

**SENSORY MICROBIAL TECHNIQUE TO DETECT PESTICIDES
AND HEAVY METALS RESIDUES IN MARKETABLE FRUITS
AND VEGETABLES**

By

Miss. Nadaf Jasmin Mansoor

(Reg. No. Ph.D. 2015/59)

A Thesis submitted to the
**MAHATMA PHULE KRISHI VIDYAPEETH
RAHURI – 413 722, DIST. AHMEDNAGAR
MAHARASHTRA, INDIA**

in partial fulfillment of the requirements for the degree

Of

DOCTOR OF PHILOSOPHY (AGRICULTURE)

in

AGRICULTURAL MICROBIOLOGY



**DEPARTMENT OF PLANT PATHOLOGY AND
AGRICULTURAL MICROBIOLOGY**

**POST GRADUATE INSTITUTE
MAHATMA PHULE KRISHI VIDYAPEETH
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MAHARASHTRA, INDIA.**

2023

CANDIDATE'S DECLARATION

I hereby declare that this thesis or part
there of has not been submitted
by me or other person to any
other University or Institution
for a Degree or
Diploma

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Date : / /2023

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This is to certify that the thesis entitled, “**SENSORY MICROBIAL TECHNIQUE TO DETECT PESTICIDE AND HEAVY METALS RESIDUES IN MARKETABLE FRUITS AND VEGETABLES**” submitted to the Faculty of Agriculture, Mahatma Phule Krishi Vidyapeeth, Rahuri Dist. Ahmednagar (M.S.) in partial fulfillment of the requirements for the degree of **DOCTOR OF PHILOSOPHY (AGRICULTURE)** in **AGRICULTURAL MICROBIOLOGY**, embodies the results of a piece of *bona fide* research work carried out by **Miss. NADAF JASMIN MANSOOR**, under my guidance and supervision and that no part of the thesis has been submitted for any other degree or diploma.

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LIST OF ABBREVIATIONS

%	:	Per cent
/	:	Per
@	:	At the rate of
µg	:	Microgram
µl	:	Micro litre
⁰ C	:	Degree Celsius
BOD	:	Biological Oxygen Demand
cm	:	Centimeter
DAI	:	Days after incubation
<i>et al.</i>	:	et alli (and other)
Etc.	:	Et cetera (and so forth)
Fig.	:	Figure (s)
g	:	Gram or gravity
g/kg	:	Gram per kilogram
gm	:	Gram
HAI	:	Hours after incubation
Hr.	:	Hour
i.e.	:	That is
<i>In-vitro</i>	:	In laboratory
kg	:	Kilogram
mg	:	Milligram
Min	:	Minute
ml	:	Mililitre
No.	:	Number (s)
ppm	:	Parts per million
Spp.	:	Species
v/v	:	Volume by volume
<i>viz.</i>	:	Videlicet (Namely)
std	:	Standard
EU	:	Europion Union
-ve	:	Negative
+ve	:	Positive

ABSTRACT

**“SENSORY MICROBIAL TECHNIQUE TO DETECT PESTICIDES AND
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Research Guide : **Dr. A.M. Navale**

Department : **Plant Pathology and Agricultural Microbiology**

The prevalence of harmful pesticide residues, heavy metals, as well as microbial contamination in fresh fruits and vegetables is a major public health problem across the globe.

The purpose of this study was to assess the residual pesticide *viz.*, Cypermethrin, Spinosad, Acephate, Carbendazim, as well as Chlorpyrifos, heavy metal *viz.*, Lead, Cadmium and Chromium, as well as the microbial contamination quality and safety of common marketable fruits and vegetables samples from various local markets in Mumbai. The current study, named "Sensory microbial Technique to detect pesticide residues as well as heavy metals in marketable fruits and vegetables," was conducted at Mahatma Phule Krishi Vidyapeeth, Rahuri, Departments of Plant Pathology and Agricultural Microbiology. The object of this study was to identify and isolate microorganisms in marketable vegetables and fruits that were sensitive to pesticide and heavy metals residues. Soil samples were taken from the railway site of Malad, Vileparle, Kandivali, Rammandir, Goregaon, as well as Andheri railway stations on Mumbai's western line. Soil samples were gathered and labelled correctly in disposable polythene bags. Ten pesticides and seven heavy metals were received from Department of

Agricultural Entomology and Department of Plant Pathology and Agricultural Microbiology, M.P.K.V., Rahuri.

As a consequence, the findings of the research revealed that the presence of residual pesticides and heavy metals in the collected samples of fruits and vegetables possess a risk when eaten raw. The methods such as Gas Chromatography (GC), HPLC High Performance Liquid Chromatography and Atomic absorption Spectroscopy (AAS) were adopted to analyse pesticide residues in fruits and vegetables before marketing to consumer. An alternative method for pesticide and heavy metal residues testing was using microorganisms to detect residues of pesticides and heavy metals, which require low cost equipments and less time.

In general, it was revealed that, ten frequently used pesticides *viz*; Cypermethrin, Chloropyriphos, Acephate, Spinosad, Captan, Carbendazim, Metalaxyl, Mancozeb, Chlorothalonil and 2-4-D as per European Union standard low concentration were screened for pure culture of different isolates. CAR-8, CTG-10 and DIM-5 showed sensitivity to Carbendazim at 2.5 ppm, Captan at 1 ppm and 2-4-D at 2.5 ppm respectively. Among the heavy metals *viz*; Copper (cu), Lead (Pb), Cadmium (Cd), Chromium (Cr), Nickel (Ni), Arsenic (As), Mercury (Hg) as per Indian standard concentration were screened for pure culture of different isolates. Isolate NIV-7 and COM-2 showed sensitivity to nickel at 0.04 ppm, Copper at 0.01 ppm respectively.

Based on morphological, cultural and biochemical characterization isolates CAR-8, CTG-10, DIM-5, NIV-7 and COM-2 were probably belonging to genus *Rhizobium spp*, *Azospirillum spp*, *Azotobactor spp*, *Pseudomonas spp* and *Paenibacillus spp* respectively.

Hence, these findings suggest that there are sensitive microbes found at low concentration of pesticide and heavy metals in soil and water sample collected from railway site of Mumbai area.

1. INTRODUCTION

Pesticides are widely used in fruits and vegetables because of their susceptibility to insect and diseases attack. Excessive use of pesticides on fruits and vegetables to protect them from damage and loss by pests increases pesticide residues in these foods. Consequently; food safety is a major public concern worldwide. During the last decade, the increasing demand of food safety has stimulated research regarding the risk associated with consumption of fruits and vegetables as they constitute major part of human diet contributing nutrients and vitamins. Therefore, residues of pesticides could affect the ultimate consumers especially when these commodities are freshly consumed. Herbicides, insecticides and fungicides are the most commonly used pesticides worldwide. Insecticides are more common in low-and middle-income countries (LMICs), whereas herbicides and fungicides are more heavily used in high income countries (HICs) (WHO 2008). Pesticide consumption is the highest in Maharashtra, followed by Uttar Pradesh, Punjab and Haryana. During the last decade, the total consumption increased in Maharashtra and Uttar Pradesh, while it slightly declined in Punjab and Haryana (Subash *et al.*, 2017).

The intensity of protection for crops, as shown by a 15-20-fold increase in pesticides used around the world, has increased significantly in order to make agriculture more productive and profitable. There are more than 1000 pesticides used around the world to protect crops against insects, fungi, weeds and other pests. Despite a clear increase in pesticide use, crop losses have not decreased significantly over the last 40 years (Oerk, 2006). The common formulation types of pesticide are liquids, wettable powder, emulsifiable concentrates and dusts, and the utilization of pesticides enters into the environment and has many different fates. Depending on the chemical structure pesticides can be classified as organochlorines (endosulfan, hexachlorobenzene), organophosphates (diazinon, omethoate, glyphosate), carbamic and thiocarbamic derivatives, carboxylic acids and their derivatives, urea derivatives, heterocyclic compounds (benzimidazole and triazole derivatives), phenol and nitro phenol derivatives, hydrocarbons, ketones, aldehydes and their derivatives, fluorine containing compounds, copper-containing compounds, metal organic and inorganic compounds, and natural and synthetic pyrethroids (Maksymiv, 2015). When they are sprayed, pesticides move

through the air and eventually end up in other parts of the environment like soil and water. Also pesticides break into ground water, soil and surface water depending upon soil type, temperature, vapor pressure, the amount of sunlight and rain, the water solubility of the pesticides and magnitude of application (Rajendra, 2003). The total dietary intake of pesticides residues that remain on agricultural commodities are known as carcinogens/or toxins and therefore it is desirable to reduce these residues (Zawiyah *et al.*, 2007). The extensive and indiscriminate use of pesticides is a major concern because of the potential harm that these compounds can cause to the environment and because of the known or suspected toxic effects in humans. Some of these toxic effects include acute neurological toxicity, neurodevelopment impairment, disturbances in the immune system, disturbances in the reproductive and endocrine systems, cancer, chronic kidney diseases and several other diseases (Guan *et al.*, 2010; Hercegova *et al.*, 2007; Sinha *et al.*, 2012).

Pesticide residues should not pose health risks if they are below the threshold of exposure known as Maximum Residue Limits (MRL). MRLs are the maximum amount of a pesticide residue allowed in food or animal feeds, expressed as milligrammes per kilogramme of the food. The presence of pesticide residues in fruits and vegetables can potentially be toxic to human health if present in quantities above the MRL (Selvaraj *et al.*, 2014). International parties such as the European Union, Codex Alimentarius Commission, and North American Free Trade Agreement have attempted to harmonize pesticide legislation by providing maximum residue limits, but globally these limits remain variable. In India, Food Safety and Standards Authority of India sets the maximum residue limits for pesticides in crops, foods, vegetables and fruits (Handford., *et al.*, 2015).

Heavy metal contamination of vegetables cannot be underestimated as these foodstuffs are important components of human diet. Fruits and vegetables are an important component of diet after cereals. Fruits and vegetables are edible plant products that are good for health which are widely used for culinary purpose. Vegetables are rich sources of vitamins, minerals, and fibers, and also have beneficial antioxidative effects. However, intake of heavy metal contaminated vegetables may pose a risk to the human health. Heavy metal contamination of the food items is one of the most important aspects of food quality assurance (Marshall, 2004; Radwan and Salama, 2006; Khan *et al.*, 2008).

Heavy metals such as Cd and Pb have been shown to have carcinogenic effects (Trichopoulos, 1997). Vegetables grown in areas polluted with dangerous metals or nearby sources of heavy metal pollution may gather greater amounts of heavy metals than other vegetables. Heavy metals are taken through the roots of plants from polluted soils and environmental wastes, entering the edible sections of plant tissues or accumulating on the surface of vegetables (Christou *et al.*, 2014). Micronutrient metals such as Cu, Mn, Mo, Ni, Co and Zn do occur naturally in fruits and vegetables as essential trace elements needed for good health (Reeves and Baker, 2000), but they could be toxic when concentrations exceed the limits of safe exposure (Reilly, 1991, Skurikhin, 1993). In general, heavy metals are those metals that have a specific density of more than 5 g/cm³ and negatively affect living organisms and the environment, but any toxic metal may be called a heavy metal, irrespective of their atomic mass or density (Singh *et al.*, 2011). Heavy metals have toxic and mutagenic effects even at very low concentration. Several cases of human disease, disorders, malfunction and malformation of organs due to metal toxicity have been reported (Avena, 1979). High concentrations of heavy metals (Cu, Cd and Pb) in fruits and vegetables were related to high prevalence of upper gastrointestinal cancer (Turkdogan *et al.*, 2002). However, the daily intake of metals (DIM) and health risk index (HRI) in that investigation were <1, indicating that the health risks might not be too serious, though the concomitant interactions of metals through dermal and inhalation exposure could exacerbate the vulnerability of humans, particularly children, to disease (Yang *et al.*, 2017).

Railway track farming are carried out from Charni road station till Borivali station along the Western line, from Byculla station till Dombivli station on central line and from Sewri station till Panvel station on harbour line of Mumbai in Maharashtra. Different types of vegetables like Spinach, Okra and Radish are grown throughout the year along these railway tracks. To save these open spaces from encroachment, the railways had allowed its employees to carry out farming on lease there under the 'Grow More Food' scheme. Also these vegetables are cultivated and sold at various markets at a low price as compared to the vegetables grown and sold by conventional farming. The water source for such agricultural activity is sewage water where their discharge of the effluents is from various industries, water from drainage

pipelines and from domestic source, which lead to the contamination of the soil, fruits and vegetables (Doshi and Zele, 2014). Sewage water is nothing but the unprocessed water collected from different sources such as domestic sources, hospitals and industries. Depending on the location, sewage water may contain an array of substances either in solution form or in solid. The different types of waste materials in sewage water include both organic and inorganic wastes, nutrients, toxic chemicals, oils and many more components. The untreated wastewater can contain disease causing microbes, certain toxins, drugs and toxic metals among other things. Soils receiving these waters accumulate heavy metals to varying degrees depending on their concentration in water and the frequency of irrigation. The heavy metals are absorbed by crops along with other essential plant nutrients.

Thus, the risk associated with consumption of food growing in areas irrigated with wastewater is extremely high (Singh *et al.*, 2009). The transfer of pathogens and diseases through vegetable consumption is noticed and documented (World Bank, 2010). Generally heavy metals are not biodegradable they have long half-life with the potential for accumulation in different body organs leading to unwanted side effects (Sathawara *et al.*, 2004). They include Pb, Cd, Zn, Cu, Co, Ni, Ar, Hg and Cr (Sharma *et al.*, 2006). They are ubiquitous in the environment through various pathways, due to natural and anthropogenic activities. Farmers use pesticides as a preventive policy against the possibility of a devastating crop loss from pests and diseases in railway track farming. However, because of their highly toxic and persistent nature, their residues still appear as pollutants in food as well as in the environment (Bempah *et al.*, 2011).

In Indian agricultural system, we do not have a system where these fruits and vegetables are assessed for their pesticides and heavy metals residues before marketing to consumers. Only those fruits and vegetables are tested for residues which have to be exported abroad (Raikwar *et al.*, 2011; Kolekar *et al.*, 2011). For residues analysis of these fruits and vegetables different methods were adapted such as Gas Chromatography (GC), High Performance Liquid Chromatography (HPLC) and Atomic absorption Spectroscopy (AAS) which require high equipments, labour and these are time consuming methods. The main objectives of the present work are to detect the pesticide residues and heavy metals in fruits and vegetables, The expensive equipment

and chemicals, are used which cannot be affordable in all the places or laboratories where these fruits and vegetables are to be tested and certified for further consumption.

To safeguard the health of our own citizens, the marketable fruits and vegetables should also be tested for their residues. However due to enormous cost of equipment required for this purpose, it is not yet feasible. Therefore, an alternative methods to these pesticide residues testing machines/instrument, has to be explore. There are very less references available on this work. Therefore, it was thought worthwhile to conduct study to detect the pesticides residues and heavy metals in marketable fruits and vegetables. Therefore, a research on “Sensory microbial technique to detect pesticide residues and heavy metals in marketable Fruits and Vegetables” is undertaken with following objectives:

- i. Isolation of micro-organisms sensitive to low concentration of pesticide residue and heavy metals.
- ii. Screening of the microbes against the low concentration of individual pesticides and heavy metals (As per Food and Drug Administration).
- iii. Detection of pesticide residue and heavy metals by using these sensory microbes in marketable fruits and vegetables.
- iv. Identification of the sensory microbes for respective pesticides and heavy metals.

2. REVIEW OF LITERATURE

In this chapter an attempt was made to review the relevant research work done on “Sensory microbial technique to detect pesticide residues and heavy metal in marketable fruits and vegetables”. However, reports that are relevant to various aspects investigated in the present study have been gathered and reviewed in the review of literature

2.1 Pesticides contamination in soil and water

Pesticides can contaminate soil, water, turf and other vegetation. Pesticide can reach surface water through run off from treated plants and soil. Contamination of water by pesticides is wider spread. The pesticides and their TPs (Transformation products) are retained by soil to different degrees, depending on the interactions between soil and pesticides properties (Aktar *et al.*, 2009). Highest persistence of these compounds is frequently found in soils with high organic matter (OM) content, which are the most vulnerable due to their high adsorption capacity. (Pose- Juan *et al.*, 2015).

Grewal *et al.* (2017) study showed that increased use of pesticides results in contamination of the environment and the excess accumulation of pesticides residues in food products, which has always been a matter of serious concern. The most consumed pesticides for vegetables, fruits and food grains in India include sulphur, endosulphan, Mancozeb, Phorate, methyl parathion, monocrotophos, cypermethrin, isoproturon, chloropyriphos, malathion, carbendazim, butachlor, quinolphos, copperoxychloride, and dichlorvos.

Farooqui *et al.* (2020) study showed that application of Ps in soil can lead to various fates depending upon its persistence and mobility. Pesticide entry in aquatic, soil, and atmosphere are main sinks of pesticides in environment. Fate of Ps involves its absorption by plant. Second fate of Ps is adsorption on soil constituents which depends upon physico-chemical properties of soil. Pesticide leaching and runoff are other fates which involve solubilization in soil solution, wind, and soil erosion.

Manjarres-Lopez *et al* (2021) studied assessment of pesticide residues in waters and soils of a vineyard region and its temporal evolution. The results reveal the presence in waters of 30 compounds from those selected (15 fungicides + 2 DP, 7

insecticides + 1 DP, and 3 herbicides +2 DP), with 14 of them at concentrations $> 0.1 \mu\text{g/L}$ water quality threshold for human consumption). The highest number of compounds was detected in summer waters and spring soils. The pesticides most frequently detected in water samples were the fungicides metalaxyl, tebuconazole, and boscalid, with the last one being the compound found in the highest number of soil samples.

2.2 Heavy metals contamination in soil and water

Wastewater irrigation is known to contribute significantly to the heavy metal contents of soils (Mapanda *et al.*, 2005).

Singh and Kumar (2006) studied heavy metal load of soil, water and vegetables in peri-urban Delhi. Peri-urban lands are often used for production of vegetables for better market accessibility and higher prices. But most of these lands are contaminated with heavy metals through industrial effluents, sewage and sludge, and vehicular emission. Heavy metal load of the soils were below the maximum allowable limits prescribed by the World Health Organization (WHO), which was higher in irrigation water and vegetable samples. The spinach and okra samples showed Zn, Pb and Cd levels higher than the WHO limits.

Varalakshmi and Ganeshamurthy (2010) studied heavy metal contamination of water bodies, soils and vegetables in peri urban areas of Bangalore city of India. Analyses revealed high concentrations of Cd and Cr in waters of all the tanks, exceeding the recommended levels of 0.01 and 0.1 mg/L respectively. Concentration of Cd was highest in waters of Bellandur (0.039mg/L) and concentration of Cr was highest in waters of Byramangala tank (0.311mg/L). Among all the tanks, Bellandur and Varthur were found to be highly contaminated with Cd, Pb and Ni. The concentration of heavy metals (mg/kg) in soils receiving sewage waters from the four tanks ranged from 1.92 - 2.90 for Cd, 47.04-68.12 for Pb, 35.08-92.78 for Cr and 48.2-57.3 for Ni. The Cd and Pb contents were highest in the soils near Varthur and Bellandur tanks, while Cr was highest in soils near Byramangala. A similar trend was observed with respect to heavy metal content of vegetables. Among all the vegetables, amaranthus and spinach accumulated higher concentrations of heavy metals followed by carrot and radish. The Cd concentration of all the vegetables grown near Varthur and Bellandur tanks exceeded the PFA safe limit.

Vazhacharickal *et al.* (2013) studied on urban and peri-urban agricultural production along railway tracks from the Mumbai metropolitan region. The result obtained showed that all the railway gardeners practiced agriculture as a primary source of income and cultivated seasonal vegetables such as lady's finger (*Abelmoschus esculentus* L. Moench), Spinach (*Spinacia oleracea* L.), Red amaranth (*Amaranthus cruentus* L.) and white radish (*Raphanus sativus* var. *longipinnatus*) which were irrigated with waste water. This irrigation water was loaded with 7–28 mg N l⁻¹, 0.3–7 mgP l⁻¹, and 8–32 mg K l⁻¹, but also contained heavy metals such as lead (0.02–0.06 mg Pb l⁻¹), cadmium (0.03–0.17mg Cd l⁻¹), mercury (0.001–0.005 mg Hg l⁻¹), and pathogens such as *Escherichia coli* (1,100 most probable number per 100 ml). Levels of heavy metals exceeded the critical thresholds in surface soils (Cr, Ni, and Sr) and produce (Pb, Cd, and Sr).

