IMPACT ASSESSMENT AND MANAGEMENT OF E-WASTE AND ISOLATION OF METAL TOLERATING BACTERIA FROM CONTAMINATED SITES

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CERTIFICATE

This is to certify that the work entitled, **"IMPACT ASSESSMENT AND MANAGEMENT OF E-WASTE AND ISOLATION OF METAL TOLERATING BACTERIA FROM CONTAMINATED SITES"** submitted by Vidhi Gupta (041513) and Parul Laul (041550) in partial fulfillment for the award of degree of Bachelor of Technology in Bioinformatics of Jaypee University of Information Technology has been carried out under my supervision. This work has not been submitted partially or wholly to any other University or Institute for the award of this or any other degree or diploma.

Syal 20/05/08

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CRT - Cathode Ray Tube
ENVIS - Environmental Information System
LCD - Liquid Crystal Display
OD - Optical Density
PWB - Printed Wire Boards
PDA - Potato dextrose agar
rpm - Rotations per minute
SIDT - Support Initiatives in Development
WEEE - Waste Electrical and Electronics Equipment

ABSTRACT

Electronic waste (e-waste) handling and disposal has become a pressing nationwide environmental issue of concern. Electronic waste, or e-waste, includes cathode ray tubes (CRTs) from televisions and computer monitors, the central processing units (CPUs) and other chips from PCs, hard drives, printers, circuit boards, keyboards, cellular and cordless phones, televisions, VCRs, and DVD players [2]. The biohydrometallurgical Techniques provide us with a better solution, i.e. to apply a bacterial leaching process ('bioleaching') for mobilization of metals from the fine-grained e-waste [1]. We did a survey of Himachal Pradesh and Chandigarh- Mohali-Dera Bassi-Panchkula-Baddi belt. E-waste is likely to be increased by tones in coming years and the problem of its disposal remains unsolved. We identified industrial sites in Delhi and soil samples were collected from these sites. 7 bacterial isolates were obtained. These isolates were able to survive high metal concentrations at the sample sites. Effect of varying metal concentrations on bacterial growth was studied in laboratory. It is a possibility that these strains can leach metals from e-waste. This new field of research can prove to be the solution to the tsunami of e-waste which is going to hit the world in coming years.

CHAPTER 1
INTRODUCTION

1.1-IN the past few years, technology advances in electronics have boosted the economy and improved the general lifestyle of a common man. The ever-growing dependence on electronic products has paved the way for an emerging environment concern called "Electronic Waste".

Electronic Waste or e-waste is the term used to describe old, end-of-life electronic appliances such as computers, laptops, TVs, DVD players, mobile phones, mp3 players etc. which have been disposed of by their original users [23]. While there is no generally accepted definition of e-waste, in most cases, e-waste comprises of relatively expensive and essentially durable products used for data processing, telecommunications or entertainment in private households and businesses [2].

Definition of e-waste according to the WEEE (Waste Electrical and Electronic Equipment) directive [19]:

- Large household appliances (ovens, refrigerators etc.)
- Small household appliances (toasters, vacuum cleaners etc.)
- Office and communication (PCs, printers, phones, faxes etc.)
- Entertainment and electronics (TVs, portable CD players etc.)
- Lighting equipment (mainly fluorescent tubes)
- E-tools (drilling machines, electronic lawnmowers etc.)
- Sports and leisure equipment (electronic toys, training machines etc.)
- Medical appliances and instruments
- Surveillance equipment
- Automatic issuing systems (ticket issuing machines etc.)

1.2-Problems caused by electronic waste

As IT firms continue to swamp India's technology, the country is starting to choke under a heap of e-waste generated from obsolete computers and discarded electronic components. The waste contains more than 1,000 different toxic substances harmful to human beings and the environment. "If we do not wake up now, in the next five years it will boomerang on us."

Electronic waste (e-waste), including all obsolete electronic products, has become the fastest growing component in the solid waste stream. Personal computers (PCs) are the most significant component in e-waste stream. Other main components of e-waste being TV sets, refrigerator and mobiles.

India along with other Asian countries like Pakistan and China are increasingly becoming a dumping ground for hazardous wastes from old computers and electronic items.
The electronic waste contains several hazardous and toxic materials like lead, mercury, cadmium, PVC plastics and brominated flame retardants, which are known to cause severe defects in human bodies [25].

After the e-revolution of the past two decades, the world is now faced with the problem of disposal of e-wastes. If handled unscientifically during disbanding and breaking down, e-waste has the potential to pollute natural resources such as water, apart from endangering the health of the workers. Some of the toxic trash also finds its way into municipal garbage dumps. If the garbage is burned, the e-waste has the potential to disastrously explode [27].

1.3 Chemical elements contained in e-waste

Substance	Occurrence in e-waste	Harmful Effects on Human Body
Halogenated compounds:		
PCB (polychlorinated biphenyls)	Condensers, Transformers	May cause Cancer in animals. May also cause effects on the immune system, reproductive system, nervous system, endocrine system and other health effects.
TBBA (tetrabromo-bisphenol-A) -PBB (polybrominated biphenyls) -PBDE (polybrominated diphenyl ethers)	Fire retardants for plastics (thermoplastic components, cable insulation) TBBA is presently the most widely used flame retardant in printed wiring boards and casings.	Toxics and Dioxins released by them can lead to severe hormonal disorders.
Chlorofluorocarbon (CFC)	Cooling unit, Insulation foam	They have a deleterious effect on the ozone layer. This results in increased incidence of skin cancer in humans and in genetic damage in many organisms.

PVC (polyvinyl chloride)	Cable insulation	When burnt, releases HCl gas which combines with water wo form HCl acid which when inhaled can cause respiratory problems.
Heavy metals and other metals:		
- Arsenic	Small quantities in the form of gallium arsenide within light emitting diodes	Its chronic exposure can lead to various diseases of the skin and decrease nerve conduction velocity and can also cause lung cancer and can often be fatal.
-Barium	Getters in CRT	Short-term exposure to barium could lead to brain swelling, muscle weakness, damage to the heart, liver and spleen
- Beryllium	Power supply boxes which contain silicon controlled rectifiers and x-ray lenses	May cause lung cancer. Also, exposure to beryllium causes a form of skin disease that is characterized by poor wound healing and wart-like bumps.

-Cadmium	Rechargeable NiCd-batteries, fluorescent layer (CRT screens), printer inks and toners, photocopying-machines (printer drums)	May have serious impacts on kidneys. Acute exposure to cadmium fumes causes flu-like symptoms of weakness, fever, headache, chills, sweating and muscular pain.
- Chromium VI	Data tapes, floppy-disks	It is irritating to eyes, skin and mucous membranes. Chronic exposure may cause permanent eye injury and DNA damage.
- Lead	CRT screens, batteries, printed wiring boards	Can affect the kidneys. Can damage nervous connections and cause blood and brain disorders in children.
- Lithium	Li-batteries	
-Mercury	Fluorescent lamps that provide backlighting in LCDs, in some alkaline batteries and mercury wetted switches	Causes brain and liver damage if ingested or inhaled.
-Nickel	Rechargeable NiCd-batteries or NiMH-batteries, electron gun in CRT	

-Selenium	Older photocopying-machines (photo drums)	Exposure to high concentrations causes selenosis. The major signs of selenosis are hair loss, nail brittleness, and neurological abnormalities.
-Rare Earth elements (Yttrium, Europium)	Fluorescent layer (CRT-screen)	
-Zinc sulphide	Interior of CRT screens, mixed with rare earth metals.	
Others:		
-Toner Dust	Toner cartridges for laser printers / copiers	It is hazardous to inhale carbon black because it can irritate the lungs as well as cause other respiratory conditions, like Asthma.
Radio-active substances		
-Americium	Medical equipment, fire detectors, active sensing element in smoke detectors.	

Table 1.1 Chemical elements contained in e-waste and there harmful effects

1.4 Components in computers

On average a computer is 23% plastic, 32% ferrous metals, 18% non-ferrous metals (lead, cadmium, antimony, beryllium, chromium and mercury), 12% electronic boards (gold, palladium, silver and platinum) and 15% glass [15]. Only about 50% of the computer is recycled, the rest is dumped. The toxicity of the waste is mostly due to the lead, mercury and cadmium – non-recyclable components of a single computer may contain almost 2 kilograms of lead. Much of the plastic used contains flame retardants, which makes it difficult to recycle.

