*Full Length Research Paper*

# Quantification of Cd, Cu, Pb and Zn from sewage sludge by modified-BCR and ultrasound assisted-modified BCR sequential extraction methods

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**Determination of the levels of heavy metals in sewage sludge is necessary prior to application of the sludge to agriculture because of the inherent risk of heavy metal toxicity to soil, plants and humans. The heavy metals (Cu, Cd, Pb and Zn) in sewage sludge were quantified using inductively coupled plasma optical emission spectroscopy (ICP-OES) following extraction with modified-BCR and ultrasonic assisted-BCR extraction methods. The quantities of heavy metals obtained from the two extraction methods were compared. The ICP-OES results indicated that in the exchangeable fraction in both extraction methods the amount of zinc released ranged from 19-227 mg kg$^{-1}$, and this represented the highest concentration of metals in this fraction. Clarifier sludge contained more zinc (2375 mg kg$^{-1}$) than raw sludge (310 mg kg$^{-1}$). An improvement in the amount of zinc (120.8 mg kg$^{-1}$) and lead (98.7 mg kg$^{-1}$) released in the reducible fraction in raw sludge samples was obtained with UA-BCR extraction. UA-BCR showed that the raw sludge contained about 17 mg kg$^{-1}$ of Cd while only about 0.625 mg kg$^{-1}$ was released using the modified BCR extraction. Some of the metals quantified were higher than the permissible levels, and the sludge not be used for soil enrichment. The results of this study demonstrated that the modified-BCR and the ultrasonic assisted-BCR extraction methods should be used in conjunction since one method alone might not be efficient in matrix breaking and releasing metal ions into solution. This may lead to incorrect conclusions about the presence or absence of metal ions in sewage sludge.**

**Key words:** Heavy metals, ultrasonication, sequential extraction, sludge, clarifier samples

## INTRODUCTION

The extraction of various targeted phases in a sample (soils, sediments, sludge and related materials) by a series of reagents in a sequential extraction process is now a well-established method for the fractionation of heavy metal content (Ure and Davidson, 2002).

Sequential extraction results provide useful information about the origin, bioavailability, occurrence, transport and potential mobility of elements in natural environments (Tuzen, 2003). The application of sequential extraction has several challenges including non-selectivity,

readsorption (Kheboian and Bauer, 1987; Belzile et al., 1989; Whalley and Grant, 1994; Gomez-Ariza et al., 1999) and the inability to compare results from different laboratories due to the use of different protocols (Mossop and Davidson, 2003). A more convenient approach has been the use of BCR (Community Bureau of Reference of the European Commission, now the Standards, Measuring and Testing Programme) procedure (Ure et al., 1993) which was designed to usher a platform to harmonize extraction protocols (Mossop and Davidson, 2003). However, since its first report, several studies have reported on the application of modified BCR extraction method (Mossop and Davidson, 2003; Cuong and Obbard, 2006; Nemati et al., 2009). Modifications were necessary to address some shortcomings of the original BCR method. The original BCR method involves the use of numerous reagents and longer extraction times. Some researchers have tried to cut down on the number of steps of extraction procedure (Pustisek et al., 2001), others have modified some steps of the BCR method by using alternative concentrations or reagents (Wang et al., 2015; Mittermüller et al., 2016), while others have tried to couple BCR with ultrasonication (Wang et al., 2015; Matong et al., 2016) or microwave techniques (Pérez-Cid et al., 1999; Vega-Morales et al., 2013) in order to improve the extraction process. Results with varying success have been reported particularly for solid samples like soils, sewage sludge and sediments.

Raw sewage contains significant concentrations of heavy metals which are not degraded by sewage treatment processes. That is, the final sewage product or effluent may contain toxic metal ions. These heavy metals may include zinc, cadmium, copper, nickel, lead, manganese, iron, mercury and chromium. Though most of the heavy metals are needed in trace amounts by growing plants, their excess concentrations can result in plant intoxication. The occurrence of heavy metals in industrial wastewater is of interest because they are often present at significant levels and if discharged into surface waters can have severe effects on the environment and public health (Masona et al., 2011). Disposed sludge volumes increase every year and this has generated interests on its usage particularly in agriculture due to the high content of nutrients. However, the presence of toxic compounds including heavy metals limits the amounts of sewage sludge to be added to soils (Jakubus and Czekala, 2001). In addition to heavy metals, sewage sludge also contains an abundance of organic matter and this could present challenges when extraction of metal ions is attempted with several reagents. This is because the organic matter in sludge can form strong complexes with metals and hence present challenges in the removal of metal ions from the sludge. It has been demonstrated that the cavitational effect generated by ultrasonication can be explored for breaking up the sample matrix, thereby exposing a fresh surface leading to faster and more aggressive extraction (Mason, 1990; Kazi et al., 2006). Hence, its application in combination with the BCR method for extraction of metal ions from solid samples is promising (Pustisek et al., 2001; Filgueiras et al., 2002). Even though the use of ultrasonification for extraction of heavy metals from solid samples has shown potential, its application is still minimal (Kazi et al., 2006). Ultrasonic agitation enhances the dissolution of heavy metals when used in combination with the BCR method and hence shortened the times of sequential extraction (Kazi et al., 2006).

