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***Full Length Article***

# Response of Soil Microbial Biomass and Enzymatic Activity in Municipal Solid Waste Compost Treated Soil

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## Abstract

Municipal solid waste compost (MSWC) can be valued in agricultural soils of Pakistan due to its many favorable properties, particularly as an organic fertilizer and soil amendment. However, higher concentrations of trace metals present in MSWC can limit its use as an organic amendment in agriculture. High concentration of Cd and Pb can alter soil microbial biomass carbon and nitrogen (SMB C and N) and enzymatic activities such as soil dehydrogenase activity (DHA). Therefore, a study was conducted to evaluate the effect of MSWC derived trace metals on SMB (C and N) and DHA. It was observed that Cd and Pb contents increased with increasing compost rates and the highest increase was observed by the application of MSWC at 20 t ha$^{-1}$. The soils amended with MSWC after 60 days incubation led to a substantial reduction in Cd (37%) and Pb (42%) concentration in soil. The MSWC applied at 20 t ha$^{-1}$ showed the highest values of SMB C (21%), N (111%) and DHA (25%) over control. The results showed MSWC soil application did not negatively affect the SMB (C and N) and soil dehydrogenase enzyme activity. 

**Keywords:** Compost; Cd; Pb; Microbes; Soil dehydrogenase activity; Incubation

## Introduction

Municipal solid waste (MSW) is mainly collected from public institutions, commerce households and various municipal areas (Sharifi and Renella, 2015). The MSW composition is mainly comprised of wood 6%, textile 9%, plastic 13%, yard timings 14%, food 15%, papers 27%, glass 4%, metals 9% and others 3% (US-EPA, 2013).

According to a recent estimate, the annual global generation of MSW is 1.3 billion tons (World Bank, 2012). In the coming twenty years, generation of MSW will likely to be doubled with the increasing world population particularly in low-income countries, leading to the generation of 2.2 billion tons of MSW per year in 2025 (Hoornweg and Bhada-Tata, 2012). In Pakistan, 59000 tons of MSW are being generated per days and growth rate of waste generation is 2.4 percent/year. The rate of waste generation varies from 0.283 to 0.613 kg/capita/days or from 1.896 to 4.290 kg/house/days (Pak-EPA, 1996).

The use of MSWC in soil as a conditioner is a good recycling practice due to its high nutrient availability and its various beneficial effects on physicochemical properties of soil (Mylavarapu and Zinati, 2009; Weber *et al*., 2014). The MSWC is very useful in nutrient depleted soils because it can improve plant growth and nutrient mineralization (Hargreaves *et al*., 2008). The use of MSWC improves SMB C and N (Margesin *et al*., 2006) by inducing changes in structure of the microbial community in soil (Saison *et al*., 2006).

However, it is also reported that soil application of MSWC, due to the presence of contaminants, could adversely affect soil health, growth of plants and ground water quality and ultimately human, animal and soil organism's health (Gomes *et al*., 2010).

The major concern regarding the use of MSWC is the presence of Cd and Pb (Bouzaiane *et al*., 2014; Alvarenga *et al*., 2015). Heavy metals toxicity can disturb soil microbial ecology because of population loss, changes in the structure, and alterations in microbial composition (Rajapaksha *et al*., 2004). The morphology, growth, and metabolism of soil microorganisms are adversely affected by toxic metals via denaturing of protein, functional disorder or damage of cell membrane (Bhattacharyya *et al*., 2003). So, heavy metals present in soil could decrease the SMB and damage the soil ecosystem. Therefore, regular monitoring of heavy metals accumulation in the soil-plant system due to the application of MSWC should be done if used on repetition basis (Riaz *et al.*, 2018). Moreover, there are a number of mechanisms through which microbes affect metals mobility in soil. A metal sink is provided by microbial biomass through biosorption to cell walls, extracellular polysaccharides, pigments, intracellular accumulation, or accumulation of metal in and around cells (Fomina *et al.*, 2008).

Data on toxic effect of Cd and Pb on soil microbes and their processes in MSWC amended soils, particularly in Pakistan, is scarce. This study was carried out to evaluate the impact of heavy metals (Cd and Pb) present in MSWC on SMB (C and N) and DHA under the alkaline calcareous soil.

