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A Review of Sequential Extraction Procedures for Heavy Metals Speciation in Soil and Sediments

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Abstract

Heavy metals are stable and persistence environmental contaminant of marine sediments. The vast increase in population growth, urbanisation and industrialisation leads to increase in of marine discharges, which results in total loads of pollutants being delivered to the sea. Heavy metal pollution in aquatic environment and subsequent uptake in food chain by aquatic organisms and humans put public health at risks. However, even at lower concentrations heavy metals like Cd, Hg, Cr and Pb may exhibit extreme toxicity under certain condition. Thus, this makes regular monitoring of aquatic environment to be more imperative and necessary. This paper therefore, review the occurrence of heavy metals and various speciation methods used for heavy speciation in soil and sediments.

Keywords: Heavy metals; Speciation; Sediments; Pollution; Marine

Introduction

Heavy metals are among the most serious environmental pollutants due to their high toxicity, abundance and ease of accumulation by various plant and animal organisms. Persistent increase of heavy metals in harbour sediments can be attributed to the contribution of effluent from waste water treatment plants, industries, mining, power stations, agriculture [1] which carry run-offs to the harbour. The increase in urbanisation and industrialisation also could lead to an increase in marine discharges and therefore results in total loads of pollutants discharges to the sea. These discharges may contain heavy metals among other pollutants [2]. In addition, ship traffic especially in and close to the harbour and repair activities are also suspected to be indicative for elevated concentration in the upper reaches of harbours.

Metal concentration in sediments can be traced to high concentration in living organisms and humans and therefore put public health at risk. The bioavailable metal load in sediments may affect the distribution and composition of benthic assemblages [3] and this will cause increase in high concentration of these pollutants in living organisms [4]. High concentrations of heavy metals in living organisms can result in morphological abnormalities, neurophysiological disturbances, genetic alteration of cells (mutation), tetratogenesis and carcinogenesis. Moreover, heavy metals can affect enzymatic and hormonal activities, as well as growth rate and an increase in mortality rate [5]. Metals accumulates in sediments from both natural and anthropogenic sources and sediments act as a scavenger agent as well as an adsorptive sink for heavy metals in an aquatic environment. Sediments can therefore be described as appropriate indicators of heavy metal pollution [6].

The accumulation of metals in sediments from both natural and anthropogenic sources occurs in the same way, thus making it difficult to identify and determine the origin of heavy metals present in the sediments [7]. Moreover, the total concentration of metals often does not accurately represent their characteristics and toxicity. In order to overcome the above mentioned obstacles it is helpful to evaluate the individual fractions of the metals to fully understand their actual and potential environmental effects [8]. Single extractions are thus used generally to provide a rapid evaluation of the exchangeable metal fraction in soils and sediments [9,10]. However, various complicated sequential extraction procedures were used to provide more detailed information regarding different metal phase associations [8,11,12].

A wide range of techniques is available whereby various extraction reagents and experimental conditions are used. These techniques involve a 5step [8], 4step (BCR, Bureau Commune de Reference of the European Commission) and 6-step [13] extraction, and are thus becoming popular and adopted methods used for sequential extraction [14,15]. Several analytical methods have been used for the determination of heavy metals contents in marine environments. These include; flame AAS [16,17], atomic fluorescence spectrometry [18], anodic stripping voltametry [19,20], ICP- AES [21] and ICP-MS [22,23].

Heavy metal mobility and bioavailability depend strongly on their chemical and mineralogical forms in which they occur [24]. Several speciation studies have been conducted to determine study different forms of heavy metals rather their total metal content. These studies reveal the level of bioavailability of metals in harbour sediments and also confirm that sediments are bio- indicators of heavy metal pollution in marine environment [1,7,25,26].

Although several studies have been conducted on heavy metal pollution of harbour sediments, this paper aims to review sources, mobility, effects remediation and analytical methods used as well as to compare results that have been collected around the world on heavy metal speciation.

Heavy metals as marine pollutants

Major and trace elements occur naturally in the environment [26]. This natural occurrence of metals in the environment due to various particle sizes for instance, complicates assessments of contaminated marine sediments because measurable quantities of metals do not automatically infer anthropogenic enrichment [26]. In addition to

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shipping traffic especially in and close harbours Industrial activities, vehicle emissions, agricultural activities and domestic waste can all act as a source of heavy metal pollution in the marine environment [7].

Many adverse effects have been done on human health by the environmental pollution of heavy metals. Heavy metals condition is problematic due to their persistence and non-degradability in the environments [27]. Metals distribution and association in marine sediments occur in various ways which include ion exchange, adsorption, precipitation and complexation. They are not permanently fixed by sediments [27]. Heavy metals pollution in aquatic environment and their uptake in the food chain by aquatic organisms and humans, put public health at risk.

