

Impacts of coagulation-flocculation treatment on the size distribution and bioavailability of trace metals (Cu, Pb, Ni, Zn) in municipal wastewater

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Abstract

This study investigated the impact of coagulation-flocculation treatment on metal form and bioavailability in municipal wastewater. Real humus effluent samples were separated into particulate, colloidal and truly dissolved fractions before and after treatment with either ferric chloride (FeCl₃) or the biopolymer Flocculan. Results revealed that both reagents effectively (≥48%) eliminated Cu, Pb and Zn from the particulate fraction

and removed Cu and Zn from the colloidal fraction in conjunction with colloidal organic carbon (COC). Although organics in the truly dissolved fraction were resistant to removal, Flocculant reduced Cu in this fraction by 72% owing to the complexation of free Cu ions to phenol and amino groups along the polymeric chains, revealing an additional removal pathway. In fact, COC removed in the CF process by Flocculant was replaced with truly dissolved compounds, input as a result of this reagent's organic composition. Flocculant, therefore, reduced the soluble concentration of Cu and Zn without changing the DOC concentration, thus reducing the bioavailability of these metals in treated effluent. FeCl₃ did not reduce the bioavailability of target metals, thus did not deliver any environmental benefit. This work provides important information for the selection and development of high performance coagulants to improve metal removal.

Keywords: Particle fractions; colloids; wastewater; bioavailability; coagulation

1. Introduction

Metals including Cu, Pb, Ni and Zn are commonly found in wastewater, with discharges of these metals influencing the chemistry and biology of receiving waters, with potentially toxic implications (Jaishankar et al. 2014). Regulatory drivers therefore typically require concentrations of these metals to be reduced in the wastewater treatment process. Within the European Union, the Water Framework Directive (WFD) (2000/60/EC) has specified bioavailability-based standards for Pb and Ni which are classified as Priority Hazardous Substances. In the United Kingdom (UK)

bioavailability-based standards have also been specified for Cu and Zn (UKTAG, 2008), which are classified as Specific Pollutants under the WFD. The concentration of metal that is bioavailable cannot easily be measured directly and consequently a predictive tool, known as a biotic ligand model (BLM), is used to convert a dissolved phase metal concentration to an equivalent bioavailable metal concentration. BLMs require site-specific information on physico-chemical characteristics as inputs, including the dissolved phase metal concentration, pH, dissolved organic carbon (DOC) and hardness (Di Toro et al. 2001).

In the United States (US), the Clean Water Act specifies Criteria Maximum Concentrations (CMC) and Criterion Continuous Concentrations (CCC) for heavy metals to protect human health and aquatic life in surface water, and the US Environmental Protection Agency (EPA) has already authorised the use of a BLM to determine water quality criteria for Cu (USEPA, 2017). Whilst improving environmental relevance, bioavailability-based standards are, nevertheless, expected to significantly reduce acceptable metal concentrations at a range of locations within the UK (Peters et al. 2009). This prospect has raised concern because wastewater treatment works (WWTWs) are traditionally designed to address problems of carbon contamination and nutrient enrichment rather than for trace metal elimination, and studies have shown that metal removal rates vary considerably at WWTWs (Gardner et al. 2013). In order to achieve removal rates to comply with more stringent discharge quality requirements, the development of novel wastewater treatment technologies is urgently required.

To enhance metal removal, a large body of previous work indicates the relevance of understanding metal form throughout wastewater treatment for the selection of suitable technologies (Chipasa, 2003; Karvelas et al. 2003; Santos and Judd, 2010). Analysis of metal form traditionally involves separation with a 0.45 μm filter. A soluble fraction passes through the filter, whilst particulates are retained on the filter (i.e. $> 0.45 \mu\text{m}$). Metals such as Cu and Pb are known to have a high affinity for the particulate fraction and, thus, can be removed as part of the wastewater treatment process by physical separation (Choubert et al. 2011). In contrast, Ni and Zn predominantly exist within the soluble fraction which potentially requires expensive and energy intensive processes such as adsorption or ion exchange for effective removal. Faced with the expectation of more stringent regulation, treatment technologies which can simultaneously remove particulate and soluble metal from wastewater at low treatment cost are desired by WWTW operators.