Therefore, an investigation of the levels of heavy metal contamination originating from industrial and residential sewage treatment plants, and their consequent effect on the food chain, is of utmost importance. The aim of the current study was to determine the heavy metal content in sewage sludge using a modified-BCR (m-BCR) (Nemati et al., 2009) and m-BCR with ultrasonication (that is, ultrasonic assisted-BCR method – UA-BCR).

## EXPERIMENTAL

### Instrumentation

Inductively coupled plasma-optical emission spectroscopy (ICP-OES) by ThermoScientific iCAP 7400 DUO (Waltham, MA) was used for the determination of the metal content after extraction. A Branston 1800 ultrasonication bath (Danbury, CT) was used for ultrasound extraction while centrifugation was accomplished in a CL10 Centrifuge, ThermoScientific provided by Labotec (Johannesburg, South Africa). A pH meter (ADWA AD111 OPR) from Adwa Instruments (Szeged, Hungary) was used to monitor the pH of the solutions. An end-over-end Labcon 3100U shaker (Maraisburg, South Africa) was used for agitation of the samples during BCR extraction.

### Chemicals and reagents

High quality grade chemicals and reagents were used without further purification in this work. Acetic acid, hydroxylammonium chloride, hydrogen peroxide (30%) were purchased from Sigma-Aldrich (Johannesburg, South Africa). Ammonium acetate, nitric acid and hydrochloric acid were purchased from Merck Chemical Co. (Johannesburg, South Africa). All solutions were prepared with ultrapure water from the LaboStar equipment (Warrendale, PA).

### *Preparation of extraction solutions*

Solution A (acetic acid, 0.11 M): A 0.43 M acetic acid solution was prepared by transferring redistilled glacial acetic acid (25±0.2 mL) to

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about 500 mL of water in a 1000 mL polyethylene volumetric flask under a fume hood. It was made up to exactly 1000 mL with ultrapure water. Then 250 mL of this solution (0.43 M acetic acid) was diluted to 1000 mL to obtain an acetic acid concentration of 0.11 M.

Solution B (hydroxylamine hydrochloride, 0.5 M, pH 1.5): Hydroxylamine hydrochloride (34.75 g) was dissolved in 900 mL of ultrapure water. The solution was acidified with concentrated nitric acid to pH 1.5 and made up to 1000 mL.

Solution C (hydrogen peroxide, 8.8 M): Hydrogen peroxide was used as supplied by the manufacturer i.e. acid-stabilized to pH 2.0-3.0.

Solution D (ammonium acetate 1.0 M): Ammonium acetate (77.08 g) was dissolved in 900 mL of ultrapure water. The solution was acidified to pH 2.0 with concentrated nitric acid and made up to 1000 mL.

## Sampling

Raw and clarifier sewage sludge samples were collected from a local municipality treatment plant in Sasolburg region of South Africa in January 2015. The samples were placed in a bag, labelled and transported to the laboratory for further analysis. Before air drying sludge samples at room temperature under exhaust suction, large objects like concrete, pieces of brick and stones were handpicked and removed. The air-dried samples were sieved through a 1 mm sieve and 1 g samples were used for sequential extraction.

## Procedures

### Aqua regia digestion

Pseudototal heavy metal content in the raw and clarifier sludge samples obtained from the local municipality treatment plant was determined following *aqua regia* digestion of the samples. *Aqua-regia* digestion was carried out (Pueyo et al., 2001) to determine the pseudototal metal content in sludge samples. One gram of the sample was added to 0.5-1.0 mL of ultrapure water to obtain a slurry. To the slurry, HCl (7 mL, 12 M) and $HNO_3$ (2 mL, 15.8 M) mixture was added dropwise to reduce foaming. The centrifuge tube with its contents was allowed to stand for 16 h (overnight) at room temperature for slow oxidation of organic matter in the sample. Thereafter, the contents were transferred into a reflux setup (Berrow and Stein, 1983) and the temperature of the reaction mixture was slowly raised until reflux conditions were reached and maintained for 2 h. The digests were then filtered, transferred into a 100 mL volumetric flask and diluted to the mark with ultrapure water.