Islam *et al.* (2018) analysis showed that soils near high traffic and industrial areas contain high concentrations of heavy metals and metalloids. Agricultural land and vegetables in sewage-irrigated areas were also found to be heavy metal and metalloid contaminated. River water, sediment, and fish from the Buriganga, Turag, Shitalakhya, and Karnaphuli rivers are highly contaminated with cadmium (Cd), lead (Pb), and chromium (Cr). Particularly, groundwater arsenic (As) pollution associated with high geological background levels in Bangladesh is well reported and is hitherto the largest mass poisoning in the world. Overall, the contamination levels of heavy metals and metalloids vary among the cities, with industrial areas being most polluted.

2.3 Pesticides residues in marketable fruits and vegetables

Bempah *et al.* (2011) studied the concentration of pesticide residues in fruits and vegetables from markets in Kumasi and to generate awareness about the lethal effects of these pesticides on human beings. The results obtained showed that 37.5 per cent of the fruits and vegetable samples analyzed contained no detectable level of the monitored pesticides, 19.0 per cent of the samples gave results with levels of insecticides residues above the MRL, while 43.5 per cent of the samples showed results below the MRL.

Rodrigues *et al.* (2011) determined five insecticide residues in fruit samples produced in Ceara State (Brazil). A sufficient recovery of 52.1, 57.0, 49.5, and

108.5% for methyl parathion, malathion, chlorpyrifos, and 2, 4-dichlorophenol was found. The compounds 2, 4-dichlorophenol, malathion, parathion methyl, and chlorpyrifos were found in all the fruit samples analyzed.

Chowdhary *et al.* (2013) investigated the presence of 19 different agricultural pesticides in 210 samples of eight type domestic vegetables collected from several growing regions in Bangladesh. Pesticide residues were detected in 51.30 per cent of total samples, and among the positive samples 38.89% contained levels above the maximum residue levels (MRLs).

Jha, R. (2013) demonstrated the prevalence of OC, OP and pyrethroid pesticides in commonly consumed fruits and vegetables. Consequently, it was recognized that regularly consumed marketable fruits and vegetables contain measurable quantities of OC, OP and pyrethroid pesticides, adopted analytical methods for quantifying amounts of these pesticide residues in the mentioned food commodities, and applied methodologies to relative samples collected from 3 markets at 3 different localities.

Sharma (2015) studied residues analysis of 75 samples of 13 vegetables indicated that 58% of the vegetable samples contained no detectable level (NDL) of the monitored pesticides, 38% samples resulted in trace level of the pesticides residue or below the minimum residue level (MRL), while 4% samples showed above MRL (EU Standard).

2.4 Heavy metals in marketable fruits and vegetables

Bagdatlioglu *et al* (2010) determined the levels of Cu, Zn, Fe, Pb and Cd in various fruits (tomato, cherry, grape, strawberry) and vegetables (parsley, onion, lettuce, garlic, nettle, peppermint, rocket, spinach, dill, broad bean, chard, purslane, grapevine leaves) grown in Manisa region. Flame and Graphite Furnace Atomic absorption spectrometry was used to estimate and evaluate the levels of these metals. Detected levels ranged from 0.56 to 329.7, 0.01 to 5.67, 0.26 to 30.68, 0.001 to 0.97 and 0 to 0.06 ug/ g for Fe, Cu, Zn, Pb and Cd, respectively. While the highest mean levels of Cu and Zn were detected in grapevine leaves, the lowest mean levels of Fe and Pb were detected in nettle. Cd was not detected in most of the fruits and vegetables studied. The estimated daily intakes of Cu, Zn, Fe, Pb and Cd through fruits and vegetables were found to be below the maximum tolerable levels recommended by FAO/WHO. The element concentrations

of fruits and vegetables analyzed in this study were within safety baseline levels for human consumption.

Mohamed and Khairia (2012) assessed the concentration of some heavy metals Fe, Mn, Cu, Zn, Pb, Cd and Hg in various vegetables grown in four major industrial and urban cities (Tabouk, Riyadh, Damamm and Jazan) in kingdom of Saudi Arabia by using atomic absorption spectrophotometer. The obtained result declared that concentrations of major studied metals were exceeding than the recommended maximum acceptable levels proposed by the joint FAO/WHO Expert committee on food Additives.

Akan *et al.* (2013) determined the concentrations of some heavy metals and anions in vegetable samples, which were freshly harvested within four agricultural locations (Mirnga, Zira, Wangaga and Malang) in Biu Local Government area Borno State, Nigeria. The result for vegetable samples showed that leaves contained much higher concentration of heavy metals and anions than roots and stems. The concentration of Cr detected in the vegetable samples ranged from 0.23 to 3.22 mg/kg; 0.23 to 3.43 mg/kg Mn; 0.23 to 3.45mg/kg Fe; 0.21 to 3.54mg/kg Ni; 0.25 to 4.56 mg/kg Pb; 0.87 to 8.34 mg/kg Zn; 0.34 to 5.44 mg/kg Cd and 0.21 to 3.22 mg/kg Cu. These values were higher than those recommended by Food and Agricultural Organization (FAO) and the WHO/EU joint limits.

Chauhan (2014) studied toxicity levels of heavy metals (Fe, Zn, Cu, Pb, Cd, Mn and Cr) found in tested vegetables (Spinach, Cabbage, Cauliflower, Brinjal, Lady's Finger, Tomato and Radish) grown in contaminated cement factory area compared with those grown in reference clean (control) area. When compared with the reference in contaminated area, water, soil and vegetables contents of all analyzed metals was significantly higher, usually over normaly content for Fe, Zn, Cu, Pb, Cd, Mn and Cr. Particularly, higher values than safe limits were found for Pb, Cd, Mn, and Cr in water and for Cd in soil and Pb, Cd and Cr were observed higher in leafy vegetable especially spinach, in contaminated areas.

Salhotra and Verma (2017) studied the concentration of heavy metals lead(Pb),cadmium(Cd), copper (Cu) iron (Fe), cobalt (Co) in ten commonly used vegetables and fruits available in local market of Jagdalpur.Comparing the results of heavy metals in vegetables and fruits by using atomic absorption spectrometer, (Thermo

scientific Pvt. Ltd. India Model No. AA 303) double beam with their respective natural level. Fe concentration varied considerably in spinach, tomato, cauliflower and lady finger are crossed permissible limits. Copper concentration in cauliflower, spinach, apple, grapes are below than the safe limit.

Mawari *et al.* (2022) studied heavy metal accumulation in fruits and vegetables and human health risk assessment finding from Maharashtra India. The mean concentrations of selected heavy metals in fruits and vegetables analyzed were: Lead (0.17 ± 0.38 mg/kg) > Mercury (0.06 ± 0.09 mg/kg) > Cadmium (0.02 ± 0.007 mg/kg) > Arsenic (0.002 ± 0.003 mg/kg). Among them, garlic showed the highest heavy metal accumulation followed by potato.

2.5 Isolation of microorganisms sensitive to low concentration of pesticidal residue and heavy metals.

Ohta and Udaka (1976) isolated cadmium and mercury sensitive mutants of *Escherichia coli*. The Cd-hypersensitive mutant, CD17P, could not grow in a chemically defined liquid medium containing 0.5 uM Cd²⁺.

Rathnayake *et al.* (2009) isolated and characterized two gram positive isolates from pristine soil. Two Gram +ve isolates were identified as *Paenibacillus sp.* and *Bacillus thuringiensis*. Both bacteria showed same pattern of metal tolerance in the order Zn⁺>Cd²⁺>Cu²⁺. When the metal tolerance in both bacteria was compared *Paenibacillus sp.* showed maximum sensitivity to Cu²⁺. Where as *B.thuringiensis* showed highest sensitivity to Cd²⁺ and Zn²⁺.

Mathivanan and Rajaram (2010) isolated bacteria from soil sample collected from around the BHEL (Bharath Heavy Electrical Limited), Tiruchirappalli. The isolated organisms present in nutrient agar medium containing mercury (II) chloride for identification of mercury resistant and sensitive bacteria. A total of fifteen colonies were isolated from the polluted soil sample. Among them colony number twelve showed better growth in high HgCl concentration, indicating the mercury resisting capacity of *Staphylococcus aureus* identified by morphological and biochemical tests like IMVIC, triple sugar ion, catalase, citrate utilization, coagulase, oxidase, starch hydrolysis, urease hydrolysis. Out of fifteen colonies, seventh colony shows no growth which was sensitive to mercury i.e. *E. coli* confirmed by the presence

of green metallic sheen observed in EMB plates. The plasmid DNA from *Staphylococcus aureus* was isolated and transformed into mercury sensitive *E. coli*.

Kacar and Kocyigit (2013) examined isolation and identification of sediment bacteria from the ship dismantling area in Aliaga (Aegean Coastline of Turkey). Thirteen isolates were identified by phylogenetic analysis using 16S rDNA sequences which indicated that the isolates belong to genus *Bacillus*. The MICs of heavy metals were different for each strain but the general order of resistance to the metals was found to be as Pb>As>Ni>Co>Cu>Zn>Cr>Cd>Hg and the toxic effects of these metals increased with increasing concentration. It can be concluded that all isolates were sensitive to Hg but were highly resistant to Pb, As, and Ni.

Mathivanan and Rajaram (2014) isolated 35 bacterial strains from the industrially polluted Cuddalore coast, on the southeast coast of India. Of these, 17 strains were cadmium resistant and the remainders were sensitive. Six strains (C-1, C-8, C-10, C-12, C-14 and N-1) were selected based on high levels of cadmium tolerance ($>150 \text{ mg L}^{-1}$) and were termed highly cadmium-resistant bacteria (HCRB). These HCRB were identified on the basis of morphological, biochemical and partial sequencing of their 16S rRNA genes. The antibiotic-susceptibility patterns and minimum inhibitory concentrations (MIC) of different metals (Cu^{2+} , Pb^{2+} and Zn^{2+}) against each HCRB were determined. Among the isolates, C-14 showed high degrees of metal and antibiotic resistance compared with other HCRB. Growth rates of HCRB at two different Cd^{2+} concentrations (50 and 100 mg L^{-1}) and under different metal conditions (Cd^{2+} , Cu^{2+} and Pb^{2+}) were also investigated. HCRB growth rates were lower in the metal treated condition than in the untreated condition. Isolates C-14 and N-1 removed $> 80\%$ of Cd^{2+} from cadmium treated broth. However, isolate C-14 removed 92.3% of Cd^{2+} compared with 86.5% for isolate N-1. Bacteria showing residual growth rates under metal stress conditions might be useful in metal removal applications under growing conditions.

Marzan *et al.* (2017) isolated the three bacterial species namely *Gemella sp.*, *Micrococcus sp.* and *Hafnia sp.* from tannery effluent in Chittagong city. Among them *Gemella sp.* and *Micrococcus sp.* showed resistance to Lead (Pb), chromium (Cr) and cadmium (Cd), where *Hafnia sp.* showed sensitivity to cadmium (Cd).

2.6 Testing sensitivity of isolates against low concentration of pesticidal residue and heavy metals

Worth and Mc Cabe (1948) reported the effect of 2-4-D on the growth of aerobic, anaerobic and facultative anaerobic micro-organisms on solid media. Growth of the aerobic bacteria was prevented by 0.0002 % 2-4-D.

Mandel *et al.* (1965) studied on the action of arsenic on *Bacillus cereus*. Trivalent sodium arsenite was found to inhibit growth at a lower concentration (0.4 mM) than pentavalent sodium arsenate (10 mM).

Focht and Joseph (1974) studied microbial activity in soils treated with acephate and monitor. With three repeated applications 20 ppm of the organophosphate insecticides, acephate (O, S-dimethyl acetylphosphoramidothioate) and monitor (O, S-dimethyl phosphoramidothioate) over a 50-day time span. Population levels of actinomycetes, bacteria, and fungi were not substantially affected by the addition of either pesticide.

Doelman and Haanstra (1979) investigated sensitivity of soil bacteria towards Pb. In the Pb-containing soils, proportionally more gram-negative rods were present while coryneform bacteria decreased.

Zevenhuize *et al.* (1979) studied inhibitory effects of copper on bacteria related to the free ion concentration. Copper-sensitive bacteria, e.g., *Klebsiella aerogenes*, were inhibited in their growth and survival in the range of 10^{-8} - 10^{-6} M Cu^{2+} ion concentrations. Copper-resistant bacteria could be isolated from a stock solution of CuSO_4 , containing 100 ppm Cu (II). They were of the *Pseudomonas* type and showed a much higher tolerance towards Cu^{2+} , up to 10^{-3} M.

Banerjee and Banerjee (1987) reported that in captan treated soil total count of fungi, bacteria and actinomycetes decreased significantly only at a relatively high fungicide concentration (1000 ug/g). Fungi and actinomycetes were more affected than bacteria.

Pozo *et al.* (1994) reported that mancozeb and maneb, at concentrations of about 2.0 to 10.0 kg/ha on an agricultural soil of silty loam texture. Total platable fungal populations, denitrifying bacteria and aerobic diazotrophs were significantly decreased in the presence of agricultural doses of mancozeb. Total platable bacteria were

not affected by the addition of mancozeb or maneb, showing that some microbial groups can tolerate agricultural doses of these fungicides.

Itoh *et al.* (1994) reported that incubation of *E. coli* cells with 10 mM of hexavalent chromium and 3 mM hydrogen peroxide caused the degradation of double-strand DNA *in vivo*, which was suppressed by the addition of mannitol.

Tu (1994) reported that that *Nitrosomonas* and autotrophic nitrifying bacteria were more sensitive to chlorothalonil than to carbendazim.

Di-ciocco and Rodriguez (1997) studied effect of fungicide captan on growth and nitrogenase activity of *Azospirillum brasilense*. The 8 h growth in rotary shaker of *A. brasilense* was inhibited with 1mg/L pure captan.

Martinez-Toledo *et al* (1998) studied the effects of the fungicide captan on some functional groups of soil microflora. Result showed that total culturable fungal populations, nitrifying bacteria, aerobic N-fixing bacteria and nitrogenase activity were significantly decreased at dose rates of 2.0 to 10.0 kg ha⁻¹. However, the presence of captan enhanced denitrifying and total culturable bacteria, showing that some microbial groups can tolerate high doses of this fungicide.

Shetty and Magu (2000) reported that low concentration of metalaxyl stimulated the bacterial and actinomycetes population, but its higher levels significantly inhibited the population. However, different doses of metalaxyl inhibited fungal and *Azotobacter* population at all slates of observation. Interestingly, 0.5 ppm and 1 ppm of metalaxyl did not affect *Nitrosomonas* and *Nitrobacter* population: however, at higher concentration it significantly inhibited the nitrification process.

Viamajala *et al.* (2004) Studies showed that the growth of *Shewanella oneidensis* was much inhibited even at a low concentration 0.015 mM of Cr (VI).

Wyszkowska *et al.* (2006) studied effect of nickel on the proliferation of the following soil bacteria suspended in liquid media: *Azotobacter spp.*, *Arthrobacter spp.*, *Rhizobium leguminosarum* bv. *viciae* and *Bradyrhizobium spp.* (lupini). Among the bacteria analysed, *Azotobacter spp.* was found to be most sensitive to nickel compounds, followed by *Arthrobacter spp.*, *Bradyrhizobium spp.* (lupini) and *Rhizobium leguminosarum* bv. *viciae*, which was most nickel-resistant

Fawole *et al.* (2009) examined that when carbendazim–mancozeb fungicidal mixture applied at concentration of 2.34 mg/kg soil had a greater ($p < 0.05$) inhibitory effect on nitrogen fixers nitrifying bacteria and cellulolytic organisms.

Pappas *et al.* (2010) the sensitivity of 36 *Septoria pyricola* single spore isolates, obtained from isolated pear orchards, to carbendazim, bitertanol, flusilazole, myclobutanil, azoxostrobin, kresoxim-methyl, pyraclostrobin, trifloxystrobin and boscalid, was studied *in vitro*. Spore suspensions were point-inoculated on media amended with various concentrations of fungicides and the minimum inhibitory concentration (MICs) of the fungicides was determined. Most isolates were highly resistant to carbendazim, forming colonies even at concentrations of 100 mg L^{-1} of the fungicide. A few isolates failed to form colonies with carbendazim concentrations of 0.1 and 10 mg L^{-1} ; these isolates were designated carbendazim-sensitive and moderately carbendazim-resistant respectively.

Krujatz *et al.* (2011) assessed the toxic effect of nickel, cadmium and EDTA on growth of the plant growth promoting Rhizobacterium, *Pseudomonas brassicacearum*. The inhibition was much greater at low concentration of Ca^{2+} (25 umol/L) and ($\text{Mg}^{2+} 100 \text{ umol/L}$) ($\text{EC}_{50} \text{ Cd}^{2+} 85 \pm 0.5 \text{ umol/L}$ and $\text{EC}_{50} \text{ Ni}^{2+}, 62 \pm 1.8 \text{ umol/L}$).

Shineyramya and Ganesh (2013) investigated the antimicrobial activity of spinosad against selected soil beneficial microbes and plant pathogenic microbes by disc diffusion method. Result showed that spinosad is harmless to soil beneficial bacteria (*Azotobacter chroococcum*, *Pseudomonas fluorescens* and *Rhizobium leguminosarum*) and fungi (*Aspergillus fumigatus*, *Penicillium chrysogenum* and *Trichoderma viride*). Whereas harmful to disease causing Plant pathogenic microbes. The maximum inhibition was 13 mm, 15 mm & 17 mm with respect to 1 ppm, 5 ppm and 10 ppm which was observed in *Xanthomonas campestris*. The zone of inhibition was 12 mm, 13 mm and 15 mm with respect to 1 ppm, 5 ppm and 10 ppm concentration in case of *Erwinia carotovora* and the inhibition of 11 mm, 11 mm & 13 mm at above said concentration (ppm) was observed in *Pseudomonas syringae*..

Walia *et al.* (2014) reported that increase in mancozeb concentrations up to 100 ppm and above decreased fungal population. Mancozeb generally decreased

actinomycetes population at all the concentrations while higher concentrations 1000 and 2000 ppm were found to be detrimental to soil bacteria.

Supreeth *et al.* (2016) reported effect of chlorpyrifos on soil microbial diversity, the soils treated with 100 and 200 ug/ g the chlorpyrifos (CP) insecticide showed decrease in the number of colony forming units (CFU) of bacteria and fungi.

Luo *et al.* (2019) reported that *Actinobacteria* are tolerant to cadmium, whereas *Proteobacteria*, *Verrucomicrobia*, and *Nitrospirae* are sensitive.

Ding *et al.* (2019) study showed that chlorothalonil reduced *Nitrosomonas* populations at the rates of 10 and 50 mg of a.i. per kg and autotrophic nitrifying bacterial populations at three application rates ($P < 0.05$).

Meena *et al.* (2019) studied that herbicide 2-4-D, atranex and agroxone inhibited the occurrence of *Rhizobium phaseoli* and *Azotobacter vinelandii* and their population further decreased with increase in herbicide concentration.

Niu *et al.* (2019) reported that lower Pb pollution increased microbial quantities and promoted the utilization of amino acids or fatty acids, while higher Pb concentrations decreased microbial quantities and metabolic activities, and promoted the utilization of carbohydrates. The bacteria (nitrogen-fixing bacteria, ammonifying bacteria, inorganic phosphorus-solubilizing bacteria, and nitrosobacteria) all increased in the 500 mg/kg treatment and decreased in the 1500 mg/kg treatment compared with the 0 mg/kg treatment, especially in rhizospheric soil.

Sultana *et al.* (2020) examined tolerance level of soil microbes *Pseudomonas* and *Penicillium* against different levels of sodium arsenite stress (1mg/L to 10mg/L). Result shown that both micobes cannot overcome higher levels of arsenic stress because in higher stress petriplates, increased inhibitory zones were observed.

McDonald *et al.* (2021) studied inhibition of select actinobacteria by the organophosphate pesticide chlorpyrifos. CPF (chlorpyrifos) dramatically inhibited the growth of most strains and/or altered colony morphologies, with 13 strains completely inhibited by CPF at 3.5ppm concentration.

Sharma *et al.* (2022) investigated impact of carbendazim on cellular growth, defence system and plant growth promoting traits of *Priestia megaterium* ANCB-12 isolated from sugarcane rhizosphere. Results showed that the increasing dose

of carbendazim gradually decreased the plant growth promoting activities of the rhizobacteria ANCB12. At a maximum 3,000 µg/ml (Carbendazim: CBZM) concentration, the activity of superoxide dismutase (SOD) declined by 82.3%, catalase (CAT) by 61.4%, glutathione peroxidase (GPX) by 76.1%, and glutathione reductase (GR) by 84.8 per cent, respectively.

