

**BIOLOGICAL TREATMENT OF HAZARDOUS BIO-MEDICAL  
WASTE ASH – A SUSTAINABLE APPROACH**

A Dissertation

Submitted in the partial fulfillment of the requirements for the award of the degree  
of

**Master of Technology**

In

**ENVIRONMENTAL SCIENCE AND TECHNOLOGY**

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**JULY 2013**

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## **DECLARATION**

I, the undersigned, hereby declare that the research work presented in the M.Tech project entitled **“Biological Treatment of Hazardous Bio-Medical Waste Ash – A Sustainable Approach”** has been carried out by me under the supervision and guidance of Dr. Anita Rajor, Assistant Professor, School of Energy and Environment, Thapar University, Patiala.

Further, I declare that no part of this Dissertation has been submitted for a degree or any other qualification of any other university or examining body in India/elsewhere.

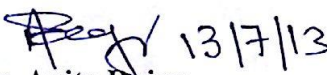
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## CERTIFICATE


This to certify that the dissertation entitled “**Biological Treatment Of Hazardous Bio-Medical Waste Ash – A Sustainable Approach**” carried out by **Ms. Shelly Heera** in partial fulfillment of the requirements for the award of **Master of technology** in **Environmental Science and Technology** at Thapar university , Patiala is an authentic piece of work carried out by her in our guidance and supervision.

To the best of our knowledge, the matter embodied in this thesis has not been submitted to any other university/institute for award of any degree/diploma.

  
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## ACKNOWLEDGEMENT

I am highly grateful to the authorities of Thapar University, Patiala for providing this opportunity to carry out the Dissertation work.

I would like to express a deep sense of gratitude and thank profusely to my thesis guide Dr. Anita Rajor (Assistant Prof., School of Energy and Environment, Thapar university) for his sincere & invaluable guidance, suggestions and attitude which inspired me to submit seminar report in the present form.

I extend my sincere thanks to Dr. M.S. Reddy, Head, DBTES and Dr. A.S.Reddy, Head, School of Energy and Environment for the providing much needed infrastructure in carrying the work

I am thankful to Kunal, Tina, Gurdeep and Akanksha Palak, Harkrishan, Shashank for the motivation and inspiration that triggered me for the report work. I am grateful to Mr. Ram Naval Yadav, Mrs. Lalita Pandit and all other Lab attendants of the department for their full support and cooperation.

I am also thankful to other faculty members of **SCHOOL OF ENERGY AND ENVIRONMENT, THAPAR UNIVERSITY**, Patiala for their intellectual support. Special thanks to my family members and friends who constantly encouraged me to complete this study.

**Shelly Heera**

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## INTRODUCTION

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Medical care is vital for our life, health and well-being. But the waste generated from medical activities can be hazardous, toxic and even lethal because of their high potential for diseases transmission. Nature has a self-cleansing property and by any means, waste generated in healthy ecosystems, becomes food for the next, in a never ending cyclic process. By combining a Clean Production approach with Zero Waste systems, communities can eliminate (or “reduce”), re-use, or recycle the vast majority of the waste. The hazardous and toxic parts of waste from health care establishments comprising infectious, bio-medical and radio-active material as well as sharps (hypodermic needles, knives, scalpels etc.) constitute a grave risk, if these are not properly treated/disposed or is allowed to get mixed with other municipal waste. Its propensity to encourage growth of various pathogen and vectors and its ability to contaminate other nonhazardous/ non-toxic municipal waste jeopardizes the efforts undertaken for overall Biomedical waste (BMW) management. Waste generated from biomedical activities represents a real problem of living nature and human world. “Biomedical waste basically means any solid and/or liquid waste including its container and any intermediate product, which is generated during the diagnosis, treatment or immunization of human beings or animals or in research pertaining thereto or in the production or testing thereof”.

India generates a huge quantity of Biomedical Waste (BMW) every year. In the past, many hospitals simply dumped all waste streams together, from reception-area trash to operating-room waste, and burned them in incinerators — and this is still common practice in many countries. Some hospitals and clinics in the developing world discard medical waste with regular trash and risk the spread of diseases among scavenger populations. Discarded needles and syringes may result in the spread of blood borne pathogens such as HIV and hepatitis. Others burn their waste in open fields or in small incinerators without pollution control, exposing communities to toxic byproducts and potentially dangerous ash. Medical waste incineration is a leading source of dioxin, mercury, lead and other dangerous pollutants that threaten human health and the environment. As health

programs expand, the problem of medical waste treatment and disposal in rural areas becomes critical.

## **1.1 PRESENT SCENARIO IN INDIA**

According to the Ministry of Environment and Forests (MoEF), gross generation of BMW in India is 4,05,702 kg/day of which only 2,91,983 kg/day is disposed, which means that almost 28% of the wastes is left untreated and not disposed finding its way in dumps or water bodies and re-enters our system (Centre for science and environment). In terms of quantum of waste generated from the states, Karnataka tops the chart with 62,241 kg/day of BMW. Uttar Pradesh, Maharashtra and Kerala come close on.

The physico -chemical and biological nature of the components of BMW, their toxicity and potential hazard are different, necessitating different methods and options for their treatment and/or disposal. In Schedule I of the Bio-medical Waste (Management and Handling) Rules, 1998 (Annexure II), therefore, the waste originating from different kinds of such establishments, has been categorized into 10 different categories :

1. Human anatomical waste (such as, tissues, organs, body parts etc.);
2. Microbiology and biotechnology waste (such as, laboratory cultures, micro-organisms, human cell cultures, toxins etc.);
3. Waste sharps (such as, hypodermic needles, syringes, scalpels, broken glass etc.);
4. Discarded medicines and cyto-toxic drugs;
5. Soiled waste (such as, dressing, bandages, plaster casts, material contaminated with blood etc.);
6. Solid waste (disposable items like tubes, catheters etc. excluding sharps);
7. Liquid waste generated from any of the infected areas;
8. Animal waste (generated during research or experimentation, from veterinary hospitals etc.);
9. Incineration ash(Ash from incineration of any biomedical waste);
10. Chemical waste (Chemicals used in production of biological, chemicals used in disinfection)

An assessment of the Biomedical waste situation obtained within a district or city hospitals as a whole is necessary before making any attempts for improvement. It means that there must be taken into account the essential steps: BMW generation; segregation; collection; storage; handling; transportation; treatment and disposal.

Given below are the observations of Bio-medical solid waste management in an Indian hospital – a case study by Patil and Pokhrel, 2005.

- the process of segregation, collection, transport, storage and final disposal of infectious waste was done in compliance with the Standard Procedures.
- the final disposal was by incineration in accordance to EPA Rules 1998.
- the non-infectious waste was collected separately in different containers.

Bio-medical waste is defined in Rule 3 (5) of the Bio-medical Waste (management and handling) Rules 1998. As per schedule 1 of this enactment the Bio-medical waste is categorized as follows and there are many specifications provided in the rules with respect the safety and protection while handling the Bio-medical waste materials. Rule 6 provides for Segregation, Packing, Transportation and storage of the Bio-medical waste. The Rule further insists that schedule 3 and schedule 4 is to be followed in all these procedures (color coding as per rule 6 as b-blue, w-white, r-red, y-yellow).

## **1.2 ENVIRONMENTAL LEGISLATION:**

Realizing the seriousness of the problem associated with the poor management of the bio-medical wastes, the Ministry of Environment and Forests (MoEF), Govt. of India, notified the Bio-Medical Waste (Management and Handling) Rules in July 1998 under the Environment (Protection) Act, 1986, through a Gazette notification [S.O. 630(E)]. Thereafter, the Bio-Medical Waste (Management and Handling) Rules were amended twice in the year 2000 and the last amendment was made in the year 2003. The first amendment was published on 6th March 2000 vide S.O. 210(E), the second amendment was published on 2nd June 2000 vide the Gazette Notification S.O. 545(E) and third Amendment was published on 17<sup>th</sup> September 2003 vide Gazette Notification S.O. 1069(E). The main objective of the rules is to ensure proper segregation, collection, transportation and disposal of the infectious BMW in order to safe guard the public health of the society.

### *Bio-Medical Waste Rules 2011: Key Provisions:*

The Rules now called the Bio Medical Wastes (Management and Handling) Rules 2011 has been notified for information of the masses and feedback received from all fronts would be considered

by the Central Government. The new Rules on BMW are elaborate, stringent and several new provisions have been added in it.

### **1.3 TECHNOLOGIES FOR WASTE TREATMENT:**

Hospitals generate between 8 to 45 pounds of waste per bed per day in the form of general trash, infectious (red bag) waste, hazardous waste, and low-level radioactive waste. Infectious waste is estimated to be about 15% or less of the overall waste.

Four basic processes are used in medical waste treatment:

Thermal; Chemical; Irradiative; Biological treatment.

**THERMAL PROCESSES** uses heat to decontaminate instruments and equipment and the temperatures in this process may rise to extremely high levels. Most of the microbes are destroyed at temperatures below 100°C (Mathur et al, 2012). It includes Autoclave; Hydroclave; Incinerator; Microwave. They can be further classified as low-heat thermal processes (operating below 350°F or 177°C), medium-heat thermal processes (between 350 to about 700°F), and high-heat thermal processes (operating from around 1000°F to over 15,000°F). The low-heat processes utilize moist heat (usually steam) or dry heat. High-heat processes involve major chemical and physical changes that result in the total destruction of the waste.

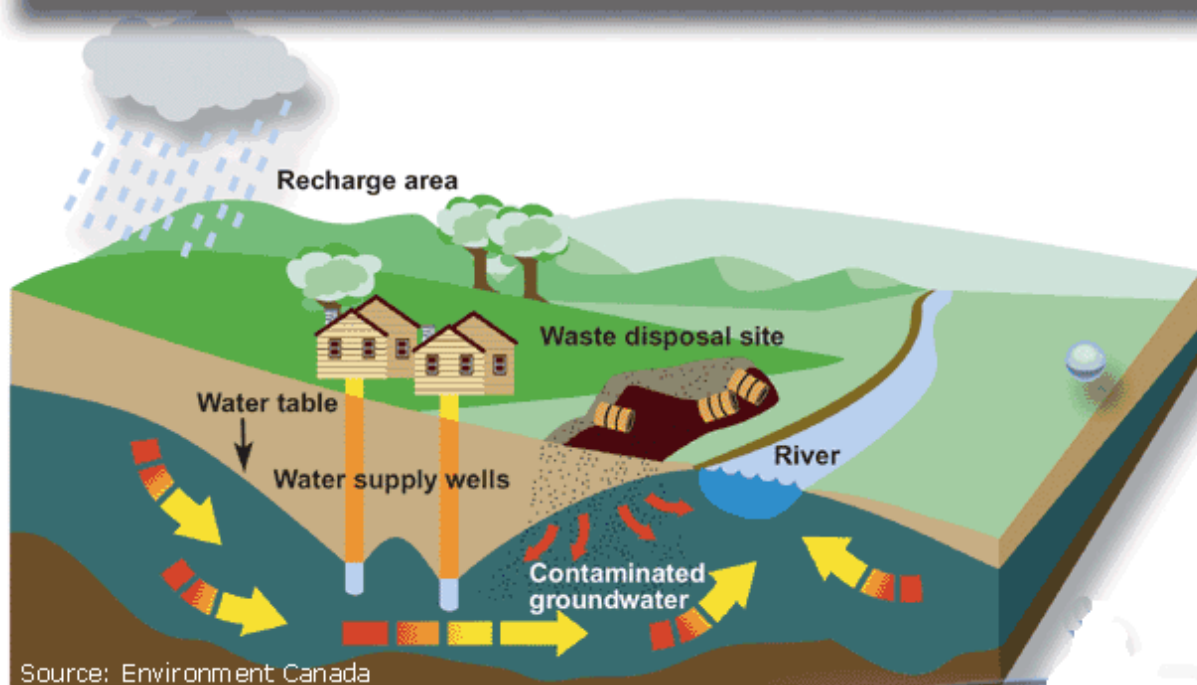
**CHEMICAL PROCESSES** employ disinfectants to destroy pathogens or chemicals to react with the waste. Safety and occupational exposures should be monitored when using any chemical technology. Chemicals are added to waste to kill or inactivate the pathogens it contains; this treatment usually results in disinfection rather than sterilization. The types of chemicals used for disinfection of health-care waste are mostly aldehydes, chlorine compounds, ammonium salts, and phenolic compounds. Powerful disinfectants are often hazardous and toxic; many are harmful to skin and mucous membranes.

