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TOTAL ANALYSIS AND ASSESSMENT OF MOBILE METAL IONS IN SEDIMENTS FROM ALGECIRAS BAY, SPAIN

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Erasmus Mundus

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This thesis is submitted by Charles Mitto Kosore to the
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This research work has been done entirely in the laboratories of the Department of Analytical Chemistry, University of Cadiz, in the Marine Geochemistry Research Group, under the supervision of Prof. Maria Dolores Galindo Riano

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Prof. Maria Dolores Galindo Riano
February 23rd 2010

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DEDICATION

To my Lord Jesus Christ for giving me victory

To my wife and children for their prayers, patience and encouragement

ABSTRACT

Total metal concentrations in surface sediments from seventeen sampling points in Algeciras Bay were determined by total acid decomposition with IR heating technique. Mobile metal ions in these sediments were determined using single extraction schemes employing 0.11M acetic acid and 0.005M DTPA + 0.01M $CaCl_2$ + 0.1M TEA (pH 7.3). Organic matter (OM) was also determined by loss on ignition (LOI) method. The results revealed that the major sources of metal pollutants in Algeciras Bay originate from the city and port of Algeciras; and the industrial zones, indicating that metals in sediments from this bay were likely to be originating from anthropogenic activities. A large area of the bay was found to be highly contaminated with Ni, whose concentration levels exceeded the high alert levels (HAL) from USEPA (1996), meaning that marine organisms might regularly be exposed to toxic effects. Cr and As concentrations exceeded the effect range-low (ERL) defined by Long, *et al*. (1995), implying that toxic effects might occasionally occur in marine organisms. DTPA and acetic acid provided information on the effect of complexation and acidification processes on metal extractability. ANOVA test showed that there existed both similarities and differences between the extractability potentials of the two conditions. The most important metals extracted in both conditions were: Co, Cu, Cd, Ni, Zn, Pb and Mn, during which Mn, Cd and Co were found to be sensitive to acid dissolution; Pb, Cu and Cd were sensitive complexation process, while Ni and Zn were sensitive to both processes. Co, Zn, Cd and Pb extracted in both conditions exceeded the low alert level (LAL) defined by Manheim and Commeau, (1981). The mobility of Cu, Zn and Pb were more pronounced in complexing condition than in acidic one, while mobility of Co and Cd were more in acidic than in complexing condition. Background levels of the most dangerous metals (Co, Cu, Zn, Cd and Pb) were violated in both conditions, although at different levels, but to the point of creating awareness that there were potential adverse effects from these metals in Algeciras Bay.

Key words: Total metals, mobile metals, surface sediments, pollutants, Algeciras Bay.

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AIMS AND OBJECTIVES

Aims

Many past studies have reported that when environmental conditions change (pH, sediment redox potential, organic matter, etc.); some of the sediment-bound metals can remobilise and be released back into the water, where they can have adverse effects on living organisms (Izquierdo, *et al*. 1997; Li, *et al* 2001; Morillo, Usero, and Gracia, 2002a; Song, *et al*. 2009). In this study, complexant-based (0.005M + 0.01M $CaCl_2$ + 0.1M TEA) and organic acid-based (0.11M acetic acid) were used as extractants for mobile and mobilizable metals in sediments in predicting the bioavailability of the metals studied. These two extractants represented the real environmental conditions; therefore, the aim of this study was to determine the status of metal pollutants and to assess the extractability potential (remobilisation and release of sediment-bound metals) of the two extractants for the prediction of bioavailabile metals in sediments from Algeciras Bay.

Specific Objectives

1. To determine the concentrations of total metals and their distributions in sediments;
2. To determine the organic matter (OM) content as one the key factors influencing distribution of metals in sediments;
3. To assess the sediment quality;
4. To determine the concentrations of mobile and mobilisable metal ions extracted in both extractants;
5. To compare the concentrations of mobile metal ions on the basis of their extractability under acidic (0.11M acetic acid) and complexing (0.005M DTPA + 0.01M CaCL2 + 0.1M TEA) conditions.

TABLE OF CONTENTS

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES

LIST OF ACRONYMS

AFNOR Association française de Normalisation

ANZECC Australian and New Zealand Environment Conservation Council

BCR (European) Community Bureau of Reference

LDPE Low Density Polyethylene

LMWOA Low Density Weight Organic Acid

NSW EPA New South Wales Environmental Protection Authority

ORP Oxidation Reduction Potential

PTFE Polytetrafluoroethylene

QA Quality Assurance

QC Quality Control

SM & T Measurements and Testing Programme

USEPA United States Environmental Protection Agency

1.0. INTRODUCTION

1.1. Sediment contamination

The distribution of metals within the aquatic environments is governed by complex processes of material exchange affected by various anthropogenic activities or natural processes including riverine or atmospheric inputs, coastal and seafloor erosion, biological activities, water drainage, discharge of urban and industrial wastewaters (Ip Carman, *et al.,* 2007; Leivouri, 1998). Coastal environments continue to attract heavy investment in various economic activities such as tourism, aquaculture, industrialization, agricultural, mining and urbanization. Therefore coastal-zone pollution problems are becoming increasingly prevalent, with potentially adverse environmental and socio-economic effects. Consequently in global context, anthropogenic fluxes of contaminants such as heavy metals now exceed the natural flux in many areas (Onyari, *et al*., 2003). The pollutants from industrial activity and urban sewage commonly reach their final destination in the coastal waters through river mouths or by direct discharge into the sea. Metals are important among these pollutants because many of them are highly toxic and that they are not biodegradable in the environment (Morillo, *et al*. 2002a; Morillo and Usero, in press).

In the study of potential mobility of metals in polluted coastal sediments in two bays of southern Spain, Morillo*, et al*. (2007) reported that large amounts of metals were accumulated in the interior of Cadiz Bay, where anthropogenic activities were prevalent and in Algeciras Bay, where highest levels of metal were found near the city of Algeciras and an area of major industrial activity. In a Lagos Lagoon, Nigeria, Okoye (1991) reported that anthropogenic heavy metal enrichment of Cd, Co, Cu, Cr, Fe, Mn, Ni, Pb and Zn was attributed to land-based urban and industrial wastes sources.

In the Rhine basin in Germany, Behrendt (1993) reported that diffuse loads of trace metals had increased from 18%, 42% and 40% to 58%, 66% and 56% of the total load of Cd, Pb and Zn in the 1970s and the late 1980s respectively.

As trace metals can have deleterious effects on aquatic ecosystems, it is important to know where biobiavailable metals are entering the systems (Gay and Maher, 2003). Sediments accumulate hazardous trace elements to levels many times higher than water column concentrations, causing a serious problem due to their toxicity and their ability to accumulate in biota (Morillo, *et al*. 2007), who also reported that a comparison of metal levels with the sediment quality guidelines suggested by Long, *et al*. (1995) showed areas in Cadiz and Algeciras Bays in which pollution was sufficient to have noxious effects on marine organisms, whereas, Vicente-Martorell, *et al*. (2009) reported high contamination of total Zn, Pb, As and Cu in sediments from the estuary of Tinto and Odiel rivers in Huelva, Spain.

When environmental conditions change (pH, sediment redox potential, organic matter, etc.); some of the sediment-bound metals can remobilise and be released back into the water, where they can have adverse effects on living organisms (Izquierdo, *et al*. 1997; Li, *et al* 2001; Morillo, Usero, and Gracia, 2002a; Song, *et al*. 2009). Metals are associated with different sediment phases (crystalline minerals, carbonates, hydrous metal oxides, organic substances, *etc.*) that determine their behaviour in the environment, mobility, bioavailability, and toxicity (Usero, *et al.* 1998). The most crucial property of metals ions is that they are bioavailable and not biodegradable in the environment and that their uptake by benthic organisms depends largely on their mobility, total concentration and chemical forms (Morillo, *et al*. 2007). Thus, to assess the environmental impact of polluted sediments, the measurement of total trace metals is not enough (Kersten and Forstner, 1987; Morillo, *et al*. 2002b).

While studying metal speciation in coastal marine sediments from Singapore, Cuong and Obbard (2006) found that all metals, except Cd were more mobile and bio-available in Kranji, where metals were present at higher percentages in the acid-soluble fractions (the most labile fraction). They found mobility order of the heavy metals studied as Cd > Ni > Zn > Cu > Pb > Cr, whereas sediments from Pulau Tekong showed the same order for Cd, Ni, Pb and Cr, but had a reverse order for Cu and Zn (Cu > Zn). Vicente-Martorell*, et al*. (2008, 2009) reported availability order of metals in the estuary of Tinto and Odiel rivers in Huelva, Spain as Cd > Zn > Cu > Pb, similar to the trend reported by Cuong and Obabard (2006) in sediments from Kranji. In the study of heavy metals in harbour contaminated sediments, Caplat, *et al*. (2005) found that Zn was the most labile metal, recovered in the first extraction stages, similar to results found by Morillo *et al*. (2004) that Zn was the most mobile (i.e., it can pass easily into the water under changing environmental conditions)

1.2. Factors influencing metal distributions in sediments

It has been validated that each environmental factor presents unique influence on metals distribution in sediment. The influences of some factors, such as pH, ORP and OM are more crucial than the others. Only a slight variation of them, the distributions of metals would be producing some significant variations. Correspondingly, some other factors (e.g. salinity, temperature) can only alter metals distribution to a less extent (Song, *et al*. 2009). In this study, only those OM will be determined.

Organic compounds in sediment, frequently existing in considerable amounts in particle form, play an important role in heavy metal transformation. Additionally, in sediment, the solubility of organic matters usually directly determines the mobility of heavy metals.

Normally, the complexation of metal ions with insoluble organic compounds can strongly lower their mobility, whereas the formation of soluble metal complexes with dissolved organic compounds would enhance their mobility (Amina, *et al*. 1999).

1.3. Sediment quality assessment (SQGs)

Total heavy metal concentrations are useful in assessing the environmental status of aquatic environments, but more important is whether the toxicants are available to biota and whether they are entering the food chain (Birch and Taylor, 1999). Trace metals can accumulate in the upper sediment by biological and geochemical mechanisms and can represent significant environmental concern such as toxic to sediment-dwelling organisms and fish resulting in decreased survival, reduced growth, or impaired reproduction, and lower species diversity (Karbassi and Amirnezhad, 2004; Okafor and Opuene, 2007; Parizanganeh, *et al*. 2007).

Quantifying the available or labile metals in the sediment provides a better indicator of sediment quality (Seen, *et al*. 2008). Many sediment quality guidelines, such as the Australian and New Zealand Environment and Conservation Council (ANZECC guidelines, 2000), have moved to a risk based approach for assessing sediment quality. Under these guidelines if a total metal analysis exceeds a sediment guideline value further testing can be undertaken to determine specifically the fraction of available or labile metal in the sediment, and to assess if this available metal concentration exceeds the guideline value.

Generally, the primary purposes of sediment quality guidelines (SQGs) are to protect the aquatic biota from the harmful and toxic effects related with sediment-bound contaminants and useful tool for evaluating the potential for contaminants within sediment to induce biological effects (MacDonald , *et al.* 2000; Spencer and Macleod, 2002; McCready, *et al*. 2006) and . These guidelines evaluate the degree to

which the sediment-associated chemical status might adversely affect aquatic organisms and are designed to assist sediment assessors and managers responsible for the interpretation of sediment quality (Caeiro, *et al*. 2005). It is also used to rank and prioritize the contaminated areas or the chemicals for further investigation (USEPA, 1992; Long, E., *et al*. 1995; Birch and Taylor, 2006; McCready, *et al*. 2006; Farkas, *et al*. 2007). Some of the sediment quality guidelines are given in Table 1.

Below ERL is not considered toxic, since adverse effects to organisms occurred in less than 10% of studies in which concentrations fell below the respective ERL values. Above ERM is considered toxic, since adverse effects to organisms occurred in more than 75% of studies in which concentrations exceeded the respective ERM values. Low alert level (LAL) from Manheim and Commeau, (1981) based on ranges found in the Atlantic continental shelf drill cores, refers to concentration range limits normally found in natural, uncontaminated sediments. High alert level (HAL) refers to estimated sediment screening values from USEPA (1996), many of these values are the same as ERM values and therefore these values can just suffice in place of ERM values since their implications and application criteria are also the same.

1.4. Sediment speciation

The ability to determine the chemical forms of metal ions in sediments is becoming increasingly important, making it necessary to assess the potential of the sediments as either a sink or a source of heavy metals in the aquatic environment (Rauret, *et al*. 1991).

Table 1: Sediment Quality Guidelines (SQGs)

Metal	Long *et al*., 1995		Manheim and Commeau, 1981	USEPA, 1996
	ERL (mg/kg)	ERM (mg/kg)	Low alert level (mg/kg)	High alert level (mg/kg)
V	NA	NA	5	350
Cr	81	370	4	370
Co	NA	NA	0.5	120
Ni	21	52	3	50
Cu	34	270	2	270
Zn	150	410	5	410
As	8.2	70	0.5	70
Cd	1.2	9.6	0.04	9.6
Ba	NA	NA	50	1000
Pb	47	220	2	218
Fe	NA	NA	NA	NA
Mn	NA	NA	NA	NA

NA means not available.

1.4.1. Types of sediment speciation

In practice, environmental studies involving sediment analysis are often based on the use of leaching or extraction procedures (e.g. single or sequential procedures) which enable broader forms or phases to be measured (e.g. `bioavailable' forms of elements) and which are, in most cases, sufficient for the purpose of environmental policy (Quevauviller, 1993, 1998). These procedures have been widely used for determining specific chemical forms of heavy metals in a range of environmental media (Stephens, *et al*., 2001; Steve, *et al*. 2001; Guevara-Riba, *et al*. 2004; Yuan, *et al*. 2004).

1.4.1.1. Sequential extraction schemes

Sequential extraction schemes have been described by several workers (Forstner, *et al*. 1981) for sediments, where consecutive leaching techniques allow us to obtain information about the mobilities of major and trace constituents under different environmental conditions such as acidic or alkaline, oxidizing or reducing behaviour, the action of chelating agents, and so on. Subjecting sediment samples to

multiple extractions has not been proved as an attractive alternative to sequential extraction; possibly because the latter approach tends to more readily identify the sediment components responsible for retaining the majority of elements of interest. The most appropriate sequence can be determined by the type of sediment being examined, the horizon involved, the elements of interest and so on (Pickering, 1986).

Sequential extraction procedures have been applied successfully to evaluate the proportion of metals bound to different phases of the sediment matrix (Pempkowiak, *et al*. 1999; Forstner and Witman, 1981; Luoma and Bryan, 1981; Trefry and Metz, 1975; Wallace, 1982; Forstner, 1989). This procedure allows obtaining the speciation of metals in the following fractions: (a) loosely adsorbed to the surface of sediment particles; (b) bound to iron and manganese hydroxides; (c) complexed by organic matter; and (d) incorporated into clay mineral lattices (Forstner and Witman, 1981; Forstner, 1989). Metal fraction (a) is considered to be the most bioavallable and important fraction (d) the least, if at all, bioavailable (Forstner, 1989).

1.4.1.1.1. BCR sequential extraction

The Standards, Measurements and Testing Programme-SM&T (formerly BCR) organized a series of inter-comparisons on extractable trace metal determination and a workshop on Sequential Extraction in Sediments and Soils (held in Spain in 1992) to discuss results of the inter-comparisons and to establish a common procedural scheme (Ure, *et al.,* 1993; Quevauviller, *et al.* 1993; Quevauviller, *et al*. 1996; Mester, *et al*., 1998; Recchia, *et al*. 2008). This project led to the optimization of a 3-step procedure (Ure, *et al*. 1993), a purely operational definition of sequential fractionation, in which the analytical significance of assay results was tied to the use of specific extraction procedures (Quenvauviller, *et al*. 1992; Ure, *et al.* 1993; Quenvauviller, *et al.* 1994) and to the production of a Certified Reference Material

(CRM 601), certified for the content of Pb, Ni, Cr, Cd and Zn in the three fractions resulting by applying the 3-step procedure (Quevauviller, *et al*. EUR report, in press).

The fractions of metals individuated by the 3-step procedure are the exchangeable/carbonatic fraction, the easily reducible fraction, and the oxidisable fraction (Mester, et al. 1998) plus a fourth final (residual fraction) stage as summarised in Figure 1. The BCR sequential extraction procedure can be carried out for the following trace metals: Cd, Cr, Cu, Ni, Pb and Zn. BCR601 was later replaced by BCR701 (Pueyo, *et al*. 2001), which included Cu on the list of certified metals. Figure 1 shows an overview of BCR sequential extraction scheme for metals.

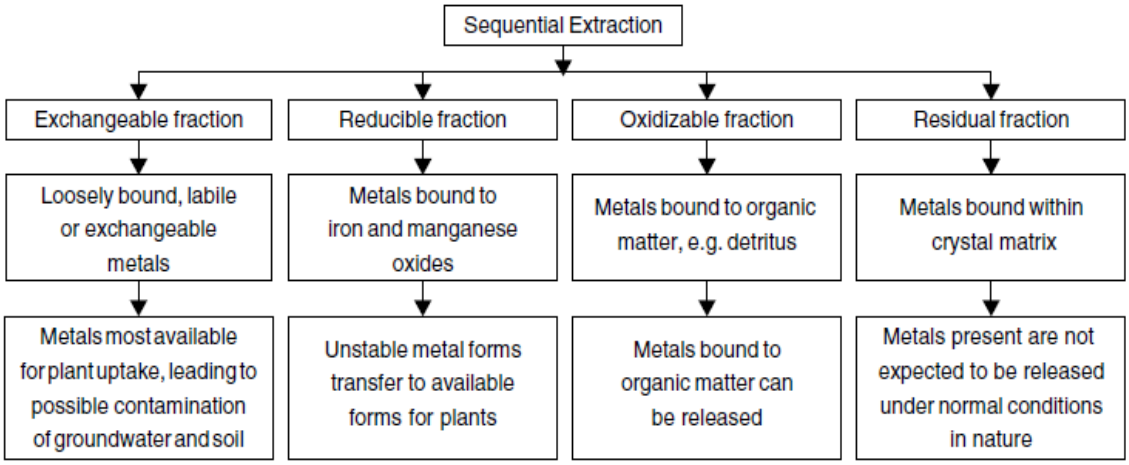


Figure 1: Overview of the BCR sequential extraction for metals, as applied to the analysis of soils and sediments (**Source:** Methods of environmental trace analysis, Dean 2003, p. 84)

1.4.1.2. Single extraction schemes

In single extraction schemes, different extracting solutions are used to assess each fraction separately. A large number of single extraction methods have been proposed in the past few decades for determination of "plant available" metals in soils and sediments (Haq and Bates, 1980; Gupta and Aten, 1993; Novozamsky, *et al*. 1993).

Mobile metal ions are the proportion of total metal ions that are available for uptake by biota. The mobility of the metals and their bioavailability are related to eco-toxicity to the plants which depend strongly on their specific chemical forms; exchangeable and carbonate bound (Fuentes, *et al*. 2006). Thus, it is of utmost importance to predict bioavailable metals rather than the total metals in order to assess toxic effects and to study bio-geochemical pathways.

