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Original Article

Estimation of culturable microbes present in heavy metal contaminated and non contaminated Agricultural soil

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Abstract

Environmental contamination and exposure to heavy metals such as mercury, cadmium and lead is a serious growing problem throughout the world. Human exposure to heavy metals has risen dramatically in the last 50 years as a result of an exponential increase the agricultural land were affected seriously. Based on the analysis of AAS five samples were showed positive for the heavy metal contamination. Estimation of Culturable microbes present in the agricultural soil showed wild difference between the heavy metal contaminated and non contaminated rhizosphere soil. The maximum microbial load was observed in the non contaminated soil sample NCS1 ($26x10^8$ CFU ml^{-1}) and contaminated soil sample CS5 ($18x10^3$ CFU ml^{-1}). Thus heavy metals used in the industries like fireworks and match work will affect microbial load present in soil of nearby agricultural land.
Keywords: Heavy metals; pollution; Culturable microbes

Introduction

Due to urbanization and land degradation, the area of agricultural land is continuously decreasing. Proficient use of available agricultural land resources is important to overcome the deficiency. Soil is a non-renewable dynamic resource and acts as an interface between agriculture and the environment (Abou *et al*., 2008). Maintaining soil quality is the vital factor to improve crop yield and productivity. Among the soil quality maintance heavy metals plays an imperative role to sustain its eminence properties (McGrath *et al*. 1995; Cheng 2003). In India many industries are using heavy metals in their process and exiled out without proper treatment. Metals are released into the environment leads wide spectrum of anthropogenic activities such as smelting of metallic ores, industrial fabrication and commercial application of metals, which are polluting our aquatic bodies.

Though, several metals are essential for biological systems and must be present in a certain concentration range. Too low concentrations lead to a decrease in metabolic activity (Vajpayee *et al*.,1995). The fireworks that are displayed in the skies to celebrate events such as Independence Day and New Years Eve, etc.contain carcinogenic sulphur-coal compounds. Fireworks spread an odor of black gunpowder and spreads radioactive barium, which makes the green sparkling color and considerable amounts of strontium, along with arsenic, mercury, cadmium, lead, copper, zinc and chromium. (Patrick, 2003). Such a pollution of the environment by toxic metals and radionuclides arises as a result of many human activities, largely industrial, although sources such as agriculture and sewage disposal also contribute. These contaminations not only affect the human and also alter the microbial community structure. (Weis, 2004). Thus, finally this may also seriously affect the nearby agricultural land and food cultivate in this. In Sivakasi area, fireworks and match work industries are predominant one and the explosion of fireworks has been discovered to be a source of intense heavy metal release. Our aim is to compare and explore the microbial load in agriculture nearby industry.

Materials and Methods

Study Area

Sivakasi is well known for crackers, printing and Match factories. The area of investigation lies in and around Sivakasi agricultural area located near fire work factory. It is located approximately in 9.28' North latitude and 77.48' East longitude. The elevation of the area of investigation is 100.07 meter above sea level. Variation in the altitude and rain fall has a bearing on the vegetation in general.

Microbial analysis of heavy metal contaminated soil

Total Culturable bacteria in the normal agricultural and heavy metal contaminated soil samples were homogenized in phosphate buffered saline solutions and serially diluted and plated on Nutrient Agar medium. After 24 h incubation at 30 °C, plates were scored for their load in terms of Colony Forming Unit (CFU) and expressed it in lane values.

Screening of heavy metals in agriculture soil

Soils samples were taken from different site of agricultural area present in Sivakasi. Plant roots were taken by loosening the soil around the root and then gently removing the plant material from the ground. The root adhering soils were used to analyze for heavy metal presence. Heavy metals were analyzed by using 20 g of soil was mixed in 100 ml of distilled water and 0.5 ml was used to find in Atomic Absorption Spectroscopic (AAS). (Sinha *et al*., 1993).

