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Influence of industrial effluents on the population of actinomycetes and *Azospirillum* sp. In the agricultural soils of Anantapuramu district in Andhra Pradesh

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Abstract

Introduction: An increasing body of evidence suggests that microorganisms are far more sensitive to industrial effluents than soil plants growing on the same soil. Setting up of new industries resulted in the disposal of industrial effluents causing air, water and soil pollution. Microbes play a crucial role in decontaminating polluted sites. The study was designed to evaluate the microbial population of soil environment contaminated with industrial pollution.

Materials and methods: Soil samples were collected from the surrounding areas (1/4th km) of Saptagir Camphor industry, Anantapuramu, Andhra Pradesh, India. Soil samples without effluent discharges served as control were collected from adjacent site (1 km) away from the same camphor industry. Triplicate soil samples of each polluted and non-polluted soil samples were withdrawn at periodic intervals such as 0, 10, 20, 30 and 40 days for the enumeration of microbial population. The population of actinomycetes is enumerated by serial dilution method using Kenknights agar medium and the estimation of population of *Azospirillum* sp. population was done by most-probable number (MPN) method.

Results and Discussion: Population of actinomycetes and *Azospirillum* sp. were significantly enhanced in polluted soil than in non-polluted soil and were increased with increase in the incubation period up to 20 days compared to 0 day, however, population count was decreased after 20 days of incubation. The population count decreases with increase in the dilution factor. The population of *Azospirillum* sp. in polluted soil is two-fold higher than in non-polluted soil.

Conclusion: Studies have shown that the ability of microbes to tolerate a definite level of pollutants under natural conditions might be owing to the complex nature of the soil environment.

Keywords: Saptagir Camphor industrial effluents, actinomycetes and *Azospirillum* sp., Polluted and Non- polluted soils

INTRODUCTION

Industrial pollutants affect the growth, morphology and metabolism of soil microorganisms, through functional disturbance, protein denaturation or the destruction of the integrity of cell membranes. Soil microorganisms are essential in the decomposition of soil

organic matter, any decrease in the microbial diversity or abundance may adversely affect nutrient absorption from the soil. The elevated levels of heavy metals in soils had significant impacts on the population size and overall activity of the soil microbial communities.

Industrial effluents entering the soil are one of major sources of environmental toxicity. It has deleterious impact on the soil microflora. The effluents of sugar and textile industry and their deleterious effects on the soil microflora. The microbial flora too is affected by it as compared to the control water sample due to the high BOD and COD values (Arminder Kaur *et al*., 2010). Cassava mill effluent has negatively affected the microbial populations and physicochemical parameters at various depths (Okechi *et al*., 2012). Above certain concentrations and over a narrow range, the heavy metals turn into toxins (Babich *et al*., 1982). Moreover, these metals adversely affect natural microbial populations leading to disruption of vital ecological processes (Brynhildsen and Rosswall, 1997). Many industrial effluents had heavy metals such as Pb, Cd, Cr, Ni and Hg as effluent constituents. These heavy metals increased their concentrations and thereby reduced soil microbial process and microbial biomass (McGrath *et al*., 1988). Nwoko and Sola (2010) reported that several chemical and biochemical properties of the investigated soil changed in response to the application of POME (palm oil mill effluents). Organic amendment produced a decrease in pH and increase in electrical conductivity. Rajannan and Kandasamy (1990) noted that the protein industry waste harboured considerable number of bacteria, actinomycetes and fungi. Application of the pulp and paper mill wastes increased the microbial population and soil fertility. In contrast, Kouchou *et al*., (2017) reported that heavy metal contaminationin alkaline soils of the reagion Fez, (Morocco) have a positive effect on bacterial population and negative effect on actinomycetes and fungal population in soil.

Population of actinomycetes

Actinomycetes are the dominant group of soil population together with bacteria and fungi. They are found with greater or less frequency in most ecological niches. They are Gram-positive bacteria having high G+C (>55%) content in their DNA and originally considered as an intermediate group between bacteria and fungi. A majority of actinomycetes are free living, saprophytic bacteria and major source for production of antibiotics (Kuster, 1967). They are wide spread in nature and found to be more in dry than wet soils. According to Alexander (1961) 70 to 90% of the actinomycetes found in virgin and cultivated soils are species of Streptomyces which are also found to occur in fresh water and marine environments. Kouchou *et al*., (2017) demonstrated that changes in soil conditions due to heavy metals (Cr, Cu and Zn) contamination have a large negative effect on actinomycetes populations of soil.

