"STUDY ON MOLECULAR CHARACTERIZATION AND ISOLATION OF HEAVY METAL REMOVING BACTERIA FROM E WASTE CONTAINING SOIL"

A Thesis

Submitted towards the Requirements for the Award of Degree of

DOCTOR OF PHILOSOPHY

IN

BOTANY

Under the Faculty of Science

Submitted By

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(Enrollment No. 161522617641)

Under the Supervision of

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P.K. UNIVERSITY

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This Piece of Work
is

Dedicated
To

My Beloved Parents

Whose blessings & love, brought me to this level.....

Mamta Prajapati.....

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Date:… … … … **Mamta Prajapati**

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Nomenclature	
As	arsenic
Cd	cadmium
CdCl2	cadmium chloride
DNA	deoxyribonucleic acid
gm	grams
Hg	mercury
M	molar
min	minutes
ml	millilitres
NCBI	National Canter for Biotechnology Information
nm	nanometers
Pb	lead
PCR	polymerase Chain reaction
ppm	parts per million
RNA	ribonucleic acid
µl	microlitres
µM	micromolar
ºC	degree Celsius

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Chapter -1

Introduction

1.1 HEAVY METALS As Pollutants

Onofre et al. (Onofre Monge-Amaya, Maria Teresa Certuchu-Barragan, Francisco Javier Almendariz-Tapia, and Gonzalo Mauricio Figueroa-Torres, 2015) demonstrate that heavy metals are natural constituents of the Earth's crust. They cannot be reduced or eradicated. Heavy metals can be categorized as either harmful or non-toxic. Densities, atomic masses, or atomic numbers are commonly utilized for its definition. Heavy metals are characterized by density in metallurgy, atomic number in physics, and chemical properties in chemistry. As a result, a definitive definition of heavy metals continues to be unattainable. Due to their propensity for bioaccumulation, they may occasionally present heightened dangers. This pertains to the gradual buildup of particular heavy metals in organisms relative to their environmental concentration.

Agricultural soils may occasionally get contaminated as a result of mining, the production of synthetic goods like batteries and pesticides, and industrial waste. Although they rarely exist at dangerous amounts, heavy metals are found in nature.

Lead, bismuth, and mercury fulfill the three previously listed criteria. Iron, copper, and tin, together with precious metals such as silver, gold, and platinum, were the first metals to be identified.

Some metals are generally non-toxic in nature but can be toxic in large amount. This type of metals are silver, ruthenium and indium, while some metals are potentially toxic like cadmium, mercury and lead. The principal sources of metal are mining and industrial waste, agricultural runoff, occupational exposure, and contact with lead-based paints.

Due to their higher density, heavy metals are often less reactive than lighter metals such as sodium. Nonetheless, certain heavy metals, such as zinc, lead, and mercury, exhibit characteristics like to lighter metals, while other light metals, including beryllium, scandium, and titanium, display features reminiscent of heavy

metals. Golf clubs, autos, plastics, solar panels, cell phones, accelerators, and antiseptics are composed of metal.

According to Doctor's Data Inc, 2012, metals such as chromium, arsenic, cadmium, mercury, and lead are the most dangerous in either their combined or elemental states. Lead is the most prevalent hazardous element on the planet. The fumes of mercury can be hazardous to humans; below is some general information regarding harmful heavy metals.

In electronic waste silver is more toxic than cadmium while cadmium is more toxic than lead. It can enter into the water body by industrial effluents.

1. Arsenic:

The sources of exposure to elevated arsenic levels include proximity to hazardous waste sites and regions with naturally high concentrations in soils, rocks, and water.

Exposure to elevated concentrations of arsenic can result in mortality.

2. Beryllium:

Exposure to this metal arises during mining, extraction, and the processing of beryllium-containing alloy metals. Beryllium exposure results in lung and skin diseases, as well as sensitization in a minority of affected workers.

3. Cadmium:

It is an extremely toxic metal found in industrial places where metals are being processed. Welders have much exposure to this metal which may cause death sometimes. It accumulates in the kidney which is responsible for the dysfunction of the kidney.

4. Hexavalent chromium:

Some chromates are human carcinogen like zink chromate, lead chromate, calcium chromate, chromium trioxide. Chromate and pigment containing chromate producing industrial workers may suffer with lung cancer.

5. Lead:

It is among the most hazardous metals to humans. Industries with significant potential exposure encompass construction, various smelting activities, radiator repair

facilities, and gun ranges. It affects the nerve system. It may affect cognitive capabilities and conduct.

6. Mercury:

This is also among the most hazardous metals. The primary sources of mercury exposure are the mining and production of mercury, along with the extraction and refinement of gold and silver ores. Elevated mercury exposure results in irreversible harm to the brain system and kidneys. Mercury tend to accumulate via food chain in the form of methyl mercury. In humans it can effect on brain and infants receive mercury via placenta causing persistent effect on children's mental development.

1.2 SOURCES OF HEAVY METAL

As mentioned by **Brian J. Alloway, 2012**, heavy metals are present in many metal sources. Like arsenic present in organic as well as inorganic components. It is present in sea living organisms in the form of Arsenocholine, Arsenosugars etc. In the same way arsenic is found in its inorganic form in organisms living on land. Industrially arsenic is used in pesticides production as well as in the production of herbicides and pesticides. It is also present in semiconductors.

Cadmium is derived from environmental sources including mining and smelting activities, pigments and paints, electroplating, electroplated components such as nuts and bolts, batteries, plastics, synthetic rubbers, photographic and engraving processes, outdated drums from copy machines, photoconductors, and photovoltaic cells.

Lead is derived from lead-based paints, batteries, industrial smelting and alloying processes, some solders, toys, ceramics, fuels, ammunition, and fishing sinkers.

Sources of mercury are dental amalgams, thermometers, barometers, laboratory equipments, batteries, fungicides, pesticides and paper industries. So all together we can say that the major source of heavy metal is electronics. According to **Neha Sadana, 2011** Electronics has undergone significant advancements in recent decades, and our daily lives increasingly incorporate electronic devices. Electronics

has significantly influenced every aspect of our lives. This can be demonstrated with the subsequent application of electronics:

Entertainment and Communication

The availability of affordable and rapid communication methods fosters a nation's advancement. Historically, the primary application of electronics was in telephone and telegraphy. Currently, we can convey any message from one location to another using radio waves, so obviating the necessity for wires. Radio and television broadcasting function as a medium for entertainment and communication. At present, electronic devices are widely employed for recreational activities.

Defense Applications

Defense applications are entirely governed by electrical circuits. RADAR, an acronym for Radio Detection and Ranging, is a significant advancement in electronics. Radar enables the detection and precise localization of adversarial aircraft. An automated control system can integrate radar and anti-aircraft guns into a cohesive unit.

Industrial Application

Electronic circuits are widely employed in industrial applications to regulate thickness, quality, weight, and moisture content of materials. Electronic amplifier circuits are employed to augment signals, thereby controlling the operations of automated door openers, power systems, and safety devices. Electronically controlled systems are utilized for heating and welding in industrial applications. The principal industrial application involves power stations producing thousands of megawatts of electricity, which are controlled by precise electronic devices and circuits.

Medical Services

Electronic systems are employed by physicians and researchers in the diagnosis and treatment of many illnesses. X-rays, electrocardiograms, shortwave diathermy devices, and oscillographs are instruments that have been employed in medical science to the present time. The application of electronics in medical science has markedly risen and is crucial for mitigating human suffering and sustaining life.

1.3 Electronic waste (e-waste)

E-waste, or electronic garbage, refers to discarded or nonfunctional electronic devices. Owing to rapid technological improvement, the majority of electronic devices become obsolete within a few years. E-waste includes electronics such as DVD players and VCRs, which have largely been supplanted by Blu-ray players, as well as older computers, telephones, computer monitors, fax machines, and printers (Bob Antonitis, Mary Backlund, B. Joslin, 2001). These electrical devices contain dangerous substances such as cadmium, beryllium, lead, mercury, chromium, arsenic, and PVC, presenting risks to workers, especially in developed nations. Hazardous electronic waste encompasses, among other items, televisions and computer monitors including cathode ray tubes, LCD desktop monitors, LCD televisions, plasma televisions, and portable DVD players equipped with LCD screens.

The disposal of electronic trash is an escalating issue due to the presence of dangerous compounds in electronic equipment. Personal computers contain lead in cathode ray tubes, mercury in switches and housings, and cobalt in steel.
According to the reports from

Mazingira Safi, 2017, The National Environmental Management Authority has issued guidelines for e-waste management in Kenya, specifying the classifications of e-waste as follows.

ICT and Telecommunication equipment

These types of equipment contain mainframes, laptop, printer, networking equipment, video cameras, video recorders, musical instruments, personal computers etc.

Office electronics

Pocket and desk calculators, electronic type writers, telephones, photocopying equipment can be included in this category.

Household Appliances

Domestic instruments may also be included in this category. Examples include refrigerators, washing machines, microwaves, electric fans, air conditioners, exhaust ventilation systems, electric heating devices, vacuum cleaners, toasters, fryers, clocks, hair dryers, water dispensers, and equipment for sewing, knitting, and weaving.

Consumer equipment's

This sector encompasses industrial and agricultural equipment. Machinery for milling, sawing, grinding, cutting, drilling, punching, folding, metal fabrication, riveting, welding, soldering, along with tools for mowing or other horticultural activities, and sewing machines, among others.

Toys, leisure and sports equipment

This encompasses all electronic toys, including electric trains, automobile racing sets, video games, cycling computers, diving apparatus, jogging devices, rowing equipment, and sporting gear with electronic functionalities.

Lighting

This category encompasses fluorescent tubes, lamps, high-intensity discharge lights, and halogen lamps.

Medical equipment

Some medical equipments like stethoscopes, cardiology equipment, laboratory equipment, dialysis equipment, scanners, pulmonary ventilators and other electric equipments can be considered here.

Automatic dispensers

This category of e-waste includes automatic beverage dispensers, temperature-controlled bottles or cans, solid items, and monetary transaction devices.

Surveillance and regulation apparatus

Smoke detectors, measurement devices, weighing machines, and certain laboratory equipment fall under this category.

Batteries

Lead, nickel and cadmium batteries are considered here.

1.4 SOURCES OF E-WASTE

According to Sushant (2011), sources of e-waste might be diverse. All electronic devices are the predominant causes of e-waste. Examples of such devices include DVD players, laptops, mobile phones, MP3 players, televisions, and several

others. In addition to IT products, several electrical and domestic appliances also constitute e-waste. These items include refrigerators, washing machines, air conditioners, and similar appliances.

1.5 E-WASTE POLLUTION

As stated by Nicole Mortillaro in 2015, e-waste contamination is a global issue today. The United Nations indicates that electronic trash is escalating by 40 million tons annually. Australia possesses around 9.2 million computers, with an additional 2.1 million recorded in 2006.

Electronic waste components, like as CPUs, contain potentially harmful substances, including heavy metals like lead, cadmium, beryllium, and mercury. These metals significantly contribute to environmental pollution.

Cathode ray tubes utilized in televisions, computer monitors, ATMs, video cameras, and many devices include lead, barium, and more heavy metals that leak into groundwater and release poisonous phosphorus.

Printed circuit boards emit air pollutants and discharge glass dust, tin, lead, beryllium, cadmium, and mercury into waterways. Chips and other gold-plated components include polycyclic aromatic hydrocarbons (PAHs), heavy metals, and brominated flame retardants, which are discharged directly into rivers, resulting in the acidification of aquatic ecosystems and flora. Heavy metals pollute both surface water and groundwater. Plastics from printers, keyboards, and monitors emit brominated dioxins, heavy metals, and hydrocarbons.

Computer wires contain polycyclic aromatic hydrocarbons (PAHs) that are emitted into the air, water, and soil.

1.6 Bioremediation of e-waste

Bioremediation is a comprehensive concept encompassing all actions aimed at biotransforming an environment that has been changed by pollutants.

P. Rajendran et al. (P. Rajendran, J. Mathkrishnan& P. Gunasekaran, 2003) demonstrate that microbiological techniques can be utilized to extract metals from e-waste materials. Certain species, particularly bacteria such as Thiobacillus thioxidans and Thiobacillus ferroxidans. Certain fungus, such as Aspergillus niger and

Penicillium simplicissimum, can proliferate in the presence of electronic waste. These organisms will produce both organic and inorganic acids to extract metals from e-waste. Both fungal strains successfully mobilized copper and sulfur by 65%, and aluminum, nickel, lead, and zinc by over 95%. Thiobacilli successfully leached over 90% of the accessible copper, zinc, nickel, and aluminum. Lead was precipitated as lead sulfate, whereas selenium was precipitated as selenium oxide.

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1.7 INDIAN SCENARIO OF E-WASTE

As shown in Sushant et al (**Sushant B. Wath, P.S. Dutt, T. Chacrabarti , 2007**), after 1990, the e-waste associated problem started increasing due to the competition between foreign and Indian companies for brand, quality, price The electrical and consumer durable industry is experiencing growth in India.

According to TAAI, there were around 113.26 million cellular customers are added in India in the year 2008 and it is continued to add average 9.5 million every month. Cellular market has been increased to 261.97 million in 2007-2008 than in 2003-2004, that was around 168.11 million. The usage and selling of every equipment like washing machines, microwave, refrigerator etc has been increased in present days than past. The usage of colored televisions surged thrice by 2007.

Solid waste management in India presents a significant challenge, further intensified by the presence of e-waste. The primary electronic waste in India consists of computer refuse. The principal states contributing to WEEE encompasses Maharashtra, Andhra Pradesh, Tamil Nadu, Uttar Pradesh, West Bengal, Karnataka,

Gujarat, Madhya Pradesh, and Punjab. The principal cities generating WEEE are Mumbai, Delhi, Bangalore, Chennai, Kolkata, Ahmedabad, Hyderabad, Pune, Surat, and Nagpur.

Maharashtra generates around 20,270.6 tones per year, while Tamil Nadu and Andhra Pradesh generate around 13,000 tones e-waste per year. Gujarat generates 8,994.3 tones of WEEE where as Lakshdweep is lowest e-waste producer with 7.4 tone.

In 2016, the global production of electronic garbage (e-waste) reached 44.7 million tons, sufficient to construct nine Great Pyramids of Giza, as reported by a United Nations-supported study in 2017. This include two million tonnes produced in India, which possesses one of the world's rapidly expanding electronics sectors. According to a Hindustan Times report from 2018, India's e-waste generation is projected to reach three million tonnes in that year. According to an Assocham-KPMG investigation, industries account for 70% of e-waste generation, while around 15% originates from homes.

Mobile and telecommunications equipment constitutes around 12% of India's total e-waste production. In January 2017, India's mobile subscriptions exceeded 1.2 billion, as reported by the Ericsson Mobility Report, while the global total reached 7.6 billion in the first quarter of the year.

1.7.1 E-WASTE MANAGEMENT SYSTEM IN INDIA

According to Rajiv Ganguly (2016), the e-waste management process in India encompasses manual collection, segregation, dismantling, recycling, and disposal. Inadequate handling of electronic waste has transpired in numerous cities, encompassing the open incineration of plastic refuse, exposure to toxic substances, disposal of acids, and widespread indiscriminate dumping. It may lead to soil pollution.

India lacks an adequate e-waste management system. The dismantling and recycling procedure conducted by individuals with limited education and lacking enough protection. Various tools such as hammers, drills, cutters, electric torches, burners, and electric drills were utilized in the disassembly process. Such operations are conducted in densely populated urban centers. The processing room lacks

adequate ventilation and illumination. The employment of these incorrect and unsuitable practices poses significant occupational health risks to workers and laborers.

The government and business sectors in India produce most of the country's e-waste, which makes up more than 70% of all trash. Home appliances make up 15%, while TVs and PCs (including computers) make up 68% and 27%, respectively. The record for the amount of e-waste in India comes from a study done by the Indian Market Research Bureau (IMRB) in 2009 on how e-waste is made at the source. One person died and six were hospitalized after the Cobalt-60 radiation event in Mayapuri, Delhi. This event showed how much toxic waste, especially electronic waste, is being produced in the country and how badly it is being disposed ofthe primary issue of industrialization and industrial advancement is the production of garbage. Generated trash necessitates effective management, prompting governments to establish regulated waste management frameworks to reconcile developmental goals with environmental sustainability.

1.7.2 Expansion of the Electrical and Electronic Sector in India

One report from 2009 says that India's first electronics business began to grow around 1965. A lot of people started using electronics like transistor radios, black-and-white TVs, calculators, and other audio goods.

The continuous and rapid growth of electronics industry had been occurred between 1989 and 1990. It is considered as ‘Golden Period’.

1. Computers and Computer Components Segment

Computers and their components are the primary catalysts of the electronics sector. The sale of personal computers had significant growth from 3.1 million in 2003-2004 to 7.3 million in 2007-2008. The main people who use the IT market are the national and state governments, as well as small and medium-sized businesses, banks, telecom companies, and schools.

2. The Consumer Electronic (Television) Segment

This chapter discusses how the emergence of LCD (Liquid Crystal Display) and plasma panels has transformed the experience of television watchers. The

Corporate Catalyst India study on the Consumer Durable Industry indicates that television sales are projected to increase from 886,000 units in 2005 to 11,795,000 units in 2010.

3. The Telecommunication Segment

The number of mobile phone users is increasing daily. Presently, there are approximately 617.53 million mobile phone customers in India, in contrast to 36.39 million fixed line subscribers. It means the number of mobile users are huge as compared to fixed line users. The cell phone users are continuously added every month and it will be maintained in coming years.

1.7.3 Projection and Recycling of E-Waste in Four Major Cities

According to the 2011 reports from the Research Unit (LARRDIS), Rajyasabha, New Delhi, Delhi and Mumbai serve as the primary centers for e-waste recycling. Hyderabad and Bengaluru are significant centers for e-waste recycling and have been pivotal in the electronic and information technology sectors.

DELHI

Delhi ranks among the top ten cities producing e-waste. The 2007 survey on garbage production in Delhi. Approximately 5000 metric tons of hazardous garbage are generated annually. Delhi is the preeminent center for e-waste processing.

The last GTZ study, which was done in 2007, found that between 10,000 and 20,000 tons of electronic waste were processed each year by about 25,000 workers. Seelampur Market is known as the biggest market in the country for disassembling electronics and is also known as the e-waste center in Delhi. The fact that garbage processing is illegal in Delhi makes it impossible for the government to get a good estimate of how much trash is made and recycled there.

MUMBAI

Mumbai is the top waste-producing metropolis among all Indian cities. The Mumbai-Pune industrial corridor is a prominent location for the manufacturing of electronic goods in the country. In Mumbai, the market for e-waste is split up into several areas, each of which is in charge of a different part of recycling. WEEE was moved from the Pune Pimpri Chinchwad Region to the Mumbai Metropolitan Region

(MMR) to be treated further. In 2009, a facility was built to recycle e-waste scientifically, and it should start running in 2010. The first part of the project will be able to handle about 7,500 tons per year, but this will later be increased.

A national e-waste assessment study says that of all the towns in India, Mumbai makes the most trash. Maharashtra makes 20,270.6 tons of electronic trash, with Navi Mumbai making 646.48 tons of that. 2584.21 tons for Pune and 1032.37 tons for Pimpri Chinch wad tons.

BENGALURU

It is the center of science in India to be in Bengaluru. E-waste recyclers send bits of broken electronics to Delhi and Mumbai every other day after they have been sorted and taken apart. In Karnataka, there are places to recycle such as Ashes Recyclers, K. G. Nandini Recyclers, Ash Recyclers, New Port Computer Services India Pvt. Ltd., and E-R3 Solutions Pvt. Ltd. Some places that recycle can only handle about three tons of trash every day, but they can process about one ton of trash every day.

IT companies and electronics makers in and around Bengaluru make between 8,000 and 10,000 tons of e-waste every year, according to industrial studies. Some people who sell scrap hire women and kids to work with this dangerous stuff. The rest of the metals that don't work are dumped in fields and waterways or burned in dangerous situations. It will hurt the people who work for scrap dealers and pollute the air, soil, and groundwater.

Every year, computers, printers, TVs, and cell phones in Bengaluru create 6,743,87 million tons of e-waste. The estimated amount of e-waste in Bengaluru was 1.71 crore kilograms, which was made up of 92,240 laptops, 15,371 TVs, and 15,982 cell phones. In the future, 130,383 kilos of e-waste is expected to be made up of 97,310 computers, 16,214 TVs, and 16,859 cell phones. To deal with this problem, the Karnataka State Pollution Control Board (KSPCB) set up a formal way to recycle electrical waste.

Bangalore's expanding technology waste. However, awareness regarding the management and processing of e-waste is exceedingly inadequate..

Individuals operating in hazardous conditions emit lead, mercury, and other toxins into the atmosphere, while employing acids to extract precious metals from devices. The residual waste is indiscriminately discarded, allowing pollutants to infiltrate groundwater.

HYDERABAD

The yearly e-waste generation is projected at 3,263,994 million tons, consisting of 3,111.25 million tons from computers, 86.46 million tons from printers, 61 million tons from televisions, and 5.284 million tons from mobile phones. In 2010, the e-waste produced by a population of 7.442 million was 98.163 kilograms, consisting of 42,869 computers, 53,581 televisions, and 1,713 mobile phones.

Andhra Pradesh is home to two official recycling facilities: Earth Sense Recycle Pvt. Ltd. and Ramky E-waste Recycling Facility. Earth Sense Recycler has constructed recycling facilities in Hyderabad in collaboration with e-Parisara of Bengaluru.

1.7.4 BEST AVAILABEL PRACTICES

Extended producer responsibility (EPR) is an environmental policy mandating that producers assume accountability for the whole life cycle of a product, especially regarding its collection, recycling, and ultimate disposal. These elements must be integrated into the legislative framework, rendering Extended Producer Responsibility (EPR) a mandatory requirement associated with the production of electronic and electrical equipment within a specified timeframe.

The RoHS rule for electronic and electrical equipment indicates a growing trend to minimize the use of hazardous compounds, including lead, cadmium, mercury, polychlorinated biphenyls, and other harmful materials for which acceptable alternatives have been identified. Numerous nations have implemented the RoHS directive in the production of electrical and electronic devices.

1.7.5 E-WASTE MANAGEMENT IMPLICATIONS

Sharon Lam (2016) states that India generates a substantial quantity of e-waste; nevertheless, there are no systematic or official methods for its handling. The prevalence of ferrous metals, nonferrous minerals, polymers, and valuable substances

in e-waste has become a notable business opportunity for many. Dismantling is the only procedure performed in the e-waste recycling process. In e-waste dismantling, several tools are utilized to separate hazardous components from waste and recover valuable resources. The physical recycling process often encompasses screening, shape separation, magnetic separation, electric conductivity-based separation, and density-based separation, among other techniques.

On Friday, the Assocham industry body announced that India's e-waste creation is projected to increase around three to fourfold from the current 1.8 million metric tons to 5.2 million metric tons annually by 2020, indicating a compound annual growth rate (CAGR) of roughly 30%. The Assocham-cKinetics analysis indicates that the worldwide e-waste volume is expected to increase from 93.5 metric tons in 2016 to 130 metric tons in 2018, representing a compound annual growth rate (CAGR) of 17.6 percent over this timeframe.

Only approximately 1.5 percent of India's total e-waste is recycled due to inadequate infrastructure, legislation, and frameworks, resulting in the depletion of natural resources and irreversible harm to the environment and the health of individuals employed in the business. More than 95 percent of e-waste is handled by an informal sector and scrap dealers, who disassemble discarded items rather than recycling them.

Approximately 400,000 to 500,000 child laborers aged 10 to 15 years are engaged in various e-waste operations without any safety precautions or safeguards.. The chamber emphasized the necessity for robust legislation to prohibit the involvement of child laborers in e-waste collection, segregation, and distribution. Approximately 213 e-waste workers in India experience respiratory issues, including dyspnea, irritation, coughing, choking, tremors, asthma, and bronchitis, attributable to inadequate safety measures and dismantle facilities.

Computers, televisions, and mobile phones are the most hazardous electrical devices due to their high concentrations of lead, mercury, and cadmium, coupled with their limited lifespans, resulting in increased disposal rates. In India, the primary contributors to electronic trash, accounting for 75 percent of waste output, are the government, public, and commercial sectors, whereas households contribute a mere

1.6 percent, with manufacturers accounting for the remainder. Households in India are significant potential generators of e-waste due to their consumption of various electrical and electronic devices.

The manufacturing of e-waste is contaminating both groundwater and air, as e-waste comprises 40 percent lead and 70 percent of other heavy metals, all of which are potential pollutants contributing to soil acidification. Extended exposure to heavy metals released during hazardous e-waste recycling results in harm to the nervous system, circulatory system, kidneys, and brain development, as well as respiratory illnesses, dermatological issues, lung cancer, and damage to the heart, liver, and spleen, the study said.

The Indian government has established new regulations regarding the responsibilities associated with the recycling of electronic components, products, and waste. We mostly have two issues. Firstly, many electronic devices include materials that are unacceptable to us. Certain light bulbs contain mercury, mobile phones contain arsenic, and Cathode Ray Tubes (CRT) contain lead. Second problem is the same electronics contain many valuable metals like there is gold in the contacts, tin in the solders, CRTs contain a little slug of tungsten. In fact, it is not an issue; however, individuals will be motivated to gather and recycle such devices. The challenge arises from the fact that it is only financially advantageous to partially recycle these items. The extraction of gold, tin, and copper is straightforward; but, post-extraction, individuals typically dispose of the residual materials by partial recycling.

In 2014, India reportedly generated about 1.7 million tons of e-waste, with an annual increase of 4 to 5 percent. A range of exports has cautioned about its environmental and health hazards. These problems occur due to the manual disassembly of outdated computers, phones, and other electronic gadgets for valuable metals, which are frequently burnt in a primitive fashion. Residues are often disposed of in rivers, sewers, and landfills, which can ultimately result in the deterioration of land and water quality, as well as neurological and dermatological problems, genetic mutations, and cancers among those who manage them.

Under EPR, all products must establish targets for e-waste collection in the forthcoming years. During announcement of new rules, Environment Minister

Prakash Javadekar stated that, "The regulations have been intensified and demonstrate the government's dedication to environmental governance." Producers are now responsible for the collection and disposal of electronic trash. The new regulations mandate that dealers and merchants must repay the amount in accordance with the take-back system or deposit reimbursement scheme instituted by the producer to the e-waste depositor. www.forbes.com

Hal Watts, a designer educated in the sustainability branch of The Royal College of Art's sustain RCA, has created a bicycle-powered gadget that recovers precious copper from electronic equipment.

The ubiquity of copper in circuit boards and most wires makes it a sought-after resource for those who scavenge e-waste to sell the recovered copper.

Watts, 2012 Most recycling systems have been modeled after large Western recycling facilities, making cost-effective technology inaccessible to smaller recyclers who lack the volume to justify investing in such expensive machinery. In many regions, recycling begins in the informal sector, where recyclers dismantle waste and sell materials like copper, plastic, and circuit boards to different buyers. These collected materials eventually reach exporters, who then supply them to recycling plants, often located in urban areas. Countries like Singapore, Belgium, and Japan operate smelting plants capable of extracting valuable metals invisible to the naked eye. In India, with widespread access to mobile phones, televisions, and computers, a growing middle class is rapidly contributing to electronic waste. Karma Recycling offers a solution by acquiring old electronic devices, either refurbishing or dismantling them to sell individual components. They also provide logistical services for handling large quantities of defective or discarded electronics. Akshat Ghiya, co-founder of Karma Recycling, stated in 2014 that e-waste is among the fastest-growing waste streams globally, necessitating scientific recycling; failure to do so will result in irreparable harm to the environment and those handling it. Companies design innovative and enhanced gadgets daily, monthly, and annually. Once all these devices have been utilized, it will exacerbate the problem of electronic waste. It is imperative for society to acknowledge that what has been utilized must be recycled or repurposed.

1.8 GLOBAL SCENARIO

As shown in Mohan and Bhamawat (D. Mohan and P.M.K. Bhamawat, 2008), Research Unit (LARRDIS), Rajya Sabha, New Delhi, 2011 and Widmer R., 2005, WEEE include any broken or unused or unwanted electrical as well as electronic equipment. It is a broad phrase including diverse types of electric and electrical devices that have lost all value to their owners. Numerous nations have established their own definitions. One of the most frequently accepted definitions is according to the EU (European Union). The definition is: 'electrical or electronic equipment that is waste, encompassing all components, subassemblies, and consumables that are part of the product at the time of disposal.'

The main challenge lies with densely populated and developing nations like China and India, where the estimated annual waste generation is around one kilogram per person. These countries have legally and illegally imported both used Waste Electrical and Electronic Equipment (WEEE) and Electrical and Electronic Equipment (EEE). The large-scale and often unethical export of e-waste from developed nations to countries like India, China, and Pakistan has shifted the environmental and health burdens of technological progress onto communities that lack the necessary infrastructure to manage such waste effectively.

The Basel Convention on the Control of Transboundary Movements of Hazardous Waste and Its Disposal plays a crucial role in regulating the increasing flow of e-waste from OECD to non-OECD countries. To mitigate the environmental hazards posed by e-waste in Europe, the EU has introduced two key directives: the Waste Electrical and Electronic Equipment (WEEE) directive and the Restriction of Hazardous Substances (RoHS) directive. The WEEE directive has been widely adopted and implemented across multiple nations to ensure responsible e-waste management.

1. UNITED KINGDOM:

E-waste generated from various electrical and electronic equipment falls under the scope of the WEEE directive, an EU legislation that was incorporated into UK law following parliamentary approval in 2007. This Act assigns the responsibilities of reporting, financing, and ensuring compliance to producer compliance scheme

operators rather than individual producers. However, each producer remains responsible for registering its members with the appropriate national regulator and providing details of the equipment they manufacture. All producers, preprocessors, and exporters must register with the producer compliance program by paying a fee, which is considered an operational cost for system administration. The national regulator determines the domestic WEEE allocation for each compliance program, and operators must ensure that the designated e-waste is processed using the most efficient treatment, recovery, and recycling methods.

2. USA:

The U.S. Environmental Protection Agency has introduced the Green National Electronics Action Plan (NEAP) to address environmental concerns related to electronic waste. This plan primarily focuses on computers, televisions, and mobile phones. However, the United States has not ratified the Basel Convention, and there is no federal legislation regulating e-waste generation, disposal, or export. Over the past two years, several states have taken initiatives to collect and recycle e-waste from residential and commercial consumers in an environmentally responsible manner. Currently, fifteen states have enacted producer responsibility laws. Additionally, California has implemented a system requiring consumers to pay an Advance Recycling Fee (ARF) at the time of purchasing new electronic products, with charges starting at $10 for items such as TVs, laptops, and monitors.

The U.S. National Center for Electronics Recycling (NCER) and MIT conducted a detailed analysis of the generation, collection, and export of obsolete electronics in the United States. In 2010, the U.S. generated approximately 258.2 million units of used computers, monitors, televisions, and cell phones, with 171.4 million units collected for recycling and 14.4 million exported. Among electronic waste exports, cathode ray tubes (CRTs) were shipped in the highest volume, while mobile phones were exported in the largest quantity compared to other electronic devices.

3. PEOPLE'S REPUBLIC CHINA

China has put legal frameworks in place to control and reduce the risk of electronic waste. The Administration of Control of Pollution from Electronic

Information Products governs e-waste management within China. Producers and designers of electronic devices are mandated to design and make their products to national industrial standards. The administration imposes penalties on importers, sellers, manufacturers, and designers who do not adhere to the regulations.

4. INDIA

In India, the Ministry of Environment and Forests (MoEF) is responsible for legislating waste management and environmental protection. Although MoEF approved guidelines for the environmentally sound management of e-waste through letter number 23-23/2007-HSDM dated March 12, 2008, there is no specific law addressing the e-waste issue. These guidelines aim to identify various sources of electrical and electronic equipment and establish protocols for producers to manage e-waste responsibly. However, most hazardous materials found in e-waste are regulated under The Hazardous and Waste Management Rules, 2008, which classify waste as either hazardous or non-hazardous.

5. ASIA

The escalation of e-waste in Asian nations is intensified by low-cost labor and lenient regulations. Approximately 47% of waste, including electronic waste, has been illicitly transported from European seaports. In 2003, the United Kingdom sent 23,000 metric tons of undisclosed electronic waste to the Far East, India, the United States, and China. Japan and South Korea illicitly export significant quantities of e-waste, comprising mobile phone chargers, computers, air conditioners, printers, cameras, and other electronic refuse, to China. The United States illicitly exports 50-80% of its garbage.

E-waste from the USA, Europe, and Japan has been discarded in Hong Kong, rendering it a dumping ground for electronic debris. Soil analyses have revealed significantly elevated levels of lead and several other hazardous elements, leading to the estimation that 10-20% of abandoned computers are disposed of in landfills. Taiwan also lacks sufficient waste management infrastructure. Delhi, India, managed between 10,000 to 20,000 tons of e-waste annually, with around 25% comprising computers. Other Asian nations dispose of approximately 12 million tons annually. Thailand produces a substantial volume of e-waste associated with mobile phones and

batteries, and the disposal locations for the abandoned devices and batteries remain unidentified.

6. AFRICA

According to data from the International Telecommunication Union (ITU), Africa experienced the fastest-growing mobile phone market globally between 1998 and 2005, with approximately 518 million mobile subscribers recorded. By 2010, the number of subscribers was projected to reach between 100 and 200 million. In 2003, Africa had an estimated 62 million televisions, 200 million radios, and between 1.5 to 7.5 million personal computers. Basel Action Network states that more than 400,000 secondhand PCs are imported into Nigeria's Lagos every month, while 1.2 to 1.5 million computers flow into the South African market each year.In South Africa, three primary companies manage e-waste: Universal Recycling Company, DescoElectronic Recyclers and African Sky have limited capacities and are unable to handle the growing volumes of e-waste effectively. Desco processes approximately 400 tons of PC boards and 2,000 tons of various electronic waste annually, while Universal Recycler processes around 1,800 tons per year, accounting for only 2% of the total waste stream. In Gauteng, a landfill receives 2.2 tons of e-waste each month, though the specific contents remain unidentified. Despite the presence of hazardous substances like arsenic and mercury in e-waste, no targeted measures are in place to mitigate environmental risks. South Africa exports a substantial portion of its processed e-waste to Asia and Europe.

7. OCEANIA

In Oceania, e-waste generation reached 0.6 million tons in 2014, with Australia, New Zealand, and Papua New Guinea being the largest contributors. These countries produced approximately 0.47 million tons, 0.09 million tons, and 0.0008 million tons of e-waste, respectively. Among them, only Australia has national regulations governing the disposal of obsolete computers and televisions. Waste management in Australia is primarily managed by state and territorial governments, which delegate responsibilities to local authorities. The Product Stewardship Act of 2011 provides a legislative framework for national product stewardship programs, requiring importers and manufacturers of televisions and computers exceeding a

certain threshold to participate in and fund a co-regulatory scheme. This regulation compels the industry to finance collection and recycling programs, with annual recycling targets set as a percentage of the estimated total television and computer waste generated in the country.

In Australia, the responsibility for disposing of mercury-containing lamps falls primarily on state and municipal governments, as there is no nationwide regulation for their collection and recycling. Many states prohibit the disposal of large quantities of these lamps in landfills. A national voluntary program facilitates the recycling of mercury-containing bulbs from commercial and public lighting sectors. Between 2012 and 2013, Australia recycled approximately 40,813 tonnes of discarded televisions and computers, achieving 98.8 percent of the national recycling target. However, this amount accounted for only 8.7 percent of the total e-waste generated across all product categories. The obligation for the disposal of mercury-containing lamps primarily lies with state and municipal governments in Australia, due to the absence of national regulations for their collection and recycling. The landfill disposal of substantial amounts of mercury-laden lamps is forbidden in numerous states. A national voluntary initiative is in place for the recycling of mercury-laden bulbs from the commercial and public lighting sectors. In Australia, almost 40,813 tons of obsolete televisions and computers were recycled in 2012 and 2013, accounting for 98.8 percent of the national recycling target. Nevertheless, this sum represents only 8.7 percent of the overall e-waste generated from all product categories.