### Sequential extraction

Step 1 (exchangeable/extractable fraction): Acetic acid (solution A, 40 mL, 0.11 M) was added to a 100 mL centrifuge tube containing 1 g of sludge sample, stoppered and shaken immediately at room temperature for 16 h (overnight). The supernatant and solid were separated through centrifugation (20 min, 3000 rpm). The supernatant was decanted into a polyethylene volumetric flask and kept for further analysis. The residual solid was rinsed with distilled water (2 x 10 mL) by shaking the solid for 15 min. Following centrifugation, the liquid was then decanted and discarded.

Step 2 (reducible fraction): A freshly prepared hydroxylammonium

chloride solution (solution B, 40 mL) was added to the centrifuge tube containing the residue from step 1. The contents were again shaken at room temperature for 16 h and then centrifuged and treated as in step 1.

Step 3 (oxidisable fraction): Hydrogen peroxide solution (solution C, 10 mL, 8.8 M) was cautiously added to the Step 2 residue and the contents were digested at room temperature for 1 h with random manual shaking. Further digestion for another 1 h was carried out at 85°C, with occasional manual shaking for the first 30 min. Thereafter, the cover was removed to allow evaporation of the solution to about 1 mL. Ammonium acetate (solution D, 50 mL, 1.0 M) was added to the moist residue once it cooled down and shaken for 16 h at room temperature (overnight). The supernatant collected after centrifugation was kept for analysis. The solid was rinsed as before and digested as detailed in step 4.

Step 4 (residue fraction): The residue remaining at the end of the step 3 was digested following the same procedure in "*Aqua regia* digestion".

### Ultrasonic assisted BCR extraction method

Ultrasound assisted-BCR sequential extraction was carried out as detailed in "Sequential extraction" with all the four steps, reagents and solutions used. However, instead of shaking the contents for 16 h at room temperature in steps 1-3, the reaction contents in centrifuge tubes were placed in an ultrasonic bath and sonicated for 60 min. All other conditions after treatments were exactly the same as detailed above. No ultrasonication was applied in step 4.

## RESULTS AND DISCUSSION

### Pseudototal metal content

For precision and accuracy, the digestions were carried out in triplicate and mean values were reported. Figure 1 shows the metal content in raw and clarifier sludge obtained after acid digestion. The amounts of Cd, Cu and Pb in the raw sludge were slightly higher than those in the clarifier sludge. However, Zn concentrations of clarifier sludge were remarkably higher than that in the raw sludge. These results reveal that zinc has the highest potential of migration and contamination (Nemati et al., 2009). The total maximum threshold levels for Cd, Pb, Cu and Zn in sludge according to the Department of Water Affairs and Forestry (DWAF) guidelines is 3, 100, 120 and 200 mg $kg^{-1}$, respectively. The maximum permissible levels (MPL) for the above metals are 5, 150, 375 and 700 mg $kg^{-1}$, respectively. Figure 1 shows that the concentrations of the investigated metal ions in the raw and clarifier sludge obtained by total digestion exceeded both the DWAF threshold and the MPL, with the exception of Cd in clarifier sludge and Zn in raw sludge. The amounts of Cu (500-700 mg $kg^{-1}$) were similar to those reported by Shamuyarira and Gumbo (2014) from Louis Trichardt and Musina sludge samples in the Limpopo Province in South Africa. Similarly, the amounts of Zn in the clarifier sludge (2300 mg $kg^{-1}$) were also similar to that reported by the same authors from Louis
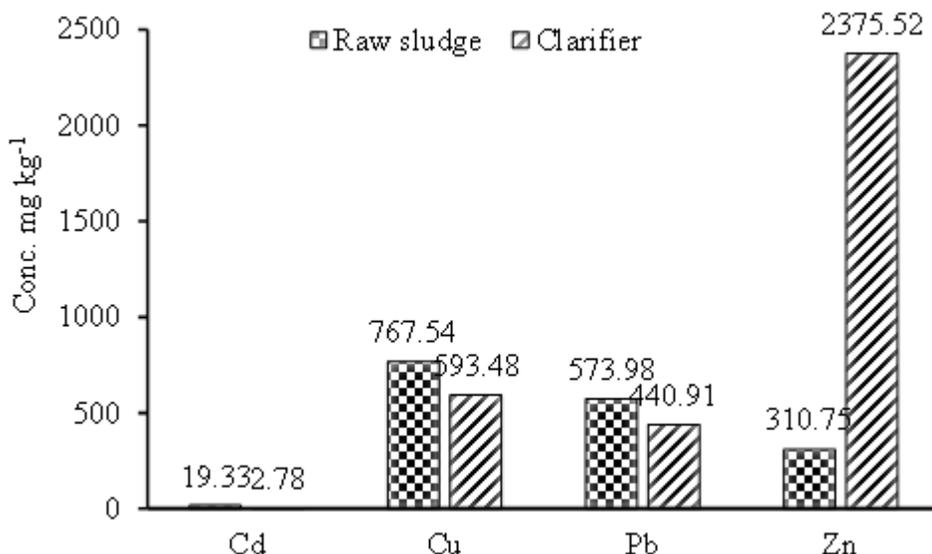
**Figure 1.** Metal content in raw and clarifier sludge obtained from acid digestion.

