JIMMA UNIVERSITY

COLLEGE OF NATURAL SCIENCES

DEPARTMENT OF BIOLOG

Evaluation of the enzymatic activities of microbial associated with solid waste dump site in Bedele town

A thesis submitted to Department of Biology College of Natural Sciences, in partial fulfillment of the requirement for the degree of Master of Science in General Biology

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DECLARATION

I, Zelalem Yohannes, at this moment present for consideration by the Biology Department within the College of Natural Sciences at Jima University, my thesis in partial fulfillment of the requirement for the degree of Masters in ‘‘Evaluation of the enzymatic activity of microbial associated with solid waste dump site in Bedele town

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Approval

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This is to certify that the thesis prepared by Zelalem Yohannes, at this moment present for consideration by the Biology Department within the College of Natural Sciences at Jimma University submitted as in partial fulfillment of the requirements for the degree of Masters in Biology.

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List of Abbreviations and acronyms

ABS Aerobic Bacteria Spore

AMB Aerobic hemophilic Bacteria

ANOVA Analysis of Variance

BPW Buffered Peptone Water

CFU Colony forming unit

ABSTRACT

Municipal waste is a major environmental problem in developing countries.. However, microbial enzymes can help reduce its negative impact. To this end the enzymatic activates of solid waste microbes were evaluated, accordingly. A total of 12 sold waste samples were collected from four area (three from each) and analysed for microbial and physiochemical properties. Results of the study showed pH (7.6-–7.833 ±0.12), moisture content (1.09 ± 0.10) were significantly higher (P< 0.05) compared to control soil samples. Similarly, dumpsite soil organic matter was significantly (P<0.05) increased with increased waste dumping moisture content. the mean microbial counts ($CFUg^{-1}$) were dominated by aerobic mesospheric bacteria count (6.8) CFU g^{-1}, S. aurous (4.4 ± 0.5) CFU g^{-1}, Enterobacteriaceae (2.96 ± 0.1 CFU g^{-1}), fungi (2.3) CFU g^{-1},the count of Coli form bacteria were 1.96 log10 CFU g^{-1} and 3.2 log10 CFU in area 2 and 3 samples, respectively .Among ..the total 120 isolates 18 Fungi and 102 bacterial isolates are identified as bacterial isolates. Bacillus spp. were the most prevalent (43.7%) among others followed by Staphylococcus spp. (24%), Pseudomonas spp. (8%), Acinetobacter spp. (6%) and Coli form (3%) The crude enzyme activity and stability study revealed that the amylase is stable within the pH 6 – 8phvalus and temperature 30˚C - 40˚Cwith the maximum activity at 37˚Ctempereture and 8 pH value. In this study, temperature and moisture content had major roles on the activity of amylase., the microbial quality of soil associated with municipal solid waste of Bedele town was poor, particularly Among the the four isolates BI16,BI5,FIA,FIH exhibited amylase production, FIH selected for amylase production with high titer value. These results have increased the scope of finding industrially important bacteria from municipal waste dump sites and FIH isolates could be vital source for the discovery of industrially useful enzymes.

Keywords: waste dump sites, bacteria, , extracellular enzyme

1. Introduction

Globally, population growth, together with economic growth and associated consumption behavior, has resulted in a significant increase in solid waste production [(UN-HABITAT,2010). In developing countries, managing municipal solid waste (MSW) is a serious problem (1996 ,Schubeler),. Urbanizations and increasing influence have resulted in a significant increase in volumes of discarded materials (Solberg,2016). MSW is defined as local waste generated by households and commercial and governmental enterprises. It includes packaging, food waste, grass clippings, clothing, paper and other solid forms of waste, but does not include hazardous and infectious waste or sewage World watch (2018). It is estimated that the volume of MSW could double from current levels of 1.3 billion tonnes annuallyto2.6 billiontonnes by 2025(World watch 2018) . Municipal solid waste management (MSWM) in developing countries accounts for between 20% and 50% of local government budgets (SchbelerC 2009). Studies show that more than 50% of developing countries' populations lack consistent access to waste collection services(UNPE,2016) . Disposal methods often include open dumping and open burning(Unpe,2009)]. The World Bank reports vast amounts of uncollected waste in urban areas; estimates suggest between 40% and 70% of discarded materials remaining uncollected(UNPE,2016) . This pollution leads to significant impacts on human health and the environment(USPA,2002) . Approximately 70-80% of MSW generated in Africa are recyclable but only 4% of its currently recycled by private sectorss (UNEP 2021).

Concurrently, the urban solid waste recycling activities in Ethiopia were not adequate; for example, only about 5% of the solid waste is recycled (Mohammed 2018). In developing countries such as Ethiopia, estimated20-50% of solid waste generated in meny urban areas are uncontrolled as it sources (Gedefaw,M 2015). This shows that Ethiopia requires several years to achieve the goal of the African Union Commission to recycle its urban waste. As a result, the MSW problem is getting worse and is one of the serious cross-cutting issues of Ethiopia . SWM in Ethiopia has mainly targeted waste collection from source and its transportation to disposal sites. This is mainly due to inadequate institutional capacity, finances, knowledge, and awareness, lack of solid waste baseline data, minimal cooperation among stakeholders, weak political commitment and prioritization, and lack of effective planning and implementation (Rehome, 2000; Dev. 2010).

The former study shows that microbiological and enzymatic parameters are among most frequently selected for soil state monitoring (Beilin's and Mocek-Płoˊciniak , 2009). The Bedele town is one among, where there is rapid growth of urban population as well as constraint in the management of solid wastes and its improper management greatly affects the public health and environment. According to previous study the problem of solid waste impact dramatically increases due to need of urbanization of many population to get work opportunity economical advantage and other advantage .As result town is susceptible for improper solid waste disposal which is easily observed in accumulation of solid waste in many areas of a town ,drain blockage and open burning and disconnected solid waste management service More over low attention has been paid for bioconversion of these organic wastes to different valuable end products such as bio fertilizer, aroma compounds, and industrially important enzymes via biological processes involving living organisms .

To cope up with the demand for new organisms with properties of production of unique enzymes for industrial application and waste degradation there have been a constant effort in isolating novel bacteria from environment. The major advantage of using microorganisms for the production of amylases is the economical bulk production capacity and the fact that microbes are easy to manipulate to obtain enzymes of desired characteristics (Kunamneni 2005). However, the growth of microorganism and the resulted amylase are strongly influenced by medium composition. (Raimbault 1998). Thus, optimization of media components and cultural parameters is the primary task in a biological process in order to obtain a good result in amylase production.

Therefore, to meet the growing demands for these enzymes in various industries, it is necessary to improve the performance of the system so as to increase the yield without increasing the cost of production especially in developing countries like Ethiopia .In order to fill some of these gaps of research, the study was aimed and designed to isolate, and characterizes potentially active hydrolytic enzymes from soil inhabiting microbial sources, such as fungal and bacterial strains from MSW soils in Bedele town with the ultimate objective of waste degradation and discovering novel bioactive compounds for industrial application . To this effect this study is focused to assess microbial community richness physic-chemical compositions and activity microbial enzyme in solid waste dump site of Bedele town which helps to formulate effective,

treatment method, discovery of potential hydrolytic enzymes impact estimation of the area and document the result for farther study.

1 .1. Statement of the problem

In many countries, particularly in developing countries due to poor infrastructure, bureaucratic, competency and limitation of institutional capacity of municipality there is no effective approach to monitoring and supervision of all activities associated with the waste management. Polluted water (leachate) flowing from waste dumps and disposal sites can cause serious pollution of water supplies (Tsai, 2007). . Such rapid increase in population coupled with rapid development of the town leads to increasing solid wastes generation rate. Moreover, the amount and type of solid wastes generated now a day is increased time to time and leads to solid waste impact (Keshaun 2018). The impacts solid waste on the environment was refelected by various indictors. Some of the indicotors are the daamage of infrastracure impacts indicters like infrastructure damage (drain blockage road destruction), respiratory infections, air and water pollution facilitate breeding site for rodent and vectors. More over the solid waste management of both municipality and private sector service is discontinuous poor facility of storage, transportation leads for anther social and economic crises and poor recycling habit that leads to other economic and contamination effect of society. This indicates solid waste management practice of the Bedele town is poorly organized and implemented. Also no previous study was conducted so far regarding its physic-chemical and enzymatic diversity of microorganisms in dump site of solid waste.

1.2 Objectives

1.2.1 General objective

the general objective this study was to:

Evaluation of the enzymatic activities of microbial associated with solid waste dump site in Bedele town

1.2.2. Specific objectives

The specific objectives of this study were to:

- determine the physicochemical properties of solid waste at dumping site
- isolate and characterize the microbes associated with solid waste at dump site in Bedele town,
- evaluate the effectiveness of microbial enzymes under various higher polymer substrates

1.3 Significance of the study

This study could be helped to provide baseline data on solid waste management through giving information about enzymatic activities of three of the most important enzymes found on bacterial and fungal isolates. Fast and reliable plate methodologies allowed to process, identify and provide semi-quantification of extracellular enzymatic activity. of microbes isolated from solid waste dump site of Bedele town.

2. Review of related litrature

2.1 Over view of solid waste management

Now days, waste management has recently been the concern of every country in the world. It is part of the important service which is sustaining our society especially in urban area. It has now become basic human right which is part of basic human needs. This basic human right involves ensuring proper sanitation, solid waste management, provision of potable water, shelter, food, energy, transport and communications which are all beneficial to society and the economy as a whole (UNEP, 2015) Solid Waste management in most of the Africa counties has two problems: lack of accurate data on waste generation, characterization and a corresponding lack of information about waste collection, processing, and disposal (Tchobanoglous et al., 2002*)* . In developing countries like Ethiopia, this problem is exacerbated by an influx of people moving to urban centers (Montgomery, 2008). Current Ethiopian MSWM is mainly focused on the collection, transport, and disposal of solid waste; (Teshome 2020). Similarly, a study conducted in Bangladesh (Ahsan et.al 2014). indicates that sustainable solid waste management strategies, such as prevention, reduction, reuse, recycling, and final disposal in many Ethiopian towns and cities, require attention from the government (Edwardess.2010, Kibrekudusan, 2017).

2.2. Source and types of Solid Wastes

Solid waste types Classified based on their origin as food waste (60%), rubbish (5%), ashes and residues (25%), demolition and construction (7%), and agriculture waste 3%. (Charlotte, 2009). Microorganisms are essential parts of biodiversity and play a crucial role in the structuring and functioning of the ecosystem on the environment (Sinha et al., 2008). Major group of microorganisms such as bacteria, fungi, and actinomycetes is found in soil which decomposes waste organic materials, of which bacteria are most abundant and predominate (Taiwo and Oso, 2004) On the other hand, also solid waste classified based on their biological characteristics as biodegradable (80%) and the other (20%) is non- biodegradable wastes. (Cheremisinoff, 2003). Solid wastes could also be classified based on the risk potentials associated with the waste as hazardous waste (16%) and non-hazardous waste (84%) (Zurbriggen, 2003).

2.3. Factors and associated problems with solid waste Management

The generation of waste and the collection, processing, transport and disposal of waste, the process of „waste management", is important for both the health of the public and aesthetic and environmental reasons (Rushton, 2018). Sites are found on the outskirts of urban areas. Collection efficiency of the existing solid waste systems in developing countries is very low due to a lack of storage bins and improper management system (Abarca et al., 2013).

Due to poor domestic waste collection and disposal systems, high amounts of waste ends up in open dumps or drainage system, threatening both surface and ground water quality and provide a breeding ground for pests (Mohammed et al., 2017). Open burnings cause air pollution and unpleasant odor to the environment more exacerbated in areas where there is lack of garbage collection containers. It also leads to loss of productive land due to the presence of non-biodegradable items and contamination of soil, ground and surface waters by leaching. Buried hazardous domestic wastes can filter down through the soil and contaminate groundwater. Pouring hazardous liquids on the ground can poison soil, plants and water (Mohammed et al., 2017).