Soluble components can be separated into two further size fractions namely, a colloidal fraction, which is composed of material found in the size range 1 kDa to 0.45 μm , and a truly dissolved fraction, which contains constituents with a molecular weight (MW) < 1 kDa (Worms et al. 2010). Metal distribution within the soluble fraction is influenced by the presence of functional groups (ligands) that may complex metal ions. It has been reported that the distribution of metals between colloidal and truly dissolved fractions is greatly influenced by the distribution of DOC within these same fractions (Worms et al. 2010). Organics found in the truly dissolved size fraction include humic substances, amino acids, carbohydrates, hydrated metal ions and synthetic ligands such as

ethylenediaminetetraacetic acid (EDTA) (Imai et al. 2002; Shon et al. 2006). Conversely, organic material in the colloidal fraction is predominantly composed of macromolecules including aminosugars, polysaccharides and proteins (Jarusutthirak et al. 2002). In a recent study (Hargreaves et al. 2017) a large proportion of Cu, Pb, Ni and Zn was observed to reside in the colloidal fraction of effluent and calculated molar ratios of metals to organic carbon indicated that a large proportion of these metals may be adsorbed to colloidal material or complexed with macromolecules present in the colloidal size fraction. Such findings suggest that technologies capable of removing organic materials within the colloidal size fraction could reduce the concentration of target metals (Cu, Pb, Ni and Zn) in effluent discharges.

One such technology is coagulation-flocculation (CF), a widely used technique for the treatment of industrial effluent. Coagulation involves combining insoluble particles and dissolved matter into larger aggregates through the addition of chemical reagents (Renault et al. 2009). Aggregates are bonded together by flocculation, which consists of a stirring/agitation procedure that encourages particles to agglomerate, thus allowing their removal in subsequent sedimentation and filtration phases (Renault et al. 2009). Mechanisms through which particulate and colloidal materials are aggregated and, thus, removed can involve a combination of adsorption, entrapment, charge neutralisation and complexation processes (Alexander et al. 2012), and are dependent on the reagent used.

Chemical reagents frequently applied in the CF process are metal salts such as ferric chloride (FeCl_3), which aggregate particles

by charge neutralisation and sweep flocculation (Duan and Gregory, 2003). FeCl_3 is commonly used to remove phosphorus from sewage effluents and studies have shown this reagent can enhance the removal of metals from combined sewer overflow (El Samrani et al. 2008), but few studies have investigated application for the treatment of municipal wastewater. On a global scale, however, waste management systems are under change, with producer responsibility and consumer accountability driving the development of sustainable and eco-friendly management systems (Zaman, 2013). An increase in the awareness of the detrimental consequences that metal salts may have to the environment, such as increasing non-target metal concentrations in treated wastewater and significant alterations to the pH of treated water (Renault et al. 2009; Vijayaraghavan et al. 2011), has resulted in ongoing investigations into alternatives.

Tanfloc, a tannin-based product with a trademark belonging to TANAC (Brazil) has attracted attention because of its renewability, biodegradability and its capacity to eliminate metals (Beltrán Heredia and Sánchez Martín, 2009). For example, zeta potential measurements have showed the cationic nature of Tanfloc and, given the polymeric structure of this coagulant, mechanisms of charge neutralisation and inter-particle bridging are suggested to be involved in the CF process (Hameed et al. 2016). Flocculan is a commercially available product which is composed of up to 30% Tanfloc and is, therefore, a practical treatment option. Studies investigating the application of this reagent to municipal wastewater are, however, limited. It is also noted that there is a scarcity of studies that assess the impact of CF on metal form and bioavailability within the effluent matrix. Specific information on the fate of metals

following CF is needed to assess the benefits of this treatment technology and to provide key information on coagulant selection for the treatment of municipal wastewater effluents. Furthermore, understanding the relationship between metal speciation and CF may help develop high performance reagents for future application to improve metal removal in wastewater treatment.

The aim of this study was, therefore, to investigate the impact of CF on metal forms (particulate, colloidal and truly dissolved fractions) and bioavailability in municipal wastewater. The study set out to compare the effect of the biopolymer Flocculan on metal removal and to compare that with the conventional coagulant, FeCl_3 . To further characterise the properties of coagulants and flocs produced from the treatment, Fourier transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM) were used.

2. Materials and Methods

2.1 Materials

Nitric acid (HNO_3), sodium hydroxide (NaOH) and hydrochloric acid (HCl) were all obtained from Fisher Scientific (Loughborough, UK) and were of reagent grade. Plastic and glassware was kept in a 2% (v/v) HNO_3 solution between uses, and were rinsed with ultrapure water prior to use. Flocculan was obtained from Lansdowne Chemicals (Oxon, UK). Ferric chloride (FeCl_3) solution (40%) was provided by Darrant Chemicals (Lincoln, UK).

2.2 Wastewater samples

Humus effluent samples were taken from a WWTWs in the UK which operated the trickling filter treatment type and which serves an urban catchment population. Contributors to the metal load entering the works include diffuse sources such as urban run-off, metals corroded from the domestic water supply infrastructure and leaching of metal from household plumbing. Samples were collected on 8 occasions over a five-month study period (from October 2016 – February 2017) with at least one collection each month. Samples were collected in 5 L plastic containers which had previously been rinsed with 0.01 M HCl and ultrapure water (0.05 μ S conductivity level). Samples were transported to, and were kept, in a refrigerated environment (4 °C) at Cranfield University laboratories prior to analysis.