1.8.1 GLOBAL E-WASTE VOLUME IN 2014

The 2014 UNU report states that global e-waste, defined as discarded electrical and electronic equipment, reached 41.8 million tons in that year. In 2014, almost 60% of global e-waste comprised discarded kitchen, laundry, and bathroom appliances. In 2014, personal information and communication technology equipment, such as cell phones, personal computers, and printers, constituted 7% of electronic waste. The specific e-waste in 2014 comprised:

1 12.8 million tons of small equipments like vacuum cleaners, microwavws, toasters, electric shavers and video cameras;

2 11.8 million tones of large equipments such as washing machines, cloths dryers, dish washers, electric stoves and photo voltaic panels;

3 7.0 million tones of temperature exchange- cooling and freezing equipments;

4 3.0 million tones of screens;

5 3.0 million tones of small information and communication technology (ICT) equipments.

6 1.0 million tones of lamps.

This electronic garbage contains around US$52 billion in potentially reusable materials; nevertheless, a little quantity has been gathered for recovery or disposed of in an environmentally responsible manner. It is estimated that fewer than one-sixth has been adequately recycled or rendered suitable for reuse.

The UNU studies estimate that e-waste discarded in 2014 contained approximately 16,500 kilotonnes of iron, 1,900 kilotonnes of copper, and 300 tonnes of gold, in addition to significant amounts of silver, aluminium, palladium, and other potentially recoverable materials, with a total estimated value of US$52 billion. It also has significant quantities of health-hazardous contaminants, including mercury, cadmium, chromium, and ozone-depleting chlorofluorocarbons (CFCs). Research indicates that in 2014, only two countries— the United States and China—accounted for roughly one-third of global e-waste disposal.

According to data collected by the United Nations Organization, in collaboration with governmental, non-governmental, and scientific entities under the 'Solving the E-waste Problem (StEP) initiative,' e-waste generation is projected to increase by one-third over the next five years, primarily driven by the United States and China. Various nations delineate e-waste in markedly distinct manners. For instance, the U.S. categorizes e-waste solely as consumer electronics like televisions and laptops, but European nations encompass all items with a battery or power cable under the e-waste classification. Last year, the world generated around 54 million tons, or 49 million metric tons, of abandoned or used electrical and electronic equipment. The average weight was approximately 43 pounds (20 kilograms), equivalent to eight bricks, for each of the 7 billion individuals on Earth.

It is anticipated that the globe will generate approximately 33 percent more e-waste, totalling 72 million tons (65 million metric tons), by the year 2017. In 2012, China was the first nation globally in e-waste generation. The generation of e-waste reached approximately 12.2 million tons, with the United States contributing over 11 million tons. In 2003, the city of Toronto, Canada commenced the transportation of its waste to a landfill located in suburban Detroit. Consequently, Michigan residents expressed grievances, and legal procedures have been initiated about the transboundary disposal of trash. The U.S. government needed to devise a way to prevent environmental litigation and allegations of class bias due to Canada's export of trash to an impoverished city like Detroit. Wastewatcher is an entity established by the EPA's Office of Environment and Compliance Assistance that oversees and provides compliance assistance information for the transportation of solid waste across the U.S.-Canadian border.

Waste Watcher primarily examines the trafficking of Canadian municipal waste into Michigan.

The distance between Canada and the United States is rather minimal comparing to other regions. Locations where the United States has been exporting its electronic garbage. The regulation of large-scale e-waste exports to foreign nations has been significantly inadequate. The exportation of e-waste to underdeveloped countries for profit has been practiced for at least two decades, however the first individual was not incarcerated for this offense until 2014. Joe Benson from the U.K. had been unlawfully shipping tons of hazardous electronic garbage to Africa, notably to Ghana, Nigeria, Ivory Coast, and the Congo. Benson received a sentence of sixteen months' imprisonment. He was convicted in 2011 for exporting hazardous garbage to Nigeria and was appealing that conviction while continuing to unlawfully sell obsolete televisions and refrigerators.

A significant portion of the world's electronic waste, emblematic of elevated living standards and economic prosperity, paradoxically accumulates in densely populated areas of underdeveloped nations. The predominant countries include China, India, Ghana, Nigeria, and the Ivory Coast. Electronic recycling is a very recent initiative in Illinois alone. A thorough recycling law was enacted in 2008, providing Illinois residents with enhanced possibilities to recycle their electronic waste. Chicago

has a permanent Chemical and Computer Recycling Facility, enabling residents to responsibly and safely dispose of electronic and hazardous products locally, rather than exporting them to poor nations and causing environmental harm.

In the late 1980s, the global community was incensed to discover 'toxic merchants', organizations that transported hazardous garbage to developing nations with dangerous and uncontrolled recycling practices. The Basel Convention, an international convention aimed at minimizing the transboundary transport of hazardous waste, was formulated and ratified as a result of this relationship.

1.8.2 E-WASTE EXPORT IS IN THE LIME LIGHT

According to a report by James et al. (James Gill, Geirdie Gill, and Hamish Gill, 2003), despite 150 countries ratifying the Basel Convention, the export of waste to unregulated recycling facilities in developing nations remains a pressing global issue.

A recent report by the Basel Action Network and the Silicon Valley Toxics Coalition highlights this concern, revealing that 50 to 80% of e-waste collected for recycling in the United States is sold to developing countries such as India, China, and Pakistan. This practice leads to environmental pollution and exposes local populations to hazardous contaminants.

Once Australia signed the Basel Convention in 1992, the exportation of hazardous waste was permitted only with a permit, issued only if it was possible to establish that the waste would be dealt with in an environmentally sound manner in the country of importation. Exportation of hazardous waste without a license is a crime under the Hazardous Waste Act, punishable with up to $1 million in fines or imprisonment for a period not exceeding five years.

Australia is expected to export an increasing volume of waste, valued at approximately $20 million annually, to China, India, and several other Asian nations. However, the country has frequently been criticized for failing to fully comply with the Basel Convention.

Objectives

The current study has been conceptualized with following objectives in the view:

1. To isolate bacteria capable of degrading e-waste.
2. To characterize and identify isolates by microscopic, biochemical and molecular method.
3. To optimize media for growth and metal removing ability of isolates.
4. To check potential of isolates to remove heavy metals under laboratory conditions.
5. To characterize potential isolates through molecular aspects

Chapter -2

Literature Review

Introduction

An alarming amount of electronic waste, or "e-waste," has accumulated as a result of the electronic industry's explosive growth in recent decades. This waste is a major source of heavy metal contamination in the environment. Toxic metals found in e-waste, including lead, cadmium, chromium, nickel, arsenic, and mercury, can seriously disturb soil ecosystems and endanger the health of people, animals, and plants. These dangerous substances are released into the soil during the disposal and breakdown of e-waste, degrading the soil's quality and altering the composition of the local microbial community. In this regard, microbial bioremediation has become a viable, economical, and environmentally beneficial substitute for traditional physical and chemical remediation methods.

Through a variety of processes, including biosorption, bioaccumulation, biotransformation, and precipitation, microorganisms—especially bacteria—are essential to the detoxification and elimination of heavy metals. In order to survive and multiply in contaminated environments, some bacterial strains have developed special adaptations, such as genes that resist heavy metals and metal-binding proteins. In addition to helping with precise identification, molecular characterization of these bacteria sheds light on their resistance mechanisms and possible uses in bioremediation.

Over the past ten years, a number of studies have been carried out worldwide with the goal of separating bacteria resistant to heavy metals from contaminated areas, such as soils from e-waste dumps. To identify and characterize microbial strains that can tolerate and remove heavy metals from polluted soils, these studies have combined conventional culture-based methods with cutting-edge molecular tools like whole genome sequencing, Random Amplified Polymorphic DNA (RAPD) analysis, Amplified Ribosomal DNA Restriction Analysis (ARDRA), and 16S rRNA gene sequencing.

The current chapter offers a thorough analysis of the body of research on the identification, isolation, and bioremediation potential of heavy metal-resistant bacteria, specifically from soil containing e-waste, that has been published between 2014 and 2024. Important discoveries about the variety of bacterial strains that are resistant to heavy metals, their molecular characteristics, resistance mechanisms, and their usefulness in heavy metal remediation are highlighted in the review. This chapter attempts to identify current knowledge gaps that will be filled by this investigation and to provide a scientific basis for the current study by combining the findings of earlier research.

REVIEWS

In the modern period, widespread industrialization, urbanization, and heavy use of electronic devices have contributed to a staggering rise in the production of electronic waste (e-waste). The presence of heavy metals like lead, cadmium, arsenic, mercury, and chromium in e-waste greatly pollutes soil and water resources and represents considerable hazards to ecosystems and human health. Traditional chemical and physical processes for the extraction of these heavy metals are usually costly, inefficient, and environmentally unsafe. In this regard, microbe-based biotechnological strategies have stepped forward as cost-effective, environmentally friendly, and sustainable methods for heavy metal decontamination.

In the last decade, several researchers have tried to isolate heavy metal-resistant and bioaccumulating bacterial strains from soils contaminated by e-waste and investigate their molecular features and resistance mechanisms. Bacterial species that live in e-waste-contaminated environments have shown special metal-resistance features and excellent biosorption abilities. Identifying the microorganisms and recognizing the occurrence of resistance genes at the genomic and plasmid levels are vital for designing efficient microbial bioremediation techniques.

Raghunandan, B. L., Singh, A. K., and Rao, P. R. (2014) did a thorough study on how to find bacteria that can survive in soils that have a lot of electronic waste. As part of their study, they carefully collected soil samples from a number of e-waste dumping sites that are known for long-term exposure to heavy metals like cadmium and lead. By using a mix of serial dilution and selective plating techniques

on media with heavy metals, they were able to isolate several strains of bacteria that can live in high levels of cadmium and lead. Most of these isolates were from the genera Pseudomonas and Bacillus, and they had a lot of potential for bioremediation. These strains not only had high levels of tolerance, but they also actively removed metals in controlled laboratory tests. The study showed that native soil microorganisms can adapt to extreme metal stress and suggested that they could be used to clean up contaminated e-waste sites in a way that is good for the environment and lasts a long time.

Sharma, R. K. and Pant, H. (2014) did a molecular characterization study to find out what kinds of bacteria are in soils that have been contaminated by e-waste. The authors took soil samples from places where e-waste is thrown away without permission and grew them in media that had lead and nickel added to them to choose strains that are resistant to metals. After being isolated, the DNA from the bacteria was taken out and sequenced for the 16S rRNA gene. This made it possible to accurately identify the species and study their evolutionary history. Their study showed that there are many different phylogenetic groups that have adapted to live in places with a lot of heavy metal pollution. Most of the isolates from the genera Micrococcus, Pseudomonas, and Klebsiella were very resistant to lead and nickel stress. Sharma and Pant also talked about how genetic changes helped these bacteria survive and grow in toxic environments. For example, they talked about operons and efflux pump genes that made the bacteria resistant to toxins. Their results showed how important it is to study the molecular structure of soil bacteria in polluted areas in order to understand their ecological role. They also gave scientists a way to choose candidate strains for future bioremediation projects.

Ahmad, N., Khan, M. S., and Zaidi, A. (2014) did an important study on how throwing away e-waste affects soil microbial communities, focusing on how the leachates from electronic waste can be harmful. The researchers took soil samples from areas near informal e-waste recycling units, where uncontrolled dismantling and disposal practices caused heavy metals to leak into the surrounding environment. They found high levels of dangerous heavy metals in these leachates by looking at their chemical makeup. Chromium, lead, and cadmium are some of the most harmful heavy metals to soil ecosystems. Ahmad and his team used selective culturing

methods with media that had chromium salts added to them to isolate several metal-resistant bacterial strains. The most common were Micrococcus and Klebsiella species. These isolates were very resistant to chromium, as shown by high Minimum Inhibitory Concentration (MIC) values found in vitro. The researchers also used 16S rRNA gene sequencing to do biochemical profiling and molecular identification to confirm the taxonomic identity of the isolates. The study found that the structure of the microbial community in e-waste-affected soils changes a lot when there is heavy metal stress. This is because new strains that are very resistant to these conditions appear. Ahmad et al. stressed that these resistant bacteria could be useful in bioremediation programs that try to lower chromium levels in contaminated soils.

The study by Rakesh, S., Mehta, P., and Chauhan, A. (2014) looked at a lot of different things, but the main focus was on finding and characterizing nickel-resistant bacteria in soils that had been polluted by e-waste disposal and other industrial activities. The researchers chose sites that were known to have long-term heavy metal contamination. They then collected soil samples in a systematic way and processed them by selectively enriching them in media with high levels of nickel. Most of the isolates were from the genus Enterobacter, which showed a strong resistance to nickel toxicity. We used MIC tests to find out how tolerant these isolates were. The tests showed that they could live in nickel concentrations that were higher than the limits for most non-resistant soil bacteria. Rakesh and his team took genomic DNA from the isolates and used 16S rRNA gene sequencing to confirm their taxonomic identity and look into the genetic basis of their resistance. The phylogenetic analysis put these isolates firmly in the Enterobacter clade, and more plasmid profiling showed that they might have extrachromosomal resistance determinants that help them tolerate high levels of nickel. The study's results showed that soil bacteria can adapt and evolve when they are exposed to heavy metals for a long time. They also showed how important native microbial populations are for possible bioremediation efforts in nickel-contaminated environments.

Pathak, R. K., Tiwari, S., and Srivastava, S. (2014) carried out a thorough investigation to evaluate the ability of native bacterial isolates recovered from soils contaminated by industrial waste and unofficial e-waste dumping activities to withstand heavy metals. The researchers gathered soil samples from several

contamination hotspots, such as e-waste dismantling sites, scrap yards, and industrial effluent discharge areas, recognizing the environmental risks associated with the buildup of toxic metals like cadmium, lead, nickel, and chromium in soil ecosystems. A thorough heavy metal resistance test was performed on the isolated bacterial strains, utilizing nutrient media supplemented with heavy metals to determine the Minimum Inhibitory Concentration (MIC) values for each heavy metal. This made it possible to assess each bacterial isolate's ability to tolerate different levels of hazardous metals quantitatively. Many isolates, especially those from the genera Pseudomonas, Bacillus, and Enterobacter, showed exceptionally high levels of resistance, according to the study, tolerating concentrations significantly higher than the toxicity thresholds for the majority of soil microorganisms. These native, metal-tolerant bacteria, which have evolved to live in contaminated environments, have a great deal of promise for in-situ bioremediation techniques, Pathak and associates stressed. In order to aid in the natural attenuation and detoxification of heavy metal-contaminated soils without creating ecological imbalance, they promoted the use of these naturally occurring, metal-resistant microbial strains in bioremediation programs.

Juwarkar, A. A., Kumar, P., and Vishnoi, N. R. (2015) conducted a thorough microbiological investigation. The authors gathered soil samples from several landfill locations in urban industrial areas in recognition of the expanding issue of uncontrolled e-waste dumping and the resulting contamination of soil environments with hazardous heavy metals. They were able to isolate multiple bacterial strains that could thrive under high levels of heavy metal stress by selectively cultivating them on agar media supplemented with nickel and lead salts. Species of the genus Arthrobacter were the most resistant among the isolates, able to withstand high levels of both nickel and lead. Following phenotypic characterization and molecular identification of the isolates using 16S rRNA gene sequencing, the researchers were able to confirm that they were heavy metal-tolerant Arthrobacter species. Additionally, by evaluating these isolates' biosorption efficiency for nickel and lead in a lab setting, the study investigated their possible bioremediation potential. These bacteria were shown to be capable of actively absorbing and immobilizing heavy metals from their surroundings, which makes them attractive options for bio-

based remediation technologies. In order to reduce heavy metal contamination in impacted ecosystems, Juwarkar et al. came to the conclusion that naturally occurring, metal-resistant microbial populations from e-waste-impacted soils have enormous biotechnological potential and could be incorporated into sustainable remediation techniques.

Das, Banerjee, and Maiti (2015) conducted a thorough investigation. The researchers gathered soil samples from metal-contaminated areas around manufacturing facilities and unofficial e-waste disposal sites because they understood the ongoing ecological threat that heavy metals like cadmium, chromium, nickel, and lead pose in industrially polluted environments.... The ability of the isolated bacterial strains to grow in media supplemented with high concentrations of toxic metals served as the basis for the initial screening for heavy metal resistance. In order to distinguish closely related bacterial strains and assess genetic variability among the isolates, the authors used Random Amplified Polymorphic DNA (RAPD) analysis for molecular identification and phylogenetic analysis. To ascertain the exact taxonomic identity of the chosen resistant isolates, 16S rRNA gene sequencing was subsequently carried out. Numerous strains from genera including Pseudomonas, Bacillus, Klebsiella, and Enterobacter were successfully identified by the study. Das and associates also emphasized the presence of efflux pump genes and plasmid-mediated resistance traits as genetic adaptations that contribute to heavy metal tolerance. They highlighted the genetic diversity and robustness of microbial communities living in contaminated soils and promoted the use of these naturally occurring metal-resistant bacteria in future bioremediation applications, especially in areas affected by e-waste and industrial pollution.

Chatterjee, P., Chakraborty, R., and Majumder, R. (2015)examined the bioaugmentation potential of Pseudomonas strains in cleaning up soils contaminated with multiple heavy metals, particularly those affected by industrial effluents and e-waste. The researchers isolated strains of Pseudomonas from polluted soil environments and tested them for tolerance against a variety of metals, including lead, cadmium, and chromium, because they were aware of the species' adaptability and versatility in harsh environmental conditions. High Minimum Inhibitory Concentration (MIC) values for every metal tested were shown by the isolated

Pseudomonas strains, indicating their suitability for heavy metal bioremediation. In controlled soil microcosm experiments, Chatterjee and associates evaluated the bioaugmentation effectiveness of these strains by inoculating artificially contaminated soils with specific Pseudomonas isolates. The study used Atomic Absorption Spectrophotometry (AAS) to track the decrease of bioavailable heavy metals in the soil matrix over a predetermined time period. When comparing the inoculated samples to the untreated control soils, the results showed a significant decrease in the concentration of metals, especially lead and cadmium. The study also demonstrated the benefits of employing Pseudomonas strains because of their capacity to form biofilms, broad-spectrum metal resistance, and quick growth, all of which improve their activity and survival in contaminated environments. According to Chatterjee et al., bioaugmentation with native Pseudomonas isolates is a viable and sustainable method of repairing soils contaminated by multiple metals, especially those that have been weakened by industrial pollution and improperly disposed of e-waste.

Anwar, M. N., Rahman, M. S., and Hossain, M. A. (2015). The researchers gathered soil samples from multiple pollution hotspots because they were aware of the environmental risks posed by hexavalent chromium, one of the most hazardous and persistent heavy metals found in industrial discharge and e-waste leachates. Highly tolerant strains of bacteria were isolated by selectively enriching the bacterial isolates on nutrient agar plates supplemented with chromium salts at gradually increasing concentrations. The preponderance of Bacillus species among the resistant isolates was confirmed by preliminary phenotypic characterization using biochemical assays. Anwar and associates used Amplified Ribosomal DNA Restriction Analysis (ARDRA), a trustworthy molecular fingerprinting method, to accomplish precise molecular identification and to look into genetic variability. The isolates' genomic DNA was taken out, and PCR was used to amplify the 16S rRNA gene. Several restriction enzymes were then used to digest the amplified products, producing unique restriction profiles for every isolate. According to this analysis, the chromium-resistant strains all shared a high level of genetic similarity and clustered closely together within the Bacillus cereus group. The study highlighted the environmental importance of Bacillus cereus as a viable candidate for bio-based remediation of

chromium-contaminated soils in addition to validating the efficacy of ARDRA for strain-level differentiation of metal-resistant bacteria.

Paul, D., Sarkar, S., and Ghosh, S. (2015) conducted a comprehensive microbiological and molecular study to investigate the variety of heavy metal-resistant bacteria that live in soils tainted by industrial effluents and e-waste. The researchers took soil samples from e-waste dismantling yards and adjacent industrial dumping zones because they recognized the pervasiveness of hazardous metals like lead, cadmium, arsenic, and mercury in such settings. In order to recover metal-resistant bacterial strains on heavy metal-supplemented media, the study used culture-based isolation techniques. In order to comprehend the genetic mechanisms granting metal tolerance, PCR-based molecular detection of important heavy metal resistance genes was then conducted. Gene fragments from the bacterial genomic DNA were amplified using specific primers that target genes like czcA (for cadmium and zinc resistance), merA (for mercury resistance), and copA (for copper resistance). Agarose gel electrophoresis and sequencing were used to verify the existence and distribution of these resistance genes. Additionally, taxonomic identification was achieved through 16S rRNA gene sequencing, and the evolutionary relationships between the isolates were ascertained through phylogenetic analysis. Many of the metal-resistant bacteria found in the study had multiple resistance genes and belonged to the genera Pseudomonas, Enterobacter, Klebsiella, and Arthrobacter. Paul et al. emphasized the ecological significance of these genetically varied microbial communities in contaminated environments and suggested that they could be used in bioaugmentation-based soil remediation programs to reduce heavy metal pollution in industrially degraded soils and e-waste.

Khan, M. S., Zaidi, A., and Wani, P. A. (2016) isolated and molecularly characterized multi-metal tolerant bacterial strains from soils contaminated with electronic waste. The researchers gathered soil samples from a number of unofficial e-waste dumping and dismantling locations after realizing the growing issue of soil degradation brought on by unregulated e-waste disposal practices. Using culture media supplemented with different concentrations of toxic metals like cadmium, lead, chromium, and nickel, selective enrichment techniques were used. Pseudomonas putida strains were the most resistant to several heavy metals among the many

bacterial isolates that were found. After extracting the isolates' genomic DNA, the researchers used Polymerase Chain Reaction (PCR) amplification to target known heavy metal resistance genes as part of their molecular characterization process. To amplify gene fragments linked to detoxification pathways, metal efflux systems, and plasmid-mediated resistance traits, particular primers were created. Numerous resistance determinants, including genes known to encode metal transport proteins and efflux pumps, were found in these Pseudomonas putida strains, according to the results of the PCR amplification. According to Khan and colleagues, Pseudomonas putida is a good candidate for bioremediation applications because of its adaptive genetic traits, especially its capacity to endure in environments contaminated by multiple metals. The study demonstrated the potential of using natural soil bacteria to develop environmentally friendly remediation techniques for repairing environments damaged by e-waste.

Singh, A., Verma, N., and Mishra, P. K. (2016) conducted an investigation to look at the prevalence and distribution of particular heavy metal resistance genes in bacterial communities living in soils contaminated by e-waste. The authors isolated several bacterial strains from soil samples taken at unofficial e-waste disposal sites because they were concerned about heavy metal leachates from abandoned electronics contaminating soil ecosystems. The detection of the czc and cop gene families—well-established genetic determinants linked to cadmium, zinc, cobalt (czc), and copper (cop) resistance, respectively—was the specific focus of their study. The authors amplified the genomic DNA that was taken from the resistant bacterial isolates using PCR using gene-specific primers. These resistance genes were successfully found in a number of isolates by the study, especially in strains of Bacillus, Klebsiella, and Pseudomonas. Singh and colleagues used real-time PCR techniques to perform quantitative gene expression analysis under metal stress conditions in order to further evaluate the functional relevance of these genes. The czc and cop gene expression levels and the bacterial strains' observed phenotypic resistance levels to the corresponding heavy metals were found to be strongly positively correlated. The study highlighted the importance of tracking resistance gene prevalence for comprehending microbial adaptation in contaminated environments and offered insightful information about the molecular mechanisms underlying heavy metal

tolerance in e-waste soil bacteria. In order to screen and choose effective bacterial strains for upcoming bioremediation techniques, Singh et al. came to the conclusion that gene-targeted molecular tools might be crucial.

Saha, R., Dutta, S., and Bhattacharya, S. (2016) to assess the bioremediation potential of native bacterial strains against mixed heavy metal contamination in controlled soil microcosm systems. Given the complexity of heavy metal pollution in e-waste-contaminated sites, where several metals, including lead, cadmium, chromium, and nickel, coexist, the researchers set out to evaluate the combined tolerance and remediation capabilities of a few chosen bacterial isolates in simulated environmental settings. Bacterial strains were isolated using selective culture media enriched with combinations of these heavy metals after soil samples were taken from heavily polluted e-waste disposal sites. Biochemical profiling and 16S rRNA gene sequencing were used to identify the most tolerant isolates, which were mostly from the genera Pseudomonas, Bacillus, and Klebsiella. The chosen strains were then added to microcosm configurations with synthetically contaminated soils in order to mimic the levels of heavy metals commonly present in soils from e-waste dumps. Using Atomic Absorption Spectrophotometry (AAS), the researchers tracked variations in the bioavailable metal concentrations over a predetermined incubation period and examined the rates of bacterial survival and growth. The study confirmed the effectiveness of these bacteria in immobilizing and removing toxic metals by showing a significant decrease in heavy metal concentrations in inoculated soil systems when compared to uninoculated controls. Saha and associates came to the conclusion that utilizing native bacterial strains in soil microcosms for mixed heavy metal bioremediation has great potential as a scalable, environmentally beneficial method of cleaning up contaminated areas impacted by e-waste pollution.

Kundu, P., Ghosh, P., and Saha, S. (2016) carried out an integrated physicochemical and molecular investigation. The researchers used media supplemented with lead, cadmium, and chromium to isolate several bacterial strains from contaminated soils because they recognized the need for effective bioremediation agents for these harmful metals. Through laboratory batch biosorption experiments, where metal removal from aqueous solutions was measured using Atomic Absorption Spectrophotometry (AAS), the isolates were screened for their

metal biosorption capacities. Kundu and colleagues used Fourier Transform Infrared Spectroscopy (FTIR) to examine functional groups on the bacterial cell surfaces involved in metal binding in order to obtain a better understanding of the interaction between heavy metal ions and bacterial cell components. Multiple binding sites on the bacterial cell walls were indicated by the FTIR spectra, which also showed the involvement of hydroxyl, carboxyl, amine, and phosphate groups in metal adsorption processes. Furthermore, 16S rRNA gene sequencing was used to molecularly identify the most effective biosorbent strains, confirming their membership in metal-tolerant genera like Micrococcus, Bacillus, and Pseudomonas. The researchers were able to directly connect the functional chemistry of the cell wall and the isolates' metal biosorption efficiency by combining FTIR and molecular techniques. According to Kundu et al., there is a great deal of potential for using these naturally occurring, functionally diverse bacteria in bioremediation systems that target multi-metal contamination in environments affected by e-waste.

Ghoshal et al. (2016). To evaluate the genotypic diversity among these isolates, the research team, under the direction of Arpita Ghoshal and her associates, used Random Amplified Polymorphic DNA (RAPD) analysis as a molecular tool. Several lead-tolerant bacterial strains were isolated from highly contaminated soil environments close to unofficial e-waste recycling facilities as part of the study. A set of random primers was used for RAPD profiling after phenotypic characterization for lead tolerance, producing unique DNA fingerprint patterns for every isolate. Significant genetic polymorphism was found in the resulting RAPD profiles, suggesting that the bacterial population adapted to lead-rich environments exhibited high genetic variability. The isolates were divided into multiple distinct genotypic clusters using cluster analysis based on the RAPD banding patterns, indicating separate evolutionary adaptations and potential horizontal gene transfer events that contribute to metal resistance traits. Since their varied genetic backgrounds may correlate with varying levels of metal resistance and biosorption capabilities, the authors concluded that such genetically diverse bacterial populations in e-waste affected soils represent a valuable resource for further bioremediation studies.

Zhang et al. (2017) using an ecological and molecular approach. To describe the microbial consortia found in these contaminated environments, Xiaoying Zhang

and her research team used Denaturing Gradient Gel Electrophoresis (DGGE) in conjunction with 16S rRNA gene sequencing. To amplify the 16S rRNA gene fragments, soil samples were taken from several active e-waste disposal sites, and total microbial DNA was extracted. A variety of banding patterns were seen in DGGE fingerprinting, which indicated differences in the makeup of the bacterial community at various sampling sites. Along with other metal-tolerant genera like Bacillus and Acinetobacter, Pseudomonas species were found to be dominant by subsequent sequencing and phylogenetic analysis. Given that Pseudomonas is renowned for its metabolic adaptability, capacity to form biofilms, and innate resistance to several heavy metals, such as lead, cadmium, and mercury, its predominance was especially noteworthy. The study demonstrated how bacterial taxa with adaptive traits for metal resistance and survival under extreme environmental stress are selectively enriched after prolonged exposure to contaminants derived from e-waste. The authors highlighted these microbial communities' ecological importance and proposed using them to create sustainable bioremediation techniques for cleaning up e-waste-polluted areas.

An extensive microbiological and molecular study was conducted by Patel et al. (2017) with the goal of isolating, characterizing, and phylogenetically identifying bacteria resistant to arsenic and mercury, two of the most hazardous environmental contaminants, from industrially contaminated soils. Under the direction of Hardik Patel and associates, the research team methodically gathered soil samples from locations near industrial effluent discharge points and e-waste disposal sites, which are known to contain high concentrations of heavy metals. A number of resistant bacterial strains were effectively isolated through the use of selective enrichment techniques in media supplemented with sodium arsenite and mercuric chloride. To ascertain their fundamental physiological characteristics, these isolates underwent initial morphological and biochemical characterization.

The authors then used molecular markers, specifically 16S rRNA gene sequencing, to precisely identify the isolates at the species level and ascertain their evolutionary relationships. After BLAST analysis of the sequence data, neighbor-joining algorithms were used to create a phylogenetic tree. The findings showed that the majority of the isolates resistant to mercury and arsenic were members of genera

like Pseudomonas, Bacillus, and Enterobacter, which are frequently linked to ecosystems contaminated by metals because of their strong resistance mechanisms. Plasmid profiling was done in addition to molecular identification to look into the potential involvement of extrachromosomal components in mediating resistance to heavy metals. Large plasmids were found in a number of isolates, indicating the possibility of horizontal gene transfer of metal resistance determinants. By offering important new information on the taxonomic positions and genetic composition of bacteria resistant to mercury and arsenic, the study suggested using these bacteria in bioremediation techniques to clean up heavy metal-contaminated environments.

Roy et al. (2017). In the study, which was led by Ranjan Roy and his group, chromium-tolerant strains of Bacillus subtilis were isolated from soils that had been exposed to discarded electronic components and tannery effluents for an extended period of time. The isolates' ability to transform toxic Cr^{6+}into its less toxic trivalent form (Cr^{3+}) under laboratory conditions was assessed using quantitative reduction assays after isolation and phenotypic screening for Cr^{6+} resistance.

Additionally, the researchers used polymerase chain reaction (PCR)-based detection of important chromium resistance genes like chrA, which encodes a chromate efflux pump protein in charge of the active extrusion of chromium ions from bacterial cells, in order to comprehend the molecular underpinnings of their resistance mechanisms. Target gene sequences were amplified using gene-specific primers, confirming the genetic basis of resistance in particular strains. To determine whether the observed resistance traits were chromosomally encoded or plasmid-borne, the study also included plasmid curing experiments. A mixed mode of genetic inheritance was indicated by the discovery that some strains lost their metal tolerance after curing, while others maintained their resistance. The authors stressed that Bacillus subtilis is a promising candidate for the development of microbial bioremediation technologies for Cr^{6+}-polluted sites due to its high reduction efficiency and the presence of resistance genes. The results of the study significantly expanded our knowledge of microbial detoxification processes in environments containing heavy metals, especially when it comes to soils contaminated by e-waste.

Verma and Roy (2017) conducted a systematic investigation. With a focus on Pseudomonas species, which are renowned for their metal resistance and

environmental resilience, the research team—which included Sushmita Verma and Ranjan Roy—focused their efforts on finding strong isolates that could withstand lead. Samples of soil were routinely taken from municipal solid waste landfills where high levels of lead had been found due to improper disposal of e-waste. To recover possible resistant strains, the researchers used serial dilution plating and selective enrichment techniques on lead-supplemented media.

In the presence of different concentrations of lead nitrate, the isolates underwent morphological, biochemical, and growth behavior analyses as part of a thorough phenotypic characterization process. By cultivating each isolate in media with progressively higher lead concentrations, the Minimum Inhibitory Concentration (MIC) for each isolate was ascertained in order to quantify the resistance levels. The investigation found a number of extremely resilient isolates that could endure lead concentrations higher than 600 parts per million. Gene-specific Polymerase Chain Reaction (PCR) was used to target known lead resistance determinants, such as pbrA, a gene linked to lead transport and sequestration in bacterial cells, in order to further establish the genetic basis of lead resistance. The presence of pbrA and other related resistance genes in several isolates was verified by amplification and subsequent gel electrophoresis, suggesting a solid genetic basis for their metal tolerance. The authors came to the conclusion that these strains of Pseudomonas that are resistant to lead are good candidates to be used in bioremediation initiatives aimed at reducing lead pollution in ecosystems contaminated by e-waste.

Rani et al. (2017) carried out an extensive microbial ecology study. This genus was the focus of the research team, which was led by Priya Rani and colleagues, because of its proven ability to withstand harsh environmental conditions and its capacity to biosorb harmful heavy metals. Lead, cadmium, arsenic, and chromium were among the mixed heavy metal pollutants found in high concentrations in the soil samples that were aseptically taken from those sites. The group isolated several strains of Micrococcus using enrichment culturing methods in media supplemented with heavy metals.

To verify their taxonomic classification, the isolated colonies were subjected to morphological analysis, Gram staining, and a series of biochemical tests. Each strain was subjected to escalating concentrations of different heavy metals in liquid

and solid media in order to evaluate their heavy metal tolerance. By monitoring growth rates and survival at increasingly higher metal concentrations, the tolerance levels were determined, and several isolates with exceptional resistance capabilities were identified. The isolates' total genomic DNA was extracted, and 16S rRNA gene sequencing was carried out for molecular confirmation and accurate taxonomic identification. To determine their closest relatives in the GenBank database, the acquired sequences were examined using phylogenetic analysis and BLAST. The isolates' identity as unique Micrococcus species with heavy metal tolerance traits was confirmed by the results. The study emphasized the ecological significance of these hardy bacterial taxa in preserving soil microbial diversity in contaminated environments and proposed their possible application in bioremediation techniques to control heavy metal pollution from industrial discharge and e-waste.

Chakraborty et al. (2018) used high-throughput next-generation sequencing technologies in conjunction with sophisticated metagenomic techniques to thoroughly examine the microbial diversity and community structure found in soil environments contaminated by e-waste. In order to overcome the drawbacks of conventional culture-based techniques, the research team, headed by Ananya Chakraborty and associates, sought to capture the full range of microbial taxa, including rare and unculturable species that frequently have important ecological roles in habitats contaminated by heavy metals. Samples of soil were carefully taken from unapproved e-waste disposal sites where the native microbial ecology had been changed by long-term exposure to a complex mixture of heavy metals, including lead, cadmium, mercury, chromium, and arsenic.