**IRRADIATION PROCESSES** involves ionizing radiation to destroy microorganisms while **BIOLOGICAL PROCESSES** use enzymes to decompose organic matter. Mechanical processes, such as shredders, mixing arms, or compactors, are added as supplementary processes to render the waste unrecognizable, improve heat or mass transfer, or reduce the volume of treated waste.

## **1.4 INCINERATION - A FAVOURABLE PROCESS:**

Incinerators are deemed as favorable in this respect because they are perceived as reducing waste to one tenth of its original volume and therefore reduce the volume of waste going to landfill sites. According to the legislation, incineration is recommended for human anatomical waste, animal waste, cytotoxic drugs, discarded medicines and soiled waste. Incineration is a high temperature thermal process involving combustion of the waste under controlled condition for converting them into inert material and gases. The large amount of ash thus generated needed to be disposed off. The landfilling of the incineration ash is the most common way for its disposal. Leaching is the process by which soluble constituents are dissolved from a solid material (such as rock, soil, or waste) into a fluid by percolation or diffusion. Thus, when fill materials come into contact with liquid (including percolating rainwater, surface water, groundwater, and liquids present in the fill material), constituents in the solid phase will dissolve into the liquid forming a leachate. The extent to which the constituents dissolve into the contact liquid will depend upon site- and material-specific conditions (chemical, physical, and biological factors) and the length of time involved. The compositions of the leachate generated from the material and its potential to impact water quality are key factors in evaluating the suitability of the material for use as fill. Due to the excessive landfilling of the incineration ash without thinking about the detrimental effects on the environment poses a threat to the nature. This landfilling is a risk to groundwater contamination.

## Groundwater contamination from a waste disposal site



**Figure 1.1: Groundwater contamination process after landfilling**

Heavy metals migration is a serious environmental problem and is a major topic of investigation and research. The retention and migration of heavy metals are highly dependent on soil pH, presence of carbonates, the degree of saturation, the influent conc. and the time duration. Effect of heavy metals contaminants on the environment depends on the metals availability and mobility in the soil and also on the soil composition and pH. It is usually the extremes of alkalinity and acidity that causes environmental problems, difficulty with growing plants or groundwater contamination. If a soil has too much acid in it, the nutrients in the soil will be dissolved too quickly, and leached away as the water drains. If a soil is too alkaline or in other words if there is not enough acid, then nutrients will not dissolve quickly enough. Thus, a neutral soil, which is neither too acidic nor too alkaline, is the preferred type of soil for plant life to thrive. Many plants will grow well in soils with a pH level between 6 and 7.5.

## 1.5 WASTE INCINERATION IS A DYING TECHNOLOGY:

The “burning” or “incineration” of waste is an age-old practice that continues in the world today. Despite the dangers to health and the environment, many governments, public health agencies, international organizations and transnational corporations continue to promote incineration technology as a waste management "solution." Medical waste incineration is a leading source of dioxin, mercury, lead and other dangerous pollutants that threaten human health and the environment. The bottom ash residues are generally contaminated with leachable organic compounds, such as dioxins, and heavy metals and have to be treated as hazardous waste. Advances have been made in the design and operation of “incinerators” to greatly reduce their impact on the environment as well as public health. The impact of years of poor disposal practices and improperly operated incinerators are still being assessed in certain areas and yet to be determined in others. Given the appropriate expenditures on air pollution control equipment and operations, incineration remains a standard method of waste disposal primarily because its greatest benefit is volume reduction. Incinerators are often billed as energy producers, since they can generate electricity. However, a detailed life-cycle analysis reveals that incinerators waste more energy than they produce. There is a need to minimize the amount and toxicity of waste generated by the health care sector, to ensure the proper management and segregation of medical waste, and to eliminate the dangerous practice of incineration by promoting and implementing alternatives.

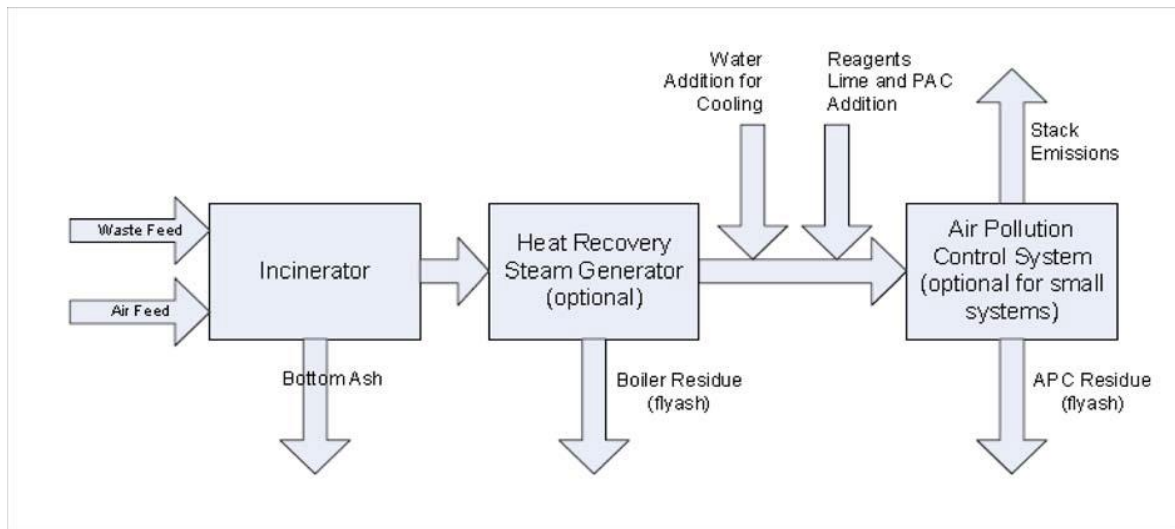


Figure 1.2: The detailed representation of the Incineration process(source: J.Industry at Thane)

### 1.5.1. INCINERATOR ASH IS POTENTIALLY HAZARDOUS:

When air pollution control equipment does function, it removes pollutants from the air and concentrates them in the fly ash, creating a hazardous waste stream that needs further treatment. Thus, the problem of pollutant releases is not solved; the pollutants are simply moved from one medium (air) to another (solids or water). Incinerator ash is highly hazardous but is often poorly regulated. Even landfill disposal is not safe, as landfills leak; but in some places the ash is left exposed to the elements or even spread in residential or food-producing areas. Landfilling of the incineration ash affects the water quality index by disturbing the three main parameters: hardness, alkalinity, chloride and heavy metal content of the groundwater. Therefore, there is a need of the biological treatment of the ash so that there is reduction in these important water parameters. Facilities have to determine the non-incineration technology that can best meets their needs while minimizing the impact on the environment, enhancing occupational safety, and demonstrating a commitment to public health. Many studies are being going on for decreasing the toxicity level of the incinerator ash so that there will be minimum health risks after landfilling of ash.

The **present work** is mainly focused on the use of micro-organisms which can act as a better tool for removing the toxicity level of the incinerator ash. The best suited micro-organism which can easily adapt to that alkaline environment can help to decrease the vulnerable effects of the ash into the groundwater. The parameters such as alkalinity, hardness, chloride and the heavy metals content if reduced to the permissible limits by the proper treatment, then, this can be a suitable tool for the toxicity reduction of the landfilled incinerator ash.

These are the objectives of the study:

1. Isolation of metal tolerating micro-organisms.
2. Optimization of metal tolerance by micro-organisms in an enriched (NB) and then in Minimal media(M9).
3. Leachate analysis of biomedical waste ash treated with isolated micro-organism. Analysis includes alkalinity, hardness, chloride and heavy metal contents.
4. Plant growth analysis in soil – ash mixtures.

## LITERATURE REVIEW

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### 2.1 INCINERATORS AS MAJOR SOURCES OF DIOXINS AND HEAVY

#### METALS:

Several methods have been employed including incineration, steam sterilization, microwave sanitation, chemical disinfection, dry heat disinfection and superheated steam disinfection but the best available technology for disposing of biomedical waste is incineration (Altin et al., 2003; Jang et al., 2005). At present, 170 common biomedical waste treatment facilities are available having 140 incinerators throughout the country. The present generation of incinerable hazardous waste is 4.16 lakhs MTA but the incinerators have only the capacity of 3.28 lakhs MTA (CPCB, 2008). According to an estimate, only 6.67% of waste is incinerated and rest of the waste going to land filled and recycled. The incineration process destroys pathogens and reduces the waste volume by 90% and weight by 75%. Incineration usually involves the combustion of mingled solid waste with the presence of air or sufficient oxygen. Typically, the temperature in the incinerator is more than 850°C and the waste is converted into carbon dioxide and water. The incineration of hospital wastes not only releases toxic acid gases (CO, CO<sub>2</sub>, NO<sub>2</sub>, SO<sub>2</sub>, etc), dioxides into the environment but also leaves a solid material called ash as residue includes bottom ash and fly ash which increases the levels of heavy metals, inorganic salts and organic compounds in the environment (Sabiha et al., 2008). Most of the ash produced is bottom ash that is the residues inside the burner after incineration. Fly ash settles on post burner equipment such as scrubbers. The incinerated hospital waste ash when melted at 1200°C then the ash is converted in to molten state and the molten ash is turned in to slag by cooling at room temperature. Metals are not destroyed during incineration, and often released into the environment along with ash. Disposal of ash in landfill without proper treatment may cause contamination of groundwater due to leachate. In spite of its advantages, the incineration of BMW produces residues, mainly the bottom ash [10% of the volume of the waste or about 250-300 kg/1000 kg of waste], the hazardous filter ash [about 25-30 kg/ 1000 kg of waste], and additional products of the flue gas cleaning processes (dry and /or wet

scrubbing) (Jakob et al., 1995). All types of incinerators release pollutants to the atmosphere in stack gases, ashes and other residues. Fly ashes from air filtration equipment of incinerators and the bottom ashes that remain after incineration contain numerous hazardous chemicals. Emissions from biomedical waste incinerators may include carbon monoxide (as a result of incomplete combustion), particulate matter, hydrogen chloride, metals (e.g. mercury, lead, arsenic, and cadmium) (Segura et al., 2004), poly-cyclic aromatic hydrocarbons (PAHs) (Levendis et al., 2001; Lee et al., 2002), dioxins (polychlorodibenzo-p-dioxin (PCDD) and furans (polychlorodibenzofuran (PCDF) (Lee et al., 1995; Brents et al., 2002; Fritsky et al., 2001; Matsui et al, 2003; Lee et al, 2004). Lorber et al. (1998) estimated that only around 2% of the dioxin emissions to air are deposited in soil near to an incinerator while the remainder is much more widely dispersed. Many of these chemicals are known to be persistent (very resistant to degradation in the environment), bio-accumulative (build up in the tissues of living organisms) and toxic. The impact of waste incinerators on health and their releases of hazardous combustion products, such as dioxins and PAHs are of great public concern (Ardevol et al. 1999). Exposure to elevated levels of lead have been associated with numerous adverse effects on renal function, development and reproduction in animals and humans (Pirkle et al. 1998, Bernard et al. 1995).

