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Effect of Heavy Metal Pollution on Total Microbial Count and Seed Germination Ability of Soil

Kirti J Mhatre*, Pratiksha R Phadke, Sonu R Tanwar, Hrutuja M Maydeo, Vishal Naik**
Department of Biotechnology, Mahatma Phule A.S.C. College, Panvel, Raigad, Maharashtra, India*
Department of Chemistry, Mahatma Phule A.S.C. College, Panvel, Raigad, Maharashtra, India**
kjmhatre2@gmail.com*, visshal.naik1@gmail.com**

Abstract: *A wide application of heavy metals in different human processes leads to its accumulation in environment. Atmospheric deposition of metals into soil ecosystem is majorly affecting the microbial count of soil and seed germination as well. In present study, soil samples were collected from metal polluted sites and agricultural fields to determine of heavy metal (Zn, Cu, Mn and Fe) content and total heterotrophic count of different soil samples. The microbial count, seed germination percentage and heavy metal concentration in agricultural field samples were found to be in permissible limit, while increased metal concentrations and lower bacterial count were observed in metal contaminated soil samples. Increasing heavy metal concentration found to be drastically affecting on total heterotrophic count of agricultural field soil samples, while high metal concentration tolerating bacterial count was found in metal contaminated soil samples. The study suggested that metal processing industries should accept new practices to avoid such metal incorporation in natural environment to save soil microbial diversity.*

Keywords: Heavy metal, Atmospheric deposition, Soil ecosystem, Total microbial count, Seed germination, Metal tolerating bacteria

I. INTRODUCTION

A group of metals which are having relatively high density is called as heavy metals. These metals are naturally present in soil ecosystem and are playing important role in Biogeochemical cycles (C, N, O and P). Some of the heavy metal like Pb, Cd and Hg are harmful to living organisms at very low concentration, while some of the heavy metal like Cu, Zn, Mn and Fe are beneficial to living things in constant range (Chu, 2017).

An increasing industrialization, anthropogenic processes, agricultural practices, Mining and release of industrial sewage in natural water bodies are leading source of heavy metal contamination in environment (Huang et al., 2021). More than 100 metal fabricators are developed in Panvel and Taloja MIDC within past two decades. The atmospheric deposition of heavy metals from such small scale industries is a major concern. Heavy metals are recalcitrant in nature. If their concentration in soil crossed the permissible limit then it will show toxic effects on living cells. They inhibit growth of microorganisms by structure deformation and by interfering into microbial metabolism. Indirectly it will affect on microbial role in decomposition of organic matter, Nutrient absorption by plant, population size of microbial community and beneficial biochemical reactions essential for living beings (Xie et al., 2016 and Vieira et al., 2005). Excessive level of Heavy metals also affects seed germination and plant growth by interfering in physiological and biochemical processes of plant cell. Many researchers had studied the effect of heavy metal accumulation on natural microbial flora. Wang et al. (2007), reported significant impact of heavy metal on soil bacterial and actinomycetic community structure.

Industrialization is need of human beings but their end products should not be threatened to our environment. It is a responsibility of researchers to determine toxic effects of heavy metals periodically which will help to develop new guidelines of metal processing practices and its disposal. Thus, the aim the study was to determine and compare the total microbial count and seed germination ability of agricultural field soil samples and metal contaminated soil samples.

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II. MATERIALS AND METHODS

2.1 Sample Collection

Agricultural field Soil samples were collected from farms of Kon, Nere and Owale villages coming under Panvel Tahsil. Metal contaminated soil samples were collected from different metal fabrication industries (10 yrs. Old) situated at Taloja and Panvel MIDC, Raigad (MS), India. 5 samples from different locations at each farm site were collected and one representative sample was prepared for further processing. At each sampling spot "V'' shaped cut was made up to 15 cm depth to collect sample. Samples were transported to the laboratory within 1h of collection and stored at below 10°C.

2.2 Determination of pH of Soil Samples

10 gm air-dried each soil sample added with 25 ml 0.01 M CaC12 and Equilibrated for 10 minutes. The pH of the suspension was measured potentiometrically with a glass electrode versus a calomel reference electrode. Before starting a series of measurements, the potentiometer is calibrated with a buffer solution of known pH (4, 7 and 10).

