

## Research Article

## Toxicology and Applied Pharmacology Insights

## Heavy Metals Speciation, Mobility, and Bioavailability in Constructed Wetland Sediment Treating Polluted River Water: Implication for Environmental Safety

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## Abstract

The presence of toxic heavy metals in sediments beyond allowable limits are often posing a serious threat to local environment. Constructed wetland sediments contaminated with heavy metals can affect sediment-dwelling biota such as benthic organisms, wetland plants, aquatic life including amphibian and reptiles. The objective of this study was to investigate heavy metals, mobility and bioavailability, level of environmental risks, and its implication for environmental safety. The study was conducted using the horizontal subsurface flow (HSSF) constructed wetland (CW) sediments designed for treating polluted Little Akaki River water. The sediment samples were collected from the CW and heavy metals speciation determined following the European Community Bureau References (BCR) sequential extraction procedures. The results showed that the highest percentage of metal fractions: Zn = 71.67%, Cr = 82.20%, Cd = 76.47% and Pb = 74.96% were bound to residual fraction (R), implying that majority of the metals were stable, immobile and not bioavailable. The mobility factor (MF) for Zn (27.60%); Cr (14.24%) Cd (18.82%) and Pb (21.53%); indicating low percentage of metals found in mobile chemical form. Furthermore, the environmental risk assessment code (RAC %) value for all heavy metals in the sediment samples were ranged: 5.37 % - 8.51%, falling in low risk category (RAC=1-10%). Low mobility, bioavailability and risk assessment code may be attributed to slight alkaline pH, low organic matter, and clay dominated sediment particles. High residual fraction occurred due to clay particles which can form strong bounding matrix with metals. Thus, metals in the CW sediments may pose low toxicity to wetland plants and sediment living biota surrounding environment.

**Keywords:** Bioavailability, Heavy Metals Fractionation, Horizontal Subsurface Flow Constructed Wetland, Mobility, Sediment, Risk Assessment Code

## 1. Introduction

Some heavy metals are toxic to plants and animals, persistent in environment, tend to accumulate in living organisms over time, and eventually reach human through food chain and cause various health repercussions [1]. When heavy metals accumulate in soil, water and plant tissues beyond allowable limits, it can impair the normal growth of plants. The principal sources of heavy metals are often categorized into point and non-point sources. Point sources include industries like tannery, textile and metal processing factories while non-point source encompasses agriculture (the application of agrochemical). Heavy metals generated either from point or non-point source are usually transported through water system and cause environmental contamination. The problem related to heavy

metals contamination increasingly become a serious health issue in Ethiopia due to lack of wastewater treatment facilities, limited regulatory control, and untreated or poorly treated industrial wastes are directly released into surface water. As a result, rivers like Beg Akaki River (BAR) and Little Akaki River (LAR) which are passing through the capital city (Addis Ababa City Administration) and receiving poorly treated and untreated wastewaters released from various industries. Some of the potential polluting industries include metal processing factories, tanneries, textile and garments, food processing, alcohol and beverage factories, vehicle battery maintenance, garage, painting and pigments [2, 3]. Metals can also be generated from natural sources such as weathering of parent volcanic rocks materials.

In order to treat polluted river water and use the water for various purposes, various technologies such as surface and subsurface constructed wetland, oxidation, sand filter method, and other chemical treatment methods can be applied. Constructed wetland (CW) is usually viewed as a viable alternative technologies to treat polluted river water, because it is relatively low cost, ease to operate, effective in pollutants removal through various processes [4]. Furthermore, the application of horizontal subsurface flow (HSSF- CW) technology allows the polluted river water to pass through the subsurface of the system and have enough contact time with wetland sediment (gravel), wetland plant roots, and micro-organisms, which all together contribute to remove contaminants through physical, chemical and biological processes [5]. In CW treatment system, sediment plays a critical role in the removal of heavy metals from the overlying water column through the process of settlement, precipitation and co-precipitation; hence, it is serving as a sink for heavy metals [6].

However, several studies have reported that determining total heavy metal concentrations in the sediment or soil provides information on overall pollution levels, but does not provide reliable information on heavy metal stability, transport, bioavailability, mobility and toxicity [6-9]. The distribution of metals in the CW sediments and their effects on sediment dwelling biota depends on their speciation and propensity for particular biogeochemical reactions [10]. According to John, metal mobility and its availability to living biota are closely associated with metal speciation [11]. Several researchers have further confirmed that the transport, stability, bioavailability, mobility, affinity, toxicity, and environmental implications of heavy metals are governed by their speciation or chemical forms [4, 12-16]. The chemical forms of a specific metal in sediment or environmental media are referred to as heavy metal speciation [6]. Thus, speciation related to specific form of metal such as isotopic composition, molecular structure or oxidation state [10].

In order to determine heavy metals speciation, the European working group has developed sequential extraction procedures usually known as the Community Bureau of Reference [17]. This method is easy to apply and to determine speciation of heavy metals found in environmental media like soil, sediment and sludge [1, 16, 18, 19]. Hence, it is a useful technique to determine mobility and bio-availability and risk of heavy metals to plants and animals, as well as, metals migration in soil, surface and ground water, and their contamination [20, 11].

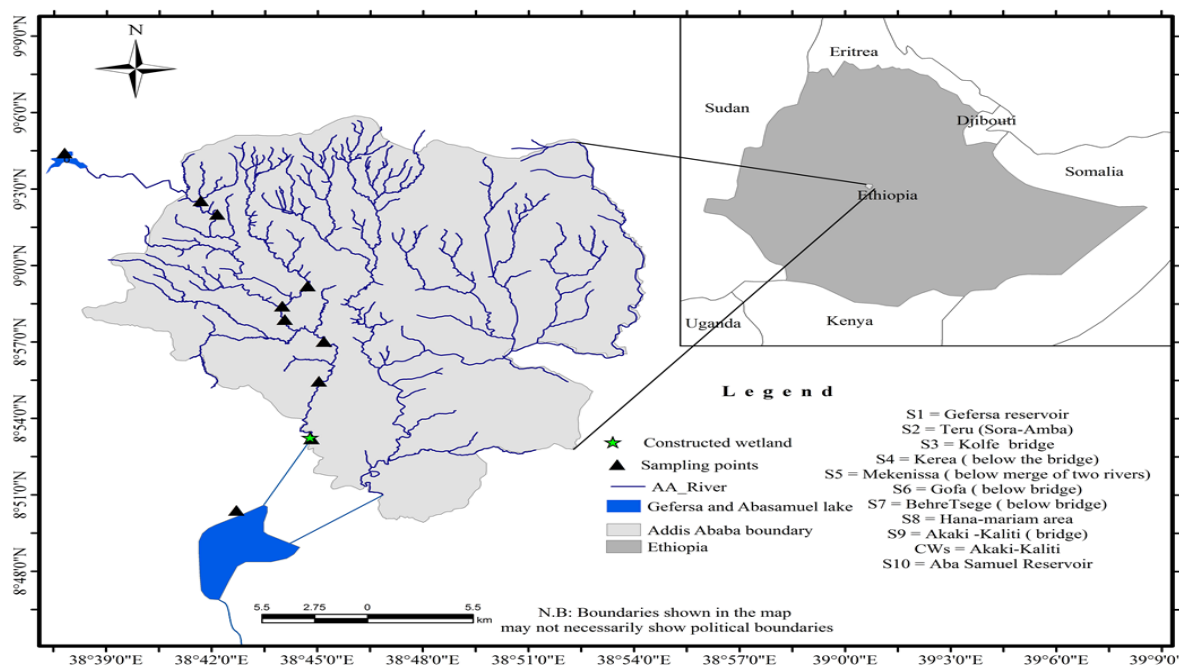
Study reports have showed that heavy metals speciation in lake and river sediments, soils, sewerage and municipal sludge, and fish meat have been studied using this sequential extraction method [3, 8, 18, 21]. However, limited studies were conducted on heavy metal speciation in CW sediments. Sullivan has conducted studies on the accumulation and fate of Zn, Pb, Fe and S in the sediments of constructed wetlands established for treating mine wastewater and they have reported that the majority of heavy metals and sulfide bound to residual fraction, mainly in the form of metal-sulfides [22]. Sundberg-Jones and Hassan have conducted studies on sediment associated Hg, Se and As using a pilot constructed wetland designed for treatment of simulated flue gas desulfurization wastewater, and investigated the distribution, characterization and toxicity to *Hyalella azteca* [10]. Thus, studies were not undertaken on HSSF-CW sediment treating polluted river water to investigate metals mobility, bioavailability, risk to environment and aquatic system. Thus, the study is a new attempt to investigate metals speciation in CW sediments treating polluted river water and environmental implications.

