

**CONVERSION OF ORGANIC BIOMEDICAL WASTE INTO  
FERTILIZER USING BIOLOGICAL METHODS**

**A THESIS SUBMITTED TO**

**D. Y. PATIL EDUCATION SOCIETY  
DEEMED TO BE UNIVERSITY, KOLHAPUR  
(Declared u/s 3 of the UGC Act. 1956)**



**FOR THE DEGREE OF  
DOCTOR OF PHILOSOPHY  
IN ENVIRONMENTAL SCIENCE  
UNDER THE FACULTY OF  
INTERDISCIPLINARY STUDIES**

**BY**

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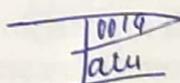
## DECLARATION

I hereby declare that the thesis entitled, "**Conversion of organic biomedical waste into fertilizer by biological methods**", is entirely original and was carried out by me independently in the D.Y. Patil Education Society (Institution Deemed to be University), Kolhapur under the supervision of Prof. (Dr) P. G. Shadija, I further declare that it has not formed the basis for the award of any degree, diploma, fellowship or associate ship or similar title of any University or institution. The extent of information derived from the existing literature has been indicated in the body of the thesis at appropriate places giving the references.

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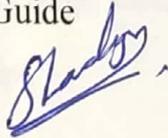
## CERTIFICATE

This is to certify that the thesis entitled "**Conversion of Organic Biomedical Waste into Fertilizer Using Biological Treatments**", which is submitted herewith for the Degree of Philosophy in Environmental Science of D. Y. Patil Education Society Deemed to be University, Kolhapur is the result of original work completed by Pooja Mahadev Patil under our supervision and guidance and to the best of our knowledge and belief the work embodied in this thesis has not formed earlier the basis for the award of any Degree or similar title of the this or any University or examining body.

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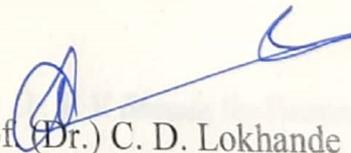
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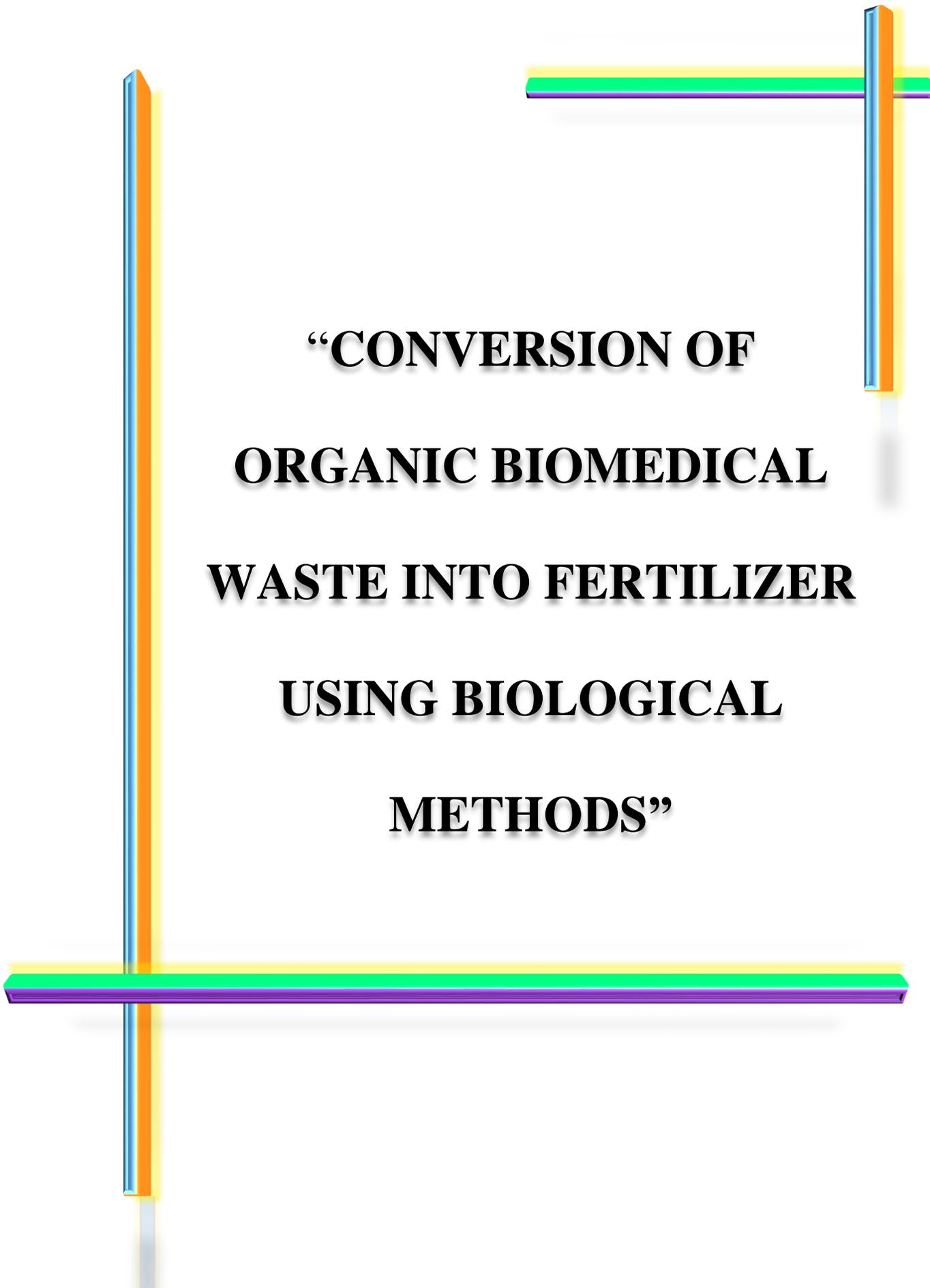
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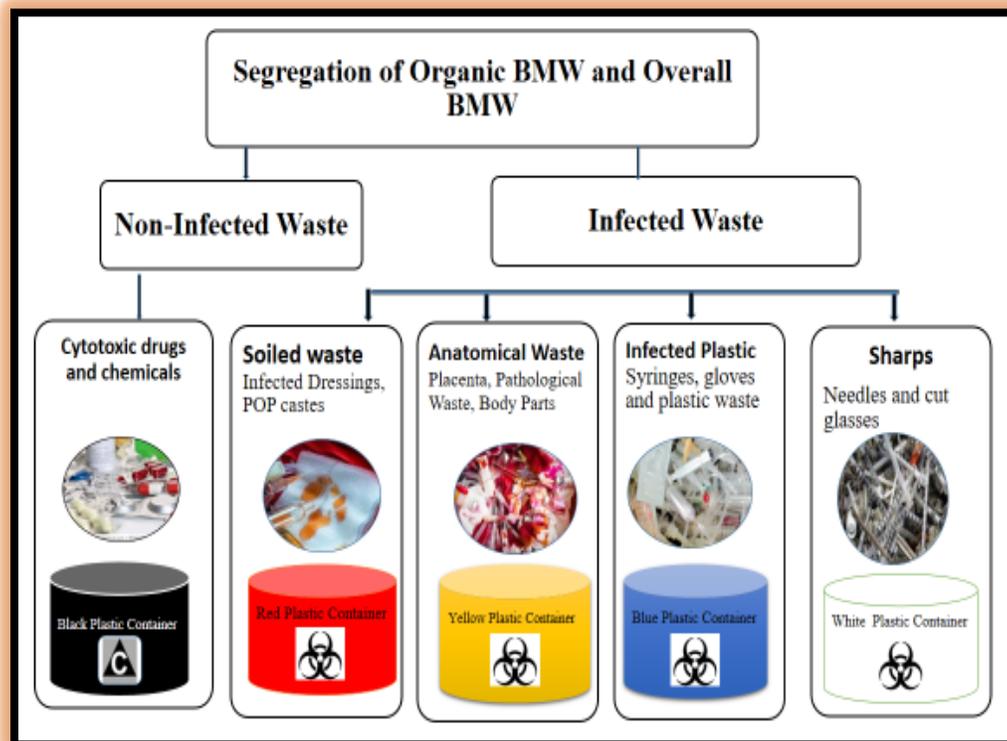


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METHODS”**

# CHAPTER 1

## CHAPTER 1

### Introduction

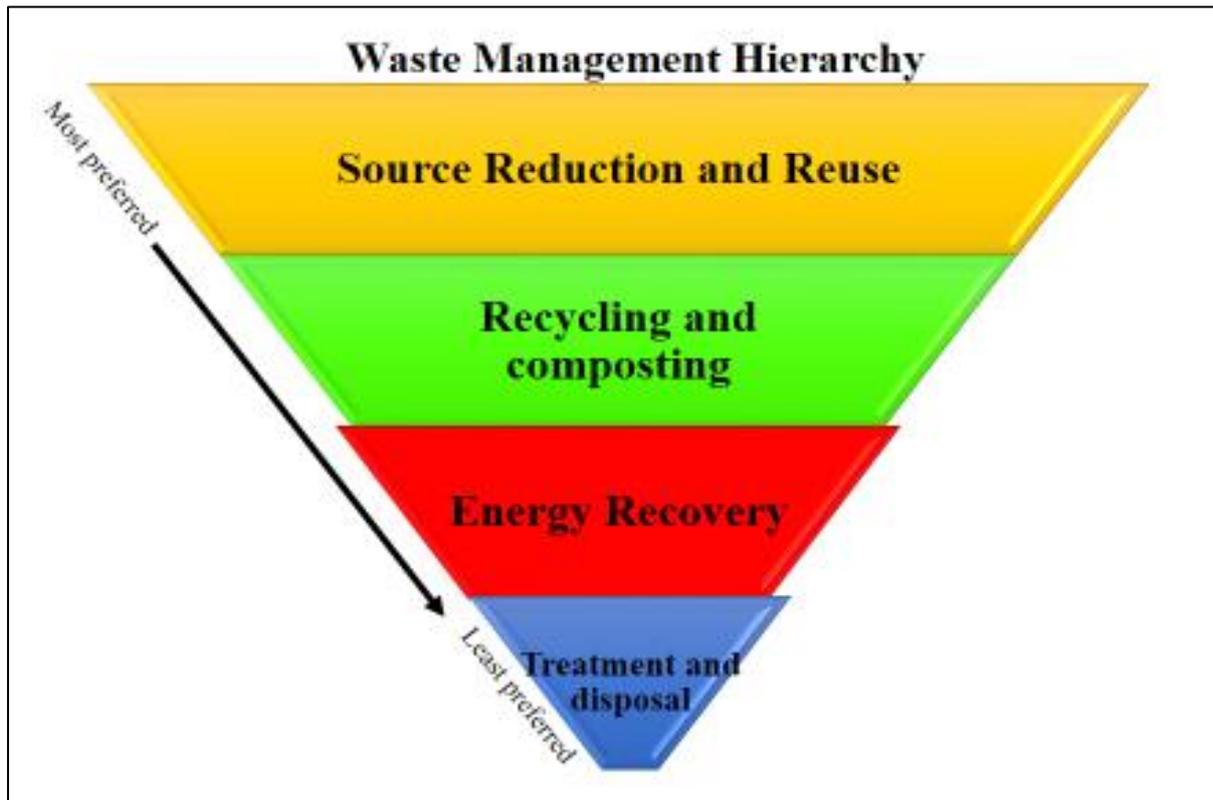


## Chapter 1: Introduction

### 1.1. Introduction of waste

Living Organisms use numerous varieties of matters which are of liquid, solid, and gaseous, for their existence (Huang et al., 2006). Some things can be utilized for longer period, some can be used for short duration, and depending upon its strength they can be either transformed into another potential material or became useless after short duration. The object which is discarded by humans or became useless or worthless is termed as waste (Zhou et al., 2014), (Xiao et al., 2007).

In the urban population encounter, enormous increments will be observed in the following 20 to 30 years (United Nations, 2014). While the World Bank in 2012 stated that this expansion of waste needs to successfully deal with a manner that is gainful for both human and ecological wellbeing. It also acknowledged that such type of strong waste management is additionally responsible for environmental change. Worldwide 5% of ozone depletion and 12% of worldwide methane (CH<sub>4</sub>) outflows due to the landfills and huge open dumping of solid waste (World Bank, 2012). In the management of such a huge waste, Integrated Solid Waste Management (ISWM) programs were created to keep human wellbeing. ISWM is a new and systematic method for the treatment of solid waste. As per the definition prescribed by the U.S. Environmental Protection Agency (EPA), ISWM is a complete waste reduction, collection, composting, recycling, and disposal system. Waste can be managed effectively to protect human health and natural environment through an effective ISWM programme which looks at how waste can be reduced, reused, recycled.



**Figure 1.1. Management of waste hierarchy established by the U.S. EPA. (<https://www.epa.gov/homeland-security-waste/waste-management-hierarchy-and-home-land-security-incidents>).**

U.S. Environmental Protection Agency (EPA) has developed a hierarchy of ISWM techniques which is shown in Figure 1.1. The hierarchy lists the various waste management techniques from the most favored to the least for the environment like reduction of source reuse, recycling and composting, energy recovery, and treatment and disposal (Basu, R. 2009). The hierarchy emphasizes the reduction, reuse, and recycling as essential to sustainable waste management.

Most human activities are waste generating (Brunner and Rechberger, 2014). Nevertheless, waste generation remains a major source of concern as it has always been since pre-memorable times. While, as per the study conducted by Vergara and Tchobanoglous in 2012, the rate and amount of waste have been increased and verity of waste has also grown as the volume of waste rises (Vergara and Tchobanoglous in 2012). In the pre-historic era, where waste was merely a source of a nuisance that required disposal and appropriate management of waste was not a big concern as the population was small, and at that time large amount of land was also available, while without any sort of degradation, the environment easily

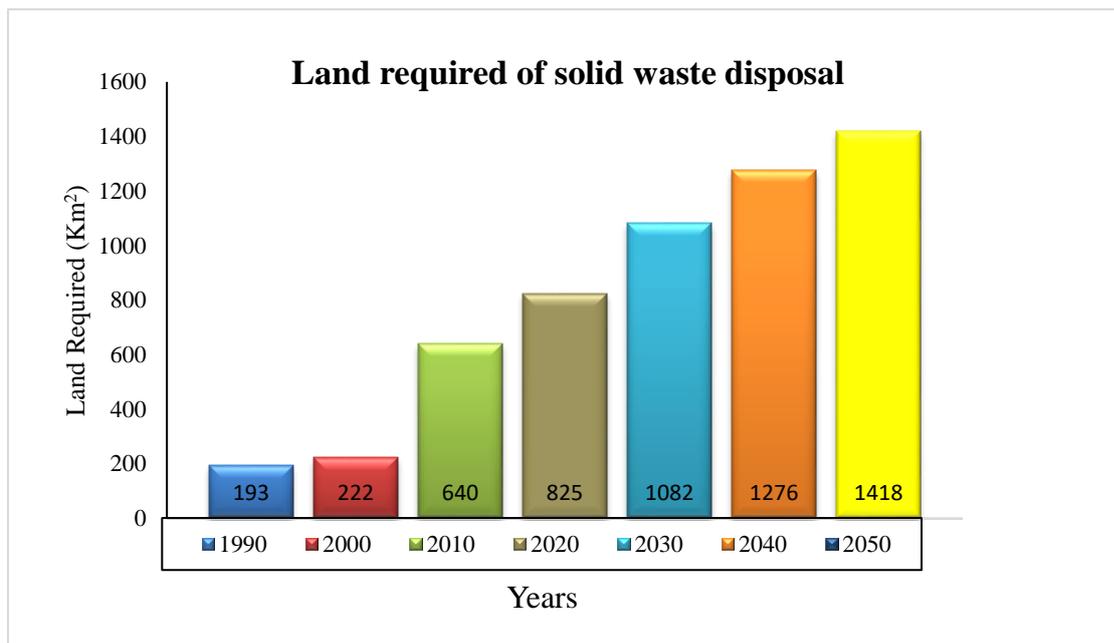
absorbed the volume of waste generated during those days (Tchobanoglous et al, 1993), (Biswas et al., 2010).

Whereas Wilson in 2007 mentioned the reason behind the generation of the huge amount of waste in the sixteenth century, according to him, people began to migrate from rural areas to cities during the industrial revolution, while this movement of people from rural to urban communities prompted population blast which turn led to an increase in the amount of waste in cities (Wilson, 2007). Many of the developing nations went through a period of eco-development. Today, however, most of these countries have tackled many of the health problems and environmental degradation issues associated with waste generation effectively (Dijkema, et al. 2000). Concerning the rising step of urbanization and growth in developing countries is now contributing to a continuation of the same historical problems that industrialized countries have to struggle with in the past (Williams, 2005). The table 1.1 describes projected the population growth and effect on waste formation.

**Table 1.1: Projected growth of population and inclusive effect on waste formation. (Annepu R K. 2012)**

<b>Year</b>	<b>Population (<math>\times 10^6</math>)</b>	<b>Per capita generation (kg per day)</b>	<b>Total waste generation (<math>\times 10^3</math> Tonnes per year)</b>
2001	197.3	0.439	31.63
2011	260.1	0.498	47.30
2021	342.8	0.569	71.15
2031	451.8	0.649	107.01
2036	518.6	0.693	131.24
2041	595.4	0.741	160.96

As per the study conducted by Kumar et al in 2017, the disposal of solid waste is at a vulnerable growth stage in India. There is a need to improve services to treat increasing volume of Municipal Solid Waste (MSW), and it is believed that more than 90 percent of India's waste is dumped unsatisfactorily. It is reported that the waste dumps occupied approximately 1400 km<sup>2</sup> in 2050 and this is projected to continue to go up in the future, this projection has been shown in the figure 1.2 (Kumar et al., 2017).

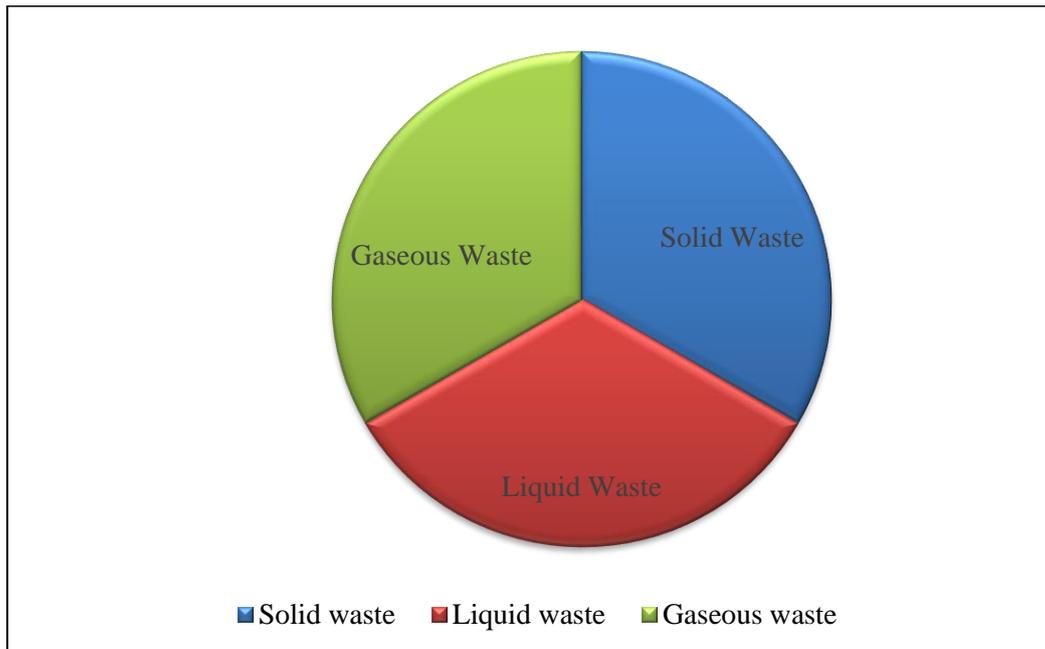


**Figure 1.2 Accumulative land required (km<sup>2</sup>) for the dumping of Solid Waste (Kumar et al., 2017).**

Concerning such a huge population of people in towns and villages, this increases volume and open dumping of waste, on the other hand, these dumps are accountable for the breeding grounds for rats and other vermin which have significant public health risks. Also, such types of toxic waste management activities led to many disease outbreaks with high death tolls (et al, 1993).

## 1.2. Types of waste

Waste can occur in different types that can be expressed in many ways. Some specific characteristics used in waste classification include physical conditions, physical properties, reusable potential, biodegradable potential, manufacturing source, and environmental effect (Demirbas, 2011), (Dixon & Jones, 2005). White et al in 1995 reported that, according to their physical states, waste can be generally divided into three main types which is shown in figure 1.3. These are liquid, solid, and gaseous waste. Though, it is clear that waste is classified into many types in different countries (White et al., 1995). Following are the most commonly used classifications of waste based on its physical state:



**Figure 1.3 Classification of waste based on the physical state of waste**

As per the various studies concluded that solid waste consists of many types, hence it is important to look briefly at the different types of solid waste.

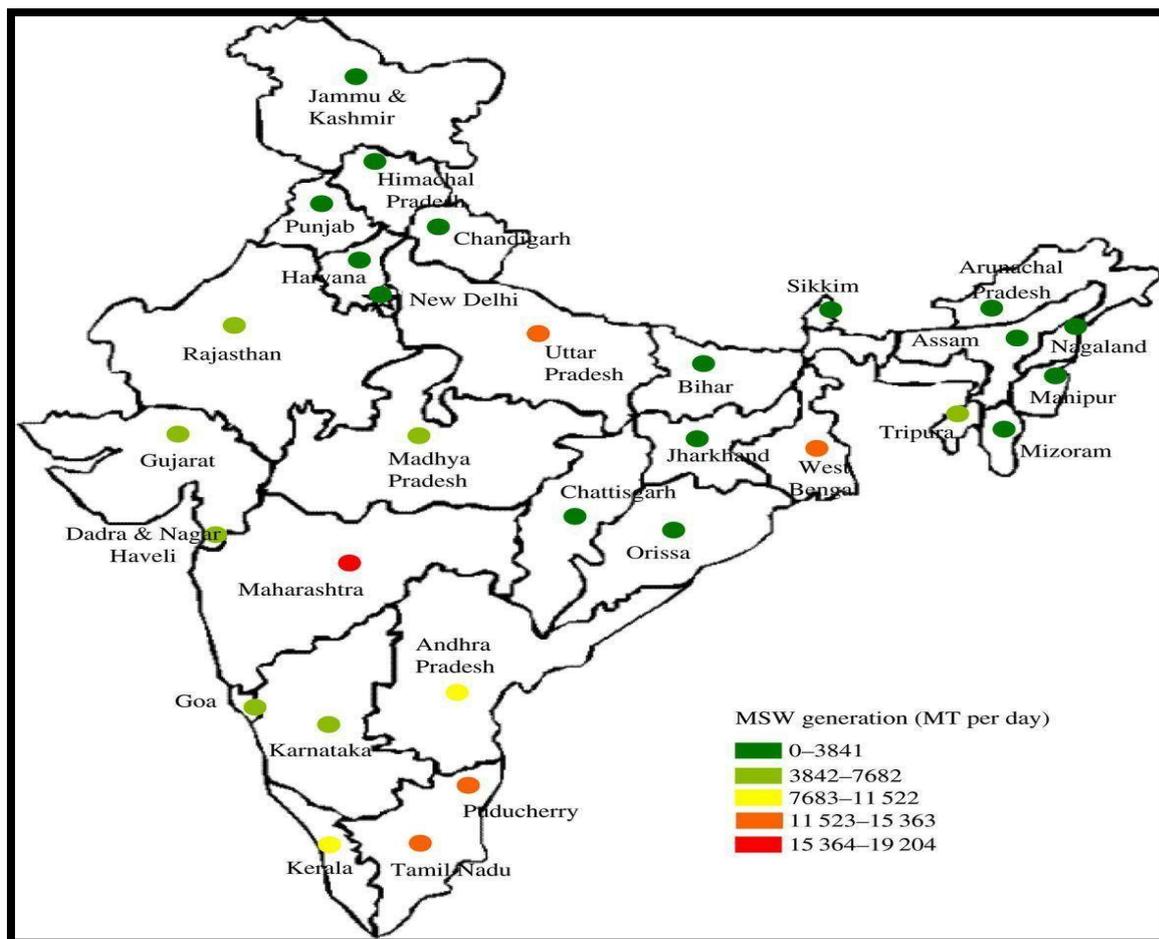
### **1.2.1 Municipal Solid Waste (MSW):**

Municipal Solid Waste is the most common and significant source of waste and it is also one of the most studied waste in the world. MSW is having several implications. Proper treatment should be given to solid waste and it need to be significantly managed as it is generated in enormous amounts (Amasuomo and Baird., 2016).

Definition of the municipal solid waste has been prescribed by Kaseva and Gupta in 1996 according to them municipal solid waste is the waste collected by the municipal authorities including household refuse, non-hazardous solids from manufacturing, commercial, institutional and non-pathogenic hospital waste (Kaseva & Gupta in 1996). The waste which is produced through domestic as well as commercial activities is also known as municipal solid waste (Buah et al. 2007). The lifestyles of people are responsible for huge urban solid waste generation and they also added improper management of MSW that adversely affect the public health and community (Jaillon, L et al. 2009). Management of MSW is difficult because of the complex component such as metal, paper, glass, and other organics which are hard to manage. The properties of MSW count mainly on its source. In the country like

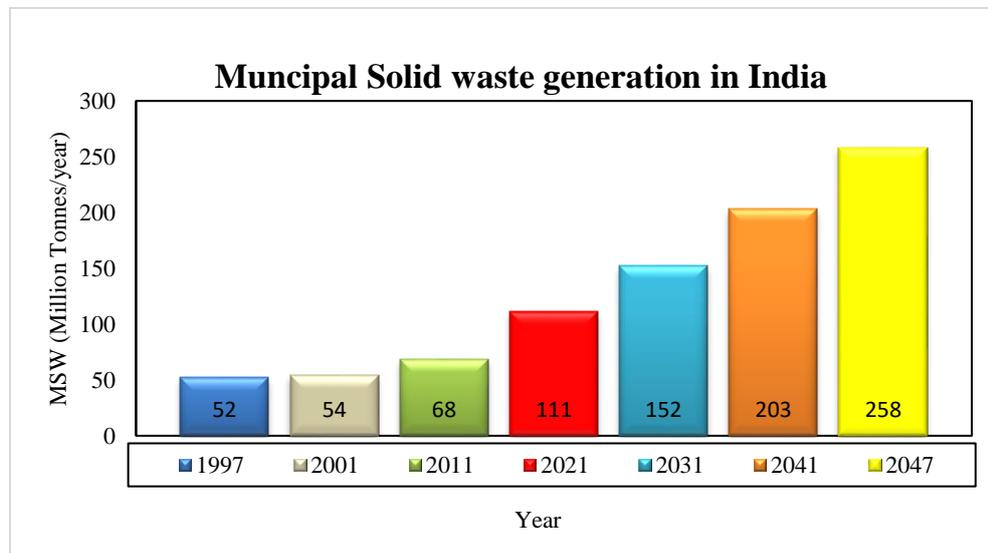
Turkey, in which nearly more than half of all MSW contains cardboard, paper, glass, and plastics make up a significant percentage of the total municipal solid waste (Berkun et al 2011), (Dixon & Jones 2005). Then the Buah et al. (2007) also prescribed the composition of MSW, according to them, MSW consists of food and garden waste, textiles, paper or cardboard, plastics, glass, and metals. As per their finding this unique composition of MSW could easily be used for energy recovery or for the fuel production (Buah et al. 2007). Compare to other more homogeneous waste sources with a decent percentage of each material, the municipal solid waste composition is complex and it will generally change from city to city and country to country (White et al. 1995).

According to the National Health Portal, The Ministry of Health and Family Welfare, Government of India, prescribed the sources of solid waste they are hazardous wastes, animal wastes, industrial waste, food wastes, non-hazardous waste, non- infectious medical wastes, mineral wastes, it also mentioned that garbage (highly degraded materials, like food), trash (immense items, like branches of tree or old piece of equipment), and rubbish (deliberately decomposable materials, like glass, paper, and metal things) also comes under the category of municipal solid waste (Kini B.S et al 2014). Mismanagement of this waste can create health-related problems to living things as well as to the environment (Lakshmikantha H. 2006).The Central Pollution Control Board (CPCB), Government of India has published State-level statistics generation of MSW in India from 2009 to 2012 which is represented in fig. 1.4.



**Figure. 1.4. State wise statistics generation of MSW in India (2009–2012). (Kumar et al., 2017)**

From fig 1.4. it is revealed that sixteen states generate 0 – 3841 metric tonnes (MT) of MSW waste per day, seven states are in the range of 3842 – 7682 (MT) of MSW waste per day, two states are in the range of 7683 – 11522 (MT) of MSW waste per day, four states are in a range of 11523 – 15363 (MT) of MSW waste per day, and one state that is Maharashtra are in the range of 15364 – 19204 (MT) of MSW waste per day.



**Figure 1.5. Municipal solid waste generation in India. (Singhal S. and Pandey S., 2001)**

Singhal and Pandey in 2001 showed the growth of municipal solid waste in India which is represented in 1.5. Fig. From the above figure it is conclude that the amount of municipal solid waste has been increased with respect to years.

**Table 1.2: State wise MSW status and required area of land for treatment in 2011 in India. (Kumar et al., 2017).**

City	Number of landfills	Area of landfills (hectare)
Chennai	2	465.5
Coimbatore	2	292
Greater Hyderabad	1	121.5
Ahmadabad	1	84
Greater Mumbai	3	140
Surat	1	200
Jabalpur	1	60.7
Delhi	3	66.4
Madurai	1	48.6

Indore	1	59.5
Greater Vishakhapatnam	1	40.5
Greater Bangalore	2	40.7
Nasik	1	34.4
Ludhiana	1	40.4
Kanpur	1	27
Jaipur	3	31.4
Srinagar	1	30.4
Chandigarh	1	18
Kolkata	1	24.7
Raipur	1	14.6
Ranchi	1	15
Guwahati	1	13.2
Meerut	2	14.2
Thiruvananthapuram	1	12.5

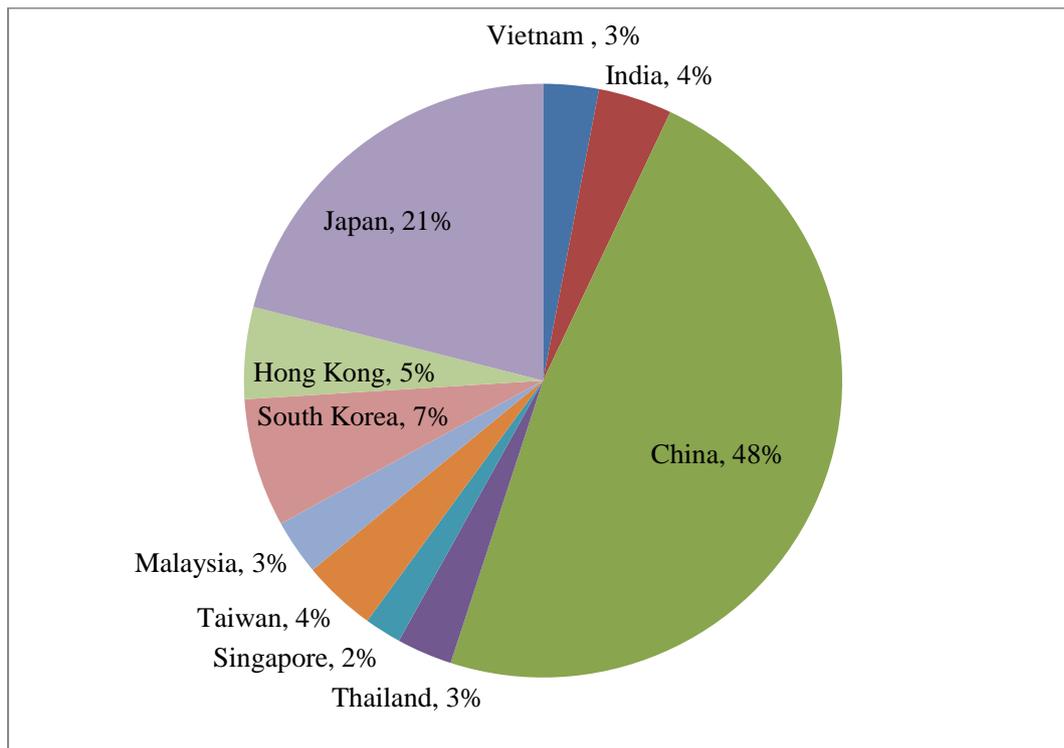
Table 1.2 Represents Planning Commission Report of Government of India in 2014. In this table the information is provided about number of required landfills and land for dumping of MSW through the nation. From this table it is revealed that the Chennai needs maximum area of land which is 465.5 hectare for handling of MSW. And Thiruvananthapuram needs minutest space of land that is 12.5 hectare for MSW treatment.

### 1.2.2. Construction Waste:

Solid waste which is generated from the construction industry is one of the main waste streams in many countries. U.S. Environmental Protection Agency (EPA) defines construction and demolition waste as waste materials consist of the debris generated during the construction, renovation, and demolition of buildings, roads, and bridges (Misra and Pandey 2005). 11<sup>th</sup> Five Year Plan of Government of India mentioned that the construction industry is second only to agriculture. In 2008, the MoEF (Ministry of Environment and Forest) Government of India had estimated that 0.53 million tons/day of waste will be generated in the country (Government of India., 2007).

In 2001, Poon et al. 2001 perform a study on construction waste which prescribed about per day 29674013 kilo grams of construction waste generates in Hong Kong. Their study has also expressed that most of the construction wastes produced in the country which included inert as well as non-inert materials (Poon et al. 2001). The same scenario can be observed in Scottish, according to the report published by SEPA in 2011, the construction industry per year contribute around £10 billion to the Scottish economy, Along with this, construction sector produced a large amount of solid waste like concrete, wood, metals, plastics, soils, glass, etc. SEPA projected that perhaps the industry produces around nine million tons of waste per annum. A similar phenomenon is experienced throughout the EU, the construction waste volume is on the rise and the waste produced is significantly high compared to the overall waste produced (Christian Fischer and Mads Werge., 2009). Eurostat statistics (2014) reported that construction waste in the UK accounted for around 100,999,493 MT<sup>2</sup> in 2008, while in 2010 it contributed about 105,560, 291 MT<sup>2</sup>of waste (Eurostat statistics 2014).

The below fig 1.6. shows the construction and demolition waste status in Asian countries which is based on the study conducted by the Asian Institute of Technology, Thailand and the different countries of Asia, published a report on management of waste in construction and demolition (C and D) on May 2008. In the analysis following countries are present such as, Japan, China, Hong Kong, Thailand, Vietnam, South Korea, Malaysia, Taiwan, and India. (Gayakwad and Sasane 2015).



**Figure 1.6. Evaluations of C and D waste in Asian nations (Gayakwad and Sasane 2015)**

### 1.2.3. Industrial Waste:

Industrial waste is well-defined as a waste that is generated while manufacturing of new products by treating raw products. These would be in plant manufacturing, mining, or even in factory and industry production (Ngoc and Schnitzer, 2009). According to some studies, in India, approximately 10 to 15 percent of industrial waste is hazardous. The Ministry of Environment, Forest and Climate Change, Government of India has described hazardous waste as any waste that is likely to harm the health of people or the environment because of its characteristics or due to its physical, chemical, or biological composition (Khan S, Faisal M 2008). In India different types of industries produce large amounts of hazardous waste; for example, petrochemicals, pharmaceuticals, plastics, fertilizers, and general engineering are one of the chief industries (An D et al., 2015), (Qdais HA, Alshraideh H 2016). It is stated that even in Malaysia, Indonesia, and Thailand, palm oil processing accounts for a huge amount of the total strong waste generation, it was also mentioned that in Thailand, from the palm oil sector, about 3.2 million metric huge quantities of solid waste are produced annually (Shafigh et al, 2014). The fig 1.6. represents disposal of untreated solid waste in open environment and submerging of industrial effluent in the water body.



**Figure 1.7. The untreated disposal of industrial solid waste on roads and mixing untreated industrial effluent into the water body. (<https://www.Indiatvnews.com/news/india-industrial-units-must-have-effluent-treatment-plant-sc-369946>), (<https://thenewdawnliberia.com/garbage-overflow-in-the-streets-of-monrovia-the-impact-on-human-health-and-the-environment>).**

#### **1.2.4. Agricultural Waste:**

Agricultural waste is characterized as unnecessary waste created from agricultural practices like manure, oil, silage plastics, poultry houses, fertilizers, pesticides and herbicides, waste from farms, and slaughterhouses; veterinary medicines, and horticultural plastics, etc (Hussein, and Sawan 2010). Fertilizers are a major source of agricultural waste whereas they involve nutritious minerals such as phosphorus, nitrogen, and potassium content that assist plants to grow more rapidly and increase production, but when exposed into the open environment that may result into the environmental pollution especially in the marine ecosystem (Sánchez et al., 2012), (Valencia et al., 2014). It was also concluded by the Seadi and Holm-Nielsen in 2004 that the improper administrative management of agriculture waste

may prompt environmental risk which may pollute surface as well as groundwater which is represented in fig. 1.8. (Seadi and Holm-Nielsen in 2004).



**Figure 1.8. The huge amount of organic agriculture wastes. This figure also represents open disposed of organic waste in the nearby water body. ([https:// www. Community food rescue. Org / the- problem /](https://www.Communityfoodrescue.Org/the-problem/)), (<https://www.exportersindia.com/omm-agro-industries/agriculture-waste-nagpur-india-1401257.htm>)**

### **1.2.5. Commercial Waste:**

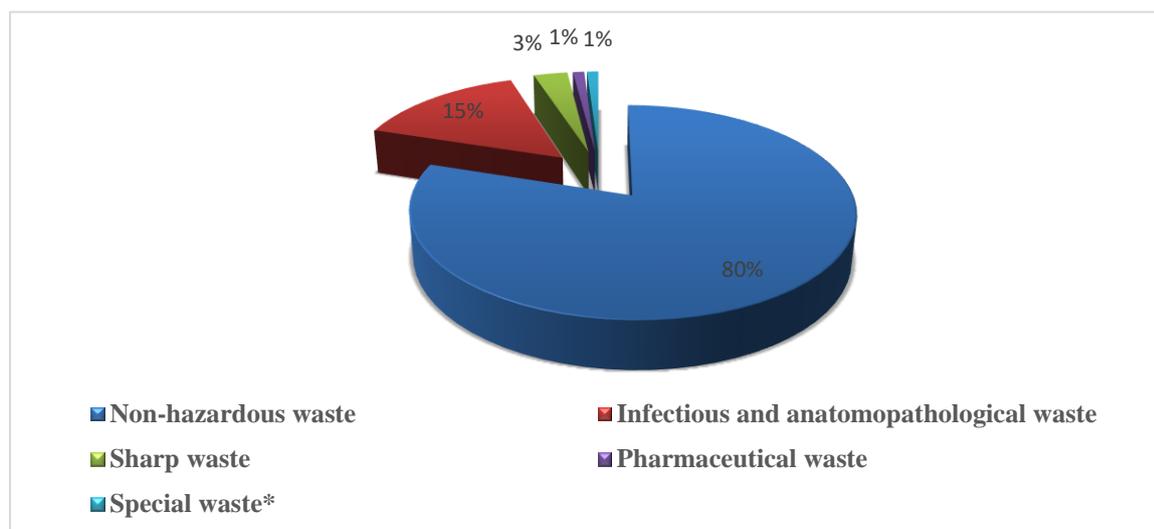
Commercial waste is generated through business premises primarily used mostly for trade, sport, entertainment or leisure. This does not encompass industrial or domestic waste; only waste produced from the commercial activity of any sort (Kasseva, and Mbuligwe 2000), (Kumar et al., 2009). The Environment and Heritage Service (2005) reported that in 2005 industrial and commercial activities produced around 1.5 million tons of commercial waste in Northern Ireland, so this report clearly showed that commercial activities can generate a maximum amount of commercial waste (Schoot Uiterkamp et al 2011). The same kind of situation was observed in England during 2002 where, Industrial and Commercial Businesses

in England represented around 11% of the total waste generated in the year 2002 (DEFRA, 2009).

### **1.3. Introduction of biomedical waste (BMW)**

The waste generated from the activities associated to the medical field such as the diagnosis, treatment or immunization of humans or animals or in medical research-related activities or in the manufacture or testing of biologicals, including the categories and in Schedule I of the BMW regulations 2016 is known to be a biomedical waste (Yadavannavar et al., 2010). During the gathering and disposal of biomedical waste there is the greatest risks to healthcare, sanitation employers as well as to the general community. The Bhagawati et al., conducted the study in 2015 and in their investigation it is confirmed that if biomedical waste disposed of without proper sterilization it may lead to AIDS, Hepatitis B & C, SARS, tetanus, psychosocial trauma, etc. (Bhagawati G, and Nandwani S, Singhal., 2015). The hazardous part of the waste presents physical, chemical, and microbiological risk to the general population and health-care workers associated with treatment, handling, and disposal of waste (Pinto et al 2014). With respect to the Indian scenario everyday approximately 2 kg/bed/ biomedical waste generates in India while, this the category of this biomedical waste includes anatomical waste, cytotoxic wastes, sharps, etc. (Aggarwal H and Kumar P, 2015). In India, the first BMW rule was notified by the Government of India, through the Ministry of Environment and forest in July 1998. Every health care establishment in the country is bound by the regulations in which by considering the hazardous nature of the biomedical waste it needs to be handled, segregated, collected, stored, transported, and disposed of safely (Hossain et al 2014).

While, as per the study done by Li and Jenq in 1995, about 10%–25% of BMW is hazardous, at the same time as 75%–95% is nonhazardous (Li CS and Jenq FT., 1995). Nikos et al., 2010 explained the structure of biomedical waste generated in the healthcare facilities which are represented in the fig 1.9. (Nikos et al., 2010).



**Figure 1.9. Structure of biomedical waste (Nikos E. Mastorakis et al., 2010)**

There is a need for proper strategies in hospitals for management of biomedical waste for those who are directly and indirectly involves in hospital work (Ola-Adisa et al 2015). Proper management often required because of inappropriate management of biomedical waste and poor incineration emission of ash may lead to environmental pollution like air, water, and soil (Aggarwal H and Kumar P, 2015).

