SPECIATION OF CHROMIUM IN ALGECIRAS BAY

by

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Abstract

"Speciation of Chromium in Algeciras Bay"

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Algeciras Bay is exposed to contamination from chromium because of the industrial activities carring out around the water, i.e Steel Industry and two Petrol Refineries can be mentioned. More over there are several activities which are being carried out including big ports, fishing and recreational activities. Most of the other trace metals in the water have been studied. Considering the environmental and health effects of this metal, study of its level and speciacion in the bay is necessary for wellbeing of all which are directly or indirectly dependent on this water.

Unfiltered samples were used for total chromium determination and filtered samples were used for the other fractions and species of chromium determinations. The samples were collected from five different sampling points and four times between 2007 and 2009. The first and third samplings were done in autumn of 2007 and 2008 respectively. The second and the fourth samplings were done in spring of 2008 and 2009 respectivelly.

Total chromium levels in the water from the bay were lower than the maximum guideline level to cause toxicity but it was higher than the background levels indicating artificial introduction of chromium to the water from industrial and/or domestic sources and activities carring out on the water. It is also found that the total and trivalent chromium level in Algeciras bay were higher than that of Mediterranean sea but there were no significant differences (α=0.05) between hexavalent chromium levels of the two waters. It is also found that the total chromium, the different fractions and species of chromium in Algeciras Bay is lower than similar water bodies. There were no significant differences among the levels of all chromium forms and species studied during the four seasons but there were significant differences in sampling points for active chromium as well as dissolved chromium. There also were significant differences in the sampling years for particulate chromium as well as dissolved chromium. The correlations among the chromium fractions and species were also studied.

Aims and Objectives

Aims

- ✗ Completion of the master's
- ✗ Earn and use practical skills on polarography and water-metal analysis
- ✗ Address the contamination level of Algeciras Bay with respect to Chromium

Objectives

- ✗ Study the concentration level of species of chromium, i.e. Dissolved Hexavalent Chromium, Dissolved Trivalent Chromium and Particulate Chromium.
- ✗ Study the variation of concentrations of the total chromium, fractions of chromium and species of chromium among sampling points, seasons and years
- ✗ Study the correlation of total chromium, fractions of chromium and species of chromium among each other
- ✗ Compare NaOH (p.a.) and NH_3 (suprapur) for use in chromium speciation
- ✗ Apply clean techniques for trace analysis in environment.

GOAL

- ✗ **Successful Completion of the Master's**

Hypothesis

- ✗ $\mathbf{H_0}$: Algeciras Bay is polluted with chromium because it is exposed to pollution from different sources
- ✗ $\mathbf{H_1}$: Algeciras Bay isnot polluted with chromium because there is flux of water from Mediterranean Sea

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1 Introduction

1.1 Water Bodies and Uses

Water body is a significant accumulation of water either artificialy or natural. This includes oceans, seas and lakes, from large water bodies, to smaller pools of water such as ponds, puddles, estuaries, bays, rivers, streams and canals. Water is vital to life. Life on this planet is completly dependent upon water. Water covers about 70% of Earth's surface. Humans and animals as well as plants use water for drinking and satisfy from trusty. Especialy for humans water gives many purpose such as saniation. People also use water for power production, industry, transportation and recreation, and often people compete for water use.

Water leaches soluble constituents from mineral matter and carries them to the ocean or leaves them as mineral deposits some distance from their sources. Water carries plant nutrients from soil into the bodies of plants by way of plant roots. Solar energy absorbed in the evaporation of ocean water is carried as latent heat and released inland. The accompanying release of latent heat provides a large fraction of the energy that is transported from equatorial regions toward Earth's poles and powers massive storms [1]. Because water is a limited resource, sometimes there is not enough water to satisfy all of these demands. This problem is complicated by pollution, which can make water unusable for almost any purpose in the future.

Water Bodies Pollution Sources and Pathways

Water bodies can be polluted in a way that water can be non-potable and treaten lives of living organisms including human beings when used for drinking, making life very

challenging. This times water pollution is a common and very series problem around the globe. Pollution kills aquatic plants and animals, and could eventually threaten plants and animals with extinction. Sea foods like fish and sell fish can accumlate high levels of pollutants like heavy metals that pose a health risk to humans. These also affect economy of one country in turn because industries related to sea food products may be closed. Additionally, water pollution affects the agriculltural sectore as farming relay on surface water for irrigation. The water used for irrigation is often pumped out directly from rivers or lakes. In the case the water used for irrigation is severly contaminated by pollutants like heavy metals, it can destroy the farm and spoil the soil for next uses. The pollution can remain in the ground for several years. This may prevent farming on these lands temporaly or permanently [2].

Pollutants of water bodies can pass through different pathways mainly from air, discharge of wastes and landfills [3] as shown in the figure below. Mainly discharge of untreated sewage and industrial runoff increase the pollution of water bodies significantly. Poorly planned landfills can also result in availablity of leakages during runoff and can extremely pollute the water bodies near and far from these fills.

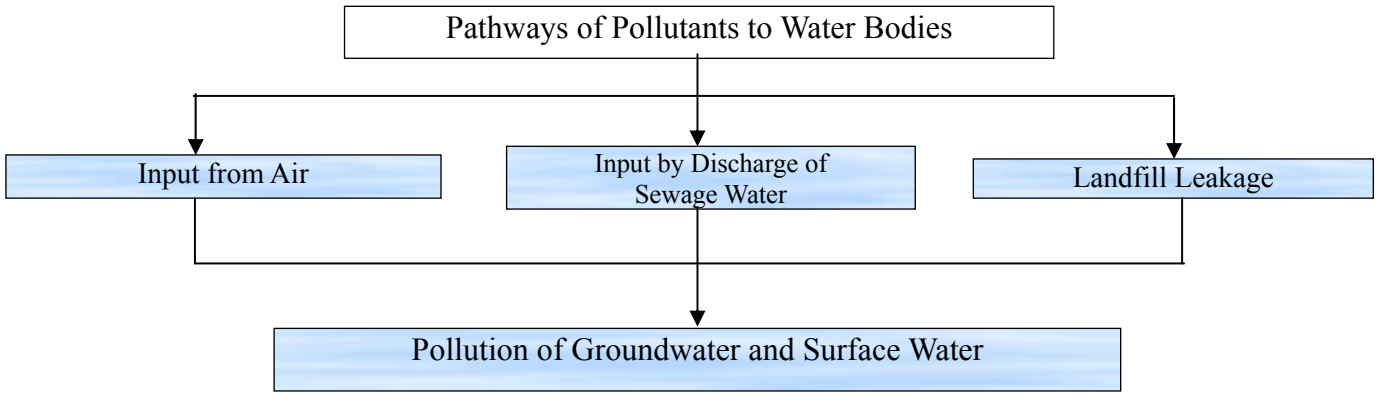

Figure 1 - Pathways of Pollutants from different sourses to Water Bodies

1.2 Algeciras Bay

Algeciras bay which is also called as Gibraltar bay as being one of the water bodies, is found in southern part of Spain (southern end of Iberian Peninsula)(see Figure 2). Its length is around 10Km and the width is around 8Km and covers an area of 75Km2. The maximum depth is 400m. The bay flows to Mediterranian Sea and Strait of Gibraltar. Several industries have been planted in the area around the bay of which the majorities are petrochemical industries. The busy ports, which are found around the bay, receive many ships daily mainly for transporting oil and goods. The heavy industries situated along the shore of the bay and the activities like ship to ship oil transfer including many other contributes for the pollution of the water and the ecosystem in general by organic pollutants, heavy metals, radioactive substances etc....

Figure 2 - Google map of Algeciras Bay; (coordinates $36^0$7'51.91"N $5^0$23'45.63"W)

1.3 Heavy metals

Contaminations of water resources have been common scenario around the world. These contaminations worsen with industralization in the last century. Among wide diversity of pollutants affecting water resources, heavy metals receive special attention because of their sever toxicity even at very low concentrations, see the background levels of heavy metals in the table below [4]. Heavy metals is a term that applies to a large group of trace elements which are both industrially and biologicaly important. Most of these metals have atomic density greater than $6g/cm^3$ [5]. In aquaitc environment, heavy metals exist as dissolved or particulated phase. (see appendix 4 for toxicity levels of heavy metals in different environments)

Heavy metals sources

Naturally heavy metals belong to different ores. These metals make their way to water bodies through different mechanisms like errosion from mineral rocks found on or/and under the benth of the water, leaching of ore deposites, volcanic entruption, solid waste disposal, domestic and industrial effluents, and surface water and under water activities, etc...[5]. Among these all, mettallurgical industries are the major source of contaminants, (see table 1 below for main industries that releases metals [6]). Generally the sources of pollutants are classified into point and nonpoint categories. The point sources include the activities of industries and domestic survival (home lawns, ornaments, pests control, etc.), while the nonpoint sources are of geological origin, from agriculture, and related activities [7].

Table 1 - Majore heavy metals from industries

	Industries	**Cd**	**Co**	**Cr**	**Cu**	**Fe**	**Mn**	**Pb**	**Zn**
1	Pulp, papermill; building paper, board mills			x	x			x	x
2	Organic chemicals, petrochemicals	x		x	x	x		x	x
3	Alkalis, chlorine, inorganic chemicals	x		x	x	x		x	x
4	Fertilizers	x		x	x	x	x	x	x
5	Petroleum refining	x		x	x	x		x	x
6	Steel works	x		x	x	x		x	x
7	Nonferrous metal works	x		x	x			x	x
8	Motor vehicle, aircraft plating	x		x	x				
9	Flat glass, cement, asbestos			x					
10	Textiles			x					
11	Leather tanning			x					
12	Steam generation			x					x

1.4 Chromium

Chromium is an element with symbol Cr and atomic number 24. It belongs to the group 6 in Periodic Table. It is a tasteless and oderless element and has melting point of 1907^0C and density of 6.3 g/cm^3. Chromium is one of the common trace metals that are toxic to living organisms at higher concentration levels depending on the oxidation state.

Chromium is a naturally occurring element found in rocks, soil, plants, animals, and in volcanic dust and gases. Chromium has three main forms chromium (0), chromium (III), and chromium (VI). Chromium (III) compounds are stable and occur naturally, in the environment. Chromium (0) does not occur naturally and chromium (VI) occurs only rarely. Chromium compounds have no taste or odor. Chromium (III) is an essential nutrient in diet, but humans need only a very small amount. Other forms of chromium are not needed by our bodies.

Uses and Toxicity of Chromium

Chromium is used in manufacturing chrome-steel or chrome-nickel-steel alloys (stainless steel) and other alloys, bricks in furnaces, and dyes and pigments for paints, for greatly increasing resistance and durability of metals and chrome plating, leather tanning, and wood preserving. Chromium is also used in cement, paper, rubber, and other materials. Manufacturing, disposal of products or chemicals containing chromium, or fossil fuel burning release chromium to the air, soil, and water. Particles settle from air in less than 10 days. It sticks strongly to soil particles; in water it sticks to dirt particles that fall to the bottom; only a small amount dissolves. Small amounts move from soil to groundwater.

Low-level exposure can irritate the skin and cause ulceration. Chromium often accumulates in aquatic life, adding to the danger of eating fish that may have been exposed to high levels of chromium. Acute toxic effects occur when breathing very high levels of chromium (VI) in air, which can damage and irritate your nose, lungs, stomach, and intestines. People who are allergic to chromium may also have asthma attacks after breathing high levels of either chromium (VI) or (III). Long term exposures to high or moderate levels of chromium (VI) cause kidney and liver damage, and damage too

circulatory and nerve tissue, damage to the nose (bleeding, itching, sores) and lungs, and can increase your risk of noncancer lung diseases. Ingesting very large amounts of chromium can cause stomach upsets and ulcers, convulsions, kidney and liver damage, and even death. Skin contact with liquids or solids containing chromium (VI) may lead to skin ulcers. Some people have allergic reactions including severe redness and swelling [8, 9, 10].

1.5 Metal Speciation

Transition metals, lanthanides, and actinides exist in different oxidation states due to the presence of empty 'd' orbitals in transition metal ions and 'f' orbitals in lanthanides and actinides. Therefore, these metal ions occur in the environment in different oxidation states and form various species, for example, Chromium (Cr) as Cr (III) and Cr (VI). The different oxidation states of a particular metal ion possess different physical and chemical properties. It is very important to observe and note that these different oxidation states have different toxicities. Basically, speciation is a concept used frequently in biological science, and it was adopted by those in analytical chemistry, expressing the idea that the specific chemical forms of an element should be considered individually [7]. The separation and identification of the different oxidation states of a particular metal ion is known as speciation. The concept of speciation dates back to 1954 when Goldberg [11] introduced the concept of speciation to improve the understanding of the biogeochemical cycling of trace elements in seawater. Since then, this development has been growing exponentially to the point that research on trace element analysis is being conducted.

Templeton et al described the speciation term as per the International Union for Pure and Applied Chemistry (IUPAC) [12]. Authors defined elemental speciation as follows:

- Chemical species or chemical element: specific form of an element defined as to isotopic composition, electronic or oxidation state, or complex or molecular structure
- Speciation analysis: separation and identification of one or more individual chemical species in a sample
- Speciation of an element: distribution of an element among defined chemical species in a system; when elemental speciation is not feasible, the term fractionation will be used
- Fractionation: classification of an analyte or a group of analytes from a certain sample according to physicochemical properties, such as size, solubility, bonding, reactivity, etc...

Methods for Metal Speciation

Metals are found in trace amount in the environment. This makes speciation of metals a difficult job. This problem worsens due to similar chemical properties of metals. Different advanced techniques were developed to overcome this problem. The analytical methods used for metal ion speciation include polarographic, spectroscopic, chromatographic, and electrochemical methods. The following graph shows the major techniques used to analyse metal species.

Table 2 - Different techniques for metal ion speciation

Speciation Techniques	Spectroscopic Methods	UV. Vis. Spectroscopy
		Atomic Absorption Spectroscopy
		Atomic Emission Spectroscopy
		Inductive Coupled Plasma
	Electrochemical Methods	Polarography
		Voltammetry
		Capillary Electrophoresis
		Gel Electrophoresis
	Chromatographic Methods	Gas Chromatography
		High Performance Liquid Chromatography
		Ion Chromatography

Among the different techniques mentioned above, the electrochemical methods and chromatographic methods has been used frequently. In the last decade, the speciation of metals using polarographic and voltammetric method has increased dramatically making these methods most used methods for speciation of metal species.

1.6 Voltammetry

Voltammetry is an analytical technique based on the measure of the current flowing through an electrode dipped in a solution containing electro-active compounds, while a potential scanning is imposed upon it. A voltammetric technique consists of three electrodes, i.e. working elctrode, reference electrode and counter (auxiliary) electrode. In general, an electrode provides the interface across which a charge can be transferred or its

effects felt. Because the working electrode is where the reaction or transfer of interest is taking place, whenever we refer to the electrode, we always mean the working electrode ald it is also adopted in this thesis. The reduction or oxidation of a substance at the surface of a working electrode, at the appropriate applied potential, results in the mass transport of new material to the electrode surface and the generation of a current [13].

The working electrode could be made with several materials. Usually, it has a very little surface in order to assume quickly and accurately the potential imposed by the electrical circuit. The electrode can be solid (gold, platinum or glassy carbon) or formed by a drop of mercury hanging from a tip of a capillary. If the electrode is formed by a drop of mercury rhythmically dropping from a capillary, the analytical technique is called *Polarography* [9].

The reference electrode provides a reversible half-reaction with Nernstian behavior, be constant over time, and be easy to assemble and maintain. The most commonly used reference electrodes for aqueous solutions are the calomel electrode, with potential determined by the reaction:

$$Hg_2\,Cl_{2\,(s)} + 2e^- = 2Hg_{(l)} + 2Cl^-$$

and the silver/silver chloride electrode (Ag/AgCl), with potential determined by the reaction

$$AgCl_{(s)} + e^- = Ag_{(s)} + Cl^-$$

Counter Electrodes: Most often the counter electrode consists of a thin Pt wire, although Au and sometimes graphite have also been used. In most voltammetric techniques the analytical reactions at the electrode surfaces occur over very short time periods and rarely produce any appreciable changes in bulk concentrations of A_{ox} or A_{red} . Thus, isolation of the counter electrode from the sample is not normally necessary.

Theory of Voltammetry

A voltammetric cell contains a solution and working electrode. The solution contains electro-active compound, A_{ox}, that can be reducible as shown in the following chemical reaction:

$$A_{ox} + ne \rightarrow A_{red}$$

A reduciable potential is imposed to the electrode respect to the reduction potential of the compound A_{ox}. The electrical current that flows through the electrode can be measured while A_{ox} is beging reduced on the electrode.

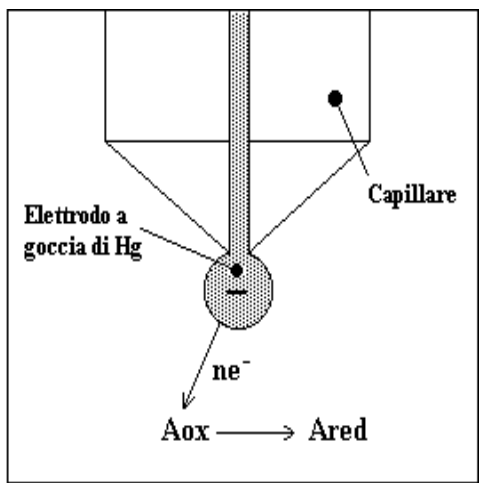

Figure 3 – Reducing process of A_{ox} on a mercury drop electrode: (the electrode has a negative charge and the correct potential that allows the flowing of electrons toward A_{ox}. In this way A_{ox} reduces itself and give raise to A_{red}.)

In order that the reduction occurs, it is necessary that A_{ox} can accept the electrons from the electrode. Thus reduction process proceeds under the influence of two kinetic factors [13]:

- the speed (v_d) of the chemical compound that reach the electrode from the bulk solution
- the speed (v_e) of the electronic exchange between the electrode and the compound.

The electron exchange speed is higher than the speed of the compound through the bulk, i.e.ve>>vd. This makes the reduction of A_{ox} on the electrode is practically instantaneous. For this reason the reduction current can depend only on v_d and not on the speed of the electronic exchange between the electrode and compound.

Motion of the particles in a solution

The processes allowing the motion of a chemical compound in solution are, principally three:

- convection
- migration
- diffusion

Convection

Convection occurs when a solution is stirred or when in the solution is present a temperature or a density gradient. In this case the molecules of the solvent and the analyte move themselves with a more or less troublesome motion, but that become more laminar in the vicinity of the electrode surface. The layer of solution closer to the electrode surface is practically stationary.

Migration

Migration is the moving due to the attraction force of the electric field generated by the electrode toward every ion having opposite charge and also due to the contemporary repulsion force of every ion having the same charge of the electrode.

Diffusion

Diffusion is the spontaneous movement of those chemical compounds subjected to a concentration gradient that means a situation in which a zone of the solution is poorer than another. With the process of diffusion the system tries to destabilise its homogeneity. During the deposition process the solution closer to the electrode will have lesser A_{ox} in respect to the bulk solution. The growing concentration gradient recalls other electro-active compound, A_{ox}, from bulk solution toward the electrode.