The purpose of the studies were to (i) determine the concentration of trace metals (Zn, Cr, Cd, and Pb) in HSSF-CW sediments, (ii) determine heavy metals speciation, bioavailability, mobility factor, and metals immobilization or retention in the CW sediment, (iii) evaluate environmental risk metals and its implications for wetland and surrounding environment and public safety. This kind of study is important to safeguard the environment and public health, because, contaminated sediment may affect water quality, wetland plants and microbes attached to gravel and plant roots, hence, pose metals toxicity to wetland biota and surrounding environment.

## 2. Materials and Methods

### 2.1 Experimental Study Site

The study was conducted in Addis Ababa City Administration. The pilot HSSF-CW was established nearby the LAR in Akaki Kaldi Sub-administration of the City, at 38°44'50"E and 8° 53'18"N at an altitude of 2060 meter above sea level (Fig1). This site was selected for pilot HSSF-CW due to the high pollution load of heavy metals released into the river system from surrounding Akaki Kaldi industrial zones and agrochemicals [3].



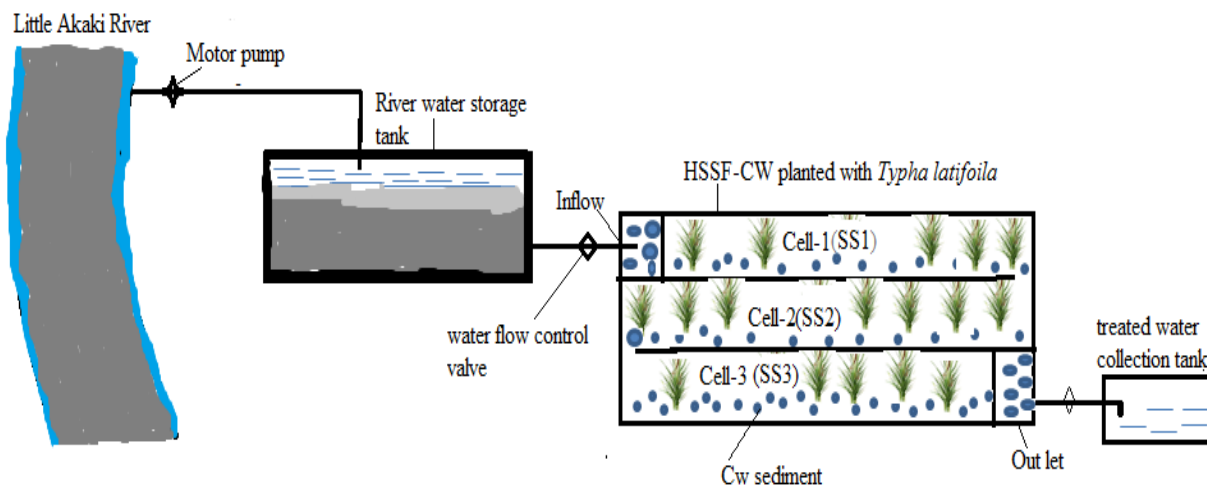
**Figure 1:** Map of the Experimental Site

## 2.2 Design and operation of HSSF-CW

The HSSF-CW with three treatment cells connected in series, and river water storage tank are the two primary components of the treatment system (Fig 2). The storage tank had a size of 9 m (length) x 5 m (width) x 0.9 m (height), and is used to store polluted Akaki River water. The river water which was loaded with industrial, municipal, and agro-chemicals was directly pumped into this tank, and then, continuously flow into the second component i.e. HSSF-CW treatment cell sediments under regulated flow condition. The HSSF-CW had three equal sized treatment cells (cell-1, cell-2, and cell-3) connected in series. The overall dimension of treatment cell is 6 m (length) x 4 m (width) x 0.6 m (depth). The treatment cells were filled with gravel (size: 2.0 mm - 31.5 mm diameter) to a depth of 0.6 m. After placing the sediment (gravel)

in the cells, the river water filled to a depth of 0.5 m. Wetland plants (*Typha latifolia*) seedlings of equal size were collected and soil bound to their roots washed with clean water and planted at spacing of 0.3 cm x 0.3cm in the gravel layer. During planting, two spades of uncontaminated soil consist of 70% clay and 30% silt were carefully placed around the root of each plant to promote their survival and growth.

The CW was operated with theoretical hydraulic retention time (HRT) of 7 days with a hydraulic loading rate (HLR) of 0.033m/d and a mass loading rate (MLR) of 2 g. /m<sup>2</sup>.d. The polluted river water from the storage tank was constantly supplied into the HSSF-CW treatment cell at an average flow rate of 0.711m<sup>3</sup>/d.



**Figure 2:** Schematic Diagram Showing Akaki River Water, Water Storage Tank, HSSF-CW Treatment Cells and Sediment Sampling (SS1, SS2 and SS3) Locations

## 2.3 Collection of Sediment Samples and Analyses

### 2.3.1 Sample Collection

The sediment samples were collected in six rounds (once per two months) between January 2019 to March 2020. However, samples were not done during wet seasons (July-September) because of flood and inaccessibility. A composite sediment samples were collected from each treatment cell of HSSF-CW: Cell-1 (sediment sample: SS1); Cell-2 (sediment sample: SS2) and Cell-3 (sediment sample: SS3). From each cell, approximately 3000 gm of composite sediment samples were collected from 0–30 cm depth and placed in high-density polyethylene plastic bags, sealed, coded, and transferred to the Addis Ababa University laboratory, where they were promptly stored at 4°C until further processing [23].

### 2.3.2 Determining Sediment Samples pH, Particle Size Composition, and Organic Matter

The pH of sediment samples determined following the procedures described in [16, 24, 25]. Accordingly, the sediment sample and deionized water were mixed at a ratio of 1: 5, and then, thoroughly stirred for 30 minutes and allowed to stay overnight. Then, pH of the suspended water was measured using a calibrated pH meter (model: PH PROBE, PHT-02 ATC, Taiwan).

The particle size composition of sediment samples were determined following the procedures described in Uwah [26]. First, the sediment samples were oven dried using oven-dry (model: GX-65B) at a constant temperature of 60°C. The dried samples were ground with a mortar and pestle and sieved with 63 µm sieve. The sediment grain size composition was determined by taking a 100 gm sieved sediment sample from each sample in such a way that particles that passed through the sieve and retained on the sieves were carefully collected and weighted. On the basis of sieve results, the sediment particles were grouped as follows: clay = <0.002 mm, silt = 0.002–0.063 mm and sand = 0.063–2 mm [27]. The percentage of grain size (%) was computed using the formula described in equation (1) [26]. The sediment texture classes were determined based on the percentage of grain size and a ternary diagram of Folk's classification.

$$\% \text{ Grain size} = \frac{\text{Sieve weight}}{\text{Total weight}} \times 100 \dots (1)$$

The organic matter (OM) content of sediment samples were determined by loss-in-ignition method [22, 28]. In this method, sediment samples were oven-dried at 105°C and weighted using an electronic balance (Model No. JD 210 - 4 CE) until constant weight were obtained. The dried samples were put in a ceramic crucible and placed in a muffle furnace (model: Sx-2.5 - 10) and

ignited at 600°C for 6 hrs, cooled and weighed. Finally, OM content was computed as the difference in weight due to ignition [28].

