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Soil pH and moisture affect cadmium fractionation in sewage sludge amended soils through humic and fulvic acid transformation

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Despite some potential issues with long-term application, sewage sludge is considered beneficial as a valuable source of plant essential nutrients but there are some potential issues with long-term application. Detrimental effects can result due to changes in cadmium (Cd) bioavailability under varying soil pH, moisture and with ageing. We examined changes in soil Cd availability and fulvic and humic like acid fractions in soil together with Cd fractionation and availability with sewage sludge application during a 135 days incubation experiment. Sewage sludge was added @ 0, 15 and 30 g kg^{-1} to three contrasting soils (variable pH, 7.10, 8.48 and 9.52) and moisture levels, 50 and 70 % of the water holding capacity, (WHC). Fourier transform infrared (FTIR) analysis revealed that aromatic compounds were the main binding sites for Cd leading to its reduced availability. However, the humic like acid fractions exhibited humification with ageing due to the existence of recalcitrant mineral compounds. The high pH amended soils (8.48 and 9.52) at both moisture regimes did not significantly affect the spectral peaks. The percentage of labile Cd fractions was decreased in high pH soils, where soil exchangeable and reducible Cd fractions were decreased by 8 and 5 %, respectively. The stable Cd fractions were consequently increased, where soil oxidizable and residual Cd fractions increased by 8 and 5 %, respectively, especially at 50 % WHC. This indicated that the sewage sludge application at 50 % WHC to high pH soils ($pH \geq 8.50$) could become a significant sink for Cd, thus decreasing Cd availability steadily over time. Our results have implications for the effective use of sewage sludge for agricultural crop production in alkaline calcareous soils of Pakistan.

Keywords: Ageing, cadmium, fractionation, organic acids, spectral analysis.

INTRODUCTION

A substantial amount of sewage sludge is produced from municipal wastewater treatment which could be used beneficially as an organic fertilizer as well as soil amendment (Antonkiewicz *et al*., 2019). Application of sewage sludge is considered beneficial because it boosts soil microbial activity and increases the concentration of both mineral nutrients and organic substances (Guo *et al*., 2019). At world scale, sewage sludge production is about 100-125 million tons and is expected to be around 150-200 million tons by 2025 (Vaithyanathan and Cabana, 2021). The production of municipal solid waste of Asia is about 760,000 tons per day which will increase to 1.8 million tons of waste per day, while total solid waste production in Pakistan is about 54,888 tons per day, out of this; only 1 % of the domestic and industrial wastewater receives treatment (WWF, 2019).

The application of fresh raw sewage sludge may be detrimental, since poor quality sludge could introduce higher concentrations of bioavailable toxic heavy metals and microbes. If properly treated, sewage sludge is widely used at farm fields, this is not usually considered to be a significant problem. Normally, sludge must be pretreated to reduce its toxicity and the health risks resulting from its use. It has been estimated that about 25 % of treated sewage sludge is used in agriculture in Europe alone (Hudcová *et al*., 2019). Because the concentration of various toxic compounds can usually be significantly decreased with ageing either due to fixation or adsorption of heavy metals (HMs) by stable humic substances. However, metal properties may be specifically related to changes in the organic matter content derived from

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the humic and fulvic acid (HA and FA) like fractions formed during aging of sewage sludge (Liu *et al*., 2019; Murtaza *et al*., 2010).

Following fresh sewage sludge application, HM availability in soil generally increased due to rapid decomposition of labile and dissolved organic matter (McBride, 2003). However, with ageing and organic matter (OM) stabilization, recalcitrant HA and FA fractions were produced, which were responsible for the binding of metals through sorption/desorption and oxidation/reduction like processes, resulting in a plateau in HM availability (Lehmann and Kleber, 2015). These humic substances contained functional groups like aromatic, amino acids, carboxyl and phenolic, which provided a greater number of adsorption sites for chelating metals. In addition, these functional groups also affected metal bioavailability through various courses including chelation and precipitation/dissolution, where the dominant process varied with the different soil regimes (Rashid *et al*., 2018; Hamidpour *et al*., 2019).

It is well-known that metal solubility especially Cd increases under oxidative conditions with a decrease in soil pH, but under reductive conditions, this phenomenon is totally reversed and Cd solubility actually decreases at lower soil pH due to the precipitation as well as the formation of stable organo-mineral complexes (Van Den Berg and Loch, 2000). Du Laing *et al.* (2009) reported increased metal mobility and translocation under low soil pH, which favors plants for greater uptake of heavy metals, especially Cd. This is a concern, because as a toxic HM, increased Cd translocation/ accumulation into the edible parts of plants could poses a risk to humans, after consuming such produce. Soil moisture content could alter the dynamics and chemical form of metals by stimulating gradual changes in the soil redox potential (Eh) and pH (Li *et al*., 2019). Ageing plays an important role in evaluating ultimate metal bioavailability and the distribution of different metals between the different soil fractions after sewage sludge application and Murtaza *et al*. (2012) has confirmed that in general metal concentrations decreased with ageing in biosolid amended soils. Thus, to avoid potential accumulation of HMs in edible crops fertilized with sewage sludge they proposed that, following sewage sludge application, soils should be left for a defined period before sowing a crop to allow all reactions of metals in sludge with soil colloids and humate complexes to occur.

