like Ca, Mn and Mg are clearly identified in a separate area and not likely to say as a cluster. It seems that at higher acid concentration their kinetics are slightly affected. Si changes its relation from cluster Fe-Ni-Zn to cluster Cr and Co. Cu still does not show any strong relation with other elements to form a cluster.

Factor F1 for sample-3 (1% HNO_3) contains 98% variability (Figure 5.4) where understanding of element fractions is quite difficult. Neverthless, PCA factor plotting (Appendix III Figure III.3) clearly distinguishes a cluster of Ca, Mg and Mn from all other elements. Therefore, Mg, Mn, Ca can be interpreted as same fractions.

Finally, two major groups can be identified, one is Ca-/Mg-group and the other is Fe group. Trace element Mn is mostly reactive with Ca/Mg fractions and Ni, Zn are mostly reactive with Fe/Mn fractions. One possible explanation can be made about the source of the exchangeable fractions that they can be leached from calcite ($CaCO_3$), dolomite ($CaMg(CO_3)_2$). Again, correlation among Fe, Mn, Zn, Ni (redox elements) may be due to remobilization from geological structure (Naimoa D. et al.,(2005), Sohlenius G. et al.(2004)), however, it can be from anthropogenic source too. Si has distinctive nature at different acid concentration, however, in correlation matrix analysis it shows a strong relation with Fe-group. Other trace elements like Co, Cd, Cr and Al exhibit relation with Si and Fe which is not straight forward as Fe, Ni and Zn. In the PCA, Cu shows distinctive relation with all elements, although in correlation matrix analysis it is observed that it has a significant relation with Cd and Cr.

5.4 Fraction analysis

The binding of metals in various fractions are analyzed with respect to different acid concentrations and pH values. Here the term ‘binding’ and ‘mobility’ are used interchangeably to express the same meaning. Generally, mobility calculation is performed on the basis of concentration of an element in each phase and in this study, the concentration is not calculated. All figures that are prepared for this analysis are attached in the Appendix-IV. Since the starting times of pH measurement are not same, for plotting intensity versus time, sample data are arranged in such a way so that point of acid application remains same for all cases. Although in this case the timing of actual measurement are altered but the time interval of each ICP-MS data remains same 5 seconds. Again, using these data and pH value, another plot intensity versus pH is drawn where time is considered as an independent variable (or constant).

Calcium (Ca) and Magnesium (Mg)

Ca and Mg exhibit similar behaviour under different acid condition. At 10% HNO_3 the mobility or bonding strength of Ca and Mg is highly significant compare with other acid strengths used. Both elements show readsorption after primary release where the adsorption phenomenon is well observed at 1% HNO_3 condition. The leaching pattern of Mg is similar to Ca which supports the idea that Ca and Mg are same factions and even at different pH level, both elements reacts in the same way. They start to leach at low pH level (<4) and the leaching continue drops from pH 2.6 to pH 2.0 and finally drastically increase the leaching at pH less than 2.0. The normalized intensity against pH plot shows strong power relationship (R^2) both Mg and Ca.

Manganige (Mn)

Mn shows significant relationship with carbonate fractions (Ca and Mg). The reaction rate at 10% HNO_3 concentration is almost ten times higher than the rate at 0.1% HNO_3. In the beginning, the leaching of Mn at 1% and 0.1% HNO_3 concentration pattern is same and after a while they decline at a different rate. A significant decrease of primary peak for sample-2 can be due to readsorption and again further increase of leaching can be a possible explanation of redistribution which supports the study of Gómez-Ariza (1999). Leaching of Mn at different pH is similar with Ca and Mg and it has significant power relation.

Iron (Fe), Nikel (Ni) and Zinc (Zn)

Under strong acid condition Fe, Ni and Zn display similar behaviour and leaching of Fe is greater than Ni and Zn. The mobility of Fe at 10% HNO_3 is more than 1000 times of its background stage. A significant release (almost 100 times) of Fe, Ni and Zn at 1% and 0.1% HNO_3 comply the result expected while using BCR method. All elements show comparatively lower sorption at 1% HNO_3 concentration which also comply the result studied by Belzile et al.(1989), Gómez-Ariza (1999). Sorption at 0.1% HNO_3 concentration is not clear and this can be due to low acid concentration and less reaction time. Very small amount of Fe, Ni and Zn are released below the pH 2.5. Maximum leaching happens below pH 2.0. A small change of leaching is observed in between pH 2.5 to pH 2.0 which may be a possible explanation of sorption. The value of R^2 is quite significant for the power relation of leaching and pH of these elements.

Cromium (Cr)

Mobility of Cr is lower than any other elements discussed above. The leaching rate of Cr is very less at lower acid concentration but certain change of leaching is observed in the higher acid condition. A sharp increase of leaching is identified at 1% HNO_3 condition. Mobility or reaction rate at 0.1% is very less. Therefore, less release of Cr inferred strong binding (Cr- complex) (Thomas et al.,1994; Usero et al.,1998). pH vs intensity plotting shows that Cr leaches at low pH (<2.0) and the changes of Cr leaching at different pH shows significant power relation.

Cobolt (Co)

Co also shows faster mobility at higher acid concentration. Compare to Cr, the mobility of Co is higher in acidic condition. Leaching of Co at 1% HNO_3 is almost 100 times greater than the background level which means Co has significant reducible fractions (Teisser et al., 1979). After primary peak of leaching curve, a sharp declination and further increase of leaching can be nothing but readsorption. Higher power relation ($R^2 = 0.9$) of Co means that it release at a very low pH. A slight decrease of leaching under pH 2.2 may be a possible explanation of adsorption.

Cadmium (Cd)

Cd starts leaching at a very low pH (<2.0) which can be an explanation of higher binding strength with ligands. Although, in the previous sections, no straight forward relationship of Cd with Ca or Fe phase has been found but leaching (almost 100 times compare to background level) of Cd in the reducible phase can comply the result expected in BCR methods (Ph. Quevauviller, 1998). Although, Tessier et al. found low level of Cd in the reducible phases (Teisser et al., 1979). No significant adsorption observed at 1% HNO_3 but there can be sorption at 0.1% HNO_3. However, Belzile et al.(1989), Rendell (1980) reported adsorption of Cd after shaking the sample. But this result is not comparable since Paul S. used river sediment and HCl. Again, Gómez-Ariza et al. (1999) did not find any adsorption in the exchangeable and carbonate phase.

Silicon (Si)

Silicon starts leaching at lower pH but the leaching is very less which might be due to strong binding (Teisser et al., 1979) of silicon-oxide groups. At higher acid concentration it shows significant change of mobility which results a strong power relation of leaching intensity and pH. Silicon does not show any signification adsorption.

Copper (Cu)

The mobility of Cu is also dependent at acid concentration. Although, the power relation is not as strong as other elements like Co, Cd, Si, higher leaching is observed at lower acid concentration (pH<2.0). For a particular pH, the mobility is higher at 10% HNO_3 concentration and less at 0.1% HNO_3. Generally, significant level of Cu is not expected at reducible phase rather oxides and sulfide phases (BCR and Teisser methods) (Ph. Quevauviller, 1998; Usero et al., 1998). The result obtains in this study, therefore, comply with those methods. The decreasing pattern of Cu in the sample can be adsorption with ligands due to stirring the sample (Paul S et. al. 1980) or due to low concentration of Al in the sample (Gómez-Ariza et al., 1999). Further decreasing of Cu during acid flux can be readsorption, however, some studies find readsorption of Cu at higher pH during the extraction of mobile and exchangeable fractions (Belzile et al., 1989; Gómez-Ariza et al., 1999).

Aluminum (Al)

Aluminum also releases at a very low pH ($R^2 = 0.673$) and it starts leaching at pH less than 3.0. A slight decreasing of leaching is occurred at pH below 2.2 and finally increases leaching at pH less than 2.0. During sample analysis, while no reagent is added to the sample, aluminum shows adsorption similar to Cu. Leaching is higher at 10% HNO_3 followed by 1% HNO_3. Although the releasing pattern is not similar with Fe and Mn , significant release of Al in the reducible phase can be explain of its binding with minerals(Matúš, 2005) and may be an effect of freezing of sample (Hjorth, 2004). Readsorption happens lately at less acid concentration 0.1% HNO_3. No significant adsorption observed after the primary peak at 1% rather it continues and increase again at low pH.

6 CONCLUSION

A continuous leaching measurement for trace and heavy metal through ICP-MS is a new technique for speciation analysis. The method proposed in this study is very sensitive and provide information on the leachability of major and trace elements and their associations.

Three different acid concentrations are used for the leaching. Results are evaluated using statistical methods to determine the association between elements. Ca and Mg are the first major elements to leach from the sediments with a maximum concentration in the aqueous phase at pH pH 2.3±0.38 and 2.4±0.43 respectively. Fe/Mn is maximum at pH 2.2±0.26 and 2.2±0.2 respectively. The results show an overlap of Mn between Ca and Fe based phases. Ni, Co and Zn are found to be associated with Fe/Mn phase and Cd, Cr, Cu is found to be less associated with Ca phase.

Significant readsorption and redistribution happens for Ca, Mg, Mn, Fe, Ni, Zn, Co, Cu and Al at different phases and it is quite clear at 1% HNO_3.

The experimental setup is very simple with less manual operation which is very important for speciation analysis. Additional care on sample preservation and further study on the development of the filtering system can increase analysis time which may provide more accurate information of the leaching and association of elements.

7 REFERENCES

Al-Merey R. Al-Masri M. S., Bozou R. (2002), Analitica chimica Acta, vol 452 pp-143-148

Baca A. J., La Ree A. B., Zhou F. and Mason A. Z. (2003), Analytical Chemistry, Vol. 75, No. 10, pp 2507 – 2511

Battiston G. A., Gerbasi R., Degetto S. and Sbrignadello G. (1993), Spectrochimica Acta Part B: Atomic Spectroscopy, Volume 48, Issue 2, Pages 217-221

Beauchemin D. (2002) Anal. Chem., vol. 74, pp 2873-2894

Beauchemin D., Kyser K. and Chipley D. (2002), Analytical Chemistry, Vol. 74, No. 15, pp 3924 – 3928

Beauchemin D., Mclaren J. W. and Herman S. S. (1987), Spectrochimica Acta Part B: Atomic Spectroscopy Volume 42, Issue 3, Pages 467-490

Belzile N., Lecomte P., Tessier A. (1989), Environ. Sci. Technol.; 23(8); 1015-1020

Benjamin M.M. (2002), 'Water Chemistry', McGrawHill, NY, pp 363-390

Bettinelli M., Baroni U. and Pastorelli N. (1989), Analytica Chimica Acta, Volume 225, Pages 159-174

Birch L., Hanselmann K. W. and Bachofen R. (1996), Water Research Volume 30, Issue 3 , , Pages 679-687

Borg H., Jonsson P. (1996) Marine Pollution Bulletin, Vol. 32, Issue 1, pp 8-21

Calace , Nicoletta, Petronio, Maria B. (2004) Annali Di Chimica, Vol. 94, Issue 7-8, pp 487-493

Das A.K, Chakraborty R., Cervera M.L and Guardia M (1995), Talanta vol 42, pp 1007-1030.

Davidson C. M. , Rhodri P. Thomas, Sharon E. McVey, Reijo Perala, David Littlejohn, Allan M. Ure (1994),Analytica Chimica Acta, vol- 291, pp. 277-286

Ebdon L., Evans E. H., fisher A., Hill S.J (1998), An introduction to analytical atomic spectrometry, Wiley NY, pp 73-134

Economou A. (2005), TrAC Trends in Analytical Chemistry, Volume 24, Issue 5, Pages 416-425

Evans E.H., Giglio J.J (1993), J. Anal. Atomic Spect. Vol-8, pp-1-18

Falciani R., Novaro E., Marchesini M. and Gucciardi M. (2000), Journal of Analytical Atomic Spectrometry, vol.15, pp 561 - 565

Fedotov P.S., Wennrich R., Stärk H.J. & Spivakov B.Ya. (2005), J. Environ. Monit. Vol 7, pp 22-28

Filgueiras V., Lavilla I. and Bendicho C. (2004), Science of The Total Environment Volume 330, Issues 1-3 , 1 September 2004, Pages 115-129

Florence T.M. (1981), Talanta, Vol. 29, pp. 345-364

Förstner U. (1992), Inter. J. Environ. Anal. Chem., Vol. 5, pp 5-23

Gatehouse S., Russell D.W. , Van Moort J.C. (1977), Journal of Geochemical Exploration, Volume 8, Issue 1-2, Pages 483-494

Gómez-Ariza J. L., Giráldez I., Sánchez-Rodas D. and Morales E. (1999), Analytica Chimica Acta, Volume 399, Issue 3, Pages 295-307

González A. E., Rodríguez M. T., Jiménez Sá J. C., Espinosa A. J. F. and Rosa F. J. B.(2000), Water, Air, & Soil Pollution, Volume 121, Numbers 1-4, Pages 11 - 29

Harris D.C. (2002), Quantitative chemical analysis' Sixth edition W.H. Freeman and Company NY. pp 494-547

Hirner A. V., Kritsotakis K., Tobschall H. J. (1990), Applied Geochemistry , Volume 5, Issue 4, Pages 491-505