2.3 Coagulation-flocculation experiments

To replicate the CF process Phipps and Bird jar test apparatus (Richmond, United States) was used. Plastic beakers (rinsed with 0.01 M HCl and ultrapure water) were filled with one litre of humus effluent, placed on the floc illuminator and once the coagulant dose was added, were agitated at a fixed intensity (100 rpm) and duration (2 min). The coagulant dose was fixed at the optimum level established in a previous study by the authors herein; 0.25 mg/L for Flocculan and 0.10 mg/L for FeCl₃ (Hargreaves et al. unpublished results). Once rapid mixing finished, the fixed intensity and duration of slow mixing (30 rpm for 30 min) was immediately established.

After slow mixing the beakers were removed from the floc illuminator and were positioned in a safe place for 30 minutes, allowing for sedimentation. After sedimentation, the supernatant was extracted from about 25 mm below the surface with a syringe. For the first 3 humus effluent samples collected, jar tests were conducted in duplicate. For the additional five samples collected, jar tests were conducted in triplicate. Therefore a total of 42 jars were treated with either FeCl₃ or Flocculan (i.e. 21 jars for each coagulant). For each sample collected a minimum of 1 beaker was processed through the jar test procedure outlined above without the addition of a coagulant, and was therefore treated by stirring only. This was used as the experimental control.

2.4 Particle size fractionation

Particle size fractionation of the samples was conducted following a variation of the method described by Barker and Stuckey (1999). To obtain the soluble phase samples were filtered through 0.45 µm polyethersulphone membranes (Millipore, Livingston, UK) by vacuum filtration (APHA, 2005). Regenerated cellulose membranes (1 kDa) (Millipore, Livingston, UK) were used to fractionate the samples into colloidal and truly dissolved fractions. Membranes were washed in sequences of ultrapure water (0.05 µS conductivity level), 0.01 M NaOH and 0.01 M HCl. This alkaline/acid cleaning sequence was repeated after each use. Truly dissolved fractionations were undertaken using an Amicon 8400 stirred pressure cell (Millipore, Livingston, UK). Filtration was driven by nitrogen gas at a pressure

of 160 kPa. To limit concentration polarisation, an integrated bar stirrer was operated and a filtrate/retentate ratio of 0.4 was adopted.

2.5 Analytical methods

The pH measurements were conducted with a Jenway 4330 pH and conductivity meter (Stone, UK). Turbidity was measured using a HACH 2100N turbidimeter (Manchester, UK). Total chemical oxygen demand (COD) and total phosphorous (TP) concentrations were determined using cell test kits (Fisher Scientific, Leicestershire, UK). Dissolved organic carbon (DOC) content was determined using a TOC-V analyser (Shimadzu, Milton Keynes, UK). Target metals (Cu, Pb, Ni, Zn) were measured by inductively coupled plasma-mass spectrometer (ICP-MS) using a Nexion 350D system (Perkin Elmer, Beaconsfield, UK). The limit of detection for all the metals was 0.01 µg/L. Prior to ICP-MS analysis, samples were digested with a CEM-MARS microwave (CEM, Buckingham, UK) in order to determine the total concentration of the target metals. Sample blanks were processed through the preparation and analytical procedures. Phase distributions were calculated as follows: particulate = total - soluble, colloidal = soluble - truly dissolved. In effluent, bioavailable metal concentrations were estimated by using the metal bioavailability assessment tool (M-BAT) (WFD and UKTAG, 2014).

Dried flocs obtained after the coagulation-flocculation process were examined under a FEI XL30 SFEG scanning electron microscope (Cambridge, UK) to reveal the morphology of flocs produced by FeCl₃ and Flocculan. The infrared absorption spectrum of

coagulants, suspended solids in the humus effluent and flocs in the treated effluent were obtained with a JASCO 4100 Fourier transform infrared spectrometer (Dunmow, UK) to characterise the molecular structure. These analyses characterised the differences in flocs formed to evaluate the mechanisms of metal removal with FeCl₃ and Flocculan.

2.6 Data analysis

Statistical analysis of the obtained data was completed with the software package Minitab 17. One- way ANOVA tests were performed to assess the differences in metal concentrations between fractions after treatment with either FeCl₃ or Flocculan. Pearson's correlation coefficient tests were used to express the relationship between the concentrations of metals and DOC. Using guidance from Evans (1996) correlation strength was determined to be weak (0.2-0.39), moderate (0.4-0.59) or strong (> 0.59). Both tests were conducted with a level of significance $p < 0.05$.