2.4 Components of Good Solid Waste Management System

Solid waste consists of many different materials. Some are combustible others are noncombustible (2%), recyclable (1%), and non-recyclable (2%), biodegradable (94%) and non-biodegradable 1% around DE Marco's Town (Zeb nay Kassa , 2010).

2.4.1 Waste Recycling and Reuse

In developing countries it is acknowledged that (85%) the recovery of materials such as iron, steel, copper, lead, paper plastic and glass will decrease the investment in importing these materials and save energy (melaku, 2008). The formal sector has concentrated on the collection and final disposal; although (10.4%) recycling is viewed as an option; its application is very weak. In the same way, the attitude of the formal waste management sector towards informal recycling often is very negative regarding it as backward, unhygienic and generally incompatible with modern waste management systems in Addis Ababa city (Wingdae, 2018).

2.4.2 Composting

Microorganisms are essential parts of biodiversity and play a crucial role in the structuring and functioning of the ecosystem on the environment (Sinha et al., 2008). Major group of microorganisms such as bacteria, fungi, and actinomycetes is found in soil which decomposes waste organic materials, of which bacteria are most abundant and predominate (Taiwo and Oso, 2004) High organic content of the waste streams of developing countries is ideal for composting, but municipal services operators do not have enough and adequate information only (17%) used for compost in Addis Ababa (Gere Emanuel, 1994). The use in agriculture, as soil conditioner or fertilizer, is one of the most usual ways to take advantage of the compost obtained with the processing of MSW; however, the quality of the product is subordinated to variables such as the design of the composting facility, type and proportions of feed stock used, composting procedure and maturation period (Jordan, 2000). Different microbial communities predominate different composting phases. The duration of each stage is dependent on the initial composition of organic material, moisture content, quantity and composition of the microbial community (Fisher et al 2012).

2.5 Physiochemical factors affecting microbial population and activity

There are known to be the fundamental indicators for estimating the level of soil nutrient contents and characteristics. Solid wasted electrical conductivity can serve as a measurement of soluble nutrients, and it is useful in monitoring the mineralization of organic matter in the soil. The availability of water content and soil pH in soil appears to be significant limitations of enzyme activity

Microbial activity is influenced by oxygen level, particle size of the feedstock material, nutrient levels and balance (indicated by carbon-to-nitrogen ratio), moisture content, temperature and acidity/ alkalinity (pH) (Rebollido et al., 2008). Any changes in these factors are interdependent; a change in one parameter can often result in changes in others. Microorganisms can only use organic molecules if they are dissolved in water (Pramanik et al., 2007). They require moisture to assimilate nutrients, metabolize new cells and reproduce. They also produce water as part of the decomposition process. If water is accumulated it is eliminated via aeration.

Water is the key ingredient that transports substances within the composting mass and makes the nutrients physically and chemically accessible to the microbes., if the moisture content is lower, the nutrients are no longer in an aqueous medium and microbial activity become decreases or dormant. If the moisture content exceeds 70% of water holding capacity, would create anaerobic conditions, aeration is hindered, nutrients are leached out, decomposition slows, which may be minimized the biological processes of microorganism (Edward et al., 2004).

The pH of a substance is a measure of its acidity or alkalinity (a function of the hydrogen ion concentration), the level of pH in the waste depends upon the decomposition rate and characteristics of feed material. The release of organic acids may decrease the pH and production of ammonia from nitrogenous compounds may raise the ph. At higher pH levels, more ammonia gas is generated and may be lost to the atmosphere. fungi thrive in a wider range of pH levels than bacteria, in general preferring a pH between 5.5 and 8 (Edwards et al., 2004). If the pH drops to acidic, microorganisms, especially bacteria die off and decomposition slows however, if the pH values increase to more basic, nitrogen is converted to ammonia and becomes unavailable to organisms. The ratio (C/N) is significant and critical factor in microorganisms. Microorganisms require specific nutrients in an available form, adequate concentration and proper ratio. (Bityutskii et al., 2002).The essential macronutrients needed by microorganisms in relatively large amounts include carbon, nitrogen, phosphorus and potassium. If the organic feed material is poor in nitrogen and C/N ratio is high, microbial activity decrease in the feed substrate. However, if there is C/N ratio is decreased it can remove nitrogen to the atmosphere in form of NH_3. This high concentration of ammonia is not suitable for better metabolic activities of microbes within the system (Pniel et al., 2008). Gases inhibit microorganisms by two mechanisms. First, they can have a direct toxic effect that can inhibit growth and proliferation. Carbon dioxide (CO_2), ozone (O_3), and oxygen (O_2) are gases that are directly toxic to certain microorganisms. This inhibitory mechanism is dependent upon the chemical and physical properties of the gas and its interaction with the aqueous and lipid phases of the food.

A second inhibitory mechanism is achieved by modifying the gas composition, which has indirect inhibitory effects by altering the ecology of the microbial environment. When the atmosphere is altered, the competitive environment is also altered. Atmospheres that have a negative effect on the growth of one particular microorganism may promote the growth of

another. This effect may have positive or negative consequences depending upon the native pathogenic micro flora and their substrate. Nitrogen replacement of oxygen is an example of this indirect antimicrobial activity. A variety of common technologies are used to inhibit the growth of microorganisms, and a majority of these methods rely upon temperature to augment the inhibitory effects. Technologies include modified atmosphere packing (MAP), controlled atmosphere packaging (CAP), controlled atmosphere storage (CAS), direct addition of carbon dioxide (DAC), and hypobaric storage (Loss and Hotchkiss 2002,).

When considering growth rates of microbial pathogens, in addition to temperature, time is a critical consideration. All microorganisms have a defined temperature range in which they grow, with a minimum, maximum, and optimum. At low temperatures, two factors govern the point at which growth stops: 1) reaction rates for the individual enzymes in the organism become much slower, and 2) low temperatures reduce the fluidity of the cytoplasmic membrane, thus interfering with transport mechanisms (Shafique 2009).

2.6 The significance of studying microbial diversity

Microorganisms play important roles in the environment. Decomposition processes are dominated by microbial activities and are as fundamental as primary production for the long-term functioning of the ecosystem (Snowball et al., 2014). Microbial diversity analyses are therefore essential in order to increase the knowledge of the diversity of genetic resources in a community as well as to understand the relative distribution of organisms also increase the knowledge of the functional role of diversity, help to understand the regulation of biodiversity and to understand the consequences and importance of biodiversity to what extent the ecosystem functioning and sustainability depend on maintaining a specific level of diversity (Jeelani et al., 2017).

Microorganisms are essential parts of the living soil and the most importance for soil health. As such they have been regarded as sensitive indicators of soil health because of the clear correlation between microbial diversity and soil health and quality (Adeleke et al., 2010). The relationship between microbial diversity and soil functionality is important; considering the fact that 80-90% of processes in soil are mediated by microbes (Sharma et al., 2014). Microorganisms play important roles in the biogeochemical cycles of the main elements as well

as the trace elements therefore critically involved in energy and nutrient exchanges within soil (Jeelani et al., 2017). Microorganisms are the original recyclers of nature, many of which are able to convert toxic organic compounds to harmless products, such as carbon dioxide and water. The discovery that microbes have the potential to transform and degrade xenobiotic, has led researchers to explore their functional diversity, especially as it relates to their potential to degrade a wide range of pollutants (Olabode et al., 2016). Motility test (stab methods) of pure colonies A motility medium was prepared using a test tube. Purified a broth culture (colonies) of bacteria were taken by sterile needle and stabbed vertically into a test tube containing motility medium and incubated at 32°C for 24 hours until a cloudy medium was observed. A positive result was grown throughout the medium away from the stabbed point, the bacteria were considered as motile. However, if the growth was restricted only to the region of stabbed, the microorganisms were non motile (Quinn et al., 2004). The results were recorded and used for the identification of isolates.

2.7 Microbial Community Structure Profiling Techniques

Identification of a bacterial species is based on many factors, including cell and colony morphology, chemical composition of cell walls, biochemical activities, and nutritional requirements. In order to begin identifying a bacterial species, you must start with a pure culture. In last week's lab, you streaked a mixed bacterial culture containing three unknown bacterial species onto an agar plate. Recall that the purpose of streaking is to place individual cells on an agar plate so that an individual cell will divide many times to produce a colony. Once you have an isolated colony, you have millions of genetically identical cells, which can be used in identification tests. In this exercise, you will examine the streaked plates from last week's lab and will attempt to identify the three unknown bacterial species found on these plates. Your preliminary identification will be done through a comparison of the characteristics of the three unknown bacteria to the characteristics of known bacterial species.

2.7.1 Gram stain reaction

An initial step in identifying a bacterial species is determining whether the cells in question are Gram-positive or Gram-negative. The Gram stain is one of the most important and widely used tools in the identification of unknown bacteria. The Gram stain reaction is dependent on the cell wall structure of the bacteria. The cell wall of Gram-positive bacteria is composed of a thick

layer of peptidoglycan that surrounds the plasma or inner membrane. In contrast, a thin layer of peptidoglycan and a second phospholipid bilayer, known as the outer membrane, surround the plasma or inner membrane of Gram-negative bacteria. These characteristically different cell wall structures permit microbiologists to classify bacteria based on the color of the stain retained by cells treated with the Gram stain (Gram,1884) .

The Gram stain is a differential stain because it divides bacteria into two groups: Gram-positive and Gram-negative, where Gram-positive bacteria stain purple and Gram-negative bacteria stain pink. The procedure requires four solutions: a basic dye (crystal violet) a mordant (Gram's iodine), a decolorizing agent (ethanol) and a counterstain safranin Bruckner MZ. (2012) .

Differences in cell wall structure cause the different staining reactions for Gram-positive and Gram-negative bacteria. When performing the Gram stain, the cells, which are stuck to a glass slide, are dipped into the crystal violet stain. The purple dye, crystal violet, is picked up by the cell wall of both Gram-positive and Gram-negative bacteria. The cells are then dipped into an iodine solution; iodine is the mordant, meaning that it is a substance that increases the affinity of the cell for crystal violet so that crystal violet is more difficult to remove from the cell. Iodine forms large complexes with crystal violet and these complexes combine with the peptidoglycan in the cell wall. This crystal violet-iodine (CV-I) complex is larger than either molecule by itself. When ethanol (the decolorizing agent) is applied, the ethanol dissolves the lipids in the outer membrane of the cell wall of Gram-negative bacteria causing the CV-I complex to leave the cells. Gram-negative cells, therefore, appear colorless after the ethanol wash. However, when Gram-positive cells are washed with ethanol, their thick peptidoglycan layer prevents the large CV-I complexes from leaving the cell. Gram-positive cells therefore stain purple, which is the color of the crystal violet dye (Sandler. 2016).

The decolorizing step in the Gram stain is the critical step in distinguishing Gram-positive from Gram-negative bacteria because the two types of bacteria differ in the rate at which they decolorize when exposed to ethanol. If Gram-positive cells are decolorized too long (i.e., exposed to ethanol for too long), they appear as Gram-negative cells. As Gram-negative cells are colorless after the ethanol wash, they are counterstained with safranin, a pink or reddish-colored dye. Therefore, Gram-negative cells appear pink after the Gram stain procedure, which enables

comparison between those cells that decolorize with ethanol and those that do not. (Sendeler. 2016).