The table 1.3. presents the types of infections determined by the contact with biomedical waste, pathogen agents and transmission

**Table 1.3. Infections affected due to biomedical waste (Nikos E. Mastorakis et al., 2010)**

Infection Type	Pathogen Agents	Transmission Path
Gastrointestinal infections	Enterobacteria: Salmonella, Shigella spp. Vibrio cholera Helminths	Feces or/and vomiting liquid
Respiratory infections	Mycobacterium tuberculosis Measles virus Streptococcus pneumoniae	Respiratory secretions, saliva
Eye infections	Herpes virus	Eye secretions
Genital infections	Neisseria gonorrhoeae Herpes virus	Genital secretions
Skin infections	Streptococcus spp.	Purulent secretions

Anthrax	Bacillus anthracis	Secretions of skin lesions
Meningitis	Neisseria meningitidis	LCR
AIDS	HIV	Blood, semen, vaginal secretions
Hemorrhagic fevers	Junin Viruses, Lassa, Ebola Marburg	Biological fluids and secretions
Septicemia	Staphylococcus species	Blood
Viral Hepatitis type A	VHA	Faeces
Viral Hepatitis type B and C	VHB, VHC	Blood, biological fluids

#### 1.4. Biomedical waste World scenario

The famous quote of Paul Russel teaches us a very informative lesson as "Nothing on earth is more international than the disease". However, there are no political or geographical boundaries to health and illness. Worldwide, biomedical waste was brought into focus in 1983. Whether in India, Tanzania, the United Kingdom, or the United States, countries around the world follow proper strategies for the management of biomedical waste in their respective countries (Hayleeyesus et al 2016). According to the World Health Organization (WHO), out of the total amount of biomedical waste generated in the world, about 85% is general, non-hazardous waste (Debalkie and Kumie 2017). Rendering to the (United Nations Development Programme) UNDP Global Healthcare Waste Project, an International level, the United Nations Stockholm Convention is to be the principal protocol for the disposal of Persistent Organic Pollutants (POPs) (Azage and Kumie 2010). Table 1.4. presents the national income level generation of biomedical waste and table 1.5. represent international generation of biomedical waste.

**Table 1.4. National income level generation of biomedical waste (Pruss et al., 1999)**

National income level	Annual waste generation (kg/head of population)
<u>High income countries:</u>	
All health-care waste	1.1-12.0
Hazardous health-care waste	0.4-5.5

Middle-income countries:

All health-care waste	0.8-6.0
Hazardous health-care waste	0.3-0.4

Low-income countries:

All health-care waste	0.5-3.0
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From the above figure it is revealed in high income countries all BMW waste generation is about 1.1-12. Kg /head of inhabitants and hazardous BMW generation is around 0.4-5.5 Kg /head of inhabitants, middle income countries generate all BMW 0.8-6.0 Kg /head of inhabitants and hazardous BMW around 0.3-0.4 Kg /head of inhabitants, and low income countries generate all health care waste around 0.5-3.0 Kg /head of inhabitants.

**Table 1.5. International level generation of biomedical waste (Pruss et al., 1999)**

Region	Daily waste generation (kg/bed)
North America	7-10
Western Europe	3-6
Latin America	3
Eastern Asia	
High-income countries	2.5-4
Middle-income countries	1.8-2.2
Eastern Europe	1.4-2
Eastern Mediterranean	1.3-3

The table 1.5. represents the international level biomedical waste generation from this figure it is conclude that North America has maximum BMW generation, daily it generate 7 to 10 kg/bed, and Eastern Mediterranean generate minimum content of biomedical waste that is 1.3 to 3 kg/bed daily.

### **1.5. Hospital waste management in developed and developing countries**

The biomedical waste associated problem in the United States is connected to the extended use of disposable items, which became prominent with the AIDS outbreak in the 1990s. (Chen, Ingfei, 2010). There are several guidelines regarding handling of biomedical waste, as per the studies which are conducted by Marinkovic et al., 2008, many developed countries impose stringent guidelines for the collection, transportation, and storage, of health-care

waste (Marinkovic et al., 2008). Concerning handling medical waste the regulatory bodies in Europe and the USA are strict. Pharmaceutical companies and hospitals are the biggest sources of healthcare waste. Such regulations prepared by countries have always helped the management of biomedical waste (Babu et al 2009). In the United States, the Medical Waste Management flea market is the one of the largest, led by Europe. It was projected by British Broadcasting Corporation (BBC) Research in 2004 that the demand for efficient management of medical waste is projected in 2018 to hit \$10.3 billion at a 4.9% cumulative annual growth rate (CAGR) (Marinković et al 2008).

Most hospitals in developed countries are beginning to look at recycling or donating leftovers that are still usable to developing countries, in fact, many of which urgently need such supplies. About a half of U.S. hospitals are sending their heir single-use items for re-processors some of it, which sterilize the items and resell them back to hospitals at a fraction of the cost (Chen and Ingfei, 2010).

The table 1.6 has shown about the category of healthcare waste and their respective treatment and disposal methods in England.

**Table 1.6. Categories of biomedical waste and their significant management procedures. (An Introductory Guide to Healthcare Waste Management in England & Wales, CIWN, 2014)**

<b>Category of Biomedical Waste</b>	<b>Treatment method</b>	<b>Disposal</b>
Infectious clinical	Alternative treatment or hazardous waste incineration	Energy from waste or landfill
Offensive waste	-	Energy from waste or Landfill
Non-medicine contaminated sharps	Alternative treatment or hazardous waste incineration	Residual ash recovery or landfill
Medicine contaminated sharps	Hazardous waste incineration	Incineration
Cytotoxic & cytostatic	Hazardous waste incineration	Incineration
Medicine waste	Hazardous waste incineration	Incineration
Medicine contaminated	Hazardous waste incineration	Incineration

infectious clinical waste

Domestic or municipal waste -	Re-use, recycle, energy from waste or landfill
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Waste management is indeed a delicate issue worldwide. Failing to disclose and ignorance has created various environmental issues; particularly in populated countries like China, India, Pakistan, and Bangladesh (Uddin et al 2014). These countries are some of the many countries documented to also have problems with waste management, especially with waste generated from health care facilities (Harhay et al., 2010),

It is observed that proper management of medical waste is essential and it is biggest problem in countries which are in developing stage. One of the important variances between developing and developed countries about the healthcare waste management have been shown by Nesli Ciplak & Songul Kaskun in 2015 as per their study-specific rules and regulations have been executed along with the endorsements for handling (collection, transportation, treatment, disposal) of healthcare waste in countless developed countries, whereas in case of developing countries such types of guidance are already published but the implementation of these guidelines are somewhere not taken seriously, it was also noted that the healthcare waste in developing countries is handled and disposed along with municipal wastes (Nesli Ciplak & Songul Kaskun 2015). The table 1.7. represents generation level of hospital waste in developing countries

**Table 1.7. Generation level of hospital waste in developing countries (Mustafa Ali et al., 2017)**

Country	Location	No. of facilities	Waste (kg/bed-day)
<b>China</b>	Nanjing	15	0.68
<b>China</b>	Shandong	23	0.6–1.5
<b>China</b>	Gansu	74	0.59–0.79
<b>China</b>	Binzhou	6	0.77–1.22
<b>Lao PDR</b>	Vientiane/Bolikhamxay	21	0.38–0.62
<b>Serbia</b>	Nisava/Tropica	3	1.9
<b>Turkey</b>	Istanbul	192	0.63
<b>Turkey</b>	Trachea	465	0.28–0.82

<b>Turkey</b>	Sivas	4	1.25–2.6
<b>Iran</b>	Fars	15	4.45
<b>Iran</b>	Tabriz	10	3.48
<b>Iran</b>	Tehran	6	2.3–3.0
<b>Iran</b>	Tehran	12	4.42
<b>Iran</b>	Tehran	8	2.75
<b>Iran</b>	Sistan/Baluchistan	14	2.76
<b>Iran</b>	Ahvaz	1	3.79
<b>Jordan</b>	North	4	1.88–3.49
<b>Jordan</b>	North	21	0.83
<b>Palestine</b>	Nablus	4	0.59–0.93
<b>Egypt</b>	El Beheira	8	0.85
<b>Sudan</b>	Khartoum	8	0.87
<b>Algeria</b>	Mostaganem	10	0.83
<b>Ethiopia</b>	Hawassa	9	3.46
<b>Nigeria</b>	Lagos	4	0.57
<b>Mauritius</b>	Port Louis/North	3	0.37–0.49
<b>El Salvador</b>	San Salvador	1	0.37
<b>Brazil</b>	Sao Carlos	1	1.07
<b>Brazil</b>	South	91	3.24
<b>India</b>	Belgaum	1	0.50
<b>India</b>	Lucknow	1	0.5
<b>India</b>	Lucknow	8	0.56
<b>India</b>	Karnataka	3	0.16–0.56
<b>India</b>	Maharashtra	14	0.08–1.04
<b>India</b>	West Bengal	8	0.19–0.51
<b>Bangladesh</b>	Dhaka	69	1.58
<b>Bangladesh</b>	Chittagong	1	1.28
<b>Pakistan</b>	Multiple	78	2.0
<b>Pakistan</b>	Gujranwala	12	0.67

## **1.6. Need of study and significance**

Hospitals and Medical facilities are very important for our society, without this it is very difficult to survive on the earth, however, waste generated through healthcare facilities creates a big problem for us (Isara et al 2015). It has proven that unscientific and inappropriate management of medical waste causes a direct health impact not only on the health of the people but also on the environment (Sabageh et al 2015). All types of waste generated by human activities are potentially hazardous to the entire environment. So, proper disposal of waste is a must, it may contaminate water, soil, and air (Ajmal and Ajmal. 2017). Among all these wastes, hospitals and other health care facilities can generate a large amount of waste which can cause infections, like HIV, Hepatitis B & C, and Tetanus, to particularly for those who handle it or come in contact with it (Radha. 2012). Therefore, hospital waste management has recently become an important and major issue not only to the hospitals, nursing home authorities but similarly to the environment (Mandal and Dutta 2009).

Chandra H, 1999 has stated some reasons behind the need of management of biomedical waste management, according to him, injuries caused due to sharps may lead to infection in all categories hospital employees, it may also have a risk for waste handlers and scavengers of infection outside the hospital at times which may infect the general public living in the vicinity of hospitals (Chandra H., 1999).

Poor regulated handling of healthcare waste is a threat to public health while, it has posed a significant danger not only to human health and safety but also to the environment as well as present and future generations too, therefore it is must to address this particular issue about the appropriate management of biomedical waste (Yenesew et al 2012). It also to be considered that the proper and effective management of biomedical waste is not only a legal requirement but also a social responsibility. Minimization of waste can be a starting of improving biomedical waste management (Ray et al 2014). While, the management of this biomedical waste can be governed by guidelines prescribed by the competent authority during the management it is essential to ensure that the health and safety precautions and preventive measures should be taken to protect health-care staff, patients as well as public (Debere et al 2013).

### **1.7. Future prospective**

The rising population of the world is accumulative the patients number, adding to the increasing amount of medicinal waste (Patil et al 2005). Moreover, there is a rising prevalence of diseases such as cancer and other chronic disorders, therefore, by considering the growing population, it is confirmed that the biomedical waste management sector has tremendous scope and importance in the future (Ozder et al 2013).

For economic perspective, healthcare waste management has tremendous scope, as per the report published by Transparency Market Research (TMR), the universal medical waste management market is projected to reach USD 22.3 Billion by 2025 with the cumulative annual growth rate (CAGR) of over 5.1% from 2017 to 2025. It was observed that over the past couple of years the demand for management biomedical waste has expanded enormously (Mbarki et al 2013). Because of the need for high-value machinery and advanced technology equipment, the need for massive capital investments is a major restraining factor for the growth of the market for medical waste management (Kumari et al 2013). The future to control over medical waste may involve robotic assistance. Cleaning of such medical products already requires robots, so robotics may be integrated into the hospital itself. An extremely contagious individual may be isolated in a room where a robot conducts some controls on vital signs, including pulse and blood pressure tests. The use of robotics for certain needs reduces the chance of spreading disease across the globe (Nemathaga et al 2008), (Mattiello et al 2013).

### **1.8. Statement of Problem**

The improper and inadequate management of biomedical waste will cause hazards not only to people but also to the environment. Several studies have been reported on negative impacts of biomedical waste on the environment as well as on human beings. According to Mohankumar and Kottaiveeran (2011), the biomedical waste produced from different sources varies regionally, internationally, and among hospitals, concerning India, due to over population in an urban area; the large quantity of biomedical waste has been generated through various healthcare facilities is attempting to keep up, it was observed that improper management of healthcare waste is highly dangerous for the environment and causes a serious health threat for inhabitants (Mohankumar and Kottaiveeran., 2011). In India, these problems are facing because there are about six lakh hospital beds in 23,000 primary health centers with 15,000

private and small hospitals due to poor management of pathological waste, also the situation will further become dangerous by the rise in population and due to the mushroom growth of private hospitals (Asadullah et al 2013).

Biomedical waste has stranded challenges in front of us to maintain the quality of water, air, and soil. It is proved that the inappropriate management of biomedical waste can have an adverse effect on the quality of the water as different pollutants that leach into the groundwater from the waste dumping sites. Improper biomedical waste can also pollute drinking water the heavy metals in leachate exceeded the standards for drinking water. Al, V, Cr, Mn, Co, Ni, Ba, Pb, and Fe 2.050, 0.9775, 2.800, 0.503, 0.128, 0.773, 0.8575, 0.130, and 39.25 mg / L, respectively (Singh et al. 2014).

In Lagos Nigeria, Oluseyi in the year of 2014, done the comparative assessment they studied the effect on the quality of neighboring groundwater near to the unrestricted waste dump site, After analysis they found that there is alteration in physico-chemical parameters of ground water, the heavy metal content of the ground was high (Oluseyi et al., 2014). It is also estimated that if biomedical waste not disposed of properly, then it may change the quality of soil which is near to the waste dumping sites (Chakraborty et al., 2014). Five heavy metals (chromium, nickel, zinc, lead, and copper) were analyzed by Abidemi and the high concentrations of heavy metals were present in the surrounding soil samples. The essential physico chemical parameters of soil are also altered due to the dumping of biomedical waste (Abidemi and Theresa 2015), (Auta and Morenikeji, 2013).

The Burning of biomedical waste can release various toxic air pollutants which will reach the altitude where it may pollute the air that is potentially harmful to human health (Chudasama et al 2017). The burning of biomedical waste is a severe problem for human health and the environment (Mane et al 2016). According to the study conducted by Javied et al. in 2008, their study it is confirmed that hospitals that generate a huge amount of waste and the waste which is treated with incineration will pollute the air by releasing contaminants like fly ash, toxins, hazardous organic compounds into the air (Javied et al. 2008). As per the study conducted by the Central Pollution Control Board (CPCB), Government of India, hazardous organic compounds such as diethyl phthalate, decane, dodecane, octane, nonane, methenamine, cyclobutane, carbon disulfide, and acetone diperoxide were determined in air samples near to the biomedical waste incineration plants (Sarkar et al 2006). Biomedical waste contains various types of hazardous contaminants which can cause damage to humans.

As per the study of Priyadarshini et al. (2016) inadequate management and treatment methods of biomedical waste can be chemically toxic, contagious, and frequently radioactive and poses a significant health threat to society (Priyadarshini et al. 2016).

**Based on above concern, the present thesis has following objectives.**

- ❖ To develop the biological methods for the treatment of organic biomedical waste.
- ❖ To analyze the physico chemical parameters like (pH, Electric Conductivity (EC), (Total Dissolved Solids (TDS), Biological Oxygen Demand (BOD), and Chemical Oxygen Demand (COD) of experimental sets.
- ❖ To analyze the physico chemical parameters like (pH, EC, organic carbon, organic matter, magnesium, calcium, water holding capacity, phosphorus, potassium, nitrogen content) of soil.
- ❖ To evaluate heavy metals content like [Manganese (Mn), Cadmium (Cd), Iron (Fe), Nickel (Ni), Copper (Cu), Cobalt (Co), Zinc (Zn), and Chromium (Cr)] in soil and plant
- ❖ To analyze the phyto-chemical parameters like (protein, chlorophyll content, polyphenol content) of plants.
- ❖ To covert organic biomedical waste in to value added fertilizer.

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## CHAPTER 2

# Knowledge and Consciousness Regarding Management of Organic Biomedical Waste

Review Article

WM&R

### Nanoparticles impact in biomedical waste management

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#### Abstract

Effectual management of biomedical waste is obligatory for healthy human beings and for a safe environment. Mismanagement of biomedical waste is a community health problem. Safe and persistent methods for the management of biomedical waste are of vital importance. This article reviews the classification of biomedical waste, sources, colour-coding system of biomedical waste and salient features of biomedical waste rules in 2016, and the future prospective of nanoparticles. The untreated disposal of biomedical waste is associated with a huge amount of risk, so the efficient treatment for biomedical waste is most imperative. The review also highlights the current methods for disposal of biomedical waste, biological treatments given to biomedical waste water in the effluent treatment plant, and impacts due to the current method. Management of biomedical waste is a great challenge in developed and developing countries. To manage the biomedical waste there is a need for cost-effective, ecofriendly and less contaminating approaches for a greener and safe environment. The awareness regarding waste management is of great interest not only for the community but also for associated employees.

## **Knowledge and Consciousness Regarding Management of Organic Biomedical Waste**

### **2.1. Introduction:**

The waste generated during the diagnosis, treatment, or immunization of human beings, animals, or research activities is known to be a bio-medical waste (Deb et al., 2017). Biomedical waste is not only generated in hospitals, laboratories, research activities but it can also be produced at homes by dialysis or using medicines, insulin injections, and even in rural areas through animal health activities (Vasistha et al., 2017; Sharma et al., 2013). The proper treatment and disposable facility must be provide to biomedical waste (Chudasama et al., 2013). If biomedical waste disposed of openly it leads to serious environmental problems and health hazards (Ramesh Babu et al., 2009). It is stated that 85% of biomedical waste is non- hazardous 15% is an infectious waste it is hazardous, radioactive, and toxic. In the USA, 15% of biomedical waste is an infectious waste but in India, it could rise from 15-35% depending on total biomedical waste generation (Rajakannan et al., 2013). The total generation of biomedical waste in India 0.115 million metric tons of waste per day and 42 million metric tons annually. There are several problems regarding the management of biomedical waste. There is plenty of confusion regarding the generation, segregation, transportation, and safe disposal of biomedical waste (Thakur and Ramesh, 2015).

International Clinical Epidemiology Network had performed a nationwide survey in that 25 districts and 20 states are involved. Form India Mumbai and Chennai had improved the management of biomedical waste (Singh et al., 2011; Kumar and Samadder, 2017). It was noted that 82% of cities are in the primary stage, 60% are in secondary stage and 54% is a tertiary stage in red category i.e. improper management of biomedical waste is observed in cities. Management of biomedical waste at source was a foremost challenge (Capoor and Bhowmik, 2017; Sapkota et al., 2014). The study was done in government and private hospitals and it is revealed that not a single doctor has knowledge regarding disposable categories of biomedical waste. A similar study

also clarifies that 26% of doctors, 43% of paramedical staff does not have awareness of the risk associated with the biomedical waste (Rudraswamy et al., 2012), (Kotasthane et al., 2017).

Management of biomedical waste is a priority issue in the hospitals, health care centers. Improper segregation, discarding of biomedical waste, and addition it with municipal waste can lead to the potential exposure of the health care employees, waste pickers, and the general public (Narang et al., 2012), (Makajic-Nikolic et al., 2016), (Mata-Alvarez et al., 2000). Landfills and incinerators are widely used methods for disposal of biomedical waste, in spite of growing recycling activities (Marinković et al., 2008). Biomedical waste management will directly relate to potential health hazards to the environment and public health (Baguma, 2016), (Ehtesham et al., 2017).

**Table 2.1: Classification of organic biomedical waste and overall biomedical waste : category, type of waste, colour coding, treatment** (Sharma and Gupta, 2017; Singh et al., 2014; Kharat et al., 2017).

Colour	Waste type	Colour coding	Treatment
Yellow	Human anatomical waste Animal anatomical waste	Yellow coloured plastics container or yellow coloured non chlorinated plastics bags.	Incineration or deep burial or plasma pyrolysis.
	Expired or discarded medicines	Yellow coloured plastics container or yellow coloured non chlorinated plastics bags.	Incineration or plasma pyrolysis.
	Soiled waste Chemical waste	Yellow coloured plastics container or yellow coloured non chlorinated plastics bags.	Incineration or deep burial or plasma pyrolysis.

	Mattresses beddings contaminated with blood or body fluids	Yellow coloured plastics container or yellow coloured non chlorinated plastics bags.	Chemical disinfection followed by incineration or plasma pyrolysis.
	Microbiological and clinical waste	Safe autoclave plastic bags or containers.	Onsite pretreatment with sterile then after incineration.
Red	Recyclable contaminated waste	Red coloured plastics container or red coloured non chlorinated plastics bags.	Onsite pretreatment with sterile then after incineration.
White	Sharps and metals	Leak proof, puncture-proof, and tamper proof containers.	Dry heat sterilization, autoclaving followed by shredding or mutilation.
Blue	Glassware and metallic body implants	Cardboard box with blue colour marking.	Autoclaving or disinfection and for recycling.

**2.1.1. Types of organic BMW and overall BMW:** (Tudor et al.,2005), (Arora et al., 2014), (Rao et al., 2004), (Matsuo et al., 2011).

**2.1.1.1 Infectious waste:** Waste which includes pathogens for example laboratory waste, blood, tissues, cotton swabs, excreta, waste from isolation wards, infected equipment.

**2.1.1.2. Pathological waste:** Waste which contains human tissues, body parts, blood samples, and other body fluids.

**2.1.1.3. Genotoxic waste:** Waste containing cytotoxic drugs used in cancer therapy and genotoxic chemicals.

**2.1.1.4. Pharmaceutical waste:** Waste containing expired medicine or no longer needed product, drugs and contaminated pharmaceuticals chemicals are known to be a pharmaceutical waste.

**2.1.1.5. The high content of heavy metals waste:** Unused batteries, broken thermometer, gauges of blood pressure, gas cylinders, pressurized containers.

**2.1.1.6. Radioactive waste:** Waste which contains infected glassware, urine, and excreta of patients treated with radionuclide is known as radioactive waste.

**2.1.1.7. Chemical waste:** Chemical waste include laboratory chemicals, solvents, and disinfectants that are expired.

**2.1.1.8. Sharps:** Sharps include broken glasses, blades, syringes, scalpels, needles, and other things that can cause a puncture.

**2.1.2. Sources of organic BMW and overall BMW:** (Franchini et al., 2004; Zimmermann, 2017; Kumari et al., 2013).

The biomedical waste is classified into two sources they are major sources and minor sources

**2.1.2.1. Major sources:**

- Government hospitals, private hospitals, health care centers, nursing homes.
- Medical colleges, veterinary colleges, and research centers.
- Animal research centers, biotechnology institutes.
- Blood banks, mortuaries, and production units.

**2.1.2.2. Minor sources:**

- Physicians and dental clinics.
- Animal and slaughter houses.
- Blood donation camps.
- Vaccination centers.
- Acupuncturists and cosmetic piercing.
- Institutes for disabled persons.

### 2.1.3. Salient Features of Biomedical Waste Rules 2016.

On 28th March 2016, Ministry of Environment, Forest & Climate Change, Government of India has published the official notification no G.S.R. 343(E) in the government gazette on biomedical waste called as "Biomedical Waste Management Rules, 2016" came into force in supersession of the Bio-Medical Waste (Management and Handling) Rules, 1998. According to the Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), Puducherry, India, published the following major salient features of Biomedical Waste Management Rules 2016 which include: (Sanjeev et al., 2014), (Datta et al., 2018), (Mathur et al., 2012), (Ministry of Environment Forest and Climate Change : Government of India, 2016).

- The rules have been extended to include various fitness camps such as blood donation camps, vaccination camps, and surgical camps.
- All health care facility should have an occupier person having organizational control over the biomedical waste management.
- Biotechnological waste, laboratory waste, and blood bags should be mandatorily pretreated before disposal. The sterilization method should be followed by the National AIDS Control Organization (NACO).
- Phase out used chlorinated bags, blood bags, gloves.
- Training should be given regarding biomedical waste management to control diseases.
- The liquid biomedical waste should be segregated and pretreatment should be given at source and then it should be disposed of.
- The operator of the biomedical waste treatment plant should notify the detail information of waste and the biomedical waste should have a barcode. Five years of records should be maintained of incineration, autoclaving, hydroclaving.
- If a common biomedical waste plant is located at a distance of seventy-five kilometers than the health care facility shall not set up onsite biomedical waste treatment plant. If a common biomedical waste treatment plant is not accessible than after occupier should establish treatment facilities by taking prior appraisal from approved authority.
- Standard limits for incineration is SPM – 50mg/nm<sup>3</sup>, standard limit for dioxins and furans – 0.1ng TEQ/Nm<sup>3</sup>, the time duration in the incineration secondary chamber is two seconds.

- The biomedical waste rules are yearly implemented by the Ministry of Environment, Forest, and Climate change. The reports of biomedical waste after every six months should be submitted to the State Pollution Control Board.

## **2.2. Compels Associated with Organic Biomedical Waste Management**

Due to indiscriminate and improper disposal of organic biomedical waste by some hospitals and health care centers, the management of biomedical waste is not up to the mark. There has been a mixing of organic biomedical waste with general waste and it has been dangerous to the environment and human beings (Mathur et al., 2012). The mixing of organic biomedical waste with urban solid waste causes environmental pollution as well as growth in rodents or worms and it eventually favors transmission and increase in diseases like hepatitis, cholera, typhoid AIDS through injuries from infected and contaminated sharps (Mandal et al., 2009). The improper treatment of organic biomedical waste also increases insects, rodents, flies as well as dogs and cats which will spread diseases like rabies and plague. So it is necessary to undertake appropriate organic biomedical waste management practices for maintaining a good environment and declining health hazards (Chitnis et al., 2005).

### **2.2.1. Steps in Organic Biomedical Waste Management**

Several steps should be followed for efficient management of biomedical waste from waste segregation till waste disposal the biomedical waste should be handled carefully. The steps for the management of organic biomedical waste are discussed below (Da Silva et al., 2005)

#### **2.2.1.1. Waste Minimization**

The prevention of waste material or waste reduction is the most significant method for organic biomedical waste management. The material which is of good quality can be reused which results in a decrease in waste production. The advanced techniques which are involved in minimization of organic biomedical waste and their application have led to commercial as well as successful replacement in product development (K. Sudhir 2006). For the waste minimization various technologies have been developed and also it has beneficial to industries that help in creating value

laterally with increasing the quality of work. In waste minimization technique reusing of contaminated material or reusing of second-hand materials, repairing of contaminated materials should be avoided. While manufacturing the products it should not be designed like one-time use reusable materials or products should be manufactured. In hospital workshops should be arranged on the management of organic biomedical waste and in that workshop information about various steps of waste minimization should be given to (Charania and Ingle 2011).

### **2.2.1.2. Waste Segregation**

Segregation is the most significant factor in waste management system. In hospitals depending upon the categories there are specific colour code containers for the segregation of organic biomedical waste in that container the waste should be stored for a specific period and then it should be transported disposal site. The waste which is to be a deep burial or incinerated should be stored in yellow colour plastic bin or bag. The waste to which is chemical treatment autoclaving, microwaving, and finally for recycling or secured landfilling should be stored in blue or red plastic containers or bags (Gautam et al., 2010). The waste sharps such as blades, needles, etc. which are used for shredding or destruction must be stored in white colour puncture proof plastic container, after final disposal that waste will be recycled. The cytotoxic waste, chemical waste, expired medicines should be stored in a black container or plastic bag with cytotoxic labels which to be disposed of as a secured landfill. All the containers and plastics bags should have biohazard labels and black colour container and the plastic bag must have the cytotoxic label (Sharma et al., 2008), (Surjit et al., 2008).

The step of organic biomedical waste management also includes reuse, reduction, and recycle. Reuse of various things like medical equipment, chemical, etc. will reduce the generation of waste and ultimately also reduces the treatment cost (Gayathri et al., 2005). Recycling of particular materials like shredded plastics contaminated materials will reduce the total generation of biomedical waste. When the segregation of waste is done at the point source eventually the contamination or infection changes to health care workers or the rack pickers will also get reduced (Hegde et al., 2007). The sharps or the blades are also must be separated from the rest of the waste and it is to be subjected in puncture proof container. Mixing of glass waste with a contaminated body part, biological waste or with cytological waste should be avoided (Kautto et al., 2004)

**2.2.1.3. Storage of Biomedical Waste.**

The storage of biomedical waste should be according to Biomedical Waste Management and Handling Rules. The storage of organic biomedical waste may occur at a point source, in a treatment source, or a disposable site. In some of the hospitals before disposal of organic biomedical waste it is stored in a refrigerator for avoiding aesthetic and contamination problems (Marinkovic et al., 2005). The locality of the storage area is present near the waste treatment site. Inflexibility to liquid is essential for walls and floor, and easily follow up of washing processes. Regular cleaning and regular disinfection of storerooms are also mandatory. For the storage of organic biomedical waste for a long period the deep freeze should be present in hospitals and research centers (Murthy et al., 2011).

**2.2.1.4. Containers and Labeling of Organic Biomedical Waste**

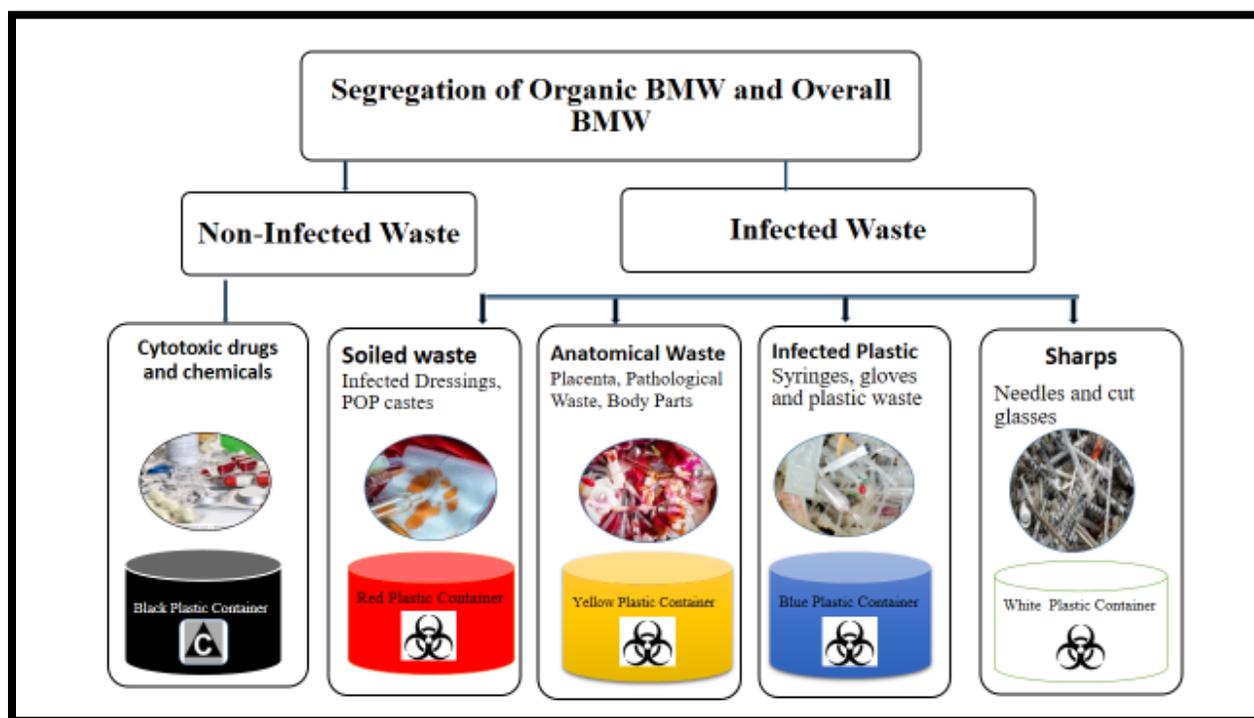
Puncture proof and non-leak plastic containers should be used with appropriate labeling and regular cleaning and maintenance of plastic containers are required. The container or the plastic bags which contain biohazard material should be sealed. The container which is having the capacity to withstand chemical, as well as thermal treatment, should be used for the storage of organic biomedical waste (Khan et al., 2001). Rigid and inflexible containers that are resistant to rupture and must be compressed well should be used for the storage sharps and blades and appropriate disposal and it should withstand the pressure of 40psi without getting ruptured. The plastic bags or the containers which are of heavy-duty should be used for non-hazardous waste and it should be labeled biohazard symbol. The bags or containers with red colour or orange colour should not be used for non-hazardous waste (Pasupathi et al., 2011). Heavy or the rigid containers which are to be autoclaved should be used for hazardous material and appropriate labeling and sealing must be done. The containers which are used for the storage of organic biomedical waste every bag or container should be appropriately labeled. The daily records of collection and storage of organic biomedical waste should also be maintained along with the name and contact details of the in charge person. Clear identification of each container of organic biomedical waste which is untreated along with appropriate colour coding and labeling should be done (Ndiaye et al., 2003).

**2.2.1.5. Handling and Transportation of Organic Biomedical Waste**

In hospitals and health care centers, a huge amount of organic biomedical waste has been generated with a broad range of characteristics and composition which has a higher prospective of contamination, injury, and infection than any other waste. The current method of organic biomedical waste management has been followed by many hospitals the proper segregation of infectious waste, construction waste, office, food, general, chemical and hazardous waste has followed but during the transportation all the segregated waste has been mixed and disposed off (Neema et al., 2002). So the collection and transportation of organic biomedical waste should be done by avoiding any possible threat to the environment and human health. The handling of organic biomedical waste must be done by a technical person who has undergone appropriate training. After the generation of organic biomedical waste it should be immediately disposed of or segregate into colour coding containers or bags (Rao et al., 2013). While handling the organic biomedical waste there is a huge risk that should be reduced by using suitable personal protective equipment. Should not mixed general waste like waste papers, kitchen waste, files, with organic biomedical waste. If in-hospital treatment plant is not present the untreated infected organic biomedical waste should be transported to another treatment site (Saurabh et al., 2006).

**For transportation of organic biomedical waste subsequent points need to be considered:**

- When the organic biomedical waste is transported from one site to another site it should be placed in a separate colour container. The person who is carrying the organic biomedical waste employing transportation separate cabin should be present for the carrier.
- The quality of the container in which the waste is to store should be checked.
- The waste storage cabin must be designed in such a way that it should be comfortably washed with disinfectants and accelerates preserving containers of organic biomedical waste in rows.
- The inner surface of the cabin must be designed smooth enough that it should minimize the stagnation of water.
- There appropriate space and side opening should be present for loading and unloading of organic biomedical waste.
- Appropriate labeling of the organic biomedical waste symbol should be done to means of transportation (Chitnis et al., 2003), (Baveja et al., 2000), (Almuneef et al., 2003).



**Figure 2.1: Segregation of biomedical waste.**

### 2.3. Treatments given to organic biomedical waste and overall biomedical waste

#### 2.3.1. Current treatments for disposal of organic biomedical waste and overall biomedical waste are as follows:

##### 2.3.1.1. Incineration.

In Incineration at the great temperature, the biomedical waste is oxidized and infectious substances which are present in waste are destroyed this method is also recognized as controlled combustion method. The temperature of incineration varies from 980 to 2000 °C. Once the waste is incinerated that waste cannot be reused, recycled, or disposed of in dumping sites. The most important feature of the incineration treatment is to diminish the amount of waste by 80–90%. In some modern incinerators, temperatures are high enough and molten material is produced, from that volume is reduced to 5% or even less than that. In the cities of India, the incineration method is not much practiced. This is due to the high moisture content, organic material, low calorific value content, and high inert content (Ranzi et al., 2011). There are three different types of incinerators they are

multiple hearth type, vogue, rotary kiln, and air types. Ensuring combustion at an ideal level both primary and secondary chambers of combustion are present in the above three incubators. Most of the biomedical waste has been incinerated but from the incineration, toxic and carcinogenic compounds are released which inclines to a huge quantity of environmental pollution, cause damage to the reproductive system and hormonal imbalance. The operating cost of the biomedical waste is 13 per kilogram (kg) and energy recovered can cost 8 per kg, thus the net cost would be Rs 7 per kg (Delmonico et al., 2018).

The waste which is not integrated for incineration is pressurized gas containers, silver salts, radiographic waste, reactive chemical waste, heavy metals, batteries, sealed ampoules, radioactive materials, unstable pharmaceuticals waste (Hiremath and Goel, 2017).

### **2.3.1.2. Autoclaving.**

Autoclaves disinfect a variety of infectious waste like cultures, medical equipment, and sharps, materials infected with blood, microbiological waste. Autoclaving is a thermal process where waste is brought into direct contact with temperature for a satisfactory duration to disinfect the waste. The autoclave should be able to hold up the repetitive build-up and liberate of steam pressures and should have constructive materials, engineered design, the accuracy of temperature and pressure should be maintained and testing must meet necessities to activate safely as per the international standards associated to a pressure vessel (Sharma, 2014). Workers who operate steam autoclaves should be systematically trained in the procedure of the apparatus. Autoclaves are generally in cylindrical vessels and have a provision for unloading and loading of waste. In the vessel, jacket steam is introduced with high pressure and temperature (Randhawa and Kullar, 2011). When the steam is transmitted rapidly heat is produce to the waste which in turn yields steam on its own. This process efficiently destroys contaminants and purifies the dry waste. The autoclave should be operated for 121<sup>0</sup>C at 105 KP a pressure for 60 min. the most used methods of autoclave are induced vacuum method and gravity displace method (Çalis et al., 2014). In induced vacuum method wherein the steam is introduced and in gravity displacement method in the chamber the air is displaced (Rajor et al., 2012).

Pathological waste, anatomical waste, organic solvents, low-level radioactive waste, chemotherapy waste, laboratory chemicals must not be autoclaved this waste is not degraded in an autoclave or at normal temperature. Organic biomedical waste which contains oxidizing agents such as sodium hypochlorite or another solvent must not be autoclaved (Jokstad and et al., 2006, Horsted 2004)

The efficiency of decontamination or detoxification of biomedical waste is reliant on the time, temperature, volume, size, and of the waste. The autoclaving cycle should be efficiently monitored either by the biological or chemical indicator (Kishore et al., 2000). The chemical indicators can only identify the temperature the time duration has not been identified, and chemical indicators are not recommended. The biological indicator found to be more consistent like the presence of *Bacillus stearothermophilus* (Al-Khatib et al., 2010). Based on its frequency the use of the autoclave should be substantiated regularly. Special consideration should be given to type plastic bags which is to be used in the autoclave. Some plastic bags can melt and certain bags hamper steam penetration during the process of the autoclave. So to assure the efficiency and integrity of plastic bags it should be assessed under working conditions. For relief and safety in operation, the autoclave should be a horizontal type and designed for the treatment of waste (Mcmanus., 2003).

### **2.3.1.3. Microwaving.**

Microwaving is a steam-based process where microwave generates energy by moist heat and steam with a cycle of 30 min to 1 hrs. The types of biomedical waste treated under microwave technology are laboratory waste, contaminated waste, infected material blood, and body fluid, sharps, spoiled waste (e.g., used cotton, bandages, and bedding). Cytotoxic, explosive compounds, dangerous waste, radioactive waste, contaminated animal cadaver, and body fragments should not be treated with microwaving technology (Bhatt et al., 2018). The toxic metals which are present in soil are remediated by the microwaving technology. In microwave thermal effect of electromagnetic radiation spectrum lying between the frequencies 300 and 300,000 MHz are given to waste and due to which microbial inactivation occurs. It has been reported that industrial and sewage sludge is treated with the microwaving treatment and excellent reduction in volume is observed. To heat

dielectric materials in many technological and scientific fields microwave technology has been applied (Manoj Kumar et al. 2018). In the field of environmental chemistry, the microwave is very valuable as it extracts a huge range of pesticides, organic compounds, and fats from soils. Microwave heating has the supplementary advantage of selective heating, shorter heating time, and no direct contact with materials as compared to the conventional heating method. The residue which is treated in microwaving is tolerable for disposal in municipal landfills specifically if waste is shredded, incapable of causing injury and if macerated to reduce it unrecognizable (Sahoo RC et al., 2013).

#### **2.3.1.4. Hydroclave.**

The hydroclave method is an innovative combination of waste fragmentation, dehydration, and waste sterilization similar to autoclaving. A hydroclave is a horizontally mounted, double-walled cylindrical vessel, with one or more top-loading doors and at the bottom a smaller unloading door and motor-driven shaft are fitted in a vessel to which the powerful mixing or fragmenting arms are attached which inside the vessel rotates slowly (Jahnavi et al., 2006). Heat is transmitted rapidly to the fragmented waste, when steam is hosted in the vessel jacket, which in turn, yields steam on its own. The resultant energetic interaction in the hydroclave is, the waste will get sterilize by high-pressure steam and temperature, as such of autoclave but heat penetration is more and faster than autoclave (Mostafa et al., 2009). Thus this treatment offers both destructions of pathogen and reduction of waste by 80% of its volume and 50% by its mass. As compared to other technologies the wealth price of a hydroclave is high. The operator training is nominal as this method does not involve a multifaceted process. The extensive permitting does not require by this technology and operation cost is also low. This equipment must not have public acceptance. The machine is presently accessible in the size range of 200 – 350 kg h (Patel et al., 2012).

**2.3.1.5. Needle Melter (for needle).**

The needles are disinfected and melted by soaking them for 30 minutes in a recently prepared 1% hypochlorite solution. The needle melter is used to destroy immediately used needle. The needle for a few seconds is put inside the hole and after putting in the holes it melts without any trace. Consequently it safeguards sterilization and due to high temperature and eradicates chances of ADIS and hepatitis (Radha et al., 2009), (Saini et al., 2005).

**2.3.1.6. Needle destroyers or cutters.**

There are two types of needle destroyers or cutters electrically or mechanical operated. The needle destroyer can be fixed on the table or bench are they are portable or light. It can be used at clinics, nursing stations, blood collection centres, pathological laboratories are at localities where needles are used. The purpose of using needle destroyers is to prevent the reuse of non-reusable needles or syringes (Schulster et al., 2003). The electrical needle destroyers can either burn the needle or shear the needle or destroy it, leaving a deposit of steel. The needle destroyer is very much effective and safer instrument but is dependent on electricity. In this device, at the time only one needle can be destroyed. Different sizes of needles can be accommodate in both types of instruments. In mechanical and electrical needle cutters the cutting time duration required is of one minute. The needle which is separated from the syringe can also be categorized under sharps and must be accordingly handled. Before disposal the needle must be disinfected. Hence, the person who is operating the instrument must wear gloves (Barbosa et al., 2014), (Botelho et al., 2012).