Bioavailability of metal is directly linked to the metal's fractionation in the soil solution, which is reliant on the fraction of metal bound to the different soil phases (Fernandez-Ondono *et al*., 2017). The distribution of metals between their different chemical forms is directly affected by different soil conditions i.e., pH, moisture and organic matter mineralization and ageing process (Li *et al*., 2016). Humic and FA are the most essential components of the soil organic matter, influencing plant growth, metal availability and microorganism activity (Guo *et al*., 2019). Thus, their analysis by FTIR is widely used during the ageing process where presence or absence of absorbance bands of characteristics functional groups e.g., aromatic, aliphatic, alcoholic, amino, carboxylic and phenolic indicates the mineralization process. It is a reliable technique to evaluate the changes in the relative intensity of spectral bands during the maturity of organic matter (Wu *et al*., 2016).

Cadmium, being highly toxic even at its very low concentration is of important concern for human, animal and microbial activities (Ruyter-Hooley *et al*., 2017). Due to weaker retention on labile humate complexes, bioavailability of Cd is higher than many other HMs (Ondrasek *et al*., 2019). Consequently, a number of different techniques have been devised to mitigate Cd risks linked with food chain contamination including, agronomic, genetic engineering approaches, phytoremediation and traditional breeding, as well as the application of plant nutrients to minimize Cd bioavailability and/or its phytoaccumulation (Sarwar *et al*., 2017). However, most of these technologies are either prohibitively expensive or require long time periods to rehabilitate contaminated soils. In comparison, application of sewage sludge to decrease Cd bioavailability is a relatively inexpensive, fast and effective alternative, but to date the specific mechanisms operating following sludge application leading to reduced Cd bioavailability has not been extensively explored. Hence, the current investigation aimed to evaluate the impact of soil moisture content and pH on the availability of Cd during transformation of HA and FA fractions following sewage sludge application. The study was conducted with the hypothesis that humic and fulvic acid transformation of sewage sludge increases with ageing. The high soil pH and moisture contents enhance the fractionation of Cd in sewage sludge amended soil.

MATERIALS AND METHODS

Collection of soils**:** Soils were collected from three locations across Faisalabad, an industrial city in Pakistan. The low pH soil 7.10 was collected from the farm area of Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad (UAF), Pakistan (N 31° 43' 10″, E 73° 06' 95″). The medium pH soil 8.48 was collected from Prokianwala village, Faisalabad (N 31° 42' 91″, E 72° 98' 33″) and the high pH soil 9.52 was collected from Dijkot, Faisalabad (N 31° 22' 66″, E 72° 99' 05″) as shown in Table 1. Sewage sludge (aerobically digested) was sampled from the sewage wastewater treatment plant of I-9 sector Islamabad, Pakistan (N 33° 65' 73″, E 73° 05' 15″). The physicochemical properties of the original soils and the sewage sludge were determined using standard methods (Sparks, 1996), and are presented in Table 1.

Experimental plan**:** The incubation study was conducted in a protected glasshouse at UAF, with an ambient temperature of 30⁄ 25 ± 3 ºC (day⁄ night) throughout the entire experiment.

Table 1. Physicochemical properties of the three soils and sewage sludge samples used in this study.

Parameters	Soil 1	Soil 2	Soil 3	Sewage sludge
Texture	Sandy loam	Sandy loam	Sandy loam	-
pH_s	7.10	8.48	9.52	7.62
EC_e (dS m^{-1})	2.09	4.84	3.33	4.90
SAR (mmol $L^{-1})^{1/2}$	10.20	17.43	22.61	-
CEC ($cmol_c$ kg^{-1})	8.40	9.60	11.10	59.10
Total K (mg kg^{-1})	41.00	56.00	34.00	240.00
Total N (g kg^{-1})	0.70	0.42	0.65	5.00
Total P (mg kg^{-1})	9.50	8.42	8.10	2396.00
Total Cd (mg kg^{-1})	0.39	0.41	0.33	5.13
OC (g kg^{-1})	8.10	7.10	6.50	326.00
Humic acid[a] (mg g^{-1})	-	-	-	14.20
Fulvic acid[a] (mg g^{-1})	-	-	-	6.50

[a] Calculated on OC (g kg^{-1}) dry wt. basis

Sewage sludge was applied at 0, 15 and 30 g kg^{-1} of soil on dry weight basis, which was equal to 0, 15 and 30 Mg ha^{-1} of soil in the upper 10 cm layer of soil. Then each soil was thoroughly mixed with amendment, equilibrated for 3 days, and then placed into plastic pots (each pot contains 200 g soil). The initial moisture content of soils (control and amended) was determined by weight loss basis. The moisture level in all 72 pots were then maintained at 50 and 70 % of soil maximum WHC by adding distilled water daily to supplement any weight-loss. All treatments were triplicated and arranged in a four factor factorial under a completely randomized design (CRD). Each experimental pot was sampled uniformly (from the top and bottom of each sample) and then thoroughly mixed afterwards. After 0, 45, 90 and 135 days, soil samples were taken periodically, i.e. to determine changes in Cd fractionation and/or OM derived HA and FA fractions transformations.