Trichardt and Polokwane. Heavy metals that exceed the DWAF guidelines is a cause for concern since this could be hazardous to both humans and the environment (Shamuyarira and Gumbo, 2014).

**BCR sequential extraction and ultrasonic assisted-BCR extraction**

Table 1a shows the comparison of the pseudototal digestion results with the sum of the 4 fractions obtained from the modified-BCR and UA-BCR method. The recoveries show that the results obtained with the two methods are in agreement, especially that of the BCR method and the pseudototal digestion. The percentage recoveries were calculated using the equation supplied below:

%Recovery = [(step 1 + step 2 + step 3 + residual)/pseudototal]*100          (1)

In the raw sludge extraction, different recoveries were obtained. The extraction recoveries of zinc were greater than 90% in both methods (modified-BCR and UA-BCR). The percentage recoveries of cadmium and lead were 88 and 62%, respectively, with UA-BCR method. m-BCR gave better recoveries (98%) for the extraction of copper. These results imply that the two methods can be used in synergy instead of individually e.g., m-BCR showed lower estimation of Pb and Cd while UA-BCR showed higher concentrations of the same metals. Cadmium and lead are soft metals that have a high affinity for soft bases such as thiol groups. Therefore, it appears that the ultrasound extraction was able to disrupt the matrix that

hold these metals and release them into solution. The individual contamination factor (Cf) was calculated as stipulated in Equation 2 (Jamali et al., 2007).

Cf = (step 1 + step 2 + step 3)/residual          (2)

The recoveries of lead and cadmium showed better extraction from the clarifier sludge with the UA-BCR method (Table 1b). However, the amounts of Cd and Pb extracted by the m-BCR method in the clarifier were much higher than those extracted from the raw sludge. This could be explained in terms on the matrix effects. The matrix effect in the raw sludge is much more complex than the matrix in the clarifier sludge, hence the higher extraction efficiency observed in the clarifier sample.

**Raw sludge extraction**

The heavy metals Cd, Cu, Pb and Zn were extracted by m-BCR and the UA-BCR sequential extraction methods from the raw sewage sludge samples. These heavy metals are known to be toxic to the *biota* and human health if found in the environment beyond its threshold concentrations. The concentrations of the heavy metals obtained with both extraction techniques were compared to the DWAF standards (Table 1). The pseudototal metal concentrations for Cd (19.33 mg kg$^{-1}$), Pb (767.54 mg kg$^{-1}$) and Cu (573.98 mg kg$^{-1}$) were found to be higher than both the DWAF threshold and MPL limits. These values suggest that raw sewage sludge has a high concentration of heavy metals. The concentration of zinc (310.75 mg kg$^{-1}$) was higher that the threshold value but lower than the MPL (700 mg kg$^{-1}$).

**Table 1a.** Pseudototal metal content, recoveries and individual concentration factors from raw sludge samples by m-BCR and UA-BCR methods.

| Metal ion | Method | Step 1 | Step 2 | Step 3 | Residual | Sum | DWAF Threshold | DWAF MPL | Pseudototal | %Recovery | Cf |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Cd | m-BCR | 0.105 (0.05) | ND | ND | 0.52 (0.66) | 0.625 | 3 | 5 | 19.33 | 3.23 | 0.2 |
|  | UA-BCR | 0.36 (0.36) | 2.14 (2.41) | 0.15 (0.13) | 14.32 (9.64) | 16.97 | 3 | 5 | 19.33 | 87.79 | 0.19 |
| Cu | m-BCR | 1.29 (0.19) | 21.38 (3.49) | 92.03 (37.30) | 639.90 (65.94) | 754.6 | 120 | 375 | 767.54 | 98.31 | 0.18 |
|  | UA-BCR | 2.95 (3.97) | 13.66 (2.20) | 6.71 (2.27) | 26.63 (10.97) | 49.95 | 120 | 375 | 767.54 | 6.51 | 0.88 |
| Pb | m-BCR | ND | 12.68 (7.51) | 209.49 (94.84) | 82.31 (27.66) | 304.48 | 100 | 150 | 573.98 | 53.05 | 2.7 |
|  | UA-BCR | 3.12 (3.98) | 98.74 (28.65) | 30.43 (6.22) | 221.54 (33.23) | 353.83 | 100 | 150 | 573.98 | 61.64 | 0.61 |
| Zn | m-BCR | 53.69 (6.34) | 65.43 (14.28) | 37.13 (3.36) | 148.78 (22.95) | 305.03 | 200 | 700 | 310.75 | 98.16 | 1.05 |
|  | UA-BCR | 19.12 (2.58) | 120.84 (46.50) | 8.31 (3.73) | 131.68 (10.63) | 279.95 | 200 | 700 | 310.75 | 90.09 | 1.13 |

