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RESEARCH ARTICLE

Effect of humic and fulvic acid transformation on cadmium availability to wheat cultivars in sewage sludge amended soil

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Abstract
The high nutrients and organic matter (OM) content of sewage sludge make it an excellent fertilizer to enhance soil fertility and crop production. However, the presence of adsorbed and precipitated forms of heavy metals, especially cadmium (Cd), can be a major problem for such a utilization of sludge. This pot study aims at producing safe food with minimal Cd concentrations from sewage sludge amended soils. Two wheat cultivars (NARC-11 and Shafaq-06) were sown in soil amended with sewage sludge with rates 0, 15 and 30 g kg^{-1} soil. Application of sewage sludge resulted in enhancement of wheat grain yield while Cd concentrations in wheat grains of both cultivars remained within permissible limits (24.1 to 58.6 μg kg^{-1} dry weight). Fourier transform infrared (FTIR) spectroscopic analysis revealed more spectral changes in fulvic acids than in humic acids, which showed a higher humification degree, making them chemically and biologically more stable for Cd retention. Sequential extraction data of Cd after NARC-11 harvest exhibited a significant decrease in mobile fractions (exchangeable and reducible fractions were reduced by 3.6 and 5.2%, respectively) and increase in immobile fraction (the oxidizable and residual fractions increased by 7 and 1.8%, respectively). It is concluded that sewage sludge application could be useful for the improvement of wheat production due to formation of stable humate complexes and decrease in Cd availability.

Keywords Cadmium · Fulvic acid · Humic acid · Metal fractionation · Organic matter transformation · Spectroscopy

Introduction

In the background of rapid industrialization and environmental risks, in the recent years, the attention of plant growers had shifted toward organic sources of nutrients such as sewage sludge rather than chemical fertilizers. High proportions of organic matter (OM), as well as macro and micro-nutrients in sewage sludge, make it an excellent soil conditioner that can boost soil microbial activity, improve soil physical conditions, and enhance crop yields (Ashraf et al. 2016). Land application of sewage sludge is increasing especially in European countries and the USA where more than 40% of the enhanced treated sewage sludge is used as fertilizer and would exceed 13 million t by 2020 (Lowman et al. 2013). Recent estimates showed that total solid sewage waste production in Pakistan is about 54,888 t per day. However, there are possible risks of soil contamination due to the presence of various toxic compounds, especially heavy metals and recalcitrant organic molecules, which can harm the soil-plant system (Zuberi et al. 2015).

It is essential to understand the processes governing the translocation and availability of heavy metals deriving from OM decomposition and uptake into plant (Lehmann and Kleber 2015). Transfer of heavy metals from sewage sludge to soil and later to plants depends on sewage sludge application rate, aging, metal interaction with soil colloids, and OM (Murtaza et al. 2012). Sewage sludge-derived OM has a major influence on redistribution and availability of heavy metals through the formation of humic and fulvic like acids fractions after humification (Chotzen et al. 2016). The bioavailability of metals from soil to plants also depends on soil properties, agronomic practices, plant growth, and genotype. Humic and fulvic acid-like fractions in sewage sludge have potential influences on the behavior of metals (Khan et al. 2006).

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Apparently, this character is due to the presence of a variety of aromatic moieties, aliphatic chains, and functional groups which make these substances a multi-ligand complex system.

Metal fractionation is a good indicator for assessing metal bioavailability. Sequential extraction procedures examine the partitioning of metals into various chemical fractions and help to estimate the potential toxicity of metals in an environment (Degryse et al. 2009; Fernandez-Ondono et al. 2017).

Fourier transform infrared (FTIR) spectroscopy is an important qualitative tool to characterize humic and fulvic acids transformations that OM undergoes during the humification process. In FTIR spectroscopy, maturity of OM is assessed through determining the relative intensity of spectral bands of various chemical (aromatic, aliphatic, alcoholic, amino, carboxylic and phenolic, etc.) groups (El Fels et al. 2015; Wu et al. 2016).

Wheat is the major staple food for more than half of the world's population with 740 million t per year production (FAO 2017). Cadmium-contaminated wheat-derived products are the main source of Cd entering human body via food chain (Rehman et al. 2015; Waqas et al. 2014). Wheat is more susceptible to Cd toxicity as compared to other metals, because Cd is mostly found in soluble and exchangeable soil fractions due to its more labile adsorption on humate complexes than other metals, especially lead and copper (Rizwan et al. 2016; Ruyter-Hooley et al. 2017). Cadmium toxicity induces oxidative stress in wheat which may lead to reduction in growth, dry matter production, and yield. Furthermore, when Cd monthly intake exceeds critical thresholds levels (7.5 μg kg^{-1} body weight), its toxicity may damage liver and lungs and can cause cancer as well as other fatal disorders (Julin et al. 2012; Moynihan et al. 2017; Khan et al. 2017). However, wheat growth could influence OM transformations and the redistribution of different Cd fractions through root exudation and alterations in organic matter and nutrients cycling (Masciandaro et al. 2013). Therefore, safe wheat productions with minimal concentrations of Cd in grains is a challenge in agriculture, since the lack of natural resources is pushing toward the use of cheap recycled sources of nutrients like sewage sludge.