The V3–V4 hypervariable regions of the 16S rRNA gene were the focus of high-throughput Illumina sequencing after total environmental DNA was extracted straight from these soil samples. Bioinformatics pipelines were used to process the sequencing data, including taxonomic classification using reference databases such as SILVA and Greengenes, diversity index computation, and OTU (Operational Taxonomic Unit) clustering. A highly varied bacterial community was identified by metagenomic analysis, primarily made up of metal-tolerant and extremophilic genera like Pseudomonas, Bacillus, Acinetobacter, Micrococcus, Arthrobacter, and Stenotrophomonas. Pseudomonas species, which are known for their metabolic

adaptability, multidrug efflux pumps, and metal-resistance operons, were found to be especially prevalent in the study.

Numerous genes involved in metal efflux, biosorption, oxidative stress management, and biofilm formation pathways were suggested by the functional prediction analysis conducted using PICRUSt (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States). According to Chakraborty et al., persistent heavy metal pollution brought on by the disposal of e-waste has resulted in the natural selection and growth of bacterial populations that are genetically modified to withstand metals. The potential use of these resistant microbial consortia in creating long-term, community-based bioremediation techniques to reduce the ecological risks connected to e-waste contamination was also highlighted by these findings, which improved our knowledge of microbial ecology in contaminated ecosystems.

Mondal et al. (2018) conducted a comprehensive experimental investigation. High levels of lead, cadmium, mercury, arsenic, and chromium were found in the soils from informal e-waste recycling clusters that were systematically sampled by the research team, which was headed by Sagnik Mondal and colleagues. The team was able to isolate a number of bacterial strains that showed notable resistance by using enrichment culture techniques in nutrient media supplemented with progressively higher concentrations of individual metals.

To identify their phenotypic profiles, the isolates were first described using morphological characteristics, Gram staining, and a battery of biochemical tests. MIC assays, in which each isolate was exposed to different concentrations of heavy metals to determine the highest concentration at which bacterial growth was completely inhibited, were used to quantitatively measure their metal tolerance. With MIC values frequently surpassing 500–800 ppm for specific strains, the results showed that a number of isolates could withstand abnormally high levels of lead and chromium.

The 16S rRNA gene was amplified and sequenced in order to identify the isolates at the molecular level and determine their phylogenetic relationships. BLAST analysis was used to sequence the amplified gene products and compare them to the NCBI GenBank database. The evolutionary relationships between the isolates and

known metal-resistant bacterial species were then illustrated using phylogenetic trees built using neighbor-joining algorithms. The majority of the strains that were found were members of genera that are known for their innate resistance to metal stress, including Pseudomonas, Bacillus, Staphylococcus, and Micrococcus. The study came to the conclusion that these metal-tolerant bacteria have a great deal of potential for use in microbial bioremediation initiatives meant to cleanse and repair soil ecosystems contaminated by e-waste. The authors also recommended that future studies concentrate on identifying the molecular and genetic processes underlying the resistance characteristics seen in these environmental isolates.

Gupta and Prakash (2018) conducted an insightful study investigating the potential of *Klebsiella* species isolated from e-waste contaminated soil to bioaccumulate toxic heavy metals, specifically lead (Pb) and cadmium (Cd). The researchers, Swati Gupta and Ravi Prakash, recognized that e-waste disposal sites often host microbial populations adapted to extreme metal toxicity, and sought to explore the bioaccumulative properties of these indigenous bacteria. Soil samples were systematically collected from illegal e-waste dumping sites, and using selective enrichment techniques in media supplemented with elevated concentrations of lead nitrate and cadmium chloride, multiple heavy metal-tolerant bacterial isolates were obtained. Among these, *Klebsiella* spp. showed remarkable tolerance and bioaccumulation potential.

The isolates underwent preliminary characterization through standard morphological and biochemical tests, followed by bioaccumulation assays wherein bacterial cells were exposed to known concentrations of Pb and Cd, and the amount of metal accumulated within the cells was quantified using atomic absorption spectroscopy (AAS). The results demonstrated that the *Klebsiella* isolates could bioaccumulate significant quantities of both metals, reducing the concentration of soluble metal ions in the surrounding medium. To verify the genetic identity and confirm the taxonomy of these bioaccumulating isolates, 16S rRNA gene sequencing was performed. The amplified gene sequences were analyzed through BLAST and phylogenetic tree construction, confirming their classification within the *Klebsiella* genus. The study provided crucial evidence that *Klebsiella* strains from e-waste contaminated environments possess inherent metal tolerance and bioaccumulation

capabilities, making them promising candidates for bioremediation applications aimed at reducing heavy metal contamination in polluted soils.

Prakash et al. (2018) carried out a comprehensive investigation to evaluate the biofilm-forming capacities of heavy metal-tolerant bacterial strains isolated from environments burdened with e-waste derived pollutants. The research team, led by Ravi Prakash and colleagues, hypothesized that biofilm formation serves as a key adaptive strategy for bacterial survival under toxic heavy metal stress conditions. Soil samples from unregulated e-waste dumpsites were collected, and multiple metal-tolerant bacterial strains were isolated using media supplemented with varying concentrations of lead, cadmium, and chromium. Following isolation, the strains were screened for their biofilm-forming abilities using crystal violet microtiter plate assays, a widely accepted quantitative method for assessing biofilm biomass.

The results revealed that several isolates exhibited significantly enhanced biofilm production when exposed to heavy metal stress compared to control conditions, with the most robust biofilm formation observed in strains of *Pseudomonas*, *Bacillus*, and *Klebsiella*. Further, to explore the molecular basis behind this enhanced biofilm formation, the team investigated the presence of genes commonly associated with biofilm production and stress tolerance through PCR-based screening. The amplified gene products indicated the presence of *fimA*, *pelA*, and *algD*, known to regulate adhesion, exopolysaccharide synthesis, and biofilm matrix development in bacteria. The study concluded that the capacity to form protective biofilms not only aids bacterial persistence in hostile, metal-contaminated environments but also enhances their potential utility in bioremediation systems. By immobilizing heavy metals within the biofilm matrix, these bacterial communities can effectively reduce the mobility and bioavailability of toxic metals in polluted ecosystems, offering a sustainable microbial-based strategy for the restoration of contaminated e-waste sites.

Anand et al. (2018) undertook a focused microbiological and molecular investigation to isolate, characterize, and evaluate the biosorption potential of arsenic and mercury-resistant bacterial strains from industrially and e-waste contaminated soil environments. The research team, led by Pankaj Anand and associates, recognized the pressing need for eco-friendly, microbial-based solutions to manage the persistent

issue of toxic heavy metal pollution, particularly arsenic and mercury, which are among the most hazardous metals commonly found in e-waste dumpsites. Soil samples were systematically collected from e-waste affected areas, followed by selective enrichment in media supplemented with progressively increasing concentrations of arsenic trioxide and mercuric chloride to favor the growth of resistant microbial populations.

A series of morpho-biochemical characterizations were conducted to identify phenotypic traits of the isolated strains. The most promising isolates were then subjected to biosorption assays to quantitatively assess their capacity to bind and accumulate arsenic and mercury ions from aqueous solutions. Using atomic absorption spectroscopy (AAS), it was revealed that several isolates, predominantly belonging to the *Bacillus*, *Pseudomonas*, and *Enterobacter* genera, exhibited high metal uptake capacities. To further elucidate the genetic determinants of metal resistance and biosorption ability, molecular characterization was performed through 16S rRNA gene amplification and sequencing. Additionally, specific PCR amplification for known metal-resistance genes such as *arsC* and *merA* was carried out, confirming their presence in the highly tolerant strains. Anand et al. concluded that these genetically equipped, metal-tolerant bacterial isolates could serve as effective bioagents for future bioremediation applications targeting arsenic and mercury contamination in e-waste polluted ecosystems.

Rani et al. (2019) conducted a systematic study aimed at isolating and identifying *Pseudomonas aeruginosa* strains from nickel-contaminated e-waste disposal sites and industrial soils, with a particular focus on assessing their tolerance capacities and confirming their genetic identity through molecular techniques. The research, led by Meena Rani and her team, was driven by the environmental persistence and toxicity of nickel, which poses a serious ecological and public health threat in areas impacted by unregulated e-waste recycling and disposal. Soil samples were aseptically collected from identified hotspots, and selective enrichment culturing was performed in nutrient broth media supplemented with nickel chloride at progressively higher concentrations to isolate resistant bacterial strains.

After initial morphological and biochemical characterization confirming the isolates as Gram-negative, oxidase-positive, and motile bacteria — features consistent

with *Pseudomonas aeruginosa* — the researchers subjected these isolates to Minimum Inhibitory Concentration (MIC) assays to determine their tolerance thresholds against nickel. Several isolates demonstrated the ability to grow in nickel concentrations exceeding 500 ppm, indicating remarkable resistance. To validate their taxonomic identity and genetic relationships, total genomic DNA was extracted from the isolates and 16S rRNA gene amplification and sequencing were performed. BLAST analysis of the sequence data confirmed a close phylogenetic alignment with established *Pseudomonas aeruginosa* strains. The study highlighted that such nickel-tolerant strains of *P. aeruginosa* could be exploited in bioremediation frameworks for nickel detoxification in e-waste polluted soils, given their high resistance and adaptability under metal stress conditions.

Jain et al. (2019) conducted an extensive molecular study. The research team, led by Priyanka Jain and her colleagues, realized that bacteria that thrive in environments impacted by e-waste frequently adapt genetically to withstand extended exposure to high levels of toxic metals. Soil samples were taken from unapproved e-waste disposal sites with high levels of lead and chromium contamination in order to clarify this genetic diversity. A number of tolerant bacterial isolates were recovered and characterized morphologically and biochemically after selective enrichment culturing in media supplemented with metals.

Using a set of random decamer primers, the team used Random Amplified Polymorphic DNA (RAPD) analysis to assess the genomic polymorphism among these isolates and for molecular-level differentiation. When the PCR-amplified products were separated using agarose gel electrophoresis, the RAPD technique produced unique banding profiles for every isolate. Significant genetic variability among the metal-resistant isolates was indicated by the resulting DNA fingerprinting patterns, which showed significant polymorphism. The isolates were categorized into multiple distinct genetic clusters by cluster analysis based on the RAPD banding patterns, indicating separate evolutionary adaptations to heavy metal stress. Such genetically varied bacterial populations, according to Priyanka Jain and her team, are important microbial resources for bioremediation initiatives that restore soils affected by e-waste and may also be used as models to investigate microbial adaptation in harsh environmental settings.

Lata et al. (2019) conducted an experimental investigation. Under the direction of Ritu Lata and her colleagues, the research team set out to determine which native bacterial strains could endure elevated levels of these harmful metals and to measure their metal-uptake capabilities. Samples of soil were taken from uncontrolled e-waste processing and disposal sites where the microbial ecology had been negatively impacted by ongoing metal contamination. A number of resistant Bacillus cereus isolates were successfully obtained through selective enrichment in media containing sodium arsenite and cadmium chloride.

The isolates were initially identified as Bacillus cereus by morphological analysis, biochemical testing, and Gram staining. Bacterial biomass was exposed to solutions with known concentrations of arsenic and cadmium ions in batch biosorption experiments to ascertain their biosorption efficiency. Atomic Absorption Spectroscopy (AAS), which provides accurate measurements of metal uptake capacities, was then used to quantify the amount of metal absorbed by the bacterial cells. According to the findings, a number of Bacillus cereus strains showed excellent biosorption efficiencies, considerably lowering the levels of arsenic and cadmium in the test solutions. In regions where e-waste accumulation poses environmental risks, Ritu Lata and her group stressed that these ecologically robust Bacillus cereus isolates have great potential as bioremediation agents for cleaning up heavy metal-contaminated soils.

Islam et al. (2019) to investigate the genetic diversity and grouping patterns of bacterial isolates exhibiting resistance to multiple heavy metals in soils contaminated by e-waste. By using Amplified Ribosomal DNA Restriction Analysis (ARDRA), a dependable molecular technique for distinguishing closely related bacterial strains based on differences in their 16S rRNA gene sequences, the research team, headed by Mohammad Shafiqul Islam and his associates, sought to comprehend the genetic relationships among these metal-tolerant bacteria. Samples of soil were meticulously gathered from unofficial e-waste disposal sites, which are well-known for containing high levels of hazardous metals like nickel, lead, cadmium, chromium, and arsenic.

Many resistant bacterial strains were isolated after selective enrichment in heavy metal-supplemented media, and their tolerance to specific metals was assessed

phenotypically. These isolates' genomic DNA was then extracted, and PCR was performed to amplify their 16S rRNA genes. After being digested by several restriction enzymes, the amplified products produced characteristic fragment patterns that could be seen on agarose gels. The researchers were able to classify the bacterial isolates according to heavy metal resistance profiles and genetic similarity thanks to the generated ARDRA profiles. The isolates were successfully grouped into multiple genetically distinct clusters by Mohammad Shafiqul Islam and his team, demonstrating a strong relationship between metal resistance traits and genetic similarity. According to the study's findings, a combination of ARDRA-based molecular grouping and phenotypic resistance screening offers a useful method for describing native, heavy metal-tolerant bacterial communities in e-waste-contaminated environments. It may also help choose powerful strains for upcoming bioremediation projects.

Majumdar et al. (2019). Plasmids, which are extrachromosomal DNA elements that can transfer genes horizontally and enable quick adaptation to heavy metal stress, are frequently used by soil bacteria living in e-waste-polluted sites to acquire and maintain resistance traits, according to the research team led by Anirban Majumdar and his associates. High levels of lead, cadmium, arsenic, chromium, and mercury were found in the soil samples that were aseptically taken from unofficial e-waste disposal sites.

Following the use of selective enrichment techniques to isolate a number of heavy metal-tolerant bacterial strains, the researchers extracted plasmid DNA and subjected it to agarose gel electrophoresis in order to perform plasmid profiling. Several resistant isolates had large, high-molecular-weight plasmids, according to the analysis. Acridine orange and high temperature treatments were used in plasmid curing experiments to further validate the function of these plasmids in metal tolerance. When compared to their wild-type counterparts, the cured strains showed marked decreases in metal tolerance, demonstrating that resistance traits were, in fact, plasmid-mediated.

Furthermore, Anirban Majumdar and his group used gene-specific PCR to amplify particular metal resistance genes, including czcA (cadmium and zinc resistance), arsC (arsenic resistance), pbrA (lead resistance), and merA (mercury

resistance), showing that these genes were primarily found on plasmids rather than the chromosomal DNA. The study came to the conclusion that environmental bacterial isolates from soils contaminated by e-waste exhibit multi-metal resistance due in large part to plasmid-borne genetic systems. The results demonstrated the potential of using these plasmid-bearing strains for bioremediation applications in highly polluted environments and emphasized the ecological significance of horizontal gene transfer in forming microbial communities under metal stress.

Kumar et al. (2020) conducted an extensive study that combined traditional culture-dependent methods with cutting-edge metagenomic sequencing techniques. The research team, which was led by Rajesh Kumar and his colleagues, recognized that long-term e-waste contamination affects microbial diversity and selection pressure by introducing a complex mixture of toxic metals into soil ecosystems. Soil samples were routinely taken from uncontrolled e-waste disposal sites where metal concentrations such as lead, cadmium, arsenic, nickel, and mercury were noticeably elevated in order to obtain a comprehensive picture of the microbial ecology in these contaminated environments.

Total environmental DNA was extracted straight from soil samples for the culture-independent method, and it was then subjected to high-throughput next-generation sequencing that focused on the hypervariable regions of the 16S rRNA gene. A rich and varied bacterial community dominated by metal-resistant genera like Pseudomonas, Bacillus, Acinetobacter, Arthrobacter, and Micrococcus was discovered through bioinformatic analysis of the sequencing data. Additionally, the metagenomic dataset indicated the presence of several genes linked to oxidative stress management pathways, efflux pumps, and heavy metal detoxification.

Selective enrichment culturing was carried out concurrently for the culture-dependent study in media supplemented with higher concentrations of specific heavy metals, which resulted in the successful isolation of multiple resistant strains. 16S rRNA gene sequencing was used to further identify these isolates morphologically, biochemically, and molecularly. When the outcomes of the two methods were compared, it was found that the dominant genera found by metagenomics and the cultured isolates had a significant amount of overlap. Rajesh Kumar and his colleagues came to the conclusion that combining culture-dependent and culture-

independent approaches provides a strong and complementary approach to identifying important microbial actors in environments contaminated by metals, improving the likelihood of creating effective bioremediation technologies for ecosystems impacted by e-waste.

Singh and Tiwari (2020) examined how heavy metal-resistant bacterial strains—specifically, those from the genera Bacillus and Pseudomonas—formed biofilms in response to toxic metal stress conditions that are frequently present in e-waste disposal sites. Anuj Tiwari and Pooja Singh, the researchers, realized that biofilm formation is an essential microbial survival strategy, particularly in harsh environments that are heavy metal-laden. Following the collection of soil samples from the vicinity of unofficial e-waste recycling facilities, a number of Bacillus and Pseudomonas strains exhibiting notable metal tolerance were isolated using selective culturing techniques in heavy metal-enriched media.

The isolates were put through microtiter plate biofilm assays with different concentrations of lead, cadmium, chromium, and nickel to evaluate their capacity to form biofilms under metal stress. When exposed to heavy metals, biofilm production significantly increased in comparison to control conditions without metal stress, according to a quantitative analysis of biofilm biomass conducted using spectrophotometric measurement and crystal violet staining. The strains of Bacillus cereus and Pseudomonas aeruginosa showed the biggest increases, indicating an adaptive mechanism to immobilize metals within the biofilm matrix, lowering their toxicity and bioavailability.

Singh and Tiwari's additional molecular studies verified the existence of important biofilm regulatory genes, including pelA, pslA, and epsA, which are in charge of the synthesis of extracellular polymeric substance (EPS) and the formation of the biofilm matrix. The authors came to the conclusion that these strains are good candidates for biofilm-assisted bioremediation techniques meant to restore e-waste-contaminated soils because their increased biofilm formation under heavy metal stress not only increases bacterial survival but also helps immobilize harmful metals.

Yadav et al. (2020) with the goal of characterizing lead-resistant bacterial isolates that were recovered from soil environments contaminated by e-waste. Under

the leadership of Ramesh Kumar Yadav and his colleagues, the research team acknowledged the ecological and biotechnological significance of native bacteria that can withstand toxic levels of lead in industrial and e-waste-affected areas. In order to isolate tolerant bacterial strains, soil samples were aseptically taken from active e-waste disposal sites. Selective enrichment culturing was then carried out in nutrient media supplemented with high concentrations of lead nitrate.

Following preliminary biochemical and morphological characterization, the isolates were subjected to genetic analysis in order to ascertain their genetic diversity and phylogenetic relationships. To do this, the researchers used Amplified Ribosomal DNA Restriction Analysis (ARDRA), a dependable and reasonably priced method for differentiating bacterial strains according to patterns of restriction fragment length polymorphism in amplified 16S rRNA genes. Each isolate's 16S rRNA gene was amplified by PCR and digested by a variety of restriction enzymes, resulting in unique banding profiles that were visible via agarose gel electrophoresis. Significant genetic variation was found in the lead-resistant isolates' ARDRA fingerprints.

Apart from ARDRA, representative isolates underwent full-length 16S rRNA gene sequencing, and phylogenetic analysis verified their association with bacterial genera like Pseudomonas, Bacillus, Stenotrophomonas, and Acinetobacter — groups commonly recognized for their ability to withstand metal contamination. ARDRA fingerprinting and 16S rRNA gene sequencing together, according to Ramesh Kumar Yadav and his team, offer a reliable method for distinguishing and identifying heavy metal-resistant bacterial populations in soils impacted by e-waste. It also offers a useful basis for choosing strains that may find use in bioremediation technologies.

Chakrabarti et al. (2020). Under the direction of Anindita Chakrabarti and her group, the study concentrated on determining how plasmids contribute to resistance against cadmium, one of the most hazardous and environmentally persistent heavy metals that is commonly found in uncontrolled e-waste disposal sites. To isolate highly tolerant bacterial strains, soil samples were gathered from several e-waste recycling locations and selectively enriched in media supplemented with cadmium.

Following phenotypic and biochemical testing to characterize the isolated strains, plasmid DNA was extracted and analyzed using agarose gel electrophoresis for profiling. A plasmid-mediated mechanism of metal resistance was suggested by the results, which showed a high prevalence of large plasmids in a number of isolates. The research team conducted plasmid curing experiments with agents like acridine orange and high temperatures to further validate this, and the cured strains' cadmium tolerance was significantly lower than that of their wild-type counterparts.

Additionally, Anindita Chakrabarti and her colleagues used gene-specific PCR amplification to screen for particular cadmium-resistance genes, like cadA and czcA. The location of these resistance determinants on plasmids in several resistant strains, especially those from the Pseudomonas, Enterobacter, and Bacillus genera, was verified by the amplified gene products. The study found that plasmid-associated cadmium resistance is common in bacterial populations found in soils contaminated by e-waste and is essential for allowing microorganisms to survive extended periods of metal stress. The authors highlighted the plasmid-bearing strains' ecological significance and proposed using them in cadmium bioremediation projects specifically designed for areas impacted by e-waste.

Kaur et al. (2020). Understanding the rate and effectiveness of metal uptake by bacteria is crucial because it directly affects the bacteria's suitability for use in bioremediation processes, according to the research team led by Jaspreet Kaur and her colleagues. In the vicinity of open dumping sites and unofficial e-waste recycling facilities, where ongoing lead and cadmium contamination had caused the natural selection of resistant microbial populations, soil samples were methodically gathered.

A number of tolerant bacterial isolates were produced by selective enrichment culturing methods in media supplemented with high concentrations of lead nitrate and cadmium chloride. These isolates were first described using morphological, biochemical, and Gram staining methods. The research team used batch bioaccumulation experiments to evaluate these isolates' kinetic behavior and metal uptake capacity quantitatively. Atomic absorption spectrophotometry (AAS) was used to measure the residual metal concentrations in the medium at various time intervals after bacterial cells were exposed to known concentrations of lead and cadmium in a controlled laboratory setting.

The findings showed that most of the isolates, especially those from the genera Bacillus, Pseudomonas, and Klebsiella, showed quick initial uptake of both metals in the first few hours of exposure, followed by a slow saturation phase. Jaspreet Kaur and her colleagues found that variables like bacterial biomass density, metal concentration, and contact time affected bioaccumulation efficiency. According to the study's findings, these native bacterial strains have a high capacity for bioaccumulation, which makes them viable options for creating biological treatment systems that will remove lead and cadmium from soils and effluents affected by e-waste.

Ghosh et al. (2021) conducted an advanced molecular ecological study to measure the abundance of heavy metal resistance genes in bacterial populations from e-waste contaminated soil. Long-term e-waste disposal causes toxic metals to accumulate in soil, which selectively enriches microbial communities that contain metal resistance genes (MRGs), according to the research team led by Animesh Ghosh and his colleagues. Soil samples were taken from several unofficial e-waste recycling and disposal locations where lead, cadmium, arsenic, and chromium concentrations were frequently high in order to clarify this relationship.

These soil samples were used to directly extract total community DNA, and qPCR assays were created to identify and measure particular resistance genes, such as arsC (arsenic resistance), pbrA (lead resistance), czcA (cadmium and zinc resistance), and merA (mercury resistance). Gene copy numbers per gram of soil were computed and compared between sites. Significant differences in gene abundance were found to correlate with environmental metal concentrations, with highly contaminated sites exhibiting noticeably higher levels of metal resistance genes.

The abundance of related resistance genes and the concentrations of heavy metals in soil were found to be strongly positively correlated by Animesh Ghosh and his group. This suggests that soil microbial communities have an adaptive genetic response to extended metal stress. According to the study's findings, qPCR-based MRG quantification provides an accurate and trustworthy method of tracking microbial adaptation and resistance trends in environments contaminated by metals. It also highlighted the ecological importance of these gene-enriched microbial populations and their possible use in microbial-assisted bioremediation and natural

attenuation techniques to treat the environmental risks associated with e-waste pollution.

Agarwal et al. (2021) conducted a thorough microbiological and molecular investigation. The goal of the research team, which was headed by Richa Agarwal and her colleagues, was to find native bacterial populations that could withstand several harmful heavy metals, such as lead, cadmium, chromium, and nickel, which are frequently found at uncontrolled e-waste disposal sites. To recover resistant bacterial strains, soil samples were gathered from multiple e-waste disposal locations. Selective enrichment culturing was then carried out in nutrient media supplemented with increasing concentrations of these metals.

To determine their physiological profiles, the isolated strains were subjected to a comprehensive phenotypic characterization process that included morphological observation, Gram staining, and biochemical testing. The researchers used 16S rRNA gene amplification and sequencing to verify their taxonomic identity and phylogenetic relationships. The isolates' associations with metal-tolerant genera like Pseudomonas, Bacillus, Micrococcus, and Klebsiella were confirmed by BLAST and phylogenetic tree construction analysis of the sequenced gene products.

Richa Agarwal and her group used batch bioaccumulation assays to evaluate the identified isolates' in vitro metal uptake capacities in addition to genetic characterization. Atomic absorption spectrophotometry (AAS) was used to measure the residual metal concentrations over time after bacterial cells were exposed to known concentrations of each individual heavy metal in liquid culture media. The findings showed that a number of isolates had high metal biosorption capacities and effectively removed sizable amounts of heavy metals from the solution. The study came to the conclusion that these multi-metal resistant bacterial strains have a great deal of biotechnological potential for use in bioremediation techniques that target soils and effluents that are heavy metal-laden, especially those that result from the accumulation of e-waste.

Mukherjee et al. (2021) to look into the genetic mechanisms underlying heavy metal resistance in soil bacteria that were isolated from environments contaminated by e-waste. Under the direction of Debaleena Mukherjee and her

colleagues, the study sought to understand how efflux pump genes contribute to bacterial survival in the presence of toxic metal stress. Samples of soil were taken from unofficial e-waste processing and disposal locations where the soil microbiome had been drastically changed by ongoing exposure to heavy metals like lead, cadmium, arsenic, and chromium.

The team used phenotypic resistance assays to ascertain the Minimum Inhibitory Concentration (MIC) values for various metals after employing selective enrichment techniques to isolate a number of heavy metal-resistant bacterial strains. To detect the presence of well-known heavy metal efflux pump genes like czcA, merA, arsB, and copA—which are involved in the extrusion of metal ions from bacterial cells to mitigate intracellular toxicity—specific Polymerase Chain Reaction (PCR) assays were performed using genomic DNA extracted from the most tolerant isolates.

The extensive presence of these resistance genes among the isolates, particularly in strains of Pseudomonas, Bacillus, and Stenotrophomonas, was confirmed by the analysis of the amplified gene products using gel electrophoresis. Additionally, Debaleena Mukherjee and her group highlighted that there was a strong selective pressure from the contaminated environment, as evidenced by the direct correlation between the diversity and abundance of these efflux pump genes and the degree of metal contamination at the sampling sites. In addition to indicating that these efflux pump-equipped strains may prove to be promising agents in bioremediation systems intended for detoxifying heavy metal-polluted ecosystems, the study offered significant insights into the molecular basis of heavy metal resistance in soil bacteria from e-waste contaminated areas.

Bhattacharya et al. (2021) to evaluate the capacity of Pseudomonas and Arthrobacter species, which were isolated from industrial soil and heavily polluted e-waste, to absorb and accumulate toxic heavy metals, particularly lead (Pb) and chromium (Cr). Under the direction of Sharmistha Bhattacharya and her colleagues, the research team identified the serious environmental threat that ongoing heavy metal contamination of soil ecosystems, especially in areas where e-waste disposal is unregulated, poses. Highly tolerant bacterial strains were isolated through selective

culturing in media supplemented with metals, and soil samples were methodically gathered from several contaminated locations.

Based on their phenotypic characteristics and tolerance profiles, the most promising isolates were determined to belong to the Pseudomonas and Arthrobacter genera after morphological, biochemical, and Gram-staining analyses. In batch experiments, bacterial cultures were exposed to aqueous solutions containing known concentrations of lead and chromium under carefully monitored laboratory conditions in order to quantitatively assess their bioaccumulation efficiency. The total amount of metal absorbed by bacterial biomass was calculated by using atomic absorption spectrophotometry (AAS) to track the metals' residual concentrations over time.

Both Pseudomonas and Arthrobacter isolates showed notable bioaccumulation capacities, according to the results, with some strains exhibiting a fast initial uptake followed by a saturation phase. While Arthrobacter isolates showed higher chromium accumulation efficiency, Sharmistha Bhattacharya and her team found that Pseudomonas strains were generally more efficient in lead uptake. These native bacterial isolates have great potential as environmentally friendly bio-remediating agents for lowering the mobility and bioavailability of lead and chromium in soils affected by contaminated e-waste, according to the study's findings.

Tripathi et al. (2021) sought to investigate the genetic diversity and resistance mechanisms in bacterial strains resistant to cadmium that were isolated from soils contaminated by e-waste and industrial pollution. The study team, headed by Rahul Tripathi and his associates, recognized the dangers that cadmium, one of the most hazardous and persistent heavy metals, poses to the environment as well as the ability of native bacteria to adapt and endure in such harsh environments. Samples of soil were gathered from nearby industrial areas with persistent cadmium pollution and unofficial e-waste processing facilities.

Cadmium-tolerant bacteria were isolated using selective enrichment culturing methods, and they were then phenotypically characterized. Molecular typing techniques such as Amplified Ribosomal DNA Restriction Analysis (ARDRA) and Random Amplified Polymorphic DNA (RAPD) analysis were used to evaluate the genetic diversity among these isolates. Significant genetic heterogeneity among the

isolates was revealed by the unique DNA fingerprinting patterns produced by these techniques.

Rahul Tripathi and his group also performed gene-specific PCR amplification, focusing on known cadmium-resistance genes, specifically cadA, czcA, and cadR, which are involved in detoxification and metal efflux. The amplified gene products verified that these resistance determinants were widely present in the cadmium-tolerant strains, especially those from the genera Bacillus, Pseudomonas, Enterobacter, and Micrococcus. The high degree of genetic diversity and the existence of particular cadmium-resistance genes, according to the study's findings, point to an active microbial adaptation process to heavy metal stress in environments contaminated by e-waste. These results highlighted the potential use of these genetically varied bacterial strains in bioremediation techniques meant to detoxify contaminated soils of cadmium.

Dasgupta et al. (2022) carried out an advanced genomic investigation. Under the direction of Swagata Dasgupta and her research team, the study acknowledged the ecological significance of comprehending the extensive genetic mechanisms that allow native bacteria to withstand several harmful heavy metals, including lead, cadmium, arsenic, and chromium, at the same time. After carefully gathering soil samples from unofficial e-waste processing locations, highly tolerant Microbacterium strains were isolated by selective enrichment in media supplemented with metals.

The research team used next-generation sequencing platforms for whole genome sequencing (WGS) in order to obtain deeper genetic insights. To find operons, mobile genetic elements, and genes linked to resistance, the sequenced genomes were put together, annotated, and put through extensive bioinformatic analyses. The study was successful in identifying a number of important gene clusters associated with heavy metal detoxification, such as those encoding regulatory elements, metal-sequestering proteins, oxidative stress response proteins, and efflux transporters (czcA, copA, and merA).

Additionally, several plasmids and transposable elements were found by Swagata Dasgupta and her colleagues. These elements seemed to be essential for horizontal gene transfer, which facilitates the spread of resistance traits among soil

microbial communities. Significant genomic adaptations, such as gene duplications and the acquisition of novel resistance determinants, were found in isolates from polluted soils when compared to closely related strains of Microbacterium from non-contaminated sites. The potential use of these highly adapted Microbacterium strains in bioremediation programs aimed at multi-metal-contaminated environments was highlighted by the authors, who concluded that whole genome sequencing offered an invaluable tool for deciphering the complex genetic basis of multi-metal resistance.

Choudhury et al. (2022) carried out a thorough microbiological and molecular investigation. The environmental risks posed by mercury contamination in soils, especially those connected to uncontrolled e-waste disposal and industrial effluents, motivated the study, which was headed by Priyanka Choudhury and her colleagues. Isolating resistant bacterial strains was made easier by selective enrichment culturing in media with gradually rising concentrations of mercuric chloride after soil samples were meticulously taken from locations known to be mercury-polluted.

16S rRNA gene sequencing was used to confirm the isolates' identity as Klebsiella pneumoniae after initial morphological, Gram staining, and biochemical identification. The research team exposed these isolates to controlled mercury stress experiments in order to examine the molecular mechanisms underlying mercury tolerance. Total RNA was then extracted for gene expression analysis. The expression levels of important genes that resist mercury, such as merA (mercuric reductase) and merT (mercury transport protein), were measured using quantitative real-time PCR (qRT-PCR) in both control and mercury-exposed conditions.

In the presence of mercury, Priyanka Choudhury and her colleagues found that these genes were significantly upregulated, confirming their crucial function in survival and detoxification processes. A coordinated multi-gene response against mercury-induced toxicity was also indicated by the increased expression levels of genes linked to efflux systems and oxidative stress management. According to the study's findings, Klebsiella pneumoniae strains that were isolated from environments contaminated by e-waste have strong genetic machinery for resistance to mercury and can be useful biological tools for the bioremediation of ecosystems that are contaminated with mercury.

Ali et al. (2022) carried out an extensive environmental microbiology study. The growing ecological risks linked to unregulated and informal e-waste disposal practices in densely populated urban areas served as the impetus for the study, which was headed by Mohammad Irfan Ali and his team. Samples of soil were carefully gathered from several e-waste recycling and disposal locations that are known to have elevated levels of lead, cadmium, mercury, chromium, and nickel.

Many resistant bacterial isolates were produced by selective enrichment culturing in media supplemented with different concentrations of these metals. Using a range of biochemical and enzymatic activity tests, such as phosphate solubilization, siderophore production, catalase activity, and biofilm formation capacity—features known to promote bacterial survival under heavy metal stress—the researchers evaluated the functional diversity of these isolates.

To find the presence of important metal resistance genes like merA, czcA, arsC, and copA, Mohammad Irfan Ali and his associates extracted genomic DNA from representative isolates and carried out gene-specific PCR assays. Significant genetic heterogeneity was found among the isolates in the study, with several strains concurrently exhibiting multiple resistance determinants. Furthermore, the prevalence of genera known for their metal tolerance characteristics, including Pseudomonas, Bacillus, Acinetobacter, and Micrococcus, was validated by molecular fingerprinting. The study found that the soil microbiomes of urban e-waste hotspots contain bacterial communities that are extremely diverse and genetically equipped to withstand high levels of metal toxicity. These bacteria also show great promise for use in site-specific bioremediation technologies in the future.

Narayan et al. (2022) with the goal of identifying heavy metal-resistant bacterial isolates from soils that have been continuously contaminated with metals derived from industry and e-waste. Under the direction of Raghav Narayan and his research colleagues, the study sought to classify these resistant bacteria's genetic variability and phylogenetic relationships according to their metal tolerance profiles. Numerous metal-tolerant bacterial strains were recovered through selective culturing in heavy metal-amended media after soil samples were methodically gathered from multiple active e-waste disposal zones.

The team used Random Amplified Polymorphic DNA (RAPD) analysis with a set of random decamer primers to evaluate genetic relatedness and polymorphism among the isolates. Significant genetic variability among the isolates was highlighted by the unique banding patterns that the RAPD-generated DNA profiles produced upon agarose gel electrophoresis. The strains were categorized into several genetic clusters based on cluster analysis of the RAPD data, each of which was linked to unique patterns of heavy metal resistance.

Raghav Narayan and his group also carried out 16S rRNA gene amplification and sequencing in order to precisely identify the isolates at the species level. The isolates' taxonomic affiliations were validated by comparative phylogenetic analysis of the obtained sequences, primarily with genera like Pseudomonas, Bacillus, Enterobacter, and Stenotrophomonas. A thorough grasp of the genetic diversity and evolutionary relationships among the heavy metal-resistant bacteria was made possible by the combination of RAPD and 16S rRNA sequencing. In order to characterize soil bacteria from metal-polluted environments and choose powerful strains for bioremediation interventions, the study found that the combined use of molecular typing and sequencing is very effective.