2.7.2. Morphological characteristics

In addition to the Gram stain, microorganisms are also classified according to colony morphology and cell morphology. Bacterial colonies grow from a single cell and are composed of millions of cells. Each colony has a characteristic size, form or shape, edge, texture, degree of opacity, and color. These characteristics describe the morphology of a single colony and may be useful in the preliminary identification of a bacterial species. Colonies with a markedly different appearance (when grown on the same medium) can be assumed to contain different bacterial specie (Fakuradin,M2015). However, since many species have similar colony morphology, the reverse (that colonies that look alike are the same species) is not always true. The composition of microbial communities during composting is dependent on the constant change in the physicochemical parameters such as temperature, moisture, C/N ratio, oxygen rate and pH (Awasthi 2017, Karanja 2019). Determination of the microbial community structure in compost is vital as changes in the structure of the microbial community can impose negative effects on the quality and yield of crops (Smith 2006). However, the understanding of microbial community structure during composting is limited due to the limitations of profiling techniques (Meng, 2019). As the traditional, cultivation-based methods have limitations to the type of strains that can be cultured, the role of conventional biochemical methods has become limited eventually shifting towards molecular techniques as the current trend. The bacterial spore stain is another technique that facilitates in the observation of bacterial Observation of bacterial end spores can be done using a light microscope (Oktari et al 2017).

For fungal identification, lacto phenol cotton blue is commonly used in examination of yeast and filamentous fungi. Staining makes septa, special mycelia and spore structures visible as light blue colored under light microscope (Sandler. T, 2016).

Culture-dependent methods of microbial community analysis

Culturing method is one of the traditionally analyses of soil microbial diversity using selective plating (Hill et al., 2000). Microorganisms can be described and classified in two major ways, namely; microscopic examination and cultural characteristics. These methods are fast, inexpensive and can provide information on the active, heterotrophic microbial population. Limitations of the culturing method include the difficulty in separation of bacteria or spores from soil particle, growth medium selections (Tobacconist et al.2000). Growth conditions (temperature, pH, light), the inability to culture a large number of bacterial and fungal species with current techniques and the potential for colony inhibition or of colony spread (Kirk et al., 2004). All of these limitations can influence the apparent diversity of the microbial community. Isolation of cultivable microorganisms is appropriate for functional analysis as it can give an indication of potential decomposer microorganisms in the soil. However, one of the major problems that makes difficult to study the microbial diversity in the environment is the inability to obtain many of microbes in culture (Sati et al., 2002). The process of bioremediation is very slow and only few species of bacteria and fungi have proven abilities to degrade pollutants. Many strains are known to be effective as bioremediation agents but only under laboratory conditions (Zambonelli C, et al 1992). The limitation of bacterial growth is under the influence of pH, temperature, oxygen, soil structure, moisture and appropriate level of nutrients, poor bioavailability of contaminants, and presence of other toxic compounds. Both bacteria and fungi rely on the participation of different intracellular and extracellular enzymes for the degradation of recalcitrant lignin and organo-pollutants (Zambonelli , et al 1992).

2.8 Enzymatic diversity in solid waste inhabitant microorganisms

Microorganisms can secrete different enzymes such as amylase, lipase, protease, cellulose and chattiness, which results rapid biochemical conversion of pretentious and cellulosic material in the waste organics residues to more soluble organic compound Enzymes are large biological molecules responsible for all those important chemical inter conversions that are required to sustain life (Rao , 2010). Enzymes are known to catalase about 4,000biochemical reactions Sheng , 2018]. Enzymes are very specific, and it was suggested by the Nobel laureate Emil Fischer in 1894 that this was because both the enzyme and the substrate possess specific complementary geometric shapes that fit exactly into one another (Steinberg, 2013). This is often

referred to as “the lock and key” model. However, while this model explains enzyme specificity, it fails to explain the stabilization of the transition state that enzymes achieve catalyst the cycling of nutrients such as carbon (C), nitrogen (N), phosphate (P), and sulfur (S) also organic matter (OM) decomposition in soils (Ten et al, 2014). At present, soil enzymology is of practical significance because of industrial waste, agro-chemicals, heavy metals, and soil fertility management that can be measured (MA koi, 2008). Enzymes in the soil are useful biological indicators due to their association with soil ecology, sensitivity, operationally practicality, ease of evaluation, and integrative (Utobo et al 2015). Enzymes come from many different soil sources, including non-living and living microorganisms, residues and roots of plants, and earth animals (Das ,2011).

The difference in soil enzyme activity is due to the variation in soil organic matter, microbial community, microbial activity related to soil biology processes affected by both the abiotic and biotic factors (Kunju ret al ,2014)]. The urease and inverses enzyme activity are essential indicators of microbial mediated functions and activities due to its rapid changes towards significant environmental changes (Logan than et al 2017). Enzymes are the critical effectors of all the alterations happening in the ecology. They also catalyze with both narrow (chemo-, region- and stereo selectivity) and broad specificity. They tend to perform processes for which no efficient chemical transformations have been developed (Rao,2010). Enzyme activity (EA) accelerates the reaction of the rate-limiting steps for organic matter degradation. Many studies have observed relationships between the plant litter decomposition, the microbial community, and enzyme activity. Naturally, enzyme reactions are sensitive towards the temperature, climatic, and geographic factors that can affect enzyme activity by changing the microbial biomass and the abiotic control of enzyme turnover and stabilization (Sheng ,2018).

Climate can significantly affect the soil enzyme activity because of their sensitivity to temperature and precipitation. Other studies indicate that some areas with harsh climatic conditions exhibit less waste input, lower decomposability, and reduced numbers of microorganisms and enzyme activity (Sheng ,2018). Both the production and turnover rates of enzymes might be affected by temperature and moisture content; hence they may differ seasonally and be affected by climate change (Steinberg, 2013). A study by Sheng et al., 2017 reported that soil pH has substantial effects on the structure and diversity of soil bacterial

communities, and a suitable pH can benefit microbial growth. They said that both climatic situations and soil pH are the primary factors affecting soil enzyme activity.

Furthermore, the outcomes revealed that most analyzed enzyme activity decreased with soil depth, but showed an increase in soil organic matter (Sheng, 2018). One of the essential soil components is soil organic carbon and organic matter because they can maintain soil fertility and crop production and prevent soil degradation, erosion, and desertification. A vital role in the decomposition of organic matter played by soil enzymes (Micatin, 2017). Most enzymes are sensitive to ph. They have a specific pH range and optimum pH of activity (Hemet 2017). Many enzymes are important for catalyzing different significant reactions necessary for soil for microorganism's life processes and the soil structure maintenance, for the breaking down of organic wastes, organic matter formation, and nutrient cycling (Makoi,2008). The activity of enzymes can be used to discover the level of pollution in soil (such as heavy metals, SO_4), assess the successional stages, and degrade pesticides in the soil environment (Das, 2011).

Several researchers have noticed the significant reasons why soil enzyme activity used as an essential soil-quality indicator. They noted that enzyme activity is often closely related to critical soil-quality parameters such as organic matter, soil physical properties, microbial activity, or biomass. Also, they can begin to change much sooner (1–2 years) than other properties (e.g., soil organic carbon); thus, they provide an early indication of the course of soil quality with changes in soil management. They can be an integrative soil biological index of past soil management (Alkorta, 2003).

Temperature and moisture can affect both the overall rate of enzyme production and the relative quality of production of different enzymes because they influence enzyme efficiency, substrate availability, and microbial efficiency. Therefore, alteration in the soil microclimate, whether they occur within hours, weeks, seasonally, or over decades as feedback to climate change, will influence the enzyme pool (Steinberg, 2013).

Most enzymes can be denatured that is, unfolded and inactivated by heating or chemical denaturants, which disrupt the three-dimensional structure of the protein. Depending on the enzyme, denaturation may be reversible or irreversible. Due to their wide range of activities based on their nature of reaction enzymes are being classified according to their enzyme catalyzing reaction. The Enzyme Commission number (EC number) is a numerical classification

scheme for enzymes, the enzymes are divided into 6 parts: oxidoreductase (EC 1), transferees (EC 2), hydrolyses (EC 3, lyase (EC 4), isomerizes (EC 5), (vi) ligase (EC 6). based on the chemical reactions they catalyze (Hemet ,2017). Dehydrogenate is an important intracellular microbial enzyme that is tightly linked to microbial oxide-reduction process and as such, serves as a potent bio indicator for the overall soil microbial activities (Brewer Ms2000). Microbial dehydrogenate (EC 1.1.1.) is an intracellular enzyme that occurs in all viable microbial cells. This enzyme functions as a measurement of the metabolic state of soil microorganisms (Tic cog 2000) . Dehydrogenates activity (DHA) is one of the most adequate, important and most sensitive bio indicators, relating to soil fertility (Brewer Ms2000). Its activity depends on the same factors which influence microbial abundance and activities (Tic cog 2000). Dehydrogenases play significant roles in the biological oxidation of soil organic matter (OM) by transferring hydrogen from organic substrates to inorganic acceptors (Ajmer MZ.2012).

Chitin is a polymer of N-acetyl glucosamine with β-1,4 bonds Kumar, R. (2000). It has a highly ordered crystalline structure, as shown by X-ray diffraction studies, and is insoluble in water Roberts, G.A.F. (1992). And generally bound to other polysaccharides and proteins Kumar, R. (2000) . Chitin chains present three forms of arrangement, denominated as α, β and γ. The α form is dominant and more stable and consists of alternating parallel and anti-parallel chains; it occurs mainly in crustaceans, insects and fungi. The β form consists of parallel chains and occurs only in marine organisms. The γ form is still being elucidated Roberts, G.A.F. (1992). Chitin is the main structural component of the cell wall of most fungi, but is susceptible to innumerable bacterial and fungal species acting as antagonists, due to their production of chitin lytic enzymes Sahai,et.al (1993). Chitins (E.C. 3.2.1.14) {Poly [1,4-(N-acetyl-β-glucosamine)]glucanohydrolase} catalysis the hydrolysis of the β-1,4 bonds of the N-acetyl-β-D-glucosamine of chitin and chitin-dextrin's. Chattiness can be used in controlling pathogenic fungi in plants and insects; in the production of biologically active chitin-oligosaccharides; in the production of single cell protein; in the preparation of Amyl lytic enzymes; and in the formation of fungal protoplasts Patil, R.S.; (2000). In the agro-technological sector, emphasis is on the bio fungicidal and bio-insecticidal effects of various chattiness produced by microorganisms. These effects relate to the hydrolysis of chitin in the fungal cell wall and insect carapace, respectively Fleuri, L.F; (2005). Chattiness are produced by various microorganisms, such as Trichoderma

sp. TUBF 781 Nampoothiri, K.M.; (2004) , Penicillium sp. (Binod, P.; (2005, Bacillus subtilis Wang, S.L.; . (2006), Trichoderma Prabavathy, V.R.; (2006) and others.

3. Materials and Methods

3.1. Description of study area and period

Bedele town administration city is located in Oromia region. It is located at about 500km south west of Addis Ababa on the high way to Mettu and Gambella . Bedele town has a total area of 2878.1-meter square. since Bedele town established a long time ago has been serving as the center for different levels of administrations at varying times. The socioeconomic of the town is mixed economy which is mainly Agriculture and the organic coffee is an important cash crop in the area. Over square kilometers are planted with coffee under tropical forest species vegetation and have also the wildlife that takes shelter in it. The dominant crop types farming on the study area are maize and sorghum It has 4(four) administrative kebeles.

The town municipal services like solid waste service begins early s1930.Topography from the slope map below the land surface terrain of the Population. Currently, it is estimated that the total population of the town to be 27,044 males and 28,824 females' total 55,868 at 2020G.C (annual report of city administration, 2020). The rainfall recorded at Bedele meteorological station indicated that the average annual rainfall is about 1000-2100mm. SSGPS coordinating reading of the town situated between 8°20′-80 35′ N and 36°15′-36°30′ E latitude and longitude respectively The temperature of Bedele area ranged of 15°c -27°c Study was conducted from t (March, 2022-April 2023).