The present study aims at evaluating humic and fulvic acid-like fractions transformation on Cd availability and their effects on wheat growth, after sewage sludge application to soil at different rates.

Materials and methods

Soil and sewage sludge

The soil used in this study was collected from the farm area of the University of Agriculture of Faisalabad (UAF), Pakistan (N 31° 43′ 10″, E 73° 06′ 95″). Aerobically digested sewage sludge was collected from the I-9 sector wastewater treatment plant of Islamabad, Pakistan. Dry samples of sewage sludge and soil were ground to get a homogenous mixture. Total metal concentrations in soil and sewage sludge were determined after digestion with aqua regia (HNO_3/HCl, 1:3; USEPA 2005) and analyzed using atomic absorption spectroscopy (AAS—model Thermo S-Series, USA). Selected initial properties of soil and sewage sludge used in the experiment are presented in Table 1.

Experimental design and raising of plants

All the experimental pots were placed in a protected glasshouse in UAF, with ambient temperature of 30/25 ± 3 °C (day/night) with relative humidity of 55–65% and bright sunlight prevailing during the experimental period. Soil was amended with sewage sludge at three rates (0, 15, and 30 g kg^{-1} soil, on dry weight basis), which is equivalent to 0, 15, and 30 Mg ha^{-1} soil (for the upper 10-cm soil layer). Six grains of each wheat cultivar viz. Shafaq-2006 and NARC-2011 were selected and were sown on December 15, 2015, in pots (which were thinned out to four plants per pot), each containing 5 kg of soil. Uprooted weeds and thinned out plants were cut into pieces and mixed in

Table 1 Physicochemical properties of soil and sewage sludge

Parameter	Unit	Soil	Sewage sludge
Texture	–	Sandy loam	–
Saturation %	–	31.1	–
pH_s	–	7.1	7.4
EC_w[b]	dS m^{-1}	2.1[c]	4.4
SAR	$(mmol\ L^{-1})^{1/2}$	10.2	–
CEC	$cmol_c\ kg^{-1}$	8.4	59.1
Total K	mg kg^{-1}	110	240
Total N	g kg^{-1}	0.70	5.00
Total P	mg kg^{-1}	–	2400
Available P	mg kg^{-1}	9.5	–
Organic carbon (OC)	g kg^{-1}	8.00	316
Humic acid[a]	mg g^{-1}	–	14.3
Fulvic acid[a]	mg g^{-1}	–	6.5
Total metal			
Cd	mg kg^{-1}	0.40	5.10
Fe	mg kg^{-1}	1642	9567
Mn	mg kg^{-1}	161	341
Ni	mg kg^{-1}	2.2	53.3
Pb	mg kg^{-1}	4.9	74.8
Zn	mg kg^{-1}	51.4	182.5
Cu	mg kg^{-1}	11.5	29.7
Cr	mg kg^{-1}	15.6	48.5

CEC cation exchange capacity, *SAR* sodium adsorption ratio, pH_s saturated paste

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

[b] Sludge/water

[c] Saturated extract (EC_e)

respective pots during crop growth. The pots were irrigated with tap water (EC_w 0.69 dS m^{-1}, SAR 1.24 and RSC 0.20 $mmol_c$ L^{-1}) as per crop water demand on visual observations. The recommended dose of NPK @ 120:100:60 kg ha^{-1} was applied through fertilizer sources as urea, diammonium phosphate (DAP), and sulfate of potash (SOP), respectively, after subtracting the NPK amount already present in the sewage sludge. All the P and K were applied at sowing while half of N was applied at sowing, and the remaining N was applied in two equal splits at tillering and booting stages. Chemical control was carried out through application of imidacloprid (20 SL) to control the attack of aphids. Treatments were replicated thrice and arranged in a three-factor factorial design under completely randomized design (CRD).

Post-harvest soil and plant analysis

Wheat crop was harvested at maturity on April 20, 2016. Harvested plant samples were washed with deionized water and dried at 70 °C till constant weight. Root, straw, and grain yield per pot were recorded by weighing. Harvest index (HI) was measured by the following formula

$$HI = \left(\frac{\text{Grain dry wt.}}{\text{Total dry wt.}} \times 100\right)$$

Root, straw, and grain samples were digested in a diacid mixture ($HClO_4/HNO_3$ 1:3 *v*/*v*) and analyzed for Cd by AAS.