Actinomycetes have been noted to serve as rich reservoirs of medicinal antibiotics and are therefore extremely relevant to scientists, pharmaceutical industries and agricultural industries (Kumar *et al*., 2010). With the increase of population of bacteria, fungi and actinomycetes, the discharge of agricultural wastewater containing chemical fertilizers and the discharge of industrial waste water such as electroplating, metallurgy and chemical industry, soil ecosystem has been seriously affected by heavy metals. Due to the persistent, toxic and non-biodegradable nature of heavy metals, the problem of heavy metals in the soil has attracted increasing attention of researchers (Dian Chu, 2018). Changes in the organic carbon also affect the actinomycetes population size and diversity associated with rhizospheric soils of non-Bt

and Bt brinjal crops. Alteration in the organic carbon between the soils of non-Bt and Bt brinjal could be one of the possible reasons for the minor fluctuations in the actinomycetes population density (Amit Kishore Singh *et al*., 2013). The post methanation distillery effluent (PME) 30 percent treatment at third irrigation had higher fungi and actinomycetes population in soil. The fungal population was in lesser number compared to bacteria or actinomycetes population. This study indicated that higher PME concentrations had a negative impact on soil microflora (Padmaja Karanam and Joshi, 2010).

The bacterial, fungal and actinomycetes populations of the soils showed that number of bacterial and fungal colonies increased significantly with sewage water irrigation, maximum being in sewage irrigated soils followed by partial sewage irrigated soils and least in ground water irrigated soils. Results also showed that microbial population (bacterial, fungal and actinomycetes) increased up to 14 days of incubation and decreased thereafter up to 56 days of incubation. Minimum population of actinomycetes was observed in sewage water irrigated soils. Soil organic matter was significantly and positively correlated with soil bacterial and fungal population, and negatively correlated with actinomycetes (Bansal *et al*., 2014). Wang *et al*., (2007) demonstrated that heavy metals pollution had a significant impact on bacterial and actinomycetic community structure. There were negative correlations between soil microbial biomass and extractable heavy metals.

Population of *Azospirillum* sp.

Among the soil microflora, *Azospirillum* sp. are susceptible to agrochemicals which are non-symbiotic, micro aerophilic, diazotrophic, heterotrophic nitrogen fixing bacterium (Charyulu and Rao, 1978). In vitro, the nitrogen-fixing rhizobacteria *Crysomonas luteola*, *Azospirillum* spp., *Azomonas* spp. and *Klebsiella pneumoniae* successfully grew in batch cultures prepared from the crude effluents. This was supported by adequate growth parameters and organic matter decomposition (Sayeda Alia *et al*., 2011). *Azospirillum* population in the rhizosphere improved significantly from 38.0 cfu g^{-1} under recommended fertilizer to 176.8 cfu g^{-1} under farm yard manure (FYM) and bioinoculants (Saini *et al*., 2004). A significant stimulation in root development and a large number of *Azospirillum* cells (108 g^{-1} washed roots) were recorded at harvest of the inoculated plants. Fertilization with 60 kg N-urea ha^{-1} stimulated yield components above controls to levels similar to inoculation with *Azospirillum* (Fulchieri and Frioni, 1994). However, meagre literature is available on the population of *Azospirillum* sp. influenced by the industrial pollutants.

MATERIALS AND METHODS

Soils

Soil samples were collected from the surrounding areas ($1/4^{th}$ km) of Saptagir Camphor industry, Anantapuramu, Andhra Pradesh, India. Soil samples without effluent discharges served as control were collected from adjacent site (1 km) away from the same camphor industry. Soil samples were air dried and mixed thoroughly to increase homogenesity and shifted to < 2 mm sieves for estimation of physico-chemical properties by standard methods. Both polluted and non-polluted soil samples of camphor industry were used for studying various microbial populations and soil enzyme activities.

Saptagir Camphor Limited: Manufacture and supply of terpene chemical and associated products. Camphor is used in traditional pooja rituals as well as pharmaceuticals. Synthetic camphor is white crystalline solid with pharma grade 95% purity.

Soil incubation for enumeration of population

Ten grams of soil samples (actinomycetes) and 5 grams (*Azospirillum* sp.) contaminated with/without effluents of Saptagir Camphor industry were transferred to test tubes (15 × 150 mm). Soil samples were maintained at 60% water holding capacity at room temperature in the laboratory (28 ± 4 ºC). Triplicate soil samples of each polluted and non-polluted soil samples were withdrawn at periodic intervals such as 0, 10, 20, 30 and 40 days for the enumeration of microbial population. The population of actinomycetes is enumerated by serial dilution method using Kenknights agar medium and the estimation of population of *Azospirillum* sp. population was done by most-probable number (MPN) method Alexander (1965) and Madhavi *et al*., (2019).