2.7 Characterization of Bacterial isolates

2.7.1 Morphological characterization

Tarrand and Dobereiner (1978) studied sixty-one strains of the root-associated nitrogen fixer *Spirillum lipoferum* exhibited a similar morphology in peptone--succinate salts medium vibrioid cells having a diameter of 1.0 micrometer. When grown in broth the cells had a single polar flagellum, but when grown on agar at 30⁰ C lateral flagella of shorter wavelength were also formed. On the basis of their characteristics it is proposed that they be assigned to a new genus, *Azospirillum*.

Singh *et al.* (2008) described the characterization of a *Rhizobium* strain isolated from root nodules of fenugreek. The *Rhizobium* isolates were rod shaped, gram negative, acid and mucous producing.

Ivanova *et al.* (2009) studied bacteria isolated from soil and the rhizosphere of agricultural plants. Bacterial cells were rod-shaped, single, 1.0–1.5 mm long and about 0.3–0.8mm in diameter, Gram-negative, Motile with three to five polar flagella. 16S rRNA gene sequence analysis indicated that the representative strain CIP 109457T had a clear affiliation with *Pseudomonas sensu stricto* groups, with the nearest relatives being *Pseudomonas brassicacearum*, *P. thivervalensis*, *P. corrugata*, *P. mediterranea* and *P. kilonensis*.

Tilak *et al.* (2010) reported that by motility test, it was found that the cells of the selected isolates are motile, which is the characteristic of *Azospirillum*.

Rathnayake *et al.* (2010) reported that bacteria isolated from a pristine soil and were characterized. Both of these bacterial isolates are rod shaped, spore formers. Two Gram positive isolates were identified as *Paenibacillus sp.* and *Bacillus thuringiensis*

Tyagi and Singh (2013) observed that bacterium isolated from Himalayan valley soil, India, bacterial cells were gram negative, short and cocci-to rod-shaped and

are 0.6–0.8 µm in width and 0.8–2 µm in length the strain ptl-3^T belonged to the genus *Azospirillum* and was closely related to *A. brasilense*.

Upadhyay *et al.* (2015) isolated forty two strains of *Azotobacter* from soil. Among 42 isolates, 7 were single cocci, 7 coccidial chain and 4 were cocci in clumps. Majority of isolates i.e. 24, were small, medium and large rod shaped. Thirty two isolates were Gram –ve in reaction. Finally from these isolates, twenty two were confirmed as *Azotobacter* strains on cyst formation.

2.7.2 Cultural characterization

Wong and Stenberg (1979) studied characterization of *Azospirillum* isolated from Nitrogen-fixing roots of harvested sorghum plants, the five isolates are slightly curve shaped. Strain S-4 appeared to be longer to slightly more curve shaped than other strains. Strains S-3 appeared circular, raised elevation with entire margin.

Chung *et al.* (2008) studied a bacterial endophyte, *Pseudomonas brassicacearum* YC5480, isolated from the root of *Artemisia sp.* producing antifungal and phytotoxic compounds. A bacteria forms mucoid colonies with regular margins.

Ahmed *et al.* (2019) studied *Rhizobium* bacteria in the roots of leguminous plants. It was yellow in color, circular form, margin was entire and rose in elevation, smooth surface and size is moderate.

Hala and Ali (2019) reported that the N₂-fixing bacteria isolated from the five soil samples of neem rhizosphere were *Azotobacter paspali* and *Azotobacter vinelandii* (Azomonas) groups. Colonies of bacteria were circular, white in colour with entire margin.

Anpalagan *et al.* (2020) isolated and characterize a bacterium *Paenibacillus dendritiformis* DDS2: which can produce thermostable alkaline protease. The colonies of the strain DDS2 at 24 h had pale colour, with irregular margin, 1.4 ± 0.3 mm in diameter, low convex elevation, moist and shiny surface.

2.7.3 Biochemical and physiological characterization

Achouak *et al.* (2000) reported that bacteria isolates phenotypically related to *Pseudomonas corrugata* have frequently been isolated from the rhizosphere of *Arabidopsis thaliana* and *Brassica napus* grown on different soils. 16S rDNA (rrs) gene sequencing, DNA–DNA hybridization, biochemical characterization and siderophore

typing showed that these isolates belong to two different species that are distinct from other species of the genus *Pseudomonas*, including *P. corrugata*. Proposed names are *Pseudomonas brassicacearum* (10 strains studied) and *Pseudomonas thivervalensis* (6 strains studied). The type strain of *Pseudomonas brassicacearum* is CFBP 11706T and that of *Pseudomonas thivervalensis* is CFBP 11261T.

Hosoda *et al.* (2003) reported that *Paenibacillus* genus did not grow at 5⁰C, 45⁰C, or in the presence of 5 % NaCl.

Chandra *et al.* (2008) studied isolation and characterization of bacterial strains ITRCS₆ and ITRCS₈. Test of gelatin hydrolysis, H₂S production and VP test for ITRCS₆ strain were negative. Positive test for urea hydrolysis, no growth at 5% NaCl. Strain ITRCS₆ was tentatively identified as *Paenibacillus sp.* based on 16S rDNA sequence analysis.

Islam *et al.* (2008) study showed that maximum growth of *Azotobacter spp* at 30⁰ C to 40⁰ C, no isolate of *Azotobacter* survived at 50⁰C.

Ivanova *et al.* (2009) isolated *Pseudomonas brassicacearum* subsp. *neaurantiaca* subsp.nov., orange-pigmented bacteria isolated from soil and the rhizosphere of agricultural plants. Organism growth was strictly aerobic; temperature range for growth is 4-37⁰ C, with optimum growth at 25⁰C. No growth at 40⁰C. Liquefies gelatin and does not hydrolyse starch, agar, aesculin, chitin or DNA.

Huang *et al.* (2012) isolated bacteria from soil; the isolate is positive for catalase, oxidase, and hydrolysis of starch and casein. Genetic analysis indicated that this strain belongs to the genus *Paenibacillus*.

Jida and Assefa (2012) reported that high tolerance to NaCl where 75% of the tested rhizobia could grow well with 1% NaCl. However, at higher concentrations, the percentage of tolerant isolate decreased with increasing salt concentration as on 11.1 % of the isolates tolerated 5 % NaCl.

Kaushal *et al.* (2013) attempted report on *Fluorescent Pseudomonas* strains were isolated from the soil samples collected from Bhojia Institute of Life Sciences, Budh, Baddi. Biochemical test results showed that test of voges-proskauer, hydrogen sulphite production and urease production were negative.

Tyagi and Singh (2014) studied bacterial strain ptl-3^T was isolated from Himalayan valley soil, India. Strain ptl-3^T belonged to the genus *Azospirillum* and was closely related to *A. brasilense* (98.7 % similarity) and *A. rugosum* (97 % similarity). Voges Proskauer's test and H₂S production test were negative for these isolates.

Hossain *et al.* (2014) described physiological characteristics of ten *Azospirillum spp* isolated from paddy fields of north Bengal. Among them growth of isolate M-1, M-2 and M-5 was absent at all concentration of NaCl 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4 per cent. These isolates identified as *Azospirillum brasilense*. High concentration of NaCl is generally inhibitory.

Roychowdhury *et al.* (2015) studied isolation and characterization of bacteria *Rhizobium* from chick pea (*Cicer arietinum*). Catalase tests of the *Rhizobium spp* strains were positive, Vp test, starch hydrolysis and hydrogen sulphide test were negative.

Hossain *et al.* (2015) described ten *Azospirillum* isolates from different paddy fields of North Bengal. Six selected isolates M-3, M-4, M-6, M-7, M-8 and M-10 were identified as *A. lipoferum*; three isolates M-1, M-2 and M-5 were identified as *A. brasilense* and one isolate M-9 was identified as *A. halopraeferns*. M-1, M-2 and M-5 observed catalase and oxidase test positive, whereas urease test positive for isolate M-2 and negative for M-1 and M-5. Starch hydrolysis negative for M-1 and positive for M-2 and M-5. Gelatin hydrolysis test were negative for all three isolates.

Chennappa *et al.* (2016) studied four *Azotobacter* species for salt tolerance and reported that *A. chroococcum*, *A. vinelandii*, and *A. salinestris* can tolerate up to 8 % NaCl concentration; however, the cell counts were reduced with high concentration of NaCl.

Qaysi *et al.* (2016) isolated *Pseudomonas brassicacearum* bacteria from rhizosphere soil of Cowpea farm in Iraq. Test of catalase, oxidase and gelatin hydrolysis were positive for bacteria, whereas test of urea hydrolysis were negative.

Wadhwa *et al.* (2017) observed the colonies of *Rhizobium* isolated from chick pea (*Cicer arietinum*) were negative for casein and urease test. Optimum temperature for rhizobial growth was found to be 28°C whereas moderate growth was observed at 35°C and minimal growth of isolates was exhibited at 5°C and 40°C.

Bag *et al.* (2017) investigated putative *Azotobacter* species were isolated from soils collected from Cooch Behar and Jalpaiguri districts of West Bengal. Test of casein and gelatin hydrolysis were negative for all 9 isolates (Az-3, Az-5, Az-8, Az-11, Az-12, Az-14, Az-16, Az-19 and Az-20), test of starch hydrolysis were negative for Az-3, Az-11 and Az-16, which support these finding. *Azotobacter spp.* is free-living aerobic bacteria dominantly found in soils.

Shaikh and Shakir (2018) studied characteristics of *Azotobacter vinelandii* in soil of various fields and Orchards. 39 isolates showed test of catalase, oxidase and H₂S production were positive.

Naga *et al.* (2018) studied biochemical characterization of six *Rhizobium* strains isolated from French bean nodules collected from different regions of Andhra Pradesh, India. All the isolates of rhizobia were positive to the urease, catalase and oxidase test. Starch hydrolysis and gelatin hydrolysis test were positive for all isolates except Rh01 and Rh3 isolates.

Hala and Ali (2019) reported that voges prosquire test was positive and urease test was negative for isolate which was belonging to *Azotobacter spp.*

Eneyi *et al.* (2020) investigated resistance pattern of the isolates at different concentration (1-3 %) of cypermethrin showed that all the isolates could grow in the presence of cypermethrin at 1 % except *Bacillus spp.* However at 2 %, only *Klebsiella spp.* grew; *Staphylococcus spp.*, *Escherichia coli* and *Bacillus spp.* were inhibited and therefore showed no growth. These findings revealed that cypermethrin had effects on the soil bacteria at higher concentration inhibiting their growth but tolerated at lower concentration.

Muthukumar *et al.* (2021) observed test of casein hydrolysis was negative and test of urease hydrolysis was positive for *Azospirillum spp.*

3. MATERIAL AND METHODS

The present investigation entitled, “Sensory microbial Technique to detect pesticide residues and heavy metals in marketable Fruits and Vegetables” was carried out at the Department of Plant Pathology and Agricultural Microbiology, Mahatma Phule Krishi Vidyapeeth, Rahuri-413 722. The aim of the study was to isolate and identify microorganisms sensitive to pesticide residues and heavy metals in marketable fruits and vegetables. The detailed information on the material used and experimental techniques adopted during the study period are presented in this chapter.

3.1 Material

3.1.2 Collection of soil samples

Soil samples were collected from railway station area of western line of Mumbai viz., Kandivali, Malad, Vileparle, Ram mandir, Goregaon, and Andheri. Soil samples were collected in polythene bags and labeled properly.

3.1.2 Collection of water samples

Water Samples were collected from nearby railway stations of Mumbai. Water samples were taken with a 250 ml polyethylene (PE) bottle either directly from the source (open sewage tunnel) or from the field.

3.1.3 Media used

Media used for isolation was Nutrient agar medium (Anon, 1957). For the preparation of nutrient agar media beef extract 3gm, peptone 5gm and Agar 20gm were added in one litre water and Ph adjusted to 6.5, further media autoclaved for sterilization at 121⁰C under pressure 1.54 kg/cm² for 15 minutes.

3.1.4 Source of chemical used

For these experiment 10 pesticides and 7 heavy metals were used which obtained from Department of Agricultural Entomology and Department of Plant pathology and Agricultural Microbiology M.P.K.V., Rahuri-413 722, which are given in the Table 1.

Table 1. Chemicals used for isolation of microorganisms

Sr. No.	Pesticide	Sr. No.	Heavy metal
1.	Cymbush 25% EC	1.	Copper Sulphate
2.	San chloro Xtra Tc	2.	Lead Nitrate
3.	TaTa Asataf 75% SP	3.	Cadmium Chloride
4.	Spintor 45%SC	4.	Chromium Trioxide
5.	Captaf 50% WP	5.	Nickel Sulphate
6.	Bavistin 50% WP	6.	Arsenic Trioxide
7.	Raxyl 35% WS	7.	Mercury Chloride
8.	Indofil M-45		
9.	Kavach		
10.	Weedoff 58 SL		

Table 2. Permissible limits for Heavy metals (mg/kg)

Indian Standard Awasthi (2000)	Cu	Pb	Cd	Cr	Ni	As	Hg
	30.0	2.5	1.5	20	1.5	1.1	NL

NL- No limit

Table 3. Maximum Residue limits of Pesticide in Fruits and Vegetables

Sr. No.	Pesticide	European Union Standard (2016) in mg/Kg
1.	Cypermethrin	0.05
2.	Chloropyriphos	0.05
3.	Acephate	0.01
4.	Spinosad	0.07
5.	Captan	0.02
6.	Carbendazim	0.1
7.	Metalaxyl	0.05
8.	Mancozeb	0.05
9.	Chlorothalnil	0.01
10.	2-4-D	0.05

3.1.5 Glasswares

The common glasswares used in the experimental work were petriplates, test tubes, conical flasks, measuring cylinder, glass rods, spirit jar, slides, beakers and pipette *etc.*

3.1.6 Equipments and instruments

The common laboratory equipments and instruments used were autoclave, incubator, laminar air flow cabinet, refrigerator, pH meter, inoculating needle,

micrometry, electronic weighing balance, research microscope, mixture grinder and spectrophotometer, *etc.*

3.1.7 Miscellaneous materials

The miscellaneous materials *viz.*, marking pencils, sticky labels, muslin cloth, blotting paper, spirit lamp, scalpel, polythene bags, sickle, scissor, sterilized water, micro-pipette and non-absorbent cotton *etc.* were used.

3.1.8 Experimental site

The present experiments were carried out in well facilitated laboratory of Department of Plant Pathology and Agricultural Microbiology, PGI, MPKV, Rahuri-413 722.

3.2 Methods

3.2.1. Isolation of Bacteria from soil and Water samples

Isolation of Bacteria were carried out by serial dilution, pour plate and food poison technique (Nene and Thapliyal, 1979)

A Ten gram soil sample from each area from western line of Mumbai was suspended in 90 ml of sterilized water blank. Serial dilutions were made from 10^{-1} to 10^{-7} . One ml aliquot of dilutions from 10^{-3} to 10^{-7} was transferred to sterilize petri plates separately. The sterilized nutrient agar medium of 500 ml of each pesticides as per EU standard concentration were prepared and poured in Petri plate just before solidification (40°C temperature) under aseptic condition and mixed the contents in plates by rotating the petri plate clock and anti-clockwise. After solidification, the plates were kept at $28 \pm 2^{\circ}\text{C}$ in bacteriological incubator for 4-5 days. Duplicate plates were made for comparison as control. Control plates contain only soil and water sample. All the plates were observed for the appearance of bacterial colonies on medium. Same procedure is followed for isolation of bacteria from heavy metals.

One ml of water sample was used for isolating the microorganisms through serial dilution and agar plate culture technique. The collected water sample was taken and it is serially diluted with distilled water. The serial dilution was done up to 10^{-9} the sterilized nutrient agar medium of 500 ml of each heavy metal as per Indian standard (Awasthi 2000) concentration were prepared and poured in petri plate just before solidification (40°C temperature) under aseptic condition. 0.5 ml of sample was collected

from 10^{-3} to 10^{-9} and was spread on nutrient agar plates and mixed the contents in plates by rotating the petri plate clock and anti-clockwise. The plates were incubated at 37°C to achieve vigorous growth. Duplicate plates were made for comparison as control.

3.2.2 Screening of isolates at low concentration of individual pesticides and heavy metals

After 4-5 days of incubation, plates were observed. Those colonies were not seen in the treated plates containing soil and water samples and low concentration of pesticides and heavy metals, but colonies seen in the control petri plates containing soil and water samples with no pesticides and heavy metals were selected. These colonies were further screened and purified.

3.2.3 Testing of isolates for their sensitivity to respective pesticides and heavy metals

Isolates which were screened for pesticides and heavy metals were tested for their sensitivity against respective pesticides and heavy metals at low concentration by streaking plate technique. The sterilized nutrient agar medium of 500 ml of each pesticides of each concentration as per given in a treatment were prepared and poured in sterilized petri plate just before solidification (40°C temperature) under aseptic condition. After solidification, the plates were streaked using isolates which were screened for particular pesticides at EU standard concentration. The sterilized nutrient agar medium of 500 ml of each heavy metal of each concentration as per given in a treatment were prepared and poured in sterilized petri plate just before solidification (40°C temperature) under aseptic condition. After solidification, the plates were streaked using isolates which were screened for particular heavy metals at Indian standard concentration. Then plates were kept at $28\pm 2^{\circ}\text{C}$ in Bacteriological incubator for 4-5 days.

3.2.4 Characterization of bacterial isolates

The five isolates CAR-8, CTG-10, DIM-5, NIV-7 and COM-2 showed sensitivity at 2.5 ppm of carbendazim, 1ppm of captan, 2.5ppm of 2-4-D, 0.04ppm of nickel and 0.01ppm of copper respectively, were subjected to morphological, cultural, and physiological and biochemical characterization (Anon. *et al.*, 1994).

3.2.4.1 Morphological characterization

The all five isolates were examined for cell shape, size, arrangement, motility test and gram reaction as per the standard procedures. Overnight grown culture of all the selected isolates were streaked onto nutrient agar and observed for colony morphology *viz.*, growth, form, margin and elevation of colonies after 48 hrs incubation at $28\pm 2^{\circ}\text{C}$. The colony morphology and Gram reaction were recorded per the standard procedures given by Barthalomew and Mittewer (1950) and Anon. (1957).

3.2.4.2 Physiological characterization (Seeley *et al.*, 1991)

i. Growth at 7 per cent NaCl

The tubes of nutrient broth 3 ml/tube containing seven per cent sodium chloride were inoculated with a loop full culture of the test isolates grown overnight in nutrient broth. The tubes were incubated at $28\pm 2^{\circ}\text{C}$ and the growth was observed after 24 hrs in terms of turbidity which was taken as positive for the test.

ii. Growth at 4°C , 41°C and 50°C

Twenty four hrs old cultures of the test organisms in nutrient broth were spotted on the trypticase soy agar plates and incubated at 4°C , 41°C and 50°C for 48 hrs and observation on growth was recorded at the end of the incubation period.

Trypticase-Soy agar media (Seeley *et al.*, 1991)

Trypticase	17 g
Phytone	3 g
NaCl	5 g
K_2HPO_4	2.5 g
Glucose	2.5 g
Agar	15 g
Distilled water	1000 ml

pH adjust to 7.3

Procedure : Agar (15 g) was melted in 500 ml distilled water and rests of the ingredients were thoroughly dissolved in 500 ml distilled water. Both the preparations were mixed and the final volume was made up to 1000 ml adding distilled water and then autoclaved.

iii. Aerobic/anaerobic test

Test tubes of yeast-extract-tryptone agar were melted and held at 100°C for 10 minutes to expel dissolved oxygen, cooled to a temperature of 42-45°C and inoculated heavily with test bacterial cultures. Tubes were solidified by placing the tubes in cool water (below 40°C) and incubated at 37°C for 2 days and observed the location and appearance of growth *viz.*, surface growth (Aerobic), growth only in depth of agar (Anaerobic) and growth throughout agar shake (Facultative).

Yeast extract tryptone medium (Seeley *et al.*, 1991)

Tryptone	10 g
Yeast extract	5 g
K ₂ HPO ₄	5 g
Glucose	1 g
Agar	15 g
Distilled water	1000 ml

Procedure : Agar (15 g) was melted in 500 ml distilled water and rests of the ingredients were thoroughly dissolved in 500 ml distilled water. Both the preparations were mixed and the final volume was made up to 1000 ml adding distilled water and then autoclaved.

