

Impact of Microbial Cultures on Soil Biological Quality and Growth of Spinach Grown in Polluted Soils

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In this present studied poly bag experiment was conducted following complete randomized block design with 12 treatments and three replications. Polluted Soil with supply of fresh water, Unpolluted soil with supply of fresh water, Unpolluted soil with supply of polluted water. The results of pot culture were reveals that the Influence of microbial cultures on biological quality and microbial population of polluted soil and spinach yield at 30 and 60 DAS was estimated. Significantly highest bacterial population was recorded in treatments T₁₂ (124.21 ×10⁷ CFU g⁻¹ soil) at 30 DAS and treatment T₈ (88.68 ×10⁷ CFU g⁻¹ soil) at 60 DAS. The highest molds population was observed in treatment T₃ (17.91, 11.32 ×10³ CFU g⁻¹ soil) at 30 and 60 DAS respectively. The treatment T₆ showed significantly highest rhizobial population at 30 and 60 DAS (29.23, 20.71 ×10³CFU g⁻¹ soil) respectively. The VAM population was highest in the treatment T₇ (24.13 ×10³ g⁻¹ soil) at 30 DAS and T₁₂ (9.0) at 60 DAS. The highest leaf fresh weight was recorded in T₈ (41.63 g plant⁻¹) at 30 DAS and (70.03 g plant⁻¹) at 60 DAS and the lowest fresh weight was recorded in T₃, T₉ at 30, 60 DAS respectively. The highest leaf dry weight was recorded in T₈ (6.62 g plant⁻¹), (4.17 g plant⁻¹) at 30 and 60 DAS respectively and the lowest values were found in T₃ at 30 and 60 DAS.

Keywords: Spinach, polluted, unpolluted soil, biological activity, microbial population,

Soil fertility is a complex concept that involves many interacting parameters. Cultivated plants may suffer nutritional stresses when the amount or availability of soil nutrients is lower than that required for sustaining metabolic processes in each growth stage. Thus, restoring of nutrients and enhancing their availability by improving soil characteristics and efficiency of plants, are the main objectives of the modern agriculture. Due to the increasing sensitivity to environmental and economic issues, researchers and consumers are more and more aware of the impact of agriculture on the environment. Pollutants such as heavy metals and chemicals in the soil, water and air are

affected by various physicochemical, biological, and environmental factors. Bioremediation is a biological process by which environmental pollutants are removed or transformed to less toxic substances. Soil amendments including fertilizer and lime, appropriate moisture levels, and periodic tilling can maximize or improve bioremediation (Brigmon *et al.* 2002). Phytoremediation specifically utilizes plants for contaminant control and has been combined with soil amendments for increasing or reducing metals uptake (Wilde *et al.* 2005).

Plants tolerant to heavy metals are able to immobilize metals by accumulation in the roots, adsorption in/onto the roots, and/or precipitation in the rhizosphere. Currently, most of what is known about aided phytostabilization of heavy metal-contaminated soils focuses on analysis of physicochemical soil properties, especially concentration of bioavailable forms of metals/

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metalloids and their accumulation in plant tissues (Wilde *et al.* 2005).

Bioindicators are the most important criterion of soil quality (Alkorta *et al.* 2010; Markert *et al.* 2003). Many definitions of soil quality have been suggested. However, a short and comprehensive definition is given by Doran and Parkin (1994) who have defined soil quality/soil health as "The capacity of a soil to function within ecosystem boundaries to sustain biological productivity, maintain environmental quality and promote plant and animal health". Phytoremediation, the use of plants to extract, sequester, and/or detoxify pollutants through physical, chemical, and biological processes (Saxena *et al.*, 1999) has been reported to be an effective, in situ, non-intrusive, low-cost, aesthetically pleasing, ecologically benign, socially accepted technology to remediate polluted soils (Alkorta and Garbisu, 2001; Garbisu *et al.*, 2002; Weber *et al.*, 2001). It also helps prevent landscape destruction and enhances activity and diversity of soil microorganisms to maintain healthy ecosystems, which is consequently considered to be a more attractive alternative than traditional methods to the approaches that are currently in use for dealing with heavy metal contamination.