Serial dilution method for enumeration of actinomycetes

Ten grams of incubated soil samples were mixed with 100 ml of distilled water and then the serial dilution blanks were prepared in test tubes and marked sequentially starting from 10^{-1} to 10^{-8} dilutions. 1 ml of soil sample solution was dissolved in 9 ml of water i.e. 10^{-1} dilution, 1 ml from this was then transferred to 9 ml of the 10^{-2} labeled test tube i.e. 10^{-2} dilution, using a sterile pipette; and this was repeated for each succeeding step till 10^{-8}. Kenknights agar medium petri plates were prepared and used for the enumeration of actinomytes. From 10^{-5}, 10^{-6}, 10^{-7} and 10^{-8} dilution tubes, 0.5 ml of dilution liquid was then spread on Kenknights agar medium petri plates in triplicates using the standard spread plate technique. The agar plates were then incubated at 30˚C for three days. After successful growth of micro organisms the colonies of actinomycetes in both polluted and non-polluted soil samples of Saptagir Camphor industry were enumerated by colony counter Nasreen *et al*., (2015).

Composition of Kenknights agar medium

Dextrose	1.0 g
Monopotassium dihydrogen phosphate KH_2PO_4	0.1 g
Sodium nitrate $NaNO_3$	0.1 g
Potassium chloride KCl	0.1 g
Magnesium sulphate $MgSO_4.7H_2O$	0.1 g
Agar Agar	15.0 g
Distilled water	1000 ml

MPN method for *Azospirillum* sp.

The population of *Azospirillum* sp. in incubated soil samples (polluted and non-polluted) were estimated by most probable number technique. Ten-fold serial dilutions of soil samples were made and the numbers were calculated using the probability tables Alexander (1965). 5 ml portions of sterile nitrogen free semi-solid malate medium Dobernier *et al*., (1976) was taken in five MPN tubes and inoculated with 0.5 ml aliquots of the suspensions from 10^{-5} to 10^{-8} soil dilutions. Inoculated soil samples were incubated at 37ºC. MPN tubes in which a typical white pellicle formed a few mm below the surface of the medium after 36 hours incubation were noticed and those are the positive for *Azospirillum* sp. Microscopic examination of the *Azospirillum* sp. shown the rods with flat droplets and very active spiral movements.

Composition of semi-solid malate medium

Malic acid	5 g
Potassium hydroxide	4 g
Dipotassium phosphate	0.5 g
Magnesium sulphate	0.2 g
Sodium chloride	0.1 g
Calcium chloride	0.02 g
Iron sulphate	0.5 g
Sodium molybdate	0.002 g
Manganese sulphate	0.01 g
5% alcoholic solution of bromothymol blue	2 ml
Agar-Agar	1.75 g
Distilled water	1000 ml
pH	6.8

RESULTS AND DISCUSSION

Physico-chemical characteristics of polluted and non-polluted soil samples of Saptagir Camphor industry

Table 1: Physico chemical properties of polluted and non-polluted soil of Saptagir Camphor industry

S. No	Physico-Chemical Properties of soil	Saptagir Camphor polluted soil	Saptagir Camphor non-polluted soil
1	Color	Red	Red
2	Water holding capacity (ml g^{-1} soil)	42.39	37.97
3	Texture		
	Sand (%)	54.3	52.2
	Silt (%)	32.4	34.5
	Clay (%)	13.3	13.3
4	pH[a]	8.58	8.5
5	Electrical conductivity (m.mhos)	223	202
6	Organic carbon (%)	1.61	0.48
7	Organic matter (%)[b]	2.76	0.82
8	Total nitrogen (%)[c]	0.079	0.050
9	NH_4^+–N (µg g^{-1} soil)[d]	7.10	6.22
10	NO_2^-–N (µg g^{-1} soil)[e]	0.64	0.41
11	NO_3^-–N (µg g^{-1} soil)[f]	0.99	0.80
12	Sulphur (%)	1.62	2.13

[a]1:1.25 (soil:water) ; [b]Walkey-Black method (Jackson, 1971) ; [c]Micro-Kjeldhal method Jackson, 1971) ; [d]Nesslerization method (Jackson, 1971) ; [e]Diazotization method (Barnes and Folkard, 1951) ; [f]Brucine method (Ranney and Bartler, 1972)