3.2.4.3 Biochemical characterization

The selected sensitive five isolates were subjected to biochemical characterization employing the standard procedures given by Seeley *et al.* (1991) and Cappuccino and Sherman (1987). Different biochemical tests were performed and protocols used are briefly outlined below.

i. Catalase test

Nutrient agar slants were inoculated with the overnight grown test bacterial isolates and were incubated at 30°C for 24 hrs. After incubation, the slants were flooded with one ml of three per cent hydrogen peroxide and observed for the formation of gas bubbles. The occurrence of gas bubbles was scored positive for catalase production.

ii. Oxidase test

Overnight grown cultures of the test isolates were spotted to the trypticase soy agar plates and the plates were incubated for 24 hrs at $28\pm 2^{\circ}\text{C}$. After incubation, two to three drops of tetramethyl-phenylenediamine dihydrochloride (TPD) was added on the growth of the test isolate. The colour change to blue was taken as oxidase positive.

iii. Gelatin liquefaction

In the pre sterilized nutrient gelatin deep tubes, the test cultures were inoculated and tubes were incubated at $28\pm 2^{\circ}\text{C}$ for 24 hrs. Following this, the tubes were kept in a refrigerator at 4°C for 30 minutes. The tubes with cultures that remained liquefied were taken as positive and those that solidified on refrigeration were taken as negative for the test.

Nutrient gelatin broth (Cappuccino and Sherman, 1987)

Peptone	5.00 g
Beef extract	3.00 g
Gelatin	120 g
Distilled water	1000 ml
Adjust pH to 6.8-7.0	

iv. Starch hydrolysis

The ability of the isolates to hydrolyse starch was examined. Triplicate plates of starch agar were inoculated with the test culture and incubated at 30°C for three days. After incubation, the plates were flooded with Lugol's iodine solution and allowed to stand for 15 to 30 minutes and observed for clear zone around the colony to indicate hydrolysis of starch.

Starch agar media (Seeley *et al.*, 1991)

Tryptone	10 g
Yeast Extract	10 g
K_2HPO_4	5 g
Soluble starch	3 g
Agar	15 g
Distilled water	1000 ml

Procedure : Agar (15 g) was melted in 500 ml distilled water and rest of the ingredients were thoroughly dissolved in 500 ml distilled water. Both the preparations were mixed and the final volume was made up to 1000 ml adding distilled water and then autoclaved.

v. Casein hydrolysis

Triplicate plates of starch casein agar inoculated with the test bacteria were incubated at 37⁰C for two days and then observed for clear zone around the colony against a black background which indicates hydrolysis of casein.

Starch Casein Agar medium

Starch	10 g
Casein	0.3 g
KNO ₃	2.0 g
NaCl	2.0 g
K ₂ HPO ₄	2.0 g
MgSO ₄ 7H ₂ O	0.05 g
CaCO ₃	0.02 g
FeSO ₄ 7H ₂ O	0.01 g
Agar	18.0 g
Distilled Water	1000 ml

Procedure : Suspended 10 g starch, 0.3g casein, 2.0 g KNO₃ , 2.0g NaCl , 2.0g K₂HPO₄, 0.05g MgSO₄ 7H₂O, CaCO₃ 0.02g , FeSO₄ 7H₂O 0.01 g and 18 g agar-agar in one liter of distilled water. Mixed well and heated with frequent agitation up to boiling point for melting of agar. Sterilized in an autoclave at 121⁰C (15 lb psi) for 20 minutes.

vi. Methyl Red – Voges Proskauer (MR-VP)

The overnight cultures were inoculated to test tubes containing 5 ml MR-VP medium and incubated for 48 hrs at 37⁰C. Then, after about one- quarter of the incubated cultures were decanted into a clean test tube added with 0.5 ml (8-10 drops) of the alpha naphthol solution (5% solution in alcohol). Decanted cultures were subsequently added to 0.5 ml of 40% KOH solution containing 0.3% creatine, shaken thoroughly and allowed to stand for 5-30 min. A pink to red colour indicate the presence of acetyl methyl carbinol and reported as positive VP test

MR-VP broth.

Peptone	7.0 g
Glucose / Dextrose	5.0 g
Potassium phosphate	5.0 g
Agar	1.8 g
Distilled water	1000 ml
pH 6.9	

Procedure : Agar (1.8 g) was melted in 500 ml distilled water and rests of the ingredients were thoroughly dissolved in 500 ml distilled water. Both the preparations were mixed and adjust the pH of medium 6.9 and the final volume was made up to 1000 ml adding distilled water and then autoclaved.

vii. Hydrogen sulfide production

The bacterial isolates were stabbed into tubes containing semisolid SIM agar .The tubes were incubated for 48 hrs at 50⁰C. After incubation, the development of black colour along the line of the stab was noted as positive for H₂S production. An uninoculated semisolid SIM agar tube served as control.

SIM agar (Cappuccino and Sherman, 1987)

Peptone	30.00 g
Beef extract	3.00 g
Ferrous ammonium Sulphate	0.20 g
Sodium thiosulphate	0.025 g
Distilled water	1000 ml
Agar	3.00 g

Adjust pH to 7.3 before adding agar

Procedure: Suspended 30 g peptone, 3g beef extract, 0.20 g ferrous ammonium sulphate, sodium thiosulphate 0.025g and 3 g agar-agar in one liter of distilled water. Mixed well and heated with frequent agitation up to boiling point for milting of agar. Sterilized in an autoclave at 121⁰C (15 lb psi) for 20 minutes.

viii. Urease activity

The overnight cultures were inoculated to test tubes containing sterilized urea broth and incubated at $50 \pm 2^{\circ}\text{C}$ for 48 hrs. The pink colour discoloration was taken as positive for the test and colour if remained unchanged was reported as a negative test. An uninoculated pre sterilized urea broth served as control.

Urea Broth (Seeley *et al.*, 1991)

Urea	10 g
Peptone	1 g
NaCl	5 g
Glucose	1 g
K ₂ HPO ₄	2 g
Phenol red	0.012 g
Distilled water	1000 ml
Adjust pH to 6.8-6.9	

3.3 Experiment details

3.3.1 Treatment

- | | |
|------------------------------|---------------------------|
| 1. Treatment + soil samples | 2. Control= Soil samples |
| 3. Treatment + Water samples | 4. Control= Water Samples |

Sr. No.	Pesticides	Treatment In (ppm)		
1.	Cypermethrin	0.05	1.25	2.5
2.	Chloropyriphos	0.05	1.25	2.5
3.	Acephate	0.01	0.25	0.5
4.	Spinosad	0.07	1.75	3.5
5.	Captan	0.02	0.5	1.0
6.	Carbendazim	0.1	2.5	5.0
7.	Metalaxyl	0.05	1.25	2.5
8.	Mancozeb	0.05	1.25	2.5
9.	Chlorothalonil	0.01	0.25	0.5
10.	2-4-D	0.05	1.25	2.5

Sr. No.	Heavy metals	Treatment In (ppm)	
1.	Copper (Cu)	30.0	0.01
2.	Lead (Pb)	2.5	0.5
3.	Cadmium (Cd)	1.5	0.07
4.	Chromium(Cr)	2.0	1.00
5.	Nickel(Ni)	1.5	0.04
6.	Arsenic(As)	1.1	0.05
7.	Mercury(Hg)	-	-

4. RESULT AND DISCUSSION

The results of the investigation conducted during 2015-2018 to detect pesticide residues and heavy metals in marketable fruits and vegetables by sensory technology at the Department of Plant Pathology and Agril. Microbiology, M.P.K.V., Rahuri-413 722, are presented in this chapter. The study covered the isolation of microorganisms sensitive to low concentration of pesticide residue and heavy metals from soil and water samples collected from western railway line of Mumbai and screening and testing of these microbes against the low concentration of individual pesticides and heavy metals.

4.1 Isolation of Bacteria from soil and water samples

A total 30 soil samples were collected from railway station area of western line of Mumbai viz., Kandivali, Malad, Vileparle, Ram mandir, Goregaon, Andheri. Twenty water samples were collected from nearby railway stations of Mumbai for isolation of microorganisms at low concentration of pesticide residues and heavy metals. The total number of isolates isolated for each individual pesticide and heavy metal are mentioned in the Table 4 and 5.

Table 4. Isolation of Bacteria from soil and water at low concentration of pesticides

Sr. No.	Pesticide	Concentration(ppm) As per EU std (2016)	Isolates
1.	Cypermethrin	0.05	21
		1.25	21
		2.5	21
2.	Chloropyriphos	0.05	16
		1.25	16
		2.5	16
3.	Acephate	0.01	11
		0.25	11
		0.5	11
4.	Spinosad	0.07	16
		1.75	16
		3.5	16
5.	Captan	0.02	22
		0.5	22
		1.0	22
6.	Carbendazim	0.1	16
		2.5	16
		5.0	16

Table 4 contd....

Sr. No.	Pesticide	Concentration(ppm) As per EU std (2016)	Isolates
7.	Metalaxyl	0.05	12
		1.25	12
		2.5	12
8.	Mancozeb	0.05	9
		1.25	9
		2.5	9
9.	Chlorothalonil	0.01	14
		0.25	14
		0.5	14
10.	2 -4-D	0.05	20
		1.25	20
		2.5	20

Table 5. Isolation of Bacteria from soil and water at low concentration of heavy metals

Sr. No.	Heavy metal	Concentration(ppm) As per Indian std (Awasthi 2000)	Isolates
1.	Copper(Cu)	30.00	30
		0.01	30
2.	Lead(Pb)	2.5	14
		0.5	14
3.	Cadmium(Cd)	1.5	10
		0.07	10
4.	Chromium(Cr)	20.0	20
		1.0	20
5.	Nickel(Ni)	1.5	10
		0.04	10
6.	Arsenic(As)	1.1	12
		0.05	12
7.	Mercury(Hg)	-	-
		-	-

4.2 Testing of isolates for their sensitivity for respective pesticides

4.2.1 Cypermethrin

The result of experiment at 0.05, 1.25 and 2.5 ppm (As per EU std 2016) concentration of cypermethrin 21 isolates were screened and tested, out of them no any isolate show sensitivity to cypermethrin at 0.05, 1.25 and 2.5 ppm by poison food technique Table 6, Table 7 and Table 8 respectively.

Table 6 Sensitivity of Bacteria at 0.05 ppm concentration of Cypermethrin

Sr. No.	Isolates	Sensitivity
1.	CYA-1(Cypermethrin Andheri-1)	-ve
2.	CYV-2(Cypermethrin Vileparle-2)	-ve
3.	CYM-3(Cypermethrin Malad-3)	-ve
4.	CYK-4(Cypermethrin Kandivali-4)	-ve
5.	CYG-5(Cypermethrin Goregaon-5)	-ve
6.	CYK-6(Cypermethrin Kandivali-6)	-ve
7.	CYR-7(Cypermethrin Rammandir-7)	-ve
8.	CYV-8(Cypermethrin Vileparle-8)	-ve
9.	CYA-9(Cypermethrin Andheri-9)	-ve
10.	CYG-10(Cypermethrin Goregaon-10)	-ve
11.	CYR-11(Cypermethrin Rammandir-11)	-ve
12.	CYM-12(Cypermethrin Malad-12)	-ve
13.	CYG-13(Cypermethrin Goregaon-13)	-ve
14.	CYR-14(Cypermethrin Rammandir-14)	-ve
15.	CYV-15(Cypermethrin Vileparle -15)	-ve
16.	CYG-16(Cypermethrin Goregaon-16)	-ve
17.	CYR-17(Cypermethrin Rammandir-17)	-ve
18.	CYA-18(Cypermethrin Andheri-18)	-ve
19.	CYM-19(Cypermethrin Malad-19)	-ve
20.	CYG-20 (Cypermethrin Goregaon-20)	-ve
21.	CYV-21(Cypermethrin Vileparle-21)	-ve

+ : Bacterial growth absent

- : Bacterial growth present

Table 7. Sensitivity of Bacteria at 1.25 ppm concentration of Cypermethrin

Sr. No.	Isolates	Sensitivity
1.	CYA-1(Cypermethrin Andheri-1)	-ve
2.	CYV-2(Cypermethrin Vileparle-2)	-ve
3.	CYM-3(Cypermethrin Malad-3)	-ve
4.	CYK-4(Cypermethrin Kandivali-4)	-ve
5.	CYG-5(Cypermethrin Goregaon-5)	-ve
6.	CYK-6(Cypermethrin Kandivali-6)	-ve
7.	CYR-7(Cypermethrin Rammandir-7)	-ve
8.	CYV-8(Cypermethrin Vileparle-8)	-ve
9.	CYA-9(Cypermethrin Andheri-9)	-ve
10.	CYG-10(Cypermethrin Goregaon-10)	-ve
11.	CYR-11(Cypermethrin Rammandir-11)	-ve
12.	CYM-12(Cypermethrin Malad-12)	-ve
13.	CYG-13(Cypermethrin Goregaon-13)	-ve
14.	CYR-14(Cypermethrin Rammandir-14)	-ve
15.	CYV-15(Cypermethrin Vileparle -15)	-ve
16.	CYG-16(Cypermethrin Goregaon-16)	-ve
17.	CYR-17(Cypermethrin Rammandir-17)	-ve
18.	CYA-18(Cypermethrin Andheri-18)	-ve
19.	CYM-19(Cypermethrin Malad-19)	-ve
20.	CYG-20 (Cypermethrin Goregaon-20)	-ve
21.	CYV-21(Cypermethrin Vileparle-21)	-ve

+: Bacterial growth absent

- : Bacterial growth present

Table 8. Sensitivity of Bacteria at 2.5 ppm concentration of Cypermethrin

Sr. No.	Isolates	Sensitivity
1.	CYA-1(Cypermethrin Andheri-1)	-ve
2.	CYV-2(Cypermethrin Vileparle-2)	-ve
3.	CYM-3(Cypermethrin Malad-3)	-ve
4.	CYK-4(Cypermethrin Kandivali-4)	-ve
5.	CYG-5(Cypermethrin Goregaon-5)	-ve
6.	CYK-6(Cypermethrin Kandivali-6)	-ve
7.	CYR-7(Cypermethrin Rammandir-7)	-ve
8.	CYV-8(Cypermethrin Vileparle-8)	-ve
9.	CYA-9(Cypermethrin Andheri-9)	-ve
10.	CYG-10(Cypermethrin Goregaon-10)	-ve
11.	CYR-11(Cypermethrin Rammandir-11)	-ve
12.	CYM-12(Cypermethrin Malad-12)	-ve
13.	CYG-13(Cypermethrin Goregaon-13)	-ve
14.	CYR-14(Cypermethrin Rammandir-14)	-ve
15.	CYV-15(Cypermethrin Vileparle -15)	-ve
16.	CYG-16(Cypermethrin Goregaon-16)	-ve
17.	CYR-17(Cypermethrin Rammandir-17)	-ve
18.	CYA-18(Cypermethrin Andheri-18)	-ve
19.	CYM-19(Cypermethrin Malad-19)	-ve
20.	CYG-20 (Cypermethrin Goregaon-20)	-ve
21.	CYV-21(Cypermethrin Vileparle-21)	-ve

+ : Bacterial growth absent

- : Bacterial growth present

The isolates which are screened at concentration of 0.05, 1.25, 2.5 ppm cypermethrin were grown on nutrient agar plates to test the sensitivity, Full growth of all isolates were observed on nutrient agar medium plates after 2-3 days of incubation. Growth of all isolates at 0.05 ppm concentration of cypermethrin showed in Plate 2.

The same results were shown Eneyi *et al.* (2020) who investigated resistance pattern of the isolates at different concentration (1-3%) of cypermethrin

showed that all the isolates could grow in the presence of cypermethrin at 1% except *Bacillus spp.* However at 2%, only *Klebsiella spp.* grew; *Staphylococcus spp.*, *Escherichia coli* and *Bacillus spp.* were inhibited and therefore showed no growth. These findings revealed that cypermethrin had effects on the soil bacteria at higher concentration inhibiting their growth but tolerated at lower concentration.

4.2.2 Chloropyriphos

In the result of experiment at 0.05, 1.25, 2.5 ppm (As per EU std 2016) concentrations of chloropyriphos 16 isolates were screened, out of them no any isolate showed sensitivity to chloropyriphos at 0.05, 1.25, 2.5 ppm by poison food technique Table 9, Table 10 and Table 11. Growth of 16 isolates at 0.05ppm concentration showed in Plate 3.

Table 9 Sensitivity of Bacteria at 0.05 ppm concentration of Chloropyriphos

Sr. No.	Isolates	Sensitivity
1.	CHA-1(Chloropyriphos Andheri-1)	-ve
2.	CHV-2(Chloropyriphos Vileparle-2)	-ve
3.	CHM-3(Chloropyriphos Malad-3)	-ve
4.	CHM-4(Chloropyriphos Malad-4)	-ve
5.	CHK-5(Chloropyriphos Kandivali-5)	-ve
6.	CHK-6(Chloropyriphos Kandivali-6)	-ve
7.	CHA-7(Chloropyriphos Andheri-7)	-ve
8.	CHG-8 (Chloropyriphos Goregaon-8)	-ve
9.	CHA-9(Chloropyriphos Andheri-9)	-ve
10.	CHR-10 (Chloropyriphos Rammandir-10)	-ve
11.	CHK-11 (Chloropyriphos Kandivali-11)	-ve
12.	CHV-12(Chloropyriphos Vileparle-12)	-ve
13.	CHM-13(Chloropyriphos Malad-13)	-ve
14.	CHK-14(Chloropyriphos Kandivali-14)	-ve
15.	CHM-15(Chloropyriphos Malad-15)	-ve
16.	CHA-16(Chloropyriphos Andheri-16)	-ve

+: Bacterial growth absent

- : Bacterial growth present

Table 10 Sensitivity of Bacteria at 1.25 ppm concentration of Chloropyriphos

Sr. No.	Isolates	Sensitivity
1.	CHA-1(Chloropyriphos Andheri-1)	-ve
2.	CHV-2(Chloropyriphos Vileparle-2)	-ve
3.	CHM-3(Chloropyriphos Malad-3)	-ve
4.	CHM-4(Chloropyriphos Malad-4)	-ve
5.	CHK-5(Chloropyriphos Kandivali-5)	-ve
6.	CHK-6(Chloropyriphos Kandivali-6)	-ve
7.	CHA-7(Chloropyriphos Andheri-7)	-ve
8.	CHG-8 (Chloropyriphos Goregaon-8)	-ve
9.	CHA-9(Chloropyriphos Andheri-9)	-ve
10.	CHR-10 (Chloropyriphos Rammandir-10)	-ve
11.	CHK-11 (Chloropyriphos Kandivali-11)	-ve
12.	CHV-12(Chloropyriphos Vileparle-12)	-ve
13.	CHM-13(Chloropyriphos Malad-13)	-ve
14.	CHK-14(Chloropyriphos Kandivali-14)	-ve
15.	CHM-15(Chloropyriphos Malad-15)	-ve
16.	CHA-16(Chloropyriphos Andheri-16)	-ve

+: Bacterial growth absent

- : Bacterial growth present

Table 11 Sensitivity of Bacteria at 2.5 ppm concentration of Chloropyriphos

Sr. No.	Isolates	Sensitivity
1.	CHA-1(Chloropyriphos Andheri-1)	-ve
2.	CHV-2(Chloropyriphos Vileparle-2)	-ve
3.	CHM-3(Chloropyriphos Malad-3)	-ve
4.	CHM-4(Chloropyriphos Malad-4)	-ve
5.	CHK-5(Chloropyriphos Kandivali-5)	-ve
6.	CHK-6(Chloropyriphos Kandivali-6)	-ve
7.	CHA-7(Chloropyriphos Andheri-7)	-ve
8.	CHG-8 (Chloropyriphos Goregaon-8)	-ve
9.	CHA-9(Chloropyriphos Andheri-9)	-ve
10.	CHR-10 (Chloropyriphos Rammandir-10)	-ve
11.	CHK-11 (Chloropyriphos Kandivali-11)	-ve
12.	CHV-12(Chloropyriphos Vileparle-12)	-ve
13.	CHM-13(Chloropyriphos Malad-13)	-ve
14.	CHK-14(Chloropyriphos Kandivali-14)	-ve
15.	CHM-15(Chloropyriphos Malad-15)	-ve
16.	CHA-16(Chloropyriphos Andheri-16)	-ve

+: Bacterial growth absent

- : Bacterial growth present

Supreeth *et al.* (2016) reported effect of chlorpyrifos on soil microbial diversity, the soils treated with 100 and 200 ug/ g the CP insecticide showed decrease in the number of colony forming units (CFU) of bacteria and fungi. McDonald *et al.* (2021) studied inhibition of select actinobacteria by the organophosphate pesticide chlorpyrifos. CPF (chlorpyrifos) dramatically inhibited the growth of most strains and/or altered colony morphologies, with 13 strains completely inhibited by CPF at 3.5ppm concentration.

