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Vipin Kumar
Department of Soil Science and Agricultural Chemistry, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India

Pramod Kumar Sharma
Department of Soil Science and Agricultural Chemistry, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India

Correspondence
Vipin Kumar
Department of Soil Science and Agricultural Chemistry, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India

Augmentation of nitrogen and phosphorous mineralization in chromium contaminated soils using organic amendments

Vipin Kumar and Pramod Kumar Sharma

Abstract
Due to wide industrial use of chromium, it is considered a serious environmental pollutant. An incubation experiment was conducted to study the nitrogen and phosphorous mineralization in chromium contaminated soils treated by organic amendments in Department of Soil Science and Agricultural Chemistry, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi during 2016-17. Five levels of chromium viz. 0, 20, 40, 60 and 80 ppm with and without three organic amendments viz. Vermicompost @ 5 ton ha^{-1}, Farm Yard Manure @ 10 ton ha^{-1} and Sewage Sludge @ 20 ton ha^{-1} treatment were applied in the experiment. Result showed that N and P mineralization significantly decreased as level of chromium increased, while application of organic amendments significantly increased the N and P mineralization as compared to their respective chromium treatment. Maximum and significantly higher increment in N and P mineralization was found with the application of vermicompost @ 5 ton ha^{-1} followed by farm yard manure @ 10 ton ha^{-1}, followed by sewage sludge @ 20 ton ha^{-1}.

Keywords: mineralization, chromium, organic amendments, vermicompost, FYM, sewage sludge, nitrogen and phosphorous

1. Introduction

Among non-essential elements, chromium is of primary concern due to its toxic effects on plants and humans (Rehman *et al.*, 2017) [24]. In the past decades, the increased use of chromium (Cr) in industrial activities i.e. electroplating and leather tanning, hexavalent chromium (Cr(VI)) has been widely detected in soil (Su *et al.*, 2016) [20]. As a result, Cr toxicity is now a major threat to agricultural land and water bodies (Choudhary *et al.*, 2012). Chromium (VI) is toxic to agronomic plants at concentration of 0.5 to 5.0 mg mL^{-1} in nutrient solution and 5–100 mg kg^{-1} in soil (Ali *et al.*, 2013; Anderson, 2003; Chrysochoou *et al.*, 2012; Wilson and Pyatt, 2007) [6, 10, 23]. The mobility and toxicity of Cr^{6+} can be reduced by converting it to the reduced state of Cr^{3+} by means of organic matter and inorganic reducing agents in the soil (Aceves *et al.*, 2007) [1]. These Organic sources may be organic manures, green manure, rural wastes, crop residues, biofertilizers and vermicompost. The positive effect of vermicompost application on soil properties is well documented and established (Kumar *et al.*, 2018) [13]. The immobilizing effect of organic amendments are thought to act through various complex processes *e.g.* formation of stable compounds with organic ligands, surface precipitation and ion exchange (Kumpiene *et al.*, 2008; Ahmad *et al.*, 2011) [18, 14]. Moreover organic amendments may enhance the soil fertility and microbial activity, leading to the amelioration of the soil quality as a whole. These overall modifications generally decrease the mobility and the bioavailability of trace elements, even if temporarily and thus promote the reestablishment of vegetation and increase plant growth (Madejon *et al.*, 2006; Branzini and Zubillaga, 2012) [15, 8]. The effect of organic amendments on the mobility and the bioavailability of metal(loid)s depends on the nature of the organic matter itself, its microbial degradability, its effects on soil chemical and physical proprieties, as well as on the particular soil type and metal(loid)s concerned (Angelova *et al.*, 2013) [7]. Immobilization of metals in contaminated soils using amendments is a remediation technique that decreases mobility and phytoavailability of metals in the soils and their uptake by plants (Sabir *et al.*, 2013; Rizwan *et al.*, 2016; Rehman *et al.*, 2017) [17, 18]. There is scarcity of research related to the effects of organic amendments on N and P mineralization in chromium contaminated soils even mineralization study is also lacking in work related to the heavy metal stress.