The effect of Saptagir Camphor industrial effluents on soil microorganisms

Lang (1993) reported that during the production process pollutants are often released into the environment. Soil is an efficient purifying medium with a great capacity to receive and decompose wastes matter by its micro flora yielding nutrients in the process (Narasimha *et al*., 1999). However, if the input of the pollutants exceeds the soil purifying limit, the effectiveness of soil microbial activity is reduced considerably. As a result, there will be an adverse change in the soil physicochemical properties which consequently affect the growth and development of the crop plants. Soil bacteria, fungi, actinomycetes, algae and other microorganisms are responsible for the cycle of C, N, P, S and other elements in the nature, they promote the decomposition of material elements and nutrient conversion. Microbial activity in POME amended soils was increased under laboratory-controlled conditions irrespective of dose and duration of incubation. However, microbial and biochemical properties examined indicated and initial stress on microbes especially at early stage of incubation, but was quickly stabilized due to buffering capacity of soil (Nwoko and Sola, 2010). Kouchou *et al*., (2017) demonstrated that quantitative analysis of soil microbial populations showed that certain groups of soil microbes (actinomycetes and fungi) were particularly sensitive to long-term contamination and revealed a strong negative correlation with

Cr, Cu and Zn. Many reports have shown that short term or long term exposure to toxic metals results in the reduction of microbial diversity and activities in soil (Lasat, 2002). Wang *et al*., (2007) found that the long-term effects of nearly 20 yrs of heavy metal contamination near a copper smelter could affect the activity and community composition of soil microorganisms. Muller *et al*., (2001) reported that the functional potential of the microbial population measured as sole carbon source utilization by Ecoplates differed between the soils, but there was no change in the number of substrates utilized. The observed changes in the different soil microbial populations are probably a combination of both direct and indirect effects of the mercury contamination.

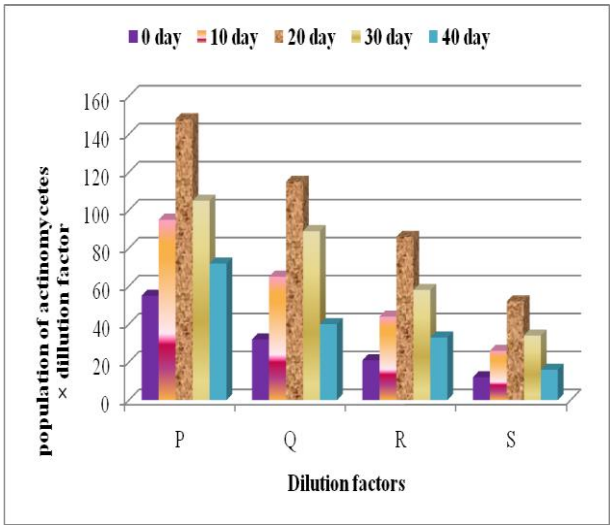
Population of actinomycetes

The overall biodiversity of soil microflora comprises bacteria, fungi, actinomycetes and photosynthetic microorganisms. Actinomycetes are a large group of microorganisms, systematically identified as bacteria, which grow as hyphae. They decompose a wide range of substances, but they are particularly important in degrading recalcitrant (difficult to degrade) compounds such as chitin, lignin, keratin and cellulose. Moreover, they produce a number of secondary metabolites such as antibiotics i.e. streptomycin or geosmine which is responsible for "earthy" smell after soil plowing. Actinomycetes are important in forming stable humus, which enhances soil structure, improves soil nutrient storage and increases water retention in soils (Alexander, 1961).

The population of actinomycetes (cfu/g) in polluted and non-polluted soils of Saptagir Camphor factory were listed (Table 2 and 3) respectively. Population of actinomycetes has been decreasing with increasing dilution factor from 10^{-5} to 10^{-8}. The population in the dilution factor 10^{-5} is higher than 10^{-8} on 0 day. The maximum population of actinomycetes was observed on 20 day in the dilution factor 10^{-5} ie. 148 cfu/g in polluted soils and 112 cfu/g in non-polluted soil of Saptagir Camphor industry. The minimum population of actinomycetes were observed on 40 day in the dilution factor 10^{-8} ie.16 cfu/g in polluted soils and 11 cfu/g in non-polluted soil of Saptagir Camphor industry. However, the population of polluted soil is comparitively higher than non-polluted soil. The same trend was observed on all the incubation days, in polluted and non-polluted soils. The population of actinomycetes on 0 day is relatively low, as the days of incubation increased the population significantly increased upto 20 days whereas, on further increasing the incubation (30 to 40 days) there was a eventual reduction in population. The population of actinomycetes on 40 day was subsided when compared to 20 day. The population of actinomycetes in dilution factor 10^{-6} on 40 day (40 and 31 cfu/g) and on 20 day (115 and 87 cfu/g) in both polluted and non-polluted soils respectively.