## Materials and Methods

The soil in bulk was collected from the research area, University of Agriculture Faisalabad and the MSW compost was acquired from Lahore compost Pvt. Ltd., Lahore, Pakistan. Soil and MSWC samples were air-dried, sieved (mesh size 2 mm) and stored for further study. Compost and soil samples were examined for basic physicochemical properties. The U.S. Salinity Laboratory Staff (1954) analytical methods were followed. The textural class of soil was sandy clay loam with saturation percentage 29.13%, $pH_w$ (1:10) 7.7, EC (1:10) 2.01 dS $m^{-1}$, organic matter 0.68%, total carbon 8.4 g $kg^{-1}$ and total nitrogen 0.7 g $kg^{-1}$. The total concentrations of Cd and Pb were 1.53 and 17.62 mg $kg^{-1}$ soil, respectively. Similarly, MSWC was also analyzed for $pH_w$ (1:10) 7.19, EC (1:10) 3.51 dS $m^{-1}$, organic matter 23.3%, total carbon 133 g $kg^{-1}$, total nitrogen 9.1 g $kg^{-1}$ and total concentrations of Cd (6.50 mg $kg^{-1}$) and Pb (370 mg $kg^{-1}$).

Soil was amended with MSWC at 0, 2.5, 5, 7.5 and 10 g $kg^{-1}$ which are equal to 0, 5, 10, 15 and 20 t $ha^{-1}$, respectively. The control and compost amended soil samples were incubated in plastic containers for 60 days. The soil moisture content was maintained at 45% of the water holding capacity (WHC) and the containers were kept at room temperature (25ºC). For maintaining approximately constant WHC during incubation, each container was periodically weighed and any weight loss was made up with distilled water. The soil samples were collected after 0, 15, 30 and 60 days of aging.

Ammonium bicarbonate-diethyltriaminepentaacetic acid (AB-DTPA) extractable Cd and Pb concentrations were analyzed with an atomic absorption spectrophotometer (Thermo AA, Solar-Series) (Soltanpour, 1985).

Fumigation-extraction method (Brookes *et al*., 1985) was used to determine SMB C and N from soil samples. 10 g (moist) soil sample was fumigated at 25ºC for 24 h with chloroform ($CHCl_3$). Then fumigants were removed and soil samples were extracted with 40 mL of 0.5 *M* $K_2SO_4$ for 30 min by shaking and then filtered. Another 10 g non-fumigated soil was also extracted. In the extracts, organic C was determined by total organic carbon analyzer (Wu *et al*., 1990). Microbial carbon ($C_{mic}$) was calculated as:

$$C_{mic} = EC/kEC$$

EC = (organic C extracted from fumigated soil)-(organic C extracted from non-fumigated soil) and kEC = 0.45

Total N in the 0.5 *M* $K_2SO_4$ extracts was determined through Kjeldahl method and microbial ($N_{mic}$) was calculated as follows:

$$N_{mic} = EN/kEN$$

EN = (organic N extracted from fumigated soil)-(organic N extracted from non-fumigated soil) and kEN = 0.54

Soil dehydrogenase activity was analyzed by the method described by Alef and Nannipieri (1995). Air dried soil (20 g) was mixed with 0.2 g of $CaCO_3$ and 6 g of this mixture was placed in each of the three test tubes. After adding 1 mL of 3% aqueous solution of Triphenyl tetrazolium chloride (TTC) and 2.5 mL of deionized water, samples were incubated at 36ºC for 24 h. Then, methanol (10 mL) was added, shaken and filtered. The red color intensity was measured by using a spectrophotometer at a wavelength of 485 nm (Alef and Nannipieri, 1995). The Completely randomized design was used to lay out the experiments with three repeats. The data obtained were analyzed statistically by performing analysis of variance technique (ANOVA), using statistix 8.1® software. The mean values were compared following LSD test at 5% probability level ($p \leq 0.05$) (Steel *et al*., 1997).

All the chemicals were of analytical grade (95% purity) and obtained from Merck (Germany). The atomic absorption spectrophotometer was standardized after feeding of every ten samples with a series of standard solutions. These standard solutions were obtained by the atomic absorption spectrophotometer manufacturer (Thermo Electron S Series, Thermo Scientific, Waltham, USA).