2. Materials and Methods

An incubation study was done in Soil Health and Plant Nutrition Lab, Department of Soil Science and Agricultural Chemistry, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi during 2016-17. The Institute of Agricultural Sciences of the Banaras Hindu University is located at a distance of about nine km from Varanasi Railway Station in the South-East part of Varanasi city, which lies in the North-East plain zone of Eastern Uttar Pradesh. Varanasi is situated at an altitude of 76.616 meters above mean sea level and located between $25^{0}18'$ North latitude and $80^{0}30'$ East longitude which enjoys a subtropical climate and is subjected to extremes of weather conditions *i.e.* extremely hot summer and cold winter having semi-arid to sub humid type of climate with moister deficit index between 20- 40.

In incubation study, bulk of alluvial soils were brought from research farm of Institute of Agricultural Sciences, BHU, processed and sieved through 2mm sieve. About hundred gram of soil was taken in plastic cups and five levels of chromium viz. 0, 20, 40, 60 and 80 ppm without and with three organic amendments viz. sewage sludge @ 20 ton ha^{-1}, farm yard manure (FYM) @ 10 ton ha^{-1} and vermicompost (VC) @ 5 ton ha^{-1} were applied as per treatment. $K_2Cr_2O_7$ solution was used to supply appropriate amount of chromium. The soil samples were taken for N and P mineralization study at 0, 15, 30, 60, 90 and 120 days interval. Moisture content of the incubated samples was maintained at field capacity. The treatments consists of T_1-Control, T_2-20 ppm Cr, T_3-40 ppm Cr, T_4-60 ppm Cr, T_5-80 ppm Cr, T_6- 0 ppm Cr + Sl, T_7-20 ppm Cr + Sl, T_8-40 ppm Cr + Sl, T_9-60 ppm Cr + Sl, T_{10}-80 ppm Cr + Sl, T_{11}- 0 ppm Cr + FYM, T_{12}-20 ppm Cr + FYM, T_{13}-40 ppm Cr + FYM, T_{14}-60 ppm Cr + FYM, T_{15}-80 ppm Cr + FYM, T_{16}- 0 ppm Cr + VC, T_{17}-20 ppm Cr + VC, T_{18}-40 ppm Cr + VC, T_{19}-60 ppm Cr + VC and T_{20}-80 ppm Cr + VC. The physico-chemical properties of soil, sewage sludge, vermicompost and farm yard manure are depicted in table 1.

Available nitrogen was determined by the method given by Subbiah & Asija (1956) [19], where 5 gram air dried soil was taken into a Kjeldahl tube; 25 ml 0.32 % $KMnO_4$ and 2.5% NaOH each were added. This was connected to automated KEL PLUS distillation unit and liberated ammonia was swiped and collected into 20 ml of 2% Boric acid. The blue colour of boric acid was titrated with 0.02N H_2SO_4 to its original brick red colour. A blank titration was also carried out for calculation.

Available phosphorous was determined by the method given by Olsen *et al.* (1954) [16], in this method phosphorous was extracted with 0.5N $NaHCO_3$ (pH 8.5). Initially reagent A was prepared by ammonium molybdate, antimony potassium tartarate and H_2SO_4. Then reagent B was prepared by using reagent A and ascorbic acid. 2.5 g soil was taken into a 150 ml conical flask, a pinch of Darco G-60 (Activated Charcoal) and 50 ml of Olsen's reagent (0.5N $NaHCO_3$) were added and contents of flask was shaken for 30 minute on a mechanical shaker. It was filtered through a Whatman No. 1 filter paper. 5 ml of aliquot was transferred into a 25 ml volumetric flask and acidified by 5 N H_2SO_4 to pH 5.0. Than 10 ml distilled water and 4 ml of reagent B were added and volume was made up to 25 ml with distilled water. The intensity of blue colour was measured spectrophotometrically at 660 nm.

For determining the significance between the treatment means and to draw valid conclusion, statistical analysis was made. The raw data observed during the whole experiment, were subjected to statistical analysis by adopting appropriate method of "Analysis of Variance". The significance of the treatment effect was judged with the help of 'F' test (Variance ratio). The difference of the treatments mean was tested using critical difference (CD) at 1% level of probability (Gomez and Gomez, 1984) by following the Complete Randomized Design (CRD) to draw the valid differences among the treatments.