Naskar et al. (2022) with an emphasis on the genetic foundation of lead and arsenic resistance in soil bacteria gathered from industrial waste disposal sites and highly contaminated e-waste. Debarati Naskar and his colleagues realized that extended exposure to heavy metals in these conditions frequently causes bacteria to develop adaptive genetic mechanisms, particularly plasmid-mediated resistance, which improves their capacity for survival and detoxification.

To isolate highly tolerant bacterial strains, soil samples were gathered from different metal-polluted zones and selective enrichment culturing was carried out in media supplemented with high concentrations of arsenic and lead salts. The isolates were subjected to plasmid profiling using agarose gel electrophoresis and alkaline lysis after morphological, Gram staining, and biochemical testing. Large, high-molecular-weight plasmids were found in a sizable portion of resistant isolates, according to the results, suggesting a plasmid-associated resistance mechanism.

Debarati Naskar and her team conducted plasmid curing experiments using curing agents such as acridine orange and elevated temperature treatments in order to validate the functional role of these plasmids. The cured strains showed a significant decrease in their tolerance to lead and arsenic, proving that plasmids play a role in resistance. Additionally, the plasmids were found to contain known metal resistance genes like arsC, pbrA, and merA, which were targeted by gene-specific PCR amplification. According to the study's findings, plasmid-mediated resistance is common among soil bacteria that live in metal-polluted e-waste sites and is essential to their ability to survive, adapt, and possibly be used in bioremediation procedures.

Patra et al. (2023). The goal of the study, which was led by Sayantan Patra and his research team, was to find and genetically describe native bacterial isolates that could tolerate a variety of harmful heavy metals and aid in the natural bioremediation of contaminated environments. In order to perform selective enrichment culturing in media supplemented with lead, cadmium, and arsenic, soil samples were methodically gathered from unofficial e-waste disposal sites.

The researchers used 16S rRNA gene amplification and sequencing to confirm the identity of the most tolerant isolates following initial morphological, biochemical, and Gram staining identification. The taxonomic identity of multiple isolates as Achromobacterdenitrificans was confirmed by comparing the obtained sequences with NCBI GenBank databases using BLAST searches. Close relationships with known heavy metal-resistant Achromobacter strains reported from other contaminated environments were found using phylogenetic tree construction.

In order to evaluate these isolates' tolerance levels, Sayantan Patra and his group also calculated the Minimum Inhibitory Concentration (MIC) values for a number of heavy metals. Plasmid screening also showed that some strains had large plasmids, which may indicate plasmid-mediated resistance mechanisms. The study found that the strains of Achromobacterdenitrificans that were isolated from soils contaminated by e-waste exhibit a high degree of genetic adaptability and resistance to heavy metals, which makes them attractive options for future bioremediation projects that target environments that are polluted by multiple metals.

Roy and Sinha (2023) used advanced multi-locus sequence typing (MLST) in a highly focused molecular genetic study to evaluate the genetic diversity of

cadmium-resistant bacteria isolated from e-waste and industrially polluted soil environments. Under the direction of Priyanka Roy and Rituparna Sinha, the study recognized the environmental risks associated with cadmium pollution in terrestrial ecosystems, especially in areas where e-waste disposal is not regulated. By using selective enrichment culturing techniques in media supplemented with cadmium, the researchers were able to isolate a number of bacterial strains that are tolerant of cadmium.

The isolates were subjected to MLST analysis, a high-resolution molecular typing technique that entails sequencing multiple housekeeping genes to establish genetic relatedness and strain-level differentiation, following initial morphological and biochemical identification. Seven conserved loci, including recA, gyrB, rpoB, atpD, and dnaK, were chosen by Priyanka Roy and Rituparna Sinha for PCR amplification and sequencing. Significant genetic polymorphism was found among the cadmium-resistant isolates, according to the allelic profiles produced for these genes, suggesting a high level of intra-species genetic diversity.

According to their individual Minimum Inhibitory Concentration (MIC) values against cadmium, the isolates were categorized into multiple clades by phylogenetic analyses based on concatenated sequence data. The study also found prominent genera with metal-resistance traits, including Pseudomonas, Bacillus, Achromobacter, and Micrococcus. In order to study the population structure and evolutionary dynamics of heavy metal-resistant bacteria, Roy and Sinha came to the conclusion that MLST provides an efficient and accurate molecular tool. This could be useful in choosing strong strains for cadmium bioremediation applications.

Das and Nandi (2023) conducted an applied microbiological study to show how bacterial consortia can remove multiple heavy metals from contaminated soils at the same time and to genetically characterize the consortia members for the presence of important resistance genes. Recognizing the shortcomings of single-strain bioremediation, the research team led by Anirban Das and Mousumi Nandi set out to create mixed bacterial consortia that would be more effective at removing lead, cadmium, chromium, and nickel.

Following the collection of soil samples from industrial effluent and e-waste disposal sites, the samples were subjected to selective enrichment culturing in media that contained a cocktail of heavy metals. Batch bioaccumulation experiments were used to test the consortia that were formed from the most tolerant isolates. The findings confirmed the bacterial consortia's synergistic action in detoxifying environments contaminated by multiple metals by demonstrating a significant and simultaneous decrease in metal concentrations over time.

Anirban Das and Mousumi Nandi used gene-specific PCR analysis to target well-known heavy metal resistance genes, such as czcA, pbrA, merA, arsC, and cadA, in order to comprehend the genetic basis of this multi-metal resistance. The presence of several resistance determinants among various consortia members was validated by the amplified products. The study came to the conclusion that bacterial consortia are better at removing metals than individual strains and that having a variety of resistance genes at the same time increases their resilience and effectiveness in treating soils contaminated by e-waste, which makes them good candidates for extensive bioremediation projects.

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treating soils contaminated by e-waste, which makes them good candidates for extensive bioremediation projects.

The goal of Ganguly et al.'s thorough metagenomic analysis from 2024 was to profile the distribution, diversity, and abundance of heavy metal resistance genes (MRGs) in the bacterial communities that live in e-waste-contaminated areas. Ananya Ganguly and her team conducted the study because heavy metal pollution in unofficial e-waste recycling zones is complex and persistent, and traditional culture-based methods frequently fall short of capturing the entire microbial diversity. In order to get around this restriction, the researchers gathered composite soil samples from several e-waste hotspots that are known to have high levels of contamination from lead, cadmium, arsenic, mercury, and chromium.

The entire community These soil samples had their DNA extracted directly, and Illumina platforms were used to carry out high-throughput shotgun metagenomic sequencing. With an emphasis on identifying heavy metal resistance gene families, the sequencing data was assembled and annotated using sophisticated bioinformatics pipelines like MG-RAST and MEGAN. A diverse range of MRGs, including those encoding metal-binding proteins, oxidative stress response proteins, and efflux pumps (czcA, copA, merA, arsC), were successfully identified by Ananya Ganguly and her group.

MRG abundance was substantially higher in soils with higher metal concentrations, according to quantitative analysis, suggesting a strong environmental selection pressure. Furthermore, these resistance genes were primarily associated with genera like Pseudomonas, Bacillus, Acinetobacter, and Stenotrophomonas by taxonomic profiling. The study highlighted the potential of metagenomic approaches to inform future bioremediation and environmental management strategies and concluded that they offer priceless insights into the complex and varied genetic reservoirs of heavy metal resistance in e-waste contaminated environments.

Sarkar et al. (2024) used 16S rRNA gene sequencing and resistance gene-specific PCR in an integrated microbiological and molecular study to characterize bacterial isolates from e-waste contaminated soils. In order to identify and genetically profile native bacterial strains that can survive in metal-rich environments brought on

by uncontrolled e-waste disposal practices, Sudipta Sarkar and her colleagues conducted the study. To isolate resistant strains, soil samples were methodically gathered from several unofficial e-waste recycling and disposal sites. Selective enrichment culturing was then performed in nutrient media supplemented with heavy metals.

Following morphological, Gram-staining, and biochemical tests for initial isolate identification, the 16S rRNA gene was amplified and sequenced for molecular identification. The presence of several heavy metal-resistant genera, such as Pseudomonas, Bacillus, Enterobacter, Klebsiella, and Micrococcus, was verified by sequence analysis using NCBI BLAST tools.

Sudipta Sarkar and her group used gene-specific PCR amplification to look into the genetic basis of their resistance. They targeted important heavy metal resistance genes like arsC (arsenic resistance), czcA (cadmium-zinc-cobalt resistance), pbrA (lead resistance), merA (mercury resistance), and cadA (cadmium resistance). The amplified products demonstrated that these genes were widely distributed among the isolates, with multiple strains exhibiting multiple resistance determinants at the same time. The study came to the conclusion that a strong and effective framework for identifying and characterizing metal-resistant soil bacteria from e-waste sites is provided by the combination of 16S rRNA gene sequencing and resistance gene-specific PCR. These isolates may prove to be useful bioremediation agents in upcoming environmental cleanup initiatives.

Khatun et al. (2024) conducted a targeted microbiological and molecular study to isolate and characterize Micrococcus luteus strains with high arsenic resistance, one of the most toxic and recalcitrant heavy metals of e-waste polluted soils. The authors, led by Nusrat Khatun along with her colleagues, attempted to isolate indigenous bacterial species capable of endurance in arsenic-contaminated environments and to confirm the genetic determinants of their resistance. Samples of soil were collected from highly arsenic-contaminated e-waste recycling and dumping areas, and subsequently selectively enriched cultured in media with increasing arsenic salt concentrations.

These monomeric strains were analyzed in detail by morphological, Gram staining, and biochemical studies, which placed them as members of species Micrococcus luteus. To determine their taxonomic status and explore their modes of resistance, Nusrat Khatun and coauthors performed 16S rRNA gene sequencing, which supported the identification with high sequence similarity to known Micrococcus luteus strains. Gene-specific PCR amplification also was utilized to screen for the presence of arsenic resistance genes, i.e., arsC (arsenate reductase) and arsB (arsenite efflux transporter), which are pivotal in arsenic detoxification in bacteria.

The findings indicated consistent amplification of the resistance genes in the various isolates as proof of their genetic potential for arsenic tolerance. In addition, the study compared the Minimum Inhibitory Concentration (MIC) of the isolates against arsenic and indicated significantly high tolerance levels relative to other previously isolated soil bacteria. Nusrat Khatun and her co-workers concluded that Micrococcus luteus strains isolated from e-waste contaminated environments are of high ecological and biotechnological significance as potential arsenic bioremediation agents for contaminated environments.

Rao et al. (2024) conducted field-level bioremediation screening to assess the efficacy of native heavy metal-tolerant bacteria under controlled microcosm conditions. Ramesh Rao and colleagues' study identified the necessity of shifting from laboratory-scale batch experiments to the verification of the applicability of native bacterial isolates in actual field conditions for mitigating heavy metal toxicity of polluted soils. E-waste dumping and industrially contaminated soil samples were collected from diverse locations and subjected to highly tolerant bacterial isolations using selective enrichment methods in multi-metal-supplemented media.

Individual strains were first identified by biochemical and phenotypic analysis, and heavy metal resistance patterns were ascertained by Minimum Inhibitory Concentration (MIC) assays for lead, cadmium, arsenic, and chromium. Strong strains, generally from genera such as Bacillus, Pseudomonas, Klebsiella, and Achromobacter, were then inoculated into controlled soil microcosm systems artificially spiked with known levels of heavy metals.

Over a monitoring period of weeks, Ramesh Rao and co-workers periodically analyzed the heavy metal content in the soil by Atomic Absorption Spectrophotometry (AAS) to evaluate metal reduction efficiency. The results showed a significant decrease in the metal content in inoculated microcosms compared to uninoculated controls, indicating effective metal immobilization and/or removal by the applied bacterial communities. The study concluded that native heavy metal-resistant bacteria from e-waste contaminated sites possess superior bioremediation potential and can be utilized effectively in in-situ soil rehabilitation programs under field-like conditions.

Karmakar et al. (2024) carried out a detailed microbiological and molecular investigation to analyze the biosorption capacity and genetic profile of multi-metal resistant Bacillus populations of extremely e-waste and industrial effluent contaminated landfill soils. The study, carried out by Soma Karmakar and her colleagues, aimed to address the critical problem of heavy metal contamination in landfill sites, where long-term pollutants such as lead, cadmium, arsenic, nickel, and chromium are key environmental and health issues. The prime objective was to screen indigenous Bacillus isolates with remarkable resistance towards a range of heavy metals and to determine their potential for bioremediation purposes.
Soil samples were collected in a routine manner from dumping sites of landfills well established for multi-metal chronic pollution. Selective enrichment culturing through the use of nutrient media with step-wise introduction of heavy metal was carried out to obtain metal-tolerant Bacillus strains. The isolates were characterized as Bacillus species by morphological study, Gram staining, and biochemical analysis. The strains were screened for Minimum Inhibitory Concentration (MIC) with various metals to select the best candidates.

To quantify their biosorption capacities, **Soma Karmakar et al**. performed batch biosorption tests, wherein bacterial biomass was subjected to heavy metal solutions of known concentration under controlled laboratory conditions. Residual metal concentrations were measured at regular time intervals by Atomic Absorption Spectrophotometry (AAS), and results showed that certain Bacillus isolates

possessed excellent biosorption efficiencies of lead, cadmium, and chromium, causing considerable metal concentration reductions in a short incubation time.

Besides, molecular identification was also performed through 16S rRNA gene sequencing to validate the taxonomic identity of the effective isolates. This was also complemented by the use of gene-specific PCR assays to detect primary heavy metal resistance genes, including czcA, cadA, arsC, and pbrA, involved in detoxification and efflux processes. Detection of these genes validated the high metal tolerance of the isolates in biosorption experiments. Soma Karmakar and colleagues concluded that the multi-metal resistant Bacillus strains from landfill soils are extremely promising candidates for eco-friendly and cost-effective bioremediation technologies to detoxify heavy metal-polluted waste lands.

Karmakar et al. (2024) carried out a comprehensive microbiological and molecular study to evaluate the biosorption capacity and genetic traits of multi-metal resistant Bacillus isolates of highly e-waste and industrial waste contaminated landfill soils. The study, carried out by Soma Karmakar and colleagues, identified the environmental risk caused by heavy metals like lead (Pb), cadmium (Cd), arsenic (V), nickel (Ni), and chromium (Cr), which remain in landfill sites and cause severe ecological and public health concerns. Their study focused on the isolation of native bacterial strains that can tolerate and biosorb multiple toxic metals and the identification of the genetic determinants of their resistance. For this purpose, soil samples were collected systematically from old landfills with a history of multi-metal contamination. A number of metal-resistant strains of Bacillus were isolated effectively by selective enrichment culturing of samples in metal-supplemented media. The isolates were screened by preliminary morphological, Gram staining, and biochemical tests, confirming them as Bacillus species. Minimum Inhibitory Concentration (MIC) for different heavy metals was determined for all the isolates, choosing the best contenders capable of surviving at high metal concentrations.

To determine their biosorption capacity, Soma Karmakar and co-workers conducted batch biosorption experiments with exposure of bacterial biomass to controlled levels of single and binary metal solutions. Metal concentration in culture supernatants at various time intervals was determined by Atomic Absorption

Spectrophotometry (AAS) and indicated that certain Bacillus isolates exhibited extremely effective biosorption with maximum removal efficiency for lead, cadmium, and chromium in a very short exposure period.

Furthermore, molecular characterization was also obtained through 16S rRNA gene sequencing that confirmed the accurate taxonomic identification of the isolates. In addition, gene-specific PCR assays for key metal resistance genes such as czcA (cadmium-zinc-cobalt efflux system), cadA (cadmium efflux ATPase), arsC (arsenate reductase), and pbrA (lead resistance ATPase) were also conducted. The amplified gene products validated the presence of these resistance determinants in effective Bacillus isolates that were responsible for their enhanced tolerance and biosorption ability under multi-metal stress conditions.

Conclusion:

Karmakar et al. (2024) provides pivotal information regarding the bioremediation ability of native multi-metal tolerant Bacillus strains isolated from soil samples of highly contaminated landfills with e-waste and industrial waste pollutants. By the use of a scientific approach with selective isolation, biosorption experiments, molecular characterization, and genetic profiling, the study satisfactorily proved that the native Bacillus strains not only endure high levels of toxic metals such as lead, cadmium, arsenic, nickel, and chromium but also actively remove them from the environment through effective biosorption processes.

The results of batch biosorption experiments also vindicated that certain of the isolated strains exhibited extremely high efficiencies of metal removal, particularly against lead, cadmium, and chromium, within a short incubation period. This validates their applied relevance in real bioremediation practice, where rapid and effective removal of a range of heavy metals is paramount. In addition, molecular identification by 16S rRNA gene sequencing confirmed the taxonomic placement of these efficient isolates in the Bacillus genus, which is well known for its toughness and resilience in stressful environments.

Gene-specific PCR analysis also confirmed the findings by checking for the presence of the significant heavy metal resistance determinants such as czcA, cadA, arsC, and pbrA in the most resistant strains. These determinants have a direct correlation with metal efflux systems, enzymatic detoxification, and metal sequestration processes responsible for the high metal resistance and biosorption capability in vitro. Together, this study indicates towards the ecologic and biotechnological potential of indigenous multi-metal resistant Bacillus populations present within metal-polluted landfill soils. The ability of the bacteria to survive and thrive in extreme metal-concentrated conditions, with their genetic resistance and high biosorption potential, makes them high-potential microbes for the establishment of eco-friendly, cost-effective, and large-scale bioremediation processes for remediation of multi-metal polluted habitats.

Karmakar et al. (2024) performed an extensive microbiological and molecular study to evaluate the biosorption capacity and genetic traits of multi-metal resistant Bacillus strains obtained from landfill soils highly polluted with e-waste and industrial contaminants. The study, conducted by Soma Karmakar and colleagues, identified the environmental risks of heavy metals like lead (Pb), cadmium (Cd), arsenic (As), nickel (Ni), and chromium (Cr), which remain in landfill environments and cause severe ecological and public health issues. Their aim was to screen indigenous bacterial isolates with the potential to tolerate and biosorb several toxic metals and to delineate the genetic determinants underlying their resistance.

For this purpose, soil samples were collected routinely from long-term landfill sites with multi-metal contamination. Multiple resistant Bacillus strains were isolated successfully by selective enrichment culturing in metal-supplemented media. The preliminary morphological, Gram staining, and biochemical tests were used to confirm the isolates as Bacillus species. The Minimum Inhibitory Concentration (MIC) for various heavy metals was established for each isolate to determine the best candidates that can survive at high metal concentration.

In order to assess their biosorption ability, Soma Karmakar and her team performed batch biosorption tests, treating bacterial biomass with solutions of

controlled concentrations of single and mixed metal solutions. Metal concentrations remaining in the culture supernatants were determined at regular time intervals by Atomic Absorption Spectrophotometry (AAS), and it was found that some Bacillus isolates showed very effective biosorption, with high removal percentages for lead, cadmium, and chromium within short contact times.

In addition, molecular characterization was performed by 16S rRNA gene sequencing, which validated the correct taxonomic identification of the isolates. Gene-specific PCR assays for key metal resistance genes like czcA (cadmium-zinc-cobalt efflux system), cadA (cadmium efflux ATPase), arsC (arsenate reductase), and pbrA (lead resistance ATPase) were also performed. The overexpressed gene products confirmed the existence of these resistance determinants in the virulent Bacillus isolates, which justified their higher tolerance and biosorption capacity under multi-metal stress conditions.

Chapter -3

Research Methodology

2.1 Site and Soil Analysis

2.1.1 Site Description

The study site was a metal-contaminated location situated in the Metoda GIDC Industrial Area, where pollutants were discharged from diverse industrial solid waste.

Different metal related works like pipe making, steel synthesis, brass synthesis etc. are being performed in different industries. The solid waste of these industries discarded on the nearby soil of that industry. So the soil of that area must be heavily contaminated by different heavy metals.

The GIDC Industrial Association occupies an area of 424 hectares, situated near state highway number 3 and NH-46 12 kilometres from Gwalior City. The estate with 1500 plots is fully equipped with infrastructural facilities required for the development of industry.

2.2 Sample Collection

Metoda GIDC is reach with different industries. Five to six different industries were selected for sample collection. Total ten samples were collected from these industries containing highly metal contaminated sites. The industries from where the samples were collected are here under.

Table-2.1 Metoda GIDC Industries

Number	Industry Name	No. of sample collected
1	Raj Enterprise	1
2	Kalsun Alloys Cast Pvt. Ltd.	1
3	Radhika Steels	1
4	Rishi Laser Ltd.	1
5	Chamunda Enterprise	1
6	L. B. Metal Industries	2
7	Balaji Steel	3

These all industries are concerned with production and processing of metal related components. So the soil of these industries must be contaminated with different heavy metals, like Iron, Copper, Brass, Lead, Mercury, Tine, some precious metals like Silver, Gold etc. Some of these companies are scrap dealer and provide metal scrap to other companies for processing or use of metal.

The samples were collected from 10 centimeter of the soil surfaces where the metal contamination might be higher, so that we can get potential organism which can grow in presence of metal. The soil was dogged with the help of sterile spatula and it has been collected into sterile sample collection bags. Then all these collected samples were stored at 4˚C in refrigerator before use.

2.3 Isolation/Screening of Microbes

After collection, the samples were processed for the isolation of different bacteria present in the soil.

1) For the isolation 1 gram soil from each soil sample was suspended into 5 mL of sterile distilled water.

2) Suspensions were allowed to stand for 5 minutes to settle down soil at the bottom of the test tube.

3) After sedimentation a loopful from the supernatant was inoculated into the N-agar plates by streaking and spread plate technique.

4) Then all Petri plates were incubated at 37˚C for 24 hours.

5) After 24 hours different colonies were selected on the basis of their different colony characteristics.

6) Total 24 isolates were obtained by colony characteristics by primary screening method.

2.3.1 Gram's staining

The detailed procedure for Gram's staining is described as under:

a) Clean and grease free slides have been taken to prepare the smear on it.

b) After cleaning a loop full of the culture was spread on the surface on all the slide with different isolates and a thin smear has been prepared.

c) After that the prepared smear was heat fixed by passing the slide over the flame.

d) After all these initial steps all slides were flooded with crystal violate solution and allowed it to remain for one minute.

e) All slides were rinsed with the tap water after one minute.

f) On the next step all slides were flooded with gram's iodine and allowed to remain for one minute.

g) After one minute all slides were rinsed off with tap water.

h) On the third step all slides were flooded with decolourizer and allowed it to stand for 4-5 seconds.

i) After five seconds the counter stain was applied to all the slides and allowed it to remain for one minute.

j) After one minute all slides were rinsed with tap water and allowed it to air dry.

k) After all slides got air dry, all the slides were observed under 100X (oil immersion) one by one.

2.3.2 Capsule Staining

The detailed procedure for capsule staining is described as under:

- Clean and grease free slides were taken and wiped it out with alcohol.
- A loop full directly from the culture plate was taken and a thick smear was prepared.
- The smear was not heat fixed because capsule get melt on the exposure of heat.
- All slides allowed to air dry.
- After that all slides were flooded with crystal violate and allowed it to remain for one minute.
- After one minute the crystal violate stain drained off. The slides were not washed with tap water, because of water the capsule can get dissolved.

- After that all slides were washed with 20% $CuSo_4$ and allowed it to air dry.
- After that all slides were observed under 100X one by one to check the capsule formation of the isolates.

2.4 Metal Tolerance of Isolates

All 24 isolates were checked for metal tolerance. Different metals like Barium, Chromium, Arsenic, Cadmium, Mercury, Lead are the dominant metals present in e-waste, So metal tolerance of all isolates was checked in different metal concentration with respect to growth.

2.4.1 Barium Tolerance

a) Barium is a primary metal present in e-waste.

b) For inoculation, microbial suspension was prepared by inoculating isolated colony in 5 ml of sterile N-broth.

c) First of all 1 ml of every isolates was inoculated into 50 ml sterile N-broth medium containing 10 ppm Barium concentration.

d) After incubation all flasks were incubated at 30˚C temperature for 48 hours.

e) After 48 hours 2 ml of culture from 10 ppm Barium containing medium was inoculated into 20 ppm Barium containing medium.

f) Like this, all isolates were transferred serially to increasing concentration like 30 ppm, 40 ppm, 50 ppm to 700 ppm.

2.4.2 Chromium Tolerance

a) Chromium is not hazardous at low concentration but it is toxic when the concentration is higher than certain levels.

b) 1 ml of inoculum of all isolates was inoculated in 50 ml sterile N-broth containing 10 ppm Chromium concentration.

c) After inoculation all flasks were incubated at 30˚C temperature for 48 hours.

d) Like Barium tolerance test, after 48 hours every isolates were serially inoculated to higher concentration like 20 ppm, 30 ppm, 40 ppm to 700 ppm.

2.4.3 Arsenic Tolerance

a) Arsenic is one of the hazardous metals present in e-waste.

b) Like chromium tolerance test, 1 ml inoculum of all 24 isolates was inoculated into 50 ml N-broth medium containing 100ppm Arsenic concentration.

c) After inoculation, all flaks were incubated at 30ºC at shaking condition for 48 hours.

d) After 48 hours organisms were inoculated to higher Arsenic concentration from 100ppm Arsenic containing medium.

2.4.4 Mercury Tolerance

- Mercury is the most poisonous metal of the e-waste. People can die due to the effect of the mercury.
- To check mercury tolerance of the isolates, 1 ml inoculum of all isolates were inoculated in 50 ml sterile N-broth medium containing 50ppm mercury concentration.
- After inoculation all isolates were incubated at 30ºC in shaking condition for 48 hours.
- After 48 hours all isolates were inoculated to higher mercury concentration containing medium.

2.4.5 Lead Tolerance

a) Lead is also a hazardous metal like mercury. It can be fatal for human being.

b) To check lead tolerance, 1 ml from bacterial inoculum was inoculated into 50 ml sterile N-broth medium containing 100ppm lead concentration.

c) All flasks were incubated at 30ºC on shaking condition for 48 hours.

d) After 48 hours 2 ml medium from 100ppm lead containing medium was added to higher lead concentration containing medium to check the highest tolerance power of the isolates.

2.4.6 Cadmium Tolerance

- This is another hazardous metal in the e-waste.
- To check the cadmium tolerance of all isolate, 1 ml culture was inoculated into 50 ml sterile N-broth medium containing 50ppm cadmium in the medium.
- After inoculation, flasks were incubated at 30ºC for 48 hours on shaking condition.
- After incubation all isolates were forwarded to higher concentration of cadmium containing medium till growth stops.

2.5 Microbial Action on Electronic Waste

2.5.1 Introduction

All 24 isolates were tested for tolerance of different heavy metals. Among 24 organisms, 12 isolates were screened which were capable to grow in the presence of all heavy metals and were giving higher growth than other isolates. All the 12 isolates have were further tested for their action on electronic waste.

CD/DVD, Mother Board (Printed Circuit Board) and Electric Cable were used as electronic wastes. All 12 isolates were tested by means of growth and removal of heavy metals present in different ewaste.

2.5.2 Microbial Action on CD/DVD

- All isolates were inoculated into 50 ml sterile N-broth medium containing 5-6 gm pieces of CD/DVD.
- After inoculation every isolates were incubated at 30ºC for 144 hours in shaking condition.
- After 144 hours growth of isolates was noted by measuring Optical Density at 520 nm.
- Damage to the surface of CD/DVD pieces was checked by Scanning Electron Microscopy (SEM).
- Medium in all flasks was subjected to total metal analysis to check the presence of metal specific to the CD/DVD in them.

2.5.3 Microbial Action on Mother Board

- All isolates were inoculated into 50 ml sterile N-broth containing 5-6 gm pieces of Mother Board.
- After inoculation all flasks were incubated at 30ºC for 144 hours in shaking condition.
- After 144 hours, growth of isolates was noted by measuring Optical Density at 520 nm.
- Damage to the surface of Mother Board pieces was checked by Scanning Electron Microscopy.
- Medium in all flasks was subjected to metal analysis to check the presence of metals specific to mother board pieces in them.

2.5.4 Microbial Action on Electric Cable

- All isolates were inoculated into 50 ml sterile N-broth containing 5-6 gm pieces of Electric Cable.
- After incubation all flasks were incubated at 30ºC for 144 hours in shaking condition.
- After 144 hours, growth of microbes was noted by measuring Optical Density at 520 nm.
- Damage to the surface of Electric Cable pieces was checked by Scanning Electron Microscopy.
- Medium in all flasks was subjected to metal analysis to check the presence of metals specific to electric cable in them.

2.6 Scanning Electron Microscope

2.6.1 Sample Preparation

- Place your sample on the aluminum holder stub using a double sticky carbon tops.
- Insulating samples must be positioned with either carbon or gold and electrically grounded. Gold coating has high conductivity but is unsuitable for

EDX investigation. Utilize silver point for the electrical grounding of the sample. Conductive samples are exempt from this treatment.

- If you possess numerous samples that resemble one other, inscribe identifiers on the sample stub or the samples themselves. Identifying analogous samples in the SEM is somewhat challenging.
- Subsequently, thoroughly dry the sample in a drying oven at 60ºC for a minimum of 3 hours, contingent upon the state of the sample. The optimal approach is to allow it to remain in the drying oven.

2.6.2 Inserting the sample into the sample holder

- Verify that the valves on both nitrogen gas cylinders are open. Each tank must contain a minimum of 100 psi of nitrogen.
- If the nitrogen level is low, press the Vent button to refill the tank.
- Once you hear a click sound, locate the lever at the bottom of the door, gently pull it up, and open the chamber door.
- Lower the sample mounting stage completely by pressing the Z-axis down arrow key on the microscope control table keyboard.
- Insert the sample holder stubs into the designated mounting apertures. Place taller samples away from the left side of the chamber, adjacent to the secondary electron detector. Utilize the yellow elongated screwdriver to secure the set screws and confirm that the sample holders are firmly affixed.
- Ensure the door is free of any obstructions before closing it gently. Then, press the EVAC button to activate the system.You will hear the rotary pump start. Wait for two minutes to confirm that the green light appears on the display.
- Allow approximately 30 to 45 minutes to achieve a high vacuum.

2.7 Total Metal Analysis by MP-AES

2.7.1 Instrumentation

- All measurements were performed using an Agilent 4200 MP-AES, with nitrogen plasma gas supplied by an Agilent 4107 Nitrogen Generator..

- The generator obviates the requirement and expense of acquiring analytical grade gases. The initial system had a double-pass cyclonic spray chamber and the One Neb nebulizer.
- An Agilent SPS 3 auto sampler was utilized to dispense samples to the instrument, enabling unattended operation of the system.
- The instrument operated in a fast sequential mode and featured a Peltier-cooled CCD detector.

Background and spectral interference were efficiently and accurately corrected using Agilent's MP Expert software. The method parameters are provided in the table.

Table 2.2: MP-AES method parameters

Parameter	Value
Replicates	3
Pump rate	15 rpm
Sample uptake delay	35 seconds
Rinse time	30 seconds
Stabilization time	15 seconds
Fast Pump during uptake and rinse	On (80 rpm)
Autosampler	Agilent SPS 3
Sample pump tubing	Orange/green
Waste pump tubing	Blue/blue

2.7.2 Sample Preparation

- Microwave digestion was used to digest the ASPAC 80 RM for the analysis of Au, Ag, Cr, Fe, Cd, and Pb by MP-AES.
- The sample (0.18 g) was subjected to a solution of 7 ml HNO_3 and 1 ml H_2O_2.
- Digestion was performed using a preloaded method on the MARS microwave system.
- After cooling, the solution was diluted to 50 ml using ultrapure water.

- No additional sample preparation was necessary, and no ionization buffers or modifiers were utilized.

2.7.3 Wavelength Selection and Calibration Range:

Continuous wavelength coverage enables the selection of lines with appropriate sensitivity for the concentration range while minimizing spectral interferences.

Table 2.3: Wavelength Selection and Calibration range

Element and wavelength (nm)	Calibration range (ppm)
Cu 333.865	1-6
Fe 259.940	6-26
Mn 257.610	6-27
Zn 213.857	1-5
Na 686.830	2-100
K 767.491	1-100
Ca 445.478	20-100
Mg 383.829	1-100
B 249.772	0.25-1.0
P 213.618	10-80

2.8 Biochemical Test of Isolates

- Biochemical screening of 12 isolates was performed by HIMedia KB003 Hi-25

Enterobacteriaceae Identification Kit.

- Isolates were screened on the basis of results of all biochemical tests.
- All details of use of the kit are as under.

2.8.1 Preparation of Inoculum

1. KB003 is inappropriate for direct application to clinical specimens; organisms must be identified and purified beforehand. Utilize exclusively pure cultures.

2. Isolate the organism on a conventional medium such as Nutrient Agar (M001/M1274) or a differential media like MacConkey Agar (M082).
3. Inoculate a solitary isolated colony into 5 ml of Brain Heart Infusion Broth and incubate at 35-37°C for 4-6 hours until the turbidity attains ≥0.1 OD at 620 nm or corresponds to the 0.5 McFarland standard. Some meticulous organisms may necessitate incubation exceeding 6 hours.
4. Alternatively, prepare the inoculum by choosing 1-3 well-isolated colonies and suspending them uniformly in 2-3 ml of sterile saline, achieving a density of 0.1 OD at 620 nm.
5. Conduct an oxidase test utilizing the Oxidase disc provided in the kit.
6. Choose a well-isolated colony and place it on an oxidase disc. A strong purple hue within 10 seconds denotes a favorable reaction, a color alteration between 10-60 seconds signifies a delayed positive reaction, and a color change after 60 seconds or the absence of color change indicates a negative reaction.

   Document the findings in the Result Entry Datasheet. The oxidase test is crucial for differentiating Enterobacteriaceae from other gram-negative bacilli.

2.8.2 Inoculation of Kit

- Open the kit in an aseptic manner. Remove the sealing foil.
- Inoculate each well with 50µl of the above inoculum by surface inoculation method.

  Alternatively, the kit may be infected by puncturing each individual well with a loopful of inoculum.

2.8.3 Incubation

- Incubate all inoculated kits at 35ºC to 37ºC temperature for 18-24 hours.

2.8.4 Interpretation of Results:

Analyze the findings according to the criteria established in the identification index. The addition of reagents, when necessary, should occur at the conclusion of the incubation phase, specifically after 18-24 hours.

Part-1 Phenylalanine-Deamination Test: Well No:5

- Incorporate 2-3 drops of TDA reagent.
- The emergence of a dark green hue within one minute signifies a favorable reaction.
- No change in colour denotes a negative reaction.

Nitrate Reduction Test:Well No:6

- Incorporate 1-2 drops of Sulphanilic acid and 1-2 drops of N,N-Dimethyl-1-Naphthylamine Reagent.
- Immediate development of pinkish red colour on addition of reagent indicates positive reaction.
- No change in colour indicates a negative reaction.

Vogus Proskauer's Test:Well No:9

- Incorporate 2-3 drops of Barit reagent A and 1 drop of Barit reagent B.
  The appearance of a pinkish-red color within 5-10 minutes signifies a favorable result.
- A lack of color change or a minor color alteration indicates an unfavorable reaction.

Methyl Red Test:Well No:10

- Add 1-2 drops of Methyl Red reagent.
- Reagent remains red in colour if the test is positive.
- Reagent decolourises and becomes yellow if the test is negative.

IndolTest:Well No:11

- Incorporate 1-2 drops of Kovac's reagent.
  The emergence of a pinkish-red hue within 10 seconds signifies a good reaction.
  The reagent retains a faint hue if the test yields a negative result.