Many air pollutants in emissions from medical waste incinerators can be significantly reduced by modern air pollution control devices if properly designed and operated. Typical air pollution control devices used at many biomedical waste incinerators include cyclones, semi-dry scrubbers, and bag-house filters (or fabric dust removers). Many devices can be modified to effectively control dioxins and furans. After incineration, the fly ash is disposed of in a hazardous waste landfill, while the bottom ash is characterized by the Leaching Test to determine appropriate final disposal methods (hazardous or non-hazardous). Current methods of hazardous waste disposal generally include dumping in landfills and incineration. The integrity of dumps can be breached, thereby causing materials to leach into surrounding water tables. In the brain storming session held during the workshop at Hyderabad (2003), it was unanimously decided that the Common Biomedical Waste Treatment Facility should emphasize more on non-burn technologies. Several new technologies, based on immobilization with cement, wet chemical treatment, or thermal treatment are in development that tries to decontaminate toxic residues and/or make them inert in the sense that they could be reused or at least be deposited without any risk. Incinerator ash was investigated for its potential use as a replacement for sand and cement in cement mortars. The

replacement of incinerator ash for sand and cement caused a reduction in concrete unit weight values. Results of Rawas et al. (2005) indicate that incinerator ash caused a reduction in slump values when it was used as a replacement for sand while an opposite trend was observed when it was used as a replacement for cement. The replacement of sand by incinerator ash up to 40% exhibited a higher compressive strength for most curing periods.

In an attempt to reduce leaching, fly ash is sometimes stabilized in cement before disposal. Although this method reduces the immediate leaching of heavy metals and other toxic chemicals, weathering and erosion over time will ultimately cause their release back to the environment. Current methods of hazardous waste disposal generally include dumping in landfills and incineration. Research has shown that soil and vegetation can be used as suitable media for monitoring contamination from atmospheric deposition of dioxins and heavy metals (Schuhmacher et al. 1997), Gutenman et al. (1992). Levels of dioxins in soils have been widely used to describe long-term exposure to these chemicals. On the other hand, vegetation is a more representative index of short-term exposure to dioxins (Schuhmacher et al. 1999). In addition, at the Fifth Intergovernmental Negotiating Committee Meeting (INC5) on the Elimination of Persistent Organic Pollutants (POPs), held in December 2000, a world-wide agreement was reached to reduce total dioxin releases, with the ultimate aim of their elimination. Incineration is listed as one of the main industrial source categories for dioxins, and requires the use of BAT (Best Available Techniques) for new installations and substantially modified existing facilities.

Xiang et al. (2003) results on chemical analysis and leaching tests suggest that the physical and chemical characterization of solid residues depends on facts such as the composition of feed MSW, the type of incinerator, the APCDs, the operating conditions. Less volatile elements with high boiling temperatures remained in the bottom ashes and grate siftings, while more volatile elements with low boiling temperature were captured by the fly ashes. Especially, the concentrations of some toxic heavy metals in the fly ashes were significantly high, which caused potential hazards. The concentrations of heavy metals in the leachates of the grate siftings and the bottom ashes were much lower than the standard, which meant they can be directly landfilled.

## 2.2 STABILISATION OF ASH CONTENTS:

Several new technologies, based on immobilization with cement, wet chemical treatment, or thermal treatment (Faulstich, 1992) are in development that try to decontaminate toxic residues and/or make them inert in the sense that they could be reused or at least be deposited without any risk. Jin et al. (2010) investigated the process of alumino-silicate formation in incinerator fly ash containing large amounts of heavy metals and treated with alkaline compounds at 375°C and examined how this process affected the mobility and availability of the metals. As a consequence of the treatments, the amount of dissolved heavy metals, and thus their mobility, was greatly reduced, and the metal leaching concentration was below the legislative regulations for metal leachability. Moreover, this process did not produce a high concentration of heavy metals in the effluent. Of great promise are thermal processes (Stark et al. 1993), which are melting the residues at temperatures around 1300-1400°C and producing a relatively inert glass. The high temperatures and long residence times of such processes lead to complete destruction of toxic organic compounds. From a leaching analysis done by Azni et al. (2005) on the slag produced proved that the melting process had successfully stabilized the heavy metals.

Vitrification is one of the technological options for stabilizing toxic waste (Cheng et al., 2002) by powder sintering technology. Results of Genazzini et al. (2003) indicate that Portland cement systems could become an alternative for the disposal of this type of ashes. Recycling may also be a way to reduce the loss of heavy metals to the environment. The Solvay Company has been working on the development of a new physicochemical treatment for bio-medical incineration fly ashes: the Revasol process. This process allows reducing the soluble fraction, fixing heavy metals and eliminating dioxins (Aubert et al., 2004).

## 2.3 UTILIZATION OF INCINERATOR ASH :

In several European countries high quantities of ash are reused for the manufacture of pavements, bridges and structural stones but also as sub-layer in the manufacture of motorways and as daily cover of landfills. *In Agriculture:* Ash, the waste product from biomedical treatment plant has potential for use in agriculture because it contains almost all macro as well as micronutrients except organic carbon and nitrogen. It may act as chemical fertilizer to increase the yield of various agricultural crops. Goswami-Giri (2007) investigated on the application of the various dose

of ash, cow, dung, urea and super phosphate for the treatment of Fenugreek and Mustard. The positive effect was observed on their average growth. The results of Aubert et al. (2004) suggest that the use of waste in concrete constitutes a potential means of adding value. The leaching tests carried out on the concrete confirm that the process makes it possible to obtain materials without major risks for the environment. Al-Mutairi et al., (2004) compared the compressive strength of mixtures made with bottom and fly hospital ash with those of micro-silica and conventional concretes in order to evaluate the effectiveness of reusing hospital incinerator ash. The effect of various percentages of micro-silica, fly ash, and bottom ash on the compressive strength was also evaluated at 25°C, 150°C, 250°C, 500°C, 600°C, and 800°C. Results showed that when 5% micro-silica and fly ash were incorporated, the compressive strength of the cubes was further increased, indicating a more significant effect. On the contrary, the replacement of cement by 15%, 20%, and 25% fly ash, bottom ash, and micro-silica resulted in lower compressive strength. This reduction in the compressive strength at higher ratios of cement replacement could be attributed to the high absorption of free water by the micro-silica which in turns reduces the workability. It could be clearly noticed that 5% replacement is optimal for silica and fly ash. As for bottom ash, its use of cement replacement did not achieve any increase in strength. This is hardly surprising since its coarser nature does not qualify it as pozzolonic material. The use of the slag produced (by melting process) as an alternative material to replace conventional aggregates for road construction was studied by Azni et al. (2005).

*As a stabilizing agent in road and asphalt pavements:* In Germany, 50% of the ash produced from incinerated waste is used for the manufacturing of sound insulation walls at National roads, as well as, sub layers on the streets. 60% of the bottom ash is used for the construction of asphalt and as a sub layer of roads in Netherlands. Above 72% of ash is reused for the manufacture of parking spaces, cycling tracks and other roads in Denmark (Reijnders et al., 2005). The results from aggregate and asphalt mix tests showed that the slag produced fulfills all the requirements of an alternative aggregate. This slag can be classified as a nonhazardous product. The use of hospital waste molten slag as an alternative aggregate for road construction showed a better performance that the required standards. Transformation of vitrified incinerated fly ash into glass ceramic products.

The products exhibited attractive physical and mechanical properties. The products can be fabricated at 900°C/2 h for construction purposes and have large application potential (Cheng et al., 2002). The heavy metals are either incorporated in the vitrified residue or separated from the residue by evaporation or by different densities of the melted constituents. Obviously, inherent safety for the glass product is achieved if the heavy metals are extracted quantitatively from the residue. The recovered heavy metal compounds themselves can be reutilized as raw materials in metallurgical processes.

#### **2.4 INCINERATOR ASH IS POTENTIALLY HAZARDOUS, THEREFORE THERE IS A NEED OF TREATMENT OF ASH BEFORE LANDFILLING:**

The public's concern for a clean environment and increasing community opposition to incineration should be paramount factors in deciding whether or not to install or continue operating a medical waste incinerator. Choosing a cleaner non-incineration technology demonstrates the health care organization's commitment to protecting public health and the environment. The contamination of groundwater by compounds that have leached from the waste, in particular, heavy metals like lead and cadmium from fly ash has been documented. In an attempt to reduce leaching, fly ash is sometimes stabilized in cement before disposal (Genazzini et al. 2003). Studies carried out on the use ash for the road construction showed that the future release of these compounds due to weathering and degradation may have detrimental consequences for man, particularly in cases where the substances may enter the food chain (Korzun et al., 1990).

Sukandar et al., (2006) studied the metal leachability from medical waste incinerator fly ash by sequential extraction and toxicity characteristics leaching procedure (TCLP) analysis in each categorized particle size. Based on the study it was observed that Ba, Cd, Ni, Pb, and Zn in the medical waste incinerator fly ash tended to bind to carbonate and exchangeable fractions and thus showed high mobility. Many studies are being going on for decreasing the toxicity level of the incinerator ash so that there will be minimum health risks after its landfilling. Micro-organisms can act as a better tool for removing the toxicity level of the incinerator ash. The best suited micro-organism which can easily adapt to that alkaline environment can help to decrease the vulnerable effects of the ash leachate into the groundwater.

### 2.4.1 MICRO-ORGANISMS ROLE:

Microbes play key geo-active roles in the biosphere, particularly in the areas of element biotransformations and biogeochemical cycling, metal and mineral transformations, decomposition, bio-weathering, and soil and sediment formation. The adaptation to heavy metal rich environments is resulting in micro-organisms which show activities for bio-sorption, bio-precipitation, extracellular sequestration, transport mechanisms, and/or chelation. Such resistance mechanisms are the basis for the use of microorganisms in bioremediation approaches. Bioremediation is the application of biological systems to clean-up organic and inorganic pollution, with bacteria and fungi being the most important organisms for reclamation, immobilization or detoxification of metallic and radionuclide pollutants. Microbes interact with metals and minerals in natural and synthetic environments, altering their physical and chemical state, with metals and minerals also able to affect microbial growth, activity and survival. Microbes possess transport systems for essential metals; inessential metal species can also be taken up. Microbes are also capable of mediating metal and mineral bio-precipitation, e.g. by metabolite production, by changing the physico-chemical micro-environmental conditions around the biomass, and also by the indirect release of metal-precipitating substances from other activities, e.g. phosphate from organic decomposition or phosphate mineral solubilisation. Microbial cell walls, outer layers, and exopolymers can sorb, bind or entrap many soluble and insoluble metal species as well e.g. clay minerals, colloids, oxides, etc. which also have significant metal-sorption properties.

Examples of geo-microbial important groups of microbes directly involved in geochemical transformations include iron-oxidizing and -reducing bacteria, manganese-oxidizing and -reducing bacteria, sulfate-reducing bacteria, sulfur-oxidizing and -reducing bacteria, and many other pro- and eukaryotes that can form or degrade silicates, carbonates, phosphates and other minerals (see Gadd et al., 2007; Kim et al., 2008; Gadd et al., 2010). Root-inhabiting rhizosphere microbes, including mycorrhizal fungi, have a major influence on plant nutrition via effects on phosphate availability but also concomitant metal circulation (Amundson et al., 2007). Indeed, during the early phases of soil formation the contribution of microbial activities (including the activities of lichens) to rock weathering, mineral dissolution and element cycling is also intimately related to metal movements and microbial strategies for metal transformations (Purvis et al., 2008; Gilmour et al., 2009; Uroz et al., 2009).

Microbiological processes were applied to mobilize metals from electronic waste materials. Bacteria (*Thiobacillus thiooxidans*, *T.ferrooxidans*) and fungi (*Aspergillus niger*, *Penicillium simplicissimum*) were grown in the presence of electronic scrap. The formation of inorganic and organic acids caused the mobilization of metals. At Guru Gobind Singh Indraprastha University, which is located in Delhi, scientist Rahul Negi and his team conducted experiments dealing with the remediation of toxic heavy metals that are produced by e-waste and industrial waste.

