EFFECT OF PROLONGED EXPOSURE TO GENERATOR FUME AND SPENT OIL ON SELECTED SOIL MICROBIAL ENZYMES.

BY

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CERTIFICATION

This is to certify that this research work titled "**Effect of prolonged exposure to generator fume and spent oil on selected soil microbial enzymes (catalase, lipase, acid and alkaline phosphatase)**" was carried out by **MBAH, GOLD OBIANUJU** with registration number **20144912658** in partial fulfillment for the award of Master of Science (M.Sc.) in the Department of Biochemistry of Federal University of Technology, Owerri.

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DEDICATION

This work is dedicated to my lovely, caring and supportive husband, Mr. Larry Anyadike, my kids (David, Barron, and Derrick), my parents HRH Eze & Ugoeze F.I. Orji and my siblings.

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ABSTRACT

The effect of prolonged exposure to generator fume and spent oil on selected soil microbial enzymes (lipase, catalase, acid phosphatase, alkaline phosphatase) was evaluated by analyzing the microbiological and enzyme activity of the soil samples. The polluted soil samples were obtained from the generator house at OkBol cyber services, popularly known as BJ services in FUTO in Ihiagwa/North-West local government area of Imo State. The samples which comprises of the top soil, sub soil and sub-sub soil were aseptically collected using plastic auger in different portions ; epicenter (where the generator was placed), 1m and 2ms away from the epicenter N (North),S (South),E(East),W(West) . Twenty–seven different soil samples were collected from generator fume polluted site known as 'BJ business center' and three soil samples from the Department of Biochemistry which serves as the control. Soil microbial load, air sampling, and enzyme activities were determined using standard procedures. The highest heterotrophic bacteria count ($7.45x10^7 \pm$ 2.58 cfu/g) was recorded from location 1(South) sub soil (2m away from epicenter sub soil) while the least ($0.9x10^7 \pm 1.1$ cfu/g) was from the location 3 (North) sub sub soil (1m away from its epi center). The result obtained showed a significant difference ($p > 0.05$) in the heterotrophic bacteria and fungi count between the control and the soil samples from the various locations. The bacterial genera isolated from the polluted site were *Bacillus spp, Enterobacter spp, Serratia spp, Moraxella spp,Micococci spp,Vibro cholera, Staphylococci spp,Pseudomonas spp,Yersinia spp, Shigella spp,Actinomyces spp,Citrobacter spp* while bacteria genera isolated from the unpolluted soil were *Escherichia coli* . The spent oil degrading bacterial genera were *Enterobacter spp, Micrococci spp, Pseudomonas spp, Vibro cholera, Alcagenes spp, Bacillus spp, Klebsiella spp, Staphylococci spp, Serratia spp, Escherichia coli, Actinomyces spp, Moraxella spp, Proteus spp, Salmonella spp, Serratia spp, Streptococci spp, Shigella spp.* The fungi genera identified were *Asperigillius fumigatus, Asperigillus niger, Coccidioides immitis, Candida spp, Penicillium spp, Fusarium spp, Scopulanopsis spp, Aspergillus flavus* while the spent oil degrading fungi genera were Scopulanopsis spp, Coccidioides immitis, *Penicillum spp, Cladosporium carronii, Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger. Micrococci spp* was the most prevalent bacteria isolate and *Moraxella spp, Escherichia coli, Citrobacter spp, Vibro cholera and Actinomyces spp* were the least. *Kiebsiella spp* was the most prevalent spent oil degrading bacteria isolate and Cladosporium *carronii, Aspergillus flavus* were the least spent oil degrading bacteria isolate. *Coccidioides immitis* was the most prevalent fungi and the prevalent spent oil degrading fungi isolate. *Fusarium spp* was the least isolated fungi while *Cladosporium carronii* and *Aspergillus niger* were the least spent oil degrading fungi isolate. The enzymatic profile revealed that the top soil had the highest acid and alkaline phosphatases activities at 1m away from epicenter locations west (w) and south(s) with the values of 0.0035 ± 0.002 and 20.26 ± 0.07 respectively while the sub soil had the highest catalase and lipase activities at 1m away from epicenter locations west (w) and south(s) with the values of 5.5 ± 0.01 and 88.50 ± 0.01 respectively. Result showed that these selected soil enzymes (catalase, lipase, acid and alkaline phosphatase) were adversely affected due to prolonged exposure of hydrocarbons released by the generators. The overall variability in enzyme activities of soil strata from different polluted locations defined the pattern of soil contamination, which could serve as biomarkers for ascertaining level of soil pollution as well as monitorial indices for bioremediation.

Keywords: Enzyme Activity, Generator fume, Hydrocarbons, Microbes, Pollution, Toxicology

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF STUDY

The use of generator in recent years has raised and continues to raise tremendous safety questions. This is because users and the environment are exposed to fumes, whose effects depend on their frequencies, durations and intensity. Fumes are by-products of combustion and are made up of different components. The compositions of fumes are derived from the material subjected to combust (Ueng *et al*., 1998). Generator fumes produced as a result of generating power through the combustion of petrol or diesel is made up of two parts; gases and soot. Each of these in turn is made up of different substances. The gaseous portion of the generator exhaust is mostly carbon monoxide, nitric oxide, nitrogen dioxide, sulphur oxides and polycyclic aromatic hydrocarbon (PAHs). The soot which is particulate in nature is made up of carbon, organic materials (including PAHs) and traces of metallic compounds (Ueng *et al*., 2004a). Fumes generated from an original source such as diesel and burning of this diesel leads to the production of pollutants such as oxides of nitrogen (NO, NO_2), oxides of sulphur (SO_2), carbon monoxide (CO), ammonia (NH_3), hydrogen sulphide (H_2S), Suspended Particulate matter (SPM), heavy metals such as lead (Pb) and polycyclic aromatic hydrocarbons that are carcinogenic (benzo (a) pyrene and 1-hydroxypyrene) in form of soot. All these are known as 'byproducts of generator fumes (Watanabe and Oonuki, 1999).

Power supply is the basic tool for industrialization of any nation and this has been epileptic in Nigeria and a serious impediment to industrial and technological growth. This has led individuals and industries to resort to internal generating plants. The increase use of diesel generator as alternative source for power has resulted to increase in emission of pollutants which negatively affects the soil environment and human health. It is known that both the gases and the soot emitted as generator

fumes affect human health. The carbon particles when inhaled are deposited in the lungs. The organic compounds from the exhaust fumes with known toxic and carcinogenic properties such as polycyclic aromatic hydrocarbon (PAH) adhere easily to the surface of the carbon particles and are carried deep into the lungs. Diesel exhaust particles (DEPS) have been demonstrated to increase the production of inflammatory cytokinins such as interleukin 1β, interleukin 8 and granulocyte-macrophage colony which in turn results to reduction in structural integrity, inhibited repair and cell death.

There are wide ranges of research documenting the ability of these generator fumes composite to affect living cells, including changes in the biochemical and molecular mechanisms of cells both *in vitro* and *in vivo*. Changes also occur in cell metabolism and proliferation, inducing potentially damaging effects in various cell components ranging from the cytoplasmic membrane, where the distribution of proteins is modified to the cytoplasm itself and the nucleus, where the activities of intracellular enzymes and molecules regulating cell growth are altered (Ueng *et al*., 2004b).

There are vast scientific, epidemiological and medical researches that affirm that exposure to generator fumes; both low and prolonged level can have profound effects on biological systems. Numerous epidemiological studies have also shown that exposure to a large amount of petroleum related particles causes an increase in morbidity and mortality which often arises from respiratory diseases and their negative impact on human health and its environment. Researchers have also proven that both solid organic matter and gaseous volatile organic compounds in petroleum related particles can trigger the mutation of cells, resulting in teratogenesis and other hazard. Generator fumes contain many known or suspected carcinogens or mutagens such as benzo (a)pyrene and 1-hydroxypyrene (Inyang *et al*., 2003).

Experiments have revealed the biological effects that occur in activities of soil isolated enzymes, cell cultures and animals after exposure to these generator fumes at low intensity. Generator fumes can

cause damage via CoHb (carboxyhaemoglobin) formation and cell death. The prolonged exposure of these generator fumes to the soil environment has negative influence on the soil microbial properties such as enzyme activities and its basal respiration rate depending on the soil pH, organic matter content and other chemical properties (Ueng *et al*., 2005). Exposure of phosphatases, important soil microbial enzymes to generator fumes transforms and alters the structural and biochemical characteristics of the enzyme, resulting to a significant change of its activities. The exposure of these generator fumes to the soil micro-organisms results to mutation arising from PAH-DNA adducts disrupting their normal DNA transcription, translation, and replication.

However, gene polymorphisms in most enzymes have also been identified in human beings and this could modulate individual cancer susceptibility. Ueng *et al*., (2004b) reported that exposure of rats to motorcycle exhaust and organic extracts of the exhaust particulate causes a dose- and time-dependent increase in cytochrome P_{450}-dependent monooxygenases as well as glutathione-S-transferase in the liver, kidney, and lung microsomes. This occurs as these enzymes metabolize the PAHs (benzo-a-pyrenes) to polar nucleophilic metabolites that bind with the adenine and guanine bases of the DNA (Ueng *et al*., 2004b).

Microbial characteristics are good indicators of the long-term contamination of soils by the generator fumes and could be useful for monitoring changes in agricultural ecosystems (Kizilkaya and Hepsien, 2004). The importance of microorganisms in the soil cannot be overemphasized. For example, the main role of mould and fungi in the soil is to breakdown the remains of plant materials using their appropriate extracellular enzymes such as pectinases, cellulases and these are further broken down through the activities of bacterial enzymes. The soil microbiological activity viz., the enzymatic activities play a key role in soil nutrient cycling, its activity is essential in both mineralization and transformation of organic matters and plant nutrients in the soil ecosystem. Through the activities of

these microbial enzymes, carbon, nitrogen, and other minerals are released to the soil for plant utilization. The soil enzyme activities undergo complex biochemical processes consisting of integrated and ecologically-connected synthetic processes, and in the immobilization and enzyme stability (Khaziyev and Gulke, 2021). In this regard, all soils contain a group of enzymes that determine soil metabolic processes which, in turn depend on its physical, chemical, microbiological and biochemical properties.

Therefore, factors like temperature, pH and substrate concentrations affect activities of soil microbial enzymes thereby negating the availability of soil minerals and soil fertility. Also, bacterial and fungal communities occupy overlapping niches in soil. Disturbing these communities, for instance through the denaturation or reduction of their enzymatic activities via exposure to generator fumes may alter the balance existing between them. The resulting imbalance may affect the influence of bacteria and fungi in their niches, and consequently, to the functional ecosystem. So, measurements of enzyme activity on polluted soils have a useful impact in examining the environmental change on soil enzyme activities (Bastida *et al*., 2008).

A better understanding of the role of these soil enzyme activities in the ecosystem will potentially provide a unique opportunity for an integrated biological assessment of soils due to their crucial role in several soil biological activities, their ease of measurement, and their rapid response to changes in soil management practices. Soil microbial enzymes are used as pollution indicators by many researchers due to agricultural practices and organic pollution (Gianfreda *et al.,* 2005), irrigation by polluted water (Zhang *et al*., 2008; Barton *et al*., 2000), sewage sludge, municipal waste application and industrial activities (Fernandes *et al*., 2005; Kizilkaya and Hepsien, 2004; Kizilkayaa and Bayrakli, 2005; Bastida *et al*., 2008).

1.2 STATEMENT OF THE PROBLEM

Environmental pollution is on the increase due to the indiscriminate and frequent release of hazardous, harmful substances to the environment. Polluted soil directly affects human health through direct contact or via inhalation of soil contaminants which have vaporized; potentially greater threats are posed by the infiltration of soil contamination into groundwater aquifers used for human consumption. Health consequences from exposure to soil contamination vary greatly depending on the pollutant type, pathway of attack and vulnerability of the exposed population. At sufficient dosages, a large number of soil contaminants can cause death by exposure via direct contact, inhalation or ingestion of contaminants in contaminated groundwater though soil.

Generator fumes have significant deleterious consequences on the ecosystems and their enzymatic activities. There are radical soil chemistry changes which can arise from the presence of many hazardous chemicals released from the generator even at low concentration. These changes can manifest in the alteration of metabolism of endemic microorganisms and anthropods resident in a given soil environment resulting to eradication of some of the primary food chain, which in turn could have major consequences for predator or consumer species. Even if the effect on the lower life form is small, the lower pyramid levels of the food chain may ingest alien chemicals which normally become more concentrated for each consuming ring of the food chain. Some of these effects are now well known, such as the concentration of persistent DDT materials for avian consumers leading to weakening of egg shells, increased chick mortality and potential extinction of species. Generator fumes typically alter plant metabolism, most commonly to reduce crop yields. This has a secondary effect upon soil conservation, since the languishing crops cannot shield the earth's soil mantle from erosion phenomena.

1.3 JUSTIFICATION OF THE STUDY

Environmental pollution in Nigeria has awakened attentions and concerns with the creation of various regulatory bodies in states and federal levels. The impact of generator fumes through petroleum processing plants from industrial activities and homes today has deleterious effects on soil microbial enzymes. This also could range from immobilizing the available soil nutrients to unconducive life conditions in the soil, thus limiting vegetation and affecting land for agricultural activities with poor crop yield.

However, most pioneer works and studies on the effect of prolonged exposure of generator fumes focus on humans and animal, while that of the soil microbial enzymes have received less attention. The problems resulting from the generator fumes to the soil, its enzyme and the health implication arising also, from the polluted soil should agitate the minds of any serious environmental researcher and scientist. Therefore, this research is of great importance as it seeks to contribute to current soil microbial enzymes dynamics.

1.4 AIM AND OBJECTIVES OF THE STUDY

The aim of this study was to evaluate the effect of prolonged exposure to generator fumes on selected soil microbial enzymes (Calase, Lipase, Acid Phosphatase & Alkaline Phosphatase).

The specific objective includes:

- To determine the components of the generator fumes.
- To determine the viable microorganisms in the polluted soil.
- To determine the viable microorganisms in the unpolluted soil.
- To determine the hydrocarbon utilizing microorganisms in the polluted soil.

- To determine the hydrocarbon utilizing microorganisms in the unpolluted soil.
- To assay the activities of selected enzymes from the polluted soil.
- To assay the activities of selected enzymes from the unpolluted soil.

CHAPTER TWO

LITERATURE REVIEW

2.1 POLLUTION

Human technological and scientific advances have caused environmental changes that are impossible to evaluate and fully comprehend. Pollution of the environment is one of the major effects of human technological advancement. Pollution results when a change in the environment harmfully affects the quality of human life including effects on animals, microorganisms and plants. Environmental pollution is the undesirable change in the physical, chemical, biological characteristics of air; water and land to such an extent that it harmfully affects the health, survival or activities of human or other living organisms. It is also the introduction of contaminants into the natural environment, thereby causing an adverse change that will alter the natural processes and these substances are known as pollutants. Pollutants may cause primary damage, with direct identifiable impact on the environment, or secondary damage in the form of minor perturbations in the delicate balance of the biological food web that are detectable only over long time periods (Ueng *et al*., 2000).