In general, heavy metals are stable and persistence environmental contaminants of marine sediments. Interest in metals like Zn, Cu, Fe, and Mn which are required for metabolic activities in organisms depends on their nutritional value and their toxicity. Metals like Cd, Hg, Cr, Pb and As may exhibit extreme toxicity even at lower concentration under certain condition. Thus this makes regular monitoring of aquatic environment to be imperative and necessary.

Occurrence of heavy metals in marine sediments

Heavy metals are stable and persistent environmental contaminants of coastal sediments. In recent years there has been growing concern over increased contamination of estuaries and harbours from various anthropogenic sources [25]. Sediments serve as the ultimate sink for many contaminants and as a result, they pose the highest risk to the aquatic life as a source of pollution [28,29]. Bruder- Hubscherv et al. [30] worked on metal speciation in coastal marine sediments from Singapore and confirmed that sediments are the main repository and source of heavy metals in the marine environment and that they play a major role in the transport and storage of potentially hazardous metals.

A number of factors have been attributed to pollutant accumulation in harbour sediments. The design of the harbour to minimize hydrodynamic energy, industrial activities (ship repairs and traffic, accidental spills, loading and unloading), agricultural activities and urban (waste water) activities can all acts as sources of heavy metal pollution in marine environment [1,2,5,31]. Heavy metal accumulation in marine sediment is due to a highly dynamic nature of the marine environment which allows rapid assimilation of these pollutants into sediments by processes such as oxidation, degradation, dispersion, dilution and ocean currents.

Phytoavailability of heavy metals depends on the characteristics of the sediment, the nature of the metal species, the interaction with sediment matrix and the duration of the contact with the surface binding. Heavy metal availability in marine organisms can be traceable to sediment characteristics such as pH, organic matter content and type, and then moisture [32]. In general, increase in population growth, rapid unplanned industrialization, urbanization, exploration and exploitation of natural resources and newly introduced modern agricultural practices are the major contributory factors responsible for the presence of heavy metals in marine sediments.

Heavy metals in water, soil and sediments

Heavy metals refer to any metallic chemical element that has a relatively high density and is toxic or poisonous at low concentration. Heavy metals occur naturally in the ecosystem with large variations in concentration. Nowadays, anthropogenic sources of heavy metals i.e. pollution, have been introduced to the ecosystem. These metals are a cause of environmental pollution (heavy-metal pollution) from a number of sources, including lead in petrol, industrial effluents and leaching of metal ions from the soil into water bodies by acid rain.

Toxic metals can be present in industrial, municipal and urban runoff, and by definition they are harmful to humans and aquatic biota. Increased urbanization and industrialization have increased the levels of trace metals, especially heavy metals in water ways. There are over 50 elements that can be classified as heavy metals, but only 17 that are considered to be both very toxic and relatively accessible. Mercury, lead, arsenic, cadmium, selenium, copper, zinc, nickel and chromium, however, should be given particular attention in terms of water pollution and discharge effects. Toxicity levels depend on the type of metals, its biological role, and the type of organisms that are exposed to it [33].

Zinc: Zinc is one of the numbers of trace elements considered essential to plant growth and the physiological function of organism. The permissible limit for zinc in portable water is 5.0ppm. At the concentrations above, 5.0ppm, zinc can cause a bitter, astringent taste and turbidity in alkaline waters. Zinc requirements of human vary because individuals zinc in adults ranges from 2-3μg. The highest concentrations are found in the urethra tract and the prostrate [34]. It has been found that various parts of the body contain zinc, relatively high concentrates are present in the skin, while the visceral organs contains approximately 30-50μg/g of fresh tissue. Most of the body zinc is in the bones where its concentration is approximately 200 μg zn/g. Excessive intake of Zn may lead to vomiting, dehydration, abdominal pains, nausea, lethargy and dehydration [34].

Cadmium: Cadmium is also one of the heavy metals found in soil and water samples. It is a by-product of the mining and smelting of lead and zinc. It is used in nickel cadmium batteries, PVC plastic and paint pigments. It can be found in soils because insecticides, fungicides sludge, and commercial fertilizers that use cadmium are used in agriculture. Cadmium may be found in reservoirs containing shell fish. Inhalation accounts for 15-20% of absorption through the respiratory system; 2-7% of ingested cadmium is absorbed in the gastrointestinal system. Cadmium toxicity is generally indicated when urine levels exceed 10 μg/dl and blood levels exceed 50 μg/dl. Cadmium sulphide and selenide are commonly used as pigments in plastics [35].