Hjorth T. (2004) Analytica Chimica Acta, Volume 526, Issue 1 , Pages 95-102

Kartal S., Aydın Z., Tokalıoglu S. (2006) ,Journal of Hazardous Materials, (Article in press)

Kebbekus B.B. and Mitra S. (1998), 'Environmental chemical analysis', Blackie A & P, London, pp 31-53

Kersten M. and Förstner U. (1986) Water Sci. Technol. 18, Issue (4-5), p. 121-130

Kheboian C. and Bauer C. F. (1987), Analytical Chemistry, Vol. 59, No. 10, pp 1417 – 1423

Kot A. and Namiesńik J. (2000), TrAC Trends in Analytical Chemistry, Volume 19, Issues 2-3, Pages 69-79

López-Sánchez J. F. (1994), Analytica Chimica Acta, Volume 286, Issue 3, Pages 423-429

MacFarlane W.R., Kyser T.K., Chipley D., Beauchemin D. & Oates C. (2005), Geochemistry: Exploration, Environment, Analysis, Vol. 5, pp. 123–134

Marin B., Valladon M., Polve M. and Monaco A. (1997), Analytica Chimica Acta, Volume 342, Issues 2-3, Pages 91-255

Martin J. M., Nirel P. & Thomas A. J.(1987), Marine Chemistry Vol 22, Issues 2-4 , pp 313-341

Matúš P., Kubová J., Bujdoš M. and Medved J. (2005), Analytica Chimica Acta, Volume 540, Issue 1 , Pages 33-43

Melaku S., Dams R., Moens L. (2005), Analitica chimica acta, vol 543 pp 117-123

Mendham J, Denney R.C, Barnes J.D, Thomas M.J.K (2000), 'Vogels quantitative chemical analysis', 6th edition, Practive hall, London, pp-617-629

Morita M., Ito H., Uehiro T. and Otsuka K.(1989), Analytical Sciences, Vol. 5, No. 5, pp-609

Morrison G.M. (1984), 'Urban stromwater pollution- Research report 8: Heavy metal speceiation studies of natural waters: A review', Chalmers techniska högskola. Göteborg, pp 4-40.

Morrison G.M.P, Batley G.E. & Florence T.M. (1989), Chemistry in Britain Vol. pp 791-796

Naimoa D., Adamo P., Imperatoa M., StanzioneD. (2005), Quaternary International, Vol 140–141, pp-53–63.

Nirel P. M. V. and Morel F. M. M. (1990), Water Research Volume 24, Issue 8, Pages 1055-1056

Nürnberg H. W. (1984),The voltammetric approach in trace metal chemistry of natural waters and atmospheric precipitation, Analytica Chimica Acta, Volume 164 , 1984, Pages 1-21

Pardo P., López-Sánchez J. F. and Rauret G. (1998), Analytica Chimica Acta, Volume 376, Issue 2, Pages 183-195

Pardo R., Barrado E., Pérez L. and Vega M. (1990), Water Research, Volume 24, Issue 3, Pages 373-379

Pardo R., Helena B.A., Cazurro C., Guerra C., Deb´an L., Guerra C.M., Vega M. (2004), Analytica Chimica Acta 523, pp-125–132

Ph. Quevauviller (1998), TrAC Trends in Analytical Chemistry, Vol. 17, Issue 5 , Pages 289-298

Ph.Quevauviller, Rauret G., Griepink B. (1993) , Int. J. Environ. Anal Chem., Vol. 51, Number-1-4, pp-231-235

Ph.Quevauviller, Ure A.M., Muntau H. & Griepink B. (1993), Int. J. Environ. Anal Chem., Vol. 51, Number-1-4, pp-129-134

Plantz M.R., Fritz J. S., Smith F. G. and Houk R. S. (1989), Analytical Chemistry , Vol. 61, No. 2, pp 149 – 153

Rauch S. (2001), 'On the Environmental Relevance of Platinum Group Elements', (a doctoral dissertation) Chalmers University of Technology, Göteborg, Sweden, pp 10-26

Rauch S., Morrison G.M., Heino M.M, etl (2000), Environ. Sci. Technol. Vol 34, pp 3119-3123.

Rauret G., Rubio R., Pineda L., López-Sanchez J. F., Beltran J. L. (1991), Analytical and Bioanalytical Chemistry, Volume 341, Number 10, Pages 631 - 635

Relic D., CorDevic´ D., Popovic´ A., Blagojevic´ T. (2005), Environment International, Vol-31, pp. 661– 669

Rendell P. S. and Batley G. E., Cameron A. J. (1980), Environmental Science & Technology, Volume 14, Number 3, pp-314-318

Roychoudhury N.(2005), Marine Pollution Bulletin (article in press).

Sahuquillo A., López-Sánchez J. F., Rubio R., Rauret G., Thomas R. P., Davidson C. M. and Ure A. M.(1999), Analytica Chimica Acta, Volume 382, Issue 3, Pages 317-327

Salomons W. and Förstner U. (1980), Environ. Technol. Letters, vol.1, pp-506-517

Salomons W.(1993), Int. J. Environ. Anal Chem., Vol. 51, Number-1-4, pp-3-4

Sanz-Medel A. and Blanco-González E. (2001), J. Anal. At. Spectrom. , vol.16, pp 957

Sastre J., Sahuquillo A., Vidal M. and Rauret G. (2002), Analytica Chimica Acta Volume 462, Issue 1, Pages 59-72

Singh K.P., Mohan D., Singh V.K., Malik A. (2005), Journal of Hydrology, vol. 312 pp-14-27.

Siuzdak G.(1996), 'Mass spectrometry for biotechnology', Academic Press. pp 32-39

Sohlenius G., Born I. O. (2004), Geoderma, vol, 122, pp-167–175

Templeton D.M., Ariese F., Cornelis R., Danielsson L.G., Muntau H., Leeuwen H. P. and Lobinski R. (2000), IUPAC, Pure and Applied Chemistry , Vol. 72, pp 1453-1470

Tanaka Y., Kuno A., Matsuo M. (2003), Journal of Radioanalytical and Nuclear Chemistry, Vol. 255, No. 2, pp- 239–243

Tessier A., Campbell P.G.C. and Bisson M. (1979), Anal. Chem., Vol 51, pp-844-851

Thomas R. (2004), 'Practical Guide to ICP-MS', NY: Marcel Dekker, cop

Thomas R. P., Ure A. M., Davidson C. M., Littlejohn D., Rauret G., Rubio R. and

Thompson M., Walsh J. N. (1989), 'Handbook of inductively coupled plasma spectrometry', NY: Chapman and Hall; Glasgow: Blackie.

Tokunaga S., Park S.W., Ulmanu M. (2005), Environmetal Tech. Vol 26 pp 673-682

Tongravee N., Shiowatana J. McLaren R. (2005), Intern. J. Environ. Anal. Chem. Vol.85 no 8 pp 567-583.

Ure A.M., Ph.Quevauviller, Muntau H. & Griepink B. (1993), Int. J. Environ. Anal Chem., Vol. 51, Number-1-4, pp-135-151

Usero J., Gamero M., Morillo J. and Gracia I. (1998), Environment International, Volume 24, Issue 4, Pages 487-496

Vandecasteele C. and Block C.B. (1993), 'Modern Methods for trace elements determination'. John wiley and sons

Vaughan M.A and Horlick G. (1986), Applied Spectroscopy, Vol-40, No-4, pp-434 Volume 19, Issues 2-3, Pages 69-79

Wallmann K., Kersten M., Gruber J. and Förstner U. (1993), Intern. J. Environ. Anal. Chem. Vol. 51, pp-187-200.

Appendix-I

SENSITIVITY CALCULATION

Table I.1 Calculation of sensitivity of for 12 elements for further concentration calculation.

	Maximum signal for standard solution (A)	**Background signal (B)**	**Sensitivity {(A-B)/2*}**
Mg(24)	3.5057	0.1630	1.6714
Al(27)	6.2635	0.2292	3.0172
Si(28)	1.2208	0.8009	0.2100
Ca(44)	5.3992	4.3146	0.5423
Cr(53)	0.6442	0.0547	0.2947
Fe(54)	0.5278	0.0847	0.2216
Mn(55)	8.8420	0.0621	4.3899
Ni(58)	3.3421	0.0624	1.6399
Co(59)	6.2887	0.0024	3.1432
Cu(63)	2.8733	0.0385	1.4174
Zn(66)	1.1702	0.0549	0.5577
Cd(111)	1.3280	0.0053	0.6614

* The concentration of standard solution was 200ppb.

Appendix-II

PHASE ANALYSIS

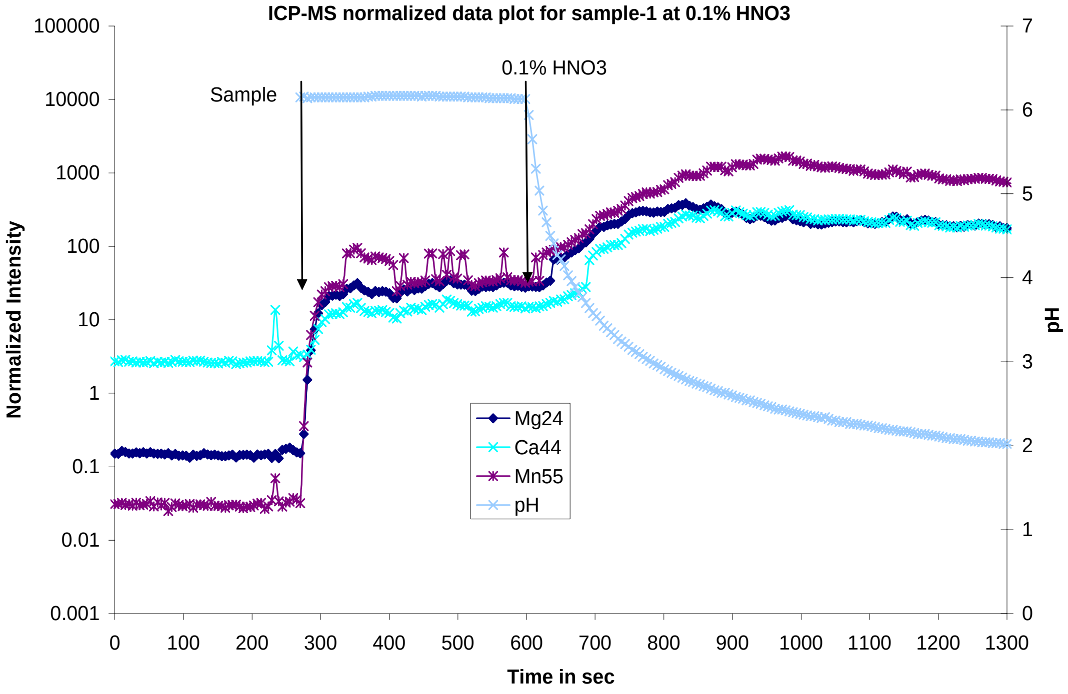

Figure-II.1 ICP-MS normalized intensity data plot for sample-1 at 0.1% Nitric acid: Carbonate and exchangeable phase.

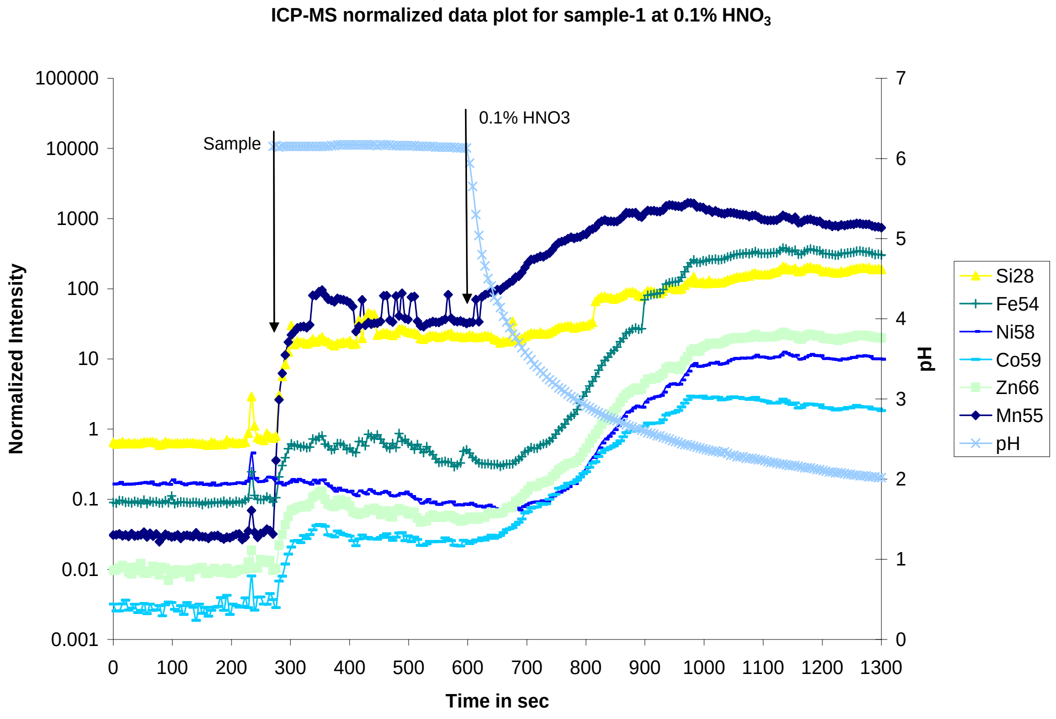

Figure-II.2: ICP-MS normalized intensity data plot for sample-1 at 0.1% Nitric acid: Iron and Mn oxide phase.

