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Microbial population dynamics and dehydrogenase activity in response to integrated use of zinc oxide and zinc solubilizers

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ABSTRACT

Soil microorganisms play a key role in nutrient transformation, nutrient cycling, organic matter decomposition, water movement and various other processes essential for maintaining soil quality. In view of scanty information, a pot experiment was carried out at the Department of Soil Science and Agricultural Chemistry, Institute of Agricultural Sciences, BHU, Varanasi, UP during *kharif*, 2018-19 to evaluate the effect of zinc solubilizers in combination with zinc oxide (ZnO) on microbial population dynamics and dehydrogenase activity of soil at 30 DAT, 60 DAT and after harvesting of rice. In this experiment, three different doses of ZnO (Z_0=0, Z_1=1.85, and Z_2=3.7 mg kg^{-1}) and three types of microbial inoculation, viz. No inoculation (M_0), *Enterobacter clocae* strain ZnPSBJ-6 (M_1) and zinc solubilizing fungi (M_2) were employed in various combinations. It has found that at 60 DAT, inoculation of M_1 resulted in 4.1% and 12.8% increase in soil bacterial count while treated with Z_1 and Z_2, respectively. Whereas, in the case of M_2, the fungal count increased by around 40% and 48% at 30 DAT while treated with Z_1 and Z_2, respectively over Z_0. After harvesting, the actinomycetes population increased significantly by 45.5% in Z_1 treated soil. At 30 DAT, dehydrogenase activity increased significantly in M_2 treated soil by 7.3% and 27.3% with increasing level of ZnO, i.e. from Z_1 to Z_2 over Z_0.

Key words: Nutrient transformation, Nutrient cycling, Organic matter decomposition, Soil dehydrogenase activity

Zn plays an important role in several metabolic and enzymatic activity in crop plants. Use of high yielding varieties (HYVs), micronutrient free high analysis fertilizer, heavy irrigation, hazardous agrochemicals in the post green revolution era not only aggravated Zn deficiency in soil but also facilitated the shift in microflora which *vis-à-vis* deteriorates the soil quality. One of the major constraint of alleviating Zn deficiency through application of chemical fertilizers that these are having very low use efficiency (2%) due to rapid conversion of soluble Zn compound to different insoluble pools like oxide bound, carbonate bound, or other occluded forms (Sarathambal *et al.* 2010). Whereas, Zn deficiency is more pronounced in case of low land rice due to submergence condition. Zinc solubilizers are special kind of plant growth promoting rhizobacteria (PGPR), which selectively solubilize Zn through production of some organic acid which lowers pH or form soluble chelate with Zn. During past few years, solubilization of insoluble Zn compounds [ZnO, $ZnCO_3$, $Zn_3(PO_4)_2$] by plant growth promoting rhizobacteria (PGPR) has been reported worldwide (Krithika and Balachandar 2016; Gontia-Mishra *et al.* 2016). Therefore, the use of zinc solubilizers are hypothesized to be an effective mean to enhance the Zn availability to plant. Apart from this, zinc solubilizing microbes are also responsible for biochemical modification of rhizospheric soil, e.g. increase in microbial count, dehydrogenase activity (Wamberg *et al.* 2003). Microbial biomass constitutes about 2 to 4% of soil organic matter (Alexander 1978). Dehydrogenase enzyme is an oxidoreductase type of enzyme, takes part in oxidation-reduction reactions and indicates soil microbial activity (Quilchano and Marañon 2002; Gu *et al.* 2009; Salazar *et al.* 2011). This enzyme is generally produced intracellularly by soil microbes (Moeskops *et al.* 2010; Zhao *et al.* 2010; Yuan and Yue 2012). It also play a significant role in the biological oxidation of soil organic matter (OM) by transferring hydrogen from organic substrates to inorganic acceptors (Zhang *et al.* 2010). Brzezińska *et al.* (1998) revealed that active dehydrogenases are capable of utilizing both O_2 and other compounds as terminal electron acceptors, although most of the dehydrogenases are produced by anaerobic microorganisms. Therefore, it reflects the rate

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Table 1 Initial characteristics of experimental soil