### 2.3.3 Preparation Samples and Determination of Heavy Metal Concentration

The sediment samples were prepared in such a way that all debris, plant materials, fallen leaves, grass and weeds were removed from the samples. The sediment samples were oven-dried at 105°C until they reached a consistent weight [14]. The dried sediment samples were crushed with a mortar and pestle, homogenized, and sieved through a 45-µm stainless steel sieve. A sub-sample of 1 gm powdered sediment sample was digested using 12 mL aqua regia (3:1, HNO<sub>3</sub> / HCl), in a microwave (Model: ETHOS ONE, MILESTONE, Italy) according to the USEPA method (3051), soil and sludge digestion procedures. The digested samples were filtered using Whatmann filter paper No. 42, and the filtrate was made 50 mL by adding deionized water, sealed, and stored in refrigerator at 4°C until analyzed. The total concentrations of each heavy metal were determined using an inductively coupled plasma optical emission spectrophotometry (ICP-OES, Model: ARCOS FHS12, Germany).

Before the analysis, ICP-OES was adjusted and calibrated according to the manufacturer's instructions. Standard solutions of 0.06, 0.11, 0.17, 0.56, 1.12, 1.68, 2.24, and 2.80 mg L<sup>-1</sup> for Zn, while for other heavy metals: Cr, Cd, and Pb; 0.03, 0.06, 0.08, 0.28, 0.56, 0.84, 1.12, and 1.40 mg L<sup>-1</sup> were used to calibrate the ICP-OES. For each metal, the total concentration of heavy metals was calculated using a linear graph of the standard concentration and its corresponding intensities. The calibration curves confirmed linearity for all measured metals in this experiment ( $R^2 = 0.99944$ ).

### 2.3.4 Determination of Heavy Metals Speciation

The modified Community Bureau of Reference (BCR) sequential extraction procedures were employed to determine heavy metals bound to different metal fractions. As described in Tokalioglu, the fractions of metal extracted in different operational steps include; water and acid soluble, exchangeable and bound to carbonates fraction (F1); bound to Fe–Mn oxides (reducible) fraction (F2); metals bound to organic matter (oxidizable) fraction (F3), and metals bound to primary and secondary minerals crystal structures as a residual fraction (R) [16]. 1 gm of dried and powdered sediment samples were used for the fractionation analysis. In each successive extraction step, the suspended supernatant was carefully separated after centrifugation at 3000 rotation per minute (rpm) for 20 minutes following the procedures described in [17, 29]. The detailed steps applied in sequential extractions of (F1), (F2) and (F3), reagents used for each step, shaking time and temperature applied are summarized and presented in Table 1.

| Fraction  | Sediment phases  | Reagents  | Shaking time                                       | Heating temperature (°C) | Extract separation from the solid residue                                      |
|-----------|--|---|--|--------------------------|--|
| <b>F1</b> | Water and acid-soluble, exchangeable and bound to carbonates | 40 mL of 0.11 M (mol. L <sup>-1</sup> ) Acetic acid                         | End-over- end shaker for 16 h, at 30 rpm           | The room temp.           | Centrifuge at 3000 rpm for 20 min.   |
| <b>F2</b> | Reducible  | 40 mL of 0.5 M Hydroxylamine hydrochloride with pH=1.5                      | End-over- end shaker for 16 h, at 30 rpm           | Room temperature         | Centrifuge at 3000 rpm for 20 min  |
| <b>F3</b> | Oxidizable   | 10 mL of 8.8 M Hydrogen peroxide H <sub>2</sub> O <sub>2</sub> , with pH=2, | 1 h with occasional shaking,                       | At room temperature      |  |
|           |  |   | 1 h with occasional shaking                        | At 85°C in a water bath  |  |
|           |  | then added 10 mL of 8.8 M Hydrogen peroxide (pH=2), cooled and,             | 1 h occasional shaking                             | At 85°C in a water bath  |  |
|           |  | Added 50 mL of 1M Ammonium-acetate" with (pH=2)                             | End-over- end shaker for 16 h, at 30 rpm overnight | Room temperature         | Centrifuge at 3000 rpm for 20 min  |
| <b>R</b>  | Residual (crystal structure)                                 | Added 10 mL Aqua regia, twice   | -  | -                        | Residue digested using microwave and heavy metal content determined by ICP-OES |

**Table1: Sequential Extraction of Heavy Metals, Reagents Used, Shaking Time, Temperature and Extract Separation**

The residual (R) fraction was digested following the procedures indicated in [3, 14, 16]. Following USEPA (3051) methods and the microwave-operating handbook for sludge and sediment analysis, the residual fraction was carefully separated from the supernatant and transferred to microwave vessels, where it was digested with 12 mL aqua- regia solution. The blank samples were also prepared and digested following the same procedures. The digest was filtered using What Mann filters paper No. 42, and the filtrate was made up to 50 mL with de-ionized water before being stored in the refrigerator at 4°C until analysis.

### 2.3.5 Evaluating Bioavailability and Mobility Factor (MF)

Bioavailability is usually described as the extents of heavy metals adsorbed or accumulated in biota [21]. According to heavy metals extracted in the first step extraction (F1) are instable and highly bioavailable, easy to transport, transform and be absorbed by biota

and cause toxicity [6, 16, 21, 30]. Heavy 13 metals occurred in reducible chemical form (F2) are also readily bioavailable. While metals occluded to organic matter (F3) are moderately bioavailable [8]. Thus, metal fractions detected in the chemical forms of F1, F2 and F3 are considered to be in the bioavailable form.

The mobility of heavy metals is highly depend on the bounding strength of metals with a matrix. Accordingly, a weak bound occurs when metal found in the chemical form of (F1), less strong bound when it is occurred in reducible form (F2) and moderately strong bound occurs when it is bound to OM (F3) [13]. The mobility factor (MF) was determined by modifying equation stated in and applied as follows [13]:

$$MF = \frac{(F1+F2+F3)}{(F1+F2+F3+R)} * 100 \dots (2)$$



Where; (F1), stands for water and acid soluble, exchangeable and carbonate; (F2) stands for reducible fraction; (F3) stands for oxidizable fractions, and (R) stands for residual fraction.

### 2.3.6 Risk Assessment Code (RAC)

The risk assessment code (RAC) is described as the percentage of metal, which is weakly occluded to water and acid soluble,

$$\text{RAC (\%)} = \frac{\text{Concentration of the first extracted fraction}}{\text{concentration of total fractions}} \dots \dots (3)$$

The percentage RAC value indicating the levels of environmental risk, which classified as follows:  $\text{RAC} < 1\%$  indicates no risk; RAC value ranged between:  $1\% < \text{RAC} < 10\%$ , indicates low risk; RAC value ranged  $11\% < \text{RAC} < 30\%$ , indicates medium risk; RAC value ranged  $31\% < \text{RAC} < 50\%$  indicates high risk, and RAC value  $> 50\%$  indicates very high risk [8, 32, 33].

### 2.4 Data Analysis and Quality Control

Descriptive statistics such as the mean and standard deviation of heavy metal concentrations in the sediment samples, the percentage of metal fractions were calculated using Micro-soft excel version 10. The graphs were performed using Origin -Pro 2017 software. Paste software version 4.02 used to perform principal component analysis (PCA).