3. Results and discussion

3.1 Humus effluent characteristics

The concentrations of target metals as well as sanitary determinands in the humus effluent are shown in Table 1. In humus effluent, the abundance of target metals in terms of total concentration was Zn > Cu > Ni > Pb (see Table 1). Other investigators (Gardner et al. 2013)

have observed similar relative abundances and metal concentrations at WWTWs elsewhere in the United Kingdom.

Table 1: Concentrations of sanitary determinands in humus effluent before and after treatment with FeCl₃ and Flocculan, and the associated rates of removal observed [Mean (standard deviation)].

Parameter	Humus Effluent	FeCl ₃	Flocculan	Rate of Removal (%)	
				FeCl ₃	Flocculan
<i>Sanitary parameters</i>					
pH	7.4 (0.1)	6.7 (0.1)	7.4 (0.2)		
Turbidity (NTU)	8.7 (1.9)	3.6 (1.3)	2.9 (1.6)	59 (15)	69 (14)
COD (mg/L)	58 (7.6)	32.8 (13.6)	43.8 (8.6)	43 (19)	22 (11)
DOC (mg/L)	13.1 (1.6)	8 (1.6)	12.9 (1.6)	42 (9)	1 (11)
Total P (mg/L)	4.7 (0.6)	0.6 (0.3)	4.3 (0.7)	86 (4)	9 (4)
<i>Metals (µg/L)</i>					
Cu (total)	19.1 (3.7)	8.5 (2.4)	3.4 (0.8)	53 (16)	81 (6)
Cu (soluble)	10.3 (2.3)	5 (1.3)	1.4 (0.4)	51 (6)	86 (2)
Pb (total)	0.8 (0.3)	0.3 (0.2)	0.3 (0.2)	58 (17)	65 (13)
Pb (soluble)	0.4 (0.2)	0.2 (0.1)	0.2 (0.1)	54 (18)	56 (16)
Ni (total)	3 (0.6)	6.4 (1.1)	2.7 (0.9)	-112 (34)	9 (28)
Ni (soluble)	1.8 (0.5)	4.8 (0.9)	1.6 (0.4)	-178 (52)	17 (24)
Zn (total)	40.3 (8.2)	26.9 (4.8)	23.1 (3.6)	36 (9)	42 (10)
Zn (soluble)	26.4 (6.8)	18 (3.9)	14.5 (2.9)	31 (13)	45 (11)

3.2 Impact of coagulation-flocculation on DOC

In the CF process, FeCl₃ removed a sizeable amount of DOC (42%) from humus effluent. Conversely, overall removal of DOC with Flocculan was negligible (1%; Table 1), as indicated by no significant ($p = 0.589$) change in the overall DOC concentration (Fig. 1). Nevertheless, in both cases significant ($p < 0.001$) removal of colloidal organic carbon (COC) was observed, with removal rates of 61 and 37% recorded for FeCl₃ and Flocculan respectively. Organic constituents in the colloidal size range are composed of macromolecules such as amino sugars, polysaccharides and proteins

(Jarusutthirak et al. 2002). In CF, co-precipitation of macromolecular substances in the colloidal size fraction is favoured, due to their large number and variety of functional groups which enhances attachment to precipitates (Haberkamp et al. 2007). In addition, constituents with higher MW can readily overcome electrostatic repulsion to become adsorbed (Wang et al. 2015). Removal of macromolecular particles in the CF process was therefore anticipated and has been observed elsewhere in the treatment of secondary effluent with FeCl₃ (Haberkamp et al. 2007).

The concentration of DOC in the truly dissolved fraction was not significantly changed following treatment with FeCl₃ (Fig. 1). Other studies (Imai et al. 2002; Shon et al. 2006) have identified that the truly dissolved fraction is composed of free metal ions and low MW organic constituents. This study, therefore, supports the findings of others (Michael-Kordatou et al. 2015) by revealing that low MW organics are removed to a minor extent in the CF process. Removal of DOC from the truly dissolved fraction was also negligible in CF with Flocculan. In fact, an increase in the concentration of DOC in this fraction was observed following treatment (1.3 to 6.1 mg/L; Fig. 1) and was likely to result from the organic substances present in this reagent.

3.3 Distribution of metals in humus effluent

The distribution of target metals within the particulate (> 0.45µm), colloidal (1 kDa – 0.45 µm) and truly dissolved (< 1 kDa) fractions in

humus effluent, the experimental control and effluent treated with either FeCl₃ or Flocculan, are presented in Fig 2.

