Project Report

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SIR P. T. SCIENCE COLLEGE

MODASA – 383315

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(Managed by THE M. L. GANDHI HIGHER EDUCATION SOCIETY) [UGC 2F, 12B RECOGNISED]- [NAAC- ACREDITED B^{++}]

CERTIFICATE

This is to certify that the project work on

Physico-Chemical and Microbiological Analysisof Soil(industrial area) of aravlli District,Gujarat,India

entitled is carried out by students mentioned below. The Project work allotted them first year Bachelor of Science during the academic year 2022-2023.

The project has been approved as it satisfies the academic requirements in respect of project work prescribed for the first year Bachelor of Science NEP 2020.

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Physico-Chemical and Microbiological Analysisof Soil (Industrial area) of Aravalli District, Gujarat, India

Summary:

Pesticides are chemical substances applied to mitigate the agricultural and domestic pests, but indiscriminate and unsafe use leads to their accumulation in the environment. Bioaccumulation of pesticides is of great public health concern due to their toxicity. The enhanced production and formulation of pesticides has posed serious problem through contaminating the nearby surroundings, which ultimately affect the biological diversity. Therefore, the present study is focused on physico-chemical and microbial characterization of pesticides contaminated industrial soil nearby pesticides industry. For this, the soil sample was collected from nearby pesticides industries from GIDC Modasa, Aravalli district of Gujarat state. The soil sample was analyzed for various physico-chemical characteristics such as temperature, pH, electrical conductivity, moisture contents, and water holding capacity, bulk density, hardness, chloride, alkalinity, sulphate, available phosphorus, total phosphorus, nitrate, nitrite, ammonium, total organic carbon and total organic matter.

For microbial characterization, isolation of bacteria was carried out using serial dilution pour plate technique and characterized morphologically, biochemically. The isolated bacteria were identified as *Bacillus spp.* and *Pseudomonas* sp. The obtain results infers that pesticides contaminated soil contains diversified bacterial species. As these bacterial species are growing in the pesticides contaminated soil and can be resistant to the toxicity of the pesticides, therefore, they may be the potential candidate for the removal of those compounds for environmental clean-up.

INTRODUCTION

Pesticides are natural or synthetic compounds that are poisonous and can kill pests including insects, nematodes and rodents etc. Over the past few decades, pesticides and other agrochemicals have become a vital component of modern agricultural system, leading to a substantial improvement in crop productivity by controlling insects and other diseases *(Carvalho 2017; Ali et al. 2019).* The state like Gujarat, agriculture is one of the most important sector as it is the primary sources of livelihood for more than half (~ 60*%*) of its workforce (Planning Commission 2004; UNDP 2004). Currently, huge amount of pesticides are being used in agricultural field for protection of crops from the pests. Due to increasing demand of pesticides, a lots of manufacturing and formulation industries has been established in Gujarat to fulfill the requirements *(Morillo and Villaverde 2017; Varjani et al. 2018).*

On the other hand, enhanced Industrial and agricultural activities in recent years, in India especially in Gujarat has led to considerable contamination of air, soil and groundwater resources due to release of large amounts of pesticides and other chemicals in the environment *(Morillo and Villaverde 2017; Varjani et al. 2018).* Indiscriminate use of pesticides for pest infestation and vector control has drawn special attention of scientific community globally due to the multifaceted toxicity, mobility, persistence and recalcitrant properties *(Fantke and Jolliet 2016; Liu et al. 2016; Varjani et al. 2018).*

The rapid increase in pesticides industries also has polluted the nearby environment severely. Nevertheless, these pesticides possess several negative impacts ranging from ecological pollution to damage of biodiversity (*Pico et al. 2018; Barbieri et al. 2019; Köck-Schulmeyer et al. 2019).* This can influences the physico-chemical properties and microbial diversity of the soil and possibly can cause a threat to both the environment and human health (Samant et al. 2018). Although, some studies show physico-chemical and microbial characterization of pesticides contaminated industrial soil from different industrial area of Gujarat *(Doolotkeldieva et al. 2018; Ravi et al. 2019),* but pesticides infused industrial soil of GIDC Modasa, Arvalli Gujarat is yet to be studied. Therefore, the present study was carried out to characterize the pesticide infused soil from Gujarat for their physico-chemical analysis and microbial diversity.