## Results

### Soil Microbial Biomass Carbon

Data shows the changes in SMB C in MSWC amended soil during a 60 days incubation period (Fig. 1). The soil amended with MSWC showed higher values of SMB C than control soil. There was also an increase in SMB C with each increasing dose (5 to 20 t $ha^{-1}$) of MSWC. During incubation, SMB C increased steadily up to 15 days, where after it declined gradually to its lowest level by 60 days of incubation. At 0 days interval, MSWC applied at 20 t $ha^{-1}$ showed the highest SMB C (176 $\mu$g $g^{-1}$) as compared to 15, 10 and 5 t $ha^{-1}$ and was lowest (135 $\mu$g $g^{-1}$) in unamended (control) soil. The SMB C increased gradually up to 15 days of incubation. The highest microbial biomass C was recorded in soil amended with MSWC at 20 t $ha^{-1}$ on 15 days interval of incubation (186 $\mu$g $g^{-1}$) as compared to control soil (159 $\mu$g $g^{-1}$). The SMB C declined after 15 days till 60 days. At 30 days interval 20 t $ha^{-1}$ (162 $\mu$g $g^{-1}$) of MSWC still showed the highest value among all treatments The MSWC at 20 t $ha^{-1}$ showed higher values (148 $\mu$g $g^{-1}$) of SMB C in soil as compared to 15, 10 and 5 t $ha^{-1}$ and control throughout the experiment.

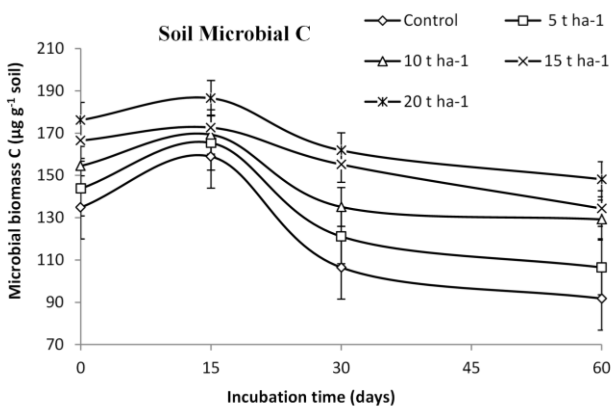


**Fig. 1:** Changes in soil microbial carbon ($\mu$g g$^{-1}$ soil) in MSWC amended soil during a 60 days incubation period. Standard error of means is shown

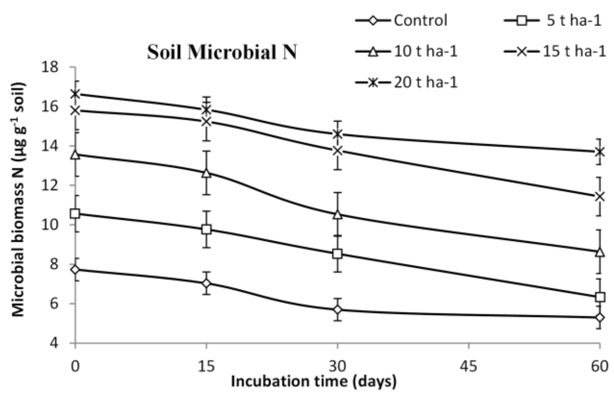


**Fig. 2:** Changes in soil microbial nitrogen ($\mu$g g$^{-1}$ soil) in a MSWC amended soil during a 60 days incubation period. Standard error of means is shown

## Soil Microbial Biomass Nitrogen

Higher values of SMB N were recorded in MSWC applied soil relative to unamended soil (Fig. 2). It was noticed that there was an increase in SMB N in MSWC enriched soil with 5 to 20 t ha$^{-1}$. During incubation from 0 to 60 days, the SMB N declined and it was at its lowest level by days 60. The SMB N in MSWC amended soil decreased with aging. MSWC application at 20 t ha$^{-1}$ resulted in higher values (16.6 $\mu$g g$^{-1}$) of SMB N in soil as compared to 15, 10 and 5 t ha$^{-1}$ MSWC amended soils and control throughout the experiment.

## Soil Dehydrogenase Activity

It was noticed that MSWC amended soil exhibited significantly higher levels of DHA than control soil (Fig. 3). A corresponding increase in DHA in soil was noticed with 5 to 20 t ha$^{-1}$. During the incubation from 0 days to 60 days, the DHA declined steadily and it was at its lowest level by days 60. Significant variation ($p \leq 0.05$) between the mean DHA values at different time periods was also observed.