#### **2.4.2. HEAVY METAL POLLUTION – BACTERIA CAN MOP IT UP!**

Heavy metals such as Cu, Zn, Ag, Cd, In, Sn, Sb, Hg, Pb, and Bi was detected in higher concentration in e-waste recycling facility soil samples (Ha et al., 2009). Due to presence of heavy metals in soil, microbes have evolved mechanisms to tolerate the presence of heavy metals by efflux, complexation, or reduction of metal ions (Gadd et al., 1990). Microbes can clear up heavy metals from waste through a method known as bio-sorption. Conventional techniques commonly applied to recover heavy metal from wastewaters have several disadvantages whereas bio-sorption has good metal removal performance from large volume of effluents.

Among the biomaterials, fungi have been reported as an efficient economic source for removal of toxic heavy metals from aqueous solutions because fungal cell wall has different functional groups which are involved in metal binding and fungal biomass is easily available which can be isolated from environment for metal sorption purposes (Ramasamy et al. 2011). In 2010, scientists from Hemwati Nandan Bahuguna Garhwal University, Uttarakhand, published an article in the Journal of Biological and Environmental Sciences on their research about four acclimated microorganisms that were isolated from soil and sludge. The four species were: *Bacillus spp.*, *Pseudomonas spp.*, *Staphylococcus spp.* and *Aspergillus niger*. They grew these microbes in cultures with various heavy metals and observed that *Bacillus* and *Pseudomonas* reduced copper and nickel to a fair amount. *Pseudomonas* reduced heavy metals more than the other microbes. *Aspergillus niger* reduced cadmium and zinc. *Staphylococcus* reduced chromium, copper and, surprisingly, reduced lead to 93 per cent! Not all heavy metals can be remediated using microbes but there are various cost-effective situations where conventional techniques cannot be used and microbial remediation is the only alternative. Earthworms could be used to extract toxic heavy metals, including cadmium and lead, from solid waste from domestic refuse collection and waste from vegetable and flower markets, according to researchers writing in the International Journal of Environment and Waste

Management. Findings from the studies done by Panwichian et.al.,2010 indicated that the resistant PNB strains, have the potential to remove HMs and Na in amounts that were found in shrimp ponds with both aerobic dark and micro aerobic-light conditions. Therefore, it will be possible to use both strains as inoculants for bioremediation of water from shrimp ponds contaminated with toxic HMs and Na. Use of Recombinant bacteria for metal removal: Metal removal by adsorbents from water and waste water is strongly influenced by physic-chemical parameters such as ionic strength, pH, the concentration of competing organic and inorganic compounds. For example genetically engineered E.coli, which expresses Hg<sup>2+</sup> transport system and metallothionin (a metal binding protein) was able to selectively accumulate 8/l mole Hg<sup>2+</sup>/g cell dry weight.

## **2.5 LEACHATE ANALYSIS:**

In environmental applications, leaching represents the source term for release of potentially hazardous substances. Assessing the leaching of pollutants to groundwater is one of the key pathways when evaluating the risk of solid wastes on human health and the environment. Leaching of waste generates leachate, a liquid that drains from the landfill and its composition varies widely depending upon the age and the waste contained landfill material. Sukandar et al., (2006) studied the metal leachability from medical waste incinerator fly ash by sequential extraction and toxicity characteristics leaching procedure (TCLP) analysis in each categorized particle size. Based on the study it was observed that Ba, Cd, Ni, Pb, and Zn in the medical waste incinerator fly ash showed high mobility. Leachability of Cd, Cr, Cu, Hg, Ni, Sn, and Zn determined by TCLP method was not statistically different among the categorized particle size. Leachability of arsenic in particle size fraction of 38µm tended to be higher than the other particle size fractions. Ba and Pb showed the highest leachability in the particle size fraction of 150-106 µm and 75-38 µm respectively. Toxicity characteristics leaching procedure (TCLP) test of medical waste incineration bottom ash conducted by Zhao et al., (2010) indicated that the leached amounts of heavy metals were well below the limits. Valavanidis et al., (2008) conducted a study to determine quantitatively the metal leachability of medical waste incineration fly ash and bottom ash by inductive coupled plasma emission spectrometry (ICPES) and by energy dispersive X-ray analysis (EDAX). Results showed that Pb, Cr, Cd, Cu and Zn have high leaching values in both the ashes extracted with water and kerosene. These results indicate that metals can become soluble and mobile if ash is deposited in landfills, thus restricting their landfilling according to EU regulations. Analysis of polychlorinated

biphenyls and polycyclic aromatic hydrocarbons showed their very low concentrations in both fly and bottom ashes. Similar study was also conducted by Gidaracos et al., (2009) in Greece.

## **2.6. POTENTIAL HEALTH EFFECTS DUE TO HIGH CHLORIDE CONTENT:**

Hutchinson (1970) suggested that elevated chloride concentrations could have an effect on persons with pre-existing cardiac (heart) or renal (kidney) problems. The chloride SMCL of 250 mg/L is based on the aesthetic consideration of taste; water with higher concentrations of chloride tastes 'salty' to most people. A greater concern might be the presence of cations with chloride, such as sodium and potassium. Sodium in drinking water can be a concern for those on low sodium diets because of cardiac, circulatory, renal or other problems (Shelton et al., 1997). Chloride in groundwater from both natural and anthropogenic sources, such as run-off containing road de-icing salts, the use of inorganic fertilizers, landfill leachates, septic tank effluents, animal feeds, industrial effluents, irrigation drainage, and seawater intrusion in coastal areas. Chlorides are important in detecting the contamination of groundwater by waste water (Purandara et al., 2003) As per Indian Drinking Water standard IS 10500:1991 the desirable limit for chloride is 250 mg/l as Cl, beyond this limit the taste become salty, corrosion and palatability affected, permissible limit in the absence of alternative source is 1000 mg/l. Chloride increases the electrical conductivity of water and thus increases its corrosivity. In metal pipes, chloride reacts with metal ions to form soluble salts (Sameer. V et al., 2010), thus increasing levels of metals in drinking-water. In lead pipes, a protective oxide layer is built up, but chloride enhances galvanic corrosion. It can also increase the rate of pitting corrosion of metal pipes (Sarwade et al., 2008). In addition leachate from land fills (Eison et al., 1980, Sharma et al., 1995, Subba et al., 1995), septic tanks and pit latrines (Olaniya et al., 1977, Craig et al., 1979, Gillison et al., 1983, Vates et al., 1986, Todd et al., 1995, Sharma et al., 1995, Polprasert et al., 1996) also contributes a significant amount of chlorides to groundwater.

**Table 2.1: Table showing the ill- effects of various contaminants.**

S.no.	Contaminant	Potential health and other effects
1.	Aluminium	Can precipitate out of water after treatment, causing increased turbidity or discolored water.
2.	Chromium	Cr III is a nutritionally essential element. Cr VI is much more toxic than Cr III and causes liver and kidney damage, respiratory damage, dermatitis, and ulcers on the skin at high concentrations.
3.	Lead	Affects red blood cell chemistry; delays normal physical and mental development in babies and young children.
4.	Silver	Can cause argyria, a blue-gray coloration of the skin, mucous membranes, eyes, and organs in humans and animals with chronic exposure.
5.	Hardness	Decreases the lather formation of soap and increases scale formation in hot-water heaters and low-pressure boilers at high levels.
6.	Chloride	Deteriorates plumbing, water heaters, and municipal water-works equipment at high levels.  Above secondary maximum contaminant level, taste becomes noticeable.

Source: water.epa.gov.

These contaminants should be removed from the environment and the course of study indicates the use of biological treatment for decreasing the detrimental effects of these contaminants. All kinds of microbes and their symbiotic associations with each other play a remarkably wide diversity of geoactive roles in the biosphere. Microbial transformations of metals and minerals are a vital part of natural biosphere processes and can also have beneficial consequences for human society if they are used for treatment of the Biomedical waste incinerator ash. Increasing our understanding of this important area of microbiology and exploiting it in applications such as bioremediation will clearly require a multidisciplinary approach.

### **EXPERIMENTAL PROCEDURE AND MATERIALS**

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It includes the materials used and methodology adopted during the research. All the chemicals used and reagents employed were of analytical grade with sufficient purity. The calibration curves were prepared prior to estimation of unknown concentration and used throughout the study.

#### **3.1. SAMPLE COLLECTION:**

1. The soil samples for the isolation of bacteria were collected from agricultural field of the Thapar University and the sludge sample was collected from textile industry.
2. The ash samples were procured from the Incineration point in Punjab (India). The samples were collected into plastic bags and sealed. The ash was greyish black in colour. The ash samples were fully autoclaved so that there would be no interference with the selected microorganism during treatment.

#### **3.2. ISOLATION OF METAL TOLERATING MICRO-ORGANISMS.**

Nutrient broth and Minimal media (M9) was used to isolate the bacteria from the soil and sediment collected. 1.0 g from each soil and sediment was added in 10ml of distilled water and placed on rotary shaker at 150 rpm for 1 hour and allowed to settle down for half an hour. One ml of supernatant was added in the nutrient broth and incubated for 24hrs 37°C at 150 rpm. After that 10<sup>-1</sup> to 10<sup>-6</sup> dilutions were made and surface spread on nutrient agar plates and repeated this process for 2-3 times. A pure culture of each isolate was inoculated finally in M9 medium at pH 12. Out of these the best grown isolates on M9 medium were selected on the basis of their growth. Finally, the strains were maintained in M9 medium and stored at 4°C until used.

**Table 3.1: M9 media composition**

S.No.	Composition	Concentration (g/l)
1.	Sucrose	10
2.	K <sub>2</sub> HPO <sub>4</sub>	2.5
3.	KH <sub>2</sub> PO <sub>4</sub>	2.5
4.	(NH <sub>4</sub> ) <sub>2</sub> HPO <sub>4</sub>	1.0
5.	MgSO <sub>4</sub> .7H <sub>2</sub> O	0.2
6.	FeSO <sub>4</sub> .7H <sub>2</sub> O	.01
7.	MnSO <sub>4</sub> . 7H <sub>2</sub> O	.007
8.	H <sub>2</sub> O	985g

Separately autoclave the Media and KCl/NaOH buffer (KCl- 4.473g/300 ml H<sub>2</sub>O and NaOH 6.336g/792ml H<sub>2</sub>O, mix both) at 121<sup>0</sup>C for 15 mins.

Finally, 32.9 ml of KCl/NaOH buffer was added with 70ml of medium to get the required 12 pH.

### **3.3. OPTIMIZATION OF METAL TOLERANCE OF ISOLATES IN AN ENRICHED (NB) AND MINIMAL MEDIA (M9).**

- a) The stock culture was sub-cultured twice to obtain an active inoculum, and then incubated for 48hrs at 37<sup>0</sup>C on rotary shaker at 150 rpm. Bacterial growth in M9 media was measured at OD<sub>600</sub> using a spectrophotometer and any isolates with growth exceeding 0.50 were selected for further screening.
- b) In order to screen isolates which are metal tolerant, each isolated culture was grown in M9 media and nutrient broth media with 25, 50, 75 ppm of different metal concentrations again with both incubating conditions as described above.

- c) Final selection for heavy metals resistant isolates were grown in M9 media containing the highest level of each heavy metal. Growth in tubes was measured by spectrophotometer at OD<sub>600</sub>.

### **3.4. IDENTIFICATION OF SELECTED BACTERIAL ISOLATES.**

#### **3.4.1. MORPHOLOGICAL AND BIOCHEMICAL CHARACTERIZATION**

Selected isolates were characterized by colony morphology on nutrient agar, gram staining and morphological characteristics according to Cappuccino and Sherman (2010). Additional biochemical tests were performed according to Aneja (2008) for taxonomic characterization which included gelatin liquefaction, starch hydrolysis, catalase test, citrate utilization test and carbohydrate utilization.

