Current Botany 2011, 2(8): 14-18
ISSN: 2220-4822
www.scholarjournals.org

CURRENT BOTANY
www.currentbotany.org

Amelioration of Sugar Mill Effluent Polluted Soil Using Microbial Isolates and its Response on Paddy

Sajani Samuel* and S.M. Muthukkaruppan

Department of Microbiology, Annamalai University, Chidambaram- 608002, Tamil Nadu, India

Article Info

Article History

Received : 20-05-2011
Revised : 29-07-2011
Accepted : 30-07-2011

*Corresponding Author

Tel : +91-9884284162

Email: sajani.samuel123@rediffmail.com

Abstract

Sugar mills play a major role in polluting the water bodies and land by discharging a large amount of wastewater as effluent. The continuous use of these effluents harmfully affects the crops when used for irrigation. As a result, a higher amount of various elements get deposited in the soil and make them polluted. Since this polluted soil reduces both the crop production as well as the soil properties, it was decided to give some bioremediation measures by treating the soil with microbial isolates. The physico- chemical analysis of the sugar mill polluted soils revealed that they are toxic in nature because they contain higher amount of micronutrients and macronutrients, organic and inorganic chemicals and heavy metals. In order to bioremediate the soil quality, PGPR and fungal isolates *(Bacillus* spp + *Pseudomonas* spp and *Aspergillus niger* + *Penicillium* spp) were cultured in the soil for 60 days. After that, the various soil properties were analysed and good percentage of pollutant reduction was observed. Germination studies were conducted in bioremediated soil and best germination was noticed under bioremediated soil when compared with polluted soil.

Key Words: Polluted soil, *Bacillus* spp, *Pseudomonas* spp, *Aspergillus* niger, *Penicillium* spp, Bioremediation, Germination

Introduction

Soil is a dynamic, living matrix that is an essential part of the terrestrial ecosystem. It is a critical resource not only for agricultural production and food security, but also towards maintenance of most life process. The functions of soil biota are central to decomposition processes and nutrient cycling. Soil pollution is a very important environmental problem. It has been attracting considerable public attention over the last decades. As a matter of fact, increasing widespread pollution has caused vast areas of land to become non- arable and hazardous for both human population and industrialization. Industrialization is an important tool for the development of any nation. Consequently, the industrial activity has expanded so much all over the world. Today, it has become a matter of major concern in the deterioration of the environment (Tiwari *et al*., 2000). During the production, industries generates useless by products and waste materials with 1 to 10% of the quantity of parent chemicals.

Among the industries, sugar industry plays a major role in producing a higher amount of water pollution because they contain large quantities of chemical elements, total hardness, total dissolved solids, biological oxygen demand, chemical oxygen demand, calcium, magnesium, sodium, iron and sulphate. In addition to that, some traceable amount of heavy metals such as zinc, copper, lead, magnesium and iron were also present in the effluent (Baskaran *et.al.,* 2009). The effluent not only affect the plant growth but also deteriorate the soil properties when used for irrigation (Maliwal, *et al*., 2004). The polluted soil becomes unsuitable for further cultivation. So, there is a need to conduct some kind of experiments to enrich the soil properties and make good source of environment in that way microorganisms play a major role in bioremediation of contaminated soil.

Bioremediation is the use of biological agents such as bacteria, fungi and plants to remove or degrade the pollutants from the contaminated soil. This technology has appeared to reduce the enormous costs and environmental disturbance that are associated with current clean up method. It is necessary to conduct experiments on the impact of these polluted soils on agricultural crops before they are used for crop cultivation. Among the microorganisms used for bioremediation, rhizosphere microorganisms that are closely associated with roots termed as plant growth promoting rhizobacteria (PGPR) play a vital role. PGPR function in three different ways: synthesizing particular compounds for the plants, facilitating the uptake of certain nutrients from the environment and protecting the plants against diseases (Glick, 2003). An extension of PGPR technology is the emerging use of bacteria with plants for environmental applications. Recent studies in this area include many different applications, such as growth promotion of soil stabilizing plants, to counteract flooding stress of plants, to aid plant growth in acidic conditions, to counteract high temperature stress, as well as for phytoremediation technology (Zhuang *et al*., 2007) also accumulation of heavy metal in the soil environment and their uptake is in growing concern. These microorganisms can be indigenous to a contaminated area (intrinsic bioremediation) or can be isolated from elsewhere and then introduced into contaminated site (bioaugmentation) (Whiting *et al*., 2001).Hence the present research has been carried out to bioremediate the sugar mill effluent contaminated soil using PGPR, fungal isolates and to study their response on paddy.