MPL - maximum permissible level.

**Table 1b.** Pseudototal metal content, recoveries and individual concentration factors from clarifier sludge samples by m-BCR and UA-BCR methods.

| Metal ion | Method | Step 1 | Step 2 | Step 3 | Residual | Sum | DWAF Threshold | DWAF MPL | Pseudototal | %Recovery | Cf |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Cd | m-BCR | ND | 1.29 (0.22) | ND | 0.27 (0.64) | 1.3 | 3 | 5 | 2.78 | 46.76 | 3.81 |
|  | UA-BCR | 0.028 (0.06) | 2.97 (0.27) | ND | ND | 2.99 | 3 | 5 | 2.78 | 107.55 | 2.99 |
| Cu | m-BCR | 1.91 (1.01) | 365.89 (59.92) | 40.25 (3.46) | 161.67 (33.89) | 569.72 | 120 | 375 | 593.48 | 96.00 | 2.52 |
|  | UA-BCR | 2.08 (2.31) | 225.99 (4.27) | 9.88 (1.59) | 32.60 (3.55) | 270.55 | 120 | 375 | 593.48 | 45.59 | 7.3 |
| Pb | m-BCR | ND | 68.23 (9.54) | 16.32 (7.39) | 57.83 (16.96) | 142.34 | 100 | 150 | 440.91 | 32.28 | 1.46 |
|  | UA-BCR | 8.36 (5.12) | 174.56 (7.42) | ND | 250.87 (55.89) | 433.79 | 100 | 150 | 440.91 | 98.39 | 0.73 |
| Zn | m-BCR | 227.08 (47.52) | 1487.59 (176.89) | 88.03 (7.31) | 362.87 (42.12) | 2165.61 | 200 | 700 | 2375.52 | 91.16 | 4.71 |
|  | UA-BCR | 125.60 (13.95) | 1042.41 (103.37) | 14.69 (6.22) | 74.77 (34.13) | 1257.47 | 200 | 700 | 2375.52 | 52.93 | 15.82 |

MPL - maximum permissible level.

## Clarifier sludge extraction

The results obtained from the m-BCR and UA-BCR sequential extraction of clarifier sewage sludge samples is shown in Table 1b. The metal concentrations were compared with DWAF threshold and MPL standards of sewage sludge. The concentration of cadmium (2.78 mg kg$^{-1}$) as determined by pseudototal metal estimation was lower than both the threshold and MPL stipulated by DWAF (Table 1b). This value is similar to that obtained in municipality sludge (3.10 mg kg$^{-1}$)
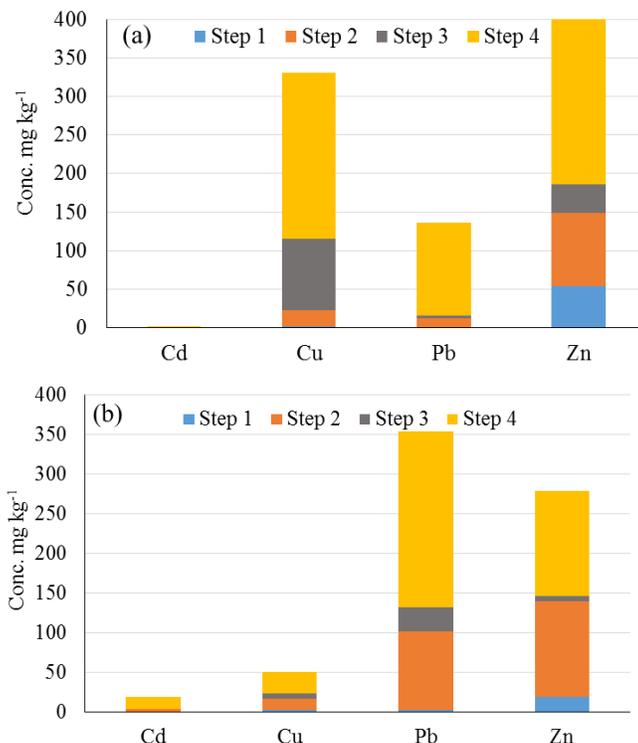
**Figure 2.** Fractionation of Cd, Cu, Pb and Zn in raw municipality sludge samples using (a) m-BCR sequential extraction and (b) UA-BCR sequential extraction modification.