Until recently in humanity's history, where pollution has existed, it has been primarily a local problem. Environmentally minded scholars such as Ocheri (2003), Gbehe (2004), as well as Aja (2005) have associated environmental pollution with human activities and albeit persistent human interaction with the environment. Research has also shown that as the population of the country increases or grows with attendant pressure on the environment especially in the wake of improved technologies, environmental abuse such as the use of generators and pollution is heightened with corresponding effects on lives of people and other living organisms (Ocheri, 2003). It has been observed further that man through industrial, agricultural and the ever-increasing urbanization

process, security and terrorist activities tend to directly and/or indirectly pollute the environment. The unrestricted use of generators, pesticides, insecticides, herbicides and indiscriminate dumping of refuse, excreta and animal dung as well as spillages from refineries, fumes from generators, large-scale bush burning are perceived as some of the leading factors of environmental pollution in Nigeria (Jande, 2005; Aja, 2005).

2.2 TYPES OF POLLUTION

The major forms of pollution are listed below along with the particular contaminant relevant to each of them (Wyszkowskwa *et al*., 2005; Bina *et al.,* 2011):

2.2.1 AIR POLLUTION: This is release of harmful chemicals and particulates into the atmosphere. Common gaseous pollutants include carbon monoxide, sulfur dioxide, chlorofluorocarbons (CFCs) and nitrogen oxides produced by industries and motor vehicles as well as toxic metals such as lead and mercury. Ozone, a gas is a major part of air pollutant. When ozone forms air pollution, it is also called smog. Particulate matter or fine dust is characterized by their micrometer size PM_{10} to $PM_{2.5}$.

2.2.2 SOIL CONTAMINATION/POLLUTION: This occurs when chemicals are released by spill or underground leakage. Among the most significant soil contaminants are hydrocarbons, heavy metals, pesticides, herbicides, chlorinated hydrocarbons, industrial and agricultural effluents.

2.2.3 NOISE POLLUTION: This is regular exposure to elevated sound levels that may lead to adverse effects in humans or other living organisms. This encompasses roadway noise, aircraft noise, noise from the generators, industrial noise.

2.2.4 WATER POLLUTION: This is as a result of discharge of wastewater from commercial and industrial waste (intentionally or through spills) into surface water; discharge of untreated

domestic sewage and chemical contaminants such as chlorine; release of waste and contaminants into surface runoff flowing to surface waters (including urban runoff and agricultural runoff, which may contain chemical fertilizers and pesticides; waste disposal and leaching into groundwater; eutrophication and littering.

2.2.5 LIGHT POLLUTION: Includes light trespass, over-illumination and astronomical interference.

2.2.6 RADIOACTIVE CONTAMINATION/POLLUTION: This is caused by natural processes such as radioactive decay of radon, nuclear power generation and nucleus weapons research, manufacture and deployment resulting from 20^{th} century activities in atomic physics.

2.2.7 PETROLEUM POLLUTION: This is associated with oil spills, refinery effluents, excavation effluents, and fumes from generators, distillation plants, and gas flaring.

2.2.8 THERMAL POLLUTION: This is a temperature change (rise) in natural water bodies caused by disposal of heated industrial waste water or water from the cooling towers of nuclear power plants.

2.3 AIR POLLUTION

Air pollution is a contamination of air by the discharge of harmful substance. A broader definition of air pollution is the presence of contaminants in the atmosphere such as dust, fumes, gas, mist, odour, smoke or vapour in quantities and of such characteristics and duration such as to be injurious to human, plant or animal life or to properly or to interfere unreasonably with the comfortable enjoyment of life and property (Lee *et al*., 2004). Some of these particles are directly emitted into the air from a variety of sources such as cars, trucks, factories, construction sites, tilled fields and burning of woods (Ueng *et al*., 2004b).

Pollutants in the atmosphere may be of natural origin such as smoke, fumes, ash and gases from volcanoes, forest fires, sand and dust from windstorms in arid regions, fog in humid low-lying areas and natural terpene haze from pine trees in mountainous region long before human induced or anthropogenic problems came on the scene. Pollutant sources are varied and may be categorized into mobile (transportations), stationary combustion, industrial process and solid waste disposal (Ueng *et al*., 2005). Regulated air pollutants are of two groups, the criteria and the hazardous air pollutants. Criteria pollutants include particulate matter, nitrogen oxides, sulphur oxides, carbon monoxide, ozone and lead while hazardous air pollutants include asbestos, beryllium, mercury, vinyl chloride, arsenic, radio nuclides, benzene and coke oven emission. Depending on their origin, pollutants are considered either primary or secondary contaminants. Primary pollutants such as hydrogen sulphide, hydrocarbon (HC) are emitted directly to the atmosphere in the form in which they were emitted. Secondary pollutants such as ozone and peroxyacetylnitrate (PAN) are those in the atmosphere by photochemical hydrolysis or oxidation reaction.

2.3.1 AMBIENT AIR QUALITY

Ambient air is the outside air that is free to move, it is the open air around us. Ambient air pollution is also referred to as tropospheric or ground level air pollution .Air pollution was a major nuisance for many and a serious concern for some in the industrializing cities of the 19th century, but concerted efforts to address ambient pollution only began in the 20^{th} century. There is considerable variation in exposure to air pollution between and within urban centres, depending on geographical factors as well as the type of activities undertaken in and around the urban centres. Ambient air pollution is usually worse in urban centres; however, overall exposure to air pollution (indoor and ambient) is higher in rural areas because most biofuel users are rural. Air pollutants are blown across state and national boarders sometimes in significant amounts.

Ambient air pollution has reached excessively high levels in many large cities in Asia, Africa and Latin America. However, as a result of higher emission standard and closer monitoring levels, certain types of air pollutants have declined in many developed countries. On the other hand, the ambient air pollution levels are a growing problem in urban cities in many developing countries. Several factors contribute to the worsening air pollution level in developing country cities including rapid growth in urban population, increasing industrialization and rising demand for energy and motor vehicles. Other factors such as poor environmental regulation, less efficient technology of production, congested roads, age and poor maintenance of vehicles; also add to the problem (Lin *et al*., 2005).

2.3.2 LOCAL AND REGIONAL EFFECTS

Smog has seriously affected more persons than any other type of air pollution. It can be loosely defined as a multisource, widespread air pollution that occurs in the air of cities. Smog, a contraction of the word smoke and fog has been caused throughout recorded history by water condensing on smoke particles, usually from burning coal (Bina *et al*., 2011). As coal economy has gradually been replaced by the petroleum economy, photochemical smog has become predominant in many cities. Its unpleasant properties result from irradiation by sunlight of hydrocarbons primarily unburned gasoline emitted by automobiles and other combustion sources or other pollutants in the air. Irradiation produces a long series of photochemical reactions. The products of the reactions include organic particles, ozone, ketones, aldehydes, ketones, peroxyacetyl nitrate and organic acids and other oxidants.

Sulfur dioxide, which is always present oxidizes and hydrates to form sulfuric acids and becomes part of the particulate matter. Also, automobiles are polluters even in the absence of photochemical reactions. They are responsible for much of the particulate material in the air, they also emit carbon monoxide which is one of the most toxic constituents of smog. With the exception of ice fog, all types

of smog decrease visibility and are irritating to the respiratory system. Photochemical smog produces eye irritation and lacrimation and thus causes severe damage to many types of vegetation, including important crops. Its acute effects include an increased mortality rate, especially among persons suffering from respiratory and coronary ailments. Air pollution on a regional scale is in part the result of local air pollution–including that produced by individual sources such as automobiles. Oxides of sulfur and nitrogen carried long distances by the atmosphere and then precipitated in solution as acid rain, can cause serious damage to vegetation, waterways and building (Venosa and Zhu, 2003).

2.4 SOIL POLLUTION

Soil contamination or pollution is caused by the presence of xenobiotics (human made) chemicals or other alteration in the natural soil environment. It is typically caused by industrial activities, agricultural chemicals, or improper disposal of waste. The most common chemicals involved are petroleum hydrocarbons, polyaromatic nuclear hydrocarbons (PAHs) such as naphthalene and benzo(a)pyrene, solvents, pesticides, lead, mercury, and soot. Contamination of soil with fumes from generators as well as with diesel oil tends to upset the biological balance of soil (Krahl *et al*., 2008; Wyszkowskwa *et al*., 2005). It usually alters the succession of microorganisms, which is directly associated with the activity of soil enzymes (Wyszkowskwa *et al*., 2005). The concern over soil contamination stems primarily from health risks, from direct contact with the contaminated soils, vapours from the contaminants, and from secondary contamination of water supplies within and underlying the soil. Agricultural soil health is linked to human health, as poor soils yield fewer crops with decreased nutritional value. Healthy soils also limit erosion, and help improve air and water quality (Brevik *et al*., 2013).

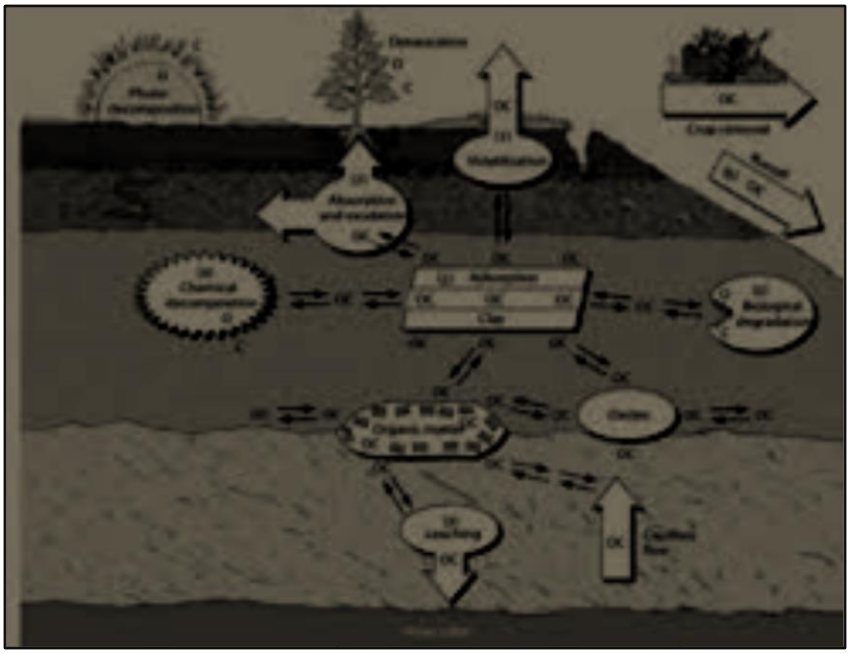


Figure 2.1: Fate of pollutants in the soil (Aja, 2005)

2.4.1 MAIN CAUSES OF SOIL POLLUTION

2.4.1.1 Soil pollution is often associated with indiscriminate use of farming chemicals such as pesticides, fertilizers etc. Pesticides applied to plants can also leak into the ground, leaving long-lasting effects (Figure 2.1). In turn, some of the harmful chemicals found in the fertilizers e.g cadmium may accumulate above their toxic levels leading to the poisoning of crops (McCormick, 2007).

Figure 2.2: Pesticide pollution (www. nunukphotos.com)

2.4.1.2 Heavy metals can enter the soil through the use of polluted water in watering crops or through the use of mineral fertilizers (Elgallal *et al.*, 2016; Woldetsadik *et al*., 2017)

2.4.1.3 Faulty landfills, bursting of the underground bins and seepage from faulty sewage systems could cause the leakage of toxins into the surrounding soils (Figure 2.3) (Rogers and Li, 2015)

Figure 2.3: Waste landfill (Rogers and Li, 2015**)**

2.4.1.4 Acid rains caused by industrial fumes mixing in rain fall on the land and could dissolve away some of the important nutrients found in the soil as such change the soil structure.

2.4.1.5 Industrial waste is one of the soil pollution factors. Iron, steel, power and chemical manufacturing plants which irresponsibly use the earth as a dumping ground often leave behind lasting effects for years to come.

2.4.1.6 Fuel leakages from automobiles which gets washed by rain, can seep into the nearby soil polluting it.

2.4.1.7 Deforestation is the major cause for soil erosion, where soil particles are dislodged and carried away by water, or wind. As a result, the soil loses its structure as well as important nutrients found in the soil.

2.4.2 EFFECTS OF POLLUTION ON SOIL

The effect of pollution on soil is quite disturbing and can result in huge disturbances in the ecological balance and health of living beings on earth. Some of the most serious soil pollution effects are:

2.4.2.1 Disturbance in the balance of flora and fauna inhabiting in the soil.

2.4.2.2 Contaminated soil decreases soil fertility and hence there is decrease in the soil yield.

2.4.2.3 Normally crops cannot grow and flourish in a polluted soil. However if some crops manage to grow, then these crops might have absorbed the toxic chemicals in the soil and might cause serious health problems in people consuming them.

2.4.2.4 Sometimes the soil pollution is in the form of increased salinity of the soil. In such a case, the soil becomes unhealthy for vegetation, and often becomes useless and barren.

2.4.2.5 When soil pollution modifies the soil structure, deaths of many beneficial and organisms e.g. earthworm in the soil could take place. Other than further reducing the ability of the soil to support life, this occurrence could also have an effect on the larger predators e.g. birds and forces them to move to other places, in search of food.

2.4.2.6 People living near polluted lands tend to have higher incidences of migraines, nausea, fatigue, skin disorder and even miscarriages. Depending on the pollutants present in the soil, some of the longer-term effects of soil pollution include cancer, leukemia, reproductive disorders, kidney and liver damage and central nervous system failure. These health problems could be as a result of direct poisoning by the polluted land eg children playing on land filled with toxic waste or indirect poisoning eg eating crops grown on polluted land, drinking water polluted by the leaching of chemicals from the polluted land to the water supply etc.

2.5 NOISE POLLUTION

Noise pollution has a relatively recent origin. It is a composite sound generated by human activities ranging from blasting stereo systems to the roar of supersonic transport jets. Although the frequency (pitch) of noise may be of major importance, most noise sources are measured in terms of intensity, or strength of the sound field. The standard unit, one decibel (dB), is the amount of sound that is just audible to the average human. The decibel scale is misleading because it is logarithmic rather than linear; for example, a noise source measuring 70 dB is 10 times as loud as a source measuring 60 dB and 100 times as loud as a source reading 50dB. Noise may be generally associated with industrial society, where heavy machinery, motor vehicles and aircrafts have become everyday items. Noise pollution is more intense in the work environment than in the general environment, although ambient noise increased an average of 1dB/year during the 1980s. The average background noise in a typical home today is between 40 and 50 decibels. Some examples of high-level sources in our environment are heavy trucks (90dB at 15m/50ft), freight trains (75dB at 15m/ 50ft), air conditioning (60dB at 6m/20ft) and generator (65dB at 10m/30ft) (Ueng *et al*., 2005).