MATERIAL AND METHODS

The soil sample was collected randomly from five sites from nearby pesticides industries from Gujarat Industrial Development Corporation **(GIDC),** Modasa, Arvalli district of north Gujarat, in sterilized polyethene zipper bag using auger up to a depth of 1- 15 cm and stored at 4 °C for further analysis The important physico-chemical properties of the soil, viz. temperature, pH, electrical conductivity, moisture contents, water holding capacity, bulk density, hardness, chloride, alkalinity, total organic carbon, total organic matter, sulphate, nitrate, nitrite, ammonium, available phosphorus and total phosphorus were analysed using standard methods (APHA1998). All the physico-chemical properties of the soil were analyzed in triplicates and their mean and standard deviation (SD) was calculated. The obtained values were described as Mean ± SD.

The Isolation of indigenous bacteria from pesticide contaminated soil was performed by serial dilution pour plate technique using nutrient agar medium. Well grown bacterial colonies were picked and further

purified by streaking. The colonies were characterized morphologically, biochemically. The colonies were counted and the average number of colonies per three plates was determined for CFU (colony forming unit) count. Morphological Characterization of isolated bacterial species: The isolated pure bacterial species were grown on nutrient agar medium and examined morphologically for their shape, size, margin, constancy, elevation, opacity, and pigmentation. The gram staining test was carried out by Gram's Method using a Gram staining kit and observed under a microscope for color and shape. Biochemical Characterization of isolated bacterial species: The isolated bacterial species were analyzed for various biochemical properties such as motility, starch hydrolysis, catalase, oxidase, urease, indole production test, nitrate reduction test, citrate utilization test, xylose, maltose, fructose, dextrose, trehalose, inositol, sucrose, L-arabinose, glycerol, melezitose, lactose, esculin hydrolysis, amylase, gelatinase, methyl red (MR) test, vogus-proskauer (VP) test, etc. using KB001 Biochemical Test Kit (HI Media, India).

RESULTS AND DISCUSSION

Physico-chemical characteristics of soil collected from GIDC Modasa,Arvalli Gujarat: The physico – chemical properties such as temperature, pH, electrical conductivity, moisture contents, water holding capacity, bulk density, chloride, alkalinity, hardness, sulphate, available phosphorus, total phosphorus, all types of nitrogen (nitrate, nitrite, ammonium), total organic carbon and total organic matter of the soil samples collected from GIDC Modas of Aravalli district, Gujarat was carried out as shown in Table 1**,** which infers temperature (°C), pH, electrical conductivity (µs cm $^{-1}$) value of 26.12 ± 0.13, 8.24 ± 0.13, 121 ± 27.65 respectively. The moisture content (%) and water holding capacity (%) was observed 15.85 ± 1.13 and 37.45 ± 0.82 respectively. The present results are supported by earlier studies in the case of rice field contaminated with pesticides *(Raman Kumar Ravi et al. 2015)* and pesticides contaminated industrial soil *(Ravi et al. 2019).*

The total organic carbon and total organic matter are the significant property of soil and play important role in its fertility *(Yennawar et al. 2013; King et al. 2020).* The result obtained of total organic carbon (%) is 0.9 ± 0.17, whereas total organic matter (%) is 1.67 ± 0.03. The recorded results for bulk density (g/ml), chloride (mg/kg), alkalinity (mg/kg), hardness (mg CaCO3/kg), sulphate (mg/kg), available phosphorus (mg/kg) and total phosphorus (mg/kg) was 1.73 ± 0.02, 236.5 ± 36.04, 203 ± 15.65, 60 ± 5.10, 3.91 ± 1.22, 0.87 ± 0.18 and 8.30 ± 0.60 respectively. The different nitrogen contents are 10.53 ± 0.71 for nitrate, 0.09 ± 0.01 for nitrite and 1.94 ± 0.38 for ammonium nitrogen.

