ORIGINAL PAPER

Assessing heavy metal accumulation in plants and soil with sewage sludge application under rice–wheat system in an Indo-Gangetic Inceptisol

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Abstract

Sewage sludge (SS) in the soil acts as a slow-release organic fertilizer, and its nutrient supplying capability and heavy metal release occur for a prolonged period depending upon the applied doses. Field experiment not only evaluated the direct impact of SS application on rice (*Oryza sativa* L.) but also tracked the accumulation of lead (Pb), cadmium (Cd), chromium (Cr), and nickel (Ni) content in succeeding wheat (*Triticum aestivum* L.) crop and next-season rice and wheat crop. The field experiment was conducted with different levels of SS (15, 30, and 45 t ha^{-1}) alone and in combination with 100% and 50% recommended doses of fertilizers (RDF). The findings indicated that the addition of SS (45 t ha^{-1}) alone and in combination with inorganic fertilizers increased heavy metal accumulation in grain and straw of rice–wheat system and also led to enhanced DTPA-extractable metal content in post-harvest soil. Heavy metal concentrations in crops (except Cr content in straw) and soil stay below the phytotoxic limits in all treatments. However, total Cd in the soil exceeds the permissible limit with the sole application of SS beyond 30 t ha^{-1}. This study admitted that one-time application of SS at the lower dose and reaping its benefits in subsequent crops can be an effective strategy of SS utilization in rice–wheat system.

Keywords Cadmium · Heavy metals · Phytotoxicity · Rice–wheat · Sewage sludge

Introduction

The semi-solid material received from the sewage treatment plant is referred to as "bio-solids" or sewage sludge (SS). Due to rapid industrialization and urbanization, a huge quantity of industrial effluents and city waste is being produced in India and its disposal has become a serious problem (Saha et al. 2018; Kumar et al. 2021). It is estimated that 38,354 million liters of sewage is generated with an equivalent amount of sludge per day currently in India (CPCB 2009; Kaur et al. 2012). Sewage sludge, being an organic waste, is a good source of plant nutrients such as nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg),

and iron (Fe), as well as other organic and mineral constituents (Latare and Singh 2013; Latare et al. 2014). In addition to nutrients, SS also provides organic matter (OM) to soil (European Commission 2010; Latare et al. 2014) which improves its physical, chemical, and biological properties (Saha et al. 2017; Urbaniak et al. 2017). Sewage sludge is considered as a slow-release fertilizer because nutrients are made available at a very slow pace leaving substantial residual value. Thus, its utilization in agriculture is gaining popularity as a source of nutrient and soil conditioner. In contrast to inorganic fertilizers, SS is relatively inexpensive and often, these materials can be obtained at a little or no cost to the farmers (Kidder 2001; Swain et al. 2021).

Sewage sludge contains high amounts of toxic heavy metals like lead (Pb), cadmium (Cd), chromium (Cr), nickel (Ni), mercury (Hg), and arsenic (As), due to the mixing of industrial wastewater with sewage (Singh and Agrawal 2007; Latare et al. 2014). The average concentrations of various heavy metals in SS are 26–154 mg kg^{-1} (Pb), 12–596 mg kg^{-1} (Ni), 66–1098 mg kg^{-1} (Cr), 2–9 mg kg^{-1} (Cd), 7–32 mg kg^{-1} (Hg), and 8–23 mg kg^{-1} (As), which

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restrict their long-term use in agriculture due to phytotoxicity and environmental concern (Sharma 2015). Besides heavy metals, other harmful toxicants, i.e., pharmaceuticals, detergents, various salts, pesticides, toxic organics, flame retardants, and hormone disruptors, can also be present in the SS (Antonious et al. 2003, 2012). The heavy metal concentration in the SS depends on sewage origin, sewage treatment processes, and sludge treatment processes. The bioavailability of the sludge borne metals to soil is influenced by soil properties like pH, redox potential (Eh), OM, sesquioxide, and clay content as well as SS application rates (Latare et al. 2014). Regular application of SS leads to soil pollution and heavy metal accumulation in soil and plant, and further affects food chain contamination (Kumar and Chopra 2016; Golui et al. 2021). Soil to plant transport of heavy metals results in bio-magnification of these metals and exerts harmful effects on crop quality, and their consumption causes human health hazard (Adimalla and Wang 2018; Kaur et al. 2018).

Previous researches have evaluated the effect of SS application on hazardous heavy metal accumulation in plants, but systematic experiments on the residual effect of SS application on rice (*Oryza sativa* L.)-wheat (*Triticum aestivum* L.) systems are not documented in India. Furthermore, there is paucity of information concerning heavy metal build-up in the soil. This study documented a unique way of one-time application of SS in the first rice crop and examines the metal build-up in succeeding crops up to two cropping cycles. Besides, the study also covered analysis of DTPA extractable and total heavy metal content in the soil to develop a full understanding of the hazardous metal build-up in the soil. Rice–wheat system is one of the most important cropping systems in India covering largest area; therefore, such data is needed to promote safe use of SS at great scale. We hypothesized that SS being a slow-release nutrient source if applied in the 1st rice crop would release the nutrients and heavy metals in safe limit for succeeding three crops. It was assumed that soil may be ready to accept the second dose of SS for the next two cropping cycles. Keeping the above hypothesis in view, this investigation was perused with the following objectives: (i) evaluate the direct and residual effect of SS application on toxic metals bioaccumulation on rice and wheat crops and (ii) an assessment of total and DTPA-extractable heavy metals accumulation in the post-harvest soil.

The manuscript has been written under four major headings i.e., Introduction, Materials and methods, Results and discussion, and Conclusions. The need for the study, novelty, and hypothesis of the manuscript has been described in the "Introduction" section. The systematic experimental setup and thorough methodology for analysis were written in the "Materials and methods" section. The data generated from the experiment and scientific interpretations of the data

have been described in the "Results and discussion" section. The "Conclusions" section presents the final outcome of the experiment.