The objective of this work was to use traditional microbiological methods based on culture techniques to evaluate the biological quality of heavy metal-contaminated soil that has been remediated with aided phytostabilization

MATERIALS AND METHODS

Soil Samples and Soil Characteristics

Soil samples of polluted and unpolluted soils were collected before sowing and analysed for the physical (pH, EC, and particle size and chemical characters like N, P, K and organic carbon parameters) and microbiological properties by adopting standard procedures at Department of Agricultural Microbiology and Bio-energy and Department of Soil Science and Agricultural Chemistry, College of Agriculture, Rajendranagar, PJTSAU, Hyderabad. Water samples were also analyzed before sowing of crop in polluted and unpolluted soils. (table 1).

Crop details

The pot culture experiment was

conducted at Department of Agricultural Microbiology and Bioenergy during 2014-15. For this investigation leafy vegetable crop, spinach beet, Pusa Jyothi variety was sown in pot experiments followed completely randomized block design with four treatments and three replications. Microbial cultures (*Pseudomonas*, VAM) collected from our laboratory. The treatments for poly bag experiment were fixed as twelve treatments each treatment with three replications were designed. All three replications were used to record observations on yield, quality parameters of spinach around 30 and 60 days after sowing.

In this context of pot culture experiment having twelve treatments and followed statistical design .in this treatment subdivided into three parts: polluted soil with supply of fresh water, unpolluted soil with supply of fresh water and unpolluted soil with supply of polluted water. Polluted soil with supply of fresh water have T1: SF Soil+FYM@12 t/ha, T2: SF Soil + FYM + VAM + *Pseudomonas*, T3: SF Soil + RDF, T4: SF Soil + RDF + FYM + VAM + *Pseudomonas*. Unpolluted soil with supply of fresh water, have T5: Soil + FYM, T6: Soil + FYM + VAM + *Pseudomonas*, T7: Soil + RDF, T8: Soil + RDF + FYM + VAM + *Pseudomonas*. Unpolluted soil with supply of polluted water, have T9: Soil + FYM, T10: Soil + FYM + VAM + *Pseudomonas*, T11: Soil + RDF, T12: Soil + RDF + FYM + VAM + *Pseudomonas*. The cleaned poly bags were filled with 8 kg soil and this soil was mixed with chemical fertilizer (0.14: 0.24: 0.37 g poly bag⁻¹ NPK), farm yard manure (78.75 g poly bag⁻¹) and Vesicular Arbuscular Mycorrhizae (100 to 150 g of infected propagules poly bag⁻¹) according to the treatments which were neatly arranged in the net house.

Chemical fertilizers

Phosphorus and potassium @ 0.24 g poly bag⁻¹ P₂O₅ and 0.37 g poly bag⁻¹ K₂O were applied through Di Ammonium Phosphate and Muriate of Potash respectively as basal application. Nitrogen was applied in the form of Urea @ 0.24 g poly bag⁻¹ after germination and after 30 and 60 days after sowing. Farmyard manure was applied @ 78.75 g poly bag⁻¹ which was mixed with soil according to the treatments requirement. EC and pH of FYM were 0.95 dS/m and 7.59 respectively and Ni, Co, Cd content in FYM was 0.91, 0.20, 0.01-0.02 respectively.

Seed Sowing and maintenance

The poly bags were sown with Pusa Jyothi variety of spinach beet at the rate of 20 seeds per poly bag. After germination, thinning was done and routine care was taken to protect the plants from pest and diseases.

RESULTS AND DISCUSSION

Microbial population (CFU g⁻¹ of Soil)

Influence of microbial cultures on biological quality of polluted soil and spinach yield at 30 DAS and 60 DAS on the microbial population in soil was estimated *viz.*, bacteria, *Rhizobium*, *Azospirillum*, *Azotobacter*, actinomycetes, *Pseudomonas*, molds and VAM show the data presented in the Table and .