Aluminium: Although aluminium is not a heavy metal (specific gravity of 2.55 -2.80), it makes up about 8% of the surface of the earth and is the third most abundant element. It is readily available for human ingestion through the use of food additives, antacids, buffered aspirin, astringents, nasal sprays and antiperspirants from drinking water [36]. Studies suggested that aluminium might have a possible connection with developing Alzheimer's and Parkinson's disease when researchers found what they considered to be significant amounts of aluminium in the brain tissue of Alzheimer's patients. Aluminium also causes senility and presenile dementia [36].

Copper: Copper is an essential substance to human life, but in high doses it can cause anaemia, liver and kidney damage and stomach and intestinal irritation. Copper normally occurs in drinking water from copper pipes, as well from additives designed to control algal growth. In humans exposure to lead can result in a wide range of biological effects depending on the level of duration of exposure [37]. Various effects occur over a bound range of doses, with the developing foetus and infant being more sensitive than the adult. High levels of exposure may result in toxic biochemical effects in humans which in turn cause problems in the synthesis of haemoglobin, effects on the kidneys and acute of chronic damage to the nervous system. Some studies suggest that there may be a loss of up to 21Q points for a rise in blood lead levels from 10 to 20 μg/dl in young children. Average daily lead intake for adults is estimated at 1.6 μg from air, 20 μg from drinking water and 28 μg from food. Copper is generally remobilized with acid based ion exchange or oxidation mechanism [37].

Mercury: Mercury is a toxic substance which has no known function in human biochemistry or physiology and does not naturally in living organisms. It is a global pollutant with complex and unusual chemical and physical properties. The major natural source of mercury is the degassing of the Earth's crust, emissions from volcanoes and evaporation from natural bodies of water. The usage of mercury is widespread in industrial processes and in various products, (e.g. batteries, lamps and thermometers). Toxicity of mercury results mental disturbance and impairment of speech, hearing, vision and movement [38].

It is also widely used in dentistry as an amalgam for fillings and by the pharmaceutical industry. Concern over mercury in the environment arises from the extremely toxic forms in which mercury can occur. Natural biological processes can cause methylated forms of mercury to form which bio-accumulate over a million fold and concentrate in living organisms especially fish. These forms of mercury: monomethylmercury and dimethylmercury are high toxic causing neurotoxicological disorders. The main pathway for mercury to humans is through the food chain and not by inhalation [38].

Effects of heavy metals on public health

Sediments house many contaminants and therefore pose the highest risk to the aquatic environment as a source of pollution [28,29]. Environmental pollution by heavy metals impacts negatively on human health. Their remediation proves to be problematic due to the persistence and non degradability of heavy metals [27]. High concentrations of heavy metals in biota can be linked to high concentration in sediments. The bioavailable metal load in sediments may affect the distribution and composition of benthic assemblages [39], and this can be linked to high concentration recorded in living organisms [40].

The most obvious effect of pollution is to reduce diversity of biological species that are not able to tolerate the toxicants. Most resistant organisms are often undesirable in human terms. Example is the blue-green algae or sewage fungus that forms slime or scum. Heavy metals are dangerous because they tend to bio-accumulate. Bioaccumulation means an increase in the concentration of a chemical in a biological organism over time, compared to the chemical's concentration in the environment. Heavy metals can cause serious health effects with varied symptoms depending on the nature and the quantity of the metal ingested [41].

Antimony is a metal used in the compound antimony trioxide, a flame retardant. There is a little information on the effect of long term antimony exposure, both lead and antimony are suspected human carcinogen [36]. Cadmium derives its toxicological properties from its chemical similarity to zinc an essential micronutrient for plants, animals and human. In human, long term exposure is associated with renal dysfunction. High exposure can lead to obstructive lung disease and has been linked to lung cancer. Cadmium may also produce bone defects (osteomalacia, osteoporosis) in human and animals. This is an intensely painful disease leading to deformity of bone [36].

The biological activity of selenium has been of interest since it is needed by humans and other animals in small amounts, but in larger amounts can cause damage to the nervous system, fatigue and irritability. Selenium accumulates in living tissue, causing high selenium content in fish and other organisms, and causing greater health problems in human over a lifetime of over exposure. Acute exposure to lead is also more likely to occur in the work place, particularly in manufacturing processes that include the use of lead symptoms include abdominal pain, convulsion, hypertension, renal dysfunction. Etc. Chronic exposure and accumulation of lead may result in birth defects, mental retardation, and autism. Lead also depresses sperm count [42].