Material and methods

Study area

The field experiment was conducted at Agricultural Research Farm, Banaras Hindu University, Varanasi, Uttar Pradesh, India (Fig. 1). Varanasi is situated at an altitude of 80.7 m above mean sea level and located between 25° 14′–25° 23′ N latitude and 82° 56′–83° 03′ E longitude. The experimental field was homogenously fertile with even topography, uniform textural make-up, and well connected with the farm irrigation channel. A proper drainage facility was also provided in order to remove the excess water during the experiment. The climate of the study area is semi-arid to sub-humid with a moisture deficit index range of 20–40. The annual rainfall of this region is 1100 mm. The maximum and the minimum temperature ranged between 20–42 °C and 9–28 °C, respectively. The mean relative humidity is about 68% which rises up to 82% during the wet season and goes down to 30% during the dry season. The soil of the experimental field was sandy loam in texture and classified as Typic Ustochrept in USDA Soil Taxonomy. Soil samples were processed and analyzed for pH and electrical conductivity (EC) (Sparks et al. 2020); organic carbon (OC) by wet digestion method (Walkley and Black 1934); available N by alkaline potassium permanganate method (Subbiah and Asija 1956); available P by $NaHCO_3$ extraction method (Olsen et al. 1954); ammonium acetate extractable K (Knudsen et al. 1982); 0.15% $CaCl_2$ extractable S (Williams and Steinbergs 1959) by turbidimetry method (Chesin and Yein 1951); and DTPA extractable Pb, Cd, Cr, and Ni (Lindsay and Norvell 1978) was analyzed by atomic absorption spectrophotometer (Agilent FS-240). Total N, P, K, and S content in SS was analyzed by the methods outlined by Peters et al. (2003). Total heavy metal (Pb, Cd, Cr, and Ni) content in soil was determined by AAS using aqua regia (HCl: HNO_3:: 3:1 v/v) digestion (Quevauviller 1998). Initially, the surface soil had pH 8.49 (1:2.5), EC 0.149 dS m^{-1}, OC 0.47%, and available N, P, and K content of 59.4, 8.17, and 57.6 mg kg^{-1}, respectively (Table 1). The DTPA-extractable Pb, Cd, Cr, and Ni contents were 0.13, 0.25, 0.37, and 2.38 mg kg^{-1}, respectively.

Experimental setup

The field experiment was chalked out in a randomized block design (RBD) taking ten treatments (Table 2) with three replications. Two cropping cycles comprised of four crops

Fig. 1 Location of the experimental field and sewage treatment plant

View of STP, Bhagwanpur

Agriculture Research Farm, BHU
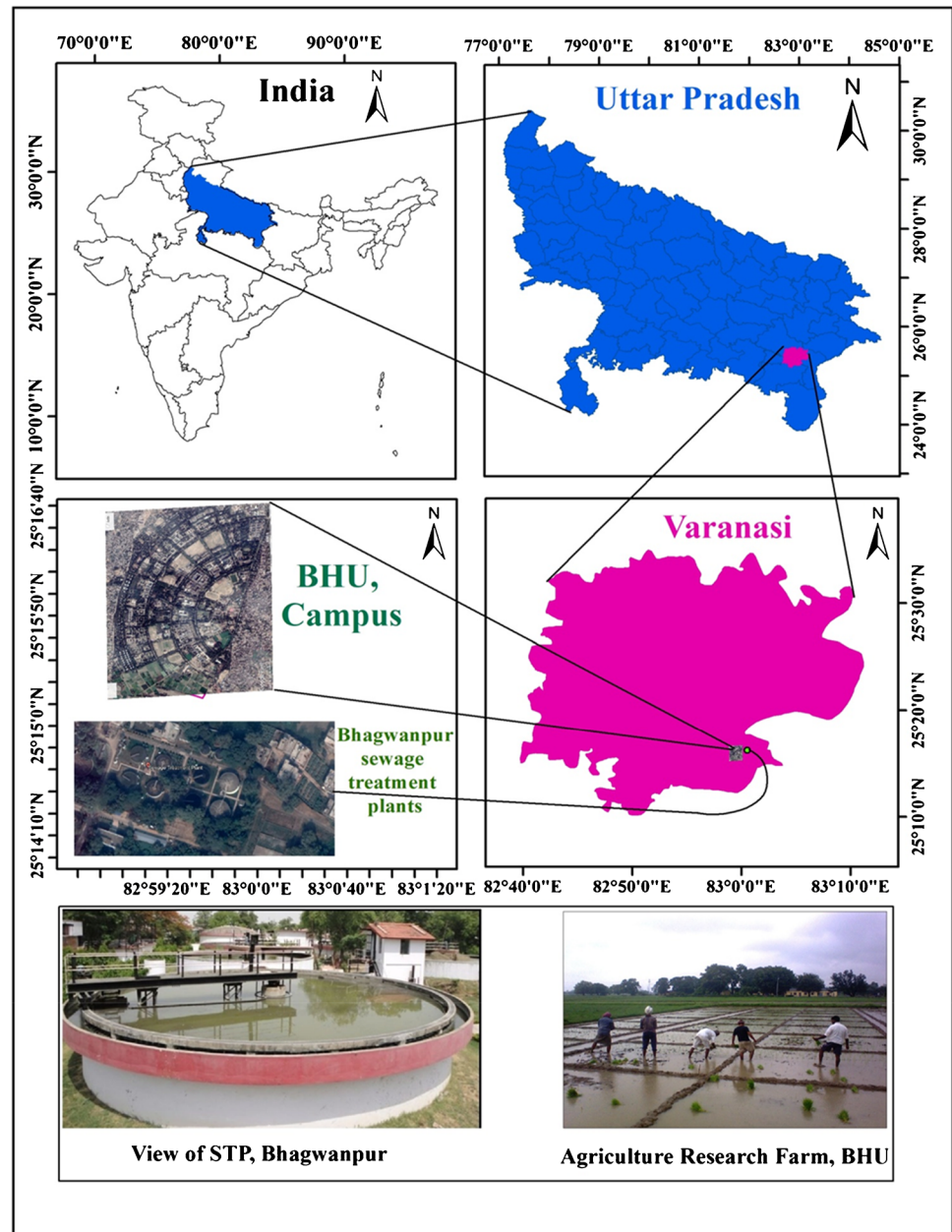
were completed during 2011–2012 (1st rice-1st wheat) and 2012–2013 (2nd rice-2nd wheat) without disturbing the experimental layout. The dimension of each experimental plot was 4×2.5 m having a zone of 0.5 m as a buffer within the plots. Each year after the onset of the monsoon, field was plowed twice followed by a harrow to obtain fine tilth. Puddling was done by the middle of July each year before the transplanting of rice. Rice (*cv.* PRH-10) nursery was prepared in the 2nd fortnight of June each year and 30-day-old seedlings were transplanted in the field during the 2nd fortnight of July each year with a spacing of 20×20 cm. After harvesting of rice in the 2nd week of November, each year, field was irrigated to get optimum moisture condition for proper germination of