Table 2: Population of actinomycetes* (CFU × 10^2) g^{-1} in polluted soil samples of Saptagir Camphor industry

Dilution factors	Saptagir Camphor Polluted Soil sample				
	Incubation in days				
	0 day	10 days	20 days	30 days	40 days
P	55a (100)	95b (173)	148c (269)	105b (191)	72e (131)
Q	32a (100)	65b (203)	115c (359)	89d (278)	40a (125)
R	21a (100)	44b (209)	86c (409)	58d (276)	33e (157)
S	12a (100)	26b (217)	52c (433)	34d (283)	16e (133)



**Dilution factor P = 10^{-5}; Q = 10^{-6}; R = 10^{-7}; S = 10^{-8}

*Number of colonies per gram soil = $\frac{\text{no. of colonies} \times \text{dilution factor}}{\text{dry weight of soil}}$

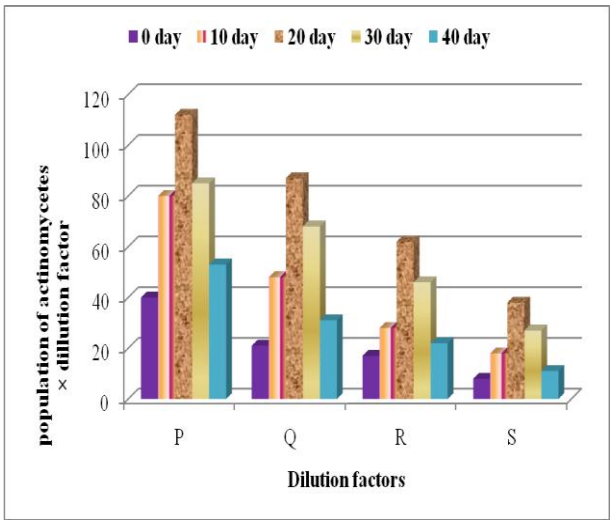
Figures, in parenthesis, indicate relative production percentages.

Values in the table and figure are means of triplicates.

Means, in each column, followed by the same letter or not significantly different ($P \leq 0.005$), from each other according to Duncan's Multiple Range (DMR) test.

Table 3: Population of actinomycetes* (CFU × 10^2) g^{-1} in non polluted soil samples of Saptagir Camphor industry

Dilution factors	Saptagir Camphor non Polluted Soil sample				
	Incubation in days				
	0 day	10 days	20 days	30 days	40 days
P	40a (100)	80b (200)	112c (280)	85b (212)	53e (132)
Q	21a (100)	48b (228)	87c (414)	68d (324)	31e (148)
R	17a (100)	28b (165)	62c (365)	46d (270)	22e (129)
S	8a (100)	18b (225)	38c (475)	27d (337)	11e (137)



**Dilution factor P = 10^{-5}; Q = 10^{-6}; R = 10^{-7}; S = 10^{-8}

*Number of colonies per gram soil = $\frac{\text{no. of colonies} \times \text{dilution factor}}{\text{dry weight of soil}}$

Figures, in parenthesis, indicate relative production percentages.

Values in the table and figure are means of triplicates.

Means, in each column, followed by the same letter or not significantly different ($P \leq 0.005$), from each other according to Duncan's Multiple Range (DMR) test.

This is because, as incubation period increases the depletion of nutrients occurs and hence the population reduces on prolonged incubation. The polluted soils of Saptagir Camphor industry show higher actinomycetes population than in non-polluted soil. But the population of actinomycetes is lower when compared to

bacterial population inhabting the same soil. This may also be attributed due to the alkaline nature of the soil, since soil actinomycetes have been known to tolerate acidic environment than the true bacteria.

Anna Lenart-Boron and Piotr Boron (2014) reported that actinomycetes were much less abundant in heavy metal polluted soils than in uncontaminated soils. Heavy metal contamination results in reduction of microbial biomass and even if they do not cause the reduction in their number they reduce biodiversity or disturb the community structure. Quantitative analysis of soil microbial populations shows a marked decrease in total culturable numbers of the different microbial groups of the contaminated soil samples (Adilia Oliveira and Pampulha, 2006). Actinomycetes have lesser degree of resistence to heavy metals. The 12 years repeated application of oil refinery treated wastewater had not significantly changed the microbial dynamics of the soil and possibly they had adapted to the changed soil environment by developing various levels of metal resistance (Hayat *et al*., 2002). Kouchou *et al*., (2017) demonstrated that changes in soil conditions due to heavy metals (Cr, Cu and Zn) contamination have a large negative effect on actinomycetes populations of soil.

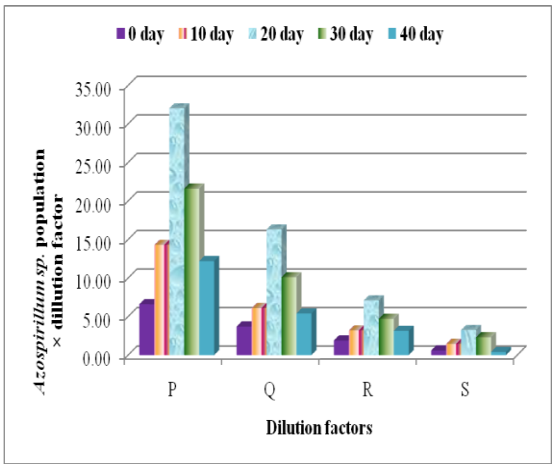
Population of *Azospirillum* sp.