Extraction of humic and fulvic acids

Thirty grams of the soil sample were treated three times with distilled water to extract non-humic substances. Each sample was then extracted with 300 mL of 0.1 M NaOH solution to separate humic substances (Helmke et al. 1996). Extraction was repeated until a clear supernatant was obtained after centrifugation at 4000 rpm for 15 min and filtered through Whatman filter paper No. 42 (125 mm). Humic acids were then precipitated out of the extracted solution with 1.5 M H_2SO_4 for 24 h at 4 °C. The precipitated humic acids were separated from fulvic acids by centrifugation (10,000 rpm for 20 min). The separated precipitates of humic acids were dissolved in 0.1 M NaOH. The separated samples of humic and fulvic acids were freeze dried and stored for FTIR analysis. The organic carbon content of humic and fulvic acids was determined by the $KMnO_4$ oxidation method (Amir et al. 2004).

FTIR analysis

The instrument used for obtaining the infrared spectra was a PerkinElmer 1600 FTIR spectrophotometer covering a wavenumber range of 600–4000 cm^{-1} at the rate of 16 nm s^{-1} by using pellets containing 2 mg of the freeze-dried humic and fulvic acids fractions with 250 mg of the dry potassium bromide (Demyan et al. 2012).

Sequential extraction procedure

The Community Bureau of Reference (BCR) sequential extraction procedure (Ure et al. 1993), which is a modified experimental form of Tessier et al. (1979), was followed to determine the different fractions of Cd in soil. Each supernatant after each fractionation step was then analyzed by AAS. The adopted extractants, the experimental conditions, and the corresponding metal fractions were

1. Exchangeable fraction (F1). Add 40 mL of (0.11 M) CH_3COOH in 1 g sample, shake for 16 h, and centrifuge.
2. Reducible fraction (F2). Add 40 mL of (0.5 M) $NH_2OH\cdot HCl$ (pH 2) to the residual from 1., shake for 16 h, and centrifuge.
3. Oxidizable fraction (F3). Add 10 mL of (8.8 *M*) H_2O_2 (pH 2–3) to the residual from 2., stay for 1 h, then add 10 mL of (8.8 M) H_2O_2 and digest at 85 °C for 2 h. Add 50 mL of (1 M) CH_3COONH_4 (pH adjusted at 2 with HNO_3), shake for 16 h, and centrifuge.
4. Residual fraction (F4). Add 2 mL of 65% HNO_3 + 6 mL 37% HCl to the residual from 3., digest at 120 °C for 2 h.

Quality assurance

All the chemicals and solvents used were of analytical reagent grade and were purchased from Merck (Darmstadt, Germany). The reliability of all the analytical procedures was verified by including blanks within every set of digested samples.

Statistical analysis

The statistical significance of experimental treatments was analyzed by subjecting the data to analysis of variance (ANOVA) using Statistix 8.1® for Windows. The least significant difference (LSD) test ($p \leq 0.05$) was used for comparison among treatment means.

Results and discussion

Cd uptake in wheat tissues

Results showed increased uptake of sewage sludge-derived Cd in wheat tissues (root, shoot, and grains) with the increase in sewage sludge rate (0, 15, and 30 g kg^{-1}). Cd mainly accumulated in roots followed by shoots and grains, both in cultivar NARC-11 and in Shafaq-06 (Table 2). Significant values

Table 2 Effect of sewage sludge application on Cd uptake in different wheat tissues

Cultivar	Sludge rate ($g\ kg^{-1}$)	Cd concentration ($\mu g\ kg^{-1}$) Root	Shoot	Grain
NARC-11	0	15.30 ± 2.1e	6.50 ± 0.3e	1.80 ± 0.2e
	15	298.6 ± 5.5d	60.3 ± 3.5d	24.1 ± 2.4d
	30	450.6 ± 14.3b	96.6 ± 2.4b	48.6 ± 3.6b
Shafaq-06	0	25.00 ± 0.8e	8.70 ± 0.5e	3.30 ± 0.2e
	15	372.6 ± 6.9c	74.2 ± 2.6c	30.3 ± 1.2c
	30	587.0 ± 13.3a	114 ± 3.8a	58.6 ± 3.1a
LSD value		12.08**	4.73**	3.16**

Values are means ± standard error ($n = 3$); mean sharing different letter(s) in a column are statistically different at $p \leq 0.05$

**Highly significant

($p \leq 0.05$) for interactions of wheat cultivars and sewage sludge rate were found for Cd uptake in wheat tissues.

The higher concentrations of Cd sequestrated in roots indicated that wheat plants reduced Cd translocation to shoots and grains by Cd-exclusion mechanisms. Koo et al. (2013) reported that root exudates, organic acids, and microbial interactions in the rhizosphere may favor Cd uptake due to the dissolution of metal-humate complexes. They credited an increased uptake of Cd at higher sewage sludge rate due to an increase in metal solubility by root exudates in the rhizosphere.