##### **3.4.1.1 MORPHOLOGICAL STUDIES:**

**(i) Gram staining:**

Thin smear of the cultures was made on separate glass slides. Smear was air dried and heat fixed. Covered the smear with crystal violet for 30 seconds then washed with distilled water. After that covered the smear with gram's iodine solution for 60 seconds and washed with decolorizer and after that with distilled water. Applied safranin for 30 seconds and washed with distilled water, stained slides were air dried and examined microscopically.

**(ii) Spore staining:**

Smears were made on separate glass slides. Smear was air dried and heat fixed. Flooded the smear with malachite green and heated the slides to steaming and steamed for 5 minutes, more stain to the smear was added from time to time. Washed the slides with distilled water and counterstained with safranin for 30 seconds. Washed smear with distilled water and examined microscopically.

### 3.4.1.2 BIOCHEMICAL TESTS:

#### (i) Gelatin Agar Medium

Table 3.2: Composition of Gelatin Agar Medium

S.NO.	Composition	Concentration(g/l)
1.	Gelatin	40
2.	Tryptic Soy broth	30
3.	Distilled water	1000ml

#### Procedure:

Stab a small inoculum of the bacterium about 3/4th of the way to the bottom of a tube of deep agar with the inoculating needle. Repeated with separate stab tubes for each of bacteria and incubated at 37<sup>0</sup>C for 24-48 hr. placed the incubated stab and the un-inoculated control into the refrigerator, for approximately 30 minutes. Compare the inoculated stab with the control by tapping the tubes gently.

#### (ii) Starch Hydrolysis:

Table 3.3: Media Composition for Starch Hydrolysis Test

S.No.	Composition	Concentration(g/l)
1.	Starch	2.0
2.	Peptone	0.5
3.	Beef extract	0.3
4.	Agar	1.5
5.	Distilled water	100ml

#### Procedure:

Inoculated the plates of starch agar with the assigned bacteria and incubated at 37<sup>0</sup>C for 24-48hrs.

Dripped a small amount of Gram's iodine on the plate around the inoculated area and a small amount in an un-inoculated area away from the inoculum. A clear zone was observed around the inoculum. Compared the inoculate area with the un-inoculate area and recorded the results.

**(iii) Citrate utilization:**

**Table 3.4: Media composition for Citrate Utilization Test**

S.No.	Composition	Concentration(g/l)
1.	(NH <sub>4</sub> ) <sub>2</sub> HPO <sub>4</sub>	0.1
2.	K <sub>2</sub> HPO <sub>4</sub>	1
3.	NaCl	5
4.	Sodium Citrate	2
5.	MgSO <sub>4</sub>	0.2
6.	Bromothymol blue	0.08
7.	Agar	2
8.	Distilled Water	1000 ml

**Procedure:**

The surface of slants of citrate agar was inoculated with the bacteria by using a small amount of inoculum and incubated at 37<sup>0</sup>C for 48hrs. the inoculated slant was then compared with control.

**(iv) Catalase Screening:**

**Table 3.5: Media composition for Catalase test**

S.No.	Composition	Concentration(g/l)
1.	Trypticase	15
2.	Phytone	5
3.	Sodium chloride	5
4.	Agar	15

**Procedure:**

Inoculated the trypticase soy agar slants and kept an uninoculated trypticase soy agar slant as control. Incubate the cultures at 37°C for 24-48 hours. Observe each culture for the appearance or absence of gas bubbles.

(v) **Carbohydrate Utilization:**

**Table 3.6: Media composition for Carbohydrate Utilization Test**

S.No.	Composition	Concentration(g/l)
1.	Peptone	10
2.	NaCl	15
3.	Sugar*	5
4.	Distilled water	100ml

Sugar: Glucose, Lactose, Sucrose, Mannitol, Xylose.

**Procedure:**

Transferred 0.1ml of inoculum of each of the bacteria into broths (: Glucose, Fructose, Sucrose, Mannitol, Maltose) and incubated the inoculated broths at 37°C for 24-48h in a rotary shaker at 150rpm. Results were observed for each type of broth and compared to the un-inoculated controls.

**3.4.1.3 PHYSIOLOGICAL TESTING:**

The optimum pH for the growth of the strains was determined:

(i) **Effect of pH on growth:**

Each specie has the ability to grow within a specific pH range that may be broad or limited, with the most rapid growth occurring within a narrow optimum range.

**Procedure:**

Inoculated each of a series of the tube with bacterial culture by adding 0.1ml of the culture.

Incubated the bacterial inoculated tubes with different pH (6-12) for 24-48hrs at 37<sup>0</sup>C in a rotary shaker at 150rpm. Examined the growth by using spectrophotometer wavelength 600nm.

### **3.5. TREATMENT OF ASH SAMPLES:**

The ash samples were then treated with selected micro-organism in the presence of molasses and glucose (0.1% conc.) as a rich carbon source and they are incubated at 37<sup>0</sup>C for 8 days and samples from 5 treatments as described below were tested periodically in triplicate for alkalinity, hardness, chloride and heavy metal content.

Where

1. Control (C :only ash)
2. Molasses + ash (MC : without microbes)
3. Molasses + ash + bacteria (MB : with microbes)
4. Glucose + ash (GC : without microbes)
5. Glucose + ash + bacteria (GB : with microbes)

10 ml bacterial culture of 1 OD was taken for the treatment and then centrifuged at 8000 rpm for 10 min for getting the pellet. The pellet was then washed twice with distilled water and re-suspended. Finally, 0.1% molasses and 0.1% glucose as a carbon sources were added respectively in different samples. These 10ml solutions were carefully added in autoclaved ash samples so that resultant mixture does not form slurry.

### **3.6. ANALYSIS OF THE ASH SAMPLES AFTER TREATMENT:**

During incubation days , the samples were tested periodically for three important water quality parameters such as the hardness, chloride, alkalinity and heavy metal content (ICP- MS) .Analysis of ash leachate was done by using ash to water ratio 1:10. After shaking the ash for half an hour on rotary shaker at 150 rpm, then flask was kept at stationary condition for half an hour until the particle settle down. The supernatant was removed from the flask and then used for the estimation of pH, alkalinity and chloride content.

#### **3.6.1. ALKALINITY TEST:**

Alkalinity of water is its acid neutralizing capacity. It is the sum of all the titrable base. The measured value may vary significantly with the end point pH used according to the APHA 1999.

### **3.6.2. HARDNESS TEST:**

An excellent way to determine water hardness is to perform a complexometric titration using a standard ethylenediaminetetraacetic acid (EDTA) solution. Due to steric hindrances, EDTA will complex with calcium and magnesium in a one-to-one molar ratio. The endpoint in this experiment will be determined using a EBT indicator ( according to APHA 1999).

### **3.6.3. CHLORIDE TEST:**

In a neutral or slightly alkaline solution, Potassium Chromate can indicate the end point of the Silver Nitrate titration of Chloride using APHA 1999.

## **3.7. PHYSICO-CHEMICAL CHARACTERIZATION OF ASH, SOIL AND THEIR MIXTURE:**

The chemical characterization of fly ash and sewage sludge samples was done as per the standard methods of Jackson(1967).

### **3.7.1 pH:**

10g sample was mixed with 100 mL of distilled water, stirred thoroughly to mix properly the solid sample in the liquid for at least half an hour and then kept in stationary conditions for another half an hour. The pH of the supernatant liquid was measured as per the method given by Jackson (1967) using a Thermo Orion Model 290 pH meter after calibration with buffer solution of three different pH 4.0, 7.0 and 9.2.

### **3.7.2 ELECTRICAL CONDUCTIVITY:**

The electrical conductivity was determined in  $\mu\text{Scm}^{-1}$  as per the method given by Jackson (1967). The sample solution was prepared by the same method as for pH measurement. Before taking reading, the conductivity meter (Orion Model 125) was calibrated with the help of potassium chloride solution (0.01 M).

### 3.7.3 AVAILABLE NITROGEN:

Total nitrogen in the supernatant liquid was estimated by the Kjeldahl method given by Piper (1960), as detailed below:

1. 5g of each sample of fly ash/sewage sludge was mixed thoroughly with sulphuric-salicylic acid followed by the addition of 5g of sodium thiosulphate. Samples were heated for 5 minutes followed by cooling up to room temperature and then 10g of the digestion mixture was added. The contents were mixed well in a Kjeldahl flask.
2. Samples were then kept in a digestion assembly at a controlled temperature of  $100\pm 2^{\circ}\text{C}$  for at least two hours.
3. A change in colour from dark brown to greenish white was observed and then contents of the samples were cooled and diluted with 300 mL distilled water.
4. 250 mL of the digested sample, 50 mL NaOH solution and glass beads were added to the distillation flasks. Contents of the flask were distilled at  $100\pm 1^{\circ}\text{C}$ .
5. The distillate was collected until the volume reached 200 mL with the help of a receiver tube in a conical flask containing 50 mL boric acid.
6. After adding two drops of the mixed indicator the distillate (200 mL) was titrated against  $\text{H}_2\text{SO}_4$  (0.02 N) until the endpoint colour changed from green to pink.

#### Calculation

$$\text{Total N (ppm)} = \frac{(T-B) \times 0.00028 \times 100 \times 10000}{5}$$

Where, T is the titre value for the sample and B is volume of sulphuric acid used for the blank.

### 3.7.4 AVAILABLE PHOSPHOROUS:

#### Reagents:

1. Ammonium fluoride (extracting solution): 22.2 g of ammonium fluoride dissolved in 41.6ml Hcl and volume made upto 2l.

2. Reagent A: 12g of ammonium molybdate in 250ml distilled water and 0.2908 g antimony potassium tartarate in 100ml distilled water. These two solutions were mixed, 1000ml of 2.5M sulphuric acid was added and volume made up to 2l with distilled water.
3. Reagent B(freshly prepared): 1.058g of ascorbic acid dissolved in 200ml of reagent A and mixed.
4. Standard P solution: 1ppm concentration.

### **Procedure**

1. 2.5 g of soil/ash was taken in a 100ml flask and 25ml extracting solution was added.
2. Solution was kept on shaker for 30 minutes and the sample was filtered through whatman filter paper no.42.
3. 2ml of aliquot of filtrate was taken in a conical flask and 20 ml of distilled water, 8ml of reagent B and added more 20ml of distilled water.
4. For standard curve 0, 2, 5, 10, 15, 20 ml of standard P solution was placed in 50ml of volumetric flask. Then, 2ml extracting solution, 8ml of reagent B, volume make up to 50ml. For blank add all the reagents except standard P solution.
5. Take absorbance at 882nm after 10 minutes.

### **3.7.5 ORGANIC MATTER:**

Total organic carbon was estimated by Walkley and Black method(Jackson 1934).

### **Reagents**

1. Potassium dichromate (1 N) solution: 49.04g of potassium dichromate was dissolved in distilled water to prepare one litre of solution.
2. Ferrous ammonium sulphate (0.5 N) solution: 198g of ferrous ammonium sulphate was dissolved in water to prepare one litre of solution.
3. Diphenylamine indicator: 0.5g of diphenylamine was dissolved in a mixture of 20 mL water and 100 mL of concentrated sulphuric acid.
4. Concentrated sulphuric acid.
5. Orthophosphoric acid (85%) and sodium fluoride (NaF).

## Procedure

1. 2g of sample was taken in a 250 mL conical flask and 10 mL of  $K_2Cr_2O_7$  (1N) solution was added. The flasks were then swirled for proper mixing of the solid sample and then reagent was added.
2. Further, addition of 20 mL of  $H_2SO_4$  was done and the flask was kept undisturbed for at least 30 minutes.
3. After 30 minutes time period 200 mL of distilled water was added to the mixture, 10mL of orthophosphoric acid, 0.5g of NaF and 1mL diphenylamine indicator were also added to the flask.
4. The contents were then titrated with freshly prepared ferrous ammonium sulphate solution (0.5 N) till the end point from (blue-violet to green) was achieved. A blank was also run without the solid sample.