Fractions and medium weight (in grams) of a personal computer [15]

Tracer item PC	Control Unit (CPU)	Monitor	Keyboard and Mouse	Printer	Total
Total in grams	8,380	12,106	1,180	5,490	27,156

Table 1.2 Average weight of a personal computer

Composition of various parts in Percentage:

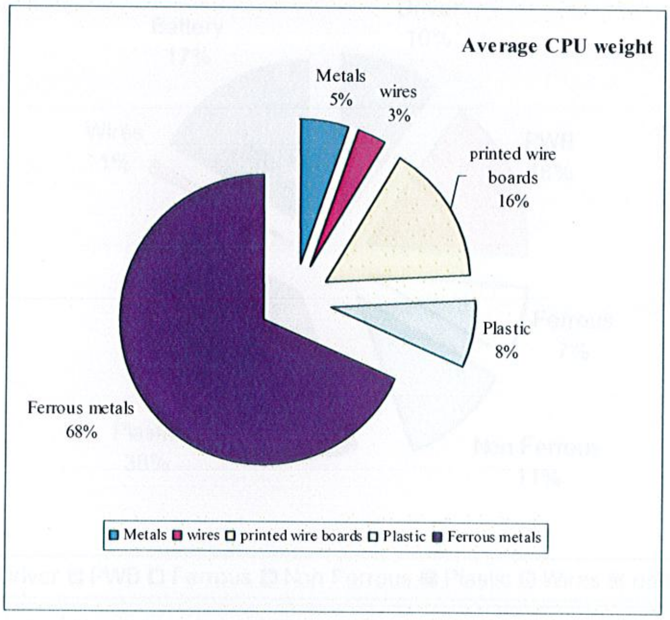


Fig1.1 Composition of CPU by weight

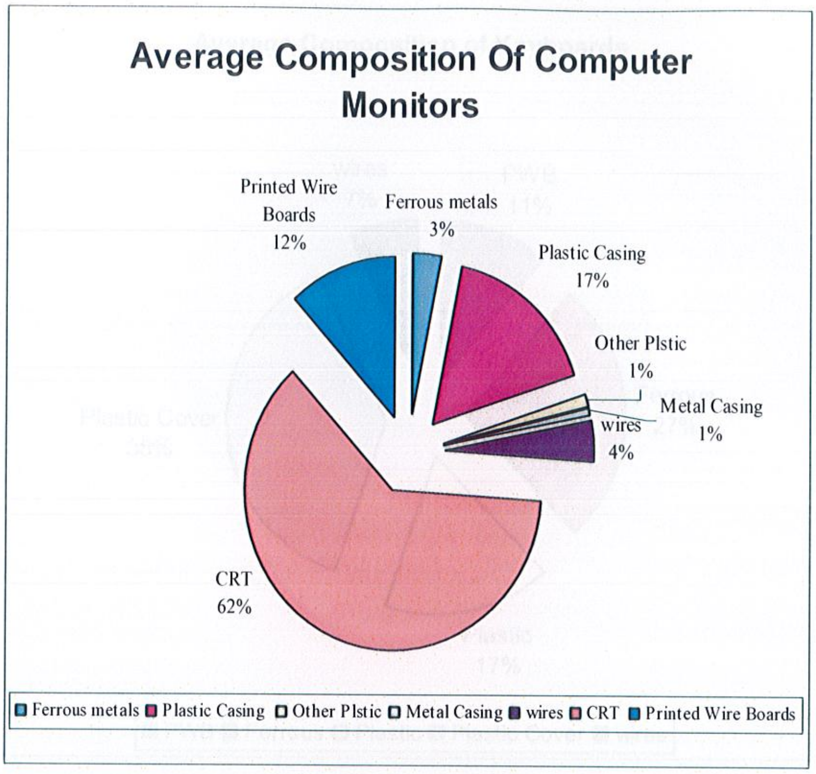


Fig1.2 Composition of Computer monitor by weight

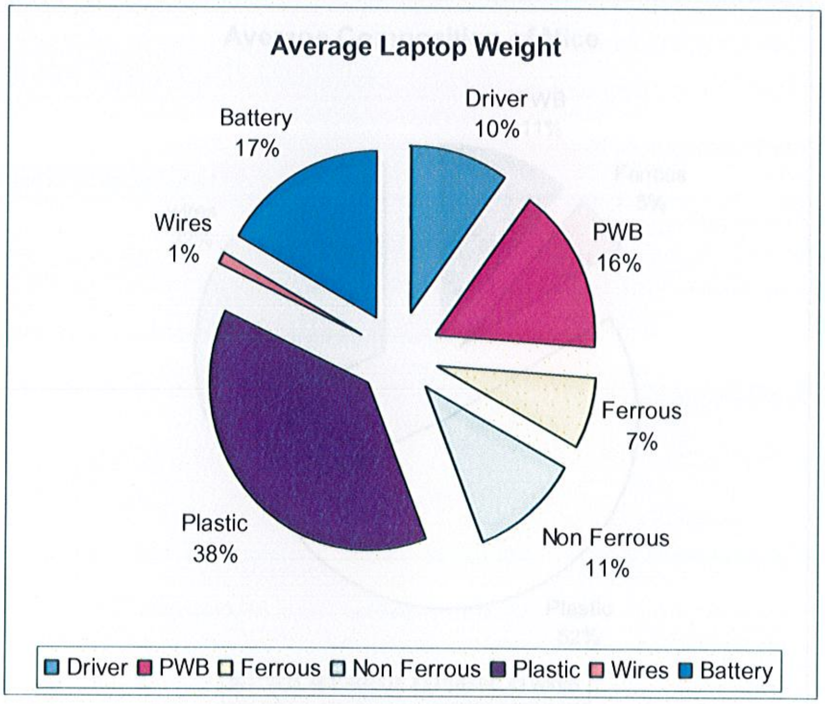


Fig1.3 Composition of Laptop by weight

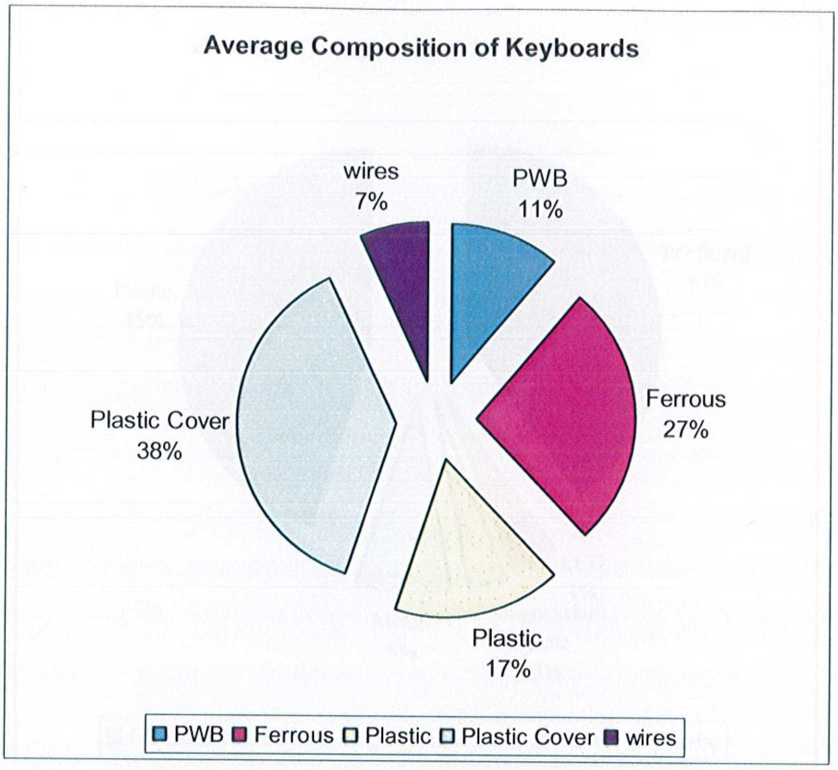


Fig1.4 Composition of Keyboard by weight

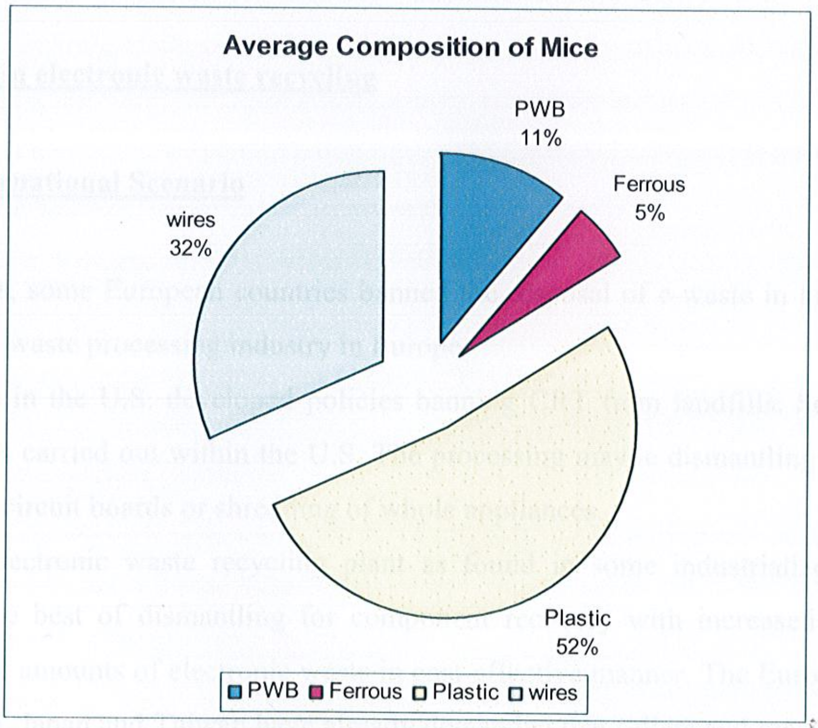


Fig1.5 Composition of Mice by weight

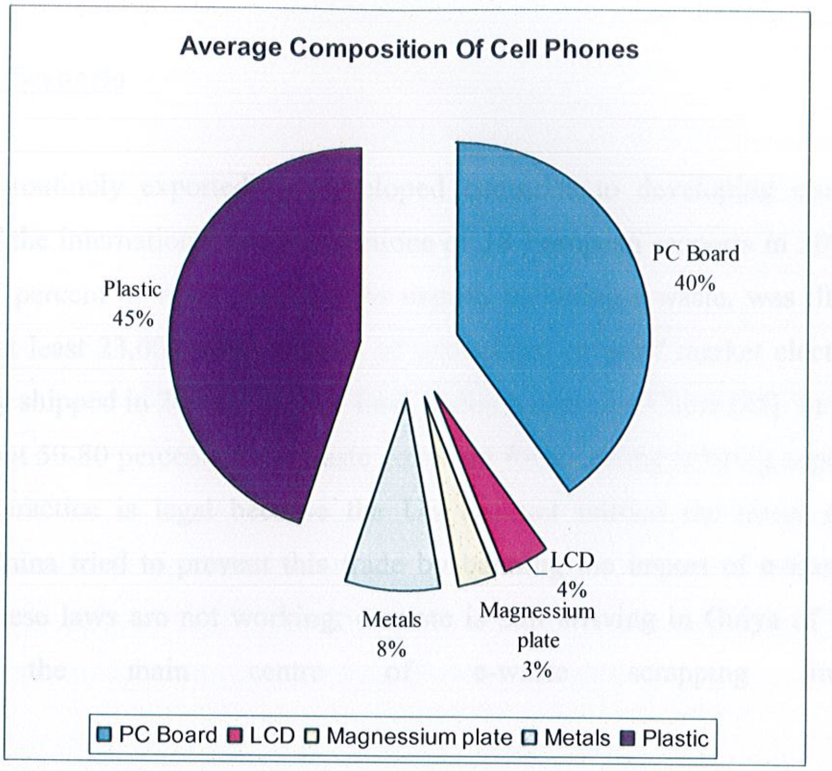


Fig 1.6 Composition of Cell Phones by weight

1.5 Trends in electronic waste recycling

1.5.1 International Scenario

In the 1990s, some European countries banned the disposal of e-waste in landfills. This created an e-waste processing industry in Europe.

Some states in the U.S. developed policies banning CRT from landfills. Some e-waste processing is carried out within the U.S. The processing maybe dismantling into metals, plastics and circuit boards or shredding of whole appliances.

A typical electronic waste recycling plant as found in some industrialized countries combines the best of dismantling for component recovery with increased capacity to process large amounts of electronic waste in cost-effective manner. The European Union, South Korea, Japan and Taiwan have already demanded that sellers and manufacturers of electronics be responsible for recycling 75% of them [20].

1.5.2 Asian Scenario

E-waste is routinely exported by developed countries to developing ones, often in violation of the international law. Inspections of 18 European seaports in 2005 found as much as 47 percent of waste destined for export, including e-waste, was illegal. In the UK alone, at least 23,000 metric tonnes of undeclared or 'grey' market electronic waste was illegally shipped in 2003 to the Far East, India, Africa and China [25]. In the US, it is estimated that 50-80 percent of the waste collected for recycling is being exported in this way. This practice is legal because the US has not ratified the Basel Convention. Mainland China tried to prevent this trade by banning the import of e-waste in 2000. However, these laws are not working; e-waste is still arriving in Guiya of Guangdong Province, the main centre of e-waste scrapping in China.

1.5.3 Recycling in India

There is a growing e-waste trade problem in India also. About 25,000 workers are employed at scrap yards in Delhi alone, where 10-20000 tonnes of e-waste is handled each year, 25 percent of this being computers. Other e-waste scrap yards have been found in Meerut, Ferozabad, Chennai, Bangalore and Mumbai [24].

There is open burning of PVC wires, de-soldering of circuit boards and glass which exposes the workers to lead, mercury and other toxic chemicals. The method of acid bath is used openly and without taking any precautions.

Most of the big companies, public and private, are disposing their waste through official tenders in newspapers. Some of them have though, in recent years, embraced the exchange policy wherein they return the old computers and get some discount on the new purchase. And in some cases, where the e-waste generation is small, the companies just sell it to the local 'kabaddiwala'.

Wipro, a leading IT company in India, will soon be offering e-waste disposal services to its customers [19]. Wipro will be among the pioneers to offer such a service, which customers can avail of free-of-cost by paying nominal freight charges. Acer India has taken the initiative to collect the e-waste. They offer an online form and people can send them the equipment through courier rather then throwing it and adding more to environmental pollution [17].

Infosys will also offer e-waste disposal services [19].

1.5.4 Slow poisoning

The trade in e-waste is camouflaged and is a thriving business in India. It is conducted under the pretext of obtaining "reusable" equipments or "donations" from developed nations. Due to lack of awareness, recyclers are risking their health and the environment as well. They use strong acids to retrieve precious metals such as gold, copper etc. Working in poorly ventilated enclosed areas without masks and technical expertise results in exposure to dangerous and slow poisoning chemicals [21].

1.5.5 Objectives