2.9 Temperature Optimization for Isolates

- On the basis of biochemical test results total six isolates were selected.
- All six isolates were tested for their optimum temperature.
- For the testing of optimum temperature 1 ml culture of all isolates was inoculated into 50 ml sterile N-broth containing e-waste.
- After inoculation all isolates were incubated at different temperature 20ºC, 28ºC, 37ºC and 42ºC for 48 hours.
- After incubation, optical density of all flasks was measured at 520 nm for the bacterial growth at different temperature.

2.10 pH Optimization for Isolates

- All six isolates were tested for their optimum pH.
- For the testing of optimum pH, 1 ml culture of all isolates were inoculated into 50 ml sterile N-broth containing different e-waste.
- The pH of medium was set to pH-5, pH-6, pH-7, pH-8 and pH-9 by using 1N HCL and 1N NaOH.

  After inoculation, all isolates were incubated at an optimal temperature of 28-37ºC for 48 hours.

  Following incubation, the optical density of all flasks was measured at 520 nm to assess bacterial growth at different pH levels.2.11 DNA Isolation of the Isolates

- The DNA isolation of all six isolates was done by the HiMedia bacterial DNA extraction kit.

The whole procedure of the kit is here under

2.11.1 Procedure

Allow the bacterial cell pellet collection tube to equilibrate at ambient temperature.

Resuspension of the cellular pellet and subsequent lysis Introduce 180 µl of Lysis solution 1 and re-suspendthe pellet through gentle pipetting.

Incorporate 20 µl of Proteinase K solution into the aforementioned collection tube. Vortex for 10-15 seconds then incubate for 30 minutes at 55ºC.

Incorporate 20 µl of RNase A solution into the aforementioned collection tube. Vortex for 10-15 seconds and incubate for 5 minutes at ambient temperature. (15-25 °C).

NOTE: This procedure facilitates the acquisition of RNA-free genomic DNA. Incorporate 200 µl of Lysis solution 2, vortex vigorously for approximately 15 seconds, and incubate at 55ºC for 10 minutes.

Observation: a homogenous mixture is crucial for effective lysis.

2.11.2 Prepare for Binding

- Incorporate 20 µl of ethanol (95-100%) into the lysate and mix completely using gentle pipetting.
  Observation: a white precipitate may develop upon the addition of ethanol. This precipitate does not hinder the DNA isolation process or any subsequent applications. It is imperative to transfer the entire precipitate to the HiElute Miniprep spin column.
- Introduce lysate into the HiElute Miniprep Spin Column Transfer the whole lysate acquired from step 5 into the HiElute Miniprep Spin Column to facilitate DNA binding. Spin in a centrifuge at 10,000 revolutions per minute for 1 minute. Eliminate the liquid flow and position the spin column into the identical 20 ml collection tube.
- Note: Employ a wide-bore pipette tip to substitute for the shearing of DNA while putting materials onto the column. Should the solution remain partially unfiltered through the membrane, centrifuge once more at 15,000 rpm until complete passage is achieved. Centrifugation at elevated speeds will not influence the yield or purity of the DNA.

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- Note: Employ a wide-bore pipette tip to substitute for the shearing of DNA while putting materials onto the column. Should the solution remain partially unfiltered through the membrane, centrifuge once more at 15,000 rpm until complete passage is achieved. Centrifugation at elevated speeds will not influence the yield or purity of the DNA.

2.11.3 Prewash

Introduce 500 µl of Prewash solution into the HiElute Miniprep Spin Column and centrifuge at 10,000 rpm for 1 minute. Eliminate the liquid flow and reutilize the identical collection tube with the column.

2.11.4 Wash

Dispense 500 µl of Prewash solution into the HiElute Miniprep Spin Column and centrifuge at 10,000 rpm for 1 minute. Cease the liquid flow and repurpose the identical collection tube with the column.

2.11.5 DNA Elution

Administer 200 µl of Elution Buffer directly into the column, ensuring no leakage on the sidewalls. Incubate for one minute at ambient temperature. Operate the centrifuge at 10,000 rpm for 1 minute to elute the sample.

DNA.

Note: For increased elution efficiency, incubate the sample at room temperature for 5 minutes after the addition of the Elution Buffer and then centrifuge. Using elution volumes less than 200 µl greatly raises the concentration of DNA in the

eluate, although it does decrease the overall DNA yield slightly. When water is used to store DNA, it can lead to acid hydrolysis.

2.116 Storage of the Eluate with Purified DNA

The eluate has pure genomic DNA. For storage for 24-48 hours, 28ºC is ideal, and -20ºC or below (-80ºC) for longer storage. To avoid denaturation of DNA, do not subject the sample to repeated thawing and freezing. The Elution Buffer stabilizes the DNA at these temperatures.

2.11.7 Agarose Gel Electrophoresis Preparation of 1x TAE

- To prepare 50 ml of 1x TAE buffer, add 10 ml of 50x TAE buffer to 490 ml of sterile distilled water.
- Mix well before use.

Preparation of Agarose Gel

- To prepare 50 ml of 0.8% agarose gel, dissolve 0.4 g of agarose in 50 ml of 1x TAE buffer in a glass beaker or flask.
- Heat the mixture using a microwave or hot plate, stirring occasionally until the agarose is fully dissolved. Ensure the flask lid is loosely placed to prevent pressure buildup.
- Allow the solution to cool to approximately 55-60ºC.
- Add 0.5 µl of Ethidium Bromide, mix thoroughly, and pour the gel solution into the tray.
- Let the gel solidify at room temperature for about 30 minutes.

Caution: Ethidium Bromide is highly toxic and mutagenic. Always handle with appropriate safety precautions, wearing latex gloves, though nitrile gloves are recommended for enhanced protection.

1. Loading of the DNA Sample

1. Prepare the sample for electrophoresis, add 2 µl of 6x gel loading buffer to 10 µl of the DNA sample.
2. Mix thoroughly by pipetting, then load the sample into the well.

3. After extracting the DNA sample, load the control DNA.

Electrophoresis

1. Attach the power cord to the electrophoretic power supply in accordance with established protocols. Red denotes the anode, whereas black signifies the cathode.
2. Conduct electrophoresis at 100-120 volts and 90 mA until the dye markers have moved an appropriate distance, contingent upon the size of the DNA to be seen..

Quantitation of DNA

- Concentration and DNA purity will be measured by spectrometric analysis and agarose gel electrophoresis.
- Dilute with Elution Buffer and zero the spectrophotometer with a sample, and measure the absorbance at 260 nm, 280 nm, and 320 nm with a quartz microcuvette.
- Absorbance at 260 nm must be between 0.1 and 1.0.
- Background absorption is adjusted using absorbance reading at 320 nm.
- Purity is determined by determining the ratio of absorbance 260 nm to 280 nm.
- DNA concentration (µg/ml) is determined by using the formula: 50 × A260 × dilution factor.
- Conduct agarose gel electrophoresis, photograph DNA bands under a UV transilluminator, and assess yield and purity with a UV spectrophotometer.

2.12 PCR of Isolated DNA

After DNA extraction from all six isolates, the DNA so extracted was used for PCR to amplify the isolated DNA.

- Components of PCR:
- The PCR reaction contains the following crucial components:

DNA Template

The double-stranded DNA (dsDNA) of interest has been isolated from the sample.

DNA Polymerase

- A thermostable Taq polymerase that remains stable at elevated temperatures (98ºC) and operates optimally at approximately 70ºC.
- Oligonucleotide Primers
- Brief segments of single-stranded DNA (about 20-30 base pairs) that are complementary to the 3' termini of the sense and antisense strands of the target sequence.
- Deoxynucleotide Triphosphate
- Single units of the bases A, T, G and C (dATP, dTTP, dGTP, dCTP) provide the energy for polymerization and the building blocks for DNA synthesis.

Buffer System

Includes magnesium and potassium to provide ideal conditions for DNA denaturation and renaturation; also essential for polymerase activity, stability, and fidelity.

PCR Procedure

The optimal amounts of Taq DNA polymerase, template DNA, primers, and MgCl2 will differ according to the specific system utilized. It may be crucial to determine the optimal conditions for each individual component. This is especially relevant to Taq DNA polymerase, cycling parameters, and MgCl2 concentration. It is recommended to titrate the enzyme and MgCl2 to determine optimal efficacy.

Choose the appropriate table for the reaction configuration: standard or readymix reagent. Introduce the reagents into a properly sized tube in the order provided in the table. A mastermix lacking the template should be produced and aliquoted into reaction tubes for several reactions. Ultimately, the template must be attached to the respective tubes.

Table 2.4 PCR Components

Amount	Component	Final Concentration
W µL	Water	
5 µL	10x PCR Buffer	1x
1 µL	Deoxynucleotide Mixture	200 µM
w µL	Forward primer (often 15-30 nucleotides in length)	0.1-0.5 µM
x µL	Reverse primer (often 15-30 nucleotides in length)	0.1-0.5 µM
0.5 µL	Taq DNA Polymerase	0.05 units/µL
y µL	Template DNA (typically 10 ng)	200 pg/µL
z µL	25mM $Mgcl_2$ (use only with buffer)	0.1-0.5 mM
50 µL	Total Reaction Volume	

Table 2.5 Readymix PCR Reaction

Amount	Component	Final Concentration
25 µL	Readymix	
w µL	Forward primer (typically 15-30 bases in length)	0.1-0.5 µM
x µL	Reverse primer (typically 15-30 bases in length)	0.1-0.5 µM
y µL	Template DNA (typically 10 ng)	200 pg/µL
z µL	Water	
50 µL	Total reaction volume	

Gently mix using a vortex and briefly centrifuge to consolidate all components at the bottom of the tube.

Add 50 µL of mineral oil to the surface of each tube to prevent evaporation when using a thermal cycler without a heated lid. The amplification settings will vary based on the primers and thermal cycler utilized. System optimization for certain primers, templates, and thermal cyclers may be necessary.

Table 2.6: Typical cycling parameters:

25-30 cycles of amplification are recommended		
Denature Template	94ºC	1 min
Anneal primers	55ºC	2 min
Extension	72ºC	3 min

The amplified DNA can be evaluated using agarose gel electrophoresis, subsequently stained with ethidium bromide. The mineral oil layer can be removed with a single chloroform extraction (1:1), obtaining the aqueous phase.

2.13 16s rRNA Sequencing

For 16s rRNA sequencing first of all PCR was performed to get multiple copies of bacterial genomic DNA.

1) Prepared Samples for PCR:

The PCR Module was optimized and used with ~25ng of genomic DNA. Three types of tubes have been used, two for control and one for sample.

Negative Control: 15µl PCR Module

15µl nuclease free water.

Positive Control: 15µl PCR Module

15 µl of the *E.coli*positive control DNA.

Samples: 15µl PCR Module

15µl of working stock.

All tubes have been caped and then placed into the thermal recycler.

2) PCR Performance:

1) The 9700 thermal cycler in 9600 emulation mode has been programmed, as follows:

Table 2.7: PCR Protocol

	Temperature	Time
Initial Steps	94ºC	3 min.
Melt	94ºC	50 sec.
Anneal	54ºC	50 sec.
Extend	72ºC	1 min.
Final Extension	72ºC	10 min.
Final Step	4º	∞

2) Set the reaction volume for thermal cycling to 30 µl, then started the run.

3) The PCR products have been stored at -15 to -25ºC until we were ready to use them.

3) PCR Products Purification:

Following gel analysis, we obtained 20 µl of the PCR product combination. Unused dNTPs and primers were eliminated from the PCR product mixture; the subsequent product was utilized:

1) 2µl ExoSAP-IT filter and 5 µl PCR product have been taken.

2) Total volume of 7 µl was put in following PCR condition:

Table 2.8 PCR Product Purification

	Temperature	Time
Step-1	37ºC	15 min
Step-2	80ºC	15 min
Step-3	4ºC	∞

4) Cycle Sequencing Reactions Preparation:

In a 2 ml micro centrifuge tube

1) 7 µl of the purified product and

2) 13 µl of the Sequencing Module were added.

All tubes were capped and then placed in the thermal cycler.

5) Cycle Sequencing Performance:

1) The 9700 thermal cycler in 9600 emulsion mode were programmed. The following thermal cycler conditions have been followed.

2) The reaction volume for thermal cycler has been set to 20 µl, then run has been started.

3) All the extension products have been stored at 4ºC overnight or -20ºC for up to one week.

Table 2.9: Thermal Cycler Conditions

	Temperature	Time
Initial Steps	94ºC	3 min
Melt	94ºC	50 sec
Anneal	54ºC	50 sec
Extend	72ºC	1 min
Final Extension	72ºC	10 min
Final Step	4ºC	∞

6) Extension Products Purification:

Following cycle sequencing, surplus dye terminators and primers were eliminated from the cycle sequencing reaction with the DyeExTM 2.0 spin kit.

This protocol was performed as followed:

1. Gently vortex the spin column to resuspend the resin.
2. Loosen the cap by a quarter turn to prevent vacuum formation inside the spin column.
3. Remove the bottom closure and place the spin column into a 2 ml collection tube. Centrifuge at 10,400 rpm for 3 minutes.
4. Carefully transfer the spin column to a sterile centrifuge tube. Slowly apply the sequencing reaction directly onto the center of the inclined gel bed surface.

5. Centrifuge again at 10,400 rpm for 3 minutes. Remove the spin column from the microcentrifuge tube. The eluate contains purified DNA, which can be directly loaded onto most sequencing devices.

6. After adding to the sequencer electrophoresis was performed.

Chapter -4

Results Analysis

3.1 Sample Collection

Total 10 samples have been collected from the industries which deal with metal related work from Gwalior. The soils of these industries usually contain higher concentration of metals. Samples have been collected into sterile sample collection bag and stored them at 4˚C in refrigerator.

3.2 Isolation/Screening of Microorganisms

In 5ml of sterile D/W 1 gm of soil has been added and suspension of these soil samples have been inoculated into N-agar plates by spread/streak plate method. On the basis of colony characters total ten colonies have been selected for further identification.

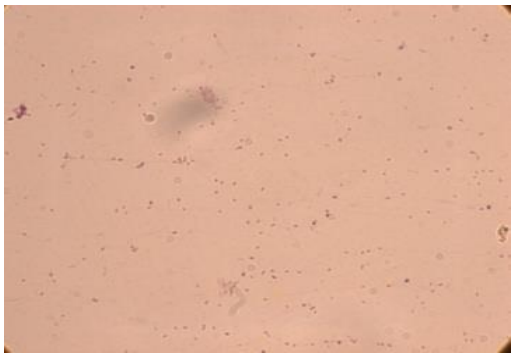
Table-3.1 Colony Characters of Isolates

Sample No	No. of colonies	Size	Shape	Colour	Elevati on	Margin	Opacity	Texture
GIDC-1	Colony-a	Medium	Circular	White	Flat	Entire	Opaque	Mucoid
	Colony-b	Small	Circular	Pink	Raised	Entire	Opaque	Mucoid
GIDC-2	Colony-a	Big	Circular	Yellow	Flat	Irregular	Opaque	Mucoid
	Colony-b	Small	Circular	White	Flat	Entire	Opaque	Dry
GIDC-3	Colony-a	Small	Circular	Yellow	Raised	Entire	Opaque	Mucoid
	Colony-b	Medium	Circular	Pinkish Yellow	Raised	Entire	Opaque	Mucoid
	Colony-c	Medium	Circular	Yellowish white	Raised	Entire	Opaque	Mucoid
	Colony-d	Big	Circular	White	Flat	Entire	Opaque	Dry
	Colony-e	Big	Circular	White	Flat	Irregular	Opaque	Dry
GIDC-4	Colony-a	Medium	Circular	Yellowish White	Flat	Entire	Opaque	Mucoid
	Colony-b	Small	Circular	White	Raised	Entire	Opaque	Mucoid
GIDC-5	Colony-a	Small	Circular	White	Raised	Entire	Opaque	Mucoid
	Colony-b	Pinpoint	Circular	Colourless	Raised	Entire	Transparent	Mucoid
GIDC-6		Medium	Circular	Colourless	Crateriform	Entire	Translucent	Mucoid
GIDC-7		Pinpoint	Circular	White	Raised	Entire	Opaque	Mucoid
GIDC-8	Colony-a	Pinpoint	Circular	White	Flat	Entire	Opaque	Mucoid
	Colony-b	Pinpoint	Circular	Yellow	Raised	Entire	Translucent	Viscous
	Colony-a	Medium	Circular	Pink	Raised	Entire	Opaque	Mucoid
	Colony-b	Big	Circular	White	Flat	Entire	Opaque	Mucoid
GIDC-9	Colony-c	Medium	Circular	White	Flat	Irregular	Opaque	Dry
	Colony-d	Small	Circular	Yellow	Raised	Entire	Opaque	Mucoid
GIDC-10	Colony-a	Big	Circular	White	Flat	Irregular	Opaque	Dry
	Colony-b	Small	Circular	White	Raised	Entire	Opaque	Mucoid
	Colony-c	Medium	Circular	Colourless	Raised	Entire	Transparent	Mucoid
	Colony-d	Small	Circular	White	Raised	Entire	Opaque	Mucoid

3.2.1 Gram's Staining

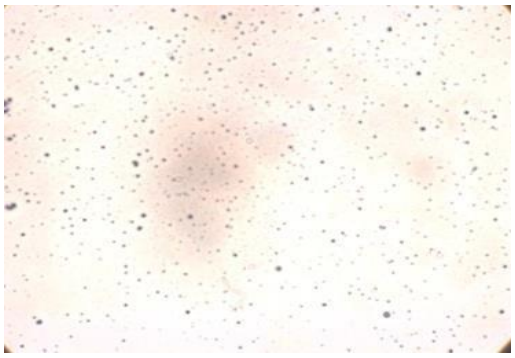
All samples were inoculated on N-agar in duplicates and from all plates two or three isolated colonies were selected for further study and identification.

Organisms from all twenty four colonies were morphologically studied by Gram's staining. Among twenty four isolates, most of the organisms were Gram positive cocci, three were Gram positive rods and three of them were Gram positive short rods.



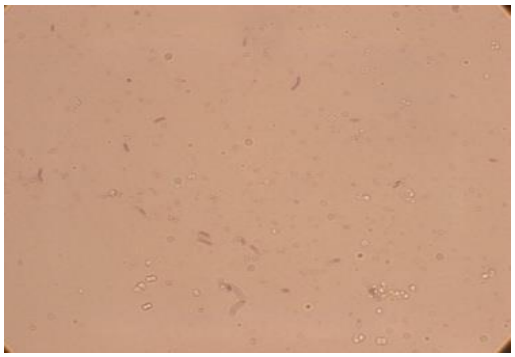
GIDC-1a

Gram positive Cocci



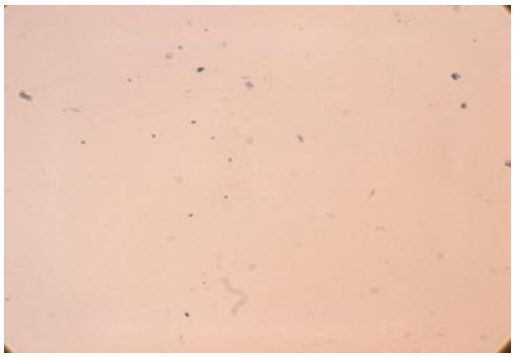
GIDC- 2a

Gram positive Cocci



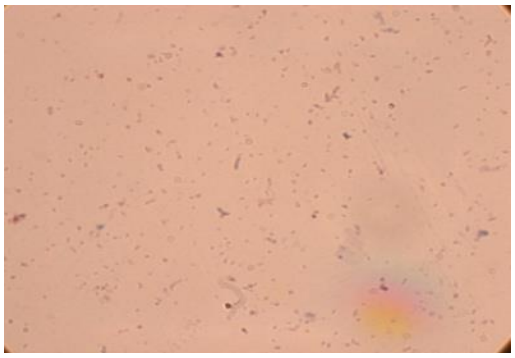
GIDC-2b

Gram positive Rods



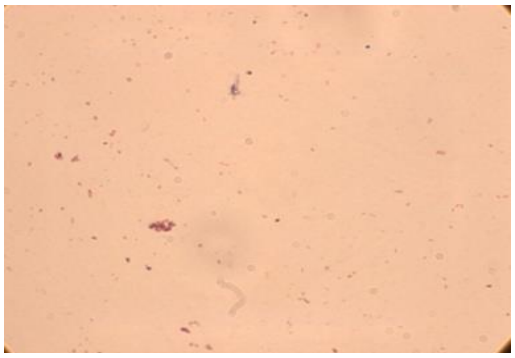
GIDC-5a

Gram positive Cocci



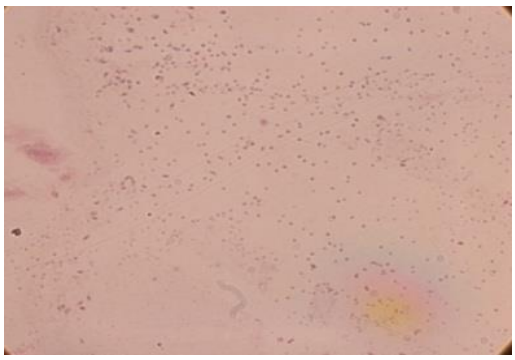
GIDC-6

Gram positive Short rods



GIDC-9b

Gram positive Cocci



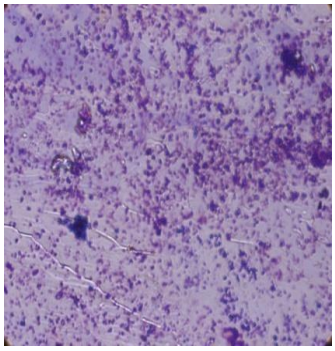
GIDC-10a

Gram positive Cocci

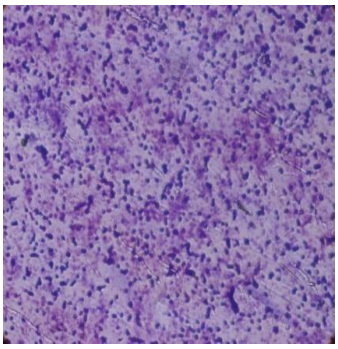
All isolates were Gram Positive and most of them have been arranged individual. GIDC-2b, GIDC-3a and GIDC-10b were Gram Positive Rods. GIDC-3c, GIDC-6 and GIDC-9a were Gram Positive Short Rods. And rest were Gram Positive Cocci.

3.2.2 Capsule Staining

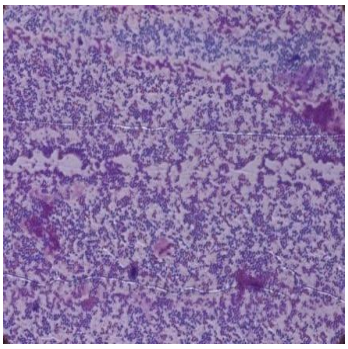
After Gram's staining all twenty four isolates have been checked for capsule formation by capsule staining. The results of capsule staining are shown in pictures below.



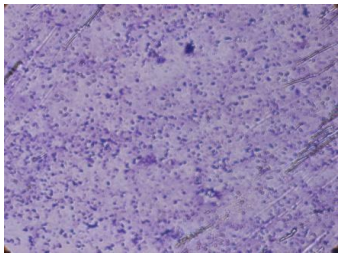
GIDC-3c



GIDC-3d



GIDC-9b



GIDC-9d

Most of the isolated were non capsulated except GIDC-2a, GIDC-3a, GIDC-4b, GIDC-5a and GIDC-10c were capsule forming isolates among all twenty four isolates.

Capsules forming isolates have been observed under 100X with purple coloured capsule, blue coloured cytoplasmic space with purple coloured background.

3.3 Metal Tolerance of Isolates

All isolates have been tested for metal tolerance by adding salts of different metals like, Barium, Chromium, Arsenic, Lead, Cadmium and Mercury.

Barium Tolerance

Barium Sulphate ($BaSo_4$) has been used as a salt of Barium to check the capability of the organisms in presence of this salt. Different concentration of Barium has been added into 20 ml sterile N-broth and incubated at 28˚C, for 48 hours in shaking condition.Growth has been tested from lower concentration to higher concentration in PPM. The concentration in the table is showing the concentration of Barium in $BaSo_4$.

Table-3.2 Barium Tolerance of Isolates

Conc.	50 Ppm	100 ppm	150 Ppm	200 ppm	250 ppm	300 ppm	350 ppm	400 ppm	450 ppm	500 ppm	550 ppm	600 ppm	650 ppm	700 Ppm
Isol.														
1a	2.1599	2.3312	1.9020	1.6653	1.3269	1.5212	1.3312	0.9482	1.1011	0.8311	0.5831	0.8250	0.1391	0.0369
1b	0.0349	0	0	0	0	0	0	0	0	0	0	0	0	0
2a	2.4135	1.7265	1.6236	0.9517	0.9572	0.9429	1.4588	1.1269	1.0804	0.9279	**0.8468**	0.8366	0.1376	0
2b	2.1168	2.3559	1.8176	1.7281	1.3786	1.3300	1.4807	1.1380	0.9050	1.0028	0.6399	0.9099	0.0828	0
3a	2.5482	2.4559	1.9760	1.8066	1.4696	1.3115	1.3726	1.1103	0.9284	1.0715	0.5641	0.5529	0.2457	0
3b	2.6047	2.4869	**2.0550**	1.7797	1.4776	1.4450	1.5117	1.1761	1.0674	0.9243	0.7041	0.8914	0.0744	0.0358
3c	2.6558	2.4215	1.8425	1.6401	1.2511	1.2490	1.3974	1.0364	0.9935	0.9867	0.7543	0.7850	0.1526	0.1331
3d	**2.8904**	2.4164	1.9789	1.8281	1.5145	1.6164	1.4498	1.1731	0.8402	1.0015	0.7565	**1.0378**	0.0755	0.1241
4a	2.4365	2.4921	1.9525	1.7754	1.6020	1.4582	1.4342	1.1236	0.7677	0.7481	0.5322	0.7752	0	0
4b	2.5754	**2.5164**	1.8270	1.7135	1.5043	1.6361	1.5021	1.1382	0.9358	0.9780	0.7231	0.9697	0	0
5a	0.2790	2.4591	1.9712	**1.8490**	1.5019	1.5008	1.4295	1.0148	0.7929	0.8760	0.6401	0.8908	0	0

5b	2.6401	2.4113	1.9360	1.7001	1.3461	1.3100	1.1805	0.935	1.0964	0.9140	0.5710	0.7959	0	0
6	1.6623	2.4039	1.9264	1.7762	1.4558	1.3769	1.3188	1.1601	1.0779	0.9902	0.5734	0.8052	0.0489	0
7	0.0537	2.3567	1.8653	1.6327	1.4753	1.3611	1.4286	1.1265	1.0776	0.9149	0.6205	0.8579	0.0825	0.1483
8a	2.059	2.4334	1.9508	1.7853	1.5028	1.6737	1.4570	1.0902	1.0110	0.9135	0.6274	0.7807	0.0972	0.0060
8b	2.0744	2.5386	1.9668	1.8055	1.4889	1.7199	1.4492	1.1170	0.9014	0.9155	0.8033	0.9111	0.2649	0.0922
9a`w	2.2086	2.4111	1.8961	1.7015	1.3171	1.2543	1.4328	1.1207	1.2347	**1.1579**	0.6983	0.8443	0.0974	0.1893
9b	2.1680	2.3786	1.9456	1.8064	**1.5421**	1.4993	1.4912	**1.1925**	1.1536	1.0960	0.6571	0.2831	**0.3054**	0.4575
9c	2.3928	1.8885	1.6024	1.3406	0.9839	0.9416	0.9340	1.1868	0.8928	0.8283	0.6740	0.4418	0.2772	0
9d	2.0579	2.3656	1.8938	1.6266	1.3696	1.7331	1.4318	1.3974	1.1864	1.1130	0.6684	0.8411	0.1565	0.1392
10a	2.5751	2.3381	1.9302	1.7939	1.5037	**1.7593**	1.4801	1.1406	1.0442	0.9026	0.5917	0.9294	0.1052	**0.5166**
10b	2.2907	2.2588	1.8021	1.6918	1.4248	1.7102	**1.5836**	1.1262	1.1538	1.0482	0.6493	1.0328	0.2744	0.1538
10c	2.3780	2.2017	1.6179	1.5698	1.2494	1.4498	1.1886	1.0038	0.9808	1.0533	0.7786	0.8869	0.1574	0.126
10d	0.4259	2.2802	1.9430	1.7790	1.2680	1.2862	1.3887	1.0473	0.9873	1.1359	0.6848	0.5037	0.1261	0.1154

On the basis of results shown in above table, highest growth and highest tolerance of barium has been shown by isolate number GIDC-3b. It has been shown growth till 600 ppm barium concentration with almost constant growth rate. GIDC-9b was the highest growing organism with Ba concentration of 250 ppm, 400 ppm and 650 ppm. On the basis of this result isolate GIDC-9b was the highest growing organism in presence of Barium. Other isolates were less tolerant to barium. After 700 ppm concentration of barium there was no growth of any isolates in 750 ppm barium concentration. The growth of isolates in N-broth medium has been shown in below images:

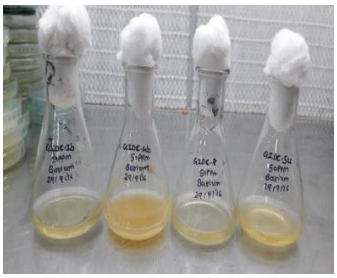
50ppm Barium concentration

GIDC-10d,10b,9a,4a

GIDC-8a,2b,3c,1a

GIDC-3a,9d,3d,2a

GIDC-1b,4b,7,5a



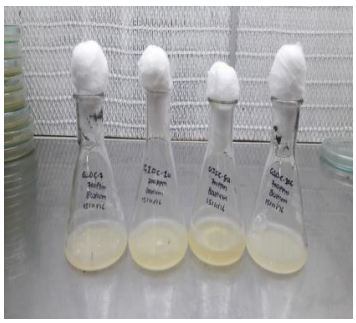
GIDC-10c,6,8b,10a

GIDC-9b,3b,9c,5b

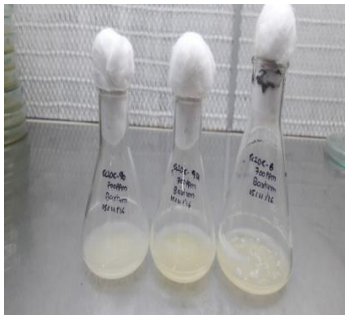
700ppm Barium Concentration

GIDC-3d,3b,3c,10a

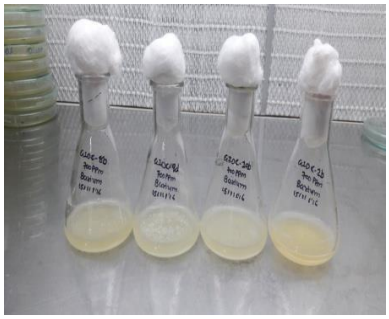
GIDC-1a,9c,10d,3a



GIDC-7,2a,8a,10c



GIDC-9b,9a,6



GIDC-8b,9b,10b,2b

Chromium Tolerance

Chromium Sulphate ($Cr_2(SO_4)_3$) has been used as a salt of Chromium to check the capability of the organisms in presence of this salt. Different concentration of Chromium has been added into

20 ml sterile N-broth and incubated at 28˚C, for 48 hours in shaking condition. Growth has been tested from lower concentration to higher concentration in PPM. The concentration in the table is showing the concentration of Chromium in the medium after adding salt.

Table-3.3 Chromium Tolerance of Isolates

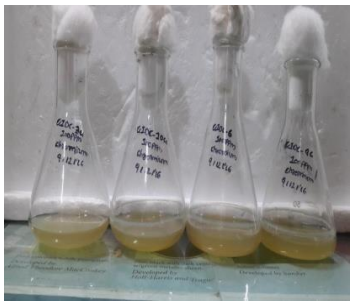
Conc. Isolates	100 Ppm	200 Ppm	300 ppm	400 ppm	500 ppm	600 Ppm
GIDC-1a	2.0814	1.9362	2.0527	2.0473	1.9711	1.3065
GIDC-1b	2.0709	2.1807	2.1136	2.1119	2.0113	1.8071
GIDC-2a	2.0075	2.0698	2.0795	2.9707	**2.4133**	1.5298
GIDC-2b	1.9488	2.0274	2.1588	2.0901	2.1997	1.7738
GIDC-3a	1.4890	1.6977	2.0208	1.7840	2.0628	0.3112
GIDC-3b	1.7469	2.0071	2.1065	1.8020	2.1805	0.8883
GIDC-3c	1.8433	2.1531	2.1132	2.1408	1.8111	1.7181
GIDC-3d	1.8803	2.1206	1.8184	2.0280	1.9205	0.9941
GIDC-4a	1.8993	2.1377	2.1852	**3.1325**	1.9984	1.6952
GIDC-4b	2.0348	2.1961	2.1806	2.0850	1.9676	1.6468
GIDC-5a	2.0324	2.0544	2.1087	2.0821	1.8798	**2.3592**
GIDC-5b	0	0	0	0	0	0

GIDC-6	2.0662	2.1153	2.1641	2.1176	2.1088	1.6550
GIDC-7	1.8942	2.2019	**2.2381**	2.0837	1.8051	0.7083
GIDC-8a	1.9270	2.0918	2.0319	2.1378	1.9323	1.8052
GIDC-8b	1.9305	2.0664	2.2366	2.0337	2.1447	2.2990
GIDC-9a	2.0805	2.0922	2.0890	2.1947	1.8964	2.1401
GIDC-9b	1.9259	**2.2177**	2.2016	2.0111	1.8993	1.1770
GIDC-9c	1.8484	2.0923	2.1476	1.9143	1.5968	1.1074
GIDC-9d	1.9764	1.9151	1.9875	2.0759	1.9815	0.8956
GIDC-10a	1.9549	1.9729	1.9681	1.9188	1.6289	1.5515
GIDC-10b	1.9612	2.1773	1.8761	1.9835	1.9331	0.9690
GIDC-10c	1.9556	2.1215	2.1735	2.3061	1.8391	1.6017
GIDC-10d	**2.1855**	2.0548	2.0970	2.0535	1.8797	1.5747

On the basis of results shown in above table, highest growth and highest tolerance of chromium has been shown by the isolate GIDC-4b. It could grow till 600 ppm chromium concentration with almost constant rate. Other isolates were less tolerant to chromium as compare to GIDC-4b. After 600 ppm not a single isolate has been able to grow in presence of chromium. GIDC-5b was the weakest isolate among all twenty four isolates. It stopped growth from 100 ppm chromium concentration.