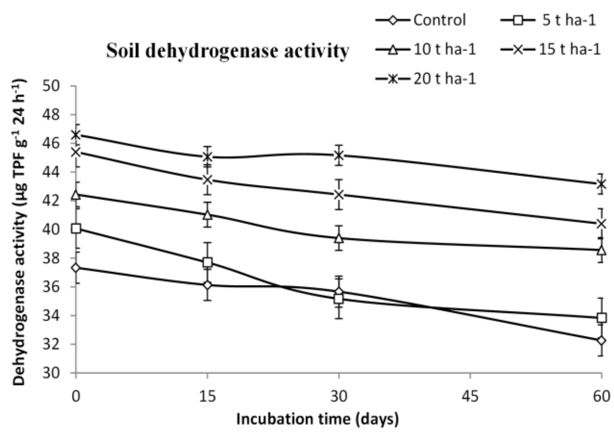


**Fig. 3:** Changes in soil dehydrogenase activity ($\mu$g TPF g$^{-1}$ 24 h$^{-1}$) in a MSWC amended soil during a 60 days incubation period. Standard error of means is shown

At 0 days interval, the highest value of DHA was found after MSWC application at 20 t ha$^{-1}$ (46.6 $\mu$g TPF g$^{-1}$ 24 h$^{-1}$) as compared to 15, 10, 5 t ha$^{-1}$ and was the lowest (37.3 $\mu$g TPF g$^{-1}$ 24 h$^{-1}$) in control soil. DHA in soil decreased throughout the incubation period. After 15 days interval, the highest value (45.06 $\mu$g TPF g$^{-1}$ 24 h$^{-1}$) of DHA was recorded in the soil amended with 20 t ha$^{-1}$ MSWC. At 30 days interval, MSWC applied at 20 t ha$^{-1}$ showed the highest value (45.1 $\mu$g TPFg$^{-1}$ 24 h$^{-1}$) of DHA than all the treatments and it was the lowest (35.6 $\mu$g TPF g$^{-1}$ 24 h$^{-1}$) in unamended control. Lowest values of DHA were recorded after 60 days aging. Soil amended with MSWC at 20 t ha$^{-1}$ at 60 days of incubation showed the highest value of DHA (43.1 $\mu$g TPF g$^{-1}$ 24 h$^{-1}$) among all the treatments.

## AB-DTPA Extractable Cadmium

The data indicates changes in AB-DTPA extractable Cd concentration in MSWC amended soil throughout 60 days duration (Fig. 4). MSWC amended Soil showed significantly ($p \leq 0.05$) higher values of AB-DTPA extractable Cd than control soil. An increase in Cd concentration was noticed with each increasing level of MSWC (5 to 20 t ha$^{-1}$). During incubation from 0 to 60 days, the Cd concentration declined and it was at lowest level by days 60. At 0 days incubation, MSWC applied at 20 t ha$^{-1}$ showed the highest value (0.55 mg kg$^{-1}$) of Cd in soil as compared to 15, 10 and 5 t ha$^{-1}$ MSWC amended soil and the lowest of that (0.16 mg kg$^{-1}$) was found in unamended control. Cadmium concentration in soil decreased with incubation time. After 15 days aging, the highest Cd was recorded in the MSWC amended soil with 20 t ha$^{-1}$ as compared to control soil (0.12 mg kg$^{-1}$). At 30 days incubation, the highest value (0.50 mg kg$^{-1}$) of Cd among all treatments was measured by MSWC application at 20 t ha$^{-1}$ and the lowest (0.10 mg kg$^{-1}$) was

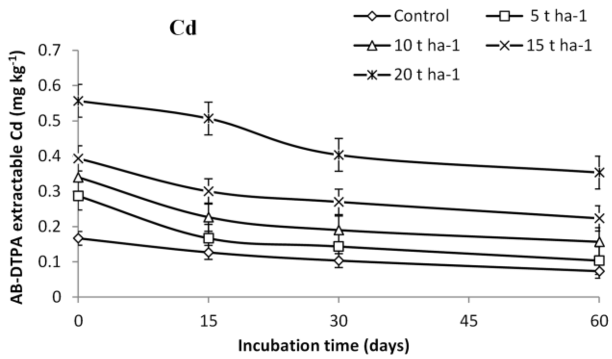


**Fig. 4:** Changes in AB-DTPA extractable cadmium concentrations in soil (mg $kg^{-1}$) in MSWC amended soil during a 60 days incubation period