To ensure data quality, the digested sediment samples were analyzed in triplicate and the average concentrations were taken. Blank samples were prepared and run to check interferences. Spiked and non-spiked samples were used to validate the method, and percentage recoveries for the four metals (Zn, Cr, Cd and Pb) were varied in between 106.30%-124.81%. The limits of detection (LOD) for each metal was determined based on three times the standard deviation and the results ranged from 0.05–0.06 mg kg<sup>-1</sup> [16]. The limit of quantification (LOQ) of the each metal was calculated from the blank samples and ten times of the blank samples and results were ranged in between 0.16 - 0.19 mg kg<sup>-1</sup>. Moreover, glassware and bottles were soaked in 10 mL HNO<sub>3</sub> diluted water for 2 days, and then, thoroughly washed and rinsed with de-ion-

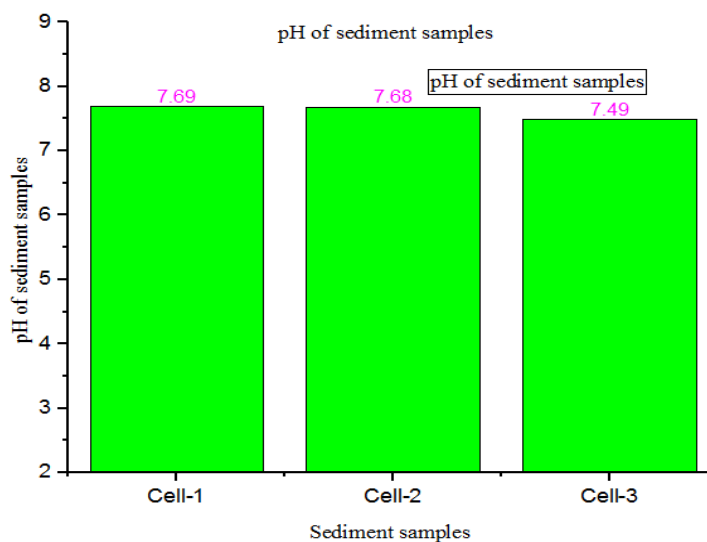
exchangeable and carbonates (F1) in relation to total trace metal fractions [15]. RAC is a common ecological risk assessment index that based on the content heavy metal available in chemical form of (F1) [8]. The potential risk of heavy metals found in the sediment can be assessed using risk assessment code (RAC) [31, 24]. According to RAC is expressed as [32].

ized water before use.

## 3. Results and Discussion

### 3.1 pH, particle size composition and organic matter content of sediment samples

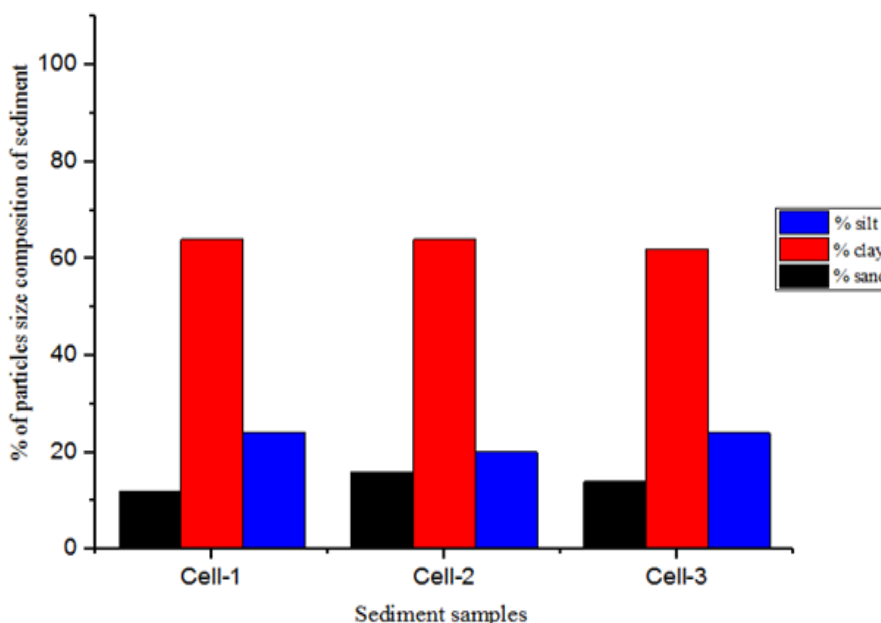
The average pH value of sediment sample taken from cell-1, cell-2 and cell-3 were 7.69, 7.68 and 7.49, respectively, indicating slightly alkaline sediment. The results of pH analyses are shown in Fig 3. The sediment pH has a capacity to influence the properties of heavy metals. For instance, the solubility, mobility and bio-accumulation of heavy metals are dependent on the pH of sediment [25, 28]. At high pH, the solubility of the heavy metals decreases; hence, heavy metals are poorly subjected to dissolution from sediment particles. Whereas, at low pH, the availability of heavy metals can increase due to increase in metals solubility under acidic conditions. Dufresne have described that change in sediment pH may leads to change in the availability and concentration of heavy metals in the sediment through time [20]. In addition, the chemical forms or speciation of heavy metals are also influenced by pH value of sediment. Mi have reported the concentration of exchangeable fraction (F1) of Cd and Pb increase with decreasing the pH of sediment [34]. In the contrary Gambrel described that sediment with pH value near neutral favor heavy metal immobilization [35]. However, in the present finding, the pH value for each cell was slightly alkaline, thus, favors immobilization of heavy metals in the sediment. This finding is comparable to the reported value for pH which is ranged 6.88-7.20 for CW sediment [10].



**Figure 3:** pH of the Sediment Samples

As shown in Fig 4, the particle size composition of the sediment samples were mainly consists of sand, silt and clay with average percentage of 14%, 22.67% and 63.33%, respectively. In all cells, the particle size of the sediment samples were predominantly composed of clay particles followed by silt particle may be due to the high clay content of soil used to promote wetland plant growth. The particle size composition of the sediment has an important effect on the chemical fractions of heavy metals. Mi have reported

that the concentration of heavy metals such as Cd and Pb increase with decreasing particle size of sediment [34]. The particle size composition of sediments can affects heavy metals assembly and mobility in sediment; for instance, coarse textured sediment has low metal affinity so that metals may be mobile, while fine textured sediments which may contains organic matter usually tends to bind and accumulate metals in the CW sediment hence, reduce metals mobility [35].



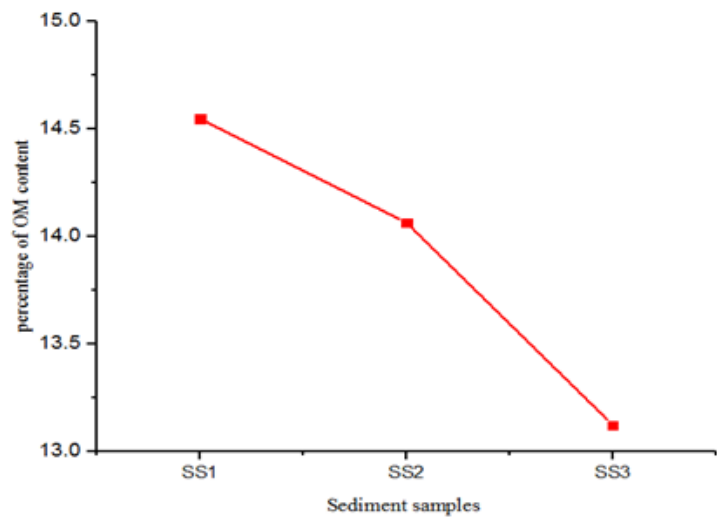
**Figure 4:** Particle Size Composition of Sediment Samples

Results of OM analysis of the sediment samples are presented in Fig 5. The OM content of sediment samples were ranged between 13.12% -14.55% with average value of 13.12%. Higher OM was found in the sediment of the first cell-1, and gradually decreased

towards the second (cell-2) and third (cell-3) of HSSF-CW sediments may be due to settling and decomposition of water carried debris, wetland plant leaves, stems, foliage by physical, chemical and biological processes. This finding is in agreement with Alayu

and Leta, have reported a decreasing OM in organic matter content along the two – stage HSSF-CW system established to treat brewery influents [36, 37]. Furthermore, cell-1 represents an inlet part of the CW and receives increased nutrient loading which promote plant growth leading to more availability of plant leaves, stems

and foliage which decompose and form more OM, as compared to cell-2 and cell-3 sediments [38]. OM content of the sediment plays an important role in sorption and binding of heavy metals. Hence, the bioavailability and mobility of heavy metals in sediment may change as a result of change in sediment OM content [20].