***Extraction of HA and FA fractions*:** For the separation of humic substances, soil (30 g) was primarily extracted with 0.1 *M* NaOH solution (300 mL) (Helmke *et al*., 1996). The extracted solution was then adjusted to pH ~ 1 by acidifying with 3 *M* H_2SO_4, added drop wise and left to stand at 4 °C for 24 h to complete the precipitation of HA and FA fractions. Prior to the solubilizing in 0.1 *M* solution of NaOH, the precipitated (HA) and supernatant fraction (FA) were separated by the process of centrifugation performed @ 4000 rpm for a period of 20 minutes and then filtered by Whatman filter paper (125 mm). These samples of both humic as well as fulvic like acid fractions were then freeze-dried (Model, Alpha, 1-4LSC, Christ, Germany) and kept in storage until analysis on FTIR. The $KMnO_4$ oxidation method was used to determine the organic carbon content of these already separated acid fractions (Amir *et al*., 2004). Potassium bromide (KBr) pellets were prepared by pressing a mixture of a ground sample (2 mg) with KBr (400 mg) under vacuum and these pellets were used to determine the infra-red spectra on a PerkinElmer 1600 FTIR spectrophotometer. Before the preparation of KBr pellets, both samples and KBr were separately dried at 105 °C to limit the interference of moisture (Demyan *et al*., 2012).

***Sequential extraction procedure*:** A modified version of the Tessier extraction procedure (Tessier *et al*., 1979), i.e. sequential extraction procedure of community bureau of reference (BCR) (Ure *et al*., 1993) was used to determine the distribution of Cd between different soil fractions. Accordingly, there were four sequential extractions for each sample, i.e. exchangeable (F1), reducible (F2), oxidizable (F3) and residual (F4) soil fractions (Table 2).

Table 2. Community Bureau of Reference (BCR) extraction procedure.

Fraction	Extraction procedure per gram of sample
F1	Add 40 mL of (0.11 *M*) CH_3COOH, shake for 16 h and centrifuge
F2	40 mL of (0.5 *M*) $NH_2OH \cdot HCl$ (pH 2) added to the residual from F1, shake for 16 h and centrifuge
F3	10 mL of (8.8 *M*) H_2O_2 (pH 2-3) added to the residual from F2, stay for 1 h, then add 10 mL of (8.8 *M*) H_2O_2 and digest at 85 °C for 2 h. Afterwards, add 50 mL of (1 *M*) CH_3COONH_4 (pH adjusted at 2 with HNO_3), shake for 16 h and centrifuge
F4	Add 2 mL of 65 % HNO_3 + 6 mL 37 % HCl to the residual from F3, digest at 120 °C for 2 h.

F1 = Exchangeable fraction, F2 = Reducible fraction, F3 = Oxidizable fraction, F4 = Residual fraction. Atomic absorption spectroscopy (Model Thermo S-Series, USA) was used to analyze the extracts and remaining solid were retained and subjected to the next extraction in this sequential extraction

Statistical analysis: Analysis of variance (ANOVA) was used to analyze the statistical significance of experimental treatments using Statistix 8.1®. Tukey's Honestly Significant Difference (HSD) method was used for comparison amongst treatment means at the 5 % probability level.

RESULTS

***Cadmium fractionation*:** The initial Cd fractionation of the three original soil and sewage sludge samples indicated that sewage sludge contained the readily available exchangeable F1 fraction (27.9 %), followed by soil 2 (0.41 %), soil 1 (0.39 %) and soil 3 (0.33 %). Soil 3 had the reducible F2 fraction (42.0 %), followed by soil 2 (30.9 %), sewage sludge (18.3 %) and soil 1 (12.8 %). Similarly, the oxidizable F3 fraction was found in soil 1 (28.2 %), followed by soil 3 (19.5 %), sewage sludge (15.0 %) and soil 2 (14.0 %). While, the residual fraction was found in soil 1 (43.6 %), followed by soil 2 (42.4 %), sewage sludge (31.8 %) and soil 3 (28.5 %).

The percent recovery of Cd was calculated by comparing the sum of four Cd fractions with the total soil Cd concentration (Lu *et al*., 2003). The percent recovery of Cd fractions was found highest for soil 3 (93.0 %), followed by sewage sludge (92.9 %), soil 2 (89.7 %) and soil 1 (87.2 %). It can be seen clearly that the sum of four steps is precise and reliable through following BCR sequential extraction procedure, as reported earlier by Yuan *et al*. (2010) by adopting same BCR procedure.

After 135 days of ageing, the distribution of exchangeable Cd fraction (F1) in sewage-sludge amended soils at 15 and 30 g kg^{-1} was changed significantly ($p \leq 0.05$) under both moisture regimes (Figs. 1 and 2). Overall, the exchangeable Cd fraction was inversely related to soil pH, i.e. a decrease in high pH soils and an increase in low pH soils. The most significant changes in F1 fraction of -8 and +5 % were observed in high pH soil (9.52) and in low pH soil (7.10), respectively where both of these changes occurred in sewage sludge amended soil at 15 g kg^{-1} of soil and 50 % soil WHC.