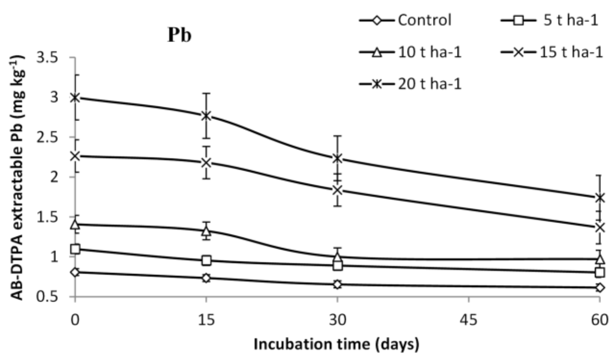


**Fig. 5:** Changes in AB-DTPA extractable lead concentrations in soil (mg $kg^{-1}$) in MSWC amended soil during a 60 days incubation period

found in control soil. Lower values of Cd were recorded after 60 days incubation. Soil amended with MSWC at 20 t $ha^{-1}$ on 60 days of incubation showed the highest value of Cd (0.35 mg $kg^{-1}$) among all treatments and control soil showed the lowest (0.07 mg $kg^{-1}$).

### AB-DTPA Extractable Lead

After 60 days aging, the AB-DTPA extractable Pb concentration remained higher in MSWC amended soil than unamended control (Fig. 5). Significant variation between the mean Pb values at different time periods was also observed with each dose of 5 to 20 t $ha^{-1}$. At 0 days interval, MSWC applied at 20 t $ha^{-1}$ showed the highest value (2.99 mg $kg^{-1}$) of Pb in soil as compared to 15, 10, 5 t $ha^{-1}$ MSWC amended soil and remained the lowest (0.80 mg $kg^{-1}$) with control soil. Pb in soil decreased with aging. After 15 days interval, the highest value (2.76 mg $kg^{-1}$) of Pb was recorded with MSWC applied at 20 t $ha^{-1}$ than control soil (0.73 mg $kg^{-1}$). At 30 days interval MSWC applied at 20 t $ha^{-1}$ still showed the highest value (2.23 mg $kg^{-1}$) of Pb among all treatments. Lowest values of Pb were recorded after 60 days interval. Soil amended with MSWC at 20 t $ha^{-1}$ on 60 days of incubation showed the highest value (1.74 mg $kg^{-1}$) of Pb among all treatments and remained the lowest (0.61 mg $kg^{-1}$) in control soil.

## Discussion

This experiment was conducted to determine the effect of Cd and Pb present in MSWC on SMB and soil dehydrogenase enzymatic activity. MSWC amended soil and control were incubated in plastic containers for the duration of 60 days. It was found that Cd and Pb concentration increased with increasing compost rates. A period of two months aging in MSWC amended soils led to substantial reductions in heavy metal availability in soil. SMB (C and N) and DHA were enhanced upon the application of MSWC. MSWC, even at a rate as high as 20 t $ha^{-1}$, did not negatively impact the SMB (C and N) and DHA.

Previous reports revealed an increase in SMB and soil enzymatic activity in the soils amended with MSWC whereas some others have reported adverse effects due to presence of heavy metals. In present study, incorporation of MSWC in soil increased the SMB C and N, and soil DHA. Similarly, Margesin *et al.* (2006) described that mixing of organic amendments in soil increased SMB, showing positive effects of organic amendments on microbial properties in MSWC amended soil. SMB C and N were higher in MSWC amended soils than control. Furthermore, Mylavarapu and Zinati (2009) stated that incorporation of organic materials including MSWC usually increases the SMB. The MSWC acted as an energy source by providing carbon contents for the microorganisms. Higher SMB in soil with increasing doses of MSWC was due to application of high amounts of carbon substrate in soil.

Beneficial effects of MSWC on SMB C were also investigated by Ros *et al.* (2006). Similarly, Bouzaiane *et al.* (2014) showed maximum microorganism's counts with MSWC application at lower rate (40 t $ha^{-1}$) than at higher rate (80 t $ha^{-1}$). Similar findings of an increase in SMB (C and N) and DHA concentration with MSWC application rate at 40 t $ha^{-1}$ were found by Jorge-Mardomingo *et al.* (2013).