Bacterial population ($\times 10^7$ CFU g⁻¹ of Soil)

Bacterial population in soil differed significantly on application of microbial cultures on biological quality of polluted soil and spinach yield .Initial bacterial population in the polluted soil was 30×10^7 CFU g⁻¹ of soil and in unpolluted soil was 40×10^7 CFU g⁻¹ of soil. At 30 DAS, treatment T₁₂ recorded significantly higher bacterial population (124.21) as compared to all

other treatments but was on par with treatment T₂ (117.85) and T₈ (123.85). The significantly lowest (82.98) bacterial population was found with the treatment T₇. At 60 DAS, treatment T₈ recorded significantly higher (88.68) bacterial population than all other treatments and treatment T₅ (83.38) and T₁₂ (84.21) was on par with T₈. The significantly lowest (46.46) bacterial population recorded in the treatment T₉.

Rhizobium population ($\times 10^3$ CFU g⁻¹ of Soil)

Rhizobial population differed significantly as influenced by application of microbial cultures on biological quality of polluted soil and spinach yield. Initial rhizobial population in polluted soil was 12.4×10^3 CFU g⁻¹ of soil and in unpolluted soil was 18.4×10^3 CFU g⁻¹ of soil. At 30 DAS, treatment T₆ recorded significantly higher (29.23) rhizobial population as compared to all other treatments and was on par with treatment T₅ (26.23), T₇ (26.58), T₈ (28.00) and T₁₁ (27.20). The significantly lowest (16.32) rhizobial population recorded in the treatment T₄. At 60 DAS, treatment T₆ recorded significantly higher (20.71) rhizobial population as compared to all other treatments and was on par with the treatment T₅ (18.66), T₇ (17.90) and T₉ (17.00). The significantly lowest rhizobial

Table 1. Effect of microbial cultures on microbial biomass carbon at harvesting stage (60 DAS) in polluted and unpolluted soils of spinach beet

Treatments	60 DAS
Polluted Soil with supply of fresh water	
T ₁ - SF Soil + FYM	83.30
T ₂ - SF Soil + FYM + VAM + <i>Psuedomonas</i>	98.30
T ₃ - SF Soil + RDF	89.31
T ₄ - SF Soil + RDF + FYM + VAM + <i>Psuedomonas</i>	103.16
Unpolluted soil with supply of fresh water	
T ₅ - SF Soil + FYM + <i>Psuedomonas</i>	109.00
T ₆ - SF Soil + FYM+ VAM + <i>Psuedomonas</i>	120.03
T ₇ - SF Soil + RDF	95.20
T ₈ - SF Soil + RDF + FYM + VAM + <i>Psuedomonas</i>	123.68
Unpolluted soil with supply of polluted water	
T ₉ - Soil + FYM	95.67
T ₁₀ - Soil + FYM + VAM + <i>Psuedomonas</i>	118.66
T ₁₁ - Soil + RDF	103.38
T ₁₂ - Soil + RDF + FYM + VAM + <i>Psuedomonas</i>	129.99
SE m \pm	2.383
C.D at 5%	6.955

SF soil = Student Farm Soil, RDF = Recommended dose of fertilizers, FYM = Farm Yard Manure

population (10.7) recorded in the treatment T₄.

Azotobacter population ($\times 10^3$ CFU g⁻¹ of Soil)

Azotobacter population differed significantly as influenced by application of microbial cultures on biological quality of polluted soil and spinach yield. Initial *Azotobacter* population in polluted soil was 3.1×10^3 CFU g⁻¹ of soil and in unpolluted soil was 4.9×10^3 CFU g⁻¹ of soil. At 30 DAS, treatment T₇ recorded significantly higher (15.13) *Azotobacter* population among all other treatments and was on par with T₆ (12.7), T₉ (14.26) and T₁₁ (15.00). The significantly lowest (9.7) *Azotobacter* population found in the treatment T₄. At 60 DAS, treatment T₅ recorded significantly higher (12.46) *Azotobacter* population as compared to all other treatments. The significantly lowest *Azotobacter* population recorded in the treatment T₃ (2.43).