from Limpopo Province (Shamuyarira and Gumbo, 2014). On the other hand, the concentrations of Pb (593.48 mg kg⁻¹), Cu (440.91 mg kg⁻¹) and Zn (2375.52 mg kg⁻¹) were higher compared to their corresponding DWAF threshold and MPL standards. The higher values of Pb, Cu and Zn in the clarifier sludge is matter of concern due to toxicity associated with these metals. Shamuyarira and Gumbo (2014) also observed high levels of Pb in the sludge of the municipalities of Polokwane (102.83 mg kg⁻¹) and Louis Trichardt (171.87 mg kg⁻¹). These values also exceeded the DWAF guidelines. Heavy traffic and the use of rubber tires in the Vaal Triangle could be the main source of cadmium pollution (Morrison et al., 2004). The amount of Zn estimated by m-BCR and UA-BCR methods in the clarifier sludge were much higher than the MPL guidelines of DWAF.

**Comparison of m-BCR and UA-BCR extraction fractions**

*Exchangeable and acid soluble fraction*

The exchangeable fraction has a greater adverse impact on the environment (Usero et al., 1998; Long et al., 2009) and is a measure of contamination that indicates the amount of heavy metal ions that could be released to the surrounding environment should conditions become more

acidic (Nemati et al., 2009), e.g. in case of contact with acid mine drainage. Figure 2(a and b) depicts the amounts (mg kg⁻¹) of Cd, Cu, Pb and Zn in raw municipality sludge obtained from the different fractions while Figure 3a, b shows the fractionation results of the same metals in the clarifier sludge samples. The concentrations of Cd, Cu and Pb obtained in the exchangeable fraction (step 1) in the raw and clarifier samples were almost negligible as determined by both m-BCR and UA-BCR methods (Figure 2a, b and 3a, b). However, the concentration of Zn in the raw sludge obtained by m-BCR and UA-BCR were 53.69±6.34 and 19.12±2.58 mg kg⁻¹, respectively. Figure 3a, b revealed that the concentration of Zn in the clarifier sludge as determined by m-BCR and UA-BCR were 227.08±47.52 and 125.60±13.95 mg kg⁻¹, respectively. These values were higher compared to values reported from the raw sludge. In both samples (raw and clarifier) the amount of Zn released using the m-BCR method was higher than that obtained by the UA-BCR method. Therefore, for the estimation of Zn in the exchangeable fraction, the m-BCR method was better as was also demonstrated by Nemati et al. (2009). The concentration of Zn (227.08 mg kg⁻¹) estimated by m-BCR method in the clarifier sludge exceeded the DWAF threshold limit. Therefore, wastewater treatments facilities need proper monitoring for Zn contamination. The higher concentration of zinc released in the clarifier sample was attributed to potential
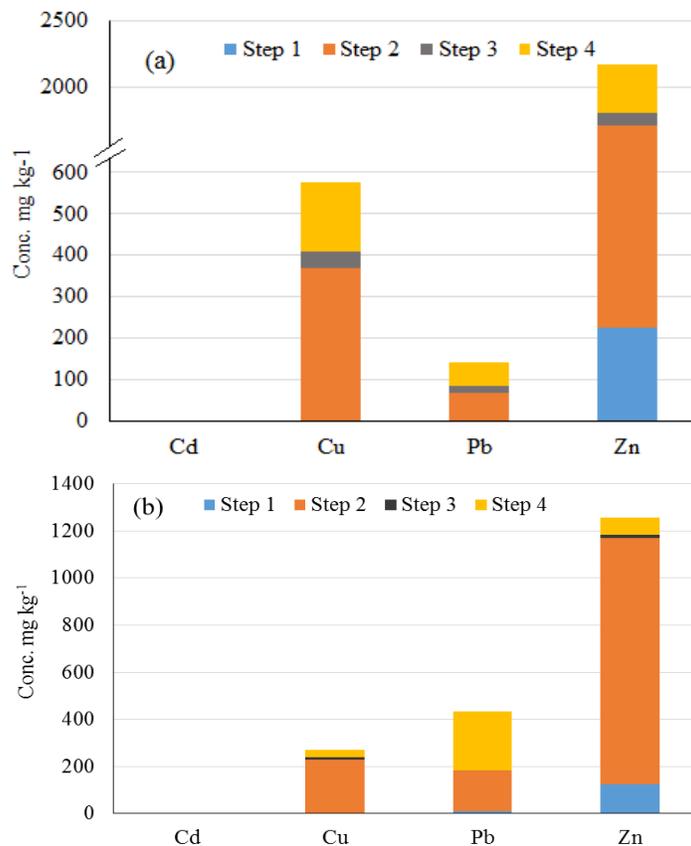
**Figure 3.** Fractionation of Cd, Cu, Pb and Zn in clarifier sludge samples using (a) m-BCR sequential extraction and (b) UA-BCR sequential extraction modification.