wheat followed by plowing to obtain proper tilth. The sowing of wheat (*cv.* HUW-234) was done in the 1st week of December with a spacing of 20×20 cm and harvested in the 2nd week of April each year. Sewage sludge was collected from the Sewage Treatment Plant (STP), Bhagwanpur, Varanasi. The SS used throughout the experiment was composed of municipal waste and treated through the sequence of processes, i.e., primary clarifier, aeration, anaerobic digestion, and sun drying, to reduce its toxicity and volume. Sewage sludge was applied only in the 1st rice crop, and the residual effects were monitored up to the succeeding fourth crop (2nd wheat). Before the puddling of the 1st rice crop, dried and ground SS was incorporated in the respective plots and mixed thoroughly to make

Table 1 Physico-chemical properties of initial soil and sewage sludge

Parameter	Soil	Sewage sludge	References
pH	8.49	6.57	Sparks et al. (2020)
Electrical conductivity (dS m^{-1})	0.15	2.57	
Organic carbon (%)	0.47	9.65	Walkley and Black (1934)
Available nutrients content (mg kg^{-1})			
Nitrogen (N)	59.4		Subbiah and Asija (1956)
Phosphorus (P)	8.17		Olsen et al. (1954)
Potassium (K)	57.6		Knudsen et al. (1982)
Sulfur (S)	15.4		Williams and Steinberg (1959)
DTPA-extractable heavy metals content (mg kg^{-1})			
Lead (Pb)	0.13		Lindsay and Norvell (1978)
Cadmium (Cd)	0.25		
Chromium (Cr)	0.37		
Nickel (Ni)	2.38		
Total nutrient content (%)			
N		1.40	Peters et al. (2003)
P		1.20	
K		0.87	
S		0.96	
Total heavy metal content (mg kg^{-1})			
Pb	7.59	44.6	Quevauviller (1998)
Cd	0.66	24.4	
Cr	2.32	51.3	
Ni	10.1	65.9	

the field homogeneous. The recommended dose of fertilizer (RDF) for rice was 150:75:75 and for wheat 120:60:60 kg N: P$_2$O$_5$: K$_2$O per ha. Half dose of N and a full dose of P$_2$O$_5$ and K$_2$O were applied at the time of transplanting/sowing through urea, di-ammonium phosphate, and muriate of potash, respectively. The remaining half dose of N was used in two equal splits at tillering and panicle/ear initiation stages of the crop. Rice and wheat crop were raised under assured irrigated conditions, and prescribed weed and pest control measures were

adopted. The data with respect to grain and straw yield of rice and wheat had been published (Latare and Singh 2013).

Characteristics of sewage sludge and its applicability in soil

Sewage sludge used in this experiment was collected in the month of May 2011. It was air-dried, ground with the wooden roller, and passed through a 2-mm sieve before field

Table 2 Treatment application details during two cycles of rice–wheat cropping system

Treatments	2011-12		2012-13	
	Ist rice	Ist wheat	IInd rice	IInd wheat
T$_1$	100% RDF*	100% RDF	100% RDF	100% RDF
T$_2$	15 t ha^{-1} SS#			
T$_3$	30 t ha^{-1} SS	No fertilizer applied	No fertilizer applied	No fertilizer applied
T$_4$	45 t ha^{-1} SS			
T$_5$	100% RDF + 15 t ha^{-1} SS			
T$_6$	100% RDF + 30 t ha^{-1} SS	100% RDF	100% RDF	100% RDF
T$_7$	100% RDF + 45 t ha^{-1} SS			
T$_8$	50% RDF + 15 t ha^{-1} SS			
T$_9$	50% RDF + 30 t ha^{-1} SS	50% RDF	50% RDF	50% RDF
T$_{10}$	50% RDF + 45 t ha^{-1} SS			

*RDF recommended dose of fertilizers; #SS sewage sludge

application. The physico-chemical properties of SS were estimated as per the standard procedures described previously for soil analysis. The SS had pH 6.57; EC 2.57 dS m^{-1}; OC 9.65%; and total N, P, K, and S contents were 1.40, 1.20, 0.87, and 0.96%, respectively (Table 1). The total Pb, Cd, Cr, and Ni were 44.6, 24.4, 51.3, and 65.9 mg kg^{-1}, respectively.

Annual pollutant loading rate of Pb, Cd, Cr, and Ni by applying the highest rate of SS. i.e., 45 t ha^{-1}, corresponds to 2.02, 1.40, 2.29, and 2.97 kg ha^{-1} year^{-1} (Table 3). For Pb, Cd, Cr, and Ni, this value is well within the maximum loading rate prescribed by USEPA (2015). The heavy metal content of SS was also within the maximum allowable limit (Table 3). Since Cd is the most toxic element, it thus had the lowest cumulative loading rate of 39 kg ha^{-1} (USEPA 2015). In this study, the highest level of SS (45 t ha^{-1}) application is once in 2 years; thus, this grade of SS can be used for more than 50 years in agricultural lands.