The mobilisable fraction which is usually assessed by complexing agents DTPA + $CaCl_2$ + TEA (pH 7.3); NH_4OAc + EDTA (acidified) or EDTA (pH 7) is suitable to predict potentially active metal fraction as shown in Table 2 (Gupta, et al. 1996). Low Molecular Weight Organic Acids (LMWOA)-based extractions e.g. 0.11M CH_3COOH are also suitable (Gupta, *et al*. 1996, Rauret, *et al*. 1999). Soluble, exchangeable and chelated metal species are quite mobile and mobilisable fractions hence are more available for plants and the food chain (Kabata-Pendias, 1993).

1.4.1.2.1. Organic Acid-Extractable Metals (acid soluble)

This phase is made up of exchangeable metals and others bound to carbonates that are able to pass easily into the water column, for example, when the pH drops. It is the fraction with the most labile bond to sediment and, therefore, the most dangerous for the environment (Morillo, *et al*. 2007).

Several mechanisms contribute to the extraction of metals from soil using an acid solution: (1) desorption of metal cations via ion exchange; (2) dissolution of metal compounds; and (3) dissolution of soil mineral components (e.g., Fe–Mn oxides) which may contain metal contaminants (Tampouris, *et al*., 2001; Kuo, *et al*., 2006). The pH of an extractant plays a significant role in the extractability of heavy metals from sediments. At low pH, the protons (H+) added can react with sediment surface

sites (layer silicate minerals and/or surface functional groups including, e.g., Al-OH, Fe-OH, and COOH groups) and enhance desorption of metal cations, which are transferred into the washing fluid (Isoyama and Wada, 2007).

The acidity induces dissolution of hydroxides and carbonates and increases overall metal solubility and mobility. Extraction with 0.11M acetic acid is the first step (for labile and easily available metals) of a three stage sequential extraction procedure has been recommended by the European Community Bureau of Reference (BCR; Davidson, *et al*. 1994, 1998). Low molecular weight organic acid (LMWOA) solutions other than the ones based on acetic acid have not yet been evaluated (Meers, *et al*. 2007). Wang, *et al*. (2003) reported that single extraction procedure using low molecular weight organic acids (LMWOAs) gave good correlation between extractable metals in soil and the ones taken up by maize roots, indicating their suitability for the assessment of mobile metals.

Acetic acid extraction method is one of the weakest chemical treatments that can be used to remove effectively the weakly bound part of the total metal concentrations in sediments (Loring, 1978; Loring and Rantala, 1988) and particulate matter (Loring and Asmund, 1989). 0.11M Acetic acid removes metals held in ion exchange positions, easily soluble amorphous compounds of iron and manganese, carbonates and those metals weakly held in organic matter.

It leaves the silicate lattices intact and does not attack the resistant iron and manganese minerals or organic compounds. The proportion of the total metal concentration *removed* by the extraction is operationally defined as the non-detrital (acid soluble) metal fraction of the sediment. The proportion of a metal *remaining* in the residual fraction is operationally defined as the detrital (acid insoluble) fraction of the material. Such fractionation allows some deductions as to the carriers, transport

mode and potential bio-availability of metals entering and within these different systems (Loring, 1981).

1.4.1.2.2. Neutral unbuffered salt-extractable metals (exchangeable metals)

Weakly adsorbed metals are considered to be labile and available for plant uptake (Kabata-Pendias, 1993). A number of extractants have been proposed to assess metal exchangeability. Unbuffered salt solutions (e.g., $CaCl_2$, $NaNO_3$, and NH_4NO_3) are widely used for the extraction of exchangeable metals (Hudson, *et al*., 2000; Novozamsky, *et al*. (1993) suggested that monovalent cations (Na^+, NH_4^+) are practically non-competitive or less competitive for the adsorption sites on organic matter, clay minerals and oxides playing an important role in ion-exchange reactions.

This, however, is not the case for Ca_2^+ and, consequently, Novozamsky, *et al*. (1993) preferred to use 0.01M $CaCl_2$ salt for prediction of bioavailable metals in soils and sediments. Houba, *et al*. (1996, 2000) elaborated on the benefits of this particular protocol. The ionic strength of the extractant is similar to that of typical soil solutions, with Ca being the predominant cation in the soil solution. Since the extraction solution is not buffered, the relevant interactions occur at the pH of the soil or sediment. The divalent cation also assures good coagulation of the colloidal material in the suspension, making higher salt concentrations such as required for monovalant cations (Na^+, K^+, or NH_4^+) unnecessary. The extractant is used in the Netherlands for assessment of nutrients and heavy metals in soils (Pueyo, *et al*., 2004). Brun, *et al*. (2001) also used the extraction protocol for assessment of metal availability to plants in analogy with Lebourg and Sterckeman (1996).

1.4.1.2.3. Complexant–extractable metals

Since chelating agents have the ability to form stable metal complexes over a wide pH range (Abumaizar and Smith, 1999; Peters, 1999; Sun, 2001; Finzgar and

Lestan, 2007), their use offers a promising approach for the extraction of metals from contaminated sediments. However, the removal of greater amounts of toxic metals has most often been observed at lower pH levels (Van Benschoten and Matsumoto, 1997).

Lindsay and Norvel, (1978) proposed a DTPA (diethylenetriaminepentaacetic acid) based extraction solution, buffered at pH 7.3 to exclude effects involving carbonate dissolution. This protocol is also widely used, predominantly for phytoavailability studies and has been adopted in the French legislation (AFNOR, 1994) for estimation of available Cu, Fe, Mn and Zn. A large number of organic complexing agents have been used in literature, showing that diethylenetriaminepentaacetic acid (DTPA) was amongst the most effective extracting ligands for metals in soils (Norvell, 1984).

DTPA (diethylenetriaminepentaacetic acid), is used to release the mobilisable forms (complexed, adsorbed and carbonate forms). Lindsay and Norvell, 1978 chose DTPA because it offered favourable combination of stability constants for the simultaneous complexing of Fe, Zn, Cu and Mn and their objective was in part achieved by buffering the extractant in a slightly alkaline pH range including soluble Ca^{2+}. Triethanolamine (TEA) was selected as a buffer because of its pKa = 7.8. DTPA is especially the best among chelating agents for Fe and Mn (Norvell, 1972) and cations forming very stable water soluble complexes (Norvell, 1984). When applying the use of simple and sequential extractions, Fuentes, *et al*. (2004) discovered that the DTPA extraction procedure is cheap and easy to perform and the obtained results were similar to those obtained with the sequential procedure. Ettler, *et al*. (2007) found that although the DTPA procedure generally extracted the highest amounts of metals during their study on assessment of single extractions, it was unsuitable for highly organic acidic forest soils, where anionic metal–DTPA complexes are assumed

to be re-adsorbed on the positively charged surfaces of soil organic matter and oxides.

Stephens, *et al*., (2001) reported that DTPA extracts performed to illustrate plant-available metal concentrations in dredged canal sediments revealed that up to 40% of the total extracted metals were in an 'available' (mobile) form. In copper-contaminated vineyard soils, Brun, *et al*. (2001) found that extraction with organic complexing agent, DTPA seemed to provide a reasonably good estimate of root Cu concentration for maize.

In a comparative study of lakes polluted with Cd, Zn and Cu from a smelter at Flin Flon, Canada, Jackson, *et al*. (1993) found that the availability of heavy metals to sedimentary micro-organisms and aquatic food-chain organisms depended on the abundance of DTPA-extractable (bio-available) metal species in the sediments rather than total metal concentrations. They reported for instance that the toxic effects of Cd on heterotrophic sedimentary microbes were more strongly dependent on DTPA-extractable Cd than on total Cd concentrations in the sediments. They also discovered that although Cd was less abundant than Zn or Cu in the DTPA extractable metal pool, it had the strongest toxic effect on microbial activity, however, the relative toxicity (mobility) increasing in the order Zn < Cu < Cd.

1.5. Selection of extractants

The choices of extractants for comparison purposes are usually based on the extraction time (the rapidity of the method is important), simple preparation of the extraction media but, at the same time, higher efficiency of the extraction and use of unbuffered inorganic salt-based extractants, organic acid-based extractants and complexation-based extractants (Ettler, *et al*. 2007). Additional important parameters considered are the molar concentration of the extractant and liquid to solid ratio of the protocol (Meers, *et al*. 2007).

Basing the criteria for choices on the above facts, single extraction scheme was chosen for this study (Table 2) because of its suitability for the extraction of mobile metal ions. The extractants employed were the first step of BCR sequential scheme (0.11M acetic acid) for acid-soluble and easily removed metals (most bioavailable and dangerous) and 0.005M DTPA + 0.01M $CaCl_2$ + 0.1M TEA (pH 7.3) for compexant-extractable metals due to their numerous suitabilities in the mobilisation and release of metal ions from sediments. Table 3 describes some of the commonly used extraction media and metal fractions found in soils and sediments.

Table 2: Single extraction procedures chosen for this study

Extractant	Ratio: g/ml	Extraction time (h)	Reference
Complexant-based extractions 0.005M DTPA + 0.01M $CaCl_2$ + 0.1M TEA (pH 7.3)	1/5	2	Sahuquillo, *et al*., 2003; Lindsay and Norvell, 1978; Hudson, *et al*., 2000; Ettler, *et al*., 2007; Cacador, *et al*., 1996
Organic acid-based extraction 0.11M CH_3COOH (BCR1)	1/40	16	Pickering, 1986; Rauret *et al*., 1999

Table 3: Commonly extraction media used and metal fractions found in soils and sediments

Fraction in sediment	Characteristic of fraction	Commonly used extracting media
Pseudo total	Non-active + potentially active (mobilisable)	Strong acid solutions: aqua ragia, boiling HNO_3, HCl
Mobilisable	-Potentially active + active -potentially bioavailable or potentially leachable	Complexing agent solution Buffered and unbuffered complexing and chelating reagents like NH_4OAc + EDTA, EDTA, acetic acid DTPA + $CaCl_2$
Mobile	Active (bioavailable and easily leachable)	Neutral unbuffered salt solutions like $NaNO_3$, $CaCl_2$, NH_4NO_3
Immobile	Pseudo total-mobilisable(non active)	See pseudo total and mobilisable

Source: Gupta, et al. (1996)

1.6. State of the art

From the scientific literature consulted, only one related study had been reported in Algeciras Bay by the time this study was being carried out. Morillo, *et al.* (2007) studied potential mobility of metals in polluted sediments from the same ecosystem using BCR sequential extraction method and reported that urban sewage and industrial zones were the main possible sources of metals in Algeciras Bay. This study looked at total metals and assessment of environmental conditions that were potentially conducive for the release of weakly sediment-bound (mobile) and mobilisable (potentially active) metals into the water column using single extraction methods employing BCR step 1 (0.11M acetic acid) and complexant-based (0.005M DTPA + 0.01M $CaCl_2$ + 0.1M TEA). The two extractants represented the water conditions below which the sediments underlie. The work of Morillo, *et al*. (2007) was quite important as a baseline data with which the results of this study were compared, especially for total metals and the BCR step 1.

2.0. MATERIALS AND METHODS

2.1. Study Area

Algeciras Bay is an important industrial area on the southern Spanish coast, located on the south-western Spanish Mediterranean Sea coast between 36^0 6' 0'' and 36^0 11' 0'' North and 5^0 26' 45'' and 5^0 21' 0'' West, very near to the city of Algeciras and strait of Gibraltar. The Bay is about 10 km wide in the interior and 8 km mouth, covering an area of about 75 square kilometres, with a depth of up to 400 m in the centre of the Bay.

The main rivers draining into the bay are rivers Guadarranque and Palmones. Large amounts of pollutants are discharged into the bay from the major population centres such as the city of Algeciras and Gibraltar from which the bay receives heavy urban sewage. The bay is also impacted by intense harbour activities from the port of Algeciras, which is one of the largest ports in Spain. Waters connected to the bay are; Strait of Gibraltar, Mediterranean Sea and Atlantic Ocean. A number of important industries have been built around the coast of Algeciras Bay, including stainless steel manufacturing plant, petrochemical and petroleum refineries, paper mills, thermal power plants, ironworks, shipyards, piers and breakwaters (Tarazona, *et al*. 1991; Carballo and Naranjo, 2002; Morillo, *et al*. 2007;).

The Bay's water has a high turnover because of its proximity to the Strait of Gibraltar, where the Mediterranean Sea and the Atlantic Ocean meet, and the strong currents that predominate in the area. These factors, together with its deep waters, mean that pollutants are dispersed in a large body of water, and their effects are considerably diminished (Morillo, et al. 2007).

However, sediments within the bay are likely to contain matter which arrives naturally, from the rivers (e.g. rivers Guadarranque and Palmones), and from man-made sources such as wastewater discharges from towns (Figure 2) and industries located around the bay and from maritime traffic within the bay. Due to all these facts, marine pollution is likely to be a serious problem in the bay. Table 4 describes the characteristics of the sampling points in Algeciras Bay. It is therefore important to raise concern about the risks of pollutants such as heavy metals, particularly in the light of the bay's ecological importance.

Figure 2: Sewage discharge point near Algeciras Mayorga Bridge

2.2. Materials

All laboratory glassware and plastic ware, low density polyethylene (LDPE) sample and reagent bottles were cleaned by soaking in a detergent solution, rinsed with ultra pure water from a Millipore Milli Q^{50} system (15.0MΩ cm resistivity TC) and soaking in a HNO_3 (2 %, v/v) bath overnight. This was followed by thorough rinsing with ultra pure water and dried before use.

Table 4: Characteristics of the sampling points in Algeciras Bay

Area	Sampling point	Potential source of metal pollution
1. Area adjacent to natural ecosystems' catchment	1.2	Least affected by human impact and considered close to pristine in Algeciras Bay, therefore could be used to establish pre-anthropogenic background concentrations of metals ions in sediments or as control.
2. Green Island and its surrounding	2.1, 2.2	Raw urban sewage disposal from the city of Algeciras and maritime traffic from port of Algeciras
3. Around the port of Algeciras and city of Algeciras.	3.1,3.2,4.1,4.2	Heavy maritime traffic from the port of Algeciras and raw urban sewage disposal from the city of Algeciras
4. Around Palmones industrial zones and the mouth of River Palmones.	5.1,5.2,6.1,6.2	Effluents from Acerinox stainless steel manufacturing plant and fluvial sources from river Palmones.
5. Around Guadarranque industrial zones and the mouth River Guadarranque; and around Mayorga bridge.	7.1,7.2,8.1,8.2	Effluents from power station, petrochemical and petroleum refineries near San Roque and fluvial sources from river Guadarranque.
6. Around the shipyard and fisheries harbour near Gibraltar.	9.1,9.2,10.1	Pollution input from the shipyard and fishing activities.

2.2.1. Chemicals and Reagents

All reagents were of Merck analytical grade or super pure quality. Standard working solutions of the elements analyzed were prepared from the corresponding 1000 mg/l Merck stock solutions using appropriate solutions employed in the digestion and extraction procedures as matrices. All standard reagent solutions were stored in low density polyethylene (LDPE) bottles.

2.2.1.1. Chemicals

- 1000 mg/l As stock standard solution, super pure, Merck, Germany.
- 1000 mg/l Ba stock standard solution, super pure, Merck, Germany.
- 1000 mg/l Cd stock standard solution, super pure, Merck, Germany.

- 1000 mg/l Co stock standard solution, super pure, Merck, Germany.
- 1000 mg/l Cr stock standard solution, super pure, Merck, Germany.
- 1000 mg/l Cu stock standard solution, super pure, Merck, Germany.
- 1000 mg/l Fe stock standard solution, super pure, Merck, Germany.
- 1000 mg/l Ni stock standard solution, super pure, Merck, Germany.
- 1000 mg/l V stock standard solution, super pure, Merck, Germany.
- 100 mg/l Mn stock standard solution, super pure, Merck, Germany.
- 1000 mg/l Zn stock standard solution, super pure, Merck, Germany.

  Calcium Chloride Dihydrate ($CaCl_2.2H_2O$), Pro Analyze, Merck, Germany
- Diethylenetriaminepentaacetic acid, also known as [(carboxymethyl)imino]bis (ethylenitrilo)tetraacetic acid, DTPA ($[(HOCOCH_2)_2NCH_2]_2NCH_2COOH$), Pro Analyze, Pancreac Chemicals SA, Barcelona, Spain.
- Glacial Acetic Acid, 100% (CH_3COOH), Pro Analyze, Germany.
- Hydrochloric Acid, 37% (HCl), Pro Analyze, Merck, Germany.
- Hydrofluoric Acid, 48% (HF), Pro Analyze, Merck, Germany.
- Nitric Acid, 65% (HNO_3), Pro Analyze, Merck, Germany.
- Nitric Acid, 65% (HNO_3), Super Pure, Merck, Germany
- Silica gel
- Triethanolamamine, $(HOCH_2CH_2)_3N$ (TEA), Pro Analyze, Merck, Germany.
- Glacial Acetic Acid, 100% (CH_3COOH), Pro Analyze, Germany.
- Hydrochloric Acid, 37% (HCl), Pro Analyze, Merck, Germany.
- Hydrofluoric Acid, 48% (HF), Pro Analyze, Merck, Germany.
- Nitric Acid, 65% (HNO_3), Pro Analyze, Merck, Germany.