Materials and Method

Collection of Sample

The sugar mill effluent polluted soil sample was collected in polythene bags from nearby industrial area of Cuddalore district, Tamil Nadu, India. The polluted soil was analysed for its various physico- chemical parameters. The PGPR and fungal isolates used for bioremediation (*Bacillus* spp, *Pseudomonas* spp, *Aspergillus niger*, *Penicillium* spp) were isolated from the rhizosphere of plants.

Experimental Method

Pot experiment was carried out using polluted soil. 90× 60 cm and 50 cm volume cement tanks were used, to about 750g polluted soil 2% microbial pollution were used along with paddy grains.

The contaminated soil was first passed through mesh sieve. Polluted soil was added to the pots and mixed with microbial agent first. Initially after 30 days the soil samples were collected and analysed to know their properties. After 60 days of treatment to the bioremediated soil paddy plants were transplanted and water was added according the regular plant growth needed. All experiments were conducted in triplicates.

Germination experiments

The bioremediated soils were analysed and they were used for germination studies of paddy (*Oryza sativa* L.). The number of seeds germinated was counted on 7th day and the germination per centage was calculated by using the following formula.

$$\text{Germination Per centage} = \frac{\text{No. of seeds germinated}}{\text{Total no. of seeds sown}} \times 100$$

Biochemical Analysis

Estimation of Chlorophyll (Arnon 1949):

Five hundred mg of fresh leaf material was taken and ground with help of pestle and mortar with 10 ml of 80% acetone. The homogenate was centrifuged at 800 rpm for 15 minutes. The supernatant was saved. The residue was re-extracted with 80% acetone. The supernatant was saved and utilized for chlorophyll estimation. Absorbance was read at 645, 663 and 480 nm in the UV- spectrophotometer.

Estimation of Protein (Lowry et.al., 1951):

Protein content was determined by the method of Lowery *et al* (1951). 0.5g of plant sample (shoot) was homogenized in 10 ml of 20% Trichloro Acetic Acid (TCA). The homogenate was centrifuged in 10 minutes for 300 rpm the supernatant was discharged and the pellet was re- extracted with 5ml of 0.1 N NaOH. One ml of the extract was taken in a test tube and 5 ml of reagent 'C' (protein reagent) was added. This solution was mixed well and kept in dark for 10 minutes. Later, 0.5 ml of folin phenol reagent was added and the mixture was kept in dark for 30 minutes. The sample was read at 660 nm in the UV- spectrophotometer.

Amino Acid (Moore and Stein, 1948):

Amino acid content was determined by the method of Moore and Stein (1948). 0.5 g of plant sample was homogenized in 10 ml of 80% ethanol. The homogenate was centrifuged for 10 minutes at 800 rpm one ml of the extract was taken in the test tube to which added 1 ml of 0.1 N of HCl to neutralize the sample. To this, one ml of ninhydrine reagent was added and heated for 20 minutes in a boiling water bath. Later, 5 ml of the diluents solution was added and heated again in water bath for 10 minutes. The test tubes were cooled and read the absorbance at 570 nm in a UV-spectrophotometer.

Estimation of Sugars (Nelson 1944):

Extraction: Five hundred mg of plant materials were weighed and macerated in a pestle and mortar with 10 ml of 80 % ethanol. The homogenate was centrifuged for 10 min at 800 rpm. The supernatant was collected and the ethanol was evaporated in a water bath at 50^0C. The net content was made upto 20 ml with distilled water and the extract was used for the estimation of reducing sugar.

Estimation: One ml of extract was taken in a 25 ml marked test tube. 1 ml of reagent 'C' was added. Then, the mixture was heated for 20 min at 100^0C in a boiling water bath, cooled and 1 ml of arsenomolybdate reagent was added. The solution was thoroughly mixed and diluted to 25 ml with distilled water. The sample was read in the UV- spectrophotometer at 520 nm. The sugar contents were expressed in mg/g fresh weight basis.