Overall, NARC-11 showed less Cd uptake in different wheat tissues as compared to Shafaq-06. Differences among wheat cultivars with respect to Cd uptake and transport have been reported earlier by Jamali et al. (2009). The present study showed Cd concentrations in wheat grains of NARC-11 and Shafaq-06 cultivars grown in sewage sludge amended soils between 24.1–48.6 and 30.3–58.6 $\mu g\ kg^{-1}$ dry weight, respectively. These values were found within safe limits (200 $\mu g\ kg^{-1}$) as set by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (2011). Corguinha et al. (2015) attributed negligible Cd contamination in wheat grains due to short-term sewage sludge application. Liu et al. (2016) attributed the low concentrations of sludge-borne Cd contamination in food grains to a "plateau response" of added sewage sludge, but they stated that long-term application could seriously contaminate soil and plants. The plateau effect occurred due to both OM stabilization and Cd immobilization on humic and fulvic acids and amorphous Fe/Mn oxides (McBride 1995; Bolan et al. 2014).

Wheat growth and dry matter production

Results revealed an increase in dry matter production of different wheat tissues with the increase in sewage sludge application rates (0, 15, and 30 $g\ kg^{-1}$) (Table 3). As regards physiological parameters, both cultivars (NARC-11 and Shafaq-06) showed a positive correlation with sludge application. In both cultivars, root and shoot length, root and shoot dry weight, grain weight, and harvest index significantly increased with sewage sludge application (Table 3) as compared to control (un-amended soil). As a whole, NARC-11 performed better than Shafaq-06 in terms of growth. Significant values ($p \leq 0.05$) for interactions of wheat cultivars and sewage sludge rate were found for the abovementioned growth parameters.

Improved soil physical properties due to sewage sludge-derived organic acids and nutrients might have caused an increase in dry matter production of the different wheat tissues (Gonçalves et al. 2014). Liu et al. (2016) also found significant increase in root, shoot, and grain dry weight of wheat, when sewage sludge was applied at 40 $t\ ha^{-1}$. They ascribed the beneficial effects of sewage sludge application to increased microbial activity, higher concentration of nutrients, and OM-derived organic acids which enhance nutrient translocation from soil to plant.

NARC-11 showed a better growth despite higher concentration of Cd added through sewage sludge. Similar findings were presented by Idrees et al. (2015) who found that NARC-11 better

Table 3 Effect of sewage sludge on wheat growth and dry matter production

Cultivar	Sludge rate ($g\ kg^{-1}$)	Shoot length (cm)	Root length	Shoot dry wt. ($g\ pot^{-1}$)	Root dry wt.	Grain dry wt.	Harvest index
NARC-11	0	15.5 ± 0.6e	11.5 ± 0.8e	10.8 ± 0.3e	1.3 ± 0.1e	7.50 ± 0.2e	38.3 ± 1.4d
	15	21.7 ± 1.1b	15.6 ± 0.5b	17.6 ± 0.5c	1.6 ± 0.1c	14.6 ± 0.2c	43.1 ± 2.2a
	30	23.7 ± 0.5a	18.8 ± 1.2a	21.8 ± 0.7a	2.0 ± 0.2a	17.2 ± 0.3a	41.9 ± 1.3b
Shafaq-06	0	13.4 ± 0.9f	9.90 ± 0.2f	10.2 ± 0.1f	1.2 ± 0.1f	7.50 ± 0.3e	39.6 ± 1.2c
	15	17.4 ± 1.3d	12.8 ± 0.4d	15.4 ± 0.6d	1.4 ± 0.2d	12.5 ± 0.2d	42.7 ± 2.1ab
	30	19.7 ± 1.2c	14.4 ± 0.3c	18.7 ± 1.1b	1.8 ± 0.2b	15.4 ± 0.4b	42.9 ± 1.7ab
LSD value		0.65**	0.95**	0.30**	0.03**	0.47**	1.06^{NS}

Values are means ± standard error ($n = 3$); mean sharing different letter(s) in a column are statistically different at $p \leq 0.05$

**Highly significant

^{NS} Non-significant

Table 4 Cadmium fractionation of original soil and sewage sludge samples (μg kg^{-1})

	Total Cd	F1	F2	F3	F4	Total fraction (TF) = (F1 + F2 + F3 + F4)	Recovery % = ($\frac{TF}{Total\ Cd}$ × 100)
Soil	390	10	50	110	170	340	87.2
Sludge	5130	1430	940	770	1630	4770	92.9

F1 = exchangeable fraction, F2 = reducible fraction, F3 = oxidizable fraction, F4 = residual fraction

tolerated Cd stress than other wheat cultivars. This higher tolerance might be due to an increased production of antioxidant enzymes, phytochelatins, and plant growth regulators. In wheat plants, excess Cd stress upregulates proteins mainly involved in biochemical reactions for Cd detoxification (Poghosyan et al. 2014; Rizwan et al. 2016). Chen et al. (2010) also reported reduced Cd mobility and translocation in different tissues of Cd-tolerant wheat cultivars as compared to Cd-sensitive cultivars.