The composition of the reducible Cd fraction (F2) changed significantly ($p \leq 0.05$) with ageing under both moisture

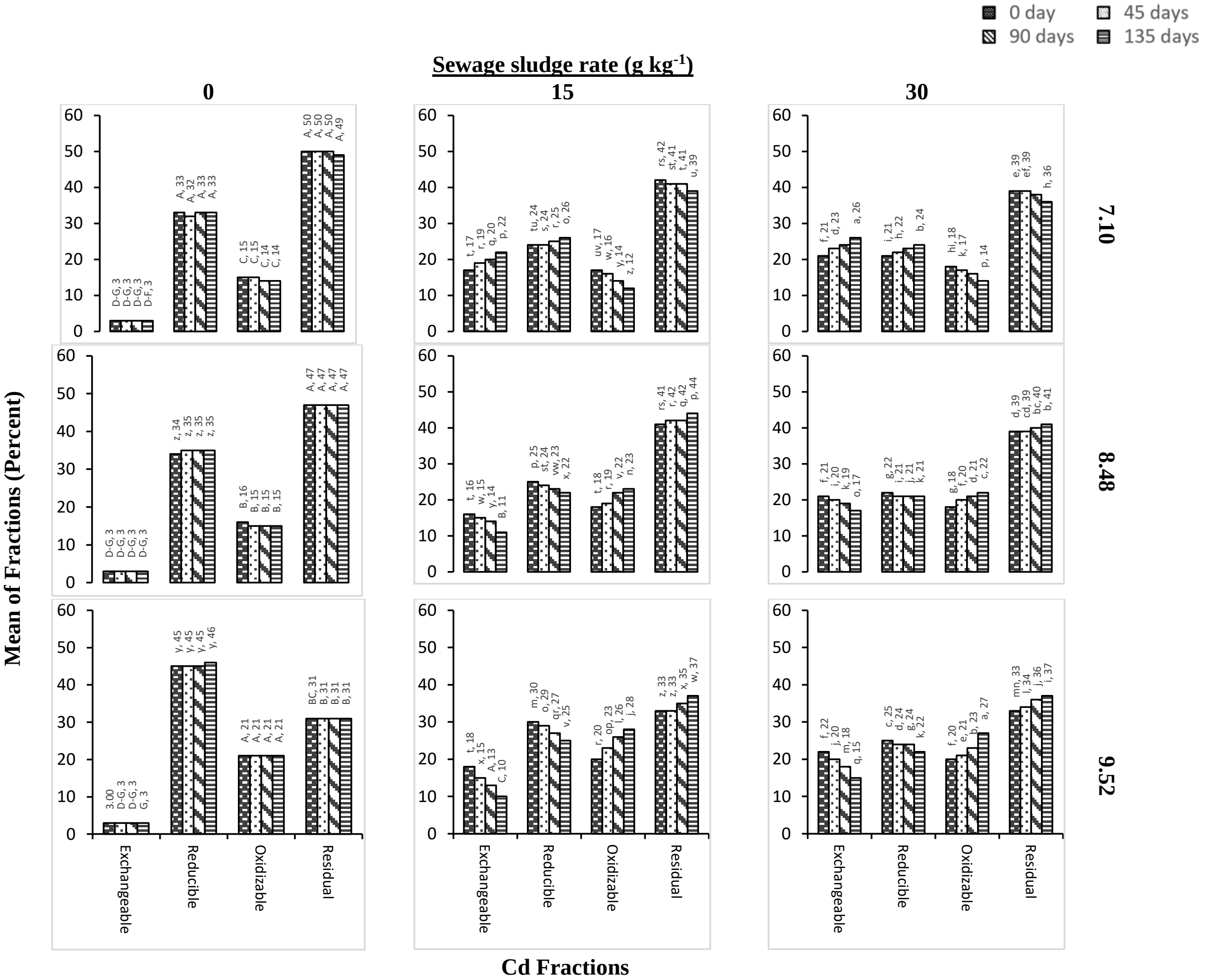

Figure 1. Effect of different soil pH and sewage sludge amendment rate on Cd fractions (%) with ageing at 50 % moisture of soil WHC (Means are statistically different at $p \leq 0.05$: ** ~ Significant LSD: F1 ~ 0.5383**, F2 ~ 0.5134**, F3 ~ 0.7149**, F4 ~ 0.5301**).