3. Result and Discussion

3.1 N mineralization

3.1.1 N mineralization at different time interval

The data associated with the effects of organic amendments on N mineralization in chromium contaminated soils are presented in Table 2. It was exhibited that application of organic amendments in chromium contaminated soil significantly affected N mineralization. N mineralization in all treatments ranged from 110.94 to 30.37 mg kg^{-1}, 242.39 to 46.56 mg kg^{-1}, 381.68 to 73.51 mg kg^{-1}, 564.88 to 106.48 mg kg^{-1}, 766.37 to 146.60 mg kg^{-1} and 1049.92 to 199.79 mg kg^{-1} at 0, 15, 30, 60, 90 and 120 days respectively. It was found that the application of vermicompost @ 5 ton ha^{-1} (T_{16}) recorded significantly highest value of N mineralization 110.94 mg kg^{-1}, 242.39 mg kg^{-1}, 381.68 mg kg^{-1}, 564.88 mg kg^{-1}, 766.37 mg kg^{-1} and 1049.92 mg kg^{-1}, at 0, 15, 30, 60, 90 and 120 days, respectively. However, application of farm yard manure and sewage sludge was found next better treatment in N mineralization at all stages. Treatment with farm yard manure was at par with the vermicompost in treatments having 40 ppm or more chromium. Among all the treatments the lowest value of N mineralization 30.37 mg kg^{-1}, 46.56 mg kg^{-1}, 73.51 mg kg^{-1}, 106.48 mg kg^{-1}, 146.60 mg kg^{-1} and 199.79 mg kg^{-1} at 0, 15, 30, 60, 90 and 120 Days, respectively was observed with treatment T_5 (80 ppm Chromium). Application of vermicompost @ 5 ton ha^{-1} farm yard manure @ 10 ton ha^{-1} and sewage sludge @ 20 ton ha^{-1} found significantly superior with their respective chromium treatments.

Further study of data confirmed that N mineralization significantly increased with increasing incubation period and decreased with increasing level of chromium (20-80 ppm) in all the treatments.

3.1.2 Net N Mineralization

The data associated to the effects of organic amendments on net N mineralization in chromium contaminated soils are presented in table 4 exhibited significant difference values of net N mineralization with application of different organic amendments in chromium contaminated soil. Net N mineralization ranged from 938.98 mg kg^{-1} to 169.41 mg kg^{-1} in all treatments. It was found that the application of vermicompost @ 5 ton ha^{-1} (T_{16}) recorded significantly highest value of net N mineralization 938.98 mg kg^{-1} among all treatment. Net N mineralization in farm yard manure treatment was at par with the vermicompost in treatment having 40 ppm or more chromium while sewage sludge treatment was at par with the FYM treatment in treatments having more than 40 ppm chromium. However significant difference was observed in both the cases till 20 ppm chromium treatments. Among all the treatments the lowest value of net N mineralization 169.41 mg kg^{-1} was observed with treatment T_5 (80 ppm Chromium). Application of vermicompost @ 5 ton ha^{-1}, farm yard manure @ 10 ton ha^{-1} and sewage sludge @ 20 ton ha^{-1} found significantly superior with their respective chromium (20-80ppm) treatments.

It was also noticed that increasing levels of chromium significantly decreased net N mineralization in all the treatments.

3.2 P mineralization

3.2.1 P mineralization at different time interval

The data related to the effects of organic amendments on P mineralization in chromium contaminated soils are presented in Table 3. It is clear from the data that application of organic amendments in chromium contaminated soil significantly influenced P mineralization. P mineralization ranged from 19.55 to 7.91 mg kg^{-1}, 23.52 to 8.37 mg kg^{-1}, 28.43 to 8.82 mg kg^{-1}, 34.05 to 9.19 mg kg^{-1}, 41.19 to 9.68 mg kg^{-1} and 48.08 to 10.50 mg kg^{-1} at 0, 15, 30, 60, 90 and 120 days, respectively. It was found that the application of vermicompost @ 5 ton ha^{-1} (T_{16}) recorded significantly highest value of P mineralization 19.55 mg kg^{-1}, 23.52 mg kg^{-1}, 28.43 mg kg^{-1}, 34.05 mg kg^{-1}, 41.19 mg kg^{-1} and 48.08 mg kg^{-1} at 0, 15, 30, 60, 90 and 120 days, respectively. However, P mineralization in farm yard manure and sewage sludge treated treatments was at par in at all stages. While P mineralization in FYM treatments was significantly lower than vermicompost and significantly higher than sewage sludge treatment at same level of chromium. Among all the treatments the lowest value of P mineralization 7.91 mg kg^{-1}, 8.37 mg kg^{-1}, 8.82 mg kg^{-1}, 9.19 mg kg^{-1}, 9.68 mg kg^{-1} and 10.50 mg kg^{-1} was observed at 0, 15, 30, 60, 90 and 120 days, respectively in T_5 treatment (80 ppm Chromium). Further study of data supported that P mineralization significantly increased with increasing incubation period. It was also seen that P mineralization decreased with increasing levels of chromium in all the treatment.