1. Survey and impact assessment of e-waste in H.P and neighboring areas.
2. Isolation of microbes from e-waste/metal dump-sites.
3. Screening and checking tolerance of microbes for metals from e-waste for recovery and reuse of these metals in industries.

CHAPTER 2

SURVEY AND ANALYSIS

2.1 Questionnaires

Survey including various questioners was done with different segments which are taken as samples for the survey. PCs (including there accessories) and mobile phones are taken as tracer elements.

Segments included were:

- Jaypee University of Information Technology, Himachal Pradesh
- ENVIS (Environmental Information System) centre, Himachal Pradesh
- SIDT (Support Initiatives in Development), Himachal Pradesh
- Computer dealers in Shimla and Solan
- Pollution Control Board, Himachal Pradesh

The questionnaires that were used are as follows:

For The Dealers

1. What are your average sales of computer?
2. What is the average no of pc's sold for personal use or house hold?
3. What is the average number of computers ordered by offices?
4. What is the average number of computers ordered by schools, colleges?
5. What is the average number of computers coming to you for up gradation?
6. What is the average number of computers coming to you for exchange?
7. While assembling whether old parts of computers are reused?
8. What is the average number of computers coming for resale?

9. What is the average number of computers dumped per year?
10. Any idea what happens to the PCs given to scrap dealer?
11. What is average lifetime of a computer?
12. What do you do with the PC parts?
13. Are these parts resold, if yes, to whom?
14. If dumped, then where?
15. Where are manufacturing units located?
16. Where are the processing units located for the computers which come for up gradation?
17. What other computer accessories are dumped by the users?
18. Can u tell us about any dumping site in Himachal Pradesh?
19. Do you deal with any regular scrap dealer?
20. What is done with unused computers parts which become obsolete with changing technology?

<u>Dealers approached</u>:

Mr. Manoj Gupta, Whiz kids, Shimla.

Mr. Ravi, Zenith, Shimla.

Mr. Rajat, R.K Enterprise, Shimla.

Access Marketing, Shimla.

Hanu Computers, Shimla.

SURVEY QUESTIONS FOR ENVIS CENTRE, H.P.

1. What comprises of e-waste according to your organization?
2. Do you classify e-waste into any kind of categories? If so, then which?
3. Since when (yr) has been E-waste dumped in Himachal Pradesh?
4. When was this e-waste recognized as a problem in HP?
5. What is done with all the different types of e-waste?
6. How many dumping sites do you have here in HP?
7. Where are all the dumping sites located?
8. Is there any site nearby Shimla which we can visit?
9. Is any e-waste recycled?
10. How is the recycling done?
11. Where is the recycling plant located?
12. Is there any impact of disposed e-waste on the environment?
13. Since when are you dedicated to e-waste management?
14. Does all the e-waste come from just HP or from other states too?
15. Approximately what quantity of e-waste is dumped in average per year?
16. Is the quantity of e-waste going up year by year? If yes, then by about how much?
17. Does ENVIS have branches in other states too which are working for e-waste management?
18. Is e-waste from foreign countries also dumped here in HP?
19. Are you taking any action against illegal dumping of e-waste? If yes, what?
20. Is e-waste dumped separately or with all the types of wastes?
21. Which NGO's are associated with you in this e-waste problem?
22. Approximately how much govt. is spending per annum on the e-waste management?
23. Are there any other organizations (both govt. and private) working on the same problem? If yes, then which?
24. Is govt. taking as much serious steps against the e-waste problem as it is in case of polythene bags?

Officials approached in ENVIS:

Mr. Sanjay Verma (Project Manager)

Mr. Umesh Kaushal (Senior Manager)

QUESTIONS FOR SIDT (Support Initiatives in Development), H.P.

1. How many PCs/laptops are present in H.P. government offices?
2. How many PCs are dumped every year?
3. What is the average life of computers in your offices?
4. Are the PCs regularly upgraded?
5. What do you do with the PC's which are obsolete?
6. If recycled, then where are the recycling units located?
7. Any idea where are the dumping sites located?
8. Which particular sector of govt. offices uses maximum number of computers?
9. Which computer parts do you change frequently?
10. Any particular scrap dealer you deal with?

