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Effect of Sewage sludge and fertilizers application on accumulation of heavy metals and yield of Indian mustard (*Brassica juncea* L.)

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Abstract

A pot experiment was conducted in net house during *Rabi* 2016-17 at Institute of Agricultural Sciences, Banaras Hindu University, Varanasi to find out the effect of Zinc, Boron, Sulphur and Sewage Sludge application on heavy metal accumulation in soil and plants of mustard [*Brassica juncea* (L.) Czern & Coss.] and its yield**.** To conduct the pot experiment, bulk soil samples (0-15 cm depth) were collected from the Agricultural Research Farm, Banaras Hindu University, Varanasi. Altogether, twelve treatments consisting of combinations of sewage sludge and chemical fertilizers, S, Zn, B, along with a control were used for pot experiment. The experiment was laid out in a completely randomized design (CRD) with three replications. The results showed that due to application of different levels of boron, zinc, sulphur and sewage sludge in mustard crop, a significant increase in grain and stover yield was obtained.The maximum seed and stover yields (20.9 and 60.5 g pot^{-1}) were found in treatment T_{10} that were 82.9 % and 69.4 % higher in comparison to control, and was accompanied by T_{12} with 19.1 and 55.8 g pot^{-1}, respectively. But both were found statistically at par with each other.The combined application of sewage sludge and inorganic fertilization also enhanced the accumulation of heavy metals in soil and mustard plant (Ni, Pb, Cd and Cr) particularly at higher rates of sludge application. The maximum content of Ni, Pb, Cd and Cr in mustard seed (13.7, 2.5, 1.57 & 0.92 mg kg^{-1}) and stover (14.7, 2.5, 1.97 and 2.2 mg kg^{-1}) as well as in post harvest soil were found under T_{10} (100% RDF + SS @ 20 t ha^{-1}) which was accompanied in the order of T_9, T_{12} and T_{11} treatments.

Keywords: *Heavy metal accumulation, mustard, sewage sludge, yield*

Introduction

India occupies the third position in area and production of rapeseed-mustard after Canada and China. It accounts for 19.29% and 10.07% of the total acreage and production of rapeseed and mustard. Mustard is gaining a wide acceptance among farmers because of its adaptability to irrigated and rainfed areas as well as suitability for sole as well as mixed cropping. Nutrient management stands among the most crucial factors in crop production. Exploitation of genetic yield potential of any crop including mustard depends on availability of all essential nutrients in balanced and optimum amounts. In addition to the major nutrients, response of mustard to application of sulphur, zinc and boron has also been reported by a number of workers. Keeping in view the ever increasing demand of oilseed production of the country, there is an urgent need to maintain adequate supply of nutrients through organic and inorganic sources, both. It has been observed in the earlier studies that sewage-sludge contains a considerable amount of micronutrients particularly Zn, Fe, Cu and Mn. Thus, sewage-sludge can be considered as a viable alternative for fertilizer and soil conditioner. Sewage sludge is a good source of macro/ micronutrients and also rich in organic matter (Golui *et al.*, 2014). Besides these beneficial aspects, sewage-sludge often also carries pollutants and heavy metals which could be phytotoxic to plants and animals (Dai *et al.*, 2007). The benefits of sewage sludge application as a nutrient source for plants are thus masked further by its potential health hazards due to transmission of pathogenic organisms, accumulation of nitrates in ground water, soil and water contamination by highly toxic organic constituents of the sludge and accumulation of toxic heavy metals in soil, water and crops. The heavy metals of major concern in sewage-sludge are- Cr, Co, Ni, Mo, Cd, Hg, Pb, As and Se. The addition of heavy metals especially to low pH soils and their uptake by plants and ingestion by animals may have health risks (Udom *et al.*, 2004). Since, heavy metals are transferable, not biodegradable and adsorbed by soil clay, the repeated addition of sewage sludge may result in build-up of heavy metal concentrations in soil and thereby, exert adverse effects on soil health (Alloway *et al.*, 1990; Sigua *et al.*, 2005). It creates the potential risk of the metal toxicity to plants and soil microbial population, and the accumulation of metals in the food chain, where man is the last sink (Amir *et al*., 2005). Thus, the heavy metal toxicity and its impact on environment and soil health are of prime concern due to use of sewage sludge. Hence, sewage sludge should be utilized judiciously to minimize the chances of these potential health and environmental hazards.