Results

Soil characteristics of pH

Soil samples from various sites of agricultural lands present near the industry areas were taken for soil analysis. From the soil pH analysis 80% of the samples showed the pH ranged from 7.2 to 8.21 and remaining 20% of them in acid soils pH<6.7. This indicates that the pH for agricultural soils in Sivakasi has a tendency to be higher than natural and acidic soils pH. Organic matter and sand, silt, and clay contents vary significantly among soil samples (data not shown).

Soil organic contents

Organic content of the soil varies from soil to soil and it is highly influenced by the microbial community and cultivated plants. In this present study the level of organic soil ranges 0.9 to 12 %.

Soil conductivity (EC)

Electrical conductivity (EC) is a measurement of the dissolved material in an aqueous solution, which relates to the ability of the material to conduct electrical current through it. Actual conductivity of the soil is highly influenced by the nutritional measurements for fertilizers may vary due to the solubility and purity of the particular fertilizer source. Monitoring the changes in the nutrient solution over time will indicate what adjustments should be made to keep the solution in balance for the crop being grown. This principle can become more useful by determining for each crop the relationships among total dissolved solids, electrical conductivity, and concentration of each essential element and stage of plant growth under similar light conditions. In this study the soil have 15 to 39% soil conductivity (Table 1). Among them 10 samples taken from the agricultural soil having >30% conductivity and 6 of them were >20% and reaming were <20% conductivity.

Screening of Heavy metal presence in agricultural soils

Total concentration of heavy metals in the agricultural soils reflects a difference in the degree of soil contamination; the percentage of studied soils with concentrations of Cd, Cu, Ni, Pb and Zn. The concentrations of Cd, Ni, and Zn in the soils of the agricultural areas near to industries were significantly higher than the concentration of these same elements in the agricultural soils far to industries. The results (Table 2) also revealed a high variability of concentration of Cd, Ni, and Zn in different land uses. The concentration of the Nickel and cadmium was higher (3.9 and 4.1 ppm respectively) in sample 1 (S1) than remaining four samples (S2, S3, S4 and S5). Similarly the concentration of the zinc was higher in sample 3 (S3) than others.

Estimation of microbial biomass in Lead and Nickel contaminated soil

Microbial load present in and around the plant roots are varied based on environmental factor like heavy metals. In this study the microbial load of culturable bacteria present in this soil also varied from contaminated and non contaminated soils. (Mohammad Iqbal *et al*., 2008).The microbial load present in the non contaminated soil sample was high than the contaminated soils. The maximum load was observed in NCS1 sample ($26x10^8$ CFU ml^{-1}). Similarly the result of microbial load was coincided with the level of heavy metal concentration. (Phipps 1981).

Sample 3 (CS3) ($11X10^3$ CFU ml^{-1}) and Sample 5 (CS5) ($18X10^3$ CFU ml^{-1}) showed degreased level of bacteria compared to other three samples (CS1, CS2, and S4) (Table 3).

Table-1: Soil characteristic feature of agriculture soil samples taken from Sivakasi

Sample	Soil pH	Organic C content	Soil conductivity (% dw)
S1	8.5 ± 0.1	0.9 ± 0.25	35.8 ± 0.7
S2	8.4 ± 0.2	10.6 ± 0.18	36.8 ± 0.7
S3	7.9 ± 0.1	12.3 ± 0.34	29.0 ± 0.8
S4	6.3 ± 0.3	3.5 ± 0.68	15.8 ± 0.9
S5	7.8 ± 0.1	11.5 ± 0.45	37.1 ± 0.7
S6	6.3±0.2	0.9 ± 0.23	22 ± 0.30
S7	7.6±0.1	11.3 ± 0.45	34 ± 0.26
S8	7.2±0.2	11.9 ± 0.33	35 ± 0.88
S9	8.7±0.1	6.7 ± 0.69	19.5 ± 0.5
S10	7.5±0.2	1.7 ± 0.68	27.1 ± 0.6
S11	6.2 ± 0.1	10.8 ± 0.34	25.3 ± 0.76
S12	7.6 ± 0.2	1.3 ± 0.78	16.8 ± 0.55
S13	7.2 ± 0.1	12.2 ± 0.76	39.0 ± 0.9
S14	7.7 ± 0.3	5.5 ± 0.63	19.8 ± 0.5
S15	7.2 ± 0.1	11.4 ± 0.54	27.4 ± 0.4
S16	6.6±0.2	8.8 ± 0.23	32.6 ± 0.6
S17	7.6±0.3	11.1 ± 0.65	33.2 ± 0.7
S18	7.1±0.2	11.0 ± 0.32	24.7 ± 0.5
S19	7.4±0.2	4.6 ± 0.61	35.3 ± 0.7
S20	6.7±0.3	3.3 ± 0.50	32.7 ± 0.2