Azospirillum population ($\times 10^3$ CFU g⁻¹ of Soil)

The population of *Azospirillum* differed significantly as influenced by application of microbial cultures on biological quality of polluted soil and spinach yield (Table &) Initial *Azospirillum* population in polluted soil was 4.2×10^3 CFU g⁻¹ of soil and in unpolluted soil was 6.5×10^3 CFU g⁻¹ of soil. At 30 DAS, treatment T₁₂ recorded significantly higher (12.1) *Azospirillum* population among all other treatments and was on

par with T₈ (10.5), T₉ (10.9), T₁₀ (11.4) and T₁₁ (12.00). The significantly lowest (8.36) *Azospirillum* population found in the treatment T₁ and T₅. At 60 DAS, treatment T₁₂ recorded significantly higher (9.4) *Azospirillum* population as compared to all other treatments and was on par with treatment T₄ (8.53). The significantly lowest *Azospirillum* population recorded in the treatment T₁₁ (4.26).

Actinomycetes population ($\times 10^4$ CFU g⁻¹ of Soil)

Actinomycetes population differed significantly as influenced by application of microbial cultures on biological quality of polluted soil and spinach yield (Table &). Initial Actinomycetes population in polluted soil was 8.2×10^4 CFU g⁻¹ of soil and in unpolluted soil was 6.2×10^3 CFU g⁻¹ of soil. At 30 DAS, treatment T₇ recorded significantly higher (41.77) actinomycetes population among all other treatments. The significantly lowest (21.48) Actinomycetes population found in the treatment T₉. At 60 DAS, treatment T₇ recorded significantly higher (34.73) actinomycetes population as compared to all other treatments and was on par with treatment T₅ (34.27). The significantly lowest actinomycetes population recorded in the treatment T₉ (15.67).

Pseudomonas population ($\times 10^4$ CFU g⁻¹ of Soil)

Pseudomonas population differed significantly as influenced by application of

Table 2. Effect of microbial cultures on fresh weight at 30 and 60 DAS in polluted and unpolluted soils of spinach beet

Treatments	Fresh weight of leaf/plant	
	30DAS	30DAS
Polluted Soil with supply of fresh water		
T ₁ - SF Soil + FYM	30.48	46.48
T ₂ - SF Soil + FYM + VAM + <i>Pseudomonas</i>	34.02	54.05
T ₃ - SF Soil + RDF	23.02	38.65
T ₄ - SF Soil + RDF + FYM + VAM + <i>Pseudomonas</i>	39.40	60.54
Unpolluted soil with supply of fresh water		
T ₅ - SF Soil + FYM + <i>Pseudomonas</i>	31.30	52.30
T ₆ - SF Soil + FYM+ VAM + <i>Pseudomonas</i>	39.89	64.87
T ₇ - SF Soil + RDF	26.61	40.32
T ₈ - SF Soil + RDF + FYM + VAM + <i>Pseudomonas</i>	41.63	70.03
Unpolluted soil with supply of polluted water		
T ₉ - Soil + FYM	26.40	38.12
T ₁₀ - Soil + FYM + VAM + <i>Pseudomonas</i>	34.71	61.03
T ₁₁ - Soil + RDF	28.82	50.37
T ₁₂ - Soil + RDF + FYM + VAM + <i>Pseudomonas</i>	41.36	68.10
SE m±	0.176	0.167
C.D at 5%	0.513	0.488

microbial cultures on biological quality of polluted soil and spinach yield.

Initial *Pseudomonas* population in polluted soil was 18.2×10^4 CFU g^{-1} of soil and in unpolluted soil was 28×10^4 CFU g^{-1} of soil. At 30 DAS, treatment T_{10} recorded significantly higher (96.10) *Pseudomonas* population as compared to all other treatments and was on par with treatment T_{12} (96.00). The significantly lowest *Pseudomonas* population recorded in the treatment T_1 (29.16). At 60 DAS, treatment T_{12} recorded significantly higher (60.25) *Pseudomonas* population as compared to all other treatments and was on par with T_7 (58.68). The significantly lowest (30.89) *Pseudomonas* population was recorded in the treatment T_{11} .