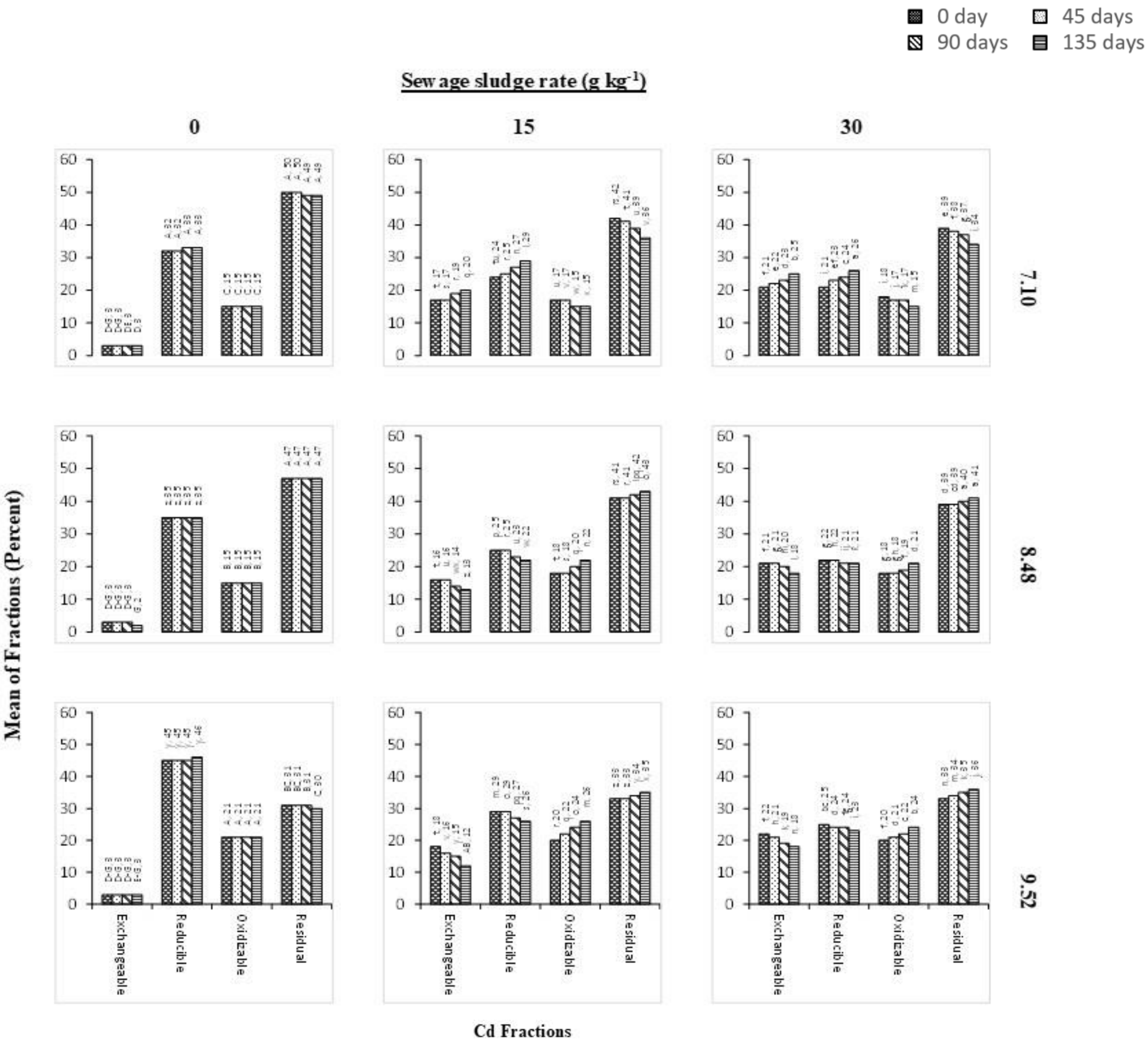

Figure 2. Effect of different soil pH and sewage sludge amendment rate on Cd fractions (%) with ageing at 70 % moisture of soil WHC (Means are statistically different at $p \leq 0.05$: ** ~ Significant LSD: F1 ~ 0.5383**, F2 ~ 0.5134**, F3 ~ 0.7149**, F4 ~ 0.5301**)

regimes (Figs. 1 and 2). Interestingly, like F1, the reducible Cd fraction exhibited an inverse relationship with soil pH. The most significant change in F2 fraction, being -5 and +5 % were observed in high pH soil (9.52) and in the low pH soil (7.10), respectively. Where again, both of these significant changes occurred at 50 % soil water holding capacity, with sewage sludge applied at 15 g kg^{-1} of soil.

The concentration of the most dynamic OM bound Cd fraction (F3) significantly changed ($p \leq 0.05$) with ageing under both moisture regimes (Figs. 1 and 2). Overall, the F3 fraction was found directly related to soil pH, i.e. F3 increased in high pH soils and decreased in low pH soils. The most significant changes in F3 fraction seen at 50 % soil WHC, i.e. +8 and -5 %, were observed in high pH soil (9.52) and low pH soil (7.10), respectively with sewage sludge at 15 g kg^{-1} soil rate.

The composition of the residual Cd fraction (F4) comprises primary and secondary minerals is unlikely to be released and bioavailable, of sewage sludge amended soils varied non-significantly ($p \geq 0.05$) after 135 days of incubation under both moisture regimes (Figs. 1 and 2). Overall, the F3 fraction underwent maximum change when the soil was treated with sewage sludge at 15 g kg^{-1} of soil. The maximum change in F4 fraction was observed to be +5 % in the high pH soil (9.52) at 50 % WHC and -6 % in the low pH soil (7.10) at 70 % soil WHC.