Supporting Electrolyte

Limitation of migration is overpassed by screening the electrode using a supporting electrolyte. Supporting electrolyte is a solution which ions do not discharge themselves at the electrode in the experimental conditions. Supporting electrolyte is added to the sample at high concentration. The supporting electrolyte surrounds the electrode with ions having the same charge of the depolarising agent, reducing in this way the electrostatic attraction toward the latter.

Supporting electrolyte has to posses the following characteristics:

- chemically inert
- do not interfere with diffusion and with the electrons exchange on the electrode surface

- have a different discharge potential (at least 100 – 200 mV)
- have an high ionic conductivity and guarantee a low electrical resistance

Faradic current and capacitive current

The electric current flowing through the working electrode has two sources:

1. faradic current, follows the Faraday laws and is due to the discharge of the electro active compound (A_{ox}),
2. capacitive current, is produced by the growth of a double electric layer on the interface between the electrode and the solution. This double layer is due to the high concentration of the supporting electrolyte in the solution and acts as a condenser with high capacity. The total current flowing through the electrode is sum of the charging current (capacitive current) of this condenser and the faradic current.

Diffusion layer

Diffusion layer is a thin layer of solution that forms near the electrode surface, during the reduction process. This layer has a thickness of about 10-100 μm if the solution is stirred, or about 500 μm if the solution is quiescent and the motion of the particles in it is due only to the diffusion. For this reason the quantity of analyte that reaches the electrode is directly proportional to its concentration in the solution and the intensity of the faradic current gets to a limit value due to the velocity with which the electro-active compound reaches the diffusion layer starting from bulk solution

Potential Scanning

During voltammetric analysis the electrode potential varies with time. This constant

increase or decrease of electrode potential with time is called *Potential Scanning*. If we consider the kinetics aspects, when an electro-active compound is subjected to a potential scanning, three possibilities could take place:

1. The exchange of electrons between the working electrode and the solution is faster than the potential variation and hence than the velocity of the diffusion ($v_s > v_d$). In this way the potential of the working electrode corresponds, in every moment, to the one expected on the basis of Nernst law. The electron exchange is then due to a rapid (or reversible) redox couple. The overpotential is absent and the reduction process is due only to the diffusion.
2. The velocity of the electron transfer is lower than the diffusion velocity ($v_s < v_d$). The redox system, in this case, is called slow or irreversible, or is characterised by high overpotential phenomenon: its potential is, in fact, "in late" respect on the one expected on the basis of Nernst law.
3. Both velocities are similar. The redox couple has intermediate characteristics and the redox process is ruled by both the diffusion and the velocity of the electron transfer.

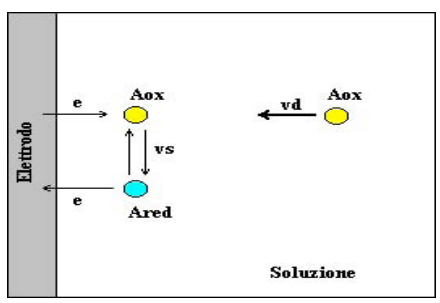

Figure 4 – Discharging process during the potential scanning. The phenomenon is ruled by two factors:

- the velocity of electron transfer (vs) from electrode and redox system
- the diffusion velocity (vd) of the motion of electro active compound toward the electrode surface.

If we consider that during reduction no adsorption process on the electrode or chemical side reactions take place and finally the thickness of diffusion layer remains constant, the potential scan and the voltametric peak can be discussed as follows.
A rapid and linear potential scanning with a rapid redox system is ruled by the Nernst law.

$$A_{ox} + ne \rightarrow A_{red}\text{-Hg}$$

What happens during potential scan is shown in Figure 5 as follows. As it can be seen from the peak there are four characterstic regions.

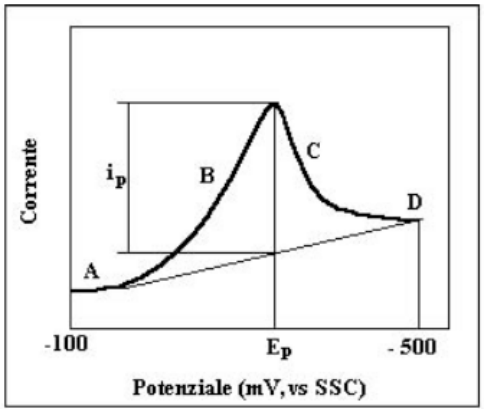

Figure 5 – A typical voltammetric peak

Ep = peak potential

Ip = peak current height

Part A: Starting background current

The potential is not sufficient to cause the reduction of A_{ox}. The measured background current is due to several causes, like the resistance of the cell, the discharge of residual oxygen, the capacitive current and the electronic noise of the electric circuit.

Part B: Ascending part of the peak

Close to the deposition potential, the curve rear up: the A_{ox} ions discharge themselves to the electrode at velocity every time faster; the diffusion layer become every time poorer and a spontaneous flow of other A_{ox} ions is established from bulk solution. The velocity of the motion of A_{ox} ions toward the diffusion layer is proportional to the concentration in the bulk.

Part C: Descending part of the peak and Part D: Deminishing part

In this part the current decrease because the potential scanning velocity is so high that the electro active compound is not able to reach early the electrode. At these values of potential, all A_{ox} ions arriving to the electrode are reduced immediately and their concentration in the diffusion layer is very low. Current trends to increase than to diminish (part D of the peak).

Peak potential, E_p

The higher point of a peak correspond to the point in which the half quantity of A_{ox} ions that reach the electrode discharge themselves, then the ratio A_{ox} /A_{red} at the electrode/solution interface become equal to 1. It can be demonstrated that the measured potential is not so far from the redox potential of the redox couple. The potential peak is then the analytical parameter that allows to make a qualitative characterisation a redox couple in a solution.

Peak height, h_{peak}

The peak current height is proportional to the concentration of the electro active compound in the solution:

$$i_p = K \quad [A_{ox}]$$

and than correspond to the analytical parameter useful for a quantitative analysis.

1.7 Laws and Equestions

There are several laws that explain the effects of the applied potential and the behavior of the redox current in voltammetry. According to the *Nernst's equetion*, the applied potential controls the concentrations of the redox species at the electrode surface (A_{ox} and A_{red}) [20]. The same potential controls the rate of the reaction (k^0), as described by or *Butler– Volmer equations*. In the cases where diffusion plays a controlling part, the current resulting from the redox process (known as the faradaic current) is related to the material flux at the electrode–solution interface and is described by *Fick's law*. The interplay between these processes is responsible for the characteristic features observed in the voltammograms of the various techniques.

Nernst equestion

Reversible reactions those types of reactions where the reaction goes to both sides and equilibrium reestablishes as soon as changes are made in the system. According to Nernst equestion for reversible system:

$$A_{ox} + ne^- \rightarrow A_{red}$$

The application of an external potential E affects the ratio of A_{ox} and A_{red} at the surface of the electrode as described by Nernst equestion:

$$E = E^0 - \frac{RT}{nF} Ln \frac{C_R^0}{C_O^0}$$

where R is the molar gas constant (8.3144 J mol–1K–1), T is the absolute temperature (K), n is the number of electrons transferred, F = Faraday constant (96,485 C/equiv), and E^0 is the standard reduction potential for the redox couple.

If the potential applied to the electrode is changed, the ratio A_{ox} /A_{red} at the surface will also change so as to satisfy above Nernst equestion. If the potential is made more negative the ratio becomes larger (that is, A_{ox} is reduced) and, conversely, if the potential is made more positive the ratio becomes smaller (that is, A_{red} is oxidized) [13].

Butler–Volmer equation

Butler–Volmer equation relates current, potential, and concentration of a voltammetric system.

$$\frac{i}{nFA} = k^0 \left\{ C_O^0 \cdot e^{-\alpha \vartheta} - C_R^0 \cdot e^{(1-\alpha)\vartheta} \right\}$$

where q = nF (E – E^0)/RT , k^0 is the heterogeneous rate constant, a is known as the transfer coefficient, i is the current and A is the area of the electrode.

Fick's law

Most of the time, the current flow also depends directly on the flux of material to the electrode surface. When new A_{ox} or A_{red} is created at the surface, the increased concentration provides the force for its diffusion toward the bulk of the solution. Likewise,

when A_{ox} or A_{red} is destroyed, the decreased concentration promotes the diffusion of new material from the bulk solution. The resulting concentration gradient and mass transport is described by Fick's law, which states that the flux of matter (F) is directly proportional to the concentration gradient:

$$\phi = -AD_o(\frac{\partial C_o}{\partial x})$$

where D_O is the diffusion coefficient of O and x is the distance from the electrode surface.

The flux of A_{ox} or A_{red} at the electrode surface controls the rate of reaction, and thus the faradaic current flowing in the cell. In the bulk solution, concentration gradients are generally small and ionic migration carries most of the current. The current is a quantitative measure of how fast a species is being reduced or oxidized at the electrode surface. The actual value of this current is affected by many additional factors, most importantly the concentration of the redox species, the size, shape, and material of the electrode, the solution resistance, the cell volume, and the number of electrons transferred.

1.8 Commonly used voltammetric techniques

There are many different types of voltammetric techniques which have been used for qualitative and quantitave purpose. Here in this part only some of them are discussed.

Stripping Voltammetry

This technique is based on preconsentrating of the metal on the electrode and subsequent stripping of the metal from the electrode to the solution. During stripping step, the electrode potental is scanned from positive potential to negative or form negative to postive depending on the type os the stripping voltammetry [13]. During the scanning of

the potential, the current is measured and plotted, so the resulting voltammogram is a peak shaped graphic. The position and the heght of this peak are related, respectively, to the type and to the concentration of the analyte. The stripping can be anodic, cathodic or adsorptive.

Anodic Stripping Voltammetry

In anodic stripping voltammetry, the poetential of the electrode is kept high positive to avoid the reduction of the metal ions the analyst is interested in. The metal ion/s in the sample solution are concentrated into a mercury electrode during a given time period by application of a sufficient negative potential. These amalgamated metals are then stripped (oxidized) out of the mercury by scanning the applied potential in the positive direction. The resulting peak currents are proportional to the concentration of the metal in the sample solution, with the position of the peak potential specific to each metal.

The use of mercury limits the working range for ASV to between approximately 0 and –1.2 V versus SCE. The use of thin Hg films or Hg microelectrodes along with pulse techniques can substantially lower the limits of detection of ASV [13].

Cathodic Stripping Voltammetry

In cathodic stripping voltammetry, the potential is scanned from postitive to negative inorder to strip the metals back to the solution [13]. This method is used to analyse substances that can form insoluble salt with mercury. This method has been used to determine inorganic anions such as halides, selenide, and sulfide, and oxyanions such as $MoO_4^{2-}$and VO_3^{5-}. In addition, many organic compounds, such as nucleic acid bases, also form insoluble mercury salts and may be determined by CSV.

Adsorptive Stripping Voltammetry

Adsorptive stripping voltametry (AdSV) is a powerful technique for determination of trace levels of metal ions and organics. Its detection limit is in ng L^{-1}. The deposition of the analyte is accomplished by adsorption on the electrode surface or by specific reactions at chemically modified electrodes rather than accumulation by electrolysis. The scanning is in the potential of the electrode in the negative direction. [14].

There are two main advantages related to adsorptive deposltlon. The first main advantage it is a fast method and any oxldatlon state can be collected rather than only the metallic state. Theoretically any element at any oxidation state can be determined by this method. The second main advantage is that the matenal is collected as a single layer (monomolecular layer) on the electrode surface, so all the matenal is instantaneously accessible to reduction (or oxldatlon), therefore, the reduction current is independent of diffusion of the reactive species and very fast potential scanmng techmques can be employed, to produce larger currents [15].

The other advantages are,

- reduction of the metal is preferable to ligand reduction as the reduction peak potential is specific to the metal which minimzes interferences by other metals [15].
- high sensitivity, low instrumentation and running costs, the possibility of analysis sailing matrices, such as seawater, without the need of prior separation [16].

Linear Sweep Voltammetry, LSV

Linear Sweep Voltammetry is a simple technique which is based on application of a rapid and linear potential scan at the working elecrode. Usualy the linear potential scanning starts before the reduction potential and stops after it. Capacitive current increases when

the velocity of scanning is increased and cannot be electronically compensated. Thus the performance of this technique is strongly restricted. Detection limits range at mg/l levels.

Cyclic Voltammetry

Cyclic Voltammetry is a technique devote widely to the study of the behaviour of redox couples for understanding reaction intermediates, and for obtaining stability of reaction products. Cyclic Voltammetry is a particular Linear Stripping Voltammetry that performs a triangular shaped scanning at the working electrode. In this way a redox couple in solution is exposed before to an oxidation and afterwards to a reduction (or vice and versa).

This technique is based on varying the applied potential at a working electrode in both forward and reverse directions (at some scan rate) while monitoring the current. For example, the initial scan could be in the negative direction to the switching potential. At that point the scan would be reversed and run in the positive direction. Depending on the analysis, one full cycle, a partial cycle, or a series of cycles can be performed.

The plot of a cyclic voltammetry consist on a close curve: reversible redox couples show both as cathodic and anodic peak, while irreversible redox systems show only one peak. The following relations can be useful to establish the standard potential of a reversible redox couple and the number of electrons involved in the discharge process:

$$E^0 \cong E_{1/2} \cong \frac{E_{pa} + E_{pc}}{2}$$

and

$$(E_{pa} - E_{pc}) \cong \frac{0.1984 \cdot T}{n}$$

where Epa = anodic peak potential , in mV e Epc = cathodic peak potential.

Staircase Voltammetry

A different variant of the Linear Stripping Voltammetric technique consist in a regular potential step scanning. The current is sampled just before the subsequent step. Thus the signal is less influenced by the capacitive current.

Differential Pulse Voltammetry

Differencial pulse voltammetry is based on a potential scanned with a series of pulses. If a series of periodical constant pulse of potential is superimposed to a linear scanning, a consistent enhancement of the signal is achieved. Current is measured at two points for each pulse, the first point just before the application of the pulse and the second at the end of the pulse. These sampling points are selected to allow for the decay of the nonfaradaic (charging) current. The difference between current measurements at these points for each pulse is determined and plotted against the base potential. In this way this differential reading of the current generates a peak shaped voltammogram.

Square Wave Voltammetry

In Square Wave Voltammetric technique a rapid step scanning of potential is applied to the electrode. Moreover on each step is superimposed an high frequency square wave. The current is sampled two times at the end of the two half waves. If the amplitude of the wave is very little and the redox system is reversible, during the first half wave the electro active

compound can be reduced (or oxidised), while, in the second half wave, at the contrary, it can be oxidised (or reduced). The two current are then summed up and so, the sensitivity is increased.

Square Wave Voltammetry has several advantages including its excellent sensitivity and the rejection of background currents. Another is the speed (for example, its ability to scan the voltage range over one drop during polarography with the DME). This speed coupled with computer control and signals averaging, allows for experiments to be performed repetitively and increases the signalto-noise ratio.

Polarography

Polarography is a voltammetric measurement whose response is determined by combined diffusion/convection mass transport. It was created in 1922 when Jaroslav Heyrovsky, *Czech Republic 1890-1967,* used dropping mercury electrode for measuring polarization curves. Encouraged by the results, he dedicated the rest of his life to the development of the method he thus initiated.

The dropping mercury electrode, DME, consists of a glass capillary through which mercury flows under gravity to form a succession of mercury drops. Each new drop provides a clean surface at which the redox process takes place, giving rise to a current increase with increasing area as the drop grows, and then falling when the drop falls. The potential when the current attains half the value of the plateau current is called the half-wave potential and is specific to the analyte's matrix. The plateau current is proportional to the concentration of analyte. The current for the polarographic plateau can be predicted by the Ilkovic equation:

$$i_d = 708nD^{1/2}m^{2/3}t^{1/6}C^0$$

where m is the rate of flow of the Hg through the capillary, t is the drop time, and c^0 is the bulk analyte concentration.

Many sub types of polarographic methods take part in deermination of trace levels of substances.

Instrumentation of a Polarograph

The main parts of a polarographic analyser are:

- The polarographic cell
- The potentiostat
- A PC with the application software

The polarographic cell is a glass or Teflon vessel in which are inserted a tube for the bubbling of nitrogen and three electrodes:

- The working electrode – A capillary connected with a mercury container or a solid electrode
- The reference electrode – A simply Ag/AgCl, saturated KCl electrode.
- The auxiliary electrode – A platinum wire inserted on a Teflon rod.
- The stirring of the sample solution is allowed by a magnetic stirrer and a magnetic rod.

The potentiostat allow to impose the desired potential scanning between working and reference electrodes while the current flowing in the circuit is recorded between working and auxiliary electrodes.

The PC is interfaced to measuring system and the software has the following functions:

- send to the potentiostatic analyser the scanning parameters
- check if the execution of the scanning is correct or not
- handle the output data (current and potential).

Mercury electrode

Mercury is the best metal for cathodic scanning because of the large overpotential that the hydrogen discharge undergoes on this element. The density of the discharge of the hydrogen on mercury is 109 time less than the platinum and gold ones. On mercury the hydrogen discharge occur at -1 V (Vs SSC, in acidic solution) or at – 2 V (Vs SSC, in alkaline solution), instead of a theoretical potential of –0.2 V (Vs SSC).

$$Hg_2^{2+} + 2e^- \leftrightarrow 2Hg$$

$$Hg^{2+} + 2e^- \leftrightarrow Hg$$

In the anodic side the operating limit is restricted by the discharge of mercury itself. Practically, it is impossible use the mercury electrode above 0.3 V (Vs. SSC). Another advantage on the use of mercury as electrode is due to the opportunity of eliminates a drop of this element at the end of the scanning. In this way the surface of the electrode can be renewed before each analysis.

1.9 Analytical Information from Voltammetry

Polarographic or voltammetric technique provides both qualitative and quantitative information.

Qualitative Information

Voltammetric techniques give rise to current signals that appear at a characteristic position on the potential scale. The potential at which the signal appears gives qualitative information about the reactant. However, the ability of the potential of the signal to identify the reactant is not very large because the position of the signal depends on the reactant conditions and the resolution is poor. Thus, a characteristic potential excludes many possibilities for the identity of the reactant; in particular, the voltammetric response absolutely excludes all nonelectroactive substances [13]. If the response is the same as that of a known substance, obtained under exactly the same conditions, then the known substance is a good hypothesis for the identity. However, in general voltammetric techniques are not good tools for qualitative identification of analytes.

Quantitative Information

The main virtue of voltammetric techniques is their good accuracy, excellent precision, sensitivity, and wide dynamic range. In the special case of stripping voltammetry, detection limits routinely are lower than the amount of signal due to contamination of sample. Another impression of voltammetric technique is the relative ability to measure small concentrations of analytes in solution [13].