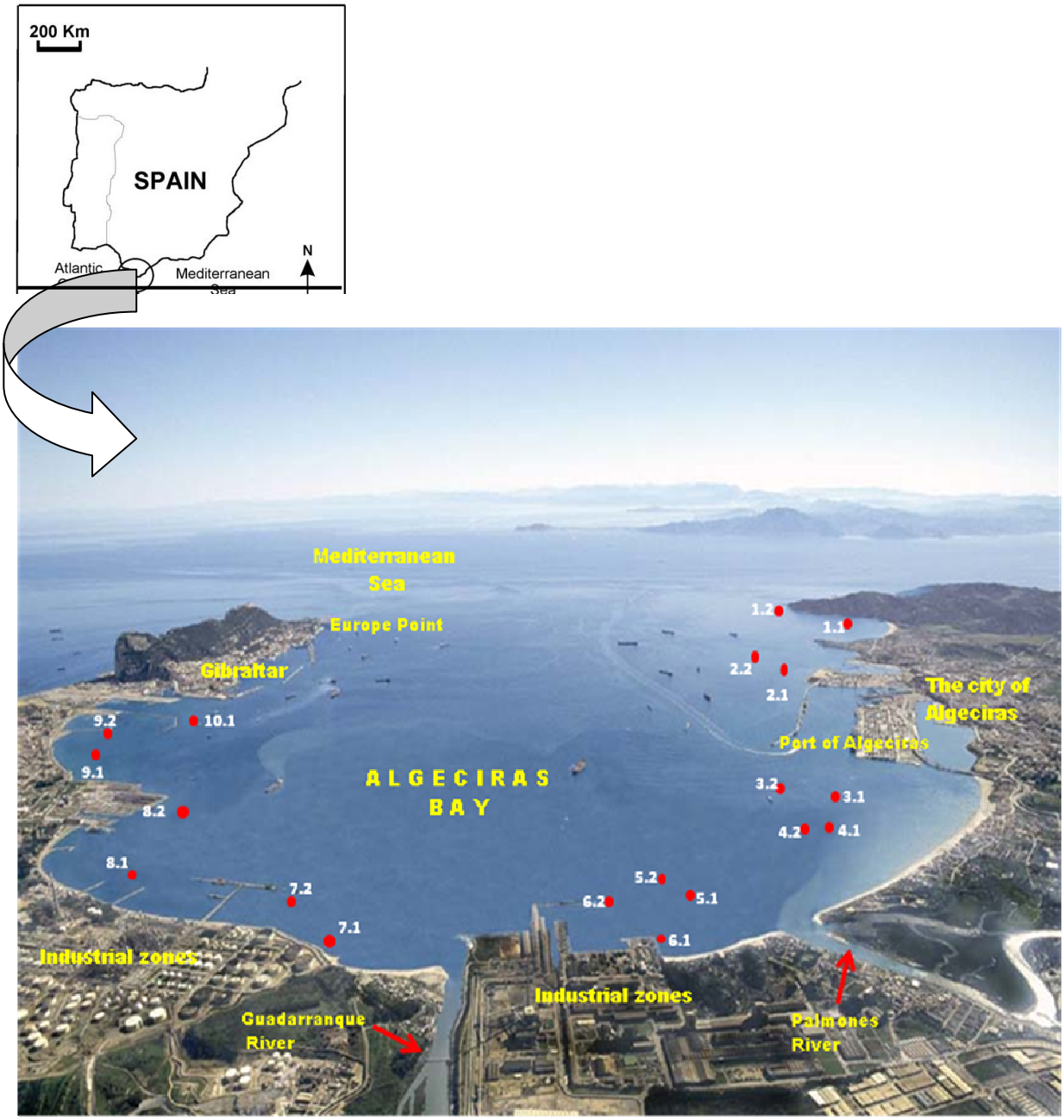


Figure 3: The map of Algeciras Bay showing sampling sites, major rivers and industrial zones

2.2.1.2. Reagents

All reagents were of super pure and pro analyze quality from Merck, Germany and Pancreac, Spain respectively. Ultrapure water from a Millipore Milli Q^{50} system (15.0MΩ cm resistivity TC) was always used. All reagents were prepared under clean laboratory environment in class 100 laminar flow cabinet, with filtered atmosphere at 0.45µ. Acids and other fuming reagents were preparing in the OR – ST 1500 fume chamber.

0.11M *acetic acid*: In OR-ST 1500 fume chamber, 25 ± 0.75ml of acetic acid was added to about 500mL of ultra pure water and made up to 1000mL with the same water to obtain 0.43M acetic acid solution. 250ml of this solution (acetic acid 0.43M) was diluted to 1000ml to obtain 0.11M acetic acid solution.

0.005M DTPA + 0.01M $CaCl_2$ + 0.1M TEA: 14.92g of TEA, 1.967 of DTPA, and 1.47g $CaCl_2.2H_2O$ were dissolved in approximately 20ml of ultra pure water. Sufficient time was allowed for the DTPA to dissolve and then diluted to approximately 900ml. The pH was adjusted to 7.30 ± 0.05 with 1N HCl using CRISON Micro pH 2001 pH meter while stirring then diluted to 1000ml. The solution was stable for several months.

2% HNO_3: This solution was prepared by dissolving 30.8 ml of nitric acid in 1000ml ultra pure water.

1% HNO_3: 1.54 ml of nitric acid was dissolved in 100 ml ultra pure water. The solution was equivalent to 1% HNO_3.

1N HCl: 1N HCl was prepared by dissolving 85 ml hydrochloric acid in 1000ml ultra pure water.

3.86. M HCl: 328 ml hydrochloric acid was dissolved in 1000 ml ultra pure water. The solution was equivalent to 3.86M HCl.

2.2.2. Apparatus and equipment

2.3.2.1. Apparatus

- Agate mortar and pestle
- $Cruma_{SA}$ Laminar Flow
- Desiccator
- Erlenmeyer flasks: 50, 100 ml capacity
- Flat bottom crucible
- Glass beakers: 1000 ml capacity
- Glass measuring cylinders: 25, 50, 100 ml capacity
- Glass volumetric flasks: 25, 50, 100, 1000 ml capacity
- Micro pipettes: 100 µl, 1ml, 5 ml.
- Nylon sieves (2000, 500, 63 µm diameter)
- Polypropylene bottles
- Polypropylene centrifuge tubes
- Polypropylene volumetric flasks: 25, 100 ml capacity
- Teflon evaporating dishes
- Teflon measuring cylinder: 25 ml capacity

2.2.2.2. Equipment

- Class 100 laminar flow cabinet
- CRISON Micro pH 2001 pH meter
- End-over-end mechanical shaker
- X-Series ICP-MS from Thermo Elemental, UK.
- Jenway 1000 hot plate and stirrer

- Mettler AE Analytical balance
- Nabertherm® Muffle furnace made in Germany
- Perkin-Elmer AAnalyst 800 FAAS
- P Selecta Drying oven
- Sigma 4K10 Centrifuge

2.3. Sampling

Seventeen (17) sampling points were located within the bay at 1.2, 2.1, 2.2, 3.1, 3.2, 4.1, 4.2, 5.1, 5.2, 6.1, 6.2, 7.1, 7.2, 8.1, 9.1, 9.2 and 10.1 as shown in Figure 3. The descriptions of the characteristics of sampling points are given in Table 5. Prior to sampling, containers and glass or plastic parts associated with the sampling equipments were cleaned with detergent and then rinsed with ultra pure water from a Millipore Milli Q^{50} system (15.0 MΩ cm^{-1} resistivity TC). Surface sediment samples were collected using Eckman-Birge grab and put in LDPE sample bottles. Since sediments usually contain iron and organic compounds that are easily oxidized, the samples were stored in a cool box at 4 ^{0}C and quickly frozen at -20ºC within 6 h after sampling until analysis (Vicente-Martorell, *et al.* 2008).

2.4. Experimental

2.4.1. Sample pre-treatment

The samples, which were previously deep frozen at approx. - 20^0 C in order to limit biological and chemical activities, were removed from the freezer, thawed and placed in glass Petri-dishes then dried in an oven at 40^0C until total dryness. The dried samples were ground using an agate mortar and pestle then sieved through clean nylon sieve of < 2mm mesh size to remove coarse material such as pebbles, coarse organic pebbles and shells.

The remaining material was sieved through a nylon sieve of <0.063 mm mesh size to obtain the fine particle-size fraction (Loring and Rantala, 1992; Tokalioglu, *et al*. 2003 and Vicente-Martorell, *et al*., 2008, 2009). The <0.063 mm fraction of sediments were used for the analyses of mobile metals due to the strong association of metal ions with fine-grained sediments (Moore, *et al.* 1989; Goh and Chou, 1997; Tam and Wong, 2000; Che, *et al*. 2003). The samples were then packed in LDPE sample bottles and stored in a cool dry place until analysis.

2.4.2. Determination of organic matter content (OM)

2.4.2.1. Principle

Organic matter content was estimated by measuring the loss of weight on ignition, the change in mass as a result of heating a sample under specified conditions. Loss on ignition method (LOI) is normally used as a qualitative measure of metals in sediments, and it is a very important parameter influencing the concentration of metals in sediments. The loss on ignition (LOI) is expressed as a weight percentage of the dry mass. A dried test sample is heated in a muffle furnace to constant mass at $(550 \pm 25)^0$ C. The difference in mass before and after the ignition process was used to calculate the loss on ignition {European Standard, TC WI: 2003 (E)}.

2.4.2.2. Procedure

The crucibles were placed in the Nabertherm® muffle furnace made in Germany and heated at $(550 \pm 25)^0$ C for at least 3 hrs. After cooling in the desiccator to ambient temperature, the crucibles were weighed to the nearest 1 mg, (*ma).* Into the crucibles, 0.5 g of the dried fine sediments were weighed to the nearest 1 mg, (*mb)* using Mettler AE analytical balance, and heated in the muffle furnace at $(550 \pm 25)^0$ C for at least 180 min. The hot crucibles containing the residue on ignition were removed from furnace and put in a desiccator to cool to ambient temperature.

After cooling, the crucibles containing the dry residue were weighed to the nearest 1 mg (*mc*). The muffle furnace was programmed as shown in Table 6.

Table 5: Time and temperature program for the Nabertherm® muffle furnace

Time 0	0 minutes	0 ^{0}C
Time 1	30 minutes	550 ^{0}C
Time 2	180 minutes	550 ^{0}C
Time 3	60 minutes	25 ^{0}C

2.4.2.3. Calculations for organic matter content

The loss on ignition of the dry mass of a solid sample expressed in percentage was calculated from the equation below.

$$W_V = \frac{(m_b - m_c)}{(m_b - m_a)} \text{ x } 100$$

Where:

W_V is the loss on ignition of the dry mass of a solid sample, in percentages;

m_a is the mass of the empty crucible, in grams;

m_b is the mass of the crucible containing the dry mass, in grams;

m_c is the mass of the crucible containing the ignited dry mass, in grams.

2.5. Sample preparation

2.5.1. Total Metal Content

Duplicates of 0.2g of fine particle-size sediments samples were weighed in 7.1 cm i.d. white Teflon plates using Mettler AE analytical balance. It was important to note the exact weights of the samples since the same weights were to be used for the calculations of the actual concentrations of metals. 5ml of 48% HF was added to each

sample using Teflon measuring cylinder and heated twice in the same reagent (48% HF) until complete dryness by IR radiation. 5ml of 65% HNO_3 was then added to the residue from the HF steps and heated twice (in 65% HNO_3) by IR radiation until complete dryness (Figure 4). These processes were performed in the OR – ST 1500 fume chamber.

The residues from the HNO_3 steps were dissolved in 20ml of 3.86M HCl and transferred into 50ml Erlenmeyer flasks and then heated with constant stirring for 1h at $85^0C \pm 5^0C$ using Jenway 1000 (heater + stirrer). After 1h, the samples were removed from the heater and left to cool to room temperature and then filtered by gravity through 110mm i.d. Filter-Lab 1238 filter papers into 50ml volumetric flasks. The Erlenmeyer flasks and filter papers were rinsed thoroughly into the volumetric flasks and made to 50 ml with ultra pure water. The sample solutions were transferred into 50 ml LDPE sample bottles and stored at 4 ^0C in the refrigerator until analyses.

2.5.2. Extraction with 0.11M acetic acid

40ml of 0.11M acetic acid was added to duplicates of 1g dry fine sediment in 100ml polypropylene centrifuge tubes. The mixture was shaken mechanically at a speed of 150 rpm for 16 h at 22 ± 5^0C (overnight) on an end-over-end shaker. After shaking for 16 h, the extract was separated from the solid phase by centrifugation at 4000 rpm for 30 minutes at 4^0C using Sigma 4K10 centrifuge then the supernatants were removed using a 5 ml micro-pipette into a polyethylene bottle. The bottles were tightly closed and stored in a refrigerator at about 4^0C until analysis.

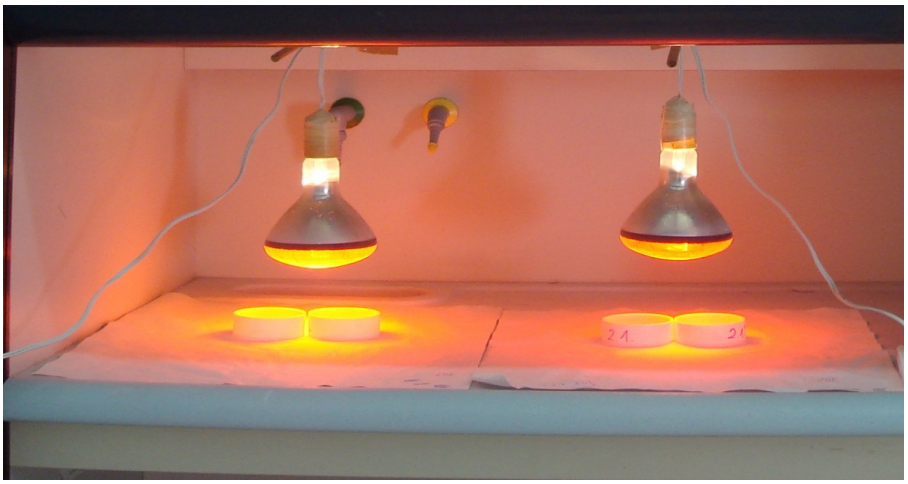


Figure 4: The heating process by IR radiation in OR-ST 1500 fume chamber

2.5.3. Extraction with 0.005M DTPA + 0.01M $CaCl_2$ + 0.1M TEA

The extraction was performed according to the original procedure (Lindsay and Norvell, 1978). 15ml of the extractant was added to 3g of fine sediment in 100ml Erlenmeyer flask and covered with stretchable Parafilm and mechanically shaken on an end-over-end shaker for 2 h at a speed of 120 rpm at room temperature. The supernatants were filtered by gravity through 110mm i.d. Filter-Lab 1238 filter papers from Filtros Anoia, S.A. Barcelona. The extracts were acidified with a drop of concentrated super pure HNO_3 to prevent adsorption to the polyethylene of the storage vessel and growth of bacteria. The acidified supernatants were put in LPDE sample bottles and stored at 4 ^{0}C until analysis.

2.5. 4. Determination of method blank

Reagents' blanks were carried out through the entire sample preparation procedure. Blank determinations for total and mobile metals were carried out in the same manner as the samples using the same acid concentrations and extractants.

2. 6. Calibration

2.6.1. Calibration standards

Considering that the composition of the samples were unknown, a qualitative scan was run prior to the preparation of standards. The standards were prepared in similar matrix to those of the samples to avoid compromising the accuracy of the results. External calibration in which commercially produced stock standards are used to create calibration curves were used to calculate the concentrations of the analytes. The advantage of using commercially produced standards to generate calibration curves is that their concentration is well characterized. In addition, they allow for the preparation of solutions that cover the compositional range in the unknown samples. Super pure individual standard stock solutions (stock A; 1000 mg/l) were purchased from Merck, Germany and stored at 4^0C.

2.6.2. Multi-element stock standard (stock B) for total metals

A combined stock B standard solution containing 20ppm Co, Cu, As and Pb; 40ppm Ni and V; 80ppm Zn and Cr; 200ppm Ba; were prepared from individual 1000 mg/l stock solutions (stock A) by pipetting 0.5, 1, 2 and 5 ml of each respectively and 0.4ppm Cd by pipetting 0.5 and 1ml from 20 ppm diluted from individual 1000ppm stock A solutions into a 25 ml volumetric flask and making the volume to 25 ml with ultra pure Milli-Q water. The multi-element stock B standard containing 250ppm Fe and 100ppm Mn was prepared from the individual 1000ppm stock A solutions by pipetting 2.5ml and 1ml Fe and Mn respectively into a 50-ml volumetric flask and made to the mark with ultra pure Milli-Q water.

2.6.3. Multi-element working standards for total metals

Five working standards for multi-element analysis of total metals were prepared

from the combined stock solution (stock B) by pipetting 125 µl, 250 µl, 500 µl, 1 ml and 2 ml in100 ml volumetric flasks. 5 ml of 3.86M super pure HCl was added into each volumetric flask and made to the 100 ml with ultra pure Milli-Q water. Five multi-element working standards for Fe and Mn were prepared from stock B by pipetting 0.5 µl, 1 ml, 2 ml, 4 ml and 8 ml into 50 ml volumetric flasks and dilutions made as above. The resulting concentrations of the working standards are given Table 6. The reagent blank for the standards was prepared by pipetting 5ml 3.86M super pure HCl into a 100ml volumetric flask and making the volume to 100ml with ultra pure water.

Table 6: Multi-element working standard concentrations for total metals

Multi-element working standard concentration (ppb); Fe and Mn (ppm)												
Std.	**Cd**	**Ba**	**Zn**	**Cr**	**Ni**	**V**	**Co**	**As**	**Pb**	**Cu**	**Fe**	**Mn**
1	0.5	250	100	100	50	50	25	25	25	25	2.5	1
2	1.0	500	200	200	100	100	50	50	50	50	5	2
3	2.0	1000	400	400	200	200	100	100	100	100	10	4
4	4.0	2000	800	800	400	400	200	200	200	200	20	8
5	8.0	4000	1600	1600	800	800	400	400	400	400	40	16

2.6.4. Multi-element stock standard (stock B) for mobile metals in 0.11M acetic acid and 0.005M DTPA + 0.01M $CaCl_2$ + 0.1M TEA extracts

A combined stock B standard solution for mobile Co, Cu, As, Pb, Ni, V, Zn, Cr, Ba and Cd, containing same concentrations as those of total metals was prepared in the same manner as for the total metals. Stock B standard solution for Fe and Mn was also prepared in the manner as for total metals.

2.6.5. Multi-element working standards for mobile metals 0.11M acetic acid and 0.005M DTPA + 0.01M $CaCl_2$ + 0.1M TEA extracts

Six working standards for multi-element analysis of Co, Cu, As, Pb, Ni, V, Zn, Cr, Ba, and Cd in 0.11M acetic acid and 0.005M DTPA + 0.01M $CaCl_2$ + 0.1M TEA extracts respectively were prepared from the combined stock solution (stock B) by pipetting

25 µl, 50 µl, 100 µl, 200µl, 400µl and 800µl into a 50 ml volumetric flask and making the volume to 50 ml with the respective extractant solutions. Five working standards for Fe and Mn in 0.11M acetic acid extracts were prepared from stock B by pippeting 0.25 µl, 0.5 µl, 1 ml and 2 ml into 50 ml volumetric flasks and made to the 50 ml with 0.11M acetic acid solution. The resulting concentrations of the working standards are given Table 7. Reagent blanks for the standards were determined using individual extractant solutions. Likewise, five working standards for Fe and Mn in 0.005M DTPA + 0.01M $CaCl_2$ + 0.1M TEA extracts were prepared from stock B by pippeting 0.4 µl, 0.8 µl, 1.6 ml, 3.2 ml and 6.4 ml into 50 ml volumetric flasks. The resulting working standard concentrations are given in Tables 5 and 6.