In humus effluent, a sizable proportion of Cu (47%), Pb (61%), Ni (37%) and Zn (33%) was distributed in the particulate fraction (Fig. 2). The distribution of metals in this fraction and trend recorded (i.e. Pb > Cu > Ni > Zn) were similar to that observed by Hargreaves et al. (2017). Soluble Cu (46%), Pb (29%), Ni (36%) and Zn (46%) were predominantly distributed in the colloidal size fraction of the humus effluent, whilst for all target metals distribution in the truly dissolved fraction was relatively low (Fig. 2). Other studies (Gagnon et al. 2014; Hargreaves et al. 2017) have reported similar distributions in effluent.

3.4 Impact of coagulation-flocculation on metal size distribution

3.4.1 Copper

With an initial total concentration of 19.1 ± 3.7 µg/L (Table 1), good overall rates of removal were observed for Cu in CF with either FeCl₃ (53%) or Flocculan (81%). High removals have been reported elsewhere for FeCl₃ (Cu: 81%; El Samrani et al. 2008) and plant based biopolymers (Cu: 90%; Beltrán Heredia and Sánchez Martín, 2009). The result was therefore in line with expectations.

For the particulate fraction, high removals were observed (FeCl₃: 60%; Flocculan: 70%), as indicated by significantly lower Cu concentrations in effluent treated with FeCl₃ or Flocculan ($p < 0.001$; Fig. 2a). In fact, particulate Cu concentrations were similar in the treated effluents (FeCl₃: 3.6 ± 2 µg/L; Flocculan: 2 ± 0.8 µg/L). Suspended particles adsorb to the surface of flocs, which are

aggregated and removed in the CF process (Renault et al. 2009). In addition, the removal of particulate Cu in conjunction with suspended particles has been reported in a previous study at the site (Hargreaves et al. 2017). Hence, removal of particulate Cu in the CF process was anticipated.

An appreciable amount of Cu was also removed from the colloidal size fraction when treated with FeCl₃ (61%) or Flocculan (88%), as indicated by significantly lower concentrations ($p < 0.001$) compared with initial humus effluent values. In fact, strong correlations were observed between colloidal Cu and COC concentrations (FeCl₃: $r = 0.704$, $p = 0.001$, Flocculan: $r = 0.715$, $p < 0.001$) in treated effluent, which indicates that removal of Cu occurred in conjunction with the elimination of organics from the colloidal size fraction. Organic constituents in the colloidal size range include proteins, polysaccharides and amino sugars (Jarusutthirak et al. 2002); such substances are highly reactive regarding metal complexation due to their polyfunctional character and large binding site density (Worms et al. 2010). Results from the present study therefore indicate that technologies capable of removing organic particles within the colloidal fraction are capable of reducing soluble Cu concentrations in effluent.

Despite FeCl₃ achieving a higher rate of removal for COC (Fig. 1), a significantly lower Cu concentration was observed in the colloidal fraction of effluent treated with Flocculan ($p < 0.001$, Fig. 2a). It was anticipated that this may arise since Flocculan has the ability to remove organic materials with functional groups that Cu readily complexes with. In fact, other studies (Hameed et al. 2016) have revealed that Tanfloc (the active ingredient in Flocculan)

contains carbonyl and amino functional groups. These are indicative of polysaccharides and proteins that can play a significant role in the complexation of Cu in effluent (Michael-Kordatou et al. 2015).

Furthermore, polysaccharides are the dominant material of extracellular polymers produced by microorganisms during biological wastewater treatment (Wang and Wu, 2009), thus were anticipated to be a prominent colloidal organic constituent in the humus effluent.

In CF with FeCl_3 , removal of Cu from the truly dissolved fraction was negligible (see Fig. 2a), Thus, FeCl_3 did not affect the Cu found within this fraction. Studies (Imai et al. 2002; Shon et al. 2006) have revealed that the truly dissolved fraction is comprised of free metal ions and organic constituents including carbohydrates and amino acids, which metals may adsorb to or complex with. Given that organic materials distributed in the truly dissolved fraction were revealed to be resistant to removal (Fig. 1), negligible removal of metals associated to them can be expected. Nevertheless, Flocculant reduced the concentration of Cu in the truly dissolved fraction by 72% (from 1.4 to 0.4 $\mu\text{g/L}$; Fig. 2a). It was, therefore, assumed that a further removal mechanism was involved in CF with Flocculant.

Flocculant is a biopolymer composed of polymeric chains along which functional groups including phenolic and amino groups are found.

Free Cu ions have a high affinity for these functional groups (Lu and Allen, 2002; Hamza et al. 2017) so it was anticipated that, in CF with Flocculant, complexation of free Cu ions with these functional groups occurred resulting in a lower Cu concentration in the truly dissolved fraction after treatment (Fig. 2a).

3.4.2 Lead and Zinc

Sizeable amounts of Pb and Zn were removed from humus effluent in the CF process (see Table 1). Such findings were expected as other researchers have reported that FeCl₃ (Johnson et al. 2008) and biopolymers (Kazemipour et al. 2008) were effective for the removal of Pb and Zn from synthetic wastewater. The residual concentrations of Pb and Zn were comparable for FeCl₃ and Flocculan (Fig. 2b and d, respectively).