Molds population ($\times 10^4$ CFU g^{-1} of Soil)

Molds population differed significantly as influenced by application of microbial cultures on biological quality of polluted soil and spinach yield. Initial molds population in the polluted soil was 15×10^4 CFU g^{-1} of soil and in unpolluted soil was 29×10^4 CFU g^{-1} of soil. At 30 DAS, treatment T_3 recorded significantly higher (17.91) molds population as compared to all other treatments. The significantly lowest (4.33) molds population was recorded in the treatment T_{10} . At 60 DAS,

higher molds population (11.32) was observed with the treatment T_3 as compared to all other treatments and was on par with treatment T_1 (10.04). The significantly lowest (1.95) fungal population recorded in the treatment with T_8 .

VAM population ($\times 10^3$ CFU g^{-1} of Soil)

VAM population differed significantly as influenced by application of microbial cultures on biological quality of polluted soil and spinach yield (Table 4.1 & 4.2, Fig 4.8). Initial VAM population in polluted soil was 4.8×10^0 CFU g^{-1} of soil and in unpolluted soil was 7.2×10^3 CFU g^{-1} of soil. At 30 DAS, treatment T_7 recorded significantly higher (24.13) VAM population among all other treatments and was on par with T_6 (20.80). The significantly lowest (7.49) VAM population found in the treatment T_3 . At 60 DAS, treatment T_{12} recorded significantly higher (9.00) VAM population as compared to all other treatments and was on par with treatment T_2 (8.04), T_6 (8.99), T_9 (8.34), T_{10} (7.88). The significantly lowest VAM population recorded in the treatment T_7 (4.14).

Microbial population recorded in the rhizosphere soil at flowering stage and harvesting stage indicated significant increase due to application of microbial cultures. Population was significantly higher under the treatment T_8 : SF Soil

Table 3. Effect of microbial cultures on dry weight at 30 and 60 DAS in polluted and unpolluted soils of spinach beet

Treatments	Fresh weight of leaf/plant	
	30DAS	30DAS
Polluted Soil with supply of fresh water		
T_1 - SF Soil + FYM	4.05	2.84
T_2 - SF Soil + FYM + VAM + <i>Psuedomonas</i>	4.73	3.24
T_3 - SF Soil + RDF	3.16	2.22
T_4 - SF Soil + RDF + FYM + VAM + <i>Psuedomonas</i>	5.55	3.58
Unpolluted soil with supply of fresh water		
T_5 - SF Soil + FYM + <i>Psuedomonas</i>	4.62	2.93
T_6 - SF Soil + FYM + VAM + <i>Psuedomonas</i>	5.82	3.80
T_7 - SF Soil + RDF	3.55	2.52
T_8 - SF Soil + RDF + FYM + VAM + <i>Psuedomonas</i>	6.62	4.17
Unpolluted soil with supply of polluted water		
T_9 - Soil + FYM	3.47	2.55
T_{10} - Soil + FYM + VAM + <i>Psuedomonas</i>	5.45	3.38
T_{11} - Soil + RDF	4.55	2.86
T_{12} - Soil + RDF + FYM + VAM + <i>Psuedomonas</i>	5.97	3.95
SE $m \pm$	0.046	0.03
C.D at 5%	0.133	0.103

Table 4. Influence of different treatments on microbial population in polluted and unpolluted soils of spinach beet at 30DAS