**Figure 5:** Organic Matter Content in the Sediment Samples along the Cells

### 3.2 Concentration of Heavy Metals in the Sediment

The results of total heavy metals concentration analyses of sediment samples are presented in Table 2. The highest average concentration was recorded for Pb ( $2.65 \pm 0.58 \text{ mg kg}^{-1}$ ). This result is may be due to Pb inputs from various sources such as used vehicle battery, used oil, fossil fuel, and garages found in the Akaki- Kaldi Industrial Zone and agrochemicals [3, 39]. The average concentration of Cr ( $1.30 \pm 0.53 \text{ mg kg}^{-1}$ ), Zn ( $1.22 \pm 0.09 \text{ mg kg}^{-1}$ ), and Cd ( $0.59 \pm 0.10 \text{ mg kg}^{-1}$ ), indicating their overall abundance in the sediment. Heavy metals like Cr largely generated from tanneries and metal works. Zn usually released from metal processing and

agrochemicals, while Cd is generated from agrochemicals, metals works and solders [3]. Heavy metals can also be generated from natural source like volcanic rocks surrounding the Addis Ababa City transported by water and accumulated in the sediment. The decreasing order of average concentration of heavy metals in sediment samples was  $\text{Pb} > \text{Cr} > \text{Zn} > \text{Cd}$ . Thus, the study results showed that Pb and Cr were relatively more abundant metals in the sediments than Zn and Cd. Generally, the total concentration of heavy metals found in the sediment samples provides information on an overall pollution status of sediments of HSSF-CW, but not toxicity, mobility and bioavailability [6, 8, 9].

| Average concentration heavy metals ( mean and SD) ( $\text{mg kg}^{-1}$ ) |   |
|---|---|
| Heavy metals  | Average concentration ( $\text{mg.kg}^{-1}$ ) |
| Zn  | $1.22 \pm 0.09$                               |
| Cr  | $1.30 \pm 0.53$                               |
| Cd  | $0.59 \pm 0.10$                               |
| Pb  | $2.65 \pm 0.58$                               |

**Table 2:** The Concentration of Heavy Metals in the Sediment

### 3.3 Speciation of Heavy Metal in Sediment Samples

The chemical speciation of heavy metals in the sediment samples are presented in Table 3.

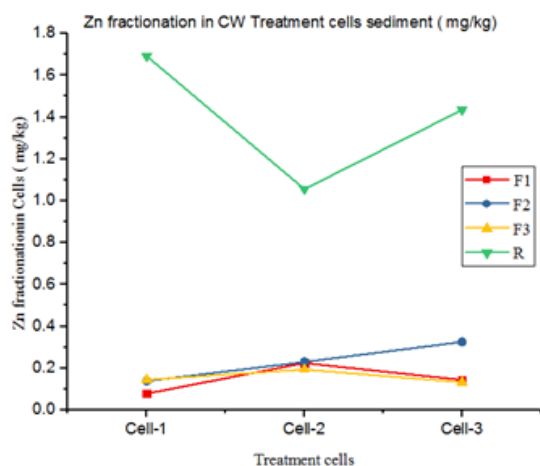


| Concentration of Zn in extracted fractions (mg kg <sup>-1</sup> ) |             |                 |                   |            |                   |                          |
|---|-------------|-----------------|-------------------|------------|-------------------|--------------------------|
| Treatment cells   | F1: Soluble | F2: Reduce able | F3: Oxi-dize-able | R: Residue | Sum of frac-tions | Total heavy metal ( THM) |
| Cell-1  | 0.078       | 0.138           | 0.144             | 1.692      | 2.052             | 1.17                     |
| Cell-2  | 0.224       | 0.229           | 0.193             | 1.056      | 1.702             | 1.32                     |
| Cell-3  | 0.143       | 0.325           | 0.132             | 1.435      | 2.035             | 1.17                     |
| Av.   | 0.148       | 0.231           | 0.156             | 1.394      |                   |                          |
| SD  | 0.073       | 0.094           | 0.032             | 0.320      |                   |                          |
| Concentration of Cr in extracted fractions (mg.kg <sup>-1</sup> ) |             |                 |                   |            |                   |                          |
| Treatment cells   | F1: Soluble | F2: Reduce able | F3: Oxi-dize-able | R: Residue | Sum of frac-tions | Total heavy metal ( THM) |
| Cell-1  | 0.046       | 0.007           | 0.173             | 1.69       | 1.92              | 0.838                    |
| Cell-2  | 0.23        | 0.031           | 0.317             | 1.671      | 2.25              | 1.877                    |
| Cell-3  | 0.046       | 0.093           | 0.15              | 1.528      | 1.82              | 1.177                    |
| Av.   | 0.107       | 0.044           | 0.213             | 1.630      |                   |                          |
| SD  | 0.106       | 0.044           | 0.091             | 0.089      |                   |                          |
| Concentration of Cd in extracted fractions (mg.kg <sup>-1</sup> ) |             |                 |                   |            |                   |                          |
| Treatment cells   | F1: Soluble | F2: Reduce able | F3: Oxidize-able  | R: Residue | Sum of frac-tions | Total heavy metal ( THM) |
| Cell-1  | 0.056       | 0.013           | 0.082             | 0.594      | 0.745             | 0.636                    |
| Cell-2  | 0.122       | 0.049           | 0.116             | 0.81       | 1.097             | 0.475                    |
| Cell-3  | 0.069       | 0.11            | 0.075             | 0.797      | 1.051             | 0.646                    |
| Av.   | 0.082       | 0.057           | 0.091             | 0.734      |                   |                          |
| SD  | 0.035       | 0.049           | 0.022             |            |                   |                          |
| Concentration of Pb in extracted fractions (mg.kg <sup>-1</sup> ) |             |                 |                   |            |                   |                          |
| Treatment cells   | F1: Soluble | F2: Reduce able | F3: Oxidize-able  | R: Residue | Sum of frac-tions | Total heavy metal ( THM) |
| Cell-1  | 0.304       | 0.151           | 0.378             | 2.785      | 3.618             | 2.233                    |
| Cell-2  | 0.649       | 0.236           | 0.77              | 3.736      | 5.391             | 2.407                    |
| Cell-3  | 0.284       | 0.612           | 0.357             | 4.6        | 5.853             | 3.321                    |
| Av.   | 0.412       | 0.33            | 0.502             | 3.707      |                   |                          |
| SD  | 0.205       | 0.245           | 0.233             | 0.908      |                   |                          |

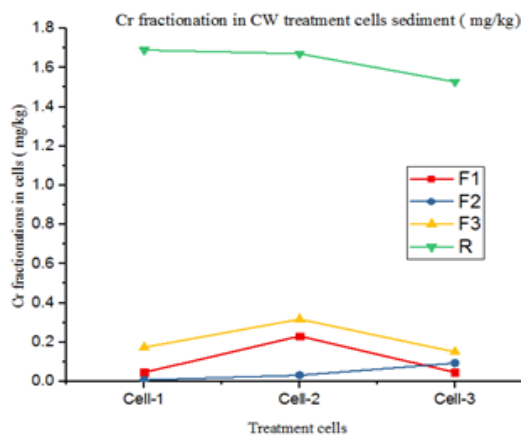
**Table 3: Concentration of Extracted Fractions of Metals in the HSSFCW Sediment**