Result and Conclusion

The result of the physico- chemical analyses of sugar mill effluent contaminated soil are presented in (Table- 1). The polluted soil is having a higher amount of pH, electrical conductivity, nitrogen, phosphorus, potassium, copper, zinc, iron and manganese present in it. At the same time, a considerable amount of nutrient changes was observed in the bioremediated soil. The polluted soil was found with excess and less amount of micro and macronutrients which will reduce the plant growth. At the same time, the bioremediated soil analysis reveals that it has a reduced amount of pollutant. The removal of elements from polluted soil may be due to accumulation of the contaminants by the isolates (Fig1-4).

The response of bioremediated soil on germination of paddy (*Oryza sativa*.L) was showed in Fig 5-7. The morphological parameters of paddy like germination percentage, seedling growth and biochemicals like chlorophyll, protein, amino acid and total sugar were observed in both polluted soil and bioremediated soil. Among them, all morphological and biochemical parameters, good germination and biochemical changes were seen increased in bioremediated soil.

From the results it could concluded that by treating the sugar mill effluent contaminated soil aerobically with microbial isolate (consortium), the physico – chemical parameters could be reduced. However, all the parameters were found to be relatively high in untreated soil and severally affected the plant and soil health. Treated soil showed better response towards other parameters such as germination percentage, chlorophyll, amino acid, protein and total sugars. Hence it is suggested that sugar mill effluent polluted soil can be aerobically treated using microbial isolate and may be suitable for agricultural purpose

Table 1:b. Physico- chemical properties of polluted soil

S.No	Soil Properties	Polluted soil
1.	pH	6.0
2.	EC(mM hos)	0.43
3.	N (Kg/ha)	92
4	P (Kg/ha)	50
5	K (Kg/ha)	150
6	Copper (ppm)	0.75
7	Zinc (ppm)	7.28
8	Iron (ppm)	21.28
9	Mangnese (ppm)	15.64
10.	Lead (ppm)	0.53

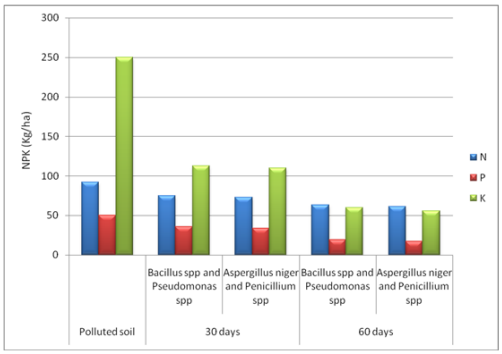

Fig 1: NPK analysis of sugar mill effluent polluted soil and bioremediated soil

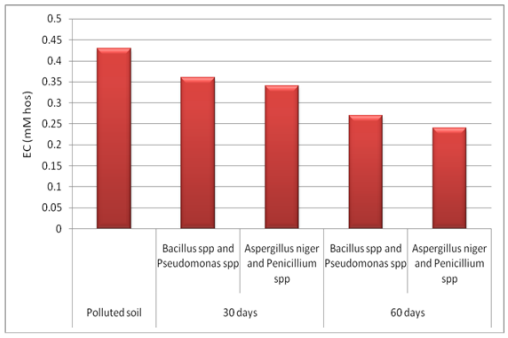

Fig 2: EC analysis of sugar mill effluent polluted soil and bioremediated soil

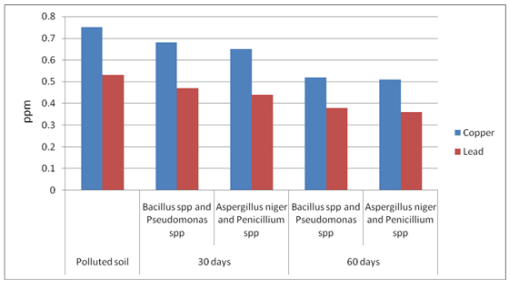

Fig 3: Copper and Lead analysis of sugar mill effluent polluted and bioremediated soil