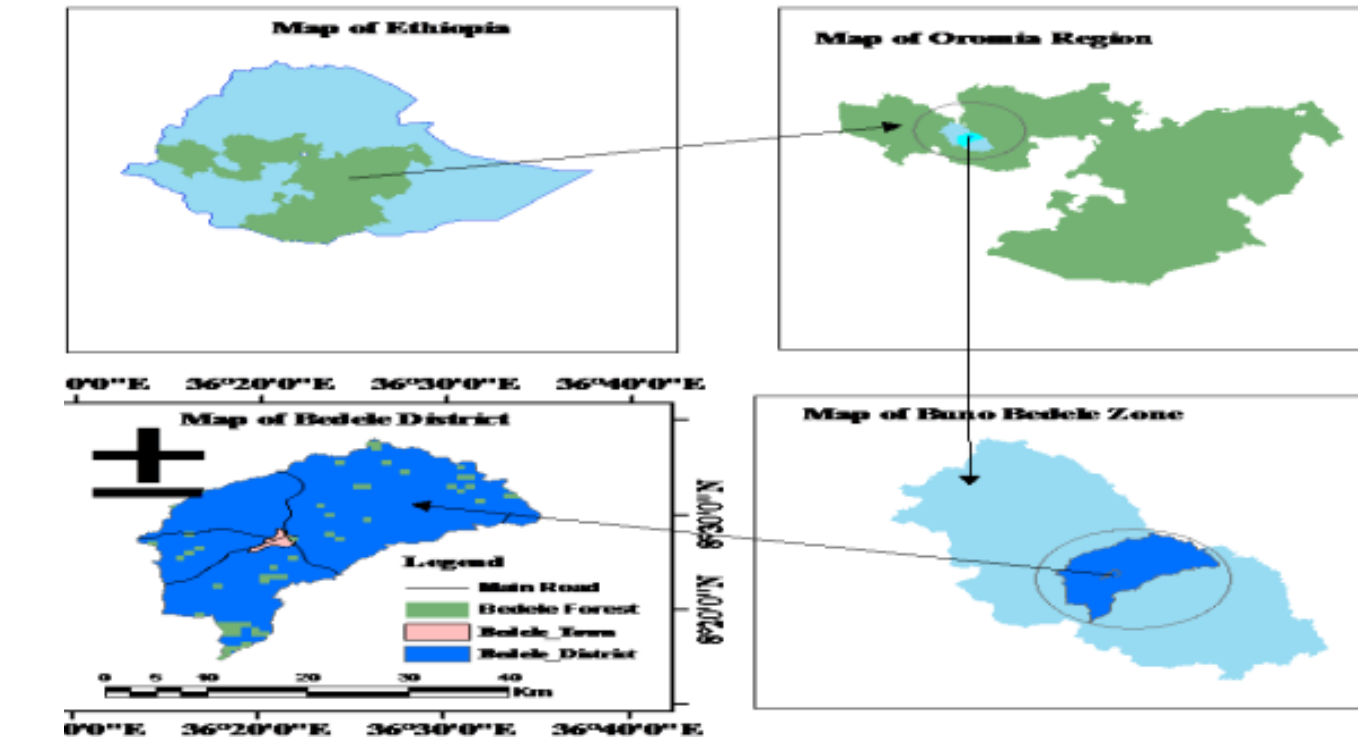


Figure 1

collected from dump site through using stainless steel and we were collected the samples at depth of 0.5-2cm. from each sample point and the top 0.5cm of surface solid waste is removed before taking sample and the composite samples added into labeled polythene and transported to Laboratory. Thereafter, simple stratified random sampling techniques followed, so as to obtain a representative homogenous sample. Sediment samples are collected from four MSW dump cells in Bedale town temperature were taken immediately. The samples are obtained in triplicates and then transported to the laboratory for further physicochemical and microbial activity

measurement.

3.2.1 Physicochemical properties of solid waste sample.

The physicochemical properties of solid waste sample were determined at spot site followed standard procedures. Jakson 1970

PH. The pH of solid waste sample in four collection sitesA1=Mixed hose hold dominant waste A2=garage dominant area A3=plastics and chat dominant area A4= none dump site area are measured using (pH-016 model) according mean concentration of soil pH in four collection sites are measured through using (pH-016model) according to method described by Bates (1954). through using glass electrode, the solid sample where disintegrated with mortar and pestle finally powdered and thoroughly mixed together in a beaker, 1.0 g of a soil sample from each site was measured and stirred with 10 mL of deionized water (1:10 soil: distilled water mixture). Before measuring the pH, the digital pH meter calibrated using a standard buffer solution at the pH value of 4.01 and 7.00. The pH readings were taken by immersing the glass

Soil Organic Carbon

The 1 g soil sample has been treated with 5 mL of concentrated H2SO4 for 4 h, then with 5 mL of 0.5 M K2Cr2O7. The mixture was heated at 150–160 ◦C for 5 min and then cooled at room temperature. The solution moved into a conical flask with 100 mL deionized water. The unreacted K2Cr2O7 was determined by titrating with 0.25 M FeSO4. The endpoint had approached when the solution changes from dark green color to blue to reddish-brown color Motsara, M.R, 2008.

Oxygen demand (BOD (water as a control and determine using a side modification of Winkler's method BOD is prepared and incubated BOD bottle is poured with mixing 2ml of orthopedic acid and shake gently and tittered with sodiyem trio sulphate to the end point where the change in color . the titer value represents dissolved oxygen and determine the BOD through subtracting the difference between dissolved oxygen in one day and day five day five to measure the initial dissolved oxygen concentration (mg/ml in each bottle and incubate at 20^0cfor five days .after five days the DO meter is used again to measure the final determine Kotut et.al 2011.

Soil Moisture Content

Soil Moisture Content In a beaker of known mass, the soil samples were added and measured the mass. The samples were oven-dried at 105◦C for 24 h until the constant mass acquired. After drying, the samples were cooled in a desiccator, and the final mass was measured Ukpong, 2013 .

Electrical conductivity

Electric conductivity of solid waste sample is determined by dissolving 5 ml of the sample in 20 ml distilled water then, 10 ml of the homogenate was pipetted into a beaker. 10 ml of each sample is directly pipetted into a beaker. For all samples, 10 ml of each sample inserted with a digital multi-parameter (Banta 900- UK) and the results recorded for determination of the soil electrical conductivity. (Jacson,1970)

3.2.2 Microbial isolation and enumeration

During this study the microbes were isolated on their respective media and enumerated, accordingly.

Sample preparation

Sample was prepared preparation for aerobic plate count, total coliform and fungal count to identify microbial community, human health and impact in biodiversity. Approximately 250 g of each garbage solid waste sample is collected from four sitesA1=Mixed hose hold dominant waste A2=garage dominant area A3=plastics and chat dominant area A4= none dump site area are to be analyzed for microbiological quality and safety which helps to know solid waste disposal practice and identify potentially risk organisms. Accordingly, 25 g of each well-mixed food sample was taken and mixed with 225 ml buffered peptone water (BPW) Oxide, homogenized in a flask for five minutes using a shaker at 160 rpm. After homogenization, 1 ml of each solid waste sample it was aseptically transferred into 9 ml of BPW, and mixed thoroughly by using vortex mixer. The homogenates were serially diluted from 10^{-1} to 10^{-8} and a volume of 0.1 ml aliquot of appropriate dilution was spread-plated on pre-solidified plates and incubated at appropriate temperature and time for microbial enumeration and identification. (Weil et al., 2006).

Aerobic hemophilic Bacteria counts (AMB)

From appropriate dilutions, 0.1 ml aliquot was spread-plated in Plate Count Agar (PCA) plates and incubated at 32°C for 48 hrs. (Weil et al., 2006).

Staphylococci counts

From appropriate dilutions, 0.1 ml aliquot was spread-plated on Mannitol Salt Agar (Oxide) plates. The culture media was incubated at 32°C for 48 hrs. after which yellow colonies were counted as staphylococci (Accor et al., 2003).

Entero bacteriaceae counts

From appropriate dilutions, 0.1 ml aliquots were spread-plated in duplicates on pre-dried surfaces of Violet Red Bile Glucose Agar (VRBGA) plates. The seeded culture plates were incubated at 32 °C for 20-24 hrs. After pink to red-purple colonies with or without haloes of bile precipitation were enumerated as members of Enterobacteriaceae (Spencer et al., 2007).

Coliforms Counts

From appropriate dilutions, 0.1 ml aliquots were spread-plated in duplicates on pre-dried surfaces of Violet Red Bile Agar (VRBA) plates. The culture plates were incubated at 32°C for 24 h. thereafter which purplish-red colonies surrounded by a reddish zone of precipitated bile were counted as coliforms (Weil et al., 2006).

Mold and Yeast Counts

From appropriate dilution, 0.1 ml of aliquots were spread- plated in duplicate on pre-solidified sterile surfaces of Potato Dextrose Agar (PDA) plates supplemented with 200mg/L of Chloramphenicol and incubated at 25^{o}C for 5-7 days. Smooth (non-hairy) colonies without an extension at the periphery were counted as yeast whereas hairy colonies with extension at the periphery were counted as molds (Spencer et al., 2007).

3.2.3. Cultural characterization of microbes

The microbial isolates cultural characterized from their respective growth medium,

Purification and microscopic observation Bacterial isolates

After enumeration of aerobic mesophilic bacteria, 10 to 15 colonies with distinct morphological differences such as color, size and shape were randomly picked from countable plates and

aseptically transferred in to a tube containing 5 ml nutrient broth. The inoculated cultures were incubated at32^0_C for 24 h. Cultures were purified by repeated plating and preserved on slants at 4^0_C for a month. Finally, the obtained organisms were characterized to genus and family levels. The characterizations of isolates were done based on John (2012).

Biochemical test bacterial classification manual. To determine the morphology and biochemical characteristics of the bacterial isolates, bacterial cells were Gram-stained (Gram, 1884). Motility test was conducted according to Shields and Cath cart (2012). Endospore test was done according to Schaeffer and Fulton (1933) method. Presence of lipopolysaccharide that is Gram positivity or negativity was determined according to Gregson (1978). Oxidation-reduction properties were determined according to Hugh and Leif's son (1953). Catalase test was conducted according to MacFadyen (1980).

Cell morphology

In order to assess the cell morphology of the pure culture, gram staining, motility test, and endosperm test were used.

Gram Staining

A smear of pure isolates was prepared on a clean slide and allowed to air-dry and heat-fix. The heat-fixed smear was flooded with crystal violet dye for 1 minute and rinsed under tap water for

3 seconds. Then, the slide was flooded with iodine solution for 1 minute and rinsed under tap water for 3 seconds. After rinsing, the smear was decolorized with 96 % of ethanol for 20 seconds and washed slide gently under tap water for 3 seconds. Thereafter, the smear was counterstained by safranin and dried with absorbent paper. Finally, the air-dried smear was observed under the oil immersion objective. At the completion of the Gram Staining, gram-negative bacteria were stained pink/red and gram-positive bacteria were stained blue/purple Bruckner MZ. (2012).

Motility Test

A motility medium was prepared using a test tube. A purified broth culture was taken by a sterile needle and stabbed straight vertically into a test tube containing motility medium to the bottom of the tube and incubated at 35°C for 24 hrs. A positive motility test was indicated by a red

turbid area diffusing away from the line of inoculation and a negative test was indicated by red growth along the inoculation line only but no further (Shields and Cath cart, 2012).

Endospore Tests

To differentiate endospore former from none former. Colonies of the test organism were picked by inoculating loop and thinned on slides. The slides were dried and heat-fixed, thereafter, the slides were placed on the mouth of a beaker containing boiling water, and the smear was flooded with 0.5% malachite green and allowed to stay on boiling water for 5 minutes. The stained smear was washed thoroughly with distilled water and counter-stained for 30 seconds with safranin. The slides were washed again with distilled water and blot-dried. The green color for the presence of endospore and the red color of the vegetative cell was observed under a microscope using an oil immersion objective. (Sandeler.Tt 2016).

Isolation of B. cereus

One ml of sample was added to 10 ml of peptone water and heated in a water bath kept 80 °C for 10 minutes and then cooled rapidly in tap water. From appropriate dilution, 0.1 ml aliquot was spread plated on the pre-dried surface of B. cereus Agar medium which is a selective medium for B. cereus, and incubated at 37^{o}C for 72 hrs. After incubation blue colonies with an opaque halo on the medium were presumptive for B. cereus. The biochemical tests for B. cereus were subjected to endospore test, Gram staining, endospore, motility, oxidase, catalase test, and insole production test. (Weil et al., 2006).