Arsenic is a highly toxic metalloid element. It is a key additive in rat poison, and with constant exposure, it is thought that arsenic may affect the chromosomes of humans and their health. However, very small amounts of arsenic could be good for humans to live and even be able to breathe. The inorganic form of arsenic found in contaminated meats, weed killers and insecticides, however can be very toxic [43]. Chromium is used in metal alloys and pigments for plants, cement, paper, rubber and other materials. Low level exposure can irritate the skin and cause ulceration. Long term exposure can cause kidney and liver damage, and damage to circulatory and nerve tissue. Chromium often accumulates in aquatic life, adding to the danger of eating fish that may have been exposed to high levels of chromium. However, under certain environmental conditions and certain metabolic transformations, chromium (III) may readily be oxidized to chromium (VI) compounds that are toxic to human health [44,45].

The vast increase in environmental pollution by heavy metals puts public health at risk. Various effects of heavy metal pollution in humans are morphological abnormalities, neurophysiological disturbances, genetic alteration of cells (mutation), tetratogenesis and carcinogenesis. The presence of heavy metals affects enzymes and hormonal activities as well as growth and in mortality rate [7].

The influence of salinity on results of heavy metal mobility of harbour sediments

Trace metals are among the most common contaminants bound to estuarine sediments. The bioavailability and toxicity of these metals to aquatic organisms depend on the physical and chemical forms of the metal as well as several physicochemical parameters such as temperature, pH, salinity, dissolved oxygen and particulates matter composition. In fresh water, pH is the controlling factors while salinity is stated as one of the controlling factors affecting the partitioning of contaminants between sediments and water in sediments in marine or estuarine environment due to the great variability of this parameter in them [46].

Several studies relating the effects of salinity and pH on heavy metals mobility in estuarine and marsh sediments are reported [47-49]. A decrease in the salinity of dredged harbour sediments may lead to a different partitioning coefficients of (ratio between metal in sediment and the interstitial water, Kd) heavy metals but depends on several predominant processes such as mobilisation of metals through complexation with seawater anions (Cl- and SO_4^{2-}-) [46Changes in salinity play a major role in metal distribution in dredged harbour sediments, especially when washing procedure is applied as a remediation technique or when dredged harbour sediments are deposited in the open air.

In related studies, [50] investigated the influence of pH, and salinity on the toxicity of heavy metals in sediments to the estuarine calm Ruditape Phillippinarium. They found out that heavy metals tend to be more bioavailable at lower salinity than at higher salinity value

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and this may be more toxic to the exposed organisms. They were able to establish that the effect of the salinity varies from metal to metal depending on the relative important of two counteracting processes, desorption from sediments to water or coagulation, flocculation and precipitation. From their results, sediments collected in area affected by chronic heavy metal contamination tend to be more efficient in trapping Zn, Cu and Pb at low salinity values. They found out that Cd tends to be more mobile as salinity increases.

In another study, [50] worked on the effect of chloride on heavy metal mobility of harbour sediments. Modified BCR- SEP was applied to harbour mobility in order to assess the extent trace element mobility (Cd, Cr, Cu, Ni, Pb and Zn) could be influenced by chloride content in sediments. Washed and non-washed sediment were compared respectively. The relative mobility order found for the six trace metals studied was not seen to be influenced by the presence of chloride in the sediments. An increase in mobility was observed for Cd and Zn (the most mobile metals) when chloride was present in the sediments. This was in agreement with findings from Riba et al. [1].

Therefore, further studies on the combine effects of pH and salinity on heavy metal mobility in marine harbour are recommended to be able to compare smaller difference in salinity values and in order to ascertain the major influence of chloride on results of heavy metals mobility

Analytical methods

Tremendous amounts of toxic pollutants have been discarded into coastal environment and the sediments of harbours represented large sink of heavy metals [51-54]. The sea and more particularly the aquatic systems are the ultimate respiratory of man's waste. Due to the dynamic nature of marine environment there is rapid assimilation of these materials by processes such as dilution, dispersion, oxidation, degradation or sequestration into sediments [2]. The release of heavy metals from sediments to water and organisms can be accelerated by processes which alter redox potentials of sediments and chemical forms of heavy metals. Toxic heavy metals are adsorbed onto organic matter and mineral surfaces in inorganic and organic forms [54,55].

Heavy metal mobility and bioavailability in sediments depend strongly on the mineralogical and chemical forms in which they occur [24]. Therefore, measurement of total metal concentrations is useful to estimate the heavy metal burden since their mobility depends on ways of binding. In other words, determination of specific chemical species or binding focus is very complex and hardly possible often. It is very imperative to study different forms of heavy metal mobility and bioavailability rather than the total concentration in order to obtain an indication of the bioavailability of metals. For this reason, sequential extraction procedures are commonly applied because they provide information on the fractionation of metals in the different lattices of the solid sample which serves as a good compromise to give information on environmental contamination risk [56,57].