The growth of all isolates in 100 ppm chromium concentration and in 600 ppm chromium concentrations are shown in the images here under:

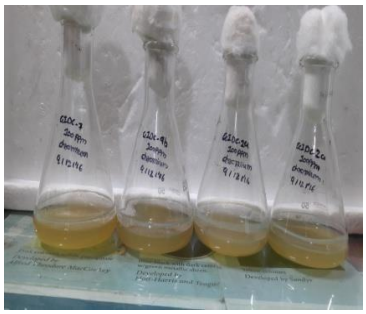
100ppm Chromium concentration



GIDC-3a,10a,6,9c

GIDC-4a,3d,10d

GIDC-9d,8b,8a,3b

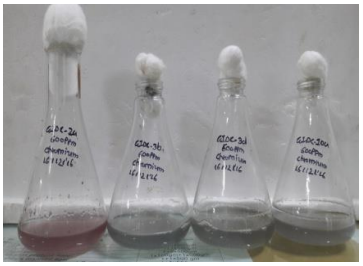
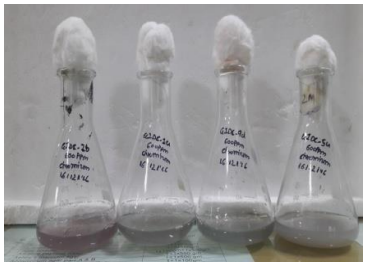




GIDC-2b,5a,1b,10b GIDC-7,9b,1a,2a GIDC-10c,9a,3c,4b

600ppm Chromium concentration



GIDC-10c,8a,6,10b GIDC-8b,7,4a GIDC-9b,1b,3c,9a

GIDC-3a,4b,10d,9c GIDC-2a,3b,3d,10a GIDC-2b,1a,9d,5a

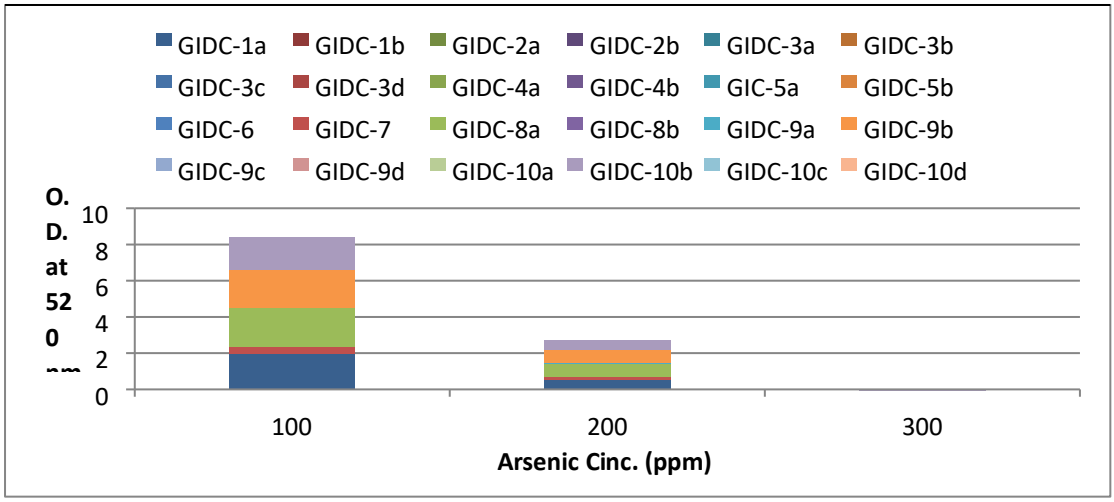
Arsenic Tolerance

Arsenic Chloride ($AsCl_3$) has been used as a salt of Arsenic to check the capability of the organisms in presence of this salt. Different concentration of Arsenic has been added into 20 ml sterile N-broth and incubated at 28˚C, for 48 hours in shaking condition. Growth has been tested from lower concentration to higher concentration in PPM. The concentration in the table is showing the concentration of Arsenic in the medium after adding salt.

Table-3.4 Arsenic Tolerance of Isolates

Concentration Isolates	100 ppm	200 ppm	300 ppm
GIDC-1a	1.9487	0.5615	0.0032
GIDC-1b	0	0	0
GIDC-2a	0	0	0
GIDC-2b	0	0	0
GIDC-3a	0	0	0
GIDC-3b	0	0	0
GIDC-3c	0	0	0
GIDC-3d	0	0	0
GIDC-4a	0	0	0
GIDC-4b	0	0	0
GIDC-5a	0	0	0
GIDC-5b	0	0	0
GIDC-6	0	0	0
GIDC-7	0.4229	0.1263	0
GIDC-8a	**2.1151**	**0.7731**	**0.0095**
GIDC-8b	0	0	0
GIDC-9a	0	0.0098	0
GIDC-9b	2.1056	0.6917	0.0048
GIDC-9c	0	0	0
GIDC-9d	0	0	0
GIDC-10a	0	0	0
GIDC-10b	1.8071	0.5150	0.0003
GIDC-10c	0	0	0
GIDC-10d	0	0	0

Graph-1 Arsenic Tolerance of Isolates

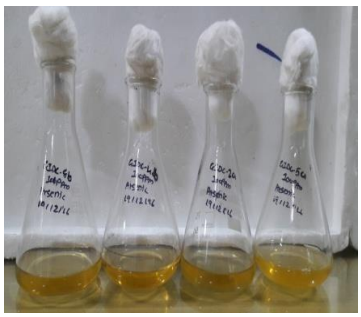


Arsenic proved as a hazardous metal on the basis of growth observed in flasks containing Arsenic in different concentration. All isolates except GIDC-1a, GIDC-8a, GIDC-9b and GIDC-10b were unable to grow in presence of Arsenic.

Isolates GIDC-8a could show highest growth in all concentrations of Arsenic. Other isolates have been proved as sensitive for arsenic presence.

The growth of above isolates has been shown in the images as follows:

100ppm Arsenic Concentration



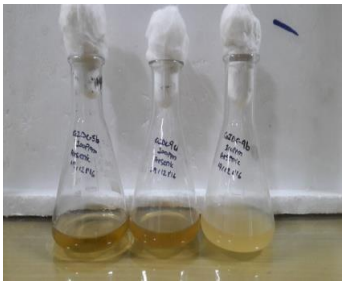
GIDC-8b,4b,2a,5a

GIDC-7,10b,1b,9d

GIDC-10a,1a,10d,2b

GIDC-10c,8a,6,9c

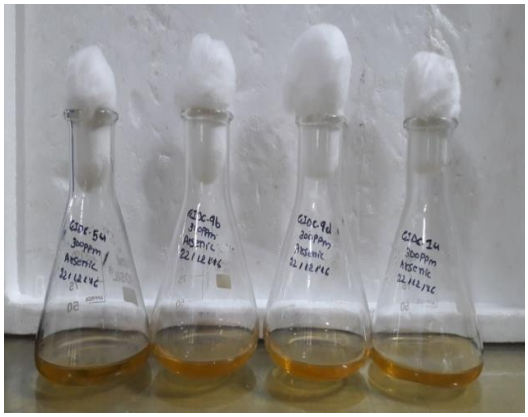
GIDC-3a,3d,3b,4a



GIDC-5b,9a,9b

300ppm Arsenic Concentration

GIDC-7,8a,9a,10b



GIDC-5a,9b,9d,1a

Mercury Tolerance

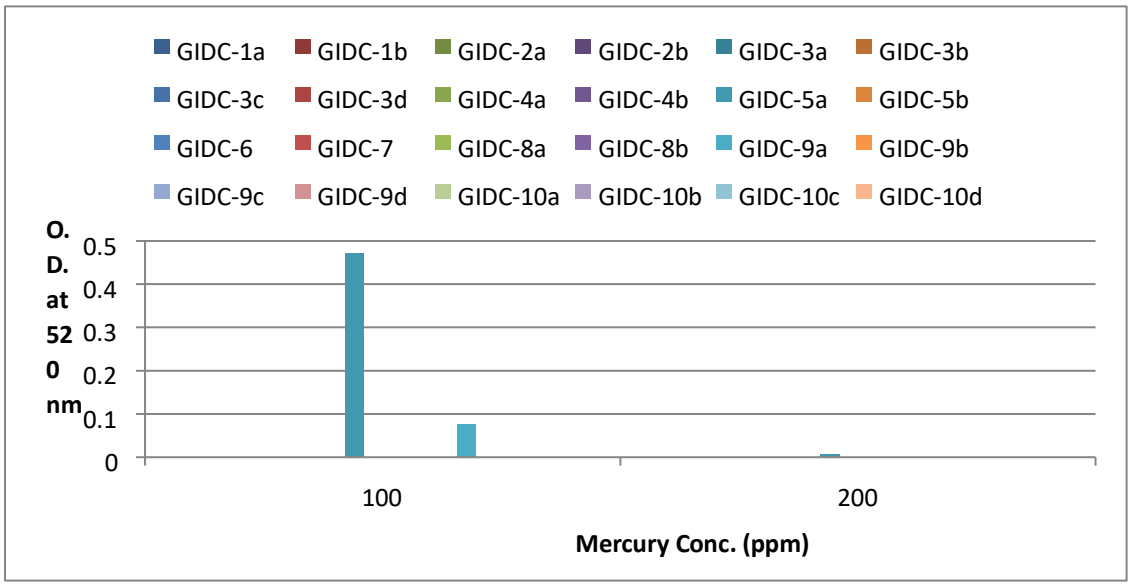
Mercury Sulfate ($HgSO_4$) has been used as a salt of Mercury to check the capability of the organisms in presence of this salt. Different concentration of Mercury has been added into 20 ml sterile N-broth and incubated at 28˚C, for 48 hours in shaking condition. Growth has been tested from lower concentration to higher concentration in PPM. The concentration in the table is showing the concentration of Mercury in the medium after adding salt.

Table-3.5 Mercury Tolerance of Isolates

Concentration Isolates	100 ppm	200 ppm
GIDC-1a	0	0
GIDC-1b	0	0
GIDC-2a	0	0
GIDC-2b	0	0
GIDC-3a	0	0
GIDC-3b	0	0
GIDC-3c	0	0
GIDC-3d	0	0
GIDC-4a	0	0
GIDC-4b	0	0

GIDC-5a	0.4707	0.0059
GIDC-5b	0	0
GIDC-6	0	0
GIDC-7	0	0
GIDC-8a	0	0
GIDC-8b	0	0
GIDC-9a	0.0751	0
GIDC-9b	0	0
GIDC-9c	0	0
GIDC-9d	0	0
GIDC-10a	0	0
GIDC-10b	0	0
GIDC-10c	0	0
GIDC-10d	0	0

Graph-2 Mercury Tolerance of Isolates



Only two isolates among all isolates were able to grow in presence of mercury and those two isolates are GIDC-5a and GIDC-9a. All other isolates got killed by adding into mercury containing N-broth medium. Between two isolates GIDC-5a is

more stronger than GIDC-9a to grow in presence of mercury. The growth of these two isolates has been shown in images here under:

100ppm **Mercury**

GIDC-3c,9a,3a

GIDC-10b,5a,10a,1a

Lead Tolerance

Lead Acetate ($Pb(C_2H_3O_2)_2$) has been used as a salt of Lead to check the capability of the organisms in presence of this salt. Different concentration of Lead has been added into 20 ml sterile N-broth and incubated at 28˚C, for 48 hours in shaking condition. Growth has been tested from lower concentration to higher concentration in PPM. The concentration in the table is showing the concentration of Lead in the medium after adding salt..

Table-3.6 Lead Tolerance of Isolates

Concentration Isolates	100 Ppm	200 Ppm	300 Ppm
GIDC-1a	0.3478	0.1437	0
GIDC-1b	0.2653	0	0
GIDC-2a	0.3370	0.0760	**0.1354**
GIDC-2b	**2.6157**	**0.8058**	0
GIDC-3a	0.1722	0	0
GIDC-3b	0.1392	0	0
GIDC-3c	0.1780	0	0
GIDC-3d	0.2425	0	0
GIDC-4a	0.2097	0	0
GIDC-4b	0.1183	0	0
GIDC-5a	0.3320	0.1528	0

GIDC-5b	0.2049	0	0
GIDC-6	0.4244	0	0
GIDC-7	0.2314	0	0
GIDC-8a	0.2761	0	0.0369
GIDC-8b	0.3719	0.1427	0.0281
GIDC-9a	0.2937	0.1381	0
GIDC-9b	0.4073	0	0
GIDC-9c	0.2147	0	0
GIDC-9d	0.3962	0	0
GIDC-10a	0.2314	0	0.0423
GIDC-10b	0.4215	0.4590	0.0355
GIDC-10c	0.2505	0.1289	0
GIDC-10d	0.2896	0.1907	0

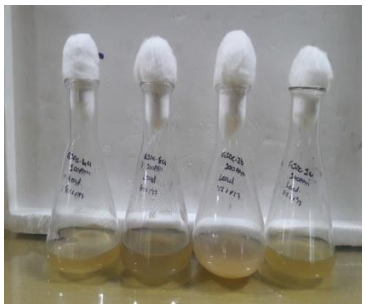
Lead is poisonous metal for all isolates.

Isolates have been unable to grow in higher concentration of lead.

Only five isolates were able to grow constantly till the concentration of 300ppm. The isolates which were able to grow in presence of lead till 300ppm concentration are GIDC-2b, GIDC-8b, GIDC-9a, GIDC-10b and GIDC-10c. GIDC-2b was the highest growing organism in all concentrations of Lead.

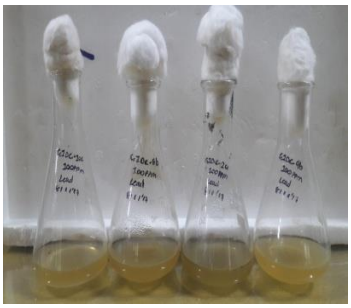
The growth of these five isolates from 100ppm lead concentration and 300ppm lead concentration has been shown in below images:

100ppm Lead Concentration

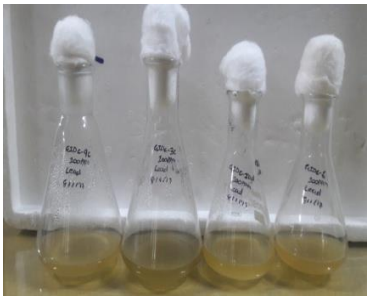


GIDC-4a,8a,2b,1a

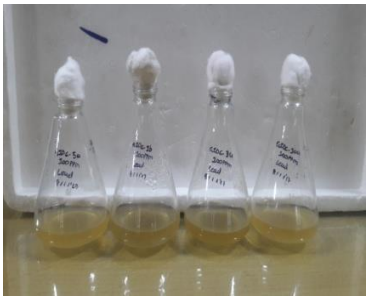
GIDC-3b,7,3d,5b



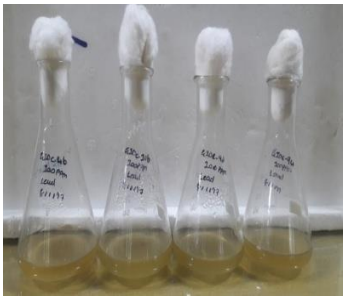
GIDC-10c,8b,2a,9b



GIDC-9c,3c,10d,6

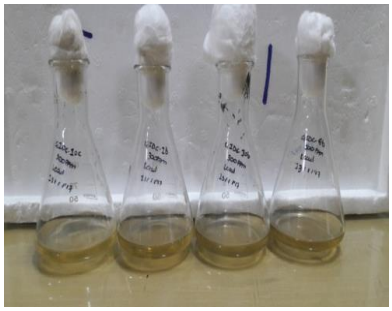


GIDC-5a,1b,3a,10a



GIDC-4b,10b,9d,9a

300ppm Lead Concentration



GIDC-10c,2b,10b,8b

Cadmium Tolerance

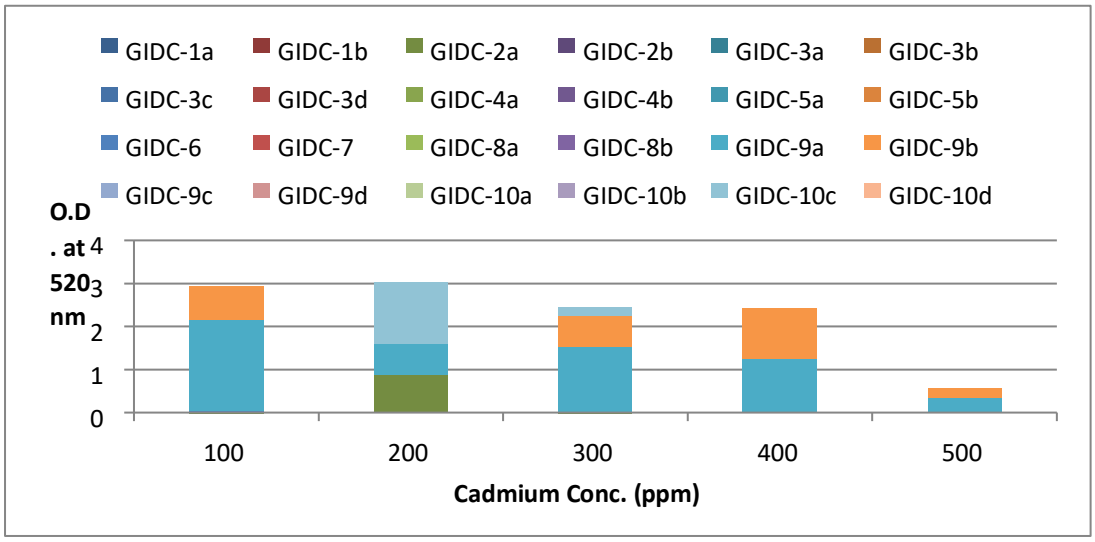
Cadmium Chloride ($CdCl_2$) has been used as a salt of Cadmium to check the capability of the organisms in presence of this salt. Different concentration of Cadmium has been added into 20 ml sterile N-broth and incubated at 28˚C, for 48 hours in shaking condition. Growth has been tested from lower concentration to higher concentration in PPM. The concentration in the table is showing the concentration of Cadmium in the medium after adding salt.

Table-3.7 Cadmium Tolerance of Isolates

Concentration Isolates	100 Ppm	200 ppm	300 ppm	400 ppm	500 Ppm
GIDC-1a	0	0	0	0	0
GIDC-1b	0	0	0	0	0
GIDC-2a	0.0315	0.0811	0.0309	0	0
GIDC-2b	0	0	0	0	0
GIDC-3a	0	0	0	0	0

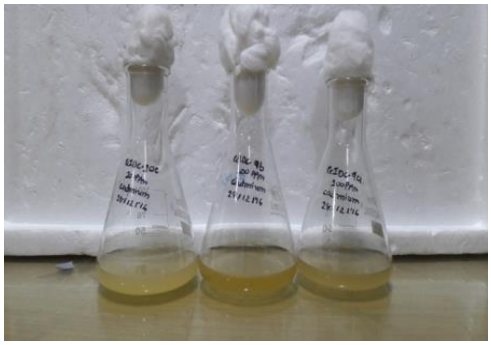
GIDC-3b	0	0	0	0	0
GIDC-3c	0	0	0	0	0
GIDC-3d	0	0	0	0	0
GIDC-4a	0	0	0	0	0
GIDC-4b	0	0	0	0	0
GIDC-5a	0	0	0	0	0
GIDC-5b	0	0	0	0	0
GIDC-6	0.0135	0	0	0	0
GIDC-7	0	0	0	0	0
GIDC-8a	0	0	0	0	0
GIDC-8b	0	0	0	0	0
GIDC-9a	**2.1189**	0.7083	**1.4849**	**1.2605**	**0.3608**
GIDC-9b	0.7698	0	0.7347	1.1642	0.2143
GIDC-9c	0	0	0	0	0
GIDC-9d	0	0	0	0	0
GIDC-10a	0	0	0	0	0
GIDC-10b	0	0	0	0	0
GIDC-10c	0	**1.4290**	0.2095	0	0
GIDC-10d	0	0	0	0	0

Graph-3 Cadmium Tolerance of Isolates



Cadmium is more hazardous for isolates. GIDC-9a and GIDC-9b were only two isolates which were able to grow in presence of cadmium. These two isolates have been able to grow till 500ppm cadmium concentration. All other isolates were very sensitive to cadmium that any of them were not able to grow in lowest concentration of cadmium. Growth of these two isolates have been shown in below images:

100ppm Cadmium Concentration 500ppm Cadmium Concentration



GIDC-10c,9b,9a

GIDC-9a,9b

3.4 Action of Selected Isolates on E-waste

On the basis of metal tolerance, total twelve isolates have been selected from twenty four isolates. All these twelve isolates have been tested for the heavy metal tolerance present in electronic waste. CD/DVD, Mother Board and Electric Cable were used as test electronic wastes. The performance of all isolates in presence of e-waste was different in all three e-wastes. The individual results for the e-waste are as under:

3.4.1 CD/ DVD as E-waste

People are widely using CD/DVD for different purpose like storage of music collection, movies, software etc, but with the advancement in technology, people stop using CD/DVD and waste of CD/DVD is started to increase day by day. The film present on CD/DVD contains different metals like Gold (Au), Silver (Ag), Aluminium (Al) so it may pollute the soil where the waste of CD/DVD has been collected. All the isolates have been tested for the action on these metals present in CD/DVD.

Microbial Action On CD/DVD

Isolates have been inoculated into 20 ml N-broth containing 2 gm of CD/DVD pieces, and incubated at 28˚C for 144 hours in shaking condition.

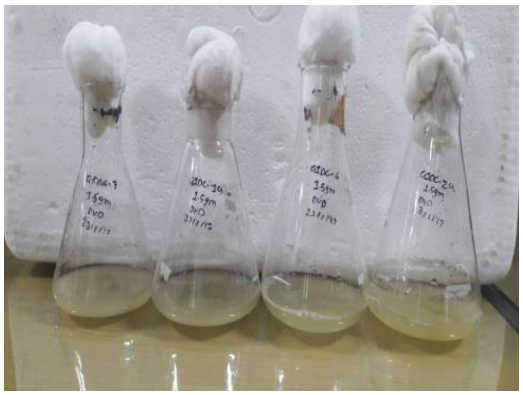
All the isolates have been able to grow in presence of CD/DVD with constant Optical Density at 520nm.

Table-3.8: Action of isolates on CD/DVD

Isolates	Set-1	Set-2	Set-3	Set-4
GIDC-1a	**2.6665**	2.5602	**2.8131**	2.6483
GIDC-2a	2.4214	2.5849	2.6003	2.7226
GIDC-2b	2.5473	2.4279	2.4729	2.7111
GIDC-5a	2.5136	2.5403	2.7964	2.7031
GIDC-6	1.3616	2.4733	2.2936	2.2196
GIDC-7	1.9914	2.5444	2.6995	2.5700
GIDC-8a	2.5518	**2.8012**	2.7876	2.5122
GIDC-8b	2.3533	2.4547	2.5014	2.7376
GIDC-9a	2.6271	2.6080	2.7047	**2.7576**
GIDC-9b	2.2621	2.3079	2.6565	2.3750
GIDC-10b	1.8357	0.3117	2.5383	2.3533
GIDC-10c	2.4398	2.2447	2.0978	2.5577

It has been shown in above table that all isolates have been able to grow in presence of DVD pieces and able to remove metals from the DVD film. GIDC-8a in set-2 and GIDC-1a in set-3 were the highly growing isolates among all isolates in presence of DVD. The growth of isolates in presence of DVD has been shown in below images:

GIDC-9b,5a,8a,1a

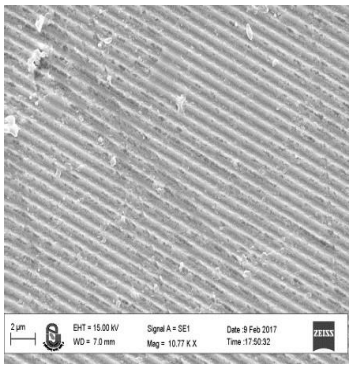


GIDC-7,1a,6,2a

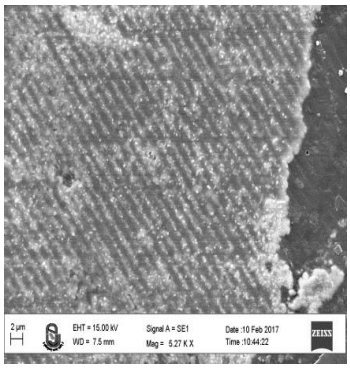
Electron Micrograph of DVD Pieces

Eight different samples of treated CD/DVD pieces including control have been selected for electron microscopy.

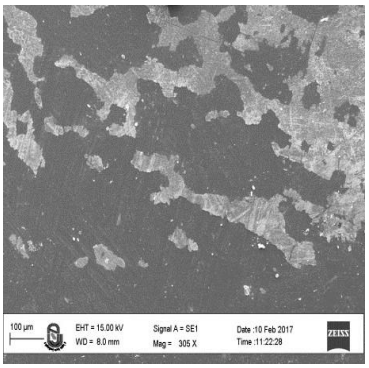
The electron micrographs have been taken from different dimensions and with different magnification to check the area of metal film utilized by isolates. The metal removal from DVD pieces could be detected from the electron micrograph taken by Scanning Electron Microscopy.



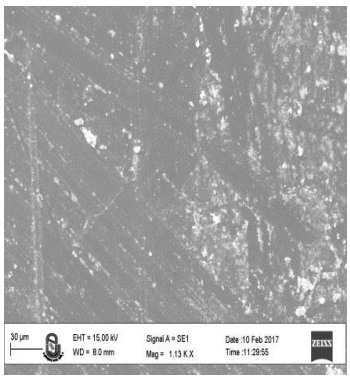
Control



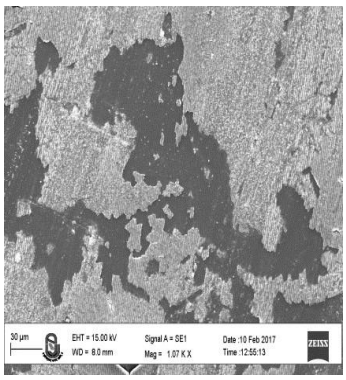
Sample-1



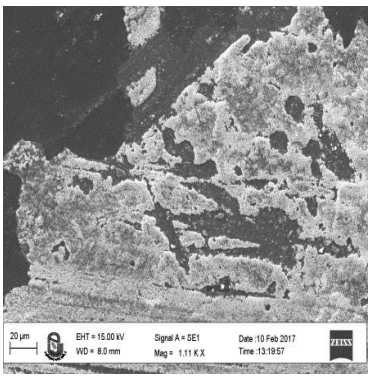
Sample-2



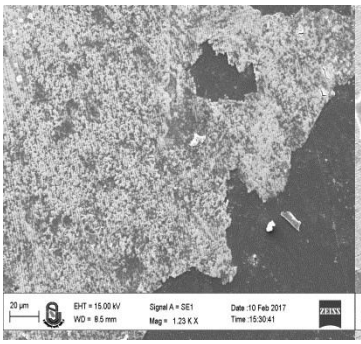
Sample-3



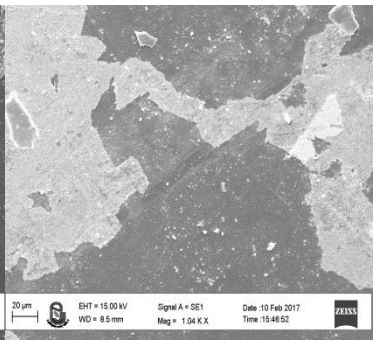
Sample-4



Sample-5



Sample-6



Sample-7

In above electron micrographs the control is used as reference for the utilization of metal film from other CD/DVD pieces by isolates, intact CD/DVD piece has been used as control and in other images the lighter area is unutilized or unremoved portion of metal film on DVD and the darker portions in the images states that the metal has been removed from the film by isolates. In sample-1 there is an utilization of metal film from the edge can be observed, while in other samples there was utilization of film from the middle of the CD/DVD pieces.

Metal Analysis

To check the presence of different metals like, Au, Ag, Dc, B, Co, Ni, Pb, Cr in treated N-broth medium, the metal analysis has been performed by MP-AES (Microwave Plasma- Atomic Emission Spectroscopy) method. After the digestion of the samples in MP-AES instrument the O.D. has been taken at different wavelengths for different metals.

Table-3.9: Metal Analysis fro, CD/DVD Containing Media

Sample	Incubation Hours	Au	Ag	B	Cd	Co	Ni	Pb	Cr
		267.595 nm	328.068 nm	249.772 nm	228.802 nm	340.512 nm	352.454 nm	405.781 nm	425.433 nm
Metal Concentration in ppm									
1a	24	2.23	20.06	19.96	ND	0.81	4.51	1.33	6.12
	144	1.37	26.69	26.59	0.12	1.25	2.23	0.91	6.21
2a	24	1.83	1.34	1.24	0.10	0.75	0.58	2.92	1.94
	144	1.85	1.32	1.22	0.14	0.64	8.71	7.22	9.47
6	24	0.031	0.48	0.38	ND	1.34	0.91	0.88	0.80
	144	0.479	23.50	23.40	ND	0.02	ND	0.01	0.01
8a	24	1.329	1.80	1.70	ND	0.19	0.58	3.15	0.95
	144	1.136	23.35	23.25	ND	ND	0.22	4.40	0.87
9a	24	0.19	1.60	1.50	ND	0.80	0.62	ND	3.71
	144	ND	44.47	44.37	ND	0.98	1.45	ND	2.68
9b	24	1.31	4.32	4.22	ND	ND	8.95	2.45	5.85
	144	0.67	13.50	13.40	ND	ND	9.02	3.67	5.52
Contortion	24	1.38	21.39	21.29	ND	ND	1.93	3.52	1.40
	144	1.67	0.87	0.77	ND	ND	44.97	13.52	1.64

In above table we can observe that almost all isolates have been able to leach different metals like Au, Ag, B, Ni, Pb and Cr etc. As per the table it can be shown that Au content has been increased in the medium containing GIDC-2a, GIDC-6 and Concentrate of isolates, while it has been decreased in the medium containing GIDC-1a, GIDC-8a, GIDC-9a and GIDC-9b after 144 hours incubation. Similarly silver and boron content has been increased in the medium containing GIDC-1a, GIDC-6, GIDC-8a, GIDC-9a, GIDC-9b while it has been decreased in the medium with the presence of isolates GIDC-2a and concentrate of isolates.

Cadmium has not been detected in any medium except two isolates GIDC-1a and GIDC-2a and the content of both the metals has been increased in both the medium after 144 hours incubation. The medium containing GIDC-1a and GIDC-9a has been shown the increased content of Cobalt while it has been decreased in the medium with GIDC-2a, GIDC-6 and GIDC-8a. GIDC-9b and concentrate of isolates has not shown presence of cobalt in the medium. GIDC-1a, GIDC-6 show the decreased content of Ni and Pb in the medium after 144 hours incubation, while GIDC-2a, GIDC-9b and concentrate of isolates show the increased concentration of Ni and Pb in the treated medium.In GIDC-8a containing medium the content of Ni has been decreased and content of Pb has been increased. Pb has not been detected in the medium with GIDC-9a while Ni has been increased. Cr concentration has been decreased in medium with isolates GIDC-6, GIDC-8a, GIDC-9a and GIDC-9b, GIDC-1a, GIDC-2a and concentrate of isolates shown increased concentration of Cr after 144 hour incubation.

3.4.2 Mother Board as E-waste

PCB (Printed Circuit Board) is the most important part of electric circuit. Mother Board used in computers is a type of PCB and also the important part of computers. Any damage to the Mother Board would make it waste. The waste PCBs are non-degradable, harmful for the soil. Many harmful metals like AU, Ag, Cu, Co etc. might be present in Mother Board and it will be the major reason for soil pollution.

Microbial Action on Mother Board

Isolates have been inoculated into 20 ml N-broth containing 2 gm of Mother Board pieces, and incubated at 28˚C for 144 hours in shaking condition

All isolates have been able to grow in presence of Mother Board pieces at almost constant OD at 520 nm.

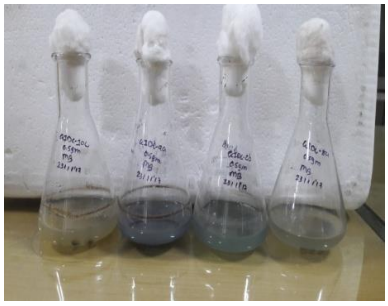
Table-3.10: Action of Isolates on Mother Board

.	Set-1	Set-2	Set-3	Set-4
GIDC-1a	2.3997	2.2386	2.3979	2.3671
GIDC-2a	**3.2671**	2.2530	2.4897	2.8658
GIDC-2b	2.7970	2.6123	2.7493	**3.0983**
GIDC-5a	3.0101	2.5883	**2.7950**	2.8428
GIDC-6	1.8208	1.9112	2.6987	2.5906
GIDC-7	2.2962	2.6076	1.9083	2.6998
GIDC-8a	2.2012	2.4043	2.2718	2.7364
GIDC-8b	2.1445	2.4999	2.2686	2.4247
GIDC-9a	3.2466	2.5463	2.7212	2.8554
GIDC-9b	2.5247	1.9519	2.3400	2.7797
GIDC-10b	0.3963	0.4618	1.5116	0.9047
GIDC-10c	2.5886	**2.7614**	2.3912	2.8890

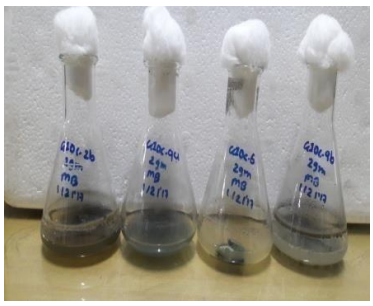
It has been shown in above table and graph that all the isolates were able to grow in presence of the pieces of Mother Board or may be can remove metal from the Mother Board pieces. GIDC2a and GIDC-9a in set-1 and GIDC-2b in set-4 were the highly growing isolates in presence of Mother Board.

The growth of isolates in presence of Mother Board has been shown in below images:

GIDC-9b,2a,8b,5a



GIDC-10c,1a,2b,8a

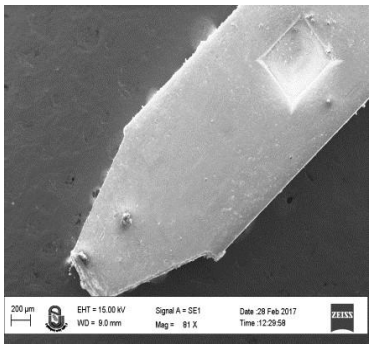


GIDC-2b,9a,6,9b

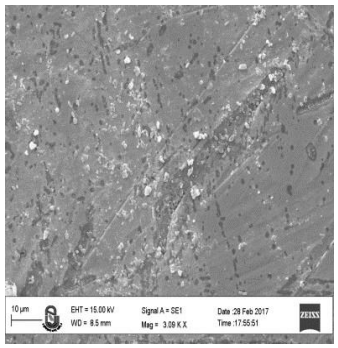
Electron Micrograph

Eight different samples of the Mother Board pieces from different flask have been taken to check removal of metal from the Mother Board pieces.

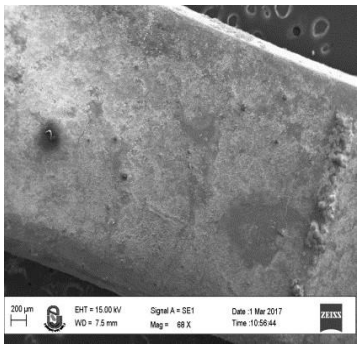
The electron micrographs have been taken from different dimension and with different magnification to check the area of metal utilized by isolates. The metal removal from Mother Board pieces could be detected from the electron micrograph taken by Scanning Electron Microscopy.