Insole Production test

A roomful of 24-hrs old pure culture of bacteria was transferred into 5 ml Tryptophan broth and incubated at 37^{o}C for 48 hrs. In order to test for insole production, 5 drops of Kodak's reagent were added directly into the tubes and the red color indicates the positive for insole production (Smyth et al., 2005).

Citrate utilization test was conducted based on the ability of an organism to use citrate as the only carbon source, ammonia, and energy. About ten milliliters (10 mL) of Simmons's citrate agar medium was dispensed into each tube and covered and then it was sterilized and allowed to cool in a slanted position. The tubes were inoculated by streaking the organisms once across the surface and incubated at 32°C for 48 H. If citrate utilization test is positive, the media was turned from green to blue or growth on medium indicated (Quinn et al., 2004). Hydrolysis of starch

This was used to assay the ability of microorganisms that can produce enzymes that degrade substrate with carbon compounds. Soluble starch was prepared and the medium was sterilized and poured into sterile plates and it was inoculated by streaking by the test isolates onto Petri plates after solidifying. The plates were incubated at 32°C for 24 to 48 H. After full growth of cultures, the Petri plates were flooded with gram's iodine. Hydrolyzed starch was formed a blue, black or purple zone with the iodine while hydrolyzed starch appeared as a clear zone (halo zone) surrounding the colonies against purple background due to amylase activity in the presence of Bacillus bacteria and reddish-brown zones around the colony was indicated partial hydrolysis of starch

Isolation of E. coli

From appropriate dilution, 0.1ml aliquot of each food sample spread on Eosin methylene blue agar and incubated at 37 °C for 24 hours. Colonies that showed typical dark red to purple red with metallic sheen were taken as E. coli (Benjamin et al., 2018). Then, the isolates were purified and further subjected to Then, biochemical tests like, urease production, citrate utilization, triple sugar fermentation, insole and sulfide production, motility, and MR-VP were conducted

Identification Fungi

Fungi from the counted PDA was purified by cutting a small portion of the edge and transferred to 5 ml PDA broth supplemented with 200m g/L chloramphenicol and incubated at 25^{o}C for 5-7 days. Then, sub-cultured until a pure colony was obtained and preserved in a slant in the refrigerator (4°C). All the fungal isolates obtained from the slant were identified based on their gross morphology such as colony growth pattern, conidial morphology, and pigmentation by slide culture techniques. The fungal isolates were identified using cultural and morphological features such as colony growth pattern, conidial morphology, and pigmentation (Taffeta et al., 2013). The identification of fungi isolates was done using stain of lacto phenol cotton blue (Oyeleke and Manga, 2008). Briefly, the identification was achieved by placing a drop of the Lacto phenol cotton blue stain on the clean slide with the aid of a mounting needle, where a small portion of the aerial mycelia from the representative fungi cultures was removed and placed in a drop of Lacto phenol. The mycelium and non-filamentous part were well spread on the slide with the help of needle and loop . A coverslip was gently placed with little pressure to eliminate air bubbles. The slide was then mounted and viewed under the light microscope with

×10 and ×40 objective lenses, gross morphology such as colony growth pattern, conidial morphology, and pigmentation by slide culture techniques. (Taffeta et al., 2013).

3.2.4 Physiological test

Effect of metal ion on amylase activity the crude enzyme was mixed with different sodium ions concentration (0 M, 0.5 M, 1 M, 1.5 M, 2 M and 2.5 M) together with soluble starch in 50mM acetate buffer pH 4.8 and incubated at 28^0c for 24 hours the growth rate of bacterial isolate was measured by spectrometerat600nm (Patailet.al2014).

Heat tolerance

For the effect of temperature lab, prepare five beakers with 40 mL of 1.0% hydrogen peroxide and 100unit/supernatant microbial amylase enzyme concentration in another beaker. Place to 40 mL of 1.0% H_2O_2 into a water bath with carrying temperatures (0° C, 10° C, 22° C, 40° C, and 65° C). Use the same procedure by placing the filter paper in the enzyme solution, then into the substrate solution of different temperatures and time how long it takes for the filter paper to rise to the surface (Millern HP ,2010).

pH tolerance

Optimization of the Enzymatic Hydrolysis Test A commercially available cellulose preparation was chosen for test optimization (α-cellulose, Sigma). The pH of the buffer solution was varied at values of 4, 5, 6, . and 8 to determine the optimal pH for the enzymatic hydrolysis. Tests at each pH were carried out in triplicate and the reported results are the mean values. (Kimura 1998).

3.3 Enzymatic activity test of microbial isolate from MSW

Catalase

Production test Catalase test was performed by taking a loop full of 24-to-48-hour old isolated bacterial colonies grown on a nutrient agar plate and flooded with 1 ml a 3% hydrogen peroxide solution to glass slide then it is placed on to a clean microscope glass slide. Finally, the mixture is stirred using sterile wire loop. The results expressed as catalase positives for the rapid and sustained production of gas bubbles and negative for not producing Tyagiet.ai (2017).

Amylase

For amylase enzyme activity, agar-agar with 1% starch is prepared aseptically. With help of sterile corkborer, 4mm size were made in which 50µl of enzyme from solid waste is inoculated then the plates were incubated for 48 hours at 37^0C. Hydrolysis of starch is visualized as clear zones around the wells of plates against deep blue brown for starch by flooded with iodine solution (Ammiol et al. 2012). Diameter of the clear zone is measured and the activity level of the microorganisms was determined by the diameter of the clear zone formed. Senite leykun and amare (2017).

Cellulose

The cellulose agar is prepared with 1% carboxyl methyl cellulose aseptically. With the help of sterile corkborer of 4mm size, wells are made in plates in which 50µl of of supernatant of microbials enzyme fromsoil sample is enzyme from garbage is inoculated in well and plates were incubated at 37^0C for 24 hours - 48 hours, the plates are flooded with 0.3% Congo red solution for 10 minutes. Then it is washed with water and flooded with 1N NaCl as distaining solution. Cellulose production is visualized by translucent zone around the colonies. Diameter of the translucent zone was measured and the activity level of the microorganisms was determined by the diameter of the translucent zone formed (Thermo gram 2016).

Protease

The protease agars prepared with 1% gelatin aseptically. With help of sterile corkborer of 4mm sizes, wells are made in plates in which 50µl of supernatant of microbial enzyme from garbage is inoculated then the plates are incubated at 37^0c for 24 hours-48 hours. After incubation plates are flooded with acidic mercuric chloride solution and are allowed to stand for 5-10 mines, excess solution is decanted. Appearance of a clear zone around the colonies demonstrated the positive result for the proteolysis hydrolysis of gelatin by the enzyme gelatinize. Diameter of the clear zone is are measured and the activity level of the microorganisms was determined by the diameter of the clear zone formed. Hydrolyzed and continuous opaque zone around the growth indicates the absence of gelatinize enzyme. Diameter of the clear zone is measured and the activity level of the microorganisms was determined by the diameter of the clear zone formed (Ammiol 2012).

Lipase

1% Tween-20 hydrolysis agar medium is prepared. With help of sterile 4mm corkborer, wells are made one plate . The wells are labelled by the name of the sample to be inoculated.50µl of each sample is added to well. The plates are at 37°C for 24 hours. After the incubation, the clear zone of hydrolysis is observed around well (Ammiol 2012).

Chitins Activity

Chitin lytic bacteria were screened according to the standered method The bacterial isolates were inoculated into colloidal chitin agar mediumChatinese (NH4SO4, 1g/l; KH2PO4, 0.2g/l; K2HPO4, 1.6g/l; NaCl, 0.1; M_{gSO4} and $FeSO_4$, 0.01g/l; $CaCL_2$, 0.02g/l; and agar, 20g/l) and incubated for five days at 28°C. Chitin lytic bacteria were screened based on clear zones of hydrolysis produced after five days of incubation. The colony diameter and clear zone diameter were calculated using the Chitin lytic Index formulag (Gupta et.al2018).

3.4 Data analyses techniques

Data analysis was carried out by using Excel and SPSS software. Data obtained from primary, secondary sources and the laboratory result were analyzed by combining the information collected by using simple descriptive statistics by both qualitative and quantitative techniques. Quantitative data includes percentages, graphical maps, charts, and tabular form. The existence of any variation among different experiments, percentage, frequency, ratio, correlation and one way ANOVA test was carried out. Also, the data collected from direct physical observation or visualization was analyzed by describing the phenomena using personal judgment and supported by photographs.

4 Result

4.1. Physicochemical analysis of solid

The soil pH is the primary factor that affects the mobility and the solubility of metals in the soil Pandey A, et al. (2000) . The soil pH testing outcomes reveal that the sample's pH ranges from slightly acidic conditions (4.84) to moderately alkaline soils (8.06) . In the present study, oven drying test data reveals that moisture content is in a very low percentage of <10%, and it was noticed to range between 1.04-1.1. The low levels at site 3 might be attributed to impact of burning land's vertical slope where the site was allocated, while on-site 1 moisture might come from some disposed garbage. The mean percentage of soil organic carbon in the study has been noted to range between the value of 1.35 and 3.94. The presence of organic carbon in soil was discovered, the mean percentage of soil organic carbon in the study has been noted to range between the value of 1.35 and 3.94 the soil from the control site .

Electrical conductivity is used mostly in the estimation of soluble salts concentration. In this study, the analyzed soil samples were recorded within the range of non-saline soil. The reported findings by Lemanowicz et al. 2018 contradict the results of this study. They said that high electrical conductivity decreases the enzymatic activity on soils, while in our study, we detected low electrical conductivity levels and very low enzyme activity Shafique et.al(2009) . The variation in mean values of electrical conductivity might be due to differences in soluble salts content in Ramesh,Lonsane (1991).

Table 1. physicochemical properties of soil associated with MSW dump site in Bedele town

Areas	Soil physio-chemical				
	MCF	PH	EC	OC	TN
A1	1.060±.010	7.833±.212	.056±0.004^{b}	3.787±.084^{b}	.323±.006^{a}
A2	1.060±.020	7.753±.118	.136±.007^{b}	1.387±.032^{b}	.110±.010^{ab}
A3	1.090±.010	7.680±.040	.112±.023^{a}	3.897±.045^{a}	.338±.008^{bc}
A4	1.075±.005	4.047±1.017	.006±.002^{b}	2.343±.120^{b}	.207±.015^{c}
P-	.053	0.000	0.000	0.000	0.000

value

MCF=moisture content, EC=electro conductivity OC=oxygen concentration TN=total nitrogen

4.2. Microbial count

The overall mean of total aerobic mesophilic bacteria in this study revealed 6.134log10 CFU/g . However, HPA (2009) indicated aerobic mesophilic count must be less than 7 log CFU/g . soil associated with MSW. high total aerobic mesophilic bacteria count reveals potential indicators for presence of pathogenic microorganisms, poor sanitation and cross contamination. According to Frew et al. (2014) observational study in Jigjig a town indicated that poor sanitation and dusty environment and full remains of slaughtered animals such as bones, horn, head and other body parts observation were correlated with total aerobic mesophilic bacterial count. Hence,

Total coli form

The mean of total coli form count from of soil associated with MSW dump site in Bedele town. 0, 1.96 log10 CFU/g ,3.2 log10 CFU/g and 0 in area 1,2,3and4 respectively. Total coli form locations ranged between 0 and 3.2 log10 CFU/g . This indicates microbial quality of each sample is in close proximity due to comparable hygienic condition and post handling practices.

Staphylococcus spp.

Staphylococcus spp. count in soil sample collected from the study sites had highly significant differences ($P<0.05$) across the study areas (fig 2). The mean value of Staphylococcus spp. count from all study areas was 3.7 , 4.4 4,2 and 2.14 log10 CFU/g for area1,3,2and4 respectively. The result of this study was relatively lower than5.5×105cfu /reported by Gebeyehu et al. (2013) in Adamma town.