Metal accumulation in sediments from both natural and anthropogenic sources is thus making it difficult to identify and determine the origin of heavy metals present in the sediment [7]. Since the early 1980s and 1990s sequential extraction methodology has been developed to determine speciation of metals in sediments [8,58] due to the fact that the total concentration of metals often does not accurately represent their characteristics and toxicity. In order to overcome the above mentioned obstacles it is helpful to evaluate the individual fractions of the metals to fully understand their actual and potential environmental effects [8]. Heavy metal pollution is a serious and widely environmental problem due to the persistent and non- biodegradable properties of these contaminants [27]. Sediments serve as the ultimate sink of heavy metals in the marine environment and they play an important role in the transport and storage of potentially hazardous metals.

To date, strong acid digestion is used often for the determination of total heavy metals in the sediments. However, this method can be misleading when assessing environmental effects due to the potential for an overestimation of exposure risk. Moreover, in order to eliminate the mobility of heavy metals in sediments, various sequential extraction procedures have been developed [59-63]. However, the number of steps in this extraction varies from 3 to 6 steps: 3[64], [65] 5 [8] to 6 [13].

Sequential extraction procedures (SEP) are operationally defined methodologies that are widely applied for assessing heavy metal mobility in sediments [66,67], soils [68] and waste materials [69]. Single extractions are thus used generally to provide a rapid evaluation of the exchangeable metal fraction in soils and sediments [9,10]. Various complicated sequential extraction procedures were used to provide more detailed information regarding different metal phase associations [8,11,12].

In addition, heavy metal speciation in environmental media using sequential extraction is based on the selective extraction of heavy metals in different physicochemical fractions of material using specific solvents [30]. These methods have been used widely in determining specific chemical forms of heavy metals in a range of environmental media which include sediments [1,27,66,67] soils [68,70,71] and waste materials [30,69].

Among a range of available techniques using various extraction reagents and experimental conditions to investigate the distribution of heavy metals in sediments and soils, the 5-step Tessier et al. [8] and the 6-step extraction method, Kerstin and Fronstier [13] were mostly widely used. Following these two basic schemes, some modified procedures with different sequences of reagents or experimental conditions have been developed [72-75]. Considering the diversity of procedures and lack of uniformity in different protocols, a BCR, Bureau Commun de Recherche (now called the European Community (EC) Standards Measurement and Testing Programme) method was proposed [64]. It harmonized differential extraction schemes for sediment analysis. The method has been validated using a sediment certified reference material BCR-701 with certified and indicative extractractable concentration of Cd, Cr, Cu, Ni, Pb and Zn [76]. This method was applied and accepted by a large group of specialists [77-82] despite some shortcoming in the sequential extraction steps [83,84].

Wang et al. [85] used a modified Tessier sequential extraction method to investigate the distribution and speciation of Cd, Cu, Pb, Fe, and Mn in the shallow sediments of Jinzhou Bay, Northeast China. This site was heavily contaminated by nonferrous smelting activities. They found out that the concentrations of Cd, Cu and Pb in sediments was to be 100, 73, 13and 7times, respectively, higher than the National guidelines (GB 18668-2002). The sequential extraction tests revealed that 39% -61% of Cd was found in exchangeable fractions. This shows that Cd in the sediments posed a high risk to the local environment. Cu and Pb were found to be at moderate risk levels. According to the relationships between percentage of metal speciation and total metal concentration, it was concluded that the distributions of Cd, Cu and Pb in some geochemical fractions were dynamic in the process of pollutants migration and stability of metals in marine sediments from

Jinzhor Bay decrease in the order Pb > Cu >Cd. Capalat et al. [86] developed a modified three-step sequential extraction procedure to examine the heavy metal mobility in harbour- contaminated sediments of Port-en-Basin, France. It was revealed that metallic contaminants associated with sediments showed various behaviours depending on physicochemical conditions. In the studied core, anoxic condition was developed a depth of 15cm. A 3-step sequential extraction procedure was applied to the anoxic sediment in order to evaluate the potential mobility of fixed metals. According to their findings zinc was the most labile metal recovered in the first extraction stages, and was associated with the non- residual fraction of sediment. Lead was found to be the least labile, with up to 70% associated with the residual fraction of the sediments. Copper was associated with organic matter, and its mobility was controlled by the concentration and degradation of the organic fraction. They finally concluded that discharge of organic rich dredged sediments at sea results in degradation of contaminated organic matter and these may affect the environmental impact of these metals significantly.

In similar studies, Yuan et al. [27] applied BCR-sequential extraction protocol to obtain metal distribution patterns in marine sediments from the East China Sea. The results showed that both the total contents and the most dangerous non-residual fractions of Cd and Pb were extremely high. More than 90% of the total concentration of V, Cr, Mo and Sn existed in the residual fraction while more than 60% of Fe, Co, Ni, Cu, and Zn were mainly present in the residual fraction. Mn, Pb, and Cd were dominantly present in the non- residual fractions in the top sediments.