Azospirillum sp. is free-living, diazotrophic, nitrogen-fixing bacterium, is most widely used as inoculant for soil fertility both in legume and non-legume crops, with a well-developed technology for its production as an inoculant. They are commonly known as plant-growth-promoting bacteria (PGPB).

The population of *Azospirillum* sp. (MPN) of polluted and non-polluted soil of Saptagir Camphor industry were presented (Table 4 and 5) respectively. Population of *Azospirillum* sp. (MPN) has been decreasing with increasing dilution factor from 10^{-5} to 10^{-8}. The population in the dilution factor 10^{-5} is higher than 10^{-8} on 0 day. The maximum population of *Azospirillum* sp. was observed on 20 day in the dilution factor 10^{-5} ie. 32.01 in polluted soils and 19.10 in non-polluted soils of Saptagir Camphor industry. The reduction in population of *Azospirillum* sp. was observed on 40 day in the dilution factor 10^{-8} ie. 0.40 in polluted soils and 0.30 in non-polluted soil. However, the population of polluted soils is two-fold higher than non-polluted soil in all the dilutions. The same trend was observed on all the incubation days, in polluted and non-polluted soil. This is because the substrate concentration is directly proportional to microbial population. The population of *Azospirillum* sp. on 0 day is relatively low, as the days of incubation increased the population significantly enhanced upto 20 day whereas on further increasing the incubation (30 and 40 days) there was an eventual reduction in population. The population of *Azospirillum* sp. on 40 day was subsided compared to 20 day. The population of *Azospirillum* sp. in dilution factor 10^{-6} on 40 day (5.40 and 3.60) and on 20 day (16.30 and 9.90) in both polluted and non-polluted soils respectively. This is because, as incubation period increases the depletion of nutrients occurs and hence the population reduces on prolonged incubation. The polluted soil of Saptagir Camphor industry shows higher population of *Azospirillum* sp. than in non-polluted soil. This may be due to the release of effluents in organic nature. The contents of total nitrogen and sulphur are also higher in polluted soils than in non-polluted soil. Scanty reports have been published on the influence of industrial effluents on population of *Azospirillum* sp.

Table 4: Population of *Azospirillum* sp. (MPN × 10^2 g^{-1}) in polluted soil samples of Saptagir Camphor industry

Dilution factors	Saptagir Camphor Polluted Soil sample				
	Incubation in days				
	0 day	10 days	20 days	30 days	40 days
P	6.60a (100)	14.30b (217)	32.02c (485)	21.60d (327)	12.20e (185)
Q	3.70a (100)	6.10b (165)	16.30c (440)	10.10d (273)	5.40e (146)
R	1.90a (100)	3.20b (168)	7.10c (374)	4.70d (247)	3.15e (166)
S	0.60a (100)	1.45b (242)	3.25c (542)	2.30d (383)	0.40e (67)



*Dilution factor P = 10^{-5}; Q = 10^{-6}; R = 10^{-7}; S = 10^{-8}

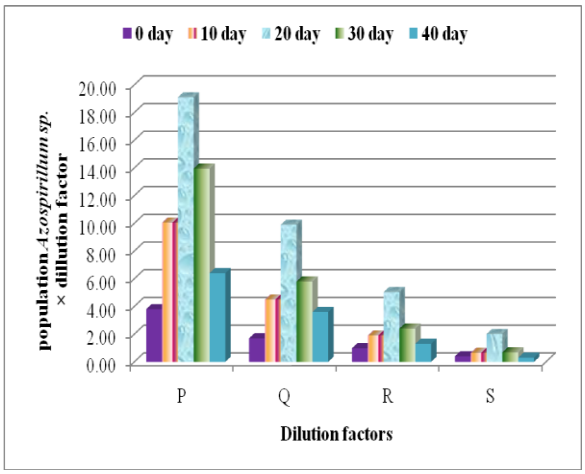
Figures, in parenthesis, indicate relative production percentages.

Values in the table and figure are means of triplicates.

Means, in each column, followed by the same letter or not significantly different ($P \leq 0.005$), from each other according to Duncan's Multiple Range (DMR) test.