Though, the sewage sludge is very rich in organic matter and nutrients, yet the information on impact of sewage sludge on crop productivity and soil health including nutrients availability, their uptake by plants and accumulation of heavy metals are scanty. The subsequent effect on the crop yield and uptake of heavy metals by plant needs to be also studied which can provide a better knowledge and understanding about the extent to which its use can be beneficial in agriculture. Mustard being highly remunerative crop can be successfully cultivated in peri-urban agriculture where sludge is produced in sufficient amount. Taking the above facts into consideration, the present investigation was carried out at BHU, Varanasi to study the effect of sewage sludge and fertilizer application on yield of mustard and heavy metal accumulation.

Materials and Methods

The investigation was carried out in a pot experiment on alluvial soils under net house at the Department of Soil Science and Agricultural Chemistry, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, U.P. (India), during *Rabi* 2016-17.Varanasi is located on the western bank of river Ganges, having an altitude of 80.71 metres above mean sea level and located between 25^0 19' North latitude and 83^0 10' East longitudes. The soils of Varanasi are formed on alluvium deposited by the river Ganges and have predominance of illite, quartz and feldspars minerals. The alluvial soil is characterized by higher silt content. Illitic nature of alluvial sediments is clearly demonstrated by higher K_2O content. The cropping history of the site from where the soil was collected is the maize-wheat. The fertility status of the soil is classed as low to medium with a slightly alkaline reaction. For the pot experiment, the soil was collected from the Agricultural Research Farm, Institute of Agricultural Sciences, BHU, Varanasi. The dry sludge was ground to get a homogenous mass. The soil (0–15 cm) used for experimentation had pH-8.15 (1:2.5), EC-0.20 dS m^{-1}, organic carbon-0.33% and available N, P and K of 95.8, 13.2 and 138 kg ha^{-1}, respectively. The DTPA extractable Fe, Mn, Cu and Zn contents of soil were 5.01, 4.29, 0.45 and 0.33 mg kg^{-1}, respectively. The experiment was laid out in a completely randomized design (CRD) with three replications. Altogether, there were twelve treatments including combinations of sewage sludge and chemical fertilizers, S, Zn, B, along with a control. The details of the treatments are: T_1- Absolute Control (no fertilizer), T_2- Control (100% RDF), T_3- 100% RDF + S @ 30 kg ha^{-1}, T_4- 100% RDF + B @ 1.5 kg ha^{-1}, T_5- 100% RDF + Zn @ 5 kg ha^{-1}, T_6- 100% RDF + S @ 30 kg ha^{-1} + B @ 1.5 kg ha^{-1}, T_7- 100% RDF + Zn @ 5 kg ha^{-1} + B @ 1.5 kg ha^{-1}, T_8- 100% RDF + S @ 30 kg ha^{-1} +Zn @ 5 kg ha^{-1} + B @ 1.5 kg ha^{-1}, T_9- 100% RDF + Sewage Sludge (SS) @ 10 t ha^{-1}, T_{10}-100% RDF + Sewage Sludge @ 20 t ha^{-1}, T_{11}- 75% RDF + Sewage Sludge @ 10 t ha^{-1}, T_{12}- 75% RDF + Sewage Sludge @ 20 t ha^{-1}.