Plant and soil analysis

For laboratory analysis, all three replicates of soil and plant samples were taken, and the mean of the three is given as the final value. For the analysis of heavy metal (Pb, Cd, Cr, and Ni) concentration in grain and straw of rice and wheat, the harvested plant samples were washed in detergent solution (0.2% liquid) followed by 0.1 N HCl and de-ionized water and dried at 65 ± 2 °C until it attains constant weight. The ground plant samples (straw and grain) were digested in a di-acid mixture (HNO$_3$: HClO$_4$:: 9:4 v/v) and analyzed for Pb, Cd, Cr, and Ni using atomic absorption spectrophotometer (AAS), model Agilent 240FS-AA (Agilent Technologies, Santa Clara, USA) as per the procedure outlined by Tandon (2001).

The post-harvest soil (PHS) samples collected after the harvesting of each rice and wheat crop were dried at room temperature and ground on a wooden plank with a wooden roller and passed through a 2-mm sieve and homogenized properly and further ground to pass through 0.5-mm sieve for chemical analysis. The Pb, Cd, Cr, and Ni contents in soil samples were determined through DTPA-extraction

in 1:2 soil:extractant ratio (Lindsay and Norvell 1978) and analyzed by AAS. After completion of the experiment (2nd wheat), total heavy metal (Pb, Cd, Cr, and Ni) content in PHS was determined by AAS using aqua regia (HCl: HNO3:: 3:1 v/v) digestion (Quevauviller 1998). The certified reference standard (CRS) for Pd (5190–8287), Cd (5190–8270), Cr (5190–8275), and Ni (5190–8298) were purchased from Agilent, USA. To control the analytical precision, quality control check samples were taken from materials with parameters of known value and set at concentrations near the midpoint of the calibration range. The recovery rate of Pd, Cd, Cr, and Ni were 97.2, 98.5, 96.4, and 98.8%, respectively.

Statistical analysis

The data were statistically analyzed with analysis of variance (ANOVA) using SPSS version 16.0 software. Duncan's multiple range test (DMRT) was performed to test the significance of the difference between the treatments at $p < 0.05$. All figures have been drawn using Excel 2016 from Microsoft Office.

Results and discussion

Heavy metal concentration in grain

Application of SS alone or in combination with inorganic fertilizers significantly increased the heavy metal accumulation in grains of rice and wheat during both the years. Application of 45 t ha^{-1} (T$_4$) SS had the highest Pb (3.75 mg kg^{-1}), Cd (1.40 mg kg^{-1}), and Cr (6.89 mg kg^{-1}) concentration in rice grain during 2011–2012 (Table 4), whereas Ni (22.2 mg kg^{-1}) was in 50% RDF + 45 t ha^{-1} (T$_{10}$). Their respective significant increases over 100% RDF (T1) were 5.43, 2.03, 3.45, and 2.64 times. The highest dose of SS (45 t ha^{-1}) alone (T$_4$) or in combination with inorganic fertilizers (T$_7$, and T$_{10}$) documented non-significant changes in heavy metal concentration in rice grain

Table 3 Quality of sewage sludge for application in agricultural lands with respect to heavy metals load

Heavy metals	Content in sewage sludge (mg kg^{-1})	Annual pollutant loading rate at the rate of application of 45 t sewage sludge ha^{-1} (kg ha^{-1} year^{-1})	Cumulative pollutant loading rates according to USEPA (2015) (kg ha^{-1})
Pb	44.6 (420)[*]	2.02 (15)[#]	300
Cd	24.4 (85)	1.40 (1.9)	39
Cr	51.3 (3000)	2.29 (150)	3000
Ni	65.9 (420)	2.97 (21)	420

[*]Figures in parenthesis show maximum allowable concentration in sewage sludge (mg kg^{-1}) according to USEPA (2015)

[#]Figures in parenthesis show maximum allowable annual pollutant loading rate (kg ha^{-1} year^{-1}) according to USEPA (2015)

Table 4 Direct and residual effect of sewage sludge application on heavy metals concentration (mg kg^{-1}) in grain of rice and wheat (values are expressed as a mean ± standard error (SE) of three replicates and different letters for each parameter column-wise show significant difference at $p < 0.05$ by Duncan's multiple range test)

Treatments	Lead (mg kg^{-1})				Cadmium (mg kg^{-1})			
	1st Rice	1st Wheat	2nd Rice	2nd Wheat	1st Rice	1st Wheat	2nd Rice	2nd Wheat
T1	0.69±0.08 a	0.61±0.18 a	0.83±0.28 a	0.77±0.06 a	0.69±0.08 a	0.58±0.03 a	0.52±0.07 a	0.57±0.10 a
T2	1.49±0.13 b	2.32±0.08 cd	1.36±0.09 b	1.07±0.49 ab	0.88±0.08 ab	0.87±0.05 ab	0.98±0.05 bc	0.94±0.05 b
T3	3.53±0.04 d	2.64±0.13 de	2.62±0.11 d	1.60±0.17 bcde	1.16±0.13 cd	1.10±0.03 bcd	1.33±0.13 cde	1.17±0.04 c
T4	3.75±0.11 d	3.03±0.34 ef	3.43±0.13 e	2.07±0.09 e	1.40±0.12 d	1.12±0.03 bcd	1.46±0.17 e	1.65±0.02 d
T5	1.41±0.08 b	1.88±0.18 bc	1.48±0.11 bc	1.42±0.10 bcd	0.84±0.05 ab	0.96±0.08 bc	1.07±0.12 bcd	0.91±0.03 b
T6	2.01±0.24 c	3.05±0.14 ef	2.01±0.36 c	1.55±0.12 bcde	1.19±0.05 cd	1.20±0.10 bcd	1.35±0.06 de	1.66±0.04 d
T7	3.36±0.15 d	3.14±0.15 ef	2.76±0.05 d	1.99±0.08 de	1.26±0.05 cd	1.30±0.03 d	1.52±0.13 e	1.70±0.06 d
T8	2.05±0.11 c	1.65±0.25 b	1.61±0.06 bc	1.34±0.05 bc	0.82±0.03 ab	0.95±0.14 bc	0.96±0.05 b	0.92±0.11 b
T9	3.62±0.21 d	2.43±0.04 cd	2.98±0.20 de	1.77±0.05 cde	1.08±0.02 bc	1.22±0.08 cd	1.43±0.20 de	1.86±0.17 d
T10	3.73±0.11 d	3.30±0.17 f	3.09±0.13 de	2.05±0.04 e	1.29±0.14 cd	1.34±0.23 d	1.71±0.05 e	1.69±0.01 d