Cd fractionation

Cadmium fractionation results for the original soil and sewage sludge are presented in Table 4. Results after wheat harvest showed that the distribution of Cd extractable fractions changed significantly ($p \leq 0.05$) in sewage sludge amended soil (Table 5). Overall, the concentrations of exchangeable, reducible, and residual Cd fractions decreased, while the concentration of oxidizable Cd fraction slightly increased after crop harvest. Both the soils sown with NARC-11 or Shafaq-06 showed a decrease in labile exchangeable (3.6 or 1.6%, respectively) and reducible (5.2 or 2.1%, respectively) Cd fractions and an increase in the immobile oxidizable (7 or 7.9%, respectively) and residual (1.8 or 1.5%, respectively) Cd fractions, when amended with sewage sludge at 30 g kg^{-1}. Moreover in all Cd fractions, significant values ($p \leq 0.05$) for interactions of wheat cultivars and sewage sludge rate were found, except for residual fraction.

The decrease in the exchangeable Cd fraction may be due to plant uptake, metal-humate complex formation, adsorption on the negative sites of organic acids (humic and fulvic-like acids), and Cd-phosphate or Cd-sulfide precipitation. The release of root exudates and the effect of CO_2 partial pressure on the precipitation/dissolution of Cd as $CdCO_3$ and $Cd(OH)_2$ might have decreased the reducible Cd fraction (Murtaza et al. 2012). Liu et al. (2016) reported a decrease in exchangeable Cd fraction and increase in OM and residual bound Cd fractions after sewage sludge application. The observed increase in the oxidizable (OM bound) Cd fraction with aging may be due to the addition of OM which provide negative adsorptive sites for Cd ions. Krishnamurti et al. (1997) and Mimmo et al. (2014) stated that the amount of inorganic (e.g., protons) and organic (organic acids, siderophores, phenolics, and enzymes, etc.) compounds in rhizosphere exuded by plants increased after sewage sludge application. These organic acids may affect microbial activity, root morphology, and mobilization of metals (e.g., Cd) through

Table 5 Effect of sewage sludge application and wheat growth on Cd fractionation (μg kg^{-1})

Cultivar	Sludge rate g kg^{-1}	Exchangeable fraction (F1)		Reducible fraction (F2)		Oxidizable fraction (F3)		Residual fraction (F4)	
		Sowing	Harvesting	Sowing	Harvesting	Sowing	Harvesting	Sowing	Harvesting
NARC-11	0	47.3 ± 1.5 g (2.8)	44 ± 2.5 g (2.7)	542 ± 11.3c (31.9)	525 ± 13.2d (30.7)	244 ± 8.5 g (14.5)	245 ± 6.3 g (15.5)	855 ± 13.2c (50.8)	837 ± 15.2e (51.9)
	15	151 ± 4.1c (7.4)	90 ± 3.0f (5.4)	598 ± 14.1b (29.3)	423 ± 15.1e (25.6)	318 ± 7.2f (15.6)	347 ± 9.6d (20.8)	970 ± 11.7b (47.6)	808 ± 16.1f (49.1)
	30	255 ± 9.1a (10.6)	126 ± 6.0d (7.0)	656 ± 17.1a (27.4)	400 ± 12.9f (22.2)	387 ± 11.1bc (16.1)	413 ± 11.1a (23.1)	1096 ± 19.0a (45.8)	853 ± 11.1 cd (47.6)
Shafaq-06	0	47.6 ± 1.2 g (2.8)	44 ± 1.9 g (2.8)	541 ± 15.5c (32.2)	528 ± 12.1d (31.1)	242 ± 6.3 g (14.5)	244 ± 7.3 g (15.5)	846 ± 23.8d (50.4)	837 ± 13.1e (50.5)
	15	157 ± 5.0b (7.5)	107 ± 3.8e (7.0)	601 ± 21.0b (29.5)	401 ± 13.5f (25.6)	316 ± 9.6f (15.5)	325 ± 12.8e (20.8)	967 ± 11.6b (47.6)	763 ± 7.9 h (48.7)
	30	256 ± 8.2a (10.6)	148 ± 8.3c (9.0)	658 ± 18.8a (27.4)	377 ± 15.3 g (25.3)	385 ± 10.8c (16.1)	394 ± 4.1b (24.0)	1096 ± 20.4a (45.7)	796 ± 5.0 g (47.2)
LSD value		5.00**		8.07**		6.82**		7.74**	

Values are means ± standard error ($n = 3$); mean sharing different letter(s) in a column are statistically different at $p \leq 0.05$; in parentheses values are percentage

**Highly significant

soil acidification, chelation, precipitation, and oxidation-reduction reactions (Terzano et al. 2015). Changes in complex rhizosphere environment and distribution pattern of Cd fractions with sewage sludge application also depend on sewage sludge composition and its repeated application on agricultural soils (Koo et al. 2013).