2 Literature review

Total concentration level of chromium and speciation of chromium in some seawater bodies and open oceans are published. But there are very few papers that presented the chromium level and speciation for monitoring purpose. Most of the other papers included this information as sub part of new method development for the same problem. The first paper we consider here discussess the speciation of chromium in the western Mediterranean Sea using cathodic stripping voltametry during the winter of 1992 and summer of 1993. [17]. The total dissolved Cr concentrations obtained were between 2 and 3.5 nM and dissolved chromium (VI) were 2 and 3 nM. For chromium (III), the vertical range obtained was between 0 and 1.4 nM.

The second paper reported the level of total chromium and chromium (VI) in river, estuarine and coastal waters of the Humber catchment in north-east England between 1993 and 2002 [18]. Total chromium concentrations in the catchment were less than 1.0 mg/L. Chromium (VI) accounted for between 27% and 100% of the total dissolved concentration, with an average of approximately 50%. Another research paper reported the levels of different species of chromium in Australian seawaters [19].

Table 3 - Chomium Level in Australian seas in µg/L

Seawater	Sampling time	Total chromium	Dissolved chromium	Chromium (VI)	Chromium (III)
Surface waters from Pahn Beach	Jan 1983	2.3	1.8	0.27	0.22
Surface waters from Pahn Beach	Jan 1984	0.6	0	0.31	0.25
Surface waters from Avaion Beach	Feb 1984	3.4	3.1	0.16	0.10
Surface waters from Avaion Beach	May 1984	0.6	0	0.27	0.29
Surface waters from Pahn Beach	Dec 1984	0.4	0	0.24	0.15
Surface waters from Pahn Beach	Jan 1985	1.8	1.2	0.19	0.43

3 Statment of the problem

What is the level of chromium species in Algeciras Bay? Are these levels of chomium species in the bay higher than the guideline limits?

Most of the trace metals in the sediment and water of Algeciras Bay were given enphasis and analysed. Chromium in sediment of the bay was also studied. Yet total chromium and its speciation in the water werenot studied. Is it because it's the water is unpolluted with respect to chromium?

Chromium is biologically essential element at a very low concentration, but it is very toxic above some concentration level and it can also be carcinogenic. Its envionmental and health effects differ with the type of its forms found in the water. Hence there is a need to study the level and speciation of this element if Algeciras bay for welbeing of the water biota and any living thing dependent on this water or directly or indirectly uses this water.

4 Materials and Instruments

Chemicals Used

- Sodium nitrate, suprapur, $NaNO_3$, 99.99% (Merk, Germany)
- Sodium acetate, suprapur, NaAc, 99.99% (Merk, Germany)
- Perchloric acid, $HclO_4$, 70% (Panreac, Spain)
- Hydrogen peroxide solution, suprapur, w(H_2O_2), 30% (Merk, Germany)
- Hydrochloric acid, suprapur, w(HCl), 30% (Merk, Germany)
- Nitric acid, suprapur, 65% (Merk, Germany)
- Sodium hydroxide pillets, p.a, NaOH, 99-100% (Merk, Germany)
- Diethylenetriaminepentaacetate (DTPA), Titriplex™ V, puriss p.a. 99% (Panreac, Spain)
- Potassium dichromate, p.a., $K_2Cr_4O_7$, 99.95 – 100.05% (Panreac, Spain)
- Ammonia, suprapur, 25% (Merk, Germany)

Equipments and Materials Used

- 746 VA Trace Analyzer with 747 VA Stand (Metrohm, Switzerland)
- 757 VA Computrace and Monitore (PC) (Metrohm, Switzerland)
- 705 UV Digester (Metrohm, Switzerland)
- Oven (SELECTA, Spain)
- pH meter, CRISON micro pH 2001
- Analytical Balance, PM 480 (METTLER, Spain)
- Analytical Balance, AE 240 (METTLER, Spain)
- Peristaltic bomb (Masterflex 7521-00, poppet head 07518-10, Cole-Parmer Instrument Co., IL, USA)

- Calyx Capsule MSI 0.45μm filter (3/8 barb,1/PK, polypropylene; Osmonics, USA)
- Teflon reactor (PTFE, 100mL, BRAND, 1305 38, Weirtheim, Germany)
- rigid Teflon pipes (FEP A-06450-07)
- flexible Tygon tubing (6424-71)
- PC software VA Computrace 2.0 installed in a personal computer using a 5326 VA Computrace Interface (Metrohm, Switzerland)
- Electrochemical portable device (Sension156, Hach Co., USA). A model 2001 pH-meter equipped with 52-02 combined glass–Ag/AgCl electrode (Crison, Spain)
- Milli-Q^{50} System (Millipore, USA)
- STATISTICA (data analysis software system) Version 7 (2004, Statsoft Inc., USA)

5 Experimental

Method

The method applied for this research was dedicated by Prof. Dolores Galindo [Appendix 1] as can be seen in the flow chart of the preocedure protocol. Metrohm methods specifically VA Application Note No. V-82 [Appendix 5] and Application Bulletin No. AB 116 were also used for specific instructions. Moreover more information concerning the handling of the voltametric procedures and manuplating the data were taken from manual of the instuments [20, 21].

The pH of the final sample is adjusted to 6.2 using NaOH (30%). The voltammetric parameters chosen for the determination are:

Table 4 - Polarographic parameters used

Parameter	**Mode/Value**
Working Electrode	HMDE
Stirrer Speed	2000 r mp
Drop Size	7
Mode	DP
Purge Time	300 sec
Deposition Time	20 sec
Deposition Potential	-1.0 V
Equilibration Time	10 sec
Pulse Amplitude	50 mV
Start Potential	-1.0 V
End Potential	-1.4 V
Voltage Step	10 mV
Voltage Step Time	0.30 sec
Sweep Rate	33.3 mV/sec
Peak Potential	-1.20 mV

Total chromium was determined after digestion of the samples in oven. The analysis was done just after the addition of the electrolyte to the digested sample to avoid inactivation of Cr (III)-DPTA complex.

Total disolved chromium represents dissolved Cr (VI) and dissolved Cr (III). The total disolved chromium was determined after digestion of the sample in UV. The analysis was

done immediatly after the addition of the electrolyte to the digested sample to avoid inactivation of Cr (III)-DPTA complex.

Total active dissolved chromium is the contribution of total Cr (VI) and free Cr (III). The analysis was done immediatly after the addition of the electrolyte to the digested sample to avoid inactivation of Cr (III)-DPTA complex.

Hexavalent chromium, Cr (VI), was determined after keeping the sample mixed with the electrolyte for 30 minutes. During this waiting time the Cr (III)-DTPA complex becomes electrochemically inactive.
Dissolved Chromium (III) is calculated from the difference between the total active dissolved chromium and dissolved chromium (VI).

Particulate chromium is calcultaed from the difference between total chromium and total dissolved chromium.

5.1 Sampling

Sampling site

The samples were taken from Algeciras Bay found in southern end of Iberian Peninsula, the southern part of Spain. The bay have length of around 10Km and the width is around 8 Km and covers an area of 75 Km^2. The maximum depth is 400m.

Sampling points and sampling time

Five sampling points were chosen and were coded as A1, A2, A3, A4 and A5. The first sampling point, A1, is found near to *Getares* beach. Sampling point A2 is located near to a

place called *Isla Verde*. This part is found were the waste descharges from the town, *Algeciras*, mixes to the bay. Samplling point A3, *Palmones,* is located near to a stainless steel manufacturing plant called *Acerinox industry*. Sampling points A4, *Guadarranque*, and A5, *Puente Mayorga,* are found near to a perto refinary industry called *Cepsa*.

The samples were collected between 2007 and 2009. Two of the samplings were done in autumn (October 2007 and October 2008) and the others were done in spring (May 2008 and May 2009), so that it allows to study the seasonal variability with respect to the total chromium, total disolved chromium, total active disolved chromium, disolved Cr(VI) disolved Cr(III) and particulate chromium.

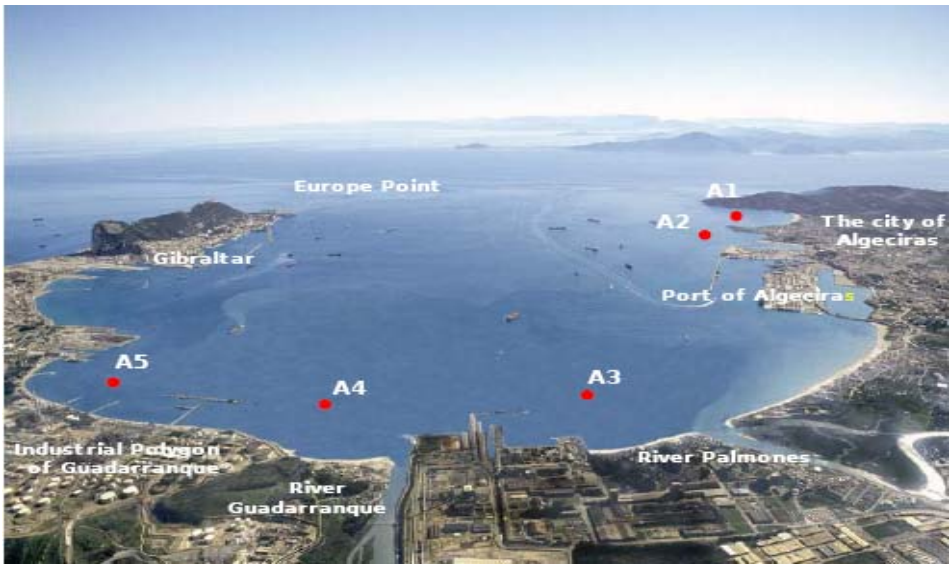

Fig. 6 - Sampling Points on Algeciras Bay

Sampling protocol

The sampling protocol was adopted from a research paper published previosly by

supervisor of this research [22]. Fishing and recreational boats were used for the sampling. As per the good sampling protocol, all the sampling were done from the bow of the boat to minimize the contamination from the engines and effect of the tide.

Fig. 7 - the boat hired for sampling purpuse

The samples were taken through a Teflon tube (tube 6429-25 of Cole-Parmer Instrument Co. Illinois U.S.A) coupled to a peristaltic pump (Masterflex 7521-00, cabezal 07518-10, Cole-Parmer (EE.UU.)). The water was let to flow through the pump and Teflone tube for few minutes before taking the samples to rinse the systems in contact with the water. The sampling bottles were rinsed with the water five times before collecting the sample. For *in situ* filtered samples, 0.45 micron pore size filteration equipment was connected to the pump. The samples were kept in ice until arrival to the labortory.

'Clean - Dirty hands' protocol was applied for this samplings. A total of three persons were involved. The first dirty hands hold the Teflon tube and had taken care of all actions done

on the surface of the water. The second dirty hands had taken care of the equipment whereas the clean hand had taken care of the filling of the water to the sampling bottles.

Fig. 8 - 'Clean-Dirty Hands' Protocol Implimentation during Sampling from Algeciras Bay

- i.e. The EPA's 'clean-dirty hands' protocol was develpoed to minimize the contamination of samples during collection. It it involves two or more persons to do the task. If three persons are chosen, two of them work as clean hands and one as dirty hand as done in this samplings. It includes the followings basic procedures. The picture below can be consulted for clarity of this protocol.

- Assign one member of the team as "clean hands" and the other as "dirty hands". "Dirty hands" opens a bag containing non-talc gloves and "Clean hands" removes a pair of clean gloves and puts them on. "Clean hands" touches sample bottle and avoids contact to any thing from the boat or the sampling materials. "Dirty hands" removes a pair of clean gloves and puts them on.

- "Dirty hand" removes an empty bagged sample bottle from the shipping container (and closes the container) and opens the outer bag. "Clean hands" opens the inner bag, removes the bottle, and folds down the inner bag. "Dirty hands" seals the outer bag and puts it back in the shipping container.

- "Clean hands" removes the bottle cap and holds the cap in one hand. With the other hand, "Clean hands" fills the sample bottle by dipping into the flowing water stream, taking care to keep their hand "downstream" of the inlet of the sample bottle. The bottle is filled, leaving a slight headspace. "Clean hands" tightly screws the cap back onto the bottle. This can also be done by two persons as clean hands.

- "Dirty hands" retrieves the bags and opens the outer bag. "Clean hands" reaches inside to re-open the inner bag, puts the sample bottle inside and seals the inner bag. "Dirty hands" seals the outer bag and places the bagged sample into the shipping container. One member of the team then records the sample bottle number with description and other relevant data.

Physical and chemical parametres of the samples which were measured *in situ* are: pH, temperature, salinity and dissolved oxygen by using a multiparameter probe.

Sample treatment and storage

Sample treatment and storage procedures were done after the samples were brought to the laboratory.

- 500mL of unfiltered sample was taken and acidified with 2mL/L of HNO_3 (Suprapur quality, Merk (Germany)). This sample was kept at room temprature for two weeks before freezing in deep freez.

- 500mL of filtered sample was kept in deep freez just after the samples were brought to the laboratory.
- To 500mL of filtered sample, 10mL of 3M acetate-acetic acid was added immediately upon the samples arival to the laboratory to make the pH of the sample 4.6. This sample was kept in deep freez.
- 1 mL of 2mL/L HNO_3 (Suprapur, Merk (Germany)) added to 500mL of filtered samples upon the arrivals of the smples to the laboratory. This sample was kept at room temprature for two weeks to make the metals that might be adsorbed on the walls of the plastic leached by the acid. After two weeks the sample was kept in deep freez at -4^0C.
- 1 mL of HCl (Suprapur, Merk (Germany)) was added to 500mL of unfiltered sample. The sample was kept in deep freez.
- 1 mL of HCl (Suprapur, Merk (Germany)) was added to 500mL of filtered sample upon arrival of the sample to the laboratory. The sample was kept in deep freez.

Sample preparation

In this part, the different techniques which were used for sample preparation are discussed which included, filteration of MQ water and Oven and UV digestions of samples, certified reference material and blanks.

5.2 Filtration MQ water

1. First the Environmental sampler equipment was connected to AC power supply and the poly tube was washed by passing MQ water first and 3M HNO_3 next. Finally it was washed again with MQ water.

2. The inlet of Watman polycap GW 0.45µm filter was connected to the outlet of the polytube.
3. The inlet of the polytube was dipped into a glass beaker which contained MQ water.
4. The Environmental sampler was made to start filtering and the filtered MQ water was collected the other end of the Watman polycap GW 0.45µm filter.

5.3 Digestion

In this research two types of digestions technique were used.

- Oven digestion was used to determine total chromium concentration in the samples, blanks and certified reference material, CRM.
- UV digestion was used to determine total dissolved chromium in the samples and blanks.

5.3.1 Oven Digestion

Total chromium concentration in the samples was determined after the samples had been digested in Oven. This procedure was used to all samples used for total chromium determination. Blank samples for total chromium were also digested in the same way to the samples.

Sample digestion by Oven

- 50mL of a sample was taken in a 50mL volumetric falsk.
- The sample was transfered to Teflon digestion bottles.
- 5mL of the sample was removed using pippetes.
- 0.2mL suprapur HNO_3 and 0.125mL suprapur $HClO_4$ were added to the sample in Telefon bottles.

- The oven temperature had been adjusted to 120^0C and waited until this temprature was attained in the oven.
- The timer of the oven was adjusted to 8 hours and the sample was kept to be digested.
- After 8 hours, the sample was removed from the oven and transfered to 50mL volumetric flask.
- The volume was adjusted to 50mL level with MQ water

Blank Sample digestion by Oven

1. Littles volume of MQ water was taken in a 50mL volumetric falsk.
2. 2 mL of suprapur HNO_3 per 1L of MQ water was added to the volumetric flask. This step was necessary becouse the samples were added with the same volume of suprapur HNO_3 during sapling to make the pH about 2.

   i.e. 2mL/L is the same as 0.1mL of suprapur HNO_3 for 50mL of MQ water
3. The volume was then adjested to 50mL with MQ water.
4. The blank was transfered to Teflon digestion bottles and 5mL of the blank was removed using pippetes.
5. 0.2mL suprapur HNO_3 and 0.125mL suprapur $HClO_4$ were added to the blank in Telefon bottles.
6. The oven temprature had been adjusted to 120^0C and waited until this temprature was attained in the oven.
7. The timer of the oven was set to 8 hours and the blank was kept to be digested.
8. After 8 hours, the blank was removed from the oven and transfered to 50mL volumetric flask.
9. The volume was adjested to 50mL level with MQ water.

Certified reference material digestion

To evaluate the quality of the method, certified reference material, CRM was analysed. For this purpose, SLEW-3, Estuarine Water Reference Material for Trace Metals was analysed by this method. Chromium concetration in the certified reference material was deterimined by using several techniques namely:

- Immobilised ligand separation coupled with GFAAS
- Co-precipitation coupled with ICP-Ms and
- Reductive precipitation separation coupled with GFAAS

Because of the fact that chromium in the certified reference material determined by these method; the concentration represents total chromium in the CRM. Due to this, the certified reference material needed to be digested in oven to determine the total chromium by stripping voltametric technique.

1. 50mL of certified reference material was taken in a 50mL volumetric falsk.
2. The CRM was transfered to Teflon digestion bottles.
3. 5mL of the certified reference material was removed using pippetes.
4. 0.2mL suprapur HNO_3 and 0.125mL suprapur $HClO_4$ were added to the CRM in Telefon bottles.
5. The oven temprature had been adjusted to 120^0C and waited until this temprature was attained in the oven.
6. The timer of the oven was adjusted to 8 hours and the certified reference material was kept to be digested.
7. After 8 hours, the CRM was removed from the oven and transfered to 50mL volumetric flask.
8. The volume was adjested to 50mL level with MQ water

5.3.2 UV Digestion

The sample used for Oxidation with UV in the 705 UV Digestor had pH between 4-6.

Sample UV Digestion

- 10mL of sample which had pH between 4-6 was taken and 50 µL of H_2O_2 was added to the sample.
- The solution was then digested for 30 min at 90^0C in 705 UV Digester.

Blanck UV Digestion

The black for UV digested samples were prepared and digested in the same was to the samples

5.4 Electrolyte solution preparation

The supporting electrolyte solution contains:

- 0.2 mol/L of sodium acetate
- 0.05 mol/L of DTPA, Diethylenetriaminepentaacetic Acid
- 2.5 mol/L of sodium nitrate

This suporting electrolyte was prepared by dissolving mixture of 1.64 g sodium acetate (suprapur), 1.96 g DTPA (p.a) and 21.3 g sodium nitrate (suprapur). The mixture was disolved in little amount of water with the help of unltasonic equipment. Finally the solution was diluted to 100mL with MQ water.

5.5 Sample and standard solutions Preparation

Final sample preparation

The final sample for analysis was prepared by diluting the sample then adding the electrolyte solution and finaly adjusting the pH of the mixture.