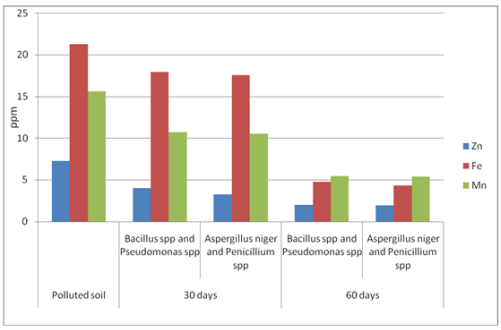

Fig 4: Zinc, Iron and Manganese analysis of sugar mill effluent polluted soil and bioremediated soil

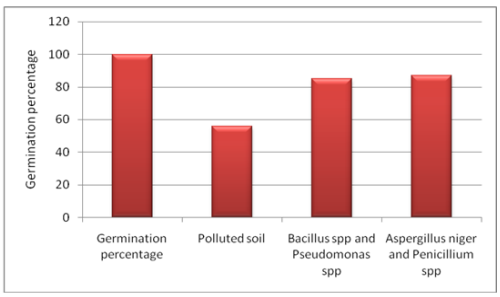

Fig 5: Germination Percentage of paddy grown under polluted soil and bioremediated soil

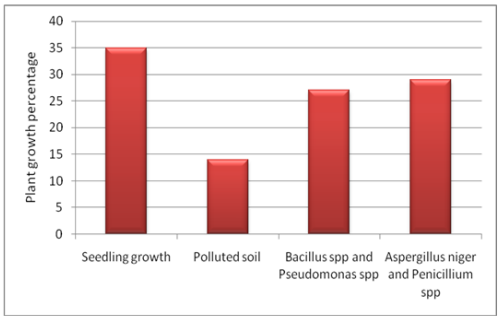

Fig 6: Seedling growth of paddy grown under polluted soil and bioremediated soil

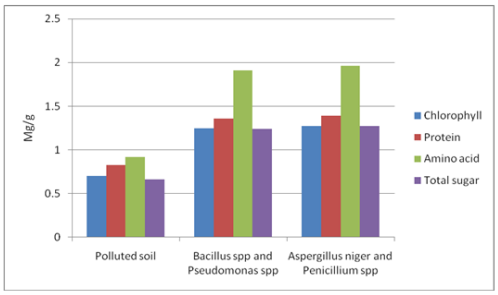

Fig 7: Chlorophyll, Protein, Amino acid and Total Sugar contents of paddy grown under polluted soil and bioremediated soil

Reference

Tiwari, P. Elecy and C.B. Indu Bhahia., 2000. Pollution studies on sugar mill effluent, physico- chemical characteristics and toxic metals. *Geobios*. 4: 20-27

Maliwal, G.L. K.P. Patel, K.C. Patel and M.N Petel., 2004. The bioavailability of chemicals in soil for the earth worms. *Poll. Res*., 13: 231-269.

Baskaran, L, P. Sundaramoorthy, A.L.A Chidambaram and K. Sankar Ganesh., 2009. Amelioration of sugar mill effluent polluted soil and its effect of green gram (*Vigna radiata* L.) *Botany research International.,* 2: 107-114

Glick BR., 2003. Phytoremediation: synergistic use of plants and bacteria to clean up the environment. *Biotech. Adv*., 21:383-393

Whiting SN, De Souza M, Terry N., 2001. Rhizosphere bacteria mobilize Zn for hyperaccumulation by *Thalaspi caerulescens*; *Environ. Sci. Techno*l. 35: 3144-3150.

Zhuang X, Chen J, Shim H, Bai Z., 2007. New advances in plant growth promoting rhizobacteria for rhizobacteria. *Environ. Int*., 33; 406-413

Arnon, D.I., 1949. Copper enzymes in isolated chloroplasts polyphenol oxidase in *Beta vulgaris. Plant Physiol*., 24:31.

Lowry, O.H., Rosenbrough, N.J., Farr A.L. and Randall, R.J., 1951. Protein measurement with Folin- phenol reagent. *J. Biol. Chem*., 193: 265-275

Moore, S and Stein, W.H. 1948. Photometric method for use in the chromatograph of amino acid. *J. Biol. Chem*., 176-388

Nelson, N., 1944. A photometric adaption of the Somogy's method for the determination of reducing sugar. *Anal. Chem.*, 3: 426- 428