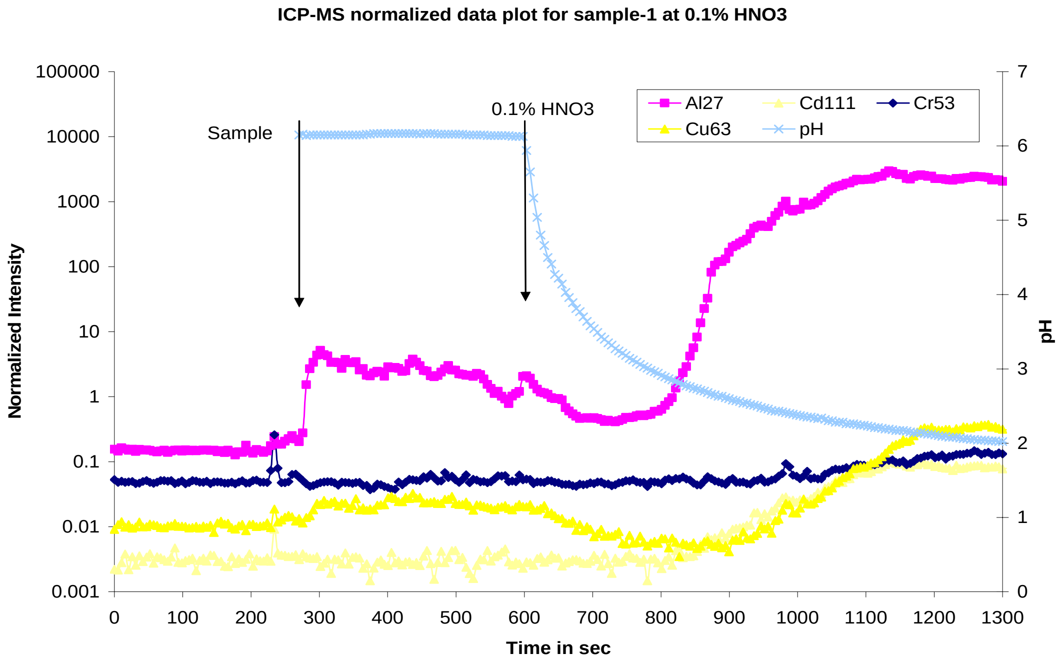

Figure II.3 ICP-MS normalized intensity data plot for sample-1 at 0.1% Nitric acid: other phase.

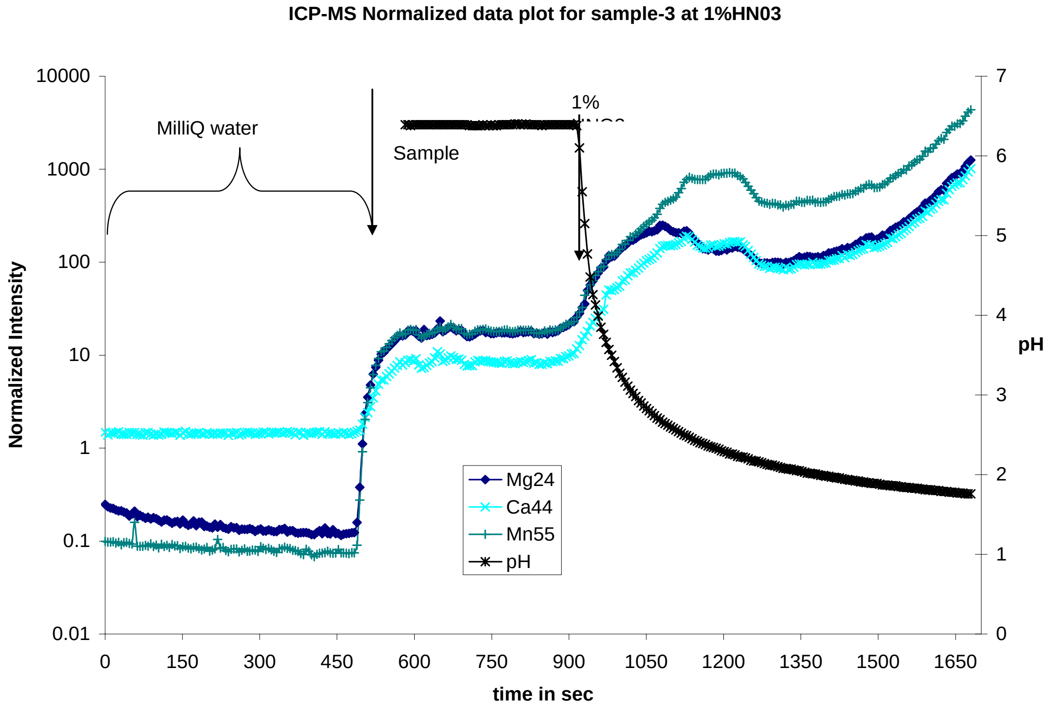

Figure II.4 ICP-MS normalized intensity data plot for sample-3 at 1% Nitric acid: Carbonate and exchangeable phase.

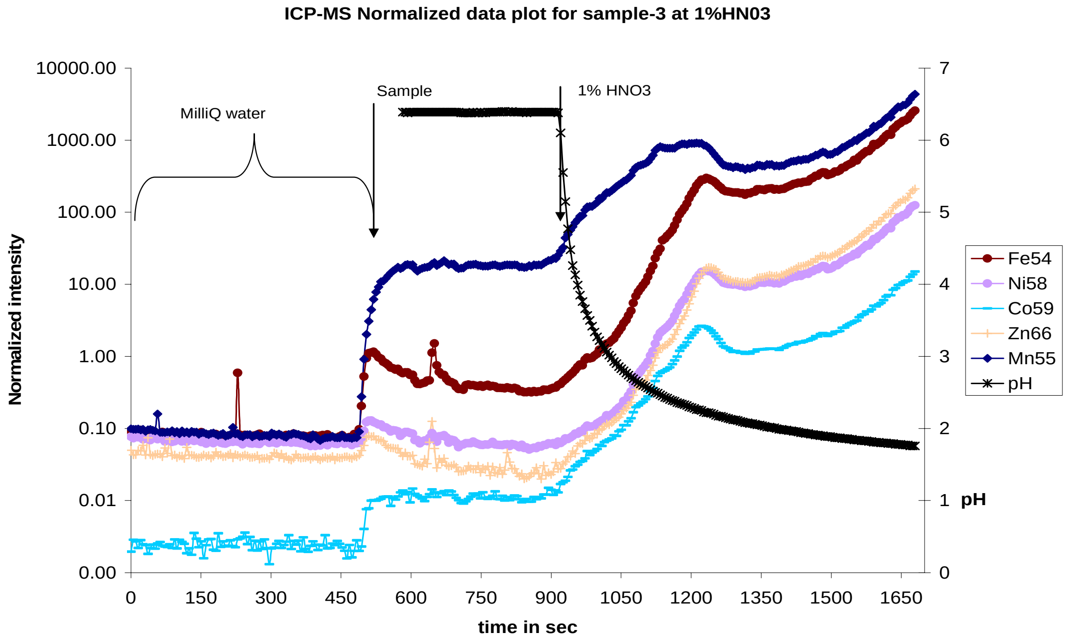

Figure II.5 ICP-MS normalized intensity data plot for sample-3 at 1% Nitric acid: Iron and Mn oxide phase.

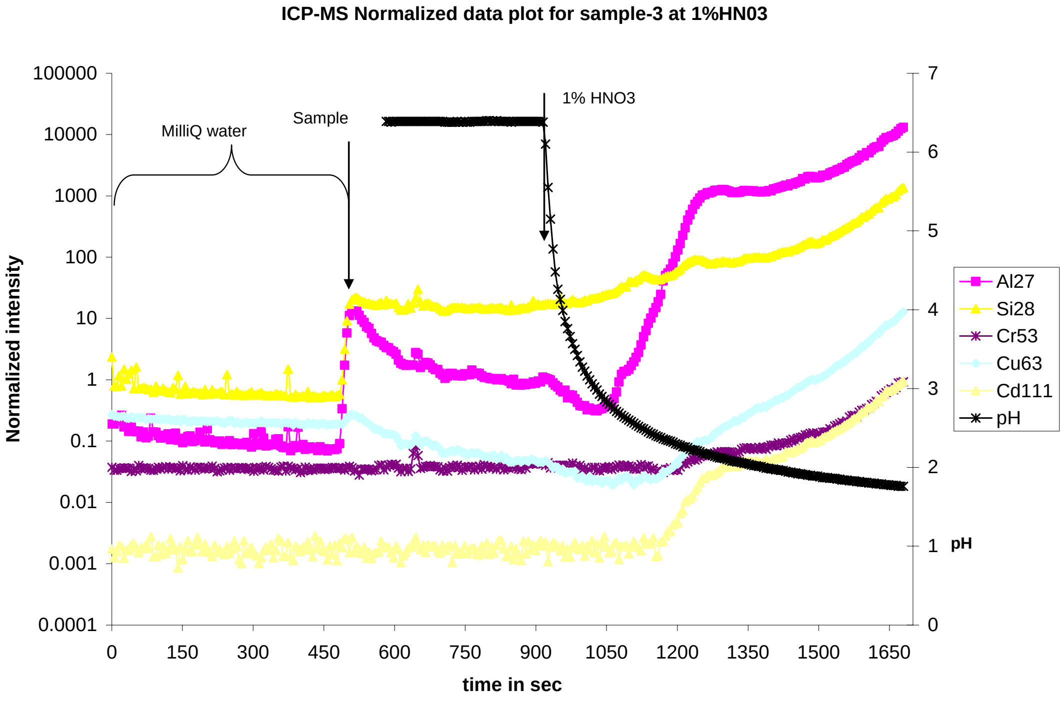

Figure II.6 ICP-MS normalized intensity data plot for sample-3 at 1% Nitric acid: other phase.

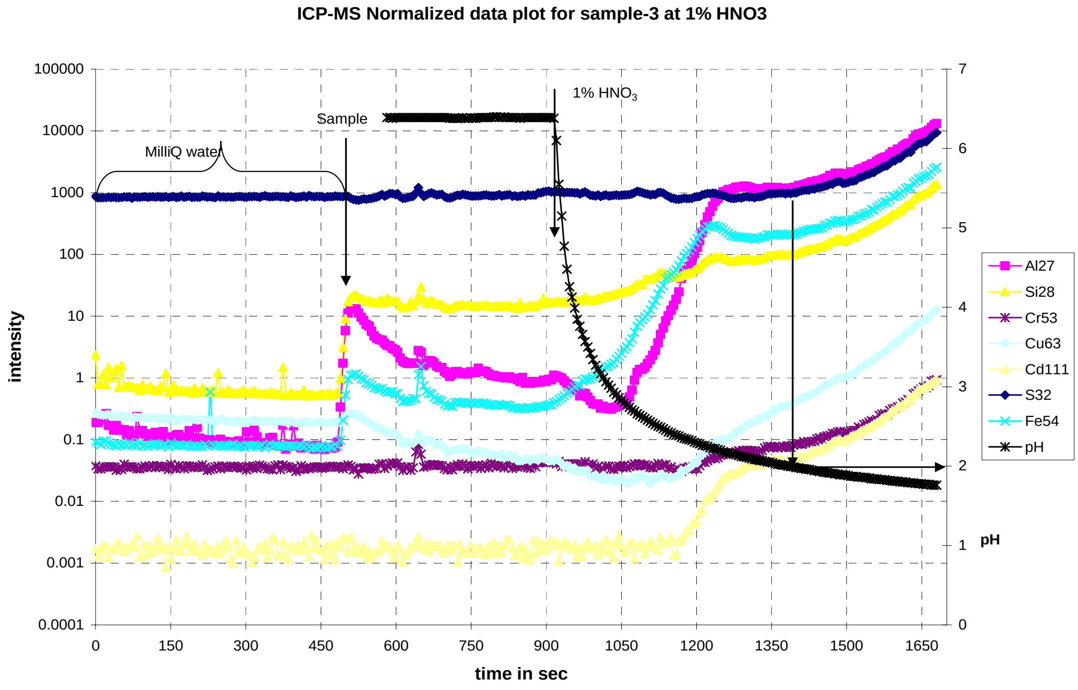

Figure II.7 ICP-MS normalized intensity data plot for sample-3 at 1% Nitric acid: relation with sulphur fractions.