Values are represented as mean ±SD of triplicate

Discussion

Soil characteristics

Soil samples from various sites of agricultural lands present near the industry areas were readily contaminated with the materials used in that particular industries. In the fire industries most of the metals which were used to make colours are heavy metals. Thus in this studies attempts were taken to analysis. In the initial analysis of soil pH, organic matters and electric conductivity analysis revealed that most of the soils are coming under alkaline condition. Though it is alkaline, most of soils harbours good organic content and it is suitable for the cultivation. (Slobodkin, 2005).

The variation between the soils are highly influenced the microbial community and cultivated plants. In this present study the level of organic soil ranges 0.9 to 12 % is an average one. Electrical conductivity (EC) is a measurement of the dissolved material in an aqueous solution, which relates to the ability of the material to conduct electrical current through it. As per this analysis in the nutrient solution of the various samples indicate the proper nutritional balance for the crop is present in the soil. (Yinglu *et al*., 2007).

Heavy metal presence in agricultural soils

Liberation of heavy metal in the environment without proper treatment may increase the concentration of this in the soil. From our results it indicates that the plant cultured in land having significant level of heavy metals and this may affect the human normal life. Previous survey (GDPEMC 1990) indicated that the heavy metal background values of natural soil in Guangzhou were Cd (0.14 mg·kg−1), Cu (13.6 mg·kg−1) Ni (22.03 mg·kg−1), Pb (42.88 mg·kg−1) and Zn (58.1 mg·kg−1). Similarly in our studies suggest that our study area are contaminated with Nickel, zinc and cadmium. (Zhang *et al*., 2007).

Microbial biomass in heavy metal contaminated soil

Microbe plays an vital role in not only in the nutrient recycling process and also it simplify certain hard and complex materials to plant. Thus, microbe improves the soil quality by increasing the nutrients and also some of them provide security with it secondary metabolites. (Diels *et al*., 1999).

If the content of the heavy metal in the soil increases automatically the diversity of the microbes will affect. The level of heavy metal increased the content of the microbes also decreased. In this study the concentration of the heavy metals are inversely proposed. Heavy metals get accumulated in time in soils and plants and could have a negative influence on physiological activities of plants (e.g. photosynthesis, gaseous exchange, and nutrient absorption), determining the reductions in plant growth, dry matter accumulation and yield (Devkota and Schmidt, 2000; Baker, 1981). In small concentrations, the traces of the heavy metals in plants or animals are not toxic (De Vries *et al*., 2007). Lead, cadmium and mercury are exceptions; they are toxic even in very low concentrations (Galas Gorchev, 1991).

Table 2. Agricultural soil analysis for Heavy metal (Nickel, Zinc and cadmium) using AAS analysis

Soil sample	Heavy metals availability	Concentration (ppm)
CS1	Nickel	3.9
	Zinc	2.5
	Cadmium	4.1
CS2	Nickel	2.6
	Zinc	3.2
	Cadmium	3.9
CS3	Nickel	2.7
	Zinc	4.1
	Cadmium	3.3
CS4	Nickel	2.9
	Zinc	3.2
	Cadmium	3.7
CS5	Nickel	3.4
	Zinc	2.8
	Cadmium	3.7

Values are represented as ppm concentration of heavy metal (Mixture of five random samples from single site).