## Calculation

$$\text{Organic carbon (\%)} = \frac{10(B - T) \times 0.003 \times 100}{BX_{soil}(g)}$$

Where:

B denotes the volume of ferrous ammonium sulphate solution required for blank titration (mL).

T denotes the volume of ferrous ammonium sulphate solution needed for fly ash/sewage sludge sample titration (mL).

## RESULTS AND DISCUSSION

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### 4.1. ISOLATION OF METAL TOLERATING MICRO-ORGANISMS.

The isolation of bacteria was done from the sample taken from the soil of Thapar University and sludge from textile industry and grown on Nutrient agar. Initially, 8 strains were selected as they showed best growth on Nutrient agar plates. These strains were then tested for their growth in Minimal medium (M9) having pH 12. At this high pH, only three colonies were able to grow and out of which only two were selected for further experimentation.

### 4.2. OPTIMISATION OF METAL TOLERANCE BY MICRO-ORGANISMS IN AN ENRICHED (NB) AND MINIMAL MEDIA (M9).

The two isolates 1 and 2 were screened for their metal tolerance capacity (Table 5.1 and 5.2) in enriched medium and minimal medium. Table 5.1 showed three concentrations 10, 25, 50 and 75 ppm of different metals in Nutrient broth (NB). It was found that Isolate 1 was found effective with Aluminum (Al) and Silver (Ag) as they showed maximum growth (1.118 and 0.834 respectively) after 4 days at 75ppm metal concentration in Nutrient broth. This strain also found to be comparatively least effective for Mercury (Hg) and Copper (Cu) as the growth after 4 days has declined to a very low level (0.002 and 0.008 respectively) at 75 ppm metal concentration.

Now, in the case of Isolate 2, the maximum growth was observed in Iron (Fe): 1.230 followed by Molybdenum (Mo): 1.065 and the least growth were found in case of copper (Cu): 0.098 and Zinc (Zn): 0.23. Santarsiero and Oltaviani in 1992 estimated heavy metals in residues from hospital solid waste incineration and found huge quantity of all of these metals present in the ash.

The data presented in table 4.1 showed different pattern with different metals. In case of Cu and Fe only, there was maximum growth observed with both isolates and the growth was decreased after increasing the concentration of metals in the medium.

**Table 4.1: Growth of microbes on different metals concentrations in nutrient broth (NB):**

S. No.	Metals	Isolate 1				Isolate 2			
		10ppm	25ppm	50ppm	75ppm	10ppm	25ppm	50ppm	75ppm
1.	Fe	1.547	0.652	0.78	0.685	1.474	0.593	0.987	1.23
2.	Mn	0.877	0.690	1.12	0.550	0.876	0.406	0.023	0.299
3.	Hg	0.823	0.799	0.338	0.002	0.563	0.304	0.045	1.007
4.	Mo	0.721	0.024	0.09	0.103	0.721	0.801	0.198	1.065
5.	Cu	1.117	1.216	0.567	0.008	1.117	1.061	0.43	0.098
6.	Zn	0.805	0.634	0.118	0.112	0.417	0.443	0.245	0.23
7.	Cr	0.221	0.429	0.765	0.089	0.224	0.068	0.098	0.652
8.	Pb	0.867	0.345	0.654	0.098	0.543	0.652	0.008	0.439
9.	Al	0.707	0.265	0.932	1.118	0.763	0.617	0.465	0.102
10.	Ag	0.654	0.234	1.09	0.834	0.943	0.843	0.005	0.174

As Nutrient broth is an enriched medium, so the metal tolerance capacity of the two isolates was checked in Minimal media (M9) also. The tolerance level was found to be 50 ppm of different metal concentrations. The maximum tolerance (Table 4.1) was found with Manganese (Mn) which was 1.705 and Molybdenum (Mo) as 1.559 and Zinc (Zn) , Chromium(Cr) showed no growth after 4 days. The isolate 2 showed maximum growth with Al and Ag as 0.612 and 0.714 respectively at 50 ppm metal concentration and the growth was totally declined in case of Cr and Hg. The growth of bacteria is depending upon several factors such as temperature, pH, etc. which are present in ash. Due to toxicity conditions in the ash , there was not fixed pattern in the growth of bacteria at different concentration of metals (Table 4.2)

This data showed that both the isolates were good for the removal of metals from the Incinerator Biomedical Waste Ash leachate.

**Table 4.2: Growth of microbes on different metal concentrations in minimal medium (M9)**

S.No.	Metals	Isolate 1		Isolate 2	
		25ppm	50ppm	25ppm	50ppm
1.	Fe	0.76	0.598	0.56	0.575
2.	Mn	0.43	1.705	0.62	0.733
3.	Hg	0.43	0.080	0.87	0.064
4.	Mo	0.62	1.559	0.63	0.174
5.	Cu	0.31	0.623	0.92	0.258
6.	Zn	0.02	-	0.73	0.549
7.	Cr	0.12	-	0.09	0.087
8.	Pb	0.23	0.210	0.03	0.098
9.	Al	0.65	0.117	0.92	0.612
10.	Ag	0.34	0.302	0.89	0.714

### **4.3 .IDENTIFICATION OF SELECTED BACTERIAL ISOLATE:**

#### **4.3.1. MORPHOLOGICAL CHARACTERIZATION:**

After testing the metal tolerance level of the two selected isolates, we selected the Isolate 1 for the further experimentation. The isolate 1 was Gram +ve, rod shaped and growing aerobically.

The isolate was able to hydrolyze starch and gelatin and exhibited the catalase and citrate test. It showed characteristic utilization of the carbohydrate substrates glucose, sucrose, and lactose. Other biochemical characters of the Isolate 1 are shown in detail in Table 4.3.

#### **4.3.2 BIOCHEMICAL CHARACTERIZATION:**

##### **4.3.2.1 Gelatin Liquefaction test:**

Gelatin is a protein produced by hydrolysis of collagen, a major component of connective tissue. Below temperature 25°C gelatin will maintain its gel properties, and exist as solid, temperature above 25°C gelatin is liquid. Liquefaction is accomplished by some micro-organisms capable of

producing a photolytic extra cellular enzyme called gelatinase which act to hydrolyze this protein to amino acid. Once degradation occur even very low temperature of 0<sup>0</sup>C will not restore the gel characteristics. The isolate 1 shows the positive results when the culture was placed in a refrigerator at 0<sup>0</sup>C for 30 minutes. Culture remain liquefied produce gelatinase and called as rapid hydrolyser, whereas isolate 1 showed gelatinase positive results.

#### **4.3.2.2. Starch Hydrolysis**

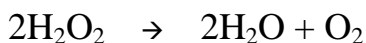
The starch hydrolytic activities include degradation of macro molecules which require coenzyme. The starch serves as the polysaccharides substrate. The detection of hydrolytic activity of starch, the starch test was performed to determine the presence and absence of starch in solid medium. Starch in the presence of iodine will impart blue color to medium and clear zone around the colony indicating presence of starch hydrolyzing bacteria. The isolate 1 showed positive result.

#### **4.3.2.3. Citrate utilization process:-**

In absence of fermentable glucose or lactose some microorganism are capable of using citrate as a carbon source for their energy this ability depend on the presence of citrate. During the growth of bacteria reaction take place and medium become alkaline O<sub>2</sub> that is generated combines with sodium and water to form sodium carbonate, an alkaline product. The presence of sodium carbonate change the bromo-thymol blue indicator incorporated into the medium from green to deep Persian blue. The strain 1 showed citrate positive results.

#### **4.3.2.4. Catalase production:-**

During aerobic respiration microorganism produce hydrogen peroxide and in some case an extremely toxic super oxide was produced. Accumulation of these substances will results in death of organism unless they can be enzymatically degraded under anaerobic condition, carbohydrate degraded for energy production organisms capable of producing catalase rapidly degraded hydrogen peroxide as:-



Catalase production can be determined by adding the substrate H<sub>2</sub>O<sub>2</sub> on agar plate. If catalase is present the chemical reaction mention is indicated by bubbles of free oxygen gas this shows a positive catalase test and the absence of bubble formation shows negative catalase test. Isolate(1)

showed positive result.

#### 4.3.2.5 Carbohydrate utilization Test:-

Most organism obtained energy through a series of orderly and integrated enzymatic reaction leading to bio-oxidation of a substrate some organism are capable of fermenting sugar such as glucose aerobically and anaerobically. In Aerobic bio-oxidation, in which molecular O<sub>2</sub> can serve as final electron acceptor where-as in anaerobic bio-oxidation, in which inorganic ions other than O<sub>2</sub> such as NO<sub>3</sub> and SO<sub>4</sub> can serve as final electron acceptor.

The ability of a microorganism to utilize carbohydrates can be key to its identification. Different carbohydrates (1% concentration) are used in testing of utilization for each strain. Isolate 1 utilizes all the 6 types carbohydrates (glucose, sucrose, lactose, mannitol, xylose & maltose) and gives the positive result.

**Table 4.3: Morphological and Biochemical characters of the bacterial Isolate 1**

<b>Morphological Test</b>	<b>Isolate 1</b>
Gram Staining	+ ve
Shape (Rod/Cocci)	Small rods
Form	Rough or round initially then irregular
Colonies	White opaque; over growth shows white glistening colony
<b>Biochemical Test</b>	
Citrate Utilization Test	+
Starch Hydrolysis Test	+
Gelatin Test	+
Catalase Test	+
<b>Carbohydrate utilization</b>	
Glucose	+
Sucrose	+
Lactose	+
Mannitol	-
Xylose	-



Figure 4.1: Figure showing morphology of the selected Isolate1.

#### 4.3.3. PHYSIOLOGICAL TEST:

##### Effect of pH on growth of bacterial strains:

Each organism has a pH range within which growth is possible. Most organisms have pH optima in 5-9 range. Very few species can grow at pH values below 2 or above 10. Organisms capable of living at low pH are called acidophiles. Those capable of living at very high pH are called alkaliphiles.



Figure 4.2: Growth of bacterial strains in M9 medium at different pH

Initially at pH 6, the growth of the isolate 1 was observed by measuring the optical density at 600 nm and it had shown OD = 0.3 and as the pH is increased, the growth increased linearly until reaches to pH = 10(OD = 1.3) and then OD showed a slight decrease till pH 12 and afterwards got totally declined to 0.45 at pH 14. This graph clearly indicated that the optimum pH level is 10-12 and is highly suitable for alkaline type of environment.

#### 4.4. HARDNESS, ALKALINITY AND CHLORIDE CONTENT RESULTS:

The characteristics of raw BMW ash leachate showed increased pH 10, alkalinity (500 mg/L), hardness (1320mg/L) and chloride (8500 mg/ L) content which are comparatively higher than the values prescribed by WHO and EPA guidelines for drinking water (alkalinity , 250-350; hardness, 300 mg/L; chloride, 250 mg/L).