3.2.2 Net P Mineralization

The data concerned with the effects of organic amendments on Net P mineralization in chromium contaminated soils is presented in Table 4. It is clear from the data that application of organic amendments in chromium contaminated soil significantly affected Net P mineralization. Net P mineralization ranged from 28.53 mg kg^{-1} to 2.59 mg kg^{-1}. It was found that the application of vermicompost @ 5 ton ha^{-1} (T_{16}) recorded significantly highest value of net P mineralization 28.53 mg kg^{-1}. Application of FYM and sewage sludge was found next better treatment. Net P mineralization in FYM treatment was significantly lower than vermicompost and significantly higher than sewage sludge treatment at same level of chromium. Among all the treatments the lowest value of net P mineralization 2.59 mg kg^{-1} was observed with treatment T_5 (80 ppm Chromium). Application of vermicompost @ 5 ton ha^{-1} farm yard manure @ 10 ton ha^{-1} and sewage sludge @ 20 ton ha^{-1} found significantly superior with their respective chromium treatments.

Aceves *et al*., (2007) [1] suggested that Cr^{6+} added alone increased the inhibition of nitrification in soil outside the canopy followed by under the canopy and cultivated soils. After 180 days incubation, the N was immobilized in under the canopy and cultivated soils amended with tannery sludge plus Cr^{3+} or Cr^{6+} and under the canopy plus Cr^{3+} alone. Soils outside the canopy showed a high inhibition of nitrification with Cr^{6+} added alone or Cr^{6+} plus tannery sludge. Alcantara *et al*. (2007) reported that compared to liming sludge (LCL), the addition of comprising very low chromium contents and a primary sludge (LCR) caused a decrease in the percentages of the accumulated mineralized nitrogen from sludge, indicating N mineralization inhibition, possible due to the high chromium content of that sludge. Vasquez-Murrieta *et al*. (2006) [22] observed that there was a significant negative correlation between microbial biomass, soil organic carbon, total N and C mineralization and the heavy metal content of the soil.

The inhibition and stimulation of nitrification throughout the incubation in soils under the canopy treated with Cr^{6+} alone or Cr^{6+} plus tannery sludge might suggest that Cr^{6+} may affect the soil microbial biomass and subsequently stimulation or inhibition of inorganic mineralization. (Dascoli *et al*., 2004; Aceves *et al*., 2007) [11, 1].

Result has also revealed that application of organic amendments significantly increased the N and P mineralization as compared to their respective chromium treatment. Maximum and significantly higher increment in N and P mineralization was found with the application of vermicompost @ 5 ton ha^{-1} followed by farm yard manure @ 10 ton ha^{-1}, followed by sewage sludge @ 20 ton ha^{-1}. Alburquerque *et al*. (2011) [3] found that heavy metal contamination can also affect negatively soil processes mediated by microorganisms, including nitrification. In their study, compost addition to the acidic soil particularly at the highest application rate had a positive effect on nitrification, leading to a significant increase in nitrate concentration at the end of the incubation period compared to the control soil. This should be related to the reduced stress/toxicity for the microorganisms. Vallini *et al*. (1997) [21] also noted the positive effect of humic acids from compost on nitrifying bacteria.

In present study, the increase in N and P mineralization might be due to positive effect of organic amendments on population and activity of microorganisms in soil as organic matter serve as the primary source of carbon and energy for microbes. Secondly organic amendments alleviate chromium toxicity to microbes by decreasing bioavailability of chromium.