Yeast and mold Yeast Yeast and mold counts of MSW soil samples collected from Bedele MSW had significant difference from soil samples collected from in soil associated with MSW of Bedele town dump site($P<0.05$).

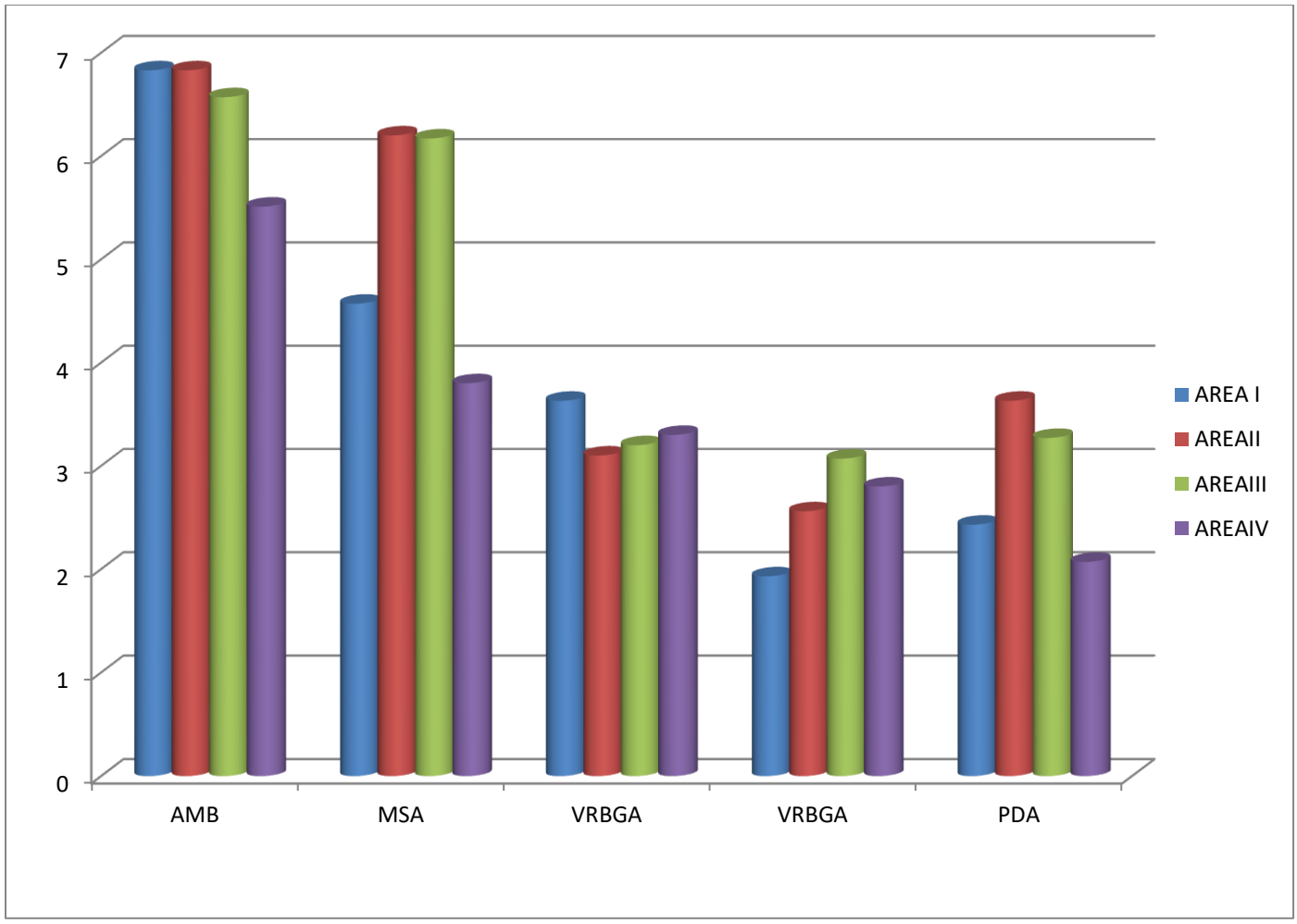


Fig 2 microbial load of solid waste isolate

4.3. Characterization of microbial isolates and microbial genera

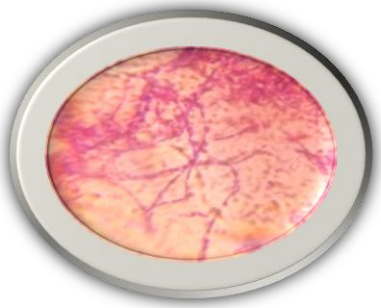
In our study 8 bacterial isolates (BI16, BI23 , BI3 , BI4, BI5, BI17, BI19and BI18 **)**were identified from a total of 102 bacterial isolates and the microbiological characterization was carried out. The results showed that BI4, BI17, and BI18 are gram Positive bacilli, BM2 is gram positive short bacilli, BI3 is gram positive diplobacilli, BI5, BI19 are gram negative short bacilli and BI16, BI23 are gram positive cocas. Different biochemical tests were performed for the 8 isolates to know their biochemical characteristics. Details of biochemical characters of the bacteria were noted in (Table 2) .The above results gave an idea of morphology, colony characteristics and biochemical nature of the isolated strains which would aid in the identification and characterization of the isolated bacterial strains in future .All these analysis

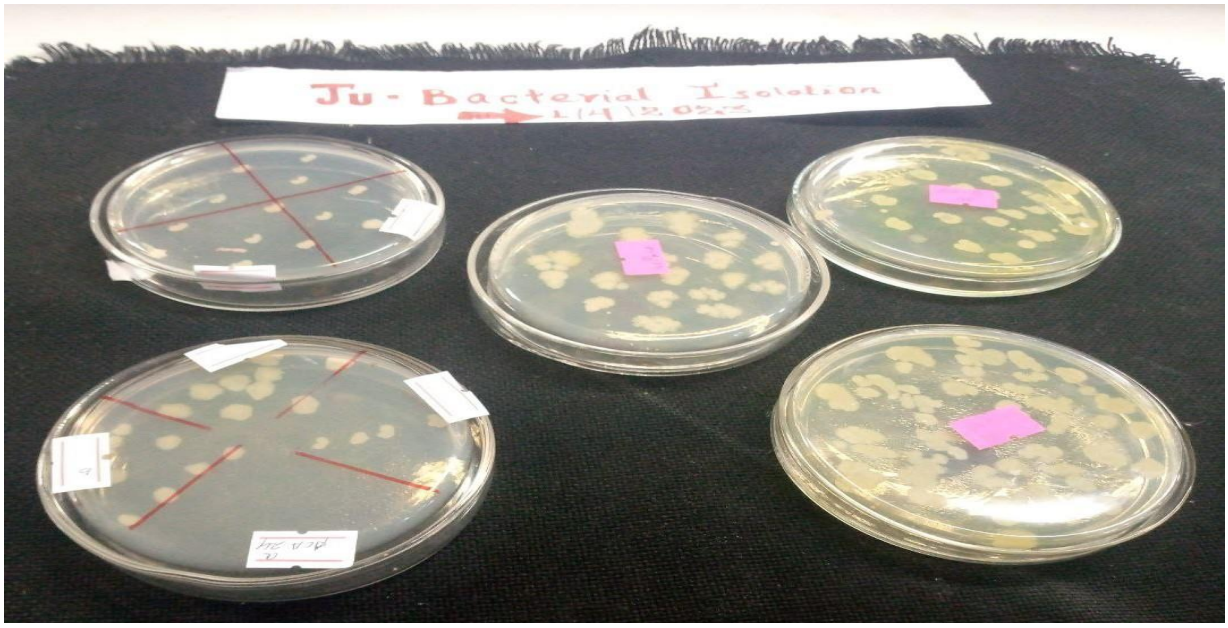
gave a clear understanding of the native environment of the bacteria and thus was the determining factor and culture of the isolates

Table 2 . Cultural characteristics of bacterial isolates in PCA

Isolate	**Cultural characteristics**		**Gram**	**shape**	**InoduleTest**	**motility**	**Spore test**	**Manitol salt agar**	**Ecosin MitaylRed EMB**
BI16	Irregular, white	+	+ vie	Rod single chain	+	motile	+	-	-
BI23	round	+	+ vie	rod	-	motile	+		-
BI3	Mucous smooth	-	+ vie	cocas		-		-	-
BI4	Creamy ,yellow	+	+ vie	cocas		-	-	+	-
BI5	Circular , grey metallic	+	-vie	cocas		motile		-	+
BI17	Round , mucous yellow white	+	+vie	Rod branched		motile	+	-	-
BI19	Large flat, Irregular merge	+	-vie	rod		motile	-	-	-
BI18	Large	+	+vie	rod		motile	+	-	-

BI5=ecoli.spp,BI16=baccilus,BI3=streptococcus. spe, BI4= streptococcus spp spp ,BI17=actinomycete spp,BI19= pseudomonas spp,BI18=clostridium spp





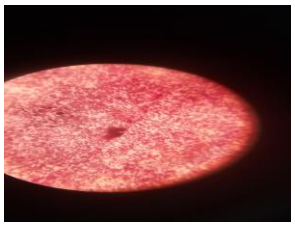


Figure 3

Table 3 Frequency of of bacterial isolates in sampling area distribution of bacterial isolates insoil associated with MSW of Bedele town dump site

	Type of sample	A1-A4	AREA1		AREA		AREA 3		AREA 4	
Isolates	code	total	Freq.	%	freq.	%	freq.	%	freq.	%
BACILLUS	BI16	12	3	25	2	16.7	5	41.7	2	16.7
BACI	BI23	13	2	15.4	7	53.9	4	30.8	0	0
STRIPTO	BI3	23	6	26.1	4	17.4	6	26.1	6	26.1
STAPHYLO	BI4	22	5	22.7	6	27.3	4	18.2	7	31.8
ECOLI 5	BI5	5	1	20	2	40	2	40	0	0
ACTINO SPECES	BI17	8	1	12.5	3	37.5	2	25	2	25
BUSIDOMONA	BI19	6	2	33.3	2	33.3	2	33.3	0	0
CLASTRIDUM	BI18	3	0	0	2	66.7	1	33.3	0	0
Atheres		10	3	30	4	40	2	20	1	10
total		102	23	22.5	32	31.4	30	29.4	17	16.1

BI5=ecoli.spp,BI16=baccilus,BI3=streptococcus. spe , BI4= streptococcus spp spp

,BI17=actinomycete spp,BI19= pseudomonas spp,BI18=clostridium spp

Cultural characterization of fungi isolates

Among total of 18 isolates identified in PDA media(FIA .FID ,FIH , FIG, FIY and FIJ isolates identified from mold and yeast culture in potato detrosemidia through observing microscopic and cultural morphology as indicated in (table4) among fungal isolates

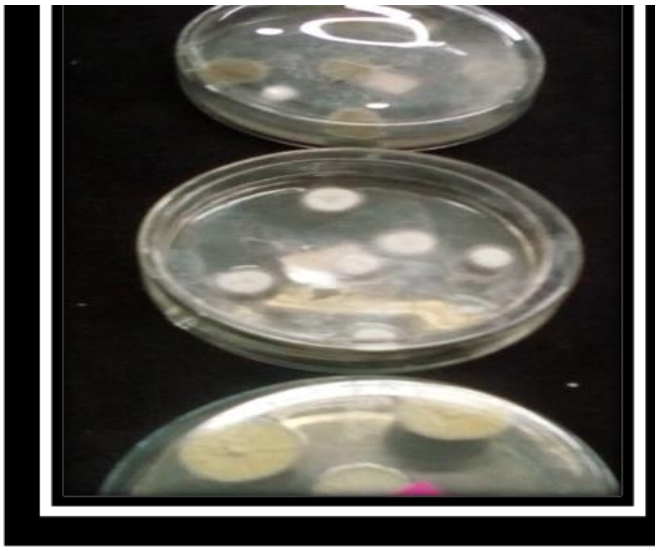
Table 4 Cultural characteristics of fungal isolates

Isolates	Macroscopic description of colony in PDA	Microscopic description and conidiophores	Suspected strains
FIA	Filamentous	Conidial pores with conical smooth globes	Asp. Spp
FID	Filamentous	Septet multinucleated	Asp. sp
FIH	Filamentous	Rough fill form	Fuzariyem. Sp
FIG	Greenish brown in reveres	Hyaline sporangia	Mucor
FIY	Smooth	Creamy	Flat
FIJ	Brush like stricture	Hype structure with flask shape	penicillium

Filamentous aspergillus isolates (FIA,FID,FIH)are a dominant spp

FIA =asarigillus spp ,FID= asparigillus spp,FIH=fusariyem spp ,FIj=pencillum.sp,FIY= yeast spp

A



B

(Fig4) .BA fungal front and back view respectively

4.4. Test of microbial enzyme

Amylase producing bacteria

In this study, about 8 bacteria were isolated and characterized based on morphological and biochemical characteristics. The bacteria were classified as gram-negative rod bacilli with the ability to form spores. Of these isolates, 18 were identified as amylase producers (Table 2). The result demonstrated that BI16-1 had the highest hydrolysis capability with a diameter zone of inhibition of 24mm, followed by BI16-2 (22.2mm), BI16-3(20.5mm), and BI5-1 (20.6mm).