Table 5: Population of *Azospirillum* sp. (MPN × 10^2 g^{-1}) in non polluted soil samples of Saptagir Camphor industry

Dilution factors	Saptagir Camphor non Polluted Soil sample				
	Incubation in days				
	0 day	10 days	20 days	30 days	40 days
P	3.80a (100)	10.05b (264)	19.10c (503)	13.95d (367)	6.40e (168)
Q	1.70a (100)	4.50b (265)	9.90c (582)	5.80d (341)	3.60e (212)
R	0.98a (100)	1.90b (194)	5.04c (514)	2.40d (245)	1.30e (133)
S	0.40a (100)	0.65b (162)	2.01c (502)	0.68b (170)	0.30e (133)



*Dilution factor P = 10^{-5}; Q = 10^{-6}; R = 10^{-7}; S = 10^{-8}

Figures, in parenthesis, indicate relative production percentages.

Values in the table and figure are means of triplicates.

Means, in each column, followed by the same letter or not significantly different ($P \leq 0.005$), from each other according to Duncan's Multiple Range (DMR) test.

CONCLUSION

Soil microbial populations such as actinomycetes and *Azospirillum* sp. were significantly higher in polluted soil samples than in non-polluted soil. Population of actinomycetes and *Azospirillum* sp. were significantly enhanced in polluted soil than in non-polluted soil and were increased with increase in the incubation period up to 20 days compared to 0 day, however, population count was decreased after 20 days of incubation. The population count decreases with increase in the dilution factor. The population of *Azospirillum* sp. in polluted soil is two-fold higher than in non-polluted soil. In fact, studies have shown that the ability of microbes to tolerate a definite level of pollutants under natural conditions might be owing to the complex nature of the soil environment.

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REFERENCES

Adilia Oliveira and Maria Elisa Pampulha. 2006. Effects of long-term heavy metal contamination on soil microbial characteristics. Journal of Bioscience and Bioengineering, Volume 102, Issue 3, September, Pages:157-161.

Alexander, M. 1961. Introduction to soil microbiology. Wiley Eastern Ltd., New Delhi.

Alexander, M. 1965. Most Probable Number method for microbial populations. In: 'Methods of Soil Analysis' (Ed. C. A. Black). Am. Soc. Agr. Madison, Wisconsin, U.S.A. Part 2. pp: 1467-1472.

Amit Kishore Singh, Major Singh and Suresh Kumar Dubey. 2013. Changes in Actinomycetes community structure under the influence of Bttransgenic brinjal crop in a tropical agroecosystem. BMC Microbiol.; 13: 122. May 29. DOI: 10.1186/1471-2180-13-122.

Anna Lenart Boron and Piotr Boron. 2014. The effect of heavy metal pollution on microbial abundance and diversity in soils. A Review . Environmental Risk Assessment of Soil Contamination. DOI. 10. 5772-57460, ch 26: 760-783.

Arminder Kaur, Siddharth Vats, Sumit Rekhi, Ankit Bhardwaj, Jharna Goel, S. Ranjeet, Tanwar, Komal and K. Gaur. 2010. Physico-chemical analysis of the industrial effluents and their impact on the soil microflora. Procedia Environmental Sciences, 2: 595-599.

Babich, H., M. Schiffenbauer and G. Stotzky. 1982. Comparative toxicity of trivalent and hexavalent chromium to fungi. Bull. Environ. Contam. Toxicol, 28: 193-202.

Bansal, O.P., Gajraj Singh and Pragati Katiyar. 2014. Effect of untreated sewage effluent irrigation on heavy metal content, microbial population and enzymatic activities of soils in Aligarh. Journal of Environmental Biology, Vol. 35, 641-647, July.

Barnes, H. and B.R. Folkard. 1951. The determination of nitrite. *Analyst*, 76: 599-603.

Brynhildsen, L. and T. Rosswall. 1997. Effects of metals on the microbial mineralization of organic acids, 94Water, Air, and Soil Pollut., p: 45-57.

Charyulu, P.B.B.N and V.R. Rao. 1978. Nitrogen fixation by Azospirillum sp. isolated from benomyl amended rice soils. Curr. Sci., 47: 822-823.

Dian Chu. 2018. Effects of heavy metals on soil microbial community. IOP Conf. Ser. Earth Environ. Sci., 113 012009.

Doberenier, J., I.E. Marreil. and N. Neyra. 1976. Ecological distribution of Spirillum lipoferum Bejerinck. Can. J. Microbiol, 22: 1464-1473.

Fulchieri, M. and L. Frioni. 1994. *Azospirillum* inoculation on maize (Zea

mays): effect on yield in a field experiment in central argentina Soil Biology and Biochemistry, Volume 26, Issue 7, Pages 921-923.

Hayata Iqbal Ahmad, S., Z.M. Azama, A. Ahmada and A. Inama Samiullaha. 2002. Effect of long term application of oil refinery wastewater on soil health with special reference to microbiological characteristics. Bioresource Technology, Volume 84, Issue 2, September, Pages: 159-163

Jackson, M.L. 1971. Soil Chemical Analysis. Prentice-Hall of India, New Delhi.