2.3 Carbendazim

In the result of experiment at 0.1, 2.5 and 5ppm (As per EU std 2016) concentrations of carbendazim 16 isolates were screened, out of them one isolate showed sensitivity to carbendazim at 2.5 ppm by poison food technique and at 0.1ppm and 5ppm all the 16 isolates showed the full growth on nutrient agar plate. Sensitivity of isolates to carbendazim at 2.5 ppm is presented in Table 13 and Plate 4.

An Isolate CAR-8 showed the sensitivity at 2.5 ppm concentration of carbendazim. Fawole *et al.* (2009) reported that when carbendazim–mancozeb fungicidal mixture applied at concentration of 2.34mg.kg⁻¹ soil had a greater ($p < 0.05$) inhibitory effect on nitrogen fixers, nitrifying bacteria and cellulolytic organisms. Pappas A. C., *etal.* (2010) observed that few isolates of *Septoria pyricola*. single spore isolate failed to form colonies with carbendazim concentration 0.1 to 10 mg.L⁻¹.

Sharma *et al* (2022) investigated impact of carbendazim on cellular growth, defence system and plant growth promoting traits of *Priestia megaterium* ANCB-12 isolated from sugarcane rhizosphere. Results showed that the increasing dose of carbendazim gradually decreased the plant growth promoting activities of the rhizobacteria ANCB12. At a maximum 3,000 µg/ml (carbendazim: CBZM) concentration, the activity of superoxide dismutase (SOD) declined by 82.3%, catalase (CAT) by 61.4%, glutathione peroxidase (GPX) by 76.1%, and glutathione reductase (GR) by 84.8%, respectively.

Table 12. Sensitivity of Bacteria at 0.1ppm concentration of Carbendazim

Sr. No.	Isolates	Sensitivity
1.	CAV-1(Carbendazim Vileparle-1)	-ve
2.	CAA-2(Carbendazim Andheri-2)	-ve
3.	CAM-3(Carbendazim Malad-3)	-ve
4.	CAA-4(Carbendazim Andheri-4)	-ve
5.	CAK-5(Carbendazim Kandivali-5)	-ve
6.	CAG-6(Carbendazim Goregaon-6)	-ve
7.	CAR-7(Carbendazim Rammandir-7)	-ve
8.	CAR-8(Carbendazim Rammandir-8)	-ve
9.	CAG-9(Carbendazim Goregaon-9)	-ve
10.	CAV-10(Carbendazim Vileparle-10)	-ve
11.	CAM-11(Carbendazim Malad-11)	-ve
12.	CAA-12(Carbendazim Andheri-12)	-ve
13.	CAA-13(Carbendazim Andheri-13)	-ve
14.	CAR-14(Carbendazim Rammandir-14)	-ve
15.	CAM-15(Carbendazim Malad-15)	-ve
16.	CAV-16((Carbendazim Vileparle-16)	-ve

+ : Bacterial growth absent

- : Bacterial growth present

Table 13. Sensitivity of Bacteria at 2.5 ppm concentration of Carbendazim

Sr. No.	Isolates	Sensitivity
1.	CAV-1(Carbendazim Vileparle-1)	-ve
2.	CAA-2(Carbendazim Andheri-2)	-ve
3.	CAM-3(Carbendazim Malad-3)	-ve
4.	CAA-4(Carbendazim Andheri-4)	-ve
5.	CAK-5(Carbendazim Kandivali-5)	-ve
6.	CAG-6(Carbendazim Goregaon-6)	-ve
7.	CAR-7(Carbendazim Rammandir-7)	-ve
8.	CAR-8(Carbendazim Rammandir-8)	+ve
9.	CAG-9(Carbendazim Goregaon-9)	-ve
10.	CAV-10(Carbendazim Vileparle-10)	-ve
11.	CAM-11(Carbendazim Malad-11)	-ve
12.	CAA-12(Carbendazim Andheri-12)	-ve
13.	CAA-13(Carbendazim Andheri-13)	-ve
14.	CAR-14(Carbendazim Rammandir-14)	-ve
15.	CAM-15(Carbendazim Malad-15)	-ve
16.	CAV-16((Carbendazim Vileparle-16)	-ve

+: Bacterial growth absent

- : Bacterial growth present

Table 14 Sensitivity of Bacteria at 5 ppm concentration of Carbendazim

Sr. No.	Isolates	Sensitivity
1.	CAV-1(Carbendazim Vileparle-1)	-ve
2.	CAA-2(Carbendazim Andheri-2)	-ve
3.	CAM-3(Carbendazim Malad-3)	-ve
4.	CAA-4(Carbendazim Andheri-4)	-ve
5.	CAK-5(Carbendazim Kandivali-5)	-ve
6.	CAG-6(Carbendazim Goregaon-6)	-ve
7.	CAR-7(Carbendazim Rammandir-7)	-ve
8.	CAR-8(Carbendazim Rammandir-8)	-ve
9.	CAG-9(Carbendazim Goregaon-9)	-ve
10.	CAV-10(Carbendazim Vileparle-10)	-ve
11.	CAM-11(Carbendazim Malad-11)	-ve
12.	CAA-12(Carbendazim Andheri-12)	-ve
13.	CAA-13(Carbendazim Andheri-13)	-ve
14.	CAR-14(Carbendazim Rammandir-14)	-ve
15.	CAM-15(Carbendazim Malad-15)	-ve
16.	CAV-16((Carbendazim Vileparle-16)	-ve

+: Bacterial growth absent

- : Bacterial growth present

4.2.4 Acephate

The result of experiment at 0.01, 0.25 and 0.5ppm (As per EU std 2016) concentration of acephate 11 isolates were screened, out of them none of the isolates showed sensitivity to acephate at 0.01,0.25 and 0.5ppm by food poison technique Table 15,Table 16 and Table 17 respectively.

It was observed that all the isolates showed full growth when streaked on nutrient agar plates at 0.01 ppm concentration of Acephate after 2-3 days of incubation Plate 5.

Focht and Joseph (1974) studied microbial activity in soils treated with acephate and monitor. With three repeated applications 20 ppm of the organophosphate insecticides, acephate (O, S-dimethyl acetylphosphoramidothioate) and monitor (O, S-dimethyl phosphoramidothioate) over a 50-day time span. Population levels of actinomycetes, bacteria, and fungi were not substantially affected by the addition of either pesticide.

Table 15. Sensitivity of Bacteria at 0.01ppm concentration of Acephate

Sr. No.	Isolates	Sensitivity
1.	ACK-1(Acephate Kandivali-1)	-ve
2.	ACA-2(Acephate Andheri-2)	-ve
3.	ACV-3(Acephate Vileparle-3)	-ve
4.	ACG-4(Acephate Goregaon-4)	-ve
5.	ACR-5(Acephate Rammandir-5)	-ve
6.	ACK-6(Acephate Kandivali-6)	-ve
7.	ACK-7(Acephate Kandivali-7)	-ve
8.	ACM-8(Acephate Malad-8)	-ve
9.	ACM-9(Acephate Malad-9)	-ve
10.	ACA-10(Acephate Andheri-10)	-ve
11.	ACR-11(Acephate Rammandir-11)	-ve

+ : Bacterial growth absent

- : Bacterial growth present

Table 16. Sensitivity of Bacteria at 0.25 ppm concentration of Acephate

Sr. No.	Isolates	Sensitivity
1.	ACK-1(Acephate Kandivali-1)	-ve
2.	ACA-2(Acephate Andheri-2)	-ve
3.	ACV-3(Acephate Vileparle-3)	-ve
4.	ACG-4(Acephate Goregaon-4)	-ve
5.	ACR-5(Acephate Rammandir-5)	-ve
6.	ACK-6(Acephate Kandivali-6)	-ve
7.	ACK-7(Acephate Kandivali-7)	-ve
8.	ACM-8(Acephate Malad-8)	-ve
9.	ACM-9(Acephate Malad-9)	-ve
10.	ACA-10(Acephate Andheri-10)	-ve
11.	ACR-11(Acephate Rammandir-11)	-ve

+ : Bacterial growth absent

- : Bacterial growth present

Table 17. Sensitivity of Bacteria at 0.5 ppm concentration of Acephate

Sr. No.	Isolates	Sensitivity
1.	ACK-1(Acephate Kandivali-1)	-ve
2.	ACA-2(Acephate Andheri-2)	-ve
3.	ACV-3(Acephate Vileparle-3)	-ve
4.	ACG-4(Acephate Goregaon-4)	-ve
5.	ACR-5(Acephate Rammandir-5)	-ve
6.	ACK-6(Acephate Kandivali-6)	-ve
7.	ACK-7(Acephate Kandivali-7)	-ve
8.	ACM-8(Acephate Malad-8)	-ve
9.	ACM-9(Acephate Malad-9)	-ve
10.	ACA-10(Acephate Andheri-10)	-ve
11.	ACR-11(Acephate Rammandir-11)	-ve

+ : Bacterial growth absent

- : Bacterial growth present

4.2.5 Metalaxyl

In experiment at 0.05, 1.25 and 2.5 ppm (As per EU std 2016) concentration of metalaxyl 12 isolates were screened, out of them none of the isolates showed sensitivity to metalaxyl at 0.05, 1.25 and 2.5 ppm by poison food technique Table 18, Table 19 and Table 20 respectively. All isolates showed full growth on nutrient agar plate at concentration 0.05 ppm showed in Plate 6.

These finding similar with results Shetty and Magu (2000) reported that low concentration of metalaxyl stimulated the bacterial and actinomycetes population, but its higher levels significantly inhibited the population. However, different doses of metalaxyl inhibited fungal and *Azotobacter* population at all slates of observation. Interestingly, 0.5 ppm and 1 ppm of metalaxyl did not affect *Nitrosomonas* and *Nitrobacter* population: however, at higher concentration it significantly inhibited the nitrification process.

Table 18. Sensitivity of Bacteria at 0.05ppm concentration of Metalaxyl

Sr. No.	Isolates	Sensitivity
1.	MEA-1(Metalaxyl Andheri-1)	-ve
2.	MEV-2(Metalaxyl Vileparle-2)	-ve
3.	MEK-3(Metalaxyl Kandivali-3)	-ve
4.	MEG-4(Metalaxyl Goregaon-4)	-ve
5.	MEA-5(Metalaxyl Andheri-5)	-ve
6.	MER-6(Metalaxyl Rammandir-6)	-ve
7.	MEG-7(Metalaxyl Goregaon-7)	-ve
8.	MEV-8(Metalaxyl Vileparle-8)	-ve
9.	MEK-9(Metalaxyl Kandivali-9)	-ve
10.	MEV-10(Metalaxyl Vileparle-10)	-ve
11.	MEA-11(Metalaxyl Andheri -11)	-ve
12.	MEA-12(Metalaxyl Andheri -12)	-ve

+ : Bacterial growth absent

- : Bacterial growth present

Table 19. Sensitivity of Bacteria at 1.25 ppm concentration of Metalaxyl

Sr. No.	Isolates	Sensitivity
1.	MEA-1(Metalaxyl Andheri-1)	-ve
2.	MEV-2(Metalaxyl Vileparle-2)	-ve
3.	MEK-3(Metalaxyl Kandivali-3)	-ve
4.	MEG-4(Metalaxyl Goregaon-4)	-ve
5.	MEA-5(Metalaxyl Andheri-5)	-ve
6.	MER-6(Metalaxyl Rammandir-6)	-ve
7.	MEG-7(Metalaxyl Goregaon-7)	-ve
8.	MEV-8(Metalaxyl Vileparle-8)	-ve
9.	MEK-9(Metalaxyl Kandivali-9)	-ve
10.	MEV-10(Metalaxyl Vileparle-10)	-ve
11.	MEA-11(Metalaxyl Andheri -11)	-ve
12.	MEA-12(Metalaxyl Andheri -12)	-ve

+ : Bacterial growth absent

- : Bacterial growth present

Table 20 Sensitivity of Bacteria at 2.5 ppm concentration of Metalaxyl

Sr. No.	Isolates	Sensitivity
1.	MEA-1(Metalaxyl Andheri-1)	-ve
2.	MEV-2(Metalaxyl Vileparle-2)	-ve
3.	MEK-3(Metalaxyl Kandivali-3)	-ve
4.	MEG-4(Metalaxyl Goregaon-4)	-ve
5.	MEA-5(Metalaxyl Andheri-5)	-ve
6.	MER-6(Metalaxyl Rammandir-6)	-ve
7.	MEG-7(Metalaxyl Goregaon-7)	-ve
8.	MEV-8(Metalaxyl Vileparle-8)	-ve
9.	MEK-9(Metalaxyl Kandivali-9)	-ve
10.	MEV-10(Metalaxyl Vileparle-10)	-ve
11.	MEA-11(Metalaxyl Andheri -11)	-ve
12.	MEA-12(Metalaxyl Andheri -12)	-ve

+ : Bacterial growth absent

- : Bacterial growth present

4.2.6 Captan

In experiment at 0.02, 0.5 and 1ppm (As per EU std 2016) concentration of captan, 22 isolates were screened, out of them one isolate showed sensitivity at 1 ppm concentration of captan by serial dilution, pour plate and food poison technique Table 23 and Plate 7. At 0.02 and 0.5 ppm concentration of captan all the isolates showed full growth on nutrient agar plates Table 21 and Table 22. Banerjee and Banerjee (1987) reported that in captan treated soil total count of fungi, bacteria and actinomycetes decreased significantly only at a relatively high fungicide concentration (1000 ug.g⁻¹). Fungi and actinomycetes were affected more than bacteria. Di-ciocco and Rodriguez (1997) studied effect of fungicide captan on growth and nitrogenase activity of *Azospirillum brasilense*. The 8 h growth in rotary shaker of *A. brasilense* was inhibited with 1mg.L⁻¹ pure captan.

Martinez-Toledo *et al* (1998) reported that total culturable fungal populations, nitrifying bacteria, aerobic N-fixing bacteria and nitrogenase activity were significantly decreased at dose rates of 2.0 to 10.0 kg ha⁻¹. However, the presence of

captan enhanced denitrifying and total culturable bacteria, showing that some microbial groups can tolerate high doses of this fungicide.

Table 21. Sensitivity of Bacteria at 0.02ppm concentration of Captan

Sr. No.	Isolates	Sensitivity
1.	CTA-1(Captan Andheri-1)	-ve
2.	CTK-2(Captan Andheri-2)	-ve
3.	CTG-3(Captan Goregaon-3)	-ve
4.	CTR-4(Captan Rammandir-4)	-ve
5.	CTM-5(Captan Malad-5)	-ve
6.	CTA-6(Captan A ndheri-6)	-ve
7.	CTK-7(Captan Kandivali-7)	-ve
8.	CTV-8(Captan Vileparle-8)	-ve
9.	CTG-9(Captan Goregaon-9)	-ve
10.	CTG-10(Captan Goregaon-9)	-ve
11.	CTK-11(Captan Kandivali-11)	-ve
12.	CTK-12(Captan Kandivali-11)	-ve
13.	CTA-13 (Captan Andheri-13)	-ve
14.	CTR-14(Captan Rammandir-14)	-ve
15.	CTM-15(Captan Malad-15)	-ve
16.	CTA-16(Captan Andheri-16)	-ve
17.	CTG-17(Captan Goregaon-17)	-ve
18.	CTR-18(Captan Rammandir-18)	-ve
19.	CTV-19 (Captan Vileparle-19)	-ve
20.	CTV-20(Captan Vileparle-20)	-ve
21.	CTA-21(Captan Andheri-21)	-ve
22.	CTV-22(Captan Vileparle-22)	-ve

+ : Bacterial growth absent

- : Bacterial growth present

Table 22. Sensitivity of Bacteria at 0.5ppm concentration of Captan

Sr. No.	Isolates	Sensitivity
1.	CTA-1(Captan Andheri-1)	-ve
2.	CTK-2(Captan Andheri-2)	-ve
3.	CTG-3(Captan Goregaon-3)	-ve
4.	CTR-4(Captan Rammandir-4)	-ve
5.	CTM-5(Captan Malad-5)	-ve
6.	CTA-6(Captan Andheri-6)	-ve
7.	CTK-7(Captan Kandivali-7)	-ve
8.	CTV-8(Captan Vileparle-8)	-ve
9.	CTG-9(Captan Goregaon-9)	-ve
10.	CTG-10(Captan Goregaon-9)	-ve
11.	CTK-11(Captan Kandivali-11)	-ve
12.	CTK-12(Captan Kandivali-11)	-ve
13.	CTA-13 (Captan Andheri-13)	-ve
14.	CTR-14(Captan Rammandir-14)	-ve
15.	CTM-15(Captan Malad-15)	-ve
16.	CTA-16(Captan Andheri-16)	-ve
17.	CTG-17(Captan Goregaon-17)	-ve
18.	CTR-18(Captan Rammandir-18)	-ve
19.	CTV-19 (Captan Vileparle-19)	-ve
20.	CTV-20(Captan Vileparle-20)	-ve
21.	CTA-21(Captan Andheri-21)	-ve
22.	CTV-22(Captan Vileparle-22)	-ve

+: Bacterial growth absent

- : Bacterial growth present

Table 23. Sensitivity of Bacteria at 1 ppm concentration of Captan

Sr. No.	Isolates	Sensitivity
1.	CTA-1(Captan Andheri-1)	-ve
2.	CTK-2(Captan Andheri-2)	-ve
3.	CTG-3(Captan Goregaon-3)	-ve
4.	CTR-4(Captan Rammandir-4)	-ve
5.	CTM-5(Captan Malad-5)	-ve
6.	CTA-6(Captan Andheri-6)	-ve
7.	CTK-7(Captan Kandivali-7)	-ve
8.	CTV-8(Captan Vileparle-8)	-ve
9.	CTG-9(Captan Goregaon-9)	-ve
10.	CTG-10(Captan Goregaon-9)	+ve
11.	CTK-11(Captan Kandivali-11)	-ve
12.	CTK-12(Captan Kandivali-11)	-ve
13.	CTA-13 (Captan Andheri-13)	-ve
14.	CTR-14(Captan Rammandir-14)	-ve
15.	CTM-15(Captan Malad-15)	-ve
16.	CTA-16(Captan Andheri-16)	-ve
17.	CTG-17(Captan Goregaon-17)	-ve
18.	CTR-18(Captan Rammandir-18)	-ve
19.	CTV-19 (Captan Vileparle-19)	-ve
20.	CTV-20(Captan Vileparle-20)	-ve
21.	CTA-21(Captan Andheri-21)	-ve
22.	CTV-22(Captan Vileparle-22)	-ve

+: Bacterial growth absent

- : Bacterial growth present

4.2.7 Spinosad

In experiment at 0.07,1.75 and 3.5 ppm (As per EU std 2016) concentration of spinosad 16 isolates were screened, out of them no any isolate showed sensitivity to spinosad at 0.07,1.75 and 3.5 by serial dilution, pour plate and poison food technique Table 24,Table 25 and Table 26 respectively.

Table 24. Sensitivity of Bacteria at 0.07 ppm concentration of Spinosad

Sr. No.	Isolates	Sensitivity
1.	SPG-1 (Spinosad Goregaon-1)	-ve
2.	SPV-2 (Spinosad Vileparle-2)	-ve
3.	SPV-3 (Spinosad Vileparle-2)	-ve
4.	SPR-4 (Spinosad Rammandir-4)	-ve
5.	SPA-5 (Spinosad Andheri-5)	-ve
6.	SPG-6 (Spinosad Goregaon-6)	-ve
7.	SPM-7 (Spinosad Malad-7)	-ve
8.	SPA-8 (Spinosad Andheri-8)	-ve
9.	SPK-9 (Spinosad Kandivali-9)	-ve
10.	SPG-10 (Spinosad Goregaon-10)	-ve
11.	SPG-11 (Spinosad Goregaon-11)	-ve
12.	SPR-12 (Spinosad Rammandir-12)	-ve
13.	SPM-13 (Spinosad Malad-13)	-ve
14.	SPV-14 (Spinosad Vileparle-14)	-ve
15.	SPA-15 (Spinosad Andheri-15)	-ve
16.	SPA-16 (Spinosad Andheri-16)	-ve

+ : Bacterial growth absent

- : Bacterial growth present

It was observed that all the isolates showed full growth when streaked on nutrient agar plates at 0.07ppm concentration of Spinosad after 2-3 days of incubation are showed in Plate 8.