Table 1. Physico-chemical Analysis of soil samples collected from GIDC Modasa, Aravalli

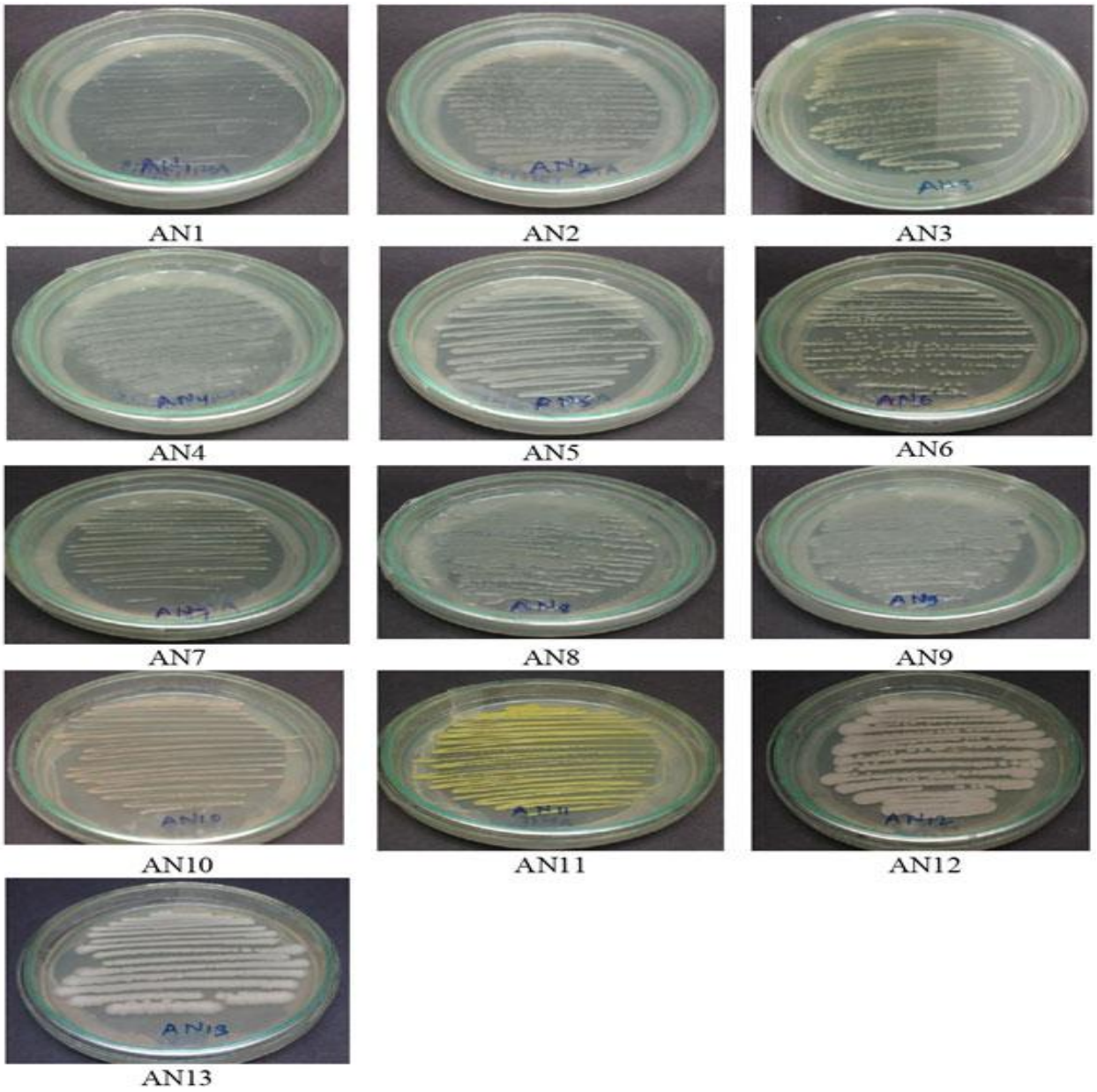
Parameters	Site 1	Site 2	Site 3	Site 4	Site 5	Average ± SD
Temperature (°C)	26.1	26	26.5	26.2	26.3	26.12 ± 0.13
pH	8.28	8	8.31	8.3	8.3	8.24 ± 0.13
Electrical Conductivity (µs cm -1)	99	104	101	159	142	121 ± 27.65
Moisture contents (%)	14.75	14.76	16.15	17.44	16.13	15.85 ± 1.13
Water Holding Capacity (%)	37.07	37.46	36.86	36.86	37.02	37.45 ± 0.82
Bulk Density (g/ml)	1.75	1.75	1.74	1.71	1.70	1.73 ± 0.02
Chloride (mg/kg)	283	216.5	200	266.5	216.5	236.5 ± 36.04
Alkalinity (mg/kg)	200	190	230	195	200	203 ± 15.65
Hardness(mg CaCO3/kg)	64	60	66	56	54	60 ± 5.10