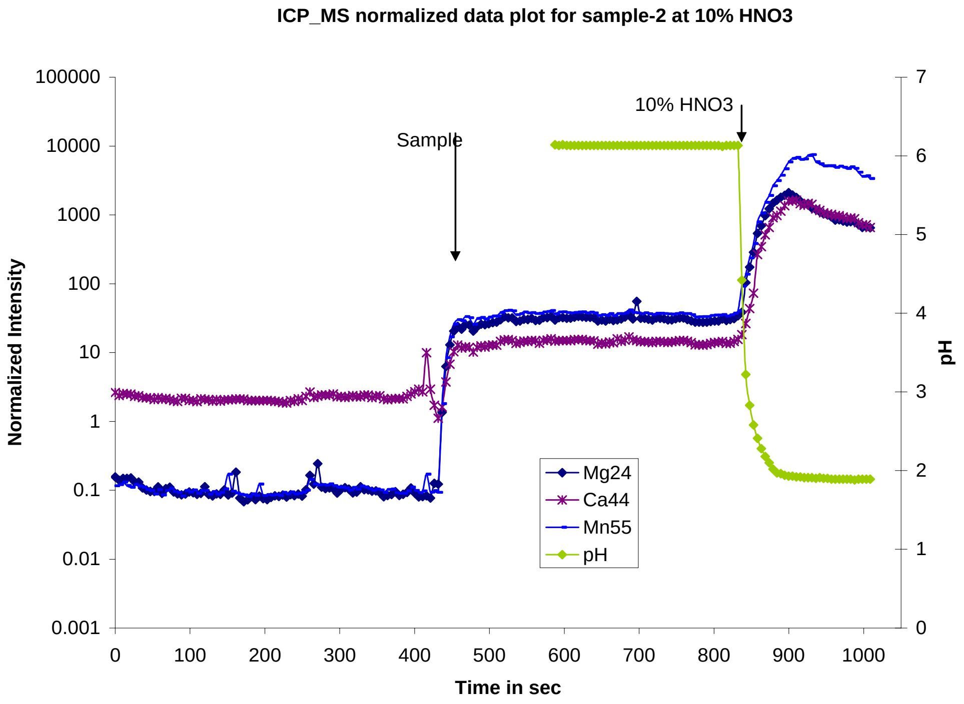

Figure II.8 ICP-MS normalized intensity data plot for sample-2 at 10% Nitric acid: Carbonate and exchangeable phase.

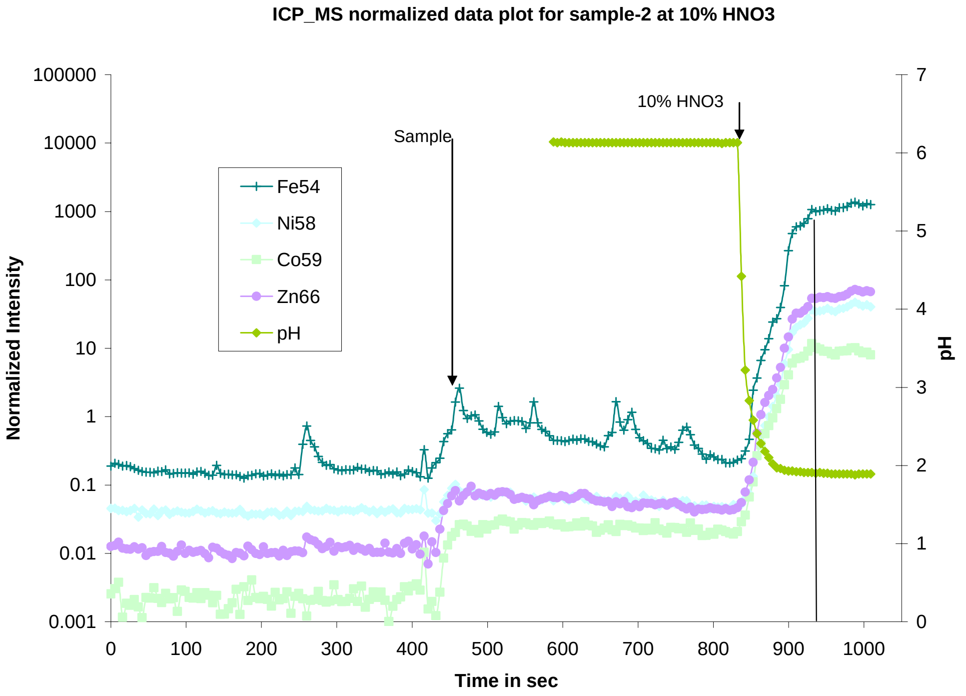

Figure II.9 ICP-MS normalized intensity data plot for sample-2 at 10% Nitric acid: Iron and Mn oxide phase.

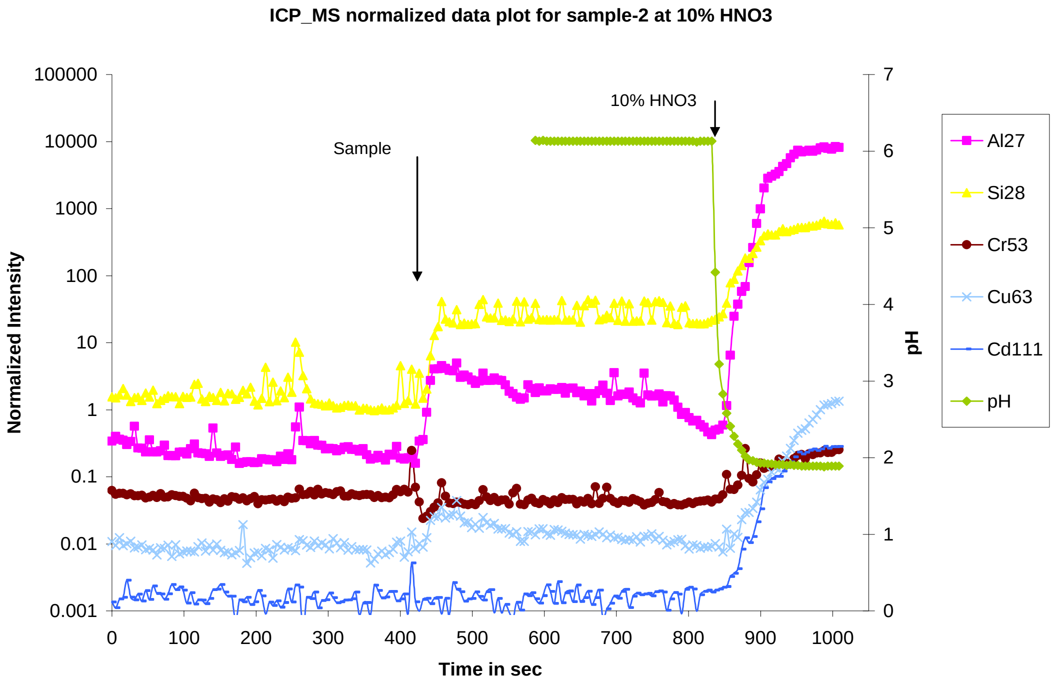

Figure II.10 ICP-MS normalized intensity data plot for sample-2 at 10% Nitric acid: other phase.