Table -3. Estimation of microbial biomass from agricultural and contaminated agricultural soils

Soil Characters	**Sample**	**CFU**
Rhizosphere soil (NC)	NCS1	$26x10^8$
	NCS2	$3x10^7$
	NCS3	$34x10^6$
	NCS4	$28x10^7$
	NCS5	$11x10^8$
Rhizosphere soil (Cont)	CS1	$15x10^5$
	CS2	$3x10^4$
	CS3	$11x10^3$
	CS4	$9x10^4$
	CS5	$18x10^3$

NC – non contaminated soil; Cont – contaminated soil; CFU – colony forming unit

Reference

Abou, R., Shanab, A., Ghanem, K and Kalaibe, A. D. (2008).The role of bacteria on heavy metal by plants growing on multi – metal contaminated soils, *world J. Microbial, Biotechnol,* 24: 253-262.

Baker, A. J. M. (1981). Accumulator and excluders: Strategies in response of plant to heavy metals. *J. Plant Nutr,* 3: 643–654.

Cheng, S., Grosse, W., Karrenbrock, F and Thoennessen, M. (2003). Efficiency of constructed wetlands in decontamination of water polluted by heavy metals, *Ecol Eng* ,18: 317–325.

Diels,L., De smet, M., Hooyberghs,L and Corbisier, P. 1999. Heavy metals bioremediation of soil, *J.molecul.biotchnol*, 149-158.

De Vries, W., Romkens, P. F., Schutze, G. 2007. Critical soil concentrations of cadmium, lead, and mercury in view of health effects on humans and animals, *Reviews of Environment Contam and Toxicol,* 191: 91–130. [Pub Med]

Devkota, B., Schmidt, G.H. 2000. Accumulation of heavy metals in food plants and grasshoppers from the Taigetos Mountains, Greece, *Agricul, Ecosyst and Environment,* 78(1): 85–91.

Galas-Gorchev, H. 1991. Dietary Intake of Pesticide Residues: Cadmium, Mercury and Lead, *Food Add Cont.* 8: 793–806.

McGrath, S.P., Zhao, F.J., Lombi, E. 1995. Plant and rhizosphere process involved in phytoremediation of metal-contaminated soils. *Plant Soil*, 232(1/2): 207-214.

Patrick. L. 2003.Toxic metals and antioxidants: Part II. The role of antioxidants in arsenic and cadmium toxicity.Altern Med Rev, *Environ Contam Taxicol,* May; 8(2): 106-28: Review.

Phipps, D. A. 1981. "Chemistry and biochemistry of trace metals in biological systems", in Effect of Heavy Metal Pollution on Plants, N. W. Lepp (Ed.), *App. Sci. Publishers.1-6.*

Sinha, S., Rai, U. N., Tripathi, R. D., Chandra, P. 1993. Chromium and manganese uptake by Hydrilla verticillata (I.f.) Royle : amelioration of chromium toxicity by manganese, *J. Environ Sci Health,* Part A 28(7): 1545–1552.

Slobodkin, 2005. Thermophilic microbial metal Reduction. *J. of Microbiol*, 74 (5): 501-514.

Vajpayee, P., Rai, U. N., Tripathi, R. D., Sinha, S., Chandra, P. 1995.Bioremediation of tannery effluent by aquatic macrophytes, *Bull Environ Contam Toxicol,* 55: 546–553.

Weis, J. S., Weis, P. 2004. Metal uptake, transport and release by wetlands plants implication for Phytoremediation and restoration. *Environ Int.* 30(5): 739–753.

Yinglu – Feng zhu-jie chen, Haihuaggan and Yenbiaogue, 2007. Chemical fractionation of heavy metals in urban soils of Guangzhau, China, *Environ monit Assess,*134: 429-439.

Zhang X-H., Liu, J., Huang H-T., Chen, J., Zhu Y-N and Wang D-Q. 2007. Chromium accumulation by the hyper accumulator plant *Leersia hexandra* . Swartz. Chemosphere 67: 1138–1143.