**2.3.1.7. Syringe Crushers**

This instrument is not known to most of the peoples, by using a syringe crusher the syringe can be safely disposed of after removal of the needle. This process is done specifically to avoid the reuse of syringe in the blood bank, pathological laboratory, etc. The syringe crushers is mostly used in health camps and in immunization camps where waste shredder is not available and a large number of disposable syringes are used (Caniato et al., 2015). The syringe crushers include two plates one

is movable and the other is fixed. By a lever a movable plate is operated. Between the two plates the syringes are kept and then they are crushed. Depending upon plates, size more than one syringe at a time can be crushed (Chen et al., 2013).

### **2.3.1.8. Shredding**

The paper and elastic waste can be destroyed by the shredder to prevent its reuse. In a shredder the waste which is disinfected can be used. The purpose of a shredder is to damage the waste which has been already disinfected. In the process of shredding is, the biomedical waste are decamped or cut into small pieces so that it makes waste unrecognizable. It supports in the prevention of recycling waste and also acts as an identifier that the biomedical waste are disinfected and secure to dispose of. The shredder is mostly used in combination with a microwave or autoclave. It can be used in common treatment or individual institutions and disposal facilities. The shredding reduces the size and bulk of waste which makes transportation easy (Sharma et al., 2013), (Gupta et al., 2009). Shredder could also be used if the diminution of a waste volume is preferred. Single or multiple shaft shredders are specially designed for biomedical waste. 80% bulk of biomedical waste is diminish by advanced shredder. The advanced shredders are normally low speed, high torque, and distinct pass shredders with easily expendable cutters and with expulsion screens that control the size of shredded biomedical waste. When this waste is treated with shredding treatment the things can be recycled or reclaimed. The waste is submerged into a hopper and a set of shafts and blades starts revolving and then waste has broken down into small pieces (Sahoo et al., 2013). This waste has gone through the mesh and at the bottom is has been collected. The superior particles which are retained on the mesh are again passed through the cutter. The problem in a shredder is mainly in blades and shafts which undergo tear and wear and periodic replacement is needed. Between the blades when particles get accumulated or in each month the shredder must be cleaned. A shredder consumes the power of 15KW and it occupies a space of 1.5 sq.m. (Lee et al., 2004), (McGain et al., 2014).

### **2.3.2. Disposal methods for organic biomedical waste**

#### **2.3.2.1. Deep Burial.**

The site of deep burial should be impermeable and no narrow must be close to the site. The deep burial should be done under dedicated and close supervision. In a deep burial method the biomedical waste is dug 2 meters deep. The waste should filled half and then covered with a 50cm surface and then with soil. When the fresh waste is submerged to the pit, to cover the fresh waste soil layer of 10cm must be added (Sanjeev et al., 2014). The burial then must be covered with galvanized iron or wire meshes. The pit should be securely away from any inhabitation, to confirm that no contamination happens to any ground or surface water source. The deep burial site must not be prone to erosion or flooding. By the prescribed authority the site location must be authorized and the institutions should maintain a record for deep of all pits (De et al., 2012).

#### **2.3.2.2. Sanitary Landfill.**

In the process of sanitary landfill the discarded waste which is completely disposed of is protected and covered with the soil layer after an operation or at the end of each day. The factors which are considered for throwing away of the waste site include i) land area availability. ii) resource recovery and processing impact iii) climatological conditions iv) haul distance v) geologic and hydrogeologic conditions vi) soil condition and topography vii) situation of the resident environment and ultimate uses for the concluded site viii) surface water hydrology (Devine et al., 2007). The sanitary landfill convention method is used when the ground is incompatible for the digging of trenches in which the waste is placed. On the surface of the land the waste is unloaded and long spread in the layer of series which differ in depth from 15 to 30 inch. After the operation of end of the each day, the cover material of the 6 to 12-inch layer is placed above a completed fill. The final layer cover material of 2 feet is applied to the disposal site when the site has extended its final capacity (Diaz et al., 2005), (Edlich et al., 2006).

### **2.3.2.3. Inertization.**

To diminish the risk of toxic constituents the inertization method is carried out. In this process the organic biomedical waste is mixed with cement and supplementary material before disposal. A distinctive concentration of the mixture is done 65 % of organic biomedical waste, 5 % water, and 15 % cement. A consistent mass is prepared and cubes are produced and then transported to suitable sites (Li et al., 1993), (Na et al., 2007).

### **2.3.3. Impacts of the current method:**

#### **2.3.3.1. Incineration:**

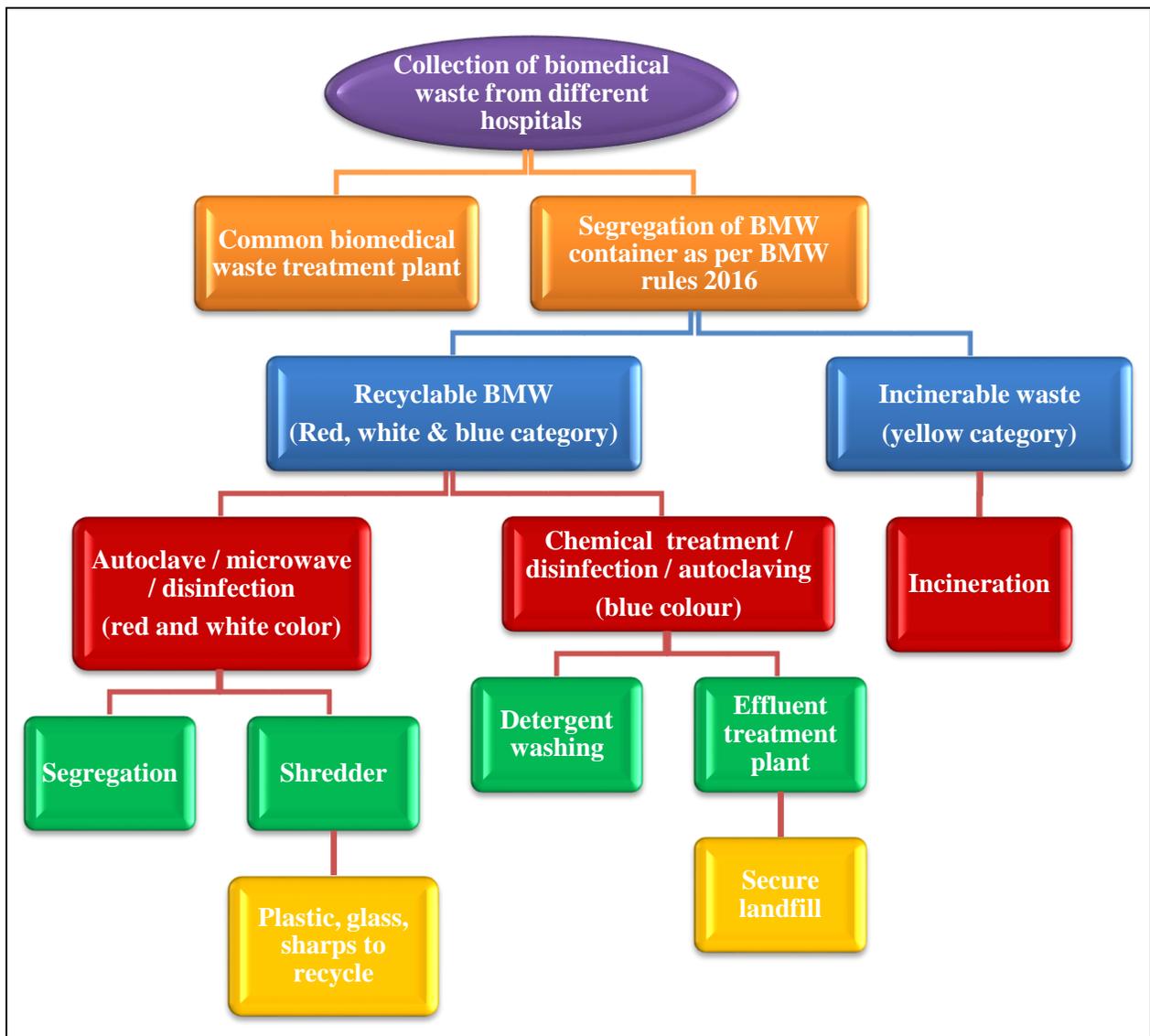
Incineration is a process based on the high temperature that destroys the pathogen and executes the microbes that exist in the waste. The number of toxins and gasses is produced due to incineration such as products of incomplete combustion and dioxins. These toxic gases are, being known carcinogenic, and cause damage to the respiratory system, immune and endocrine system of the human (Komilis et al., 2012). The ash produces after the incinerator is also harmful so there is a need to check the level of toxin before being sent to landfills. Therefore, keeping these points in deliberation, the majority of the countries are shifting to environmentally friendly treatments for discarding of organic biomedical waste (Çalış et al., 2014), (Marinković et al., 2008).

#### **2.3.3.2. Chemical-based technology:**

Various chemicals used for disposal of biomedical waste are currently underdeveloped. The cultures, sharps, liquid waste, human waste, research laboratory waste, and soft waste are treated with chemical-based technology. The toxic byproducts formed due to large scale chlorine and hypochlorite used for the decontamination of waste and often produces an offensive odour (Mattiello et al., 2013), (Oweis et al., 2005).

**2.3.3.3. Landfilling:**

Gas and leachate formation are unavoidable consequences due to the discarding of hospital waste in landfills. The spreading of gas and leachate away from the landfill area and their exposure to the environment will cause serious environmental concerns like health hazards, fires and explosions, unpleasant odours, damage to vegetation, global warming, air and groundwater pollution. (Windfeld et al., 2015), (El-Fadel et al., 1997).



**Figure 2.2: Diagrammatic representation showing various methods of disposal of biomedical waste.**

**2.3.4. Current treatments given to biomedical waste water in the effluent treatment plant:****2.3.4.1. Preliminary treatment:**

The preliminary treatment is mainly done for the removal of solid particles and a large particle which is often present in the raw biomedical waste water. In preliminary treatment, there is a reduction and removal of suspended particles, floating particles present in waste water (Rajan et al., 2018), (Azuma et al., 2016). These solid particles consist of plastics, papers, etc. that are removed by preliminary treatment. In preliminary treatment filtration or screening is done for separation of solid particles present in the biomedical effluent (Martínez et al., 2018), (Pauwels et al., 2006). Screening is usually made with a stainless steel net with varying pore sizes. After completion of preliminary treatment of waste water screens are regularly cleaned (Saliba et al., 2017), (Rajasulochana et al., 2016).

**2.3.4.2. Primary treatment:**

In primary treatment, there are two types of methods physical method and chemical method and further, these two are divided into the following types. In the physical method, sedimentation and flotation treatment are carried out and in chemical method neutralization, coagulation, and flocculation treatments are given to waste water. The main objective of primary treatment is to remove organic and inorganic particles present in waste water. Approximately 60-65% of oil and grease, 60-70% of total suspended solids, 40-50% of biochemical oxygen demand are removed throughout primary treatment. During the process of sedimentation heavy metals, organic nitrogen, and organic phosphorus are removed. In the sedimentation process sludge settle down at the bottom of the tank and effluent is transferred to another tank. Thus primary treatment plays a crucial role in waste water treatment (Chandra et al., 2011; Carraro et al., 2016; Chitnis et al., 2004; Hong et al., 2018; Kajitvichyanukul et al., 2006).

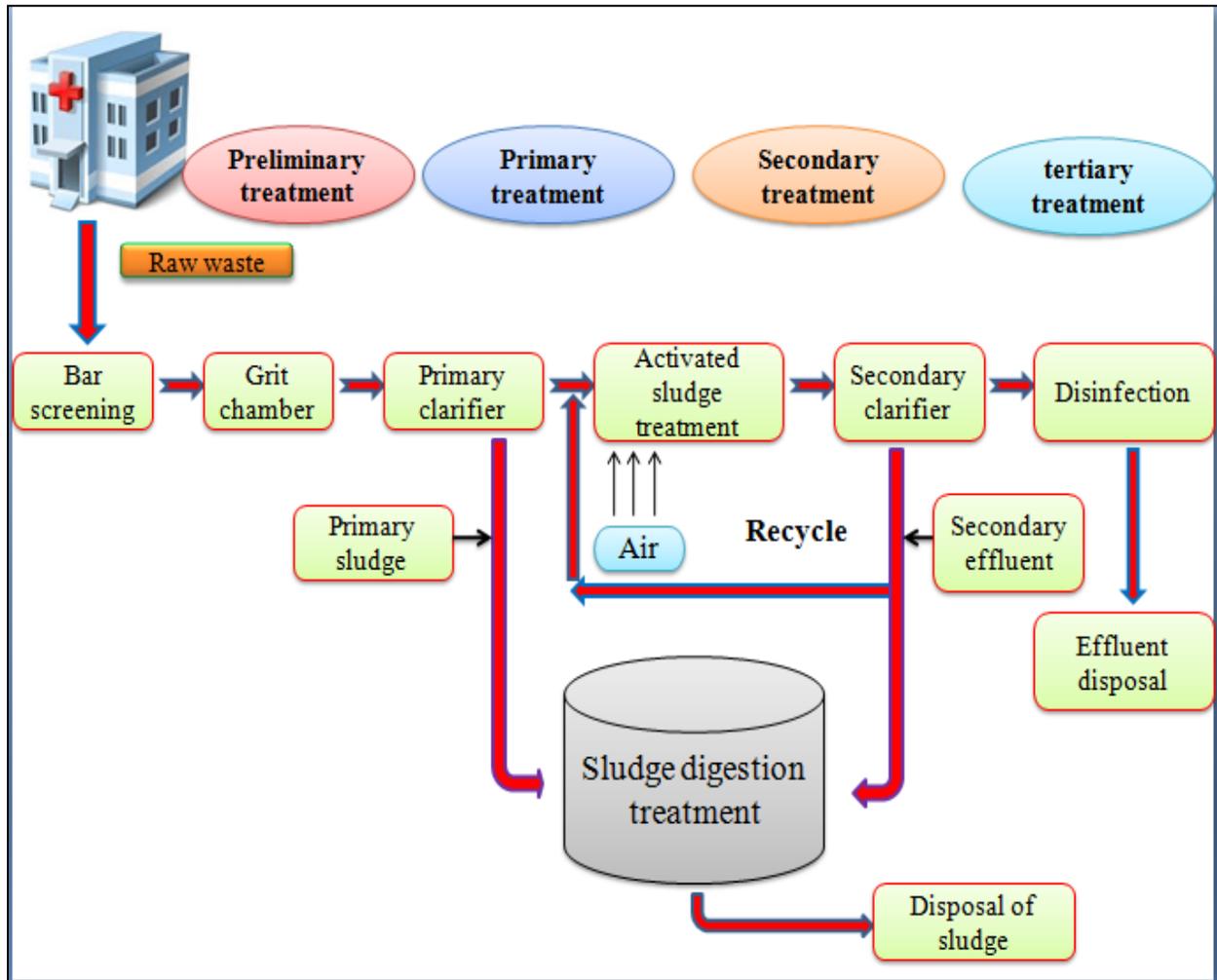
**2.3.4.3. Secondary treatment:**

In the secondary treatment, there are a number of methods carried out for the management of waste water they are activated sludge, aerated lagoons, trickling filter, anaerobic lagoons, stabilization

tank. In secondary treatment, various types of microorganisms are used in a prohibited environment. Secondary treatment is intended for exclusion of suspended and residual organic matter present in waste water. Approximately 60-65% dissolved solids, 30-35% suspended particles, 85-95% of biological oxygen demand are removed in secondary treatment only 5-10% colloidal particles are present in the secondary waste water (Mackul'ak et al., 2015), (Lee et al., 2016). In primary treatment, the clarifiers are used for the removal of organic and inorganic particles. Therefore the primary waste water contains more amount of organic and inorganic particles and colloidal forms (Oli et al., 2016), (Suarez et al., 2009). The waste water standards and water quality need a superior amount of removal of organic matter from effluent and primary treatment can alone accomplish it. Supplementary elimination of organic matter can be accomplished by secondary waste water treatment (Nkwonta et al., 2010), (Chonova et al., 2016).

#### **2.3.4.4. Tertiary treatment:**

A number of methods are carried in tertiary treatment like carbon absorption, iron exchange, sand filtration, denitrification, ultrafiltration, and reverse osmosis. After secondary process the subsequent step of waste water treatment is tertiary treatment. Tertiary treatment is also called as a final treatment or advanced method (Zhang et al., 2008), (Larsson et al., 2007). In tertiary treatment, the pathogenic bacteria are demolished and the residues left after the secondary treatment are removed (Puri et al., 2012),. The main objective of tertiary treatment is to use the treated water for the land application or straightly liberate it into lakes, rivers, ponds, etc. The tertiary treatment removes infectious agents, reduces total dissolved solids from the secondary treated waste water (Ajo et al., 2018), (Balasubramanian et al., 2006).



**Figure 2.3: Treatments given to biomedical waste water in the effluent treatment plant:**

### 2.3.5. Organic methods given to organic biomedical waste:

#### 2.3.5.1. Degradation of waste using cow dung.

Cow dung is not waste material, but it is a disinfectant of all waste in the environment. Cow dung is a gold mine due to its spacious appliances in the field of medicine, environment management, energy source, agriculture, beneficial applications. In cow dung the affluent amount of micro flora is present and it is cheap and easily accessible (Sharma et al., 2015). The cow dung contains proteins, fibres, and minerals such as potassium, nitrogen, magnesium, cobalt, calcium, etc. The cow dung contains 100 species of yeast and protozoa, 60 species of bacteria, fungi naturally. The

organic substance present in the waste sample is broken down by bacteria, fungi, and other microorganisms and degrades the waste (Shrivastava et al 2014)

The organic biomedical waste samples like bandages, used pieces of cotton were kept for degradation by using cow dung for a specific time interval. After ten days it was observed that *Periconiella* species of fungus were responsible for the degradation of cotton (Anupriya pandey et al., 2008). The waste includes plenty of organic substances, nutrients, and also includes numerous kinds of contaminated materials. The inappropriate disposal of waste would produce environmental pollution and possible hazard to human health. Composting technology is a kind of biological treatment, which has been extensively acknowledged as a substitute method to recycle organic substances and generate a potential soil fertilizer (Wang et al., 2016).

Taking indication from Ayurveda, cow dung performs as a brilliant bioremediation method. It is an inexpensive, and efficiently viable alternative and is close by accessible in the rural areas of India. A lot more exhaustive researches are essential to bioremediate the active biomedical waste particularly the ones which are non-degradable in the environment. Thus, the unfavorable effects of these compounds on the environment can be diminished for a healthy and secure future (Kaur et al., 2011).

Management of organic biomedical waste is becoming challenging day by day as it has become global quandary (Patil et al., 2019). Patil and et al have converted organic biomedical waste into a potential fertilizer isolating microorganisms and fungus from cow dung. In this research work, organic biomedical waste samples (dressing swabs blood swabs, and soiled waste) were collected from the hospital then the collection of cow dung was done in the sterile container to avoid contamination instantaneously transported to the laboratory for checking of any intestinal parasitic infection, fecal occult blood gastrointestinal infection and routine bacterial culture then after from mixed culture preparation of pure culture were done. The microorganisms and fungus were individually subjected to organic biomedical waste for degradation. After 288h degradation the treated sample of organic biomedical waste was mixed in soil and physico chemical parameters of soil were analyzed. After analysis of physico chemical parameters tomato plants were planted and phytochemical parameters were studied. It was revealed that the fungus and organisms show admirable degradation of organic biomedical waste. It was found that the physico chemical

parameters soil, treated experimental set and phytochemical parameters of tomato plants were increase as compared to control. It has been proved that pathogens that are present in the organic biomedical waste can be effectively destroyed by the fungus and organisms (Patil et al., 2019), (Wang et al., 2018 ).

### **2.3.5.2. Degradation of organic biomedical waste using plant extracts.**

The secure and professional methods for management and disposal of organic biomedical waste are important. The most significant feature in the management of organic biomedical waste is the obliteration of pathogens for which sure conventional approach can be used.

The subsistence of polyphenols content in herbal extracts is accountable for the degradation of any organic matter present waste sample. Enormous research is approved a wide range of herbal extracts and its chemical components. The polyphenols content is used as a green approach for biodegradation and treatment purposes (Devatha et al., 2018). Herbal extracts were preferred based on the existence of polyphenols, neem shows 82.35% deduction of chemical oxygen demand. Neem shows the satisfactory reduction of organic matter present in waste samples as compared to other herbal extracts. On the 11<sup>th</sup> day, there is an 82.35% reduction in COD of the waste sample due to polyphenols content in leaf extracts. The study proved that the exploitation of herbal extracts for the degradation of organic waste is an agreeable, cost-effective, and eco-friendly method (Devatha et al., 2016).

Jayanthi et al., have used 'neem' extract for the devastation of pathogens present in biomedical waste which was collected from a hospital. From the results it was observed that lime solution has efficiently destroyed the pathogens present in the biomedical waste. The superior degradation of biomedical waste was observed in fresh neem leaves extract. However, the viability and performance of organic treatment have to be recognized, by amplifying the use of related kinds of resources either alone or in the mixture and based on broad researches carried out on biomedical waste (Jayanthi and Sarojini 2010).

Patil. et al in year 2019 used neem and tobacco extract for degradation of organic biomedical waste. The organic biomedical samples were collected from hospital and experimental sets were arranged in the airtight container. The individual sets of neem and tobacco extracts organized and

a combination of both was also done. After giving treatment of neem and tobacco extracts the physicochemical samples and biological parameters were analyzed. From the physico chemical and biological parameters it is concluded that there was 86.15% of reduction in COD by using neem and tobacco extract. Superior results were observed when the biomedical waste was treated with a combination of neem and tobacco extracts. Treatment given to biomedical waste with a combination of tobacco and neem extracts was three times cost effective than the current methods used for the treatment of biomedical waste ( Patil et al., 2019).

### **2.3.5.3. Degradation of organic biomedical waste using earthworms**

Vermicompost has been a richer in various nutrients than compost that are produced from other composting process. Vermicompost reestablish the microbial population which comprises phosphate solubilizers, nitrogen fixers (Kitturmath el al 2007), (Garg et al 2006). Dinesh et al., collected biomedical waste and it was initially subjected to cow dung slurry for the decomposition process in the culture tubes. Then after the initially decomposed organic biomedical waste was subjected to the three different species of earthworm (*E. eugeniae*, *E. fetida*, and *P. excavates*) and placed in a separate container and one set was of the mixed earthworm and natural composting set was labeled as control. After decomposition of organic biomedical waste it is revealed that the mixed culture of all the three species of earthworm shows excellent as that of *E. fetida* species. This study proves that the vermicomposting process is an ecofriendly and efficient method for organic biomedical waste management as compared to other technologies (Dinesh et al 2010).

Mathur et al., have designed vermicomposting beds for treatment of organic biomedical waste made up of one layer earthen tiles on the cemented floor having ceiling asbestos sheets. Twelve sets of experiments were carried out with the help of beds. In these beds infected organic biomedical waste like cotton gauzes, blood swabs, dressing swabs, soiled waste were subjected. It was further covered with dry leaves and then *Eudrilus eugeniae* and *Eisenia fetida* species of earthworm with an adequate number were subjected. Then the beds of vermicomposting were left in undisturbed condition for a specific time interval. After periodic time interval the compost was formed and then that compost was transferred to aerobic microbial culture for a specific time

interval till complete composting took place. Thus composting is one of the easiest, simple, eco-friendly methods for degradation or the management of biomedical waste, and it has been a trend since immemorial time. Vermicomposting plays a vital role in the management of biomedical waste where stabilization and biooxidation of organic waste are carried out by submerging various species of earthworm. From this study, it has been revealed that there is a marked decrease in the net content of the organic biomedical waste and vermicomposting converts infected organic biomedical waste containing infected and pathogenic microorganisms to an inoffensive waste (Mathur et al., 2006). Vermicompost produce worthy aeration to soil, thereby cumulative root growth and prevent soil erosion. (Suthar et al., 2007), (Jeyaraaj et al., 2005).

#### **2.4. Conclusions.**

From the above discussion, it is concluded that the persistent and safe techniques for management of organic biomedical waste are of great importance. Minimization of waste at source will improve the management of organic biomedical waste. Executing and managing organic biomedical waste is one of the most important collective responsibilities of the public, state officials, and government. Due to a lack of apprehension and awareness, there is the inappropriate management of organic biomedical waste. Therefore the training must be given in society; the public must be educated regarding health hazards that are associated with organic biomedical waste. The innovative and ecofriendly methods must be developed for the treatment of organic biomedical waste. Eventually, for the fortification of an environment and our wellbeing sensitizing ourselves is most significant. Hence the awareness regarding waste management, minimization of waste at source, segregation of waste, proper transportation of waste, and proper treatment is of great interest not only for associated employees but also for the community. This will make sure preservation of ecological stability, biodiversity as well as the physical condition of the global population as a whole.

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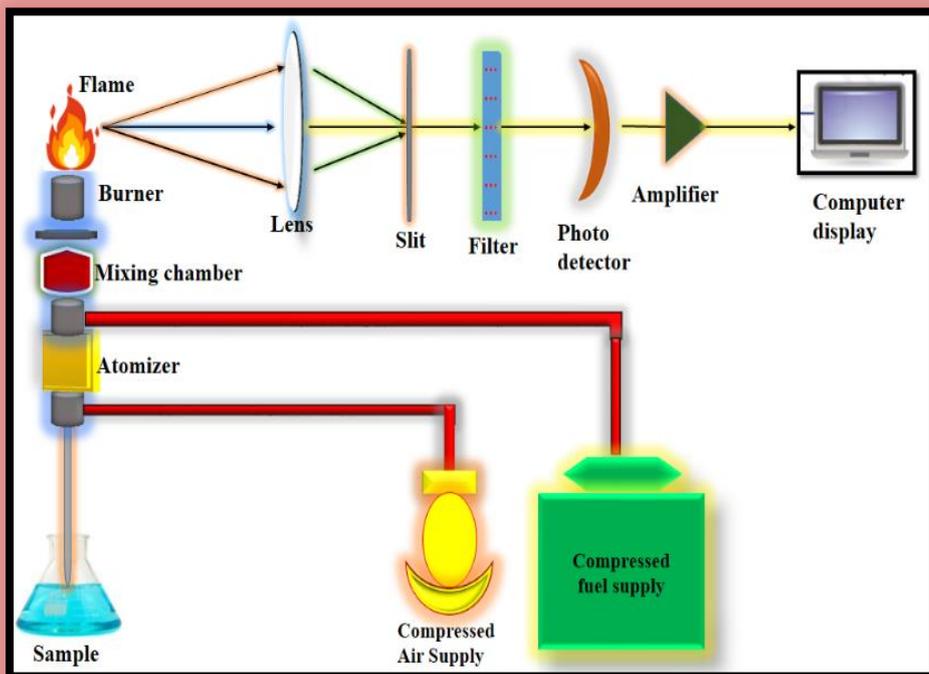
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# CHAPTER 3

## CHAPTER 3

### Experiment Techniques



## Chapter 3

### 3.1. Introduction of Biomedical Waste:

In current years, both the transboundary movements of biomedical waste and public health impact endure to be the hot issues over the world. These wastes are now threatening the public as the healthcare foundations are located in the heart of the city and therefore medical waste can cause dangerous infection if not properly managed (Patwary et al., 2009). In a country main concern about the hospital waste management is a health and environmental effects, uncertainty regarding regulations and negative perceptions by waste handles (Sanida et al., 2010). The revised hospital waste management strategy is compliant with the Rules on Biomedical Waste (management and handling) (second amendment) 2000 by the Ministry of Environment and Forests, Government of India (Patil et al., 2005).

The latest changes in health care systems are being made specifically for community health prevention and safety. Sophisticated methods have come into existence for treating disease in different operations (Diaz et al., 2005). Such developments and advancements in scientific understanding have resulted in the per capita production of waste in health-care units per patient. (Jindal et al., 2013).

American Dental Association and Centre for Disease Control Reported that medical waste disposal must be carried out in harmony with guidelines. Suitable treatment handling, and disposal of health care wastes are indispensable elements of biomedical waste management programme (Hegde et al., 2007) Precise techniques helps protect health care employees, local society, and the patients. Waste products from health care should be regarded as a source of pathogenic microorganisms, which would lead to contamination and cause infection (Bdour et al., 2007). These microorganisms can be transmitted if the waste is treated inadequately. It can pose a serious threat to human health and to the environment by direct contact, or through the air, or through a variety of vectors (Soliman and Ahmed., 2007). In the community every member has the right to complain or inform about mismanagement of biomedical waste. So there is need to educate the community regarding detrimental effects of biomedical waste, or to aware the community on the subject of appropriate treatments of biomedical waste. In the management of BMW or regarding the generation of BMW (total quantity) the institutes or

the hospitals should have a holistic transparent approach (Radha et al., 2009). The most crucial part in the management of biomedical waste is the treatments or the methods should be in organic, and environmental friendly (Mathur et al., 2012).

### **3.2. Introduction of soil**

Soil, the natural medium for the growth of land plants, is in its usual sense. Soils are all unconsolidated earth's crust content in which land plants can grow if water and temperature are sufficient. Plants can also grow when they get sufficient nutrients and when there is low concentration of harmful (Berthrong et al., 2009). The soil is a natural body of mineral and organic materials differentiated into horizons that differ among themselves as well as from the underlying material in morphology, physical make-up, chemical compositions, and biological characteristics. A soil analysis is a process by which elements such as P, K, Ca, Mg, Na, S, Mn, Cu, and Zn are chemically extracted from the soil and measured for there "plant available" content within the soil sample (Don et al., 2011).

Soil is a natural body comprised of solids, liquids, and gases that occurs on the land surface. It occupies space and is characterized by one or both of following: horizons or layers that are distinguishable from the initial material as a result of additions, losses, transfers, and transformations of energy and matter, or the ability to support rooted plants in a natural environment (Hungate et al., 2009)

Final untreated disposal of biomedical waste generally causes inevitable soil pollution, however, with proper treatment of this waste can minimized its impact on the soil. Soil pollution from biomedical waste is due to infectious waste, discarded medicines, chemicals used during treatment processes. Heavy metals in the waste are absorbed by plants into the ecosystem and contaminate the food chain (Dwivedi A. K, Pandey S. Shashi, 2009). While, Sahar and Ahmed in 2007 stated that the biomedical waste includes radioactive waste causing contamination to the soil, bodies, protective clothing, paper absorbent, used in nuclear imaging medicine workshop, etc. (Sahar and Ahmed., 2007)

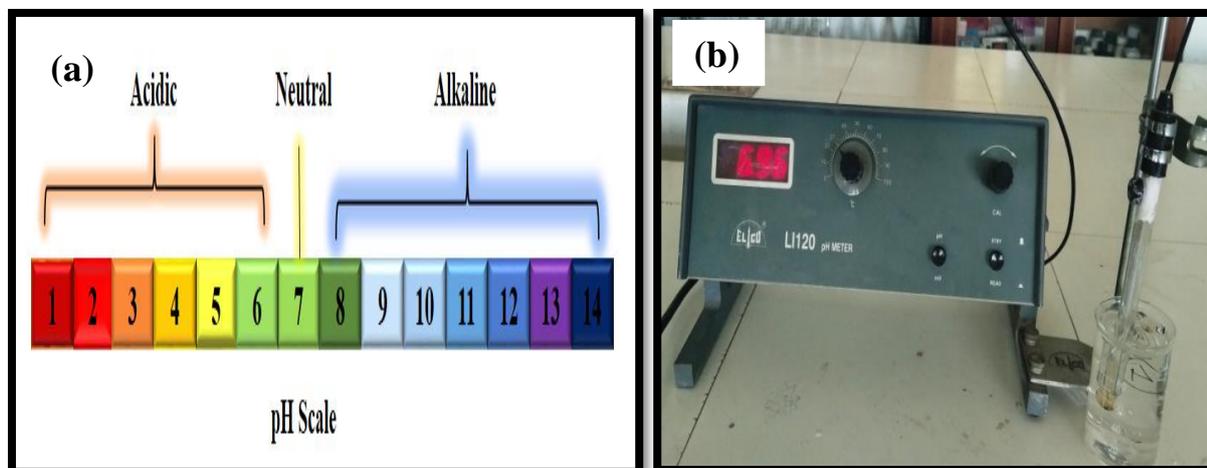
### 3.3. Physicochemical parameters of sample and soil:

#### A) pH

The pH value is a measure of the activity of the hydrogen ions. The most widely used indicator electrode in the digital pH meter is a glass electrode, which follows the Nernst equation and depends directly on the behavior of the hydrogen ions (Si et al., 2006). pH is an important property of any sample as it determines the availability of nutrients, microbial activity, and physical condition of the sample (Barbosa et al., 2001).

pH is a measure of the aqueous solution's hydrogen ion concentration or activity and each aqueous solution can be calculated to determine its pH value. This value ranges from pH 0-14 with values below pH 7 showing acidic characteristics and values above pH 7 showing basic or alkaline characteristics. pH 7 is neither acidic nor basic (Lima-Rodriguez et al., 2003). The pH scale can be traced to a set of standard solutions, the pH of which is set by international agreements, while, Mathematically, pH is the negative logarithm of the [solved] hydronium ion activity, more commonly expressed as the hydronium ion concentration measurement (Zheng et al., 2010).

Digital pH meter LI 120 was used for the determination of pH for all samples, a commercial glass electrode was used to analyze the pH, before analyzing any sample, pH meter always calibrated with known value buffer solutions like pH 4.0, pH 9.0. The 10 gram sample was taken in a 100 ml glass beaker 50 ml of double distilled water was added in it. Then for one hour beaker was kept in a mechanical shaker and further allowed to stand, till particles settle down. Then the electrode was dipped in the supernatant and pH of was measured.



**Fig. 3.1. (a) Range of pH acidic to basic (pH scale) (b) Digital LI 120 pH meter.**

### **B) Total Dissolve Solids**

Total Dissolved Solids (TDS) can be found in all freshwater systems. It is found to be low levels in the water quality of freshwater bodies like streams, rivers, lakes, while it can be found a high-level scale in recirculating water systems (Abudu et al., 2012). TDS is considered as one of the quality indicators for drinking water and it measure an existence of chemicals (Antonopoulos et al., 2001). The term total dissolved solids refer to materials that are completely dissolved in water. These solids are filterable in nature and; it can be defined as residue upon evaporation of the filterable sample. The term total suspended solids can be referred to materials which are not dissolved in water and are non filterable (Chenini et al., 2009). So, it is defined as residue upon evaporation of non-filterable sample on a filter paper. Total dissolved solids are determined as the residue left after the evaporation of the filtered sample (Etemad-Shahidi et al., 2009). The total dissolved solids is recognized on the weight of the residue on evaporation after exposing to a temperature of 105<sup>0</sup>C to 110<sup>0</sup>C. (1, 5) or at 180<sup>0</sup>C (Ghavidel et al., 2014).

In water the assessment of total dissolved solids analysis have always been taken to represent the amount of dissolved as well as suspended material in the water. The difference between the residual weight at the evaporation of the water's unfiltered and filtered samples was often taken as an indicator of the suspended matter. The loss at ignition was also taken as a sign of the organic matter in the water. Comparison of reported determinations of total solids and reduced ignition with the results of the analysis of the actual mineral content indicates, however, that in some analyses the figures for total solids and loss on ignition have no real significance.

In the freshwater body, TDS is generally found to be low, at less than 500 ppm, while, seawater and brackish (mixed fresh and sweater) water, contain TDS about 500–30,000 and 30,000–40,000 ppm respectively. Conductivity, which is proportional to TDS, is a quicker method to use. Another method of determination of TDS is by weighing a filtered sample and drying at 105<sup>0</sup>C. In the present study, TDS determination of samples was done by using the filter sample method (Monney et al., 2013).

**Calculation:**

$$\text{TDS, mg/L} = \frac{(A - B) \times 1000 \times 1000}{\text{Volume of sample}} \quad \text{————— (3.3.1)}$$

Where,

A= Final weight of the dish in gm

B= Initial weight of the dish in gm

V= Volume of sample taken in ml

**C) Chemical Oxygen Demand**

Chemical Oxygen Demand (COD) is the amount of oxygen consumed by oxidizable water under defined procedural conditions; it may be organic (biodegradable as lipids, proteins, carbohydrates, urea, or refractory chemicals as aromatic compounds) or mineral (ferrous iron, ammonia) (Areerachakul et al., 2013). COD determines the quantity of oxygen required to oxidize the organic matter in a waste sample, under specific conditions of an oxidizing agent, temperature, and time (Maier et al., 2010). Since the test utilizes specific chemical oxidation the result has no definite relationship to the Biochemical Oxygen Demand (BOD) of the waste or the Total Organic Carbon (TOC) level. The test result should be considered as an independent quantity of organic matter in the water sample, rather than as a substitute for the BOD or TOC test (Cox et al., 2003). The method can be applied to domestic and industrial waste samples having an organic carbon concentration greater than 50 mg/L. For lower concentrations of carbon such as in surface water samples, the low-level modification should be used. When the chloride concentration of the sample exceeds 2000 mg/L, the modification for saline waters is required (Choi et al., 2017).

The organic matter present in the water sample is oxidized by potassium dichromate in the presence of sulfuric acid, while, silver sulfate mercury sulfate to produce carbon dioxide (CO<sub>2</sub>) and water (H<sub>2</sub>O). The quantity of potassium dichromate used is calculated by the difference in the volume of ferrous ammonium sulfate (FAS) consumed in blank and sample titrations. The quantity of potassium dichromate used in the reaction is equivalent to the oxygen (O<sub>2</sub>) used to oxidize the organic matter of wastewater. Figure 3.2 represent standard protocol of COD.

By using the formula COD can be calculated,

Calculate the COD in the sample in mg/L as follows:

$$\frac{(b - a) \times \text{Normality of FAS} \times 8000}{\text{ml of sample taken}} \quad \text{—————} \quad (3.3.2.)$$

Where, a = ml of a titrant with sample.

b = ml of a titrant with blank.

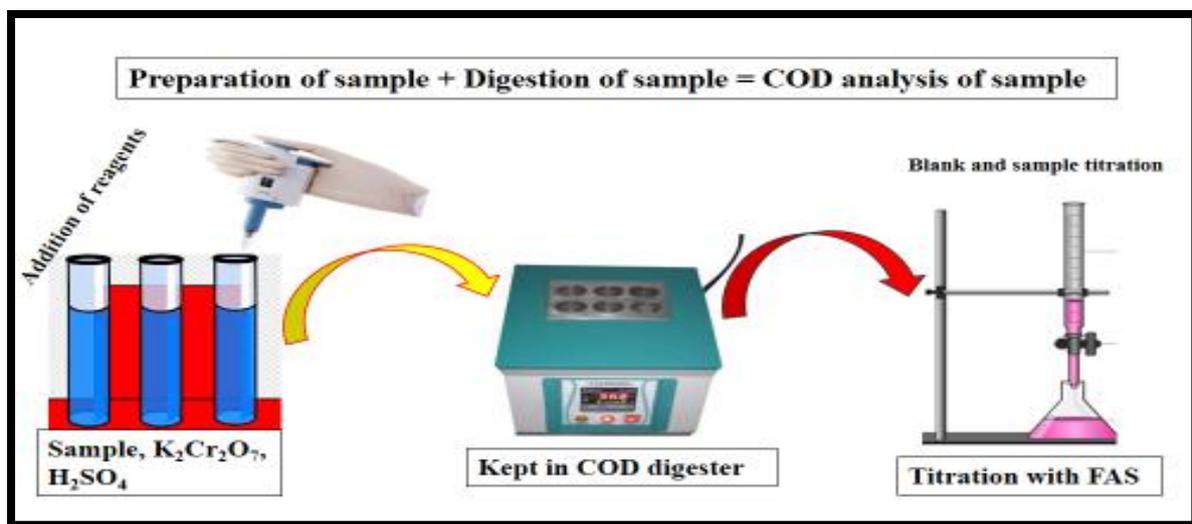


Fig. 3.2. The standard protocol for Chemical Oxygen Demand analysis.

#### D) Biological Oxygen Demand (BOD)

BOD is the quantity of the decomposable biological material existing in a sample and can be defined as the quantity of oxygen prerequisite by the microorganisms in stabilizing the biologically degradable organic matter under aerobic conditions (Dogan et al., 2009). This characterization is not a definite quantifiable parameter, even though it is extensively used as a quality water indication (Dehghani et al., 2013). The principle of the method involves, quantifying the alteration of the oxygen among the preliminary sample and subsequently 5 days of incubation at 20°C (Fu et al., 2015).

BOD is expressed as weight of oxygen used per unit volume of water during 5 days at 20°C; BOD is related to the quantity of degradable biological matter in water sample; oxidative

decomposition of biological matter, aerobic micro-organisms in which it consume oxygen existing in water as dissolved gas (Antanasijević et al., 2014), (Hore et al., 2008).

In definite volume of sample has its initial dissolved oxygen quantity. The sample is taken out after an incubation interval of 5 days at 20°C, and final dissolved readings are noted. From the quantity of sample occupied and the BOD has been calculated

### **Calculation of BOD:**

BOD, mg/L =  $(D_0 - D_5) \times$  dilution factor

$$\text{Dilution Factor} = \frac{\text{Bottle Volume}}{\text{Sample Volume}} \quad \text{————— (3.3.3.)}$$

Where,  $D_0$  = Initial DO in the sample

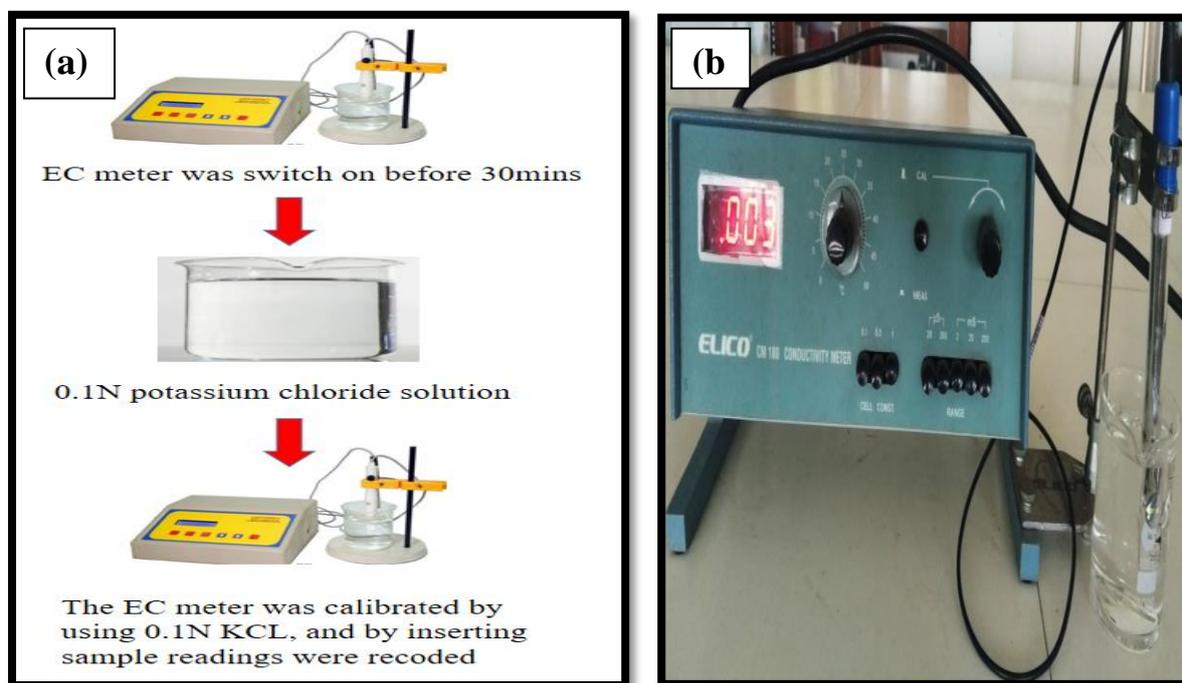
$D_5$  = DO after 5 days

### **E) Electrical Conductance (EC)**

EC is the capability of a constituent to conduct the electric current. In water, it is the property caused by the presence of various ionic species. It is generally measured with the help of a conductivity meter having a conductance cell containing electrodes of platinum-coated with carbon (Bai et al., 2013). These electrodes are mounted rigidly and placed parallel at a fixed distance. When the measurement of conductance between the electrodes having a surface area of 1 cm<sup>2</sup> and placed at a distance of 1 cm, is termed electrical conductivity and it is the property of the sample, rather than the measuring system (Bertermann et al., 2017), (Corwin et al., 2005). The term specific conductance is too used in place of electrical conductivity but is an obsolete term. The unit of conductivity measurement is Siemens (S) cm<sup>-1</sup> is now rarely used. The conductivity of most waters is generally low so the unit  $\mu$  S cm<sup>-1</sup> shall be much appropriate (Corwin et al., 2005), (Friedman et al., 2005).

