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Distribution of micronutrient in different parts of rice grown on sludge treated and metal polluted soils

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Abstract
An experiment was conducted on basmati rice (var. Pusa Basmati 1121) in 2016 to study the distribution of micronutrient in different parts of rice on sludge treated and metal polluted soils. The different doses of sludge were used as treatments 0, 10, 20, 30, 40 and 50 g/kg in IARI soil whereas, Debari soil is used as such treatment. The results indicated that total (aqua regia extractable) Zn, Cu, Fe and Mn varied from 94.4-1032 mg/kg, 17.1-24.2 mg/kg, 1.12-1.93% and 590-652 mg/kg, respectively throughout the treatments with corresponding values for EDTA extractable micronutrients were 3.94-329, 3.03-6.18, 81.5-167 and 47.3-95.6 mg/kg, respectively. DTPA extractable micronutrients in post- harvest soil were significantly higher in sludge application @ $\geq$ 30 g/kg treatments. Distribution of Zn and Cu content in rice crop found in the following order straw > grain > husk whereas, Fe and Mn found with the following sequence straw > husk > grain. Finally, it can be concluded that zinc and iron content in brown rice was increased with increase rates of sludge application, which may help in alleviating the deficiency of these two micronutrients in human.

Keywords: sludge, micronutrients, rice, polluted soils

Introduction
Application of sewage-sludge (SS) as good source of available plant nutrients and soil conditioner is increasing day by day in our country as well whole the world. Overgrowing population, industrialization and urbanization have led to increased production of sewage-sludge per day basis. The total amount of sewage generated in the country from urban areas is around 72368 million litres per day (MLD), out of which only about 51% is treated. In Delhi, a total of 38 sewage treatment plants (STPs) have been operational with total treatment capacity of 2896 MLD (CPCB 2021) [1]. Use of sewage-sludge in agriculture as a source of organic material and plant nutrients may be another effective option. Besides, important soil properties were reported to improve with the application of sludge (Verma *et al.* 2021) [9]. Currently, 43 million ha of land is under the cultivation of rice crop in our country with the total production of 122 million tonnes (Annual Report 2021-22, Ministry of Agriculture and Farmer Welfare). Limited information is available on suitability of use of sludge in rice crop in relation to soil quality and food chain contamination by metals. The present investigation was undertaken to study distribution of micronutrient in different parts of rice plants grown on sludge amended and metal polluted soils.

Materials and Methods
Location and collection of soil samples
To achieve the objectives of the present investigation, two bulk surface (0-15 cm) soil samples were collected from Debari (24°36′ N, 73°48′ E; 502 m amsl), Udaipur District, Rajasthan and experimental farm of IARI (28˚30´ N, 77˚10´ E; 250 m amsl), New Delhi. Soil samples collected from Debari belongs to Typic Haplustept located in the sub-humid southern plain and aravalli hill climate (precipitation >720mm). On the other hand a soil of IARI farm belongs to Typic Haplustept in sub-tropical semi-arid agro-climatic zone (precipitation 651 mm) of Upper Gangetic Plain.
An experiment was conducted on basmati rice (var. Pusa Basmati 1121) in 2016 to study the distribution of micronutrient in different parts of rice on sludge treated and metal polluted soils. Sludge was collected from sewage treatment plants of Delhi, i.e. Okhla, in the month of March and analysed for chemical properties using standard procedure and sludge consisted pH (6.73±0.02) and EC (3.92±0.03 dS/m). Total micronutrients in sludge were 0.95±0.03% Fe, 1186±87.1 mg/kg Zn, 94.1±2.29 mg/kg Cu and 128±2.11 mg/kg Mn.