Shineyramya and Ganesh (2013) reported that spinosad is harmless to soil beneficial bacteria (*Azotobacter chroococcum*, *Pseudomonas fluorescens* and *Rhizobium leguminosarum*) and fungi (*Aspergillus fumigatus*, *Penicillium chrysogenum* and *Trichoderma viride*). Whereas harmful to disease causing plant pathogenic microbes. The maximum inhibition was 13 mm, 15 mm & 17 mm with respect to 1 ppm, 5 ppm and 10 ppm which was observed in *Xanthomonas campestris*. The zone of inhibition was 12 mm, 13 mm and 15 mm with respect to 1 ppm, 5 ppm and 10 ppm concentration in case of *Erwinia carotovora* and the inhibition of 11 mm, 11 mm & 13 mm at above said concentration (ppm) was observed in *Pseudomonas syringae*.

Table 25. Sensitivity of Bacteria at 1.75 ppm concentration of Spinosad

Sr. No.	Isolates	Sensitivity
1.	SPG-1 (Spinosad Goregaon-1)	-ve
2.	SPV-2 (Spinosad Vileparle-2)	-ve
3.	SPV-3 (Spinosad Vileparle-2)	-ve
4.	SPR-4 (Spinosad Rammandir-4)	-ve
5.	SPA-5 (Spinosad Andheri-5)	-ve
6.	SPG-6 (Spinosad Goregaon-6)	-ve
7.	SPM-7 (Spinosad Malad-7)	-ve
8.	SPA-8 (Spinosad Andheri-8)	-ve
9.	SPK-9 (Spinosad Kandivali-9)	-ve
10.	SPG-10 (Spinosad Goregaon-10)	-ve
11.	SPG-11 (Spinosad Goregaon-11)	-ve
12.	SPR-12 (Spinosad Rammandir-12)	-ve
13.	SPM-13 (Spinosad Malad-13)	-ve
14.	SPV-14 (Spinosad Vileparle-14)	-ve
15.	SPA-15 (Spinosad Andheri-15)	-ve
16.	SPA-16 (Spinosad Andheri-16)	-ve

+ : Bacterial growth absent

- : Bacterial growth present

Table 26. Sensitivity of Bacteria at 3.5 ppm concentration of Spinosad

Sr. No.	Isolates	Sensitivity
1.	SPG-1(Spinosad Goregaon-1)	-ve
2.	SPV-2(Spinosad Vileparle-2)	-ve
3.	SPV-3(Spinosad Vileparle-2)	-ve
4.	SPR-4(Spinosad Rammandir-4)	-ve
5.	SPA-5(Spinosad Andheri-5)	-ve
6.	SPG-6(Spinosad Goregaon-6)	-ve
7.	SPM-7(Spinosad Malad-7)	-ve
8.	SPA-8(Spinosad Andheri-8)	-ve
9.	SPK-9(Spinosad Kandivali-9)	-ve
10.	SPG-10(Spinosad Goregaon-10)	-ve
11.	SPG-11(Spinosad Goregaon-11)	-ve
12.	SPR-12(Spinosad Rammandir-12)	-ve
13.	SPM-13(Spinosad Malad-13)	-ve
14.	SPV-14(Spinosad Vileparle-14)	-ve
15.	SPA-15(Spinosad Andheri-15)	-ve
16.	SPA-16(Spinosad Andheri-16)	-ve

+ : Bacterial growth absent

- : Bacterial growth present

4.2.8 Chlorothalonil

In the experiment at 0.01,0.25 and 0.5ppm (As per EU std 2016) concentration of chlorothalonil 14 isolates were screened, out of them no any isolate showed sensitivity to chlorothalonil at 0.01,0.25 and 0.5 ppm by serial dilution, pour plate and poison food technique are presented in Table 27,Table 28 and Table 29 respectively.

Ding *et al* (2019) study showed that chlorothalonil reduced *nitrosomonas* populations at the rates of 10 and 50 mg of a.i. per kg and autotrophic nitrifying bacterial populations at three application rates ($P < 0.05$). Tu (1994) reported that that *nitrosomonas* and autotrophic nitrifying bacteria were more sensitive to chlorothalonil than to carbendazim.

Table 27. Sensitivity of Bacteria at 0.01 ppm concentration of Chlorothalonil

Sr. No.	Isolates	Sensitivity
1.	CLG-1(Chlorothalonil Goregaon-1)	-ve
2.	CLK-2(Chlorothalonil Kandivali-2)	-ve
3.	CLG-3(Chlorothalonil Goregaon-3)	-ve
4.	CLV-4(Chlorothalonil Vileparle-4)	-ve
5.	CLK-5(Chlorothalonil Kandivali-5)	-ve
6.	CLM-6(Chlorothalonil Malad-6)	-ve
7.	CLR-7(Chlorothalonil Rammandir-7)	-ve
8.	CLM-8(Chlorothalonil Malad-8)	-ve
9.	CLV-9(Chlorothalonil Vileparle-9)	-ve
10.	CLV-10(Chlorothalonil Vileparle-10)	-ve
11.	CLM-11(Chlorothalonil Malad-11)	-ve
12.	CLA-12(Chlorothalonil Andheri-12)	-ve
13.	CLA-13(Chlorothalonil Andheri-13)	-ve
14.	CLG-14(Chlorothalonil Goregaon-14)	-ve

+: Bacterial growth absent

- : Bacterial growth present

Table 28. Sensitivity of Bacteria at 0.25ppm concentration of Chlorothalonil

Sr. No.	Isolates	Sensitivity
1.	CLG-1(Chlorothalonil Goregaon-1)	-ve
2.	CLK-2(Chlorothalonil Kandivali-2)	-ve
3.	CLG-3(Chlorothalonil Goregaon-3)	-ve
4.	CLV-4(Chlorothalonil Vileparle-4)	-ve
5.	CLK-5(Chlorothalonil Kandivali-5)	-ve
6.	CLM-6(Chlorothalonil Malad-6)	-ve
7.	CLR-7(Chlorothalonil Rammandir-7)	-ve
8.	CLM-8(Chlorothalonil Malad-8)	-ve
9.	CLV-9(Chlorothalonil Vileparle-9)	-ve
10.	CLV-10(Chlorothalonil Vileparle-10)	-ve
11.	CLM-11(Chlorothalonil Malad-11)	-ve
12.	CLA-12(Chlorothalonil Andheri-12)	-ve
13.	CLA-13(Chlorothalonil Andheri-13)	-ve
14.	CLG-14(Chlorothalonil Goregaon-14)	-ve

+: Bacterial growth absent

- : Bacterial growth present

Table 29. Sensitivity of Bacteria at 0.5ppm concentration of Chlorothalonil

Sr. No.	Isolates	Sensitivity
1.	CLG-1(Chlorothalonil Goregaon-1)	-ve
2.	CLK-2(Chlorothalonil Kandivali-2)	-ve
3.	CLG-3(Chlorothalonil Goregaon-3)	-ve
4.	CLV-4(Chlorothalonil Vileparle-4)	-ve
5.	CLK-5(Chlorothalonil Kandivali-5)	-ve
6.	CLM-6(Chlorothalonil Malad-6)	-ve
7.	CLR-7(Chlorothalonil Rammandir-7)	-ve
8.	CLM-8(Chlorothalonil Malad-8)	-ve
9.	CLV-9(Chlorothalonil Vileparle-9)	-ve
10.	CLV-10(Chlorothalonil Vileparle-10)	-ve
11.	CLM-11(Chlorothalonil Malad-11)	-ve
12.	CLA-12(Chlorothalonil Andheri-12)	-ve
13.	CLA-13(Chlorothalonil Andheri-13)	-ve
14.	CLG-14(Chlorothalonil Goregaon-14)	-ve

+: Bacterial growth absent

- : Bacterial growth present

4.2.9 Mancozeb

In the experiment at 0.05,1.25 and 2.5 ppm (As per EU std 2016) concentration of mancozeb 9 isolates were screened, out of them none of the isolates showed sensitivity to mancozeb at 0.05,1.25 and 2.5 ppm by serial dilution, pour plate and poison food technique are presented in Table 30,Table 31 and Table 32.

Walia *et al.* (2014) reported that increase in mancozeb concentrations up to 100 ppm and above decreased fungal population. Mancozeb generally decreased actinomycetes population at all the concentrations while higher concentrations 1000 and 2000 ppm were found to be detrimental to soil bacteria. Pozo *et al.* (1994) reported that mancozeb and maneb, at concentrations of about 2.0 to 10.0 kg/ha on an agricultural soil of silty loam texture. Total platable fungal populations, denitrifying bacteria and aerobic diazotrophs were significantly decreased in the presence of agricultural doses of mancozeb. Total platable bacteria were not affected by the addition of mancozeb or maneb, showing that some microbial groups can tolerate agricultural doses of these fungicides.

Table 30. Sensitivity of Bacteria at 0.05 ppm concentration of Mancozeb

Sr. No.	Isolates	Sensitivity
1.	MAR-1(Mancozeb Rammandir-1)	-ve
2.	MAR-2(Mancozeb Rammandir-2)	-ve
3.	MAK-3(Mancozeb Kandivali-3)	-ve
4.	MAK-4(Mancozeb Kandivali-3)	-ve
5.	MAA-5(Mancozeb Andheri-5)	-ve
6.	MAA-6(Mancozeb Andheri-6)	-ve
7.	MAV-7(Mancozeb Vileparle-7)	-ve
8.	MAG-8(Mancozeb Goregaon-8)	-ve
9.	MAM-9(Mancozeb Malad-9)	-ve

+ : Bacterial growth absent

- : Bacterial growth present

Table 31. Sensitivity of Bacteria at 1.25 ppm concentration of Mancozeb

Sr. No.	Isolates	Sensitivity
1.	MAR-1(Mancozeb Rammandir-1)	-ve
2.	MAR-2(Mancozeb Rammandir-2)	-ve
3.	MAK-3(Mancozeb Kandivali-3)	-ve
4.	MAK-4(Mancozeb Kandivali-3)	-ve
5.	MAA-5(Mancozeb Andheri-5)	-ve
6.	MAA-6(Mancozeb Andheri-6)	-ve
7.	MAV-7(Mancozeb Vileparle-7)	-ve
8.	MAG-8(Mancozeb Goregaon-8)	-ve
9.	MAM-9(Mancozeb Malad-9)	-ve

+: Bacterial growth absent

- : Bacterial growth present

Table 32. Sensitivity of Bacteria at 2.5 ppm concentration of Mancozeb

Sr. No.	Isolates	Sensitivity
1.	MAR-1(Mancozeb Rammandir-1)	-ve
2.	MAR-2(Mancozeb Rammandir-2)	-ve
3.	MAK-3(Mancozeb Kandivali-3)	-ve
4.	MAK-4(Mancozeb Kandivali-3)	-ve
5.	MAA-5(Mancozeb Andheri-5)	-ve
6.	MAA-6(Mancozeb Andheri-6)	-ve
7.	MAV-7(Mancozeb Vileparle-7)	-ve
8.	MAG-8(Mancozeb Goregaon-8)	-ve
9.	MAM-9(Mancozeb Malad-9)	-ve

+: Bacterial growth absent

- : Bacterial growth present

4.2.10 2-4-D

In the experiment at 0.05, 1.25 ppm (As per EU std 2016) concentration of 2-4-D 20 isolates were screened, out of them none of the isolates showed sensitivity to 2-4-D at 0.05, 1.25 by serial dilution, pour plate and poison food technique are presented in Table 33 and 34 . At 2.5 ppm concentration isolate DIM-5 showed sensitivity to 2-4-D which is presented in Table 35 and Plate 9.

Worth and Mc Cabe (1948) reported the effect of 2-4-D on the growth of aerobic, anaerobic and facultative anaerobic micro-organisms on solid media. Growth of the aerobic bacteria was prevented by 0.0002 % 2-4-D. Rath *et al.* (1998) reported a significant structural change in the microbial community of an anaerobic microbial community of flooded soil contaminated by different 2, 4-D concentration (0.75, 1.5, 7.5

e 15 g g⁻¹). The authors reported an overall increase in the microbial biomass at lower 2, 4-D concentration (0.75 and 1.5) and a decrease in higher concentrations. However, in the majority of the cases, the number of 2, 4-D degraders increased.

Meena *et al.* (2019) studied that herbicide 2-4-D, atranex and agrozone inhibited the occurrence of *Rhizobium phaseoli* and *Azotobacter vinelandii* and their population further decreased with increase in herbicide concentration. It has been suggested by several authors that 2, 4-D concentrations lower than 2 mg kg⁻¹ may not significantly affect microbial production, biomass, respiration and nitrification (Whelp and Brummer 1999; Lupwayi *et al.* 2004). Concentrations higher than 2 mg kg⁻¹ of 2, 4-D has shown the potential to cause significant disturbances on the function of distinct microbial communities (Wardle and Parkinson 1990; Prado and Airoidi 2000).

Table 33. Sensitivity of Bacteria at 0.05ppm concentration of 2-4-D

Sr. No.	Isolates	Sensitivity
1.	DIG-1(2-4-D Goregaon-1)	-ve
2.	DIG-2(2-4-D Goregaon-2)	-ve
3.	DIK-3(2-4-D Kandivali-3)	-ve
4.	DIA-4(2-4-D Andheri-4)	-ve
5.	DIM-5(2-4-D Malad-5)	-ve
6.	DIM-6(2-4-D Malad-6)	-ve
7.	DIM-7(2-4-D Malad-7)	-ve
8.	DIA-8(2-4-D Andheri-8)	-ve
9.	DIV-9(2-4-D Vileparle-9)	-ve
10.	DIR-10(2-4-D Rammandir-10)	-ve
11.	DIR-11(2-4-D Rammandir-11)	-ve
12.	DIK-12(2-4-D Kandivali-12)	-ve
13.	DIA-13(2-4-D Andheri-13)	-ve
14.	DIA-14(2-4-D Andheri-14)	-ve
15.	DIG-15(2-4-D Goregaon-15)	-ve
16.	DIG-16(2-4-D Goregaon-16)	-ve
17.	DIK-17(2-4-D Kandivali-17)	-ve
18.	DIK-18(2-4-D Kandivali-18)	-ve
19.	DIK-19(2-4-D Kandivali-19)	-ve
20.	DIK-20(2-4-D Kandivali-20)	-ve

+: Bacterial growth absent

- : Bacterial growth present

Table 34. Sensitivity of Bacteria at 1.25ppm concentration of 2-4-D

Sr. No.	Isolates	Sensitivity
1.	DIG-1(2-4-D Goregaon-1)	-ve
2.	DIG-2(2-4-D Goregaon-2)	-ve
3.	DIK-3(2-4-D Kandivali-3)	-ve
4.	DIA-4(2-4-D Andheri-4)	-ve
5.	DIM-5(2-4-D Malad-5)	-ve
6.	DIM-6(2-4-D Malad-6)	-ve
7.	DIM-7(2-4-D Malad-7)	-ve
8.	DIA-8(2-4-D Andheri-8)	-ve
9.	DIV-9(2-4-D Vileparle-9)	-ve
10.	DIR-10(2-4-D Rammandir-10)	-ve
11.	DIR-11(2-4-D Rammandir-11)	-ve
12.	DIK-12(2-4-D Kandivali-12)	-ve
13.	DIA-13(2-4-D Andheri-13)	-ve
14.	DIA-14(2-4-D Andheri-14)	-ve
15.	DIG-15(2-4-D Goregaon-15)	-ve
16.	DIG-16(2-4-D Goregaon-16)	-ve
17.	DIK-17(2-4-D Kandivali-17)	-ve
18.	DIK-18(2-4-D Kandivali-18)	-ve
19.	DIK-19(2-4-D Kandivali-19)	-ve
20.	DIK-20(2-4-D Kandivali-20)	-ve

+: Bacterial growth absent

- : Bacterial growth present

Table 35. Sensitivity of Bacteria at 2.5 ppm concentration of 2-4-D

Sr. No.	Isolates	Sensitivity
1.	DIG-1(2-4-D Goregaon-1)	-ve
2.	DIG-2(2-4-D Goregaon-2)	-ve
3.	DIK-3(2-4-D Kandivali-3)	-ve
4.	DIA-4(2-4-D Andheri-4)	-ve
5.	DIM-5(2-4-D Malad-5)	+ve
6.	DIM-6(2-4-D Malad-6)	-ve
7.	DIM-7(2-4-D Malad-7)	-ve
8.	DIA-8(2-4-D Andheri-8)	-ve
9.	DIV-9(2-4-D Vileparle-9)	-ve
10.	DIR-10(2-4-D Rammandir-10)	-ve
11.	DIR-11(2-4-D Rammandir-11)	-ve
12.	DIK-12(2-4-D Kandivali-12)	-ve
13.	DIA-13(2-4-D Andheri-13)	-ve
14.	DIA-14(2-4-D Andheri-14)	-ve
15.	DIG-15(2-4-D Goregaon-15)	-ve
16.	DIG-16(2-4-D Goregaon-16)	-ve
17.	DIK-17(2-4-D Kandivali-17)	-ve
18.	DIK-18(2-4-D Kandivali-18)	-ve
19.	DIK-19(2-4-D Kandivali-19)	-ve
20.	DIK-20(2-4-D Kandivali-20)	-ve

+: Bacterial growth absent

- : Bacterial growth present

4.3 Testing of isolates for their sensitivity for respective Heavy metals

4.3.1 Nickel

In the experiment of 1.5 and 0.04 ppm (As per Indian std Awasthi, 2000) concentration of Nickel 10 isolates were screened, out of them none of the isolates showed sensitivity to Nickel at 1.5 ppm, except NIV-7 showed sensitivity at 0.04 ppm concentration by serial dilution, pour plate and poison food technique are presented in Table 37 and Plate 10.

Similarly, Wyszowska *et al.* (2006) studied effect of nickel on the proliferation of the following soil bacteria suspended in liquid media: *Azotobacter spp.*, *Arthrobacter spp.*, *Rhizobium leguminosarum* *bv. viciae* and *Bradyrhizobium spp.* (lupini). Among the bacteria analysed, *Azotobacter spp.* was found to be most sensitive to nickel compounds, followed by *Arthrobacter spp.*, *Bradyrhizobium spp.* (lupini) and *Rhizobium leguminosarum* *bv. viciae*, which was most nickel-resistant. Krujatz *et al.* (2011) assessed the toxic effect of nickel, cadmium and EDTA on growth of the plant growth promoting Rhizobacterium, *Pseudomonas brassicacearum*. The inhibition was much greater at low concentration of Ca^{2+} (25 $\mu\text{mol/L}$) and Mg^{2+} 100 $\mu\text{mol/L}$ (EC 50 Cd^{2+} $85 \pm 0.5 \mu\text{mol/L}$ and EC Ni^{2+} , $62 \pm 1.8 \mu\text{mol/L}$). The major toxicity of Ni to bacterial cells include: (1) replacement of essential metal of metalloproteins; (2) attachment to catalytic residues of non-metalloenzymes; (3) allosteric inhibition of enzyme; (4) oxidative stress that enhanced DNA damage, protein impairment, lipid peroxidation along with increased titers of oxidative stress defense systems. (Fashola *et al.* 2016).

Bacteria generally possess two types of uptake system for heavy-metal ions: one is fast and unspecific and driven by the chemiosmotic gradient across the cytoplasmic membrane and another type is slower, exhibits high substrate specificity, and is coupled with ATP hydrolysis (Nies *et al.*, 1995). Low concentrations of metals may have only imperceptible effects on total viable counts of natural populations, the balance of species, and thus the metabolic characteristics of the population, may be drastically affected.

Table 36. Sensitivity of Bacteria at 1.5 ppm concentration of Nickel

Sr. No.	Isolates	Sensitivity
1.	NIR-1(Nickel Rammandir-1)	-ve
2.	NIA-2(Nickel Andheri-2)	-ve
3.	NIA-3(Nickel Andheri-3)	-ve
4.	NIK-4(Nickel Kandivali-4)	-ve
5.	NIM-5(Nickel Malad-5)	-ve
6.	NIV-6(Nickel Vileparle-6)	-ve
7.	NIV-7(Nickel Vileparle-7)	-ve
8.	NIV-8(Nickel Vileparle-8)	-ve
9.	NIG-9(Nckel Goregaon-9)	-ve
10.	NIG-10(Nickel Goregaon-10)	-ve

+ : Bacterial growth absent

- : Bacterial growth present

Table 37. Sensitivity of Bacteria at 0.04 ppm concentration of Nickel

Sr. No.	Isolates	Sensitivity
1.	NIR-1(Nickel Rammandir-1)	-ve
2.	NIA-2(Nickel Andheri-2)	-ve
3.	NIA-3(Nickel Andheri-3)	-ve
4.	NIK-4(Nickel Kandivali-4)	-ve
5.	NIM-5(Nickel Malad-5)	-ve
6.	NIV-6(Nickel Vileparle-6)	-ve
7.	NIV-7(Nickel Vileparle-7)	+ve
8.	NIV-8(Nickel Vileparle-8)	-ve
9.	NIG-9(Nckel Goregaon-9)	-ve
10.	NIG-10(Nickel Goregaon-10)	-ve

+ : Bacterial growth absent

- : Bacterial growth present

4.3.2 Lead

In the experiment of 0.5 and 2.5 ppm (As per Indian std Awasthi, 2000) concentration of lead 14 isolates were screened, out of them no any isolate showed sensitivity to lead by serial dilution, pour plate and poison food technique are presented in Table 38 and Table 39.