The role of sewage sludge as metal immobilizing agent through chelation has been reported earlier by Keiluweit et al. (2015). They found that the composition of chelated complexes is directly related to OM decomposition through the action of soil microbes and root exudates (including organic acids). Organic matter decomposition may favor Cd release into soil solution and promote its translocation into plants (Billingham 2015). On the other hand, aging and humification of OM may cause increased metal retention by humic and fulvic acids through precipitation and chelation (Torri and Correa 2012).

Organic matter transformations

Fulvic acids

At sowing, FTIR spectra of fulvic acids obtained from sewage sludge amended and un-amended soils showed similar bands (Fig. 1). Four dominant regions of absorbance appeared: (a) a broad band around 3300–3400 cm^{-1} exhibiting the presence of H-bonded OH groups of alcohols, phenols, and H-bonded NH groups of amides and carboxylic acids; (b) a well-pronounced sharp peak at 1650 cm^{-1} showed the presence of aromatic and olefinic C=C, C=O in amide I, ketone, and quinone groups; (c) a small but sharp peak at 1250 cm^{-1}confirmed the presence of amide III, aliphatic CH_2 or alcohols, and C=O stretch of aryl ethers, as well as organo-sulfur compounds; and (d) a peak at 1040 cm^{-1} relates to the presence of polysaccharide and Si-O stretch of silicate impurities (Coates 2000; Wu et al. 2016).

Both the cultivars showed variations in fulvic acids spectra around 1000–1300 cm^{-1} spectral region in sewage sludge amended soils (Fig. 1a, b), while no obvious changes were observed in un-amended soil (Fig. 1c). These transformations in the OM of sewage sludge amended soil might be due to indigenous microbes which used labile aliphatic and carbohydrate molecules as a source of carbon and energy (Blume et al. 2016). El Fels et al. (2015) referred the disappearance of peaks at 1050–1300 cm^{-1} region due to polycondensation reactions of COOH groups. It might be also due to the increased OM addition through sewage sludge which favors the dissolution of soluble amides, aliphatic and carbohydrate groups and facilitate chelation, precipitation, and adsorption of metals on stable metal-humate complexes (Amir et al. 2010; Krishnamurti and Naidu 2008).