The most readily measurable physiological effect of noise pollution is damage to hearing which may be either temporary or permanent and as well causes disruption of normal activities or just general annoyance. The effect is variable, depending upon individual susceptibility, duration of exposure, nature of noise (loudness), and time distribution of exposure (such as steady or intermittent). On the average an individual will experience a threshold shift (a shift in an individual‘s upper limit of sound detectability) when exposed to noise levels of 75 to 80dB for several hours. A second physiologically important level is the threshold of pain, at which even short-term exposure will cause physical pain (130 to 140dB). Any noise sustained at this level will cause permanent threshold shift or permanent hearing loss. At the uppermost level of noise (greater than 150dB), even a single –term blast may

cause traumatic hearing loss and physical damage inside the ear. Although little information are available on the physiological effects of increased noise levels. Many researchers attribute increased irritability, lower productivity, decreased tolerance levels, increased incidence of ulcers, migraine headaches, fatigue, and allergic responses to continued exposures to high –level noises in the workplace and the general environment (Lee *et al*., 2004).

2.6 GENERATOR FUMES

The epileptic power supply in Nigeria and the increase in industrialization have led to an increase in the use of diesel generators as alternate source for power, resulting in increase in the emission of pollutants which negatively affects the environment and the human health. The fumes that are emitted from these generators are termed 'generator fumes'. These fumes are generated from its original source diesel. The burning of diesel leads to the production of pollutants as earlier stated in introduction, page 6. All these are known as 'byproducts of generator fumes'. Generators fumes are also termed the ''silent killer" because of its composition. Naturally, generators produce smoke as its product of combustion which could vary in color; blue, black and white smoke, respectively.

Generator fumes contain many known or suspected carcinogens or mutagens such as benzo(a)pyrene and 1-hydroxypyrene.

2.6.1 THE DELETERIOUS EFFECTS OF GENERATOR FUMES:

2.6.1.1 EFFECTS ON LIVING CELLS:

There are wide ranges of research documenting the ability of these generator fumes composite to affect living cells, including changes in the biochemical and molecular mechanisms of cells both *in vitro* and *in vivo.* Changes also occur in cell metabolism and proliferation, inducing potentially damaging effects in various cell components ranging from the cytoplasmic membrane, where the

distribution of proteins is modified to the cytoplasm itself and the nucleus where the activities of intracellular enzymes and molecules regulating cell growth are altered. Researchers have also proven that both solid organic matter and gaseous volatile organic compounds in petroleum related particles can trigger the mutation of cells, resulting in teratogenesis and other hazard.

2.6.1.2 EFFECTS ON THE SOIL MICROBIAL ENZYMES:

Soil contains free enzymes, immobilized extracellular enzymes and enzymes within microbial cells. They are indicator of biological equilibrium (Frankenberger and Bingham, 2012), fertility (Nannipieri *et al.,* 2020), quality, and changes in the biological status of soil due to pollution (Nannipieri *et al.*, 2020; Trasar- Cepeda *et al*., 2000). The role of soil microbial enzymes and their activities are defined by their relationships with soil and other environmental factors (e.g., acid rain, heavy metals, generator fumes, pesticides and other industrial chemicals) that affect the activities. Generator fumes reaching the soil may disturb local metabolism or enzymatic activities (Liu *et al.*, 2005). The prolonged exposure of these generator fumes to the soil environment has negative influence on the soil microbial properties such as enzyme activities. For example, the exposure of phosphatases (acid and alkaline), important soil microbial enzymes to generator fumes transforms and alters the structural and biochemical characteristics of these enzymes, resulting in significant changes of their activities . Negative impacts of pollutants on soil microbial enzymes like catalases, hydrolases, lipases and dehydrogenases activities has been widely reported in the literature. A number of factors, for example chemical components of the generator fumes, concentration used, microbial community structure, type of soil, and soil conditions can contribute to divergent research findings. Enzyme activity is influenced by soil conditions such as organic matter content, moisture, and temperature (Tscherko *et al.*, 2003). The effect on fumes on soil enzymes particularly extracellular enzymes are not clear due to their multidimensional behavior in the complex soil medium and the

greater complexity of soil microbial and biochemical interactions. For this reason, some researchers are faced with difficulties in discerning the effect of some pollutants such as fumes on extracellular enzymes activities in soil (Nannipieri *et al.*, 2020).

2.6.1.3 EFFECTS ON THE MICRO ORGANISMS/HUMANS:

Soil microoganisms including bacteria, fungi, actinomyces, and algae mediate many of the processes that influence soil fertility. For example, microorganisms are dynamically involved in many basic ecological processes such as the biogeochemical cycling of elements, and the mineralization of carbon, nitrogen, sulfur, and phosphorus (ie organicmatter decompostition. A decrease in the number of species or diversity of microorganisms affects the cycling of plant nutrients in the soil. Similarly, any disturbance in the soil ecosystem can disturb the microbial activity and hence the availability of nutrients. Therefore, the presence and activity of soil microrganisms are of fundamental importance to the productivity of agricultural soils. It has been observed that soils contaminated with generator fumes affect microbial population, community structure (biodiversity), microbial activities and processes e.g decomposition and mineralization.

Microbial communities in soils are thought to be highly diverse. A gram of soil may contain as many as 13,000 species of bacteria and an unknown diversity of fungi and algae. It is highly unlikely that a test using a single organism or group of organisms from such a complex system could represent all the variety of effects that a pollutant might have on the system on the whole.

The toxicity exerted by fumes may suppress or even kill sensitive members of the microbial community and lead to a shift in community structure. Some species may be eliminated while others may appear in larger numbers because of reduced competition for substrate. Microbial populations in soils close to the mechanic workshop contained greater proportions of bacteria able to grow.

Generator fumes in soil undergo a variety of degradative, transport, and absorption/ desorption processes depending on the chemical compostion of the fumes and soil properties. Generator Fumes interacts with soil microorganism and their metabolic activities and may alter the physiological and biochemical behavior of soil microbes (Chen *et al*., 2004). Microbial biomass is an important indicator of microbial activities and provides direct assessment of the linkage between microbial activities and the nutrient transformations and other ecological processes. Generally, a decrease in soil respiration reflects the reduction in microbial biomass or increase in respiration implies the enhanced growth of bacterial population. Some microbial groups are capable of using generator fumes as a source of energy and nutrients to multiply, whereas the generator fumes may be toxic to other organisms. Likewise, sometimes, release of generator fumes reduces microbial diversity but increases functional diversity of microbial communities even sometimes demonstrate the tendency of reversible stimulatory / inhibitory effects on soil microorganisms. Generator fumes may also inhibit or kill certain group of microorganisms and outnumber other groups by releasing them from the competition. Sometimes, initially microbial population is affected by fumes but with time after a period of acclimation, the population merely returns to normal or even increases .This is an indication of changes in microbial degradation capabilities or due to a change within the microbial community.

The exposure of these fumes to soil microorganism results to mutation arising from PAH-DNA adducts disrupting their normal DNA transcription, translation, and replication. However, gene polymorphisms in most enzymes have also been identified in human beings and this could modulate individual cancer susceptibility. Ueng *et al*, (2004b) reported that exposure of rats to motorcycle exhaust and organic extracts of the exhaust particulate cause a dose- and time-dependent increase in cytochrome P_{450}-dependent monooxygenases as well as glutathione-S-transferase in the liver, kidney, and lung microsomes. This occurs as these enzymes metabolize the PAHs (benzo-a-pyrene) to polar

nucleophilic metabolites that bind with the adenine and guanine bases of the DNA (Ueng *et al.*, 2004b).

Numerous epidemiological studies have also shown that exposure to a large amount of petroleum related particles causes an increase in morbidity and mortality which often arises from respiratory diseases and their negative impact on human health and its environment. These fumes pose health hazard if unchecked. For instance, the problem of CO (Carbon monoxide) is a chronic one. i.e. constant exposure to CO causes decrease of oxygen carrying capacity of the blood; it also causes physiological stress and visual problems. Likewise, prolonged exposure to SO_2 poses a lot of damage; it is absorbed in the mucus membrane of the upper respiratory tracts usually in the presence of the suspended particulate matter (SPM). Its likely symptoms are sneezing, coughing, shortness of breath together with long-term effect, such as bronchospasm (Ueng *et al.*, 2005).

2.6.1.4 EFFECTS ON AMBIENT AIR QUALITY

Due to the trans-boundary movement of these fumes in the air, it makes the problem of air pollution a worldwide concern. This is because air is the fastest means through which air borne hazardous particles or diseases can circulate. Any increase or decrease in concentration of these atmospheric constituents may have adverse global effects.

2.6.1.5 OTHER EFFECTS

Plants are affected by these generator fumes. Sulphur oxide (SO_2) which causes acid rain damages trees and causes harm on animals, fish and other wild life. Both hydrogen sulphide (H_2S) and nitrogen dioxide (NO_2) in high concentration have been shown to be harmful to citrus trees and ornamental plants (Thurston, 2006). Oxides of nitrogen can injure vegetation at certain concentrations resulting to bleaching or killing plant tissue, causing leaves to fall and reducing growth rate .

2.7 FACTORS AFFECTING THE IMPACT OF GENERATOR FUMES ON MICROBIAL DIVERSITY

The effect of generator fumes on soil microorganisms is controlled by numerous environmental factors in addition to the persistence, concentration, toxicity of the fume and its bioavailability. One of the major factors contributing to the net impact of released generator fumes is its bioavailability in soil environment. Absorption and desorption processes regulate concentration of a fume in soil solution and hence its bioavailability, bioactivity, and degradability in soil environment (Lee *et al*., 2004).

2.8 EFFECT OF GENERATOR FUMES ON BIOCHEMICAL REACTIONS IN SOIL

Soil microorganisms have the ability to carry out biochemical transformations of various elements like nitrogen (N), phosphorus (P), sulphur(S), and carbon (C). Generator fumes may directly or indirectly affect the vital biochemical reactions such as mineralization of organic matter, nitrogen fixation, nitrification, denitrification, and ammonification by activating/deactivating specific soil microorganism and/or enzymes. Information on possible effects of generator fumes on all biochemical processes is sparse.

2.8.1 NITROGEN FIXATION

Biological Nitrogen Fixation (BNF) is an efficient and natural source of nitrogen, and the total BNF has been estimated twice (175 million tones) as compared to the total nitrogen fixation by non-biological processes. Rhizobial symbioses with over 100 agricultural important legumes contribute nearly half the annual quantity of BNF entering the soil ecosystem. Generator fumes may influence the nodulation and BNF in legumes either by affecting virulence of attacking nodular bacteria, the root fibers of the plants in which the infection occurs or both (Morin *et al*., 2000).

2.8.2 MINERALIZATION OF ORGANIC COMPOUNDS AND AVAILABILITY OF NITROGEN, PHOSPHORUS AND POTASSIUM IN SOIL

Organic matter is one of the most critical properties of soil that affects soil quality, the productivity of soil and the emission of trace gases to atmosphere. Much changes in the levels and the dynamics of organic matter is controlled by biological activities in soil, and the quantity and quality of plant residues returned to the soil. Reseachers have reported inhibitory effects of generator fumes on rates of decomposition of organic matter and mineralization in agricultural and grassland ecosystems, forest areas, and a desert ecosystem. In contrary, others have advocated the stimulatory effect of fumes on mineralization process (Krahl *et al*., 2008).

2.8.3 NITRIFICATION, DENITRIFICATION, AMMONIFICATION

Prolonged exposure to generator fumes can stimulate nitrification, denitrification and ammonification by switching on bacterial communities responsible for carrying out these biological processes and vice versa. Nitrous oxide (N_2O) and nitric oxide (NO), which are environmentally suignificant trace gases produced in soil by the processes of nitrification and denitrification, are inhibited by fumes at prolonged concentrations. In most cases, prolonged exposure to generator fumes can disturb microbial equilibrium and cyling of biological elements (Krahl *et al*., 2006).

2.9 CARBON MONOXIDE (CO)

Ever since man learned how to break stone by heat or to burn wood with resisted flow of air to produce charcoal, he has been affected by carbon monoxide. Whilst smoking is the major source of carbon monoxide for humans, most of the hazards at work connected with gas come from motor vehicles. During their repairs, testing, raising or servicing. Blasting, fire-fighting, methanol production, wood distillation and cooking over charcoal have similar hazards. Carbon monoxide unlike many toxic gases is a colourless, odourless and life-threatening gas can be breathed without

giving any warning to the victim. It is a poisonous gas produce by the partial combustion of wood, coal, tobacco, car emission, gas water heater, leaking chimney and furnaces, generators and other gasoline powered equipment (Kooter *et al*., 2011).

Carbon monoxide is the next most abundant atmospheric pollutants in the troposphere. Emission of carbon monoxide caused by man far exceeds those of other pollutants. Sources of air pollutants are diverse and are often impossible to discriminate between them. Domestic sources often surround industrial sources and power or heat generation by particular activities without making distinction between industrial and domestic apportionment (Krahl *et al*., 2006).

In areas away from major sources, the atmospheric level of carbon monoxide is fairly constant which meant that significant natural sinks exist to remove this vast excess of gas. The natural sinks and their relative importance are the oxidation to carbon dioxide in the troposphere by hydroxyl radicals. Wood, stubble, dunk and grass are use daily in about half of the world's household as energy for cooking. In most part of the third world, they are burnt in open fire or inefficient stoves in poorly ventilated kitchen. The result is toll in death and ill health far greater than the outdoor air pollution. The first of severe poisoning is loss of consciousness and further inhalation of high concentrations readily lead to death. During normal combustion each atom of carbon in the burning fuel joins with two atoms of oxygen forming a harmless gas called carbon dioxide. When there is lack of oxygen to ensure complete combustion of the fuel, each atom of carbon links up with only one atom of oxygen forming carbonmonoxide gas (Morin *et al*., 2000)

Carbon monoxide inhibits the blood capacity to carry oxygen in our lungs. Carbon monoxide quickly passes into our blood stream and attaches itself to haemoglobin (oxygen carrying pigment in red blood cell). Haemoglobin readily accepts carbon monoxide even over the life giving oxygen atom (as 210 times as readily as oxygen), forming a toxic compound known as carboxyhaemoglobin

(COHb); consequently, disrupting oxygen transport to the tissues. Depending on the amount inhaled, CO impedes coordination, worsen cardiovascular conditions and produce fatigue. The elderly, the fetus and persons with cardiovascular and pulmonary diseases are particularly sensitive to elevated CO level. Methylene chloride found in some common household products such as paint strippers can be metabolized to form carbon monoxide which combines with the haemoglobin to form carbonyl haemoglobin. Carbonyl haemoglobin levels of the order 60% COHb saturation are normally fatal. Lower concentration of 40% saturation can also be lethal for young or elderly people. The health effects associated with exposure to carbon monoxide at low concentration include fatigue and chest pain in people with heart diseases (WHO, 2011). At higher concentration impaired vision and coordination, heart aches, permanent damage to central nervous System, dizziness, confusion, nausea and death may occur. At moderate concentration, impaired vision and reduced brain function in pregnancy result in low birth weight and prenatal death. The health effect of exposure is depended upon the carbon monoxide content of the blood, the air breathed, the duration of exposure of temperature, the working rate or the general fitness (WHO, 2011).