Table 7: Multi-element working standard concentrations for 0.11M acetic acid and 0.005M DTPA + 0.01M CaCl2 + 0.1M TEA extracts

	Multi-element working standard concentration (ppb)									
Std.	**Cd**	**Ba**	**Zn**	**Cr**	**Ni**	**V**	**Co**	**As**	**Pb**	**Cu**
1	0.2	100	40	40	20	20	10	10	10	10
2	0.4	200	80	80	40	40	20	20	20	20
3	0.8	400	160	160	80	80	40	40	40	40
4	1.6	800	320	320	160	160	80	80	80	80
5	3.2	1600	640	640	320	320	160	160	160	160
6	6.40	3200	1280	1280	640	640	320	320	320	320

Table 8: Multi-element working standard concentrations for Fe and Mn in 0.11M acetic acid and 0.005M DTPA + 0.01M CaCl2 + 0.1M TEA extracts

	0.11 acetic acid (ppm)		**0.005M DTPA + 0.01M $CaCl_2$ + 0.1M TEA (ppm)**	
Standard	**Fe**	**Mn**	**Fe**	**Mn**
1	1.25	0.5	2	0.8
2	2.5	1	4	1.6
3	5	2	8	3.2
4	10	4	16	6.4
5	20	8	32	12.8

The actual concentrations of total and mobile metals were calculated using the following formula:

3.6.6. Calculation of concentrations (mg kg^{-1})

$$\text{Concentration (mg kg}^{-1}) = \left\{ \left[\frac{\text{Volume of sample (ml)}}{\text{Weight of sample (g)}} \right] \text{x Measured concentration (ppb)} \right\} /1000$$

2.7. Instrumentation

Multi-element analysis of total and mobile V, Cr, Co, Ni, Cu, Zn, As, Cd, Ba and Pb concentrations were performed using X-Series ICP-MS from Thermo Elemental, UK equipped with Xi interface and a concentric nebulizer to deliver a sample at the rate of 1 ml/min using ASX-510 autosampler from CETAC. The X-Series ICP-MS instrument operating parameters are given in Table 9. Perkin-Elmer AAanalyst 800 flame atomic absorption spectrophotometer (FAAS) with atomic absorption background correction (AA-BG) and Perk-Elmer multi-element (Cu-Fe-Mn-Zn) hollow cathode lamp was used for the analysis of total and mobile Fe and Mn using a fuel-lean air-acetylene flame. The operational parameters for Perkin-Elmer AAanalyst 800 FAAS are given in Table 10.

2.8. Quality Control and Assurance (QC and QA)

All laboratory glassware and plastic ware (LDPE) sample and reagent bottles were cleaned by soaking in a detergent solution, rinsing with ultrapure water from a Millipore Milli Q^{50} system (15.0MΩ cm resistivity TC) and soaking in a HNO_3 (2 %, v/v) bath overnight. This was followed by thorough rinsing with ultra pure water and drying before use. Preparation of reagents and standards were performed under clean laboratory environments in Cruma$_{SA}$ Class 100 Laminar Flow Cabinet (2.2.1.2).

The X-Series ICP-MS was calibrated using ^{71}Ga, ^{103}Rh and ^{209}Bi as internal standards in order to minimize interferences due to matrix effect and baseline drifts. Prior to sample analyses, the instrument was optimized using blanks spiked with In

Table 9: X-Series ICP-MS operating parameters

Parameter	Total Metals	DTPA extracts	Acetic acid extracts
Ext.	-220	-220	-220
L1	2	2	2
Focus	4	9	9
D1	-29.8	-16.5	-16.5
PB	-9	7	7
Hex.	-10	-10	-10
Nebulizer	0.79	0.79	0.79
Depth	80	100	100
L2	-34.5	-34.5	-34.5
L3	-75.3	-75.3	-75.3
Fwd	1400	1400	1400
Hor	80	80	80
Vert.	290	290	290
D2	-90	-90	-90
DA	-30.2	-30.6	-30.6
Cool	14	14	14
Aux	0.8	0.8	0.8
CCT1	0	0	0
CCT2 (NH_3/He)	4	1.7	1.15

Table 10: Perkin-Elmer AAanalyst 800 FAAS operating parameters

	Element	
	Fe	Mn
Wavelength (nm)	248.3	279.5
Slit width (H)	0.2	0.2
Time (s)	3	3
Delay time (s)	0	0
Lamp current (mA)	15	15
Air flow-rate (l/min)	17	17
Acetylene flow-rate (l/min)	2	2

and Ur, during which the recoveries were found to be in the acceptable range of < 20 ppb, while solutions of CeO/Ce and Ce^{2+}/Ce were used to reduce the risks of interferences to the expected < 2% and < 3 % respectively when checking interferences due to oxides and doubly charged elements. Collision cell technology (CCT) was applied in order to minimize the risks of polyatomic interferences. The Perkin-Elmer AAanalyst 800 FAAS was calibrated by rotating the burner until the linear range for the determination Fe and Mn was automatically obtained.

The QC standards, blanks and samples were run in triplicate between each 10 sets of samples to check precision of the methods. The average values of % RSD

obtained from the triplicate samples were generally < 10% and were considered satisfactory for environmental analysis as recommended by Morillo, *et al*. (2004 2007), except Cd and As, which had a wide spread (21-61 and 11-15% RSD respectively). Although Cd is one of the most important toxic elements to be determined in marine sediments; it has however, proved to be a difficult element to be determined with good precision and accuracy (Loring and Rantala, 1988). Calibration coefficients were confirmed to have at least three 9s before proceeding with samples (r = 0.999).

2.9. Method validation

Accuracy and repeatability of the analytical process for total metals were checked using estuarine sediment standard reference material® 1646a from National Institute of Standards and Technology (NIST), during which satisfactory accuracy and repeatability were realised, with recovery rates of between 84% and 161% as shown in Table 11. Standard reference material CRM® 701 was used to check the accuracy and repeatability of the analytical process for the 0.11M acetic acid extracts and gave good recoveries of between 91% and 132% (Table 12). Graphical representation of percent recoveries are given by Figures 5 and 6 respectively. No reference material was available for 0.005M DTPA + 0.01M $CaCl_2$ + 0.1M TEA; however, good recoveries of between 85% and 105% were obtained from samples spiked with known concentrations of metals studied, indicating that the method was accurate for the analyses of samples to proceed.

Table 11: LODs (3 * SD of blank), Blanks (± SD, n=10), Recoveries of estuarine sediment SRM® 1646a (±SD, n=6)

Metal	LOD(mg/kg)	Blank (± SD mg/kg)	Values (±SD) of SRM® 1646a (mg/kg)	Recovery (±SD, n=10) values (mg/kg)	% Recovery
V	1.26	-0.72±0.42	44.84 ± 0.76	43.75 ± 1.59	98
Cr	0.72	3.32±0.24	40.9 ± 1.9	37.05 ± 1.75	91
Co	0.09	0.01±0.03	5	4.7 ± 0.2	94
Ni	0.66	0.01±0.22	23	22.1 ± 0.8	96
Cu	0.51	0.08±0.17	10.01 ± 0.34	9.9 ± 0.5	99
Zn	0.96	0.59±0.32	48.9 ± 1.6	46.75 ± 1.84	96
As	0.87	0.08±0.29	6.23 ± 0.21	5.2 ± 0.8	84
Cd	0.24	0.03±0.08	0.148 ± 0.007	0.24 ± 0.11	161
Ba	0.42	0.05±0.14	210	211.78 ± 7.3	101
Pb	0.12	0.96±0.04	11.7 ± 1.2	11.8 ± 0.5	101

NB: SRM® 1646a was not analysed for Fe and Mn.

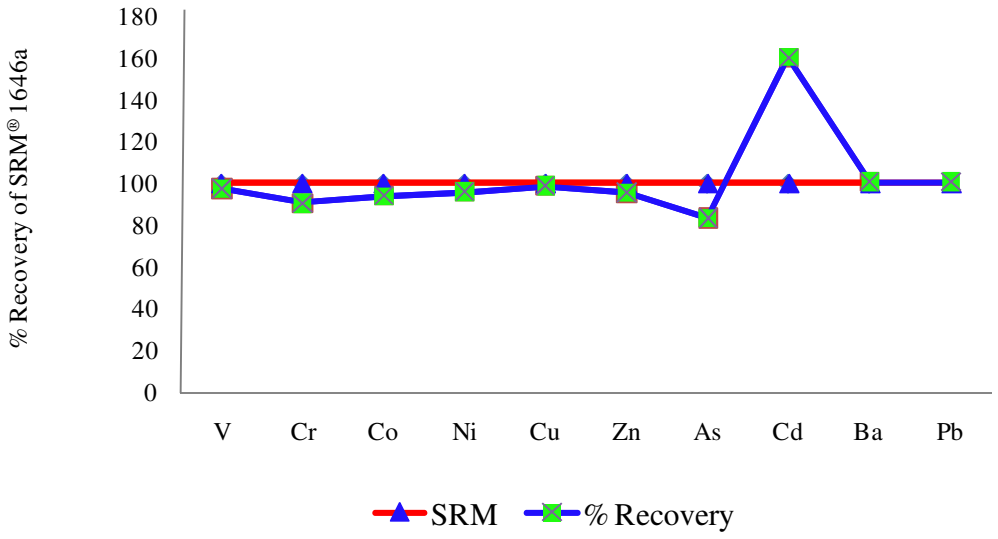


Figure 5: Method validation (accuracy and repeatability) for total metals showing % recoveries of estuarine sediment SRM® 1646a

Table 12: LODs (n=10), Blanks (± SD, n=10), Recoveries of CRM BCR® 701 (±SD, n=6)

Metal	Blank		CRM BCR® 701	Recovery	
	LD	Blank (mg/kg)	certified value (mg/kg)	Recovered value (mg/kg)	% Recovery
Cd	0.12	0.00±0.04	7.34±0.35	7.39±0.35	101
Cr	3.27	0.33±1.09	2.26±0.16	2.99±0.16	132
Cu	0.6	0.00±0.19	49.3±1.7	63.05±1.70	128
Ni	1.26	0.34±0.42	15.4±0.9	16.13±0.90	105
Pb	0.6	1.5±0.2	3.18±0.21	2.90±0.21	91
Zn	24.9	54.4±8.3	205±6	190.63±5.99	93

NB: Co, As, Ba, V, Fe and Mn are yet to be certified by BCR for 0.11M acetic acid (BCR 1) extracts

2.10. Statistical analysis

The analytical results were compiled to form a multi-elemental database using Microsoft Excel 2007. Data statistical treatments were performed using STATISTICA version 7 software package (2004, Statsoft Inc., USA) and Sirius version 8.0 from Pattern recognition Systems (PRS), Bergen, Norway.

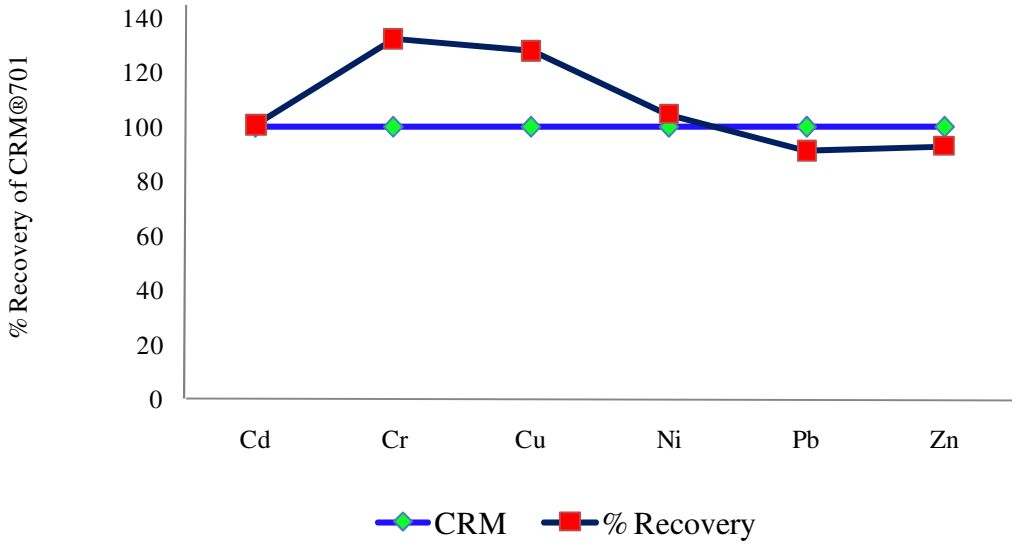


Figure 6: Method validation (accuracy and repeatability) for mobile (in 0.11M acetic acid extracts) metals showing % recoveries of CRM BCR®-701

3.0. RESULTS AND DISCUSSIONS

3.1. Total Metal and organic matter content

Total concentrations (mg/kg) of V, Cr, Co, Ni, Cu, Zn, As, Cd, Ba, Pb, Fe, Mn and organic matter content (%OM) in surface sediments from Algeciras Bay are given in Table 14, in which the mean concentrations of total metals at each sampling point and the averages of total concentrations of total metals and their ranges (minimum-maximum values) in the whole system are given. The distribution patterns of total metals are shown in Figures 9 and 10. The distribution of organic matter content is given Figure 8. The average concentrations of total metals and organic matter in Algeciras Bay were: V (66 mg/kg); Cr (112 mg/kg); Co (11 mg/kg); Ni (65 mg/kg); Cu (17 mg/kg); Zn (73 mg/kg); As (11 mg/kg); Cd (0.3 mg/kg); Ba (196 mg/kg); Pb (24 mg/kg); Fe (28129 (mg/kg); Mn (534 mg/kg) and OM (10 %).

The concentration ranges were: V (36-94 mg/kg); Cr (30-251 mg/kg); Co (5-22 mg/kg); Ni (19-144 mg/kg); Cu (5-25 mg/kg); Zn (33-117 mg/kg); As (8-23 mg/kg); Cd (0.1-0.7 mg/kg); Ba (93-263 mg/kg); Pb (12-39 mg/kg); Fe (12255-42756 mg/kg); Mn (234-967 mg/kg) and % OM (7-15%). It was observed that Cd had the lowest minimum-maximum and also the lowest range values, with Fe having the highest minimum-maximum and highest range values. Some of the concentration ranges were comparable to those found in other past studies such as: Co (18-95 mg/kg) reported by Morillo, *et al*., (2007) in Algeciras Bay; (52.3–179 mg/kg) reported by Marmolejo-Rodriguez, *et al*. (2007) at the pacific coast of Mexico. The ranges for Cd (0.17-0.59 mg/kg and 0.53-0.84 mg/kg); Pb (9.1-33 mg/kg and 4.6-35 mg/kg) reported by Morillo, *et al*. (2007) in Cadiz and Algeciras Bays respectively. Cr (2007) and Morrillo, *et al*. (2004) in Algeciras Bay and in Southwest Coast of Spain

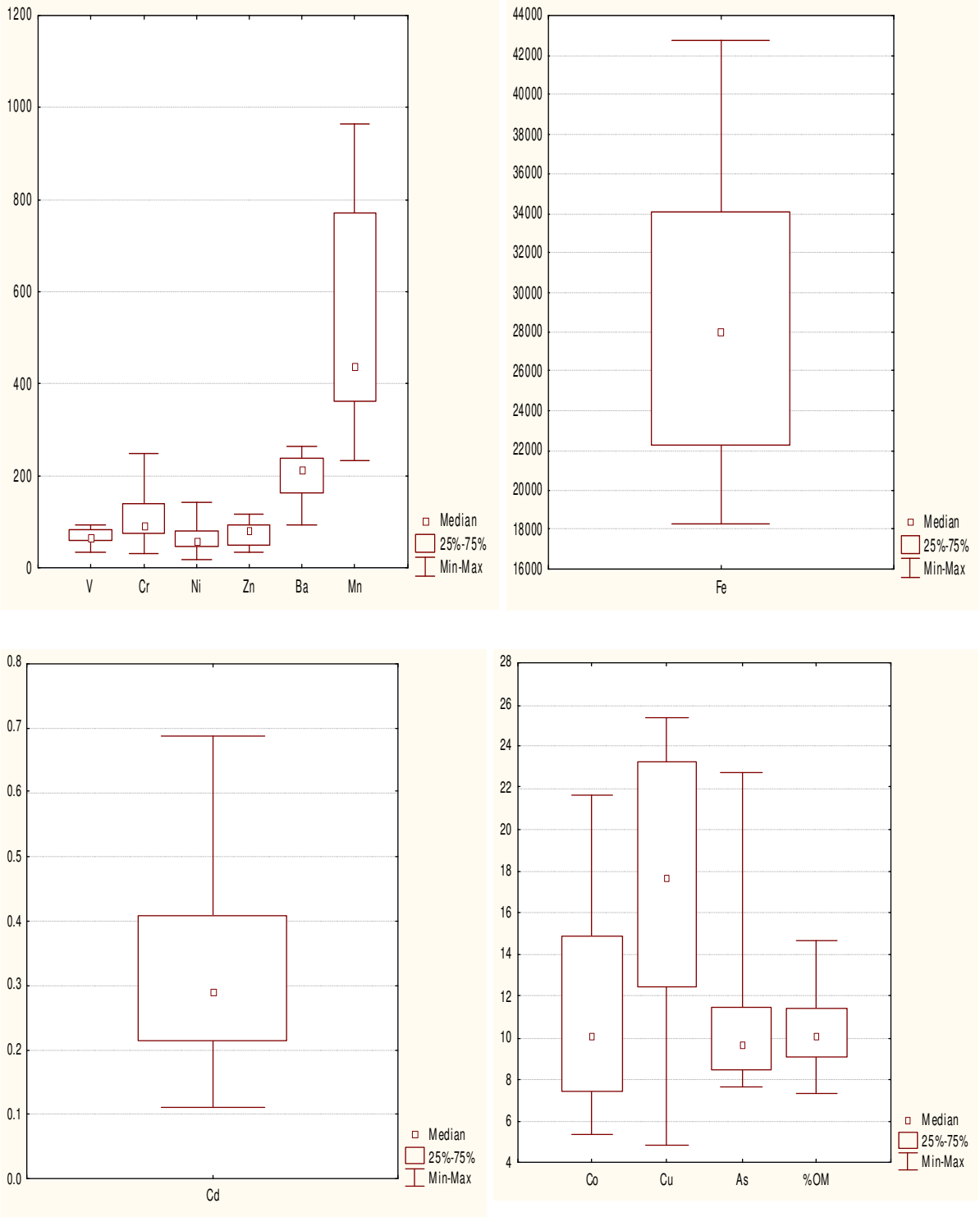


Figure 7: Box-Whisker plots showing average concentration ranges of total metals in sediments from Algeciras Bay, including 25- and 75-percentile of the values.

was comparable to (20-148 mg/kg and32-92 mg/kg) reported by Morillo, *et al*. respectively, while Ni was comparable to (18-100mg/kg); Fe and Mn were comparable to (27800-42600 mg/kg and 93-463 mg/kg) reported by Morillo, *et al*. (2007); %OM was (7-15%). Figure 7 shows the minimum and maximum metal contents in sediments as well as the 25th and 75th percentile of the values

The results identified pollution hotspots for each metal as follows: V was at point 3.2 (94 mg/kg); Cr, Ni and Fe were at point 6.1 (251 mg/kg, 144 mg/kg and 42756 mg/kg respectively). Cu, Zn, Cd and Ba were at point 6.2 (25 mg/kg, 117 mg/kg, 0.7 mg/kg and Ba mg/kg respectively). Hotspots for Co was point 7.1 (22 mg/kg); Pb at point 7.2 (39 mg/kg); As at point 2.1 (23 mg/kg) and Mn at point 10.1 (967 mg/kg). These results revealed that points 6.1 and 6.2, which are located adjacent to the stainless steel manufacturing industry, were the most important sampling points in terms of potential pollution by most metals in Algeciras Bay. Other important hotspots are points 7.1 and 7.2 located near the petrochemical industries. Points 10.1, 3.2 and 2.1 located near the shipyard (10.1) and port of Algeciras were important hotspots for Mn, V and As respectively. The hotspot for organic matter was point 7.2 located near river Quadarranque. Pollution hotspots for each metal and organic matter are highlighted in red as shown in Figures 8 - 10.