Initial pH was 8.0 and 7.73 for IARI soil and Debari soil respectively, with the corresponding values of electrical conductivity (EC) of 0.24 and 1.64 dS/m. Mechanical analysis indicates that soil of IARI and Debari soil belong to sandy loam and sandy clay loam textural classes, respectively. Organic carbon content was 3.8 and 9.7 g/kg in IARI soil and Debari soil respectively. DTPA extractable Zn, Cu, Fe and Mn contents in IARI soil were 2.06, 1.24, 4.11 and 7.26 mg/kg, respectively, whereas, Debari soil was found to contain 21.3, 1.39 4.67 and 15.4 mg/kg of Zn, Cu, Fe and Mn, respectively. For this purpose plastic pots of 8 kg capacity were filled with 5 kg of soil. The sludge was added @ 0, 10, 20, 30, 40 and 50 g/kg of soil and one treatment from polluted soil. All seven treatments were arranged in completely randomised design (CRD) replicated thrice. A dose of N: P_2O_5: K_2O @ 75: 40: 30 mg/kg soils were added in solution form to the soil of each pot through urea, DAP and muriate of potash, respectively. Half of N and full dose of P and K were applied at the time of transplanting of rice and remaining N fertilizer was applied in two equal splits at tillering and flower initiation stage. Plants were harvested at maturity and recorded yield data. After harvest of the crop, soil samples were collected and analysed for pH (1:2:: soil: water), electrical conductivity (EC), organic carbon (OC) (Walkley and Black 1934) [10]. Aqua regia extractable micronutrients with HNO_3-HCl (1:3), EDTA extractable micronutrients with 0.05 M EDTA solution adjusted at pH 7.0 (soil: extractant: 1:10) (Quevauviller, 1998) [5], DTPA extractable Zn, Fe, Cu and Mn (Lindsay and Norvell 1978) [4] in soil samples were analysed. Micronutrient contents in the samples were determined using ICP-MS. Different part of rice (straw husk and grain) samples were digested with HNO_3 in a microwave digester and content of micronutrients in the digested materials were determined using ICP-MS. Statistical analysis of data was done adopting through completely randomized design (Snedecor and Cochran 1967) [7].

Result and Discussion

Effect of sludge application on *Total (aqua regia), EDTA and* DTPA extractable micronutrient contents in soil

Total (aqua regia extractable) micronutrients

Total (aqua regia extractable) micronutrients as influenced by sludge addition on soil after harvest of rice crop is presented in table 1. Results indicate that total Zn was observed as 94.4, 119, 137, 156, 168 and 184 mg/kg with the addition of sludge at the rate of 0, 10, 20, 30, 40 and 50 g/kg, respectively. Significant increases in total Zn in soil was recorded where 30, 40 and 50 g of sludge per kg of soil was added. Metal polluted soil had 1032 mg/kg of total Zn. Total Cu in soil was recorded as 17.1, 19.5, 21.3, 21.6, 22.8 and 24.2 mg/kg, where sludge was added @ 0, 10, 20, 30, 40 and 50 g/kg, respectively. There was a significant increase in total Cu content in soil over control, when sludge was added beyond 20 g/kg. Total Cu content in metal polluted soil was 22.5 mg/kg. On an average, total Fe content in soil was observed as 1.12, 1.18, 1.29, 1.34, 1.36 and 1.40% at 0, 10, 20, 30, 40 and 50 g of sludge addition per kg of soil, respectively. Significant increase in total Fe content in soil were recorded, where 50 g of sludge per kg was added. In metal polluted soil was observed to have as 1.93%. Total Mn in soil was recorded as 638, 639, 644, 649, 650 and 652 mg/kg with the addition of sludge at the rate of 0, 10, 20, 30, 40 and 50 g kg-1 of soil, respectively. There was a significant increase in total Mn in soil, when sludge was applied as 40 and 50 g per kg of sludge-amended pots. Total Mn in metal polluted soil was 590 mg/kg.

EDTA extractable micronutrients

EDTA extractable micronutrients as influenced by added sludge in soil after harvest of rice is presented (Table 1). Results indicate that on an average, EDTA extractable Zn increased by 1.61, 4.24, 6.56, 8.76 and 13.96 mg/kg at 10, 20, 30, 40 and 50 g/kg of applied sludge, respectively over control. More or less similar effect of sludge addition on EDTA extractable Cu was recorded as observed in case of Zn. On an average, EDTA extractable Cu content in soil was increased by 0.36, 0.72, 1.12, 1.77 and 3.15 mg/kg due to application of sludge @ 10, 20, 30, 40 and 50 g/kg, respectively as compared to control. There was a significantly increased in Cu content in soil were recorded for all sludge treated pots over control. Metal polluted soil had 329 mg/kg for EDTA extractable Zn and 3.06 mg/kg for EDTA extractable Cu. EDTA extractable Fe content in soil increased from control (81.5 mg/kg) to the highest rate of sludge addition (167 mg/kg). Significant increase in EDTA extractable Fe was noticed when sludge was added @ ≥ 10 g/kg over control. EDTA extractable Fe content in metal polluted soil was observed as 123 mg/kg. On an average, EDTA extractable Mn content in soil was recorded as 50.7, 64.1, 69.4, 77.3, 88.2 and 95.6 mg/kg with the addition of sludge @ 0, 10, 20, 30, 40 and 50 g/kg of soil, respectively. There was a significant increase in EDTA extractable Mn, when sludge was applied @ ≥ 10 g/kg. In metal polluted soil EDTA extractable Mn was observed as 47.3 mg/kg.