2.10 HYDROGEN SULPHIDE (H_2S)

Hydrogen Sulphide is a colourless, toxic and flammable gas. Small amount of Hydrogen Sulphide occur in crude petroleum but natural gas can contain up to 28%. Volcanoes and hot spring emit some H_2S about 10% of the total emissions of H_2S are due to human activities. By far the largest industrial route to H_2S occurs in petroleum refineries. Other anthropogenic sources of Hydrogen Sulphide include coke, ovens, paper mills (using the sulphate method) and tanneries. H_2S arises from virtually anywhere elemental sulfur come into contact with organic material especially at high temperature, H_2S can be present naturally in well water, in such cases; ozone is often used for its removal. Appreciable quantity of Hydrogen Sulphide and other reduced sulfur compounds are formed during

treatment of sewage by the anaerobic bacteria decomposition of organic matter in the presence of the sulphate (SO_4^{2-}) ion (Morin *et al*., 2000).

Hydrogen Sulphide has offensive that is detectable at low concentration below 8ug/m3 at a concentration of 50 – 150 ug/m^3. It has a deceptively sweet smell, above this range; it deadens the sense of smell. Its concentration varies from 0.1 – 1.0 ug/m^3 in ambient air, although concentration above 100 ug/m^3 has been reported in industrial plants. Hydrogen Sulphide is considered a broad-spectrum poison - meaning that it can poison several different systems in the body, although the nervous system is most affected. It forms a complex bond with iron in the blood, thereby blocking oxygen from binding and stopping cellular respiration. However, since Hydrogen Sulphide occurs naturally in the environment and the gut, enzymes exist in the body capable of detoxifying it by oxidation to harmless sulphate. Chronic exposure to low level H_2S (about 2ppm) has been implicated in increased miscarriage and reproductive health tissues amongst Russian and Finish wood pulp workers.

Different methods have been employed for the determination of hydrogen sulphide gas in the environment. A sensitive spectrophotometric method has been use for the determination of H_2S in environmental sample after its fixation as zinc sulphide. The suitability of the method for monitoring H_2S in atmosphere air in the vicinity of possible sources such as a sewage treatment plant and in waste water has been evaluated. Colourimetric, stain length, personal dosimeters, operating by gas diffusion has been developed by. To determine worker exposure for up to an 8-hour period for several inorganic air borne contaminants in the range of their threshold limit values, H_2S in air was measured (Morin *et al*., 2000).

2.10 SULPHUR DIOXIDE (SO_2)

Sulphur dioxide has long been recognized as an important parameter in the determination and control of ambient air quality. SO_2 gas is the main product from the combustion of sulphur compounds and is of significant environmental concern. SO_2 is produced in volcanoes and in various industrial processes. Since coal and petroleum contain various amounts of sulphur compounds, their combustion generates Sulphur dioxide. Further oxidation of SO_2 usually in the presence of a catalyst, such as NO_2 forms H_2SO_4 and thus acid rain. Sulphur dioxide is a colourless, non-flammable gas with a strong suffocating odour. It has a taste threshold of 784 ug/m^3 (0.3 ppm) and an odour threshold of 1306ug/m3 (0.5 ppm). Its high solubility in water causes it to be irritating to the eyes and upper respiratory tract. It may oxidize to sulphur trioxide (SO_3) which then dissolves in water to produce sulphuric acid (Wood, 2017). The United States has witnessed a 33% decrease in emission between 1983 and 2002. This improvement resulted from glue gas desulphurization, a technology that enables SO_2 to be chemically bound in power plants burning sulphur containing coal or oil. New fuel additive catalyst such as ferox, are being used in gasoline and diesel engines in order to lower the emission of sulphur dioxide gas into the atmosphere (WHO, 2011). Epidemiology studies indicate that chronic exposure to SO_2 is associated with increased respiratory symptoms and decrease in pulmonary function. Clinical studies have shown that some asthmatics respond with bronchus constriction to brief exposure of SO_2 levels as low as 0.4ppt (part per trillion). Its concentration in air may range from 0.01 to several parts per million, and it is responsible for the decay of building and monuments, acid rains and human discomfort and disability (Frank and Malkomes, 2013). Different methods have been employed to determine the concentration of sulphur oxides in the atmosphere collected and determined sulphur dioxide by permeation of the gas through a membrane into a catalytic oxidizing solution, which stabilizes the sulphur dioxide as sulphate, which is then analysed turbidmetrically by precipitation (Morin *et al*., 2000). Liu *et al* (2008) employed gas permeation continuous flow

colometric analysis for determining high of sulphur dioxide. Sulphur dioxide in air was determined on the basis of the catalysed autoxidation of CO (II) in azide medium. The method provides result comparable with those obtained with a colometric monitor based on the I_2/I-System (Wood, 2017).

2.11 LEAD (Pb)

Lead is very toxic and has very chronic health implication even at low concentration. United States Environmental Protection Agency (USEPA) classified lead as being potentially hazardous and toxic to most form of life (USEPA, 1999). It has been found to be responsible for quite a number of ailments in human such as chronic neurological problems and mental retardation in children, colic anemia and renal diseases. Lead replaces Ca in the bone. Its effect is cumulative and long-term exposure has been quoted to cause serious health hazard which include inhibition of the synthesis of haemoglobin and also adversely affect the central and peripheral nervous system as well as the kidney. Lead has been found to be bio-accumulated by benthic bacteria, plants and other aquatic biota. The ubiquity of this metal in the environment could be traced to wide increase in industrial activities and continual use of leaded petrol in many developing nations. Leaded petrol is still in used in Nigeria. It has been pointed out that African's contribution to global lead pollution has increased from just 5% in 1980s to 20% in 1996. Other inputs of lead into the environment include wastes form used lead batteries, atmospheric deposition from automotive and industrial emission (generators). The level of lead in Nigeria's super grade gasoline is 600 – 800mg per litre which is much higher than permissible levels in some pollution conscious countries. Lead levels in soils vary depending on the location and nearness to lead based activities and vehicular density (Naplekova and Bulavko, 2013).

2.12 ENZYMES

Enzymes are defined as biological catalysts for specific reactions, which depend on several biotic and abiotic factors such as: pH, temperature, presence or absence of inhibitors, soil organic matter

composition, cultivation technique, and other factors, which can directly influence the chemical reactions of these molecules in the soil (Shukla and Chandel 2008). Enzymes are biologically produced proteinic substances, having specific activation in which they combine with their substrates in such a stereoscopic position that they cause changes in the electronic configuration around certain susceptible bonds. Their significance in all spheres including soil is worth tested and reported. In plant nutrition, their role cannot be substituted by any other substance and their function is quite pragmatic in solubilizing and dissolving the much-needed food in ionic forms for the very survival of the animal and plant kingdom.

Enzymes are the key to understanding underground biochemistry and the role of soil in the global carbon cycle. These biological mediators of change are active both within living soil organisms and independently as extracellular proteins that are actively secreted into the soil by roots and fungi or released as prokaryotic and eukaryotic cells that die and decompose. These enzymes can persist in the soil for weeks (Tabatabai, 2012).

2.12.1 ENZYME ACTIVITY

This is the measure of the quantity of active enzyme present and is thus dependent on conditions, which should be specified. It is also the moles of substrate converted per unit time which is equal to rate times reaction volume (i.e= rate x reaction time). The S.I unit is katal, 1katal=1mol^{-1}, but this is an excessively large unit. A more practical and commonly used value is enzyme unit (U) = 1µmol min^{-1}. 1U corresponds to 16.67 nanokatals.

2.12.2 SPECIFIC ENZYME ACTIVITY

This is the activity of an enzyme per milligram of total protein (expressed in µmol min^{-1}mg^{-1}). This gives a measurement of enzyme purity in the mixture. It is the moles of product formed by an enzyme

in a given amount of time (minutes) under given conditions per milligram of total proteins. Specific activity is equal to the rate of reaction multiplied by the volume of reaction divided by the mass of total protein. The S.I unit is katalkg^{-1}, but a more practical unit is µmol mg^{-1} min^{-1}. It is also referred as a measure of enzyme processivity, at a specific (usually saturating) substrate concentration, and is usually constant for a pure enzyme.

2.12.3 COFACTORS

A cofactor is a non-protein chemical compound or metallic ion that is required for a protein's biological activity to occur. These proteins are commonly enzymes and cofactors can be considered ''helper molecules'' that assist in biochemical transformations. Cofactors are often classified as inorganic substances that are required for, or increase the rate of catalysis. They are subdivided into either one or more inorganic ions, or a complex organic or metallo organic molecule called a coenzyme; most of which are derived from vitamins and from required organic nutrients in small amounts. An inactive enzyme without the cofactor is called an apoenzyme, while the complete enzyme with cofactor is called a holoenzyme (Rogers and Li, 2015).

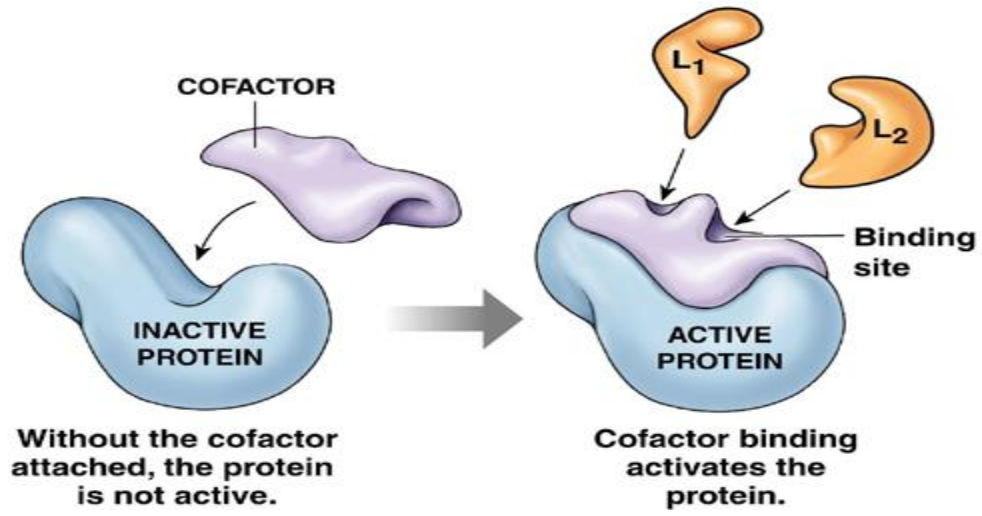


Figure 2.4: Cofactor (Rogers and Li, 2015).

2.12.4 COENZYMES

Coenzymes are non-protein organic molecules that are required by certain enzymes to carry out catalysis. They bind to the active site of the enzyme and participate in catalysis but are not considered substrates of the reaction. Coenzymes often function as intermediate carriers of electrons, specific atoms or functional groups that are transferred in the overall reaction. For example, the role of NAD in the transfer of electrons in certain coupled oxidation reduction reactions. A coenzyme that is tightly or even covalently bound is termed a prosthetic group while cosubstrates refer to loosely bound coenzymes that are released in the same way as substrates and products (Rogers and Li, 2015).

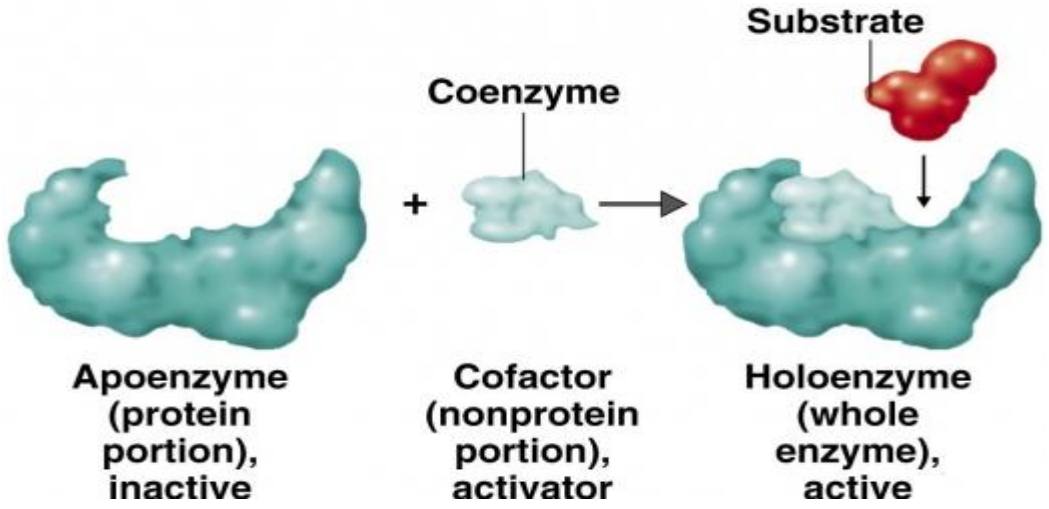


Figure 2.5: Coenzyme (Rogers and Li, 2015).

2.13 SOIL

The soil is the place where hazardous residues deriving from anthropogenic activities are very often released with dangerous and frequently irreversible effects on its safety and consequently on human health. Often, these human activities have produced desertification, deficit or even loss of biodiversity, alteration of the soil matrix, deficiency of organic matter and nutrients. Soil is also a complex mixture of minerals, nutrients, organic matter and living organisms upon which all other

terrestrial trophic systems are dependent**.** (Perez-de- Mora *et al*., 2006). It is a vital resource for sustaining basic human needs such as food, fibre and shelter (Branzini *et al*., 2009)

Soil is a major component of the Earth's ecosystem. From ozone depletion and global warming to rain forest destruction and water pollution, the world's ecosystems are impacted in far- reaching ways by the processes carried out in the soil. Soil is the largest surficial global carbon reservoir on earth, and it is potentially one of the most reactive to human disturbance and climate change. As the planet warms, soils will add additional carbon dioxide into the atmosphere due to its increased biological activity. Thus, soil carbon losses likely have a huge positive feedback response to global warming (Brady and Nyle, 2014).

Soil acts as an engineering medium, a habitat for soil organisms, a recycling system for nutrients and organic wastes, a regulator of water quality, a modifier of atmospheric composition, and a medium for plant growth. Since soil has a tremendous range of available niches and habitats, it contains most of the earth's genetic diversity. A handful of soil can contain billions of organisms, belonging to thousands of species. Soil has a mean prokaryotic density of roughly 10^{13} organisms per cubic meter, whereas the ocean has a mean prokaryotic density of roughly 10^{8} organisms per cubic meter. Carbon content stored in the soil is eventually returned to the atmosphere through the process of respiration which is carried out by heterotrophic organisms that feed upon the carbonaceous material in the soil. Since plant roots depend on the process of respiration, ventilation is an important characteristic of the soil. This ventilation can be achieved via networks of soil pores, which absorb and hold rainwater making it readily available for plant uptake. Since plants require a nearly continuous supply of water, but most regions receive sporadic rainfall, the water-holding capacity of soil is vital for plant survival (Dominguez-Rosado and Pichtel, 2014).