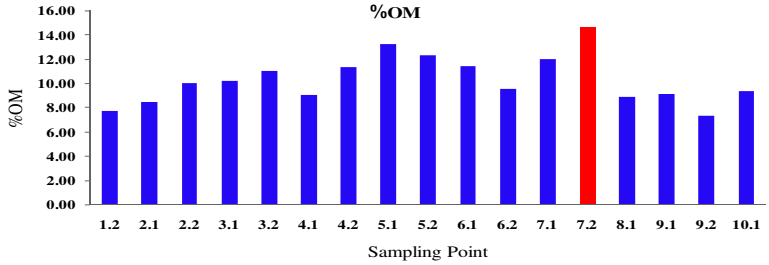


Figure 8: Distribution patterns and pollution hotspots (in red) for organic.

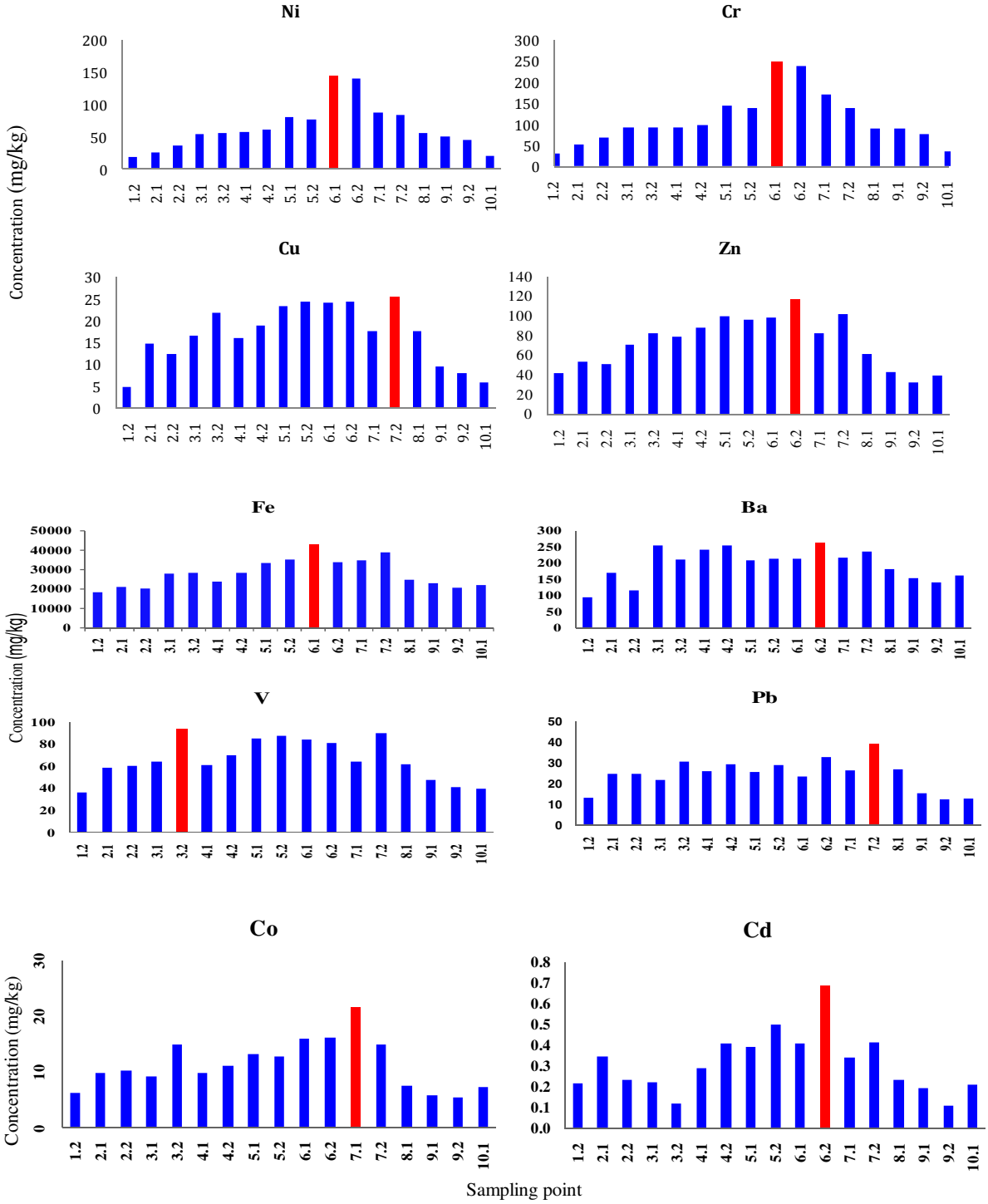


Figure 9: Distribution patterns and pollution hotspots (in red) for Ni, Cr, Cu, Zn, Fe, Ba, Pb, Co and Cd.

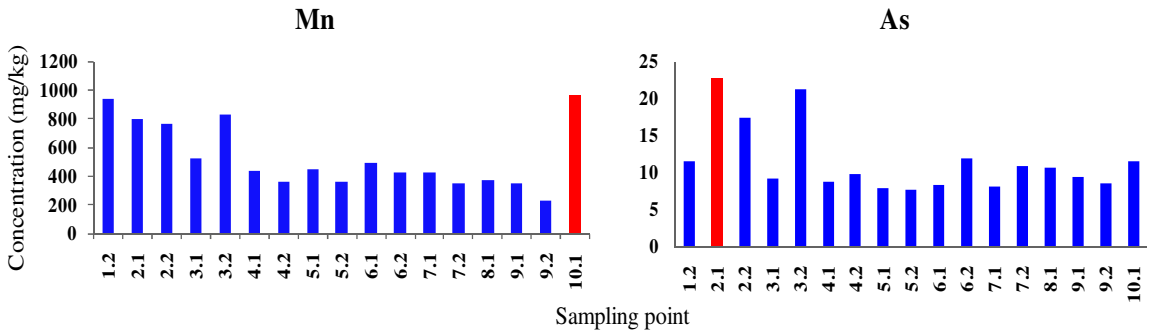


Figure 10: Distribution patterns and pollution hotspots (in red) for Mn and As Cd.

In order to obtain more information, the data was tested for normality using *Shapiro-Wilk W test*. The hypothesis that the respective metals were normally distributed was rejected if the *W statistic* was significant at $p < 0.05$. The normality test results given in Table 13 showed that V, Cr, Co, Cu, Zn, Ba, Pb, Fe, Cd and % OM were normally distributed, while Ni, As and Mn were not before transformation. Ni and Mn were normally distributed after log transformation, while As was after 1/As transformation as shown in Table 13. For further data analysis, the data for V, Cr, Co, Cu, Zn, Ba, Pb, Fe, Cd and % OM were used directly, while log Ni and Log Mn were used for Ni and Mn and for As, 1/As was used.

Some of the concentrations (mg/kg) of total metals in sediments from one of the hotspots, point 6.1 were converted to concentration (ppm) and compared with results reported by Tarazona, *et al*. (1991) for dissolved metals in Algeciras Bay. The dissolved concentrations of the selected metals at point 6.1 were: Zn (0.024 ppm); Cd (0.065 ppm); Cu (0.076 ppm) and Pb (0.425 ppm). The total concentrations of the same metals in sediments at the same sampling point were; Zn (98860 ppm); Pb (23508 ppm); Cu (17734 ppm) and Cd (410 ppm).

Comparison between these results revealed that the total concentrations of these metals in sediments were respectively in the magnitude of ~ 4000000; 200000; 50000 and 6000 times higher than those dissolved in the water column. This was in agreement with the fact that sediments accumulate hazardous trace elements to levels many times higher than water column concentrations, causing a serious problem due to their toxicity and their ability to accumulate in biota (Morillo, *et al*. 2007).

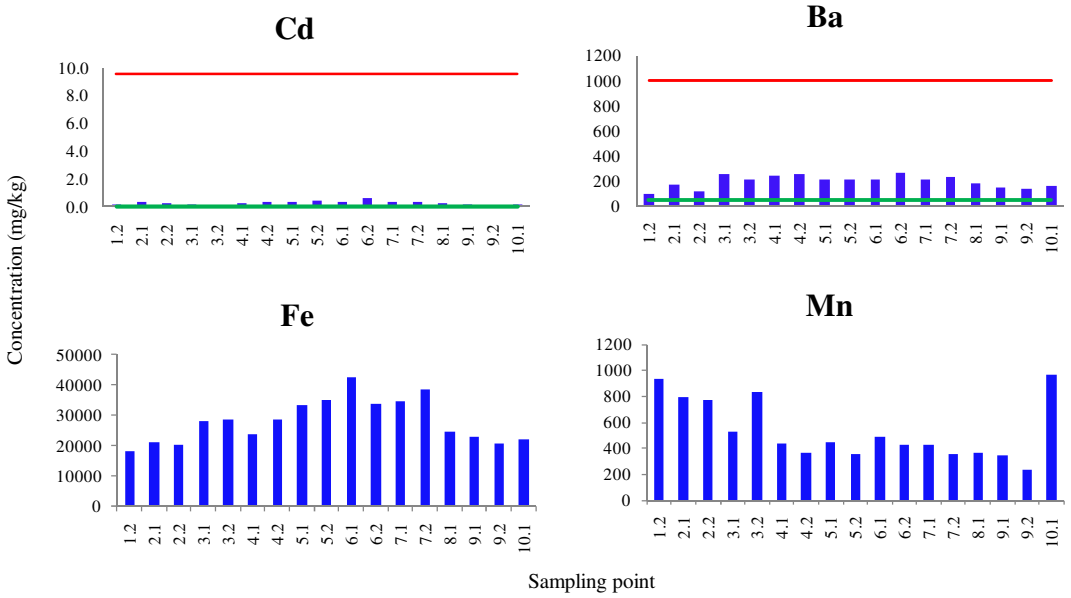
Table 13: Shapiro-Wilk normality test for total metals and % OM (W statistic was significant at $p < 0.05$)

Parameter	W statistic p-value	Distribution
V	0.268	Normal
Co	0.366	Normal
Cr	0.052	Normal
Ni	0.047	Not normal
Cu	0.182	Normal
Zn	0.362	Normal
As	0.000	Not normal
Ba	0.330	Normal
Cd	0.204	Normal
Pb	0.309	Normal
Fe	0.305	Normal
Mn	0.010	Not normal
%OM	0.797	Normal
Log Ni	0.508	Normal
Log Mn	0.100	Normal
1/As	0.097	Normal

3.2. Sediment quality assessment

The concentrations of total metals were compared with sediment quality guidelines (SQGs) as shown in Figures 11 and 12. Estimates of toxicity were made using the biological-based criteria of Long, *et al.* (1995); Manheim and Commeau, (1981) and USEPA, (1996). The low alert range (LAL) was exceeded by all metals throughout the bay, except at point 1.2 where Cu was below LAL. Sampling point 1.2 was close to the natural ecosystem catchment meaning that this point was expected to be pristine; however, the results showed that in future, the whole ecosystem in Algeciras Bay might be endangered by these metal pollutants, probably due to

urban -based industrial processes (city of Algeciras and the industrial zones around the bay) as reported by Galloway, *et al*. (1982) and Gray, *et al*. (2003). These pollution sources are considered to have the potential of affecting even the most undeveloped areas as suggested by Birch and Taylor, (1999).



Note: Low Alert Levels (LAL) values from Manheim and Commeau, (1981); Effect- Range Low (ERL) and High Alert Levels (HAL) sediment quality guidelines (SQGs) values from Long *et al.* (1995) and USEPA, (1996) respectively. HAL guidelines were used here since many of these values are the same as Effect-Range Medium (ERM) values from Long *et al*. (1995).

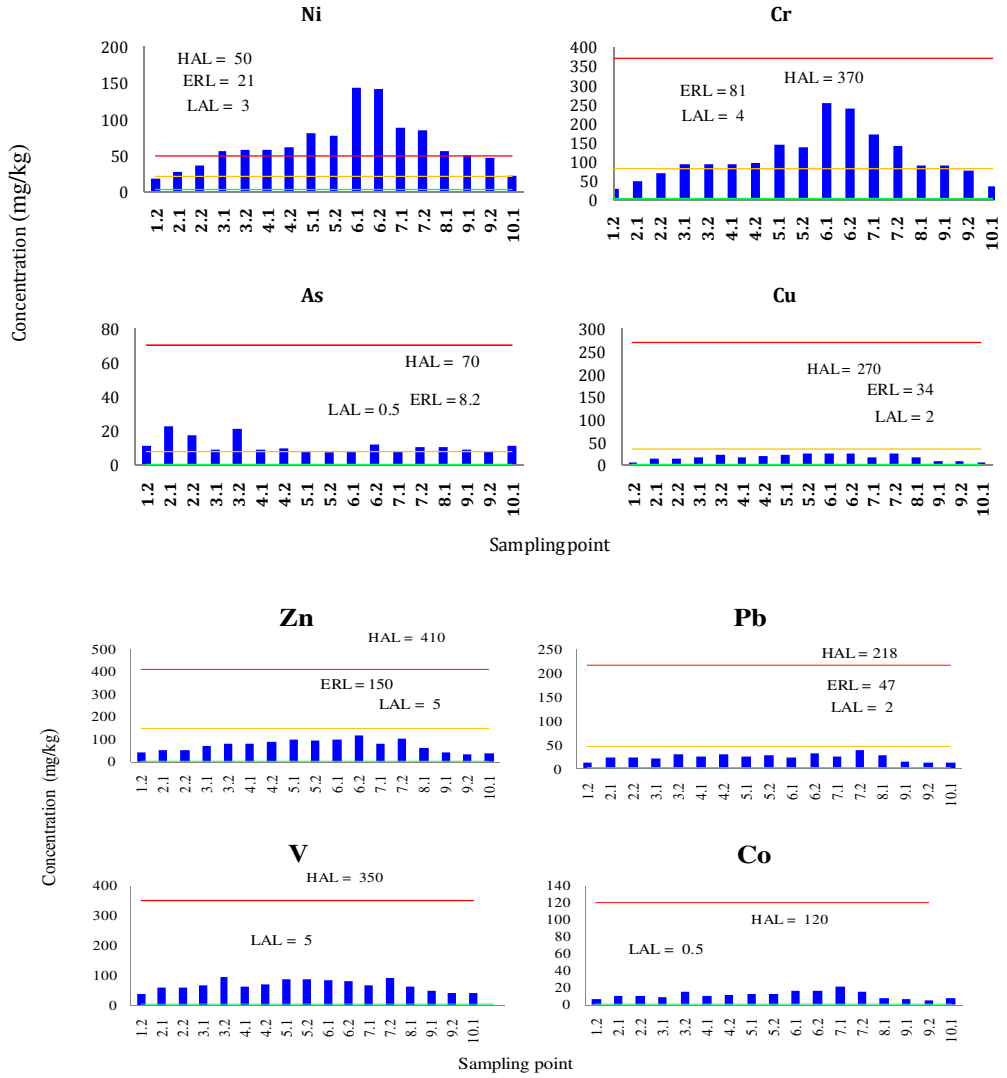
HAL **ERL** **LAL** **Metal concentration (mg/kg)**

Figure 11: Distribution patterns of total Cd, Ba, Fe and Mn concentrations (mg/kg dry mass) and HAL, ERL, and LAL sediment quality guidelines. ERL was not defined for Cd and Ba, while HAL, ERL and LAL were not defined for Fe and Mn.

Table 14: Mean (± SD; n = 6) of total metal concentrations (mg/kg dry mass) and percent organic matter in sediments

Sampling point	V	Cr	Co	Ni	Cu	Zn	As	Cd	Ba	Pb	Fe	Mn	%OM
1.2	36 ± 2	30 ± 1	6 ± 1	19 ± 1	4.8 ± 0.3	41.4 ± 2.4	11.9 ± 1.1	0.21 ± 0.09	92.8 ± 4.7	13.4 ± 1.3	18285±550	940.7±2.2	7.7
2.1	59.1 ± 2.3	50.8 ± 1.5	9.8 ± 0.4	27 ± 1	15 ± 1	53.0 ± 1.6	22.7 ± 2.2	0.34 ± 0.12	170 ± 6.7	24.5 ± 0.5	12255±356	792.7±0.4	8.5
2.2	60.4 ± 5.8	68.3 ± 0.9	10.1 ± 0.3	36.3 ± 1.7	12.5 ± 0.7	50.6 ± 3.9	17.4 ± 0.6	0.23 ± 0.12	116 ± 5.3	25 ± 2	29230±337	769±3	10.1
3.1	64.7 ± 1.6	93.3 ± 2.2	9.1 ± 0.3	55 ± 1	16.5 ± 0.5	70.1 ± 3.9	9.2 ± 0.8	0.22 ± 0.07	256 ± 11.3	21.7 ± 0.2	27947±198	526.4±0.1	10.2
3.2	**94 ± 7**	92.6 ± 2.5	14.9 ±.1.4	56.9 ± 4.7	21.7 ± 0.9	82 ± 6	21.3 ± 1.2	0.12 ± 0.05	211 ± 20	31 ± 2	28522±462	835±1	11
4.1	61.2 ± 1.9	91.8 ± 8.9	9.7 ± 0.4	57.3 ± 0.9	16.1 ± 0.7	79.3 ±2.7	9 ± 1	0.3 ± 0.1	242 ± 10	26.2 ± 1.4	23995±176	437.9±4.4	9.1
4.2	69.7 ± 4.6	98 ± 8	11.1 ± 0.4	61.5 ± 5.3	19 ± 1	87.6 ± 8.8	9.7 ± 0.7	0.4 ± 0.1	256 ± 31	29.5 ± 2.4	28511±341	362±1	11.3
5.1	85.5 ± 3.7	143 ± 4	13.1 ± 0.5	80.9 ± 1.9	23 ± 1	100 ± 3.6	7.9 ±1.2	0.4 ± 0.1	210 ± 7	26 ± 1	33308±435	446±2	13.3
5.2	87 ± 5	139 ± 7	12.7 ± 0.6	77.7 ± 3.7	24.4 ± 1.9	96 ± 5	7.8 ± 1.1	0.5 ± 0.2	215 ± 12	29 ± 2	35093±89	360.4±0.4	12.4
6.1	84.6 ± 2.1	251 ± 3	15.8 ± 1.2	144 ± 3	24.1 ± 0.9	98.9 ± 3.4	8.2 ± 0.9	0.4 ± 0.1	214 ± 4	23.5 ± 0.4	42756±220	487±3	11.4
6.2	81.3 ± 6.5	238.3 ± 12.1	16.1 ± 0.7	141.5 ±11.6	25 ± 1	117 ± 2	11.9 ± 1.6	0.69 ± 0.24	263 ± 13	33 ± 2	34012±456	425.3±0.9	9.6
7.1	65 ± 2	172.3 ± 3.9	21.7 ± 0.7	88.6 ± 2.6	18 ± 1	81.9 ± 3.7	8.0 ± 0.9	0.3 ± 0.1	216± 9	26.3 ± 1.9	34766±191	423±5	12
7.2	89.9 ± 1.7	140 ± 5	14.9 ± 0.5	88.8 ± 2.9	25.4 ± 0.7	102 ± 2	10.7 ± 1.7	0.42 ± 0.16	236 ± 3	39.2 ± 0.3	38824±151	353.1±1.2	15
8.1	62 ± 2	91 ± 1	7.4 ± 0.7	55.7 ± 2.2	17.7 ± 0.8	61 ± 3	10.7 ± 0.5	0.23 ± 0.14	180 ± 4	27.1 ± 0.9	24640±285	372±2	8.9
9.1	48 ± 7	89.1± 9.3	5.7 ± 0.8	51.1 ± 6.5	9 ± 1	42.8 ±7.6	9.4 ± 1.1	0.19 ± 0.04	154 ± 13	15.3 ± 0.2	23082±297	351±4	9.2
9.2	41 ± 2	77.3 ± 2.8	5.4 ± 0.2	45.9 ± 1.9	8.1 ± 0.5	32.5 ± 3.5	8.5 ± 1.3	0.11 ± 0.06	142 ± 6	12.3 ± 0.3	20748±385	235±3	7.3
10.1	39.7 ± 3.1	37.2 ± 1.4	7.2 ± 0.5	21.5 ± 1.8	6.0 ± 0.4	39.9 ± 2.6	11.5 ± 1.7	0.21 ± 0.06	162 ± 6	13 ± 2	22225±88	967±1	9.4
Mean	66	112	11	65	17	73	11	0.3	196	24	28129	534	10
Range	36-94	30-251	5-22	19-144	5-25	33-117	8-23	0.1-0.7	93-263	12-39	12255-42756	234-967	7-15

Note: The red highlights are the pollution hotspots for each metal.