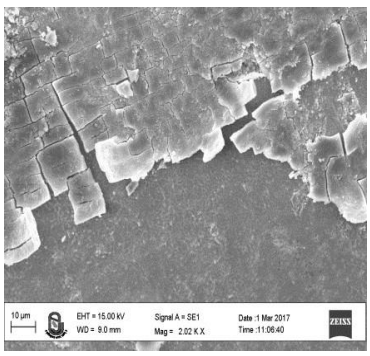
Control



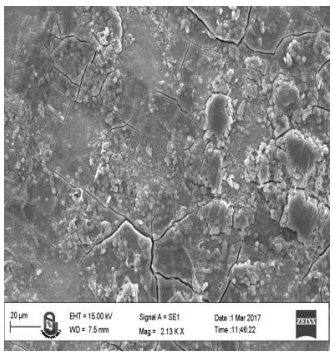
Sample-1



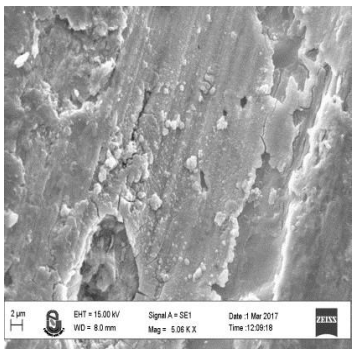
Sample-2



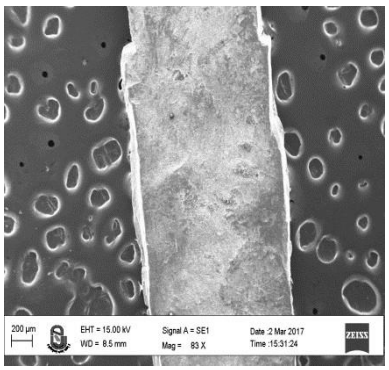
Sample-3



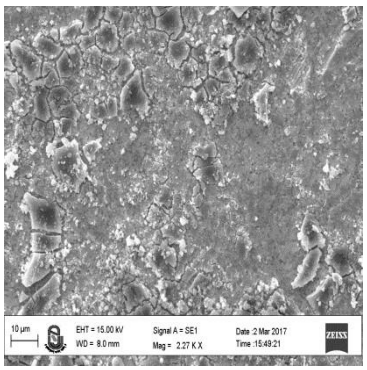
Sample-4



Sample-5



Sample-6



Sample-7

In above micrographs 1st microgram taken as control and other Mother Board pieces have been treated with isolates. In the electron microscopy images lighter and darker portion have been observed. The lighter portion meant that the metal of Mother Board was intact at that spot and darker area suggested that the metal from the pieces has been removed by microbial action for example we can observe in sample-3 microgram there is metal utilization at the edge of the piece, while in sample-4 the metal utilization has been initiated as there are some cracks on the metal layer observed in microgram and that metal could be present into the medium or it might be utilized by the organism itself.

Metal Analysis

To check the presence of different metals like, Au, Ag, Dc, B, Co, Ni, Pb, Cr in treated N-broth medium, the metal analysis has been performed by MP-AES (Microwave Plasma- Atomic Emission Spectroscopy) method. After the digestion of the samples in MP-AES instrument the O.D. has been taken at different wavelengths for different metals.

Table-3.11 Metal Analysis of Mother Board Containing Sample

Sample	Incubation Hours	Au	Ag	B	Cd	Co	Ni	Pb	Cr
		267.595 nm	328.068 nm	249.772 nm	228.802 nm	340.512 nm	352454 Nm	405.781 Nm	425.433 nm
		Ppm	ppm	ppm	ppm	ppm	Ppm	ppm	Ppm
1a	24	1.19	15.58	15.48	ND	1.00	2.30	1.74	4.60
	144	1.46	140.73	140.63	ND	0.71	13.01	2.56	13.35
2a	24	1.99	3.51	3.41	0.37	0.69	1.67	5.07	2.44
	144	2.24	ND	ND	ND	0.45	21.21	3.55	17.91
6	24	ND	ND	ND	ND	ND	ND	ND	ND
	144	0.157	6.77	6.67	ND	ND	0.04	0.86	0.65
8a	24	5.97	21.65	21.55	ND	0.82	0.51	3.54	1.06
	144	0.47	12.78	12.68	ND	1.51	0.33	1.24	0.40
9a	24	ND	2.71	2.61	ND	0.97	0.03	ND	0.14
	144	ND	10.81	10.71	ND	1.26	0.06	ND	0.09
9b	24	1.28	ND	ND	ND	ND	9.01	2.81	5.03
	144	1.23	ND	ND	ND	ND	8.05	2.27	4.90
Conc.	24	2.29	1.08	0.98	ND	ND	84.97	24.98	1.41
	144	2.07	ND	ND	ND	0.69	2.73	1.34	2.22

As per above table Cd has been observed in the medium containing isolate GIDC-2a after 24 hours incubation but after 144 hours incubation this Cd has been disappeared, so we can say that organisms might be utilized Cd. Despite of Cd, GIDC-2a show increased concentration of Au, Ni, and Cr while Ag, B, Co and Pb have been decreased in the medium after 144 hours incubation. GIDC-1a and GIDC-6 show the increased concentration of Au, Ag, B, Ni and Cr and Pb was decreased in both the isolated Co was not detected in the medium of isolate GIDC-6 while it was decrease in the medium of isolate GIDC-1a. Au, Ag, B, Ni and Cr, while Co and Pb were increased after 144 hours were decreased in the medium containing isolate GIDC-8a. Cd was not detected in that medium. GIDC-9a containing medium contained icreased concentration of Ag, B, Co and Ni, while Au, Cd and b were not detected. Same medium show the decreased concentration of Cr. Au, ni and Pb have been decreased in the medium of GIDC-9b and concentrate of isolates. Cd was not detected in both the medium; Ag and B were not detected in the medium of isolate GIDC-9b while it was decreased in concentrate of isolates.

3.4.3 Electric Cable as E-waste

Electric cables are the connecting part of every electronic circuits from which the electricity will pass. Electric cables may contain some metals like Cu, Ag, Au, Ni, Cr etc.

Microbial Action on Electric Cable

Isolates have been inoculated into 20 ml N-broth containing 2 gm of Electric Cable pieces, and incubated at 28˚C for 144 hours in shaking condition

All isolates have been able to grow in presence of electric cable pieces at almost constant OD at 520 nm.

Table-3.12: Microbial Action on Electric Cable

Isolates	Set-1	Set-2	Set-3	Set-4
GIDC-1a	1.6824	2.6103	2.9950	1.0308
GIDC-2a	2.7764	3.2824	2.9823	**2.5826**
GIDC-2b	2.7797	2.1895	1.8761	2.2283
GIDC-5a	2.8930	2.0210	2.3385	2.6089

GIDC-6	1.8979	2.6242	2.1849	1.5230
GIDC-7	2.0644	**3.0127**	**3.2077**	1.2983
GIDC-8a	1.9032	1.5024	3.0387	1.8806
GIDC-8b	2.4919	2.3770	2.6801	2.0984
GIDC-9a	**2.9645**	2.9666	4.9218	2.3892
GIDC-9b	2.3841	2.6450	2.7765	1.1903
GIDC-10b	0.6762	2.6513	2.1898	0.3588
GIDC-10c	2.7538	2.5422	3.1881	2.5208

It has been shown in above table and graph that all isolates have been able to grow in presence of pieces of electric cable or may be they can remove the metals from the pieces of electric cable. GIDC-9a in set-3 was the highly growing isolate in presence of pieces of electric cable as compared to other isolates.The growth of isolates in presence of electric cable has been shown in below images:

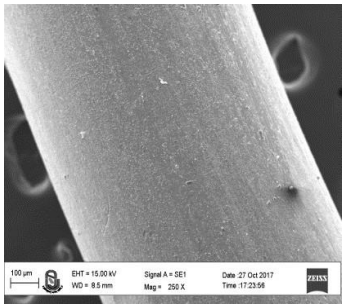
GIDC-10b,5a,9a,7

GIDC-8b,9a,7,2b

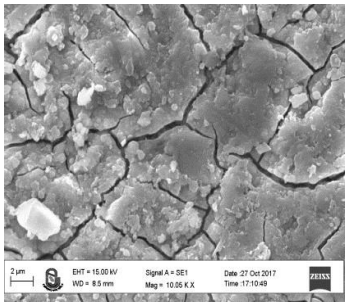
Electron Micrograph

Eight different samples of the electric cable pieces from different flasks have been selected to check removal of metal from an electric cable pieces.

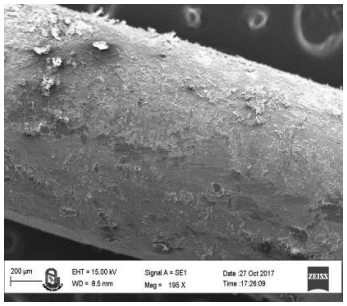
The electron micrographs have been taken from different dimension and with different magnification to check the area of metal removed by isolates. The metal removal from electric cable pieces could be detected from electron micrograph taken by Scanning Electron Microscopy.



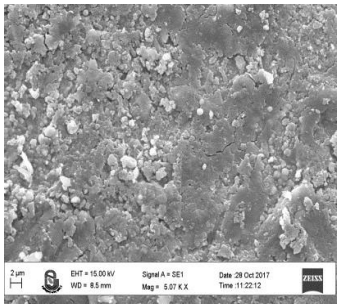
Control



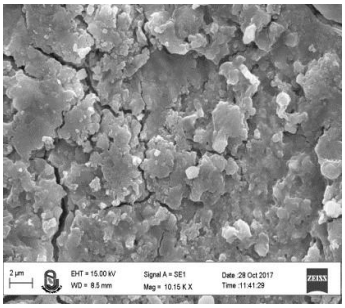
Sample-1



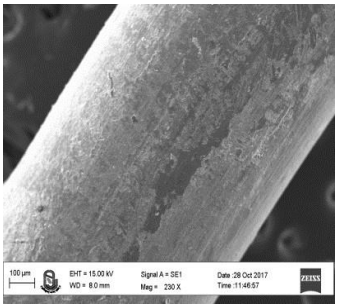
Sample-2



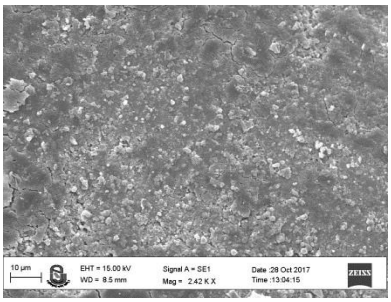
Sample-3



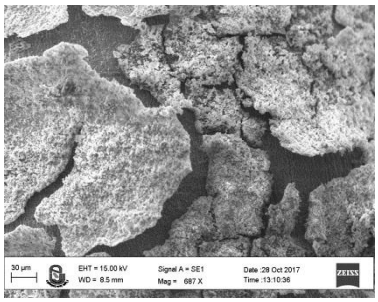
Sample-4



Sample-5



Sample-6



Sample-7

In above electron microscopy images the 1st micrograph is control and in other images sample-1 to sample-7 two areas are there, dark and light. Darker area suggests that the metal from the electric cable pieces has been removed by the action of isolates, for example in sample-7 there are bigger cracks have been observed with blackish coloured area, while the lighter area suggests that the metal has not been removed from the electric cable pieces by the action of isolates.

Metal Analysis

To check the presence of different metals like, Au, Ag, Dc, B, Co, Ni, Pb, Cr in treated N-broth medium, the metal analysis has been performed by MP-AES (Microwave Plasma- Atomic Emission Spectroscopy) method. After the digestion of the samples in MP-AES instrument the O.D. has been taken at different wavelengths for different metals.

Table-3.13: Metal Analysis of Electric Cable Containing Media

Sample	Incubation Hours	Au	Ag	B	Cd	Co	Ni	Pb	Cr
		267.595 nm	328.068 nm	249.772 nm	228.802 nm	340.512 nm	352.454 nm	405.781 nm	425.433 nm
		Metal concentration in ppm							
1a	24	1.70	2.33	2.23	ND	0.45	1.25	3.38	3.55
	144	1.65	2.73	2.63	0.10	1.21	12.82	1.57	11.47
2a	24	2.013	0.05	ND	0.14	0.78	0.74	2.35	3.56
	144	0.244	29.10	29.00	ND	1.41	1.73	2.11	4.15
6	24	ND	7.32	7.22	0.38	ND	0.51	1.46	0.88
	144	0.139	0.42	0.32	ND	ND	0.26	3.43	0.56
8a	24	0.58	20.14	20.04	ND	1.35	0.21	ND	0.29
	144	5.02	19.46	19.36	ND	8.12	7.11	12.04	27.38
9a	24	ND	ND	ND	ND	1.18	0.54	0.99	0.24
	144	ND	1.90	1.80	ND	ND	0.22	2.50	3.26
9b	24	1.37	ND	ND	ND	ND	8.21	2.77	5.22
	144	1.57	ND	ND	ND	ND	8.81	2.49	5.18
Consortium	24	1.46	ND	ND	ND	0.37	2.60	2.00	1.67
	144	1.35	28.75	28.65	ND	0.78	3.32	0.81	2.13

From above table we can say that in the medium of the isolate GIDC-1a show the increased Ag, B, Cd, Co, Ni and Cr has been observed, while it show decreased concentration of Au and Pb after 144 hour incubation. In this medium Cd has been detected and was increased after 144 hour incubation so we can say that this isolate was leaching Cd in the medium at that time. In GIDC2a containing medium there was increase in the concentration of Au, Ag, B, Co and Cr. Cd, Ni and Pb has been decreased after 144 hours. Co has not been detected in the medium of GIDC-6, all other metals have been decreased except Au and Ni. Cd has not been detected in the medium containing GIDC-8a, rest of the metals have been increased after 144 hours in same medium. GIDC-9a shown increased concentration of Ag, B, Pb and Cr, decreased concentration of Co and Ni. Rest of the metals were not detected in the

medium. Ag, B, Cd and Co were not detected in the medium containing GIDC-9b Au and Ni have been increased and Pb and Cr have been decreased after 144 hour. All metal concentration has been increased except Au and Pb in the medium containing concentrate of isolates.

3.5 Temperature Optimization

All the isolates have been checked for their optimum temperature. All isolates have been inoculated into 20 ml of sterile N-broth containing pieces of e-waste like CD/DVD, Mother

Board and Electric Cable and incubated at different temperatures 20˚C, 28˚C, 37˚C and 40˚C in shaking condition for 144 hours.

Table-3.14 Temperature Optimization of Isolates

Isolates	20˚C	28˚C	37˚C	40˚C
GIDC-1a	0.0809	2.2398	2.1819	0.1980
GIDC-2a	0.0032	2.5211	2.0318	0.0091
GIDC-2b	0.0310	2.6301	2.0030	0.0832
GIDC-5a	0.1108	2.6852	1.8988	0.1289
GIDC-5b	0.0003	2.4423	1.5308	0.0025
GIDC-6	0.2801	2.4077	2.3689	0.0381
GIDC-7	0.3284	2.5023	2.2091	0.9191
GIDC-8a	0.0330	2.6592	2.8902	0.0882
GIDC-8b	0.0186	2.7933	1.9983	0.0019
GIDC-9a	0.0935	2.3767	2.1923	0.0892
GIDC-9b	0.3988	2.8043	2.9308	0.0118
GIDC-10b	0.1303	2.4088	2.5831	0.0326
GIDC-10c	0.0099	2.6250	2.2384	0.0382

In above graph it was clear that all the isolates have been able to grow on all different temperature conditions, but temperature 28˚C and 37˚C was the most suitable temperature for all isolates to grow than other two temperature conditions.

3.6 pH Optimization

All the isolates have been checked for their optimum pH. All isolates have been inoculated into 20 ml of sterile N-broth containing pieces of e-waste like CD/DVD, Mother Board and Electric

Cable and incubated at different temperatures 28˚C with the medium pH of pH-5, pH-6, pH-7, pH-8 and pH-9 in shaking condition for 144 hours.

Table-3.15 pH Optimization of Isolates

Isolates	pH-5	pH-6	pH-7	pH-8	pH-9
GIDC-1a	0.0830	0.1212	2.5665	0.5284	0.0035
GIDC-2a	0.0342	0.1193	2.2414	0.0982	0.0381
GIDC-2b	0.0181	0.0320	2.7352	0.3210	0.0083
GIDC-5a	0.0628	0.2834	2.5236	0.0984	0.0992
GIDC-5b	0.0019	0.2532	2.3816	0.5289	0.1130
GIDC-6	0.0038	0.0589	2.1991	0.1238	0.0920
GIDC-7	0.0825	0.0363	2.5516	0.0663	0.0386
GIDC-8a	0.0283	0.1793	2.5535	0.9820	0.0998
GIDC-8b	0.0091	0.0881	2.6762	0.3280	0.0209
GIDC-9a	0.0338	0.2894	2.4261	0.5389	0.0284
GIDC-9b	0.0059	0.0928	2.8352	0.2298	0.0528
GIDC-10b	0.0383	0.2230	2.3381	0.3885	0.3283
GIDC-10c	0.0883	0.0353	2.7430	0.0932	0.8821

Growth was observed in all acidic and basic pH, but it was much weaker growth in pH-5, 6, 8 and 9 than in pH-7. According to graph all isolates were able to grow in neutral pH (pH-7).

3.7 Biochemical Test

All twelve isolates were checked for biochemical processes to find out their biochemical reactions over provided substance. Sugar fermentation, protein utilization

etc. have been checked by providing different sugar, Urea etc as substance. The table of results for all biochemical tests are as under:

Table-3.16 Biochemical Test Results Strip-I

Sample	ONPG	Lysine	Ornithine	Urea se	TDA	Nitrate	H2S	Citrate Utilization	VP	MR	Indol	Malonate
1a	-	-	-	-	-	-	-	+	-	+	-	-
2a	-	-	-	-	-	+	-	-	-	-	-	-
2b	+	-	-	V	-	-	-	-	-	-	-	V
5a	-	-	-	-	-	-	-	-	-	-	-	-
5b	+-	-	-	V	V	+	-	+	-	-	-	-
6	-	-	-	-	-	+	-	-	-	-	-	-
7	+	-	-	-	-	V	-	-	-	-	-	V
8a	V	-	-	+	-	-	V	+	-	-	-	V
8b	-	-	-	-	-	+	V	-	-	V	-	-
9a	+	-	+	-	-	-	V	-	-	+	-	V
9b	+	-	-	-	-	+	-	-	-	-	-	-
10b	-	-	-	V	-	+	-	--	-	-	-	-
10c	+	-	-	-	-	-	-	-	-	-	-	V

Table-3.17 Biochemical Test Results Strip-II

Sample	Esculin Hydrolysis	Arabinose	Xylose	Adonitol	Rham-nose	Cello-biose	Melibise	Saccharose	Reffi-Nose	Tre-halose	Glucose	Lactose
1a	+	+	+	V	+	+	V	+	V	+	+	V
2a	+	V	V	V	V	+	V	+	+	V	+	V
2b	+	-	-	-	-	V	-	V	-	-	V	-
5a	+	-	-	-	-	+	+	+	+	+	+	+
5b	+	V	-	-	-	-	-	+	V	+	+	-
6	+	+	+	+	V	+	V	+	V	+	+	V
7	+	V	-	-	-	+	V	+	-	+	-	V
8a	+	V	-	-	-	+	-	+	V	V	+	V
8b	+	V	-	-	V	+	V	+	V	+	+	V
9a	+	+	+	+	+	V	V	+	+	+	+	V
9b	+	+	-	-	V	+	V	+	+	+	+	V
10b	+	+	V	V	V	+	V	+	V	+	+	V
10c	+	V	-	-	-	V	-	+	-	V	+	V

In above table of biochemical test of isolates (+) sign indicates positivetest for particular organism, (-) sign indicates the test is negative and the sign (V) indicates that the test is neither positive nor negative.

GIDC-1a

GIDC-2a

GIDC-2b

GIDC-5a

GIDC-5b

GIDC-6

GIDC-7

GIDC-8a

GIDC-8b

GIDC-9a

GIDC-9b

GIDC-10b

GIDC-10c

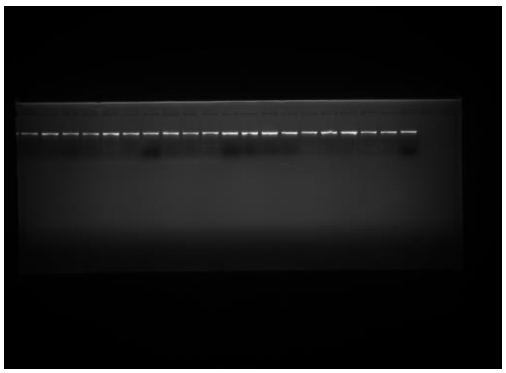
The HiMedia*Enterobacteriaceae* identification kit has been used to test the biochemical nature of the isolates. There were two strips for biochemical tests. Different tests like ONPG, Lysine utilization, Ornithin utilization, Urea test, Phenylalanine deamination, Nitrate reduction, H_2S production, Citrate utilization,

Voges Proskauer's test, Methyl Red, Indol test, Malonate utilization in strip-I, while in strip-II included tests for all sugar fermentation and oxidase test.

On the basis of biochemical test results four isolates GIDC-1a, GIDC-2a, GIDC-6 and GIDC-9b were selected for 16s rRNA sequencing on the basis of Bergey's Manual. As per Bergey's Manual, the biochemical test results of the isolates GIDC-1a, GIDC-2a, GIDC-6 and GIDC-9b resembeled with *Paenibacillus*, *Bacillus*, *Bacillus* and *Paenibacillus* respectively.

3.8 DNA ISOLATION

On the basis of biochemical results four isolates GIDC-1a, GIDC-2a, GIDC-6 and GIDC-9b have been selected for further identification (molecular identification). DNA of the selected isolates has been isolated by centrifugation followed by Agarose Gel Electrophoresis. Following are the images of isolated DNA of all four isolates by Agarose Gel Electrophoresis.



3.9 Sequence Alignment

The isolated DNA have been sequenced through Sanger's Dideoxy Method. After sequencing, all the sequences have been submitted to GenBank for sequence alignment. The organisms identified by sequence alignment as follows:

GIDC-1a *Bacillus megaterium* (Accession-MH031303)

GIDC-1aR *Bacillus circulans* (Accession-MH031304)

GIDC-2a *Paenibacillusmacerans* (Accesion-MH236120)

GIDC-6 *Paenibacillusmacerans* (Accession-MH249998)

GIDC-9b Pantoea agglomerans (Accession-MH250000)

Chapter -5

Discussion and Conclusion

- **Sample Collection**

We obtained 24 different isolates from 10 soil samples, indicating that large population of bacteria were present in the soil samples collected from GIDC, Gwalior.

- **Isolation and Screening**

All the isolates were screened on the basis of colony characters. Some colonies were amost same with each other, so we can conclude that the organism might be from the same genus.

- **Gram's Staining & Capsule Staining**

As all the isolates were Gram's positive with different shapes like cocci, rods and short rods and almost all isolates were non capsule forming except GIDC-3a, GIDC-4b, GIDC-5a and GIDC-10c, we can say that none of the isolates is pathogenic or harmful.

- **Metal Tolerance**

All the isolates were checked for tolerance of different metals like Berium, Chromium, Lead, Cadmium, Arsenic and Mercury. On the basis of results, we can say that not all isolates were able to grow in presence of every metal.

- **Barium:**

As per the results, we can conclude that Barium is not a poisonous metal, so that almost all organisms except GIDC-1b were able to grow in the medium which contain different concentration of Barium, while GIDC-1b was sensitive to Barium.

- **Chromium**

On the basis of optical density for Chromium containing medium, it can be concluded that, like Barium, Chromium is not the hazardous metal as all the organisms except GIDC-5b were able to grow in presence of different concentration of Chromium.

- **Arsenic**

As the results shown, it is clear that Arsenic is a hazardous metal for most of the isolates as compare to Barium and Chromium, as not a single isolate can grow no more than 300 ppm Arsenic Concentration. GIDC-1a, GIDC-8a, GIDC-9b and GIDC-10b were totally sensitive to Arsenic.

- **Mercury**

Mercury was proved as the most poisonous metal to all isolates except GIDC-5a and GIDC9a. These both organisms had potential to grow in presence of 100 ppm of Mercury.

- **Lead**

As per the data obtained from Lead tolerance test, we can say that Lead is less hazardous than Mercury as all isolates were able to grow in 100 ppm Lead containing medium. Not all organisms were able to tolerate higher concentration of Lead except GIDC-2b, GIDC-8b, GIDC-9a, GIDC-10b and GIDC-10c. They saw growth till 300 ppm concentration of Lead.

- **Cadmium**

Cadmium is also an hazardous metal as no isolates were able to grow in presence of Cadmium except GIDC-9a and GIDC-9b. These both isolates have the potential to grow till 500 ppm concentration of Cadmium in the medium.

Microbial Action on E-waste

The pieces of CD/DVD, Mother Board and Electric Cable were used as E-waste to test the action of isolates on the metals present in those E-wastes.

CD/DVD as E-waste

On the basis of results, we can say that all the isolates have great potential to grow in the presence of the pieces of CD/DVD

Electron Micrograph

As shown in the picture of Scanning Electron Microscopy, it is clear that isolates were acted on the CD/DVD pieces as there was damage observed in electron micrographs.

✦ **Total Metal Analysis**

On the basis of total metal analysis results (Table-3.11), metals like Au, Ag, B, Co, Ni, Pb and Cr were detected in the medium after incubation for 144 hours, so we can say that organisms might accumulate it from the CD/DVD pieces

✦ **Mother Board as E-waste**

As shown in the table, organisms were able to grow in the presence of mother board pieces.

There were constant growth observed in all four sets.

✦ **Electron Micrograph**

As shown in electron micrographs of mother board pieces, there was damage on some metal parts was observed.

✦ **Total Metal Analysis**

As per the table-3.12, different metals like Au, Ag, B, Cd, Co, Ni, Pb, Cr were detected in total metal analysis so it can be concluded that organisms might leach these metals.

✦ **Electric Cable as E-waste**

Metal part of the electric cable was added to the medium. As per the results shown in the table-3.14, all isolates were able to grow in presence of small pieces of electric cable.

✦ **Electron Micrograph**

As shown in the electron micrographs, there was visible damage observed in electric cable sample- 1, 4 and 7, while slight damage observed in sample-2 and sample-5 in comparison with control, so we can say that organisms might removed metals from electric cable.

✦ **Total Metal Analysis**

As per metal analysis data, there were some metals like Au, Ag, b, Cd, Co, Ni and Pb were detected, so we can conclude that organism might leach those metals in the medium.

Temperature and pH Optimization

From the data shown in table-3.15 and 3.16 as well as graphs, we can conclude that all the isolate give best growth in temperature range 30˚C-37˚C and pH-7

On the basis of biochemical tests, DNA isolation and DNA sequencing it can be concluded that GIDC-1a, GIDC-2a, GIDC-6 and GIDC-9b are the potential isolates which were giving rich growth in metal tolerance tests and having noticeable action on all Electronic Wastes. By sequence alignment on GenBank, the selected isolates were identified as following Genus and Speices:

- GIDC-1a *Bacillus megaterium* (Accession-MH031303)
- GIDC-1aR *Bacillus circulans* (Accession-MH031304)
- GIDC-2a *Paenibacillusmacerans* (Accesion-MH236120)
- GIDC-6 *Paenibacillusmacerans* (Accession-MH249998)
- GIDC-9b Pantoeaagglomerans (Accession-MH250000)

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A Study of Potential Bio-Inoculant for Enhancing Agricultural Productivity and Stress Resilience

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Abstract

Recent discoveries in the field of Plant Growth-Promoting Bacteria (PGPB) have revealed significant contributions to nitrogen fixation, stress tolerance, and plant growth enhancement. Key findings include the identification of nitrogen-fixing bacteria like *Rhizobium* and *Azospirillum*, and the role of PGPB in mitigating both abiotic stresses (such as drought, heat, salinity) and biotic stresses (pathogens and pests) through mechanisms like hormone production, biocontrol, and enzyme activity. This study examined the effect of bacterial inoculation on plant growth, focusing on wheat, tomato, and chilli plants, using *Bacillus licheniformis SS15* for inoculation. Various assays, including root colonization, plant growth promotion, and antagonistic activity against plant pathogens were conducted. Additionally, the production of secondary metabolites like indole-3-acetic acid and siderophores was assessed for their potential role in plant growth enhancement. *Bacillus licheniformis SS15* demonstrated significant plant growth promotion (PGP) effects on wheat, tomato, and chilli, including increased root and shoot length, biomass, and nitrogen content. It also exhibited strong root colonization potential and persistence in the rhizosphere, enhancing nutrient uptake. The bacterium showed antagonistic activity against several plant pathogens and produced beneficial metabolites like indole-3-acetic acid (IAA) and siderophores. These traits suggest its potential as a bio-inoculant to improve agricultural productivity and reduce reliance on chemical fertilizers.

Keywords: Plant growth promotion, antagonistic activity, *Bacillus*, Bio-inoculant, Siderophores

Introduction

Sustainable agriculture is a critical area of research aimed at improving agricultural productivity while minimizing environmental degradation. The growing global population and increasing demand for food require innovative approaches to enhance crop yields, improve soil health, and mitigate the adverse effects of climate change. One such promising approach is the use of Plant Growth-Promoting Bacteria (PGPB), which can provide multiple benefits, such as improving soil fertility, enhancing plant growth, and mitigating the effects of both abiotic and biotic stresses [1]. Among these beneficial microorganisms, *Bacillus* species, including *Bacillus licheniformis*, have attracted considerable attention for their plant growth-promoting potential and ability to suppress plant pathogens.

PGPB are known to enhance plant growth through a variety of mechanisms, including nitrogen fixation, hormone production (such as indole-3-acetic acid, IAA), phosphate solubilization, siderophore production, and enzyme activities [2] (Bashan et al., 2014). In addition to promoting plant growth, many PGPB also exhibit antagonistic properties, suppressing plant pathogens through the production of antimicrobial compounds or by outcompeting pathogens for resources in the rhizosphere [3] (Van der Heijden et al., 2008). The interaction between these bacteria and plants, especially in the rhizosphere, plays a crucial role in enhancing plant health and resilience.

The focus of this study is on *Bacillus licheniformis* SS15, a strain isolated from agricultural soil, and its potential as a bio-inoculant for enhancing plant growth and stress resilience. This bacterium has shown promise in promoting growth in various crops, including wheat, tomato, and chili, and possesses several beneficial traits, including nitrogen fixation, IAA production, siderophore production, and antagonistic activity against plant pathogens. In this study, we aim to investigate the plant growth-promoting (PGP) effects and antagonistic activity of *Bacillus licheniformis* SS15, with the goal of evaluating its potential as a bio-inoculant for improving agricultural productivity and reducing dependency on chemical fertilizers.

This study aims to explore the plant growth-promoting potential of Bacillus licheniformis SS15 and its application in sustainable agriculture. Specifically, the study investigates its ability to enhance the growth of wheat, tomato, and chili plants through the production of beneficial metabolites such as indole-3-acetic acid (IAA) and siderophores. Furthermore, it evaluates the antagonistic activity of Bacillus licheniformis SS15 against common plant pathogens, emphasizing its potential as a biocontrol agent. Additionally, the study examines the persistence and colonization efficiency of Bacillus licheniformis SS15 within the rhizosphere, providing insights into its role in promoting plant health and productivity.

Materials and Methods

The isolation and identification of *Bacillus licheniformis SS15* began with collecting soil samples from agricultural fields across various regions. These samples were diluted in sterile water to reduce bacterial concentration and then spread onto Nutrient Agar plates, which were incubated at 30°C for 48 hours. After incubation, distinct bacterial colonies were observed and selected for screening. The screening process focused on identifying plant growth-promoting traits such as nitrogen fixation, phosphate solubilization, hormone production, and antagonistic activity against plant pathogens. Among the isolates, one demonstrated significant plant growth-promotion abilities and was selected for further study.

For precise identification, molecular techniques were employed. The DNA of the selected isolate was extracted, and the 16S rRNA gene was amplified using polymerase chain reaction (PCR). The amplified gene was sequenced, and the resulting nucleotide sequence was compared with sequences in the GenBank database. Based on sequence similarity, the isolate was identified as Bacillus licheniformis SS15. This identification confirmed the isolate's species and its potential applications in promoting sustainable agricultural practices.

Plant Growth Promotion

Wheat, tomato, and chili seeds were surface sterilized to remove any contaminants and sown in sterilized soil pots to ensure controlled growth conditions. The plants were inoculated with *Bacillus licheniformis SS15*, which had been cultured in nutrient broth. After culturing, the bacterial cells were suspended in a solution suitable for plant inoculation. For comparison, a control group of plants was treated with sterile water instead of the bacterial suspension.

After 30 days of growth, various growth parameters were measured, including root and shoot length, biomass, and nitrogen content in the plants. These measurements provided insights into the effects of Bacillus licheniformis SS15 on plant growth and nutrient uptake. This approach follows established protocols for evaluating plant-microbe interactions and assessing the efficacy of plant growth-promoting bacteria (PGPB) in agricultural applications [1-2].

Root Colonization and Persistence

To determine the ability of *Bacillus licheniformis* SS15 to colonize plant roots, root samples were collected at various time intervals (7, 14, 21, and 30 days) after inoculation. The root tissues were washed, surface sterilized, and plated on nutrient agar. The bacterial colony-forming units (CFUs) were counted to assess the persistence and colonization ability of the bacterium in the rhizosphere.

Secondary Metabolite Production

The production of indole-3-acetic acid (IAA), a key phytohormone involved in plant growth, was measured using the Salkowski method as described by Gordon and Weber (1951) [4]. This colorimetric assay detects IAA by forming a pink complex when IAA reacts with Salkowski reagent under acidic conditions. Siderophore production, which enhances iron availability to plants, was assessed using the Chrome Azurol S (CAS) assay, a method established by Schwyn and Neilands (1987) [5]. The CAS assay identifies siderophore activity based on a color change resulting from the removal of iron from the CAS dye complex.

Both IAA and siderophore production were quantified to evaluate their respective roles in promoting plant growth. These biochemical traits are key mechanisms by which Bacillus licheniformis SS15 contributes to enhancing nutrient availability and stimulating plant development.

Antagonistic Activity Against Plant Pathogens

To evaluate the antagonistic activity of Bacillus licheniformis SS15, several plant pathogens, including *Fusariumoxysporum, Rhizoctoniasolani,* and *Pythiumaphanidermatum,* were used. The pathogen inoculation was carried out using the dual culture method [6] (Dennis and Webster, 1971). In this method, the pathogen was inoculated on the surface of the agar plate, and *Bacillus licheniformis* SS15 was placed at a distance from the pathogen. The zone of inhibition was measured after incubation to determine the antimicrobial activity.

Statistical Analysis

The collected data were statistically analyzed using one-way analysis of variance (ANOVA) to evaluate differences in growth parameters (e.g., root length, shoot length, biomass, and nitrogen content) among the treatments. Following the ANOVA, Tukey's post-hoc test was performed to identify specific pairs of treatments with significant differences. This combination of statistical methods ensures robust comparison across multiple groups while controlling for Type I error. The level of statistical significance was set at $p<0.05p<0.05$, indicating that differences were considered significant if the probability of error was less than 5%. These analyses provide a reliable basis for assessing the impact of Bacillus licheniformis SS15 on plant growth.

Results

Inoculation with *Bacillus licheniformis* SS15 significantly promoted plant growth in wheat, tomato, and chili plants. The treated plants showed increased root and shoot length, biomass, and nitrogen content compared to the control group. For wheat, the average shoot length increased by 25%, while the root length increased by 30% in the treated group. Similarly, tomato and chili plants exhibited improvements in both root and shoot length, as well as biomass (Figure 1).