DTPA extractable micronutrients

Data on the effect of sludge application on DTPA extractable micronutrient of soil after harvest of rice is presented (Table 1). On an average, DTPA extractable Zn content in soil was significantly increased as 3.55, 5.99, 6.16, and 11.05 at 20, 30, 40, and 50 g/kg of added sludge, respectively over control. More or less similar effect of sludge addition on DTPA extractable Cu was recorded as observed in case of Zn. DTPA extractable Cu ranged from 1.65 mg/kg in control to 4.01 mg/kg in the highest rate of sludge addition. Significant increase in DTPA extractable Cu was noticed when sludge was applied @ ≥ 20 g/kg. Metal polluted soil had DTPA extractable Zn and Cu as 59.2 and 1.99 mg/kg, respectively. On an average, DTPA extractable Fe progressively increased from 4.75 mg/kg (control) to 23.6 mg/kg due to addition of up to 50 g/kg sludge. Significant increase in DTPA extractable Fe with increasing rates of sludge addition was noticed. In metal polluted soil DTPA extractable Fe was recorded as 6.15 mg/kg. DTPA extractable Mn content in soil was recorded as 8.02, 10.6, 12.8, 15.6, 19.7 and 24.1 mg/kg with the addition of sludge at the rate of 0, 10, 20, 30, 40 and 50 g/kg of soil, respectively. Significant increase in DTPA extractable Mn in sludge treated soil was noticed, when sludge was added @ ≥ 10 g/kg over control. Aqua regia extractable, EDTA extractable and DTPA extractable Zn, Cu, Fe, and Mn increased with increase rates of sludge application. Such increase in extractable micronutrient pool as a result of sludge application can be considered as a positive effect. However, DTPA extractable Zn exceeded the phyto toxicity limit, i.e. 10 mg/kg. Several researchers also reported that sludge application increased the extractable metals content in post-harvest soil (Ray *et al.* 2013, Golui *et al.* 2014, Verma *et al*. 2020) [6, 3, 9].

Table 1: Effect of sludge application on Aqua regia, EDTA and DTPA extractable micronutrients soil harvest of rice

Rates of sludge addition (g/kg)	Zinc (mg/kg)			Copper (mg/kg)			Iron (mg/kg)			Manganese (mg/kg)		
	Aqua regia	EDTA	DTPA	Aqua regia	EDTA	DTPA	Aqua regia (%)	EDTA	DTPA	Aqua regia	EDTA	DTPA
0 (control)	94.4	3.94	2.45	17.1	3.03	1.65	1.12	81.5	4.75	638	50.7	8.02
10	119	5.55	3.73	19.5	3.39	1.93	1.18	104	6.48	639	64.1	10.6
20	137	8.18	6.00	21.3	3.75	2.79	1.29	117	9.11	644	69.4	12.8
30	156	10.5	8.44	21.6	4.15	3.43	1.34	144	14.2	649	77.3	15.6
40	168	12.7	8.61	22.8	4.80	3.89	1.36	147	17.8	650	88.2	19.7
50	184	17.9	13.5	24.2	6.18	4.01	1.40	167	23.6	652	95.6	24.1
Metal polluted soil	1032	329	59.2	22.5	3.06	1.99	1.93	123	6.15	590	47.3	16.2
LSD (P=0.05)	43.8	1.82	1.78	3.41	0.28	0.76	0.25	16.7	1.28	11.7	9.94	1.65

LSD value as per Tukey's HSD test at P = 0.05

Effect of sludge application on micronutrients content in different plant parts of rice

***Micronutrients* content in rice straw**

On an average, Zn content in rice straw was increased by 21.7, 30.8, 35.2 and 42.3 mg/kg with the addition of sludge @ 20, 30, 40 and 50 g/kg, respectively as compared to control. There was progressive increase in Cu content in straw from control (6.73 mg/kg) to the highest rate of sludge application (12.9 mg/kg). Significant increase in Cu content in straw of rice was recorded at ≥ 20 g of sludge addition per kg of soil. Zn and Cu content in straw of rice grown on metal polluted soil were 235 and 8.33 mg/kg, respectively. On an average, Fe content in rice straw was observed as 509, 552, 595, 620, 687 and 721 mg/kg when sludge was added @ 0, 10, 20, 30, 40 and 50 g/kg, respectively. There was a significant increase in Fe content in rice straw with the addition of sludge @ 20 to 50 g/kg. Iron content in straw of rice grown on metal polluted soil was recorded as 513 mg/kg. Manganese content in rice straw was increased from 111 (control) to 154 mg/kg at the highest rate of sludge addition. Significant increase in Mn content in straw of rice was recorded when sludge was added at the rate of ≥ 10 g/kg. Manganese content in straw of rice grown on metal polluted soil was observed as 169 mg/kg (Table 2).