1. After washing the voltametry electrodes and cell, the cell was put on an analytical balance. The balace was then adjusted to zero.
2. 5mL of sample was put on the cell and the exact volume was recorded.
3. This sample was diluted to 10mL with MQ water.
4. 2.5mL of electrolyte solution was added to the diluted sample
5. The pH of the mixture was adjusted to 6.2 ± 0.1 using 30% NaOH (p.a) and the final volume was recorded, see pH adustment of the samples below.
6. This sample was then analysed with stripping techniique and HMED electrode

Synthetic Sample Preparation

50 µL of 50 ng/L chromium solution was added to 10mL of MQ water to make the concentration of chromium in the synthetic sample 250 ng/L.

pH of the solution

The volume of NaOH (30%) required to adust the pH to 6.2 ± 0.1 differs to all types of samples, reference materials, accordingly to the blancks. The pH was adjested with pH Meter (CRISON micro pH 2001). The pH meter was calibrated at pH 7 and pH 2 everytime before using it for adjestment.

- In all the cases, 39µL of NaOH (30%) was added to 2.5 mL of electrolyte solution prior to mixing with the diluted sample.
- In all the cases, 16µL of NaOH (30%) was added after mixing the electrolyte with the diluted sample whose pH is modified as follows.
- For total chromium determination, 71µL of NaOH (30%) was required for samples.

Preparation of standard solutions

Even though stock solutions of chromium can be found commercialy, I decided to prepare the stock as well as standard chromium solution in the laboratory. For this purpose solid potassium chromate was used.

Stock Solution, 1000 mg/L

Chromium stock solution is prepared from dried potassium chloride powder as follows:

- Solid potassium dichromate was dried in an oven at 130 $^{\circ}$C for 2 hours. Then it was cooled in desicator over P_2O_5.
- 0.2828 gm of dried $K_2Cr_2O_7$ was dissolved in 100mL of MQ water to make the solution 1000mg/L with respect to chromium.

Standard solution

Standard solution of 0.1mg/L was used throughout the analysis. This standard solution prepared daily by dilution of stock solution. First 10mg/L was prepared from which 1000mg/L standard solution could be prepared.

10mg/L: 0.5mL of chromium stock solution (1000mg/L) was diluted to 50mL with MQ water. The resulting solution had concentration of 10 mg/L with respect to chromium.

0.1mg/L: 0.5mL of 10mg/L chromium solution prepared in the previous step was diluted to 50mL with MQ water. The resulting solution had concentration of 0.1g/L with respect to chromium.

All the dilutions were done in 50mL volumetric flask and transfered immediatly to polythyne bottles to avoid adsorbtion of chromium on the walls of the flasks. All flasks were cleaned before analysis according to the glassware and flask cleaning step.

5.6 Procedure

Here is presented the analytical procedure for the final measurment and for detection limit investigation.

Sample Anaytical pocedure

In this part we call samples all the solutions subjected to anaysis, i.e samples, reference materials and blanks.

All samples were anaysed in similar ways concerning the voltammetric parameters and dilution. All the samples were diluted by taking 5mL and diluting to 10mL. After dilution, 2.5mL of electrolyte solution was added to each samples and the pH was ajested to 6.2 ± 0.1 using 30% NaOH (p.a.). This solutions was analysed by stripping voltammetry and using 50

Detection Limit Determination

This determination was done to findout the lower detection limit of the method used. For this purpose, sample was prepared in the same way as for other anaysises. The intensity if the sample peak (in current) was measured 10 times and two replicates for each measurments. The data collected were used to calculate the lower detection limit.

5.7 Material cleaning

Glasswares and Flasks cleaning

All glasswares used in trace metal analysis are cleaned as follows:

1. All the glasswares were first washed three times with tap water and detergents.
2. Then they were rinsed with Milli Q water three times and more
3. They were filled full with cleaing solution of 2M HCl/2M HNO_3 and were kept atleast for over night.

   i.e. One litre of 2M HCl/2M HNO_3 cleaning solution was prepared as follows.

   - 168 mL of conc HCl, i.e. 98%, and 139 mL of conc. HNO_3, i.e. 68%, were taken in 1 L volumetric flask and diluted it to the 1 L level with Milli Q water.
4. The glasswares were emptied and were rinsed them with Milli Q water several times a minimum of 3 times.
5. The glasswares were put upside down to dry
6. All glasswares used were cleaned this way before every analysis
7. No glassware was used in consequative analysis

Volumetry Cell and Electrode Cleaning

- The electrodes were washed several times with Milli Q water. The water portion was disposed to the waste tank and the lower portion which had mercury drops were disposed to the mercury waste tank.

- The glass cell was washed with Milli Q water for several times a minimum of 3. The cell was filled with Milli Q water ($3/4^{th}$ of the volume) and 100nL of Suprapur HCl solution plus 100nL of suprapur HNO_3 was added in to the cell.
- The cell was replaced to its position in the voltammetre and it was stirred for 40 min or more to clean before the next analysis.
- The glass cell was removed and the electrodes were washed with Milli Q water. The water portion was disposed to the waste tank and the lower portion which had mercury drops was disposed to the mercury waste tank incase it contained mercury dropletes.
- The glass cell was washed several times with a minimum of 3 with Milli Q water.
- The Voltammetric cell and electrodes were cleaned in this way every time before analysis, between analysis and at the end of the analysis.

6 Result and Discussion

6.1 Reports of Chromium in Sediments of Algeciras Bay

The chromium level in sediments of Algeciras Bay was analysed previously by a reseach group in the department. It is found that to all points the total concentration level is higher than the backgroud level for unpolluted seawaters. This is the primary reason for the need to study total and speciation of chromium in the Alegeciras Bay. As it is reported, the concentration of chromium in the bay ranges from 45 mg/L to 395 mg/L. From the graph it can be easily seen that only few samples are below the safe (unpolluted) limit, 81 mg/L, where adverse biological effects on estuarine bottom organisms are rarely observed [23]. One sampling point had concentration level above the higher toxicity limit, 370 mg/L, where toxic effects are frequently observed [23]. Most of the results obtained are between the safe limit and toxicity limit. In this region, toxicity effects occasionally occure. During the development of these limits, the incidence of effects was 10% or less in the minimal-effects ranges and 11%-47% in the possible-effects ranges from all of the trace metals. The incidence of adverse effects exceeded 75% in the probable-effects ranges for chromium

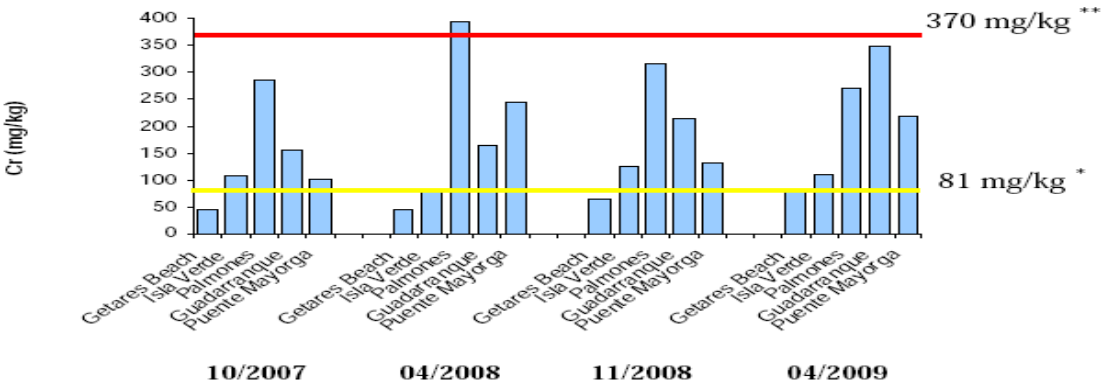

Fig. 9 - Total chromium levels obtained in previous studies on Algeciras Bay Sediments

* ERL reference to Toxic Effects-Range Low, from Long and others (1995, 1998). Below this level isnot considered toxic, since adverse effects to organisms occure in less than 10% of the studies in which concentrations fell below the respective ERL values

** ERM reference to Toxic Effects-Range Medium, from Long and others (1995, 1998). Above this level is considered toxic, since adverse effects to organisms occure in more than 75% of the studies in which concentrations exceeded the respective ERM values

6.2 Synthetic Samples

Synthetic samples were analysed to get practice on the method prior to analysing the real samples. The samples were produced by dissolving 50 µL of 50 µg/L of chromium solution in 10 mL of MQ water.

Syntheic samples, pH adjusted with NaOH (p.a, 30%)

The true concentration of chromium in the syntheic sample is:

$$[Cr] = \frac{50\mu L \times 50\mu gL^{-1}}{10mL}$$

$$= \mathbf{250\ ngL^{-1}}$$

The result found after analysing the synthetic sample is:

$$= \mathbf{255ngL^{-1} \pm 77ngL^{-1}\ (\alpha=0.05)}$$

The synthetic sample result which was obtained after analysis is in good agreement with the true concentrations of the synthetic samples.

Table 5 - Blank for synthetic sample 1, of which pH was adjusted by NaOH (p.a, 30%)

Experiment No	result (ng/L)	stand. Dev	Average (ng/L)
1st	264	6.4	260 ± 19 (α=0.05)
2nd	265		
3rd	258		
4th	252		

Table 6 - Synthetic Sample, the pH was adjusted to 6.2 ± 0.1 with NaOH (p.a., 30%)

Experiment No	result (ng/L)	stand. Dev	Average (ng/L)	Blank (ng/L)	real conc (ng/L)
1st	537	22.7	515 ± 69 (α=0.05)	260 ± 19 (α=0.05)	255 ± 77 (α=0.05)
2nd	491				
3rd	516				

Synthetic samples, pH adjusted with NH_3 (suprapur, 25%)

The blank sample for the synthetic sample of which the pH was adjusted to 6.2 ± 0.1 with suprapur ammonia had chromium concentration less than the detection limit of the method making the concentration of the blank equals to zero. The result of the synthetic sample after analysis is 228 ngL^{-1} ± 22 ngL^{-1}(α=0.05) which is in good agreement with the true concentration of chromium in the synthetic samples.

Table 7 - Synthetic Sample, the pH is adjusted to 6.2 ± 0.1 with NH_3 (suprapure, 25%)

Experiment No	result (ng/L)	stand. Dev	Average (ng/L)	Blank (ng/L)	real conc (ng/L)
1st	236	7	228 ± 22 (α=0.05)	0	228 ± 22 (α=0.05)
2nd	221				
3rd	226				

6.3 Comparison of NaOH with NH_3 for adjusting the pH

During analysis of synthetic sample, result was both (NaOH, p.a. 30%; NH_3, suprapur, 25%) were used and they are proved to give correct result for determination of chromium in the synthetic samples. However when real samples were analysed with these basic solutions, NH_3 didnot give peak of chromium while NaOH did. This proves that NH_3 makes complex with chromium ions in real samples and the chromium isnot available to bind with the ligand Diethylenetriaminepentaacetic Acid, DTPA. Thus ammonia, although it gives zero chromium concentration for blank which is desirable, isnot proper solution to adjust the pH of samples for chromium determinations.

6.4 Quality Control

Certified Refeence Material

A certified reference material, CRM SLEW-3, was analysed to check the applicablity of the method and the quality of the results obtained for the samples. The CRM has chromium concentration of *183 ngL^{-1} ±19 ngL^{-1} (α=0.05)*. The result obtained is the same as the true value of the certified reference material.

Table 8 - SLEW-3 Reference material

Experiment no	result (ng/L)	stand. Dev	Average (ng/L)	Blank (ng/L)	real conc (ng/L)
1st	709	15.98	725 ± 49 (α=0.05)	552 ± 14 (α=0.05)	173 ± 50 (α=0.05)
2nd	741				
3rd	726				

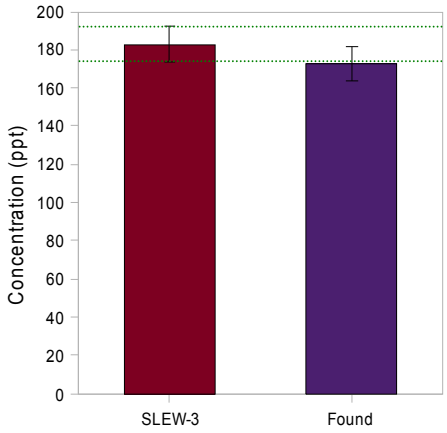

Fig. 10 - Certified Reference Materia, SLEW-3, and Found Concentrations

Limit of Detection and Limit of Quantification

The method's limits of detection (LOD) as well as limit of quantification (LOQ) were determined from the slop of a calinration curve ploted. For this purpose, 10 results were collected each in two replicates.

Table 9 - Results collected for LOD and LOQ determinations

Expt. No	1	2	3	4	5	6	7	8	9	10
Result (nA)	-107.57	-105.85	-104.95	-104.05	-101.69	-106.65	-105.37	-104.81	-103.10	-105.78
	-109.29	-107.51	-105.41	-106.22	-108.13	-108.15	-108.04	-108.50	-107.35	-107.14
Average(nA)	-108.43	-106.68	-105.18	-105.14	-104.91	-107.40	-106.71	-106.66	-105.23	-105.23

LOD and LOQ

The limit of detection can be calculted by using the standard deviation of the averages of these results and the slop of the calibration curve.

LOD = 3*s.d/slop

s.d = 1.15 nA

slop = 0.52nA L/ng

LOD = **6.63 ng/L**

LOQ = 3* LOD
= **19.9 ng/L**

6.5 Statistical treatment of the data

Statistical treatment of the data was done using STATISTICA (version 7) software. The normality of the data was checked and the data which didnot show normality behavior was standardized using standardization methods until a normal distibuted data is obtained. The similarities of the variances were also checked to know if the set of the data are coparable in the subsequent step. The anova tests were done to know if the data are similar or not. For the data which exhabited significant differences, another statistical test wass carried out to diffeenciate the points of the differences. Finaly the coorlestions of the variables were also studied.

Raw Data

The raw data obtained from the investigation is found in this part. Each results were obtained from average of three replicates of results. All the raw data and other statistical variables of the data are given in the appendix 6. Here can be seen that results for dissolved chromium (VI) are also given after standardizing it in two ways. The reason for this will be explained in latter parts.

Table 10 – Raw data for total chromium, fraction of the total and species. Loarithmic and root transformed reults for chromium (VI). All concentrations are in ng/L

Sampling Point	Year	Season	Total Cr	Dissolved Cr	Active D. Cr	Dissolved Cr (VI)	Dissolved Cr (III)	Particulate Cr	Log Dis Cr(VI)	R Dis Cr(VI)
1	2007	1	200.48	188.40	165.94	93.30	72.64	12.08	1.97	9.66
1	2008	2	304.41	136.85	128.37	103.99	24.39	167.55	2.02	10.20
1	2008	1	407.61	211.25	136.94	113.23	23.70	196.36	2.05	10.64
1	2009	2	324.05	237.27	176.12	108.69	67.42	86.78	2.04	10.43
2	2007	1	422.40	238.81	199.15	92.99	106.16	183.59	1.97	9.64
2	2008	2	555.12	257.73	225.31	106.21	119.10	297.39	2.03	10.31
2	2008	1	425.80	245.87	233.58	172.76	60.82	179.94	2.24	13.14
2	2009	2	392.36	337.48	254.25	165.16	89.09	54.89	2.22	12.85
3	2007	1	309.92	302.00	205.37	126.76	78.62	7.59	2.10	11.26
3	2008	2	478.15	269.06	146.63	75.65	70.98	209.09	1.88	8.70
3	2008	1	286.78	267.83	205.89	173.00	32.89	18.95	2.24	13.15
3	2009	2	383.21	331.18	250.64	179.70	70.94	52.04	2.25	13.41
4	2007	1	492.70	298.64	211.00	118.25	92.75	194.06	2.07	10.87
4	2008	2	519.78	295.27	166.18	107.15	59.02	224.51	2.03	10.35
4	2008	1	537.50	239.07	228.10	178.16	49.93	298.43	2.25	13.35
4	2009	2	394.65	346.31	254.66	92.64	162.03	48.34	1.97	9.62
5	2007	1	344.21	162.98	136.95	90.82	46.00	181.23	1.96	9.53
5	2008	2	494.99	95.91	77.26	61.15	16.10	399.08	1.79	7.82
5	2008	1	476.81	205.80	181.98	122.96	59.02	271.00	2.09	11.09
5	2009	2	302.36	196.13	163.83	72.94	90.88	106.22	1.86	8.54

Basic statistics

With the aim of finding out the basic statistical variables od the data (i.e. Mean, median, minimum, maximum and standard deviation) this study was carried out. The results obtained are shown as follow.

Table 11 – Basic statistical parametres, all concentrations are in ng/L

Variable	Valid N	Mean	Median	Minimum	Maximum	Std.Dev.
Total Cr	20	402.66	401.13	200.48	555.12	96.43
Dissolved Cr	20	243.19	242.47	95.91	346.31	66.94
Active D. Cr	20	187.41	190.57	77.26	254.66	47.89
Dissolved Cr (VI)	20	117.78	107.92	61.15	179.70	37.00
Dissolved Cr (III)	20	69.63	69.18	16.10	162.03	35.10
Particulate Cr	20	159.46	180.58	7.59	399.08	109.17

6.5.1 Histograms and Shampiro-Wilk test for normality

To check the normal distribution of the data along the mean, histrograms were drawn using Shampiro-Wilk test to each variable. According to the null hypothesis, the distribution is normal if the 'p' value is lower than 0.05 (assuming significance level of 0.05 for all cases of my research). Otherwise the null hypothesis wil be rejected and the distribution is regaarded as not normal distribution.

Total chromium and Shampiro-Wilk test

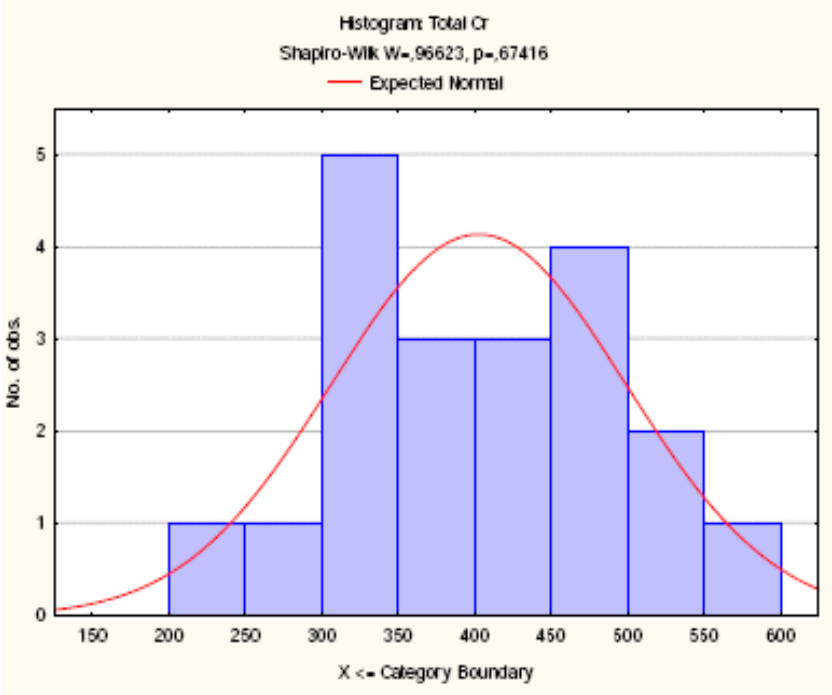

Fig. 11 – Normal Distribution Curve of Total Chromium

The data for the total chromium has 'p' value of 0.67416 which is greater than the significant level (0.05). Since 'p' > 0.05, the null hypothesis wilnot be rejected. Thus the data are normaly distributed.