2.3 Measurement of Heavy Metal Concentration

20 gm air dried soil sample sieved through a 1mm-pore size screen. Sieved sample treated with 40 ml DTPA solution in shaking condition at 100 rpm at R.T. (±25°C) for 2h. Treated soil sample filtrate was used for determination of Fe, Mn, Zn and Cu content in sample. [DTPA : (a) 0.1 M triethanolamine (TEA) = 14.9 gm, (b) 0.005 M diethylenetriamine Pentaacetic acid (DTPA) = 1.967 gm, (c) 0.01 M calcium chloride CaCl2 . 2H2O= 1.47 gm, a + b + c compounds were dissolved in 800 ml D/W, adjusted pH to 7.3 using diluted HCl 1:1 (10 ml). Made the total volume of 1L using D/W]

Heavy metal content of soil samples was determined by using Atomic absorption spectrophotometer (model-Shimadzu AAS3500). Total amount of heavy metals present in unknown soil samples was calculated by calibration curve method. Standards of 2ppm, 5ppm and 10 ppm were used for calibration. Blank of extracting solution was used for AAS study.

2.4. Enumeration of Total Microbial Count

Total viable count of heterotrophic aerobic bacteria, Fungi and asymbiotic nitrogen fixing bacteria was determined by standard Spread plate method. Nutrient agar, Potato dextrose agar and Jensen's agar medium was used for total plate count of respective microorganisms (Hayat et al., 2002, Ahmad et al., 2005). Following is the medium composition (gm/L) used for standard plate count : a) Nutrient agar: (Peptone-5 gm; Nacl-5 gm; beef extract-1.5 gm; yeast extract-1.5 gm; agar 30gm, pH- 7.0± 0.2);b) Potato dextrose medium (Potato infusion - 200 gm; Dextrose-20 gm, agar 20gm, pH, 5.6 ± 0.2); c) Jensen's medium (Sucrose- 20 gm; KH2Po4-1 gm; MgSo4-0.5 gm; Nacl0.5gm; FeSo4- 0.1 gm; Na-molybdate-0.005 gm; CaCo3- 2gm; agar- 30gm, pH- 6.8 ± 0.2). For total heterotrophic bacterial count the plates were incubated at R.T. for 24 h, while for fungal and aymbiotic nitrogen fixing bacterial count, plates were incubated R.T. for 2 to 7 days

2.5 Effect of Heavy Metal Concentration on Seed Germination

The seeds of Trigonellafoenum-graecum (Fenugreek), Viciafaba (Broad beans) and Vignaunguiculata (Blacked eye Pea) are selected for the study as these are commonly growing vegetables of Panvel region. 25 gm of each soil sample was placed in clean and dry plastic pots. 100 seeds of each plant were soaked in waterfor 12 h. Well soaked 50 seeds of each plant were placed in potted soil samples. After 4-5 days the total no. of germinated seeds were measured and germination percentage was calculated.

III. RESULT AND DISCUSSION

3.1 Determination of pH and Heavy Metal Concentration

The pH of agricultural field soil samples is ranging in between 6.5 to 7.5, while pH of metal contaminated soil sample is ranging in between 7.5 to 8.0. The total quantity of Fe, Cu, Mn and Zn metals present in farm soil samples

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was found in between the range of 13-19 ppm, 10-27 ppm, 4 to 11ppm and 1-5 ppm respectively. Soil samples collected from metal contaminated sites showed increased in Fe, Cu, Mn and Zn concentration i.e. 150-200 ppm , 12 to 28 ppm , 11 to 17 ppm and 30 to 75 ppm respectively. The exact values of heavy metal concentration in each soil were represented in table 3.1. It indicates the atmospheric deposition of metal powders and residues generated during metal processing in fabrication industries.

Different environmental factors is responsible for Physiochemical characteristic of soil. Many scientist were evaluated the total microbial count. But there is large variation was observed in values. The Indian CPCB standards for every region of India are available in literature and thus the obtained values of heavy metals in present study were not comparing with the standards.