The determination of EC is made with a conductivity cell by measuring the electrical resistance of a 1:5 soil: water suspension. Digital EC meter was used for the determination of EC for all samples. Proper calibration of the EC meter was performed before the analysis of

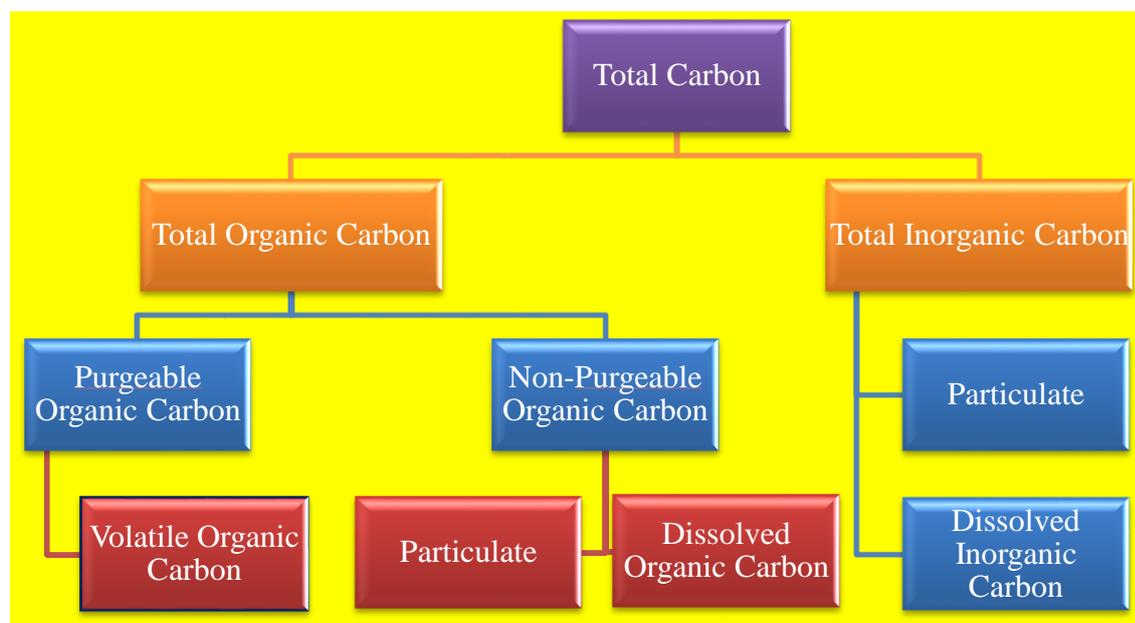
the samples. The standard protocol for analysis of EC and digital electric conductivity meter is represented in fig. 3.3.



**Fig. 3.3. (a) The standard protocol for analysis of electric conductivity and (b) digital electric conductivity meter.**

#### **F) Organic Carbon and Organic Matter:**

In soil and water plentiful categories of carbon are existing. The Total carbon (TC) is known to be existence of all carbon, comprising inorganic and biological carbon. In inorganic carbon only inorganic carbon, bicarbonate, dissolved carbon dioxide and carbonate is present (Smebye et al., 2016). In organic carbon the degradable biological material, bacteria or other biological elements are present. Purgeable or volatile organic carbon (POC) these elements are same as volatile biological elements which can be determined by gas chromatography (Spark et al, 2002). Non Purgeable organic carbon (NPOC) is the carbon that is remain after sample acidification of sample. Dissolved organic carbon is remain in the even when sample is filtered with 0.45 micrometer filter and suspended organic carbon which is too large in size the principal quantity of biological carbon do not pass through a filter (Movassaghi, Karim et al., 2006). The relation of all above carbon is represented in following figure.



**Fig. 3.4. The flow chart represents the types of carbon and its relation.**

Organic carbon and organic matter is one of the most extensively performed rapid soil tests for the assessment of available nitrogen, it is based upon the estimation of readily oxidizable organic carbon which roughly represents 58% of the soil biological matter, and the base of nitrogen in the soil. This technique has been found to work fairly well unless the bulk of the organic matter is non-humic and the organic carbon values are on the very high side. Various methods are available for the identification of biological carbon, utmost extensively accepted methods is Walkley - Black, which also determine the organic matter by using the Van Bemmelen correction factor. In this procedure following reaction take place The excess  $\text{Cr}_2\text{O}_7^{2-}$  back titrated with 0.50 N  $\text{FeSO}_4$  solution by using diphenylamine-4-sulfonic acid barium salt indicator until change in colour from blue to green in the sample. The organic carbon which is samples was oxidized by an oxidizing sulphuric acid and by potassium dichromate ( $\text{K}_2\text{Cr}_2\text{O}_7$ ). After the oxidation of carbon, with ferrous ammonium sulphate (FAS) is black titrated with the excess of  $\text{Cr}_2\text{O}_7^{2-}$ . The  $\text{Cr}_2\text{O}_7^{2-}$  which is reduced during reaction with sample equivalent to the organic carbon (Walkley & Black., 1934).

**Calculation:**

$$\% \text{ Organic Carbon} = \frac{(\text{BTV} - \text{STV}) \times \text{Number of FAS} \times 0.003 \times 100}{\text{Weight of soil sample} \times 0.77} \quad \text{————— (3.3.4)}$$

$$\% \text{ Organic Matter} = \% \text{ Organic Carbon} \times 1.724 \quad \text{—————} \quad (3.3.5)$$

Where,

BTV = Blank titrate value

STV = Sample titrate value

0.003 = Conversion factor from ml of dichromate to gram of carbon

0.77 = Efficiency factor of the analytical method

1.724 = Factor for converting organic carbon to organic matter

### **G) Water Holding Capacity (WHC)**

The determination of WHC of the soil is important as it gives an idea of the capacity of soil to hold water for the use of crops (Celik., 2005). The light soil which does not hold much water required more frequent irrigation than heavy clay soil, well-decomposed organic matter increases the matter which increases the water holding capacity (Chen et al., 2016). A soil's WHC is of great value to the terrestrial ecosystem as it provides a simple means of determining the moisture content required in soils for the growth of good plants (Wall et al., 2003), (Dong et al., 2012) .

Air dry soil was crushed and passed from sieve of 2 mm. then round filter paper was placed and fixed it in an internal bottom of circular brass box. Then the weight of box and filter paper was noted. The box was then filled with soil and weight was noted and it was placed in enamel tray where water was poured in enamel tray at half of height of box, in which water may rise in box through bottom and moist the soil to its capacity. Then water was kept for 5 to 6 hours. Then after box was placed on filter paper sheet, to drain out excess water. The box containing moist soil was weighted and the weight was noted (Akola et al 2000).

#### **Observation:**

- a) Weight of empty box + filter paper = A
- b) Weight of empty box + filter paper + air dried soil = B
- c) Weight of box + filter paper + wet soil = C

### Calculations:

$$\text{Water Holding Capacity (\%)} = \frac{(C-A) - (B-A)}{(B-A) \times 100} \quad \text{————— (3.3.6)}$$

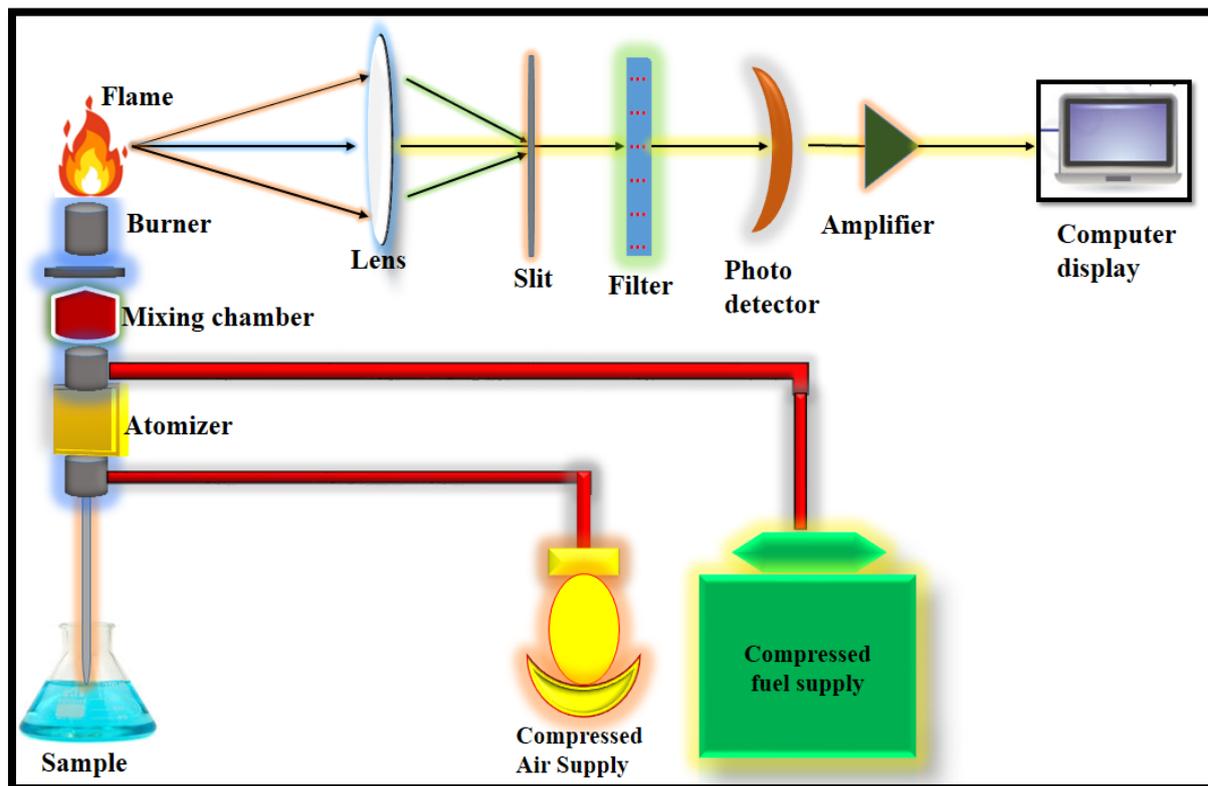
### H) Potassium

Potassium is one of the essential nutrient for animal, plants, and humans. Through the budding season it is one of the major nutrient and it need in large amount. For plant development it is very crucial nutrient. It increase root growth and improved nutrient uptake, potassium reduces plant brackling and builds cellulose. Potassium activates sixty plant enzymes which are necessary for growth of plants (Bansal et al 2002), (Bhattacharyya et al., 2008). Potassium maintain the balance of water in plant cells, and it also maintain the chlorophyll content in plant. Potassium also increases plants patience to foliar diseases low potassium content in soil will reduce the yields of crop and profitability (Datta. 2005), (Jalali. 2006). Exhaustive cropping such as maize, cereals, grass on grown deficient potassium soil outcome in lower yields of crop (Mohammed. 2006).

For analysis of samples the standard solutions were prepared by diluting stock solutions by using glass pipettes and 100ml volumetric flask. Then the 2.5 gram of sample was weighted which was previously ground with mortar and pestle. Then it was transferred in a beaker and 125ml of double distilled water and 50ml of ammonium oxalate was added in it, then it was boiled gently for 30 minutes then the suspension was cooled and ammonium hydroxide solution was added in it. Then the sample was cooled and it was transferred in 250ml volumetric flask and volume was diluted. Then the solution was filtered through dry Whatmann filter paper No. 30 and 25ml of aliquot was pour in 500ml volumetric flask and it was diluted with double distilled water and mixed thriving. Then in flame photometer blank reading was taken then the instrument was set to read 100 by using 20ppm potassium solution to consecutively aspirate 10, 12, 14, 16 and 18ppm solutions, for three times to take its mean reading for graph calibration. After standard readings then sample readings were recorded and calibration graph were prepaid to calculate concentration of potassium in the sample. Fig. 3.4. represents the digital flame photometer

**Calculations:**

$$\text{Potassium (\%)} = \frac{\text{ppm of potassium in sample solution} \times 20}{\text{Volume of aliquot}} \quad \text{————— (3.3.7)}$$



**Fig. 3.4. Schematic representation of the Digital Flame Photometer**

**I) Phosphorus (P)**

The term available phosphorus (P) refers to the inorganic form, occurring in the soil, which is absolutely orthophosphate. This orthophosphate exists in several forms and combinations and the only fraction quantity of phosphorus may be accessible to floras, which is of direct relevance in assessing the P fertility level (Bhadoria et al., 2002), (Ahemad et al., 2014). The phosphate concentration in solution is governed by heterogeneous equilibrium in which it takes part (Blaisdell et al., 2003).

Phosphorus is a major plant macronutrient that is incredibly important for plant growth and development (Djodjic et al., 2002). Enormous cultivation of vegetables and other crops along

with natural hazards, cause depletion of the natural phosphate content in soil accordingly. Phosphate fertilizers are used to overcome this wear and tear for soil enriches (Johnston et al., 2016).

The available P is considered to be a fairly good indicator/measure of the P-supplying capacity of a soil. Knowledge of available P content in the soil is important for determining its critical limit (Kuchenbuch et al., 2011). The evaluation of the soil critical limit of P would help in developing P-fertility classes for an effective fertilizer recommendation schedule. The critical limit of soil P would help eventually in predicting crop response to the applied P (Sharpley et al., 2013).

Determination of P from the soil samples was done with the help of a method which is prescribed by Olsen et al, 1954 by extracting the available soil P from soils. Olsen's method is the most accurate method for soils in the range of mild acidic to basic pH. When 30ppm of phosphorus is present in soil then the phosphorus content is extremely high, when it is in 22.5 to 30 then it is known to be high, when it is between 15 to 22.5 then the soil has medium content of phosphorus and below 15 then the phosphorus content is low in soil. Fig. 3.5. (a) represents UV- visible spectrophotometer and (b) represents digital UV- visible spectrophotometer.

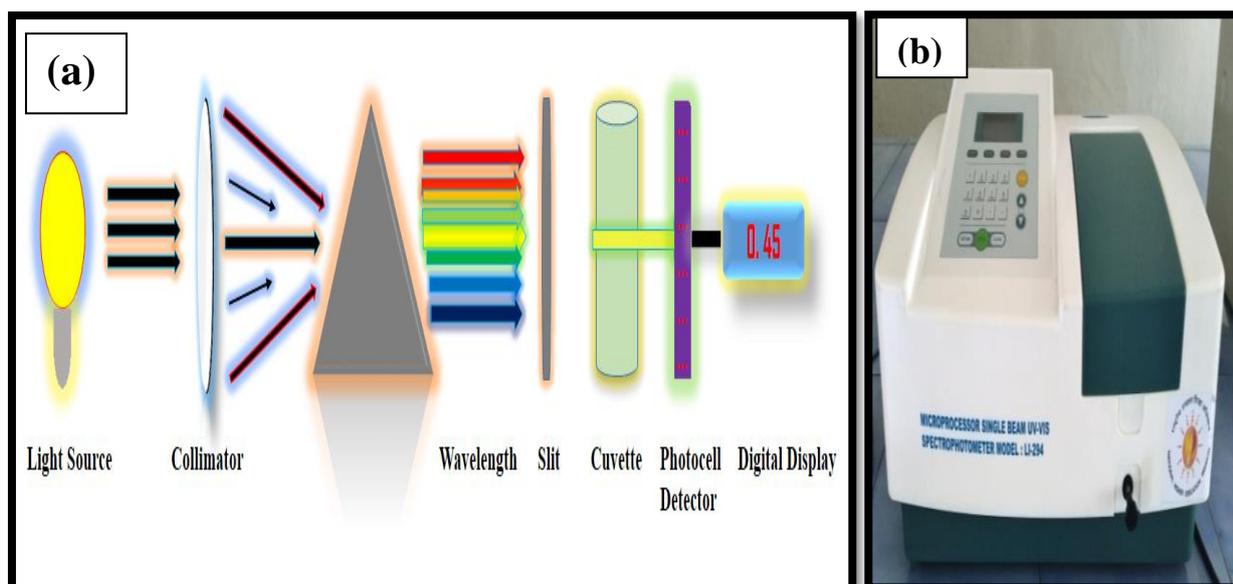
Calculation:

$$\text{Available Phosphorus (\%)} = \text{P (conc)}/50$$

$$\text{Available Phosphorus (ppm)} = \text{P (conc)} \times 200$$

$$\text{Available Phosphorus (kg/ha)} = \text{P (ppm)} \times 2.24$$

} (3.3.8)



**Fig. 3.5. (a) Schematic representation UV- Visible Spectrophotometer (b) Digital UV- Visible Spectrophotometer**

#### **J) Total Nitrogen**

In the soil solution, organic N is gradually transformed into ammonia ( $\text{NH}_4^+$ ) then nitrite ( $\text{NO}_2^-$ ), and last to nitrate ( $\text{NO}_3^-$ )-N by microbial processes. Organic – N is, in itself, of very little use to plants as it cannot be absorbed or available N (Bateman et al., 2005), (Cosandey et al., 2003). The  $\text{NO}_3^-$ -N together, hardly, exceeds 1% of the total N in normal soil. The available N in soil refers to a fraction of total N which is converted into forms accessible to the plant. This constitutes on average only 0.5-2.5% (rarely 5%) of the total nitrogen in the soil at any given time (De et al., 2016). N is generally, adsorbed by plants as  $\text{NO}_3^-$  under oxidized environment and as  $\text{NH}_4^+$  under reduced condition.  $\text{NO}_2^-$  is sometimes, detectable but the amount is generally very small (Dixon et al., 2010).

Nitrogen is one of the elements that are widely distributed in nature. It's available in the lithosphere, hydrosphere also found in the atmosphere. The atmosphere is the main reservoir of nitrogen (Galloway et al., 2008). The soil accounts for only a minute fraction of lithospheric nitrogen and of this soil nitrogen, only a very small proportion is directly available to plants (Liu et al., 2017). The chemical analysis of the soil for N is less precise when the requirement for this element needs to be forecast over a longer period as they vary not only with species but with the phase of growth and season as well (Qin et al., 2017).



**Fig. 3.6 Kjeldahl Distillation instrument (<https://www.laboreszkozkatalogus.hu/velp-kjeldahl-desztillalok-vizgoz-desztillalok.html>)**

A given weight of soil is treated with an excess of alkaline  $\text{KMnO}_4$  and distilled water.  $\text{KMnO}_4$  is a mild oxidizing agent in an alkaline medium. The organic matter present in the soil is oxidized by the nascent oxygen liberated by  $\text{KMnO}_4$  in the presence of  $\text{NaOH}$  and thus, the ammonia released is distilled water and adsorbed in a known volume of a standard acid. The excess is then titrated with a standard alkali using methyl red as the indicator. N estimated by this method is considered to be hydrolyzable N or potentially available N.

**Observation:**

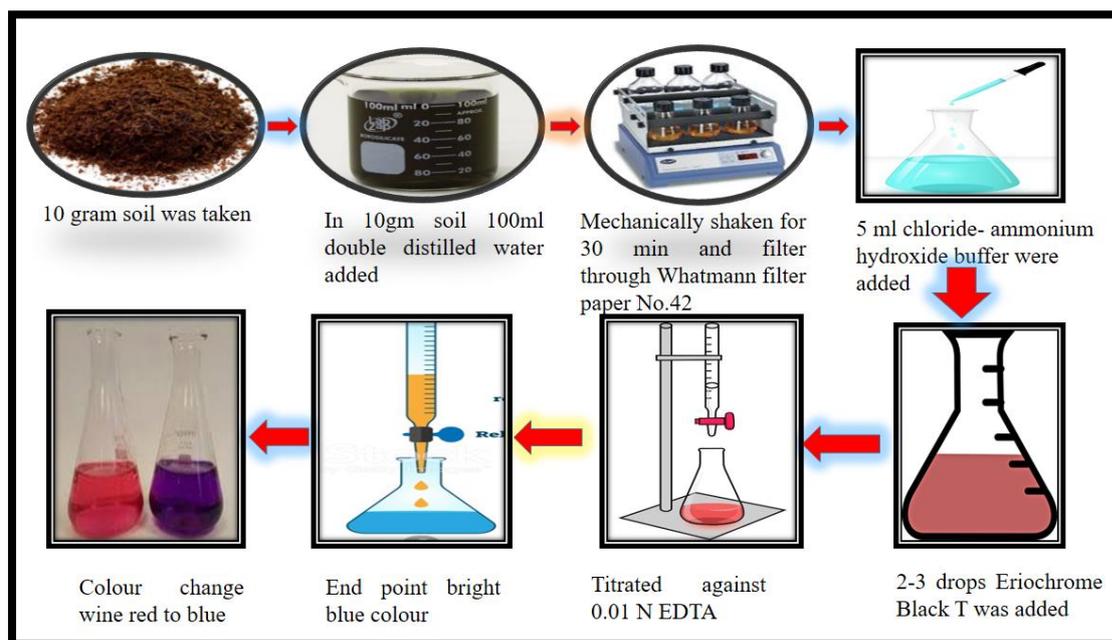
- a) Weight of soil taken = 20 gm
  - b) Volume of N/50  $\text{H}_2\text{SO}_4$  taken = 25 ml
  - c) Volume of N/50  $\text{NaOH}$  used (titrate-value) = X ml
  - d) Volume of N/50 acid used for  $\text{NH}_3$  absorption = 925-X ml
- (1 ml of N/50  $\text{H}_2\text{SO}_4$  = 0.02 meq. of N = 0.28 mg N = 0.00028 gm N)

**Calculation:**

- a) Nitrogen Available in (%) =  $(25-X) \times 0.00028 \times 100/20$
  - b) Available N in the soil (ppm) =  $e \times 10,000$
  - c) Available N in the soil (Kg/ha) =  $f \times 2.24$
- } (3.3.9)

### K) Calcium:

Calcium (Ca) and Magnesium (Mg) are the two most abundant alkaline earth cations in soils. The calcium and magnesium occur in soluble ions, exchangeable ions and non-exchangeable ions in primary and secondary minerals and in inorganic material (Abrahams, 2002). Soils from arid-regions are richer in Ca (1-2% CaO) while those from humid zones have a low content (0.01-0.2% CaO) (Baldock and Skjemstad 2000). On the other hand exchangeable Mg is found in very small quantities in calcareous soils. Sometimes in acid soil soils  $Mg^{2+}$  represents the largest quantity of sum of exchangeable bases (10-30%). Quantitatively the lowest amount of Mg occurs in podzolized and sandy soils (Bélanger et al., 2008). In general the supply of Ca and Mg to plants is very much governed by their cation exchange equilibria in soils (Cailleau et al., 2005). That is their concentration in the soil solution is fairly well buffered either by cation exchange reactions or by the solubility product of their sparingly soluble compounds arid soils (Cailleau et al., 2004). Fig. 3.7. represents standard protocol for calcium analysis



**Fig. 3.7. Represents the standard protocol for analysis of calcium.**

### L) Magnesium:

In soil magnesium acts like calcium. In humid areas both are simply leached. Magnesium conservation depends on soils cation exchange characteristic. The climate situations and age of soil effect the presence of magnesium and capacity of cation exchange (Cakmak 2013). Magnesium is involved in numerous biochemical and physiological process, it is a crucial nutrient for plant improvement and progress and plays a vital role in abiotic stress condition during plant defense mechanisms (Cakmak and Kirkby 2008). The magnesium's most crucial function in plants is to perform as the chlorophyll dominant atom fragment of chloroplasts in the light absorbing complex and it contribute to carbon dioxide photosynthetic fixation (Huber and Jones 2013). Looking towards the importance of magnesium in soil the content of magnesium has been analyzed by using standard protocol which is represented in figure 3.8.

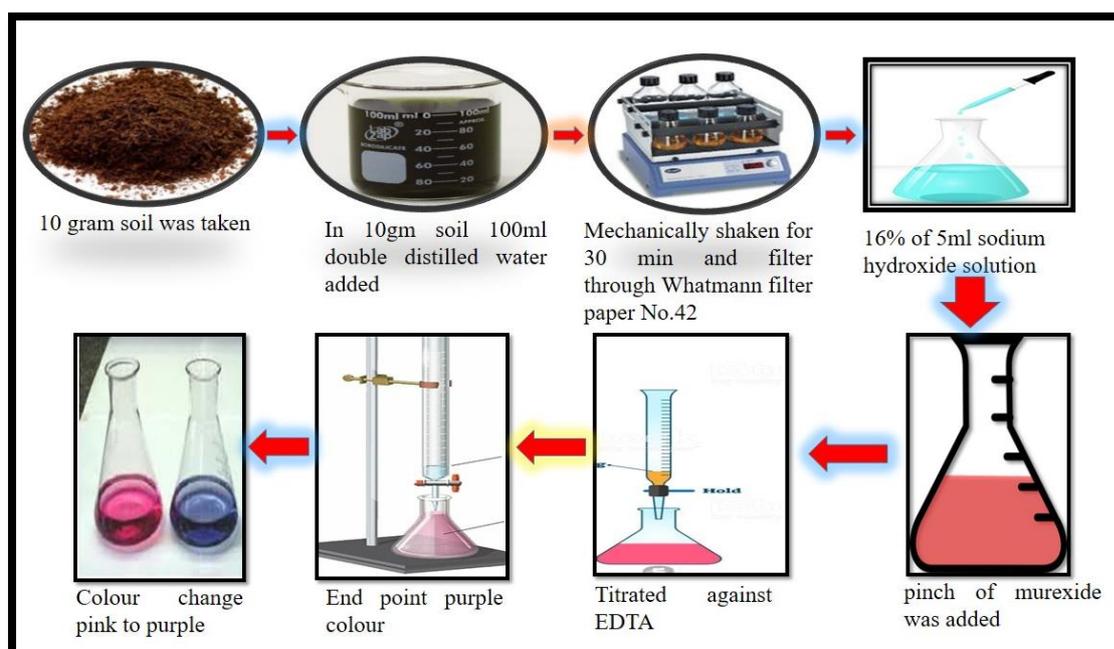


Fig. 3.8. Represents the standard protocol for analysis of Magnesium.

### M) Heavy Metals

The heavy metal pollution of the soil as well as surface water mainly caused due to industrial, traffic, and municipal wastes such as solid, liquid, and gaseous ones (Chiroma et al., 2014). Soil is both a principal source of trace elements entering the food chain and a major sink for pollutant elements (Ahmad et al., 2016). So the analysis of heavy metal is most important as

beyond the limit it is toxic for the environment and also for living organisms (Mahar et al 2016), (Ahmad et al., 2018).

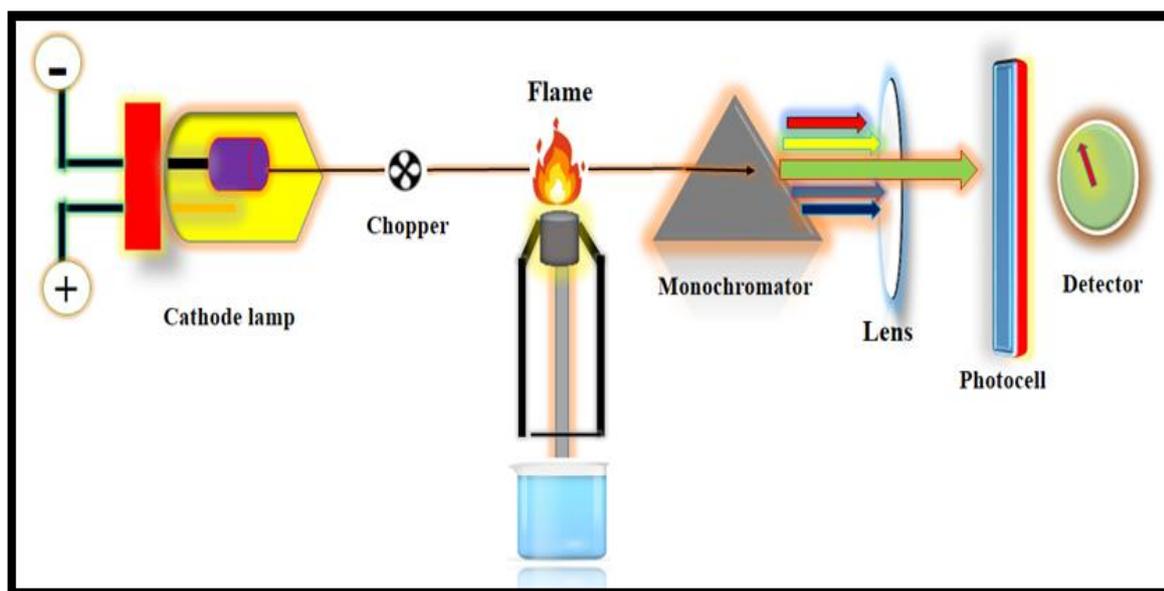
For heavy metal analysis few gram of soil was shake with a buffered solution, containing DTPA (diethylene triamine penta-acetic acid). This chemical acts as a mild chelating agent, which extracts zinc, iron, copper, and manganese. The extracting solution is buffered at pH 7.3 by the dissolution of calcium carbonate. These conditions permit the right amount of zinc, copper, iron, and manganese to be dissolved and  $\text{CaCl}_2$  is to stabilize the pH of the extractant. The dissolved elements in the extract are then measured by the atomic absorption spectrophotometer, where the extracted sample is converted first into an atomic vapour, usually by flame and irradiated by the metal being required. The absorption of the light by the vaporized sample is associated to the concentration of the desired metal in it. 10 gm of soil added in 20 ml of the extractant. It was shake continuously for 2 hours on a horizontal shaker and filtered it through Whatman No. 42 filter paper. 10 standards were prepared by using DTPA as the matrix for each element with a range of 0 to 3 ppm for few heavy metals, and for other metals 0 to 20 ppm standards was prepared. The first reading was taken of the blank then of standards then elements were measured from the filtrate by atomic absorption spectrophotometer. A blank solution containing all reagents excepting the soil was run to correct for contamination. Fig. 3.9. Represents the schematic representation of instrument atomic absorption spectrophotometer.

**Observation:**

- a) Weight of Soil = 10 gm
- b) Volume of DTPA extract made = 20 ml
- c) Concentration (ppm) of heavy metal as read from Standard curve against T (for sample) = C
- d) Concentration of heavy metal in the blank solution =  $C_b$

**Calculations:**

- a). Dilution factor =  $20/10 = 2$  times
- b). Now, available heavy metal in the soil (ppm) =  $(C - C_b) \times 2$  } (3.3.10)  
Available heavy metal in the soil (Kg/ha) =  $(C - C_b) \times 2 \times 2.24$  }



**Fig. 3.9.** The schematic representation of instrument atomic absorption spectrophotometer.

### 3.4. Microbial Characterization

#### A) Preparation of pure culture:

In 10 ml of sterile physiological saline one Gram of the sample was mixed and shaken in a vortex vigorously for 3-5 minutes and incubated for 20-30 minutes at 37°C for activation of microorganisms. Then for pure culture by standard dilution method samples were prepared by using sterile distilled water. In serial dilution method each tube contains 9 ml of distilled water and one ml of activated suspension from which the suspension has been transferred from the first tube till the last tube (Patil and Deshmukh, 2016).

From each test tube 0.1 ml of suspension was transmitted to the sterile nutrient agar plate and evenly spread in the plates by using a sterile glass rod by using the spread plate method. Then the plates were incubated for 24 hours at 37°C. Based on the colony characteristics and colony morphology the organisms are separated and by using the streak plate method. Then the colonies were inoculated in MacConkey agar and Blood agar plates and incubated for 24 hours at 37°C (Gupta & Rana 2016).



**Fig. 3.10. Isolation of pure culture (*E.coli*, *Bacillus subtilis*, *Pasturella canis*) by using MacConkey agar and Blood agar**

**B) Isolation of Fungus:**

For isolation of the fungus from the cow dung pour plate technique was performed. In 9 ml of sterile saline solution one gram of cow dung was mixed and diluted serially from  $10^1$  till  $10^6$  (Patil and Deshmukh, 2016). Then further 1ml of solution from each serial dilution was added into Sabouraud dextrose agar (SDA) plates and incubated for 72 hours at  $28^\circ\text{C}$  and then fungal spore-forming unit (sfu/g) and then calculation was done. Triplicate sets were performed for easy identification and confirmation of pure culture.

After accomplishing pure culture of fungal isolate, culture characteristics were macroscopically studied and microscopic observation was performed by using the Lactophenol Cotton Blue method. Slides were observed under a microscope at 45x and 10x magnification. Further by 18s rRNA sequencing confirmation was done (Accession Number LC413613.1)



**Fig. 3.11. Isolation of Fungus (*mucor circinelloides*) by using Sabouraud dextrose agar (SDA)**

### **C) Identification of bacterial isolates:**

The bacterial isolates were identified by performing standard biochemical reactions such as the Oxidase test, Catalase test, Voges Proskauer test, Triple sugar Iron test, Indole Test, Urease test, Citrate utilization test Methyl red test and automated Vitek 2 compact test (Biomeriux). Table one represents the results of the biochemical test.

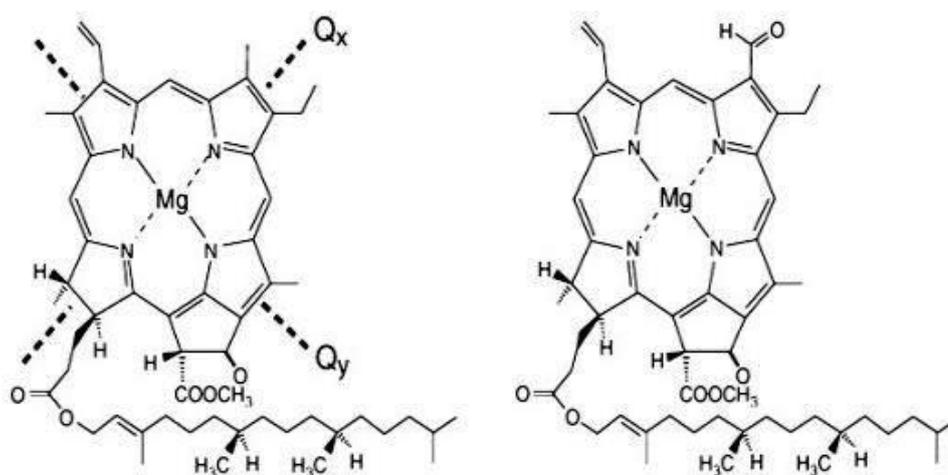
## **3.5. Phytochemical and morphological parameters of (tomato plant)**

### **A) Chlorophyll**

As an indicator of chloroplast content, photosynthetic mechanism, and of plant metabolism, a leaf chlorophyll concentration is an important parameter that is regularly measured (Hawkins et al., 2009). Chlorophyll is antioxidant compounds that are present and stored in the chloroplast of green leaf plants and mainly it is present in the green area of leaves, stems, flowers, and roots (Naus et al., 2010).

The chlorophyll is the essential component for photosynthesis and occurs in chlorophyll as a green pigment in all photosynthetic plant tissues. Chemically, each chlorophyll molecule contains a porphyrin (tetra pyrol) nucleus with the chelated magnesium atom at the center and a long-chain hydrocarbon (phytyl) side chain attached through a carboxylic acid group. There are at least five types of chlorophyll in plants (Pruzinska et al., 2005), (Richardson et al., 2002). However, as per the research of Srichaikul et al., 2011, chlorophyll production is mainly depended on the penetration of sun light and it is the main source of energy for plants (Srichaikul et al., 2011).

The molecular structure of (A) chlorophyll a and (B) chlorophyll b which is prescribed by Chen and Blankenship in 2011 has shown in fig. 3.12.



**Fig. 3.12 The molecular structure of (A) chlorophyll a and (B) chlorophyll b. (Chen, M. and Blankenship, R. E. 2011)**

The chlorophyll content in the leaf of plant was estimated by the method of Witham *et al.* (1971). The methodologies used for chlorophyll extraction in plant materials are almost always based on methods that destructively extract leaf tissue using organic solvents that include acetone, dimethylsulfoxide (DMSO), methanol, N-dimethyl formamide, and petroleum ether. During the extraction and dilution, significant pigment losses may occur thus leading to high variability in the results. Chlorophyll is extracted in 80% acetone and the absorbance is measured at 645nm and 663nm. The amount of chlorophyll is calculated using the absorption coefficient (Witham *et al.*, 1971). Chlorophyll was extracted from 1g of the sample using 20ml of 80% acetone. The supernatant was transferred to a volumetric flask after centrifugation at 5000 rpm for 5 minutes. The extraction was repeated until the residue became colorless. The volume in the flask was made up to 100ml with 80% acetone. The absorbance of the extract was read in a spectrophotometer (Genesys 10-S, USA) at 645 and 663nm against 80% acetone blank.

### Calculations:

The amount of total chlorophyll in the sample was calculated using the formula.

$$\text{Total chlorophyll} = 20.2 (A_{645}) + 8.02 (A_{663}) \times \frac{V}{1000 \times W} \quad (3.5.1.)$$

Where,

V = final volume of the extract

W = fresh weight of the leaves

### B) Polyphenol

Phenols, the aromatic compounds with hydroxyl groups are widespread in the plant kingdom. They found in all parts of the plants (Brighente et al 2007). Grains containing a high amount of polyphenols are resistant to disease and pest in plants. Total phenols estimation can be carried out with the Folin- Ciocalteu reagent. Phenols react with Phosphomolybdic acid in Folin-Ciocalteu reagent in alkaline medium and produce blue-colored complex (molybdenum blue) (Siddhuraju et al., 2002).

Exactly 0.5 to 1.0gm of sample weighed and ground with a pestle and mortar in 10 times volume of 80 % ethanol followed by centrifugation was done of a sample at 10,000 rpm for 20 min. supernatant saved and re-extracted the residue with five times the volume of 80% ethanol, the supernatant was evaporated to dryness, while residue was dissolved in a known volume of distilled water (5 ml). Different aliquots (0.2 to 2 ml) were pipette out into test tubes, with the help of water volume which made up in each tube to 3 ml. 0.5 ml of folin-Ciocalteu reagent and 2 ml of 20% sodium carbonate were added after 3 min respectively, absorbance was measured at 650 nm. The standard curve was prepared by using different concentrations.

### C) Protein

Proteins are polymers of amino acids. There are 20 various types of amino acids observed naturally in proteins. Concerning the type, number, and sequence of amino acids that make up the polypeptide backbone, proteins differ from each other (Lizarazo et al., 2015). Hence, they have different molecular structures, nutritional attributes, and physiochemical properties. Also, proteins are always considered one of the important constituents of foods due to various

reasons. Protein contains a variety of amino-acids, like lysine, tryptophan, methionine, leucine, isoleucine, and valine, all these amino acids are required for better growth of human health (Multari et al., 2016). While it can also be found that many food proteins are enzymes that are capable of enhancing the rate of certain biochemical reactions (Pihlanto et al., 2017).

Protein can be estimated by different methods as described by Lowry and also by estimating the total nitrogen content. Hydrolyzing the protein and estimating the amino acid alone will give the exact quantification. The method developed by the Lowry *et al* (1951) is sensitive enough to give a moderately constant value and hence largely followed. The protein content of enzyme extracts is usually determined by this method.

The phenolic group of tyrosine and tryptophan residues (amino acid) in a protein will produce a blue-purple color complex, with maximum absorption in the region of 660 nm wavelength, with Folin- Ciocalteu reagent which consists of sodium tungstate molybdate and phosphate (Schauser et al., 2005). Thus the intensity of color depends on the amount of these aromatic amino acids present and will thus vary for different proteins (Amamcharla et al., 2010). Most protein estimation techniques use Bovin Serum Albumin (BSA) universally as a standard protein, because of its low cost, high purity, and ready availability. The method is sensitive down to about 10µg/ml and is probably the most widely used protein assay despite its being only a relative method, subject to interference from Tris buffer, EDTA, nonionic and cationic detergents, carbohydrate, lipids, and some salts. The reaction is dependent on pH and a working range of pH 9 to 10.5 is essential. Extracted protein is to be measured at 660 nm on a spectrophotometer. With the help of the standard curve graph, the concentration of protein content in the leaves sample can be calculated (Schauser et al., 2005).

#### **D) Height of the Plants**

In a green revolution new potential, much higher yields are related to using dwarf varieties that will be able to feed high levels of nutrients without the rank growth that would overthrow the productivity above ground. Plants are mirror images that are nearly identical at the top and the bottoms. The root biomass below ground is compromised which makes the plants less able to get scarce soil nutrients rather than a fertilizer. Height and branching can be of great value to defensive characters but dwarf singular stemmed plants are often favored based on

the mechanical harvest uniformity. The height of the tomato crops is measured with the help of measuring tape from ground level to leave the base of the highest fully expanded leaf.