Total dissolved chromium and Shampiro-Wilk test

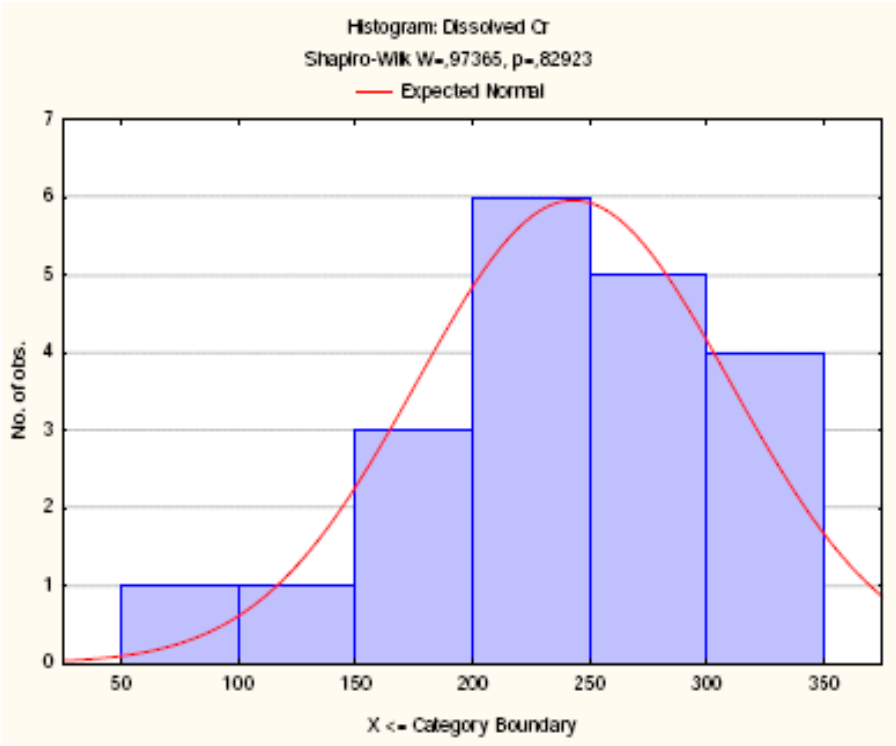

Fig. 12 – Normal Distribution Curve of Total Dissolved Chromium

The data for the total dissolved chromium has 'p' value of 0.82923 which is greater than the significant level (0.05). Since 'p' > 0.05, the null hypothesis wilnot be rejected. Thus the data are normaly distributed.

Total active dissolved chromium and Shampiro-Wilk test

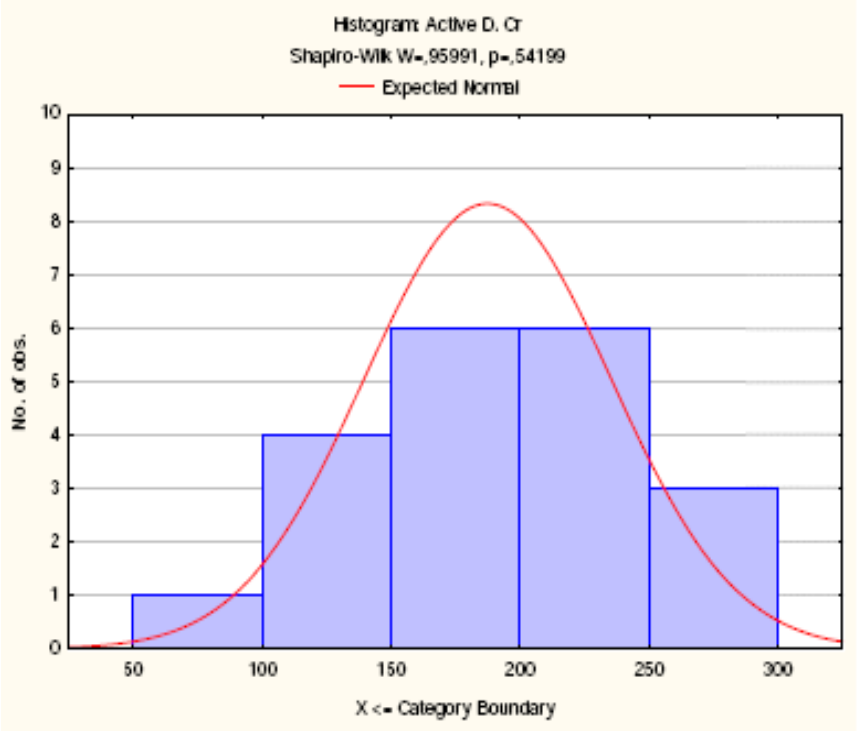

Fig. 13 – Normal Distribution Curve of Total Active Dissolved Chromium

The data for the total active dissolved chromium has 'p' value of 0.54199 which is greater than the significant level (0.05). Since 'p' > 0.05, the null hypothesis wilnot be rejected. Thus the data are normaly distributed.

Dissolved hexavalent chromium and Shampiro-Wilk test

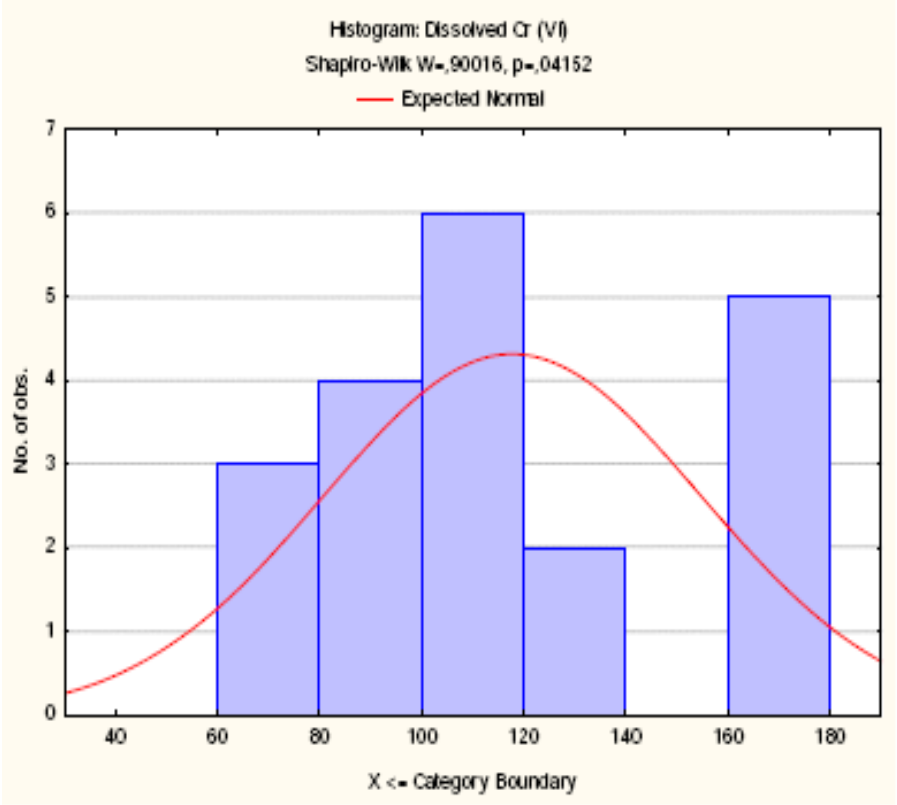

Fig. 14 – Normal Distribution Curve of Hexavalent Chromium

The data for the total active dissolved chromium has 'p' value of 0.04152 which is less than the significant level (0.05). Since 'p' < 0.05, the null hypothesis will be rejected. Thus the data arenot normaly distributed. Here there is a need to normalize these data untill a normally distributed is obtained. For this reason logarthmic and square root transformation was carried out.

Logarithmic transformation for Hexavalent Chromium

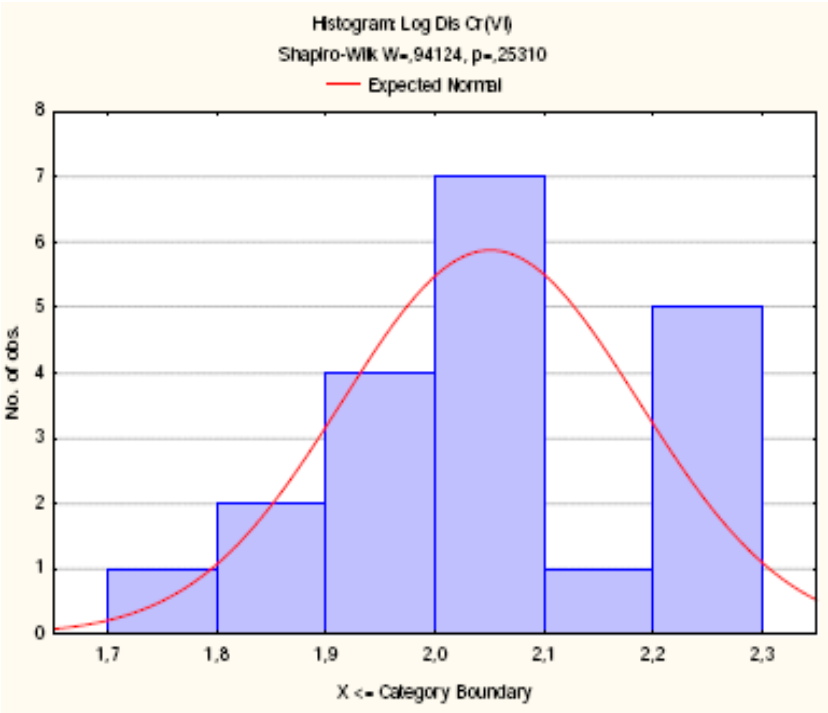

Fig. 15 – Normal Distribution Curve of Hexavalent Chromium (logarthmic transformed)

The logarithmic transformed data of the dissolved hexavalent chromium has 'p' value of 0.25310 which is greater than the significant level (0.05). Since 'p' > 0.05, the null hypothesis wilnot be rejected. Thus these transformed data are normaly distributed.

Square root transformation

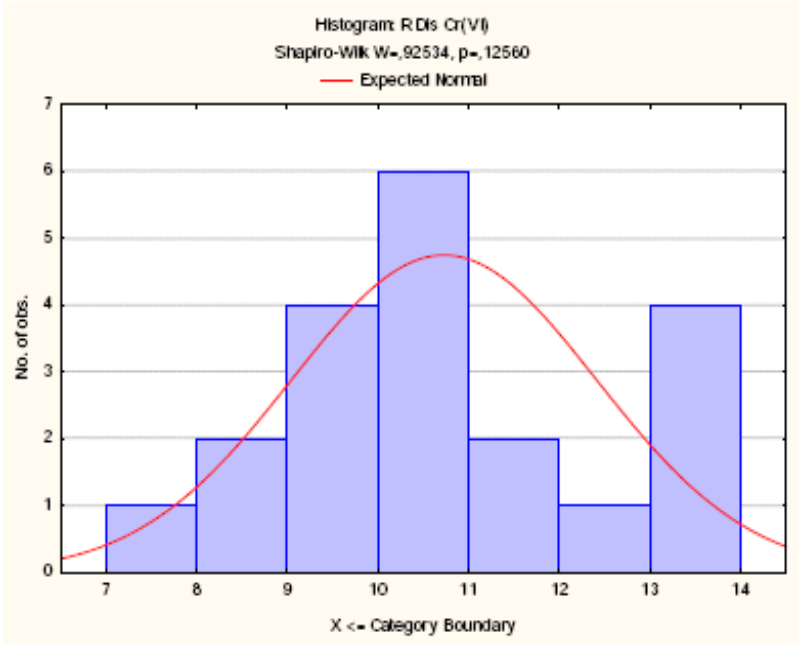

Fig. 16 – Normal Distribution Curve of Hexavalent Chromium (Root transformed)

The root transformed data of the dissolved hexavalent chromium has 'p' value of 0.12560 which is greater than the significant level (0.05). Since 'p' > 0.05, the null hypothesis wilnot be rejected. Thus these transformed data are normaly distributed.

Both transformed data of dissolved chromium (VI) are normally distributed along the mean. We will use the logarismic transformed one from this point onwards as values of dissolved chromium (VI). This is because the 'p' value of the logarithmic transformed data is higher than that of the root transformed data. Moreover the logarismic transformation is more common used than root transformaton.

Dissolved trivalent chromium and Shampiro-Wilk test

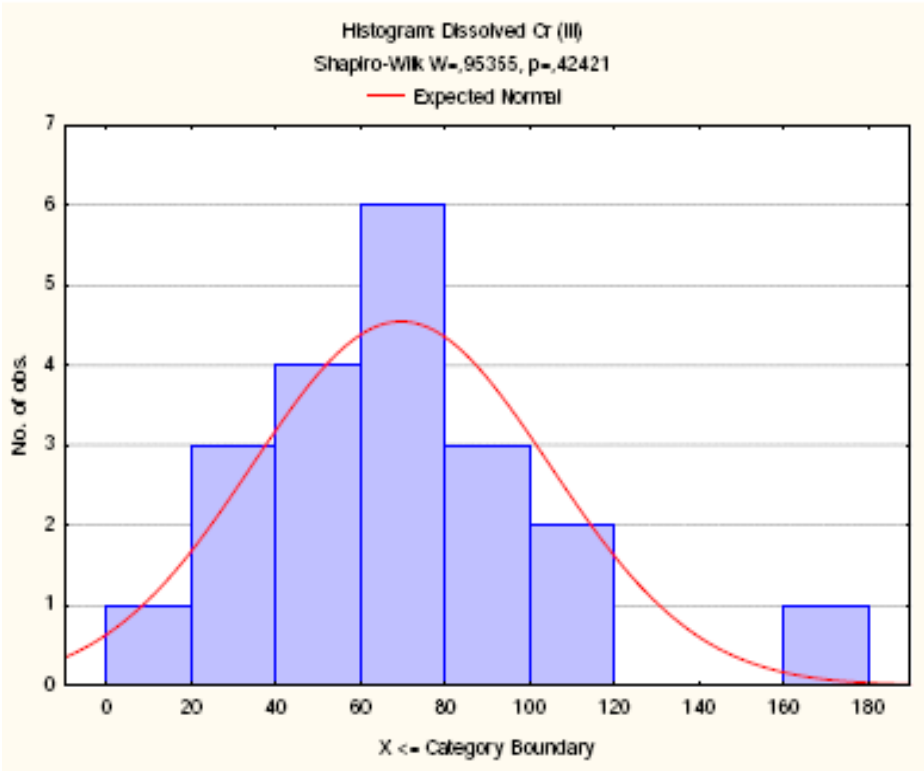

Fig. 17 – Normal Distribution Curve of Trivalent Chromium

The data for the dissolved chromium (III) has 'p' value of 0.42421 which is greater than the significant level (0.05). Since 'p' > 0.05, the null hypothesis wilnot be rejected. Thus the data are normaly distributed.

Particulate chromium and Shampiro-Wilk test

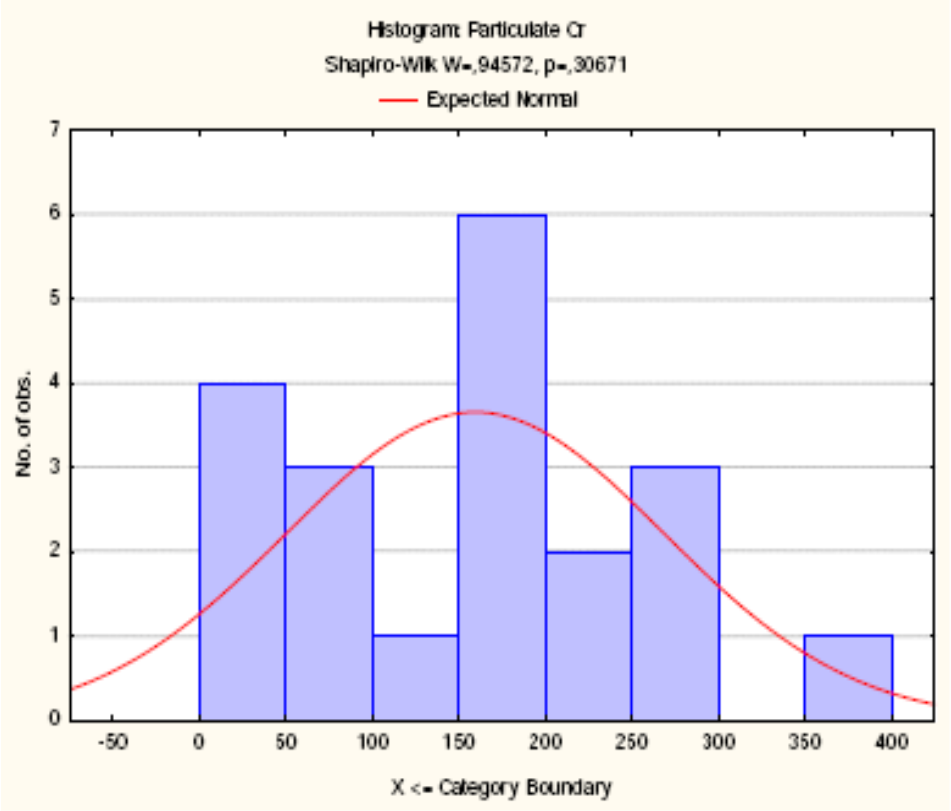

Fig. 18 – Normal Distribution Curve of Particulate Chromium

The data for the Particulate chromium has 'p' value of 0.30671 which is greater than the significant level (0.05). Since 'p' > 0.05, the null hypothesis wilnot be rejected. Thus the data are normaly distributed.

6.5.2 Box and Whisker Plot

Box and Whisker plot is helpfull to get basic information about the distribution of data. These information included median and range of the data. Normally distributed data can be ploted using the mean of the data while for non-normlly distributed data, median will be used.

Normally distributed data

The Box and Whisker plot for normally distributed data are shown below. Data of total chromium, total dissolved chromium, total active dissolved chromium dissolved chromium (III) and particulate chromium are symetrically distributed along the means and there is noe skewed data.