As shown in this Table 3, the concentrations of Zn fraction (F1: water and acid soluble, exchangeable and carbonate) in cell-1, cell-2 and cell-3, ranged 0.078-0.224 mg kg<sup>-1</sup>; (F2) (reduce able fraction) ranged: 0.138-0.325 mg kg<sup>-1</sup>; F3 (oxidize able fraction) ranged: 0.132-0.193 mg kg<sup>-1</sup> and residue (R) ranged: 1.056-1.692 mg kg<sup>-1</sup>. The highest average Zn fraction ( $1.394 \pm 0.320$  mg kg<sup>-1</sup>)

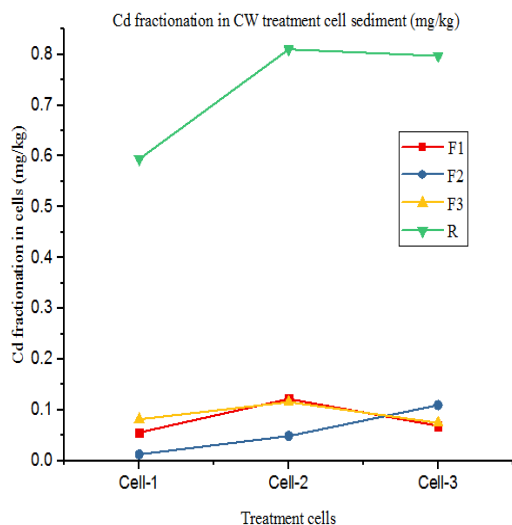
1) was recorded for (R) which may attributed to metal adsorption to clay soil particles. Moving from cell-1 through cell-3, (F1) and (F3) showed a decreasing trend, may be due to slight decreasing OM content of sediment while (F2) showed slightly increasing may be due to a slight decreasing in the pH of sediment which cause release of metals bound to Fe-Mn oxides (Fig- 6(a).



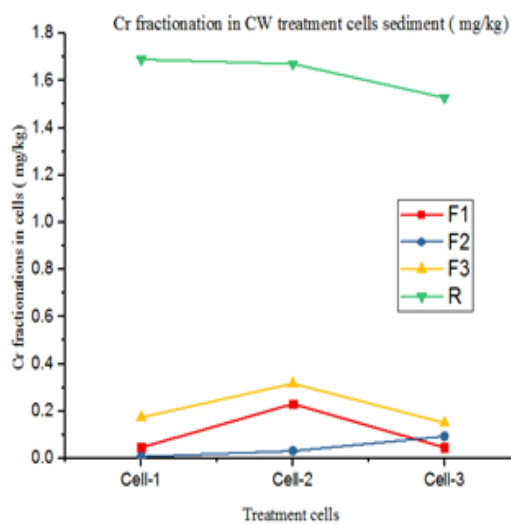
**Figure 6 (a):** Zn fractionation in CW sediment



**Figure 6 (b):** Cr fractionation in CW sediment



**Figure 6 (c):** Cd fractionation in CW sediment



**Figure 6 (d):** Pb fractionation in CW sediment

The residual fraction (R) showed decreasing trends between cell-1 and cell-2 may be due to Zn absorption to plant tissues and bounding to clay soil structure, while increasing from cell-2 to cell-3 may be due to decomposition of plant materials and release Zn in to sediment.

The Cr fraction that occurred in the chemical form of (F1), in cell-1, cell-2 and cell-3, ranged 0.046-0.230 mg kg<sup>-1</sup>; (F2) (reduce-able fraction) ranged: 0.007-0.093 mg kg<sup>-1</sup>; (F3) (oxidize-able fraction) ranged: 0.15-0.317 mg kg<sup>-1</sup>, and (R) (residue) ranged 1.528-

1.690 mg kg<sup>-1</sup>. The highest average Cr fraction ( $1.630 \pm 0.089$  mg kg<sup>-1</sup>) was recorded for (R). This result may attributed metal binding ability of small particles of clay and silt particles of the sediment. As shown in Fig-6 (b), moving from cell-1 to cell-3, the abundance of chemical fractions that occurred in the form of (F1), (F3) and (R) exhibits decreasing trends due to slight decreasing in OM content of sediment that bind metals in the sediment [35].

The concentration of Cd fraction that occurred in the chemical form of (F1) in cell-1, cell-2 and cell-3 were ranged: 0.059-0.122

mg kg<sup>-1</sup>; (F2) ranged: 0.013-0.110 mg kg<sup>-1</sup>; (F3) ranged: 0.075-0.116 mg kg<sup>-1</sup> and (R) ranged: 0.594-0.810 mg kg<sup>-1</sup>. The chemical fractions of Cd which occurred in the form of (F1) and (F3) were showed slight decreasing trends between cell-2 to cell-3 (Fig 6 (c)) may be due to decrease in OM content which can bound metals. On the contrary, (R) fractions were showed increasing trends between cell-1 to cell-2, which may be due to high affinity of clay and silt particles for metals [34].

The chemical fractions of Pb that occurred in the form of (F1) in cell-1, cell-2 and cell-3 were ranged: 0.284-0.649 mg kg<sup>-1</sup>; (F2) ranged: 0.151-0.612 mg kg<sup>-1</sup>; (F3) ranged: 0.357-0.770 mg kg<sup>-1</sup> and (R) ranged: 2.785-4.60 mg kg<sup>-1</sup>. Considering the average of three cells, the highest average Pb fraction occurred in the form of (R) (3.707 ± 0.908 mg kg<sup>-1</sup>), followed by (F3) (0.502 ± 0.233 mg kg<sup>-1</sup>) may due to metal affinity and bound to clay and silts, and metal bound with OM respectively. As shown in Fig 6 (d), as moving from cell-1 through cell-3, the abundance of chemical fractions occurred in the form of (F2) and (R) exhibits slightly increasing

trends may be due to Pb bounding to clay soil particle structure.

Generally, the highest average fraction for Zn (1.394 ± 0.320 mg kg<sup>-1</sup>), Cr (1.630 ± 0.089 mg kg<sup>-1</sup>), Cd (0.734 ± 0.121 mg kg<sup>-1</sup>), and Pb (3.707 ± 0.908 mg kg<sup>-1</sup>) were found in the chemical form of a residual fraction (R). As indicated in table-4, 71.67% of Zn; 82.20% of Cr; 76.47% of Cd and 74.96 % of Pb were bound to residual fractions (R), while lower metals fraction occurred in the form of water and acid soluble and exchangeable (F1), reduce able form (F2) and oxidizable (F3). The high residual fractions of metals are attributed to the presence of silt and clay soil in the CW sediment, which have high affinity for heavy metals. Unlike (F1), (F2) and (F3), (R) fraction held with strong crystal structure and is forming very stable matrix in the CW sediment. Toxic metals bound to (R) fraction are immobile, not bioavailable, not cause metal toxicity; due to strong bounding with clay and silt structure; hence they have positive environmental and public health safety implication.