Doelman and Haanstra (1979) investigated sensitivity of soil bacteria towards Pb. In the Pb-containing soils, proportionally more gram-negative rods were present while coryneform bacteria decreased. Niu *et al.* (2019) reported that lower Pb pollution increased microbial quantities and promoted the utilization of amino acids or fatty acids, while higher Pb concentrations decreased microbial quantities and metabolic activities, and promoted the utilization of carbohydrates. The bacteria (nitrogen-fixing bacteria, ammonifying bacteria, inorganic phosphorus-solubilizing bacteria, and nitrosobacteria) all increased in the 500 mg/kg treatment and decreased in the 1500 mg/kg treatment compared with the 0 mg/kg treatment, especially in rhizospheric soil.

Table 38. Sensitivity of Bacteria at 0.5 ppm concentration of Lead

Sr. No.	Isolates	Sensitivity
1.	LEV-1(Lead Vileparle-1)	-ve
2.	LEV-2(Lead Vileparle-2)	-ve
3.	LEV-3(Lead Vileparle-3)	-ve
4.	LEM-4(Lead Malad-4)	-ve
5.	LEK-5(Lead Kandivali-5)	-ve
6.	LEK-6(Lead Kandivali-6)	-ve
7.	LEK-7(Lead Kandivali-7)	-ve
8.	LEK-8(Lead Kandivali-8)	-ve
9.	LEG-9(Lead Goregaon-9)	-ve
10.	LEG-10(Lead Goregaon-10)	-ve
11.	LER-11(Lead Rammandir-11)	-ve
12.	LER-12(Lead Rammandir-12)	-ve
13.	LER-13(Lead Rammandir-13)	-ve
14.	LER-14(Lead Rammandir-14)	-ve

+: Bacterial growth absent

- : Bacterial growth present

Table 39. Sensitivity of Bacteria at 2.5 ppm concentration of Lead

Sr. No.	Isolates	Sensitivity
1.	LEV-1(Lead Vileparle-1)	-ve
2.	LEV-2(Lead Vileparle-2)	-ve
3.	LEV-3(Lead Vileparle-3)	-ve
4.	LEM-4(Lead Malad-4)	-ve
5.	LEK-5(Lead Kandivali-5)	-ve
6.	LEK-6(Lead Kandivali-6)	-ve
7.	LEK-7(Lead Kandivali-7)	-ve
8.	LEK-8(Lead Kandivali-8)	-ve
9.	LEG-9(Lead Goregaon-9)	-ve
10.	LEG-10(Lead Goregaon-10)	-ve
11.	LER-11(Lead Rammandir-11)	-ve
12.	LER-12(Lead Rammandir-12)	-ve
13.	LER-13(Lead Rammandir-13)	-ve
14.	LER-14(Lead Rammandir-14)	-ve

+: Bacterial growth absent

- : Bacterial growth present

4.3.3 Copper

In the experiment of 0.01 and 30 ppm (As per Indian standard Awasthi 2000) concentration of copper 16 isolates were screened, out of them COM-2 showed sensitivity to copper at 0.01 ppm by poison food technique are presented in Table 40 and Plate 11.

These finding similar with Rathnayake *et al.* (2009) reported that *Paenibacillus spp* isolated from pristine soil showed highest sensitivity to Cu^{2+} at concentration 0.011mg.L^{-1} i.e. 0.01 ppm of Cu^{2+} . Zevenhuize *et al.* (1979) studied inhibitory effects of copper on bacteria related to the free ion concentration. Copper-sensitive bacteria, e.g., *Klebsiella aerogenes*, were inhibited in their growth and survival in the range of 10^{-8} - 10^{-6} M Cu^{2+} ion concentrations. Copper-resistant bacteria could be isolated from a stock solution of CuSO_4 , containing 100 ppm Cu (II). They were of the *Pseudomonas* type and showed a much higher tolerance towards Cu^{2+} , up to 10^{-3} M.

Copper toxicity is as a result of its harmful effects on the bacterial cell membranes and nucleic acid structure as well as its ability to alter enzyme specificity and disrupt cellular functions (Fashola *et al.* 2016). For most bacteria, copper enters the cell via passive diffusion along its chemical gradient. Since biological membranes are relatively impermeable to copper, the cytosol of most bacterial species constitutes a copper-poor environment. In Gram negative bacteria, copper homeostasis is mainly played out in the periplasm, where most of the intracellular copper is located. (Macomber *et al.*, 2007; Parmar *et al.*, 2018).

Table 40. Sensitivity of Bacteria at 0.01 ppm concentration of Copper

Sr. No.	Isolates	Sensitivity
1.	COM-1 (Copper Malad-1)	-ve
2.	COM-2 (Copper Malad-2)	+ve
3.	COM-3 (Copper Malad-3)	-ve
4.	COA-4 (Copper Andheri-4)	-ve
5.	COA-5 (Copper Andheri-5)	-ve
6.	COA-6 (Copper Andheri-6)	-ve
7.	COA-7 (Copper Andheri-7)	-ve
8.	COG-8 (Copper Goregaon-8)	-ve
9.	COG-9 (Copper Goregaon-9)	-ve
10.	COR-10 (Copper Rammandir-10)	-ve
11.	COR-11 (Copper Rammandir-11)	-ve
12.	COV-12 (Copper Vileparle-12)	-ve
13.	COV-13 (Copper Vileparle-13)	-ve
14.	COK-14 (Copper Kandivali-14)	-ve
15.	COK-15 (Copper Kandivali-15)	-ve
16.	COK-16 (Copper Kandivali-16)	-ve

+ : Bacterial growth absent

- : Bacterial growth present

Table 41. Sensitivity of Bacteria at 30 ppm concentration of Copper

Sr. No.	Isolates	Sensitivity
1.	COM-1 (Copper Malad-1)	-ve
2.	COM-2 (Copper Malad-2)	-ve
3.	COM-3 (Copper Malad-3)	-ve
4.	COA-4 (Copper Andheri-4)	-ve
5.	COA-5 (Copper Andheri-5)	-ve
6.	COA-6 (Copper Andheri-6)	-ve
7.	COA-7 (Copper Andheri-7)	-ve
8.	COG-8 (Copper Goregaon-8)	-ve
9.	COG-9 (Copper Goregaon-9)	-ve
10.	COR-10 (Copper Rammandir-10)	-ve
11.	COR-11 (Copper Rammandir-11)	-ve
12.	COV-12 (Copper Vileparle-12)	-ve
13.	COV-13 (Copper Vileparle-13)	-ve
14.	COK-14 (Copper Kandivali-14)	-ve
15.	COK-15 (Copper Kandivali-15)	-ve
16.	COK-16 (Copper Kandivali-16)	-ve

+: Bacterial growth absent

- : Bacterial growth present

4.3.4 Cadmium

In the experiment of 0.07 and 1.5 ppm (As per Indian std Awasthi, 2000) concentration of cadmium 10 isolates were screened, out of them no any isolate showed sensitivity to cadmium by serial dilution, pour plate and poison food technique Table 42 and Table 43. All isolates showed full growth on nutrient agar plate at concentration 0.07 ppm of Cadmium showed in Plate 12.

Ohta and Udaka (1976) isolated cadmium and mercury sensitive mutants of *Escherichia coli*. The Cd-hypersensitive mutant, CD17P, could not grow in a chemically defined liquid medium containing 0.5 uM Cd²⁺. Luo *et al.* (2019) reported that *Actinobacteria* are tolerant to cadmium, whereas *Proteobacteria*, *Verrucomicrobia*, and *Nitrospirae* are sensitive.

Table 42. Sensitivity of Bacteria at 0.07 ppm concentration of Cadmium

Sr. No.	Isolates	Sensitivity
1.	CDA-1 (Cadmium Andheri-1)	-ve
2.	CDA-2 (Cadmium Andheri-2)	-ve
3.	CDA-3 (Cadmium Andheri-3)	-ve
4.	CDA-4 (Cadmium Andheri-4)	-ve
5.	CDR-5 (Cadmium Rammandir-5)	-ve
6.	CDR-6 (Cadmium Rammandir-6)	-ve
7.	CDM-7 (Cadmium Malad-7)	-ve
8.	CDM-8 (Cadmium Malad-8)	-ve
9.	CDM-9 (Cadmium Malad-9)	-ve
10.	CDV-10 (Cadmium Vileparle-10)	-ve

+ : Bacterial growth absent

- : Bacterial growth present

Table 43 Sensitivity of Bacteria at 1.5 ppm concentration of Cadmium

Sr. No.	Isolates	Sensitivity
1.	CDA-1 (Cadmium Andheri-1)	-ve
2.	CDA-2 (Cadmium Andheri-2)	-ve
3.	CDA-3 (Cadmium Andheri-3)	-ve
4.	CDA-4 (Cadmium Andheri-4)	-ve
5.	CDR-5 (Cadmium Rammandir-5)	-ve
6.	CDR-6 (Cadmium Rammandir-6)	-ve
7.	CDM-7 (Cadmium Malad-7)	-ve
8.	CDM-8 (Cadmium Malad-8)	-ve
9.	CDM-9 (Cadmium Malad-9)	-ve
10.	CDV-10 (Cadmium Vileparle-10)	-ve

+ : Bacterial growth absent

- : Bacterial growth present

4.3.5 Chromium

In the experiment of 1 and 20 ppm (As per Indian std Awasthi, 2000) concentration of chromium 20 isolates were screened, out of them no any isolate showed sensitivity to chromium by serial dilution, pour plate and poison food technique are presented in Table 44 and Table 45.

Viamajala *et al.* (2004) studies showed that the growth of *Shewanella oneidensis* was much inhibited even at a low concentration 0.015 mM of Cr (VI). Incubation of *E. coli* cells with 10 mM of hexavalent chromium and 3 mM hydrogen peroxide caused the degradation of double-strand DNA *in vivo*, which was suppressed by the addition of mannitol (Itoh *et al.*, 1994).

Table 44. Sensitivity of Bacteria at 1ppm concentration of Chromium

Sr. No.	Isolates	Sensitivity
1.	CRR-1 (Chromium Rammandir-1)	-ve
2.	CRR-2 (Chromium Rammandir-2)	-ve
3.	CRR-3 (Chromium Rammandir-3)	-ve
4.	CRA-4 (Chromium Andheri-4)	-ve
5.	CRA-5 (Chromium Andheri-5)	-ve
6.	CRA-6 (Chromium Andheri-6)	-ve
7.	CRV-7 (Chromium Vileparle-7)	-ve
8.	CRV-8 (Chromium Vileparle-8)	-ve
9.	CRM-9 (Chromium Malad -9)	-ve
10.	CRM-10 (Chromium Malad -10)	-ve
11.	CRM-11 (Chromium Malad -11)	-ve
12.	CRM-12 (Chromium Malad -12)	-ve
13.	CRK-13 (Chromium Kandivali-13)	-ve
14.	CRK-14 (Chromium Kandivali-14)	-ve
15.	CRK-15 (Chromium Kandivali-15)	-ve
16.	CRK-16 (Chromium Kandivali-16)	-ve
17.	CRG-17 (Chromium Goregaon-17)	-ve
18.	CRG-18 (Chromium Goregaon-18)	-ve
19.	CRG-19 (Chromium Goregaon-19)	-ve
20.	CRG-20 (Chromium Goregaon-20)	-ve

+: Bacterial growth absent

- : Bacterial growths present

Table 45. Sensitivity of Bacteria at 20ppm concentration of Chromium

Sr. No.	Isolates	Sensitivity
1.	CRR-1 (Chromium Rammandir-1)	-ve
2.	CRR-2 (Chromium Rammandir-2)	-ve
3.	CRR-3 (Chromium Rammandir-3)	-ve
4.	CRA-4 (Chromium Andheri-4)	-ve
5.	CRA-5 (Chromium Andheri-5)	-ve
6.	CRA-6 (Chromium Andheri-6)	-ve
7.	CRV-7 (Chromium Vileparle-7)	-ve
8.	CRV-8 (Chromium Vileparle-8)	-ve
9.	CRM-9 (Chromium Malad -9)	-ve
10.	CRM-10 (Chromium Malad -10)	-ve
11.	CRM-11 (Chromium Malad -11)	-ve
12.	CRM-12 (Chromium Malad -12)	-ve
13.	CRK-13 (Chromium Kandivali-13)	-ve
14.	CRK-14 (Chromium Kandivali-14)	-ve
15.	CRK-15 (Chromium Kandivali-15)	-ve
16.	CRK-16 (Chromium Kandivali-16)	-ve
17.	CRG-17 (Chromium Goregaon-17)	-ve
18.	CRG-18 (Chromium Goregaon-18)	-ve
19.	CRG-19 (Chromium Goregaon-19)	-ve
20.	CRG-20 (Chromium Goregaon-20)	-ve

+: Bacterial growth absent

- : Bacterial growths present

4.3.6 Arsenic

In the experiment of 0.05 and 1.1 ppm (As per Indian std Awasthi, 2000) concentration of arsenic 12 isolates were screened, out of them no any isolate showed sensitivity to arsenic by serial dilution, pour plate and poison food technique Table 46 and Table 47.

Similarly, Mandel *et al.* (1965) studied on the action of arsenic on *Bacillus cereus*. Trivalent sodium arsenite was found to inhibit growth at a lower concentration

(0.4 mM) than pentavalent sodium arsenate (10 mM). Sultana *et al.* (2020) examined tolerance level of soil microbes *Pseudomonas* and *Penicillium* against different levels of sodium arsenite stress (1mg/L to 10mg/L). Result shown that both micobes cannot overcome higher levels of arsenic stress because in higher stress petriplates, increased inhibitory zones were observed.

Table 46. Sensitivity of Bacteria at 0.05 ppm concentration of Arsenic

Sr. No.	Isolates	Sensitivity
1.	ARM-1 (Arsenic Malad-1)	-ve
2.	ARM-2 (Arsenic Malad-2)	-ve
3.	ARM-3 (Arsenic Malad-3)	-ve
4.	ARG-4 (Arsenic Goregaon-4)	-ve
5.	ARG-5 (Arsenic Goregaon-5)	-ve
6.	ARG-6 (Arsenic Goregaon-6)	-ve
7.	ARR-7 (Arsenic Rammandir-7)	-ve
8.	ARR-8 (Arsenic Rammandir-8)	-ve
9.	ARA-9 (Arsenic Andheri-9)	-ve
10.	ARA-10 (Arsenic Andheri-10)	-ve
11.	ARA-11 (Arsenic Andheri-11)	-ve
12.	ARV-12 (Arsenic Vileparle-12)	-ve

+ : Bacterial growth absent

- : Bacterial growth present

Table 47. Sensitivity of Bacteria at 1.1 ppm concentration of Arsenic

Sr. No.	Isolates	Sensitivity
1.	ARM-1(Arsenic Malad-1)	-ve
2.	ARM-2 (Arsenic Malad-2)	-ve
3.	ARM-3 (Arsenic Malad-3)	-ve
4.	ARG-4(Arsenic Goregaon-4)	-ve
5.	ARG-5(Arsenic Goregaon-5)	-ve
6.	ARG-6(Arsenic Goregaon-6)	-ve
7.	ARR-7(Arsenic Rammandir-7)	-ve
8.	ARR-8(Arsenic Rammandir-8)	-ve
9.	ARA-9(Arsenic Andheri-9)	-ve
10.	ARA-10(Arsenic Andheri-10)	-ve
11.	ARA-11(Arsenic Andheri-11)	-ve
12.	ARV-12(Arsenic Vileparle-12)	-ve

+ : Bacterial growth absent

- : Bacterial growth present

Table 48 Nomenclature of selected Five isolates with GPS locations

Sr. No	Isolate Code	Railway station	Location	GPS Location	
				N	E
				Latitude	Longitude
1.	CAR-8 (Carbendazim Rammandir-8)	Ram mandir	Near Laxmi nagar	19.1428256	72.8505748
2.	CTG-10 (Captan Goregaon-10)	Goregaon	Near Pandurang wadi	19.1695332	72.8493742
3.	DIM-5 (2-4-D Malad-5)	Malad	Central Ordnance depot	19.1954715	72.8506905
4.	NIV-7 (Nickel Vileparle-7)	Vile parle	Near Parle colony	19.1076454	72.8453640
5.	COM-2 (Copper Malad-2)	Malad	Chincholi footover bridge	19.177513	72.848626

4.4 Characterization of Bacterial Isolates

4.4.1 Morphological characterization

The five isolates CAR-8, CTG-10, DIM-5, NIV-7 and COM-2 isolates were examined for morphological characterization. The cells of bacterial isolates CAR-8, DIM-5, NIV-7 and COM-2 were rod shaped, motile (Plate 13 and 17). Whereas cell of CTG-10 was vibrioid in shape and motile (Plate 15). Out of five isolates isolate COM-2 was Gram positive rods and another isolates were Gram negative in reaction. Size of isolate CAR-8 (0.8 x 1 μm), CTG-10 (0.6 x 3 μm), DIM-5 (2 x 5 μm), NIV-7 (0.8x 2.2 μm) and COM-2 (0.6 x 3 μm). Among the isolates isolate CTG-10, DIM-5, COM-2 formed endospore or cyst (Table 49).

These results are in agreement with the findings of Singh *et al.* (2008) describes the characterization of a *Rhizobium* strain isolated from root nodules of fenugreek. The *Rhizobium* isolates were rod shaped, gram negative, acid and mucous producing.

Similarly, Tyagi and Singh (2013) bacterium isolated from Himalayan valley soil, India, bacterial cells were gram negative, short and cocci-to rod-shaped and are 0.6–0.8 μm in width and 0.8–2 μm in length the strain ptl-3^T belonged to the genus *Azospirillum* and was closely related to *A. brasilense*. Tilak *et al.* (2010) reported that by

motility test, it was found that the cells of the selected isolates are motile, which is the characteristic of *Azospirillum*. Which support these finding.

Upadhyay *et al.* (2015) isolated forty two strains of *Azotobacter* from soil. Among 42 isolates, 7 were single cocci, 7 coccidal chain and 4 were cocci in clumps. Majority of isolates i.e. 24, were small, medium and large rod shaped. Thirty two isolates were Gram –ve in reaction. Finally from these isolates, twenty two were confirmed as *Azotobacter* strains on cyst formation. Ivanova *et al.*, (2009) bacteria was isolated from soil and the rhizosphere of agricultural plants. Bacterial cells were rod-shaped, single, 1.0–1.5 mm long and about 0.3–0.8mm in diameter, Gram-negative. Motile with three to five polar flagella. 16S rRNA gene sequence analysis indicated that the representative strain CIP 109457T had a clear affiliation with *Pseudomonas sensu stricto* groups, with the nearest relatives being *Pseudomonas brassicacearum*, *P. thivervalensis*, *P. corrugata*, *P. mediterranea* and *P. kilonensis*.

In support of these finding, Rathnayake *et al.*, (2010) reported that bacteria isolated from a pristine soil and were characterized. Both of these bacterial isolates are rod shaped, spore formers. Two Gram positive isolates were identified as *Paenibacillus sp.* and *Bacillus thuringeinsis*.

Table 49. Morphological characteristics of five isolates

Isolates	Shape	Size	Gram reaction	Endospore/cyst	Motility
CAR-8	Rods	0.8×1 µm	-ve	-	+
CTG-10	Vibroid	0.6 x 3µm	-ve	+	+
DIM-5	Rods	2x 5µm	-ve	+	+
NIV-7	Rods	0.8×2.2 µm	-ve	-	+
COM-2	Rods	0.6 x 3µm	+	+	+

4.4.2 Cultural characterization

On nutrient agar medium, two sensitive isolates CAR-8 and CTG-10 formed circular colonies with entire margin, raised, slight to moderate growth. DIM-5 formed circular colonies with entire margin, convex, moderate growth. NIV-7 formed mucoid colonies with entire margin, low convex, slight to moderate growth. Isolate COM-2 formed irregular colonies, undulate margin, low convex, moderate growth (Table 50).