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## CHAPTER 4

# Conversion of Organic Biomedical Waste into Potential Fertilizer Using Isolated Organisms from Cow Dung for a Cleaner Environment

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RESEARCH ARTICLE



Conversion of organic biomedical waste into potential fertilizer using isolated organisms from cow dung for a cleaner environment

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## **Conversion of Organic Biomedical Waste into Potential Fertilizer Using Isolated Organisms from Cow Dung for a Cleaner Environment**

### **4.1. Introduction:**

Hospitals are complex institutions in which peoples frequently visit them without any race, religion, sex, age (Rajakannan et al., 2013). In health care centers and hospitals mostly patients, the staff is present. The generation of biomedical waste is not only limited to health care centers, hospitals or staff, patients but it has its huge serious impact on the environment, animals, plants, and human beings (Deb et al., 2017), (Sharma et al., 2013). Around the world development in hospitals and health care centers has led to substantial improvement in developed countries for the management of biomedical waste in order to avoid infections and to safeguard the environment. The Ministry of Environment and Forest has formulated and notified the biomedical waste rules in 1998 and amended at specific time interval according to need which should be followed by all hospitals, healthcare centres where biomedical waste generated, and accordingly, treatment should be given (Vasistha et al., 2018).

Direct discharge of biomedical waste in open areas without any preliminary treatment is one of the main concerns (Ramesh Babu et al., 2009). In the hospitals or healthcare centres various chemical, hazardous substances are used like pharmaceuticals, disinfectants, radionuclides, and other solvents for treatment of patients like for diagnostics, for research purposes, disinfection (Chakraborty et al., 2013). Due to the mixing of biomedical waste into general waste various hazardous compounds are found in surface water which contaminates and disturbs the entire ecosystem and then that toxic compounds accumulate in the entire food chain and bioaccumulation occurs (Thakur and Ramesh, 2015). To avoid the further problem of bioaccumulation and biomagnification the significant management of biomedical waste by using effective treatment is needed (Rudraswamy et al., 2012).

The existing method harms the environment and living beings and also causes a huge amount of pollution which disturbs the total environment, so for the treatment of biomedical waste alternative

options should be needed which is to be eco-friendly and organic. So various researches have used cow dung for treatment of biomedical wastes as it has tremendous bioremediation property. It is eco-friendly, economically viable, cheap, and locally accessible (Anitha and Jayaaj, 2012). Composting is a well known traditional process which recycles the organic waste and breaks complex organic matter into simple form and converts into the value-added product (Quan Wang et al., 2018). Due to its expansive applications in the area of energy generation, environmental protection, and in medicine field cow dung is considered as "gold mine" (Randhawa and Kullar, 2011). Cow dung is a mixed culture and numerous organisms, bacteria, and fungus are present in it which is responsible for the degradation of biomedical waste which has huge benefits without prevailing any dangerous trace effect on a community (Pandey et al., 2011), (Gupta et al., 2016), (Patil et al., 2019). So there is essential to develop the management of biomedical waste by using an appropriate method.

In the current research the innovative process for the treatment of biomedical waste was developed. The pure cultures of fungus and bacteria were isolated from cow dung. The physicochemical parameters of experimental sets and soil were analyzed. Heavy metals and phyto-chemical parameters of plants were evaluated to confirm that organic biomedical has been converted into potential fertilizer.

## **4.2. Materials and Methods:**

### **4.2.1. Collection of samples (for each set):**

The samples were collected from D. Y. Patil medical college hospital and research institute Kolhapur. 50gram of fresh organic biomedical waste samples (10gm dressing swabs, 30gm blood swabs, and 10gm used cotton) were collected and then at 121<sup>0</sup>C samples were doubled autoclaved and prior to use.

#### **4.2.2. Collection of Cow dung:**

Three cow dung samples were collected on three successive days by using the sterile container. A cow was maintained with the same diet pattern for 4 days. After collection of samples it were labeled properly and straightly transported to the laboratory and for the screening of any gastrointestinal infection with the help of microscopy for bacterial culture, a faecal occult blood test for any intestinal bleeding and intestinal parasitic infection. (Gupta & Rana 2016).

#### **4.2.3. Preparation of pure culture:**

In 10 ml of sterile physiological saline one gram of the sample was mixed and it was shaken in a vortex vigorously for 3-5 minutes and incubated it for 20-30 minutes at 37<sup>0</sup>C for activation of microorganisms. Then for making pure culture, serial dilution method was used. In serial dilution method each tube contains 9 ml of distilled water and one ml of activated suspension then the suspension was transferred from the first tube till the last tube (Patil and Deshmukh, 2016).

From each test tube, 0.1 ml of suspension was transmitted to the sterile nutrient agar plate and evenly spread in the plates by using a sterile glass rod. Then the plates were incubated for 24 hours at 37<sup>0</sup>C. Based on the colony characteristics and colony morphology the organisms were separated and by using the streak plate method bacterial cultures were purified. Then the colonies were inoculated in MacConkey agar and Blood agar plates and incubated for 24 hours at 37<sup>0</sup>C (Gupta & Rana 2016).

#### **4.2.4. Isolation of Fungus:**

For isolation of fungus from the cow dung, pour plate technique was performed. In 9 ml of sterile saline solution one gram of cow dung was mixed and sample was diluted serially from 10<sup>1</sup> till 10<sup>6</sup> (Patil and Deshmukh, 2016). Then further 1ml of solution from each serial dilution was added into Sabouraud Dextrose Agar (SDA) plates and incubated for 72 hours at 28<sup>0</sup>C and then fungal spore-

## Chapter 4

### Conversion of Organic Biomedical Waste into Potential Fertilizer Using Isolated Organisms from Cow Dung for a Cleaner Environment

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forming unit (sfu/g) and then calculation was done. Triplicate sets of pour plate technique were performed for easy identification and confirmation of fungus culture.

After accomplishing pure culture of fungal isolate, culture characteristics were macroscopically studied and microscopic observation was performed by using the Lacto-phenol Cotton Blue method. Further by 18s rRNA sequencing confirmation was done (Accession Number LC413613.1). Fig. 4.1. represents isolation of organisms and fungus in petri plates.



**Fig 4.1. Isolation of organisms and fungus in petri plates (pure culture).**

#### 4.2.5. Identification of bacterial isolates:

The bacterial isolates were identified by performing standard biochemical reactions such as the oxidase test, catalase test, voges proskauer test, triple sugar iron test, indole test, urease test, citrate utilization test methyl red test and automated vitek 2 compact test (biomeriux). Table 4.1. represents the results of the biochemical test.

**Table 4.1. Biochemical reaction of isolated bacteria.**

Test	Organisms		
	<i>E.coli</i>	<i>Bacillus subtilus</i>	<i>Pasterulla canis</i>
Catalase	+	+	+
Oxidase	-	-	+
Indole test	+	+	+
Methyl red test	+	-	+
Voges Proskauer test	-	+	-
Citrate utilization test	-	-	-
Urease Test	-	-	-
TSI	Acid/Acid	Alkaline/ Alkaline	Acid /Acid

From the above table the biochemical results of organisms were confirmed. The biochemical test help for identification of micro-organisms.

#### 4.2.6. Preparation of experimental sets:

After the collection of the sample, the triplicate sets of experiments were arranged and kept for 288 hours in anaerobic conditions.

**Table 4.2. Preparation of experimental sets.**

Sr. No	Name of Set	Composition
1	A	Control (50gm of BMW + 30ml d/w).
2	B	<i>E.coli</i> sample ( <i>E.coli</i> suspension 15ml + organic BMW 50 gm + d/w 30ml).
3	C	<i>Bacillus subtilus</i> sample ( <i>Bacillus subtilus</i> suspension 15ml + organic BMW 50gm + d/w 30ml).
4	D	<i>Pasterulla canis</i> sample ( <i>Pasterulla canis</i> suspension 15ml + organic BMW 50gm + d/w 30ml).
5	E	<i>Mucor Circinelloides</i> sample ( <i>Mucor Circinelloides</i> suspension 15ml + organic BMW 50gm + d/w 30ml).
6	F	An equivalent amount of organic BMW was incinerated by the standard method

d/w- Distilled water, BMW- Biomedical waste.

#### 4.2.7. Mixing of experimental sets into the soil:

The soil was taken from agriculture farm which is situated near Panchganga River. The treated organic biomedical waste were submerged in the soil and then the physico chemical parameters of soil sets were analyzed. The control experimental set (A) has not submerged as the physico-chemical results are beyond the limit of Central Pollution Control Board (CPCB).

**Table 4.3. Submerging of experimental sets into the soil:**

Sr. No	Name of Set	Composition
1	A1	Control (1000gm of soil)
2	B2	50gm set B submerged in 1000gm of soil, it is referred to as B2
3	C3	50gm set C submerged in 1000gm of soil, it is referred to as C3

4	D4	50gm set D submerged in 1000gm of soil, it is referred to as D4
5	E5	50gm set E submerged in 1000gm of soil, it is referred to as E5
6	F6	50gm set incinerated ash submerged in 1000gm of soil, it is referred to as F6

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#### **4.2.8. Plant selection for the experiment:**

The *S.lycopersium* species (tomato plants) of fifteen days, because it has a short duration were chosen for plantation.

#### **4.2.9. Physicochemical parameters of sample:**

The samples are evaluated by performing their physicochemical parameters like pH, Biochemical Oxygen demand, Total Dissolved Solids (TDS), Chemical Oxygen Demand (COD).

#### **4.2.10. Physicochemical parameters of soil:**

The physicochemical characterization of a soil sample was done like pH, organic matter, organic carbon content, EC, magnesium, calcium, nitrogen, water holding capacity, potassium, phosphorus, and heavy metals.

#### **4.2.11. Phytochemical analysis of plants:**

The phytochemical and morphological parameters of tomato plants were also studied like polyphenol content, protein content, chlorophyll content.

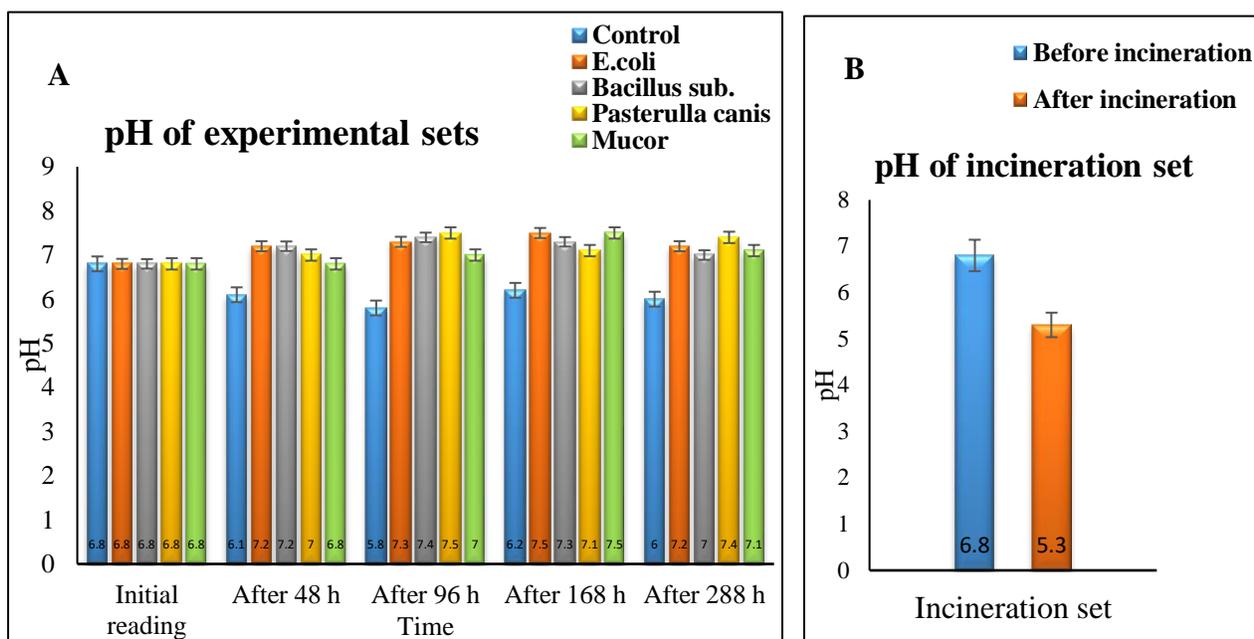
### **4.3. Results and Discussions**

#### **4.3.1. Physicochemical parameters of experimental sets.**

##### **4.3.1.1. pH.**

It has been reported that the excellent degradation of organic waste is obtained in the neutral pH value and 70-90% of degradation of organic waste and of total dissolved solid was seen at 6-8 pH value (Dinamarca et al., 2018). Katheem Kiyasudeen S et al also revealed that neutral pH supports

the growth of microorganisms and enhance microbial activity to degrade solid waste or organic waste (Katheem Kiyasudeen S et al 2016).

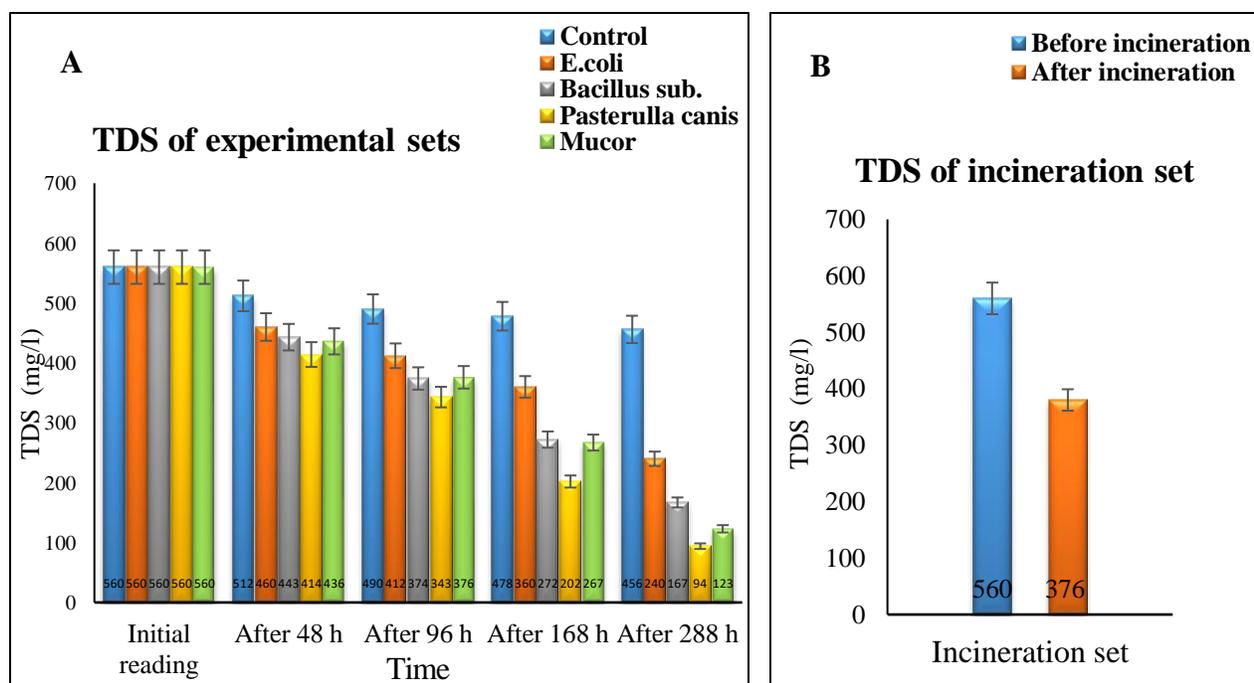


**Fig 4.2.** Figure (A) The pH result of experimental sets at the different time intervals of various organisms like *E. coli*, *Bacillus subtilis*, *Pasterulla canis* *Mucor Circinelloides* and of control. Figure (B) The pH readings of incineration sets (before and after).

The most of the microbial activity is observed in the neutral pH and degradation rate was superior in the neutral pH value. After a specific time interval, the pH of the experimental sets were analyzed. In the set of control to some extent slightly acidic pH was seen, whereas in *Bacillus subtilis*, *E. coli*, *Mucor Circinelloides* and in *Pasterulla canis* the pH was neutral at different time interval. Whereas in figure 4.2. (B) The biomedical waste was incinerated and after incineration of organic biomedical waste acidic pH was observed.

#### 4.3.1.2. Total dissolved solids (TDS)

Total dissolved solids is a measure of all dissolved elements like organic, inorganic, ionized, molecular or micro granular suspended form. They are determined by evaporating dryness of the filtered sample, and the dry residue mass of the sample is calculated. Total dissolved solids is not been considered as primary pollutants but it has been used as a sign of aesthetic features of water (Atekwana et al., 2004). It has been reported that when organic matter treated with the cow dung due to mixed culture degradation of TDS has been observed. S. K. Bansal et al treated organic waste with cow dung in pilot-scale batch reactor and 68.75% degradation of TDS has been observed respectively, within the eight days (Bansal et al., 2012). The boy's hostel waste was treated with the vermicomposting technique and nearly 85% degradation in TDS was observed (Lakshmi et al., 2014).



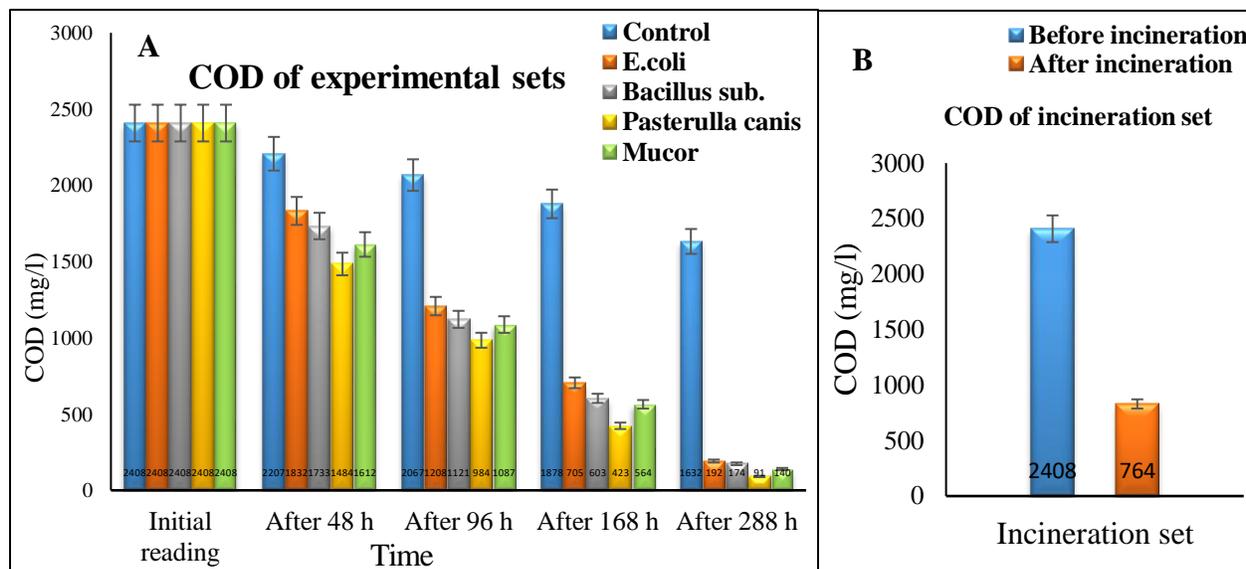
**Fig 4.3. Figure (A) The TDS of experimental sets at the specific time interval five results of experimental sets are shown control, *E. coli*, *Bacillus subtilus*, *Pasterulla canis*, and *Mucor circinelloides*. Figure (B) The readings of TDS incineration sets (before and after incineration).**

To make it eligible for the fertilizer application the TDS of the particular sample must be less than 700mg/l. The experimental sets were analyzed at a different time interval the results of TDS are represented in Fig 4.3. After analysis over 288 hours of the operational period it was confirmed that there were excellent decrease in TDS in all the samples except control. The fungus and the bacteria have effectively decrease the total dissolved solids which are present in the experimental sets and all the samples are eligible to apply as a fertilizer. The progressive decrease in TDS was observed in the case of *Pasterulla canis* and it was 560 to 094mg/l which was extremely significant than that of other sets.

#### 4.3.1.3. Chemical Oxygen Demand (COD)

Chemical oxygen demand (COD) is a standard method in which the quantity of the polluted material which is biologically not oxidized in the sample is measured indirectly (Singh et al., 2012). The chemical oxygen demand process is based on the degradation of an inorganic substance, organic substances, or chemical elements that are present in the sample (Ali and Yasmin, 2014). It has been revealed that when the organic waste submerged in the cow dung for the treatment purpose there was 35.75% degradation in COD respectively (Bansal et al., 2012). When the leachate of the solid waste is treated with the ash of cow dung in neutral pH condition than 79% COD removal was achieved so the cow dung is a good source for COD absorbent and it should be significantly used for removal of a pollutant from waste (Kaur et al., 2016). Divya C Das et al ayurvedic hospital waste with a help of microorganisms and then further it is treated in vermifiltration unit after treatment it was revealed that there is a 98.03% decrease in the COD after reduction again it was disinfected and after disinfection of waste, it used for irrigation (Das et al., 2015). For the purpose of the bio decomposition, Jianzheng Li et al the industrial sludge waste is treated with cow dung for four weeks and excellent degradation of COD was observed (Li et al., 2011). . It has been reported that the *Mucor circinelloides* can effectively degrade the hydrocarbons and also degrade the compounds which are present in diesel oil (Olga Marchut et al., 2015). So moving towards the organic approach we have designed the work and various parameters were

analyzed to confirm the biodegradation rate of organic biomedical waste. Fig. 4.4. represents the COD values of experimental sets at a different time interval (mg/l).

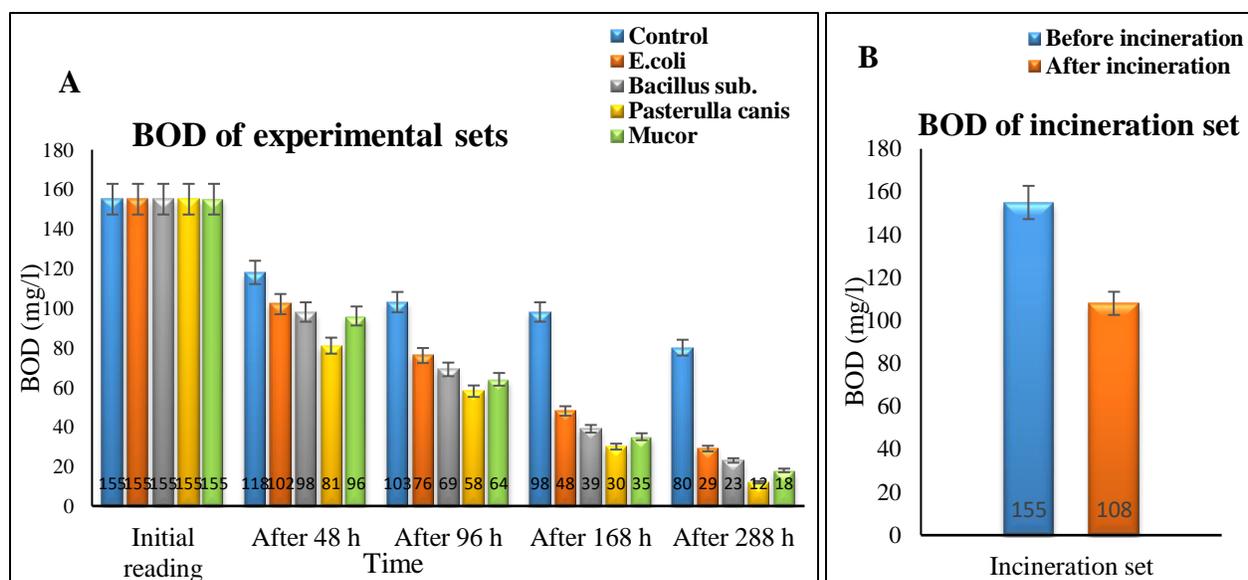


**Fig 4.4.** Figure (A) the results of control, *E. coli*, *Bacillus subtilis*, *Pasterulla canis*, and *Mucor circinelloides* are represented. Figure (B) The readings of COD incineration sets (before and after incineration).

From fig 4.4. (A), it is revealed that when the biomedical waste is treated with fungus and organisms sufficient degradation of biomedical waste is observed in 288 hours except in the control set. In the set first that is of control the COD value decreased from 2408 to 1632 mg/l, in the second set of (*E. coli*) it was decreased from 2408 to 192 mg/l, in the third set (*Bacillus subtilis*) it was decreased from 2408 to 174 mg/l, in the fourth set (*Pasterulla canis*) it was decreased from 2408 to 91 mg/l, in the fifth set (*Mucor Circinelloides*) the COD was decreased from 2408 to 140 mg/l and in the set of incineration, the level of COD was decreased from 2408 to 764 mg/l. This significant decreased in the level of COD and in all other characterization reveals that the organisms and fungus which are isolated from cow dung can degrade the organic biomedical waste with respect to time.

#### 4.3.1.4. Biological Oxygen Demand (BOD)

Biochemical oxygen demand is the quantity of the amount of dissolved oxygen (DO) that is used by aerobic microorganisms when they are decomposing organic matter which is present in a waste sample. Biochemical oxygen demand is also referred to as biological oxygen demand. From the results of BOD we can identify the quality of the waste or effluent. The control waste sample or waste water usually has high biological oxygen demand (Singh et al., 2012). If the BOD of the sample is high the greater amount of organic matter or the biological matter is present in the sample. Then the bacteria consume the available oxygen present in the waste. In the water body the depletion in the DO content can cause stress on aquatic organisms and phytoplanktons, which disturbs the entire ecosystem making the environment inappropriate for life (Ali and Yasmin, 2014). Fig. 4.5. represents the COD values of experimental sets at different time interval (mg/l).

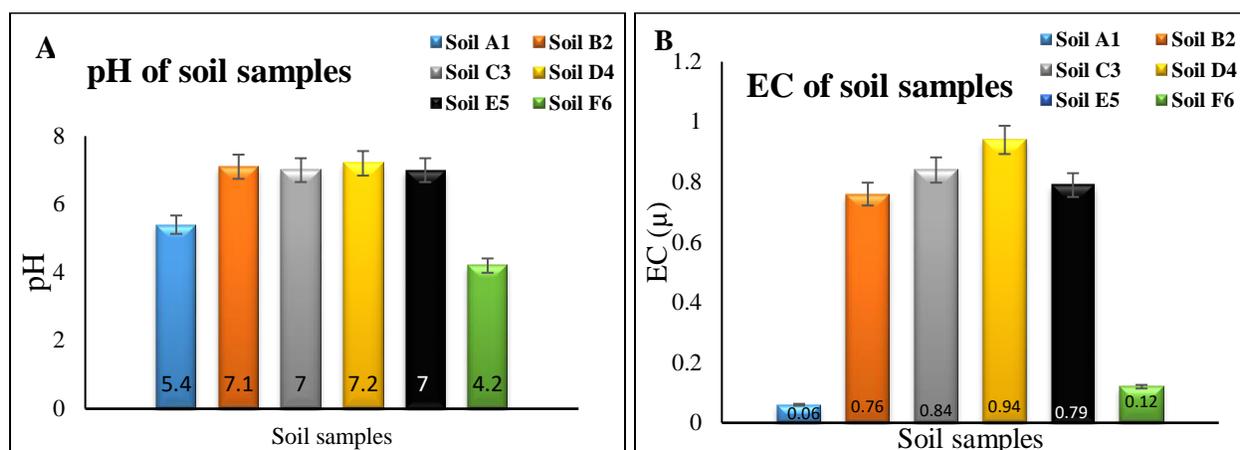


**Fig 4.5. (A) The results of control, *E. coli*, *Bacillus subtilis*, *Pasterulla canis*, and *Mucor circinelloides* are represented. (B) The readings of BOD incineration sets (before and after incineration).**

Figure 4.5. represent the results of biological oxygen demand from these results we can conclude that the treated experimental sets are within the permissible limit that is 30mg/l given by Central Pollution Control Board (CPCB). The set of control and incineration are beyond the permissible limit. The *Pasterulla canis* has acceptably degraded organic biomedical waste as compare to *E.*

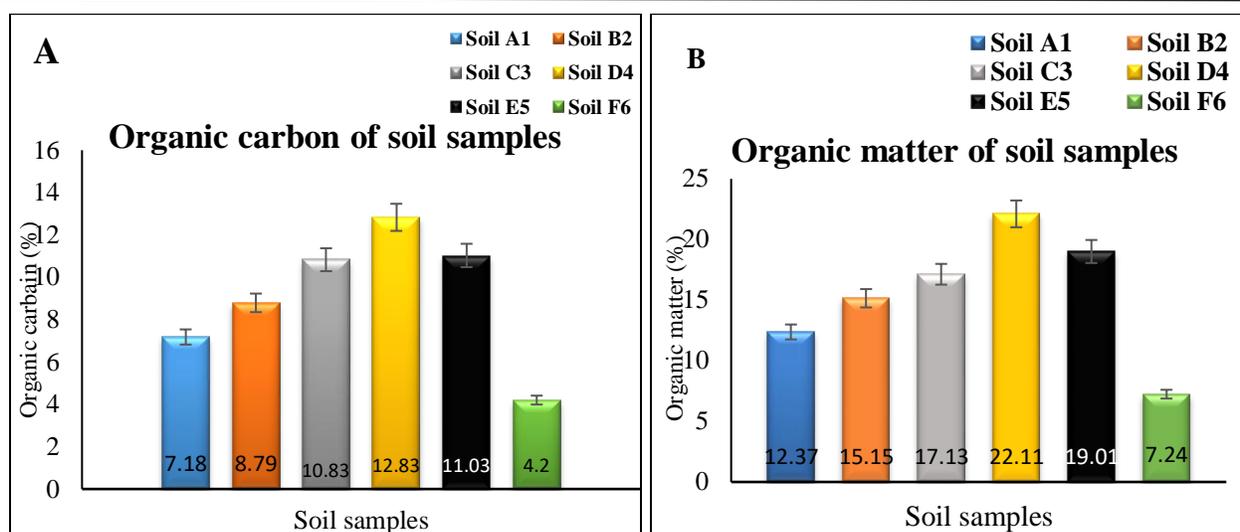
*coli*, *Bacillus subtilis*, *Mucor Circinelloides*. Due to the degradation of biomedical waste by organisms and fungus BOD level of experimental sets have been reduced.

#### 4.3.2. Physicochemical parameters of the soil sample.



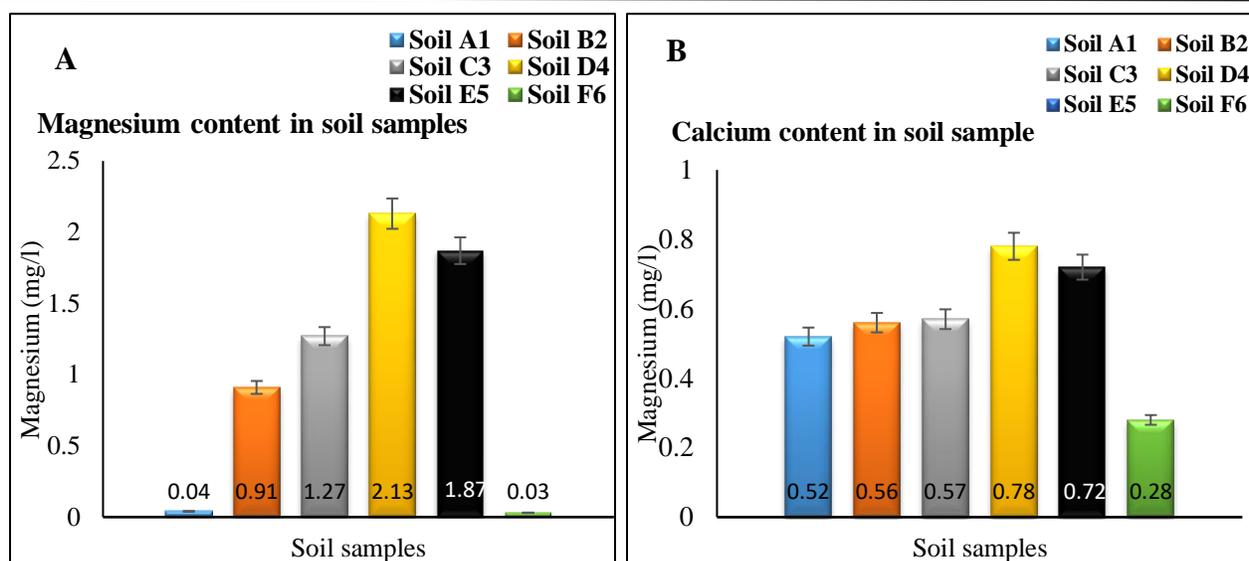
**Fig 4.6. (A) pH of soil samples and (B) EC content of soil samples.**

Due to soil pH several chemicals, biological routes in soil are disturbed so soil pH is recognized as the most vital parameter. In soil and in plants the finest value of pH is between 5.5 to 7.5 (Randhawa and Kullar, 2011). Fig. 4.6. represents the pH and EC values of soil samples. After the addition of residue in the soil the soil sample A1 has slightly acidic pH, and in soil sample F6 acidic pH value was observed due to submerging of incinerated ash in it. In the B2, C3, D4, E5 sample have neutral pH values due to microbial activity in the soil samples. Depending upon the presence of moisture content the electric conductivity of the soil may vary. The change in electric conductivity will impact crop yield. (Corwin and Lesch, 2005). The presence of EC in the soil depends upon the particle size, soil texture, and moisture content in soil (Xu et al., 2006). The content of EC in the F6 soil sample is low than that of further. The supplementary EC content is observed in soil sample D4 due to converting the complex organic material into simple organic material and producing the soil porosity by organisms.



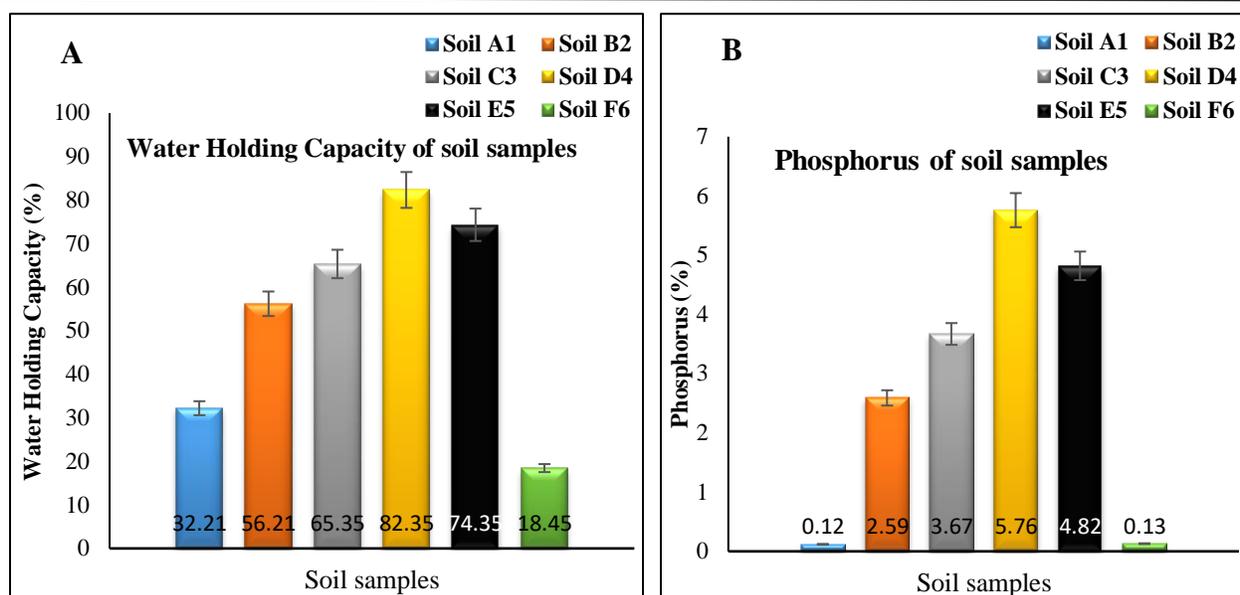
**Fig 4.7. Figure (A), (B) physico chemical parameters of soil samples organic carbon and organic matter content.**

Organic content exerts numerous positive things on the soil to continue the regulatory ecosystem (Campion et al., 2015). Fig. 4.7. represents the organic carbon and organic matter values of soil samples. After submerging the treated organic biomedical waste into a soil the bacteria and fungus degraded the biological content, cells, tissues of the organisms, and synthesized material which are present in the soil and in organic biomedical waste and converted it into organic matter. The *Pasterulla canis* has degraded high content of organic constituent so more organic matter is present in soil sample D4, than that of E5 (*Mucor Circinelloides*), C3 (*Bacillus subtilis*), B2 (*E. coli*), and A1 (Control) soil sets respectively. Then the incinerated ash has been mixed in F6 soil so most of the organic constituent has been burned, and due to the process of incineration less organic matter is present in the F6 soil sample.



**Fig 4.8. Figure (A), (B) physico chemical parameters of soil samples magnesium and calcium content in the soil.**

Fig. 4.8. represents the magnesium and calcium content of soil samples. The magnesium and calcium contents which are present in the organic biomedical waste (blood swabs or cells) have been degraded by the organisms and fungus and when the soil is submerged, it is transmitted in the soil. Due to the degradation of organic biomedical waste, and submerging of bacteria and fungus into the soil the contents of calcium and magnesium have been enhance. For the development and growth of the plant each and every plant require calcium as it helps to maintain the salinity of the soil, it balances the chemical process in soil and also improves the water penetration level. Magnesium plays a wide key role and it performs the numerous functions in plants. In photosynthesis, process magnesium plays a vital role, as it is building block of chlorophyll pigment, and the leaves of plants appear green due to the presence of magnesium (Karhu et al., 2011). In the set of control less calcium and magnesium contents have been seen as organic matter is not added in it and in the set of incineration (F6) the cells or biological matter has burned so less content of magnesium and calcium content is present. And in the D4 set high magnesium and calcium content in present followed by E5, C3, and B2 soil samples.

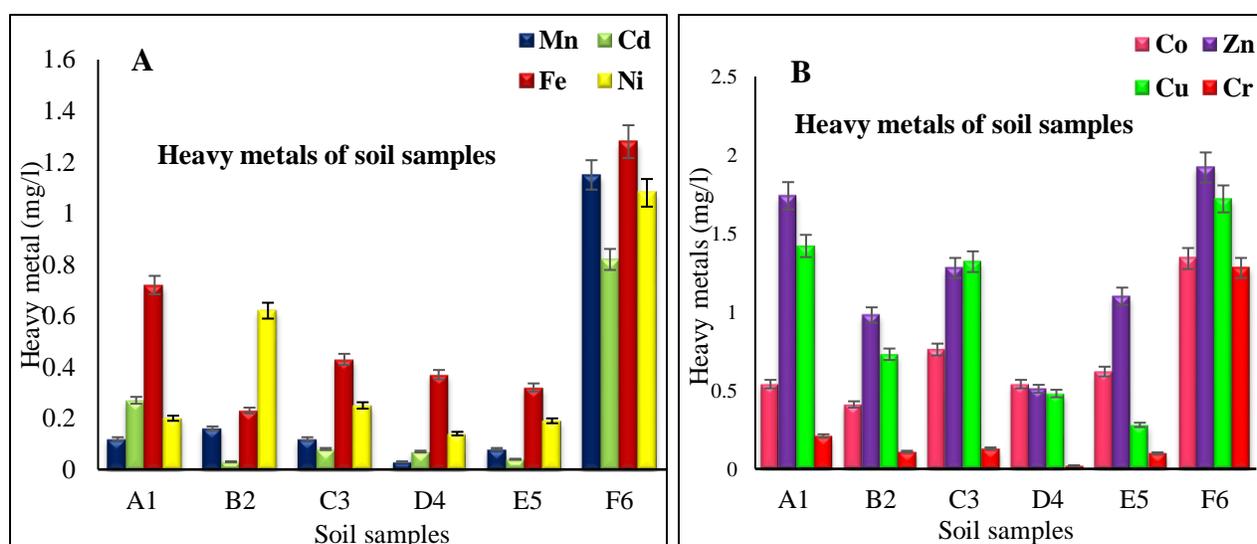


**Fig 4.9. Figure (A), (B) physico chemical parameters of soil samples water holding capacity and phosphorus content in the soil samples.**

Fig. 4.9. represents the water holding capacity and phosphorus of soil samples. The quantity of water by which the given soil can hold is known as the water holding capacity of the soil. The presence of organics matter increases the water holding capacity of the soil. When the organic matter is high the water holding capacity increases, when organic matter is less the water holding capacity decreases. The organic matter of D4 soil set is high so the water holding capacity of D4 soil sample is high followed by E5, C3, B2, A1 and F6 soil sample respectively. The phosphorus plays a vital role in the metabolic process like in energy transmission, breakdown, photosynthesis, and amalgamation of carbohydrates. (Bennett et al., 2001). The organisms break down the presence of complex organic material into simple organic and inorganic molecules which are present in soil and in the organic biomedical waste. So the organisms has broken down the complex organic matter and added organic and inorganic nutrient to the soil sets. After degradation and submerging of biomedical waste in soil the content of phosphorus in D4 soil sample is high followed by E5, C3, B2, F6 and A1 soil sample respectively

### 4.3.3. Heavy metals of the soil sample.

In soil the heavy metal is present due to the disposal of used products like pigmented plastics, batteries, electronics products, glasses, paints, ceramics, etc. The heavy metal is removed by the several mechanisms and process they can be removed by particulate settling and trapping, chemical precipitation, binding to organic substances they can also be removed by plant uptake, accumulation into plant tissues, filtration, adsorption of soil components, sedimentation of suspended particles, etc (Wuana and Okieimen, 2011). Fig 4.10. represents the heavy metal content of the soil sample.