Amyl lytic activity of fungal isolates

The present study shows that it is possible to isolate amylase producing fungi from MSW dump site area. Additionally, the isolated fungi strains were found to belong to aspergillums genus(FIA)and FIH (Fusarium SP.) with enzymatic activity of 2.6cm and3.2cmclear zone diameter of clear zone. Enzyme produced from the isolated fungal strain had been stabilized with changes of temperature and pH on the activity and stability of amylase have taken as an advantage for enzymatic liquefaction of starch industrially at moderately acidic condition of pH around 5.

Chitnolyticenzayemactivity of fungal isolates

Similarly the isolated fungi strains were found to belong to aspergillums genus(FIA)and FIH (*Fusarium* spp.)showes enzymatic activity of2.1 and 1.6cmcler zone diameter respectively.

Table 5 Enzymatic activity of amylase and chitin lytic microbes isolated from soil sample of study area

Amylase producing Isolates	Diameter of Clear zone	Chitinase producing	Diameter of Clear zone
BI5M	2.6cm	FIA	2.1cm
BI16	2.2cm	FID	1.6cm
FIA	2.6cm		
FIH	3.2cm		

FIA =asarigillus spp ,FID= asparigillus spp,FIH=fusariyem spp, BI5=ecoli.spp,BI16=baccilus,

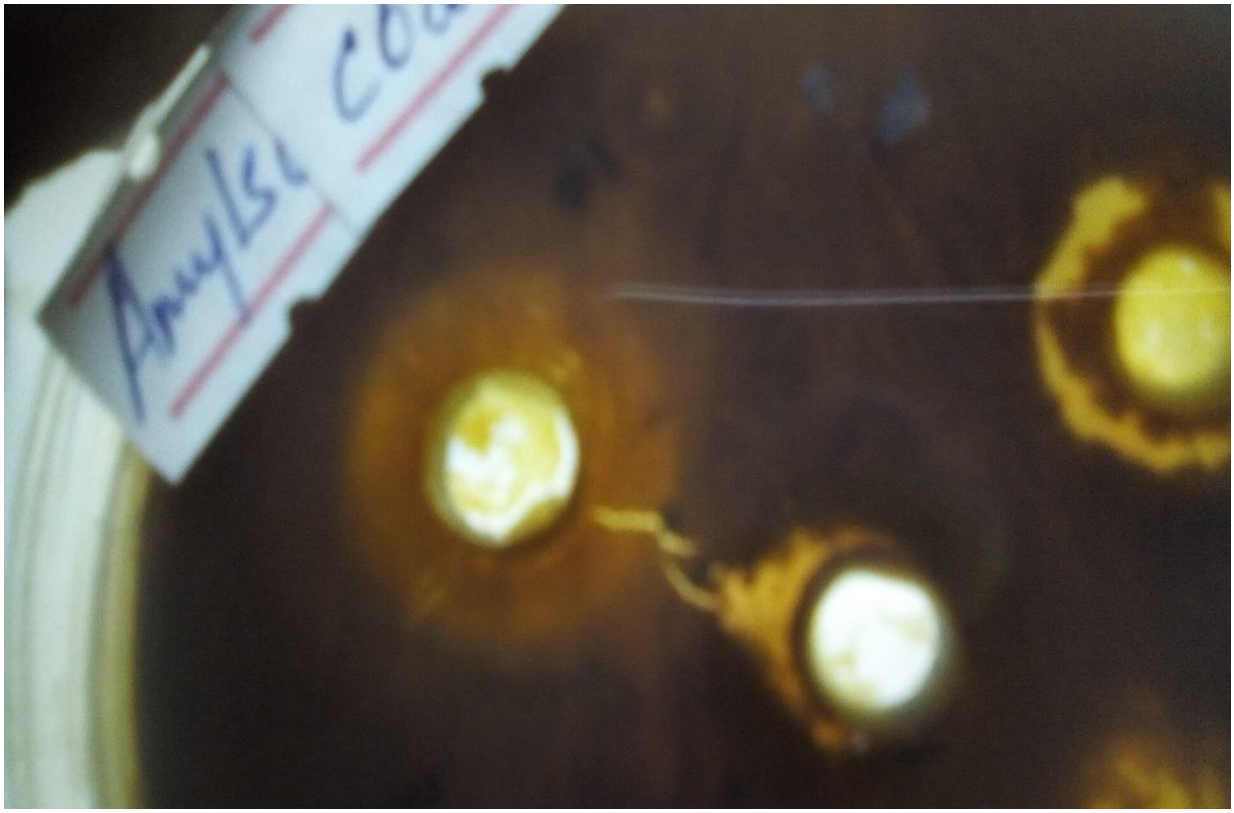


Fig 5 amyloid activity of microbial isolates

The enzyme activity for different pH of the production medium was presented in Figure 4 . Based on the pH values, the bacterial species produces amylase enzyme activity ranged from

2.04-24.85, 2.62-.2.55 and for B. subtitles, and B. cereus respectively. The bacterial species are alkalophilic as they present better enzyme activity between pH 7 and 9.31 The optimal enzyme activity across all the bacterial species was at pH 7.

4.5. Evaluation the effectiveness of some selected microbial enzyme

Effect of salt on concentration on amylase activity: results for E. coli grown in cultures of varying NaCl concentration at 37°C. A significant difference was observed in growth with decreasing turbidity from 0% (No salt enhancement- control) to 1.0% NaCl concentration. The observed steady decrease in growth of E. coli as the NaCl concentration increases have also been reported by Abdelkarim et al. Kunamneni , Permaul, Singh (2005). The growth isolates was increased when sodium chloride concentration increased up to 1% and their growth was sharply declined when its concentration increased beyond 2% In general, this result implies both isolates showed poor growth on either side of the optimum value. This may suggest that *S. aurous* It was observed that the optimum growth recorded was in a decreasing order for both E. coli (0.556, 0.534 and 0.486) and *S. aurous* (0.583, 0.553 and 0.495) at NaCl levels 0%, 1% and 3% respectively. These shows that the higher the NaCl concentration the lesser both bacteria will reach their optimal growth. These decreasing optimal growths as a result of increasing salt concentration, was also reported by Hajmeer and Abdelkarim et al.(2009). The enzyme activity in the presence of different sodium metal ions concentration was analysed and it was found that the highest (100%) enzyme activities occurred in the absence of NaCl and subsequently decrease in their activity as the sodium ion concentration increases. Similarly, the activity of amylase was lost when NaCl concentration increased Zambare N (2010). This might be the distraction of ion linkages due to interaction of enzyme with sodium ion at the surface of their natural structure therein liquid culture were selected on the bases of the area of clearance and OD results of supernatant

Effect of pH on stability of amylase: The production of

The production of amylase in the fermentation medium affected by different pH value and have very essential role in the synthesis, secretion and stability of amylase. Dubnovitsky AP, Kapetaniou EG, (2005) . After 1hr of incubation pH stability of fungal amylase was evaluated by assaying the enzyme and showed broad stability to pH range of 4.0 to 7.0 in which the enzyme retained a residual activity of at least 83% .

Effect of temperature on stability of amylase:

Temperature stability of amylase was determined by incubating the enzyme at a range of (20-60° C) for 30min and followed the standard assay method of amylase. The result shows that amylase retained a residual activity of 96%, 91.8% and 85% related to incubation temperature of 20, 30 and 40 °C for 30 min respectively. It maintained more than 50% of its residual activity after it was incubated at temperatures below 60° C for 30min. It also maintained about 75% of its original residual activity at its maximum temperature of activity (50° C) after 30 min of incubation The residual activity of this enzyme decreased with increase in incubation.
Temperature.

5. Discussion

The obtained soil pH has been noted to falls within the normal range set by WHO for both sites, except for one soil sample collected at sitearea4, which was recorded slightly below the allowable limits. It has been reported by (Nena1.35 2019) that enzymes mostly become more active between pH of 6.8 to 8.0 Bradley (1976) . In this study, the results disagree with the written finding because, in this study, enzyme activity levels were detected in very seamounts and ranged below 5%. The mean values of soil organic carbon were observed to vary significantly from one area site to another, and values randomly differ within the site; there is no consistent sequence. We can report that soil organic carbon directly affects enzymes' activity due to the low enzyme activity observed. The study by(Zhao et al. 2018). reported similar findings, and that supports our results. The organic matter content was found to be 27.84% . Also, in this study, the moisture content had recorded in a low amount, so low moisture in soil limits C's downward movement from waste materials to soil, resulting in the Water occurs and available naturally on soils and profoundly affects soil behavior and the degree of compaction on the earth.. Furthermore, this study's moisture content levels were generally very low because of the lack of rainfall during the collection period (climate conditions and seasonal changes). The ground surface in these sites tends to evenly downwards and might results in low moisture content because water is hardly absorbed on the surface during the runoff or rainy days. Low water levels in soil (drying of soil) can decrease or complete soil enzyme activity Ramesh MV, Lonsane (1991) . Our experimental study shows that low moisture content levels directly affected the activity of enzymes in soil, confirmed by minimal amounts of enzyme activity detected in this study.

Electrical conductivity is a very liable test for soil salinity. Tiwari , Jadhav , Fatima (2007) . It represented the conduction current ability of soil solution and sustained ion concentration increase under electric field effects that leads to conductivity increase Zambare (2010) . The salinity test on this study reveals that soil samples have a low electrical conductivity that's is noticed to range between.006±.002- .136±.007 Low electrical conductivity in this study shows that they can't conduct electrical current and affect plant yield and health. The electrical conductivity is temperature dependent; if the temperature increases, electrical conductivity also increases. Nusrat, Rahman (2007) . Therefore, low levels of electrical conductivity in this study might be due to low-temperature levels during the sampling period

(which was a winter season). The overall mean of total aerobic mesophilic bacteria in this study revealed 6.134log10 cfu/g . However, HPA (2009) indicated aerobic mesophilic count must be less than 7 log cfu/g soil associated with MSW. high total aerobic mesophilic bacteria count reveals potential indicators for possible presence of pathogenic microorganisms, poor sanitation and cross contamination. All these analyses gave a clear understanding of the native environment of the bacteria and thus was the determining factors in the isolation and culture of the strains. The higher coli form counts correlate with the higher levels of foodborne pathogens of fecal origin (Jay, 2000). But less total coli form count was observed contrast to Francis et al. (2015) who reported the mean of total coli form count of raw beef collected from local super market in Ghana was 7.23log10cfu/g. The present result was higher than that of Flew et al (2014The overall mean of total coliform count in this study was1.73 log10 CFU/g . The higher coli form counts correlate with the higher levels of foodborne pathogens of fecal origin (Jay, 2000). But, less total coli form count was observed contrast to Francis et al. (2015) .