Appendix-III

PCA PLOT

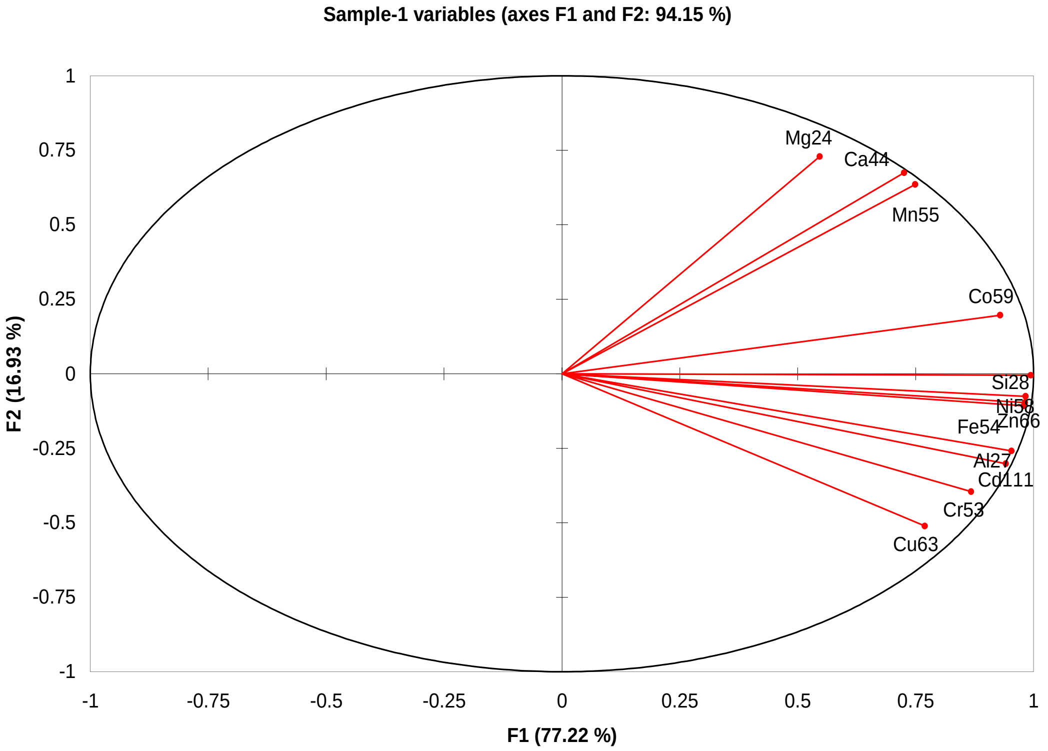

Figure III.1 PCA Factor plot for variable F1 and F2 for sample-1 at 0.1% HNO_3

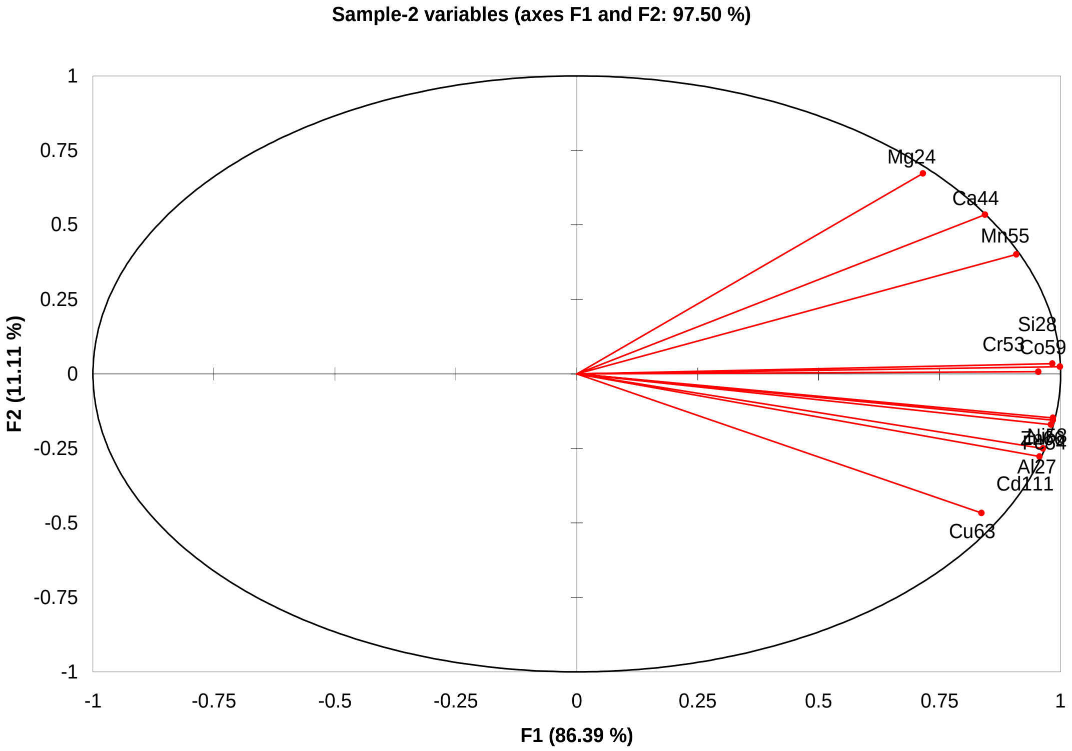

Figure III.2 PCA Factor plot for variable F1 and F2 for sample-2 at 10% HNO$_3$

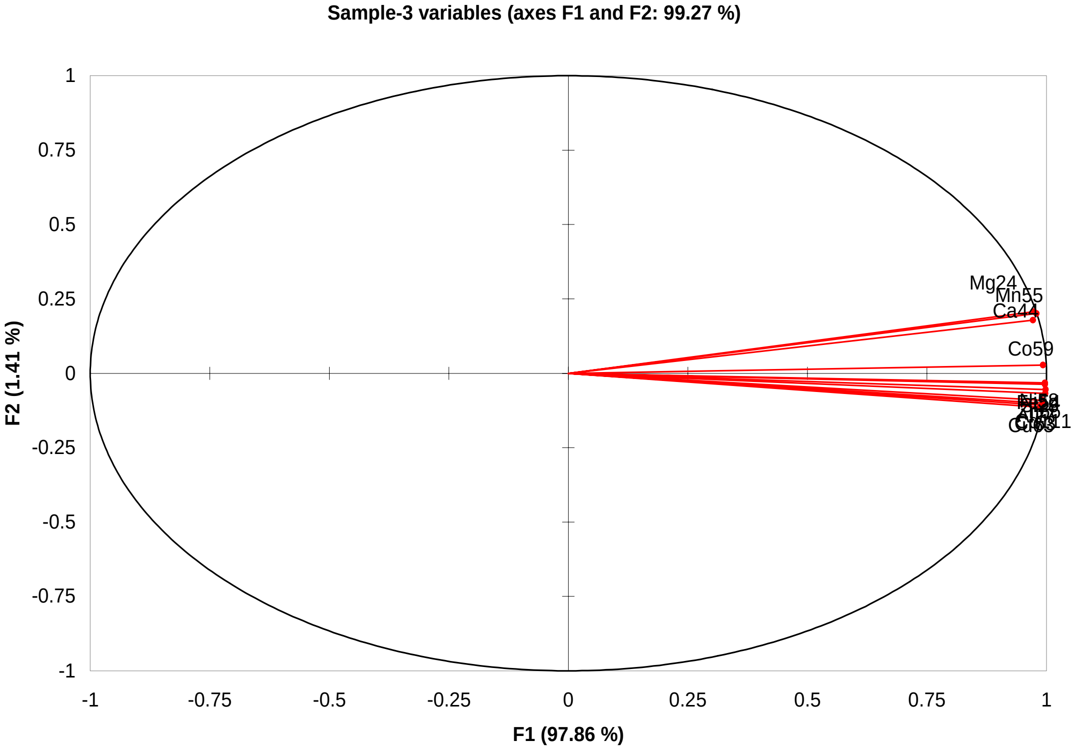

Figure-III.3: PCA Factor plot for variable F1 and F2 for sample-3 at 1% HNO$_3$

Appendix-IV

FRACTION ANALYSIS

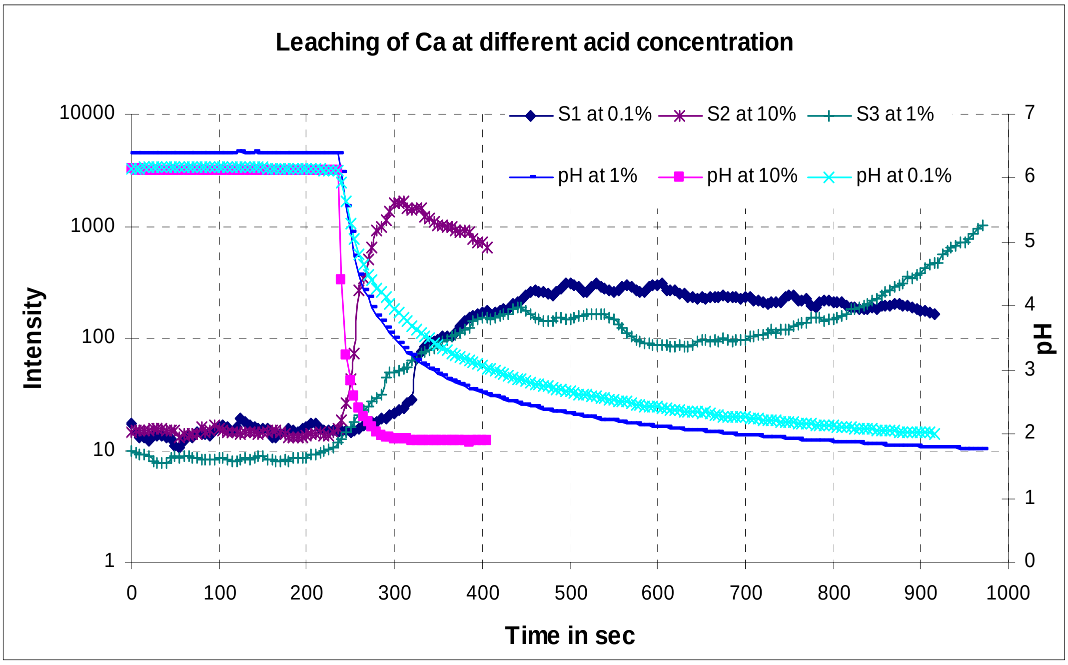

Figure IV.1.a Leaching of Ca at different acid concentration

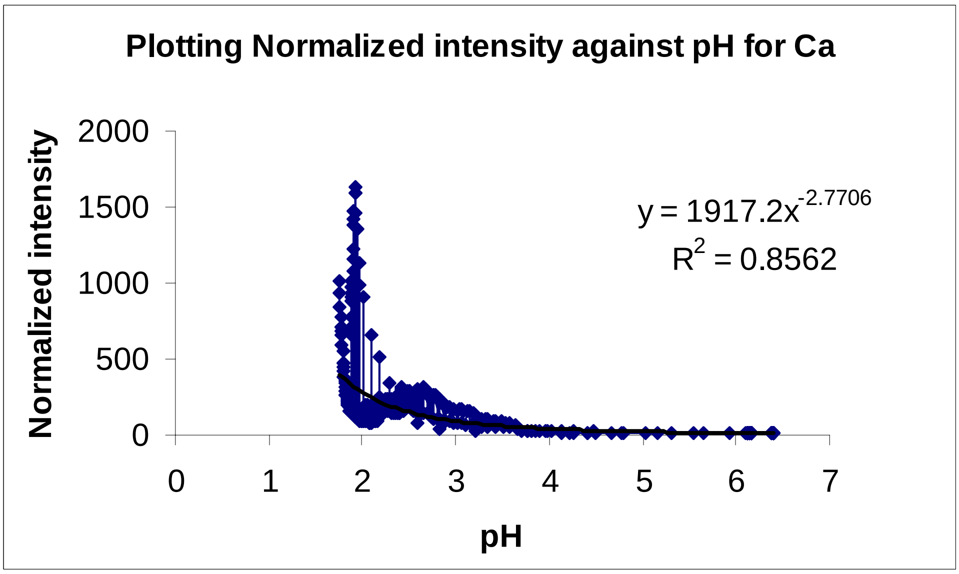

Figure IV.1.b Normalized intensity against pH for Ca

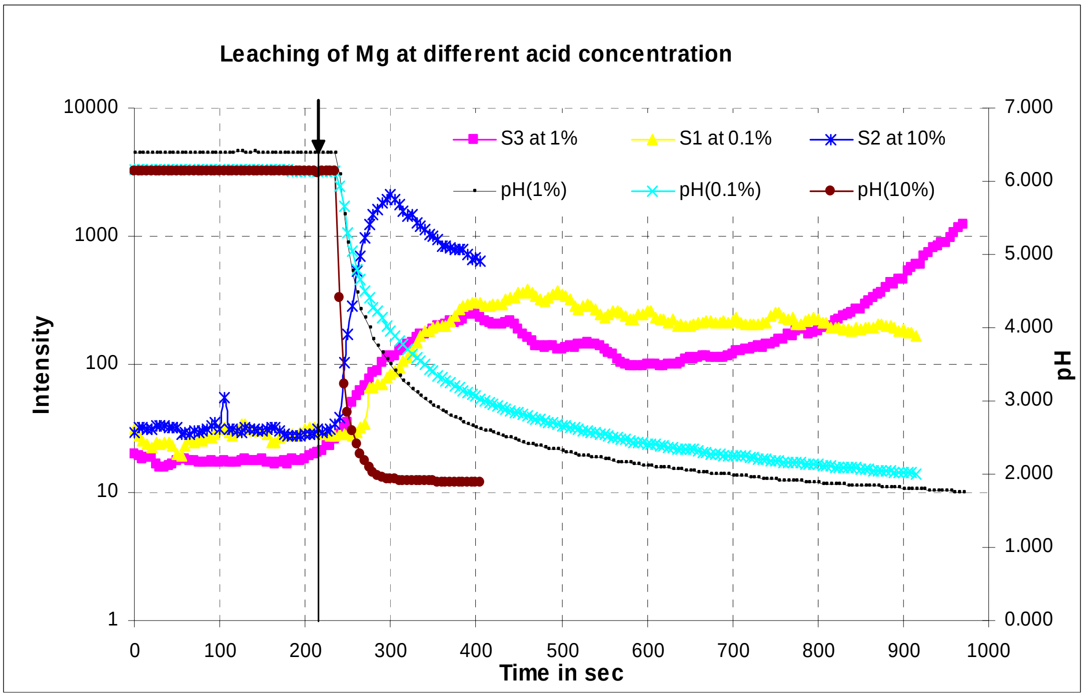

Figure IV.2.a Leaching of Mg at different acid concentration