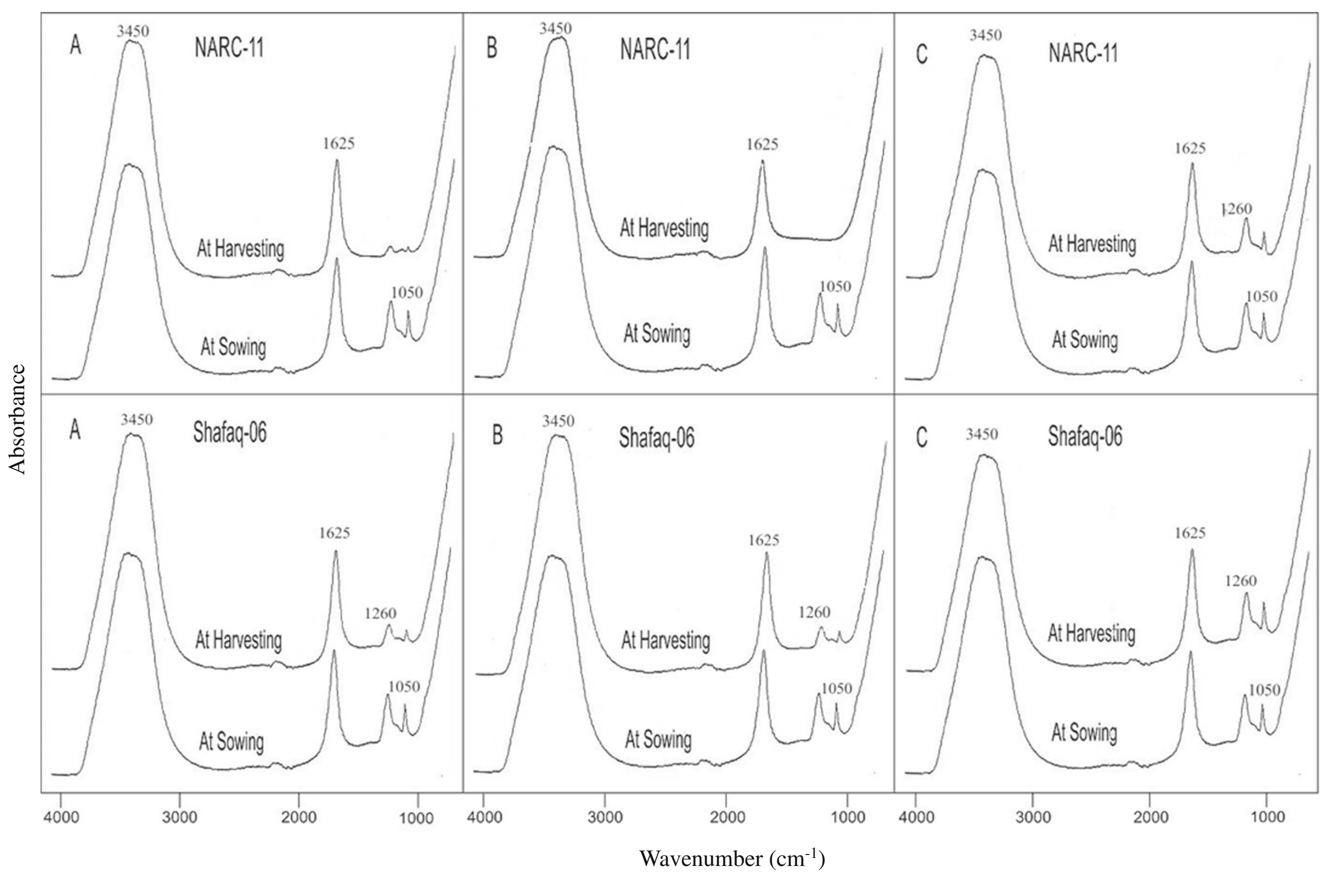

Fig. 1 Effect of sewage sludge application at 30 g kg^{-1} (**a**), 15 g kg^{-1} (**b**), and 0 g kg^{-1} (**c**) of the soil on fulvic acid spectra

The influence of fulvic acids on metal speciation has been studied by Popovic et al. (2011), who reported that metal complexes with fulvic acids are non-available for plant uptake. Similar results were obtained by Nigam et al. (2001), who found an increased Cd-humate complex interactions in the soil-plant system and designated fulvic acid transformation as a key factor in minimizing Cd availability.

Humic acids

Spectral analysis of humic acids at sowing showed similar spectral bands as noted for fulvic acids, since both are characterized by the same functional groups in their chemical structure (Fig. 2). The absence of bands at 1250 and 1040 cm^{-1} in humic acids spectra showed its higher aromatic character (Zhang et al. 2015).

Slight variations in humic acids spectra with aging might have appeared due to the presence of recalcitrant compounds like lignin, minerals, and silicates (Fig. 2a, b). An increased stability in humic acids spectra after crop harvest was confirmed by the slight increase of the bands at 1650 cm^{-1}. On the contrary, the band at 3400 cm^{-1} slightly decreased after crop harvest (El Fels et al. 2015). Sewage sludge addition and plant growth favor root exudates, microbial activity, and soluble metal-humate interaction, which decrease the hydrophilic character and increased the hydrophobic character of humic acids like fractions (Negrea et al. 2004). The increased hydrophobic character (aromatic) of humic acids led to a decreased Cd availability (decrease in the exchangeable Cd fraction and increase in OM and residual bound Cd fractions) through Cd chelation and adsorption on stable humate complexes.

The role of humic acids in reducing metal mobility in soil has been reported earlier by Harter and Naidu (2001), who attributed it to covalent bonding and the chelating capacity of the humic material. Billingham (2015) and Krishnamurti et al. (1997) reported that humic acids have the ability to decrease metal mobility with aging due to the presence of carboxyl and phenyl groups which favors the formation of stable complexes with metals, especially Cd. Humic acids like fractions could also act as a buffer for metal solubility by preventing rapid changes in soil pH. It might be due to enrichment of humic acids with aromatic compounds (sorption to soil particles and interactions with anions/cations) in sewage sludge amended soils (Pawar 2015).