Officials contacted:

Mr.Rajendra Verma (Manager)
Mr.Ashok Shandil (Assistant Manager)

2.2 Analysis and estimation in Himachal Pradesh

APPROXIMATE NUMBER OF COMPUTERS IN USE IN SCHOOLS AND COLLEGES OF HIMACHAL PRADESH IN 2007

- UNIVERSITY COMPUTERS IN USE:

UNIVERSITY	TOTAL COMPUTERS
HPU, Summer Hill, Shimla	2000
Dr. Y.S. Parmar Univ. of Horticulture and Forestry, Nauni, Solan	1000
C.S.K. H.P. Krishi Vishwa Vidyalaya, Palampur	1500
JUIT, Waknaghat, Solan	2100

- COMPUTERS IN USE IN OTHER INSTITUTES IN H.P:

INSTITUTE	TOTAL COMPUTERS
IIAS, Shimla	500
HP Board of School Education, Dharamshala	200
SCERT, Solan	100

- COMPUTERS IN USE IN DENTAL COLLEGES:

COLLEGE	TOTAL COMPUTERS
Govt. Dental College, Shimla	1000
DAV Dental College, Solan	1000
Bhojia Dental College and Hospital, Baddi	600
Dental College, Sundar Nagar	500

- COMPUTERS IN USE IN MEDICAL COLLEGES:

COLLEGE	TOTAL COMPUTERS
IGMC, Shimla	1000
Dr. Rajender Prasad Govt. Medical College, Kangra	200

- COMPUTERS IN USE IN ENGINEERING COLLEGES

COLLEGE	TOTAL COMPUTERS
NIT, Hamirpur	2000
HPU-Institute of Information Technology, Shimla	1500
IIET, Baddi	450
Green hills college, Kumarhatti	350
Engineering College, Kala Amb	600

- COMPUTERS IN USE IN POLYTECHNIC COLLEGES:

COLLEGE	TOTAL COMPUTERS
Govt. Polytechnic College, Sundernagar	200
Govt. Polytechnic College (Women), Kandaghat, Solan	100

- COMPUTERS IN USE IN GENERAL COLLEGES:

NO. OF COLLEGES	TOTAL COMPUTERS
64	3200

- COMPUTERS IN USE IN MIDDLE SCHOOLS:

NO. OF SCHOOLS	TOTAL COMPUTERS
1056	10560

- COMPUTERS IN USE IN HIGH/HIGHER SECONDARY SCHOOLS:

NO. OF SCHOOLS	TOTAL COMPUTERS
1339	26780

TOTAL COMPUTERS IN HP SCHOOLS AND COLLEGES:

TOTAL COMPUTERS	**56640**

Table 2.1 Total number of computers in use in schools and colleges of Himachal Pradesh in 2007

Total male working population of Himachal Pradesh 1,686,658 [22].

Assuming 60% of them are having personal computers, so total house hold computers in HP are 1011995.

Total computers in Himachal Pradesh are approximately 10, 68,635.

2.3 Analysis and estimation of number of computers in Chandigarh-Mohali-Dera Bassi-Panchkula- Baddi belt

Currently the following peripherals are being manufactured in India:

1. Dot Matrix Printers
2. Line Printers/Daisy Wheel Printers
3. Floppy Disc Drives Hard Disc Drives
4. Cartridge Tape Drives (CTD)
5. Terminals, Monitors and Key Boards
6. Plotters and Digitizers
7. Magnetic Ink Character Recognizers (MICR)

2.3.1 Computer Manufacturing Units to be established in H.P in near future

1. Chirag Computers
2. (Approx 40 crores project in Baddi, Parwanoo)
3. Lenovo (Baddi)
4. Intex (Baddi)
5. Spice (Baddi)
6. eSYS Technologies (1,200,000 units per annum and around Rs.250 crores project in Baddi)

2.3.2 Computer Industries in Chandigarh Technology Park

Rajiv Gandhi Chandigarh Technology Park (RGCTP), was conceived in 2001 by Chandigarh administration. Located in the foot hills of Shivaliks Close to Sukhna lake. The Technology park is now full of big IT players like [18, 25, 26, 27]

- Infosys Technologies Ltd.
- IDS Infotech Limited
- Bebo Technologies Pvt. limited
- QASource India Inc.
- IBM Daksh
- Miracle Studios
- Net Solutions
- Outerbay (Now HP India)
- Virsa Systems (now SAP Labs)
- ICICI Prudential (for Software Development)
- FCS Software Solutions Ltd.
- Webart Softech
- Netsoft Informatics
- Dell

OTHER COMPUTER MANUFACTURING INDUSTRIES IN AND AROUND CHANDIGARH:

1. eSys (Capacity approx. 250 PCs per day in 2003).
2. Cogniter Technologies
3. HCL technologies
4. Wipro
5. Bharti- Airtel
6. Techmahindra
7. ST Microelectronics
8. Hughes and Convergys
9. Quark (Mohali)
10. Reliance
11. Satyam
12. Autoronica (Panchkula)
13. Semiconductor Complex Ltd. (SCL)
14. C-DAC
15. IDS Infotech (Mohali)
16. TCS
17. Teledata Informatics
18. Asterisk Electronics Private Ltd. (Panchkula)
19. Golden Computer
20. TVSE

14 companies are already working in Chandigarh IT park and 13 more to set up there units there [15].

CHAPTER 3

IMPACT ASSESSMENT

3.1 Data Analysis of Himachal Pradesh

3.1.1 Computers and laptops

It has been found that average life of a computer is 3-4 years after this either consumer goes for up gradation or it keeps on lying as e-waste and consumer buys the new set. Approximate sale of PCs and laptops in Himachal is about 1700 per month.

As per above data in coming years there will be more than 2 lacs of computers which would be dumped.

According to Mr. Sanjay Verma (Project Manager) of ENVIS problem of e-waste in Himachal mainly compromises of PCs and old TV sets. Till now all these keep on lying in store rooms and there is no method available to dispose them, and this waste is increasing approximately 20-30% of e-waste every year.

3.1.2 Mobile phones

There are about 8.5 lacs mobile users in Himachal Pradesh. Average life of mobiles here is 2-3 years. People prefer to resale the mobiles so till date mobiles are not adding to e-waste but in coming 4-5 years it will. It is predicted that after 3-4 years there will be tsunami of about 6 lacs mobiles being dumped in Himachal itself.

3.2 Data Analysis of Chandigarh-Mohali-Dera Bassi-Panchkula- Baddi belt

- Computers (including their accessories) are taken as tracer elements.
- We investigated the life cycle of computers and assessed the future impacts of pervasive computing on health and the environment. A major finding was that, due to continually increasing technology, these products are increasingly being thrown away which could lead to harmful effect on environment.
- This assessment is done through internet.

Total Domestic Computers in Chandigarh

Total working male population of Chandigarh is 3, 83,311 [14]

Assuming 60% of them are having personal computers, so total house hold computers in CHD are 2, 29,986.

Total working Female population of Chandigarh is 2, 69,470 [14]

Assuming 40% of them are having personal computers, so total house hold computers in CHD are 1, 07,788.

Total domestic computers = 3, 37,774 (approx)

Total Computers in industries in Chandigarh Belt.

Total computers being used in industries=16,500

Total computers in CHD belt= 3, 54, 274 (approx.)

Assuming that 30% of PCs are dumped every year, e-waste generated = 13,816 tons (approx.)

MEASURES TAKEN SO FAR TO HANDLE E-WASTE

Though many dumping sites have been identified by various districts some of them are in Una, Mandi (Pandoh) and Lahoul n Spiti. Government is not that much concerned about e-waste problem till now as from 2002-07, only 3 meetings regarding the e-waste management have been held.

Himachal Pradesh is setting up a Treatment Storage and Disposal Facility (TSDF) at Majra village near Nalagarh for disposal of all hazardous waste from the entire state. The facility is being set up at a cost of Rs.35 crore with joint initiative of Baddi, Barotiwala and Nalagarh Industries Association (BBNIA).

UT Administration is planning to develop a site near Dera-Bassi for proper disposal of e-waste, in collaboration with the Punjab Government [16].

Recycling in Chandigarh

- Chandigarh Administration has been asked to adhere to the guidelines for the maintenance and environmental upkeep of the Rajiv Gandhi Chandigarh Technology Park, with a special reference to the e-waste [15].
- The city authorities have been specifically asked to create a special space for storage of computers, floppies and compact discs (CDs) [14].
- The Administration has been asked to dispose of the e-waste as per the guidelines of the Central Pollution Control Board.
- In December, nominated councilor Arshad Khan had introduced an agenda in the House on the disposal of e-waste.
- UT Administration has also decided to take care of domestic e-waste [15].
- Infosys and Wipro for setting up the recycling plant [14].

CHAPTER 4

METAL LEACHING FROM ELECTRONIC SCRAP

4.1 Abstract

Microbiological processes were applied to mobilize metals from metal salts. Bacteria (*Pseudomonas sp.*) was grown in the presence of metals. *Pseudomonas sp.*, which was available in lab, was taken for experiments because according to researchers, some strains of this genus are being used for bioleaching [3,9,11,12]. Initial experiments showed that the microbial growth was inhibited at the higher concentrations of the metals.

4.2 Introduction

Relatively short lifetimes of electrical and electronic equipment result in the production of increased amounts of waste materials. Though most of the electronic scrap can be recycled, the dust residues have to be disposed in landfills or incinerated. However, these residues can contain metals in concentrations, which might be of economical value [1].

To extract these metals, we can use Biohydrometallurgical techniques which allow metal cycling by processes similar to natural biogeochemical cycles [1, 6]. Using biological techniques, the recovery efficiency can be increased, whereas thermal or physiochemical methods alone are less successful.

One of the objectives of this project is to apply a bacterial leaching process (Bioleaching) for the mobilization of metals from different metal containing solid wastes such as fine grained e-waste [7].