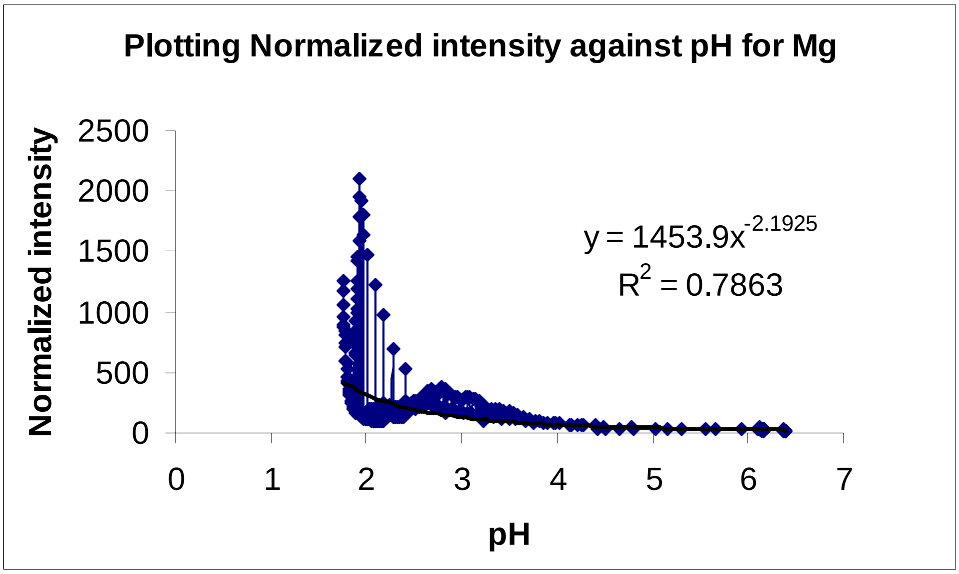

Figure IV.2.b Normalized intensity against pH for Mg

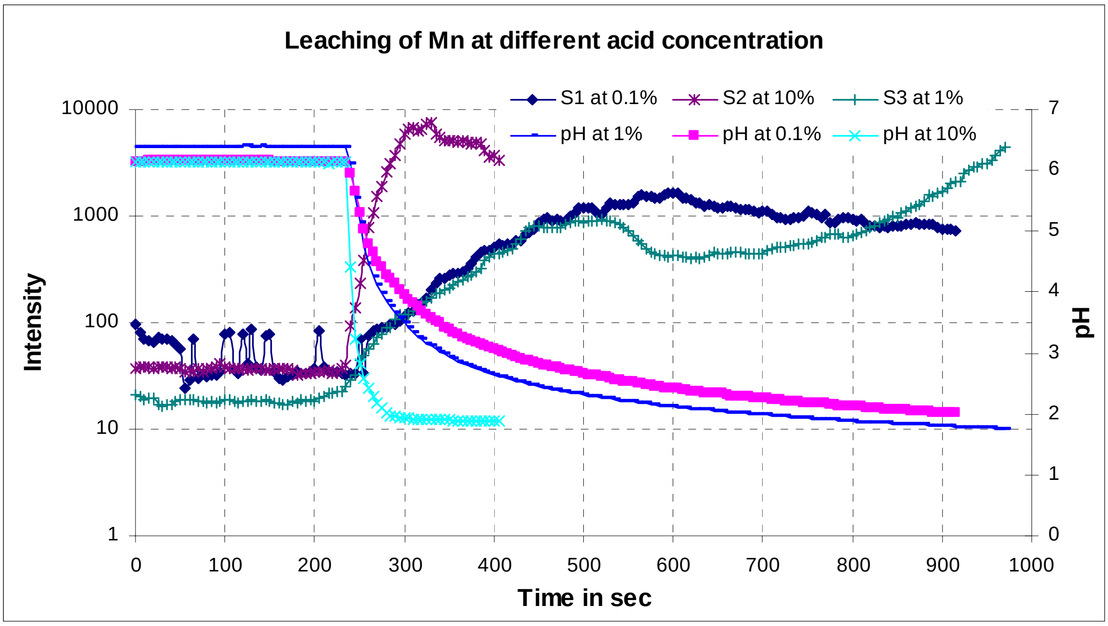

Figure IV.3.a Leaching of Mn at different acid concentration

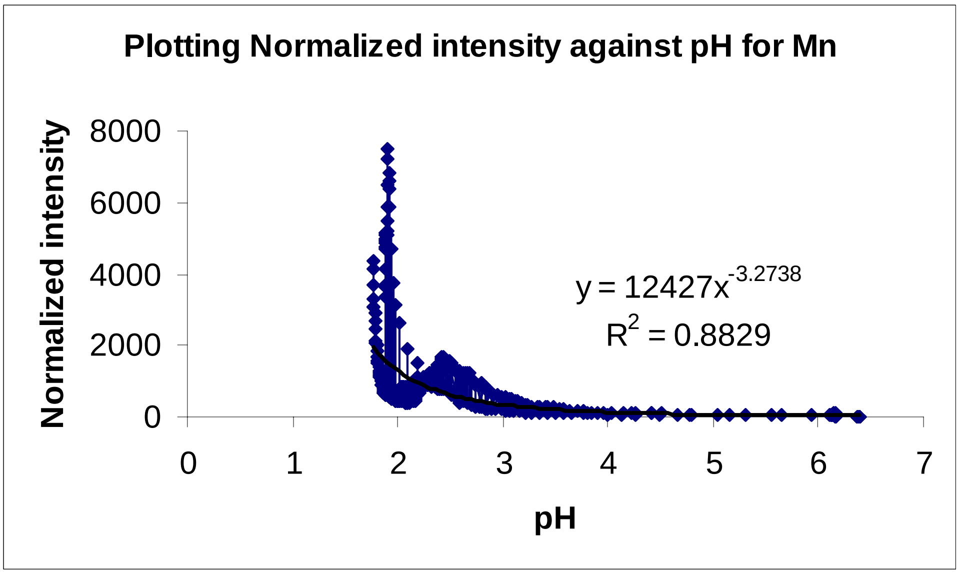

Figure IV.3.b Normalized intensity against pH for Mn

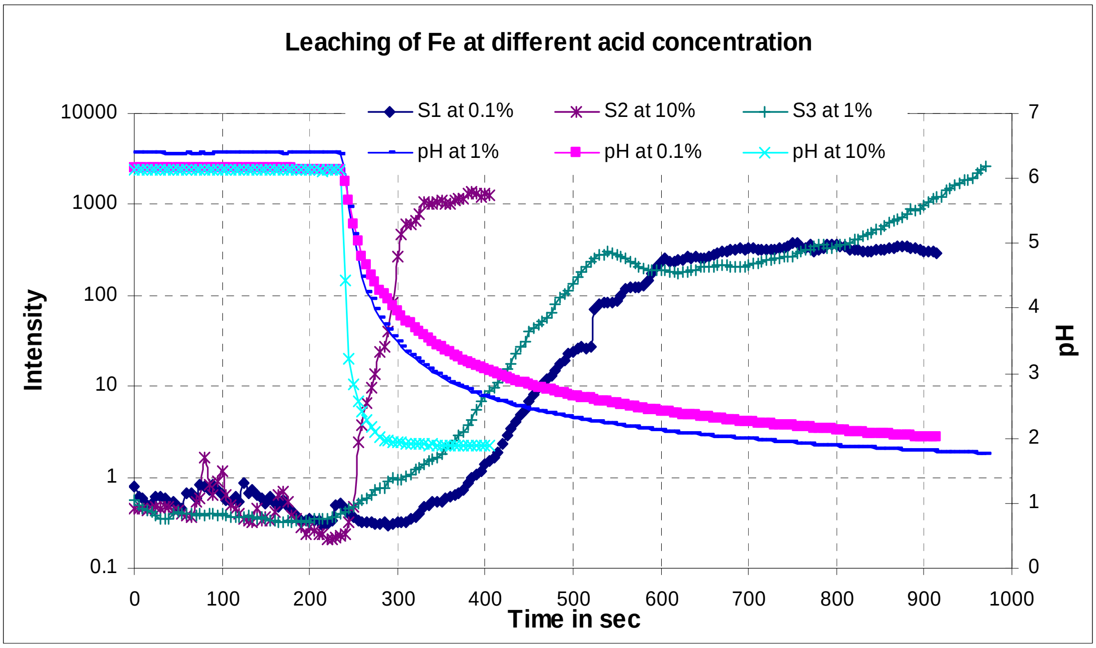

Figure IV.4.a Leaching of Fe at different acid concentration

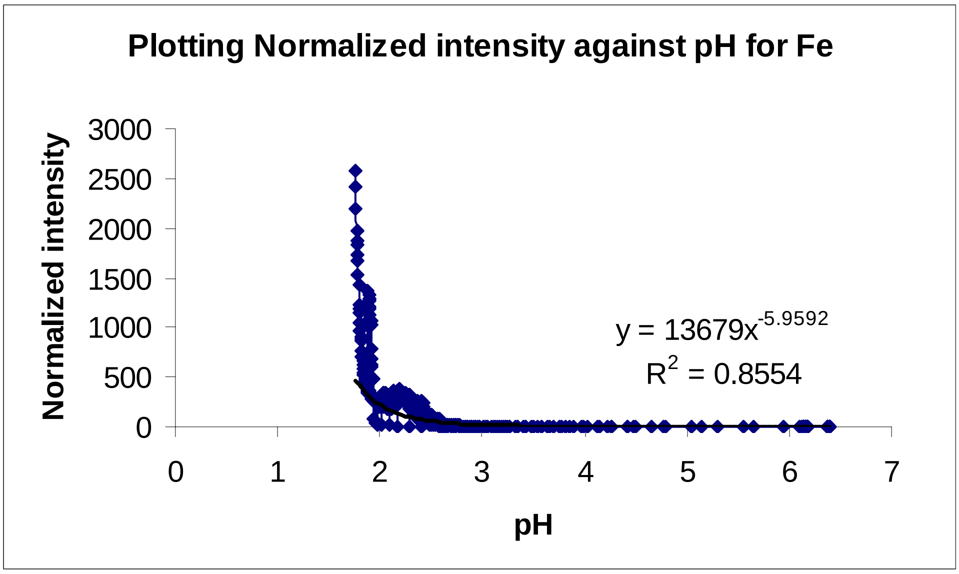

Figure IV.4.b Normalized intensity against pH for Fe

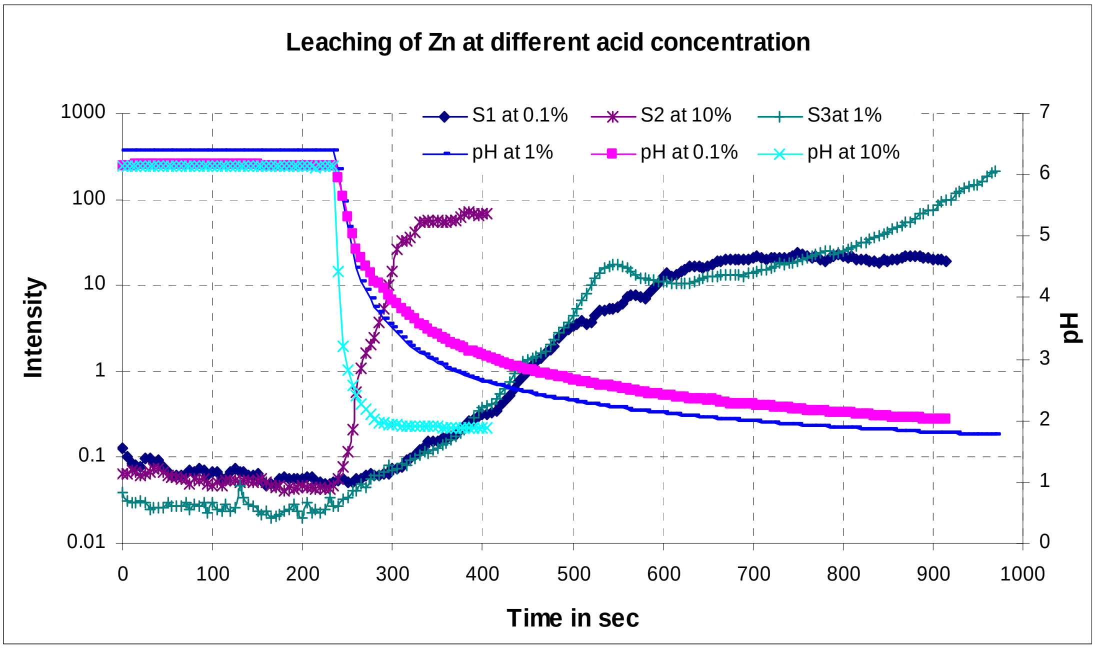

Figure IV.5.a Leaching of Zn at different acid concentration

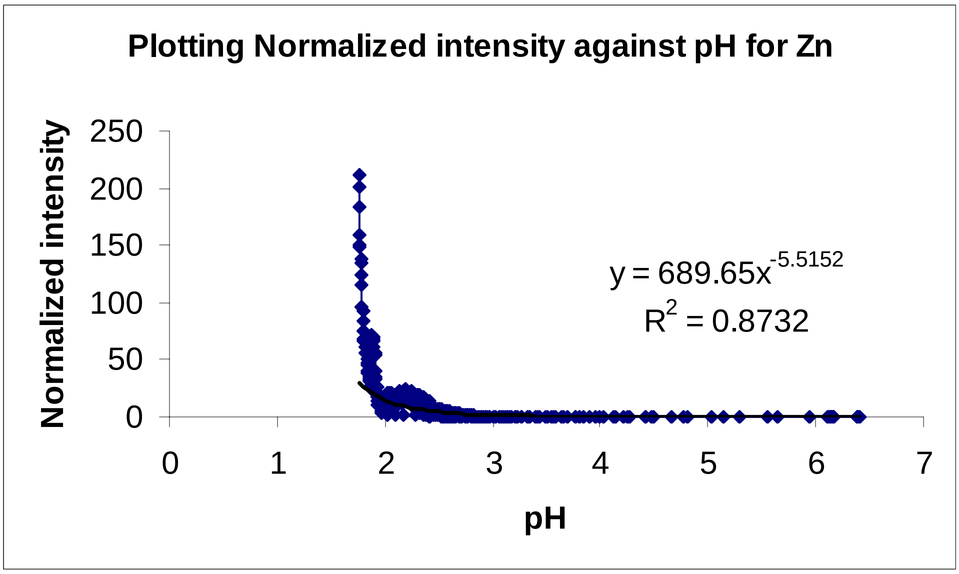

Figure IV.5.b Normalized intensity against pH for Zn