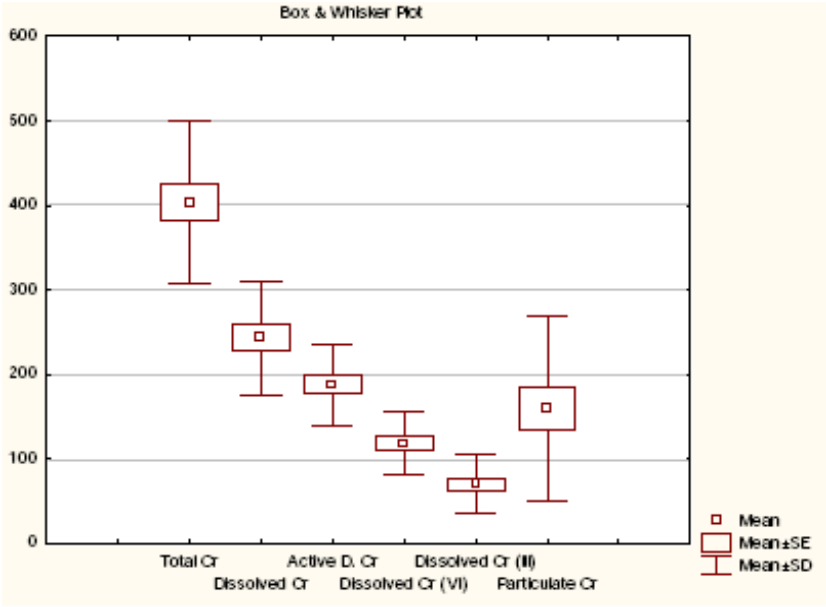

Fig. 19 – Box and Whisker Plot of normally distributed data

Non-normally distributed data

Box and Whisker plot can also be applied to non-normally distributed data, i.e dissolved chromium (VI). The data are symetrically distributed along the median of the group only a little skewed to the right.

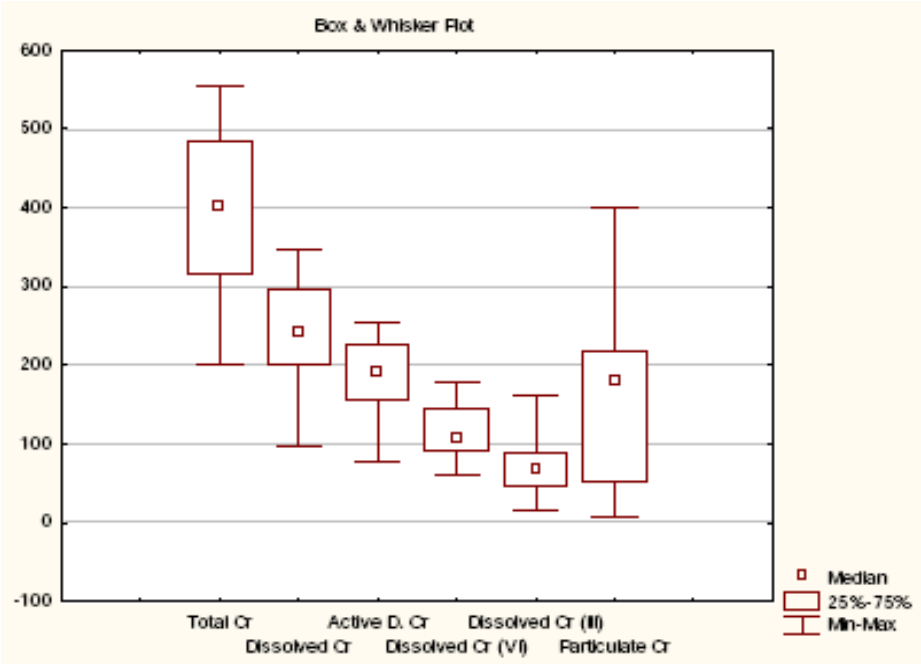

Fig. 20 – Box and Whisker Plot of non-normally distributed data

6.6 ANOVA test for the Data

Analysis of variance test was applied to the data inorder to check if the groups are equal or belongs to one one population. The null hypothesis assumes the groups are the same and 'p' is greater than the significance level (α=0.05). Thus if the 'p' value is less than the siginificance level, the null thypothesis will be rejected otherwise the null hypotheisis will be retained.

ANOVA of sampling points

Table 12 shows all 'p' values for different variables and sampling points after ANOVA test using STATISTICA software.

Table 12 - ANOVA of sampling points

Variable	Square Sum	Mean Square	F(cal)	p
Total chromium	77268	19317	2.9	0.057
Dissolved chromium	57491	14373	7.8	0.0013
Active chromium	24572	6143	4.8	0.010
Log chromium (VI)	0.11	0.03	1.6	0.22
Chromium (III)	7455	1864	1.8	0.19
Particulate chromium	69453	17363	1.7	0.21

For total chromium, log [Cr^{+6}], chromium (III) and particulate chromium, the 'p' values are less than the significance level (0.05) thus the null hypotheisis will be retained. Thus there is no difference among the results of these variables among all sampling points.

Dissolved chromium and active chromium have 'p' value which is less than 0.05 and then the null hypothesis will be rejected. Thus there are significant differences among results of these vaiables at some or all of the sampling points. To check the exact sourse of the differences, Tukey HSD test will be applied in te subsequent steps after ANOVA.

ANOVA of Seasons

Some parametrs of the variables in the goup year including 'p' values for different variables and seasons after ANOVA test using STATISTICA software are given in the table below.

Table 13 - ANOVA of season

Variable	Square Sum	Mean Square	F(cal)	p
Total chromium	2998	2998	0.31	0,58
Dissolved chromium	1016	1016	0.22	0.65
Active chromium	190	190	0.079	0.78
Log chromium (VI)	0.037	0.037	2.16	0.16
Chromium (III)	1087	1087	0.88	0.36
Particulate chromium	527	527	0.042	0.84

For all the variables, the 'p' values are less than the significance level (0.05) thus the null hypotheisis will be retained. Thus there is no difference among the results of these variables at all seasons. There is no variation of the levels of the total chromium and chromium fractions in the seasons the samples for this study were collected.

ANOVA of Years

Table 14 - ANOVA of years

Variable	Square Sum	Mean Square	F(cal)	p
Total chromium	42449	21224	2.69	0.097
Dissolved chromium	15226	7613	1.85	0.19
Active chromium	7417	3709	1.7	0.20
Log chromium (VI)	0.009	0.0045	0.225	0.80
Chromium (III)	7210	3605	3.78	0.044
Particulate chromium	94480	47240	6.08	0.01

All the variables except chromium (III)and particulate chromium, have 'p' values which are less than the significance level (0.05) thus the null hypotheisis will be retained. In conclusion there is no difference among the results of these variables at all years. There is no variation of the levels of the total chromium, dissolved chromium, active chromium, and chromium (VI) in the years 2007, 2008 and 2009 when the samples for this study were collected.

Chromium (III) and particulate chromium have 'p' value which is less than 0.05 and then the null hypothesis shall be rejected. Thus there are significant differences among results of these vaiables between years. The years which have diffeences will be check by Tukey HSD.

6.7 Tukey HSD Test

Tukey HSD test is used to findout which mean is significantly different from the other means. It is generally used in conjection with ANOVA.

Tukey HSD test for Active Chromium: sampling points

Under the group sampling points, dissolved chromium had shown signifcance difference. The output of the Tukey HSD test for this variable in the group is given in table below. As can be seen from the table, the significance difference is between sampling point A2 and A5. The possible reason for this difference is because the latter sampling point is near to the the place the bay mixes with Mediterannean sea. At this point the organic matters of the water (Humic acid and Fulvic acid) are lower because of the flow or flux of the water around this place. This results in reduction of the active chromium level of the water around this are. In contrary to this, sampling poiint A2 is feed with the domestic waste of Algeciras town. The waste isnot pretreated before disposing it to the water and hence it contains alot of organic maters. Thus this area of the bay contains high active chromium.

Table 15 - Tukey HSD test for Active Chromium against sampling point

Cell No	Tukey HSD test; Variable Active D. Cr					
	Sampling Point	{1} 151.84	{2} 228.07	{3} 202.14	{4} 214.98	{5} 140.00
1	1		0.055845	0.312935	0.140763	0,988999
2	2	0.055845		0.837466	0.983982	0.023024
3	3	0.312935	0.837466		0.985047	0.150605
4	4	0.140763	0.983982	0.985047		0.061200
5	5	0.988999	0.023024	0.150605	0.061200	

As can be seen from the table, the significance difference for active chromium is between sample A2 and A5.

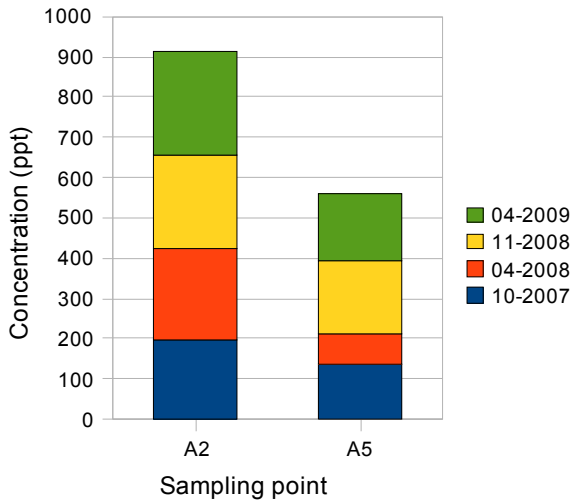

Fig. 21 – Plot of active chromium against Sampling Points A2 and A5

Tukey HSD test for Dissolved chromium: sampling points

Under the group sampling points, dissolved chromium had shown signifcance difference. The output of the Tukey HSD test for this variable in the group is given in table below. There are five significant differences anoung variables of the sampling time. If we consult the bar graphs of the pai of sampling points which shows significant diferences in level of chromium, ther is sampling point A1 or A5 as one of the poirsbut none have two of them. These sampling points, A1 and A5, are least exposed to pollution because of their positions. These is no nearby artificial pollution sourse for A1 and as explained above A5 is present near to the Meditranean sea where a large water flux is there. In opposite to this,

the other three sampling points have pollution sourse in nearby places. So these places are expected to contain high level of dissolved chromium.

Table 16 - Tukey HSD test for Dissolved Chromium against sampling point

Cell No	Tukey HSD test; Variable Dissolved Cr					
	Sampling Point	{1} 193.44	{2} 296.97	{3} 292.52	{4} 294.82	{5} 165.21
1	1		0.138050	0.036077	0.031252	0.880899
2	2	0.138050		0.942891	0.920886	0.025275
3	3	0.036077	0.942891		0.999992	0.006073
4	4	0.031252	0.920886	0.999992		0.005259
5	5	0.880899	0.025275	0.006073	0.005259	

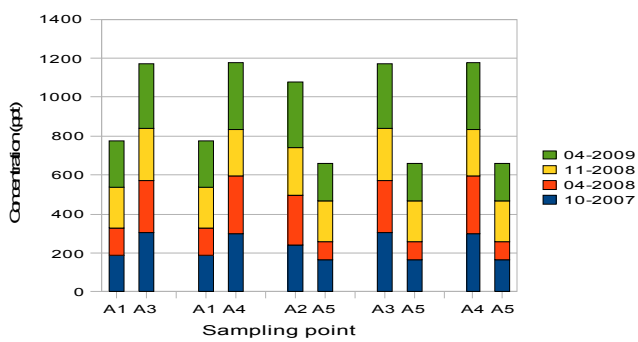

Fig. 22 – Plot of dissolved chromium against Sampling Points

Tukey HSD test for Prticulate chromium: Year

In years particulate chromium had shown signifcance difference. As can be seen from the bar graph below, in spring of 2009, the particulate matter of dramatically reduced from the levels obtained for the previous year. To know exactly what caused this variation, deep

study on the bay is necessary. This may have desirable and undesirable reasons. If it reduced because of remidation or natual mechanisms, the approach that caused this may be studied and implimented to other water bodied to get similar effect. If the reduction cause is the conversion of this for to other more toxic forms like dissolved chromium (VI), it is undesireable and monitoring purpose shall be implimented. Finally I would like to recommend to future studies on the particulate chromium variation in the bay.

Table 17 - Tukey HSD test for Particulate Chromium against year

Cell No	Tukey HSD test; Variable Particulate Cr			
	Year	{1} 115.71	{2} 226.23	{3} 69.652
1	2007		0.084693	0.692219
2	2008	0.084693		0.012669
3	2009	0.692219	0.012669	

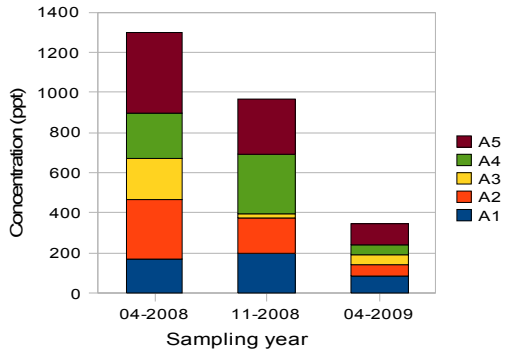

Fig. 23 – Plot of particulate chromium against year 2008 and 2009

Tukey HSD test for Dissolved chromium: Year

Dissolved chromium had shown signifcance difference in the level in 2008 and 2009. Consulting the bar graph below, the level of total dissolved chromium in the bay increased in 2009 from its level in 2008. This could be because of two main reasons. More domestic and industrial wastes join the water everytime so that the total dissolved chromium level should increase with time due to accumulation of chromium in the bay. The other possible reason is in 2009, wastes which contain higher dissolved chromium were disposed to the water. This included not pretreated wastes from industrial sourses.

Table 18 - Tukey HSD test for Dissolved Chromium against year

Cell No	Tukey HSD test; Variable Dissolved Cr(III)			
	Year	{1} 79.234	{2} 51.597	{3} 96.074
1	2007		0.258697	0.670345
2	2008	0.258697		0.044062
3	2009	0.670345	0.044062	

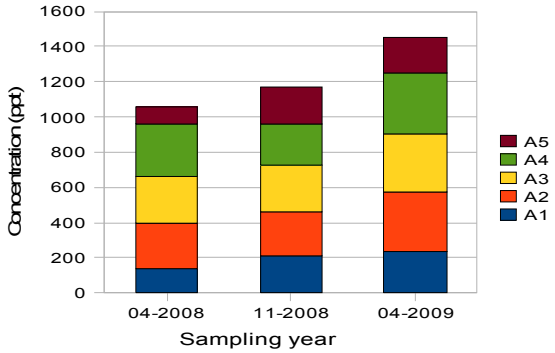

Fig. 24 – Plot of dissolved chromium against year 2008 and 2009

6.8 Pearson correlation

Correlation is a technique for investigating the relationship between two quantitative variables. Pearson's correlation coefficient is a measure of the strength of the association between the two variables.

The result of Pearson correlation for our data is given in the table below.

Table 19 – Pearson coorelation parameters for total chromium, fraction of chromium and species

	Total Cr	Dissolved Cr	Active D. Cr	Dissolved Cr (III)	Particulate Cr	Log Dis Cr(VI)
Total Cr	1,0000	,1446	,0955	,0950	,7948	,0181
	p= ---	p=,543	p=,689	p=,690	p=,000	p=,940
Dissolved Cr	,1446	1,0000	,8313	,6224	-,4856	,5202
	p=,543	p= ---	p=,000	p=,003	p=,030	p=,019
Active D. Cr	,0955	,8313	1,0000	,6415	-,4254	,7074
	p=,689	p=,000	p= ---	p=,002	p=,061	p=,000
Dissolved Cr (III)	,0950	,6224	,6415	1,0000	-,2978	-,0758
	p=,690	p=,003	p=,002	p= ---	p=,202	p=,751
Particulate Cr	,7948	-,4856	-,4254	-,2978	1,0000	-,3030
	p=,000	p=,030	p=,061	p=,202	p= ---	p=,194
Log Dis Cr(VI)	,0181	,5202	,7074	-,0758	-,3030	1,0000
	p=,940	p=,019	p=,000	p=,751	p=,194	p= ---

Fom the value of the Pearson's correlation coefficient and significance level, the following conclussions are made.

In the output of the correlation, there are 7 significant coorelations found ('p' < 0.05).

- Total chromium with particulate chromium has no coorelation. This is becsuse the level of the particultae chromium is very low comparing to the total chromium concentration. More over the seasonal increase of particulated chromium is insignificant comparing to that of total chromium.

- Dissolved chromium had positive coorelations with:
    - active chromium
    - chromium (VI)
    - chromium (III)

  This indicates that these these three fractions of chromium were resulted from total dissolved chromium and have direct coorelation with it.
    - and it has beed been found out that total dissolved chromium has a weak negative coorelation with particulate chromium.
- Active chromium also have positive coorelations with:
    - chromium (VI) and
    - chromium (III)

  This positive coorelation of active chromium with chromium (VI) and chromium (III) indicated chromium (VI) and chromium (III) are fractions of active chromium and have direct coorelation with it.

6.9 Results of total and species of chromium in Algeciras Bay

The total chromium and fractions of the total chromium are analysed in this research. The fractions of the total chromium include: total dissolved chromium, active dissolved chromium, dissolved hexavalent chromium, dissolved trivalent chromium and particulate chromium. 20 samples were analysed for each samples, the total and the fractions, and thre replicate results were collected for each samples. Thus 60 results were collected for the total and for each fractions of the total chromium. All results collected are given in the appendix 6.

6.9.1 Comparing the chromium level of Algeciras Bay with Guideline limits

The backgound levels of chromium in different environmental matrix are given in the table below [12]. The bachground level is given for the total chromium concentration thus it can be compared with the total chroum obatined in Algeciras Bay. The obtained total chromium concentration ranges from 200 ng/L in autumn 2007 to 555 ng/L in spring 2008 as shown in table 20.

As can be seen in the bar graph below, figure 25, all sampling points at all sampling times have higher concentration level of chromium from the background level. This showes that the water bay in exposed artificially to chromium. This is mainly from industrial activities around the bay, (two petrol refinaries and one steel industry) and several big ports along with domestic wastes.

Table 20 – Total chromium, all concentrations are in ng/L

Sampling point	Sampling Time			
	10-2007	04-2008	11-2008	04-2009
A1	200	304	408	324
A2	422	555	426	392
A3	310	478	287	383
A4	493	520	538	395
A5	344	495	477	302

Table 21 - Background concentrations of chromium found in water, sediment and soil

Environmental Matrix	Concentration	Reference
Seawater (open ocean)	0.057-0.234 ug/L	Bryan and Langston 1992
Freshwater	1-30 ug/L	USPHS 1997
Drinking water	0.4-8.0 ug/L	USPHS 1997
Marine sediment	30-200 mg/kg	Bryan and Langston 1992
Freshwater sediment / suspended particulates	1-500 mg/kg	USPHS 1997
Soil	<1-100 mg/kg 4-80 mg/kg	Alloway 1990 Dudka and Adriano 1997

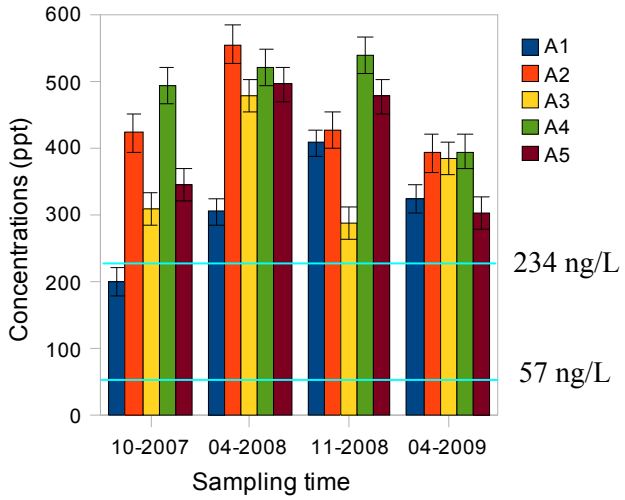

Fig. 25 – Total Chromium and background levels

6.9.2 Toxicity level of chromium in Algeciras Bay

Hexavalent chromium is the most toxic species amoung the forms of chromium in water. Thus toxicity of chromium is usually given by the concentration of hexavalent chromium. These levels are reported by Prof, M.F. Buchmann in NOAA HAZMAT Report 99-1 [23] and are also found in the table 21 below. As report by Prof. M.F. Buchmann the limit for chronic toxicity of chromium is 0.6 µg/L, appendix 4. The total chromium concentration level above from is regarded as toxic for marine living things. As can be seen in the table 20 above, no sampling point exhibited higher level of chromium from this limit in the sampling years. Yet many high results were observed. This indicates that there is a need to monitor the amount of chromium mixes to the bay at all sampling points.