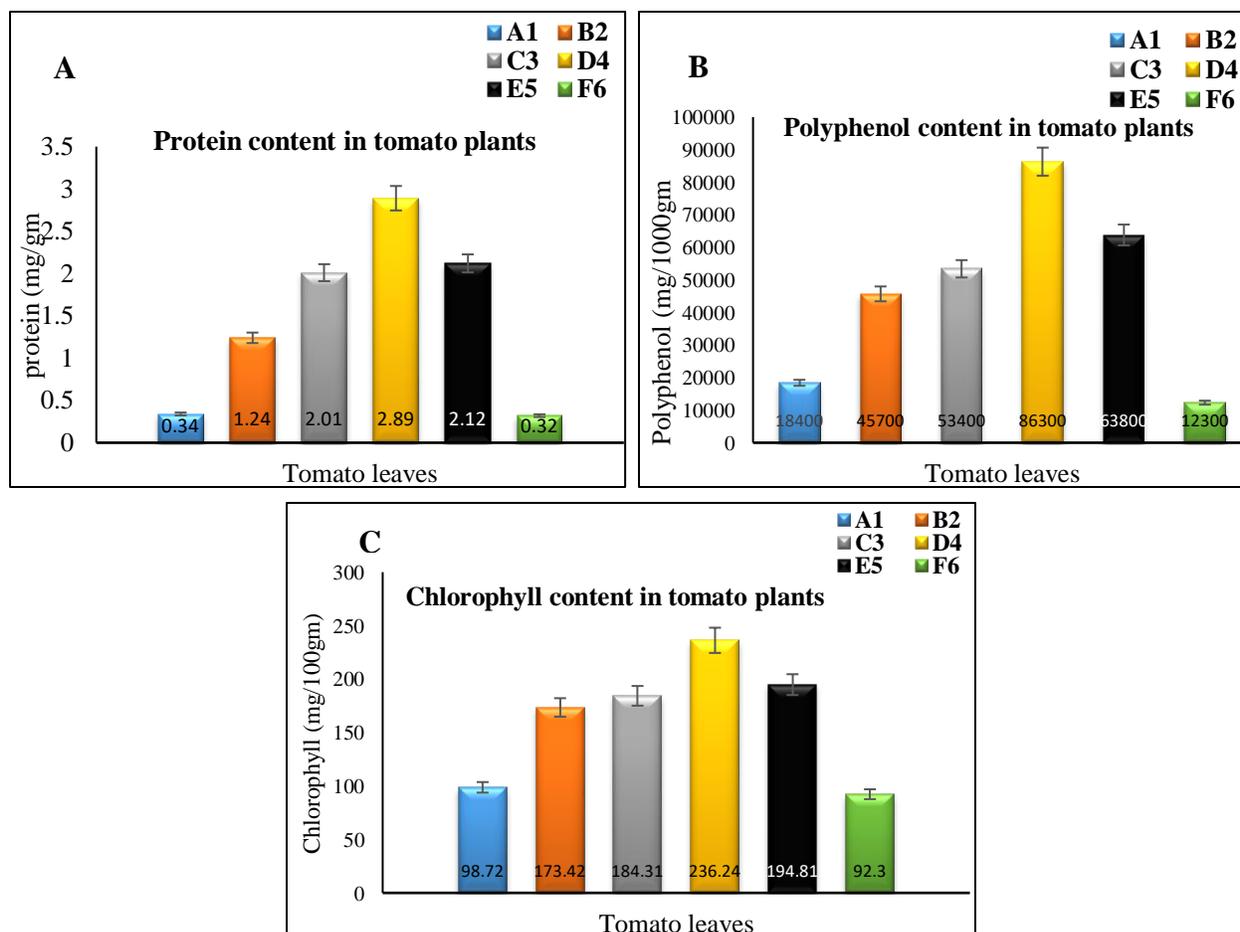


**Fig 4.10. (A) The heavy metal content of the soil sample (Mn, Cd, Fe, Ni) and figure (B) represents (Co, Zn, Cu, Cr).**

After analyzing the heavy metal content in the soil sample it has been concluded that the existence of heavy metals in the treated sets were within the permissible limit given by CPCB. The maximum permissible limits of CPCB for the organic compost are as follow (Cd-1mg/l, Fe-1mg/l, Ni-3mg/l, Zn-5mg/l, Cu-1.5mg/l, and Cr-2mg/l). As the heavy metal contents in the treated soil samples are within the limit there is no risk of contamination to the environment. But in the set of incineration the content of the heavy metal is more than that of others. So while disposing of the incinerated ash the proper analysis and treatment should be given to it.

#### 4.3.4. Phytochemical parameters of (Tomato plant).

After the sowing of tomato plants the phytochemical parameters of tomato plants were analyzed and results are represented in fig 4.11. and fig 4.12 represents plantation of tomato plants. In the process of photosynthesis chlorophyll pigment plays a significant role. During the process of photosynthesis, chlorophyll captures the rays of the sun, and energy or sugary carbohydrates are created which allows the growth of plants (Baglieria. et al., 2014). The tannins which are condensed are known as the abundant polyphenol, which originates in all families of plants. Most of the polyphenols are found in the flowers, leaf tissue, and fruit. These polyphenols which are present in the leaves or in the plants play an important role in the degradation of organic material. Proteins that originate in the plant are highly complex elements. Proteins originate in the cell of plants and carry out a diversity of cellular functions because the amino acid and proteins form the large constituent of plant life.



**Fig 4.11. phyto chemical parameters of tomato plants. (A) chlorophyll content, (B) polyphenol content and (C) protein content in tomato plant.**

The phyto chemical parameters play a vital role in the enlargement and development of the plant so it has been analyzed and results of phyto chemical parameters are in fig 4.12. As phyto chemical parameters plays a vital role in the process of photosynthesis so phyto chemical parameters were analyzed it has been revealed that the plant D4 has high chlorophyll, polyphenol and protein content followed by E5, C3, B2, A1, and F6 because all essential nutrients which require for the development and growth of plants like magnesium, calcium, NPK content are high in D4 soil set followed by E5, C3, B2, A1, and F6 soil sample respectively.



**Fig 4.12. Plantation of tomato plants.**

#### **4.4. Conclusions.**

It has been found that the fungus and the organisms isolated from cow dung shows exceptional degradation of organic biomedical waste. The physicochemical parameters of treated experimental sets, submerged soil sets, and phyto chemical parameters of the tomato plants are also enhanced. The heavy metal content in soil are within the limit. From the above result it is revealed that organisms can effectively degrade organic biomedical waste with respect to time. The microbiological method used for the treatment of organic biomedical waste is an organic, biological, and ecofriendly method the objectives are achieved without causing solitary harm to the environment. By using organisms (*E. coli*, *Bacillus subtilus*, and *Pasterulla canis*) and fungus (*Mucor circinelloides*) the organic biomedical wastes can be converted into a workable product.

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# CHAPTER 5

## CHAPTER 5

# Conversion of Organic Biomedical Waste into Worth Added Product Using Organic Approach



## **Conversion of Organic Biomedical Waste into Worth Added Product Using Organic Approach**

### **5.1. Introduction**

Biomedical waste is well defined as the waste produced during the immunization or treatment of animals, human beings, diagnosis, or through the research. If biomedical waste not treated appropriately may cause severe health hazards and environmental problems (Anitha and Jayaaj, 2012). The biomedical waste invites flies, insects, dogs, rodents, and cats that are responsible for the spread of contagious diseases (Kotasthane et al., 2017). Interaction with polluted materials and acquaintance to infected body fluids while handling hospital waste has a maximum risk of blood-borne contaminations (Jacob et al., 2017). In India the total biomedical waste generation by hospitals is around 397 kg/day. In India each city generates approximately 0.115 million tons of waste per day and annually 42 million metric tons (Narang et al., 2012). In India numerous hospitals are giving ayurvedic or herbal treatments to the patients by using traditional methods (Peltzer et al., 2016). Roughly 10-25% of hospital waste is harmful and infectious while 75-90% of hospital waste is non-infectious if these two hospital wastes submerged then the entire waste converts into harmful and infectious waste (Deb et al., 2017).

Incineration is used as the most existing treatment for discarding of biomedical waste (Vasistha et al., n.d.). Incineration leads to the enormous extent of air pollution and disclosure to harmful gases like polychlorinated dibenzo furans and polychlorinated dibenzo p-dioxins which hormonal imbalance, damage to the reproductive system, cancer and also cause the respiratory diseases (Prakash et al., 2017). In some cities, hospital waste has been discarded into open dumps. Due to the open dumping of biomedical waste there is an enormous threat of soil and groundwater contamination, as well as it declines the quality of air (Ali and Yasmin, 2014), (Mor et al., 2006). Poor management of hospital waste is having a negative impact on the environment and affects the entire society (Ramesh Babu et al., 2009). The biomedical waste which is disposed of in the open areas or the garbage bins may be mixed with municipal solid waste and converts entire waste

into hazardous waste. (Gupta and Boojh, 2006). Therefore, there is a necessity to develop safe work, transport, collection, handling, and biomedical waste (Chaudhuri et al., 2017).

In recent times organic approach for biomedical waste management was applied by authors in that they have treated the organic biomedical waste with the *Periconiella* species of fungus which is isolated from cow dung. Then further this species was submerged in the organic biomedical waste for a specific time interval which shows admirable degradation of organic biomedical waste (Pandey et al., 2011). Rajakannan et al. have used plant extracts for the disintegration of organic biomedical waste the degradation rate was also superior but the work has been restricted to degradation and further parameters were not analysed (Rajakannan et al., 2013). So looking towards the organic and eco-friendly approach the development of innovative method were done for degradation or decomposition of organic biomedical waste by using the extract of plants (Tobacco) and (Neem) as due to incineration enormous amount of pollution has been caused which disturbs the environment. As neem extract degrade the pathogen which is present in biomedical waste. The neem extract is responsible for the superior degradation of biomedical waste. The experimental sets were arranged and kept for the degradation of organic biomedical waste for the definite time interval. The physicochemical characterization of experimental sets was done to estimate the performance of tobacco and neem extract. After a definite time interval the degraded sets of biomedical waste, were submerged in the soil in equivalent proportions. Then further soils physicochemical parameters were evaluated to confirm its role of potential fertilizer. Then a heavy metal analysis of plants and soil was done because adulteration of heavy metals in plants and soil may pose risks and hazards to the environment and living organisms. The phytochemical and morphological parameters of plants were analysed and from the result it was revealed that organic biomedical waste has been converted into potential fertilizer.



**Fig. 5.1.** Diagrammatic representation and of degradation of organic biomedical waste and conversion into value added products.

## 5.2. Materials and Methods

### 5.2.1. Collection of the organic biomedical waste sample (each set):

50g of samples (12g dressing swabs, 6g of used cotton, 32g blood swabs) were collected from D. Y. Patil medical college hospital and research institute, Kolhapur. After collection of organic biomedical waste samples the sample were double autoclaved at 121<sup>0</sup>C for 2 hours samples are prior to use. Then the experiment sets were as shown in table 5.1.



**Fig.5.2. Experimental sets of organic biomedical waste.**

### 5.2.2. Preparation of tobacco and neem extract:

Forty grams of two *Nicotiana tabacum* (Tobacco) leaves were collected and in that 30 ml of distilled water were added in it and then after ground with mortar and pestle which forms 70 ml of extract. Identically eighty-five to ninety leaves of *Azadirachta Indica* (Neem) were collected and in that 30 ml of distilled water were added. Then after it has been ground with mortar and pestle which forms 70 ml of plant extract.

### 5.2.3. Preparation experimental sets:

The triplicate experimental sets were prepared after the collection of samples and kept for 96 hours in anaerobic degradation in an airtight container.

**Table 5.1. Preparation of experimental sets**

Sr.No	Name of Set	Composition
1	<b>A</b>	<b>(Untreated)</b> 50g of BMW + 40ml d/w
1	<b>B</b>	Tobacco extract 20ml + 50g of BMW + 40ml d/w
2	<b>C</b>	Tobacco extract 25ml + 50g of BMW + 40ml d/w
3	<b>D</b>	Neem extract 20ml + 50g of BMW + 40ml d/w
4	<b>E</b>	Neem extract 25ml + 50g of BMW + 40ml d/w

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5	<b>F</b>	Tobacco extract 20ml + Neem extract 20ml + 50g of BMW + 40ml d/w
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d/w- Distilled water, BMW- Biomedical waste.

#### 5.2.4. Submerging of experimental sets into the soil:

The soil was taken from the agriculture farm which is situated near Panchganaga River. 1000 grams of soil was mixed with 35 gram of residue to confirm their role as a potential fertilizer as shown in table 5.2. The untreated experimental set (A) has not submerged as the physico-chemical results are beyond the limit of Central Pollution Control Board (CPCB).

**Table 5.2. Mixing of experimental sets into the soil:**

Sr.No	Name of Set	Composition
1	<b>A<sub>1</sub></b>	<b>(Control)</b> 1000g of soil
2	<b>B<sub>2</sub></b>	35g set <b>b</b> mixed with 1000g of soil
3	<b>C<sub>3</sub></b>	35g set <b>c</b> mixed with 1000g of soil
4	<b>D<sub>4</sub></b>	35g set <b>d</b> mixed with 1000g of soil
5	<b>E<sub>5</sub></b>	35g set <b>e</b> mixed with 1000g of soil
6	<b>F<sub>6</sub></b>	35g set <b>f</b> mixed with 1000g of soil

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#### 5.2.5. Selection of plant for the experiment:

The two weeks species *S.lycopersium* (Tomato) plants were carefully chosen for plantation because its lifespan is short.

#### 5.2.6. Physicochemical parameters of sample:

The physicochemical characterization of the organic biomedical waste sample was analysed like Total Dissolved Solids (TDS), pH, and Chemical Oxygen Demand (COD).

### 5.2.7. Physicochemical parameters of soil:

The physicochemical parameters of a soil sample were analysed such as EC, pH, organic matter, organic carbon, potassium, total nitrogen, magnesium, calcium, phosphorus, and water holding capacity.

### 5.2.8. Phytochemical parameters of plants:

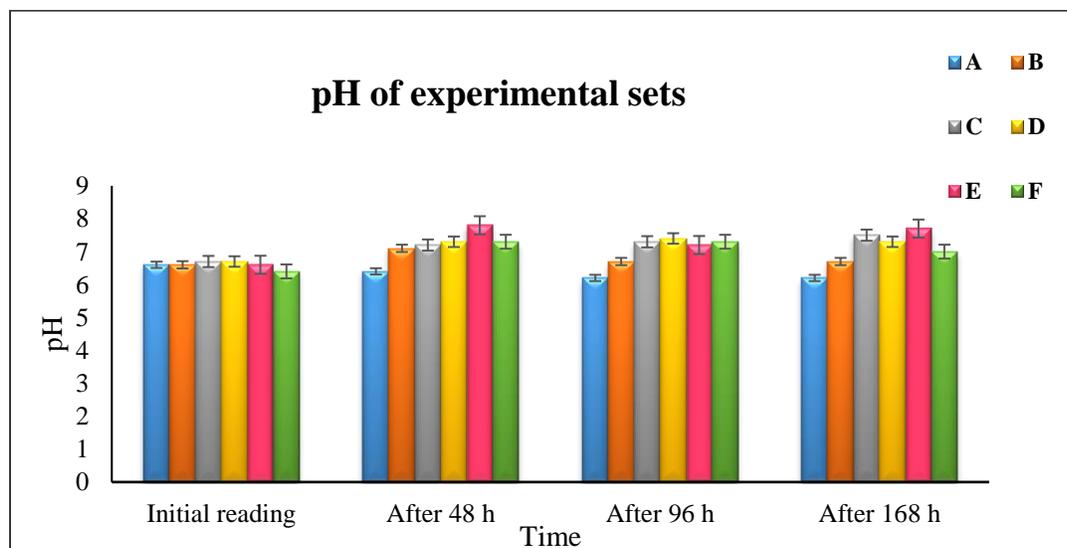
The phytochemical characterization of tomato plants was studied such as estimation of, polyphenol content, chlorophyll content, and protein content.

## 5.3. Results and Discussions

### 5.3.1. Physicochemical parameters of experimental sets.

#### 5.3.1.1. pH.

Generally, it has been revealed that the degradation rate of biomedical waste favours the neutral pH the key challenge was to retain the neutral pH during the experiment because the degradation rate of organic matter was more efficient in the neutral pH than that of acidic or basic (Nakasaki et al., 1993), (Nicol et al., 2008). Fig.5.3. represents the pH of experimental sets at different time intervals.

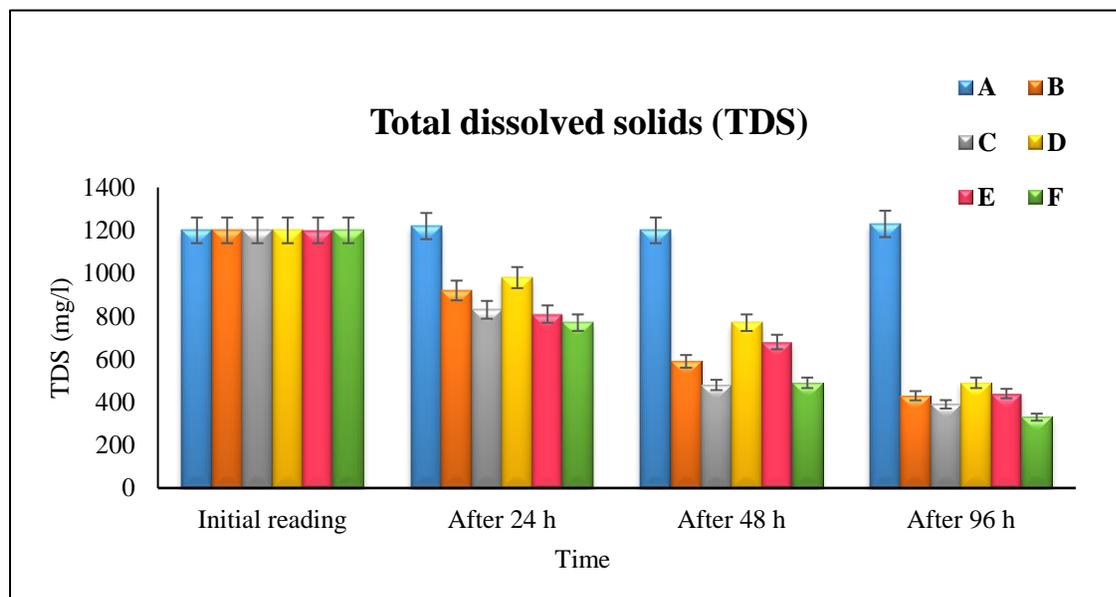


**Fig.5.3. The pH of experimental sets (A, B, C, D, E, and F) at different time intervals.**

It is reported that the extreme removal of COD and TDS occurs at the neutral pH. By the neutral pH the problem of odour will effectively reduce due to retardation it has been caused and it will also skillfully shorten the necessary time (Nakasaki et al., 1993). Therefore the experimental sets were analysed for pH at different time intervals and the results revealed that from the initial to final level all the experimental set are in the neutral range of pH.

### 5.3.1.2. Total dissolved solids (TDS).

All the inorganic and organic substance which are present in a sample and a suspended micro granular form are considered as TDS. In TDS salts such as sodium chloride, calcium, iron sulphates are present (Kasam et al., 2016). The chief aspects of changing the TDS and COD are due to the presence of organic and inorganic matter, the alteration in climatic conditions, and period of the solid waste. (Atekwana et al., 2004).



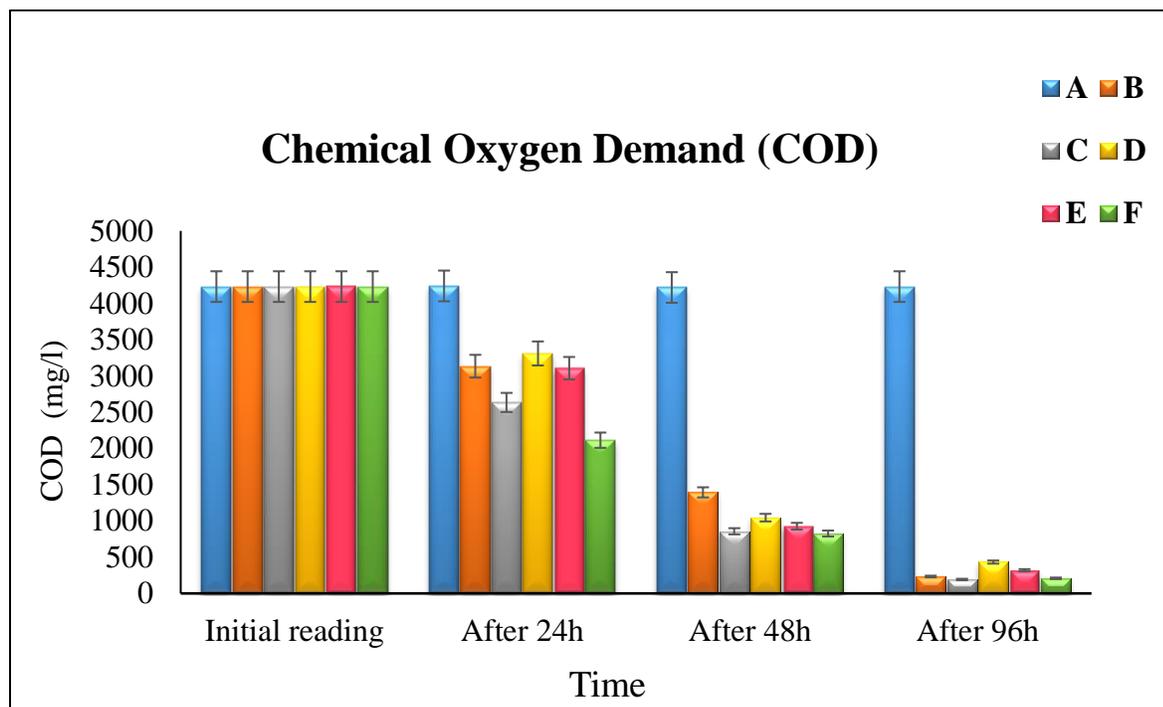
**Fig.5.4. TDS of experimental sets (A, B, C, D, E, and F) at different time intervals.**

The total dissolved solids content is used to explain the minute quantity of organic matter and inorganic salts in solution. Depending upon the type of waste the concentration of TDS may vary. Fig.5.4. represents the TDS of experimental sets with a different time interval. The result reveals that at the end of 96 hours except for untreated there was a progressive decrease in TDS all the experimental sets because of decomposition of organic biomedical waste. While in the case of

sample F the TDS value was drastically decreased from 1200 to 330mg/l this value is far significant than that of the required value for fertilizer.

### 5.3.1.3. Chemical oxygen demand (COD).

The COD and BOD determine the presence of a total concentration of organic matter or total organic constituents which is present in the waste sample. If the COD level is high the oxidizable organic matter must be present in a greater amount which ultimately decreases the level of dissolved oxygen (DO) (Mata-Alvarez et al., 2000). The most important application of COD is to analyze the number of oxidizable pollutants present in the waste (Khwairakpam and Bhargava, 2009). Fig.5.5. represents the COD of experimental sets at different time interval.



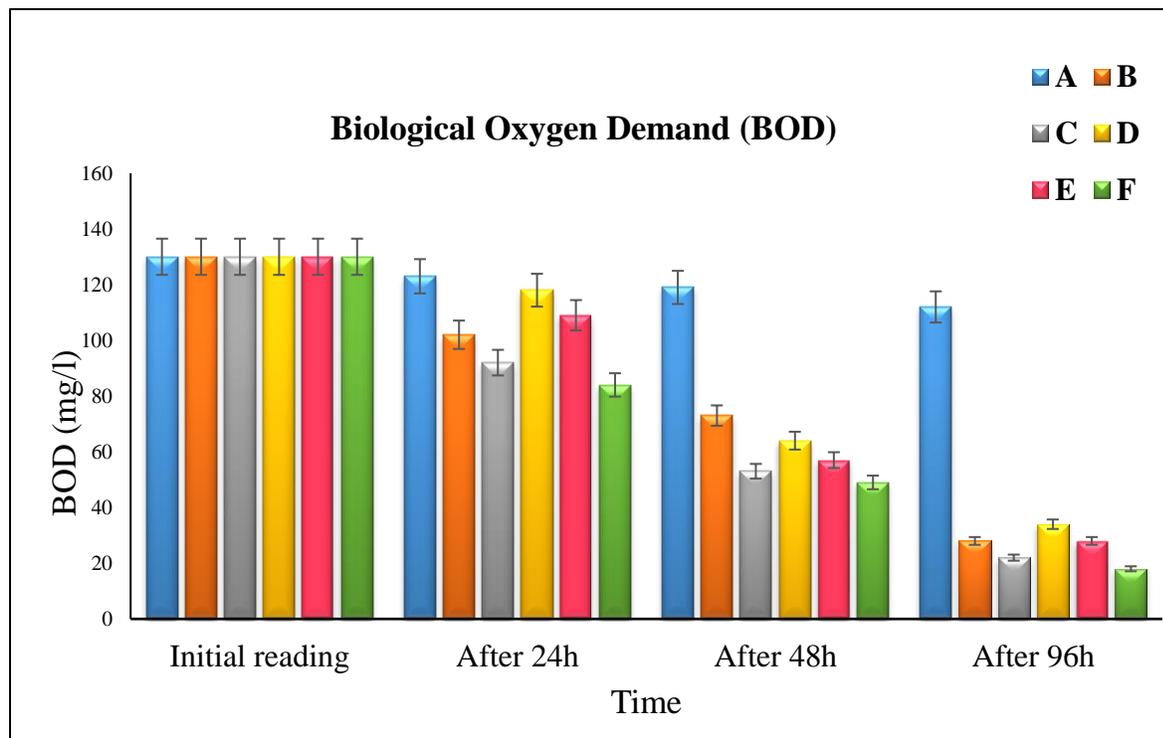
**Fig.5.5. COD of experimental sets (A, B, C, D, E, and F) at different time intervals.**

The experimental sets were analysed for COD after different time intervals from this result it has been concluded that the sample range for COD was initially more and as the time increases the level of COD decrease. In all the samples there was a significant decrease in COD at the end of 96 hours except in untreated set. These results show that there is a vital role of neem and tobacco extract for the degradation of organic biomedical waste. This reduction or degradation has

effectively converted organic biomedical waste into potential fertilizer and add value to the end product. As it is reported that when organic biomedical waste is treated with the neem extract in 120 hours 90% of COD has been reduced (Rajakannan et al., 2013).

#### 5.3.1.4. Biological oxygen demand (BOD).

The biochemical oxygen demand (BOD) is an essential environmental factor to determine the suitable oxygen necessities in the effluent, contaminated waste, and in the waste (Khwairakpam and Bhargava, 2009). It controls the molecular oxygen used during a definite incubation time for the reduction of organic matter and the oxygen which is used to oxidize inorganic matter (Mohabansi et al., 2011), (Trujillo et al., 2006). The substantial aspect of compost superiority is recognized by the fraction of readily available biodegradable organic material (Rastogi et al., 2003). After smearing the compost to the soil for crop practice, biological processes will last so care must be taken because later maintenance of compost soil nutrients can strip (Kalamdhad, 2013).

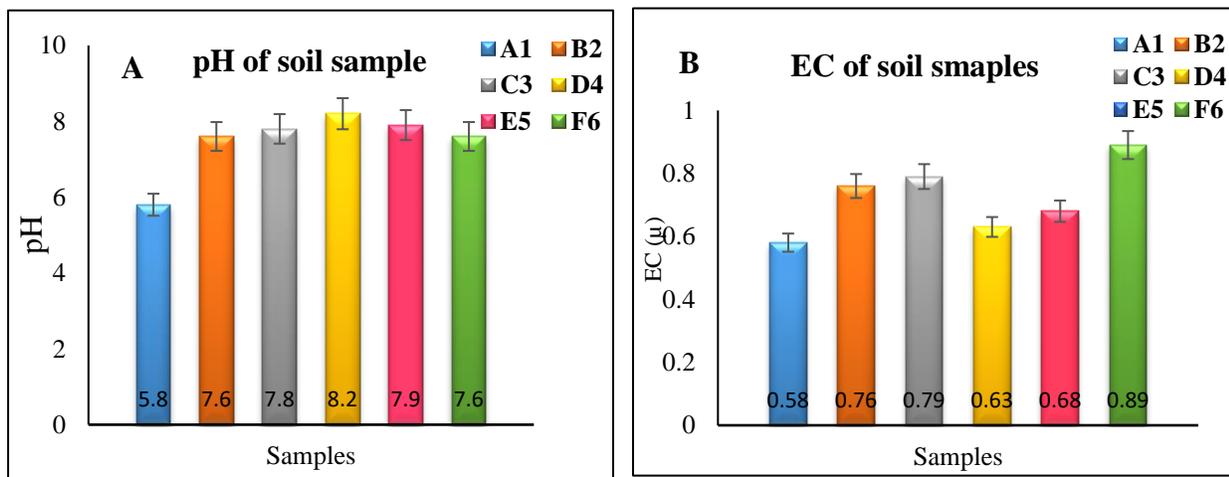


**Fig.5.6.BOD of experimental sets (A, B, C, D, E, and F) at different time intervals.**

Fig. 5.6. represent biological oxygen demand (BOD) of experimental sets. The experimental sets were analysed at a different time interval. The readings of BOD were initially increased as time increases the level of BOD decreases. At the end of 96 hours the results of BOD were significant because the polyphenol content which are present in the plant extracts are responsible for degradation of organic biomedical waste. In sample **F** the level of BOD was drastically decreased from 130 to 18mg/l from these results it has been stated that the value of BOD is far significant than that of required value that is 30mg/l for fertilizer. In the case of untreated the value of BOD is far high than that of required value for fertilizer, in this experimental set the level of BOD has not been decreased because the biomedical waste has not been degraded.

### 5.3.2. Physicochemical parameters of the soil sample.

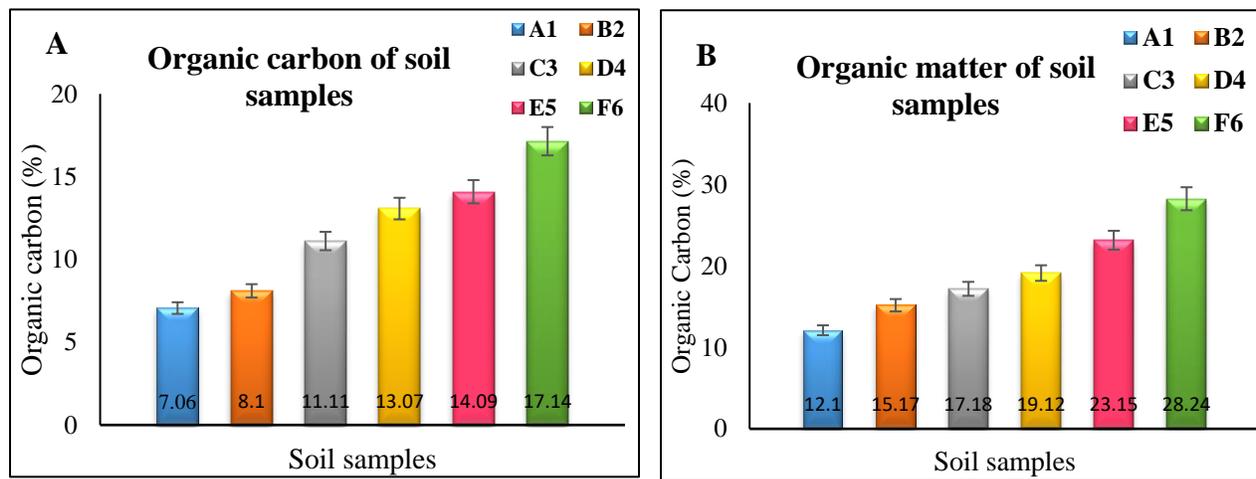
The physicochemical characterization of soil samples such as electrical conductivity (EC), pH, organic matter, organic carbon, water holding capacity, potassium, phosphorus, calcium, nitrogen, and magnesium are important parameters and must be present in an ideal range. These parameters play an important role in the development and growth of plants.



**Fig.5.7. Represents physicochemical parameters of all soil samples pH, EC.**

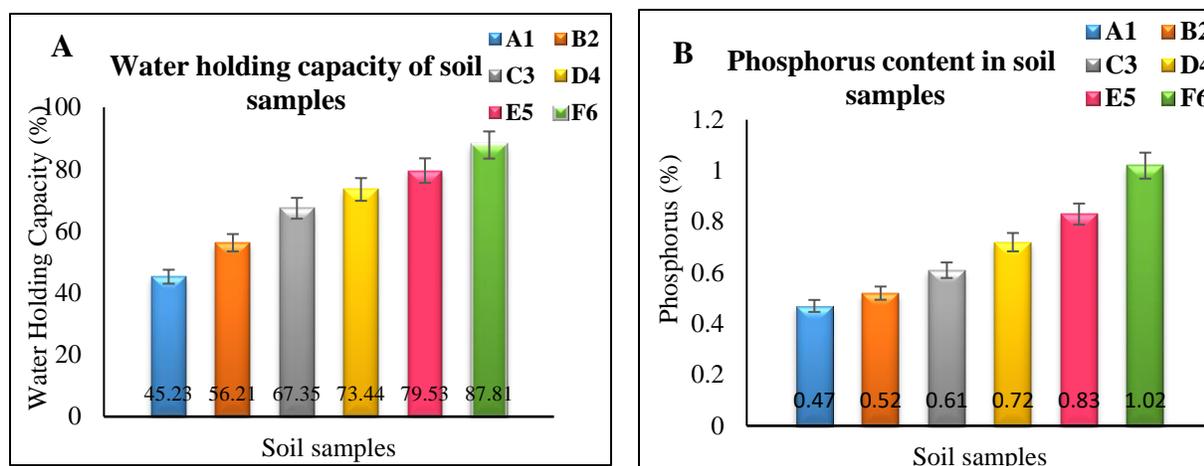
Fig.5.7. represents the pH and EC of soil sets. There must be a tolerable range of pH in soil and it must be within the tolerable limit (5.5-9.0) to bacteria fungus and the microorganisms. Numerous growth of the crops are in the neutral range of pH and alteration in the pH also affects the other

parameters which are present in the soil (Steiner et al., 2007). In the control soil slightly acidic pH was observed and in the treated soil sets the range of pH was neutral. Electrical conductivity (EC) of the soil is an quantity that correlates the physical characteristics of soil which disturbs the efficiency of crop, soil's texture, salinity, cation exchange capacity, biological matter, drainage conditions, and soil possessions (Grisso et al., 2009), (Brevik et al., 2006). The soil samples were analysed for the EC content the maximum EC content was observed in **F<sub>6</sub>** due to high content of decomposable matter in experimental set **F**. The less EC content was in the **A<sub>1</sub>** soil set it is due to less organic content in the control set.



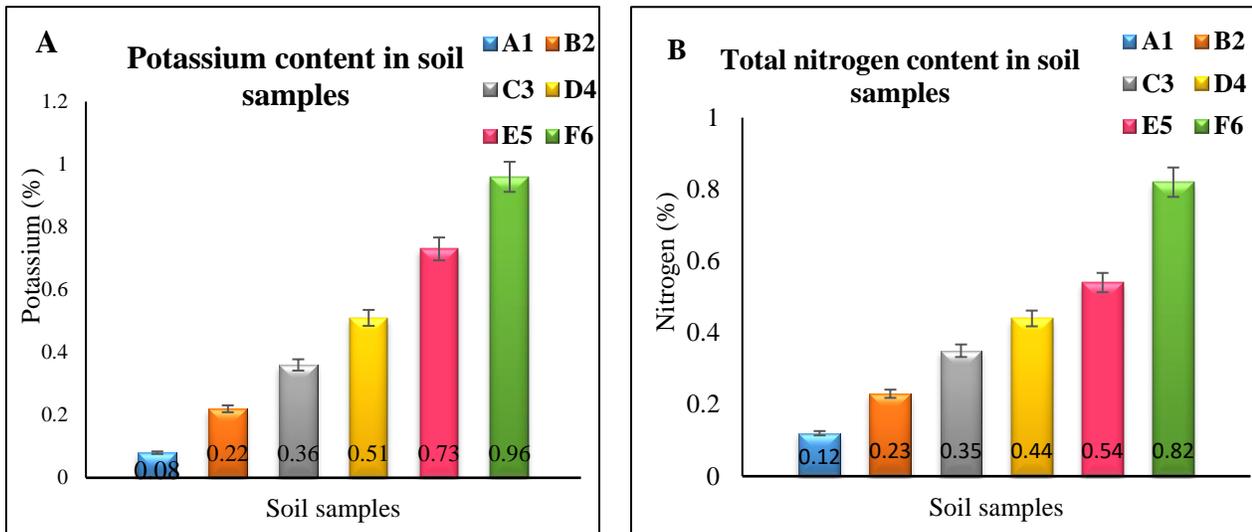
**Fig.5.8. Represent physicochemical parameters of all soil samples organic carbon, organic matter.**

Fig.5.8. represents the organic carbon and organic matter of soil sets. After submerging the residues into the soil, the presence organic waste has been converted from complex to a simple form. When the soil is rich in biological constituent or the organic carbon its water holding capacity will get increased, hydraulic and aggregation capacity will also get increased and bulk density will decrease (Carolina, 1981), (Six et al., 2002). Due to the decomposition of organic matter the treated soil sets are rich in organic carbon and organic matter. The soil set **F<sub>6</sub>** has high content of organic matter because it has rich biological constituent followed by **E<sub>5</sub>**, **D<sub>4</sub>**, **C<sub>3</sub>**, **B<sub>2</sub>**, and **A<sub>1</sub>** soil set respectively.



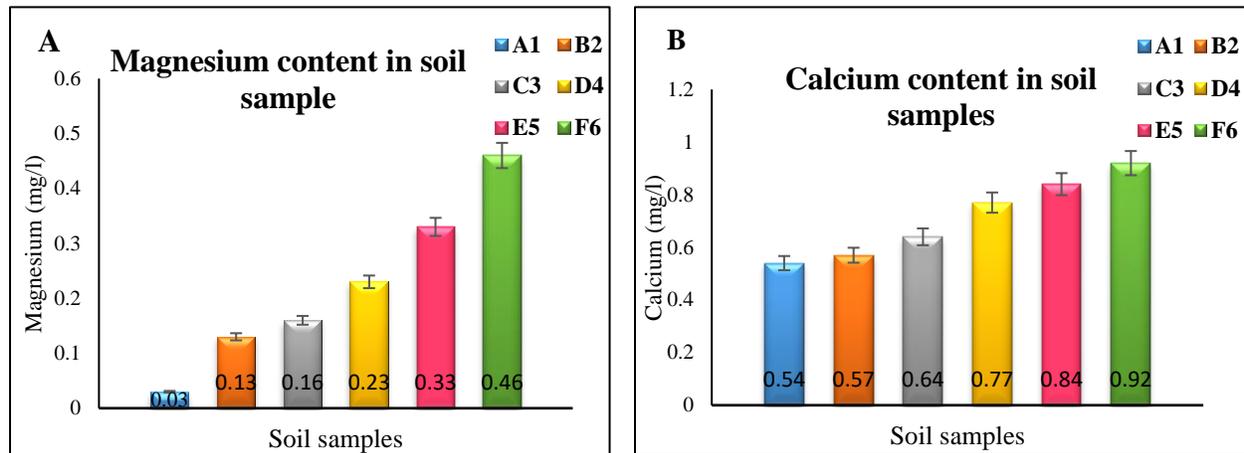
**Fig.5.9. Physicochemical parameters of all soil sample water holding capacity (WHC) and phosphorus content.**

The WHC of the soil depends upon the quality of soil and the presence of biological constituent in it. The designing of management practices will improve the WHC of the soil and develop the soil structure (Evanylo et al., 2008), (Karhu et al., 2011). Fig.5.9. represents the WHC and phosphorus content of soil sets. After the addition of residue into the soil the presence of organic matter, WHC, as well as the physico chemical parameters of the soil, has enhanced as compared to control. The excellent results are shown by the sample F6 (neem + tobacco) amongst all. In recent year's utilization of organic waste and converting it into fertilizer has become widespread. The disposal of solid waste or the disposal of garbage waste has become a well-known problem so after treating that waste and converting this waste into fertilizer will contribute to minimizing the waste (Lynch, 2011). After applying the compost to the soil the phosphorus content has maintain its superior level and increase the yield of crops. After submerging of treated biomedical waste into the soil it has been converted into the soluble and insoluble nutrient. The soil set **F6** has high content of phosphorus followed by **E5**, **D4**, **C3**, **B2**, and **A1** soil set respectively.



**Fig.5.10. Physicochemical parameters of all soil samples potassium and nitrogen content.**

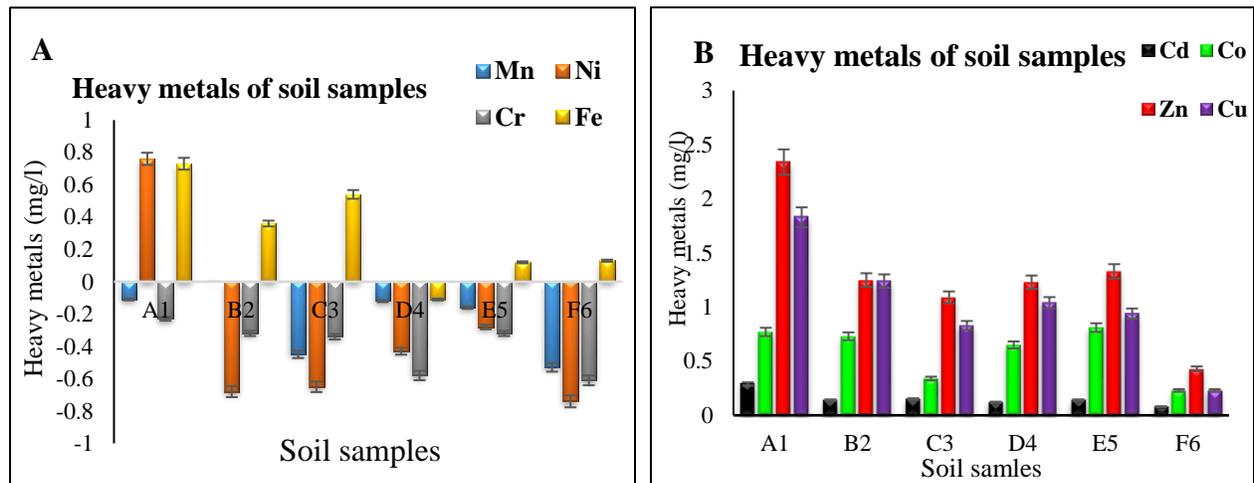
In the soil 95 to 99% of nitrogen is present because of biological residues of plant and animal, or due the microorganism or bacteria present in the soil (Ashley et al., 2006). Plants required much more nitrogen than supplementary nutrients (Bodelier and Laanbroek, 2004). Fig.5.10. represents the potassium and nitrogen content of soil sets. After submerging treated organic biomedical waste the microorganisms which are present in soil converts that organic component into the nutrients. As experimental set **F** has high decomposable material the content of potassium and nitrogen is high in **F6** followed by **E5**, **D4**, **C3**, **B2**, and **A1** soil set respectively.