Ten kg of processed soil was filled in each pot after grinding to pass through 2 mm sieve. The whole quantity of soil was placed on a polythene sheet and according to the treatments, the required quantities of sewage sludge material was incorporated and mixed thoroughly with the soil. The recommended dose of fertilizer (RDF) for mustard (*Brassica* sp.) was $N:P_2O_5:K_2O$ :: 80:40:40 kg ha^{-1}. To supply the recommended dose of fertilizer, a nutrient solution was prepared which supplied 40 mg N, 20 mg P_2O_5 and 20 mg K_2O kg^{-1} soil. The nutrient solution was prepared using urea as N source, analytical grade Diammonium phosphate (DAP) as the source of phosphorus and potassium chloride (KCl) as the source of potassium. Water was added to the soil samples to raise the moisture content to 50% of the field capacity. The moist samples were then transferred into the pots and kept in the greenhouse.The seeds of mustard were sown on 10th November, 2016. Ten seeds were sown in each pot and the upper soil layer was moistened with water to ensure proper germination. After germination of seeds, thinning was done to maintain four plants per pot. The plants were maintained in the pot culture and care was taken to ensure proper growth. Irrigation was given as and when required. The growth and yield attributes were estimated as per the standard procedure in each pot. At maturity, the plants were harvested and seeds were separated from the plant, kept in paper bags and dried in hot air oven at 60 ± 2^0C till the weight became constant and seed yield (g pot^{-1}) was computed. After harvesting of crop, seeds were separated and plant samples were kept in paper bags, dried in hot air oven at 60 ± 2^0C till the weight became constant and stover yield (g pot^{-1}) was recorded.

Plant and soil analysis

Harvested plant samples were washed in detergent solution (0.2% liquid), 0.1 N HCl solution and deionised water in sequence and dried at 70 ^oC till the constant weight. Straw and grain (1.0 g) were taken in a conical flask of 150 ml capacity. About 10 ml of di-acid mixture (HNO_3:$HClO_4$ :: 9:4) was added to each sample and digested on a hot plate at 180-200 °C till the solution became clear but not completely dry. This extract was used for determination of P, K, Fe, Zn, Cu and Mn, subsequently. Heavy Metals (Ni, Pb, Cd and Cr) in the extracts were determined by an atomic absorption spectrophotometer (Agilant FS-240) as outlined by Tandon, (2001).The soil samples were collected after the harvest of mustard crop. The soil samples were analyzed for pH and Electrical conductivity (Jackson, 1973), organic carbon (Walkley and Black, 1934), available nitrogen (Subbiah and Asija., 1956), available phosphorus (Olsen *et al*., 1954), available potassium K (Hanway and Heidel, 1952) and DTPA extractable Fe, Cu, Mn, Zn, Cd, Cr, Ni and Pb (Lindsay and Norwell, 1978) by atomic absorption spectrophotometer following the procedure outlined in Sparks (1996).

The harvest index was calculated by using the following formula:

$$\text{Harvest Index (\%)} = \frac{\text{Economic yield}}{\text{Biological yield}} \times 100$$

Statistical analysis

Data recorded from the laboratory and as well as from the pot experiment were assessed by Duncan's Multiple Range Tests (DMRT) with a probability $P< 0.05$ by using the SPSS program (SAS version 17.0).

Results and Discussion

Effect on heavy metal contents (Ni, Pb, Cd and Cr) in seed and stover of mustard

Nickel (Ni): There was significant increase in content of heavy metal in seed and stover of mustard with graded application of RDF and sewage sludge. The Ni content in seed (Table 1) varied from 1.87 to13.67 mg kg^{-1}. The maximum content of Ni in seed (13.67 mg kg^{-1}) was recorded under the treatment T_{10} and the minimum (1.87 mg kg^{-1}) was observed in T_1. Similarly, the maximum content of Ni in stover (14.73 mg kg^{-1}) was recorded in T_{10} whereas, the minimum (2.93 mg kg^{-1}) was observed in treatment T_1.

Lead (Pb): The Pb content in seed of mustard varied from 0.15 to 2.25 mg kg^{-1}. The highest content of 2.25 mg Pb kg^{-1} was recorded under application of 100% RDF + SS @ 20 t ha^{-1} (T_{10}) and the minimum (0.15 mg kg^{-1}) was under control (Table 1). The values of lead content in mustard stover ranged from 0.47 (control) to 2.53 mg kg^{-1} (100% RDF + SS @ 20 t ha^{-1}).

Cadmium (Cd): The data pertaining to Cd content in seed and stover of mustard have been presented in Table 1. Results showed that the highest content of Cd in seed and stover of mustard (1.57 and 1.97 mg kg^{-1}) were recorded under the treatment T_{10} that were higher by 1.40 mg kg^{-1} over absolute control (T_1) wherein, the minimum values of 0.17 and 0.57 mg kg^{-1} Cd contents were recorded.