**Table 4.4 : Initial analysis of BMW ash leachate :**

S.NO.	PARAMETER	VALUE
1.	pH	10
2.	EC	5.47 mS
3.	Alkalinity	500 mg/L CaCO <sub>3</sub>
4.	Hardness	1320 mg/L CaCO <sub>3</sub>
5.	Chloride	8500 mg/L CaCO <sub>3</sub>

**Table 4.5: WHO and EPA(2012) Drinking Water Standards:**

S.NO.	PARAMETER	VALUE
1.	pH	6.5-8.5
2.	EC	50-800 $\mu$ S
3.	Alkalinity	200-600 mg/L CaCO <sub>3</sub>
4.	Hardness	300-600 mg/L CaCO <sub>3</sub>
5.	Chloride	250-1000 mg/L CaCO <sub>3</sub>

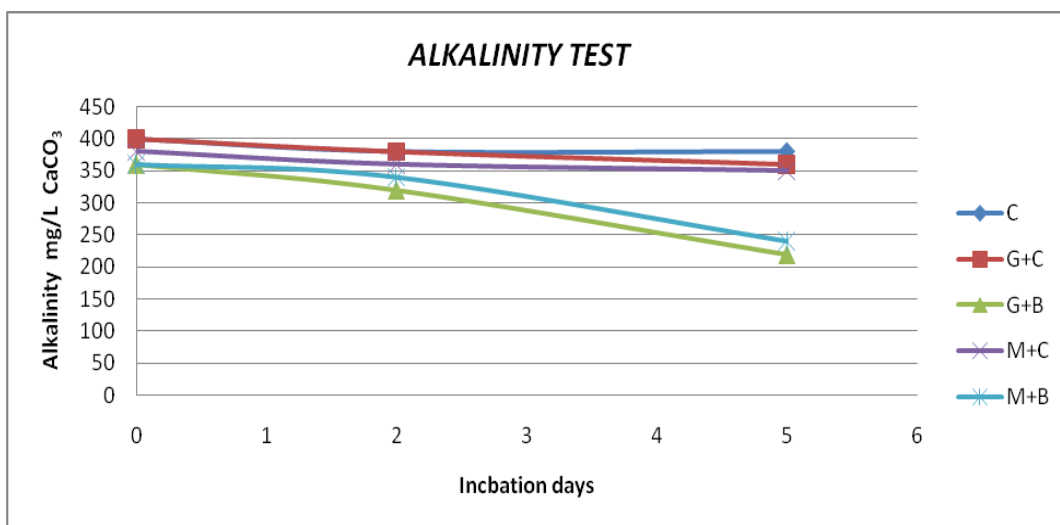
Table 4.6: Characterization of treated BMW ash leachate showing alkalinity, hardness and chloride contents.

S.NO.	SAMPLE	ALKALINITY		
		0 day	2 <sup>nd</sup> day	5 <sup>th</sup> day
1.	C	400	380	380
2.	G+C	400	380	360
3.	G+B	360	320	220
4.	M+C	380	360	350
5.	M+B	360	340	240

S.NO.	SAMPLE	HARDNESS		
		O day	2 <sup>nd</sup> day	5 <sup>th</sup> day
1.	C	1320	1310	1310
2.	G+C	1340	1320	1300
3.	G+B	1290	1040	1000
4.	M+C	1380	1360	1320
5.	M+B	1360	1240	1160

S.NO.	SAMPLE	CHLORIDE		
		O day	2 <sup>nd</sup> day	5 <sup>th</sup> day
1.	C	8500	8450	8600
2.	G+C	8750	8150	7650
3.	G+B	9200	7600	3500
4.	M+C	9550	9300	8100
5.	M+B	8900	7050	3850

In alkalinity tests, there was 33% reduction in alkalinity of BMW ash leachate after 5 days of treatment in which molasses was used as a carbon source and 38.8% reduction when glucose was used as a carbon source whereas there was no considerable amount of reduction in Control experiments. On 2nd day, it was observed that there was 11.11% decrease in the alkalinity of the leachate whereas on 5th day, the results have shown 31.25% decrease. In hardness tests, there was 22.4% reduction (glucose as a carbon source) in hardness content of the leachate and 14.7% reduction when molasses as a carbon sources. It means glucose acted as readily available carbon source for the microbes during treatment of the ash. On 2nd day, the value has reached down to 1040mg/L from 1290 mg/L and on 5th day, the value has sufficiently decreased to 1000mg/L. Also, the chloride content of the treated BMW ash leachate after 5 days, showed sufficient amount of decrease 61.0% in case of glucose and 56.76% in case of molasses. The control in all the tests showed no considerable decrease indicating that the microbes was responsible for reducing the various parameters of the leachate and made it less toxic.



**Figure 4.3 : Graphs showing reduction in alkalinity content of the BMW ash leachate after treatment**

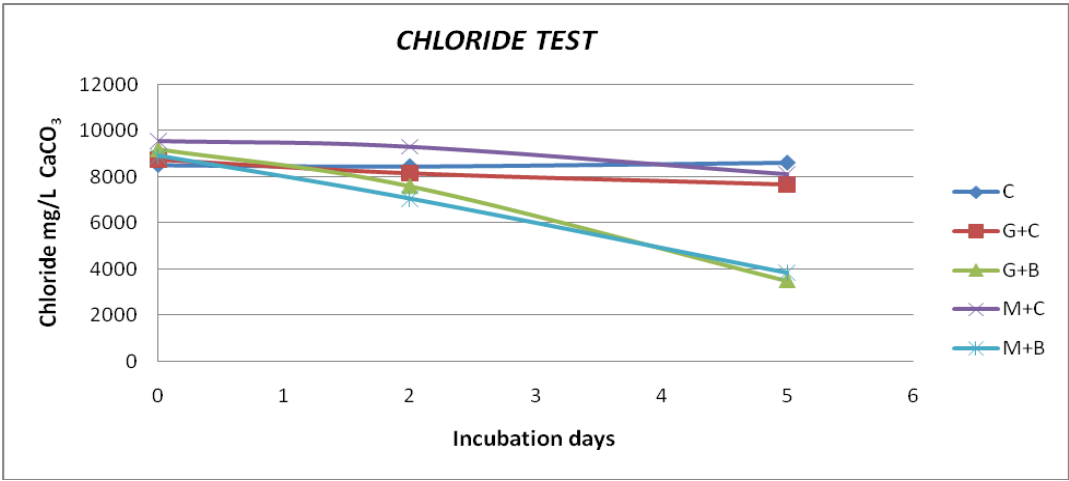
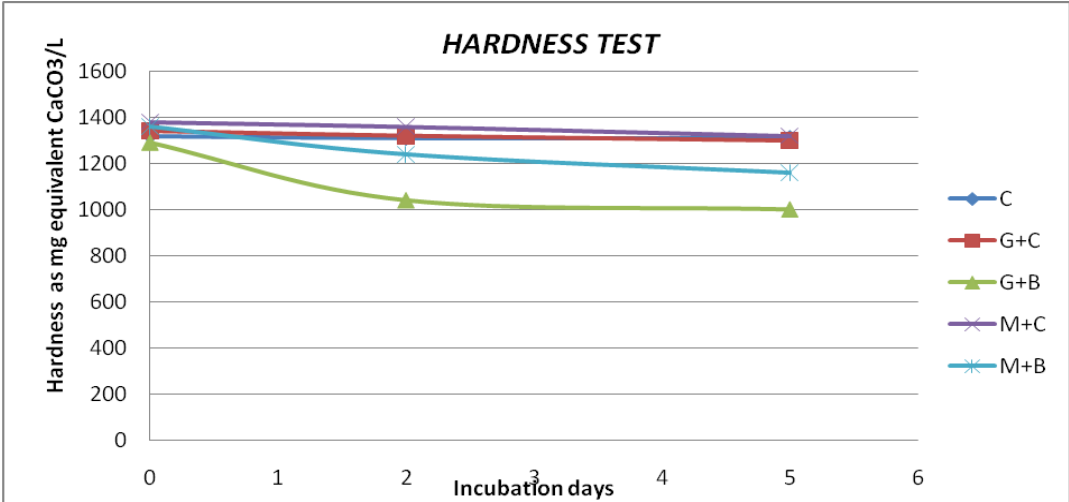


Figure 4.4: Graphs showing reduction in hardness and chloride contents of the BMW ash leachate after treatment.

Analysis by using APHA standard methods proved that there is reduction in 50% alkalinity, 25% hardness and 60% chloride within 5 days of treatment compared to control when suitable carbon source (glucose and molasses) was given. The treatment of the microorganism with ash had given some positive response. There is reduction in alkalinity, hardness and chloride within 5 days of treatment compared to control when suitable carbon source (glucose and molasses) was given. Glucose is an easily available carbon source as it can be easily metabolized by the bacteria as compared to molasses which contains several sugars such as glucose, fructose, saccharose, raffinose which may/may not be readily utilized by the bacteria. This reduction in the alkalinity may impart less negative impact on environment in terms of groundwater, surface water and soil contamination.

#### 4.5. HEAVY METAL ANALYSIS:

Table 4.7: ICP-MS analysis of the BMW ash leachate after treatment (in ppm):

S.NO.	ANALYT E (metals)	C (only ash)	M+B (1 <sup>st</sup> day)	M+B (10 <sup>th</sup> day)	G+B ( 1 <sup>st</sup> day)	G+B (10 <sup>th</sup> day)	WHO STANDARDS (ppm)
1.	Al	9.543	8.4601	7.5508	10.447	7.12	5.0
2.	Cr	5.989	4.6359	3.1788	4.5811	2.618	2.0
3.	Pb	0.876	0.52761	.2709	.7836	.3305	0.1
4.	Ag	2.643	2.76628	2.5498	2.2193	1.9076	1.2

When molasses was used as a carbon source for the biological treatment of the BMW ash leachate, there was comparatively less reduction in heavy metals toxicity as compared to the case in which glucose was used as a carbon source. In case of aluminium, maximum retention(50%) was found in the isolate when we have used glucose as carbon source and in case of molasses, there was only 10% retention. The chromium content in BMW ash leachate was comparatively very high and after 10 day treatment with the selected isolate, the results had shown considerable amount of decrease(42%) in case of glucose as a carbon source. Lead which is highly poisonous in nature was also taken up by the microbes in a sufficient amount(57%) after treatment and this case had shown better results. Silver metal had shown least amount of retention as compared to others as there was only 7.4% decrease. It means the uptake of silver metal by the isolate was not that efficient as compared to others.

The treatment of the ash by the use of the isolate 1 showed positive response on the reduction of the toxicity level of the heavy metals. There is 50%, 42%, 57%,40% reduction in Al, Cr, Pb, Ag content when 0.1% glucose was used as a carbon source and 10%, 32%, 60%, 60% reduction in Al, Cr, Pb, Ag content when 0.1% molasses was used as a carbon source.

Valvandalis et al., 2008 also studied on metal leachability, polycyclic aromatic hydrocarbons and polychlorinated biphenyls in fly ash and bottom ash of Biomedical Waste Incinerator Ash and maximum metals were removed by leachability. The selected isolate might have different mechanisms to respond to the contaminants. Some can bind the metal ions present in the medium at the cell surface or transport them into the cells and detoxify them in various ways (Ariskina et al., 2004; Gavrilesco et al., 2004; Mallick et al., 2004). It is possible that the selected isolates may use more than one mechanism to remove metal ions like biosorption (adsorption of metal ions onto the cell surface without requirement of energy) or bioaccumulation (absorption of metal ions into the cells with an energy requirement).It is likely that biosorption would be the main mechanism that the biomass could use in the absence of an energy demanding accumulation inside the cells.