Treatments	Chromium (mg kg^{-1})				Nickel (mg kg^{-1})			
	1st Rice	1st Wheat	2nd Rice	2nd Wheat	1st Rice	1st Wheat	2nd Rice	2nd Wheat
T1	2.00±0.09 a	1.09±0.08 a	1.19±0.39 a	0.74±0.10 a	8.40±1.59 a	8.31±0.53 a	8.10±0.33 a	7.97±0.30 a
T2	4.14±0.02 b	3.48±0.11 b	4.56±0.36 b	0.89±0.05 a	9.33±0.73 a	13.4±0.55 bc	9.69±0.29 abc	8.72±0.29 ab
T3	5.00±0.27 bcd	4.77±0.03 cd	6.18±0.33 c	2.65±0.29 b	16.3±1.36 b	18.7±4.48 de	11.4±0.58 c	12.4±0.57 cd
T4	6.89±0.35 e	5.01±0.49 de	8.01±0.22 d	3.42±0.16 c	20.7±1.20 c	21.7±0.82 e	18.0±0.71 e	13.7±0.29 de
T5	4.24±0.09 bc	3.70±0.25 b	3.87±0.37 b	0.87±0.04 a	10.2±0.45 a	12.7±0.28 abc	8.54±0.36 ab	9.68±0.27 abc
T6	5.17±0.71 bcd	4.62±0.32 bcd	4.38±0.20 b	2.29±0.04 b	14.1±1.07 b	15.8±1.00 cd	9.38±0.49 abc	11.4±0.66 bcd
T7	6.52±0.38 de	4.92±0.14 de	7.33±0.49 cd	3.62±0.51 c	21.0±1.36 c	21.9±1.11 e	15.3±0.57 d	15.3±0.58 e
T8	3.89±0.15 b	3.96±0.39 bcd	4.73±0.29 b	0.84±0.06 a	14.4±1.45 b	9.85±0.20 ab	8.57±0.40 ab	9.82±0.29 abc
T9	4.91±0.83 bc	4.29±0.19 bcd	6.33±0.59 c	2.20±0.49 b	15.0±1.32 b	16.0±0.35 cd	10.3±0.55 bc	13.4±1.63 de
T10	5.80±0.93 cde	6.04±0.85 e	7.06±0.28 cd	3.67±0.02 c	22.2±0.75 c	20.0±0.84 de	15.0±1.40 d	14.0±1.74 de

Please see Table 1 for treatment details

during the first year of the experiment. In the case of 2nd rice (2012–2013), the maximum Pb (3.43 mg kg^{-1}) and Cr (8.01 mg kg^{-1}) concentration in grain was in T_4 although Cd (1.71 mg kg^{-1}) was in T_{10}, and Ni (15.0 mg kg^{-1}) in T_7. The corresponding significant increase in Pb, Cd, Cr, and Ni concentration was 4.13, 3.29, 6.73, and 1.89 times as compared to T_1. As regards to wheat, during the first year (2011–2012), the highest concentration of Pb, Cd, and Cr was in T_4 whereas Ni in T_7 (Table 4). The last crop in the sequence was 2nd wheat and its grain had the maximum concentration of 2.07 mg kg^{-1} (Pb), 1.86 mg kg^{-1} (Cd), 3.67 mg kg^{-1} (Cr), and 15.3 mg kg^{-1} (Ni), respectively, in treatments T_4, T_9, T_{10}, and T_7. It was found that in 1st wheat, the significant enhancement of Pb, Cd, Cr, and Ni concentration was 5.40-, 2.31-, 5.54-, and 2.64-folds and in 2nd wheat, it was 2.69-, 3.26-, 4.96-, and 1.92-folds, respectively, over 100% RDF (T_1). Further, the residual effect of SS application in the treatments T_4, T_7, and T_{10} was statistically at par with respect to heavy metal accumulation in 2nd rice, and wheat in both the years. The increase in heavy metal concentration in rice and wheat grain was attributed to the higher concentration of heavy metals in SS. According to Kabata-Pendias (2011), the phytotoxic range for Pb, Cd,

Cr, and Ni in the plant was 30.0–300, 5.00–30.0, 10.0–100, and 40.0–246 mg kg^{-1}, respectively; therefore, all the heavy metal concentration in grain were found within the safe limits. We found that concentrations of heavy metals were higher in the grain of rice as compared to wheat. This could be explained by the higher availability and release of heavy metals from freshly applied SS to rice and reduced condition prevailing during its cultivation compared with the wheat crop. Latare et al. (2014) and Riaz et al. (2018) also reported a similar increase in heavy metal concentration in the different plant parts of rice and wheat with the addition of SS. The findings of the present investigation were lined with the findings of Shahbazi et al. (2017) in wheat, Eid et al. (2017) in spinach, Yadav et al. (2020) in mustard, and Swain et al. (2021) in spinach. It has been found that crops adopt some resistance mechanisms such as blocking the absorption of heavy metals, extracellular complexation, cytoplasmic complexation, chelation, and the expression of stress-inducible proteins to tolerate heavy metal resistance in crops (Yu et al. 2019). However, detoxification of heavy metals by sequestering them inside the vacuole after complexation with small peptides, e.g., phytochelatins, is the predominant model of heavy metal detoxification in plants (Dubey et al. 2018).