Overall, the humification of OM after wheat growth decreased Cd availability by transferring available Cd fractions into less available Cd fractions and this is related to the formation of metal-humate complexes mainly from humic acids (Masciandaro et al. 2013). This short-term trial has shown that the uptake of many metals into crops decreased due to

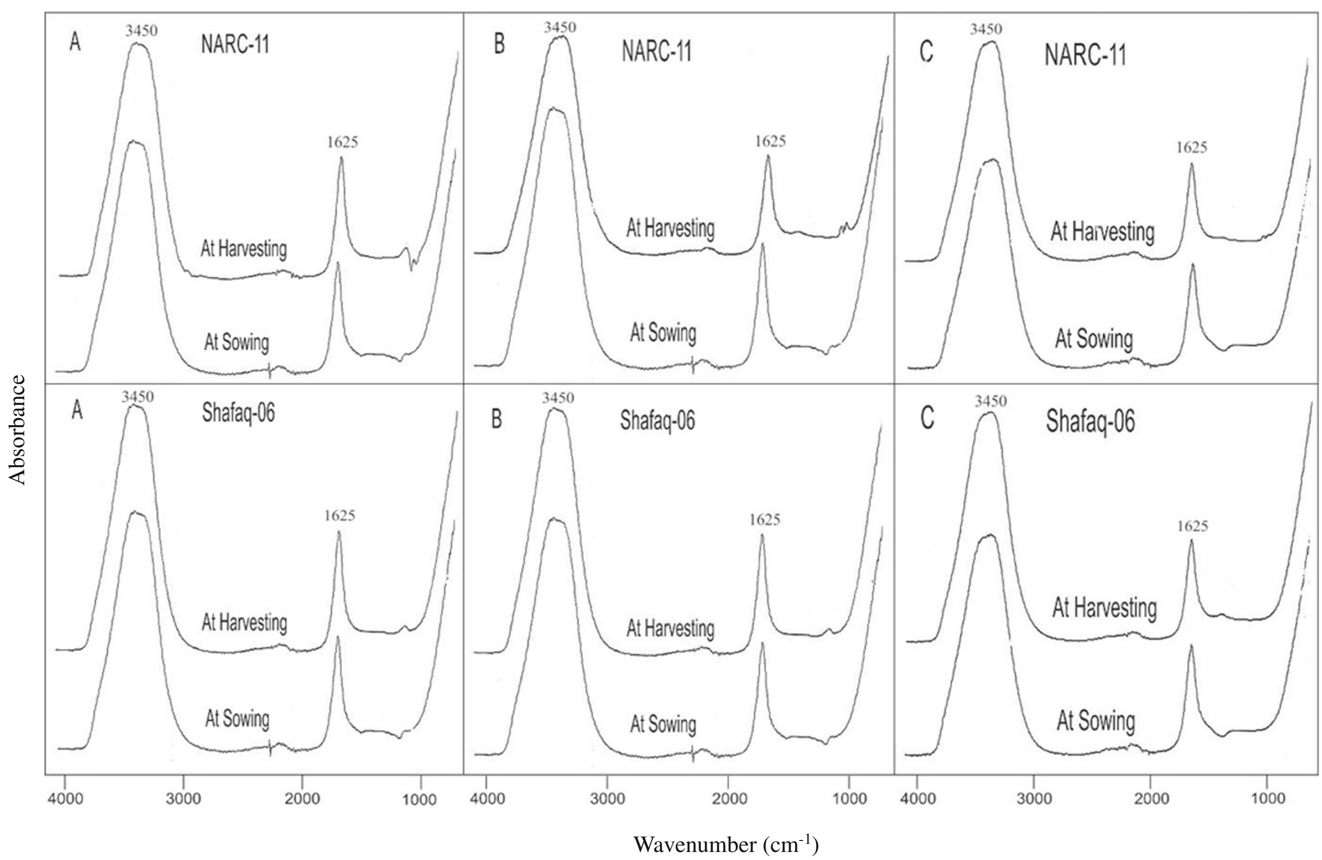

Fig. 2 Effect of sewage sludge application at 30 g kg^{-1} (**a**), 15 g kg^{-1} (**b**), and 0 g kg^{-1} (**c**) of the soil on humic acid spectra

adsorptive behavior of sewage sludge, a protection governed by the added organic matter (McBride 1995). Stevenson (1994) also suggested that humification of organic matter leads to increased condensation and re-polymerization reactions of humic and fulvic acids like fractions with soil primary (mica and silicates, etc.) and secondary (clay minerals, calcite, iron, and aluminum oxides, etc.) minerals.

Conclusions

The cultivars, NARC-11 and Shafaq-06, showed better growth and dry matter production in sewage sludge amended soils. Cadmium uptake in different plant tissues (root, shoot, and grains) was found to be lower in NARC-11 than Shafaq-06 at different rates of sewage sludge application. The Cd concentrations remained within permissible limits in wheat grains of both cultivars grown in sewage sludge amended soil. Sewage sludge-derived Cd availability decreased due to increase in oxidizable Cd fraction. Fulvic acids showed more changes in FTIR spectra than humic acids which exhibited higher stability due to the presence of more aromatic groups. Nevertheless, regular monitoring is needed for metal buildup in soil and plants after continuous applications of sewage sludge.

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