Soil can effectively remove impurities, kill disease agents, and degrade contaminants. Typically, soils maintain a net absorption of oxygen and methane, and undergo a net release of carbon dioxide and nitrous oxide. Soil offer plants physical support, air, water, temperature, moderation, nutrients and protection from toxins. Soils provide readily nutrients to plants and animals by converting dead organic matter into various nutrient forms. Soils supply plants with mineral nutrients held in place by the clay and humus content of the soil. For optimum plant growth, the generalized content of soil components by volume should be roughly 50% solids (45% mineral and 5% organic matter), and 50% voids of which half is occupied by water and half by gas (Dominguez-Rosado and Pichtel, 2014).

The percent soil water and organic content is typically treated as a constant, while the percent soil water and gas content is considered highly variable whereby a rise in one is simultaneously balanced by a reduction in the other (Voroney, 2006). The pore space allows for the infiltration and movement of air and water, both of which are critical for life in soil. Compaction, a common problem with soils, reduces this space, preventing air and water from reaching the plant roots and soil organisms. Given sufficient time, a soil will evolve into a soil profile which consists of two or more layers, referred to as soil horizons that differ in one or more properties such as in their texture, structure, density, porosity, consistency, temperature, color and reactivity. The horizons differ greatly in thickness and generally lack sharp boundaries (Figure 2.6).

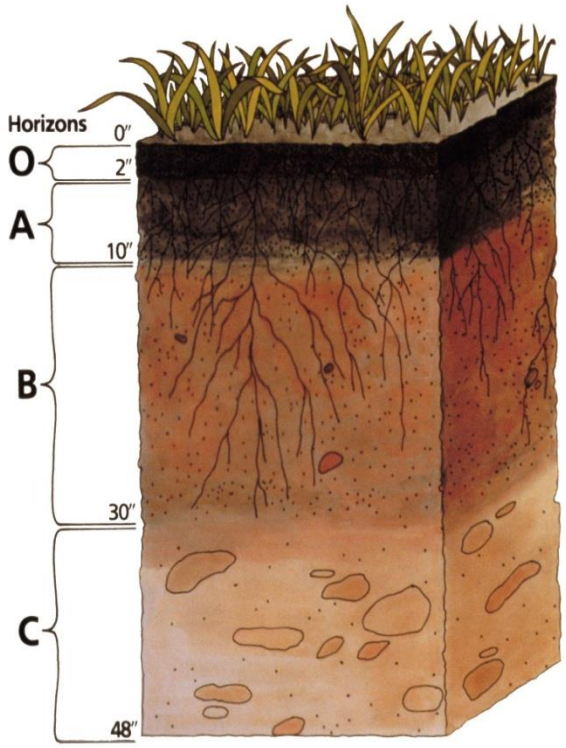


Figure 2.6: Darkened topsoil and reddish subsoil layers (Voroney, 2006)**.**

The soil texture is determined by the relative proportions of sand, silt, clay in the soil. The addition of organic matter, water, gases and time causes the combination to develop into a larger soil structure called an aggregate. At that point a soil can be said to be developed that can be described further in terms of color, porosity, consistency, reaction. Of all the factors affecting the evolution of soil, water is the most powerful due to its involvement in the solution, erosion, transportation and deposition of the materials of which a soil is composed. The mixture of water and dissolved and suspended materials is called the soil solution. Since soil water is never pure water, but contains hundreds of dissolved organic and inorganic substances, it may be more accurately called the soil solution. Water is central to the solution, precipitation and leaching of minerals from the soil profile. Finally, water affects the type of vegetation that grows in a soil, which in turns affects the development of the soil profile (Dominguez-Rosado and Pichtel, 2014).

The most influential factor in stabilizing soil fertility are the soil colloidal particles, clay and humus, which behave as repositories of nutrients and moisture and so act to buffer the variations of soil solution ions and moisture. Their contributions to soil nutrition are out of proportion to their part of the soil. Colloids act to store nutrients that might otherwise be leached from the soil or to release those ions in response to changes of soil pH (Chesworth and Ward, 2008).

The greatest influence on plant nutrition is soil pH, which is a measure of the hydrogen ion (acid-forming) soil reactivity, and is in turn a function of the soil materials, precipitation level, and plant root behaviour. Soil pH strongly affects the availability of nutrients. Most nutrients, with the exception of nitrogen, originate from minerals. Some nitrogen originates from rain, but most of the nitrogen available in soils is the result of nitrogen fixation by bacteria. The action of microbes on organic matter and minerals may be free nutrients for use, sequester them, or cause their loss from the soil by their volatilization to gases or their leaching from the soil. The nutrients may be stored on soil colloids, and live or dead organic matter, but may not be accessible to plants due to extremes of pH. The organic matter of the soil has a powerful effect on its development, fertility and available moisture. Following water and soil colloids, organic material is next in importance to soil's formation and fertility (Osuji. and Nwoye, 2007).

2.14 SOIL MICRO ORGANISMS

The soil represents a favourable habitat for microorganisms and is inhabited by a wide range of microorganisms, including bacteria (nitrobacter, nitrosomonas), fungi (mushroom), algae, viruses and protozoa. Cultivated soil has relatively more population of microorganisms than the fallow land, and the soils rich in organic matter contain much more population of microorganisms than sandy and eroded soils. Microbes in the soil are important to us in maintaining soil fertility, cycling of nutrient elements in the biosphere and as sources of industrial products such as enzymes, antibiotics, vitamins,

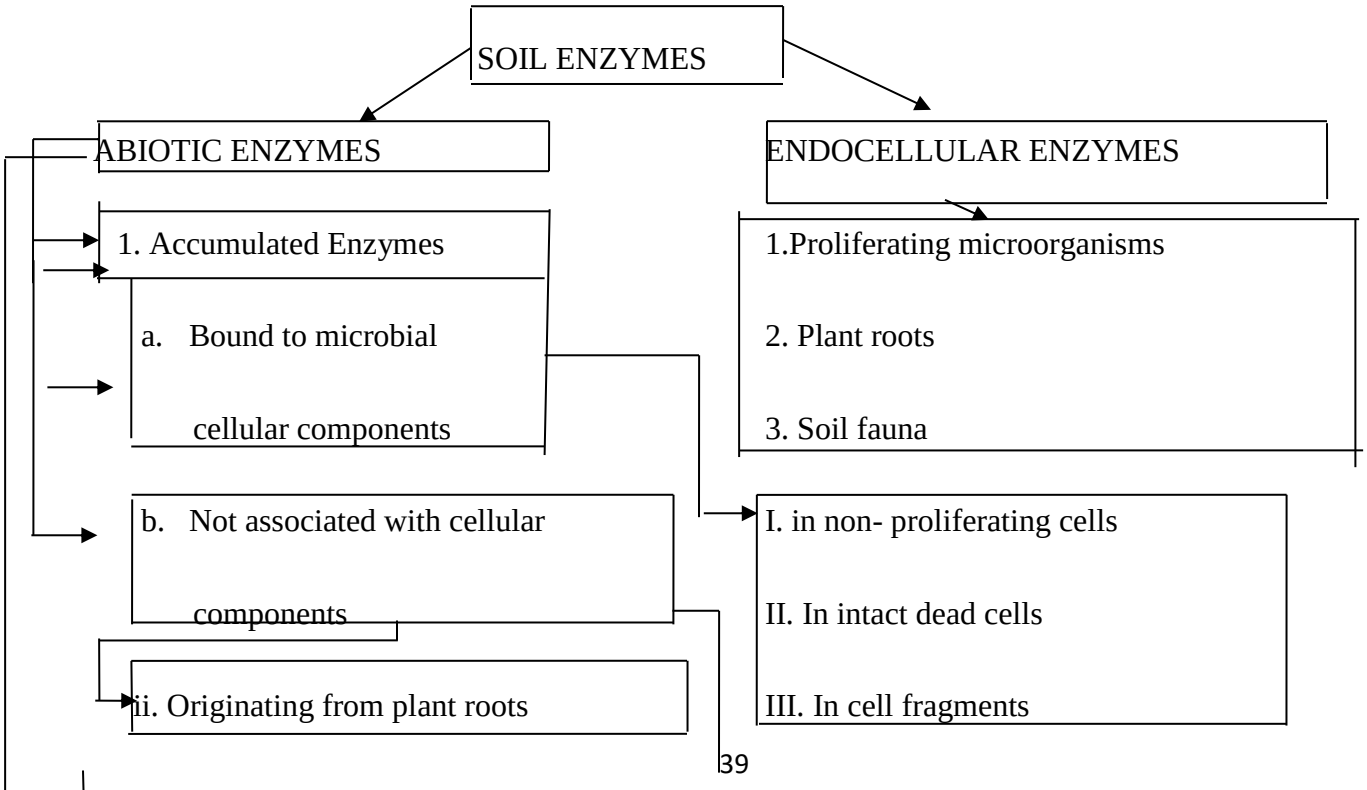
hormones, organic acids etc. But certain microbes in the soil are the causative agents of various human and plant diseases. The plant and animal remains deposited in the soil contribute organic substances (Cottenie *et al*., 2012).

Through biodegradation, soil micro-organisms breakdown a variety of organic materials and use a portion of these breakdown products to generate or synthesize a series of compounds that make up humus, a dark coloured amorphous substance composed of residual organic matter not readily decomposed by microorganisms. Bacteria bring about a number of changes and biochemical transformations in the soil and thereby, directly or indirectly help in the nutrition of higher plants growing in the soil. The important transformations and processes in which soil bacteria play vital roles are: decomposition of cellulose and other carbohydrates, ammonification, nitrification, denitrification. Many microorganisms are capable of using molecular nitrogen in the atmosphere as their nitrogen source. The conversion of molecular nitrogen into ammonia by soil microorganisms is known as nitrogen fixation by bacterium (*Rhizobium*) found in soil, root of the leguminous plants associated with the biosynthesis of nitrate (Abou-Seeda *et al*., 2017).

2.15 SOIL MICROBIAL ENZYMES/SOIL ENZYMES

Soil enzymes are synthesized by microorganisms and act as biological catalysts to facilitate different reactions and metabolic processes to decompose organic pollutants (dead plants) and produce essential compounds such as Carbon (C), Nitrogen (N), and Phosphorus (P) for both microorganisms and plants. Various soil enzymes originate from different plants and organisms that grow in soil and a major origin of soil enzymes is generally from microbial populations present in soil. Enzymes in soil show variation or alteration due to various biotic and abiotic factors in environment. These factors include microbial communities, temperature, pH, nutrient availability and chemicals already present in soil. These enzyme acts as tool microbes use to fulfill their roles as material recyclers in the global

carbon and nutrient cycles. Increased Nitrogen and Phosphorus concentration in plant material promotes enzymes production which increases decomposition and nutrient recycling. Soil enzymes play key biochemical functions in the overall process of organic matter decomposition in the soil system (Burns, 1983; Sinsabaugh *et al*., 2002). They are important in catalyzing several important reactions necessary for the life processes of micro-organisms in soils and the stabilization of soil structure, the decomposition of organic waste, organic matter formation and nutrient cycling. These enzymes are constantly being synthesized, accumulated, inactivated and /or decomposed in the soil, hence playing an important role in agriculture and particularly in nutrients cycling .The activity of these enzymes in soil undergoes complex biochemical processes consisting of integrated and ecologically –connected synthetic processes, and in the immobilization and enzymes stability. In this regard, all soils contain a group of enzymes that determine soil metabolic processes which, in turn depends on its physical, chemical, microbiological and biochemical properties. Soil enzyme activities are often used as indices of microbial growth and activity in soils (Skujis 2018).



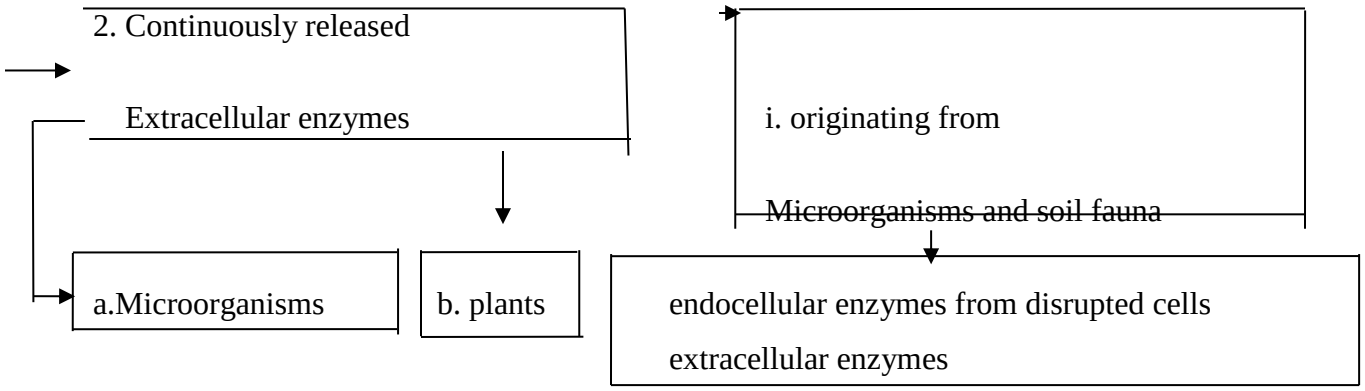


Figure 2.7: Sources of enzymes in soils (Skujis 2018)

Categories of soil enzymes include (Skujis 2018)**:**

1. Enzymes associated with living, metabolically active cells in soil; found in cell's cytoplasm, bound to cell wall or as extracellular enzymes that have been recently produced by the cell.
2. Enzymes associated with viable but non-proliferating cells (such as spores).
3. Enzymes that are attached to dead cells or to cell debris, or which have diffused away from dead /dying cells that originally produced them.
4. Enzymes that are "permanently" immobilized on soil clay and humic colloids. Such enzymes can remain active for long periods of time. Such immobilized soil enzymes can arise from either eukaryotic or prokaryotic cells. Binding of enzymes to soil surfaces (especially clay and humic materials) may take place via ionic interactions, covalent bonds, hydrogen bonding, entrapment of enzyme by soil colloids and other mechanisms.