Table 5 Direct and residual effect of sewage sludge application on heavy metals concentration (mg kg^{-1}) in straw of rice and wheat (values are expressed as a mean±standard error (SE) of three replicates and different letters for each parameter columns wise show significant difference at $p < 0.05$ by Duncan's multiple range test)

Treatments	1st Rice Lead (mg kg^{-1})	1st Wheat	2nd Rice	2nd Wheat	1st Rice Cadmium (mg kg^{-1})	1st Wheat	2nd Rice	2nd Wheat
T_1	4.20±0.21 a	3.92±0.36 a	3.26±0.35 a	3.74±0.42 a	0.73±0.09a	0.75±0.12 a	0.59±0.09 a	0.27±0.04 a
T_2	6.53±0.64 bc	7.94±0.72 bc	5.34±0.04 bcd	5.78±0.16 cde	0.97±0.23ab	1.05±0.20 a	1.19±0.17 b	0.33±0.15 a
T_3	10.2±0.97 d	9.40±0.14 d	5.69±0.29 cd	6.07±0.24 def	1.54±0.14b	1.17±0.16 a	1.30±0.14 b	1.09±0.22 ab
T_4	19.3±1.36 e	12.1±0.71 ef	7.39±0.13 e	6.73±0.21 e	2.08±0.17c	2.37±0.11 b	2.96±0.12 c	1.86±0.17 d
T_5	7.60±0.51 c	6.96±0.19 b	4.85±0.35 b	4.41±0.17 b	1.20±0.14ab	0.97±0.23 a	1.14±0.07 b	0.85±0.32 ab
T_6	11.4±0.57 d	9.14±0.43 cd	5.13±0.08 bc	5.21±0.24 c	1.30±0.19ab	1.11±0.14 a	1.08±0.19 b	1.18±0.30 ab
T_7	17.2±0.34 e	9.46±0.41 d	5.97±0.08 d	5.71±0.16 cde	2.27±0.26 cd	2.47±0.05 b	2.73±0.10 c	1.37±0.06 abc
T_8	5.19±0.42 ab	7.05±0.24 b	4.74±0.25 b	4.45±0.17 b	1.25±0.15ab	1.18±0.16 a	1.03±0.19 b	1.08±0.19 ab
T_9	11.1±0.34 d	11.2±0.33 e	5.32±0.11 bcd	5.45±0.13 cd	1.19±0.18ab	1.08±0.17 a	1.14±0.16 b	1.45±0.31 abc
T_{10}	18.2±0.73 e	12.8±0.28 f	7.72±0.26 e	6.26±0.10 ef	2.63±0.16d	2.46±0.18 b	2.97±0.07 c	1.57±0.04 cd
	Chromium (mg kg^{-1})				Nickel (mg kg^{-1})			
T_1	8.36±0.49 a	7.57±1.04 a	7.52±0.46 a	5.12±0.62 a	17.4±0.48 a	11.9±0.24 a	15.0±0.25 a	10.2±1.34 a
T_2	11.4±1.25 b	11.5±0.61 b	9.22±0.64 ab	5.84±0.37 ab	21.6±0.74 b	14.9±0.67 b	20.2±0.88 bc	12.2±0.77 ab
T_3	14.5±0.57 cde	12.7±0.85 b	12.0±0.46 cde	7.51±1.02 bc	24.8±1.19 c	19.2±1.20 d	22.5±0.66 cd	16.5±1.10 c
T_4	17.1±0.44 ef	16.3±1.09 cd	14.5±0.38 fg	13.8±0.64 ef	32.9±1.06 d	27.3±0.50 e	28.4±0.79 e	24.6±0.59 d
T_5	12.2±1.01 bcd	11.0±0.90 b	10.0±0.68 bc	10.9±0.52 d	23.7±0.77 bc	17.0±0.32 bc	20.8±1.55 cd	14.3±0.24 bc
T_6	16.4±1.30 ef	13.8±1.62 bc	12.5±1.17 def	11.1±0.20 d	24.9±0.41 c	18.9±0.56 cd	23.1±0.66 d	16.2±0.48 c
T_7	19.0±0.33 f	17.5±0.32 de	13.6±0.45 ef	14.8±0.30 f	34.7±1.62 d	28.8±0.82 ef	29.0±0.34 e	27.7±0.48 e
T_8	11.7±1.26 bc	12.3±1.12 b	10.5±0.64 bcd	8.84±0.36 c	21.3±0.64 b	15.8±0.65 b	18.0±0.91 b	14.1±0.48 bc
T_9	14.9±0.85 de	16.3±0.51 cd	11.5±0.64 cde	12.6±0.47 de	26.3±1.22 c	20.2±1.01 d	22.6±0.62 cd	16.3±1.19 c
T_{10}	18.1±1.34 f	19.9±0.81 e	15.8±0.77 g	14.7±0.70 f	34.7±1.25 d	30.3±0.65 f	28.1±0.35 e	26.6±1.03 de