Note: Low Alert Levels (LAL) values from Manheim and Commeau, (1981); Effect- Range Low (ERL) and High Alert Levels (HAL) sediment quality guidelines (SQGs) values from Long *et al.* (1995) and USEPA, (1996) respectively. HAL guidelines were used here since many of these values are the same as Effect-Range Medium (ERM) values from Long *et al.* (1995).

HAL **ERL** **LAL** **Metal concentration (mg/kg)**

Figure 12: Distribution patterns of total Ni, Cr, As, Cu, Zn, Pb, V and Co concentrations (mg/kg dry mass) and HAL, ERL, and LAL sediment quality guidelines. ERL was not defined for V and Co.

All metals with defined effect range-low (ERL) and high alert level (HAL) values were below these guideline levels, except Ni, Cr and As. A large area, between sampling points 3.1 and 8.1 located in the inner parts of the Bay were highly contaminated with Ni and slightly by Cr and As (Figure 12). Ni, whose concentration levels were above high alert level suggested by USEPA (1996), indicated that marine organisms would regularly be exposed to toxic effects of Ni. Cr concentrations exceeded effect range-low suggested by Long, *et al*. (1995), while the concentrations of As exceeded or were at the peak of this guideline throughout the whole system, implying that effects of the two metals might occasionally occur in marine organisms. It was noteworthy that the concentration of As at sampling point 1.2 (pristine point) was above ERL guidelines, suggesting that As could have been originating from the maritime traffic at the port of Algeciras. These observations were in agreement with those reported by Morillo, *et al*. (2007). The results revealed that Ni, Cr and As seem to be the most important metals in the bay subject to confirmation that their levels were sufficient to be available to marine organisms.

Spearman's rank linear correlation analysis was performed in order to check for the existence of any correlations among metals at different sampling areas and to determine whether the said correlations existed between total metals and organic matter. Output for total metals and organic matter is given in Table 15. Organic compounds in sediments play an important role in heavy metal transformation (1.2). The analysis revealed significant (at $p < 0.05$) correlations with high positive correlations between metals (e.g. Cu vs. V = 0.936; Zn vs. Cu = 0.966; Fe vs. Cu = 0.919; log Ni vs. Cr = 0.980 and log Ni vs. Fe =.926).

Table 15: Spearman's Rank Order linear correlation analysis of total metals and %OM in sediments (correlations marked in red are significant at p < 0.05; n=17)

	V	Cr	Co	Cu	Zn	Cd	Ba	Pb	Fe	%OM	1/As	Log Ni	Log Mn
V	1.000												
Cr	0.794	1.000											
Co	0.775	0.826	1.000										
Cu	**0.936**	0.882	0.836	1.000									
Zn	0.892	0.890	0.858	**0.966**	1.000								
Cd	0.578	0.696	0.708	0.787	0.824	1.000							
Ba	0.640	0.740	0.608	0.716	0.752	0.630	1.000						
Pb	0.782	0.591	0.721	0.819	0.794	0.630	0.664	1.000					
Fe	0.860	0.929	0.811	**0.919**	0.880	0.694	0.701	0.642	1.000				
%OM	**0.816**	0.755	0.757	0.775	0.770	0.578	0.515	0.564	**0.826**	1.000			
1/As	0.216	0.500	0.100	0.240	0.230	0.221	0.221	-0.100	0.444	0.390	1.000		
Log Ni	0.779	**0.980**	0.833	0.890	0.895	0.723	0.728	0.652	**0.926**	0.718	0.475	1.000	
Log Mn	-0.189	-0.355	0.002	-0.309	-0.203	-0.225	-0.243	-0.252	-0.314	-0.157	-0.507	-0.402	1.000

The results indicated that these metals had the same distribution characteristics. However, no significant correlations were observed between Log Mn and 1/As and the rest of total metals, implying that these two metals showed different distribution characteristics from the others. The Spearman's analysis showed significant correlations between total metals and organic matter as by the positive correlations (e.g. %OM vs V = 0.816 and %OM vs Fe = 0.826), meaning that organic matter had some influence on the distribution of metals in sediments (Amina, *et al*. 1999)

In order to understand more about the distribution behaviour of total metals and organic matter, factorial analysis and principal component analysis (PCA) with Varimax raw factor loadings' rotation was applied. Table 16 and Figure 13 show the factor loadings and factor scores principal component analysis plot. Two factors(representing behaviours) were identified for the 13 variables (metals) in sediments represented by 17 cases (n=17) corresponding to eigenvalues > 1 and

cumulative variance of 79.16%. Most of the correlations were > 0.7, implying that the extraction of the two factors was reasonable for this study. Factor 1 (most important) accounted for 65.69% of the total variance explained and grouped V, Zn, Cu, Pb, Co, Fe, %OM and Ba, which were positively intercorrelated and had the highest loadings (0.958, 0.941, 0.936, 0.925, 0.920, 0.831, 0.800 and 0.745, respectively). The results showed that organic matter once again displayed similar distribution behaviour to those of V, Zn, Cu, Pb, Co, Fe and Ba as supported by the positive correlations (0.800). Factor 2, which accounted for 13.47% of the total variance explained, grouped Mn and As, meaning that these metals displayed similar distribution behaviour.

Factor loadings plot of principal component analysis (PCA) for total metals and organic matter in sediments are shown in Figure 13. This plot grouped the metals and organic matter according their distribution behaviour. It was observed that V, Zn, Cu, Pb, Co, Fe, Ni, Cr, Cd, Ba and %OM were grouped together. This grouping was also reflected by the factor loadings as shown in Table 16, although Cr, log Ni and Cd were not significant at > 0.7. Ni and Cr belong to factor1 grouping; however, the two metals appear to be along each other, an observation which was also illustrated by their identical distribution patterns displayed in Figure 9. It was observed that organic matter could be playing a role in influencing the behaviour of metals in Algeciras Bay sediments.

To obtain a general idea about the potential sources of metal pollutants and organic matter in Algeciras Bay, principal component analysis (without varimax rotation) was applied to the total metals and organic matter. Two principal components were obtained for 17 objects (sampling points) and 13 variables (total metals and organic matter) scores. The two principal components accounted for a total of 67.9% variance explained. Figure 14 contains a biplot of the principal components showing the distribution of the objects and variables Comp.1-Comp. 2 (principal component 1-principal component-2) axes.

Table 16: Factor Loadings (Varimax raw rotation) of Principal components for total metals and %OM in sediments (n=17; Marked loadings are significant at > 0.700)

Variable	Factor 1	Factor 2
V	0.958351	0.060103
Cr	0.691859	0.568660
Co	0.919894	0.038976
Log Ni	0.693989	0.571673
Cu	0.935802	0.185142
Zn	0.941497	0.239998
1/As	0.102604	-0.922504
Cd	0.652906	0.323063
Ba	0.745409	0.346961
Pb	0.924626	-0.064949
Fe	0.831284	0.464456
Log Mn	-0.216528	-0.800675
%OM	0.800478	0.153286
Eigenvalue	8.595848	1.824764
Cumulative variance (%)	66.12191	80.15855

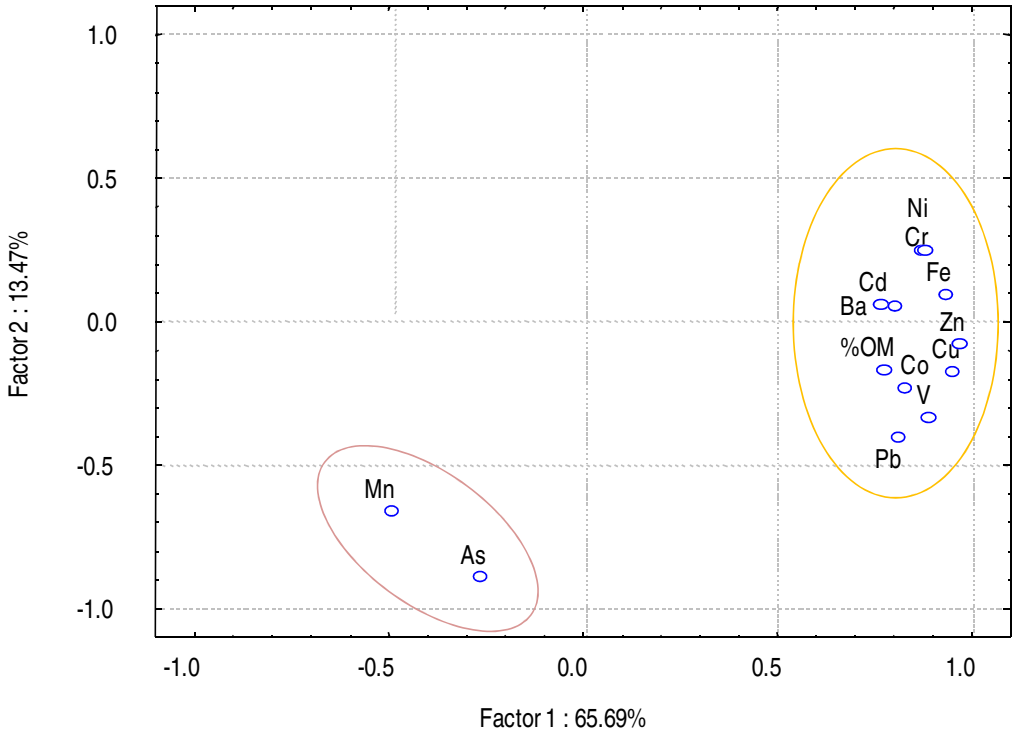


Figure 13: Factor loadings (factor 1 vs. factor 2 plot), varimax raw rotation Principal component analysis plot for total metals and organic matter.

The plot shows that component 1, the most important, accounted for 38.7% of the total variance explained among the total metals and organic matter. The component was categorised by significant positive contributions of variables (total metals) for Pb, V, Cu, Zn, Co, Ba, Cd, Ni, Cr and Fe. Component 2 accounted for 29.2 % of the total variance explained with positive contributions by As, %OM and Mn.

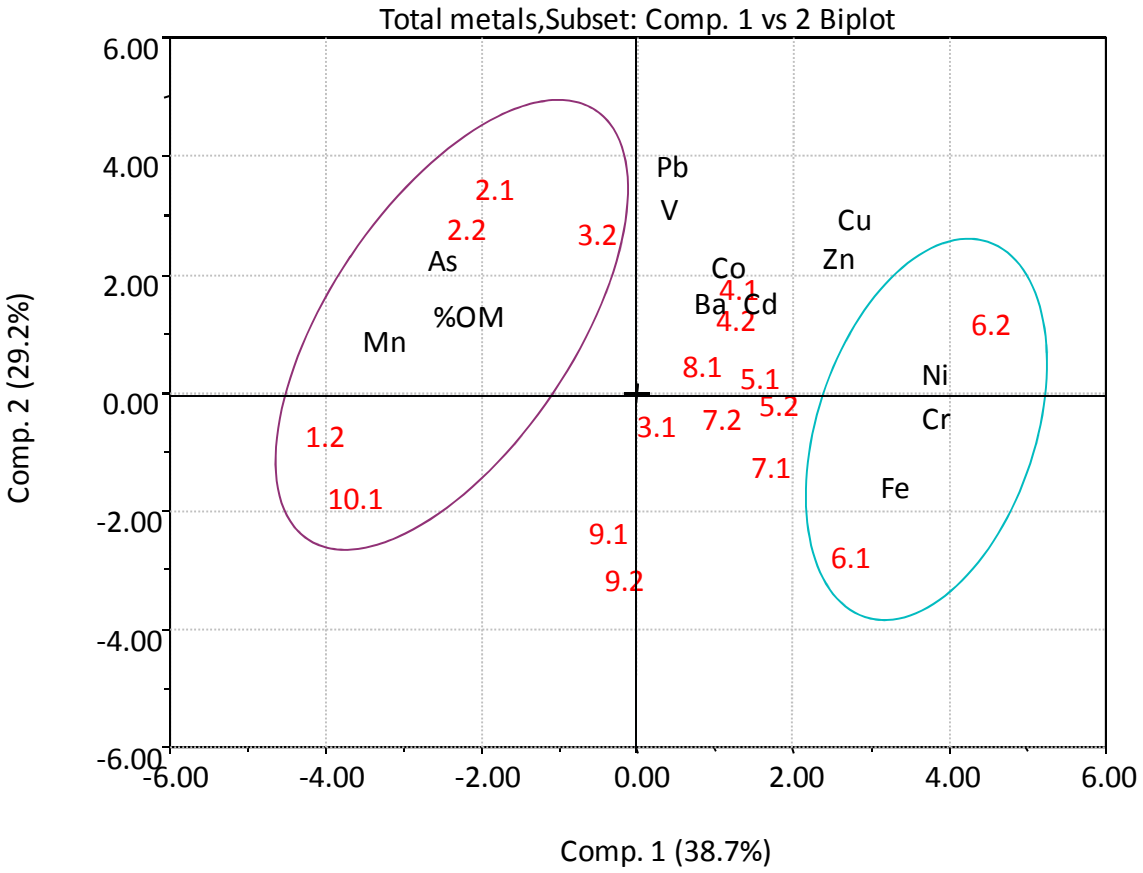


Figure 14: Biplot principal component analysis (PCA) for objects (sampling points) and variables (metals).

Sampling point 6.2 presented the highest value for principal component 1. This point is located near the stainless steel manufacturing plant and was the most important pollution hotspot for Cd, Zn and Ba (Figure 9).

Sampling point 6.1, which is located in the same area as 6.2 but nearer to the steel manufacturing plant, presented the second highest value in component 1. This point was an important pollution hotspot for Ni, Cr and Fe (Figure 9). The other metals in component 1 were found around 7.1 and 7.2, which are located near the petrochemical industries in the Guadarranque zone. Sampling point 3.2, located near the port of Algeciras and Mayorga Bridge (Figure 2) was an important pollution hotspot for V (Figure 9). Sampling point 1.2, 2.1, 2.2, 3.2 and 10.1 were associated with component 2 in which As, %OM and Mn were grouped together. Mn and As were grouped together probably due to the fact that they were found in high levels at points 2.1, 2.2 and 3.2 although the most important sampling points for Mn were 10.1 and 1.2 located near a shipyard and the natural ecosystem catchment respectively.

It was indeed observed that it was at sampling points 10.1 and 1.2 that the highest concentrations of Mn were found, with 10.1 being the hotspot as revealed by Figure 9. This indicated that the shipyard was the potential source of Mn at point 10.1 and that the highest concentration of Mn found at point 1.2 could have been due to overflow from the heavy maritime activities from the port of Algeciras. Arsenic was mostly found at points 2.1 and 3.2 located near the port of Algeciras and Mayorga Bridge respectively, in which sampling point 2.1 was the hotspot for As (Figure 9). Mn, As and %OM were grouped together due to their relationships at sampling point 3.2 indicating that the port of Algeciras and urban sewage discharge at the Mayorga Bridge appeared to be responsible for the high levels of these variables in Algeciras Bay.

From the observations, it was understood from the principal component analysis that the industrial zones represented by component 1 and the shipyard; city and port of Algeciras, represented by component 2 are the main potential sources of metal pollutants and organic matter in Algeciras Bay, indicating

that the contents of metals in sediments of the bay were clearly of anthropogenic origin. Similar observations were reported by Morillo, et al. (2007).

3.3. 0.11M acetic acid extraction

Table 17 shows the results of 0.11M acetic acid extractions in which the mean concentrations of metals extracted at each sampling point (mean ± SD; n =6); average of total concentrations of metal ions extracted and their ranges (minimum-maximum values); average percentage of each total metal extracted and their ranges in the whole ecosystem are given. The average total concentrations of metal extracts were: V (0.2 mg/kg); Cr (0.2 mg/kg); Co (1.6 mg/kg); Ni (2.3 mg/kg); Cu (0.3 mg/kg); Zn (9.2 mg/kg); As (ND); Cd (0.03 mg/kg); Ba (ND); Pb (4.2 mg/kg); Fe (119 mg/kg); Mn (173 mg/kg).

The concentration ranges of the extracts were: V (0.1-0.3 mg/kg); Cr (0-0.7 mg/kg); Co (0.3-4.3 mg/kg); Ni (0.5-9.5 mg/kg); Cu (0.02-0.7 mg/kg); Zn (nd-30 mg/kg); As (ND); Cd (0.03-0.04 mg/kg); Ba (ND mg/kg); Pb (3-7 mg/kg); Fe (ND-375 mg/kg) and Mn (49-325 mg/kg). The highest concentrations of some selected total metals extracted with 0.11M acetic acid were realised as follows: Zn (30 mg/kg) and Ni (9.5 mg/kg) at point 6.2; Pb (7 mg/kg) at point 2.1 and Mn (325 mg/kg) at point 1.2. It was observed that the points at which the concentrations of total Zn and Co were extracted (6.2 and 7.1 respectively), were the same points identified as hotspots for these metals from the results of total metals indicating that the sources could be due to anthropogenic input from the industrial zones and the port of Algeciras. Figures 15 and 16 shows the ranges (minimum and maximum) of mobile metal ions extracted in acetic acid, including the 25th and 75th percentile of the values.

The metal extracts in 0.11M acetic acid were compared with total metal concentrations. The average total percentage of total metal extracted were as follows: V(0.3%); Cr (0.1%); Co (14%); Ni (4%); Cu (1.5%); Zn (13%); As (ND); Cd (0.3%); Ba (ND); Pb (17%); Fe (0.4%) and Mn (32%). The ranges of percent total metals extracted were: V (0.1-0.4%); Cr (ND-0.7%); Co (2-38%); Cd (5.8-27%); Ni (0.7-15%); Cu (0.1-4%); Zn (ND-41%); As (ND); Cd (0.3-0.3%); Ba (ND); Pb (12-28%); Fe (ND-1.3%) and Mn (9-61%). The results showed that significant percentage of total Co, Ni, Cu, Zn, Pb and Mn were extracted with 0.11M acetic acid in the following decreasing order: Mn > Zn > Co > Pb > Cd > Ni > Cu. However, the extractions of total V, Cr, As, Fe and Ba were insignificant (<4%).