These enzymes may include catalase, lipase, phosphatase (acid and alkaline), dehydrogenase released from plants and animals, organic compounds and microorganisms, and soils (Gupta *et al*., 2013)

2.15.1 CATALASE (E.C.3.1.1.3)

Some bacteria can reduce diatomic oxygen to hydrogen peroxide or super oxide. Both of these molecules are toxic to bacteria. Some bacteria, however poses a defense mechanism, which can

minimize the harm done by the two compounds. These resistant bacteria use two enzymes to catalyze the conversion of hydrogen peroxide and super oxide back into diatonic oxygen and water. One of these enzymes is catalase. We rely on oxygen to power our cells, but oxygen is a reactive molecule that can cause serious problems if not carefully controlled. One of the dangers of oxygen is that it is easily converted into other reactive compounds. One of such reactive compounds is hydrogen peroxide. Fortunately, cells make a variety of antioxidant enzymes to fight the dangerous side effects of reactive oxygen species. Two important layers are superoxide dismutase, which converts superoxide radicals into hydrogen peroxide and catalase, which converts hydrogen peroxide into water and oxygen gas.

Catalase (EC 1.11.1.6) is thus a common enzyme found in nearly all living organisms exposed to oxygen which catalyses the decomposition of hydrogen peroxide (H_2O_2) to water and oxygen. Catalase is a tetramer of four polypeptide chains, each with over 500 amino acids long. It contains four porphyrin heme (iron) groups that allow the enzyme to react with the hydrogen peroxide. Catalase has one of the highest turnover numbers of all enzymes; one catalase molecule can convert millions of molecule of H_2O_2 to water and oxygen each second. Catalase is an iron porphyrin enzyme which catalyses very rapid decomposition of hydrogen peroxide to water and oxygen (Nelson and Cox, 2000). The enzyme is widely present in nature which accounts for its diverse activities in soil. Catalase activity alongside with the dehydrogenase activity is used to give information on the microbial activities in soil. Both catalase and dehydrogenase activity are very sensitive to heavy metal pollution. The optimum pH for catalase is approximately neutral pH. Bacteria cannot protect themselves from the lethal effects of hydrogen peroxide, which is accumulated as a product of carbohydrate metabolism. Catalytic decomposition of hydrogen peroxide involves the reduction of trivalent ions (Fe^{3+}) to (Fe^{2+}) and the reoxidation of the latter by the oxygen.

The action of catalase is shown below:

$$2H_2O_2 \xrightarrow{\text{catalase}} 2H_2O + O_2$$

2.15.2 PHOSPHATASES (ACID AND ALKALINE)

Phosphatases are broad group of enzymes that are capable of catalyzing hydrolysis of esters and anhydrides of phosphoric acid. Apart from being good indicators of soil fertility, phosphatases play key roles in the soil system. Acid and alkaline phosphatases are the two forms of active phosphatases. These phosphatases are ubiquitous in plants, animals and microorganism. Both phosphatases in plants play a major role in the supply and metabolism of inorganic phosphate for the maintenance of cellular metabolism (Mishra and Dubey, 2008). Acid phosphatase (EC 3.1.3.2) and alkaline phosphatase (EC 3.1.3.1) are enzymes that catalyze the removal of inorganic phosphate (orthophosphate) from organic phosphate esters, in acidic and alkaline media respectively. Acid and alkaline phosphatases have been traditionally classified as being acid and alkaline, due to their optimum pH activity, above pH 7.0 or below pH 7.0 (Sharma *et al.,* 2004).

2.15.2.1 ALKALINE PHOSPHATE (ALP) (EC 3.1.1)

Alkaline phosphatase (EC 3.1.3.1) is a hydrolase enzyme responsible for removing phosphate groups from many types of molecules, including nucleotides, proteins, and alkaloids. The process of removing the phosphate group is called dephosphorylation. As the name suggests, alkaline phosphates are more effective in an alkaline environment. In humans, alkaline phosphatase is present in all tissues throughout the entire body, but is particularly concentrated in liver, bile duct, kidney, bone and placenta.

2.15.2.2 ACID PHOSPHATASE (ACP) (EC 3.1.3.2)

Acid phosphatase (ACP) (3.1.3.2) is a hydrolase that catalyses the same type of reactions as alkaline phosphatase (ALP). Acid phosphates are more effective in an acidic environment. In human, acid

phosphatase is found in the prostate, bone, liver, spleen, kidney, erythrocytes and platelets. The prostate is the richest source with many times the activity found in other tissues.

In soil ecosystems, both enzymes (acid and alkaline phosphatase) are believed to play critical roles in P cycles as evidence shows that they are correlated to P stress and plant growth.

2.15.3 LIPASE (EC 3.1.1.3)

Lipase or triacylglycerol (TAG) acylhydrolases (EC 3.1.1.3) is an important hydrolytic enzyme excreted to the environment by plant roots and microorganism. Under physiological conditions, this enzyme catalyses hydrolysis of oils and fats, as the biological role of lipase is metabolism of lipids (Nelson and Cox, 2004).

Besides, lipases catalyze various reactions other than hydrolysis, under controlled conditions. The capability of catalyzing various reactions makes lipases very useful biocatalysts for modification oil and fats, and other synthetic chemistry. Many pollutants such as organophosphorus pesticides and synthetic polyesters and polyamides can be degraded by lipase (Marten *et al*., 2003). In soil, enzymatic reactions occur in a heterogeneous rather than homogeneous environment. Catalytic function of lipase, an extracellular enzyme, is associated with soil clays. Consequently, properties and kinetic behavior such as enantioselectivity and activity of enzyme-clay complexes will differ from those of the free enzyme.

Lipase (and esterases) catalyzes three types of reactions. The catalytic action of lipases is reversible. They catalyze hydrolysis in an aqueous system, but also esterification (reverse reaction of hydrolysis) in a micro aqueous system, where water content is very low. Transesterification is categorized into four subclasses according to the chemical species which reacts with the ester. Alcoholysis is the reaction with an ester and an alcohol, while acidolysis is the one with an ester and an acid. Interesterification is a reaction between two different esters, where alcohol and acid moiety is

swapped. In aminolysis, an ester is reacted with the amine, generating an amide and an alcohol. There are big differences between the chemical and lipase catalyzed reactions.

Lipase degrades lipids derived from a large variety of microorganisms, animals, and plants. Recent works have shown that lipase is closely related with the organic pollutants present in the soil. Lipase activity was responsible for the drastic reduction in total hydrocarbon from contaminated soil (Margesin *et al.*, 2000; Riffaldi *et al.,* 2006). Lipases have been extracted from bacteria, plants, actinomycetes, and animal cell. Lipase enzymes can catalyze various reactions such as hydrolysis, interesterification, esterification, alcoholysis and aminolysis (Prasad and Manjunath, 2011). Lipases are ubiquitous enzymes which catalyze the hydrolysis of triacylglycerols to glycerol and free -fatty acids. Lipolytic reactions occur at the lipid water interface, where lipolytic substrates usually form equilibrium between monomeric, micellar and emulsified states. Lipases have been classified into two types on the basis of criteria such as (a) enhancement in enzyme activity as soon as the triglycerides form an emulsion and (b) lipases with the loop of protein (lid) covering on the active site (Sharma *et al*., 2011). Triglyceride is the main component of natural oil or fat. This can hydrolyze consecutively to diacylglycerol, monoacylglycerol, glycerol and fatty acids. Lipase adsorbs on the oil-water interface in the bulk of the water phase. The lipase then breaks the ester bonds of triolein to produce consecutively reaction stage. The glycerol formed is hydrophilic and thus dissolves into the water phase (Hermansyah *et al*., 2007).

Lipase activity was found to be the most useful indicator parameter for testing hydrocarbon degradation in soil (Margesin *et al.*, 2000; Riffaldi *et al.*, 2006).

2.16 ADVANTAGES OF SOIL MICROBIAL ENZYMES

- Release of nutrients into the soil by means of organic matter degradation.
- Identification of soil types.
- Identification of microbial activity.
- Serve as bio indicators of ecological change.

2.16.1 MECHANISM OF THE ADVANTAGES OF SOIL MICROBIAL ENZYMES.

2.16.1.1 RELEASE OF NUTRIENTS INTO THE SOIL BY MEANS OF ORGANIC MATTER DEGRADATION.

Bacteria degrade compounds by producing very specific enzymes. Bacteria are extremely efficient "enzyme factories". They recognize the organics present in an environment and respond by producing the enzymes required to degrade those specific organics. The breakdown of waste (biodegradable) is a complicated metabolic pathway in which each step requires a specific enzyme. For example, a triglyceride is initially cleaved by lipase into a glycerol and three fatty acids. The glycerol and each fatty acid are then broken into smaller and smaller components by specific enzymes until eventually these compounds are reduced to carbon dioxide and water (Nelson and Cox, 2004).

Due to the insolubility of waste (biodegradable), microorganisms degrade waste (biodegradable) by acting at the oil/water interface. Bacteria themselves may produce biosurfactants that assist in the degradation by making the waste more available by emulsification. Microbes tend to collect at the surface of waste and oil due to the oleophilic nature of the cell wall and due to a property termed "chemo taxis". Chemo taxis is the ability whereby bacteria recognize compounds as food sources, and, if motile, move in the direction of that food source. Bacteria increase contact with particulate food sources by the production of extracellular polymers that allow bacteria to adhere to surface such

as waste, oil, scum, and walls of collection systems. Once attached, the organisms produce enzymes to breakdown waste globules into molecules small enough to be transported into the bacterial cell to be broken into progressively smaller particles.

A consortium of bacteria, rather than a single strain, is most effective in breaking down waste and other complex wastes. A synergistic blend of selectively adapted microorganisms added to the indigenous population increases the speed and scope of degradation.

Each time waste is broken down into a smaller particle, the bacterium gains energy. This energy is utilized to produce more enzymes and for cell growth. Attached microorganisms continue to grow and produce enzymes for biodegradation. Some new cells are released into the liquid stream while others further colonize the biofilm.

2.16.1.1.1 MECHANISM:

Bacterial or enzyme digestion is the process of bacteria, consuming organic matter. Enzymes act to break the organic matter into water soluble nutrients, which the bacteria digest. Using complex chemical reactions, the organic waste is metabolized down to water and carbon dioxide (the final metabolic waste products), providing the bacteria with energy for growth and reproduction. Microbes excrete specialised enzymes that digest biodegradable waste constituents (e.g. cellulose and other complex polysaccharides, proteins and fats) into simple nutrients (e.g. sugars, amino acids, fatty acids), which they absorb. As the microbes grow and proliferate a significant proportion of this is converted into heat, carbon gases and water, which can result in large losses in mass during biological treatment (Riffaldi *et al.*, 2006). It may be simply shown by the following equation:

Aerobic Digestion:

Organic waste + water -----**Enzyme**----> water soluble nutrients + oxygen ----**Bacteria**---> water + carbon dioxide

Anaerobic Digestion:

Organic waste + Water -----Enzyme------>Water Soluble Nutrients ---------Bacteria-------> Water + biogas (Carbon Dioxide+ Methane CH_4)
40% **60%**

Organic waste is consumed by the bacteria, used as nutrients by the bacteria, and is no longer present to produce odors, sludge, pollution, or unsightly mess. Anaerobic digestion (AD) is a natural process in which micro-organisms break down the organic matter found in wet biomass waste (such as sewage sludge, animal manure and slurry and waste food) in the absence of oxygen, to produce biogas (mainly a mixture of around 60% methane and 40% carbon dioxide) and digestate (a nitrogen rich fertilizer). The biogas can be burned directly in a gas boiler to produce heat or burnt in a combined heat and power (CHP) unit to produce heat and electricity. Alternatively, the biogas can be cleaned to remove the carbon dioxide and other substances, to produce biomethane. This can be injected into the national gas grid to be used in the same way as natural gas, or used as a vehicle fuel (Riffaldi *et al.,* 2006).

2.16.1.1.2 PROCESS STAGES

The four key stages of anaerobic digestion involve hydrolysis, acidogenesis, acetogenesis and methanogenesis. The overall process can be described by the chemical reaction, where organic material such as glucose is biochemically digested into carbon dioxide (CO_2) and methane (CH_4) by the anaerobic microorganisms.

$C_6H_{12}O_6 \rightarrow 3CO_2 + 3CH_4$

2.16.1.1.2.1 HYDROLYSIS

In most cases, biomass is made up of large organic polymers. For the bacteria in anaerobic digesters to access the energy potential of the material, these chains must first be broken down into their smaller constituent parts. These constituent parts, or monomers, such as sugars, are readily available to other bacteria. The process of breaking these chains and dissolving the smaller molecules into solution is called hydrolysis. Therefore, hydrolysis of these high-molecular-weight polymeric components is the necessary first step in anaerobic digestion. Through hydrolysis the complex organic molecules are broken down into simple sugars, amino acids, and fatty acids.

Acetate and hydrogen produced in the first stages can be used directly by methanogens. Other molecules, such as volatile fatty acids (VFAs) with a chain length greater than that of acetate must first be catabolised into compounds that can be directly used by methanogens (Nelson and Cox, 2004).

2.16.1.1.2.2 ACIDOGENESIS

The biological process of acidogenesis results in further breakdown of the remaining components by acidogenic (fermentative) bacteria. Here, VFAs are created, along with ammonia, carbon dioxide, and hydrogen sulfide, as well as other byproducts. The process of acidogenesis is similar to the way milk sours.

2.16.1.1.2.3 ACETOGENESIS

The third stage of anaerobic digestion is acetogenesis. Here, simple molecules created through the acidogenesis phase are further digested by acetogens to produce largely acetic acid, as well as carbon dioxide and hydrogen.

2.16.1.1.2.4 METHANOGENESIS

The terminal stage of anaerobic digestion is the biological process of methanogenesis. Here, methanogens use the intermediate products of the preceding stages and convert them into methane, carbon dioxide, and water. These components make up the majority of the biogas emitted from the system. Methanogenesis is sensitive to both high and low pH and occurs between pH 6.5 and pH 8 (Riffaldi *et al.,* 2006). The remaining, indigestible material the microbes cannot use and any dead bacterial remains constitute the digestate.

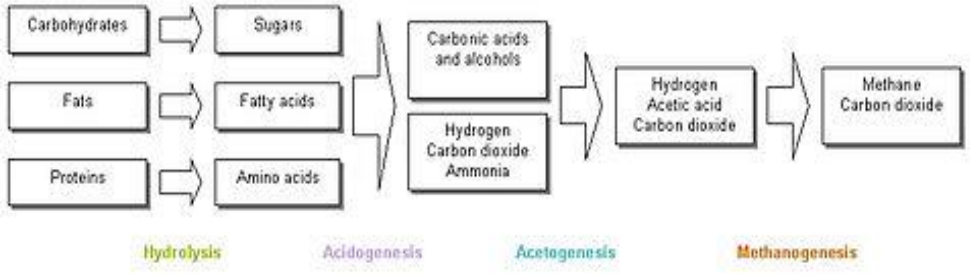


Figure 2.8: The key stages of anaerobic digestion (Riffaldi *et al.,* 2006)

2.16.2 IDENTIFICATION OF SOIL TYPES

Different soil types possess different physical properties such as air content, water retention, texture, nutrient content etc. These physical properties affect the kinds and number of microbes that are found in the soil. Hence, affects the type and quantity of the microbial enzyme produced (Waldrop *et al*., 2000).