| Percent of Zn in each fraction |             |              |             |              |
|--------------------------------|-------------|--------------|-------------|--------------|
| Treatment cells                | F1%         | F2 %         | F3%         | R%           |
| Cell-1                         | 3.80        | 6.73         | 7.02        | 82.46        |
| Cell-2                         | 13.16       | 13.45        | 11.34       | 62.04        |
| Cell-3                         | 7.03        | 15.97        | 6.49        | 70.52        |
| Av. (%)                        | <b>8.00</b> | <b>12.05</b> | <b>8.28</b> | <b>71.67</b> |

| Percent of Cr in each fraction |             |             |              |              |
|--------------------------------|-------------|-------------|--------------|--------------|
| Treatment cell                 | F1%         | F2 %        | F3%          | R%           |
| Cell-1                         | 2.40        | 0.37        | 9.03         | 88.20        |
| Cell-2                         | 10.23       | 1.38        | 14.10        | 74.30        |
| Cell-3                         | 2.53        | 5.12        | 8.26         | 84.09        |
| Av. (%)                        | <b>5.05</b> | <b>2.29</b> | <b>10.46</b> | <b>82.20</b> |

| Percent of Cd in each fraction |             |             |             |              |
|--------------------------------|-------------|-------------|-------------|--------------|
| Treatment cell                 | F1%         | F2 %        | F3%         | R%           |
| Cell-1                         | 7.52        | 1.74        | 11.01       | 79.73        |
| Cell-2                         | 11.12       | 4.47        | 10.57       | 73.84        |
| Cell-3                         | 6.57        | 10.47       | 7.14        | 75.83        |
| Av. (%)                        | <b>8.40</b> | <b>5.56</b> | <b>9.57</b> | <b>76.47</b> |

| Percent of Pb in each fraction |             |             |              |              |
|--------------------------------|-------------|-------------|--------------|--------------|
| Treatment cell                 | F1%         | F2 %        | F3%          | R%           |
| Cell-1                         | 8.40        | 4.17        | 10.45        | 76.98        |
| Cell-2                         | 12.04       | 4.38        | 14.28        | 69.30        |
| Cell-3                         | 4.85        | 10.46       | 6.10         | 78.59        |
| Av. (%)                        | <b>8.43</b> | <b>6.34</b> | <b>10.28</b> | <b>74.96</b> |

**Table 4: Percentage of Zn, Cr, Cd and Pb Bound to Different Fractions in Each Treatment Cell Sediment**

### 3.4 Bioavailability and mobility of heavy metals

#### 3.4.1 Bio-available fractions of heavy metals in sediment samples

The bioavailable fractions of heavy metals in CW sediment samples presented in Fig 7. The percentage of metals bound to water and acid soluble, exchangeable and carbonate (F1) were: Zn (8%); Cr (5%); Cd (8%) and Pb (8. %), indicating a low percentage of metals exists in readily bioavailable chemical forms. Heavy metals extracted in the first step extraction (F1) are instable and highly bioavailable [30, 6].

According to Nkinahamia, heavy metals that exist in the chemical form of (F1) are easily released into the environment [8]. Hence easy to transport, transform and are absorbed by biota and cause metal toxicity [21]. Thus, toxic heavy metals present in this form can be easily taken up by wetland plants or sediment inhibiting biota; and cause hazardous to plants, animals and the ecosystem [6]. However, due to low percentage of metals that occurred in the chemical form of (F1), their adverse impacts on biota and environment may not expected to be very serious.

Heavy metals bound to Fe and MN oxides form (F2) were: Zn (12%), Cr (3%), Cd (6%) and Pb (7%), are also indicating small percentage of heavy metals occurred in reducible chemical forms (F2). Metals occurred in this chemical form are less readily bioavailable as compared to metals occurred in the chemical form of (F1). The percentage of metals bound to organic matter (OM) and occurred in the form (F3) were also low; Zn (8%), Cr (10%), Cd (9.57%) and Pb (10 %). Heavy metals that occluded to OM (F3) are moderately bioavailability as compared to metals bound to (F1) and (F2) fractions. Metals bound to OM are usually released when OM is decomposed or when acidic condition occurred.

Generally, low percentage of metals fractions occurred in the forms of (F1), (F2), (F3) could be attributed to the low percentage of organic matter in the sediment [6]. Presence of clay and silt particles in the sediment scavenging trace metals and strongly bound the sediment and metals, and enhance, metals adsorption under alkaline pH condition [30, 11]. This result have environmental implications; low bioavailable metal fractions decreases chance of uptake of toxic metals by plants and animals, and reduce environmental risks.

The highest percentage residual fraction (R): Zn (72%), Cr (82%), Cd ( 76%), and Pb (75%), indicating that most heavy metals are not exist in bioavailable forms may be due to the presence of small size particle (clay and silt) sediment texture that can absorb metals [34].

In this respect, Klink reported that sediment that contains a higher percentage of small particles such as clay has a high surface area, and it has an affinity to bind metals in the sediments [33]. Authors have described that metals bound to residual fractions are not bioavailable due to strong bound within the crystalline lattice structure of clay minerals by isomorphous substitution [12, 14, and 23]. The presence of heavy metals largely bound to residual fractions of the HSSF-CW sediment have positive implication for environment, because, toxic heavy metals are immobilized, stable and not reactive in terms of biological processes [40]. Klink have also reported that high percentage of metals that found in the chemical form of (R) implies that the CW sediment was relatively less unpolluted with heavy metals. In addition, heavy metals bound to (R) are not easily taken up by wetland plants because of strong bounding with clay structure [33].

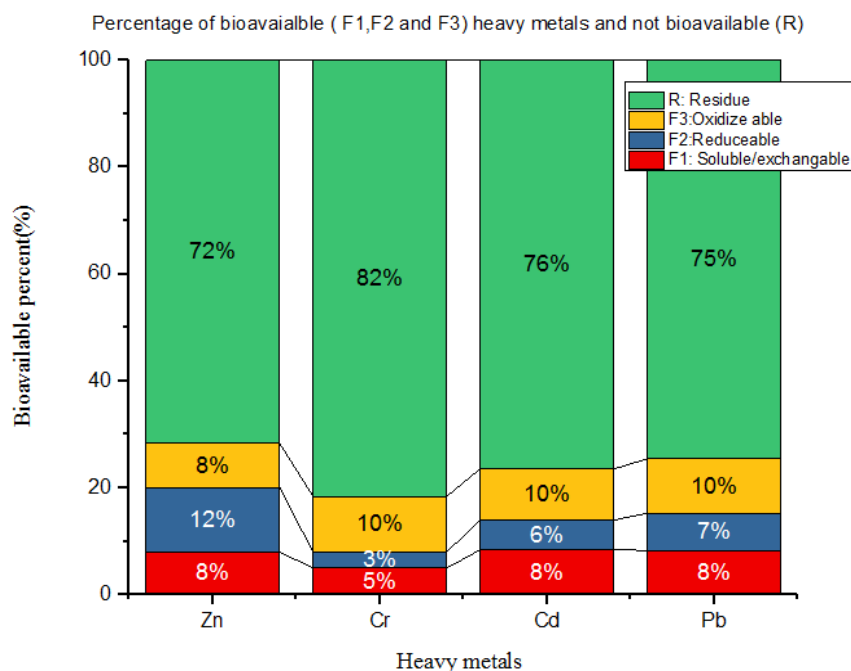


Figure 7: Bioavailable Heavy Metal Fractions in the Sediment Samples

### 3.4.2 Mobility Factor (MF) of Heavy Metals in the Sediment Samples

The MF of heavy metals found in the HSSF-CW sediment samples are presented in Table 5.

| Heavy metals     | MF of Heavy metals in CW sediment samples |             |             |              |
|------------------|---|-------------|-------------|--------------|
|                  | F1 (%)                                    | F2 (%)      | F3 (%)      | MF (%)       |
| Zn               | 7.06                                      | 11.72       | 8.82        | 27.60        |
| Cr               | 2.91                                      | 1.20        | 10.13       | 14.24        |
| Cd               | 6.84                                      | 3.39        | 8.59        | 18.82        |
| Pb               | 7.40                                      | 4.87        | 9.26        | 21.53        |
| <b>Average</b>   | <b>6.05</b>                               | <b>5.30</b> | <b>9.20</b> |              |
| <b>Av.MF (%)</b> |   |             |             | <b>20.55</b> |