Table 3.1: pH and Heavy metal concentration in collected soil samples

Soil Sample	pH	Fe (ppm)	Cu (ppm)	Mn (ppm)	Zn(ppm)
Kon Village- Farm 1	7.63	19.7537	10.8223	5.5045	1.7837
Kon Village-Farm 2	7.75	21.7868	15.6180	9.2655	2.9691
Owale Village-Farm 1	7.07	26.1544	15.4244	5.8264	5.5253
Owale Village-Farm 2	6.57	62.8493	27.0637	10.6364	3.8933
Nere Village- Farm1	7.57	37.6029	26.7029	9.0964	5.6067
Nere Village- Farm 2	6.32	13.1801	0.8170	4.0400	0.4551
A.K. Fabrication	7.89	152.2167	12.1923	12.4367	51.5621
Shivank Fabrication	8.21	145.6754	19.4183	16.9452	87.4893
Shree Ram Samarth fabrication works	7.90	186.5678	15.1247	13.3985	29.4659
Maharashtra fabrications works	7.62	196.5462	27.7634	11.8836	74.5478

3.2 Enumeration of Total Microbial Count

The total no. of heterotrophic bacteria, Fungi and asymbiotic nitrogen fixing bacteria present in fertile soil sample was found to be much higher as compare to total no. bacteria present in metal contaminated soil samples. Table 3.2 represents the total microbial count of collected soil samples.

Heavy metals have a strong affinity towards with some biological macromolecules such as enzyme activity center, electron-donating groups, nucleotides and phosphate molecule which results in the structural instability of biological macromolecules and thus bacteria are get inhibited in presence of high concentrations of heavy metals (Chu *et al.*, 2017). Similar to our results, Oliveira *et al.* (2005) also observed the reduction of total microbial count in metal containing soil samples. The bacterial count of metal contaminated soil samples is actually a count of metal resistant bacteria count. Because, numerous studies revealed that high heavy metal concentration in soil responsible for the transitions of bacteria

Table 3.2: Total microbial count of collected soil samples (CFU/ml)

Soil Sample	Total Heterotrophic count	Total fungal count	Total asymbiotic nitrogen fixers
Kon Village- Farm 1	$8.7x10^7$	$6.4x\ 10^6$	$4.2x10^6$
Kon Village-Farm 2	$9.2x10^7$	$7.3x10^6$	$4.3x10^6$
Owale Village-Farm 1	$7.5x\ 10^7$	$4.5x10^6$	$5.3x10^6$
Owale Village-Farm 2	$6.9x10^7$	$3.2x10^5$	$4.1x10^6$
Nere Village- Farm1	$8.4x10^7$	$6.2x10^6$	$6.1x10^6$
Nere Village- Farm 2	$9.6x10^7$	$5.6x10^6$	$5.0x10^6$
A.K. Fabrication	$3.4x10^6$	$4.2x10^4$	$2.4x10^4$
Shivank Fabrication	$2.8x10^6$	$3.6x10^4$	$4.3x10^4$
Shree Ram Samarth fabrication works	$4.3x10^6$	$4.6x10^4$	$7.3x10^4$
Maharashtra fabrications works	$3.7x10^6$	$4.8x10^4$	$6.8x10^4$

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3.3 Effect of Heavy Metal Concentration on Seed Germination

More than 90% seed germination of all plants was observed in farm soil sample however, less that 60% seed germination was observed in metal contaminated soil samples. The study suggests that, Farm soil samples promoted the seed germination while metal contaminant is interfering in seed germination process. Table 3.3 represents the seed germination percentage of three different plants.

Microbial soil flora bacteria, algae, actinomycetes and fungi carried out many of the processes that increase the soil fertility. If the total microbial count is decreased, it affects the diversity and biochemical reactions carried out by variety of microorganisms. The process like Nitrogen fixation, organic matter decomposition and mineralization are important in cycling of plant nutrients in the soil. Any disturbance in the soil ecosystem it will directly affect the productivity of agricultural soils. Thus in present study, seed germination was found to be very low in metal contaminated soil samples.

Table 3.3: Represents the seed germination percentage of three different plants

Soil Sample	*Trigonellafoenum-graecum* (Fenugreek seeds)	*Viciafaba* (Broad beans)	*Vignaunguiculata* (Blacked eye Pea)
Kon Village- Farm 1	98	91	94
Kon Village-Farm 2	89	90	96
Owale Village-Farm 1	95	88	86
Owale Village-Farm 2	92	94	91
Nere Village- Farm1	89	92	97
Nere Village- Farm 2	96	88	88
A.K. Fabrication	42	34	56
Shivank Fabrication	34	36	39
Shree Ram Samarth fabrication works	41	24	43
Maharashtra fabrications works	46	21	41

IV. Conclusion

Metal processing small scale industries are one of the sources of heavy metal contamination in environment which is affecting on total microbial count of heterotrophic, fungal and nitrogen fixing bacteria. Prevention of seed germination is not a good sign to environment sustainability. Thus there should be development of new techniques to remediate heavy metal form environment.

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