2.16.3 IDENTIFICATION OF MICROBIAL ACTIVITY

Soil enzymatic activities have been related to soil physio-chemical characteristics, microbial community structure (Waldrop *et al.,* 2000), disturbance and succession.

Also microbial activities can be identified by measuring activities against target substrates from the major nutrient resources, distinguishing different reaction mechanisms to activities within a given

enzyme function (e.g proteolysis) and the possible determination of enzyme sources. Plant growth should be the cause of concern on enzyme activities in soils in terms of evaluating the impact of fertilizers.

2.16.4 SOIL MICROBIAL ENZYMES SERVES AS BIO INDICATORS OF ECOLOGICAL CHANGE

Changing land use from one type to another generally affects the soil ecosystem status. Land use and management practices alter the total amount and composition of soil organic matter and significantly changes the enzyme activity. Natural systems changed to agricultural systems not only affect vegetation, but also biological properties are altered in soil ecosystem. Fertilization of soil is conducted in soils by using different fertilizers such as mineral, manure, compost and vermin compost, farm yard manure enhances microbial biomass, urease, deaminase and alkaline phosphate activities in soil compared with other treatments under rotations. Organic amendments influence soil microbes and biochemical properties in different ways depending on the nutrient content. Xenobiotics are of crucial concern in the soil environment as they could affect many biological and biochemical reactions in the soil.

2.17 OTHER CHARACTERISTICS OF THE SELECTED POLLUTED SITE

2.17.1 HYDROCARBON POLLUTED SITE

Pollution caused by crude oil is the most prevalent problem in the environment. The contamination of the environment (mainly terrestrial and aquatic) by crude oil is therefore referred to as crude oil pollution and it is estimated that 80% of crude oil pollution is as a result of spillage. Unrefined petroleum or crude oil is a complex substance found in deposits throughout the world. In a natural state, this material consists of thousands of distinct hydrocarbon compounds. All chemical

compounds known as hydrocarbons are composed of various arrangements of carbon and hydrogen atoms, but those constituting crude oil can be divided into three main groups; saturated and unsaturated aliphatic and aromatic hydrocarbons.

Saturated hydrocarbons also known as alkanes have the maximum number of hydrogen atoms possible and therefore no double bonds. Alkanes can be linear, branched or cyclic structures. Unsaturated compounds do not possess the full complement of hydrogen atoms, but instead have formed either one double carbon bond (alkenes), two double carbon bonds (dienes) or at least triple carbon bonds (alkynes). Aromatic hydrocarbons consist of an aromatic ring, which contain six carbon atoms connected with three alternating double bonds .The BTEX compounds namely benzene, toluene, ethylbenzene, xylene are examples of aromatic hydrocarbons. Aromatic molecules which contain two or more fused aromatic rings are referred to as polycyclic aromatic hydrocarbons or PAHs.

Crude oil also contains some inorganic elements such as sulphur, nitrogen, phosphorus; trace elements such as vanadium, nickel, aluminum, copper, and some heavy metals like cadmium and lead. There are a variety of pollutants affecting soil and subsoil such as fuel and oil products, hydrocarbon residues, crude oil, other products resulting from the operation (saturated and unsaturated aliphatic hydrocarbons, and the polycyclic aromatic). These types of products (mainly hydrocarbons) have a harmful risk, affecting the quality of groundwater, which become unfit for use for a long time (drinking water, irrigation, and different industrial use). It also poses risks to human health, biological environment and vegetation. Aromatic compounds having a strong feature of mutagenic and carcinogenic and not least, affect the environment security, presenting risks of explosion and fire, when the floating oil reach the groundwater in the basement of various buildings (Engelking, 2000).

2.17.1.1 IMPACT OF CRUDE POLLUTION

A review of studies of the effect of crude oil pollution showed the widespread presence of constituents of crude oil in the bio-physical environment of the impacted areas. The presence and quantity of these constituents are known to be capable of exerting some acute and long-term adverse health effects. Known carcinogens like benzo(a)pyrene and polycyclic aromatic hydrocarbons (PAHs) were respectively, found in the surface water and soil of the impacted communities. Like other known carcinogens, they do not have any safe levels, as even a few molecules of these can be genotoxic.

Oil spills affect plants by creating conditions which make essential nutrients like nitrogen and oxygen needed for plant growth unavailable for them. Plants germinate, develop and grow in soil medium where water, air, and nutrients resources supply plants for healthy growth for productive and profitable agriculture. Frequent crude oil spillage on agricultural soils and the consequent fouling effect on all forms of life; render the soil (especially the biological active surface layer) toxic and unproductive. The soil renders the soil's fertility such that most of the essential nutrients are no longer available for plants and crops utilization (Engelking, 2000). Spilled crude-oil which is denser than water reduces and restricts permeability: organic hydrocarbons which fill the soil pores expels water and air, thus depriving the plant roots the needed water and air.

CHAPTER THREE

MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Equipment and Apparatus

Table 1: Showing the underlisted equipment, their model and manufacturer used in carrying this research.

Equipment and Apparatus	MODEL	Manufacturer
Dry oven	DHG-9101-ISA	Searchtech,UK
Electrothermal incubator	DNP	Searchtech, UK
UV-VIS spectrophotometer	STECRRUMLAB 755S	Searchtech, UK
pH meter	PHS-3	Searchtech,China
Deionizer	DEIONIZER 50	Searchtech, UK
Binocular microscope	B-BRAN	Searchtech, UK
America pressure sterilizer	AUTOCLAVE	Wisconsin aluminium foundry,US

3.1.2 Chemicals and Reagents

Table 2: The underlisted chemicals and reagents are used in carrying this laboratory analysis

Chemicals and Reagents	MANUFACTURER
Sabourand dextrose agar	LABM
Nutrient agar	LABM
Sulphuric acid	BDH
Hydrochloric acid	BDH
Sodium hydroxide	M and B
Petroleum ether	MERCK
Methyl red	BDH
Bromocressol green	M and B
Iodine	MERCK
Safranine	BDH
Mineral salt agar	LABM
Potassium permanganate	MERCK
Tetraoxosulphate (IV)acid	MERCK
Hydrogen peroxide	TRODAT CHEMICALS
4-Aminoantipyrine	MERCK
Potassium ferricyanide	MERCK
Ethyl alcohol	TRODAT CHEMICALS
Alcohol phenolphthalein	MERCK
Deionized water	FISHER SCIENTIFIC

3.2 METHODS

3.2.1 Study area

Gases and Soil samples used for this study were collected from different locations in Federal University of Technology, Owerri (FUTO) in ihiagwa/ North -West local government area of Imo State. Seven different gas samples (CO, NO_2, H_2S, SO_2, Pm_{10}, CH_4 (flammable gas), NH_3) were collected from four different locations; Publc Procument office (location 1), Okbol cyber services popularly known as BJ services (location 2), Mr Umunnakwe business center (location 3) and business center behind the old registry . The selected areas for this investigation were areas with high traffic and business activities. These areas were busy within the hours of 8.00am –5.30pm at the close of work and business activities. Four sample points were chosen in each of the locations in order to capture concentration of pollutants within that location.

Location one (1) was the Public procurement office with latitude 05^o 22.808'N and longitude 006^o 59.803' E with 224.0ft elevation. Location two (2) was 'BJ Business center' with latitude 05^o 22.772' N and latitude 006^o 59.792'E with 202.5ft elevation. Location three (3) was Mr. Umunnakwe business center with latitude 05^o 22.773'N and longitude 006^o 59.774'E with 195.9ft elevation and Location four (4) was the business center behind the old registry with latitude 05^o 22.729' N and latitude 006^o 59.840'E with elevation 210.6ft for 5 days (13^{th} -17^{th} June, 2016) at morning and evening sessions. Location one (1) known as public procurement office was characterized by offices. Location two (2) otherwise known as BJ business services was characterized with offices (administrative and internet services), shops with regular use of generator and other gasoline-powered equipment. The sale of food was also noticed around the site and it has around it a T-junction and a mini-park for cars and motorcycles. Location three (3) was an enclosure that houses a business (mainly administrative work) center and stationary shops. Location four was an enclosure that houses

a business center. It also had around it a football viewing center and the sale of food was carried out there.

Twenty –seven (27) different soil samples were also collected from generator fume polluted site at location two (2) known as 'BJ business centre' i.e 9 different soil samples from the top soil, 9 different soil samples from 1 m depth sub soil and 9 different soil samples from 1m depth sub-sub soil. 'BJ business centre' was situated at the northern apex area of Federal University of Technology Owerri (FUTO) between latitude 05^{o} 22.772' N and longitude 006^{o} 59.792' E. This location was characterized by high population density, high energy demand and erratic power supply, thus making it a suitable location for this study. The control was collected from the Department of Biochemistry surroundings at Federal University of Technology, Owerri (FUTO), which was regarded as an unpolluted site. All these areas lie entirely within Imo State in the Southeastern Nigeria. The soil samples were labeled accordingly.

3.2.2 Preparation of Soil Samples

Each soil sample was divided into two; one set of the samples from each pair was isolated and inoculated for the microbial (bacteria and fungi) identifications. The other set for enzyme identification was stored in polyethylene plastic bags at cold temperature of below 15 ^{o}C to avoid the loss of moisture, inhibition of microbial activities and enzymatic reactions.

3.2.3 Collection of Gas Samples

The concentrations of carbon monoxide, hydrogen sulphide and sulphur dioxide, ammonia, nitrogen oxide, particulate matter and flammable gases were determined from four different sites around the business location in Federal University of Technology Owerri (FUTO). A total of four hundred and twenty (420) readings were taken for the seven (7) gases using the automatic gas sampler (EEx)

between 13th June and 17th June, 2016 with sampling times between 8:00am and 5:30pm. Appropriate gas sensor models were used for different gases.

3.2.4 ASSAY OF ENZYMATIC ACTIVITIES

3.2.4.1 Assay of Soil Lipase Activity

Principle

The titration method of Lorentz, (1998) for lipase assay was used. Lipase activity was measured by titrating the fatty acid released with 0.1mol NaOH using 0.1 % alcoholic phenolphthalein as indicator. In this procedure, native substrate (triacylglycerol) was hydrolyzed to yield fatty acid. Reactivity of sample was quenched after few minutes by addition of ethanol. The amount of fatty acids released during the reaction was determined by direct titration with 0.1mol NaOH to an alcoholic phenolphthalein end point. Fresh palm oil was used as source of glyceride.

Procedure

Ten grams of soil sample was mixed with 10ml of 1.107M of acetate buffer for enzyme extraction. The mixture was stirred with stirring rod and centrifuged at 3000 rev/mins for 10 mins. The supernatant served as source for crude enzyme. Into a glass stopper Erlenmeyer flask, 10 ml of acetate buffer, 1ml of hexane, and 1ml of fresh palm oil as source of glyceride were added. The contents were stirred for 5 mins for thorough mixing. Two millilitre of the crude enzyme solution was added with vigorous shaking. The set up was allowed to stand for 20 mins with continuous shaking manually for hydrolysis to take place.

At the end of the hydrolysis, 20 ml of ethanol was added. The liberated fatty acid was titrated against 0.1 M NaOH. The determination of blank was also carried out the same way without the addition of the enzyme solution.

Calculation

The activity of the enzyme was expressed in arbitrary units- one arbitrary unit of enzyme activity corresponds to 1ml of 0.1 M NaOH required to neutralize the fatty acid liberated during the incubation period of 20minutes.

3.2.4.2 Assay of Soil Alkaline Phosphatase Activity

Alkaline phosphatase activity was determined by Kind and King Method (1954).

Principle

Alkaline phosphatase (ALP) at an alkaline pH of 9.8 catalyzes the degradation of its substrate (phenyl phosphate) to phenol, phosphate and water. The phenol condenses with aminoantipyrine and then oxidized with alkaline ferricyanide to give red complex, whose intensity at 520 nm is proportional to the alkaline phosphate activity.

Phenyl Phosphate $\xrightarrow[\text{pH 9.8}]{\text{Alkaline Phosphatase}}$ **Phenol + Phosphate + water**

Phenol + 4-Aminoantipyrine $\xrightarrow[K_3Fe(CN)_6]{\text{Alkaline medium}}$ **Red coloured complex**

Procedure

Three different tubes were labeled as follows: T (Test), S (Standard), and B (Blank). One milliliter of phosphate buffer was mixed with 1.0 ml of phenyl phosphate while 1.0ml of phosphate buffer and 1.0 ml of distilled water was added to the standard and the blank test tubes. The mixtures were allowed to incubate for 5 mins at 37 ^{o}C. Then, 0.1 ml of the soil enzyme solution was added to the test, while 0.1 ml of distilled water was added to both standard and blank. The mixtures were also allowed to incubate for 5 mins at 37 ^{o}C. Then, 1.0 ml of alkaline phosphatase and 1.0 ml of distilled

water was added to both standard and the blank test tubes, respectively. The mixtures were incubated again at 37 ºC for 15 mins and 0.8 ml of 0.5N NaOH was added to the tubes and the mixtures was allowed to cool. Then 1.2 ml of $NaHCO_3$, 1.0 ml of 4-aminoantipyrine and 1.0 ml of potassium ferricyanide were added to each of the test tubes. The absorbance of the test and standard mixtures were read against the reagent blank after 5 mins using 1 cm light path cuvette in a spectrophotometer at 410 nm.

Calculation:

$$\text{ALP activity } (1.U.L^{-1}) = \frac{\textbf{Absorbance of test x Conc. of standard}}{\textbf{Absorbance of standard}}$$

3.2.4.3 Assay of Soil Acid Phosphatase Activity

Acid phosphatase activity was assayed by Kind and King, (1954)

Principle

Acid phosphatase (ACP) at pH of 4.85 catalyzes the degradation its substrate (phenyl phosphate) to phenol, phosphate and water. The phenol condenses with aminoanti-pyrine and then oxidized with alkaline ferricyanide to give complex, whose intensity at 520 nm is proportional to the acid phosphatase activity.

Phenyl phosphate $\xrightarrow{\text{Acid phosphatase}}$ **Phenol + phosphate +water**

Phenol + 4-Aminoantipyrine $\xrightarrow[K_3Fe(CN)_6]{\text{Acid medium}}$ **Red coloured complex**

Procedure

Three different test tubes were labelled as follows: T (test), S (Standard), B (Blank). One milliliter of citrate buffer was mixed with 1.0 ml of phenyl phosphate, while 1.0 ml of citrate buffer and 1.0ml

of distilled water were added to the standard and blank test tubes. The mixtures were allowed to incubate for 5 mins at 37 ^{o}C. Then 1.0 ml of soil enzyme solution was added to the test, while 0.1 ml of distilled water was added to both the standard and the blank. The mixtures were also allowed to incubate for 5 mins at 37 ^{o}C. Then 1.0ml of acid phosphatase and 1.0 ml of distilled water was added to the standard and the blank test tubes respectively. The mixtures were incubated again at 37 ^{o}C for 15 mins and 0.8 ml of 0.5 N NaOH was added to the tubes and the mixture was allowed to cool. Then 1.2 ml of $NaHCO_3$, 1.0 ml of 4-aminoantipyrine and 1.0 ml of potassium ferricyanide were added to each of the test tubes. The absorbance of the test and standard mixtures were read against the reagent blank after 5mins using a 1cm light path cuvette in a spectrophotometer at 410 nm.