Kouchou, A., N. Rais, F. Elsass, J. Duplay, N. Fahli, N.E.L. Ghachtouli. 2017. Effects of long-term heavy metals contamination on soil microbial characteristics in calcareous agricultural lands (Saiss plain, North Morocco). JMES, Volume 8, Issue 2, Page: 691-695

Kumar, N., R.K. Singh and S.K. Mishra. 2010. Isolation and screening of soil actinomycetes as sources of antibiotics active against bacteria. International Journal of Microbiology Research, 2: 12-16.

Kuster, E. 1967. The actinomycetes. In: " Soil Biology", eds. Burges (A.) and Raw (F.), 111-124. Academic Press, London.

Lang, I. 1993. Environmental management andsustainable development. In B. Nath, L. Hens and D. Devuyst (Eds.). Environmental Management Instruments for Implementation Brussels: VUB University Press, VUB University.3.2.

Lasat, M.M. 2002. Phyto extraction of toxic metals: A review of biological mechanisms. J. Environ. Qual., 31: 109-120.

Madhavi, A., B. Anuradha and V. Rangaswamy. 2019. Influence of Pesticides on *Azospirillum* sp. Population and Its Nitrogen Fixation in Groundnut (*Arachis hypogaea* L.) Soils., 15(4) : 1-12.

Mc Grath, S.P., P.C. Brookes and K.E. Giller. 1988. Effect of potentially toxic metals in soil derived from past application of sewage sludge on nitrogen fixation by Trifolium repens L. Soil Biol. Biochem., 20: 415-424.

Muller, A.K., K. Westergaard, S. Christensen and S. Johannes Sorensen. 2001. The effect of long-term mercury pollution on the soil microbial community. FEMS Microbiology Ecology, 36: 11-19

Narasimha, G., G.V.A.K. Babu and B. Rajasekhar Reddy. 1999. Physico-Chemical and biological properties of soil samples collected from soil contaminated with effluents of cotton ginning industry. J.Env.Biol., 20: 235-239.

Nasreen, C., G. Jaffer Mohiddin, M. Srinivasulu, B. Manjunatha and V. Rangaswamy. 2015. Interaction effects of insecticides on microbial populations and dehydrogenase activity in groundnut (*Arachis hypogeae* L.) planted black clay soil.Int J. Curr Micribiol Appl Sci., V.4: 135-146.

Nwoko, Chris.O. and Sola Ogunyemi. 2010. Effect of Palm Oil Mill Effluent (POME) on Microbial Characteristics in a Humid Tropical Soil under Laboratory Conditions. International Journal of Environmental Science and Development, Vol. 1, No. 4, October 2010.ISSN: 2010-0264. pg: 307-314.

Okechi, R.N., C.E. Ihejirika, N.A. Chiegboka, E.I. Chukwura and I.J. Ibe. 2012. Evaluation of the effects of cassava mill effluent on the microbial populations and physicochemical parameters at different soil depths. International Journal of Biosciences (IJB) Vol. 2, No.12, p: 139-145.

Padmaja Karanam, V. and H.C Joshi. 2010. Application of distillery effluents to agricultural land: Is it a win-win option for soils and environment © 2010 19th World Congress of Soil Science, Soil

Solutions for a Changing World 1-6 August, Brisbane, Australia. Published on DVD.

Rajannan, G., and D. Kandaswamy. 1990. Environmental pollution of pykara river by protein industry effluent Ind. J. Environ. Hlth., 32: 230-337.

Ranney, T.A and R.J. Bartlett. 1972. Rapid field determination of nitrate in natural waters. Communications in Soil Science and Plant Analysis, 3: 183-186.

Saini, V.K., S.C. Bhandari. 2004. Comparison of crop yield, soil microbial C, N and P, N-fixation, nodulation and mycorrhizal infection in inoculated and non-inoculated sorghum and chickpea crops. Field Crops Research, Volume 89, Issue 1, Pages: 39-47.

Sayeda M. Alia Shawky, Z. Sabaea, M. Fayezb, M.M. Nabil, A. Hegazib. 2011. The influence of agro-industrial effluents on River Nile pollution. Journal of Advanced Research, Volume 2, Issue 1, Pages: 85-95.

Walkley, A and I.A. Black. 1934. An examination of the Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. Soil Sci., 37: 29-38.

Wang, Y.P., J.Y. Shi, H. Wang, Q. Lin, X.C. Chen and Y.X. Chen. 2007. The influence of soil heavy metals pollution on soil microbial biomass, enzyme activity, and community composition near a copper smelter. Ecotoxicology and Environmental Safety, Volume 67, Issue 1, Pages: 75-81.