4.3 Experiments done with *Pseudomonas aeruginosa*

In order to check the ability of *Pseudomonas aeruginosa* for leaching metals from e-waste, we started off with the following experiments:

4.3.1 Checking the tolerance of *Pseudomonas aeruginosa* for different metals

The tolerance of bacteria *Pseudomonas aeruginosa* was checked against 10 different metal salts.

The Soya bean Casein Digest Medium Agar plates with the following metals of concentration 1mM were prepared:

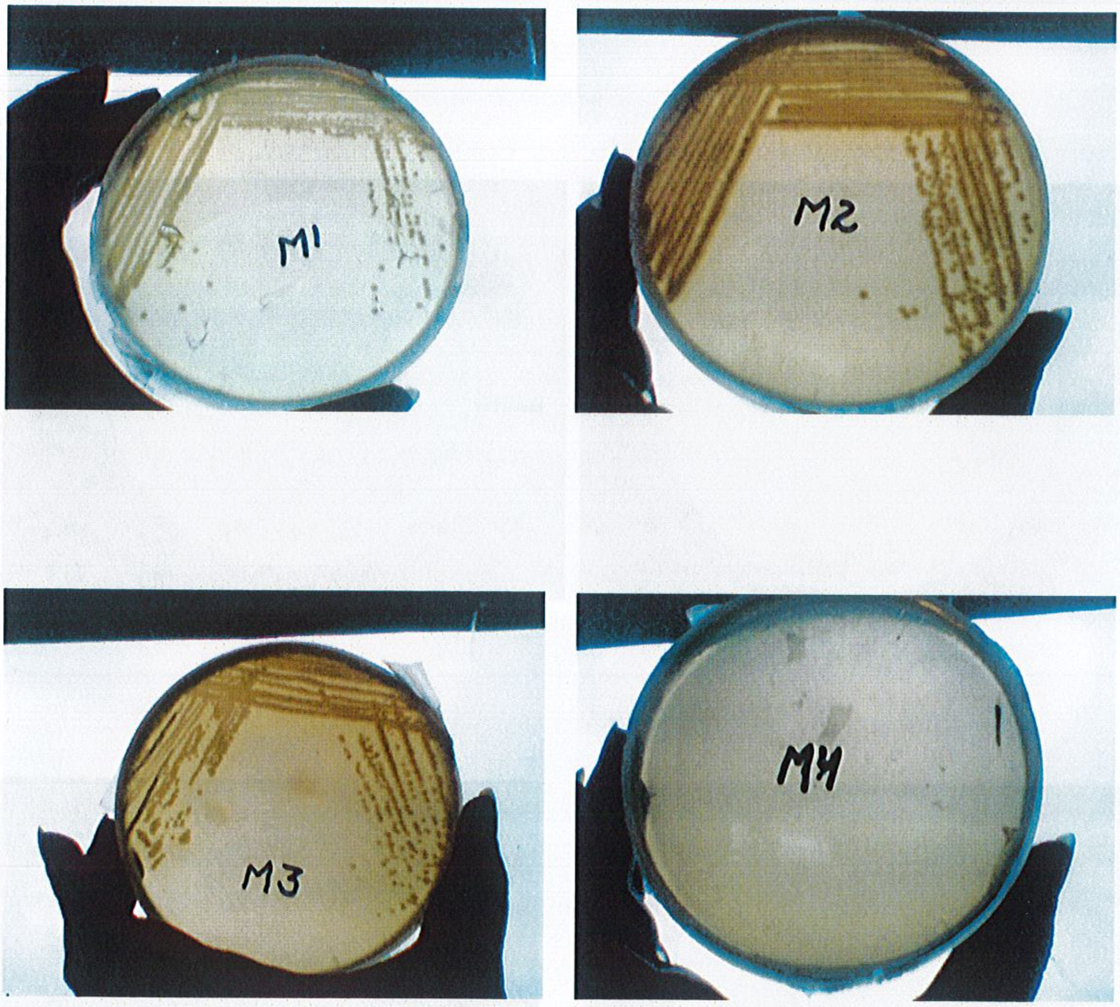
1. 1mM Zinc sulfate
2. 1mM Cupric Sulfate
3. 1mM Potassium dichromate
4. 1mM Mercury(II) chloride
5. Contained all the above metals (from 1-4) with concentration 1mM.
6. 1mM Barium chloride
7. 1mM Lithium lactate
8. 1mM Nickel chloride
9. 1mM Iron sulfate
10. Contained metals from 6-9 with concentration 1mM.

The plates were streaked with the bacteria *Pseudomonas aeruginosa.*

Observations after incubating the plates for 1 day

- Media 1: Growth observed till 3 quadrants.
- Media 2: Growth observed till 3 quadrants.
- Media 3: Growth observed till 3 quadrants.
- Media 4: No growth observed.
- Media 5: No growth observed.

- Media 6: Growth only in 1st quadrant.
- Media 7: Good growth in all quadrants.
- Media 8: Good growth in all quadrants.
- Media 9: Little growth only in 1st quadrant.
- Media 10: Little growth only in 1st quadrant.



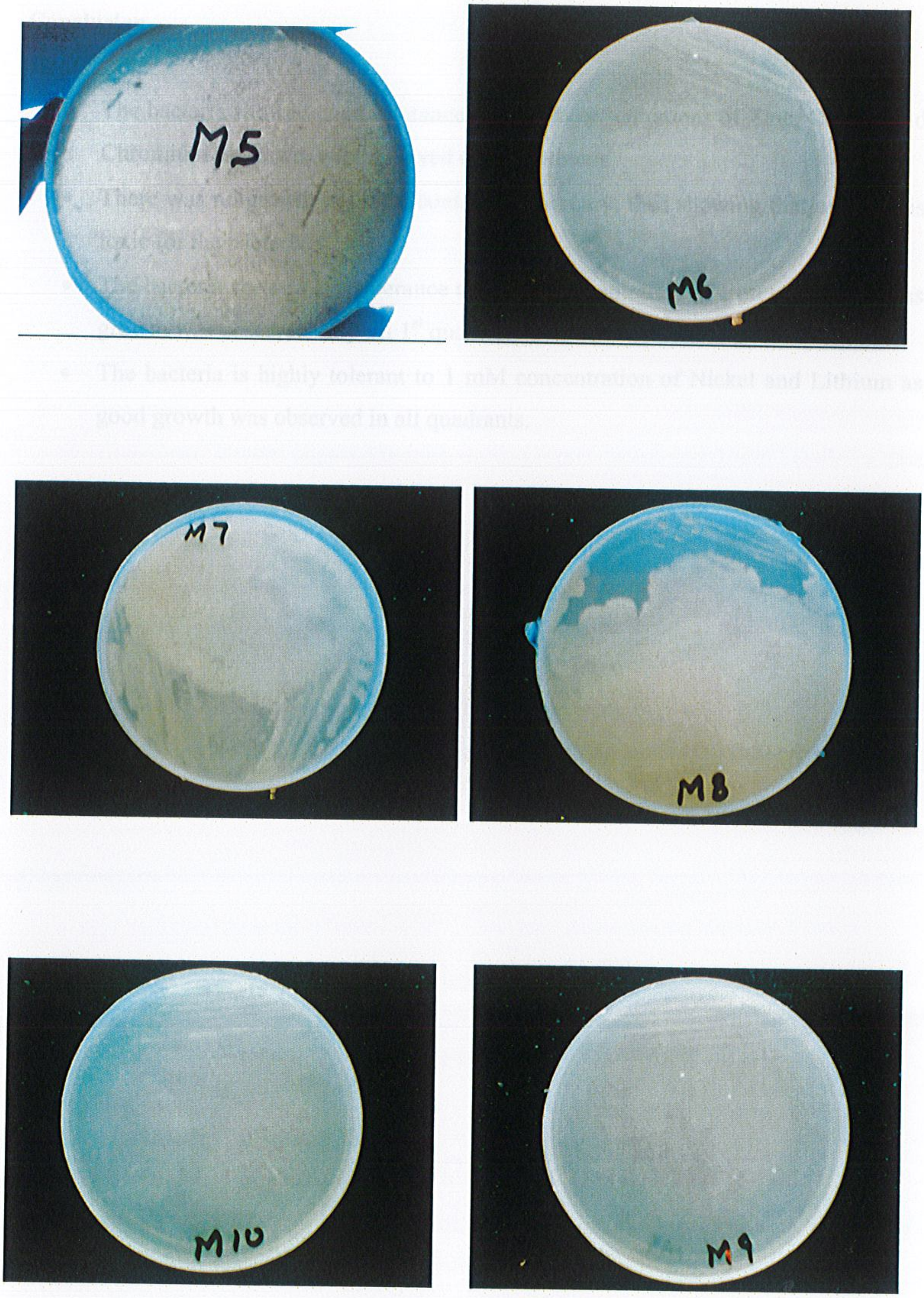


Fig4.1 *Pseudomonas aeruginosa* on different metal plates

Conclusion

- The bacteria showed good tolerance to 1 mM concentrations of Zinc, Copper and Chromium as growth was observed till 3 quadrants.
- There was no growth in plates containing mercury, thus showing that mercury is toxic for the bacteria.
- The bacteria showed less tolerance to 1 mM concentration of Iron and Barium, as growth was observed only till 1st quadrant.
- The bacteria is highly tolerant to 1 mM concentration of Nickel and Lithium as good growth was observed in all quadrants.