**Table 5: Heavy Metals Mobile Factor for Sediment Sample**

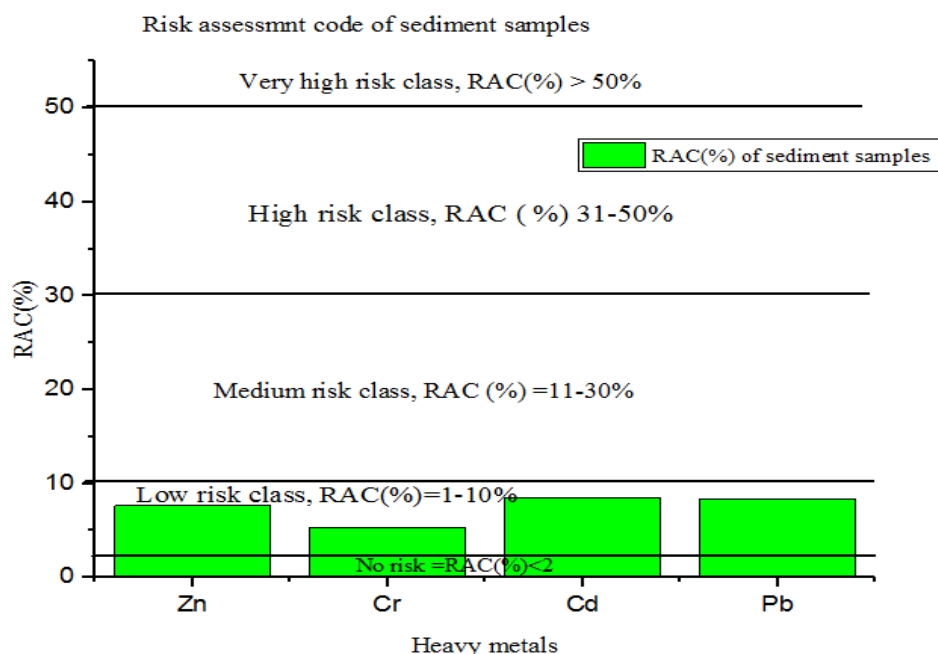
The MF of heavy metals in sediment samples were: 27.6% for Zn, 14.24% for Cr, 18.82% for Cd, and 21.53% for Pb. The average MF of all metals under the study was 20.55%, indicating low mobility of metals fractions. The decreasing order of heavy metals MF: Zn > Pb > Cd > Cr. Even though, the MF of all metal fractions are low, Zn and Pb are relatively more mobile than that of Cr and Cd. Hence, due to higher MF, Zn and Pb can move horizontally in the HSSF-CW sediment and cause sediment contamination, and ultimately, they pose adverse effects on wetland plants and other organisms found in the sediment.

Several authors have described that heavy metals which are weakly bound to water and acid soluble, exchangeable and carbonate (F1) and reducible fraction (F2) are considered to be highly mobile, while oxide-able fraction (F3) is moderately mobile [9, 13, 14, 34]. Thus, heavy metals which are available in the chemical forms of F1, F2, and F3 are mobile, migrate through soil and sediment may cause contamination of wetland treatment system and affects environmental quality when aged sediments (gravels and

soils) of the HSSF-CW sediment is disposed into surrounding environment [40]. However, in this study low average percentages of MF (20.55) was found. This result shows that the majority of heavy metals in sediments were not in mobile chemical forms and they are bound to residue (R) (table 4). Thus, due to the low mobility, toxic heavy metals may not cause high contamination of sediment, wetland plants, soil and surrounding environment.

### 3.4.3 Risk Assessment Code

The results of RAC of sediment samples are presented in Fig 8. The percent RAC for Zn, Cr, Cd and Pb in the sediment samples were: 7.67%, 5.37%, 8.51%, and 8.32%, respectively. According to Jakubus and Klink risk classification, the RAC % value for all metals fall in low risk (1% - 10%) category; indicating a lower risk to the environment [32, 33]. This implies that the majority of metals fractions found in the HSSF-CW sediments were in the chemical form of (R) fraction, and strongly bound to clay particles and become immobilized. Thus, the HSSF-CW sediment may not be highly polluted with heavy metals.



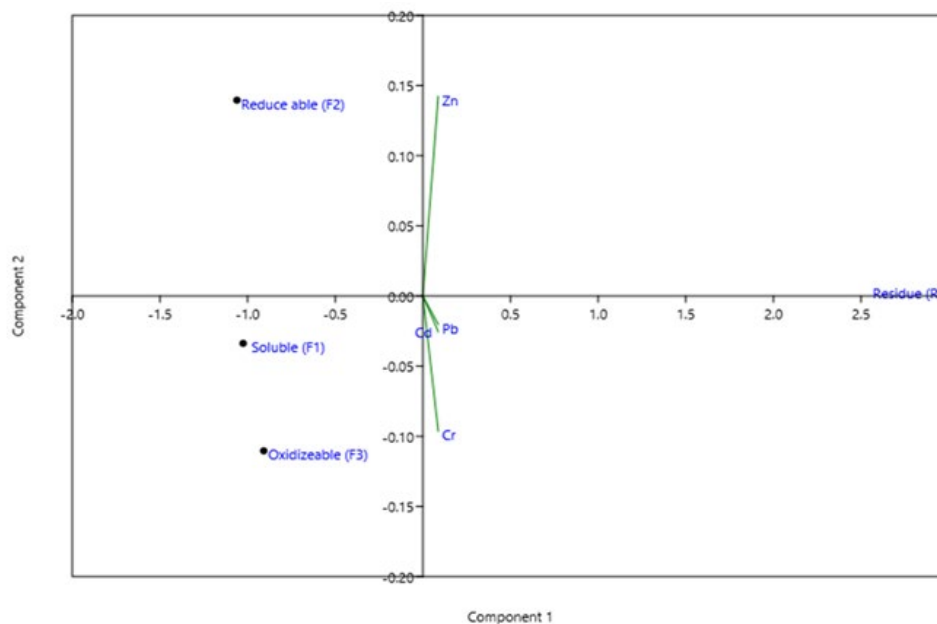
**Figure 8:** Risk Assessment Code and Risk Classes of Sediment Samples

### 3.5 Principal Component Analysis

The principal component analysis (PCA) was applied to identify sources of pollutants and to describe the variations of the data set [40, 41]. The result of (PCA) is presented in Fig 9. The number of principal components (PC) to be retained was selected on the basis of scree plot [42, 43]. The extracted eigenvectors of PCA indicated that the first principal component (PC1) represents 99.7% variation in the total data set of sediment samples with an eigenvalue of 3.99. The PC1 also showed that Cr, Cd and Pb were associated with the residual fraction (Fig 9) may be due to high metals scavenging and strong binding capacity of clay soil. PC1 showed

a high similarity with the loadings ( $> 0.50$ ), indicating a similar source of Cr, Cd and Pb that generated from anthropogenic and geological source and transported through river water, but strongly bound to clay soil crystal structure in the sediment.

High loading of Zn was associated with reducible fraction, indicating anthropogenic source. Zn can be generated from galvanized metals, application of Zn oxide in rubber manufacturing industries and agrochemicals. The eigenvalue (0.01) of PC2 was lower than 1; hence, it has no significant contribution and ignored.



**Figure 9:** PCA of Sediment Samples



#### 4. Conclusion

Heavy metals can be found in different chemical forms in constructed wetlands sediments. The study conducted using HSSF-CW sediment showed that low concentration of heavy metals occurs in the chemical forms of (F1), (F2) and (F3) implying that the bioavailability and mobility of metals in sediments is low. The high concentration of metals fraction bound to residual fraction (R) indicating that most metals are strongly bound to clay soil and immobilized. The risk assessment code (RAC %) of heavy metals falls in the low risk category. Thus, it can be concluded that heavy metals in the CW sediment may not pose a serious adverse impact on the surrounding environment.

Hence, heavy metals identified in HSSF-CW sediment are unlikely to cause significant sediment pollution. In general, the research has improved our understanding of heavy metal speciation in CW sediments or substrates, as well as, the environmental ramifications. It is therefore, highly recommended to use small amount of clay soil along with treatment media (gravel) to reduce contamination of sediment and local environment with heavy metals.

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