Parameter	Value
$pH_{1:2}$	7.8
$EC_{1:2}$ (dS m^{-1})	0.48
Clay %	15.7
Silt %	23.1
Sand %	61.2
Texture	Sandy loam
Organic carbon (g kg^{-1})	16
Cation exchange capacity [cmol (p^+) kg^{-1}]	20.3
Dehydrogenase activity (µg TPF g^{-1} soil day^{-1})	21.64
DTPA extractable Zn	0.54
Soil bacterial count (cfu $g^{-1} \times 10^6$)	13.2
Soil fungal count (cfu $g^{-1} \times 10^4$)	12.6
Soil actinomycetes count (cfu $g^{-1} \times 10^4$)	10.2

of metabolic activity of soil and its activity has always found to be proportional to soil microbial biomass. Keeping the above fact in mind, the present study was undertaken with following hypothesis, i.e. to check influence of Zn solubilizing microbes in combination with varying level of zinc oxide (ZnO) on microbial population dynamics and dehydrogenase activity in soil.

MATERIALS AND METHODS

Collection of soil sample: One bulk surface (0-15 cm) soil (Typic Haplusteps) was collected from the Agricultural Research Farm, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, UP.

Pot experiment: A pot experiment was conducted at Department of Soil Science and Agricultural Chemistry, Institute of Agricultural Sciences, BHU, Varanasi, UP during *kharif*, 2018-19 with a rice cultivar characterized as dwarf non-aromatic high yielding variety (HYV), *viz.* Swarna-sub-1. Seedlings of four-leaf stage were obtained from the Agricultural Research Farm, IAS, BHU. For this purpose, earthen pots were filled with 10 kg of processed soil. Zinc oxide (ZnO) was added at the rates of 0 (Z_0), 1.85 (Z_2) and 3.7 (Z_3) mg kg^{-1} of soil, which are equivalent to field application of 0, 4.16 and 8.33 kg ha^{-1}. Seedling root dip was done with desired microbial inoculation, *viz.* No inoculation (M_0)*, Enterobacter clocae* strain ZnPSBJ-6 (M_1) and a zinc solubilizing fungi (M_2) with the aid of some adhering substances like jaggary and gum acacia. Transplanting was done in such a way that each pot consists of four hills and each hill contains two to three seedlings. All treatment combinations (nine) replicated thrice and the experiment was laid out in factorial completely randomized design. The uniform basal dose of N : P_2O_5: K_2O @ 40.1: 40.2: 26.8 mg kg^{-1} were added in solution form in each pot through urea, potassium dihydrogen phosphate and muriate of potash, respectively. After establishment of plant, thinning was done to maintain a uniform population of four plants per pot. Water level was maintained at 3 cm above the soil surface until harvest. Two top dressings of N, each at the rate of 20.1 mg kg^{-1} were done at 30 and 60 days after transplanting (DAT). For enumerating the microbial population of soil, composite soil samples were prepared by mixing together the samples of soil from the pots under each treatment.

Culture media: Three distinct medium, *viz*. Thornton medium (Thornton 1922), Rose Bengal Agar Medium (pH 7.2), Kenknight and Munaier's medium (Ram *et al.* 2013), were used for proliferation of bacteria, fungi, actinomycetes and zinc solubilizing bacteria, respectively.

Total microbial count by serial dilution and standard plate count method: Total bacteria, fungi, actinomycetes and Zn solubilizing bacterial population were determined by serial dilution and pouring technique. Serial dilution was done in laminar flow chamber. The 10 g of test soil sample under aseptic conditions was weighed in a sterile paper and transferred it to 250 ml conical flask containing 90 ml sterilized 0.85% solution NaCl and shaken for thorough mixing. From this suspension, 1 ml of suspension was transferred to first culture tube with the help of sterile pipette containing 9 ml of sterile saline water and thoroughly shaken. Here dilution was 10 times. From this, further dilution was made by transferring 1 ml to another 2nd, 3rd and 4th sterile culture tube containing 9 ml sterilized saline water aseptically to desired level as 10^{-4} and 10^{-5}.