Treatments (30 days)	Bacteria 10 ⁷ CFU g/soil	Rhizobium 10 ³ CFU g/soil	Azotobacter 10 ³ CFU g/soil	Azospirillum 10 ³ CFU g/soil	Actinomycetes 10 ³ CFU g/soil	Pseudomonas 10 ³ CFU g/soil	Molds10 ³ CFU g/soil	VAMX10
Polluted Soil with supply of fresh water								
T ₁ - SF Soil + FYM	101.1	19.31	10.16	8.36	28.18	29.16	13.79	9.58
T ₂ - SF Soil + FYM + VAM + <i>Pseudomonas</i>	117.85	17.59	11.2	9.36	22	78.68	9.73	12.92
T ₃ - SF Soil +RDF	91.82	19.59	9.7	9.63	32.28	59.75	17.91	7.49
T ₄ - SF Soil + RDF + FYM+VAM+ <i>Pseudomonas</i>	83.31	16.32	10.43	10.46	23.61	71.43	7.88	15.15
Unpolluted soil with supply of fresh water								
T ₅ - SF Soil +FYM	101.45	26.23	12.63	8.36	24.51	84.13	12.30	11.55
T ₆ - SF Soil + FYM+ VAM + <i>Pseudomonas</i>	84.92	29.23	12.7	9.13	26.31	85.72	11.51	20.80
T ₇ - SF Soil + RDF	82.98	26.58	15.13	9.4	41.77	74.40	9.59	24.13
T ₈ - SF Soil + RDF+ FYM+ VAM+ <i>Pseudomonas</i>	123.85	28.00	11.46	10.5	31.35	55.48	6.01	14.46
Unpolluted soil with supply of polluted water								
T ₉ - Soil+FYM	93.1	19.6	14.26	10.9	21.48	47.04	9.80	16.40
T ₁₀ - Soil+FYM+VAM+ <i>Pseudomonas</i>	85.1	23.3	11.26	11.4	22.16	96.1	4.33	13.85
T ₁₁ - Soil+RDF	93.81	27.2	15	12	32.14	43.85	10.41	14.29
T ₁₂ - Soil+RDF+FYM+VAM+ <i>Pseudomonas</i>	124.21	23.80	12.73	12.1	32.01	96.00	5.16	15.39
SE _m ±	2.794	1.063	0.841	0.546	1.732	1.657	0.758	1.326
C.D at 5%	8.155	3.103	2.454	1.594	5.054	4.837	2.213	3.871

Table 5. Influence of different treatments on microbial population in polluted and unpolluted soils of spinach beet at harvesting stage (60DAS)

Treatments (30 days)	Bacteria 10 ⁷ CFU g/soil	Rhizobium 10 ³ CFU g/soil	Azotobacter 10 ³ CFU g/soil	Azospirillum 10 ³ CFU g/soil	Actinomyces 10 ⁴ CFU g/soil	Pseudomonas 10 ⁴ CFU g/soil	Molds10 ³ CFU g/soil	VAMX10
Polluted Soil with supply of fresh water								
T ₁ - SF Soil+FYM	60.68	12.5	4.3	5.43	21.16	38.3	10.04	4.86
T ₂ - SF Soil+FYM+VAM+ Pseudomonas	53.25	11.96	9.2	4.76	19.01	37.17	5.60	8.04
T ₃ - SF Soil +RDF	49.05	11.41	2.43	6.3	24.86	41.23	11.32	4.29
T ₄ - SF Soil+RDF+FYM+VAM+Pseudomonas	63.78	10.7	9.8	8.53	16.55	34.68	2.98	6.16
Unpolluted soil with supply of fresh water								
T ₅ - SF Soil +FYM	83.38	18.66	12.46	4.43	34.27	54.24	8.55	6.16
T ₆ - SF Soil + FYM+ VAM+Pseudomonas	55.41	20.71	2.73	6.5	26.58	45.48	5.85	8.99
T ₇ - SF Soil+RDF	49.57	17.90	5.1	8	34.73	58.68	4.45	4.14
T ₈ - SF Soil+RDF+FYM+VAM+Pseudomonas	88.68	15.79	5.63	7.2	22.94	50.86	1.95	7.27
Unpolluted soil with supply of polluted water								
T ₉ - Soil+FYM	46.46	17.00	9.5	5.4	15.67	25.42	5.43	8.34
T ₁₀ - Soil+FYM+VAM+Pseudomonas	59.14	16.53	9.9	5.5	19.44	53.98	2.56	7.88
T ₁₁ - Soil+RDF	50.59	15.59	9.43	4.26	24.41	30.89	5.85	5.14
T ₁₂ - Soil+RDF+FYM+VAM+Pseudomonas	84.21	15.92	10.43	9.4	23.63	60.25	3.41	9.00
SE m±	1.473	1.337	0.523	0.392	2.040	1.857	0.335	0.580
C.D at 5%	4.299	3.902	1.526	1.144	5.956	5.420	0.978	1.692