The result of present findings is supported with the finding Ahmmed *et al.*, (2019) studied *Rhizobium* bacteria in the roots of leguminous plants. It was yellow in color, circular form, margin was entire and rose in elevation, smooth surface and size is moderate. Wong and Stenberg (1979) studied characterization of *Azospirillum* isolated from Nitrogen-fixing roots of harvested sorghum plants, the five isolates are slightly curve shaped. Strain S-4 appeared to be longer to slightly more curve shaped than other strains. Strains S-3 appeared circular, raised elevation with entire margin, which is similar to these finding.

Hala and Ali (2019) reported that the N₂-fixing bacteria isolated from the five soil samples of neem rhizosphere were *Azotobacter paspali* and *Azotobacter vinelandii* (Azomonas) groups. Colonies of bacteria were circular, white in colour with entire margin.

Anpalagan *et al.* (2020) isolated and characterize a bacterium *Paenibacillus dendritiformis* DDS2: which can produce thermostable alkaline protease. The colonies of the strain DDS2 at 24 h had pale colour, with irregular margin, 1.4 ± 0.3 mm in diameter, low convex elevation, moist and shiny surface.

Chung *et al.*, (2008) studied a bacterial endophyte, *Pseudomonas brassicacearum* YC5480, isolated from the root of *Artemisia sp.* producing antifungal and phytotoxic compounds. A bacteria forms mucoid colonies with regular margins, which support these finding.

Table 50 Cultural characteristics of five isolates

Sr. No.	Isolate	Colony characters			
		Growth	Form	Margin	Elevation
1	CAR-8	Moderate	Circular	Entire	Raised
2	CTG-10	Moderate	Circular	Entire	Raised
3	DIM-5	Moderate	Circular	Entire	Convex
4	NIV-7	Moderate	Circular	Entire	Low Convex
5	COM-2	Moderate	irregular	undulate	Low Convex

4.4.3 Biochemical and physiological characterization

All sensitive five isolates were tested for their biochemical characters viz., catalase, oxidase, hydrolysis of gelatine, starch and casein, growth at 4⁰C, 41⁰C and 50⁰C, voges-proskauer (VP) test, required NaCl 7%, H₂S production, aerobic/ anaerobic test, and urease activity etc. (Table 51,52 and 53).

Test of catalase and oxidase were positive for all five sensitive isolates. Test of urease activity positive for CTG-10 and COM-2 rest of three isolates showed urease negative (Plate 16,14,19 and 18). VP test and hydrogen sulphide production tests were negative for all isolates except one isolate DIM-5 (Plate 18). CAR -8 and NIV-7 showed positive test for hydrolysis of gelatin (Plate 19), whereas remaining three isolate showed negative test of hydrolysis of gelatin (Plate 16,18 and 20). Test of starch hydrolysis positive for CTG-10 and COM-2. Whereas isolates CAR-8, DIM-5 and NIV-7 negative for starch hydrolysis. Casein hydrolysis test negative for all isolates except one COM-2. Growth of all five isolates (CAR-8, CTG-10, DIM-5, NIV-7 and COM-2) decreased with 7% NaCl Concentration. All the five isolates were aerobic. Isolate CAR-8 well grow at 4⁰ C and 40⁰ C. Isolate CTG-10,DIM-5,and NIV-7 exhibited growth at 4⁰ C. Growth of isolate COM-2 at 4⁰, 40⁰, 41⁰ and 50⁰ C temperature were negative.

Similar results were also reported by Roychowdhury *et al.*, (2015) studied isolation and characterization of bacteria *Rhizobium* from chick pea (*Cicer arietinum*). Catalase tests of the *Rhizobium spp* strains were positive, VP test, starch hydrolysis and hydrogen sulphide test were negative. Similarly, Naga *et al.*, (2018) studied biochemical characterization of six *Rhizobium* strains isolated from French bean nodules collected from different regions of Andhra Pradesh, India. All the isolates of rhizobia were positive to the, urease, catalase test and oxidase test. Starch hydrolysis and gelatin hydrolysis test were positive for all isolates except Rh01 and Rh3 isolates. Wadhwa *et al.*, (2017) observed the colonies of *Rhizobium* isolated from chick pea (*Cicer arietinum*) were negative for casein and urease test. Optimum temperature for rhizobial growth was found to be 28°C whereas moderate growth was observed at 35°C and minimal growth of isolates was exhibited at 5°C and 40°C. Jida and Assefa (2012) also reported high tolerance to NaCl where 75% of the tested rhizobia could grow well with 1% NaCl. However, at higher concentrations, the percentage of

tolerant isolate decreased with increasing salt concentration as on 11.1% of the isolates tolerated 5% NaCl.

Hossain *et al.*, (2015) studied ten *Azospirillum* isolates from different paddy fields of North Bengal. Six selected isolates M-3, M-4, M-6, M-7, M-8 and M-10 were identified as *A. lipoferum*; three isolates M-1, M-2 and M-5 were identified as *A. brasilense* and one isolate M-9 was identified as *A. halopraeferns*. M-1, M-2 and M-5 observed catalase and oxidase test positive, whereas urease test positive for isolate M-2 and negative for M-1 and M-5. Starch hydrolysis negative for M-1 and positive for M-2 and M-5. Gelatin hydrolysis test were negative for all three isolates, which support these finding. Muthukumar *et al.* (2021) observed test of casein hydrolysis was negative and test of urease hydrolysis was positive for *Azospirillum spp.* Tyagi and Singh (2014) studied bacterial strain ptl-3^T was isolated from Himalayan valley soil, India. Strain ptl-3^T belonged to the genus *Azospirillum* and was closely related to *A. brasilense* (98.7 % similarity) and *A. rugosum* (97 % similarity). Voges proskauer's test and H₂S production test were negative for these isolates. Hossain *et al.* (2014) studied physiological characteristics of ten *Azospirillum spp* isolated from paddy fields of north Bengal. Among them growth of isolate M-1, M-2 and M-5 was absent at all concentration of NaCl 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4 %. These isolates identified as *Azospirillum brasilense*. High concentration of NaCl is generally inhibitory. The activity decreases with increase temperature above 40⁰ C Khan *et al.*, (2001) which also support these finding.

Shaikh and Shakir (2018) studied characteristics of *Azotobacter vinelandii* in soil of various fields and Orchards. 39 isolates showed test of catalase, oxidase and H₂S production were positive. Hala and Ali (2019) reported that Voges proskaure test was positive and urease test was negative for isolate which was belonging to *Azotobacter spp.* Similarly, Bag *et al.*, (2017) investigated putative *Azotobacter* species were isolated from soils collected from Cooch Behar and Jalpaiguri districts of West Bengal. Test of casein and gelatin hydrolysis were negative for all 9 isolates (Az-3, Az-5, Az-8, Az-11, Az-12, Az-14, Az-16, Az-19 and Az-20), test of starch hydrolysis were negative for Az-3, Az-11 and Az-16, which support these finding. *Azotobacter spp.* is free-living aerobic bacteria dominantly found in soils. Chennappa *et al.*, (2016) who studied four *Azotobacter* species for salt tolerance and reported that *A. chroococcum*,

A. vinelandii, and *A. salinestrus* can tolerate up to 8% NaCl concentration; however, the cell counts were reduced with high concentration of NaCl. Islam *et al.*, (2008) study showed that maximum growth of *Azotobacter spp* at 30⁰ C to 40⁰ C, no isolate of *Azotobacter* survived at 50⁰C.

Table 51. Biochemical characteristics of five isolates

Name of isolates	Catalase	Oxidase	Urease activity	H ₂ S Production	Voges-Proskauer Test
CAR-8	+	+	-	-	-
CTG-10	+	+	+	-	-
DIM-5	+	+	-	+	+
NIV-7	+	+	-	-	-
COM-2	+	+	+	-	-

Table 52. Biochemical characteristics of five isolates

Sr. No.	Isolate	Hydrolysis		
		Gelatine	Starch	Casein
1	CAR-8	+	-	-
2	CTG-10	-	+	-
3	DIM-5	-	-	-
4	NIV-7	+	-	-
5	COM-2	-	+	+

Table 53 Physiological characteristics of five isolates

Isolates	Growth at 7% NaCl	Aerobic/ anaerobic	Growth at			
			4 ⁰ C	40 ⁰ C	41 ⁰ C	50 ⁰ C
CAR-8	-	+	+	+	-	-
CTG-10	-	+	+	-	-	-
DIM-5	+	+	+	-	-	-
NIV-7	-	+	+	-	-	-
COM-2	-	+	-	-	-	-

Ivanova *et al.* (2009) isolated *Pseudomonas brassicacearum* subsp. *neaurantiaca* subsp. nov., orange-pigmented bacteria isolated from soil and the rhizosphere of agricultural plants. Organism growth was strictly aerobic; temperature range for growth is 4–37°C, with optimum growth at 25°C. No growth at 40°C. Liquefies gelatin and does not hydrolyse starch, agar, aesculin, chitin or DNA. Similarly, Qaysi *et al.*, (2016) isolated *Pseudomonas brassicacearum* bacteria from rhizosphere soil of Cowpea farm in Iraq. Test of catalase, oxidase and gelatin hydrolysis were positive for bacteria, whereas test of urea hydrolysis were negative. Kaushal *et al.*, (2013) *Fluorescent Pseudomonas* strains were isolated from the soil samples collected from Bhojia Institute of Life Sciences, Budh, Baddi. Biochemical test results showed that test of voges-proskauer, hydrogen Sulphite production and urease production were negative, which support present finding.

Hosoda *et al.*, (2003) reported that *Paenibacillus* genus did not grow at 5°C, 45°C, or in the presence of 5% NaCl. Huang *et al.*, (2012) isolated bacteria from soil; the isolate is positive for catalase, oxidase, and hydrolysis of starch and casein. Genetic analysis indicated that this strain belongs to the genus *Paenibacillus*.

Chandra *et al.* (2008) studied isolation and characterization of bacterial strains ITRCS₆ and ITRCS₈. Test of gelatin hydrolysis, H₂S production and VP test for ITRCS₆ strain were negative. Positive test for urea hydrolysis, no growth at 5% NaCl. Strain ITRCS₆ was tentatively identified as *Paenibacillus sp.* based on 16S rDNA sequence analysis, which support present finding.

On the basis of biochemical characters performed in the laboratory the probable genus of sensitive bacterial isolates are *Rhizobium*, *Azotobacter*, *Azospirillum*, *Paenibacillus* and *Pseudomonas spp.*

5. SUMMARY AND CONCLUSIONS

Vegetables and fruit are vulnerable to insects and disease attack and pesticides are routinely employed on them. As a result, food hygiene is a significant public issue across the globe. The rising need for food safety has sparked study into the risks linked with the intake of fruits and vegetables, which make up a large portion of the human diet and provide minerals and vitamins. As a result, pesticide residues may damage final consumers, particularly when such commodities are eaten fresh.

Because of the potential damage these chemicals might cause to the environment as well as known or suspected harmful effects on people, the widespread as well as indiscriminate utilization of pesticides is a significant source of worry. Severe neurological poisoning, neuro developmental impairment, immune system abnormalities, reproduction as well as endocrine system disturbances, cancer, chronic renal disease, and a variety of other disorders are among the hazardous consequences.

As vegetables are essential part of the human diet, heavy metal poisoning of these foods should not be overlooked. Vegetables are high in vitamins, minerals, and fiber and they also have antioxidative properties. Intake of heavy metal-contaminated plants, on the other hand, may represent a health risk to humans. One of the most critical areas of food quality assurance is heavy metal contamination of food products.

From Charni road station to Borivali station on the Western line, from Byculla station to Dombivli station on the Central line, and from Sewri station to Panvel station on the Mumbai Harbour line, railway track farming is carried out. Along these railway rails, various crops such as spinach, bhendi and radish are producing year round. These vegetables produced are sold at a reduced cost at numerous market places. The water supply for such agricultural activities is sewage water, which contains effluents from different businesses, water from drainage pipes, and water from home sources, all of which pollute the soil, fruits, and vegetables. We do not have a framework in place in the indian agricultural sector to test these vegetables and fruit for pesticides as well as heavy metal residues prior to selling to consumers. Only certain vegetables and fruit that must be shipped overseas are checked for residues.

The results of the investigation conducted during 2015-16 to detect pesticide residues and heavy metals in marketable fruits and vegetables by sensory microbial technology were presented at the Department of Plant Pathology and Agril Microbiology, M.P.K.V. Rahuri-413 722. The study covered the isolation of micro-organisms sensitive to low concentration of pesticide residue and heavy metals from soil and water samples collected from western railway line of Mumbai of Maharashtra state, screening and testing of these microbes against the low concentration of individual pesticides and heavy metals and identification of bacterial isolates.

5.1 Pesticides residues

The total number of isolates identified for each herbicide and heavy metal are listed. The results of the experiment at 0.05, 1.25, and 2.5 ppm (as per EU std) concentrations of cypermethrin were screened and tested on 21 isolates, and none of them showed sensitivity to cypermethrin at 0.05, 1.25, or 2.5 ppm by poison food method. The results of the experiment at 0.05, 1.25, and 2.5 ppm (as per EU standard) concentrations of chloropyriphos were screened and tested on 16 isolates and none of them showed sensitivity to chloropyriphos at 0.05, 1.25, or 2.5 ppm by poison food method. The results of the experiment at 0.1, 2.5, and 5 ppm (as per EU standard) concentrations of carbendazim were screened, and isolate CAR-8 exhibited sensitivity to carbendazim at 2.5 ppm using the poison food method, while at 0.1 and 5 ppm all 16 isolates showed complete growth on nutrient agar plate. The results of the experiment at 0.01, 0.25, and 0.5 ppm (as per EU std) concentrations of acephate were tested on 11 isolates, and none of them showed sensitivity to acephate at 0.01, 0.25, or 0.5 ppm using the food poisoning approach. The results of the experiment at 0.05, 1.25, and 2.5 ppm (as per EU standard) concentrations of metalaxyl were evaluated on 12 isolates, however none of them showed sensitivity to metalaxyl at 0.05, 1.25, or 2.5 ppm using the poison food technique. The results of the experiment at 0.02, 0.5, and 1 ppm (as per EU std) concentrations of captan on 22 isolates, one isolate CTG-10 which showed sensitivity at 1 ppm concentration of captan using the food poison technique. The results of the experiment at 0.07, 1.75, and 3.5 ppm (as per EU std) concentrations of spinosad were examined, and no isolate showed sensitivity to spinosad at 0.07, 1.75, or 3.5 using the poison food technique. The results of the experiment at 0.01, 0.25 and 0.5 ppm (as per EU std) concentrations of

chlorothalonil, no any isolate showed sensitivity to chlorothalonil at 0.01, 0.25 and 0.5 ppm using the poison food technique. The results of the experiment at 0.05, 1.25, and 2.5 ppm (as per EU std) concentrations of mancozeb were examined, and no isolate showed sensitivity to mancozeb at 0.05, 1.25, or 2.5 ppm using the poison food technique. Experiment results at 0.05, 1.25 ppm (as per EU std) concentration of 2-4-D were screened against 20 isolates, none of which showed sensitivity to 2-4-D at 0.05, 1.25 using the poison food technique. Isolate DIM-5 demonstrated sensitivity to 2-4-D at a concentration of 2.5 ppm, as shown in Table 19.

5.2 Heavy metals residues

The results of the experiment at 1.5 and 0.04 ppm (as per Indian standard Awasthi, 2000) concentrations of nickel were tested on 10 isolates. No isolate exhibited sensitivity to nickel at 1.5 ppm concentration, however isolate NIV-7 demonstrated sensitivity at 0.04 ppm concentration using the poison food technique. The results of an experiment with 14 isolates at 0.5 and 2.5 ppm (as per Indian standard Awasthi 2000) concentrations of lead were screened, and none of them showed sensitivity to lead using the poison food technique. The results of an experiment with 16 isolates at 0.01 and 30 ppm (as per Indian standard Awasthi *et al.*, 2000) concentrations of copper, isolate COM-2 showed sensitivity to copper at 0.01 ppm using the poison food method. The results of the experiment at 0.07 as well as 1.5 ppm (as per Indian standard Awasthi *et al.*, 2000) concentrations of cadmium were tested, and no isolate showed sensitivity to cadmium using the poison food approach. The results of the experiment at 1 and 20 ppm (as per Indian standard Awasthi, 2000) concentrations of chromium were tested, and no isolate showed sensitivity to chromium using the poison food technique. The result of an experiment with 12 isolates at 0.05 and 1.1 ppm (as per Indian standard Awasthi, 2000) concentrations of arsenic. None of the isolates showed sensitivity to arsenic using the poison food method.

5.3 Characterization of bacterial isolates

The result of morphological characterization of the five isolates CAR-8, CTG-10, DIM-5, NIV-7 and COM-2 showed that the cells of bacterial isolates CAR-8, DIM-5, NIV-7 and COM-2 were rod shaped, motile. Whereas cell of CTG-10 was vibrioid in shape and motile. Out of five isolates isolate COM-2 was gram positive rods

and another isolates were gram negative in reaction. Size of isolate CAR-8 (0.8 x 1 μm), CTG-10 (0.6 x 3 μm), DIM-5 (2 x 5 μm), NIV-7 (0.8x 2.2 μm) and COM-2 (0.6 x 3 μm). Among the isolates isolate CTG-10, DIM-5, COM-2 formed endospore or cyst. Result of cultural characterization on nutrient agar medium showed that two sensitive isolates CAR-8 and CTG-10 formed circular colonies with entire margin, raised, slight to moderate growth. DIM-5 formed circular colonies with entire margin, convex, moderate growth. NIV-7 formed mucoid colonies with entire margin, low convex, slight to moderate growth. Isolate COM-2 formed irregular colonies, undulate margin, low convex, moderate growth.

Biochemical and physiological characterization of five isolates showed that test of catalase and oxidase were positive for all five sensitive isolates. Test of urease activity positive for CTG-10 and COM-2 rest of three isolates showed urease negative. VP test and hydrogen sulphide production tests were negative for all isolates except one isolate DIM-5, CAR -8 and NIV-7 showed positive test for hydrolysis of gelatin, whereas remaining three isolate showed negative test of hydrolysis of gelatin. Test of starch hydrolysis positive for CTG-10 and COM-2. Whereas isolates CAR-8, DIM-5 and NIV-7 negative for starch hydrolysis. Casein hydrolysis test negative for all isolates except one COM-2. Growth of all five isolates (CAR-8, CTG-10, DIM-5, NIV-7 and COM-2) decreased with 7% NaCl concentration. All the five isolates were aerobic. Isolate CAR-8 well grow at 4⁰ C and 40⁰ C. Isolate CTG-10, DIM-5, and NIV-7 exhibited growth at 4⁰ C. Growth of isolate COM-2 at 4⁰, 40⁰, 41⁰ and 50⁰ C temperature were negative.

Conclusion-

1. The result obtained in this study generally revealed that many of bacteria which were isolated to detect the sensitivity for pesticides and heavy metal residue found in marketable fruits and vegetables showed normal growth on nutrient agar medium.
2. These isolates when used to detect sensitivity for respective pesticide and heavy metal residue at low concentration out of them very few Isolates CAR-8, CTG-10, DIM-5 showed sensitivity to carbendazim at 2.5ppm, captan at 1ppm and 2-4-D at 2.5ppm respectively and NIM-7 and COM-2 showed sensitivity to heavy metals like nickel at 0.04ppm and copper at 0.01ppm concentration.

3. Based on morphological, cultural, biochemical characterization isolates CAR-8, CTG-10, DIM-5, NIV-7 and COM-2 were found probably belonging to genus *Rhizobium spp*, *Azospirillum spp*, *Azotobactor spp*, *Pseudomonas spp* and *Paenibacillus spp* respectively.
4. Hence these finding of the study suggest that there are sensitive microbes found at low concentration of pesticides and heavy metals in soil and water sample collected from western railway track area. However the sensitive bacteria was found at higher concentration of carbendazim i.e 2.5 ppm and 2-4-D i.e 2.5ppm in soil and water sample collected from Rammandir and Malad of western railway track of Mumbai.
5. As it is simple alternative, easy, cheap and effective method require less equipment's for detection of pesticides and heavy metals residues, it has been concluded that the contamination of marketable fruits and vegetables of western railway track area can be detected by using these sensory microbial technique.

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*** Originals are not seen**

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in

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