Plating: With the help of sterilized pipette, 1 ml of suspension from desired dilution was transferred to petri plates. About 20 m1 of specific media was poured into petri plates in three replication followed by rotating the plate clock wise and anti-clock wise. After solidifying the media, petri plates were inverted and kept in BOD Incubator at 28±1°C. Microbial counts were recorded when colonies were developed by observing their specific colony characteristics.

Counting: Microbial colonies were counted depending upon morphological features. Colonies characterized as slimy and shiny with smooth boundary were counted as bacteria. For the fungal colony, cottony appearance was observed and for actinomycetes those colonies were counted which were appeared in the media having concentric ring with star appearance. ZSB colonies were counted by observing the clear halo zone around the colony. The colonies were counted and expressed by multiplying colony forming unit (cfu) by dilution factor. Colony forming unit in the plates for each soil were counted and averaged. Population was expressed as number per gram of oven dry basis.

Dehydrogenase activity: Soil dehydrogenase activity was determined by the standard procedure given by Casida *et al.* (1964). The 1 g of air-dried soil sample was taken in air tight screw capped test tube (15 ml capacity). 1.5 ml of 1% glucose solution and 1 ml of 3% triphenyl tetrazolium chloride (TTC) solution and 2.5 ml distilled water were added in each of the tubes. Incubation was done at 28°C for 24 hours. After incubation, 10 ml of methanol was added and shaken vigorously then allowed to stand for 6 hours. Clear pink coloured supernatant was removed and readings were taken with a spectrophotometer at a wave length of 485 nm (blue filter). Results were estimated by multiplying with dilution factor and expressed in terms of µg TPF $g^{-1}day^{-1}$ soil.

Satistical analysis: Data obtained from the experiments were subjected to statistical analysis by adopting Factorial Completely Randomized Design. Interpretation of the data was carried out in accordance with Panse and Sukhatme (1985). The critical difference values were calculated wherever the 'F' test values were significant at 5% level of significance.

RESULTS AND DISCUSSION

Soil bacterial count: It was found that at 60 DAT, bacterial count has increased in treatment inoculated with M_1 (27.3 cfu $g^{-1} \times 10^6$) as compared to M_2 (18.5 cfu $g^{-1} \times 10^6$) when treated with similar doses of Zn, i.e. 3.7 mg kg^{-1} (Table 3). Even higher level of Zn (Z_2) also resulted significant increase in bacterial count, i.e. 25.2 cfu $g^{-1} \times 10^6$ when inoculated with M_1 as compared to lower dose of Zn (Z_1). Bacterial count was found to be drastically reduced in T_9 as compared to absolute control (T_1). This may be due to the fact that, zinc solubilizing fungi in case of T_9 had somehow resulted in lowering of pH (data not presented) which was not conducive for survival of soil bacteria. Bacterial proliferation follows a typical sigmoidal pattern over total crop growing period which is may be due to the fact that optimum survival condition such as pH, temperature, soil wetness or lesser degree of competition prevail at 60 DAT. Similar result was found by Poonam *et al.* (2014). The results pertaining to soil bacterial population followed invariably similar kind of trend even at 30 DAT (Table 2) as well as after harvesting (Table 4) which is in parallel with the findings reported by Ghodpage *et al.* (2009).Treatment interaction also significantly increases the bacterial population because it is probable that Zn plays a catalytic activity in microbial metabolism.

Soil fungal count: Results showed that soil fungal count at 30 DAT, 60 DAT as well as after harvesting has increased significantly due to integrated use of ZnO and zinc solubilizers. At 30 DAT, in case of M_1 treated soil fungal count increased significantly with increasing level of zinc, i.e. from Z_1 (13.5 cfu $g^{-1} \times 10^4$) to Z_2 (15.5 cfu $g^{-1} \times 10^4$) over T_2 (13.3 cfu $g^{-1} \times 10^4$) where no zinc was applied (Table 2). Maximum fungal population (18.5 cfu $g^{-1} \times 10^4$) was observed in T_9 where zinc solubilizing fungi was inoculated with higher level of zinc (i.e. ZnO at the rate 3.7 mg kg^{-1}). However, in case of T_3 fungal population declined significantly and found to be minimum (12.5 cfu $g^{-1} \times 10^4$) as compared to absolute control (13.3 cfu $g^{-1} \times 10^4$). The results followed invariably similar kind of pattern at 60 DAT (Table 2) as well as after harvesting (Table 4) which is in parallel with the findings reported by Kelly *et al.* (1999). This is may be due to the fact that, high level of zinc along with zinc solubilizing microbes induces microbial shift in soil as well as microbial tolerance towards high concentration of zinc.