contamination or that the matrix of the clarifier was less complex and released Zn quite easily. The percentage of zinc that could be released in acidic conditions as calculated by m-BCR and UA-BCR methods was 21.45 and 43.06% of total zinc in raw sludge and, 68.69 and 82.89% of total zinc in clarifier sludge, respectively. Therefore, zinc has a greater potential to be released and cause contamination especially in clarifier sludge which has a less matrix complexity than raw sludge. The heavy metal content obtained in the exchangeable fractions with both techniques was below the DWAF MPL guideline limit.

### Reducible fraction

Figures 2 and 3 also illustrate the amounts of Cd, Cu, Pb and Zn obtained with m-BCR and UA-BCR methods in the reducible fraction in raw and clarifier sludge. In BCR extraction, the reducible fraction is characterised as the fraction showing the content of heavy metals bound to manganese and iron oxides which could be released into the environment under reducing conditions (Panda et al., 1995). In our study, the reducible fraction was extracted

following the optimised BCR conditions reported in Nemati et al. (2009). The concentration and pH of hydroxylammonium chloride solution for optimum Zn extraction were 0.5 M and pH 1.5. The concentration of Cd in raw sewage sludge was below detection limits with m-BCR and $2.14\pm2.41$ mg $kg^{-1}$ by the UA-BCR method. These values were below the DWAF threshold limit of 3 mg $kg^{-1}$. The amounts of Cu, Pb and Zn released in step 2 in raw sludge using the m-BCR method were $21.38\pm3.49$, $12.68\pm7.51$ and $65.43\pm14.28$ mg $kg^{-1}$, respectively. The concentrations of the same metals obtained with the UA-BCR method were $13.66\pm2.20$, $98.74\pm28.65$ and $120.84\pm46.50$ mg $kg^{-1}$, respectively. In clarifier sludge samples, the amounts recorded for Cd, Cu, Pb and Zn were $1.29\pm0.22$; $365.89\pm59.92$; $68.23\pm9.54$ and $1487.59\pm176.89$ mg $kg^{-1}$ respectively, when the m-BCR method was used. The UA-BCR method produced the following concentrations for Cd, Cu, Pb and Zn ($2.97\pm0.27$, $225.99\pm4.27$, $174.56\pm7.42$ and $1042.41\pm103.37$ mg $kg^{-1}$), respectively, in the clarifier sludge.

According to Shamuyarira and Gumbo (2014) the source of high concentrations of Zn in the sludge could be due to the use of brass as a household cleaning

material. The leaching of galvanized steel water distribution pipes is another possible source of Zn in sludge samples (Pawlowski et al., 2013). The Zn source from pipes makes better sense for our study as there was more Zn in clarifier sludge than in raw sludge which has undergone movement through pipes. The UA-BCR method gave higher recoveries of Pb (98.78 mg kg$^{-1}$) and Zn (120.84 mg kg$^{-1}$) compared to the m-BCR method. In this case, m-BCR under quantified the amounts of Pb and Zn in the reducible fraction.

### Oxidable fraction

Step 3 (oxidisable fraction) of the m-BCR sequential extraction method represents the heavy metal ion content bound to organic matter and sulphur compounds (Nemati et al., 2009). This fraction of the metal could only be leached to the environment under oxidising conditions. Only less than 0.15±0.13 mg kg$^{-1}$ of Cd was obtained in both samples. In terms of environmental pollution, the amounts of heavy metals quantified by m-BCR in the clarifier sludge were below the DWAF threshold limit. The oxidative environment results in increased solubility of copper and zinc (Long et al., 2009; Nemati et al., 2009). This can be explained on the basis that organic matter tends to degrade under oxidising conditions leading to release of bound metal ions to a complex with ligands present in the system (DiPietro et al., 1989; Johnson et al., 1996; Nemati et al., 2011; Li et al., 2013).