Micronutrients content in rice husk

Zinc content in husk of rice was increased as 2.0, 3.8, 4.2, 5.1 and 7.7 mg/kg with corresponding sludge addition @ 10, 20, 30, 40 and 50 g/kg, over control**.** There was a significant increase in Zn content in rice husk when sludge was added at the rate ≥ 20g/kg. Copper content in husk of rice was increased from 2.58 in control to 5.28 mg/kg associated with the highest rate of sludge addition. Significant increase in Cu content in husk of rice was recorded when sludge was applied @ ≥ 20 g/kg. Iron content in husk of rice was assessed as 103, 112, 143, 148, 156 and 169 mg/kg due to application of sludge @ 0, 10, 20, 30, 40 and 50 g/kg, respectively. Significant increase in Fe content was recorded @ ≥ 20 g/kg sludge addition. Manganese content in husk of rice was increased by 8.8, 18.5, 22.5, 24.5 and 29.5 mg/kg over control due to application sludge @ 10, 20, 30, 40 and 50 g/kg, respectively. There was a significant increase in Mn content in rice husk when sludge was added beyond 20 g/kg. Zinc, Cu, Fe and Mn content in husk of rice grown on metal polluted soil were observed as 43.9, 3.83, 167 and 133 mg/kg, respectively (Table 2)

Micronutrients content in grain rice

There was a progressive increase in Zn content in rice grain from control (21.5 mg/kg) to the highest rate of sludge applied (38.4 mg/kg). Significant increase in Zn content in grain of rice was observed when sludge was applied @ ≥ 20 g/kg. Copper content in rice grain was increased by 0.44, 1.25, 2.02, 2.64 and 3.71 mg/kg with the addition of sludge @ 10, 20, 30, 40 and 50 g/kg, respectively as compared to control. Significant increase in Cu content in grain of rice was recorded when sludge was added at the rate of ≥ 20 g/kg. Iron content in rice grain was as 6.61, 9.28, 10.6, 15.1, 17.2 and 19.5 mg/kg as a result of addition of sludge @ 0, 10, 20, 30, 40 and 50 g/kg, respectively. There was a significant increase in Fe content in rice grain with increased level of sludge addition from 10 to 50 g/kg. Manganese content in rice grain was progressively increased from 9.58 mg/kg (control) to 19.8 mg/kg due to application of sludge @ 50 g/kg (Table 18). There was a significance increase in Mn content in rice grain when sludge was added beyond ≥ 10 g/kg. On an average, Zn, Cu, Fe and Mn content in grain of rice grown on metal polluted soil were observed as 56.2, 3.87, 8.81 and 21.9 mg/kg, respectively (Table 2). By and large, a consistent increase in metal content in different parts (grain, straw and husk) rice was observed as a result of concomitant increased rate of sludge application. This is attributed to the fact that considerable amount of Zn, Cu, Fe and Mn was added to soil through sludge, which in turn enhances the available pool of metals in soil. Apart from extractable metal in soil, pH and organic carbon have been considered as the most important factors govern the solubility of metals in soil (Datta and Young 2005 [2]; Verma *et al*. 2020[9]).

Table 2: Effect of sludge application on micronutrients content in different plant parts of rice

Rates of sludge addition (g/kg)	Straw (mg/kg)				Husk (mg/kg)				Grain (mg/kg)			
	Zn	Cu	Fe	Mn	Zn	Cu	Fe	Mn	Zn	Cu	Fe	Mn
0 (control)	35.5	6.73	509	111	19.7	2.58	103	88.5	21.5	2.82	6.61	9.58
10	45.9	8.27	552	128	21.7	2.91	112	97.3	25.3	3.26	9.28	11.5
20	57.2	9.82	595	133	23.5	3.35	143	107	26.8	4.07	10.6	14.5
30	66.3	11.8	620	140	23.9	3.60	148	111	31.1	4.84	15.1	16.7
40	70.5	12.8	687	148	24.8	5.05	156	113	35.2	5.46	17.2	18.4
50	77.8	12.9	721	154	27.4	5.28	169	118	38.4	6.53	19.5	19.8
Metal polluted soil	235	8.33	513	169	43.9	3.83	167	133	56.2	3.87	8.81	21.9
LSD (P=0.05)	20.4	1.59	81.2	16.6	3.99	0.49	15.9	11.4	4.85	0.77	1.82	1.88

LSD value as per Tukey's HSD test at P = 0.05

Conclusion
Based on findings, it can be concluded that sludge is a good source of plant nutrients to enhanced crop productivity and soil fertility. Rice crop responded positively to sludge application. Zinc and iron content in brown rice was increased with increase rates of sludge application, which may help in alleviating the deficiency of these two micronutrients in human.

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