Table 22 - CMC and CCC values for chromium (VI)

Seawater µg/L	
CMC*	CCC**
1100	50

* **CMC (Criteria Maximum** Concentration) is the water quality criteria to protect against acute effects in aquatic life and is the highest instream concentration of a priority toxic pollutant consisting of a short-term average not to be exceeded more than once every three years on the average.

****CCC (Continuous Criteria Concentration) is the water quality** criteria to protect against chronic effects in aquatic life and is the highest in stream concentration of a priority toxic pollutant consisting of a 4-day average not to be exceeded more than once every three years on the average.

Since the samples of Algeciras Bay were collected in autumn of 2007 and 2008 and spring of 2008 and 2009, it is only posible to use CMC to check the toxicity level of chromium in the bay. All levels of chromium (VI) in Algeciras Bay are lower than the CMC value for toxicity. Thus the chriterea for level of chromium to be regarded as toxic by CMC werenot observed in Algeciras Bay for these sampling times. The concentration of chromium in the bay is even less than the maximum limit for lake water used for drinking purpose according to European Drinking Water Directive Guideline [24]. The guideline restricts the maximum concentration to 50 μg/L of chromium.

6.9.3 Comparing the chromium level of Algeciras Bay with other seawater levels

The results papers and resultes used to compare with Algeciras bay levels are discussed in the litrature summary part of the introduction. Accordingly the levels of total chromium in river, estuarine and coastal waters of the Humber catchment in north-east England between 1993 and 2002 [16] is much higher than (1 mg/L) that of Algeciras Bay (maximum is 0.555 μL). More over the level of chromium (VI) is also higher (0.25mg/L to 1mg/L) to the level of chromium (VI) in Algeciras Bay (0.061 ppb to 0.180ppb).

The study of Australian seawaters have much higher concentration levels of total chromium, total dissolved chromium, chromium (VI) and chomium (III) when comparing to Algeciras bay. The Algecira Bay results can be consulted from appendix 6.

Table 23 - Chromium Level in Australian seas in µg/L

Seawater	Sampling time	Total chromium	Dissolved chromium	Chromium (VI)	Chromium (III)
Wirface waters from Pahn Beach	Jan 1983	2.3	1.8	0.27	0.22
Wirface waters from Pahn Beach	Jan 1984	0.6	0	0.31	0.25
Surface waters from Avaion Beach	Feb 1984	3.4	3.1	0.16	0.10
Surface waters from Avaion Beach	May 1984	0.6	0	0.27	0.29
Wirface waters from Pahn Beach	Dec 1984	0.4	0	0.24	0.15
Wirface waters from Pahn Beach	Jan 1985	1.8	1.2	0.19	0.43

6.9.4 Comparing with the levels of the Mediterranean Sea

As reported in the paper for the levels of chromium in the Mediterranen sea [17], the total dissolved chromium levels were between 131 ng/L and 177 ng/L. Chromium (VI) were between 112 ng/L and 165 ng/L while chromium (III) levels were between 8 to 45 ng/L. All the data collected for total chromium, chromium (VI) and chromium (III) are obtained from the research paper and are given below. In this part the similarity or differences of the results between Mediteranean Sea and Algeciras Bay for the three types of chromium levels are compared. In all the cases, significant level of 0.05 is assumed.

Table 24 - Chromium Level in Mediteranean sea in µg/L

Total Chromium	**Chromium (VI)**	**Chromium (III)**
131	112	19
149	123	26
158	113	45
161	132	29
166	125	42
163	138	24
157	124	33
140	122	18
136	112	24
149	140	8
168	151	17
177	165	12

The following statistical variables help to compare:

Table 25 – Statistical parameters of chomium Level in Mediteranean Sea

	Mean Medit	Mean Alg. Bay	Strd Dev. Medit. (n=12)	Strd Dev Alg. B. (n=20)	F (cal)	F (tab)	d.f	t(cal)	t(tab)
Total Chromium	152.55	402.66	13.88	96.43	48.28	2.34	20.43	11.4	2.09
Chromium (VI)	126.55	117.78	16.36	37.00	5.11	2.34	29.51	0.92	2.05
Chromium (III)	25.91	69.63	11.19	35.10	9.83	2.34	25.45	5.15	2.06

F-test

To know which method to use, there is a need to check the similarity of the variances by F-test. The null hypothesis, H_0, is:

$$H_o : s_1^2 = s_2^2 \qquad F_{cal} \leq F_{tab(\alpha, n_1 - 1 \text{ and } n_2 - 1 \text{d.f.})}$$

Thus the null hypothesis will be rejected if F(cal) is greater than F(tab) the null hyphothesis shall be rejected.

As can be seen from the table F(cal) for all chromium forms are higher than F(tab). Thus the variations of each pair of results found from Mediterranean Sea and Algeciras Bay are significant.

T-Student test

Since the variances of the pairs of variables significantly differ from eachother, the appropriate statistical tests shall be used for this types of popullation to check if the means of each pair of variables differet. For this purpose the t-student and degree of freedom are calculated using the following formulea. This degree of freedom shall be used to determine t (table) value. The null hypothesis for student t-student test is:

$$H_o : \overline{x_1} \leq \overline{x_2} \qquad t_{cal} \leq t_{tab(\alpha, \text{d.f.})}$$

Thus if t-calculated is greater than t-table, the null hypothesis shall be rejected.

$$t_{cal} = \frac{\overline{x_1} - \overline{x_2}}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}} \qquad d.f. = \frac{\left(\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}\right)^2}{\frac{\left(s_1^2/n_1\right)^2}{n_1 + 1} + \frac{\left(s_2^2/n_1\right)^2}{n_2 + 1}} - 2$$

T-calculated value for total chromium and chromium (III) ae greater than the t-tabulated value as can be seen from table 24 above. Thus there are significant differences between total chromium levels in Mediterranean Sea and Algeciras Bay as well as chromium (III) levels of the two water bodies. Hence the total chromium and chromium (III) levels of Algeciras Bay are higher than Mediterranean Sea, from the average levels obtained from the table above.

For the case of chromium (VI), the t-calculated is less than t-table (see table 24 above). This shows that there is no significant diferences between the chromium (VI) levels in the two water bodies.

Conclusion

- The chromium levels of Algeciras Bay were lower than the acute toxicity level, CMC level, untill spring 2009. However the bay's chromium levels were higher than the background level indicating the fact that there were artificial supplies of chromium to the water from industrial and domestic wastes and from activities on the water.
- There werenot significant diffeences of total choromium or a fraction of the total chromium (dissolved chromium, active chromium, chromium (VI), chromium (III) and particulated chromium) among seasons. There were significant diffeences (α=0.05) of:
- Dissolved chromium as well as active chromium among sampling points.
- Chromium (III) and particulate chromium between 2008 and 2009.
- Ammonia cannot be for speciatio of chromium in natural waters to adjest the pH of the system as it make complex with chromium and the chromium willnot be avialable to give peak.
- There is no coorelation between total chromium and particulate chromium. Dissolved chromium has a positive correlation with active chromium, chomium (VI) and chromium (III) and a weak negative coorelation with paticulate chromium. Similarly active chromium has a positive coorelation with chromium (VI) and chromium (III).
- It has been found out that particulate chromium had been decreasing in all seasons since spring of 2008. I would like to recommend this for further studies how or why it could happen.
- This thesis can be used as a starting point for future monitoring and study of chromium speciation in Algeciras bay. It can also be used to compare chromium species level of other seawaters.

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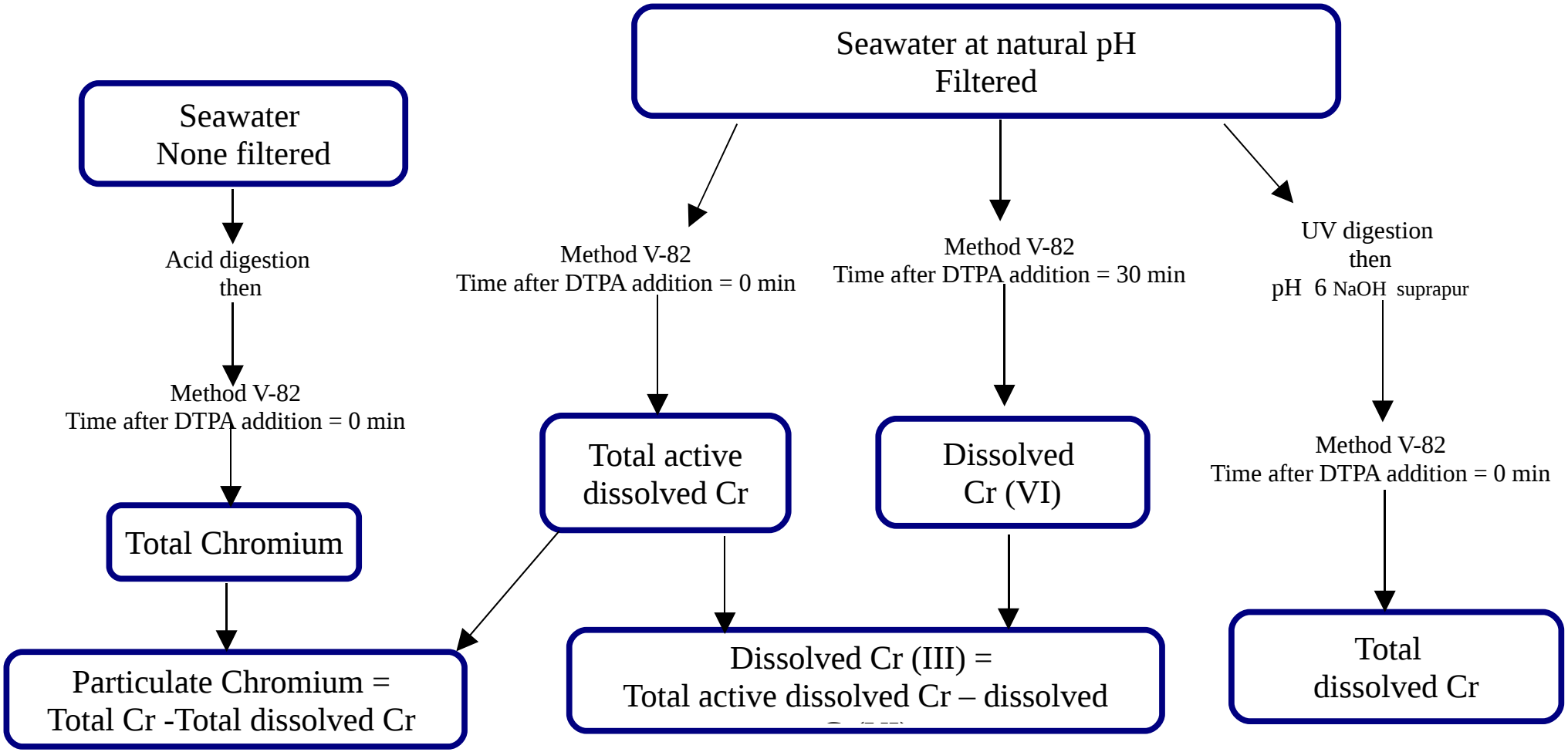
Appendices

Appendix 1; Scheme of Chromium speciation in seawater



Appendix 2; Certified Reference Material SLEW-3

National Research Council Canada — Conseil national de recherches Canada

Certified Reference Material

SLEW-3

Estuarine Water Reference Material for Trace Metals

The following table shows the twelve metals for which certified values have been established. Certified values are based on the results of determinations by at least two independent methods of analysis. The uncertainties represent 95 percent confidence limits for an individual subsample. That is, 95 percent of samples from any bottle would be expected to have concentrations within the specified range 95 percent of the time.

Trace Metal Concentrations
(micrograms/litre)

Arsenic (n,r)	1.36	± 0.09
Cadmium (i,m,o,r)	0.048	± 0.004
Chromium (m,o,r)	0.183	± 0.019
Cobalt (o,q,r)	0.042	± 0.010
Copper (i,m,o,r)	1.55	± 0.12
Iron (i,m,o)	0.568	± 0.059
Lead (m,o,r)	0.0090	± 0.0014
Manganese (d,i,q)	1.61	± 0.22
Molybdenum (c)	(5.1)*	
Nickel (i,m,o,s)	1.23	± 0.07
Silver (m,o)	(0.003)*	
Uranium (m)	(1.8)*	
Vanadium (q,r)	2.57	±0.31
Zinc (i,m,o)	0.201	± 0.037

*information value only

c - Direct determination by isotope dilution inductively coupled plasma mass spectrometry (ICPMS)
d - Direct determination by graphite furnace atomic absorption spectrometry (GFAAS)
i - Immobilized ligand separation, GFAAS determination
m - Immobilized ligand separation, isotope dilution ICPMS
n - In situ hydride generation, GFAAS determination
o - Co-precipitation, ICPMS determinatior
q - Immobilized ligand separation, ICPMS determination
r - Reductive precipitation separation, GFAAS determination
s - Solvent extraction, GFAAS determination

NRC · CNRC

Appendix 3; Physical properties of water of Algeciras Bay

Sampling 1: 30/10/07

Sampling points	GPS	Time	COT (mg/L)	Conductivity	pH	O_2	Tº Water	Salinity	TDS
A1	N 36° 05`207` W 5° 26`004´	14:10	1,72	54,1 mS/cm	8,34	47,7% 4,04 mg/L	21,2 °C	35,6‰	34,4 g/L
A2	N 36° 07`196` W 5° 25`682`	13:30	1,52	53,8 mS/cm	8,27	48,3% 4,09 mg/L	22,1 °C	35,5‰	34,2 g/L
A3	N 36° 10`374` W 5° 25`505`	12:40	1,39	56,4 mS/cm	8,25	43,7% 3,99 mg/L	20,1 °C	37,2‰	36,0 g/L
A4	N 36° 10`660 W `5° 24`606	12:00	1,53	53,3 mS/cm	8,24	45,2% 4,03 mg/L	21,3 °C	35,2‰	33,9 g/L
A5	N 36° 10`610` W 5° 23`468`	11:15	6,23	52,3 mS/cm	7,03	44,5% 4,08 mg/L	21,0 °C	34,4‰	33,1 g/L

Sampling 1: 30/10/07

Water

Sampling points	Mean Density (g/ml)	Density of Particulate Materials. (g/mL)	Density of Organic Materials. (g/mL)	g OM /g PM
A1	1,0257865	3,16891E-05	1,14081E-05	0,360
A1		2,16356E-05	6,43917E-06	0,298
A2	1,02431675	2,27775E-05	2,84718E-06	0,125
A2		2,54505E-05	5,55815E-06	0,218
A3	1,028164	2,16011E-05	5,77703E-06	0,267
A3		2,67718E-05	7,01167E-06	0,262
A4	1,02634325	2,73846E-05	6,26875E-06	0,229
A4		3,58635E-05	1,01885E-05	0,284
A5	1,01680675	3,64491E-05	1,18671E-05	0,326
A5		4,43728E-05	1,6573E-05	0,373

Sampling 2: 08/05/08

Sampling points	GPS	Time	COT (mg/L)	Conductivity	pH	O_2	Tº Water	Salinity	TDS
A1	N 36° 05`207` W 5° 26`004´	15:08	0,4134	52,8 mS/cm	8,42	85,8% 6,55 mg/L	18,3 °C	34,8%o	33,8 g/L
A2	N 36° 07`196` W 5° 25`682`	15:49	0,5943	51,6 mS/cm	8,42	90,4 % 7,03 mg/L	19,7 °C	33,9%o	32,5 g/L
A3	N 36° 10`374` W 5° 25`505`	16:45	0,6664	49,6 mS/cm	8,56	88,8 % 6,85 mg/L	20,2 °C	32,4%o	31,1 g/L
A4	N 36° 10`660 W `5° 24`606	17:15	0,7188	51,5 mS/cm	8,51	89,6 % 6,94 mg/L	20,9 °C	33,8%o	32,5 g/L
A5	N 36° 10`610` W5° 23`468`	18:00	0,5563	51,3 mS/cm	8,58	88,5% 6,85 mg/L	18,9 °C	33,5%o	32,4 g/L

SAMPLING 2: 08/05/08

Sampling points	Mean Density (g/ml)	Density of Particulate Materials (g/mL)	Density of Organic Maters (g/mL)	g OM/g PM
A1	1,02627	1,36044E-05	2,04998E-06	0,151
A1		1,48241E-05	1,56043E-06	0,105
A2	1,02498	1,49642E-05	2,46246E-06	0,165
A2		1,46463E-05	2,92926E-06	0,200
A3	1,02523	1,59674E-05	2,72614E-06	0,171
A3		1,59779E-05	1,55882E-06	0,098
A4	1,02584	1,44593E-05	2,50627E-06	0,173
A4		1,48012E-05	3,41566E-06	0,231
A5	1,02583	1,48475E-05	2,66494E-06	0,179
A5		1,71391E-05	3,42781E-06	0,200

Sampling 3: 04/11/08

Sampling points	GPS	Time	COT (mg/L)	Conductivity	pH	O_2	Tº Water	Salinity	TDS
A1	N 36º 05`207` W 5º 26`004´	9:15	1,74	50,3 mS/cm	7,98	77,5% 5,68 mg/L	14,9 ºC	32,9 ºoo	31,7 g/L
A2	N 36º 07`196` W 5º 25`682`	10:00	1,03	51,7 mS/cm	8,03	75,1% 5,44 mg/L	15,9 ºC	32,8 ºoo	32,8 g/L
A3	N 36º 10`374` W 5º 25`505`	10:25	0,6581	51,0 mS/cm	8,06	67,7% 4,67 mg/L	15,4 ºC	32,2 ºoo	32,2 g/L
A4	N 36º 10`660 W `5º 24`606	11:00	1,67	49,6 mS/cm	8,04	73,5% 5,27 mg/L	15,3 ºC	31,2 ºoo	31,2 g/L
A5	N 36º 10`610` W5º 23`468`	11:25	1,52	48,5 mS/cm	8,07	66,8% 4,58 mg/L	14,3 ºC	32,4 ºoo	30,4 g/L