4. Conclusion

It was observed that chromium has negative impact on N and P mineralization in soils. N and P mineralization in soil decreased significantly as level of chromium increased. Organic amendments found better option to mitigate chromium toxicities on N and P mineralization as they supply organic matter which enhances microbial population and simultaneously reduces the toxicity of chromium by decreasing availability of chromium in soil. Among the organic amendments vermicompost exhibited superior efficacy in alleviating the effect of chromium.

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Table 1: Physico-chemical properties of the initial soil, vermicompost, sewage sludge and farm yard manure

Parameter	Initial Soil	Sewage Sludge	FYM	Vermicompost
pH_w (1:2.5)	7.86	6.55	6.74	7.17
EC_w (1:2.5) (dS/m)	0.13	2.48	3.73	6.18
Organic Carbon (%)	0.43	8.65	9.63	11.26
Available Content (mg kg^{-1})				
N	70	1195	476	537
P	15	1210	490	550
K	104	4322	3288	1258
S	15	54	28	415
Total Content (%)				
N	-	1.54	0.48	1.88
P	-	1.26	0.24	1.04
K	-	0.84	0.46	1.82
S	-	0.98	0.03	0.42
DTPA Extractable (mg kg^{-1})				
Cr	0.39	9.94	1.31	1.43
Fe	42.65	70.65	118.53	159.42
Mn	14.96	31.21	80.21	116.22
Cu	1.74	23.08	6.83	13.16
Zn	1.38	25.14	14.22	16.12

Table 2. Effect of organic amendments on Nitrogen mineralization (mg kg^{-1}) in chromium contaminated soils

Treatment	0 Day	15 Days	30 Days	60 Days	90 Days	120 Days
T_1-Control	84.37	171.77	270.48	400.31	543.10	744.04
T_2-20 ppm Cr	67.50	137.42	216.38	320.25	434.48	595.23
T_3-40 ppm Cr	53.15	103.06	162.29	240.19	325.86	446.42
T_4-60 ppm Cr	42.19	71.51	113.60	168.13	228.10	312.50
T_5-80 ppm Cr	30.37	46.56	73.51	106.48	146.60	199.79
T_6- 0 ppm Cr + Sl	97.68	213.42	336.06	497.38	674.78	924.45
T_7-20 ppm Cr + Sl	78.63	175.01	275.57	407.85	553.32	758.05
T_8-40 ppm Cr + Sl	62.03	134.76	212.19	314.04	426.06	583.70
T_9-60 ppm Cr + Sl	49.33	97.02	152.78	226.11	306.76	420.26
T_{10}-80 ppm Cr + Sl	35.65	61.67	101.36	149.83	202.20	278.24
T_{11}- 0 ppm Cr + FYM	104.22	227.71	358.57	530.68	719.97	986.35
T_{12}-20 ppm Cr + FYM	84.42	189.00	297.61	440.47	597.57	818.67
T_{13}-40 ppm Cr + FYM	66.70	147.42	232.14	343.56	466.11	638.56
T_{14}-60 ppm Cr + FYM	53.15	107.62	169.46	250.80	340.26	466.15
T_{15}-80 ppm Cr + FYM	38.56	70.51	113.90	169.21	228.71	314.31
T_{16}- 0 ppm Cr + VC	110.94	242.39	381.68	564.88	766.37	1049.92
T_{17}-20 ppm Cr + VC	90.42	201.18	316.79	468.85	636.08	871.43
T_{18}-40 ppm Cr + VC	71.56	156.92	247.10	365.70	496.15	679.72
T_{19}-60 ppm Cr + VC	57.13	114.55	180.38	266.96	362.19	496.19
T_{20}-80 ppm Cr + VC	41.60	75.56	120.32	180.87	245.08	335.41
SEm±	1.59	3.20	4.76	7.07	9.38	13.71
CD (P=0.01)	6.07	12.26	18.22	27.05	35.88	52.44

Cr = Chromium, Sl = Sewage Sludge, FYM = Farm Yard Manure, VC = Vermicompost, CD = Critical Difference, SEm± = Standard error of mean

Table 3: Effect of organic amendments on Phosphorous mineralization (mg kg^{-1}) in chromium contaminated soils