Chromium (Cr): like Ni, Pb and Cd, the content of Cr in seed and stover of mustard was also enhanced

Table 1:Effect of sewage sludge and fertilizer application on heavy metal content (mg kg^{-1}) in seed and stover of mustard

Treatments	Ni (mg kg^{-1})		Pb (mg kg^{-1})		Cd (mg kg^{-1})		Cr (mg kg^{-1})	
	Seed	Stover	Seed	Stover	Seed	Stover	Seed	Stover
T1	1.87^{h}	2.93^{g}	0.15^{g}	0.47^{g}	0.17^{h}	0.57^{g}	0.22^{c}	0.43^{e}
T2	2.77^{fgh}	3.83^{efg}	0.55^{ef}	0.83^{fg}	0.37^{fg}	0.77^{fg}	0.24^{c}	0.63^{de}
T3	2.37^{gh}	3.43^{fg}	0.65^{ef}	0.93^{ef}	0.67^{e}	1.07^{de}	0.25^{c}	0.63^{de}
T4	3.07^{fg}	4.13^{efg}	0.45^{f}	0.73^{fg}	0.27^{gh}	0.67^{fg}	0.22^{c}	0.63^{de}
T5	2.27^{gh}	3.33^{fg}	0.55^{ef}	0.83^{fg}	0.37^{fg}	0.77^{fg}	0.26^{c}	0.53^{de}
T6	3.47^{ef}	4.53^{def}	0.75^{e}	1.03^{ef}	0.87^{d}	1.27^{cd}	0.24^{c}	0.73^{d}
T7	4.17^{de}	5.23^{de}	0.75^{e}	9.03^{a}	0.47^{f}	0.87^{ef}	0.26^{c}	0.63^{de}
T8	4.67^{d}	5.73^{d}	1.05^{d}	1.33^{de}	0.97^{d}	1.37^{c}	0.29^{c}	1.03^{c}
T9	11.67^{b}	12.73^{b}	1.85^{b}	2.13^{bc}	1.37^{bc}	1.77^{ab}	0.72^{ab}	2.03^{ab}
T10	13.67^{a}	14.73^{a}	2.25^{a}	2.53^{b}	1.57^{a}	1.97^{a}	0.92^{a}	2.23^{a}
T11	8.77^{c}	9.83^{c}	1.45^{c}	1.73^{cd}	1.27^{c}	1.67^{b}	0.62^{b}	1.83^{b}
T12	9.37^{c}	10.43^{c}	2.05^{ab}	2.33^{b}	1.47^{ab}	1.87^{ab}	0.82^{ab}	2.23^{a}
CV (%)	10.74	13.18	11.76	12.28	10.00	12.10	21.61	15.28

Different letters for each parameters show significant different at p<0.05.

Table 2: Effect of sewage sludge and fertilizer application on heavy metal contents (mg kg-1) in post-harvest soil

Treatments	Heavy Metals (mg kg^{-1})			
	Ni	Pb	Cd	Cr
T_1 (Absolute Control),	2.11^{h}	0.20^{e}	1.15^{g}	0.12^{fg}
T_2 (100 % RDF)	2.29^{fgh}	$0.34d^{e}$	1.29^{f}	0.18^{ef}
T_3 (100% RDF+ S 30 kg ha^{-1})	2.21^{fgh}	0.43^{cde}	1.23^{fg}	0.19^{e}
T_4 (100% RDF+ B 1.5 kg ha^{-1})	2.35^{efg}	0.37^{de}	1.29^{f}	0.11^{g}
T_5 (100% RDF+Zn 5 kg ha^{-1})	2.19^{gh}	0.41^{cde}	1.27^{fg}	0.17^{efg}
T_6 (100%RDF+ S 30 kg ha^{-1} + B 1.5 kg ha^{-1})	2.43^{ef}	0.48^{cde}	1.43^{e}	0.17^{efg}
T_7 (100% RDF + Zn 5 kg ha^{-1} + B 1.5 kg ha^{-1})	2.57^{de}	0.53^{cd}	1.35^{ef}	0.22^{de}
T_8 (100% RDF+ S 30 kg ha^{-1} + Zn 5 kg ha^{-1}+ B 1.5 kg ha^{-1})	2.67^{d}	0.68^{c}	1.63^{d}	0.28^{cd}
T_9 (100% RDF + SS 10 t ha^{-1})	4.07^{b}	1.28^{ab}	2.23^{b}	0.39^{ab}
T_{10} (100% RDF + SS 20 t ha^{-1})	4.47^{a}	1.44^{a}	2.37^{a}	0.42^{a}
T_{11} (75% RDF + SS 10 t ha^{-1})	3.49^{c}	1.12^{b}	2.07^{c}	0.34^{bc}
T_{12} (75% RDF + SS 20 t ha^{-1})	3.49^{c}	1.12^{b}	2.07^{c}	0.34^{bc}
CV (%)	4.70	24.57	4.90	15.95