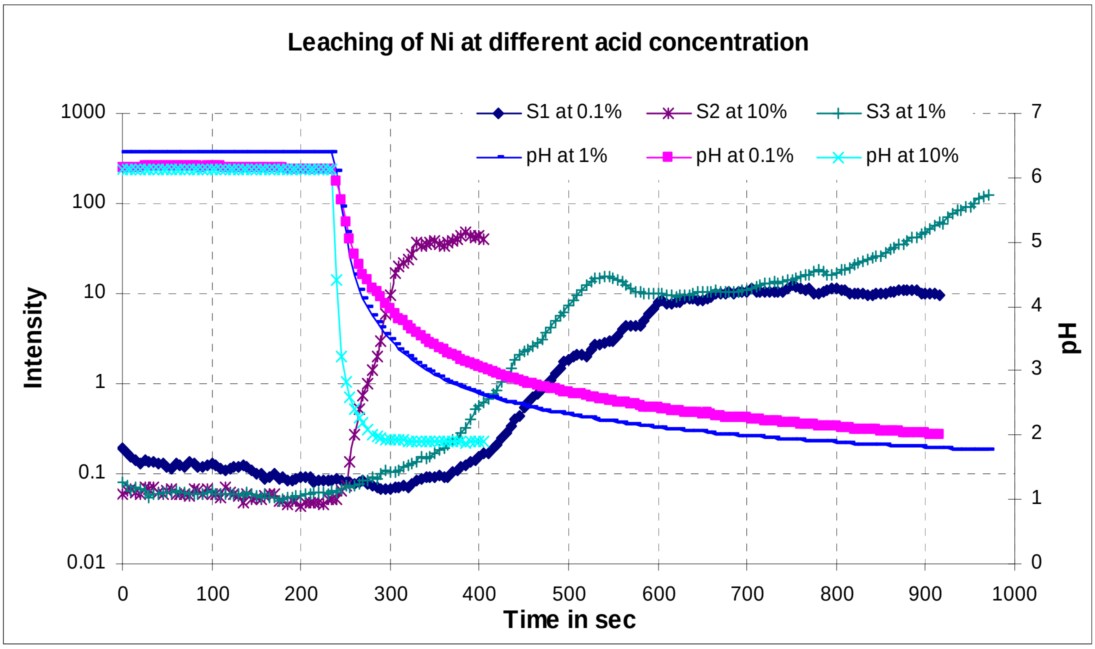

Figure IV.6.a Leaching of Ni at different acid concentration

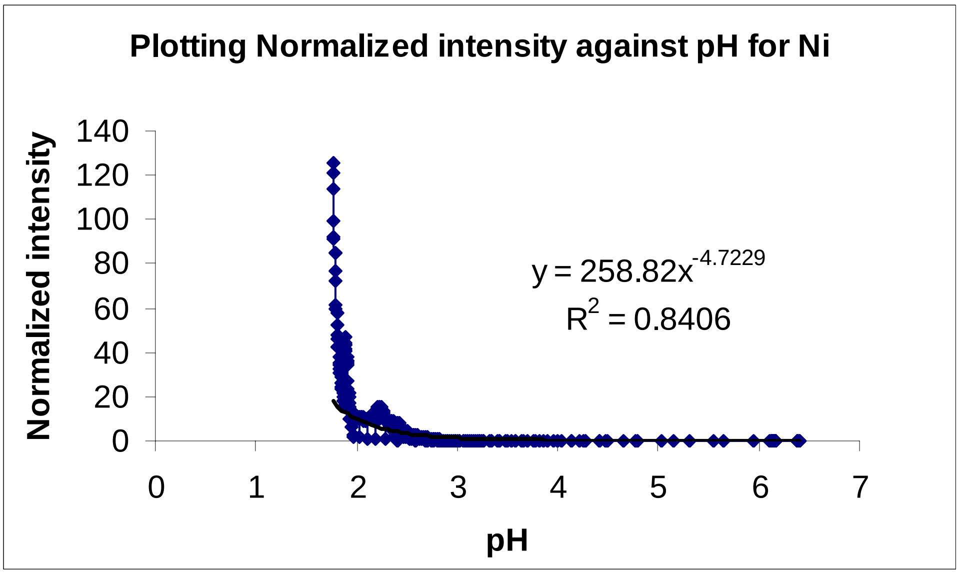

Figure IV.6.b Normalized intensity against pH for Ni

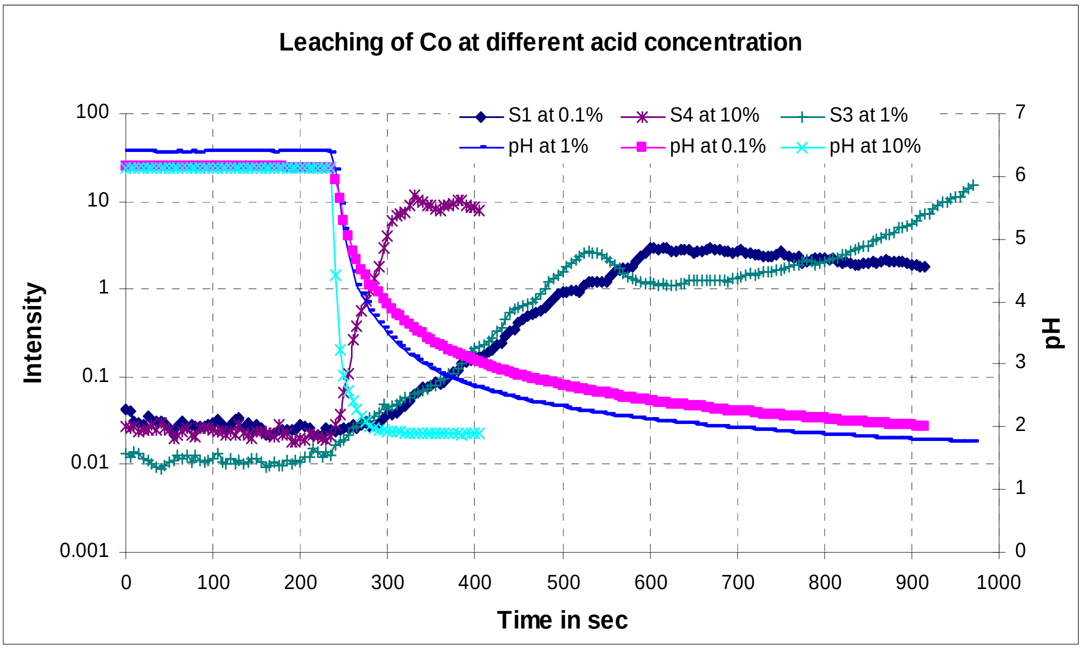

Figure IV.7.a Leaching of Co at different acid concentration

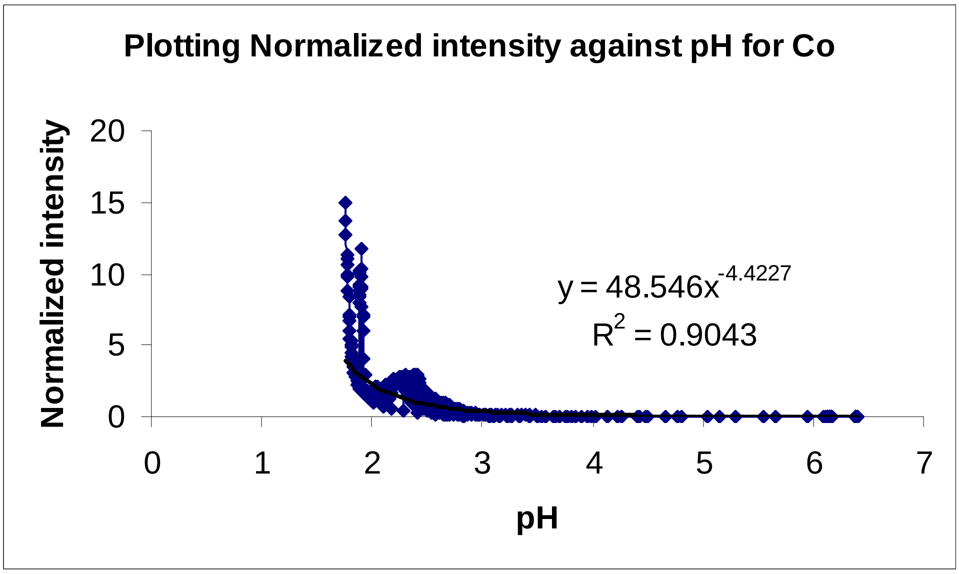

Figure IV.7.b Normalized intensity against pH for Co

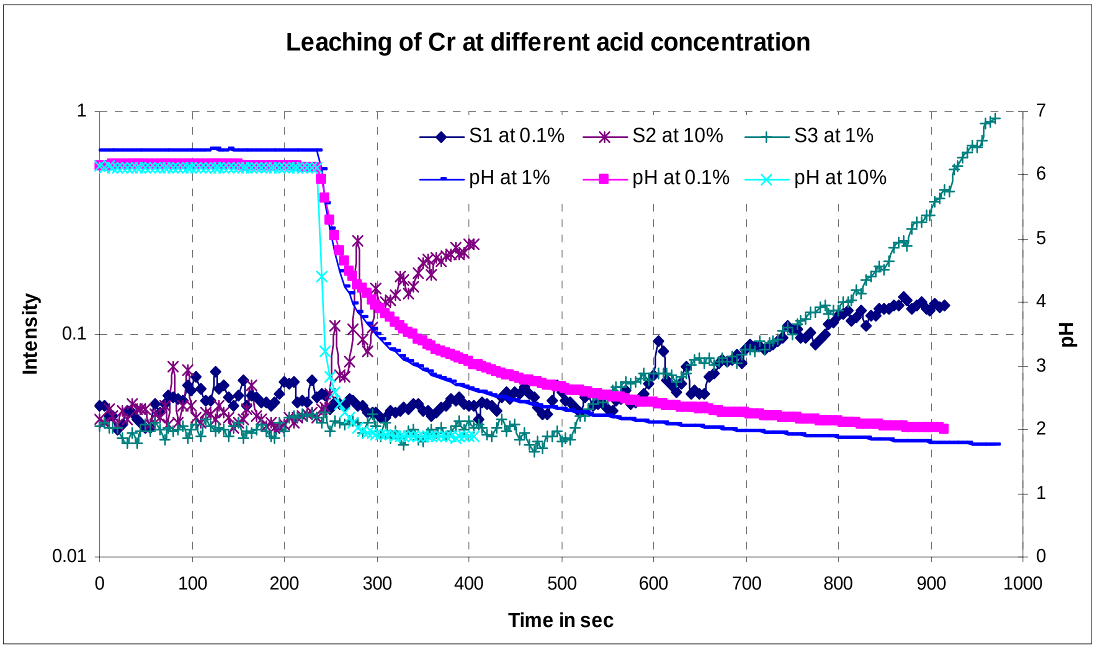

Figure IV.8.a Leaching of Cr at different acid concentration

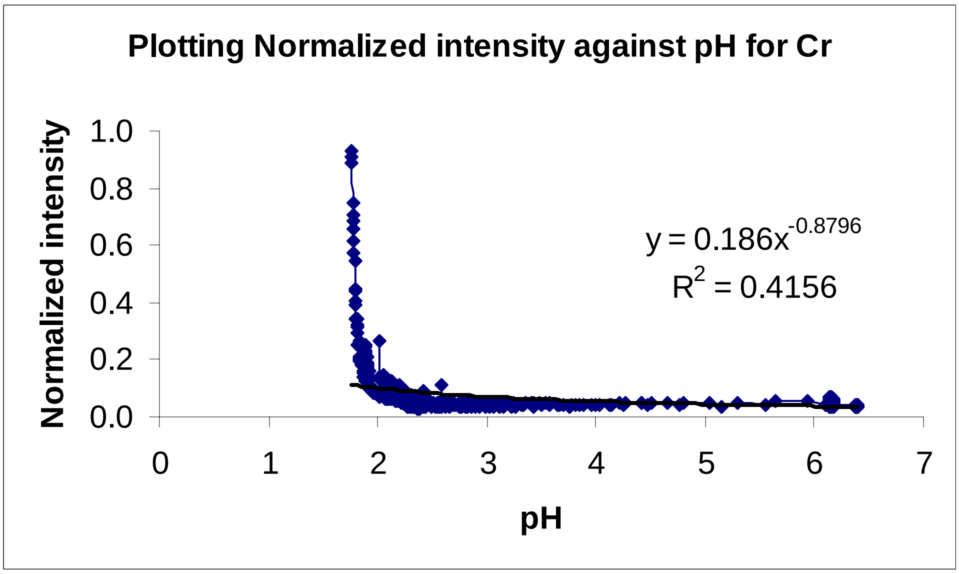

Figure IV.8.b Normalized intensity against pH for Cr

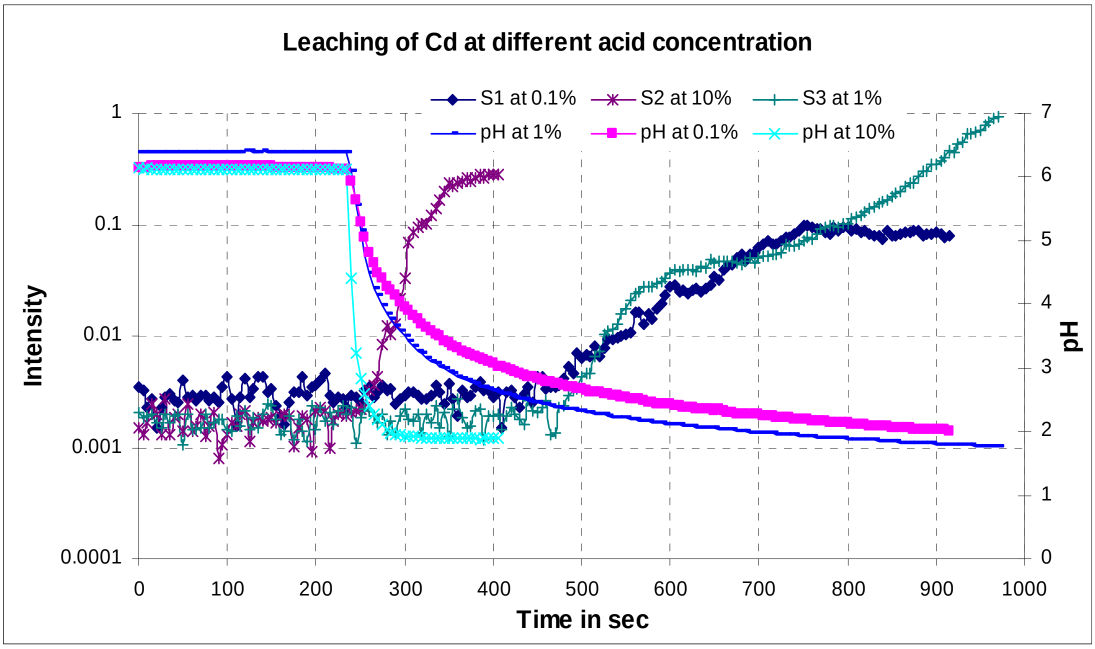

Figure IV.9.a Leaching of Cd at different acid concentration