Significant ($p < 0.001$) removal of Pb occurred from the particulate fraction in CF with either FeCl₃ (89%) or Flocculan (83%) (Fig. 2b). Lead was predominantly distributed in the particulate fraction of humus effluent (Fig. 2) and, when in this form, it is known to be removed in conjunction with suspended particles (Hargreaves et al. 2017). Hence, such finding was in line with expectations. In the case of Zn, the respective removals from the particulate fraction for FeCl₃ and Flocculan were 42 and 35% (Fig. 2d), as indicated by significantly lower concentrations following treatment ($p < 0.05$). Nonetheless, Zn removals were lower than those observed for Cu (FeCl₃: 60%, Flocculan: 70%) and Pb (FeCl₃: 89%, Flocculan: 83%), as a relatively low proportion of Zn was distributed in the particulate fraction of humus effluent. Such trend in metal removal from the particulate fraction supports the findings of other studies at WWTWs elsewhere (Karvelas et al. 2003).

As Fig. 2d shows, a large proportion of Zn in the colloidal size fraction was eliminated after CF with FeCl₃ (48%) and Flocculan (54%). Moderate correlations (FeCl₃: $r = 0.461$, $p = 0.035$; Flocculan: $r = 0.509$, $p = 0.006$) were observed between colloidal Zn and COC

concentrations indicating that, as for Cu, Zn was eliminated in conjunction with COC. Other studies (Louis et al. 2014; Constantino et al. 2015) have indicated specific ultraviolet absorbance (SUVA) to be a poor predictor of Zn complexation capacity, which suggests that macromolecules distributed in the colloidal size fraction may be significant for the binding of Zn ions in effluent. Nevertheless, removal rates in the colloidal fraction were much lower for Zn (FeCl₃: 48%, Flocculan: 54%) compared with Cu (FeCl₃: 61%, Flocculan: 88%). This was anticipated to result from the adverse impact of alternate competing soluble metals. At municipal WWTWs effluent contains a large number of metallic species beyond those tested in this study, for example Ca and Mg. Hence, a highly competitive process occurs between metal ions and binding sites in effluent: metal ions compete with each other for complex formation and thus stability constants provide information on adsorption equilibria (Turhanen et al. 2015). The conditional stability constants for Zn with dissolved organic matter were revealed to be lower than that of Cu in effluent at municipal WWTWs (Chaminda et al. 2013). Zinc may, therefore, be more readily displaced at the surface of binding sites by competing soluble metals, and such behaviour has been supported by the findings of others. For example, Zhao et al. (1999) revealed that Ca and Mg may have a negative effect on the removal of Zn via competitive sorption to binding sites on the surface of biomass. In contrast, it has been reported that even in the presence of high Ca and Mg concentrations, Cu may be capable of binding to complexing agents (Turhanen et al. 2015). Thus, Cu can readily outcompete other metals for binding sites on the surface of colloidal organic compounds, which were removed in the CF process.

The difference between the concentration of colloidal Pb in humus effluent ($0.3 \pm 0.1 \mu\text{g/L}$) and that observed in humus effluent treated with FeCl_3 ($0.2 \pm 0.1 \mu\text{g/L}$) or Flocculan ($0.1 \pm 0.1 \mu\text{g/L}$) was negligible. Furthermore, no significant change ($p > 0.05$) in the concentration of Zn and Pb in the truly dissolved fraction was observed (Fig. 2b and d). These findings indicate that FeCl_3 and Flocculan had little or no effect on the truly dissolved Pb and Zn.

3.4.3 Nickel

The concentration of Ni in the humus effluent was not reduced following treatment with either FeCl_3 or Flocculan (see Fig. 4c). Ni is highly soluble in wastewater, with a sizeable proportion (27%) of Ni distributed in the truly dissolved fraction of humus effluent (Fig. 2c), and because of its thermodynamic properties (i.e. slow rate of reaction, low stability constant) its removal in the CF process is low especially compared with Cu, Pb and Zn (see Table 1). Similar removal trends have been observed elsewhere in conventional wastewater treatment processes (Karvelas et al. 2003).

A substantial increase in the concentration of Ni was observed in CF with FeCl_3 , which was attributed to impurities in this reagent. Other studies (Buzier et al. 2006; Chys et al. 2013) have reported that FeCl_3 contains substantial amounts of Ni and contributes toward the presence of this metal in treated effluent. In this study Ni concentrations in the truly dissolved fraction were significantly increased by 72% ($p < 0.001$) after addition of FeCl_3 . This is of concern as this fraction is considered the most bioavailable to aquatic life (Gagnon et al. 2014), therefore when used in

wastewater treatment FeCl_3 may pose a risk to receiving watercourse ecology.