Jones and Turki [87] worked on distribution and speciation of heavy metals in surface sediments from the Tees estuary, North East England. Tessier et al. [8] metal speciation scheme modified by Ajay and van Loon [88] was used for the study. They found out that the sediments were largely organic- rich clayey silts in which metal concentrations exceed background levels, and which attain peak values in the upper and middle reaches of the estuary. Cr, Pb and Zn were associated with the reducible, residual, and oxidizable fractions. Co and Ni were not highly enriched while Cu is associated with the oxidizable and residual fractions. Cd is associated with the exchangeable fractions.

Pempkowlak et al. [89] investigated the speciation of heavy metals in sediments and their bioaccumulation by mussels. They used a 4-step sequential extraction procedure adapted from Forstner and Watmann [31]. Their investigation which was characterized by varying metal bioavailability was aimed at revealing differences in the accumulation pattern of heavy metals in mussel inhabiting that inhabit in sediments. The bioavailabilities of metals were measured using the contents of metals adsorbed to sediments and associated with iron and manganese hydroxides. The biovailable fraction of heavy metals contents in sediments collected from Spitsbergen represented a small proportion (0.37% adsorbed metals and 0.11%, are associated with metals hydroxides). It was also revealed that the percentages of metals adsorbed and bound to hydroxides of the sediments ranged from 1 to 46% and 1 to 13%, respectively.

Wepener and Vermeulen [25] worked on the concentration and bioavailability of selected metals in sediments of Richards Bay harbour, South Africa Sequential extraction of sediments was carried out according to Tessier et al. [8] methods. The following metals were investigated: Al, Cr, Fe, Mn, and Zn respectively. Their studies revealed that metals concentrations in sediments samples varied only slightly between seasons, but showed significant spatial variation, which was significantly corrected to sediment particle size composition. Highest metal concentration was recorded in sites with substrates dominated by fine mud. Mn and Zn had more than 50% of this concentration in reducible fraction while more than 70% of the Cr was associated with the inert fractions and the concentration recorded at some sites were still above action levels when considering only the bioavailable fractions. They also concluded that the concentration of zinc recorded was not elevated their results were compared with the historic data. Coung and Obbard [90] used a modified 3-step sequential extraction procedure to investigate metal speciation in coastal marine sediments from Singapore as described by the European Community Bureau of Reference (ECBR). Highest percentages of Cr, Ni, and Pb were found in residual fractions in both Kranji (78.9%, 54.7% and 55.9% respectively) and Pulang Tokong (82.8%, 77.3% and 62.2% respectively). This means that these metals were strongly bound to sediments. In sediments from Kranji, the mobility order of heavy metals studied were Cd > Ni > Zn > Cu > Pb > Cr while sediments from Pulan Tekong showed the same order for Cd, Ni, Pb and Cr. but had a reverse order for Cu and Zn (Cu > Zn). The sum of the 4-step s (acid soluble, + reducible + oxidizable + residual) was in good agreement with the total metal content, which confirmed the accuracy of the microwave extraction procedure in conjunction with the GFASS analytical method. Analytical methods used for heavy metal speciation are summarized in Table 1 and Table 3 respectively.

A critical appraisal of different sequential fractions

Exchangeable fraction: This fraction involves weakly adsorbed metals retained on the solid surface by relatively weak electrostatic interaction, metals that can be released by ion-exchangeable processes etc. Remobilisation of metals can occur in this fraction due to adsorption-desorption reactions and lowering of pH [96,97]. Exchangeable metals are a measure of those traces metals which are released most readily to

Locations	Extraction techniques	Analytical methods	References
Barcelona harbour, Spain	BCR-3STEP Sequential Extraction	ICP-MS, ICP-AES and Single-beam-flame AAS.	[1].
Kranji and Pulau Tekong harbour Singapore	Modified BCR-3STEP Sequential Extraction	GFAAS	[90].
Townsville harbour, Queensland,	Modified Tessier Extraction	AAS	[26].
Richards Bay Harbour	Tessier Extraction step	Flame AAS	[25].
East China Sea	BCR-3STEP Sequential Extraction	ICP-MS	[27].
Norwegian Sea and Baltic Sea	4- Step Sequential Extraction	AAS	[89].
Huelva Estuarine	3-Step Sequential Extraction	AAS	[81].
Southwest Coast of Spain	BCR-Sequential Step	GFAAS	[91].
Urban and suburban agricultural soils from China	BCRSEP Optimized	ICP-MS	[92].
Polluted soil and sediments from Morocco	BCRSEP optimized	FAAS	[93].
Soil affected by an accidental spills in Spain	BCRSEP	ICP-MS	[94].
Agricultural soil from Chile	New SEP developed	AAS	[95].