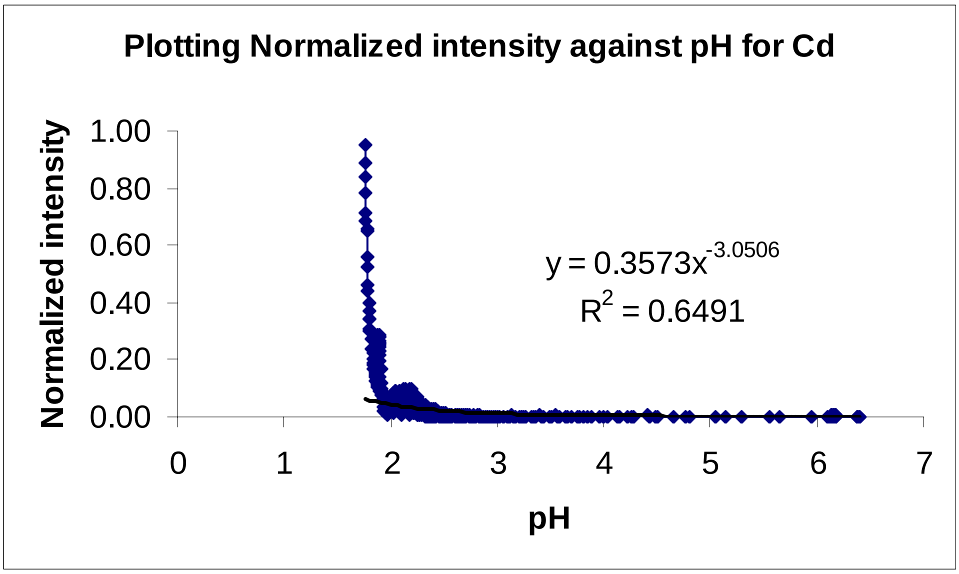

Figure IV.9.b Normalized intensity against pH for Cd

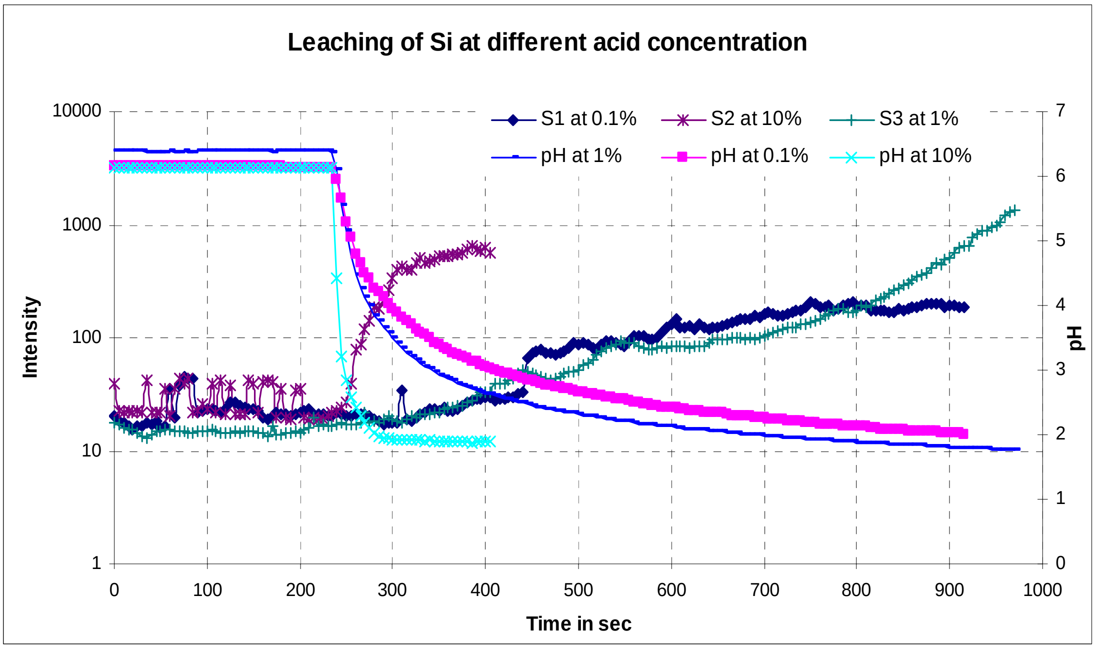

Figure IV.10.a Leaching of Si at different acid concentration

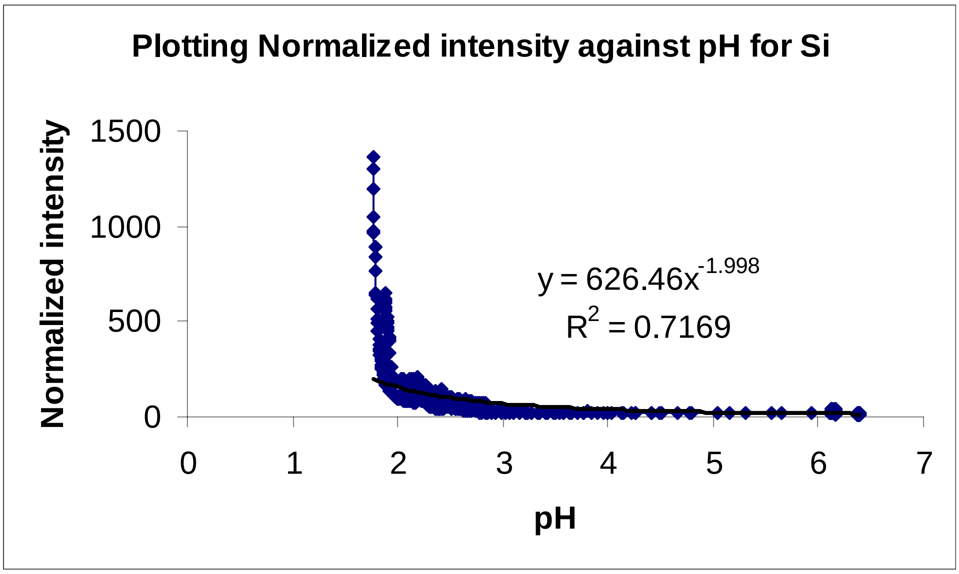

Figure IV.10.b Normalized intensity against pH for Si

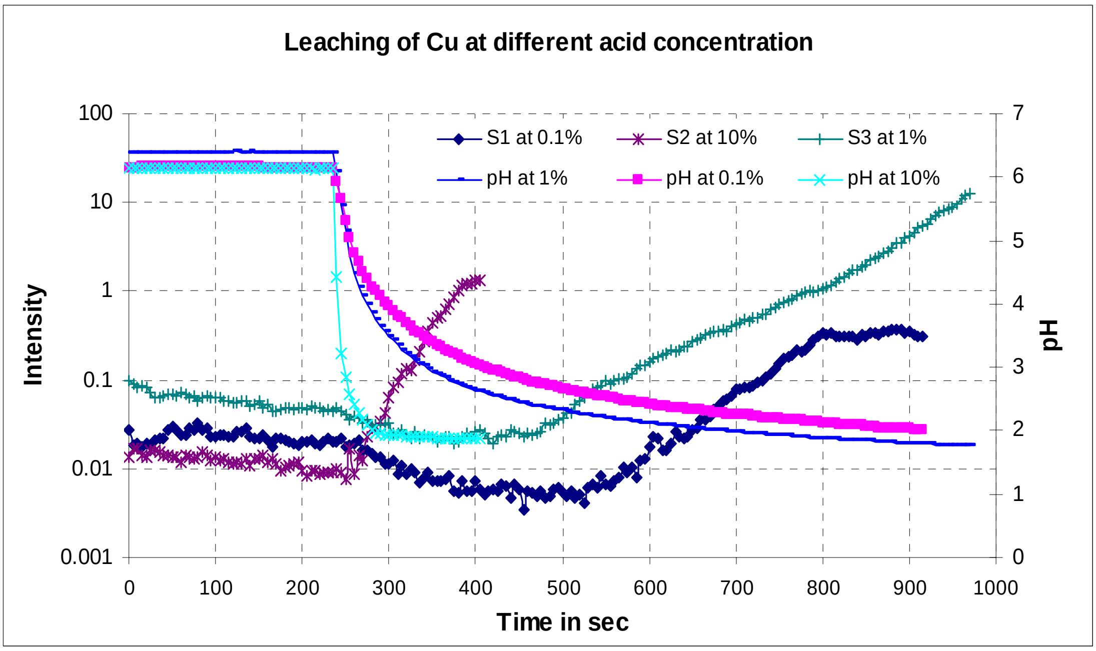

Figure IV.11.a Leaching of Cu at different acid concentration

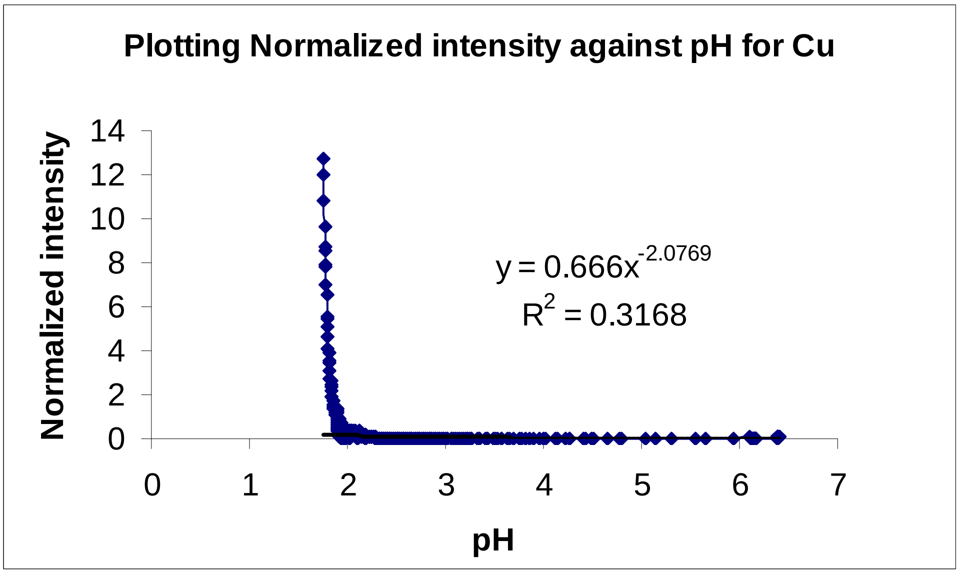

Figure IV.11.b Normalized intensity against pH for Cu

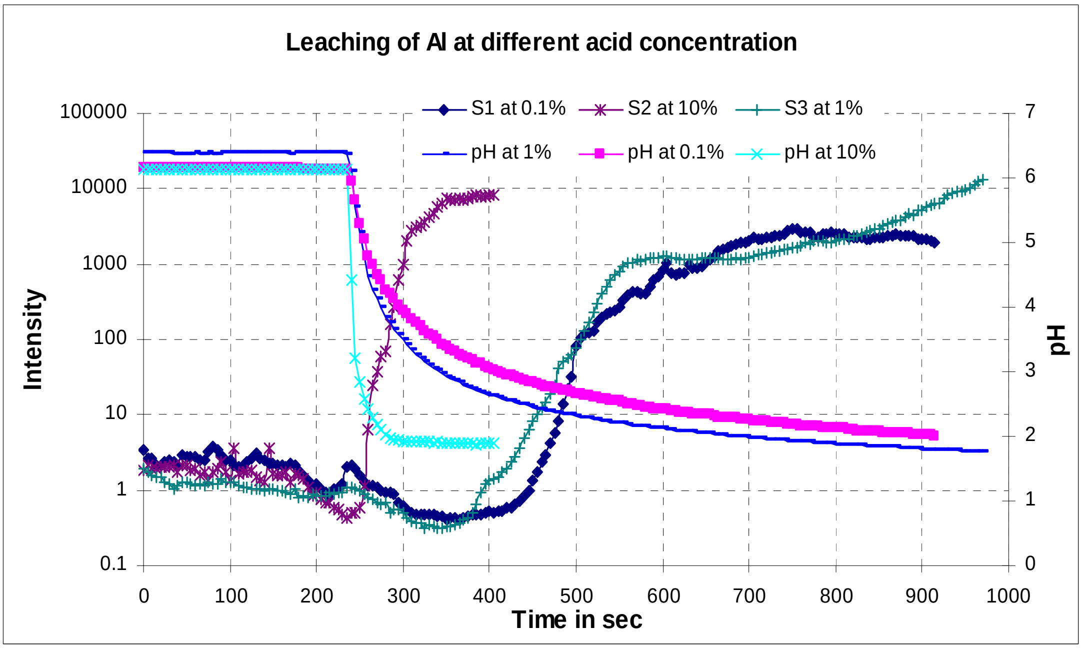

Figure IV.12.a Leaching of Al at different acid concentration

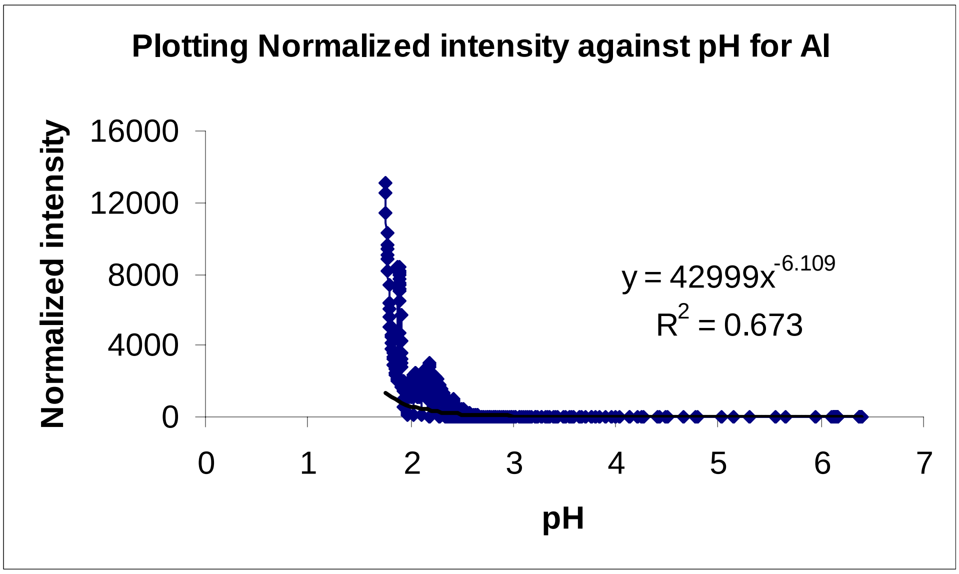

Figure IV.12.b Normalized intensity against pH for Al