Sulphate (mg/kg)	4.43	5.39	4.21	2.15	3.38	3.91 ± 1.22
Available Phosphate (mg/kg)	0.84	0.58	0.92	1.06	0.93	0.87 ± 0.18
Total Phosphorus (mg/kg)	9.08	8.61	7.73	7.66	8.40	8.30 ± 0.60
Nitrate (mg/kg)	11.21	9.55	10.05	11.11	10.73	10.53 ± 0.71
Nitrite (mg/kg)	0.11	0.09	0.08	0.09	0.10	0.09 ± 0.01
Ammonium (mg/kg)	2.09	1.30	2.23	2.17	1.91	1.94 ± 0.38
Total Organic Carbon (%)	1.2	1.05	1.2	1.35	0.9	0.9 ± 0.17
Total Organic Matter (%)	1.64	1.70	1.65	1.69	1.68	1.67 ± 0.03

Note: SD – Standard Deviation

Microbiological Characterization of soil collected from GIDC Modasa, Aravalli (North Gujarat): The bacterial species were isolated from contaminated soil using serial dilution pour plate technique on nutrient agar medium. A total of 13 bacterial species were isolated and pure cultured from collected soil sample of GIDC Modasa, Aravalli (North Gujarat (Fig. 1).

Figure 1: Bacteria isolated from GIDC Modasa soil, Aravalli



Morphological characterization of isolated bacterial species: The isolated pure culture of bacteria was characterized by several morphological properties like shape, size, margin, elevation, pigmentation, optical character, surface, and consistency by growing them on nutrient agar plates (Table 2)

Table 2. Morphological characterization of bacteria isolated from GIDC Modasa soil, Aravalli.

Isolates	Shape	Size	Margin	Elevation	Pigment	Optical character	Surface	Consistency
AN1	Irregular	Large	Undulate	Flat	Whitish	Opaque	Smooth	Moist
AN2	Elliptical	Intermediate	Undulate	Flat	Whitish	Opaque	Smooth	Moist
AN3	Round	Large	Undulate	Convex	Whitish	Opaque	Smooth	Moist
AN4	Round	Intermediate	Entire	Convex	Creamy	Sebaceous	Smooth	moist
AN5	Elliptical	Intermediate	Curled	Unbonate	Creamy	opaque	Smooth	moist
AN6	Round	Small	Entire	Convex	White	sebaceous	Smooth	moist
AN7	Round	Small	Entire	Convex	Yellowish	sebaceous	Smooth	moist
AN8	Round	Intermediate	Entire	Convex	Yellow	opaque	Smooth	moist
AN9	Round	Small	Entire	Convex	Orange	opaque	Smooth	moist