Meena *et al.* (2016) also described gradual increase followed by decrease in SMB, in soil treated with MSWC. The gradual increase in SMB up to 30 days may have been related to the availability of carbon for biomass stimulation (Lakhdar *et al*., 2008). While the decrease in SMB after 30 days of incubation can be associated to the carbon shortage (Araujo *et al*., 2015). At 60 days of incubation MSWC treated soils had higher SMB than did the soil sample with no input. Higher SMB levels, at all incubation stages, in MSWC amended soil than control indicate an improvement in the soil microbiological activity. Apparently there was no harmful effect of Cd and Pb on soil microbial activity observed in MSWC amended soil.

Results showed that DHA values increased with increasing amounts of MSWC incorporated in soil. Similarly, Crecchio *et al.* (2004) who observed an increase in soil dehydrogenase activity after MSWC addition in soils. Kizilkaya and Bayrakli (2005) found that soil enzymes are mainly organic carbon dependent. MSWC provide high amount of organic carbon resulting in increased enzyme activity (DHA).

Furthermore, in this study, the MSWC application at all levels enhanced the total Cd and Pb in soil. Zhang *et al*. (2006) found that Cd and Pb concentrations were increased with the addition of MSWC in soil. Alvarenga *et al*. (2015) found no changes in Cd concentration in soil amended with the low MSWC rate. AB-DTPA extractable Cd in MSWC was found greater than in control. This can be inferred as potential Cd availability in soil due to its mobility (Hargreaves *et al*., 2008). The AB-DTPA extractable Pb was higher in the MSWC amended soil than control. The results of Carbonell *et al*. (2011) also showed that MSWC enhanced Cu, Pb and Zn concentrations in soil. The metal toxicity, solubility and availability in soil are significantly affected by soil pH (Nwuche and Ugoli, 2008).

The results also showed significant decrease in Cd and Pb concentrations during a 60 days incubation period (Table 1). Bio-sorption of microbial biomass onto microbial cell walls could significantly decrease heavy metal translocation and bioavailability (Fomina *et al*., 2008). It is also found that microbial are responsible for the sorption of heavy metals and consider it a vital process for living and dead organisms in the biosphere (Wang and Chen, 2009). But other factors such as precipitation/dissolution, oxidation/ reduction, nucleation and bio-mineral formation also affect the mobility of metals (Gadd, 2009).

The MSWC application improved the soil quality parameters; however, heavy metal concentrations were increased in soil. The results showed that AB-DTPA extractable Cd and Pb did not negatively impact SMB (C and N) and DHA in MSWC amended soil during a 60 days incubation period. The results were in contrast with literature reported by Garcıa-Gil *et al*. (2000) and Araujo *et al*. (2015), negative impact of MSWC borne heavy metals to SMB and enzymatic activities in long-term field studies. This could be due to the potential buildup of heavy metals over time due to repeated application of MSWC.

## Conclusion

It was observed that Cd and Pb concentrations increased with increasing compost rate. The highest increase was observed with MSWC application at 20 t $ha^{-1}$. Two months aging for MSWC amended soils led to substantial reductions in heavy metal availability in soil. The SMB (C and N) and soil dehydrogenase activity were increased by the application of MSWC. However, repeated applications of MSWC in soil could possibly result in a potential buildup of heavy metals. Therefore, a systematic monitoring of Cd and Pb buildup in the soil and plant system should be practiced if MSWC used on repetition basis.

**Table 1:** Reduction (%) in concentrations of Cd and Pb (compared with time 0 days) in a MSWC amended soil during a 60 days incubation period

| MSWC application Rate | Cd Reduction (%) from time 0 days | | | Pb Reduction (%) from time 0 days | | |
|---|---|---|---|---|---|---|
| (t $ha^{-1}$) | 15 days | 30 days | 60 days | 15 days | 30 days | 60 days |
| 0 | 24±1.15c | 38±1.51c | 56±2.01b | 9±1.08ab | 15±2.08d | 24±3.05b |
| 5 | 42±2.08a | 50±1.58a | 64±1.52a | 13±2.05a | 19±1.52c | 27±2.75b |
| 10 | 33±1.52b | 44±2.02b | 54±3.60c | 6±bc1.52 | 29±1.63a | 31±4.26b |
| 15 | 24±0.57c | 31±2.5d | 43±2.51d | 4±c1.15 | 25±1.73b | 40±3.57a |
| 20 | 9±2.64d | 28±1.17e | 37±2.88e | 8±bc1.52 | 20±2.11c | 42±2.64a |

Means followed by the same letter (s) in the same column do not differ significantly at $p \leq 0.05$; Values are means ± standard deviation

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