Please see Table 1 for treatment details

Heavy metal concentration in straw

The concentration of heavy metals in straw was higher as compared to grain in rice and wheat crops. Application of graded doses of SS alone or in combination with N, P, and K significantly enhanced the heavy metal concentration in the straw of both the crops. We observed that in case of 1st rice, the maximum Cd (2.63 mg kg^{-1}) and Ni (34.7 mg kg^{-1}) concentration in straw was in T_{10} whereas Pb (19.3 mg kg^{-1}) in T_4 and Cr (19.0 mg kg^{-1}) in T_7. In comparison to T_1, a significant increase in Pb, Cd, Cr, and Ni concentrations were 4.60, 3.60, 2.27, and 2.92 times, respectively (Table 5). During 2011–2012, the direct effect of SS application (1st rice) on heavy metal concentrations in rice straw was statistically comparable in T_4, T_7, and T_{10}, whereas in 2nd rice (2012–2013), the maximum Pb (7.72 mg kg^{-1}), Cd (2.97 mg kg^{-1}), and Cr (15.8 mg kg^{-1}) concentration in straw was in T_{10} and Ni (29.0 mg kg^{-1}) in T_7. The highest accumulation of Pb, Cr, and Ni in the straw of the 1st wheat crop was observed in T_{10} but Cd in T_7 (Table 5). During the second year (2012–2013) of the experiment, the highest values of Pb (6.73 mg kg^{-1}) and Cd (1.86 mg kg^{-1}) was in T_4 whereas Cr (14.8 mg kg^{-1}) and Ni (27.7 mg kg^{-1}) in T_7 in wheat straw. In 1st wheat, a significant increase of Pb, Cd, Cr, and Ni concentration was 3.27, 3.29, 2.63, and 2.55 times and in 2nd wheat, it was 1.80, 6.89, 2.89, and 2.72 times, respectively, over 100% RDF (T_2). As regards to the residual effect of SS, the treatments receiving the highest dose (45 t ha^{-1}) alone (T_4) or in combination with 50% RDF (T_{10}) documented non-significant changes in heavy metal accumulation in rice straw during the second year of the experiment. The Cr concentration in the straw of 1st rice and wheat exceeded the phytotoxic limit (> 10 mg kg^{-1}) in all SS applied treatments and in case of 2nd rice and wheat, treatments receiving SS beyond 30 t ha^{-1} alone or in combination with inorganic fertilizers recorded toxic level of Cr in straw (Kabata-Pendias 2011). The probable reason could be the higher content of Cr (51.3 mg kg^{-1}) in the applied SS which upon decomposition released Cr in soil thus absorbed by the crops. This hazardous accumulation of Cr in straw, makes it unfit for use for fodder purposes. Shahbazi et al. (2017) and Eid et al. (2017) reported that a higher rate of SS application causes greater accumulation of heavy metals in wheat and spinach shoots, respectively, which was a major concern for food chain contamination. The study further revealed that all heavy metal (except Cr) accumulations in straw were within the acceptable limit and did not cross the phytotoxic threshold level (Kabata-Pendias 2011). It has been reported that application of SS significantly increased the Ni concentration in straw by 738% in rice (Singh and Agrawal 2010) and 48% in wheat (Lakhdar et al. 2010) as compared to unamended
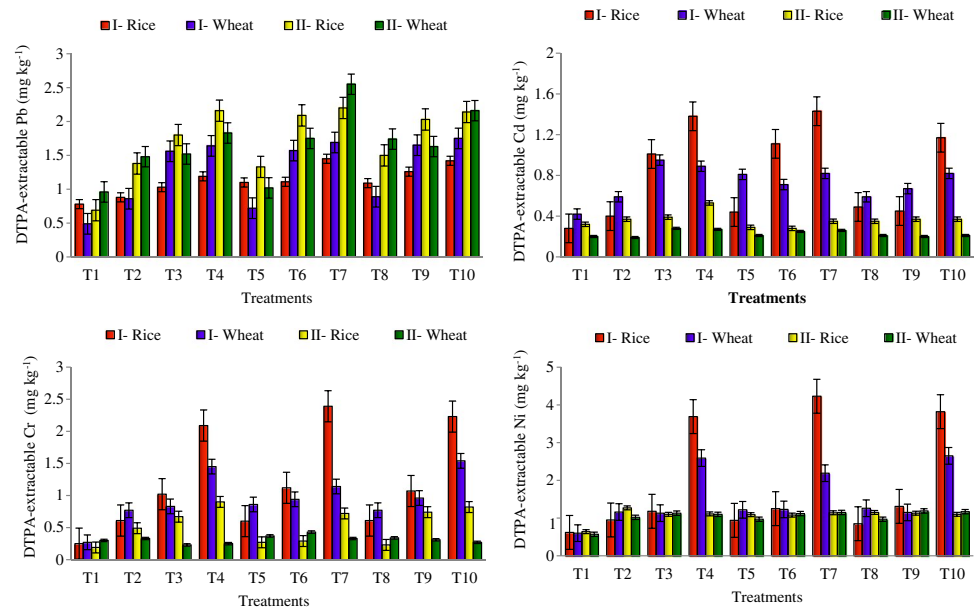
treatment. It was also found (Singh and Agrawal 2010) that SS incorporation at the rate of 12 kg m^{-2} in rice increased the Cd concentration by 903% in stem over the control. Our findings are in line with many studies that have reported that heavy metals are largely retained in shoots (Kchaou et al. 2018; Eid et al. 2020; Swain et al. 2020; Kumar et al. 2021). Moreover, H$^+$-ATPase activity is also involved in promoting metal compartmentalization contributing to alleviate heavy metal toxicity (Chen et al. 2014; Kharbech et al. 2020).