Amyl tic activity of bacteria isolates: In the present investigation, we have successfully isolated a pure bacterial strain from municipal waste. The maximum amylase activity was found in Municipal waste require disposal by incineration or land filling, from which eventually many social and environmental problems may arise. The conversation of organic waste through microbial process decreases land filling by waste disposal and also produces ecofriendly compost which can be used as fertilizer Nusrat , Rahman (2007) . Most importantly, enzymes are playing an important role as biological catalyst in the chemical transformation of this waste without affecting the environment. Amylase producing bacteria which are able to convert the starchy materials into different sugars can be one of the remarkable molecular devices in this context. The bacteria themselves were intentionally isolated from the municipal waste as they can innately tolerant to adverse environment like high pH, large amount of heavy metal etc. and can be grown luxuriously in the solid wastes for their biochemical conversion. Undoubtedly, our present investigation on amylase producing bacteria can be successfully used for large scale bioconversion of starchy materials present in municipal waste which has a beneficial role in waste management. The bacteria were able to tolerate the adverse condition like wide range of pH and temperature and have significant toxic metal ion tolerance. On the other hand, Amylase from fungal source is the first industrially produced enzyme used as a pharmaceutical aid for treatment of digestive disorder. Although they can be isolated from different sources like plant, animals; amylases from microbial sources have greater stabilities which meet the industrial demands in food and textile

processing. With the advent of new frontiers in biotechnology, the spectrum of amylase application has broadened in many other fields such as chemical, analytical and medicinal chemistry. In our study the organisms were giving amylases which are able to tolerate temperature up to 40˚C and pH 6 - 8. These characteristics are good enough to make them applicable for industrial purpose. Future study on isolation of more thermos stable bacteria from municipal waste will definitely propitious for both industrial use and waste management purposes across the globe. . This is important to get the enzyme in very short time and thus reducing the cost of production and risk of contamination. Amyl lytic property of the enzyme helps to convert starch into smaller unit and value-added product for human and animal consumption. The enzyme is highly active at optimum temperature of 50° C and pH 5. It is also active in broad pH and temperature ranges. The enzyme has a great potential for starch industry because of its amyl lytic potential and environmentally friendly approach.

The selected amylotic Isolates are need for solid waste management for bio conversation of starch in to ethanol, starch fermentation loss of viscosity and partial hydrolysis of solid state fermentation of starch and production of glucose and maltose. Djekrit (2005)

6 Conclusions

From this investigation, it can be concluded that

- fungal and bacterial isolates obtained are good producers of alpha-amylase enzyme, chitin lytic and the sourcing of microorganisms with high amylase activity
- Understand the native environment of amylase and chitin lytic microbial which is important for effective isolation and culture of the strains from cheap, affordable, easily available sources should be recommended.
- Bacteria can tolerate a soil reaction between pH levels 4 and 10,
- but the most favorable pH for the majority is just an alkaline side to neutrality.
- Enzyme produced from the isolated fungal is highly active at optimum temperature of 50° C and pH 5. It is also active in broad pH and temperature ranges.

The enzyme is highly efficient for starch hydrolysis.

The results show that there is a need for the implementation of waste management programs, which will help the municipality to prioritize issues of illegal dumping so that there will be an improvement in economic growth, living conditions of humans in affected areas, and reduction of unfavorable influence on the soil environment..

Moreover, it can be an imperative resource for bio prospecting novel/ rare species which could yield valuable bioactive molecules necessary for ecofriendly degradation of waste and can also act as a good substitute in chemical processes in industry.

7 Recommendation

The current findings were forwarded, the following recommendations:

- Awareness creation by the concerned body should be provided for solid wast collector ,trancporter, and users about microbial contamination waste allow them to take an active role in infection control.
- Government or any concerned body should make a persistent inspection onMSW management practice the practice. So that, the vendors keep personal and environmental hygiene to avoid cross-contamination.
- Therefore, continuous microbiological surveillance is needed to enhance discovery of economically use ful isolates and monitor these organisms and put them under control.
- Implementation of waste management programs, which will help the municipality to

  Prioritize issues of illegal dumping so that there will be an improvement in economic growth, living conditions of humans in affected areas,

- Reduction of unfavorable influence on the soil environment The true identity and diversity of microbes associated with solid as well as their metabolite needs molecular approach.

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ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
AmB	Between Groups	.000	3	.000	25.815	.000
	Within Groups	.000	8	.000		
	Total	.000	11			
Entro	Between Groups	.000	3	.000	1802.908	.000
	Within Groups	.000	8	.000		
	Total	.000	11			
Coluf	Between Groups	.000	3	.000	134.982	.000
	Within Groups	.000	8	.000		
	Total	.000	11			
Fungi	Between Groups	.000	3	.000	7.463	.010
	Within Groups	.000	8	.000		
	Total	.000	11			
MsA	Between Groups	.000	3	.000	1.783	.228
	Within Groups	.000	8	.000		
	Total	.000	11			

		Mean	Std. Deviation	Std. Error
AmB	mixed household	.000006000	.0000005000	.0000002887
	garage dominant	.000005800	.0000002000	.0000001155

	plastics dominant	.000005833	.0000002887	.0000001667
	none dump site	.000004000	.0000002000	.0000001155
	Total	.000005408	.0000008959	.0000002586
Entro	mixed household	.000004100	.0000001732	.0000001000
	garage dominant	.000003067	.0000003055	.0000001764
	plastics dominant	.000003200	.0000001000	.0000000577
	none dump site	.000286667	.0000115470	.0000066667
	Total	.000074258	.0001281824	.0000370031
Coluf	mixed household	.000000000	.0000000000	.0000000000
	garage dominant	.000196000	.0001508907	.0000871168
	plastics dominant	.002100000	.0002645751	.0001527525
	none dump site	.000000000	.0000000000	.0000000000
	Total	.000574000	.0009330825	.0002693577
Fungi	mixed household	.003133333	.0003055050	.0001763834
	garage dominant	.001293333	.0016523115	.0009539625
	plastics dominant	.002700000	.0001732051	.0001000000
	none dump site	.000223333	.0000251661	.0000145297
	Total	.001837500	.0014039692	.0004052910
MsA	mixed household	.000003700	.0000011269	.0000006506
	garage dominant	.000004333	.0000005774	.0000003333
	plastics dominant	.000004767	.0000001528	.0000000882
	none dump site	.000114667	.0001431829	.0000826667

The mean difference is of AmB among the four areas (mixed household, garage dominant, plastics dominant and none dump site) is highly statistically significant (P<0.001). the lowest

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
MCF	Between Groups	.002	3	.001	3.960	.053
	Within Groups	.001	8	.000		
	Total	.003	11			
PH	Between Groups	30.986	3	10.329	37.727	.000
	Within Groups	2.190	8	.274		
	Total	33.176	11			
EC	Between Groups	.031	3	.010	67.593	.000
	Within Groups	.001	8	.000		
	Total	.032	11			
OC	Between Groups	13.113	3	4.371	712.641	.000

	Within Groups	.049	8	.006		
	Total	13.162	11			
TN	Between Groups	.104	3	.035	325.092	.000
	Within Groups	.001	8	.000		
	Total	.104	11			

MCF	mixed household	3	1.06000	.010000	.005774
	garage dominant	3	1.06000	.020000	.011547
	plastics dominant	3	1.09000	.010000	.005774
	none dump site	3	1.07500	.005000	.002887
	Total	12	1.07125	.016804	.004851
PH	mixed household	3	7.83333	.211975	.122384
	garage dominant	3	7.75333	.118462	.068394
	plastics dominant	3	7.68000	.040000	.023094
	none dump site	3	4.04667	1.017120	.587235
	Total	12	6.82833	1.736668	.501333
EC	mixed household	3	.05600	.003606	.002082
	garage dominant	3	.13567	.006658	.003844
	plastics dominant	3	.11167	.023288	.013445
	none dump site	3	.00567	.001528	.000882
	Total	12	.07725	.053701	.015502
OC	mixed household	3	3.78667	.083865	.048419
	garage dominant	3	1.38667	.032146	.018559
	plastics dominant	3	3.89667	.045092	.026034

	none dump site	3	2.34333	.120139	.069362
	Total	12	2.85333	1.093853	.315768
TN	mixed household	3	.32333	.005774	.003333
	garage dominant	3	.11000	.010000	.005774
	plastics dominant	3	.33833	.007638	.004410
	none dump site	3	.20667	.015275	.008819
	Total	12	.24458	.097455	.028133

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Multiple Comparisons

LSD

Dependent Variable	(I) AREA	(J) AREA	Mean Difference (I-J)	Std. Error	Sig.
MCF	mixed household	garage dominant	.000000	.010206	1.000
		plastics dominant	-.030000*	.010206	.019
		none dump site	-.015000	.010206	.180
	garage dominant	mixed household	.000000	.010206	1.000
		plastics dominant	-.030000*	.010206	.019
		none dump site	-.015000	.010206	.180
	plastics dominant	mixed household	.030000*	.010206	.019
		garage dominant	.030000*	.010206	.019
		none dump site	.015000	.010206	.180
	none dump site	mixed household	.015000	.010206	.180
		garage dominant	.015000	.010206	.180
		plastics dominant	-.015000	.010206	.180

PH	mixed household	garage dominant	.080000	.427220	.856
		plastics dominant	.153333	.427220	.729
		none dump site	3.786667*	.427220	.000
	garage dominant	mixed household	-.080000	.427220	.856
		plastics dominant	.073333	.427220	.868
		none dump site	3.706667*	.427220	.000
	plastics dominant	mixed household	-.153333	.427220	.729
		garage dominant	-.073333	.427220	.868
		none dump site	3.633333*	.427220	.000
	none dump site	mixed household	-3.786667*	.427220	.000
		garage dominant	-3.706667*	.427220	.000
		plastics dominant	-3.633333*	.427220	.000
EC	mixed household	garage dominant	-.079667*	.010017	.000
		plastics dominant	-.055667*	.010017	.001
		none dump site	.050333*	.010017	.001
	garage dominant	mixed household	.079667*	.010017	.000
		plastics dominant	.024000*	.010017	.043
		none dump site	.130000*	.010017	.000
	plastics dominant	mixed household	.055667*	.010017	.001
		garage dominant	-.024000*	.010017	.043
		none dump site	.106000*	.010017	.000
	none dump site	mixed household	-.050333*	.010017	.001
		garage dominant	-.130000*	.010017	.000
		plastics dominant	-.106000*	.010017	.000

OC	mixed household	garage dominant	2.400000*	.063944	.000
		plastics dominant	-.110000	.063944	.124
		none dump site	1.443333*	.063944	.000
	garage dominant	mixed household	-2.400000*	.063944	.000
		plastics dominant	-2.510000*	.063944	.000
		none dump site	-.956667*	.063944	.000
	plastics dominant	mixed household	.110000	.063944	.124
		garage dominant	2.510000*	.063944	.000
		none dump site	1.553333*	.063944	.000
	none dump site	mixed household	-1.443333*	.063944	.000
		garage dominant	.956667*	.063944	.000
		plastics dominant	-1.553333*	.063944	.000
TN	mixed household	garage dominant	.213333*	.008416	.000
		plastics dominant	-.015000	.008416	.113
		none dump site	.116667*	.008416	.000
	garage dominant	mixed household	-.213333*	.008416	.000
		plastics dominant	-.228333*	.008416	.000
		none dump site	-.096667*	.008416	.000
	plastics dominant	mixed household	.015000	.008416	.113
		garage dominant	.228333*	.008416	.000
		none dump site	.131667*	.008416	.000
	none dump site	mixed household	-.116667*	.008416	.000
		garage dominant	.096667*	.008416	.000
		plastics dominant	-.131667*	.008416	.000

*. The mean difference is significant at the 0.05 level.