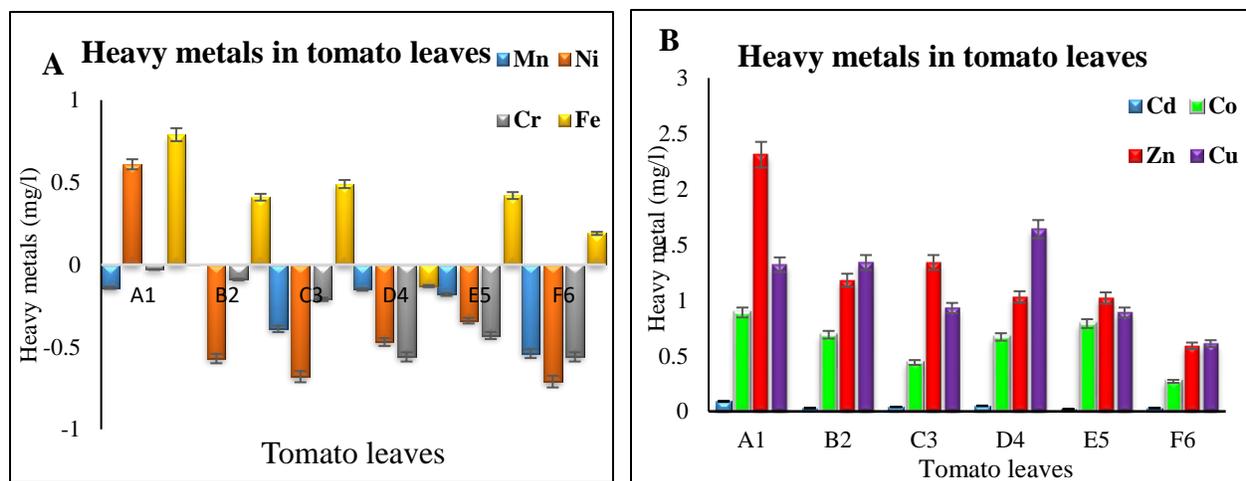
**Fig.5.11. Physicochemical parameters of all soil sample water magnesium and calcium content.**

Magnesium and calcium have numerous nutrients that can fulfil the demands of plants. When there is a sufficient amount of calcium and magnesium then the yield of crops will increase and the root hairs will also proliferate (Cole et al., 2016), (Maathuis., 2009), (Raij et al., 1986) . Fig.5.11. represents the magnesium and calcium content of soil sets. After degradation of organic biomedical waste and after addition of residue into the soil there is an enormous difference in all the above parameters. The results revealed that the readings of calcium, magnesium, and nitrogen have also enhanced as compared to control **A1**. Among all the experimental sets the **F6** (neem + tobacco) shows an admirable result as rate of degradation of organic biomedical waste was high in it.

## 5.3.2.1. Heavy metals of the soil sample.



**Fig.5.12. Heavy metal content in soil samples magnesium, nickel, chromium, iron, cadmium, cobalt, zinc, and copper.**

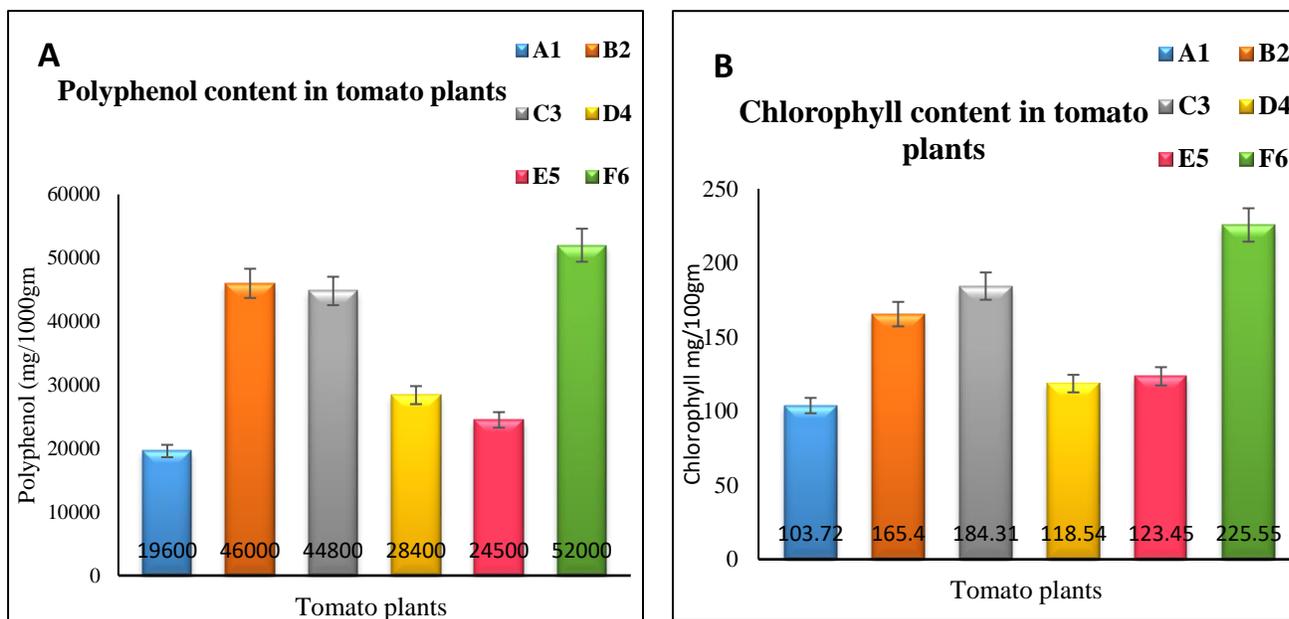


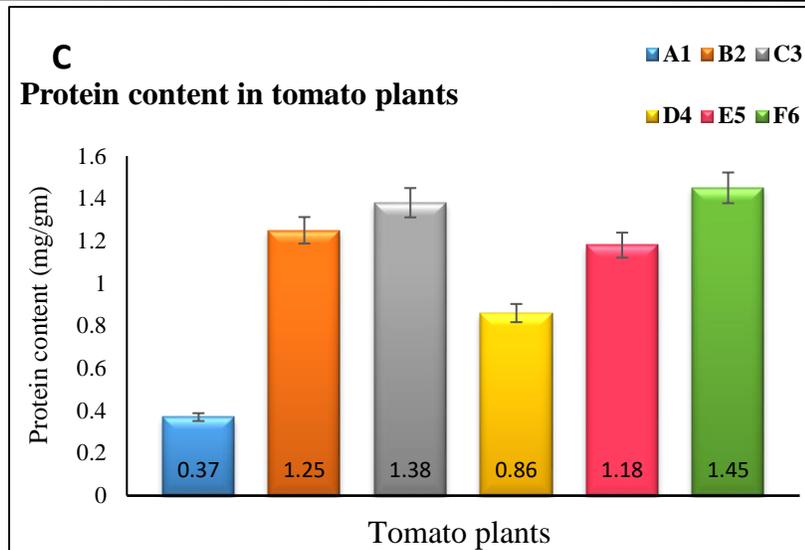
**Fig.5.13. Heavy metal content in tomato leaves magnesium, nickel, chromium, iron, cadmium, cobalt, zinc, copper.**

The biodegradation or bioremediation of organic pollutants can be strictly inhibited by the presence of toxic metals in soil (Mihailovic and Gajic, 2008). Heavy metal adulteration in the soil can pose risks and hazards to the surrounding and the living organisms through the straight intake or by contact with polluted soil, water, or by food chain, etc. Most of the heavy metals are essential for living organisms and they have multiple biochemical functions as they are crucial micronutrients for living beings (Wuana and Okieimen, 2011), (Abdel-daim, 2018). Though these

heavy metals are necessary to living organisms, they can be toxic and harmful when they are beyond the limit. The necessity and the toxicity of the heavy metals will differential from species to species (Liu et al., 2013). The plants possessed the homeostatic mechanisms by which in an accurate concentration the heavy metals will be present in cellular compartments and minimize the harmful effects (Chibuike and Obiora, 2014). After submerging the degraded organic biomedical waste in the soil. The treated soil and the tomato plants were examined to determine the occurrence of heavy metals (Cd- Cadmium, Mn- Manganese, Fe- Iron, Co- Cobalt, Cr- Chromium Zn- Zinc Ni- Nickel, Cu- Copper). Fig 5.12., 5.13. represents the heavy metal contents of the soil samples and tomato plants respectively. From the results of heavy metals it has been revealed that the tomato plants and the soil samples are not contaminated with the heavy metals and they are in the permissible limit given by CPCB. The maximum permissible limits of heavy metals by CPCB are as follow (Cd-1mg/l, Fe-1mg/l, Ni-3mg/l, Zn-5mg/l, Cu-1.5mg/l, and Cr-2mg/l). So we can conclude that there is no hazard or risk to the environment or the living organism as there is no adulteration of heavy metals in the tomato plants and the soil samples.

### 5.3.3. Phytochemical of tomato plant





**Fig.5.14. The phyto chemical and morphological parameters of plants (A) polyphenol, (B) chlorophyll, (C) protein.**

The plantation of tomato plants was done in treated soil and in untreated soil to confirm its role as a potential fertilizer. Phenols are derivative of secondary plant metabolism of the shikimic acid pathway, malic acid pathway, or both phenylalanines and are the pioneers for phenolic compounds. Phenolics have numerous functions in the growth of the plants (Hartmut K and Ursula, 1988). Chlorophyll is the pigment that provides plants their characteristics green colour. Chlorophyll pigment inhabits an exclusive role in the productivity, economy, and physiology of plants. For the photosynthesis chlorophyll is essential, it allows plants to absorb energy from light (Xu et al., 2006). From the smaller molecules called amino-acids derives the proteins. Proteins play a vital role in the enlargement and progression of the plants (Waters et al., 1996). Fig.5.14. represents the phyto chemical parameters of plants and fig. 5.15. represents plantation of tomato plants. From the figure 5.14 it has been revealed that the phyto chemical and morphological parameters were maximum in **F6** tomato plants as essential nutrient like calcium, organic matter, potassium which is required for the development of plants has been provided by **F** soil sample followed by **C3**, **B2**, **E5**, **D4**, and **A1** soil set respectively.



**Fig.5.15. Plantation of tomato plants.**

#### **5.4. Mechanisms of action.**

The presence of polyphenols content in plant extracts is accountable for the degradation of organic matter. Vast research has been carried out regarding the composition of plant extract and its chemical composition (Devatha et al., 2018). When waste is treated with the herbal extract there is excellent degradation of organic biomedical waste. When the neem extract was submerged in the waste 82.35% of chemical oxygen demand has been reduced. It has been reported that neem also shows adequate degradation of organic matter. Devatha and et al treated the organic waste with leaf extract and it has been reported that on 11<sup>th</sup>-day huge reduction in chemical oxygen demand has been observed due to the presence of polyphenol content in neem extract. From this study it has been revealed that the submerging herbal extract in the organic waste and converting them into compost is a cost-effective, ecofriendly, and agreeable method (Devatha et al., 2016). The usage of herbal extract for the treatment of organic waste is a traditional and natural method. As we know that every single neem part has valuable properties and it has been stated that neem extract has antifungal, antiviral, antibacterial, and due to presence of polyphenols content, phytochemical elements tobacco extract also displays admirable results in degradation of organic

biomedical waste so and by referring research articles the combinations of tobacco and neem extracts have made for the treatment of organic biomedical waste and outstanding results were obtained (Ravva and Korn, 2015).

### **5.5. Conclusion.**

The present work proves that by using neem and tobacco extract the biomedical waste can be converted into potential fertilizer. The tobacco and neem extract show admirable results in the reduction and degradation of organic biomedical waste. There is a 95.30% reduction of COD, 86.15% reduction of BOD and 63.33% of removal of TDS was observed at the end of 96 hours. The physicochemical and phytochemical parameters of soil and plants are also enriched as paralleled to control. The heavy metal analysis of soil sample and the tomato plants has done and they are within the limit specified by WHO. So from the above results it has been concluded that the treated waste is a worthy source of fertilizer and there is no hazard and risk to the environment and human being. So the use of herbal extracts for degradation of organic biomedical waste and converting them into potential fertilizer on the source of nutrient content is an imperious indication that this process will decrease the load of synthetic fertilizer.

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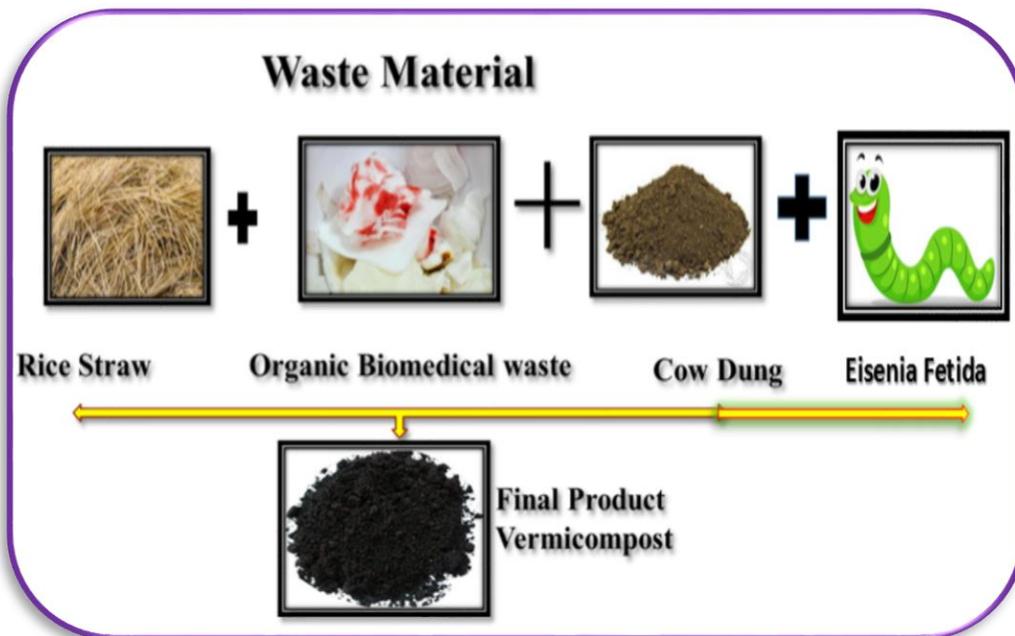
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# CHAPTER 6

## CHAPTER 6

**The Organic Biomedical Waste, Rice Straw, and Cow Dung Converted into Vermicompost by Employing Earthworm *Eisenia Fetida***



## **The Organic Biomedical Waste, Rice Straw, and Cow Dung Converted into Vermicompost by Employing Earthworm *Eisenia Fetida***

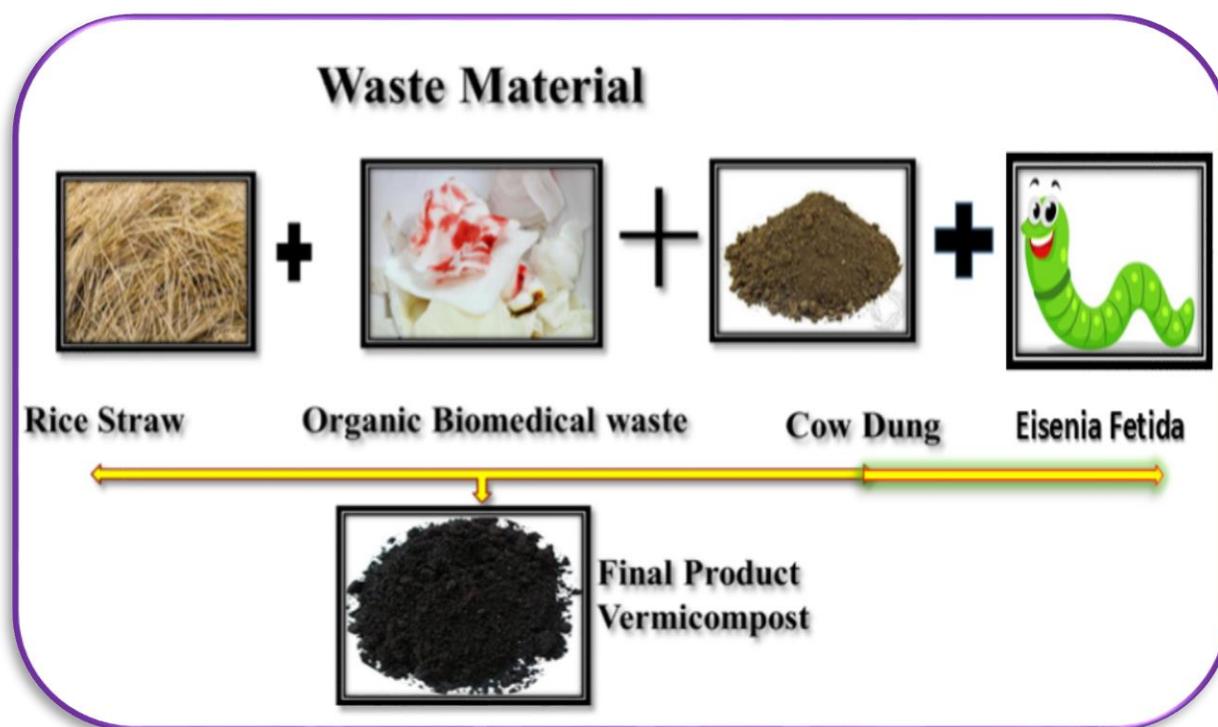
### **6.1. Introduction**

Significant quantities of organic biomedical waste are produced through patient treatment, in hospitals, health care treatment, mortuaries, research centers that subsidize pollution, climate change, and disturbs the ecological balance (Singh et al. 2016). An enormous amount of agro residues are generated as waste mainly in the form of the husk, crop straw, stalks, cobs, etc. Universally, the prevalent agricultural crop is rice straw which associates about 45% of the total volume in rice production (Lim and Wu, 2016). India is the second-largest producer of rice which annually generates around 112MMT of rice straw. Around 80% of rice straw major fraction remains left and unutilized in the fields (Gupta and Garg, 2009). In India and Asian countries the rice straw burning in the agricultural is a traditional and common practice. The burning of rice straw generates numerous problems like air pollution, smog, and also destroys the nutrients which are present in rice straw (Yan et al. 2013). Several problems connected with rice straw waste which demands sustainable management and recycling of waste. Intended for vermicomposting the leftover rice straw and agro residues is prospective feedstocks which recycle the nutrient in the agriculture field and maintain the health of soil (Abbasi et al 2015).

Vermicomposting is the organic practice in which the relations between the microbes and earthworm is observed which proficiently convert numerous type of organic waste into value-added manure (Pigatin et al. 2016), (Amouei et al. 2017). The interaction between microorganism and earthworm alters the physico chemical, and biological features of waste and convert it into vermicompost (Hussain et al 2016). Vermicompost is the homogenous, stabilized, odorless, peat-like substance which contains a significant amount of nutrient with less level of toxicants. The vermicomposting practice is suitable because it is cost-effective, simplicity, and sufficiently degrades all organic waste (Ewusi-Mensah et al 2015), (Nath et al 2008). Numerous types of agriculture residues, agro-industrial waste, and crop residue are efficiently converted into nutrient-rich compost due to vermicomposting. In the current investigation the gradual biomedical waste

and rice straw are used for vermicomposting and it is mixed with cow dung (Mupambwa et al 2016).

In the hospitals, clinics, and health care centers the biological biomedical waste is mixed with the inorganic waste and directly incineration treatment is given to the entire waste. For the management of biomedical waste proper segregation and sorting is required the improper segregation leads to mismanagement of biomedical waste. Keeping all these points in mind research was formulated to investigate the prospective of organic biomedical waste and rice straw. The various experimental sets were designed for different concentrations. The physico chemical parameters of experimental feedstock were analyzed. Then further the physico chemical analysis and the heavy metal analysis of the treated set were done. The phytochemical analysis of tomato plants were also evaluated. The results revealed that both the rice straw and biological biomedical waste can be an excellent food for the earthworms.



**Fig.6.1. Diagrammatic representation of degradation of organic biomedical waste by using rice straw and cow dung by vermicomposting method and conversion it into nutrient-rich compost.**

## 6.2. Materials and methods

### 6.2.1. Samples collection (for each set):

From D. Y. Patil medical college hospital and research institute, Kolhapur. Organic biomedical waste samples like 12g dressing swabs, 32g blood swabs, and 6g used cotton total 50g were collected. Then the biomedical waste samples were double autoclaved and it has been used for the experiment.

### 6.2.2. Collection of waste material and earthworms

Rice straw was collected from agricultural fields situated nearby, Ajara (India). Cow dung was collected from Sidhigiri math, Kolhapur. India. Earthworm, *Eisenia fetida* were collected from Kolhapur using cow dung as a culture medium.

### 6.2.3. Preparation of experimental media

Vermicomposting was carried out in plastic vermibin with a dimension of 50 cm (diameter) and 25 cm (depth). Four different sets were conducted using organic biomedical waste, rice straw, and cow dung. Organic biomedical waste and rice straw were cut and chopped into the small pieces and submerged into the cow dung into various concentrations as given in table 6.1. Moisture content was periodically monitored and about 60-70% moisture content was maintained by manually sprinkling water.

**Table 6.1. Preparation of experimental sets**

Sr.No	Name of Set	Composition	Ratio of Cow dung: Rice straw
1	<b>A</b>	<b>(Untreated)</b> 50g of BMW + 35 ml d/w	0:0
2	<b>B</b>	50g of BMW + five earthworms of average size 300 mg + 45g cow dung + 5g Rice straw.	90:10
3	<b>C</b>	50g of BMW + five earthworms of average size 300 mg + 35g cow dung + 15g Rice straw.	70:30

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4	<b>D</b>	50g of BMW + five earthworms of average size 50:50 300 mg + 25g cow dung + 25g Rice straw.
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**\*60-70% of the moisture is maintained.**

#### **6.2.4. Earthworms development**

The earthworms development, mortality, survival rate was observed. The earthworms were separated by hand from the mixture of feed on 15, 30, 45, 60, 75, 90 days and then it is washed with water. Further it is weighted and counted and then submerged back into their feed mixture.

#### **6.2.5. Physico chemical characteristics of feedstock and vermicomposting.**

Various parameters were analyzed to calculate the changes in physico-chemical parameters of feedstock the parameter include pH, electrical conductivity (EC), organic carbon, organic matter, content of potassium, total nitrogen content, phosphorus, and content of heavy metal.

#### **6.2.6. Selection of plant for the experiment:**

The two weeks species *S.lycopersium* (Tomato) plants were selected for plantation because it has a short lifespan.

#### **6.2.7. Submerging of residue into the soil:**

50gram of residue was mixed with 1000gram of soil to confirm its role as a potential fertilizer. The details are shown in table 6.2.

**Table 6.2. Mixing of residue into the soil:**

Sr.No	Name of Set	Composition
1	<b>A<sub>1</sub></b>	<b>(Control )</b> 1000g of soil
2	<b>B<sub>2</sub></b>	50g set <b>A</b> mixed with 1000g of soil
3	<b>C<sub>3</sub></b>	50g set <b>B</b> mixed with 1000g of soil
4	<b>D<sub>4</sub></b>	50g set <b>C</b> mixed with 1000g of soil
5	<b>E<sub>5</sub></b>	50g set <b>D</b> mixed with 1000g of soil

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### 6.2.8. Phytochemical parameters of plants:

The phytochemical parameters of tomato plants were analyzed such as estimation of, chlorophyll content, polyphenol content, and protein content.

## 6.3. Results and Discussion

### 6.3.1. Analysis of physicochemical parameters.

Biomedical waste, cow dung, rice straw were initially evaluated and their physico chemical characterization was done. The samples were dried and stored at room temperature before analysis. The pH and EC content were analyzed by using pH and EC meter double distilled water was used for all the analyses. Nitrogen content was determined by using the Kjeldahl method. By using a flame photometer the potassium content was determined and the heavy metals were monitored by using flame atomic absorption spectrophotometer. The results of the physico chemical parameters are given in table two.

**Table 6.3. Represent initial physico-chemical characteristics of feed mixtures (cow dung, organic biomedical waste and rice straw) used for vermicomposting**

Sr. No	Parameter	Cow dung	Organic biomedical waste	Rice straw
1	pH	7.16 ± 0.03	6.01 ± 0.02	7.42 ± 0.02
2	EC mS/cm	6.87 ± 0.04	4.12 ± 0.03	2.32 ± 0.03
3	Total Organic Carbon (%)	48.82 ± 0.13	49.93 ± 0.17	52.91 ± 0.16
4	Total Organic Matter (%)	83.92 ± 0.26	85.79 ± 0.31	91.89 ± 0.28
5	Potassium (%)	0.21 ± 0.03	0.54 ± 0.05	0.69 ± 0.06
6	Phosphorus (%)	0.43 ± 0.02	0.79 ± 0.17	0.67 ± 0.03
7	Total nitrogen (%)	0.37 ± 0.04	0.51 ± 0.07	0.83 ± 0.07
8	Fe (mg/l)	0.12 ± 0.01	0.23 ± 0.02	0.28 ± 0.02
9	Ni (mg/l)	0.26 ± 0.03	0.45 ± 0.03	0.13 ± 0.03

10	Zn (mg/l)	$2.26 \pm 0.09$	$2.98 \pm 0.13$	$1.75 \pm 0.18$
11	Pb (mg/l)	$1.45 \pm 0.08$	$2.13 \pm 0.12$	$2.90 \pm 0.09$
12	Cu (mg/l)	$3.12 \pm 0.10$	$2.16 \pm 0.14$	$1.99 \pm 0.08$

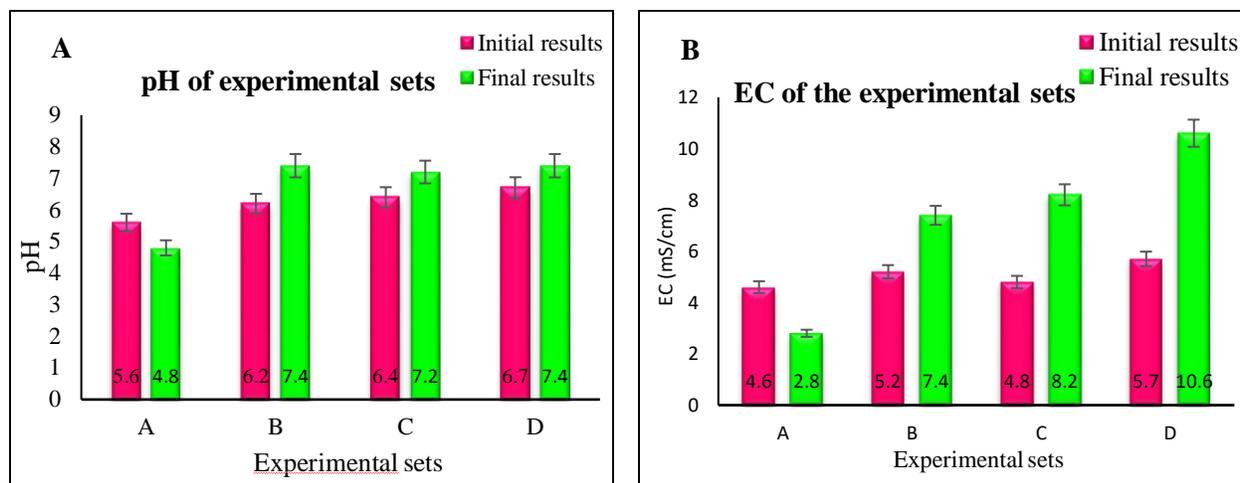
From table 6.3. it is revealed that the pH of the cow dung and rice straw is in the neutral range the pH of biological biomedical waste is slightly acidic as compare to others. The EC content is high in the cow dung than that of biological biomedical waste and rice straw. The total organic carbon and organic matter is high in rice straw then after in biological biomedical waste and then after in cow dung. The content of potassium and phosphorus content was also analyzed and from the results it has been revealed that the high content of potassium and phosphorus is in organic biomedical waste then after in cow dung then in rice straw respectively. The more amount of nitrogen content is found in rice straw than in organic biomedical waste than in cow dung. The content of heavy metal in organic biomedical waste, cow dung, and rice straw was also analyzed.

### 6.3.2. physico chemical parameters of experimental sets.

#### 6.3.2.1. pH and EC content

During vermicomposting the variation in pH is observed due to the biochemical feature of the feedstock. During the process, the alteration in pH are due to the formation of ammonia and organic acid. The range of pH between 6.82 to 7.52 is considered as the optimum range for application in the field of agriculture. In vermicomposting the pH ranges decreases due to the conversion of phosphorus and nitrogen into orthophosphate and nitrates. In accumulation the conversion of organic biomedical waste into organic acid may reduce the feedstock of pH (Ravindran et al. 2016). During the vermicomposting process earthworm balance the range of pH by secreting ammonia and intestinal calcium which neutralizes phenolic and carboxylic groups generate during the process (Pramanik et al. 2007). Release of various soluble mineral salts existing forms and biological matter decomposition can proliferates the EC content in vermicompost (Kaviraj and

Sharma, 2003). The available EC content in the sample depends upon the size of the particle, soil texture, and presence of moisture content in the soil.

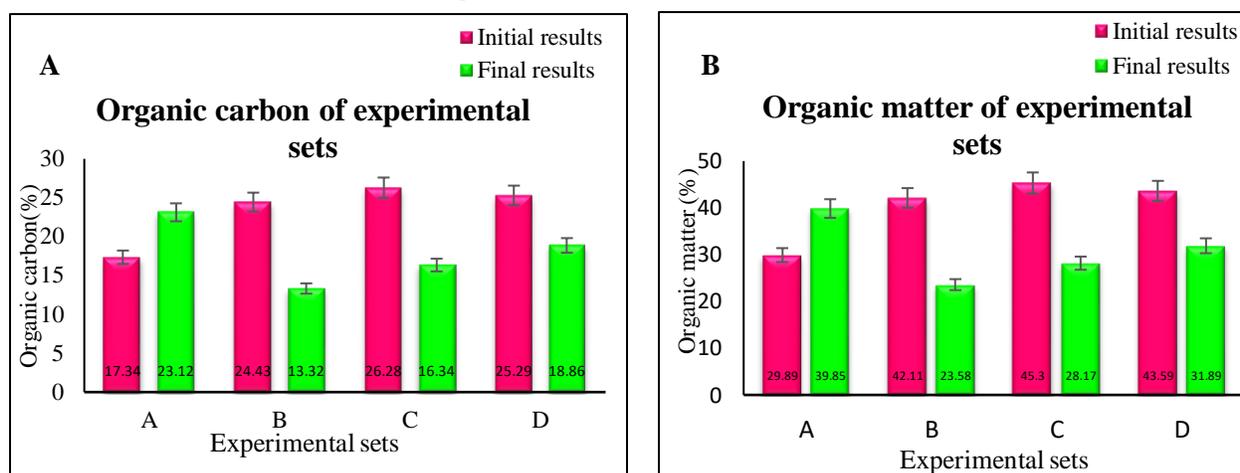


**Fig. 6.2. (A) The initial and final results of pH in different vermibins and (B) the initial and final results of EC (mS/cm) in different vermibins.**

Figure 6.2. reveals that the experimental sets were analyzed initially and finally. As it is reported that earthworm balance and maintain the pH during the process of vermicomposting. Initially slightly acid pH was observed in set A but at the final analysis acidic pH was seen due to submerging of organic biomedical waste in soil. In the other experimental sets (B, C, D) neutral pH was observed during the entire process of vermicomposting. pH plays a vital role in the process of vermicomposting neutral pH reduces the problem of odour and effectively increases the rate of reaction. The EC measured salt and minerals content in the soil. In figure 6.2. (B) the results of EC content are observed. In set A initially and finally there is minimum EC content as organic matter has not been degraded. The experimental set D has more EC content followed by D, C, B, and A as earthworms and has degraded the organic matter and converted it into nutrient form.

**6.3.2.2. Organic carbon and organic matter:**

The total organic carbon includes organic or biological carbon like oxygen, hydrogen, and carbon. The earthworm used organic carbon content as a feed and convert it into its biomass (Sharma and Garg, 2017a). The organic carbon content is also reduced due to reduced microbial respiration and carbon is lost and carbon dioxide is released. The reduction in total organic results in degradation of feedstock which converts into a stabilized and sustainable product (Song et al. 2014). The existence of biological matter increases the stability of soil and ultimately the quality of compost will also increase (Sharma and Garg, 2017a).



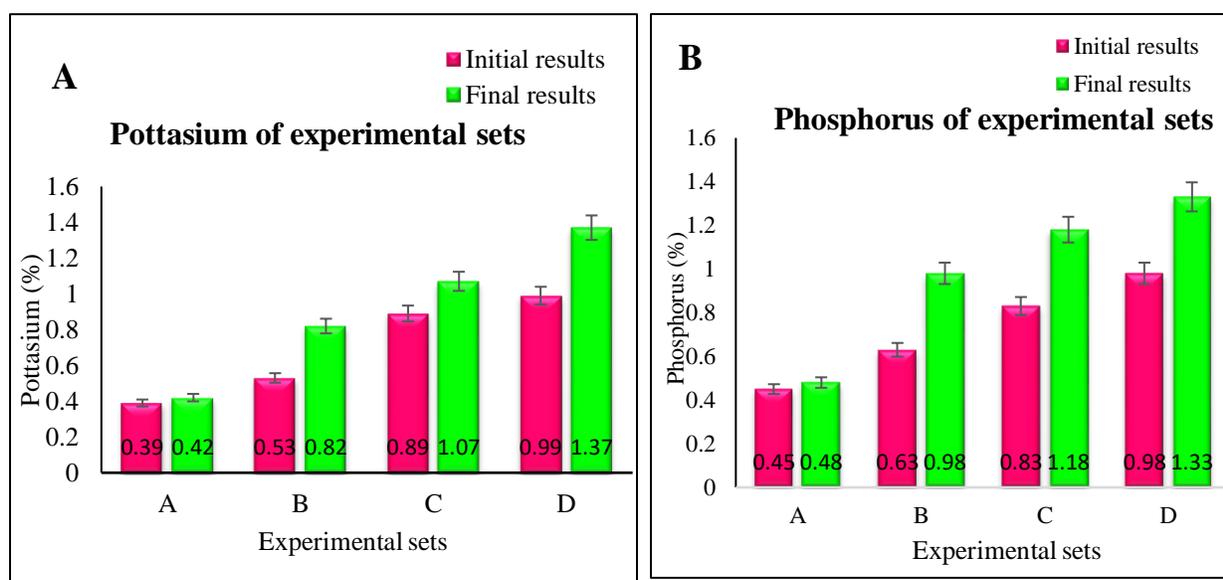
**Fig. 6.3. (A) The initial and final results of organic carbon content in different vermibins and (B) represents the initial and final results of organic matter content in different vermibins.**

During vermicomposting the changes in total organic carbon and total organic matter were studied and the results are encapsulated in figure 6.3. There is a lessening in the concentration of total organic carbon and biological matter in all the vermibins except control. The degree of reduction varied concerning its feedstock. It has been observed that when there is a high percentage of rice straw the reduction of organic carbon and biological matter was high. In all the vermibins the total organic carbon is reduced due to mineralization of the organic compound as earthworm consumed carbon as a source of feed and convert it into biomass. Carbon is also reduced during microbial respiration as it outcomes in the form of carbon dioxide. This decrease in the organic carbon and

organic matter in all the sets except control designates that feedstock has degraded effectively and it has been converted into a sustainable product.

### 6.3.2.3. Potassium and phosphorus content:

In vermicomposting the phosphorus and potassium content is increased due to the activity of the enzyme (Singh and Kalamdhad, 2016), (Unuofin. et al. 2016). Potassium and content of phosphorus is needed for the progress of plant and in the vermicompost, their rise concentration makes an appropriate promoter for plant growth (Pramanik et al. 2016). Higher potassium and phosphorus reduced the weight of organic waste and degrades the organic matter which is present in the vermibin and releases the carbon dioxide gas (Swarnam et al. 2016). Phosphorus released from the feedstock is mainly due to phosphatase enzyme which is present in the intestine of the earthworm and due to phosphate solubilizing microorganisms (Malinska et al. 2016).

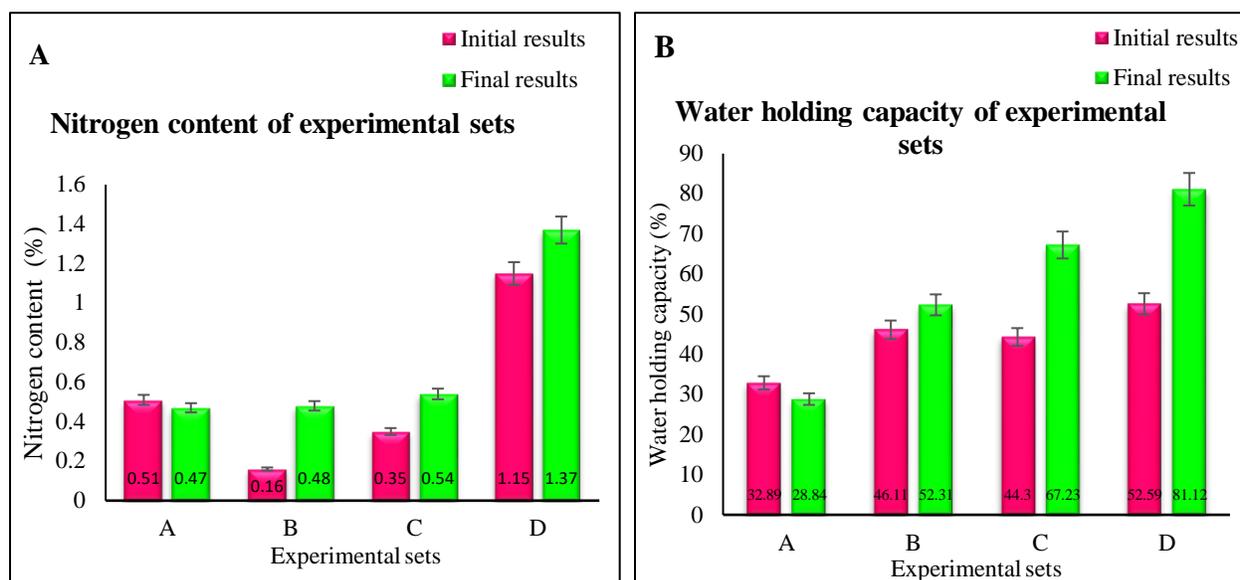


**Fig. 6.4. (A) The initial and final results of potassium (%) in different vermibins and (B) represents the initial and final results of phosphorus (%) in different vermibins.**

As it is reported that increasing the level of phosphorus and potassium adds nutrient value to the product. The initial and final potassium content of vermicompost were analyzed and reported in figure 6.4. In the experimental set A, initial potassium content is 0.39% and the final is 0.18% which is very less as compared to other experimental sets. In B, C, and D initially, 0.53, 0.89, and 0.99% potassium content is respectively there and at the final stage in the set B 0.82%, in the set C, 1.07% and in the set D 1.37% potassium content is present. Whereas in D experimental set high percentage of phosphorus and potassium is present as earthworm and microorganisms which are present in cow dung have degraded the organic material and converted that organic material into nutrients. The set A has low content of phosphorus and potassium as organic material and earthworm are not submerged in that set A.

#### **6.3.2.4. Nitrogen and water holding capacity:**

Nitrogen is a significant building block of nucleic acids, proteins, and other cellular components which are necessary for all life forms (Atiyeh et al 2001). Nitrogen is an essential nutrient for plant and it should be carefully managed if it is not managed properly it may lead to severe environmental problems. In most plants nitrogen is available in the inorganic form it is also known as mineral nitrogen. In the above-ground tissues of healthy plants 3 to 4 percent of nitrogen is present (Sharma, K., & Garg, V. K., 2018b). As compared to other nutrients this is much higher concentration. Water holding capacity plays a crucial role in the production of crops. The water holding capacity is well-defined as the quantity of water that a particular soil can hold for the use of crop (Tauseef et al., 2014). Biological matter and consistency of soil are the substantial constituents that identify the water holding capacity of a particular sample (Arancon, and Edwards 2005).



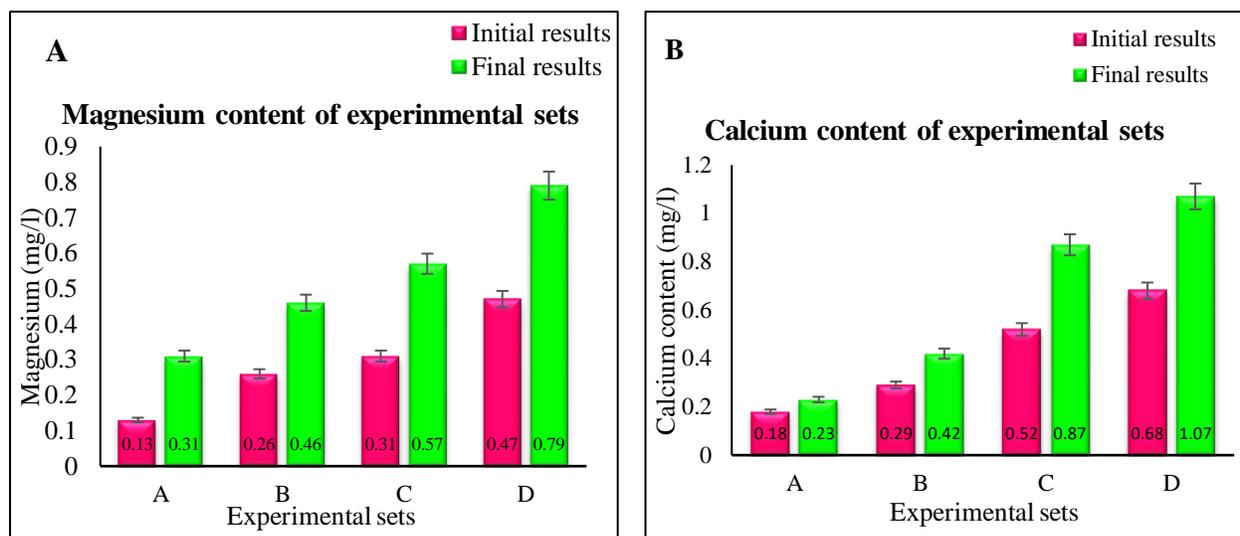
**Fig. 6.5. (A) The initial and final results of nitrogen content (%) in different vermibins and (B) initial and final results of water holding capacity (%) in different vermibins.**

Fig. 6.5. Represent the initial and final results of nitrogen content and water holding capacity. From the fig.6.5. it is revealed that at the final stage the high content of nitrogen and water holding capacity is in the set D followed by C, B, and A respectively. The results are obtained because the earthworms have degraded the organic biomedical waste and the biological constituent and converted it into rich nutrients which adds value to the soil.

### 6.3.2.5. Magnesium and calcium content:

Magnesium is deemed as a secondary micronutrient it is indispensable for plant health and growth. It is one of the most constituents of chlorophyll so is essential for the process of photosynthesis (Atiyeh et al 200). It is also a constituent of numerous plant enzymes and supports in their function. The plants which are grown in cold or acidic soil in those plants deficiency of magnesium were found (Gholami et al 2018). Calcium is a secondary micronutrient for plant and it plays a vital role in maintaining the strength of plants. It is essential for the formation of new cells it is also necessary for the growth of leaves, stems, and leaves (Joshi et al 2013), (Savci et al 2012). Calcium is used by the plants when they respond to disease attacks and pests. Deficiency in calcium can affect

negatively the legume's ability to companion with nitrogen-fixing bacteria. There are numerous plant enzymes which require calcium (Mahaly et al 2012). Fig. 6.6. Represents the initial and final results of magnesium and calcium content.