Table 1: Analytical methods used for speciation of heavy metals.

Method	Metals species analyzed
Liquid Phase	
Electcroanalysis	Free ionic concentration
Ion selective electrodes voltametry	Free ions and labile complexes
Spectroscopy	Specific forms inorganic and organometallic species, different oxidation state (Sn, As, Se, Te
Chromatography	
GC or LC	Oragonometallic compound of mercury, tin and lead
Physico-chemical fractionation	
Ion-exchange resins	Free ions and liable complex
UV radiation	Organic complex
Solvent extraction	Organic complex
Solid phase	
Ion –exchange resins	Reagent soluble fraction
Single reagent leaching	Reagent soluble fraction

Table 2: Speciation of the analyzed heavy metals in sediment of different coastal system.

Metals		F1	F2	F3	F4	F5	References
Cr	Acheloos river estuary, Greece	2	1.5	7.1	1.1	88.3	[112].
		0	6.9	6.4	8.3	78.4	[113].
		0.3	0.7	16.5	3.5	79.0	[114].
Fe	Acheloos river estuary, Greece	0.3	5.2	12.2	4.2	78.1	[112].
	Coastal sediment, Baja, USA	0	0.4	6	1.6	92	[115].
	Barbate River Salt marshes	<0.1	0.8	17.4	2.8	79	[112].
Cu	Humber estuary, UK	2	6.1	8.2	53.1	30.6	[116].
	Mersey estuary, UK	1.8	12.9	10.5	37.4	37.4	[116].
	Barbate Rivers salt marshes	1.1	6.9	8.8	20.3	62.9	[114].
Zn	Vigo estuary, Spain	0.0	10.0	34.0	11.0	39.0	[117].
	Huelva estuary, Spain	15.7	18.9	28.8	12.9	24.3	[118].
	Barbate River salt marshes	0.0	3.6	15.4	16.1	64.8	[114].
Cd	Humber estuary, UK	31.9	37.7	26.1	4.3	0	[116].
	Mersey estuary, UK	34.2	29.1	22.8	6.3	7.6	[116].
	Barbate River Salt marshes	9.9	27.6	30	7.7	24.8	[114].
Pb	Mersey estuary, UK	5.3	16.8	55	9.2	13.7	[116].
	Vigo estuary, Spain	0	9	31	8	53	[117].
	Barbate River Salt marshes	1.1	13.8	18.9	7	59.2	[114].
Mn	Acheloos River estuary, Greece	1.1	72.1	11.2	13.4	13.4	[112].
	Huelva estuary, Spain	9.3	0.25	25	58.6	58.6	[118].
	Cadiz	11.7	41.1	16.2	22.8	22.8	[113].

Key: Results are expressed in % the total metal concentration. F1 to F5 represents fractions of the sequential extraction techniques of Tessier et al. (1979) [8].

Table 3: Other Analytical techniques applied for metal speciation Track et al. (1995) [119].

the environment. Corresponding metals in the exchangeable fraction represent a small fraction of the total metal content in soil, sewage sludges and sediments and can be replaced by neutral salts [98]. This fraction generally accounted for less than 2% of the total metals in soils presents, the exceptions to this microelement, K, Ca, and Mn [99]. Exchangeable fraction is also known as non-specifically adsorbed fraction, it can be released by the action of cations such as K, Ca, Mg or (NH$_4$) displacing metals weakly bond electrostatistically organic or inorganic sites [100]. The common reagents used for the extraction of metals in this fraction are MgCl$_2$, sodium acetate (pH 5.4) by acetic acid [8]. Reagents used for this purpose are electrolytes in aqueous solution, such as salts of strong acids and bases or salts of weak acids and bases at pH 7. Other reagents showing similar properties have seldom been used, such as nitrate salts (to avoid complexation that is too strong) or calcium salts (Ca 2+ being more effective than Mg^{2+} or NH^{4+} in removing exchangeable ions). Results obtained with these reagents give good correlation with plant uptake [101].

The carbonate phase: Carbonate tends to be a major adsorbent for many metals when there is reduction of Fe-Mn oxides and organic matter in the aquatic system. The most popular use reagent for the extraction of trace metals from carbonates phases in soils and sediments is 1M sodium acetate adjusted to pH 5.0 with acetic acid [8]. The carbonate fraction is a loosely bound phase and bound to changes with environmental factors such as pH [102]. The time lag for the complete solubilisation of carbonates depends on some factors such as the type and amount of the carbonate in the sample, particle size of the solid [102]. Extraction of metals from carbonates phases enhances the leaching of metals specifically sorbed to organic and inorganic substrates [8]. In general, this fraction is sensitive to pH changes, and metal release is achieved through dissolution of a fraction of the solid material at pH close to 5.0 [101].