SAMPLING 3: 04/11/08

Sampling points	Mean Density (g/ml)	Density of Particulate Material (g/mL)	Density of Organic Materials (g/mL)	g OM/g PM
A1	1,02401	2,91035E-05	7,17621E-06	0,247
A1		2,88656E-05	4,06558E-06	0,141
A2	1,02459	1,74468E-05	1,62296E-06	0,093
A2		1,74978E-05	2,78375E-06	0,159
A3	1,02396	2,40046E-05	3,60068E-06	0,150
A3		1,93169E-05	4,02435E-06	0,208
A4	1,03229	2,31281E-05	4,78512E-06	0,207
A4		2,38797E-05	5,26162E-06	0,220
A5	1,02677	2,09312E-05	4,02523E-06	0,192
A5		1,70695E-05	2,77875E-06	0,163

Sampling 4: 22/05/09

Sampling points	GPS	Time	COT (mg/L)	Conductivity	pH	O_2	Tº Water	Salinity	TDS
A1	N 36º 05`207` W 5º 26`004´	9:35	0,9197	47 mS/cm	7,11	88,0% 7,5 mg/L	18,2 ºC	30,1 ºoo	29,1 g/L
A2	N 36º 07`196` W 5º 25`682`	10:00	2,51	45,4 mS/cm	6,96	83,5% 7,20 mg/L	18,3 ºC	29,3 ºoo	28,2 g/L
A3	N 36º 10`374` W 5º 25`505`	10:30	0,4068	46,6 mS/cm	7,58	95,5% 7,9 mg/L	18,2 ºC	30,3 ºoo	28,9 g/L
A4	N 36º 10`660 W `5º 24`606	11:00	1,05	45,5 mS/cm	7,37	88,4% 7,47 mg/L	18,6 ºC	29,5 ºoo	28,5 g/L
A5	N 36º 10`610` W5º 23`468`	11:30	1,14	45,6 mS/cm	7,42	94% 7,72 mg/L	18,5 ºC	29,7 ºoo	28,6 g/L

SAMPLING 4: 22/05/09

Sampling points	Mean Density (g/ml)	Density of Particulate Maters (g/mL)	Density of Organic Maters (g/mL)	g OM/g PM
A1	1,02454	2,08712E-05	3,03581E-06	0,145
A1		2,11267E-05	2,34741E-06	0,111
A2	1,02538	2,1828E-05	4,28763E-06	0,196
A2		2,18917E-05	2,73646E-06	0,125
A3	1,02491	2,20791E-05	3,2115E-06	0,145
A3		2,37677E-05	3,50671E-06	0,148
A4	1,02408	2,0789E-05	1,96123E-06	0,094
A4		2,23825E-05	3,53408E-06	0,158
A5	1,02431	1,85993E-05	-1,18719E-06	-0,064
A5		2,1821E-05	1,55864E-06	0,071

Appendix 4; Toxicity levels of heavy metals in environment

Metal	Lake Water (µg L-1)		UK Project (c)			Web (ppb by weight) (h)			Soil (µg g-1)			
	Norway (a)	Japan (b)	Marine Water Column (ppm)	Marine Sediment (ppm)	Earth's Crust (ppm)	Sea Water	Crustal Rock	Stream	Florida (d)	Alaska (e)	China (f)	North Carolina (g)
Ag	NA	NA	NA	NA	NA	0.1	80	0.3	0.07–2.50	NA	0.03–0.41	NA
Al	NA	1.2	NA	NA	NA	5	82×10^6	400	NA	NA	NA	NA
As	NA	NA	2–3	<7	NA	2.3	2,100	1	0.02–7.01	1.26–35.8	2.52–33.6	3–12
Ba	NA	NA	NA	NA	NA	30	340×10^3	25	1.67–112	213–1659	266–761	NA
Be	NA	NA	NA	NA	NA	0.0006	1900	0.1	0.04–4.15	0.68–3.33	0.85–3.91	NA
Cd	0.1-0.5	<0.006	<0.005–0.1	0.03–1.0	0.1 to 15#	0.05	150	NA	0.00–0.33	NA	0.02–0.33	0.1–1.0
Co	NA	<0.004	NA	NA	NA	0.08	30,000	0.2	NA	NA	NA	NA
Cr	NA	NA	<1.0	NA	NA	0.6	140×10^3	1	0.89–80.7	12.5–200	19.3–150	1.5–40
Cu	0-2.0	0.069	1.0	NA	NA	3	68×10^3	6	0.22–21.9	7.33–78.6	7.26–55.1	1–50
Fe	NA	2.6	0.0028/0.029–0.224/1.228	20,800	NA	3	63×10^6	670	NA	NA	NA	NA
Hg	NA	NA	NA	NA	NA	0.05	67	0.07	0.75–39.6	NA	5.9–270	NA

Metal	Lake Water (µg L-1)		UK Project (c)			Web (ppb by weight) (h)			Soil (µg g-1)			
	Norway (a)	Japan (b)	Marine Water Column (ppm)	Marine Sediment (ppm)	Earth's Crust (ppm)	Sea Water	Crustal Rock	Stream	Florida (d)	Alaska (e)	China (f)	North Carolina (g)
Mn	NA	0.76	NA	NA	NA	2	1.1 x10^6	5	1.74–236	70–3718	134–1740	NA
Mo	NA		NA	NA	NA	10	1,100	0.8	0.13–6.76	0.14–5.29	0.15–9.82	NA
Ni	NA	0.027	0.2–0.7	NA	0.008%	2	90 x10^3	0.3	1.70–48.5	5.1–113	7.73–70.9	NA
Pb	0-2.0	0.05	NA	NA	NA	0.03	10 x10^3	3	0.69–42.0	3.96–36.3	9.95–56.0	4–12*, 200–500**
Sb	NA	NA	NA	NA	NA	0.2	200	2	0.06–0.79	NA	0.377–2.98	NA
Se	NA	NA	NA	NA	NA	0.45	50	0.2	0.01–1.11	NA	0.047–0.99	NA
Sn	NA	NA	NA	NA	NA	0.01	2,200	0.06	NA	NA	NA	NA
Ti	NA	0.06	NA	NA	\NA	1	6.6 10^6	3	NA	NA	NA	NA
V	NA	0.15	1–<20	NA	Not Occur	1.5	190x 10^3	1	NA	NA	NA	NA
Zn	0.5-12.0	0.63	NA	NA	NA	5	79 x10^3	10	0.89–29.6	26–188	28.5–161	NA

(a) Reported by Henriksen, A. and Wright, R.F., Water Res., 12, 101, 1978.

(b) Reported by Nojiri, Y., Kawai, T., Otsuki, A., and Fuwa, K., Water Res., 19, 503, 1985.

(c) Reported by UK Marine SACs (Special Areas of Conservation) Project. http://www.ukmarinesac.org.uk/index.htm. 2001 (# in Sedimentary rock).

(d) Reported by Chen, M., Ma, L.Q., and Harris, W.G., Annual progress report. University of Florida, Gainesville, FL, 1998. N=448.

(e) Reported by Gough, L.P., Severson, R.C., and Shacklette, H.T., Element concentrations in soils and other surficial materials of Alaska. US Geological Survey Professional Paper 1458. U.S. Government Printing Office, Washington, DC, 1988. N=437.

(f) Reported by Bradford, G.R., Chang, A.C., Page, A.L., Bakhtar, D., Frampton, J.A., and Wright, H., Background Concentrations of Trace and Major Elements in California Soils, Kearney Foundation Special Report, University of California, Riverside, CA, 1996. N=50.

(g) Reported by Tucker, M.R., Hardy, D.H., and Stokes, C.E., Heavy metals in North Carolina soils: occurrence and significance. North Carolina Department of Agriculture and Consumer Services, Agronomic Division, Raleigh, NC, 2005, 2 pp. NÂ¼5000 (* rural areas, ** metropolitan or suburban areas or near highways)

(h) Reported by http://www.webelements.com/(371). WebElements, the periodic table on the WWW. Mark Winter, Department of Chemistry, The University of Sheffield and WebElements Ltd., England. Http:// www.webelements.com/. Document served: 5 August, 2006.

NA, data not available.

Appendix 5; Metrohm method for speciation of chromium

VA Application Note No. V-82

Title:	**Different chromium species in sea water**

Summary:	Cr(III) forms an electrochemically active complex with diethylenetriaminepentaacetic acid (DTPA), so does Cr(VI) after in situ reduction on the surface of the HMDE. Depending on the sample preparation procedure and the waiting time after the addition of the complexing agent, the different chromium species can be differentiated: • **Total active chromium** [total concentration of Cr(VI) and free Cr(III)]: The measurement is carried out immediately after the addition of DTPA. • **Cr(VI):** Between the addition of DTPA and the start of the analysis a minimum waiting time of 30 min is necessary. During this waiting time the Cr(III)-DTPA complex becomes electrochemically inactive. • **Cr(III):** The difference between the total active Cr and Cr(VI). • **Total chromium:** Determination of total active Cr after UV digestion.

Sample:	Sea water
Sample preparation:	UV digestion for the determination of total chromium

All Cr species:	
Electrolyte:	Acetate buffer + DTPA + sodium nitrate (pH = 6.2)
AE:	Pt
RE:	Ag/AgCl/KCl (3mol/L)
Parameters:	DPAdSV at HMDE Deposition: –1000 mV (20 s) Determination: –1000 mV to –1400 V Peak potential Cr: –1200 mV

Results:	Cr(total)	Cr(total active)	Cr(VI)	Cr(III)
	1.74 µg/L	0.68 µg/L	0.47 µg/L	0.21 µg/L

Appendix 6; raw results from speciation of chromium

Total Cr
Samples NF 10-07

Sampling point	result (ppt)	stand. Dev	Average (ppt)	Blank(ppt)	real conc(ppt)	Final Conc (ppt)
A1	1308	14.49	1310	1130	180	200
	1326					
	1297					
A2	1518	11.19	1510	1130	380	422
	1515					
	1497					
A3	1419	15.14	1409	1130	279	310
	1416					
	1391					
A4	1571	1.72	1573	1130	443	493
	1573					
	1575					
A5	1423	17.97	1440	1130	310	344
	1438					
	1459					

Total Cr
Samples NF 04-08

Sampling point	result (ppt)	stand. Dev	Average (ppt)	Blank (ppt)	real conc (ppt)	Final Conc (ppt)
A1	1393	9.37	1404	1130	274	304
	1409					
	1410					
A2	1622	7.69	1629	1130	500	555
	1637					
	1629					
A3	1563	9.27	1560	1130	430	478
	1568					
	1550					
A4	1589	9.62	1598	1130	468	520
	1596					
	1608					
A5	1594	16.3	1575	1130	445	495
	1567					
	1565					

Total Cr
Samples NF 11-08

Sampling point	result (ppt)	stand. Dev	Average (ppt)	Blank(ppt)	real conc(ppt)	Final Conc (ppt)
A1	1492	9.51	1497	1130	367	408
	1508					
	1490					
A2	1519	16.68	1513	1130	383	426
	1526					
	1494					
A3	1388	11	1388	1130	258	287
	1399					
	1377					
A4	1616	20.48	1614	1130	484	537
	1633					
	1592					
A5	1541	17.42	1559	1130	429	477
	1560					
	1576					

Total Cr
Samples NF 04-09

Sampling point	result (ppt)	stand. Dev	Average (ppt)	Blank(ppt)	real conc(ppt)	Final Conc (ppt)
A1	1411	9.62	1421	1130	292	324
	1423					
	1430					
A2	1468	15.87	1483	1130	353	392
	1481					
	1500					
A3	1489	13.58	1475	1130	345	383
	1474					
	1461					
A4	1461	21.47	1485	1130	355	395
	1502					
	1492					
A5	1397	10.46	1402	1130	272	302
	1395					
	1414					

Total Dissolved Cr
Samples F 10-07

Sampling point	result (ppt)	stand. Dev	Average (ppt)	Blank (ppt)	real conc (ppt)
A1	723	16.09	720	531	188
	702				
	733				
A2	789	16.74	770	531	239
	764				
	757				
A3	818	13.76	834	531	302
	845				
	837				
A4	850	18.27	830	531	299
	824				
	815				
A5	690	7.61	694	531	163
	690				
	703				

Total Dissolved Cr

Samples F 04-08

Sampling point	result (ppt)	stand. Dev	Average (ppt)	Blank (ppt)	real conc (ppt)
A1	664	8.76	668	531	137
	678				
	662				
A2	792	3.2	789	531	258
	786				
	789				
A3	795	14.84	800	531	269
	789				
	817				
A4	830	23.66	827	531	295
	801				
	848				
A5	628	5.86	627	531	96
	621				
	633				

Total Dissolved Cr

Samples F 11-08

Sampling point	result (ppt)	stand. Dev	Average (ppt)	Blank (ppt)	real conc (ppt)
A1	753	16.12	742	531	211
	724				
	750				
A2	759	21.23	777	531	246
	801				
	771				
A3	805	7.34	799	531	268
	801				
	791				
A4	771	4.23	770	531	239
	774				
	766				
A5	727	21.36	737	531	206
	723				
	762				

Total Dissolved Cr

Samples F 04-09

Sampling point	result (ppt)	stand. Dev	Average (ppt)	Blank (ppt)	real conc (ppt)
A1	791	26.9	769	531	237
	739				
	775				
A2	873	15.73	869	531	337
	882				
	851				
A3	884	18.62	862	531	331
	851				
	853				
A4	894	14.61	878	531	346
	871				
	868				
A5	746	16.41	727	531	196
	722				
	714				

Total Active Dissolved Cr

Samples F 10-07

Sampling point	result (ppt)	stand. Dev	Average (ppt)	Blank(ppt)	real conc(ppt)
A1	726	24.29	718	552	166
	691				
	738				
A2	749	19.36	752	552	199
	772				
	734				
A3	775	28.53	758	552	205
	725				
	773				
A4	743	22.39	763	552	211
	787				
	760				
A5	686	24.69	689	552	137
	666				
	715				

Total Active Dissolved Cr

Samples F 04-08

Sampling point	result (ppt)	stand. Dev	Average (ppt)	Blank (ppt)	real conc (ppt)
A1	710	25	681	552	128
	666				
	666				
A2	797	21.2	778	552	225
	755				
	782				
A3	706	19.95	699	552	147
	676				
	714				
A4	731	18.71	719	552	166
	697				
	727				
A5	646	18.75	630	552	77
	634				
	609				

Total Active Dissolved Cr

Samples F 11-08

Sampling point	result (ppt)	stand. Dev	Average (ppt)	Blank (ppt)	real conc (ppt)
A1	698	11.77	689	552	137
	676				
	694				
A2	777	8.61	786	552	234
	788				
	793				
A3	774	14.63	758	552	206
	746				
	755				
A4	782	4.17	780	552	228
	776				
	784				
A5	741	18.26	734	552	182
	714				
	748				

Total Active Dissolved Cr

Samples F 04-09

Sampling point	result (ppt)	stand. Dev	Average (ppt)	Blank (ppt)	real conc (ppt)
A1	731	7.38	729	552	176
	720				
	734				
A2	805	7.88	807	552	254
	800				
	815				
A3	801	18.06	803	552	251
	822				
	786				
A4	785	26.21	807	552	255
	836				
	800				
A5	710	17.05	716	552	164
	703				
	736				

Dissolved Cr (VI)

Samples F 10-07

Sampling point	result (ppt)	stand. Dev	Average (ppt)	Blank (ppt)	real conc (ppt)
A1	606	17.88	596	503	93
	576				
	607				
A2	616	17.39	596	503	93
	589				
	583				
A3	629	10.16	630	503	127
	620				
	640				
A4	621	6.31	621	503	118
	628				
	615				
A5	591	2.83	594	503	91
	595				
	596				

Dissolved Cr (VI)

Samples F 04-08

Sampling point	result (ppt)	stand. Dev	Average (ppt)	Blank (ppt)	real conc (ppt)
A1	587	17.54	607	503	104
	621				
	612				
A2	631	19.24	609	503	106
	595				
	601				
A3	563	17.47	579	503	76
	576				
	597				
A4	615	5.92	610	503	107
	603				
	612				
A5	561	19.72	564	503	61
	546				
	585				

Dissolved Cr (VI)

Samples F 11-08

Sampling point	result (ppt)	stand. Dev	Average (ppt)	Blank (ppt)	real conc (ppt)
A1	603	12.33	616	503	113
	618				
	627				
A2	662	18.68	676	503	173
	668				
	697				
A3	674	8.6	676	503	173
	685				
	668				
A4	697	14.69	681	503	178
	668				
	679				
A5	616	8.29	626	503	123
	631				
	631				

Dissolved Cr (VI)

Samples F 04-09

Sampling point	result (ppt)	stand. Dev	Average (ppt)	Blank (ppt)	real conc (ppt)
A1	607	4.46	612	503	109
	613				
	615				
A2	675	14.1	668	503	165
	652				
	677				
A3	680	11.55	683	503	180
	695				
	672				
A4	595	11.8	596	503	93
	607				
	584				
A5	581	13.65	576	503	73
	587				
	560				

Dissolved Cr (III)

Samples F 10-07
All the concentrations are in ppt

Sampling Point	T. Act. Diss. Cr	Diss. Cr(VI)	real conc (ppt)
A1	166	93	73
A2	199	93	106
A3	205	127	79
A4	211	118	93
A5	211	91	109

Dissolved Cr (III)

Samples F 04-08
All the concentrations are in ppt

Sampling Point	T. Act. Diss. Cr	Diss. Cr(VI)	real conc (ppt)
A1	128	104	24
A2	225	106	119
A3	147	76	71
A4	166	107	59
A5	77	61	16

Dissolved Cr (III)

Samples F 11-08
All the concentrations are in ppt

Sampling Point	T. Act. Diss. Cr	Diss. Cr(VI)	real conc (ppt)
A1	137	113	24
A2	234	173	61
A3	206	173	33
A4	228	178	50
A5	182	123	59

Dissolved Cr (III)

Samples F 04-09
All the concentrations are in ppt

Sampling Point	T. Act. Diss. Cr	Diss. Cr(VI)	real conc (ppt)
A1	176	109	67
A2	254	165	89
A3	251	180	71
A4	255	93	162
A5	164	73	91

Particulate Cr

Samples F 10-2007
All the concentrations are in ppt

Sampling Point	T. Cr	T. Diss. Cr	real conc (ppt)
A1	200	188	12
A2	422	239	184
A3	310	302	8
A4	493	299	194
A5	344	163	181

Particulate Cr

Samples F 04-08
All the concentrations are in ppt

Sampling Point	T. Cr	T. Diss. Cr	real conc (ppt)
A1	304	137	168
A2	555	258	297
A3	478	269	209
A4	520	295	225
A5	495	96	399

Particulate Cr

Samples F 11-08
All the concentrations are in ppt

Sampling Point	T. Cr	T. Diss. Cr	real conc (ppt)
A1	408	211	196
A2	426	246	180
A3	287	268	19
A4	537	239	298
A5	477	206	271

Particulate Cr

Samples F 04-09
All the concentrations are in ppt

Sampling Point	T. Cr	T. Diss. Cr	real conc (ppt)
A1	324	237	87
A2	392	337	55
A3	383	331	52
A4	395	346	48
A5	302	196	106