AN10	Round	Intermediate	Entire	Convex	Creamy	Sebaceous	Smooth	moist
AN11	Round	Small	Entire	Convex	Yellowish	sebaceous	Smooth	moist
AN12	Round	Intermediate	Entire	Convex	Pinkish	opaque	Smooth	moist
AN13	Round	Small	Entire	Convex	Orange	opaque	Smooth	moist

Biochemical characterization of bacterial species: Several biochemical analyses were carried out to describe the isolated bacterial species. All the bacterial species were found gram-positive except isolate AN12 (gram negative) (Table 3). All the bacterial species were rod-shaped except isolate AN11. All the bacterial species were negative for urease, starch hydrolysis, lactose, xylose, galactose, raffinose, sodium gluconate, inositol, dulcitol, arabitol, erythritol, α-methyl -D glucoside, rhamnose, α-methyl- D mannoside, xylitol, esculin hydrolysis, malonate utilization and sorbose test, while positive for catalase and dextrose test. The isolate AN2, isolate AN4, isolate AN5, isolates AN9, isolate AN11 and isolate AN12 were able to reduce nitrates.

The isolate AN2, isolate AN3, isolate AN4, isolate AN7, isolate AN9, isolate AN10 and isolate AN11 were found positive for oxidase test. The isolate and isolate AN10 were able to utilize citrate and adinitol, therefore positive for that test. The isolate AN1, isolate AN6, isolate AN8 and isolate AN13 were positive for maltose, methyl red, sucrose, trehalose and melibiose test.

Table 3. Biochemical characterization of isolated bacteria from GIDC Modasa soil, Aravalli

Tests	**AN 1**	**AN 2**	**AN 3**	**AN 4**	**AN5**	**AN 6**	**AN 7**	**AN 8**	**AN 9**	**AN 10**	**AN 11**	**AN1 2**	**AN 13**
Gram stain	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve

Shape	Rods	Rods	Rods	Rods	Rods	Rods	Rods	Rods	Rods	Rods	Cocci	Rods	Rods
CFU counts (N x 10^5 cfu/ g)	1.90	1.41	2.15	1.65	2.42	1.12	1.12	2.01	1.48	2.30	1.69	2.72	1.56
Motility test	–	–	+	+	–	+	–	–	–	+	+	–	+
Catalase test	+	+	+	+	+	+	+	+	+	+	+	+	+
Oxidase test	–	+	+	+	–	–	+	–	+	+	+	–	–
Urease test	–	–	–	–	–	–	–	–	–	–	–	–	–
Indolete st	–	–	+	–	–	+	–	–	–	+	–	–	+
Starch hydroly sis	–	–	–	–	–	–	–	–	–	–	–	–	–
Nitrate reductio n	–	+	–	+	+	–	–	–	+	–	+	+	–

Methyl Red test	+	+	+	+	+	+	–	+	+	+	+	+	+
V-P test	–	+	+	+	–	+	+	–	+	+	+	–	+
O-F test	O-/F-	O+/F-	O-/F-	O-/F-	O+/F+	O-/F-	O-/F-	O-/F-	O+/F-	O-/F-	O-/F-	O+/F+	O-/F-
Triple Sugar Iron	K/A	K/A	K/A	K/A	K/A	K/A	K/A	K/A	K/A	K/A	K/A	K/A	K/A
Glucose	+	+	+	+	+	+	V	+	+	+	+	+	+
Sorbinol	V	–	–	–	–	–	–	V	–	–	–	–	–
Lactose	–	–	–	–	–	–	–	–	–	–	–	–	–
Xylose	–	–	–	–	–	–	–	–	–	–	–	–	–
Maltose	+	–	+	+	+	+	+	+	–	+	+	+	+
Fructose	+	+	–	+	+	–	–	+	+	–	+	+	–
Dextrose	+	+	+	+	+	+	+	+	+	+	+	+	+
Galactose	–	–	–	–	–	–	–	–	–	–	–	–	–