4.3.2 Checking the tolerance of *Pseudomonas aeruginosa* at different concentrations of Copper

Prepared culture of *Pseudomonas aeruginosa* in Soya bean Casein Digest Medium broth. Separated bacterial cells by centrifuging 1ml of growing culture at 5,000 rpm for 10 minutes. Discarded supernatant and washed cell pellets with 0.9% NaCl to completely remove traces of any carbon source. The pellet was then resuspended in 1ml of 0.9% NaCl and spread on Soya bean Casein Digest Medium agar plates whose cfu/ml was calculated as $2X10^7$ cfu/ml.

Media prepared:

- Soya bean Casein Digest Medium broth (20ml) containing 2mM Cupric sulfate (0.00996 gm)
- Soya bean Casein Digest Medium broth (20ml) containing 4mM Cupric sulfate (0.01992 gm)
- Soya bean Casein Digest Medium broth (20ml) containing no metal as control

Added 20 μl of dissolved pellets in each flask containing the above media.
Kept all the flasks in Shaker at 120 rpm, 28°C for 7 days.

Observations

No growth was observed in any of the flask (except control) for 2 days.
After 4 days, growth was observed in control as well as broth containing 2 mM cupric sulfate. No growth was observed in broth containing 4 mM cupric sulfate.

Conclusion

The experiment showed that bacteria is intolerant to higher concentrations (4mM) of copper.

4.3.3 Checking the tolerance of *Pseudomonas aeruginosa* at different concentrations of Zinc Dust

Prepared culture of *Pseudomonas aeruginosa* in Soya bean Casein Digest Medium broth. Separated bacterial cells by centrifuging 1ml of growing culture at 5,000 rpm for 10 minutes. . Discarded supernatant and washed cell pellets with 0.9% NaCl to completely remove traces of any carbon source. The pellet was then resuspended in 1ml of 0.9% NaCl and spread on Soya bean Casein Digest Medium agar plates whose cfu/ml was calculated as $2X10^7$ cfu/ml.

Media prepared:

- Soya bean Casein Digest Medium broth (100ml) containing 1% Zinc dust (1 gm)
- Soya bean Casein Digest Medium broth (100 ml) containing no metal as control

Added 30 µl of dissolved pellets in each flask containing the above media.

Kept all the flasks in Shaker at 120 rpm, 28°C for 3 days.

Observations

Growth was observed in both the flasks.

Zinc dust was stuck at the bottom of the flasks containing 1% zinc dust.

Conclusion

As the zinc dust was stuck at the bottom of the flasks, it did not effect the growth of the bacteria. Thus, this experiment did not give us any useful result regarding the tolerance of bacteria to zinc dust. So, we repeated the experiment with zinc sulfate in media.

4.3.4 Checking the tolerance of *Pseudomonas aeruginosa* at different concentrations of Zinc sulfate

Prepared culture of *Pseudomonas aeruginosa* in Soya bean Casein Digest Medium broth. Separated bacterial cells by centrifuging 1ml of growing culture at 5,000 rpm for 10 minutes. . Discarded supernatant and washed cell pellets with 0.9% NaCl to completely remove traces of any carbon source. The pellet was then resuspended in 1ml of 0.9% NaCl and spread on Soya bean Casein Digest Medium agar plates whose cfu/ml was calculated as $2X10^7$ cfu/ml.

Media prepared:

- Soya bean Casein Digest Medium broth (100ml) containing 1.0 mM Zinc sulfate (0.0287 gm)
- Soya bean Casein Digest Medium broth (100ml) containing 1.5mM Zinc sulfate (0.0431 gm)
- Soya bean Casein Digest Medium broth (100ml) containing 2.0 mM Zinc sulfate (0.0575 gm)
- Soya bean Casein Digest Medium broth (100ml) containing 2.5 mM Zinc sulfate (0.0718 gm)

Observations

Zinc sulfate settled at the bottom of the flasks as it did not dissolve in the media.

Conclusion

As the zinc salt did not dissolve in the media, it would not have effected the growth of bacteria. Thus, we did not inoculate the media with *Pseudomonas aeruginosa.*

4.4 Experiments done with soil samples

The soil samples from 4 different industrial sites were collected. As e-waste dumping sites are not yet listed, so we chose these industries as they were using metals and metallic salts extensively. We assumed that the microorganisms which were prevalent in these sites might be tolerant to metals present in e-waste.

Soil Samples were collected from the following places in Delhi:

1. Jeans Washing and Dyeing Factory, Rohini.
2. Gas Stove Plant, Rohini.
3. Metal Forging Factory, Rohini.
4. Welding Workshop, Rohini.

History of Sample Sites:

1. Jeans Washing and Dyeing Factory

- Approximately 5 years old.
- Uses a lot of chemicals having metal salts having metals like Copper, Chromium, Arsenic etc.
- Sample was collected from the drain.

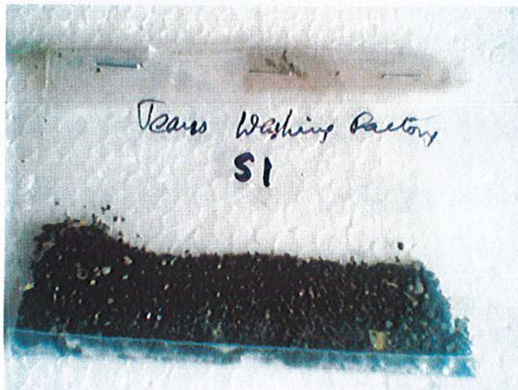


Fig4.2 (a) Jeans Washing and Dying factory sample and site

2. Gas Stove Plant

- More than 10 years old.
- Parts of various metals like Brass (an alloy of Copper and Zinc), Steel (an alloy of Iron and Carbon), and Iron are made.
- Sample was collected from the site where the parts were being made.

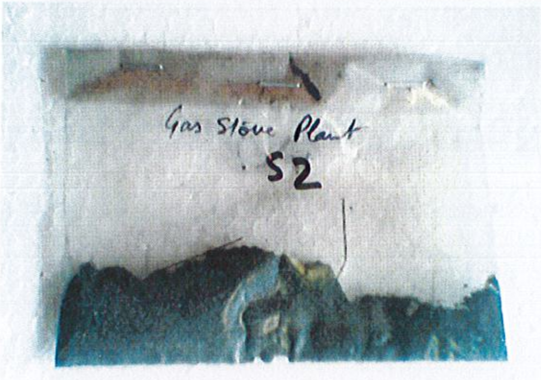


Fig 4.2(b) Gas stove plant sample

1. **Metal Forging Factory**

- About 27 years old.
- Parts of Brass (an alloy of Copper and Zinc) are made in this factory.
- Sample was collected from the site where the parts were being made.

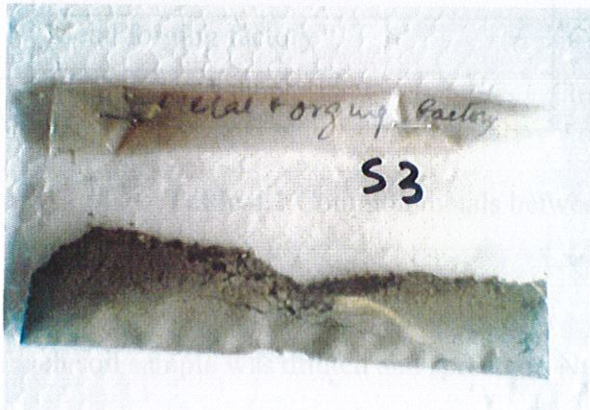


Fig4.2(c) Metal forging factory sample

4. Welding Workshop

- More than 10 years old.
- Various metals like Iron, Steel (an alloy of Iron and Carbon), and Brass (an alloy of Copper and Zinc) are welded.
- Sample was collected from the site where welding takes place.

Fig4.2 (d) Welding workshop sample and site

METALS COMMON BETWEEN THE SAMPLE SITES AND E-WASTE

Sample Site	Metals common with e-waste
Jeans washing and dyeing factory	Arsenic, Copper, Chromium.
Gas stove plant	Iron, Copper, Zinc.
Metal forging factory	Copper, Zinc.
Welding workshop	Iron, Copper, Zinc

Table 4.1 Common metals between the sample sites and e-waste

Each soil sample was diluted and spread on Nutrient Agar plates.

COLONY CHARACTERISTICS OF BACTERIAL ISOLATES

SAMPLE NO.	SIZE	SHAPE	ELEVATION	COLOR	SURFACE
S1 W	Medium	Circular	Raised	Off white	Smooth, glistening
S2 W	Medium	Circular	Raised	White	Smooth, glistening
S2 Y	Small to Medium	Circular	Raised	Yellow	Smooth, glistening
S3 W	Medium	Circular	Convex	Peach	Smooth, glistening
S4 O	Large	Circular	Convex	Orange	Smooth, glistening
S4 W	Large	Circular	Raised	Off-white	Smooth, glistening

Table 4.2 Colony characteristics of bacterial isolates from soil samples

- All the cultures were found to be Gram negative by KOH String test.