***Organic acids transformations*:** After 135 days of organic matter constancy and maturity, the HA and FA fractions derived FTIR spectra exhibited somewhat similar spectra in the control and sewage sludge amended soils. It means different soil moisture and pH treatments applied had only slight effect on HA and FA spectra. Therefore, we represent only the spectra of HA and FA fractions when amended with sewage sludge at 15 g kg^{-1} of soil. At day 0, the initial FTIR spectra of the fulvic acid extracts displayed similar spectral bands in all soils (Fig. 3). The broad band around 3200-3400 cm^{-1} correspond to the presence of H-bonded OH groups of alcohols, organic acids, phenols, and H-bonded NH groups of amines and carboxylic acids. The broad width of this peak confirmed the presence of hydroxyl groups. The well-pronounced peak at 1600-1700 cm^{-1} indicated the existence of olefinic and aromatic C=C, C=O in amide I, ketone and quinoine groups. A small but sharp peak at 1250 cm^{-1} corresponded to aliphatic CH_2, amide III, organo-sulphur compounds and O-H deformation of carboxylic groups of carbohydrates. The appearance of small peak at 1040 cm^{-1} region might be due to the presence of C=O stretching of polysaccharide/ aromatic ethers compounds and Si-O stretching of impurities of silicate. Likewise, at day 0 the initial FTIR spectra of humic acid extracts also displayed similar spectral bands in all the three soils (Fig. 4). The spectral bands of fulvic like acid fraction showed non-significant changes ($p \leq 0.05$) with ageing at both moisture regimes (Fig. 3) in all amended soils. However, some changes were more pronounced in high pH soils compared to low pH soils.

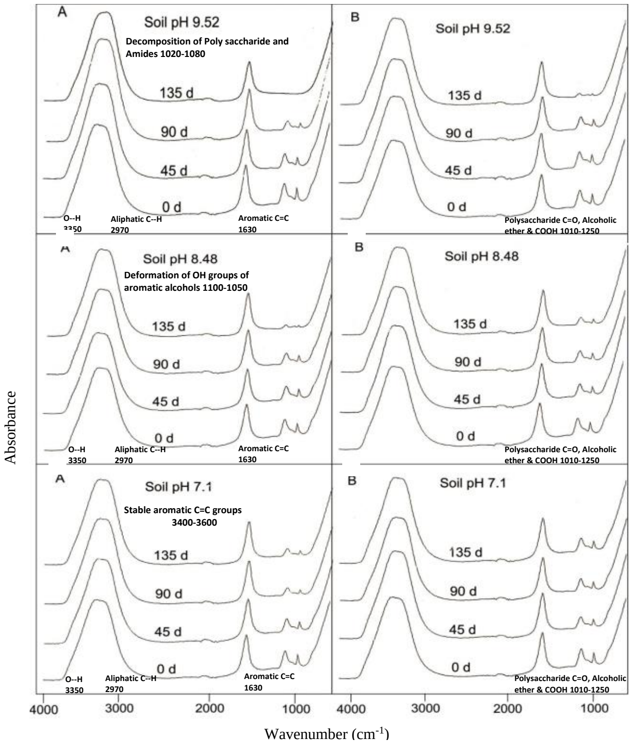

Figure 3. Influence of different soil pH and moisture contents (A) 50 % and (B) 70 % on fulvic acid spectra in sewage sludge amended soil (15 g kg^{-1}).

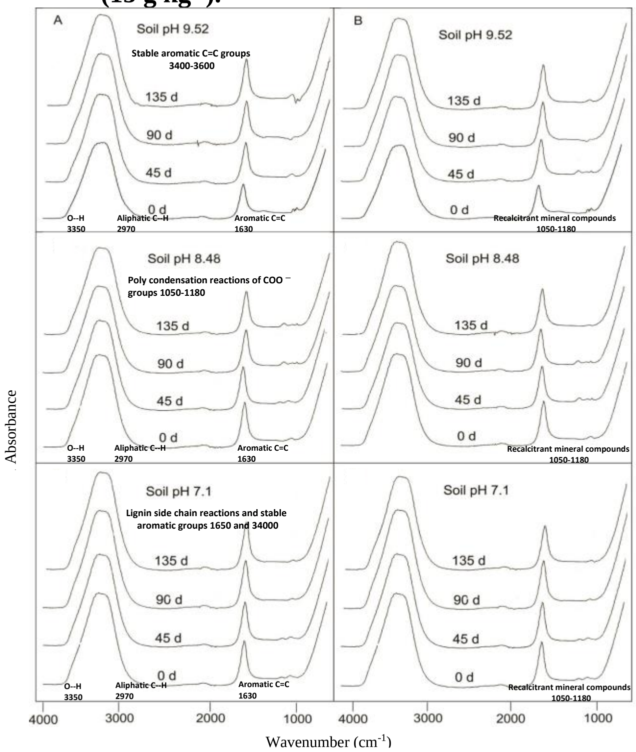

Figure 4. Influence of different soil pH and moisture contents (A) 50 % and (B) 70 % on humic acid spectra in sewage sludge amended soil (30 g kg^{-1}).