Different letters for each parameters show significant different at $p<0.05$.

significantly due to different treatments (Table 1). Increase in level of Cr was observed higher under higher levels of sewage sludge applied treatments. Application of 100% RDF in conjunction with sewage sludge @ 20 t ha^{-1} registered the highest content of 0.92 mg kg^{-1} Cr in seed and 2.23 mg kg^{-1} in stover of mustard. However, it showed statistical equivalence with treatment T_{12} in enhancing Cr content in seed and stover (0.82 and 2.23 mg kg^{-1}). The treatments T_9 and T_{11} also witnessed significantly higher values of chromium content in seed and stover of mustard in comparison to control.

The increase in heavy metal contents of mustard seed and stover might be directly associated with the application of sewage sludge. The application of sewage sludge might have enhanced the content of heavy metals in plant parts, due to higher amount of heavy metals in it (Rattan *et al*., 2005). The suitability of sludge application to agricultural land depends on extractable trace element like Ni, Cd, Pb and Cr to a great extent. There was significant increase in Ni, Cd, Pb and Cr content in plant stover and seed due to sludge amended soil over control. Several researchers also reported that sludge application increased the extractable metal and metalloid content in soil (Karami *et al*., 2009; Ray *et al*., 2013; Golui *et al*., 2014; Latare *et al*., 2014;).

Effect on heavy metal contents (Ni, Pb, Cd and Cr) in post-harvest soil

The DTPA extractable Ni, Pb, Cd and Cr increased in post harvest soil with different levels of RDF with conjoint application of sewage sludge at varying levels. The content of Ni, Pb, Cd and Cr in soil ranged from 2.11 to 4.47, 0.20 to 1.44, 1.15 to 2.37 and 0.12 to 0.42 mg kg^{-1}, respectively. The higher content of heavy metals in post harvest soil was observed under the treatments receiving higher levels of sewage sludge application. The application of sewage sludge @ 20 t ha^{-1} along with 100% RDF resulted in the highest content of 4.47, 1.44, 2.37 and 0.42 mg kg^{-1} of Ni, Pb, Cd and Cr, respectively in post harvest soil, that were 111.8, 620.0, 106.1 and 250 percent higher over control, respectively. Application of 100% RDF + SS @ 10 t ha^{-1} (T_9) also recorded 92.9, 540.0, 93.9 and 225 percent higher contents of Ni, Pb, Cd and Cr over control and thus found at par with treatment T_{10}. Application of sewage sludge either at 10 or 20 t ha^{-1} combined with 75% RDF also resulted significant enhancement in heavy metal contents in post harvest soil.

The increase in DTPA extractable heavy metals in post harvest soil might be due to direct addition of metal rich sewage sludge application in soil. The application of sludge might have enhanced the inherent supplying capacity of the soil with respect to heavy metal in extractable form as a result of sludge application (Korboulewsky *et al.,* 2002). There was significant increase in Ni, Cd, Pb and Cr content in sludge content in sludge amended soil over control. Several researchers also reported that sludge application increased the extractable metal and metalloid content in soil (Ray *et al*., 2013; Golui *et al*., 2014; Latare *et al*., 2014; Latare *et al*., 2017).