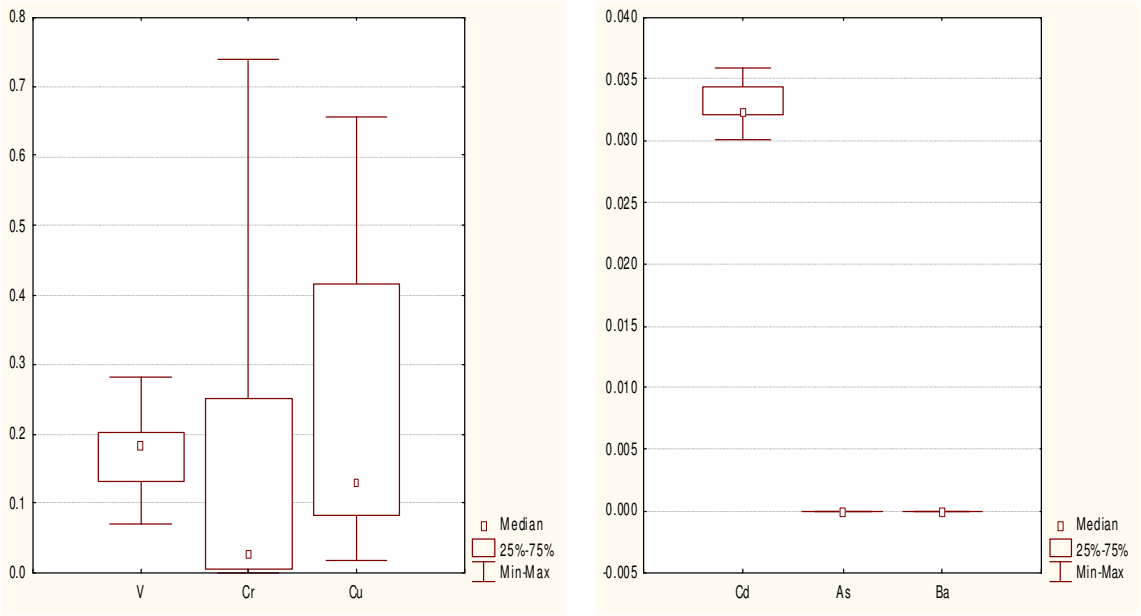


Figure 15: Box-Whisker plots showing average concentration ranges of mobile V, Cr, Cu, Cd, As and Ba in acetic acid, including 25- and 75-percentile of the values.

These results were comparable to the extractions of Co, Zn and Ni [Co (0–35%), Zn (0–25%) and Ni (3–16%)] reported in a past study by Marmolejo-Rodriguez, *et al*. (2007).

The negligible values (< 1%) observed for Cr and Fe were acceptable since these two metals are well known to accumulate in the residual fraction ((Sahuquillo, *et al*. 2003; Morillo, *et al*. 2007).

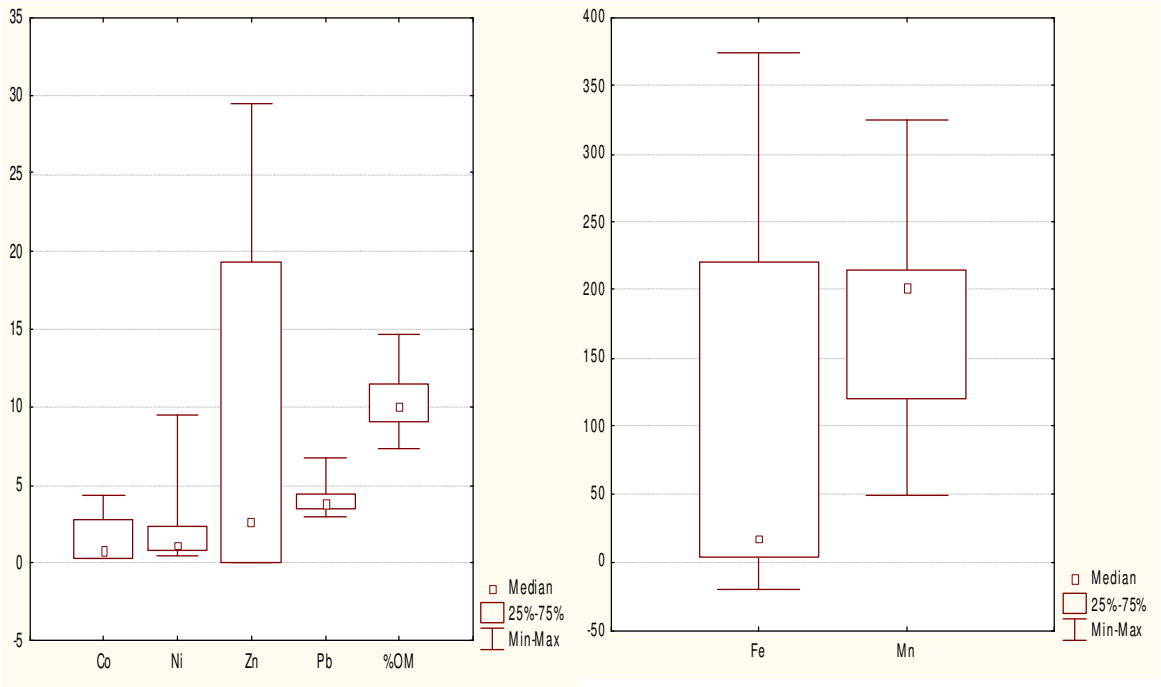


Figure 16: Box-Whisker plots showing average concentration ranges of %OM and mobile Co, Ni, Zn, Pb, Fe and Mn extracted in acetic acid, including 25- and 75-percentile of the values.

3.4. 0.005M DTPA + 0.01M $CaCl_2$ + 0.1M TEA extraction

Table 18 shows the results of 0.005M DTPA + 0.01M $CaCl_2$ + 0.1M TEA extraction in which the mean concentrations of metals extracted at each sampling point (mean ± SD; n =6); average of total concentrations of total metals extracted and their ranges; average percentage of each total metal extracted and their ranges in the whole system are given.

The average concentrations of each metal extracted were: V (0.2 mg/kg); Cr (0.03 mg/kg); Co (0.6 mg/kg); Ni (1.1 mg/kg); Cu (3.6 mg/kg); Zn (12 mg/kg); As (0.1 mg/kg); Cd (0.1 mg/kg); Ba (0.2 mg/kg); Pb (6.5 mg/kg); Fe (144 mg/kg); Mn (13 mg/kg).

The concentration ranges and corresponding percentage of each total metal extracted with 0.005M DTPA + 0.01M $CaCl_2$ + 0.1M TEA were: V (0.1-0.5 mg/kg; 0.3-0.5%); Cr (0-0.1 mg/kg; 0-0%); Co (0.1-2.4 mg/kg; 2-11%); Ni (0.1-7.9 mg/kg; 0.3-5.5%); Cu (0.1-17 mg/kg; 2-68%); Zn (2-54 mg/kg; 6-47%); As (0.02-0.1 mg/kg; 0.4-0.5%); Cd (0.01-0.3 mg/kg; 5-38%); Ba (0.1-0.3 mg/kg; 0.1-0.1%); Pb (1.4-15 mg/kg; 11-38%); Fe (59-248 mg/kg; 0.3-0.6%) and Mn (4-29 mg/kg; 1.7-3%). Significant extractions were realised for Ni, Co, Cu, Cd, Pb, Zn in decreasing order as follows: Cu > Zn > Cd = Pb > Co > Ni. The rest of the metals V, Cr, As, Fe, Mn and Ba yielded insignificant (< 3%) concentrations with of 0.005M DTPA + 0.01M $CaCl_2$ + 0.1M TEA extraction. It was observed that the highly extracted metals, Cu (68%); Zn (47%); Cd (38%) = Pb (38%); Co (11%) and Ni (5.5%) shared a common hotspot at point 6.1, indicating that industrial zone, especially the stainless steel manufacturing plant was the potential source of these metals. Figure 17 and 18 shows the ranges (minimum and maximum) of mobile metal ions extracted in EDTA as well as the 25th and 75th percentile of the values.

3.5. Comparison between acidic and complexation conditions

The extractability potential for the two extractants were compared with the view of assessing their individual ability to effect the remobilisation and release of the weakly sediment-bound (mobile) and mobilisable (potentially mobile) metals ions.

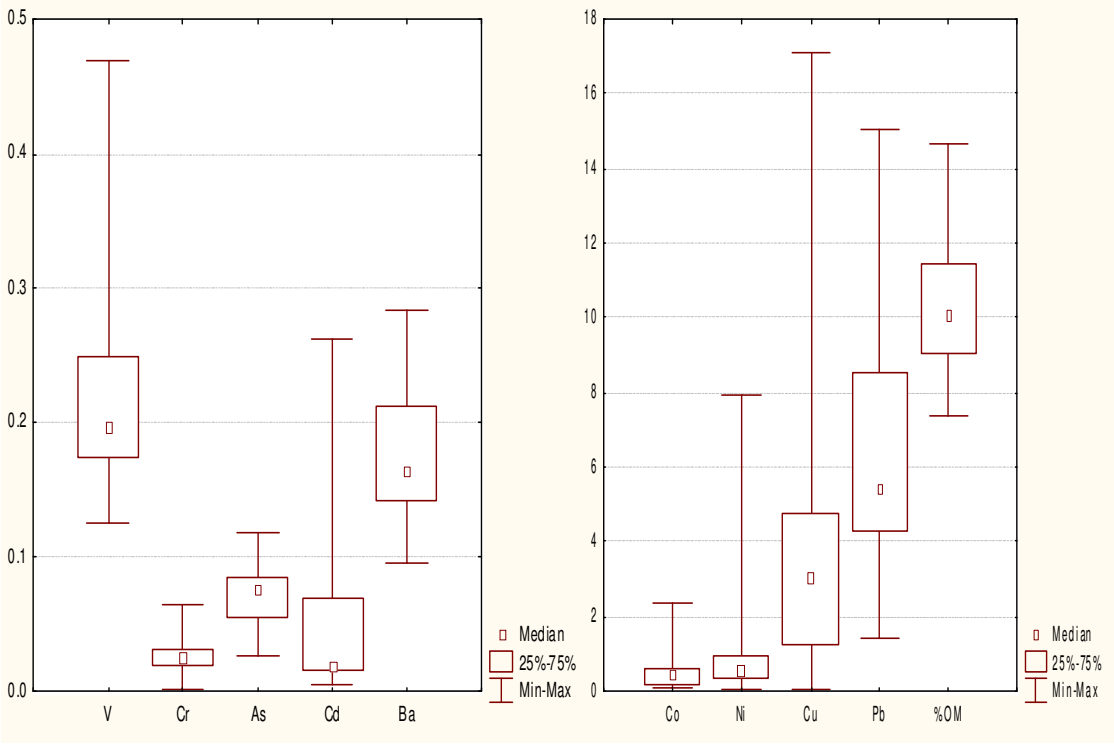


Figure 17: Box-Whisker plots showing average concentration ranges of %OM and mobile V, Cr, As, Cd, Ba, Co, Ni, Cu and Pb extracted in DTPA, including min, max; 25- and 75-percentile of the values.

It was noteworthy here that an assumption was made that these extractants represented the real environmental conditions (water column conditions) in Algeciras Bay.

Type VI (unique) one-way ANOVA was performed in order to study whether there were any differences existing between the two extractants. Table 19 gives the one-way ANOVA univariate results, which showed that there existed some similarities and differences between the two conditions. It was observed that their extractability potentials were different for the majority of the metals (Cr, Co, Cu, As, Cd, Ba, Pb and Mn), while similarities were found with V, Ni, Zn, and Fe, although V and Fe yields were negligible (< 1%). The results obtained by the one-way ANOVA test were indeed confirmed to be correct as demonstrated by the graphical

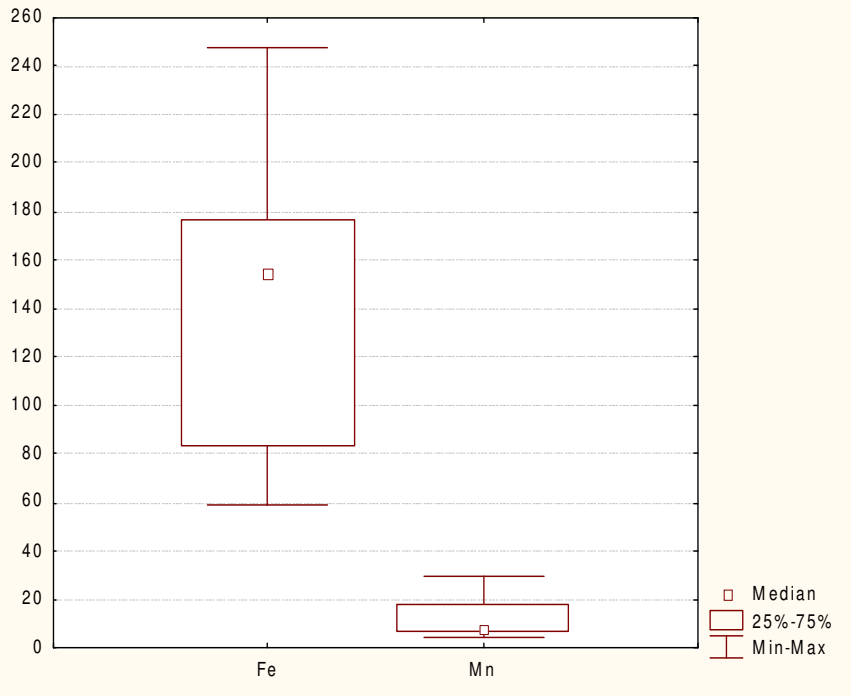


Figure 18: Box-Whisker plots showing average concentration ranges of mobile Fe and Mn extracted in DTPA, including min, max, 25- and 75-percentile of the values.

representation in Figure 19. It was revealed that acidic condition (0.11M acetic acid) had a higher potentiality to extract Co and Mn, whereas complexing condition (0.005M + 0.01M CaCl2 + 0.1M TEA) proved to be better in the extraction of Pb, Cd and Cu. The higher percentage of Mn in acetic acid was probably due to its known close association with carbonates (Dassenakis, *et al.,* 2003). This agrees with the results of other past studies of marine sediments (Kiratli and Ergin, 1996; Marin and Guiresse, 2001; Morillo, Usero, and Gracia, 2004, 2007) in which a high proportion of Mn was found in the acid-soluble fraction.

Table 17: Mean (± SD; n = 6) of mobile metal concentrations (mg/kg dry mass) in 0.11M acetic acid extracts

Sampling point	V	Cr	Co	Ni	Cu	Zn	As	Cd	Ba	Pb	Fe	Mn
1.2	0.23±0.06	ND	0.32±0.01	0.5±0.0	0.02±0.00	0.04±0.01	ND	0.03±0.00	ND	3.95±0.08	3.7±2.1	325.4±1.3
2.1	0.202±0.005	0.008±0.001	0.26±0.01	0.7±0.1	0.082±0.001	ND	ND	0.04±0.00	ND	6.7±0.1	3.7±1.7	67±1
2.2	0.282±0.004	0.023±0.001	0.26±0.01	0.78±0.03	0.09±0.01	ND	ND	0.03±0.00	ND	4.4±0.1	2.3±0.9	120±1
3.1	0.114±0.003	0.002±0.001	0.79±0.07	1.1±0.1	0.083±0.003	2.7±0.1	ND	0.03±0.00	ND	5.01±0.18	7.3±1.6	172.1±1.2
3.2	0.158±0.001	0.01±0.00	0.53±0.04	0.9±0.1	0.082±0.003	1.27±0.03	ND	0.04±0.00	ND	5.9±0.1	5.9±4.1	205.7±2.4
4.1	0.14±0.01	0.25±0.01	1.67±0.02	1.9±0.1	0.44±0.04	18±1	ND	0.03±0.00	ND	3.98±0.11	6.5±2.1	202±2
4.2	0.18±0.01	0.18±0.02	1.6±0.1	2.3±0.1	0.33±0.01	19±1	ND	0.04±0.00	ND	3.9±0.2	246.8±1.7	220.5±2.1
5.1	0.118±0.001	0.345±0.004	2.9±0.1	4.6±0.1	0.60±0.02	21.97±0.19	ND	0.03±0.00	ND	3.6±0.1	196.8±0.3	212±2
5.2	0.132±0.003	0.28±0.02	2.9±0.1	4.96±0.11	0.66±0.01	23.1±0.4	ND	0.04±0.00	ND	3.5±0.1	269.8±1.3	215±2
6.1	0.09±0.01	0.74±0.06	2.9±0.1	9.1±0.4	0.64±0.02	25±1	ND	0.03±0.00	ND	3.8±0.2	325.9±0.5	221±2
6.2	0.071±0.001	0.27±0.03	2.6±0.1	4.997±0.198	0.41±0.01	30±2	ND	0.04±0.00	ND	4.3±0.2	374.7±5.2	212±1
7.1	0.196±0.004	0.17±0.01	4.3±0.1	2.23±0.02	0.21±0.02	7.7±0.2	ND	0.03±0.00	ND	3.2±0.1	220.2±0.1	129±2
7.2	0.19±0.01	0.18±0.01	4.2±0.1	2.21±0.03	0.22±0.02	7.4±0.3	ND	0.03±0.00	ND	3.4±0.1	192±1	137±2
8.1	0.183±0.004	0.003±0.003	0.26±0.01	0.69±0.02	0.13±0.01	ND	ND	0.03±0.00	ND	3.5±0.1	ND	50±2
9.1	0.184±0.003	0.016±0.001	0.25±0.02	0.9±0.1	0.058±0.001	ND	ND	0.03±0.00	ND	2.95±0.08	ND	49±2
9.2	0.26±0.01	0.027±0.001	0.32±0.01	1.2±0.1	0.098±0.015	0.017±0.002	ND	0.03±0.00	ND	3.8±0.1	18.2±1.6	96±1
10.1	0.25±0.01	ND	0.29±0.01	0.5±0.1	0.028±0.002	0.04±0.03	ND	0.03±0.00	ND	4.9±0.2	ND	313±2
Mean	0.2	0.2	1.6	2.3	0.3	9.2	ND	0.3	ND	4.2	118	173
Range	0.2-0.3	0-0.7	0.2-4.3	0.5-9.5	0.02-0.7	0-30	ND	0.03-0.04	ND	3-7	ND-375	49-325
Mean % total extracts	0.3	0.1	14	4	2	0.3	ND	0.3	ND	17	0.4	32
% Range	0.1-0.4	ND-0.7	2-38	0.7-15	0.1-4	ND-41	ND	5.8-27	ND	12-28	ND-1.3	9-61

Notes:

1. ND means not detected 2. The red highlights are the pollution hotspots for some metals.

Table 18: Mean (± SD; n = 6) of mobile metal concentrations (mg/kg dry mass) in 0.005M DTPA + 0.01M $CaCl_2$ + 0.1M TEA extracts

Sampling point	V	Cr	Co	Ni	Cu	Zn	As	Cd	Ba	Pb	Fe	Mn
1.2	0.13 ±0.01	0.02±0.00	0.135±0.004	0.15±0.02	0.34±0.00	2.2±0.1	0.07±0.01	0.005±0.002	0.21±0.01	2.8±0.1	80.82±0.03	19.7±0.2
2.1	0.18 ±0.01	0.019±0.001	0.28±0.01	0.28±0.02	1.6±0.1	2.98±0.16	0.09±0.02	0.012±0.002	0.27±0.01	4.5±0.2	98.3±0.1	29.2±0.1
2.2	0.18±0.01	0.04±0.00	0.204±0.016	0.35±0.02	1.23±0.03	2.2±0.1	0.09±0.01	0.016±0.002	0.18±0.02	4.3±0.2	82.2±0.2	25.9±0.2
3.1	0.25±0.01	0.02±0.00	0.37±0.02	0.51±0.03	3.1±0.1	6.7±0.2	0.08±0.01	0.019±0.002	0.15±0.01	5.5±0.1	167.4±0.2	6.2±0.2
3.2	0.28±0.01	0.03±0.00	0.46±0.02	0.58±0.02	3.3±0.1	6.6±0.3	0.12±0.01	0.019±0.001	0.16±0.01	6.8±0.2	149.1±0.1	17.9±0.1
4.1	0.2 ±0.0	0.014±0.003	0.45±0.01	0.59±0.01	3.7±0.1	12.5±0.3	0.08±0.01	0.063±0.002	0.14±0.01	7.2±0.1	176.5±0.5	5.8±0.1
4.2	0.47±0.01	0.033±0.003	0.55±0.01	0.96±0.02	4.8±0.1	19±1	0.102±0.008	0.084±0.002	0.18±0.01	11.8±0.2	169.7±0.3	6.33±0.14
5.1	0.13±0.01	0.02±0.00	0.83±0.02	1.53±0.01	5.98±0.11	16.8±0.3	0.04±0.01	0.068±0.001	0.10±0.01	6.3±0.1	248±1	15.6±0.1
5.2	0.25±0.01	0.028±0.001	0.904±0.013	1.91±0.03	6.9±0.2	19.04±0.63	0.06±0.01	0.081±0.002	0.15±0.01	8.6±0.3	211±3	16.9±0.2
6.1	0.2±0.0	0.06±0.01	2.4±0.1	7.9±0.1	17.1±0.3	54±1	0.08±0.02	0.26±0.01	0.28±0.02	15.1±0.2	238.3±0.4	22.8±0.2
6.2	0.26±0.01	0.03±0.00	0.63±0.02	1.6±0.1	4.6±0.1	25±1	0.06±0.01	0.096±0.006	0.11±0.01	10.1±0.2	182±1	6.9±0.2
7.1	0.16±0.01	0.016±0.001	1.59±0.05	0.73±0.03	0.08±0.08	8.8±0.1	0.04±0.01	0.062±0.002	0.14±0.01	4.98±0.07	157.9±0.1	6.4±0.2
7.2	0.34±0.01	0.026±0.001	0.65±0.03	0.74±0.03	5.01±0.16	9.5±0.4	0.09±0.01	0.031±0.004	0.16±0.01	11.2±0.1	154.9±0.3	6.85±0.2
8.1	0.21 ±0.01	0.025±0.003	0.165±0.002	0.28±0.00	1.65±0.04	3.03±0.03	0.06±0.01	0.014±0.001	0.18±0.01	4.8±0.1	118.8±0.1	7.03±0.17
9.1	0.14±0.00	0.002±0.002	0.11±0.01	0.35±0.01	1.01±0.03	1.84±0.03	0.03±0.01	0.018±0.004	0.11±0.01	1.39±0.02	83.2±0.1	3.9±0.3
9.2	0.19±0.02	0.07±0.00	0.12±0.01	0.42±0.03	1.5±0.1	2.4±0.1	0.04±0.01	0.018±0.001	0.27±0.02	2.05±0.03	79.3±0.1	6.7±0.1
10.1	0.18±0.01	0.021±0.004	0.12±0.01	0.06±0.00	0.30±0.01	2.2±0.1	0.08±0.01	0.005±0.001	0.28±0.01	3.18±0.05	59.2±0.1	14.1±0.1
Mean	0.2	0.03	0.6	1.1	3.6	11.5	0.1	0.1	0.2	6.5	145	13
Range	0.1-0.5	0-0.1	0.1-2.4	0.1-7.9	0.1-17	1.8-54	0.03-0.2	0.01-0.3	0.1-0.3	1.4-15	59-248	4-29
Mean % total extracts	0.3	ND	5.2	1.7	22	16	0.6	16.4	0.1	27	0.5	2.4
% Range	0.3-0.5	ND	2-11	0.3-6	2-68	6-47	0.4-05	5-38	0.1-0.1	11-38	0.3-0.6	1.7-30

Note: The red highlights are the pollution hotspots for some metals.

Table 19: One-way ANOVA univariate results for the comparison of 0.11M acetic acid and 0.005M + 0.01M CaCl2 + 0.1M TEA (red highlights were significant at $p < 0.05$).

Variable	Df	ANOVA Type VI (Unique) SS	MS	F	p
V	1	0.016	0.016	2.941	0.096
Cr	1	0.123	0.123	6.428	0.016
Co	1	7.927	7.927	6.358	0.017
Ni	1	12.846	12.846	2.819	0.103
Cu	1	98.343	98.343	11.946	0.002
Zn	1	43.510	43.510	0.296	0.590
As	1	0.011	0.011	36.055	0.000
Cd	1	0.023	0.023	11.540	0.002
Ba	1	0.275	0.275	150.078	0.000
Pb	1	46.240	46.240	6.081	0.019
Fe	1	5589.7	5589.7	0.502	0.484
Mn	1	218899.9	218899.9	64.275	0.000

The results were also in agreement with the fact Cd has been reported as being among the easily removed (Sahuquillo, *et al*. 2003) and most labile (Marmolejo-Rodriguez, *et al*. 2007) metals, while Zn being reported in past studies of polluted sediments that high percentages of its total concentrations in sediments have been associated with more labile fractions (Lopez-Sanchez, *et al*. 1996), indicating that their high levels in acidic condition in this study were justified. These two metals were also found to be high in the complexing condition, which was in concurrence with the suggestion of Lindsay and Norvel (1978).

The results were also justified due to the fact that 0.005M DTPA + 0.01M $CaCl_2$ + 0.1M TEA extractant contains a high concentration of $CaCl_2$, of which Ca^{2+} may exchange rapidly with bivalent cations, especially Cd^{2+} and Zn^{2+} from soils and that $CaCl_2$ is the primary component of soil electrolytes. Additionally, the exchangeable cations and heavy metals may also be displaced by the basic cations (Ca^{2+}) commonly present in extraction solution (Wang, *et al.* 2004). It also contains triethanolamine (TEA), which is protonated at pH 7.3 and could exchange with cations from the exchange sites.

The highest differences were observed between Co, Cu, Pb and Mn, in which Cu and Pb were highly sensitive to complexation processes. The greatest proportion

of Cu in the 0.005M DTPA + 0.01M $CaCl_2$ + 0.1M TEA extracts implies that this extractant had the ability of mobilising Cu from the potentially active fractions (Gupta, *et al*. 1996), mostly from the oxidisable fraction (coinciding with organic and sulphur compounds) since this metal can easily form complexes with organic matter due to the high stability constant of organic-Cu-compounds as suggested by Morillo, *et al*. (2004). These suggestions were confirmed by the correlation realised in this study between organic matter and Cu as shown in Figure 20.

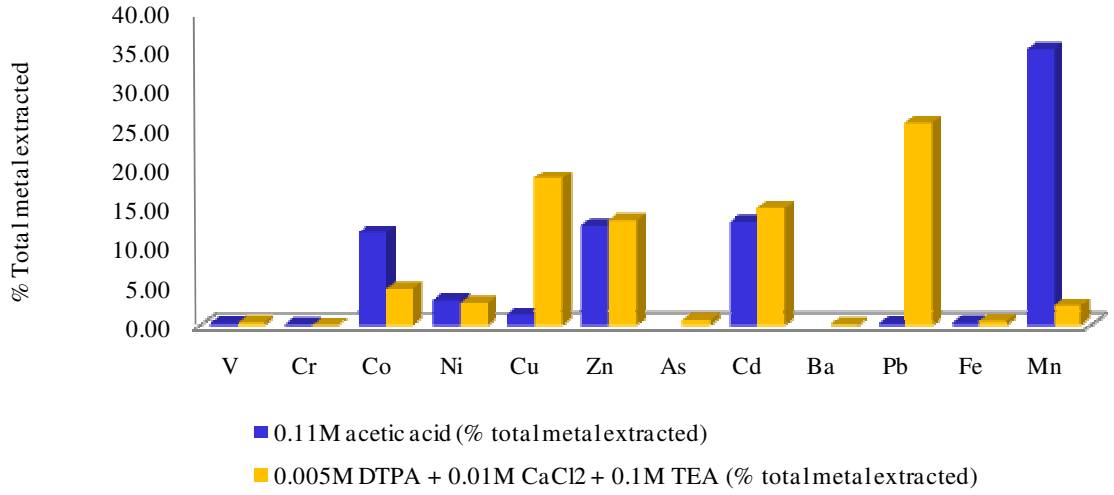


Figure 19: Comparison of extractability of mobile metals under acidic (0.11M acetic acid) and complexing (0.005M DTPA+ 0.01M CaCl2 + 0.1M TEA) conditions.

The ability of DTPA to extract metals complexed by organic matter was reported by Piccolo, (1989); Ure, (1996) and McGrath, (1996). Greatest percentage (68%) of total Cu in 0.005M DTPA + 0.01M $CaCl_2$ + 0.1M TEA was found in the inner part of the Bay at point 6.1 located in the vicinity of Palmones River mouth and adjacent to the Palmones industrial zones from which large amounts of organic matter enter the bay (Tarazona, *et al.* 1991).

Similar observations were made by Scoullos, *et al*. (2003).

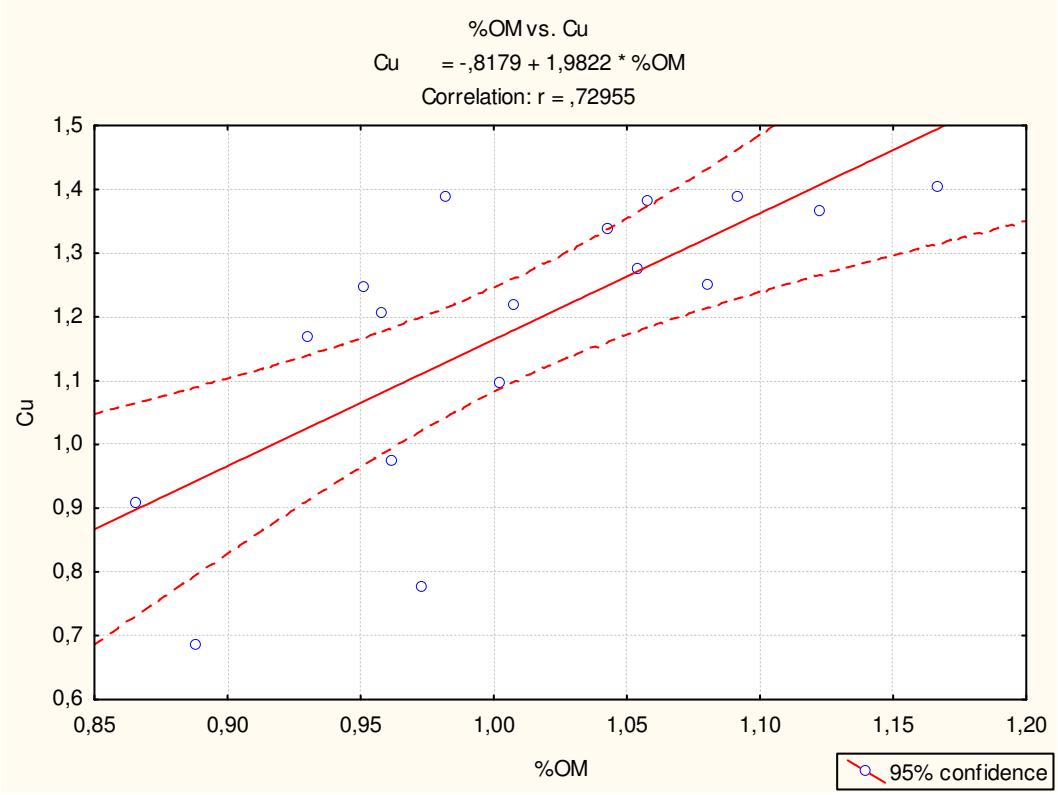
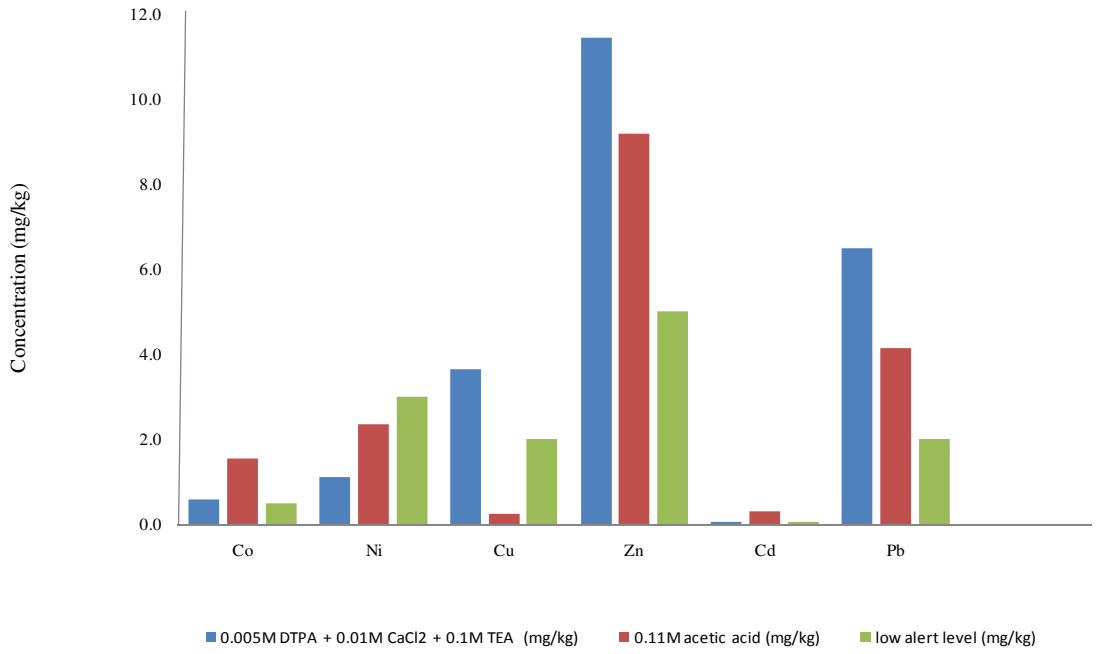


Figure 20: Correlation between percent organic matter and Cu.

In order to predict the potential bioavailability of metals studied, the averages of total concentrations in the whole system were compared with low alert-level sediment quality guidelines defined by Manheim and Commeau, (1981) as shown in Figure 21. Low alert level (LAL) refers to concentration range limits normally found in natural, uncontaminated sediments. Co extracted with both extractants exceeded the low alert level, while the Cd extracted in 0.11M acetic acid exceed the low alert level. Cu, Zn and Pb extracted in 0.005M DTPA + 0.01M $CaCl_2$ + 0.1M TEA exceeded low alert level guideline. These results helped to predict the potential bioavailability of the above metals, implying that had the water been in a condition similar to that of 0.11M acetic acid, then Co, Zn, Cd and Pb would have been the potentially mobile metals. Had it been that the water condition been complexing similar to that of

0.005M DTPA + 0.01M $CaCl_2$ + 0.1M TEA, then the same metals including Cu would have been the potentially mobile metals. It is noteworthy that the mobility of Cu, Zn and Pb were observed more in complexing condition than in acidic one, while mobility of Co and Cd were observed more in acidic than in complexing condition. The results disclosed that background levels of the most dangerous metals (Co, Cu, Zn, Cd and Pb) were violated; indicating that had there been frequent changes in environmental conditions that would favour acidic and complexing conditions, then Algeciras Bay would be adversely affected by the metal pollutants.



Note: Low Alert Levels (LAL) values from Manheim and Commeau, (1981); Effect- Range Low (ERL) and High Alert Levels (HAL) sediment quality guidelines (SQGs) values from Long *et al.* (1995) and USEPA, (1996) respectively. HAL guidelines were used here since many of these values are the same as Effect-Range Medium (ERM) values from Long *et al*. (1995).

Figure 28: Comparison of low alert level sediment quality guidelines defined by Manheim and Commeau, (1981) and the average of extracted concentrations of Co, Ni, Cu, Zn, Cd and Pb in both conditions.

CONCLUSIONS

The results of this study have disclosed that the major sources of metal pollutants in Algeciras Bay originate from the city and port of Algeciras and the industrial zones, indicating that metals in sediments from the bay were mostly originating from anthropogenic activities. A large area of the Bay was highly contaminated with Ni, whose concentration levels were above the high alert levels (HAL) from USEPA (1996), meaning that marine organisms might regularly be exposed to toxic effects. Cr and As concentrations also exceeded the effect range-low (ERL) defined by Long, *et al*. (1995), implying that toxic effects might infrequently occur in marine organisms. DTPA and acetic acid provided some information on the effect of complexation and acidification processes on metal extractability. ANOVA test showed that there existed both similarities and differences between the extractability potentials of the two conditions. The most important metals extracted by both conditions were: Co, Cu, Cd, Ni, Zn, Pb and Mn, during which Mn, Cd and Co were found to be sensitive to acid dissolution; Pb, Cu and Cd were sensitive complexation process, while Ni and Zn were found to be sensitive to both processes. Co, Zn, Cd and Pb extracted in both conditions were above low alert level. The mobility of Cu, Zn and Pb were observed more in complexing condition than in acidic one, while mobility of Co and Cd were observed more in acidic than in complexing condition. These results helped to predict the potential bioavailability of the above metals. The background levels of the most dangerous metals (Co, Cu, Zn, Cd and Pb) were violated; indicating had there been frequent changes in environmental conditions favouring acidic and complexing conditions, then Algeciras Bay would be adversely affected by these metal pollutants. The results disclosed that both conditions effected the remobilisation and release of metals ions, although at different levels, but to the point of creating awareness that there were potential adverse effects from metal pollutants in Algeciras Bay. It was recommended that this status should be contained through proper environmental management.

FUTURE WORK

- Discussion of all results from total, mobile metal concentrations, speciation and their correlations with levels of metal concentrations in water and biota using chemometric tools.
- Apply the knowledge acquired during this master's course for further studies and in environmental pollution monitoring in my country.

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