Actinomycetes count: A distinct kind of response was found in case of soil actinomycetes count while inoculated with different type microbial strain along with varying doses of Zn in all the three stages, i.e. at 30 DAT, 60 DAT, and after harvesting. At 30 DAT, in case of M_1 treated soil, actinomycetes count increased significantly with increasing level of zinc, i.e. from Z_1 (14.5 cfu $g^{-1} \times 10^4$) to Z_2 (16.5 cfu $g^{-1} \times 10^4$) over Z_0 (13.6 cfu $g^{-1} \times 10^4$) (Table 2). Maximum actinomycetes population (18.2 cfu $g^{-1} \times 10^4$) has been observed in T_9 where *Enterobacter clocae* strain ZnPSBJ-6 and zinc solubilizing fungi were inoculated with higher level of zinc (i.e. ZnO at the rate 3.7 mg kg^{-1}). However, after harvesting, in case of M_2, actinomycetes population increased significantly by 45.5% with the ZnO dose at the rate 1.85 mg kg^{-1} but further increment in Zn level could not make any significant difference (Table 4). On the other hand, treatments like T_1, T_2 and T_8 have found to be statistically at par with T_3, T_4 and T_9 respectively. A similar kind of trend has observed at 60 DAT as well as after harvesting which was in consonance with the findings of Kelly *et al.* (1999). This may be due to the fact high level of zinc has stimulated proliferation soil microbes.

Dehydrogenase activity: Data pertaining to the effect of ZnO in combination with zinc solubilizers reveal that at 30 DAT, both the level Zn had not been able to enhance soil dehydrogenase activity of soil treated with M_1. On the other hand, at 30 DAT, in case of M_2 treated soil dehydrogenase activity increased significantly by 7.3% and 27.3% with increasing level of zinc, i.e. from Z_1 (24.7 µg TPF g^{-1} day^{-1}) to Z_2 (29.3 µg TPF g^{-1} day^{-1}) over Z_0 (23 µg TPF g^{-1} day^{-1}). A similar kind of trend was observed at 60 DAT as well as after harvesting. It is well known, that redox potential (Eh) play a crucial role in regulating microbial activity as well as community structure (Song *et al.* 2008) and affecting on soil enzymatic activity, especially dehydrogenase activity. Apart from Eh of soil system, there

Table 2 Effect of ZnO and zinc solubilizers on soil microbial count and dehydrogenase in soil at 30 DAT

Treatment	Bacterial count (cfu $g^{-1} \times 10^6$)	Fungal count (cfu $g^{-1} \times 10^4$)	Actinomycetes count (cfu $g^{-1} \times 10^4$)	Dehydrogenase activity (µg TPF g^{-1} day^{-1})
T_1 (M_0Z_0)	14.0	13.3	12.5	19.4
T_2 (M_1Z_0)	15.5	13.3	13.6	26.3
T_3 (M_2Z_0)	14.4	12.5	12.5	23.0
T_4(M_0Z_1)	15.2	15.3	13.5	23.5
T_5(M_1Z_1)	18.4	13.5	14.5	27.3
T_6(M_2Z_1)	13.5	17.5	17.4	24.7
T_7(M_0Z_2)	15.5	15.8	15.5	23.5
T_8(M_1Z_2)	18.6	15.5	16.5	26.6
T_9(M_2Z_2)	13.5	18.5	18.2	29.3
SEm±	0.16	0.24	0.18	0.73
CD(0.05)	0.46	0.70	0.54	2.16

SEm±: Standard error of mean (both positive and negative), CD (0.05): Critical difference at 5% level of significance

Table 3 Effect of ZnO and zinc solubilizers on soil microbial count and dehydrogenase activity at 60 DAT of rice