3.5 Impact of coagulation-flocculation on metal bioavailability

The bioavailable concentrations of target metals in humus effluent, the experimental control and effluent treated with either FeCl_3 or Flocculan are presented in Fig. 3. In order to calculate bioavailable metal concentrations, BLMs account for the interaction of trace metals with DOC which contains a variety of ligands that bind metal ions, thereby reducing their ability to interact with organisms. Thus, increasing the DOC concentration significantly reduces the bioavailability of the metal. At low metal:DOC ratios, there is an excess of complexation capacity so that free metal ion activity is reduced (Doig and Liber, 2007). For example, Pernet-Coudrier et al. (2008) reported that Cu toxicity towards *Daphnia magna* was reduced in the presence of dissolved organic matter derived from wastewater treated effluent. More recently, a study by Constantino et al. (2017) demonstrated that effluent-derived DOC offers a relatively greater complexation capacity per milligram of DOC in comparison with DOC obtained from natural sources. As seen in Fig. 1, COC removed in the CF process by Flocculan was replaced with truly dissolved compounds as a result of the organic nature of this reagent. Flocculan was, therefore, able to reduce the soluble concentration of Cu (86%), Pb (56%) and Zn (45%) without significantly ($p = 0.589$) reducing the concentration of DOC in treated effluent (Fig. 1). Thus, a significant reduction in the concentration of bioavailable Cu ($p < 0.001$), Pb ($p = 0.001$) and Zn ($p < 0.001$) was observed (Fig. 3a, b

and d, respectively). These findings indicate that Flocculant was capable of simultaneously reducing the concentration and bioavailability of these metals in treated effluent. The environmental benefit of utilising Flocculant should therefore be considered in decision making on municipal wastewater treatment. The bioavailable Ni concentration was unchanged ($p = 0.101$) following treatment with Flocculant (Fig. 3c).

In CF with FeCl_3 a sizeable amount of DOC (42%) was removed (Table 1). Therefore, despite the ability to remove modest amounts of soluble Cu (51%), Pb (54%) and Zn (31%) from effluent (Table 1), no significant reduction was observed in the bioavailable metal concentration following treatment as shown in Fig. 3 panels a, b and d, respectively ($p > 0.05$). These findings indicate that FeCl_3 had no environmental benefit with regards to the toxicity of metals in effluent. Furthermore, an increase in the concentration of Ni coupled with DOC removal significantly increased the bioavailability of Ni in effluent treated with FeCl_3 ($p < 0.001$; Fig. 3c). Based on these results CF with FeCl_3 may not be recommended for application in wastewater treatment for the removal of trace metals from municipal wastewater.

The pH of effluent is also a component input into BLMs. Nevertheless, the difference in the pH of effluent treated with FeCl_3 and Flocculant was not substantial (see Table 1) and was therefore unlikely to have significantly influenced the bioavailability of the residual metal.

3.6 Characterisation of flocs

3.6.1 Structural characteristics

Features observed in Fig. A1 were representative of FeCl₃ (0.81 FDM library match score). On the other hand, the spectrum generated for Flocculan (see Fig. 4a) showed bands representative of organic compounds. Peaks were assigned in accordance with their respective wavenumbers (cm⁻¹) as reported in literature. A broad stretching peak around 3010 cm⁻¹ was a combined effect of O-H and N-H groups (Hameed et al. 2016). The occurrence of stretching vibrations over a broad range of frequencies indicated the presence of bonded O-H bands of carboxylic acids and 'free' hydroxyl groups (Gnanasambandam and Proctor, 2000). Peaks at 1599 and 1458 cm⁻¹ indicated the presence of asymmetric and symmetric stretching vibrations of ionic carboxylic groups (COO⁻) (Iqbal et al. 2009). The aliphatic acid group vibration at 1227 cm⁻¹ can be assigned to deformation vibration of C=O and stretching formation of O-H groups of carboxylic acids and phenols (Guibaud et al. 2003). The effect of bending of C-O group was shown at the peak 1092 cm⁻¹ (Hameed et al. 2016). It is well indicated from FTIR spectrum of Flocculan that hydroxyl, carboxyl and amino groups were present and, such groups, could be involved in coordination with metal ions.

Figure 4 also includes the FTIR spectra of suspended solids from the humus effluent and flocs produced after CF treatment. The spectra of the suspended solids from humus effluent (see Fig. 4b) exhibited a broad band at 3254 cm⁻¹ which represents O-H functional groups and O-H stretching of polymeric compounds. Major bands at the wavelengths 2979 and 2888 cm⁻¹ correspond to C-H bonds of aliphatic acids (Farinella et al. 2007). The band at 1645 cm⁻¹ corresponds to the C=C stretching that is associated with C-C

bonds and COO^- asymmetric stretching's (Farinella et al. 2007). The peak at 1387 cm^{-1} represents aliphatic group vibration and COO^- symmetric stretching (Pavia et al. 1996), whilst the band at 1076 cm^{-1} is indicative of the C-O group (Farinella et al. 2007).