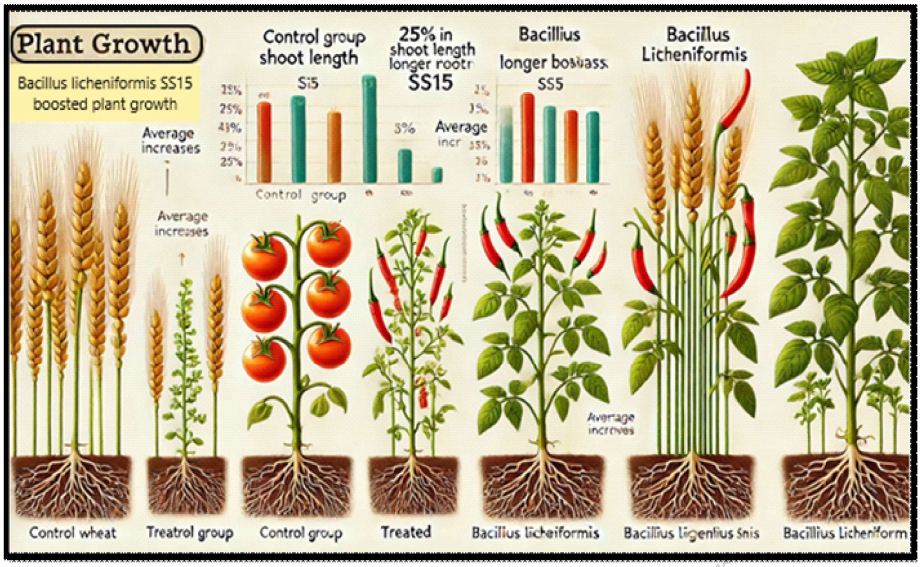

***Figure 1: Bacillus licheniformis* SS15 enhanced growth in wheat, tomato, and chili, improving root and shoot length, biomass, and nitrogen content.**

The bacterial isolates demonstrated a substantial impact on wheat growth parameters, as detailed in Tables 1 and 14. Comparisons between the uninoculated control (with Hoagland N and P) and Bacillus licheniformis SS15 revealed marked enhancements. The bacterial isolates increased shoot and root lengths by 25–45% and 29–52%, respectively, compared to the control. Correspondingly, shoot and root biomass showed significant improvements, ranging from 2–62% and 100–172%, respectively (Figures 2 A, B).

Moreover, bacterial inoculation significantly enhanced nitrogen (N) content in both shoots and roots relative to the uninoculated control (Figure 3), with increases ranging from 22–76% in shoots and 10–32% in roots. Among the isolates, Bacillus licheniformis SS15 exhibited superior performance compared to other well-studied bacteria, showcasing its robust potential for promoting plant growth. Notably, the inoculation effects were more pronounced in root parameters than in shoot characteristics, underscoring the efficacy of Bacillus licheniformis SS15 as a powerful growth-promoting agent.

Parameters	Control	*Bacillus licheniformis SS15*
Shootlength (cm)	22	26
Shootfreshweight (mgplant^{-1})	90	129
Shootdryweight (mgplant^{-1})	42	68
Rootlength (cm)	15.3	22.15
Rootfreshweight (mgplant^{-1})	25	42
Rootdryweight (mgplant^{-1})	7	16

Table 1. Effect of Bacillus licheniformis SS15 on the growth characteristics of wheat grown in pouches under axenic conditions.

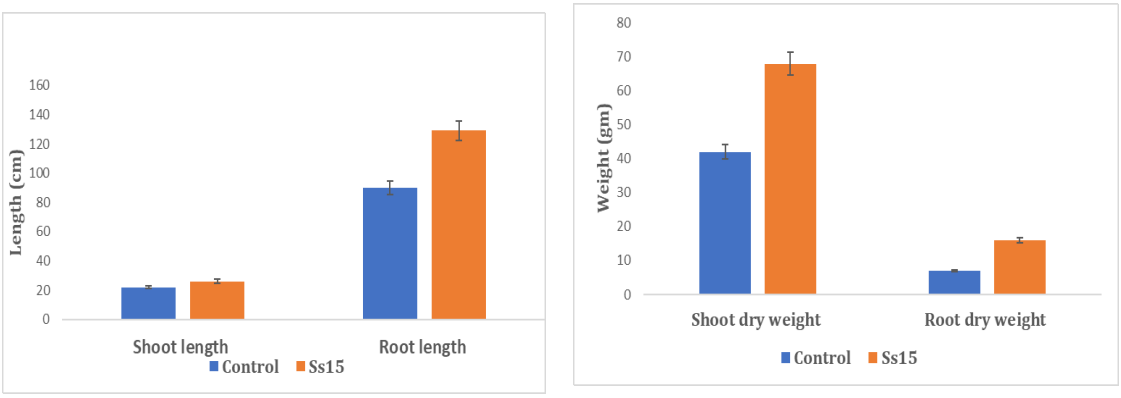

Fig 2. Effect of PGPB inoculation on shoot and root length (A) and shoot/root dry weight (B) of wheat variety Inqlab grown in growth pouches under axenic conditions. The error bars represent the least significant difference among treatments at P ≤ 0.05.

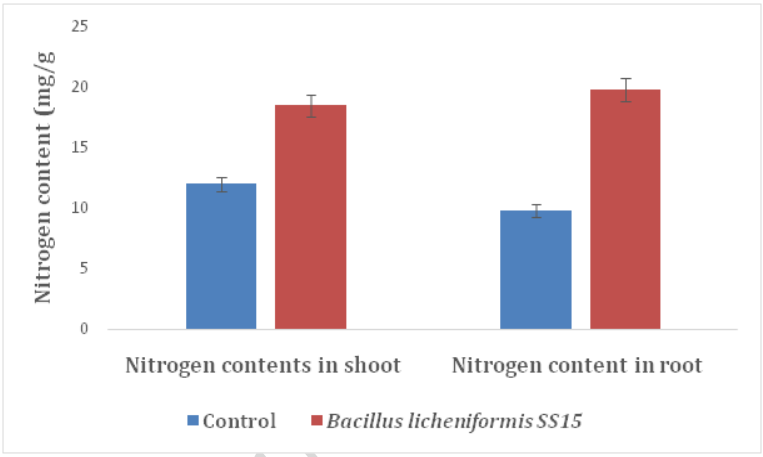

Fig 3. Nitrogen contents (mg g-1) in shoots and roots (A). The error bars represent the least significant difference (LSD) among different treatments at P ≤ 0.05 of PGPR-inoculated wheat variety Inqlab grown in growth pouches under axenic conditions.

Bacillus licheniformis SS15 significantly increased shoot and root length, shoot and root dry weight, and also enhanced the N contents of inoculated wheat seedlings. The plant growth promotion could be the result of the beneficial functions of applied PGPR isolate, like plant growth hormone production, nitrogen fixation, and P solubilization. As the inoculated plants were not supplied with any additional source of N or any form of soluble P, the higher amount of N detected in the shoot or roots of inoculated plants as well as growth promotion may be attributed to the bacterial-assisted growth enhancement phenomenon. In addition to some other parameters positively influenced the growth of plant, auxin production by the isolates is proposed as a major means of attaining growth promotion [7]. Furthermore, the inoculation of Bacillus licheniformis SS15 having multi-functional traits is better than having single traits [8]. IAA is involved in root initiation, cell division, cell enlargement, increases root surface area, and conse quent access to soil nutrients by enhanced formation of roots [9-10]. Auxin production has been proposed as a major means of attaining early growth promotion in

wheat [11] along-with P-solubilization [12]. The response of plants to Bacillus licheniformis SS15 was variable which may be attributed to their individual traits and rhizospheric competencies. Bacillus licheniformis SS15 showed good survival and persistence in the rhizosphere. The significant increase in growth and N level both in shoot and root upon isolates application is a clear indicative of the fact that the Bacillus licheniformis SS15 has been able to provide better nutrient flux to the plant host which resulted in the increase of the plant biomass and N accumulation. The increase in root length due to the applied isolates may also contributed to increase N uptake in plant shoot as both the parameters were significantly correlated in the study.

Nitrogen Content

The analysis revealed a significant increase in nitrogen content in plants treated with *Bacillus licheniformis SS15* compared to the control group. Wheat plants demonstrated the highest response, with a 20% increase in nitrogen content, whereas tomato and chili plants showed a 15% increase each. These results highlight the effectiveness of *Bacillus licheniformis SS15* in enhancing nitrogen availability or uptake in plants, contributing to improved nutritional status and overall growth. The data are visually represented in Figure 1, which illustrates the comparative nitrogen content across the treatments, emphasizing the beneficial impact of the bacterial inoculation (Figure 3).

Root Colonization and Persistence

The root colonization ability of Bacillus licheniformis SS15 was evident throughout the 30-day growth period. Colony-forming units (CFUs) of the bacterium were consistently detected in root samples, confirming its persistence in the rhizosphere. The CFU counts increased over time, with the highest numbers recorded after 30 days of plant growth. This trend indicates effective colonization and the ability of Bacillus licheniformis SS15 to establish and maintain a stable presence in the rhizosphere, thereby contributing to sustained plant growth promotion. These findings are illustrated in Figure 3, which depicts the CFU dynamics over the 30-day period, emphasizing the bacterium's colonization efficiency.

Secondary Metabolite Production

Bacillus licheniformis SS15 produced significant amounts of indole-3-acetic acid (IAA) and siderophores. The IAA production ranged from 25 to 35 µg/mL, and the siderophore production was high, as indicated by the CAS assay. These metabolites likely contributed to the observed plant growth promotion. The plant growth-promoting effect of *Bacillus licheniformis* SS15 is linked to its production of IAA, which enhances root growth and nutrient uptake, and siderophores, which improve iron nutrition and inhibit pathogens. Exploring native beneficial microbes is crucial for sustainable agriculture, especially as concerns over chemical fertilizers grow.

Antagonistic Activity

Bacillus licheniformis SS15 demonstrated strong antagonistic activity against the phytopathogenic fungi *Fusarium oxysporum, Rhizoctonia solani,* and *Pythium aphanidermatum*. This was evidenced by the formation of significant zones of inhibition around the bacterial colonies in dual culture assays. These clear zones indicate the production of antifungal metabolites or other inhibitory substances by the bacterium, which suppress fungal growth. The results highlight the

potential of Bacillus licheniformis SS15 as a biocontrol agent capable of mitigating the impact of these pathogens on crops. The extent of the inhibition is presented in Figure 5, showcasing its efficacy in pathogen suppression.

Discussion

The plant growth-promoting effects of *Bacillus licheniformis* SS15 can be attributed to several mechanisms. The production of IAA plays a critical role in promoting root elongation, improving nutrient uptake, and enhancing plant growth [13]. Siderophore production helps in chelating iron and making it available to the plants, which is essential for plant growth, particularly under iron-deficient conditions [14]. Additionally, nitrogen fixation contributes to improved soil fertility, supporting the growth of plants by providing a natural source of nitrogen [15].

Antagonistic Properties and Biocontrol Potential

The ability of *Bacillus licheniformis* SS15 to inhibit the growth of plant pathogens suggests its potential as a biocontrol agent. The antagonistic activity against common pathogens such as *Fusarium oxysporum* and *Rhizoctonia solani* could help in reducing the reliance on chemical pesticides, which often have negative environmental and health impacts [16]. The production of antimicrobial compounds, such as bacteriocins and lytic enzymes, may be responsible for the observed biocontrol activity [2].

Potential as a Bio-Inoculant

The results of this study highlight the potential of *Bacillus licheniformis* SS15 as a bio-inoculant for enhancing agricultural productivity. Its ability to promote plant growth, improve nutrient uptake, and suppress plant pathogens makes it a promising candidate for sustainable agriculture. Additionally, its persistence in the rhizosphere ensures long-term benefits, supporting its use as an effective bio-inoculant in various agricultural systems.

Conclusion

Bacillus licheniformis SS15 demonstrates significant plant growth-promoting and antagonistic activities, making it a valuable candidate for use as a bio-inoculant in sustainable agriculture. The bacterium enhances plant growth by producing beneficial metabolites such as IAA and siderophores, improving nitrogen content, and promoting root colonization. Its ability to suppress plant pathogens further adds to its potential as a biocontrol agent. The findings of this study suggest that *Bacillus licheniformis* SS15 can contribute to improving agricultural productivity and reducing the reliance on chemical fertilizers and pesticides.

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An analysis of Plant Growth-Promoting Bacterium for Sustainable Agriculture

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Abstract

Sustainable agriculture, focusing on plant growth-promoting bacteria (PGPB), enhances soil fertility, reduces pathogens, and promotes plant growth. PGPB help plants tolerate abiotic stresses like drought and temperature fluctuations by producing hormones, antioxidants, and improving nutrient uptake, ultimately boosting crop yield and resilience to environmental challenges. Moreover, Plant Growth-Promoting Bacteria (PGPB) enhance plant growth through mechanisms like producing phytohormones, siderophores, lytic enzymes, and phosphate solubilization. In this study, soil samples were collected from various farms in India and bacterial isolates with plant growth-promoting traits were identified through morphological, biochemical, and molecular techniques, including 16S rRNA sequencing. The isolates' growth characteristics, such as response to pH, temperature, and salinity were also studied to assess their potential for agricultural applications. Bacillus licheniformis SS15, isolated from agricultural fields, demonstrated significant plant growth-promoting traits, including nitrogen fixation, IAA production, and phosphate solubilization. It showed optimal growth in moderate temperatures (30-37°C), alkaline pH (7-9), and moderate salinity (up to 18% NaCl). Molecular identification confirmed its species as *Bacillus licheniformis*.

Keywords: Plant growth promoting bacteria, sustainable agriculture, phosphate solubilization, *Bacillus*

Introduction

Sustainable agriculture has emerged as a cornerstone for addressing the dual challenges of ensuring global food security and preserving environmental integrity. With the global population expected to reach nearly 10 billion by 2050 [1], agricultural systems face mounting pressure to increase productivity without exacerbating environmental degradation. In this context, the integration of biological agents, particularly plant growth-promoting bacteria (PGPB), has garnered significant attention as a viable and eco-friendly alternative to conventional farming practices that rely heavily on chemical fertilizers and pesticides. PGPB play a multifaceted role in enhancing crop performance and soil health through their diverse mechanisms of action. These include nitrogen fixation, phosphate solubilization, hormone production, pathogen suppression.

Certain PGPB, such as *Rhizobium* and *Azospirillum*, convert atmospheric nitrogen into a bioavailable form for plants, reducing the dependency on synthetic nitrogen fertilizers [2]. This process not only enhances crop yields but also minimizes nitrate leaching and greenhouse gas emissions, contributing to environmental sustainability.Phosphorus is a critical nutrient for plant growth but is often present in insoluble forms in the soil. PGPB such as *Pseudomonas* and *Bacillus* species secrete organic acids that solubilize phosphate, making it accessible to plants and reducing the need for phosphate-based fertilizers [3].PGPB synthesize phytohormones like indole-3-acetic acid (IAA), gibberellins, and cytokinins, which promote root elongation, shoot growth, and overall plant vigor [4]. These hormonal changes enhance nutrient uptake and improve plant resilience under stress conditions.Through the production of antimicrobial compounds and the induction of systemic resistance in plants, PGPB effectively suppress soil-borne pathogens. This biocontrol ability reduces the reliance on chemical pesticides, mitigating their detrimental effects on non-target organisms and ecosystems [5].

The utilization of PGPB aligns seamlessly with the principles of sustainable agriculture, offering a dual benefit of enhancing productivity and maintaining ecological balance. For instance, studies have demonstrated that the application of PGPB not only improves crop yield but also enriches

soil microbial diversity, a key indicator of soil health and resilience [6].Advancements in biotechnology and genomics have unlocked new potentials for PGPB in agriculture. The development of bioinoculants tailored to specific crops and soil conditions is an area of active research. Furthermore, integrating PGPB with other sustainable practices, such as crop rotation and organic farming, could amplify their benefits and pave the way for a more holistic approach to sustainable agriculture.

Among the numerous plant growth-promoting bacteria (PGPB), *Bacillus* species, including *Bacillus licheniformis*, have gained recognition for their remarkable ability to enhance plant growth and improve soil health. These bacteria play a vital role in mitigating the adverse effects of abiotic stresses, such as drought, salinity, and temperature fluctuations, which are increasingly impacting agricultural productivity due to climate change [7]. The versatile mechanisms of *Bacillus licheniformis* include the production of phytohormones, solubilization of soil nutrients, and secretion of antimicrobial compounds that suppress plant pathogens. This study examines *Bacilluslicheniformis SS15*, a strain isolated from agricultural fields, and explores its potential application in sustainable agriculture. By characterizing the physiological and biochemical properties of this strain, the research aims to elucidate its role in promoting crop resilience, reducing dependence on chemical inputs, and contributing to environmentally friendly farming practices. The findings underscore the promise of integrating such biological agents into agricultural systems as a sustainable solution to current and emerging challenges in food production.

The Aim of this study are to explore the plant growth-promoting traits of isolated *Bacillus licheniformis* SS15 and to characterize its growth under varying environmental conditions. Specifically, the research focuses on examining bacterial growth responses to different pH levels, temperatures, and salinity conditions, which are critical factors influencing microbial activity and survival. Additionally, the study aims to evaluate the potential application of *Bacillus licheniformisSS15* in enhancing plant growth within agricultural systems, thereby contributing to sustainable farming practices and improved crop productivity.

Materials and Methods

Soil samples were collected from agricultural fields across various regions in India to ensure a diverse and representative study of microbial populations. These sites were carefully chosen based on differences in soil properties such as pH, organic matter content, and texture, as well as the variety of crops cultivated in each location. This diversity in sampling conditions helps capture a wide range of Bacillus licheniformis strains that may exhibit different plant growth-promoting traits. By selecting varied agricultural environments, the study aims to identify a strain like SS15 that not only possesses robust growth-promoting abilities but also demonstrates adaptability to different soil and environmental conditions. This approach ensures that the findings are broadly applicable and relevant to the diverse agricultural landscapes in India.

Isolation of Bacteria

The collected soil samples were subjected to a serial dilution process to reduce microbial density, enabling the isolation of individual bacterial colonies. This step involves systematically diluting the soil samples with sterile water to obtain a range of concentrations. Aliquots from each dilution were then spread on nutrient agar medium, a general-purpose growth medium suitable for cultivating a wide variety of bacteria. The plates were incubated at 30°C for 48 hours, a temperature conducive to the growth of many soil bacteria, including *Bacillus spp.*

Following incubation, colonies exhibiting distinct morphological characteristics, such as variations in size, shape, color, and texture, were carefully selected. This approach ensures the selection of diverse bacterial isolates for further analysis, as these morphological traits often indicate potential differences in bacterial species or strains. The chosen colonies were then subjected to additional tests to identify their plant growth-promoting traits and evaluate their suitability for agricultural applications.

Screening for Plant Growth-Promoting Traits

The bacterial isolates were systematically screened for various plant growth-promoting traits to identify their potential for enhancing agricultural productivity.Nitrogen fixation, a critical trait for supporting plant growth, was assessed using a semi-solid nitrogen-free medium. This method enables the detection of bacterial isolates capable of converting atmospheric nitrogen into bioavailable forms, a key process that reduces dependence on synthetic nitrogen fertilizers.Indole-3-acetic acid (IAA) production, an important phytohormone involved in root elongation and plant development, was measured using the colorimetric method developed by Gordon and Weber (1951) [8]. This method involves reacting the bacterial culture with specific reagents to produce a color change, the intensity of which is quantified to determine the level of IAA production.

Phosphate solubilization, another vital plant growth-promoting trait, was tested using Pikovskaya's agar medium [9-10]]. This medium contains insoluble phosphate compounds, and bacterial isolates capable of solubilizing phosphate create clear halos around their colonies, indicating their ability to make phosphorus bioavailable to plants.These tests collectively provided a comprehensive evaluation of the isolates' capabilities, identifying those with strong potential for application in sustainable agriculture.

Molecular Identification

For identification of the bacterial isolate 16S rRNA sequencing wascarried out. The selected potential isolate was grown in nutrient broth andits genomic DNA was extracted using phenol: chloroform method. Qualityof the genomic DNA was evaluated by performing gel electrophoresis on1.0 % agarose gel. Polymerase chain reaction (PCR) was carried out toamplify the fragment of 16S rRNA gene using universal primers 16SrRNA-Fand 16SrRNA-R. The amplified PCR product was purified to remove theunwanted contaminants. Forward and reverse DNA sequencing reaction ofPCR product was carried out with 16SrRNA-F and 16SrRNA-R primersusing BDT v3.1 Cycle sequencing kit on ABI 3730xl Genetic Analyzer. Finalconsensus sequence of 16S rRNA gene was generated from forward andreverse sequence data using aligner software. Lastly, the 16S rRNA genesequence was used to carry out BLAST (Basic Local Alignment Search Tool)with the 'nr' database of NCBI (National Center for Biotechnology)GenBank database. Based on maximum identity score first ten sequenceswere selected and aligned using multiple alignment software programClustal W. Distance matrix and phylogenetic tree was constructed using MEGA 10 software[11].

Growth Characteristics

The growth characteristics of *Bacillus licheniformis SS15* were evaluated under varying environmental conditions to understand its adaptability and resilience, crucial factors for its application in diverse agricultural settings.To assess the effect of pH, the bacterium was cultured in media with

pH values ranging from 5 to 10. This range encompasses acidic, neutral, and alkaline conditions, enabling the determination of the pH range and optimum level at which Bacillus licheniformis SS15 exhibits optimal growth.The impact of temperature was studied by incubating the bacterium at temperatures between 20°C and 45°C. This range represents typical environmental temperatures encountered in agricultural fields. Testing across this gradient allowed for the identification of the temperature range within which the bacterium thrives and its tolerance to temperature fluctuations [12].

Salinity tolerance was evaluated by growing the bacterium in media containing varying concentrations of sodium chloride (NaCl): 0%, 5%, 10%, and 18%. This test assessed the bacterium's ability to survive and grow in both non-saline and saline conditions, which is critical for its potential application in soils affected by salinity, a common issue in agriculture.These experiments collectively provided a detailed understanding of the environmental conditions that influence the growth and performance of *Bacillus licheniformis SS15*, laying the foundation for its practical use in sustainable farming practices.

Results

When cultured on nutrient agar, *Bacillus licheniformisSS15* exhibited translucent, cream-colored colonies that were flat, medium-sized, and characterized by uneven margins with a dry consistency. In contrast, on LB agar, the colonies transformed dramatically into white, mucoid forms with gelatinous secretions, highlighting a significant variation in colony morphology depending on the growth medium (Figure 1). Gram's staining confirmed SS15 as a Gram-positive bacterium, displaying long-chain rod morphology (Figure 2). Further analysis using Schaeffer-Fulton staining revealed the presence of endospores in 48-hour-old cultures, whereas Anthony's capsule staining confirmed the absence of a capsule. This morphological adaptability underscores the dynamic nature of SS15 under varying cultural conditions.

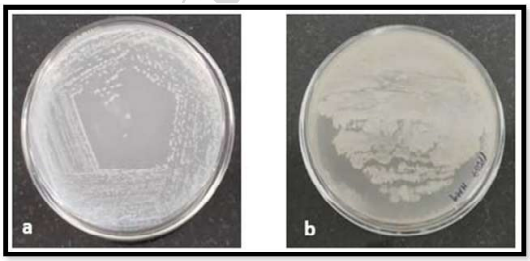

Fig 1. Morphological characteristics of SS15 isolate on a) nutrient and b) LB agar plates

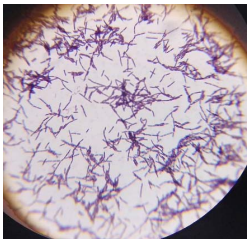

Fig 2: Microscopic view of SS15 bacterial isolate after Gram's staining

Molecular Identification of isolated PGPB

Bacillus licheniformis SS15 was successfully isolated from the soil samples and identified using 16S rRNA sequencing, a reliable molecular technique for bacterial identification. This

method involves amplifying and sequencing the 16S ribosomal RNA gene, a highly conserved region across bacterial species with variable regions that provide species-specific signatures.

Fig 3. Genomic DNA and 16S rRNA amplicon of SS15 bacterialisolate resolved on agarose gel

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Bacillus licheniformis strain DSM 13 16S ribosomal RNA, partial sequence	2615	2615	100%	0	99.11%	NR_118996.1
Bacillus haynesii strain NRRL B-41327 16S ribosomal RNA, partial sequence	2610	2610	100%	0	99.04%	NR_157609.1
Bacillus sonorensis strain NBRC 101234 16S ribosomal RNA, partial sequence	2608	2608	99%	0	99.17%	NR_113993.1
Bacillus licheniformis strain ATCC 14580 16S ribosomal RNA, partial sequence	2604	2604	100%	0	98.97%	NR_074923.1
Bacillus licheniformis strain NBRC 12200 16S ribosomal RNA, partial sequence	2597	2597	99%	0	98.96%	NR_113588.1
Bacillus licheniformis strain BCRC 11702 16S ribosomal RNA, partial sequence	2593	2593	99%	0	99.10%	NR_116023.1
Bacillus swezeyi strain NRRL B-41294 16S ribosomal RNA, partial sequence	2582	2582	100%	0	98.69%	NR_157608.1
Bacillus aerius strain 24K 16S ribosomal RNA, partial sequence	2569	2569	99%	0	98.56%	NR_042338.1
Bacillus subtilis subsp. inaquosorum strain BGSC 3A28 16S ribosomal RNA, partial sequence	2532	2532	100%	0	98.08%	NR_104873.1
Bacillus atrophaeus strain JCM 9070 16S ribosomal RNA, partial sequence	2532	2532	100%	0	98.08%	NR_024689.1

Fig 4. Sequences producing significant alignments after BLAST

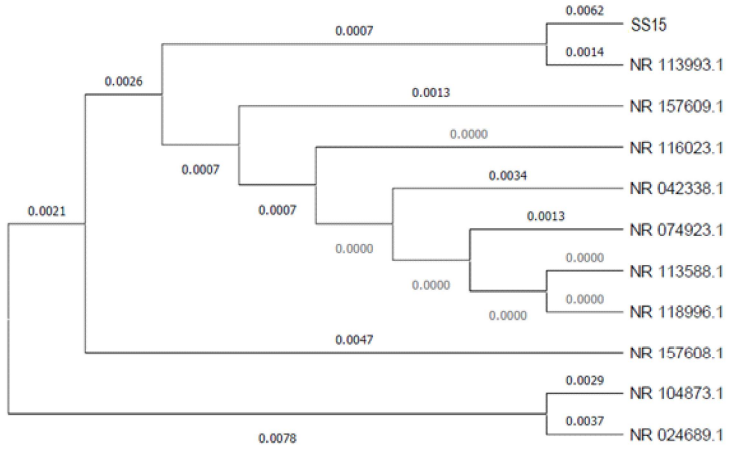

Fig 5. Phylogenetic tree of the SS15 bacterial isolate

The sequencing results revealed a 99% similarity to Bacillus licheniformis strains listed in the GenBank database (Fig. 3-5), confirming the identity of the isolate. The high degree of similarity indicates a close genetic relationship to previously characterized Bacillus licheniformis strains, validating the accuracy of the identification process. This molecular identification not only establishes the taxonomic position of SS15 but also aligns it with a well-studied bacterial group known for its diverse plant growth-promoting capabilities and resilience under various environmental conditions.

Growth Promotion of Plants

The inoculation of wheat, tomato, and chili plants with *Bacillus licheniformis* SS15 resulted in significant improvements in growth parameters compared to the control. In wheat, the average shoot length increased by 20%, and root length showed a 25% increase. Tomato and chili plants also exhibited similar improvements, with shoot length and biomass increasing by up to 30% and 15%, respectively.

Nitrogen Content

The nitrogen content in the inoculated plants was significantly higher than in the controls. Wheat plants showed an increase of 25% in nitrogen content, while tomato and chili plants exhibited increases of 18% and 20%, respectively.

Root Colonization

Root colonization studies revealed that *Bacillus licheniformisSS15* was able to effectively colonize the root systems of wheat, tomato, and chili plants. The bacterial population remained high throughout the 30-day period, with the highest CFUs observed at 30 days.

Secondary Metabolite Production

Bacillus licheniformisSS15 produced significant amounts of IAA (28 µg/mL) and siderophores, which were consistent with its ability to promote plant growth. The results also confirmed its phosphate solubilization activity, indicated by the clear zones formed on Pikovskaya agar.

Antagonistic Activity

Bacillus licheniformisSS15 exhibited strong antagonistic activity against *Fusariumoxysporum*, *Rhizoctoniasolani*, and *Pythiumaphanidermatum*. The largest zones of inhibition were observed against *Fusariumoxysporum* and *Rhizoctoniasolani*, indicating its potential as a biocontrol agent.

Plant Growth-Promoting Traits

Plant growth-promoting traits are characteristics exhibited by certain bacteria that enhance plant growth and productivity, often through natural, eco-friendly processes. These traits include nitrogen fixation, where bacteria convert atmospheric nitrogen into a form that plants can use; production of phytohormones such as indole-3-acetic acid (IAA), which promotes root and shoot development; and phosphate solubilization, where bacteria convert insoluble phosphorus compounds in the soil into forms accessible to plants. By utilizing these traits, plant growth-promoting bacteria like *Bacillus licheniformis* can reduce the need for synthetic fertilizers, promote healthier crops, and contribute to more sustainable agricultural practices.

Nitrogen Fixation

The isolate exhibited a positive result for nitrogen fixation, as evidenced by its ability to grow in a nitrogen-free medium. This indicates that Bacillus licheniformis SS15 can convert atmospheric nitrogen into bioavailable forms, a vital trait for reducing dependence on synthetic nitrogen fertilizers in agricultural practices.

IAA Production

The bacterium demonstrated the production of significant levels of indole-3-acetic acid (IAA), a key phytohormone involved in promoting plant root and shoot development. The concentration of IAA ranged from 10 to 20 µg/mL, depending on the incubation period and environmental conditions, showcasing the isolate's ability to enhance plant growth under varied scenarios.

Phosphate Solubilization

Bacillus licheniformis SS15 exhibited phosphate-solubilizing activity, as indicated by the formation of clear zones around its colonies on Pikovskaya's agar medium. This ability to convert insoluble phosphates into forms accessible to plants highlights its potential to improve phosphorus availability in soils, reducing the need for chemical phosphate fertilizers.These traits collectively underline the potential of Bacillus licheniformis SS15 as a beneficial bioinoculant for sustainable agricultural practices.

Growth Characteristics

The optimal growth of *Bacillus licheniformis* SS15 occurred at an alkaline pH of 7 to 9, with reduced growth observed at pH values below 7 or above.

Temperature

The bacterium grew optimally at temperatures ranging from 30°C to 37°C, with minimal growth observed below 25°C or above 40°C.

Salinity

The bacterium demonstrated resilience to moderate salinity, with significant growth observed at up to 18% NaCl concentration, which is considered high for most soil bacteria.

Effect of pH, temperature and salinity on bacteria growth

The growth of *Bacillus licheniformis SS15* was evaluated under varying environmental factors, including temperature, pH, and salinity, to identify its optimal growth conditions and tolerance limits. In terms of temperature, SS15 exhibited optimal growth within the range of 30°C to 37°C, indicating its preference for moderately warm conditions typical of temperate environments or agricultural settings (Fig. 3a). Beyond 50°C, growth was significantly inhibited, establishing an upper thermal tolerance limit for the strain.Regarding pH, SS15 thrived in alkaline conditions, with peak growth observed between pH 7.0 and 9.0, suggesting its adaptation to neutral to slightly basic soils (Fig. 3b). Conversely, acidic conditions (pH 4.0 to 5.0) severely hindered its growth, highlighting the strain's sensitivity to low pH environments.Salinity tolerance analysis revealed that SS15 is moderately halotolerant, capable of robust growth in salt concentrations up to 18% NaCl, with optimal growth occurring between 3% and 9% NaCl (Fig. 4). Growth was entirely inhibited at higher salinity levels, such as 21% NaCl, indicating a limit to its salt tolerance. In summary, Bacillus licheniformis SS15 demonstrates remarkable adaptability to moderately warm temperatures (30°C-37°C), alkaline pH (7.0-9.0), and moderate salinity (up to 18% NaCl). These characteristics position SS15 as a promising candidate for agricultural applications, particularly in regions experiencing moderate salinity stress or alkaline soil conditions.

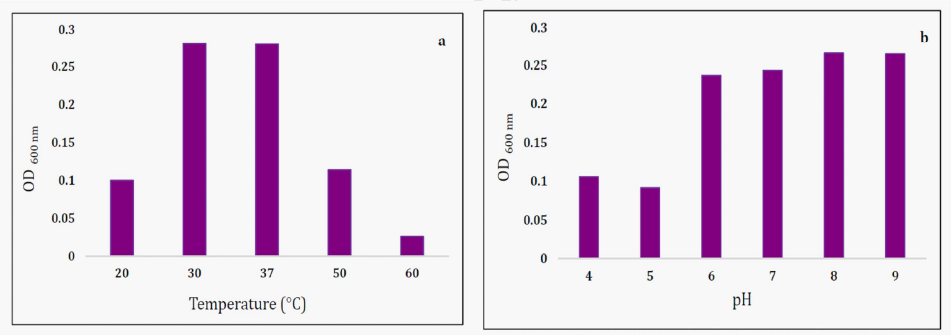

Fig 6 Effect of various a) temperatures and b) pH on growth of SS15

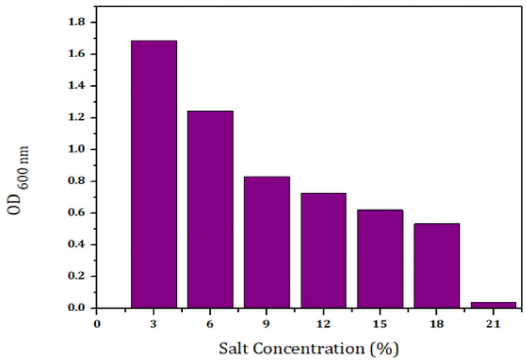

Fig 7 Effect of various salt concentration on growth of SS15

Discussion

Bacillus licheniformis SS15 demonstrated key plant growth-promoting mechanisms, including nitrogen fixation, IAA production, and phosphate solubilization. Nitrogen fixation plays a crucial role in improving soil fertility by converting atmospheric nitrogen into a form that is available to plants [2]. The production of IAA, a key phytohormone, is essential for root elongation and overall plant growth [4]. Phosphate solubilization is another important trait that aids in improving the availability of phosphorus, a vital nutrient for plant growth [3].

Environmental Tolerance

The ability of *Bacillus licheniformis* SS15 to grow in a broad range of pH, temperature, and salinity conditions suggests its potential for use in diverse agricultural environments. The bacterium's resilience to high salinity is particularly important in regions affected by soil salinization, a growing concern in sustainable agriculture [7].

Application in Sustainable Agriculture

The results of this study highlight the potential of *Bacillus licheniformis* SS15 as a biofertilizer and biocontrol agent in sustainable agriculture. Its ability to enhance nutrient availability, promote plant growth, and tolerate environmental stresses makes it a promising candidate for use in various agricultural systems.

Antagonistic Properties

The antagonistic activity exhibited by *Bacillus licheniformis* SS15 against common plant pathogens supports its potential as a biocontrol agent. The bacterium's ability to produce antimicrobial compounds or directly outcompete pathogens for nutrients in the rhizosphere is a key factor in its biocontrol potential [13]. The dual-culture assays demonstrated that *Bacillus licheniformis* SS15 could effectively suppress the growth of soilborne pathogens, reducing the need for chemical pesticides.

Conclusion

Bacillus licheniformis SS15 exhibits a range of significant plant growth-promoting traits, including nitrogen fixation, production of indole-3-acetic acid (IAA), and phosphate solubilization, all of which contribute to improved plant growth and soil health. Additionally, its ability to tolerate various environmental stresses, such as temperature fluctuations, salinity, and pH changes, enhances its suitability for diverse agricultural conditions. These characteristics position Bacillus licheniformis SS15 as a promising candidate for integration into sustainable agricultural practices, offering a natural alternative to chemical fertilizers and pesticides. However, further field trials are essential to evaluate its long-term impact on crop yield, soil health, and overall ecosystem balance, providing a comprehensive understanding of its practical applications in agriculture.

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