+ RDF + FYM + VAM + *Pseudomonas* and T₁₂ = Soil + RDF + FYM + VAM + *Pseudomonas*. This is due to application of VAM and *Pseudomonas* along with FYM will enhance high number of cells in the rhizosphere which will compete with the nature genera.

Microbial Biomass Carbon

Microbial biomass carbon differed significantly at harvesting stage (60 DAS) as influenced by application of microbial cultures on biological quality of polluted soil and spinach yield. At 60 DAS, treatment T₁₂ recorded significantly higher (129.99) microbial biomass carbon as compared to all other treatments and treatment T₈ (123.68) was on par with T₁₂. The significantly lowest microbial biomass carbon recorded in the treatment T₁ (83.3). The MBC was higher in unpolluted soil compared to polluted soil individually that the microbial activity and mass is reduced in polluted soil due to the accumulated pollutants.

Leaf fresh weight (g plant⁻¹)

The data presented revealed that the leaf fresh weight was significantly affected by different treatments with RDF, combination of inorganic, organic manures (FYM, and biofertilizer) at 30 DAS and 60 DAS of crop. The highest leaf fresh weight plant⁻¹ was recorded in treatment T₈ (41.63 g plant⁻¹) than the rest of treatments at 30 DAS in unpolluted soils. The lowest leaf fresh weight per plant was showed in T₃ (23.02 g plant⁻¹) at 30 DAS in polluted soils. The highest leaf fresh weight was observed in T₈ (70.03 g plant⁻¹) and the lowest value observed in T₉ (38.12 g plant⁻¹) at 60 DAS in unpolluted soil. It was observed that the treatment T₈ (70.03 g plant⁻¹) comprising RDF + FYM + VAM and *Pseudomonas* showed highest values at 30 DAS, 60 DAS in unpolluted soils over other treatments.

Leaf dry weight (g plant⁻¹)

The data presented revealed that the leaf dry weight was significantly influenced by recommended dose of fertilizers, combination of inorganic, organic manures (FYM) and biofertilizers (VAM and *Pseudomonas*) at 30 DAS and 60 DAS. The highest leaf dry weight plant⁻¹ was observed in T₈ (6.62 g plant⁻¹) and lowest value in T₃ (3.16 g plant⁻¹) was observed at 30 DAS. The highest leaf dry weight was observed in T₈ (4.17 g plant⁻¹) and the lowest in T₃ (2.22 g plant⁻¹) at 60 DAS. Among

all the treatments, T₈ comprising RDF, FYM, VAM and *Pseudomonas* was showed highest dry weight of leaf per plant at 30 DAS & 60 DAS in unpolluted soils. In same way, the lowest dry weight of leaf was found in T₃ at 30 and 60 DAS in polluted soils. Similar results were reported by Madhvi *et al.* (2014). It was reported that increased leaf area and leaf dry weight in spinach was due to application of chemical fertilizers along with organic manures and biofertilizers.

CONCLUSIONS

Taken together the results obtained in the present study clearly indicate that use of the polluted soil or polluted water for raising spinach beet crop gave reduced yield in terms of leaf fresh weight and dry weight. The yield was significantly highest in the treatment (T₈) with FYM, RDF and microbial cultures VAM & *Pseudomonas* in a normal soil irrigated with fresh water. The microbial populations viz. bacteria, molds were more influenced by the application of FYM and chemical fertilizers irrespective of the soil or water pollution.

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