The FTIR spectrum for flocs formed by Flocculan (see Fig. 4c) shared similar peak locations with the dried humus effluent suspension (Fig. 4a), with bands around 3254, 2979, 1387 and 1076 cm^{-1} observed in both spectra. Such findings indicate that the polymeric chains within this reagent were capable of bridging organic molecules present in humus effluent together, incorporating them in flocs formed. Although Flocculan contributed to the spectrum, introducing the peak which appeared at 1247 cm^{-1} (carboxylic acids and phenols), the spectrum, overall, appears representative of the material collected from the humus effluent. This is not surprising, given that Flocculan itself (Fig. 4a) has similar structural characteristics compared with the dried humus effluent suspension (Fig. 4c).

Conversely, the FTIR spectrum for flocs produced by FeCl_3 (see Fig. 4d) was strongly affected by this coagulant's chemistry. Reduced intensity of the peak around 2979 and 2888 cm^{-1} indicated suppression of non-charged components. In addition, peaks at 3254, 1645 and 1076 cm^{-1} shifted to 3232, 1631 and 1007 cm^{-1} , respectively. These shifts may be attributed to adsorbed carbohydrates or polysaccharide-like constituents (Gu et al. 1994); this indicates that complexation between C-O functional groups of suspended humus effluent particles and the surface of ferric hydroxide precipitates occurred. Hence, the removal of particles in a growing hydroxide precipitate, known as sweep flocculation (Duan

and Gregory, 2003), played an important role in the CF process with FeCl_3 .

3.6.2 Morphological properties

From the SEM micrographs shown in Fig. 5 it is clear that the flocs produced after the treatment of humus effluent with either FeCl_3 (Fig. 5a) or Flocculan (Fig. 5b) were compact and clustered, indicating the presence of aggregates formed through the CF of particles by these reagents. In comparison, flocs which were formed by Flocculan exhibited a more compact structure with a smoother surface as a result of the formation of larger, denser and easier settling flocs. These characteristics are typical of flocs produced in CF with biopolymers through interparticle bridging, and are desirable for good settling and dewatering (Liao et al. 2006) and the formation of a low volume of sludge. Other studies (Renault et al. 2009) have revealed that salts hydrolyse rapidly when added to wastewater, producing abundant sludge that is difficult to dehydrate. Hence, explaining the visibly lower floc density observed for FeCl_3 in Fig. 5b.

4. Conclusions

This study was one of the first of its kind to assess the influence of CF on metal size distribution and bioavailability. The experimental findings herein indicate that CF with either FeCl_3 or Flocculan can simultaneously remove particulate and soluble metal from wastewater. In the CF process, both FeCl_3 and Flocculan removed Cu

and Zn from the colloidal fraction in conjunction with the elimination of COC. Such findings indicate that technologies capable of removing organic materials within the colloidal fraction can reduce the concentration of Cu and Zn in effluent. Despite FeCl_3 achieving a higher rate of removal for COC, a significantly lower Cu concentration was observed in the colloidal fraction of effluent treated with Flocculan. It was anticipated that Flocculan removed organic compounds (such as polysaccharides and proteins) with functional groups that Cu readily complexes with. Furthermore, Flocculan removed Cu from the truly dissolved fraction through the complexation of Cu ions with functional groups (phenol and amino groups) present in the polymeric chains. This additional removal mechanism helped Flocculan outperform FeCl_3 in the removal of soluble Cu. The bioavailability of metals in effluent was strongly influenced by the concentration of DOC. Flocculan was able to remove soluble Cu, Pb and Zn without reducing the concentration of DOC, thus was capable of simultaneously reducing the concentration and bioavailability of these metals in treated effluent. Flocculan should, therefore, be considered for its environmental benefits in decision making on municipal wastewater treatment. In CF with FeCl_3 a substantial amount of DOC was removed, therefore despite achieving modest rates of removal for Cu, Pb and Zn, FeCl_3 did not reduce the bioavailability of target metals in treated effluent. Such findings suggest that failure to account for the effect of treatment technologies on DOC may result in the application of a technique which reduces metal concentrations in effluent discharges, but which does not deliver any environmental benefit. The CF performance data and proposed mechanisms of metals removal (i.e. sweep flocculation

for FeCl₃; inter-particle bridging for Flocculan) were further supported by analysis of flocs formed, which included FTIR and SEM.

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