DISCUSSION

The observed decrease in exchangeable Cd fractions with ageing in high pH amended soils (soil pH 8.48 and 9.52), might result from an increase in the number of pH dependent negative functional groups Cd $(OH)^+$ during hydrolysis and precipitation of metal oxides (McBride, 2000). High pH soils favor metal sorption on humate complexes and soil colloids, either by altering the metal concentration, or by increasing the number of negative sites. In contrast, low pH soil (7.10) showed an increase in the exchangeable Cd fraction due to decrease in metal-ligand complex stability and increase in H^+ concentration (Caporale and Violante, 2016). This might be due to the changes in soil surface charge, i.e., the surface becomes more positive at low pH and more negative at high pH (Lwin *et al*., 2018). Kashem and Singh (2001) and Murtaza *et al*. (2012) reported increased metal availability in low pH soils and conversely, decreased metal translocation in high soil pH_S, which they attributed to adsorption and precipitation of metal on soil and humate surfaces. They accredited low Cd availability in high pH sludge amended soils to decreases in soil redox potential and an increase in the soil solution pH. Golui *et al*. (2016) observed greater increases in the exchangeable Cd soil fraction at low soil pH compared to high pH (alkaline) soil, when soils were maintained at high moisture. They reported higher Cd availability and redistribution under increased moisture regime and microbial respiration which leads to decreases in Eh, and reduction in Fe and Mn oxides contents, among the solid-phase components.

The observed decline in reducible Cd fractions in high pH soil amended with sewage sludge, could be due the precipitation of Cd with amorphous hydrous oxides, sulfates and carbonates like anions, which generally have a strong sorption capacity (Martınez and McBride, 2001; Deng *et al*., 2019). Although, Naidu *et al*. (1997) attributed the lowering of Cd translocation in high pH soils due to the pH-dependent surface charge present on Fe, Al, and Mn oxides, precipitation of Cd hydroxides and chelation through organic matter (Naidu *et al*., 1997). Hence, with increasing soil pH, the functional groups in humic substances e.g., alcoholic, carbonyl, carboxyl, and phenolic dissociate to form chelate complexes with Cd (Bolan *et al*., 2014). However, the increased Cd availability at soil moisture content, i.e., 50 % WHC, might have resulted from the dissolution of soluble metal humate complexes (Ok *et al*., 2010; Mahar *et al*., 2015). The reducible Cd fraction increased in low pH soil as the soil moisture increased, which might be due to decrease in cation exchange capacity (Tang *et al*., 2008). The phenomenon behind Cd release in acidic soils was due to release of Fe and Mn oxides (via hydrolysis and oxidation) and pH-dependent metal adsorption reactions under anaerobic conditions.

The organic matter bound Cd fraction is the most dynamic fraction and hence undergoes the greatest increases in high pH soil, probably because of the addition of sportive phases as organic matter and dependence of the number of negative OM sites on pH, phosphates and silicates (Chavez *et al*., 2016; Zheng and Zhang, 2011). The oxidizable Cd fraction increases might be linked due to increased adsorption on the negative surface sites of humus colloids and amphoteric metal oxide (McBride, 2000). Wang *et al*. (2019) reported an increased proportion of inorganic and organic compounds after sewage sludge application which actively participated in the translocation and re-distribution of metals (especially Cd) through oxidation-reduction reactions, where increases in this fraction mostly occurred at high pH. Conversely, in the low pH soil, decreases in the oxidizable fraction were probably caused by the rapid decomposition of labile moieties in OM. In agreement with this result, Sun *et al*. (2007) had previously reported decreases in the oxidizable Cd concentration with increased humification of OM via sewage sludge application, where the reduction was due to an increase in the number of negative adsorptive sites for Cd.

This increase in residual Cd fraction in high pH soils was probably due to the increased complexation of Cd with recalcitrant soil minerals. In addition, as observed here, Qi *et al*. (2014) and Spark (1997) also reported increases in the residual Cd fraction as soil moisture decreased. They accredited this due to increased Cd adsorption, primarily to silicates and mica, and secondly to calcite, clay, aluminum and iron oxide minerals, forming stable inner-sphere cadmium complexes, i.e. CdO, $Cd(OH)_2$, $CdCO_3$, $Cd(PO_4)_2$ and $CdHPO_4$. The stabilization and humification of organic matter derived humic substances redistributes potentially available fractions (exchangeable and reducible) into inert fractions (oxidizable and residual), thus significantly decreasing Cd availability (Deng *et al*., 2019; Vaca *et al*., 2008).