Photograph Showing Isolates From Soil Samples

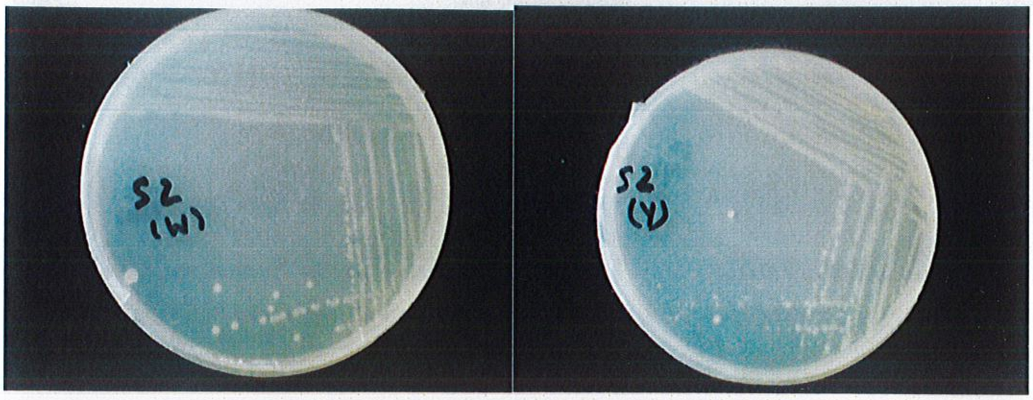


Fig. 4.3(a) Soil isolates of Gas stove plant

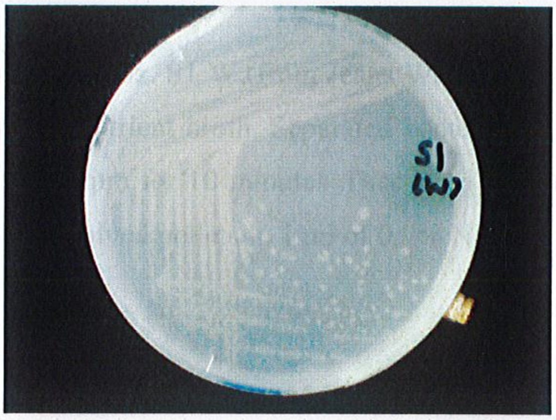


Fig. 4.3(b) Soil isolate of Jeans washing factory

Fig. 4.3(c) Soil isolate of Metal Forging factory

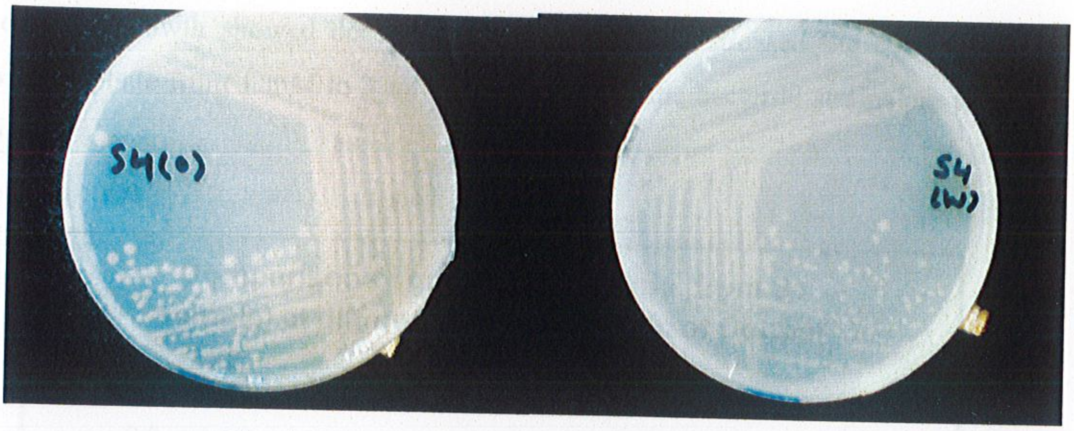


Fig. 4.3(d) Soil isolates of Welding workshop

4.4.1 Checking Tolerance of Bacterial Strains for Copper

Prepared culture of 2 samples, viz. S1 W (from Jeans Washing Factory) and S3 W (from Metal Forging Factory), in Nutrient broth. Separated bacterial cells by centrifuging 1ml of growing culture at 5,000 rpm for 10 minutes. Discarded supernatant and washed cell pellets with 0.9% NaCl. Dissolved pellets in 1 ml of 0.9% NaCl.

Media prepared:

- Nutrient broth (100ml) containing 1mM Copper sulfate
- Nutrient broth (100ml) containing 2mM Copper sulfate
- Nutrient broth (100ml) containing 3mM Copper sulfate
- Nutrient broth (100 ml) containing no metal as control

Added 20 μl of dissolved pellets in each flask containing the above media.
Flasks were kept in shaker at 37C and 200 rpm for 7 days.

Observations

As shown in Fig 4.4(a) and Fig 4.4(b), the control showed the highest growth, whereas flask containing 3mM Copper Sulphate showed the lowest growth for both the bacteria. Flask containing 1mM copper Sulphate showed growth approximately equivalent to that of control. Growth showed decreasing trend when we increased the concentration of copper sulphate from 1 mM to 3 mM. However both the bacterial strains are tolerant to copper.

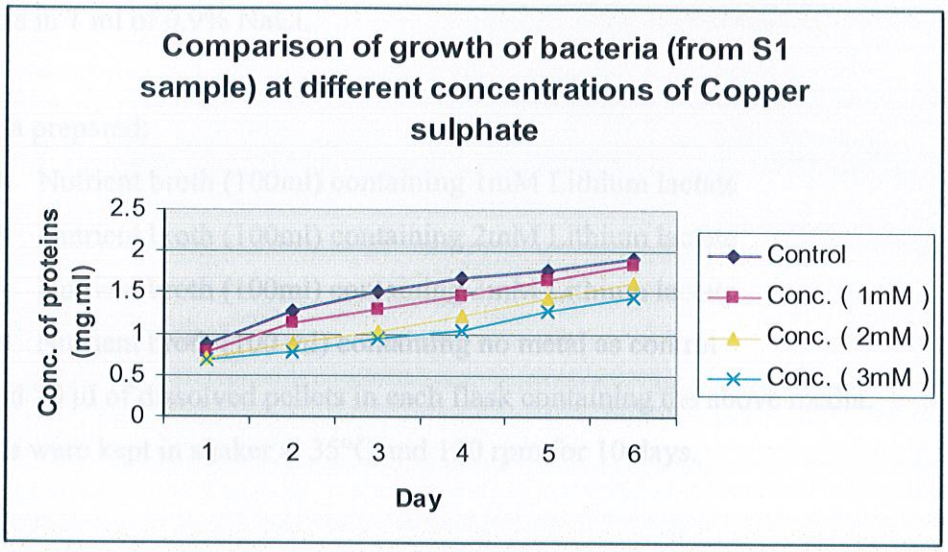


Fig. 4.4(a) Growth of soil isolate S1 W from soil sample S1 (from Jeans washing factory) on increasing the concentration of Copper sulphate

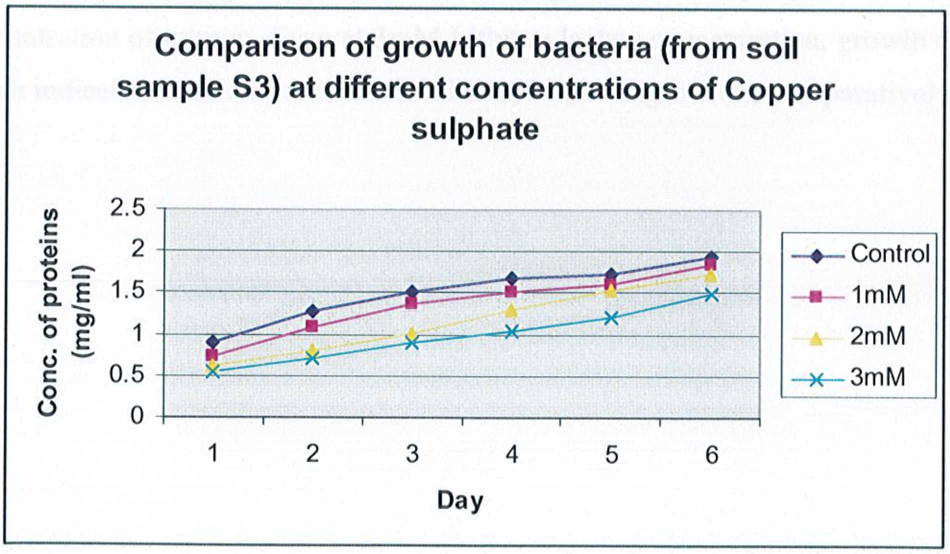


Fig. 4.4(b) Growth of soil isolate S3 W from soil sample S3 (from Metal forging factory) on increasing the concentration of Copper sulphate

4.4.2 Checking Tolerance of Bacterial Strains for Lithium

Prepared culture of S1 W (from Jeans Washing Factory) sample in nutrient broth. Separated bacterial cells by centrifuging 1ml of growing culture at 5,000 rpm for 10 minutes. Discarded supernatant and washed cell pellets with 0.9% NaCl. Dissolved pellets in 1 ml of 0.9% NaCl.

Media prepared:

- Nutrient broth (100ml) containing 1mM Lithium lactate
- Nutrient broth (100ml) containing 2mM Lithium lactate
- Nutrient broth (100ml) containing 3mM Lithium lactate
- Nutrient broth (100 ml) containing no metal as control

Added 20 μl of dissolved pellets in each flask containing the above media.

Flasks were kept in shaker at 35°C and 130 rpm for 10 days.