Iron and Manganese oxides phases: This is referred to as sink for heavy metals. Scavenging by these secondary oxides, present as coating on mineral surfaces or as fine discrete particles. This can occur as a combination of the precipitation, adsorption, surface complex formation and ion exchange [103]. Extraction of metals in Fe-Mn oxides phases with 0.1M hydroxylamine when compared with the extraction with 0.5M hydroxylamine. There is a variation accounted, 0.1M release metal mainly from amorphous manganese oxide phases with less attack on the iron oxide phase [104]. Extract with 0.5M gives effective attack on the iron oxide phase while still release metals from manganese oxide phase. Different reagent has been used for metal extraction in Fe-Mg oxide phases amongst are sodiumdithionate in combination with sodium citrate and sodium bicarbonate in a varying concentration range [100]. Extraction with ascorbic acid / ammonium oxalate reagent offers great merits over others because high purity degree is achieved and does not attack silicates. However, the most successful reagents for evaluating the total amount of metal ion associated with these minerals contain both a reducing reagent and a liable ligand able to retain released ions in a soluble form, the efficiency of the reagent being determined by its reduction potential and its ability to attack the different crystalline forms of Fe and Mn oxyhydroxides. Hydroxylamine, oxalic acid and dithionite are the most commonly used reagents [101].

Organic phases: The bioaccumulation or complexation process being the primary source in which trace metal get associated with organic material such as living organisms, detritus etc. In aquatic systems, organic substances tends to have high degree selectivity for individuals ions compared to monovalents ions into organic matter being Hg > Cu > Pb > Zn > Ni > Co [105]. In organic phase, metallic pollutant bound to this phase are assumed to stay in the soil for longer periods but may be immobilized by decomposition process [106]. Under oxidizing conditions, degradation of organic matter can lead to a release of soluble trace metals bound to this component. The extracts obtained during this step are metals bound to sulphides [107]. The organic fraction released in the oxidisable step is considered not to be bioavailable due to the fact that it is thought to be associated with stable high molecular weight humic substances that release small amount of metals in a slow manner [105]. The most commonly used reagent for the extraction of metals in organic phases is hydrogen peroxide with ammonium acetate readsorption or precipitation of released metals [108]. Other reagents such as H$_2$O$_2$ / ascorbic acid or HNO$_3$ + HCl have been used which can dissolve sulphides with enhanced selectivity, but on the other hand, silicates are attacked to some extent [109]. Oxidation with sodium hypochlorite has also been recommended [104] but fraction of organically bond metals released showed considerable variability in different soil horizons [110].

Strong acid-Extractable fraction: Residual phase: Residual phase serve as a useful tool in the assessment of the long-term potential risk of heavy metal or toxic metals entering the biosphere. Digestion in strong acid such as nitric acid, hydrochloric acid or mixture such as aqua regia that do not dissolve the silicate matrix have been commonly used to leach out the recasistrant metals that are bound to the sediment in the residual phase. Residual phase give an estimate of the maximum amount of elements that are potentially mobilisable with changes environmental conditions. ISO 11466 [111] aqua regia digestion procedure is the known well procedure with a legal back in some European countries and had been used for the standard reference material of soil and sediments. Moreover, primary and secondary minerals containing metals in the crystalline lattice constitute the bulk of this fraction. Its destruction is achieved by digestion with strong acids, such as HF, $HClO_4$, HCl and HNO_3. The amounts of associated metals are also associated by some authors as the difference between the total concentration and the sum of the fractions of the metals extracted during the previous steps [101]. Results of heavy metal speciation in sediment in different fractions collected from different coastal system worldwide are presented on Table 2.

Conclusions

In this paper, we have reviewed the analytical methods for chemical speciation of heavy metals in marine harbour sediments. Heavy metal is one of the most serious environmental pollutants because of its high toxicity, abundance and ease of accumulation by various plant and animals. This review highlighted various effects, occurrence and different speciation methods to determine concentration of heavy metals and their mobility in different fractions. Also, we were able to highlight different activities that take place in and around harbours such as loading and offloading of goods, cleaning, ballasting, fuelling, maintenance practise which include painting of ships, motors and engines emission, agricultural activities, urban runoff to be contributing factors to the dumping of pronounced amounts of wastes directly into the seas. Although, a considerable amount of work has been conducted on the heavy metal pollution in marine water and sediments and their effects on aquatic life's. Very limited data are available on the effects of these heavy metals on human.

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