DTPA-extractable (available) heavy metal content in soil

The DTPA-extractable heavy metal content in PHS of rice and wheat (Fig. 2) had a significant variation with respect to SS application rate during both years. In the first year (2011–2012), 100% RDF + 45 t SS ha^{-1} (T_7) had the highest availability of Pb (1.45 mg kg^{-1}), Cd (1.43 mg kg^{-1}), Cr (2.39 mg kg^{-1}), and Ni (4.23 mg kg^{-1}) in the PHS of 1st rice and the statistically significant corresponding percent increase over 100% RDF (T_1) was 86, 410, 856, and 582%. The direct effect of SS application in treatments T_4, T_7, and T_{10} was statistically comparable with respect to heavy metal content in PHS of rice during the same year. As regards to the residual effect of SS application in PHS of 2nd rice (2012–2013), the maximum Pb content (2.20 mg kg^{-1}) was in T_7, whereas Cr (0.90 mg kg^{-1}) in T_4, and Ni (1.27 mg kg^{-1}) in T_2. The treatment T_{10} had the highest Pb, Cr, and Ni content in the PHS of wheat during the first year (2011–2012). The PHS of the last crop (2nd wheat) had the maximum content of Pb (2.55 mg kg^{-1}), and Ni (1.19 mg kg^{-1}) were noted in treatments T_7, and T_9, respectively. It was found that the significant increase in the content of Pb, Cr, and Ni was 257, 470, and 342% in 1st wheat and Pb and Ni was 166 and 109% in 2nd wheat, respectively, over 100% RDF (T_1) (Fig. 2). Cadmium content in PHS of 2nd rice, and wheat in both the years exhibited a non-significant variation across all the treatments, and similar result was also noticed in for Cr content in PHS of 2nd wheat. It was also found that the residual effect of SS application in T_4, T_7, and T_{10} was statistically at par with respect to Pb and Ni content in PHS of 2nd rice, and wheat during both the years. An increase in the content of heavy metals in soil due to SS application could be attributed to high content of heavy metals in SS which tend to release after decomposition. The possible argument presented by earlier researchers has been elevated application rate of SS increased soil heavy metals content, due to its slow decomposition and release (Latare et al. 2014; Eid et al. 2017; Angin et al. 2017). Roy et al. (2013) and Swain et al. (2021) observed that integrated use of SS with inorganic fertilizers have fastened the decomposition of SS, thus releasing more

Fig. 2 Effect of sewage sludge application on DTPA extractable Pb, Cd, Cr, and Ni content ($mg\ kg^{-1}$) in post-harvest soils (values are expressed as a mean ± standard error (SE) of three replicates and different letters for each parameter show significant difference at $p < 0.05$ by Duncan's multiple range test). Error bars identify standard errors of different treatments

Total heavy metal content in soil

The total heavy metal content in PHS was analyzed after the completion of the two cycle of the experiment, i.e., after harvesting of 2nd wheat. The total content of Pb, Cd, Cr, and Ni ranged between 8.78 and 17.0, 0.59 and 2.43, 2.61 and 8.95, and 11.9 and 18.9 $mg\ kg^{-1}$, respectively (Fig. 3). The average total content of heavy metals follow the order Ni > Pb > Cr > Cd. The treatment T_4 had the maximum total Pb, Cd, Cr, and Ni content with a significant per cent increase of 94, 312, 243, and 59, respectively, as compared to 100% RDF (T_1). We found that application of SS at the rate of 45 t ha^{-1} (T_4) without inorganic fertilizers documented a significantly higher Cd, and Cr build-up in the soil as compared to T_7, and T_{10} (inorganic fertilizers + 45 t ha^{-1} SS). This may be due to more vegetative growth in the treatments where SS was applied with inorganic fertilizers resulting higher uptake of easily bio-available Cd and Cr. However, treatments T_4, T_7, and T_{10} were statistically at par with respect to Pd, and Ni build-up in soil due to the lower bio-availability of these elements. Jamali et al. (2009) also found that the soil amended with sludge increased both total and bio-available forms of Pb, Cd, Cr, and Ni in soil. It has

nutrients and also heavy metals along with it in the soil. However, Zhang et al. (2016) reported that SS application in combination with NPK fertilizers was a suitable strategy for phytostabilization of heavy metals in soil. The present research result corroborates with the findings of Latare and Singh (2013) and Ankush et al. (2021).

also been reported that increasing levels of SS increased the total heavy metal status in the soil (Latare et al. 2017; Bedwal 2019; Yadav et al. 2020). The permissible limits for total Pb, Cd, Cr, and Ni in soil are 90.0–300, 1.60–3.00, 100–200, and 48.0–75.0 $mg\ kg^{-1}$, respectively (Kabata-Pendias 2011); thus, all the heavy metal content in PHS stay below the toxic limits except Cd in T_3 and T_4. This is because high dose of SS (> 30 t ha^{-1}) having Cd content of 24.4 $mg\ kg^{-1}$ increased soil Cd above the toxic level, due to slow decomposition and release of bioavailable Cd from SS (Latare et al. 2014; Eid et al. 2017).
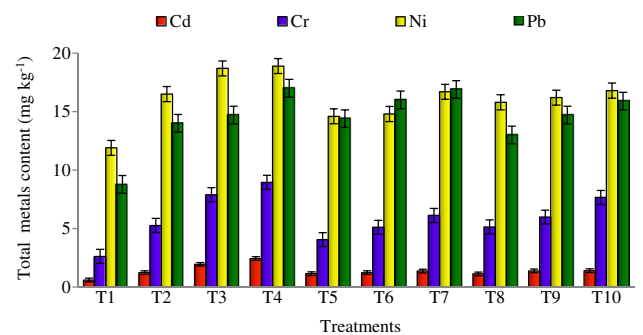
Fig. 3 Effect of sewage sludge application on total Pb, Cd, Cr, and Ni content ($mg\ kg^{-1}$) in post-harvest soil after completion of the experiment (values are expressed as a mean ± standard error (SE) of three replicates and different letters for each parameter show significant difference at $p < 0.05$ by Duncan's multiple range test). Error bars identify standard errors of different treatments

Conclusions

The study clearly documented that the application of higher dose of SS (45 t ha^{-1}) alone or in combination with inorganic fertilizers increased heavy metal concentration in grain and straw of rice and wheat. It has also resulted in build-up of DTPA-extractable heavy metal content in PHS. It was noticed that heavy metal concentration in grain and straw (except Cr) stay below the phytotoxic limits in all SS-treated plots. Total Cd build-up in soil significantly increased in treatments amendment solely with 45 t SS ha^{-1} and exceeded the permissible limit. Therefore, one-time application of SS in a lower dose (30 t ha^{-1} or less) appears to be a feasible proposition up to two cycles of the rice–wheat system. The soil treated with SS should be regularly monitored in order to keep the heavy metal content within safe limits. However, this study needs to be verified under long-term experiments in various agro-climatic regions before final recommendation.

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Declarations

Conflict of interest The authors declare no competing interests.

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