Observations

As shown in Fig 4.5, the bacterial growth decreases with increase in metal concentration. The control showed the highest growth, whereas flask containing 3mM concentration of lithium lactate showed the lowest growth for both the bacteria. Flask containing 1mM and 2mM Lithium lactate showed comparable growth showing tolerance towards higher concentration of lithium. Even at 3mM Lithium lactate concentration, growth is observed which indicated its tolerance towards lithium, however growth is comparatively less.

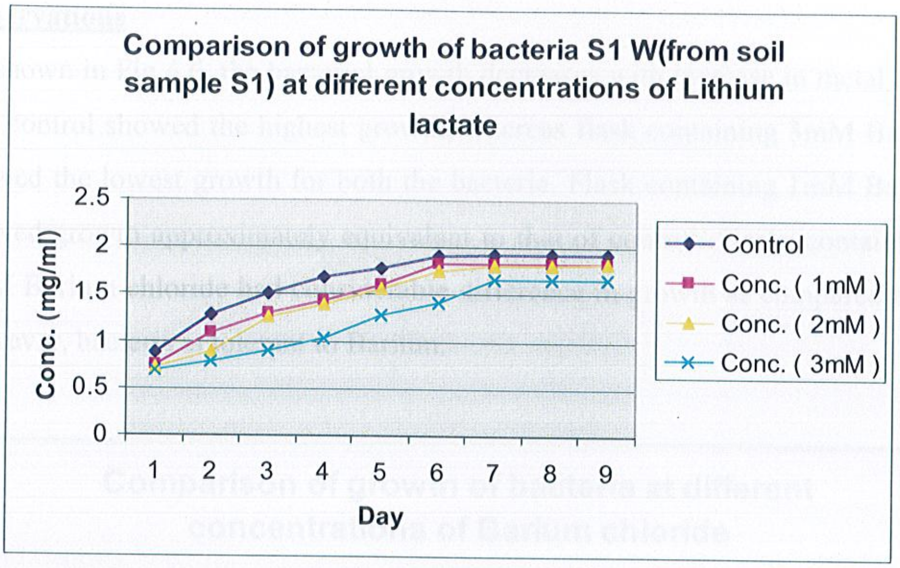


Fig. 4.5 Growth of soil isolate S1 W soil sample S1 (from Jeans washing factory) on increasing the concentration of Lithium Lactate

4.4.3 Checking Tolerance of Bacterial Strains for Barium

Prepared culture of S1 W (from Jeans Washing Factory) sample in nutrient broth.

Separated bacterial cells by centrifuging 1ml of growing culture at 5,000 rpm for 10 minutes. Discarded supernatant and washed cell pellets with 0.9% NaCl. Dissolved pellets in 1 ml of 0.9% NaCl.

Media prepared:

- Nutrient broth (100ml) containing 1mM Barium chloride
- Nutrient broth (100ml) containing 2mM Barium chloride
- Nutrient broth (100ml) containing 3mM Barium chloride
- Nutrient broth (100 ml) containing no metal as control

Added 20 µl of dissolved pellets in each flask containing the above media.

Flasks were kept in shaker at 35°C and 130 rpm for 10 days.

Observations

As shown in Fig 4.6, the bacterial growth decreases with increase in metal concentration. The control showed the highest growth, whereas flask containing 3mM Barium chloride showed the lowest growth for both the bacteria. Flask containing 1mM Barium chloride showed growth approximately equivalent to that of control. Flasks containing 2mM and 3mM Barium chloride had considerable difference in growth as compared to the control. However, bacteria is tolerant to Barium.

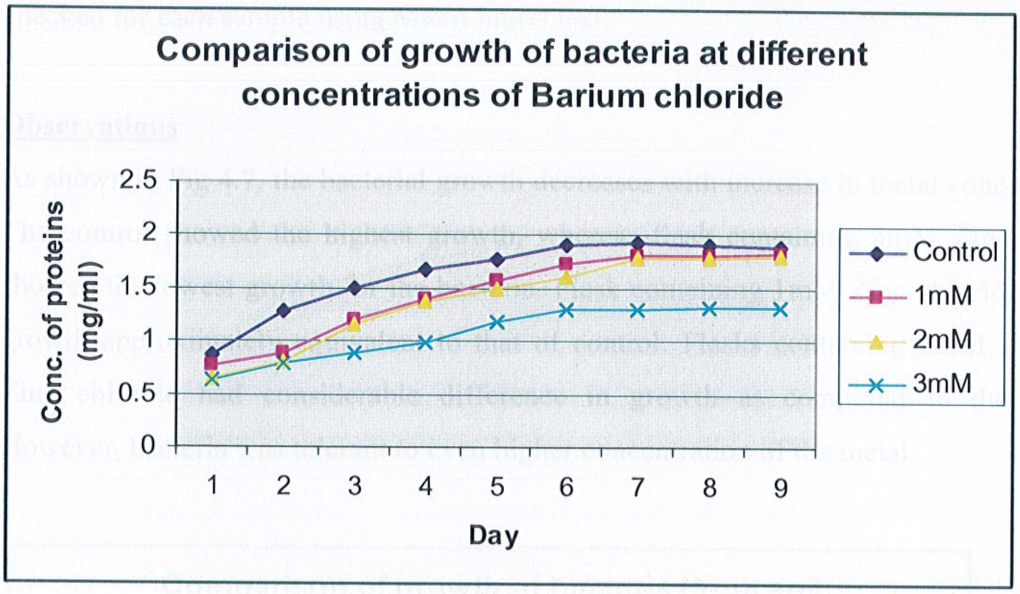


Fig. 4.6 Growth of soil isolate S1 W from soil sample S1 (from Jeans washing factory) on increasing the concentration of Barium chloride

4.4.4 Checking Tolerance of Bacterial Strains for Zinc

Prepared culture of S1 W (soil isolate from Jeans Washing Factory) sample in nutrient broth. Separated bacterial cells by centrifuging 1ml of growing culture at 5,000 rpm for 10 minutes. Discarded supernatant and washed cell pellets with 0.9% NaCl. Dissolved pellets in 1 ml of 0.9% NaCl.

Media prepared:

- Nutrient broth (100ml) containing 1mM Zinc chloride
- Nutrient broth (100ml) containing 2mM Zinc chloride
- Nutrient broth (100ml) containing 3mM Zinc chloride
- Nutrient broth (100 ml) containing no metal as control

Added 20 μl of dissolved pellets in each flask containing the above media.

Flasks were kept in shaker at 35°C and 130 rpm for 10 days. Bacterial growth was checked for each sample using Micro biuret test.

Observations

As shown in Fig 4.7, the bacterial growth decreases with increase in metal concentration. The control showed the highest growth, whereas flask containing 3mM Zinc chloride showed the lowest growth for the bacteria. Flask containing 1mM Zinc chloride showed growth approximately equivalent to that of control. Flasks containing 2mM and 3mM Zinc chloride had considerable difference in growth as compared to the control. However, bacteria was tolerant to even higher concentration of the metal.

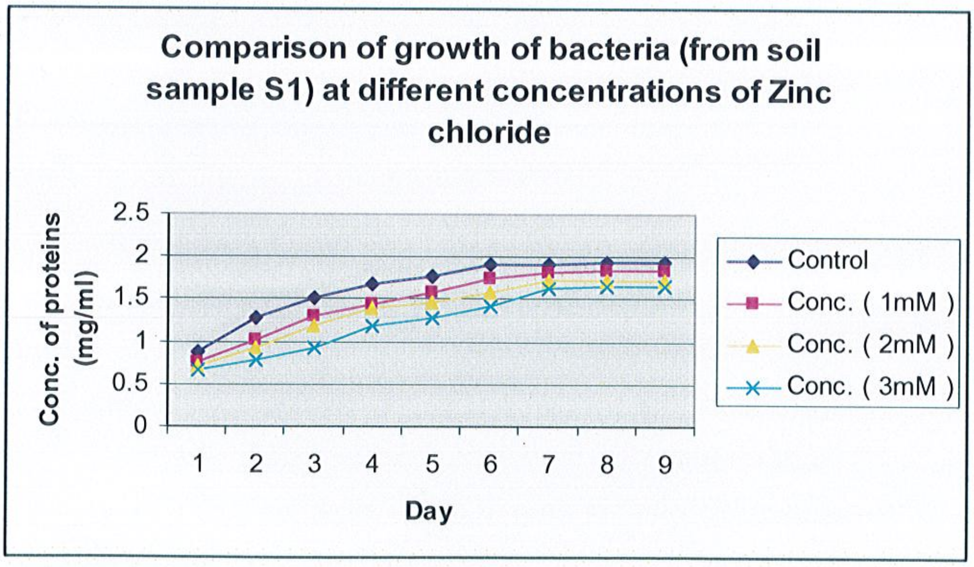


Fig. 4.7 Growth of soil isolate S1 W from soil sample S1 (from Jeans washing factory) on increasing the concentration of Zinc chloride

Conclusion

We were able to isolate metal tolerating bacteria from industrial soils which can further be utilized for bioleaching of metals from e-waste. As these bacteria were well adapted to the industrial environmental conditions, they were able to tolerate high metal concentrations at the sample sites. The soil isolate was able to grow on various concentrations of metals in laboratory, suggesting that it can possibly leach the metals out of e-waste. Further studies relating to bioleaching could not be performed as the appropriate apparatus was not available.

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