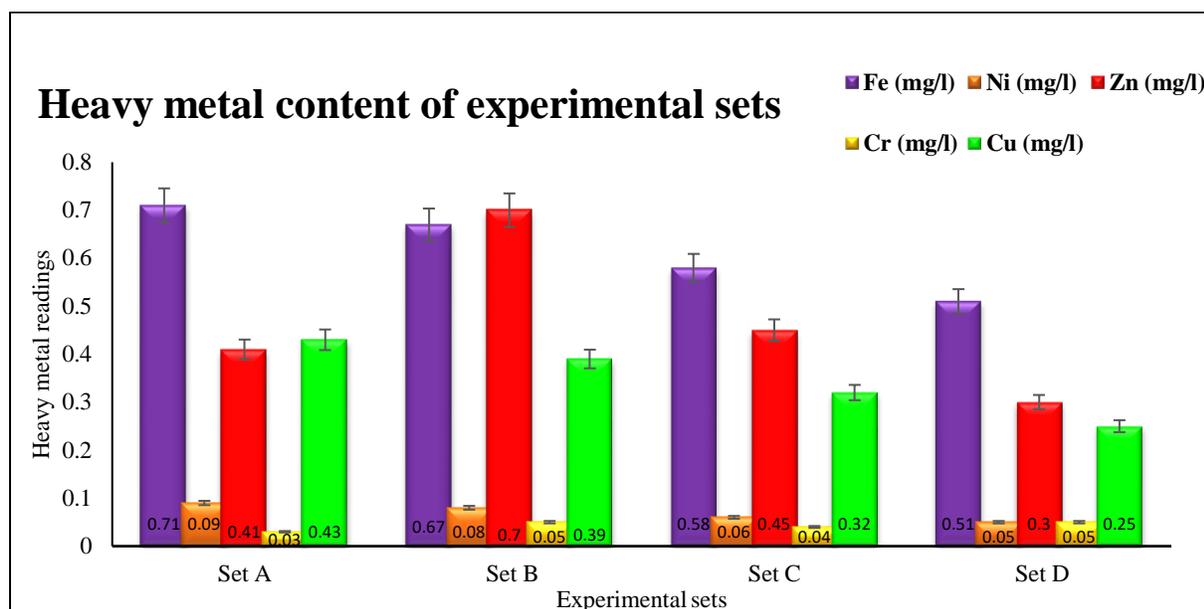
**Fig. 6.6. (A) The initial and final results of magnesium content (mg/l) in different vermibins and (B) initial and final results of calcium (mg/l) in different vermibins.**

Initially and finally the magnesium and calcium content of all the experimental sets were analyzed. Initially there is less content of magnesium and calcium content in all the experimental sets but at the final stage the enhancement of magnesium and calcium was found in all the experimental sets. The high content of magnesium and calcium was found in set D then after in set C than in set B than in set A.

#### 6.3.2.6. Heavy metal content:

The heavy metal is defined as any metallic element which has a comparatively high density and poisonous or toxic at low concentrations. Cadmium (Cd), mercury (Hg), thallium (Tl), chromium (Cr), lead (Pb), and arsenic (As) are the examples of heavy metals (Babu et al 2013), (Boominathan, and Doran 2002). Heavy metals are the natural element of the Earth's crust which cannot be destroyed or degraded. They can enter our bodies to a small extent through drinking

water, air, and food (Callahan et al 2006). To maintain the human body's metabolism some heavy metals are essential they are selenium, zinc, and copper. But, at higher concentrations it may lead to poisoning (Caregnato et al 2008). Heavy metals tend to bioaccumulate so they are dangerous. In many research, the reduction of heavy metals was found due to the accumulation of heavy metals in the tissues of an earthworm but in some research, it is revealed that there is a higher content of heavy metals after vermicomposting (Cherian et al 2005), (Garbisu and Alkorta, 2001).



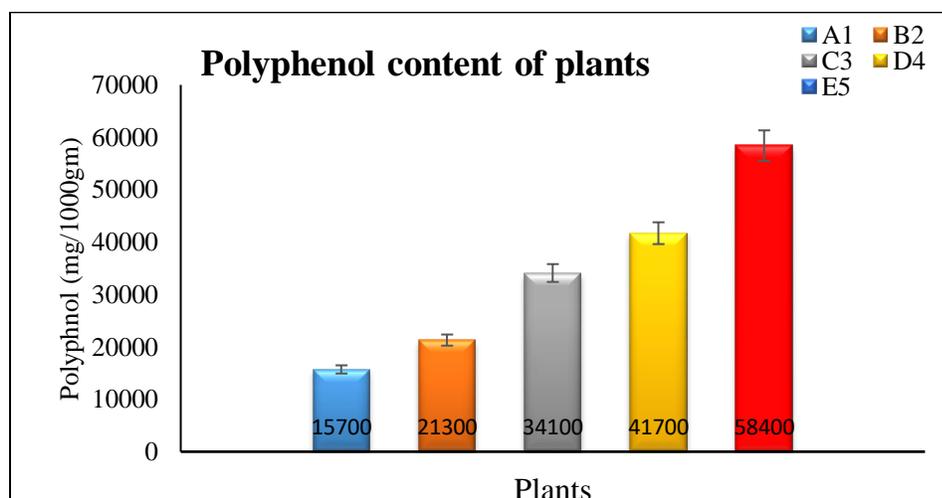
**Fig. 6.7. The heavy metal content in experimental sets (iron, nickel, zinc, lead, and copper).**

The heavy metal content of all the experimental sets were analyzed the results are represented in fig.6.7. From the results it is revealed that the heavy metals content in experimental sets are not in the dangerous stage or the poisonous form they are within the limit given by CPCB. The maximum permissible limits of heavy metals by CPCB are (Fe-1mg/l, Ni-3mg/l, Zn-5mg/l, Cu-1.5mg/l, and Cr-2mg/l). The heavy metals which are present in the feedstock earthworms accumulates the heavy metals in their tissue are reduces the concentration of heavy metals.

### 6.3.3. Phytochemical parameters of tomato plant:

#### 6.3.3.1. Polyphenol content:

Phenolic compounds are important secondary class metabolites in plants which include more polyphenols aromatic rings with involved hydroxyl groups in its structures or one phenolic acid. Its capacity of antioxidants is connected to phenolic rings and hydroxyl groups (Losada-Barreiro and Bravo-Díaz 2017), (Tungmunnithum et al 2018). In spite of its antioxidant activity, they consume numerous other beneficial properties on human health. The antioxidant property, which is present in the plants has a vital role in the lipid oxidation-reduction in animal and plant tissues, when they incorporate in the diet of human not only they conserve the food quality, but they also diminish the danger of emerging diseases (Dudonné et al 2009), (Madamanchi et al 2005), (Joshi and Joshi 2000).

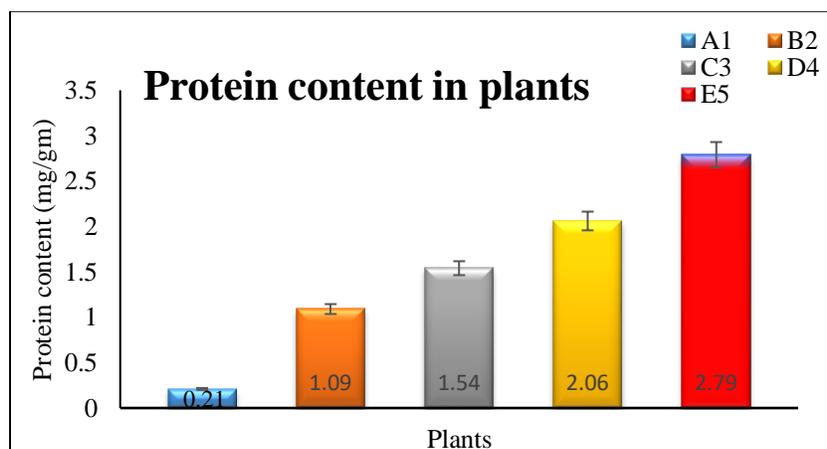


**Fig. 6.8.** The polyphenol content in tomato plants.

The residue is submerged in the soil and tomato plants were planted. The phytochemical parameters were analyzed to the confirmed vermicomposting role as a sustainable fertilizer. From figure 6.8. it is revealed that **A<sub>1</sub>** set has less polyphenol content as the required nutrients are not in soil **A<sub>1</sub>** set so less polyphenol content are present in set **A<sub>1</sub>** than that of other plants and in the **E<sub>5</sub>** set high polyphenol content are present due to presence of nutrient content in the soil.

### 6.3.3.2. Protein content:

Proteins are present in the plant cell which carry out numerous crucial and important cellular functions. In any plant cell there are thousands of proteins having a variety of structures, functions, and sizes (Zhu et al 2010). Proteins are the peak important structural elements of the cell wall. Proteins are abundant and complex of the macromolecules (Galili et al 2005). In cells, numerous proteins purpose as enzymes in the catalysis of metabolic reactions, whereas others assist as transport molecules, electron carriers, structural components, and storage proteins of the cell (Karau, and Grayson, 2014), (Galvez et al 2008). Proteins are most important to seeds, the proteins make up 40% seeds weight and for developing an embryo it serves as amino acid (Wenefrida et al 2009). Fig. 6.9. Represents the protein content in tomato plants.

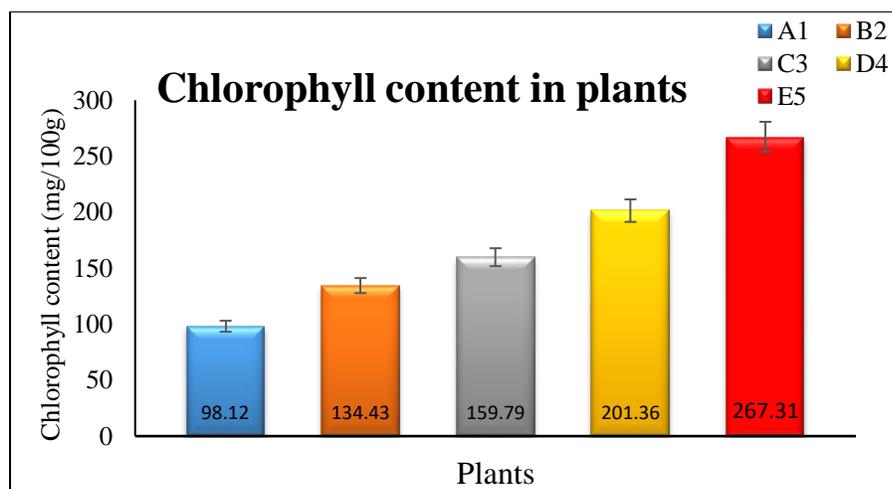


**Fig. 6.9. The protein content in tomato plants.**

As we know that protein is a structural unit of cell they play a crucial role in the development of plants so the protein content of tomato plants was analyzed. In A<sub>1</sub> tomato plant 0.21 (mg/gm) protein content is available in B<sub>2</sub> tomato plant 1.09 is (mg/gm) protein content is present in C<sub>3</sub>, D<sub>4</sub> and E<sub>5</sub> 1.54, 2.06, 2.79 (mg/gm) protein content is present respectively. The essential nutrient are given by vermicompost to the soil due to the presence of essential nutrients there is significant development in the tomato plants.

### 6.3.3.3. Chlorophyll content:

Chlorophyll is an important constituent for photosynthesis, they allow plants to fascinate energy from light (Adebooye et al 2008). Chlorophyll controls the potential of photosynthesis by catching light energy from the sun (Cannella et al 2016). Chlorophyll represents one of the most significant photosynthetic pigments. Chlorophyll, which exists in the chloroplasts of plants, which is green pigment is essential for plants that converts water and carbon dioxide, using sunlight into glucose and water (Houborg et al 2015), (Holt et al 2005). Fig. 6.10. Represents the chlorophyll content in tomato plants.



**Fig. 6.10.** The chlorophyll content in tomato plants.

As chlorophyll content is essential for the photosynthesis process. Chlorophyll is mostly present in the thylakoids of the chloroplast. The chlorophyll content of tomato plants was analyzed the supreme chlorophyll is present in the E<sub>5</sub> followed by D<sub>4</sub>, C<sub>3</sub>, B<sub>2</sub> and A<sub>1</sub> tomato plants because the vermicompost which is added in the soil have rich nutrients and tomato plants have absorbed that essential nutrients and phytochemical parameters of plants have enhanced.



**Fig. 6.11. Plantation to tomato plants.**

#### **6.4. Conclusions:**

The present study initiates that rice straw, biomedical waste, and cow dung can proficiently be converted into vermicompost by employing earthworms. Earthworm vermicompost is ascertaining to be an extremely nutritive organic fertilizer and supplementary dominant growth promoter. After vermicomposting the physico chemical, and biological parameters of vermicomposting sets are enhanced as compare to untreated set. The vermicompost sets are also rich in the content of (nitrogen, potassium, phosphorus) NPK. Vermicompost systematically provides a marvel growth promoter and also a plant protector from diseases and pests. So with the help of vermicomposting process feedstock and biomedical waste is converted into a justifiable material that can be used in the field of agriculture to develop soil strength and crop efficiency.

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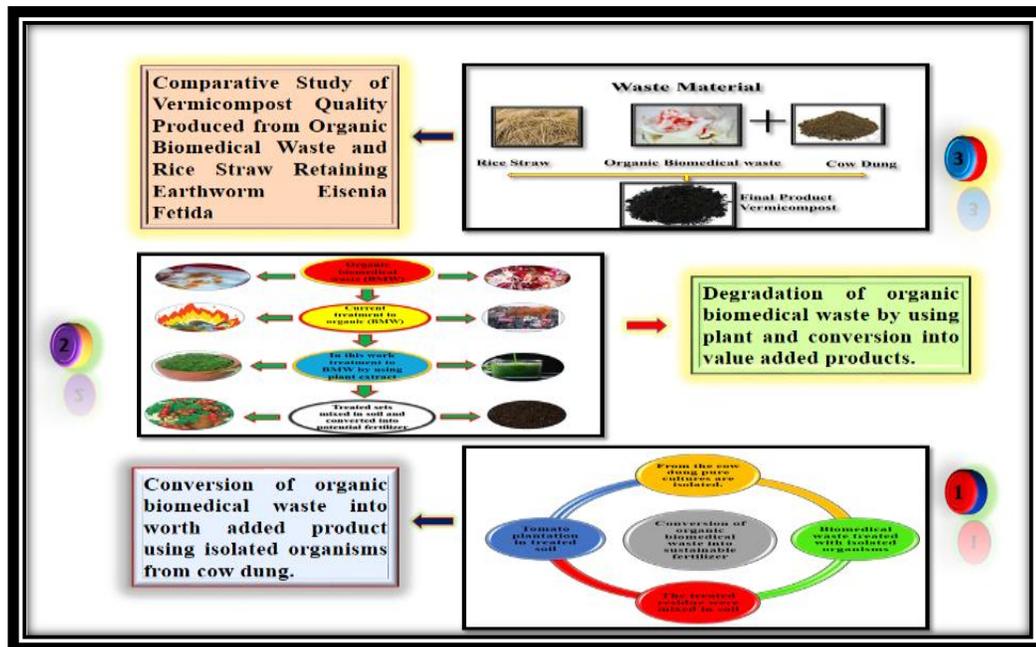
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# CHAPTER 7

## SUMMARY AND CONCLUSIONS



## 7.1. Introduction

Biomedical waste management is the peak crucial practice which should be dispensed with diligently. The management of biomedical waste needs particular regulations and knowledge and it should be carried out by field specialists. In the developed nations heaps of litter are disposed to dumping sites. And in developing countries, most of the biomedical waste is disposed of on open roads and open environment. The untreated disposal of biomedical waste in an open environment bears a health risk and financial cost for citizens of that area and also causes a huge amount of pollution. The rise in pollution leads to change in environment, climate change, health hazards to living organisms, and a negative impact on infrastructure so there is a great need for management of biomedical waste. Recently the proper management of biomedical has caught pronounced attention of public and government all over the world. The management of biomedical waste includes collecting, segregating, processing, transporting, recycling, and reusing materials.

The management of biomedical waste postures a foremost problem in numerous countries. In current years, biomedical waste disposal has postured additional difficulties with the appearance of disposable syringes, needles, and other related items. According to constituents and density, the World Health Organization has categorized biomedical waste into numerous categories. Depending upon the category of biomedical waste disposable treatments are specified which can efficiently treat biomedical waste. As infectious biomedical waste results hepatitis B, C, and HIV/AIDS virus. These viruses are mostly transmitted through injuries from sharp, needles, and used syringes, which are contaminated with blood. However, some various other infections and diseases could transmit by contaminated biomedical waste. Respiratory tract infection, Urinary tract infection, skin infection, wound infections and bacterial infections can also transmit through biomedical waste. The most crucial objective of biomedical waste management is to prevent transmission of disease from patients to hospital care workers, from one patient to another or from health care worker to patient. Management of biomedical waste can be relatively effective when designing appropriate planning with the help of an expert is properly done. The industrial biomedical waste management pilot project has been launched by refining the proper segregation of hazardous and non-hazardous waste by which the risk and health hazards are minimized and the total volume of biomedical waste is also reduced. According to rule, the waste special insulated

container is designed for the disposal of hazardous and infectious waste. For management and reduction of hazardous waste hospitals or institutes should appoint an onsite training officer who performs as an in charge of the management section.

The proper management of biomedical waste is a big concern and it has become a humanitarian issue worldwide. Risky and unsafe management of biomedical waste has become a difficulty of concern predominantly in the light of its special effects which are extreme reaching affecting living organisms and human health and to the environment. With reference to this issue, the present thesis is focused on the development of an innovative, organic, and eco-friendly method for the management of organic biomedical waste which effectively degrades and decomposes the organic biomedical waste. Because the current method (incineration) causes a huge amount of pollution which interrupts the environment. So the organic biomedical waste is treated with a different biological methods, the physicochemical characterization of experimental sets was analyzed. The heavy metal analysis was also done because contamination of heavy metals may have threats and dangers to animals, humans and the environment. The morphological and phytochemical characterization were also analyzed. And finally, organic biomedical waste has been converted into a sustainable value-added product.

## **7.2. Competent Components of Thesis**

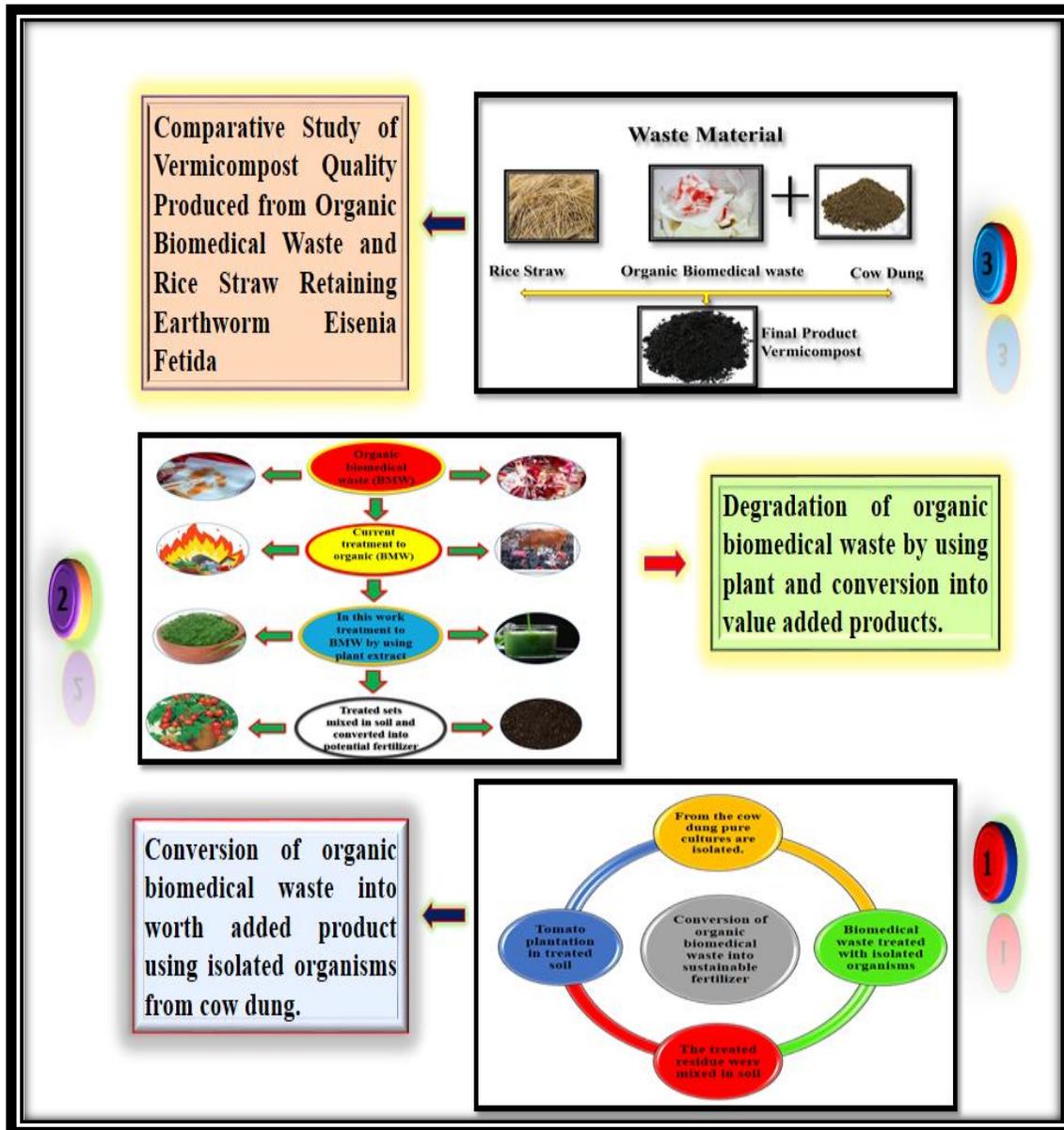
The overall goal of this thesis is to develop an innovative and organic method for the treatment of organic biomedical waste as the management of biomedical waste is a big concern and day by day it is becoming to confront. The transformation of organic biomedical waste into potential fertilizer is of pronounced concern. In this research, the samples of organic biomedical waste (dressing swabs, used cotton, and blood swabs) were collected from D. Y. Patil hospital then cow dung was also collected in the sterile container and transported immediately to the laboratory and screened for intestinal parasitic infection, gastrointestinal infection by using microscopy, fecal occult blood for intestinal bleeding, and bacterial culture. Then the pure cultures were prepared of bacteria and fungus and biomedical samples were submerged to degradation by using isolated bacteria and fungus for 288h. And a definite quantity of organic biomedical waste was treated with incineration. Then further physicochemical analysis organic biomedical waste samples were done.

Then the treated experimental sets were submerged in soil and physico chemical and heavy metal analysis of soil were done to confirm its role as potential fertilizer. Then tomato plants were planted in treated soil and phytochemical and morphological characterization of tomato plants were done. From this research, it is revealed that the fungus and organisms show admirable degradation of organic biomedical waste. The D4 (*Pasterulla canis*) experimental set has physicochemical parameters. The phytochemical and morphological parameters of the D4 plant also high as paralleled to others. The fungus and organisms can efficiently destroy pathogenic biomedical waste with respect to time. This is one type of biological method, and to recycle organic substances it is an alternative process. So by isolating organisms and fungus from cow dung organic biomedical waste has been converted into sustainable fertilizer for cleaner surroundings.

Secondly, the decomposition of organic biomedical waste was done by using plant extract (neem) and (tobacco) as due to the current method vast extent of pollution has been caused and the environment is getting disturbed. The plant extracts content polyphenol which degrades the organic waste effectively and it is reported that 82.35% of reduction in chemical oxygen demand is observed by submerging neem extract in the waste. The utilization of herbal extract for the degradation of organic biomedical waste is agreeable, effective, natural, traditional, and ecofriendly. And most of the plant extracts have antibacterial, antifungal, and antiviral properties. So beholding the organic approach the samples of organic biomedical waste (12 g dressing swabs, 32 g blood swabs, and 6 g used cotton) were treated with extracts of *Nicotiana tabacum* and *Azadirachta indica* kept for 96-hours degradation at various concentrations, and followed by evaluation of physico chemical characterization of experimental sets. Then after the specific quantity of treated residues were submerged in an equivalent amount of soil and soil physicochemical characterization were evaluated. Soils heavy metal content was also analyzed because adulteration of heavy metals in soil can pose threat and hazards to living organisms and the environment. Then in the specific quantity of soil, the tomato plantation was done and morphological and phytochemical parameters of tomato plants were evaluated. From this result, it is revealed that there is a 63.33% decrease in TDS and 95.30% and 86.15% reduction of COD and BOD, respectively at the end of 96hours. As paralleled to control the physicochemical parameters of soil are also enriched and heavy metal in the soil is within the limit. Phytochemical and morphological parameters of plants are also enhanced as compared to control. So the plant extracts

which are used for the conversion of organic biomedical waste into fertilizer on the root of the nutrient content are an imperious indication that this process will decrease the burden of synthetic fertilizer.

In most health care institutes and in hospitals the non-infectious biomedical waste is mixed with infectious waste and was directly discarded for incineration treatment without any segregation which tends mismanagement of biomedical waste. So to avoid the mismanagement the segregated organic biomedical waste was collected from the hospitals and it was doubled autoclaved for 121<sup>0</sup>C. The work was formulated to examine the potential of rice straw and organic biomedical waste. For different concentrations, the experimental sets were designed. The physico chemical parameters of feed material which is used for the process of vermicomposting were analyzed. Then the heavy metal content and physico chemical parameters of treated residues were evaluated. And from this work, it initiates that biomedical waste, rice straw, and cow dung can competently convert into vermicompost by retaining earthworms. The vermicompost is determining to be an exceptionally nutritive organic fertilizer and complementary principal growth developer. As vermicompost also contains plant growth enzyme hormone and beneficial soil microbes and micronutrients. After treatment, the biological and physico chemical characterization of vermicomposting is enriched as compared to the untreated set. The vermicompost is also rich in potassium, nitrogen, and phosphorus content. From the results, it is also concluded that by using *Eisenia Fetida* earthworms the biomedical waste and feedstock has been converted into a justifiable product which can be used in agriculture field to develop the strength of soil and productivity of the crop.



*Thesis Representation of competent components.*

### **7.3. Summary of thesis**

The current thesis is divided into 7 chapters. The summary of the thesis chapter is mentioned below.

#### ***Chapter 1***

In this chapter all the essential part is briefly introduced like the introduction of waste, and types of waste like (municipal solid waste, industrial waste, construction waste, agriculture collected, and commercial waste). A brief introduction about biomedical waste is mentioned in this chapter. It describes the biomedical waste world scenario and management of biomedical waste in the developed nations and countries which are in the developing stage. Lastly, the need and significance of management of biomedical waste and future perspective are explained in brief.

#### ***Chapter 2***

This chapter describes the classification of biomedical waste according to its type, category, and colour. The major and minor sources of biomedical waste are also described in this chapter. In brief, the silent features of the biomedical waste rule are also explained. The current methods are given to biomedical waste like incineration, autoclaving, microwaving, hydroclave, needle melter (for needle), needle destroyers or cutters, syringe crushers, shredding, and disposal methods like deep burial, sanitary landfill, inertization and impacts due to current methods are explained in brief. Treatment given to biomedical waste water like preliminary treatment, primary treatment, secondary treatment, tertiary treatment is described. The current chapter also includes organic treatment given to the waste by using cow dung, plant extracts, and earthworms. Lastly, the compels associated and steps in management of biomedical waste like waste minimization, waste segregation, stowage of biomedical waste, canister and labeling, handling, and carrying of biomedical waste are described in this chapter.

### ***Chapter 3***

This chapter focuses on experimental techniques that are used to analyze the sample and soil. The physico chemical parameters of soil are enlisted along with its principle, and benefits. The heavy metal content and the microbiological characterization are briefly discussed in this chapter. The phytochemical characterization techniques and morphological parameters are discussed.

### ***Chapter 4***

This chapter deals with the conversion of organic biomedical waste into potential fertilizer using isolated organisms from cow dung for a cleaner environment. The organisms (*E. coli*, *Bacillus subtilus*, *Pasterulla canis*) and fungus (*Mucor Circinelloides*) were isolated from cow dungs. After isolation of pure culture the organisms were submerged in the organic biomedical waste for degradation purpose. Then further the physicochemical parameters of organic biomedical waste and soil were analyzed. The heavy metal content and phyto-chemical parameters of plants were also analyzed. From the physicochemical parameters of organic biomedical waste and soil, heavy metal content and phyto-chemical parameters of plants it was confirm that organic biomedical has been converted into potential fertilizer.

### ***Chapter 5***

This chapter focuses on compacts conversion of organic biomedical waste into the worth-added product using an organic approach. The experimental sets were arranged and kept for the degradation of organic biomedical waste for the definite time interval. The physicochemical characterization of experimental sets was done to estimate the performance of tobacco and neem extract. After a definite time interval the degraded sets of biomedical waste, were submerged in the soil in equivalent proportions. Then further soils physicochemical parameters were evaluated to confirm its role of potential fertilizer. Then a heavy metal analysis of plants and soil was done because adulteration of heavy metals in plants and soil may pose risks and hazards to the environment and living organisms. The phytochemical and morphological parameters of plants

were analyzed and from the result it was revealed that organic biomedical waste has been converted into potential fertilizer.

### ***Chapter 6***

This chapter deals with the comparative study of vermicompost quality produced from organic biomedical waste and rice straw retaining earthworm *Eisenia fetida*. The experimental sets of different concentration were designed. The physico chemical parameters of feedstock, organic biomedical waste, and soil were evaluated. The phytochemical analysis of tomato plants were also studied. From the physico chemical, phytochemical results it is revealed that the rice straw and biological biomedical waste can be an exceptional food for the earthworms.

#### **7.4. Major conclusions**

The results obtained from the present thesis work lead to the following conclusions.

- ❖ The fungus and organisms isolated from cow dung show outstanding degradation of organic biomedical waste and convert it into a workable product.
  - The physicochemical characterization of treated experimental sets are enhanced.
  - The heavy metal contents in soil are within the limit in experimental sets. The phyto chemical and morphological characterization of tomato plants are also enhanced when treated organic biomedical waste submerged in the soil sets.
  - The treatment used for degradation of organic biomedical waste is a biological, organic, and ecofriendly treatment the objectives were achieved without causing solitary damage to the surrounding.

- ❖ The tobacco and neem extracts convert the organic biomedical waste into sustainable fertilizer virtuously profitable to the farmer.
  - The neem and tobacco extract shows the admirable result in the degradation and reduction of organic biomedical waste.
  - There is 63.33% of removal of TDS, 86.15% reduction of BOD, 95.30% reduction of COD, was revealed at the end of 96 hours.
  - The physicochemical parameters of treated soil sets and phytochemical parameters of tomato plants are also improved.
  - The heavy metals present in soil are within the permissible limit given by Central Pollution Control Board (CPCB).
  - From the results, it is concluded that the waste which is treated with the plant extracts is the worthy fertilizer source.
  - The degradation of organic biomedical waste by using plant extract and converting them into value-added fertilizer on the nutrient content source is an imperious sign that this method will decrease the synthetic fertilizer load.
  
- ❖ In the study rice straw, organic biomedical waste, and cow dung can proficiently be converted into vermicompost by employing *Eisenia fetida* earthworms.
  - After specific time interval biological and physico chemical characterization of experimental sets were studied and from result it is revealed that physico chemical characterization of experimental sets are enhanced at final analysis.
  - The heavy metal content of compost were also analyzed and they are within the limit.
  - Phyto chemical parameters of plants were also analyzed and the treated sets has more content of protein, polyphenol, chlorophyll content that of untreated set.

- With the vermicomposting process the organic biomedical waste, feedstock, and cow dung are converted into worth-added material that can be utilized in the field of agronomy to improve productivity and health of the soil.

### 7.5. Future Scope

#### **Synthesis and characterization of TiO<sub>2</sub> nanoparticles by Sol-gel method for waste water treatment.**

The titanium dioxide has a abundant applications because of its non-toxicity, high stability, and low cost. The nanoparticles have capacity to detect and capture the bacteria at a preliminary stage. Catalytic applications of TiO<sub>2</sub> have been considered for the eradication of environmental pollutants. Most of the pollutant which is existent in air, water, and soil, are degraded by using photocatalytic processes.

#### **Green synthesis of titanium dioxide nanoparticles using *Azadirachta Indica* leaf extracts for photocatalytic degradation of waste water**

Green synthesis of nanoparticles is an eco-friendly, emerging, and simple approach and recently, fascinating scientific community from all over the world. Green synthesis is less toxic to synthesizing nanoparticles from decomposable materials like plant extracts, enzymes, and microbes. By using plant extracts the synthesis of nanoparticles is the greatest useful strategy as it reduces the chance of accompanying contamination while decreasing the reaction period and sustaining the cell structure.

## Abbreviation

### **B:**

BBC: British Broadcasting Corporation

BOD: Biological Oxygen Demand

BMW: Biomedical Waste

BTV: Blank titrate value

BSA: Bovin Serum Albumin

### **C:**

Ca: Calcium

CAGR: Cumulative Annual Growth Rate

C and D: Construction and Demolition

Cd: Cadmium

COD: Chemical Oxygen Demand

Co: Cobalt

CPCB: Central Pollution Control Board

Cr: Chromium

Cu: Copper

### **D:**

DMSO: Dimethylsulfoxide

### **E:**

EC: Electric Conductivity

*E.coli: Escherichia Coli*

E. fetida: Eisenia fetida

EPA: Environmental Protection Agency

E. eugeniae: Eudrilus eugeniae

### **F:**

FAS: Ferrous Ammonium Sulfate

Fe: Iron

**I:**

ISWM: Integrated Solid Waste Management

**J:**

JIPMER: Jawaharlal Institute of Postgraduate Medical Education and Research

**M:**

Mg: Magnesium

Mn: Manganese

MSW: Municipal Solid Waste

MoEF: Ministry of Environment and Forest

**N:**

NACO: National AIDS Control Organization

Ni: Nickel

NPOC: Non Purgeable organic carbon

**P:**

POC: Purgeable organic carbon

POPs: Persistent Organic Pollutants

P: phosphorus

**S:**

SDA: Sabouraud Dextrose Agar

STV: Sample titrate value

**T:**

TC: Total carbon

TDS: Total Dissolved Solids

TOC: Total Organic Carbon

TMR: Transparency Market Research

**U:**

UNDP: United Nations Development Programme

**W:**

WHC: Water Holding Capacity

WHO: World Health Organization

**Z:**

Zn: Zinc

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Birth Date: 01/06/1993.  
Gender: Female  
Nationality: Indian.  
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## ACADEMIC PROFILE

❖ **Ph.D. Environmental Science**

**July 2016 to till date**

**Department: Center for Interdisciplinary Research, D.Y. Patil Education Society (Deemed to be University), Kolhapur.**

**Topic: Conversion of organic biomedical waste into fertilizer by biological methods.**

**Guide: Prof. (Dr.) P. G. Shadija**

**Pre. Ph.D. ("A" Grade)**

**Submitted thesis in July 2020**

**M.Sc. Environment Science (CGPA: 6.9, 67.08%)**

**April 2016**

**Department of Environmental Science, Shivaji University, Kolhapur, Maharashtra- 416003, India.**

M.Sc. with subjects including Environmental Biology, Environmental Chemistry, Environmental Engineering, Environmental Pollution, Environmental Monitoring and Energy Studies, Natural Resources and their Management, Environmental Policy and Legislation, Environmental Statistics and Computer Application,

Environmental Toxicology and Public Health, Socio-Economic Aspects of Environment, Environmental Hazards and Disaster Management, Environment Planning and Management, Environmental Biotechnology.

**Dissertation:** “Biochemical analysis of pressmud and its amendment with dairy waste and their response to soil and plants” under the guidance of **Prof (Dr.) Pallavi Bhosale.**

**Training Details: One month internship at “Menon & Menon International LTD.”**

**B.Sc. Microbiology (72.36%)**

**April 2014**

**Vivekanand College, Shivaji University, Kolhapur.**

B.Sc. with subjects including Microbiology, Biochemistry, Immunology, Genetics.

## **EXPERIENCE:**

### **1) Assistant Professor**

M.Sc. (Environment & Safety) & M.B.A. (Environment Management). Dept. of Environment Management, Chhatrapati Shahu Institute of Business Education & Research (CSIBER) (An Autonomous Institute), Kolhapur, Maharashtra, India  
01-07-2019 to 30-05-2020

### **2) Teaching Assistant**

M.Sc. Medical Biotechnology and M.Sc. Stem Cell Center for Interdisciplinary Research/Medical Biotechnology, D. Y. Patil Education Society (Deemed to be University), Kolhapur, Maharashtra, India

21-12-2018 to 19-06-2019

### **3) Technical Incharge of VITEK and RTPCR**

RICH Cell D. Y. Patil Hospital, Kolhapur, Maharashtra, India

01-06-2018 to 01-12-2018

### **COMMITTEE MEMBER OF CONFERENCES:**

- Organizing Committee Member in International Conference “Sustainable Development Goals for 21<sup>st</sup> Century” jointly organized by CSIBER, Kolhapur, Maharashtra Pollution Control Board (MPCB), Govt. of Maharashtra held on 10<sup>th</sup> January 2020 at CSIBER, Kolhapur,

### **RESEARCH INTEREST:**

- Management of biomedical waste, Solid waste using ecofriendly approach.
- Treatment of waste water by using innovative microbiology and nanotechnology technique.
- Biodiversity and its conservation, Ecology and its environment.
- Environmental Pollution Control Technology and Management.

### **CONFERENCE AND WORKSHOP PRESENTED / ATTENDED:**

- ❖ Attended and Participated in Five Days Research Methodology Workshop Organised by IQAC, during 08<sup>th</sup> to 12<sup>th</sup> April 2019 held in D. Y. Patil Education Society (Deemed to be University), Kolhapur, India.
- ❖ Participated in Workshop on Applications of Animal Model in Research Organized by Department of Stem Cell and Regenerative Medicine Centre for Interdisciplinary Research, D. Y. Patil Education Society (Deemed to be University), Kolhapur, India held on 08-05-2019.
- ❖ Presented a Poster in the International Conference on “Nanotechnology Addressing the Convergence of Material Science, Biotechnology and Medical Science”. Organized by Centre for Interdisciplinary Research, D. Y. Patil Education Society (Deemed to be University), Kolhapur, India held on 9-11-2017.
- ❖ Participated a National Conference on “Emerging Trend in Nanomaterials and their Applications”. Organized by Department of Medical Physics, Centre for

Interdisciplinary Research, D. Y. Patil Education Society (Deemed to be University), Kolhapur, India held on 2,3<sup>rd</sup> June 2017.

- ❖ Presented research in Anveshan-2017 Organized by Centre for Interdisciplinary Research, D. Y. Patil Education Society (Deemed to be University), Kolhapur, India held on 27<sup>th</sup> December 2017.
- ❖ Attended Conference on “National Conference on Religion and Environment with Special Reference to Jainism.” Organized by Department of Environment Science, Shivaji University Kolhapur, India held on 23,24<sup>th</sup> Feb 2016.
- ❖ Attended one day workshop on Prime Minister’s Fellowship Scheme for Doctoral Research held on 25<sup>th</sup> November 2016 organized by DST-SERB and CII New Delhi D. Y. Patil Education Society Deemed University, Kolhapur, India.
- ❖ Attended the National Conference on Resource Planning and Development: Indian Scenario. Organized by Department of Environment Science, Shivaji University Kolhapur held on 20, 21<sup>th</sup> Feb 2015.
- ❖ Attended the National Conference on NCRUNA Organised by Department of Microbiology D. Y. Patil Medical College, Kolhapur, India.
- ❖ Presented a Poster in the International Conference on Angiogenesis: Targeted Anti Angiogenic Therapy. Organized by Centre for Interdisciplinary Research, D. Y. Patil Education Society (Deemed to be University), Kolhapur, India.

❖ **PUBLICATIONS:**

**Patil PM, Mahamuni PP, Shadija PG, Bohara RA.** (2018). Conversion of organic biomedical waste into value added products using green approach. *Environment Science and Pollution Research* 26, 16. <https://doi.org/10.1007/s11356-018-4001-Z>.

**Patil PM**, Mahamuni PP, Shadija PG, Bohara RA. (2019). Conversion of organic biomedical waste into value added products using green approach. *Environment Science and Pollution Research*. <https://doi.org/10.1007/s11356-019-05795-7>.

**Pooja M Patil** and Raghvendra A Bohara. (2020). Nanoparticles impact in biomedical waste management. *Waste Management & Research* 1 –15. <https://doi.org/10.1177/0734242X209>.

Pranjali P.Mahamuni-Badiger, **Pooja M Patil**, Manohar V.Badiger, Pratiksh RPatel, Bhagyashi S. Thorat- Gadgil, AbhayPandit, Raghvendra A. Bohara. (2019). Biofilm formation to inhibition: Role of zinc oxide-based nanoparticles, *Materials Science and Engineering: C*. <https://doi.org/10.1016/j.msec.2019.110319>.

Mahamuni, P. P., **Patil, P. M.**, Dhanavade, M. J., Badiger, M. V., Shadija, P. G., Lokhande, A. C., & Bohara, R. A. (2019). Synthesis and characterization of zinc oxide nanoparticles by using polyol chemistry for their antimicrobial and antibiofilm activity. *Biochemistry and biophysics reports*, 17, 71-80.

Mahamuni-Badiger, P. P., **Patil, P. M.**, Patel, P. R., Dhanavade, M. J., Badiger, M. V., Marathe, Y. N., & Bohara, R. A. (2020). Electrospun poly (3-hydroxybutyrate-co-3-hydroxyvalerate)/polyethylene oxide (PEO) microfibers reinforced with ZnO nanocrystals for antibacterial and antibiofilm wound dressing applications. *New Journal of Chemistry*.

Bedge PA., Bohara RA., **Patil PM.**, Joshi MG., Bohara DA. (2019). Current cancer therapies: Focus on hyperthermia and immunotherapy, *Hybrid Nanostructures for Cancer Theranostics*. 3. 42-61. <https://doi.org/10.1016/B978-0-12-813906-6.00003-2>.

**LANGUAGE PROFICIENCY:**

- English
- Hindi
- Marathi

**SELF ASSESSMENT:**

Self-motivated, Hard worker, Strong will power to learn and achieve.

Yours Faithfully

**Place: Kolhapur**

**Date: 29/08/2020**

A handwritten signature in blue ink that reads "Pooja Patil". The signature is written in a cursive style with a horizontal line above the name.

**POOJA MAHADEV PATIL.**