In raw sludge, the amount of Cu, Pb and Zn obtained by m-BCR were 92.02±37.30 mg kg$^{-1}$; 209.49±94.84 mg kg$^{-1}$; 37.13±3.36 mg kg$^{-1}$ and 6.71±2.27 mg kg$^{-1}$; 30.43±6.22 mg kg$^{-1}$; 8.31±3.73 mg kg$^{-1}$, respectively. High metal concentrations were estimated in the m-BCR method. In this case, UA-BCR method under quantified the concentrations of metals. Only the Pb (209.49 mg kg$^{-1}$) exceeded the DWAF MPL guidelines. In the clarifier sludge, m-BCR method gave better recoveries for Cu (40.25±3.46, Pb (16.32±7.39 mg kg$^{-1}$ and Zn (88.03±7.31 mg kg$^{-1}$) than the UA-BCR method. This suggests that heavy metals (Cu, Pb, Zn) are better quantified with m-BCR in the oxidisable fraction or that the conditions used in step 3 did not favour ultrasonication, that is, oxidation of metals is a slow process.

### Residual fraction

Figure 2a, b showed that substantial amounts of Zn, Cu and Pb were extracted in the raw sewage sludge. Interestingly, the UA-BCR methods showed higher concentrations of Pb (221.54±33.23 mg kg$^{-1}$) extracted from the raw sewage sludge compared to m-BCR. A similar trend was observed in the clarifier sludge extractions where 250.87±55.89 mg kg$^{-1}$ of Pb was released in the residue fraction by the UA-BCR method

and only 57.83±16.96 mg kg$^{-1}$ in m-BCR. The cavitation phenomenon of ultrasonication was enough responsible for breaking the crystalline structures holding the Pb ions. On the other hand, UA-BCR was not a good method for the extraction of Zn and Cu from the residue materials. The concentration of Cd, Cu and Zn determined by the m-BCR method were 0.52±0.66; 639.90±65.64; 148.78±22.95 mg kg$^{-1}$ in the raw sludge while in the clarifier sludge the amounts were 0.27±0.64; 161.67±33.89, 57.83±16.96 mg kg$^{-1}$, respectively. The concentrations of the same metals as determined by UA-BCR in the raw sludge were 14.32±9.64; 26.63±10.97; 221.54±33.23 mg kg$^{-1}$ while in the clarifier sludge they were: ND; 32.60±3.55 and 74.77±34.13 mg kg$^{-1}$, respectively. Both the sludge samples contained substantial amounts of Zn and Cu, especially as determined by the m-BCR method. The UA-BCR method was better suited for extraction of Pb in both samples.

### Environmental implications

Research has shown that the Cf (individual contamination factor) can be used to draw conclusions on the environmental implications of the extracted metal ions (Kazi et al., 2005; Jamali et al., 2007). The Cf was calculated as detailed in Equation 2. The Cf values obtained in raw sludge when the m-BCR was used ranged from 0.2 to 2.7, while for UA-BCR method the values ranged between 0.2 and 1.1. In clarifier sludge, the Cf values were between 1.14 and 4.71 for m-BCR method and 0.73 and 15.82 for UA-BCR method. These results revealed that metal ions were more easily released in the clarifier sludge than in the raw sludge and that Cu and Zn were the major elements. In some cases, especially using UA-BCR, a greater amount of Pb was released in both the raw and clarifier samples. The higher release of metals in the clarifier sludge could be attributable to the less complex matrix of clarifier sludge compared to that of raw sludge or contamination due to corrosion of pipes used in the wastewater treatment plants.

### Conclusion

It is paramount to study the heavy metal ion content in sludge samples before it is discharged into the environment. The metal content of sludge will dictate whether a particular sludge is fit for agricultural or landfill use. The amount of heavy metal released from sewage sludge was ascertained following two methods, the modified-BCR and the ultrasound assisted-BCR. The results for both methods were compared with the DWAF limits of exposure, and the results showed that certain elements were above the specified limits (e.g., Zn) in both methods, depending on the fraction or the extraction

stage. The results also showed that the ultrasonic assisted extraction method was more favourable for the quantification of Pb since more Pb was released with the UA-BCR method in reducible and residual fractions of raw and clarifier sludge. In both samples, the use of UA-BCR method showed that more Pb ions were released into solution, particularly the reducible fraction (step 2) and the residual fraction (step 4). Lead is poisonous and high concentrations of this metal in wastewater need to be treated before being discharged into the environment. The *aqua regia* extraction showed that almost all metal ions investigated exceeded the DWAF guidelines.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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