Treatment	0 Day	15 Days	30 Days	60 Days	90 Days	120 Days
T_1-Control	14.27	15.05	15.90	16.63	17.56	18.86
T_2-20 ppm Cr	12.95	13.66	14.43	15.08	15.92	17.12
T_3-40 ppm Cr	10.85	11.46	12.10	12.63	13.33	14.37
T_4-60 ppm Cr	8.60	9.10	9.59	10.00	10.54	11.41
T_5-80 ppm Cr	7.91	8.37	8.82	9.19	9.68	10.50
T_6- 0 ppm Cr + Sl	15.95	18.40	21.31	24.43	28.31	33.11
T_7-20 ppm Cr + Sl	14.64	16.90	19.56	22.42	25.98	30.41
T_8-40 ppm Cr + Sl	12.53	14.47	16.74	19.19	22.22	26.04
T_9-60 ppm Cr + Sl	10.36	11.97	13.84	15.85	18.34	21.54
T_{10}-80 ppm Cr + Sl	7.88	9.12	10.53	12.04	13.91	16.41
T_{11}- 0 ppm Cr + FYM	17.69	21.30	25.74	30.81	37.27	43.53
T_{12}-20 ppm Cr + FYM	16.42	19.77	23.88	28.59	34.58	40.40
T_{13}-40 ppm Cr + FYM	14.27	17.18	20.76	24.83	30.03	35.12
T_{14}-60 ppm Cr + FYM	12.01	14.47	17.47	20.89	25.25	29.57
T_{15}-80 ppm Cr + FYM	9.67	11.66	14.06	16.80	20.30	23.82
T_{16}- 0 ppm Cr + VC	19.55	23.52	28.43	34.05	41.19	48.08

T_{17}-20 ppm Cr + VC	18.25	21.97	26.55	31.79	38.46	44.90
T_{18}-40 ppm Cr + VC	16.13	19.42	23.47	28.09	33.97	39.69
T_{19}-60 ppm Cr + VC	13.92	16.76	20.25	24.22	29.29	34.25
T_{20}-80 ppm Cr + VC	11.51	13.87	16.74	20.02	24.20	28.34
SEm±	0.32	0.43	0.47	0.48	0.61	0.66
CD (P=0.01)	1.21	1.66	1.81	1.85	2.32	2.54

Cr = Chromium, Sl = Sewage Sludge, FYM = Farm Yard Manure, VC = Vermicompost, CD = Critical Difference, SEm± = Standard error of mean

Table 4: Effect of organic amendments on Net Nitrogen and Phosphorous mineralization (mg kg^{-1}) in chromium contaminated soils

Treatment	Nitrogen	Phosphorous
T_1-Control	659.67	4.59
T_2-20 ppm Cr	527.74	4.17
T_3-40 ppm Cr	393.27	3.52
T_4-60 ppm Cr	270.31	2.81
T_5-80 ppm Cr	169.41	2.59
T_6- 0 ppm Cr + Sl	826.77	17.17
T_7-20 ppm Cr + Sl	679.42	15.77
T_8-40 ppm Cr + Sl	521.67	13.51
T_9-60 ppm Cr + Sl	370.93	11.19
T_{10}-80 ppm Cr + Sl	242.59	8.53
T_{11}- 0 ppm Cr + FYM	882.13	25.83
T_{12}-20 ppm Cr + FYM	734.25	23.98
T_{13}-40 ppm Cr + FYM	571.86	20.85
T_{14}-60 ppm Cr + FYM	413.00	17.56
T_{15}-80 ppm Cr + FYM	275.75	14.15
T_{16}- 0 ppm Cr + VC	938.98	28.53
T_{17}-20 ppm Cr + VC	781.02	26.65
T_{18}-40 ppm Cr + VC	608.16	23.56
T_{19}-60 ppm Cr + VC	439.06	20.34
T_{20}-80 ppm Cr + VC	293.81	16.83
SEm±	12.12	0.35
CD (P=0.01)	46.37	1.32

Cr = Chromium, Sl = Sewage Sludge, FYM = Farm Yard Manure, VC = Vermicompost, CD = Critical Difference, SEm± = Standard error of mean

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