The FTIR spectroscopy is a single beam technique, which gives information about organic matter decomposition and its relation with metal. The initial FTIR spectra of humic acid extracts displayed similar spectral bands in all the three soils. The humic like acid fractions generally contained the same functional groups as the fulvic like acid fractions, except the absence of a sharp peak at 1250 cm^{-1} region (Coates, 2000; Wu *et al*., 2016). The observed changes in the 1000-1050 cm^{-1} region with ageing correspond to the decomposition of polysaccharide compounds and the changes at 1140 cm^{-1} were probably due to oxidative coupling, deformations of OH groups of aromatic alcohols and complex esterification/ etherification reactions (El Fels *et al*., 2015). The decrease in carboxylic groups and aromatic structures indicates maturity of organic matter and extensive decomposition and degradation for cellulose, polysaccharides and polypeptides derived from sewage sludge addition. It could also be linked to the higher proportion of stable peptide and aromatic groups after intense microbial breakdown of alcoholic and acidic structures at ambient moisture (50 % of WHC) and high soil pH (9.52 and 8.48). A higher degree of polymerization and a strong relative abundance of aromatic functional groups at 1450 cm^{-1} indicated stable aromatic character (Liu *et al*., 2019). This spectral peak vanished with ageing in the high pH soil especially, at 50 % WHC. This might be due to the action of indigenous microbe, being added via sewage sludge. As a source of energy and carbon requirement, these indigenous microbes might have used labile aliphatic and carbohydrate molecules (Jindo *et al*., 2016).

However, with ageing the absorbance peaks at 3100-3400 and 1650 cm^{-1} region remained unchanged at both moisture regimes due to the continued presence of stable aromatic C=C groups. Popovic *et al*. (2011) confirmed non-significant changes at 1650 and 3400 cm^{-1} for fulvic like acid spectral bands which they attributed to lignin side chain oxidation reactions and microbial breakdown of alcoholic structures, thus generating a higher proportion of stable peptide and aromatic groups. The presence of non-significant peak at 3100 cm^{-1} region showed reduction in alcoholic and organic (CH_3 and CH_2) groups which confirms the decrease in aliphatic compounds, thus endorsing the stabilization of humic substances. Overall, the decrease of labile aliphatic compounds, fats, polypeptides and aromatic components confirmed the mineralization and maturity of organic matter at 50 % WHC.

Gonzalez Perez *et al*. (2008) described that microbial activity and moisture changes were important variables contributing to soil OM stabilization and heavy metal retention. They found that 50 % WHC was a more effective moisture regime for increasing OM humification and metal fraction redistribution. Alloway (1997) found that changes in moisture regimes, from field capacity to submergence, increased Cd availability due to an increase in precipitation reactions. Furthermore, small changes in the spectral bands of fulvic like acid fractions with ageing could be a key factor in reducing Cd availability due to increased metal-humate interactions (Li *et al*., 2019; Rashid *et al*., 2018). As, Harter and Naidu (2001) reported earlier, that covalent bonding and chelating capacity of the functional groups present in humic materials made them excellent sorbents for slowing metal release and OM mineralization.

During the 135 days incubation, spectral bands of HA like fractions remained stable and only slight variation in the associated spectral bands occurred (Fig. 4). However, amended high pH soil with 50 % WHC caused slightly more transformations in spectral bands than soils with 70 % WHC. It might be due to the presence of recalcitrant mineral compounds at 1050 cm^{-1} region (Smidt and Meissl, 2007). The involvement of carboxyl groups during poly-condensation reactions of humification could be another factor for this stability and aromaticity with ageing (El Fels *et al*., 2015). In addition, increased hydrophobic character (aromaticity) of HA like fraction led to decreased Cd availability (decrease in exchangeable Cd fraction) through chelation and adsorption reactions on stable humate complexes. The stability of peaks at 1650 and 3400 cm^{-1} coupled with only small variations at 1050-1250 cm^{-1} region was linked to sustained decomposition of amides, aliphatic moieties and carboxylic compounds, as reported earlier by Smidt and Meissl (2007).

It was observed that as the humification of OM proceeded, it led to an increase in active fulvic like acid fractions and stable humic like acid fractions as these are highly linked with aliphatic C–H stretching, alcoholic stretching vibration, and C–C stretching. It is due to the fact that HA fractions reduced metal mobility with ageing due to the existence of considerable amounts of phenyl and carboxyl groups which formed stable complexes with metals, especially Cd, under ambient soil pH and moisture conditions. It could also act as buffer for metal solubility by preventing rapid changes in pH of the soil matrix due to presence of aromatic and carboxylic groups. (Billingham, 2012: Lehmann and Kleber, 2015).

Conclusions: At high pH (≥ 8.50) and ambient soil moisture level (50 % WHC), the release of Cd from sewage-sludge amended soil was significantly decreased with ageing. In addition, labile Cd fractions were transformed to more stable fractions over time due to the ageing effect of soil HA and FA fractions. The chemical structure of the humic fractions became more stable, while for the fulvic fractions, the carboxylic groups (COOH) decreased and the number of amino groups increased especially in the 1050 to 1280 cm^{-1} region. Based on these results, it was concluded that in high pH soils, sewage sludge could be used as an effective bio-sorbent to minimize Cd contamination in food chain.

***Conflict of interest*:** All the authors declare that there is no conflict of interest.

***Authors' contribution statements*:** IR executed the pot trial research, GO improved language, TH and AM assisted in laboratory and statistical analyses, whereas GM conceived the idea and supervised the work.

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