Raffinose	–	–	–	–	–	–	–	–	–	–	–	–	–
Trehalose	+	–	+	+	+	+	+	+	–	+	+	+	+
Melibiose	+	–	–	–	–	+	–	+	–	–	–	–	+
Sucrose	+	+	–	+	+	+	–	+	+	–	+	+	+
L-Arabinose	–	–	–	–	+	–	–	–	–	–	–	+	–
Mannose	–	+	–	+	+	+	–	–	+	–	+	+	+
Inulin	–	–	–	–	+	–	–	–	–	–	–	+	–
Sodium gluconate	–	–	–	–	–	–	–	–	–	–	–	–	–
Glycerol	–	+	+	–	–	+	–	–	+	+	–	–	+
Salicin	–	–	+	–	+	+	–	–	–	+	–	+	+
Dulcitol	–	–	–	–	–	–	–	–	–	–	–	–	–

Inositol	–	–	–	–	–	–	–	–	–	–	–	–	–
Sorbitol	–	–	–	–	–	–	–	–	–	–	–	–	–
Mannitol	–	+	–	–	+	–	+	–	+	–	–	+	–
Adonitol	–	–	+	–	–	–	–	–	–	+	–	–	–
Arabitol	–	–	–	–	–	–	–	–	–	–	–	–	–
Erythritol	–	–	–	–	–	–	–	–	–	–	–	–	–
α-methyl D glucoside	–	–	–	–	–	–	–	–	–	–	–	–	–
Rhamnose	–	–	–	–	–	–	–	–	–	–	–	–	–
Cellobiose	–	–	+	–	+	+	–	–	–	+	–	+	+
Melezitose	+	–	–	–	+	–	–	+	–	–	–	+	–

α-methyl-D-mannoside	–	–	–	–	–	–	–	–	–	–	–	–	–
Xylitol	–	–	–	–	–	–	–	–	–	–	–	–	–
ONPG	+	–	–	–	–	–	–	+	–	–	–	–	–
Esculin hydrolysis	–	–	–	–	–	–	–	–	–	–	–	–	–
D-Arabinose	–	–	–	–	+	–	–	–	–	–	–	+	–
Citrate utilization	–	–	+	–	–	–	–	–	–	+	–	–	–
Malonate utilization	–	–	–	–	–	–	–	–	–	–	–	–	–
Sorbose	–	–	–	–	–	–	–	–	–	–	–	–	–

Note: (+ = Positive, – = Negative, V= 11-89% positive, O^{+}/F^{-}= only oxidative; O^{+}/F^{+} = Oxidative and fermentative; O^{-}/F^{-} = glucose not metabolised; A/A= Glucose, lactose & sucrose fermentation; K/A = Glucose fermentation; K/ K = Non fermentative)

The present study show that slight variation in the physico-chemical properties of soil around the pesticides industry are occurred, which provide better environmental condition for the bacterial diversity to grow. The study shows that the soil around the pesticides industry of GIDC, Modasa Aravalli district contains diversity of bacterial species. The soil is mainly dominated by different species of genus *Bacillus*. This finding was also supported was earlier studies for the soil contaminated with pesticides *(Doolotkeldieva et al. 2018; Ravi et al. 2019).*

CONCLUSION

The pesticides industries are adding different types of pesticides to environment which bring changes in natural properties of the soil. Pesticides are observed to influence the physico-chemical and biological properties of soil. The present study has indicated slight variations in observed parameters. The soil samples from five different sight exhibited similar pattern in selected parameters which indicates the changes brought about by deposition from industries. The microbial analysis show diversified bacterial species, mainly dominated by different species of *Bacillus*. As these bacterial species are growing in the pesticides contaminated soil, therefore, this study will help to determine the variations and possibilities for remediation.

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