Yield and harvest index

Results presented in table 3 which clearly showed that seed and stover yield of mustard was significantly influenced due to application of varying levels of boron,

Table 3: Effect of sewage sludge and fertilizer application on seed yield, stover yield and harvest index of mustard

Treatments	Seed Yield (g pot^{-1})	Stover Yield (g pot^{-1})	Harvest Index (%)
T_1 (Absolute Control),	11.43^{g}	35.70^{g}	24.24^{a}
T_2 (100 % RDF)	13.30^{f}	42.37^{f}	23.89^{a}
T_3 (100% RDF+ S 30 kg ha^{-1})	16.43^{de}	48.18^{de}	25.43^{a}
T_4 (100% RDF+ B 1.5 kg ha^{-1})	14.87^{ef}	46.46^{e}	24.23^{a}
T_5 (100% RDF+Zn 5 kg ha^{-1})	14.93^{ef}	47.24^{e}	24.02^{a}
T_6 (100%RDF+ S 30 kg ha^{-1}+ B 1.5 kg ha^{-1})	16.97^{cd}	50.72^{cd}	25.07^{a}
T_7 (100% RDF + Zn 5 kg ha^{-1}+ B 1.5 kg ha^{-1})	16.63^{cd}	49.53^{cde}	25.12^{a}
T_8 (100% RDF+ S 30 kg ha^{-1}+Zn 5 kg ha^{-1}+ B 1.5 kg ha^{-1})	18.97^{b}	54.67^{b}	25.82^{a}
T_9 (100% RDF+ SS 10 t ha^{-1})	18.20^{bc}	54.17^{b}	25.15^{a}
T_{10} (100% RDF+ SS 20 t ha^{-1})	20.90^{a}	60.49^{a}	25.71^{a}
T_{11} (75% RDF+ SS 10 t ha^{-1})	17.53^{bcd}	52.90^{bc}	24.88^{a}
T_{12} (75% RDF+ SS 20 t ha^{-1})	19.13^{b}	55.82^{b}	25.49^{a}
CV (%)	6.01	4.06	4.80

Different letters for each parameters show significant different at $p<0.05$.

zinc, sulphur and sewage sludge. The maximum seed and stover yields (20.90 and 60.49 g pot^{-1}) were found in treatment T_{10} that were 82.9 % and 69.4 % higher in comparison to control, respectively. Treatment T_{12} recorded the seed and stover yields of 19.13 and 55.82 g pot^{-1} , respectively. Which were higher by 67.4 and 56.3 percent over unfertilized control, respectively. However, these two treatments were found statistically at par with each other. Being at par among themselves, T_8, T_9 and T_{11} also increased the seed yield of mustard by 66.0, 59.2 and 53.4 percent, respectively over control and found the next superior treatments. The corresponding increase in stover yield was 53.1, 51.7 and 48.2 percent. The lowest seed and stover yield was found in absolute control (T_1) with 11.43 and 35.70 g pot^{-1}, respectively. The maximum value of harvest index (25.82%) was obtained under the treatment T_8 and was closely followed by T_{10} (25.71%), T_{12} (25.49%) and T_9 (25.15%), respectively.

This increase in yield might be due to the fact that optimal and balanced supply of nutrients from sewage sludge along with application of sulphur, zinc and boron led to higher growth and development of plants which ultimately resulted in higher seed and stover yield of mustard as compared to control and chemical fertilization, alone (Tripathi *et al.* (2011). Moreover, integrated use of sewage sludge and inorganic fertilizers improved the physical, chemical and biological health of soil, which further provide an optimum environment for higher growth and development of plants and led to higher yield of crop (Indoria *et al.* (2013). Latare and Singh, (2013) also recorded significant increase in seed and stover yield with conjoint application of sewage sludge and fertilizers.

Conclusion

Addition of sewage sludge to the soil along with recommended dose of fertilizers significantly improved the seed and stover yield of mustard and harvest index but the higher doses of sewage sludge addition increased the heavy metal contents in seed and stover of mustard and post harvest soil. This warrants a need to apply lower doses of sewage sludge in combination with chemical fertilizers for sustaining productivity of crops and avoiding food chain contamination. Application of sewage sludge is good for improving crop production and build-up of nutrients and organic matter in soil but a regular monitoring of metals build-up in soil and plant parts should be taken into consideration while its regular use.

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