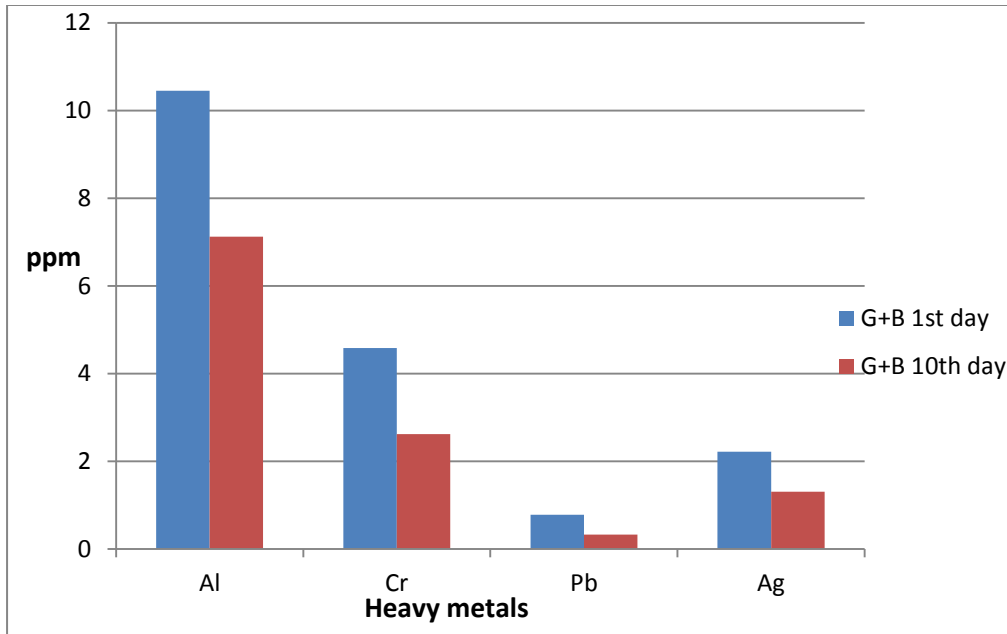


Figure 4.4: Graph showing decrease in metal toxicity after 10 day treatment(ppm)(glucose as a carbon source):

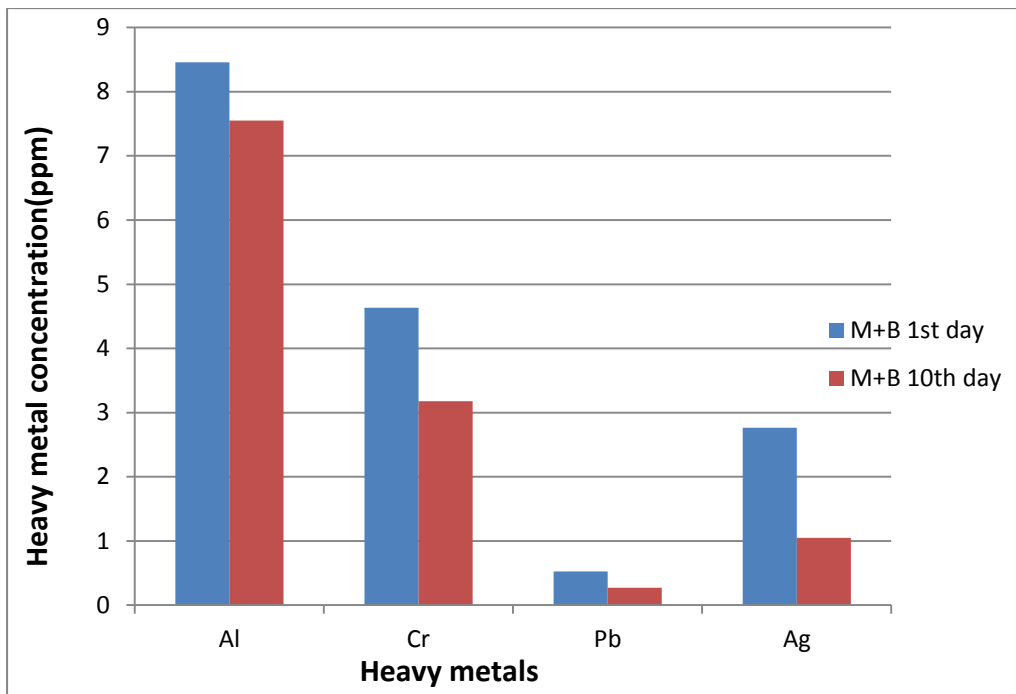


Figure 4.5: Graph showing decrease in metal toxicity after 10 day treatment(ppm)(molasses as a carbon source):

#### 4.6 PHYSICO-CHEMICAL CHARACTERIZATION OF ASH AND SOIL AND THEIR MIXTURE:

Analysis of the ash and soil was separately done for pH, electrical conductivity, Available phosphorous, Available Nitrogen, and organic matter and then utilized in different ratios(2%, 4%,6%, 8%, 10%) for growth of ladyfinger seeds in pots.

#### CHARACTERIZATION OF LEACHATE OF BIOMEDICAL WASTE ASH :

The hospital wastes are mainly managed by burning in incinerators because other methods are not suitable due to high toxicity and pathogenicity in the waste. Biomedical waste after incineration causes significant scale damage. Zhao et al (2008) said that the type of biomedical waste which comes out after incineration needs special treatment before land filling as it contains various amounts of toxic materials. The ash having pH 10 and COD is 18 which is quite less indicating that the toxicity is responsible for the less COD value. The electrical conductivity is 5.47 mS where as hardness is 1320 mg/L. The alkalinity was 400mg/L where as the chloride content was also on the higher side (8500 mg/L) indicating that it can create trouble for prolonged used. The available nitrogen and phosphorous present in soil was 18.48 and 9.5 mg/L respectively where as it was 15.6 mg/L and 1.7 mg/L respectively in case of ash. The organic matter is very high in soil as 23.85% where as in ash it was only 0.3%.

**Table 4.8: Characterization of leachate (soil and raw ash)**

S.NO.	PRAMETERS	SOIL	ASH
1.	pH	7.2	10
2.	Electrical conductivity	0.25 dS/m	5.45 dS/m
3.	Available nitrogen(ppm)	18.48	15.6
4.	Available phosphorous(ppm)	9.5	1.7
5.	Organic matter(%)	23.85	0.3

Table 4.9: Characterization of leachate (mixture of soil and ash) after 15 days of growth of plants.

S. NO.	Parameters	Soil + Ash Mixtures									
		2%		4%		6%		8%		10%	
		1 <sup>st</sup> day	15 <sup>th</sup> day	1 <sup>st</sup> day	15 <sup>th</sup> day	1 <sup>st</sup> day	15 <sup>th</sup> day	1 <sup>st</sup> day	15 <sup>th</sup> day	1 <sup>st</sup> day	15 <sup>th</sup> day
1.	pH	7.4	7.6	8.2	8.44	8.5	8.8	8.9	9.5	9.4	9.9
2.	Electrical conductivity (dS/m)	1.45	1.9	2.4	2.7	3.2	3.8	4.5	4.6	5.1	5.23
3.	Available nitrogen (ppm)	24.08	18.5 (23 %)	22.4	15.4 (33%)	19.6	14 (28%)	14	10 (18%)	12.3	10 (18%)
4.	Available Phosphorous (ppm)	15	10.0 (33%)	13.5	9.5 (29%)	11.7	8.0 (31%)	10.7	8.0 (25%)	9.4	7.0 (25%)
5.	Organic matter (%)	26.25	18 (31%)	18.75	12.5 (33%)	16.95	12.5 (29%)	13.95	10.25 (26%)	12.3	10.0 (18%)

\*Whereas the values in percentages shows % reduction after 15 days.

#### 4.7: PLANT GROWTH ANALYSIS:

The soil(150g) used for the plant growth shows pH 7.2, electrical conductivity as 7.8 mS, available nitrogen as 18.48 ppm, available phosphorous as 9.5ppm, organic matter as 23.85%. This indicated that the nutritional level of the soil is appropriate for the study and makes it suitable for growing crops.

#### AVERAGE GROWTH OBSERVATIONS OF LADYFINGER:

- a) The germination of seeds depends on atmospheric temperature, moisture, available soil characteristics and nature of crop. The amount of soil used for germination purpose was 150g.
- b) The number of seeds sown in each pot were four in number. In control, where only soil was placed (control 1) showed 100% germination and when the soil was mixed with different ash percentages 2%, 4%, 6%, 8%, 10% , they showed varied amount of germination(Table 4.10). In control 2, where only ash was used, showed no germination.
- c) To analyse the growth of seedling and crop growth, the observation from 4 day to 15 days majority include length measurement of plumule and radicals and number of leaves in case of ladyfinger. The seeds were irrigated with suitable amount of water after every alternate days. The height of the shoot is in the range of 2-6.5 cm and the leaves ranges from 2-6 in number.



Figure 4.7: Growth of ladyfinger seeds after 15 days.

Table 4.10: Plant growth analysis:

S. No.	Sample	No. of seeds	% germination	Plant height		No. of leaves
				7 <sup>th</sup> day	15 <sup>th</sup> day	
1.	Control 1 (only soil)	4	100%	6.5	10.45	6
2.	Control 2 (only ash)	4	-	-	-	-
3.	2%	4	75%	3.5	7	4
4.	4%	4	30%	2.75	7.2	4
5.	6%	4	25%	2.75	7.2	3
6.	8%	4	25%	2.0	2.35	2
7.	10%	4	-	-	-	-

Ready and Borse (2001) applied sewage on seed germination and found that lower concentration increases the percentage seed germination where as higher concentration of use of sewage hinders the seed germination. The seed germination (table 4.12) was found 100% when only soil was used for germination where-as when only biomedical waste incinerator ash was used, no growth was observed and the minimum germination (25%) was observed in case of 8% of mixture.

The plant height was observed after 7<sup>th</sup> and 15<sup>th</sup> days and the maximum height 6.5 cm was observed in control 1 (only soil) within 7 days which reached to 10.45cm within 15 days. This table indicates that the plant height keep on decreasing as the concentration of ash in the soil ash mixture was increased. At 2% mixture, the height was 3.5 cm on 7<sup>th</sup> day which got doubled 7 cm on 15<sup>th</sup> day where-as with 4% mixture, the growth increased more on 15<sup>th</sup> day to 7.2 cm and in

case of 6% mixture even on 15<sup>th</sup> day, the length was found only 4.0 cm. Bnisra et al., 2002 studied distillery effluent for irrigation of black gram crop in laboratory conditions and found inhibition when raw influent was used in both growth as well as seed germination. Pandey et al., (2006) used fertilisers factory waste in soil as such and found that EC, BOD, COD, PO<sub>4</sub>, Chloride was increased as compared to control. Except PO<sub>4</sub>, other parameters found inhibitory for plant growth. The number of leaves were maximum in soil and minimum number three was found in 8% mixture. Thus, table indicated that for agricultural purpose, 4% soil and ash mixture is optimum where as higher percentage showed inhibition in seed germination as well as in growth of plant.

### CONCLUSIONS AND FUTURE SCOPE

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Ash analysis and characterization of incinerator ash is an important tool to predict the behaviour of ash during the biological treatment of specific waste ash but also the reactivity of ash during its disposal in landfill areas or its use as a base material for construction purpose.

During landfilling the leachate from the ash may create ground water contamination and surface water due to high alkalinity, hardness, chloride and heavy metals content. Thus, to reduce these parameters from the BMW ash leachate, biological treatment was employed which is effective and acts as green technology in terms of non-toxic and economical than chemical treatment where chemicals are used which in turn proves toxic, costly and may affect the health of the workers associated with it. The reduction in the alkalinity, hardness, chloride and heavy metals content may impart less negative impact on environment in terms of groundwater, surface water and soil contamination.

Use of metal tolerant micro-organisms for the treatment and disposal of generated ash material is an economical and environment friendly way. The treated ash do not pose any threat to ground water, as, ground water is *less susceptible to bacterial pollution* than surface water because the soil and rocks through which ground water flows screen out most of the bacteria.

Also, the treated ash can be used in combination with soil in a different percentages for agricultural purposes as the germination of ladyfinger had shown good effective results up till 4% soil and ash mixture.

The future aspect would be dependent on using both the strains to obtain a higher percentage of HMs removal and by increasing the exposure time (incubation period) and biomass dose including the use of optimal conditions for pH and temperature.

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## **PUBLICATIONS:**

Work done has been presented in National Seminar on Green technologies for Sustainable Environmental Management (GTSEM' 13) organized by Doon University, Dehradun.

Research paper titled "BIOLOGICAL TREATMENT OF HAZARDOUS BIO-MEDICAL WASTE ASH – A SUSTAINABLE APPROACH " is communicated to be published in Journal of Environmental Science and Sustainability