Treatment	Bacterial count (cfu $g^{-1} \times 10^6$)	Fungal count (cfu $g^{-1} \times 10^4$)	Actinomycetes count (cfu $g^{-1} \times 10^4$)	Dehydrogenase activity (µg TPF g^{-1} day^{-1})
T_1 (M_0Z_0)	22.5	19.5	14.6	29.0
T_2 (M_1Z_0)	24.2	19.7	15.6	33.6
T_3 (M_2Z_0)	23.2	19.3	15.3	30.9
T_4(M_0Z_1)	24.4	21.3	16.2	31.3
T_5(M_1Z_1)	25.2	20.6	16.2	31.3
T_6(M_2Z_1)	27.2	24.5	21.1	33.0
T_7(M_0Z_2)	25.7	22.2	19.5	31.4
T_8(M_1Z_2)	27.3	22.4	19.5	33.3
T_9(M_2Z_2)	18.5	26.3	21.6	37.0
SEm±	0.16	0.16	0.26	0.68
CD(0.05)	0.47	0.49	0.77	2.01

SEm±: Standard error of mean (both positive and negative), CD (0.05): Critical difference at 5% level of significance

Table 4 Effect of ZnO and zinc solubilizers on soil microbial count and dehydrogenase activity after harvesting of rice

Treatment	Bacterial count (cfu $g^{-1} \times 10^6$)	Fungal count (cfu $g^{-1} \times 10^4$)	Actinomycetes count (cfu $g^{-1} \times 10^4$)	Dehydrogenase activity (µg TPF g^{-1} day^{-1})
T_1 (M_0Z_0)	18.3	17.6	13.6	27.1
T_2 (M_1Z_0)	19.5	18.4	14.4	26.7
T_3 (M_2Z_0)	18.5	17.4	13.6	26.2
T_4(M_0Z_1)	20.4	19.4	14.7	28.1
T_5(M_1Z_1)	20.5	18.4	15.3	29.5
T_6(M_2Z_1)	18.2	21.4	19.8	27.7
T_7(M_0Z_2)	21.3	20.3	18.8	26.4
T_8(M_1Z_2)	22.5	20.4	19.9	28.9
T_9(M_2Z_2)	15.5	23.4	19.9	32.9
SEm±	0.17	0.12	0.36	0.78
CD(0.05)	0.49	0.35	1.08	2.32

SEm±: Standard error of mean (both positive and negative) CD (0.05): Critical difference at 5% level of significance

are several factors mediated by microbial activity such as moisture content, oxygen diffusion rate (ODR) also play a major role governing dehydrogenase activity. Nihorimbere *et al.* (2011) reported that microbial activities increase the dehydrogenase activity in the rhizosphere with increasing availability of food materials for its growth.

It has found that soil microbial count increased gradually and reached to maximum at 60 DAT in response to various treatments due to higher microbial activity during reproductive stage of crop growth. At 60 DAT, inoculation of M_1 resulted in 4.1% and 12.8% increase in soil bacterial count while treated with Z_1 and Z_2, respectively (Table 2). However, this degree of increment is less conspicuous at 30 DAT as well as after harvesting. However, in case of M_2, fungal count increased by around 40% and 48% at 30 DAT while treated with Z_1 and Z_2, respectively, as compared to Z_0. Whereas, after harvesting, in case of M_2, actinomycetes population increased significantly by 45.5% with the ZnO dose at the rate 1.85 mg kg^{-1} but further increment in Zn level could not make any significant difference. Whereas at 30 DAT, in case of M_2 treated soil dehydrogenase activity increased significantly by 7.3% and 27.3% with increasing level of zinc, i.e. from Z_1 (24.7 µg TPF g^{-1} day^{-1}) to Z_2 (29.3 µg TPF g^{-1} day^{-1}) over Z_0 (23 µg TPF g^{-1} day^{-1}). Therefore, it can be concluded that, application of zinc solubilizing microbes in combination with practically realizable doses of ZnO has a positive influence on soil microbial population *vis-à-vis* soil quality, which should be exploited by farmers particularly in the alluvial plain of UP.

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