Calculation

ACP activity ($1.U.L^{-1}$) = $\frac{\textbf{Absorbance of test x Conc. of standard}}{\textbf{Absorbance of standard}}$

3.2.4.4 Assay of Soil Catalase Activity

Catalase activity was assayed according to Cohen *et al*., 1970.

Procedure

0.5 ml Aliquot of extract –homogenate was added to ice cold tubes. Reaction started by adding 5 ml of 30 mM H_2O_2. Tube contents were then mixed thoroughly by inversion. Reaction was stopped after 3 mins with 1 ml of 6 M H_2SO_4. 7 ml of 0.01 M potassium permanganate ($KMnO_4$) was then added and absorbance read at 480 nm within 30-60 sec.

Calculation

Catalase enzyme activity = $\frac{\textbf{Absorbance/min x V x1000}}{\textbf{M x v x W}}$

V= total volume of the reaction mixture.

M= molar extinction which is 40.0.

W= Weight of tissue.

v = volume of sample used.

3.2.5 ENUMERATION OF MICROORGANISMS

Enumeration of hydrocarbon utilizing microorganism was done using the mineral salt medium of described by Okpokwasili and Okorie (1988).

Procedure

The mineral salt agar comprising per distilled water, NaCl 10 g, $MgSO_4$ $7H_2O$ 0.42 g; KCl 0.29 g; K_2HPO_4 1.2 g, KH_2PO_4 0.83 g; $NaNO_3$ 0.42 g, agar 15 g, pH 7.2 was used.

To 990 ml of the mineral salt medium in conical flasks was added 10ml of spent engine oil which served as sources of carbon. However, of the hydrocarbon utilizing fungi, the medium was supplemented with an antibiotics chloramphenicol. The hydrocarbon utilizers were then enumerated after plating in duplicate using pour plate technique, 0.1 ml of the appropriate dilutions of the samples on the petri dishes. The molten mineral salt agar medium and the ones containing antibiotics at 45 ^oC were poured into the petri dishes for the isolation of hydrocarbon utilizing bacteria and fungi respectively. These were swirled to mix, allowed to solidify and incubated. Enumeration of the hydrocarbon utilizers were performed after incubation at 25 ^oC for fungi for 7days and 37 ^oC for bacteria. Colonies of the hydrocarbon utilizing bacteria growing on the agar plates were counted, isolated, purified by streaking on nutrient agar plates and kept on nutrient agar slant as stock cultures for characterization and identifications. In the case of hydrocarbon utilizing fungi, the isolates were streaked to purity onto sabourand dextrose agar plates and kept on sabourand and agar slants as stock cultures for characterization and identification.

3.2.6 AIR QUALITY ANALYTICAL METHODS

Procedure

The air pollutants SO_2, CO, NO_2, H_2S, flame, NH_3 and $PM_{10,}$ commonly used in air quality index (AQI) assessment were sampled two (2) times a day (morning and evening). Gasman potable digital Air Quality monitoring pieces of equipment were used for the monitoring of air pollutants in the study area. At each designated position the instrument was held at arm's length in an open space. The knob was adjusted to TEST position and allowed to stand for two (2) minutes. The instrument was then adjusted to GAS position and reading taken in triplicates when the display on LCD was steady. The model of instruments used and their respective gases monitored are shown in table 3. Suspended Particulate Matter (SPM) was measured in locations studied using Haz- Dust ^{TM} 10µ/m^3 particulate monitor. Particulate matter was determined by the difference in weight of filter before and after following the instructions of the manufacturer.

Table 3: Equipment used in determining the Air pollutants

Parameters	Equipment's Model	Range	Alarm level
Nitrogen (IV) Oxide (NO_2)	NO_2 gas monitor Gasman model 1983IH	0-10 ppm	3 ppm
Sulphur (IV) Oxide (SO_2)	SO_2 gas monitor model19648H	0-10 ppm	2 ppm
Hydrogen Sulphide(H_2S)	H_2S gas monitor model 19502H	0-50 ppm	50 ppm
Carbon (II) Oxide	CO gas monitor model 19252H	0-500 ppm	50 ppm
Ammonia (NH_3)	NH_3 gas monitor model 19812H	0-5 ppm	25 ppm
Suspended particulate Matter (SPM)	Haz-Dust TM 10 μ/m^3 particulate monitor	0.01-200 $\mu g/m^3$	1.50 $\mu g/m^3$

3.2.7 STATISTICAL ANALYSIS

The data collected was subjected to statistical analysis using the graph pad prism software version 5.0. One Way Anova was used in the analyses with significant level set at $p \leq 0.05$.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 RESULTS

4.2 ENZYME ACTIVITIES IN THE SOIL SAMPLE

The prolonged exposure to generator fumes altered soil catalase activity significantly at $p< 0.05$. In figure 4.1 (top soil-1m away), there was significant increase ($p< 0.05$) in the activity of catalase on polluted soils (loc W(west) when compared with control (unpolluted) site. Also, there was a significant reduction at $p<0.05$ at loc S(south), E(east) and no significant difference at $p < 0.05$ at loc N(north) and Epi(epicenter) when compared with control (unpolluted) site. In figure 4.4 (top soil-2m away), there was significant increase at $p< 0.05$ at loc W, S, and significant reduction at $p<0.05$ at loc E and N when compared with control (unpolluted soil). No significant difference at loc epi. In figure 4.2 (sub soil-1m), there was significant increase at $p< 0.05$ at loc S, W and significant reduction at $p< 0.05$ at loc E, N and epi when compared with the control. In figure 4.5 (sub soil -2ms), there was a significant difference (increase) at $p<0.05$ at W, N and significant reduction at $p<0.05$ at loc S, E and epi when compared with the control (unpolluted) soil. In figure 4.3 (sub-sub soil -1m apart), there was a significant difference (increase) at $p<0.05$ at loc W and a significant reduction at $p<0.05$ at loc S, E, N, epi when compared with the unpolluted soil (control). In figure 4.6 (sub-sub soil-2meters apart), there was a significant increase at $p<0.05$ at loc W, S and a significant reduction at $p<0.05$ at loc E, N, and epi when compared with the control (unpolluted) soil.(See Appendix).

In figure 4.7 (top soil (1m apart), there was significant difference in the activity of lipase at $p< 0.05$ when control (unpolluted soil) was compared with loc S(South), E(East), W(West) and epi(epicenter) but no significant difference at $P<0.05$ when compared with loc N(North). There was significant increase at $p<0.05$ at loc E, W, S and epi. In figure 4.10 (2m apart -top soil), there was significant

increase at p <0.05at loc E, W, significant reduction at p< 0.05 at loc epi and no significant difference at p > 0.05 at loc S, N when compared with control (unpolluted soil). In figure 4.8 (Sub soil -1m apart), there was significant difference (increase) at p< 0.05 at loc loc E, W, epi when compared with control (unpolluted soil). There was a significant reduction at p < 0.05 at loc epi and no significant difference at p> 0.05 at loc N and S when compared with control (unpolluted soil). In figure 4.11 (2meters apart- sub soil, there was significant difference (increase) at p< 0.05 at loc E, W when compared with control (unpolluted soil). There was also a significant reduction at p < 0.05 at loc epi. In figure 4.9 (Sub-Sub soil -1m apart), there was significant difference (increase) at p <0.05 at loc E, W, Epi when compared with control (unpolluted soil). Also, there was a significant reduction at p < 0.05 at loc S, N. In figure 4.12 (sub-sub soil-2 meters apart, there was a significant increase at p < 0.05 at E, W, Epi, N and significant reduction at p < 0.05 at loc S when compared with the control (unpolluted soil).

In figure 4.13 (top soil -1m apart), there was significant difference (increase) in the activity of acid phosphatase at P< 0.05 when control (unpolluted soil) was compared with sample loc W but no significant difference at P<0.05 at loc S, E, N and epi. In figure 4.16 (top soil-2ms apart, there was a significant reduction at p<0.05 when sample location S was compared with control (unpolluted soil) but no significant difference at p> 0.05 at sample loc E, W, N and epi. In figure 4.14 (sub soil -1m apart), there was significant difference (increase) at P< 0.05 with sample location N and a significant decrease at p< 0.05 with sample location E, W, epi when compared with the control (unpolluted soil). No significant difference at p> 0.05 with sample loc S when compared with the control. In figure 4.17 (sub soil-2ms apart, there was a significant increase at p<0.05 at loc W and a significant reduction at p<0.05 at loc S, E, N, epi. In figure 4.15 (sub -sub soil -1m apart), there was a significant difference (increase) at p<0.05 at loc S, a significant reduction at p<0.05 at loc E, W, N and no significant difference at P<0.05 at loc epi when compared with the control (unpolluted soil). In figure

4.18 (sub sub-2ms apart, there was a significant reduction ($p<0.05$) at loc S, E, W, N, when compared with control (unpolluted soil). No significant difference at $p> 0.05$ at loc epi when compared with control.

The effect of prolonged exposure to generator fumes on soil alkaline phosphatase activity was significantly ($p<0.05$) different. In figure 4.19 (top soil -1m apart), there was a significant difference (increase) at $p<0.05$ at sample loc S and a significant reduction at $p<0.05$ at loc E, W and epi when compared with control (polluted soil). No significant difference at $P<0.05$ at loc N. In figure 4.22 (top soil-2ms apart, there was a significant difference (reduction) at $p<0.05$ at loc W, N and epi when compared with control (unpolluted soil). Also, no significant difference at $p< 0.05$ at loc S and E when compared with the control. In figure 4.20 (sub soil -1m apart), there was a significant difference (increase) at $p< 0.05$ at sample loc E, a significant reduction at $p<0.05$ at sample loc N when compared with the control. No significant difference at $p< 0.05$ at loc S, N and epi when compared with the control (unpolluted soil). In figure 4.23 (sub soil-2ms apart, there was a significant difference (reduction) at $p<0.05$ at sample loc W, N and epi, also no significant difference at $p<0.05$ at sample loc S and E when compared with the control (unpolluted) site. In figure 4.21 (sub-sub soil -1m apart), there was a significant difference (increase) at $p< 0.05$ at loc E and W when compared with control. No significant difference at $p<0.05$ at sample loc S, N and epi. In figure 4.24 (sub -sub soil - 2ms apart, there was a significant difference (increase) at $p<0.05$ at sample loc E and W when compared with control. No significant difference at $p<0.05$ at sample loc S, N and epi when compared with the control (See appendix).

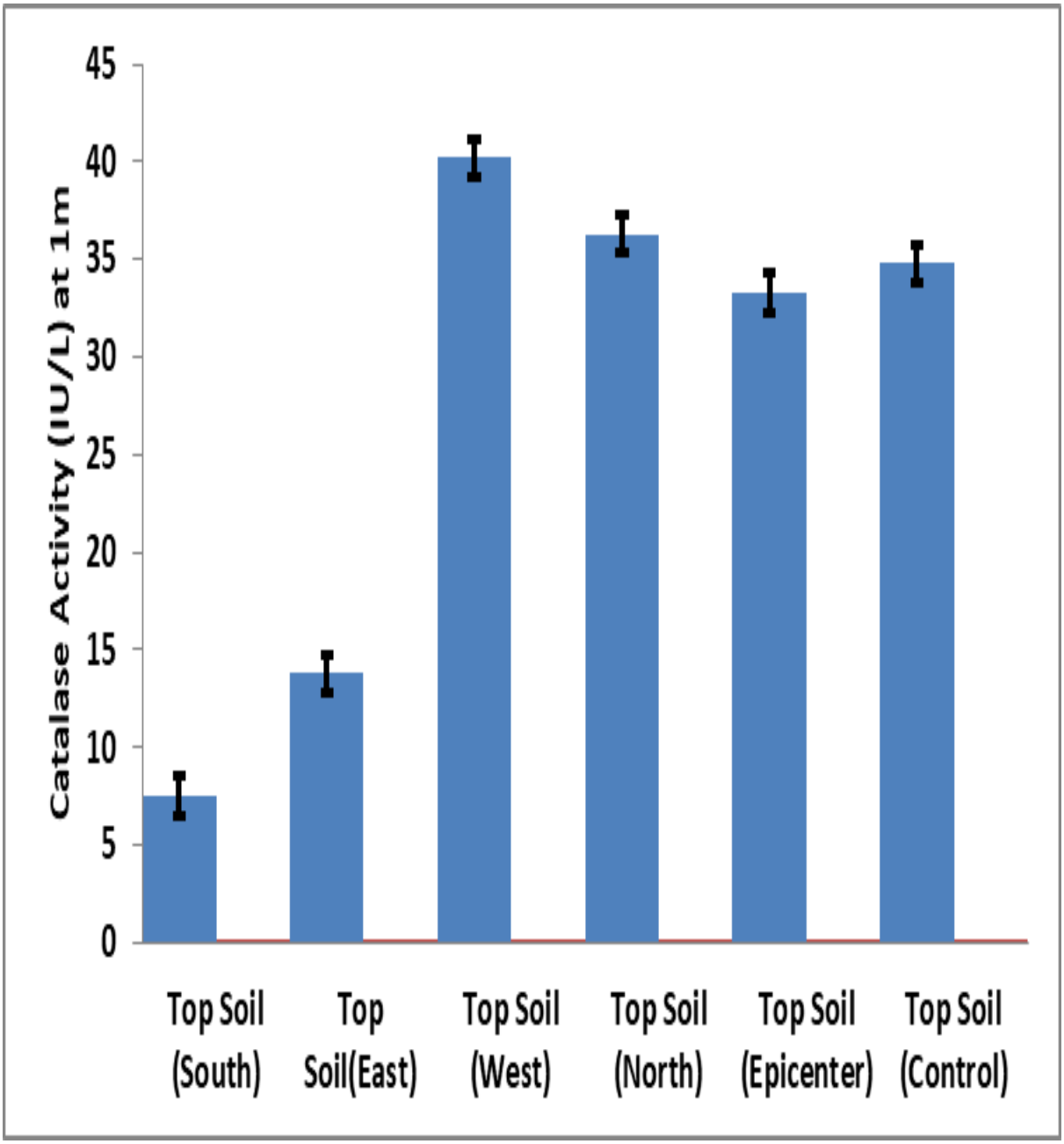


Figure 4.1: Effect of prolonged exposure to generator fume on soil catalase activity in top soil (1m apart).

Bars are presented as means± standard errors of mean (SEM) of triplicate determinations at $p < 0.05$.

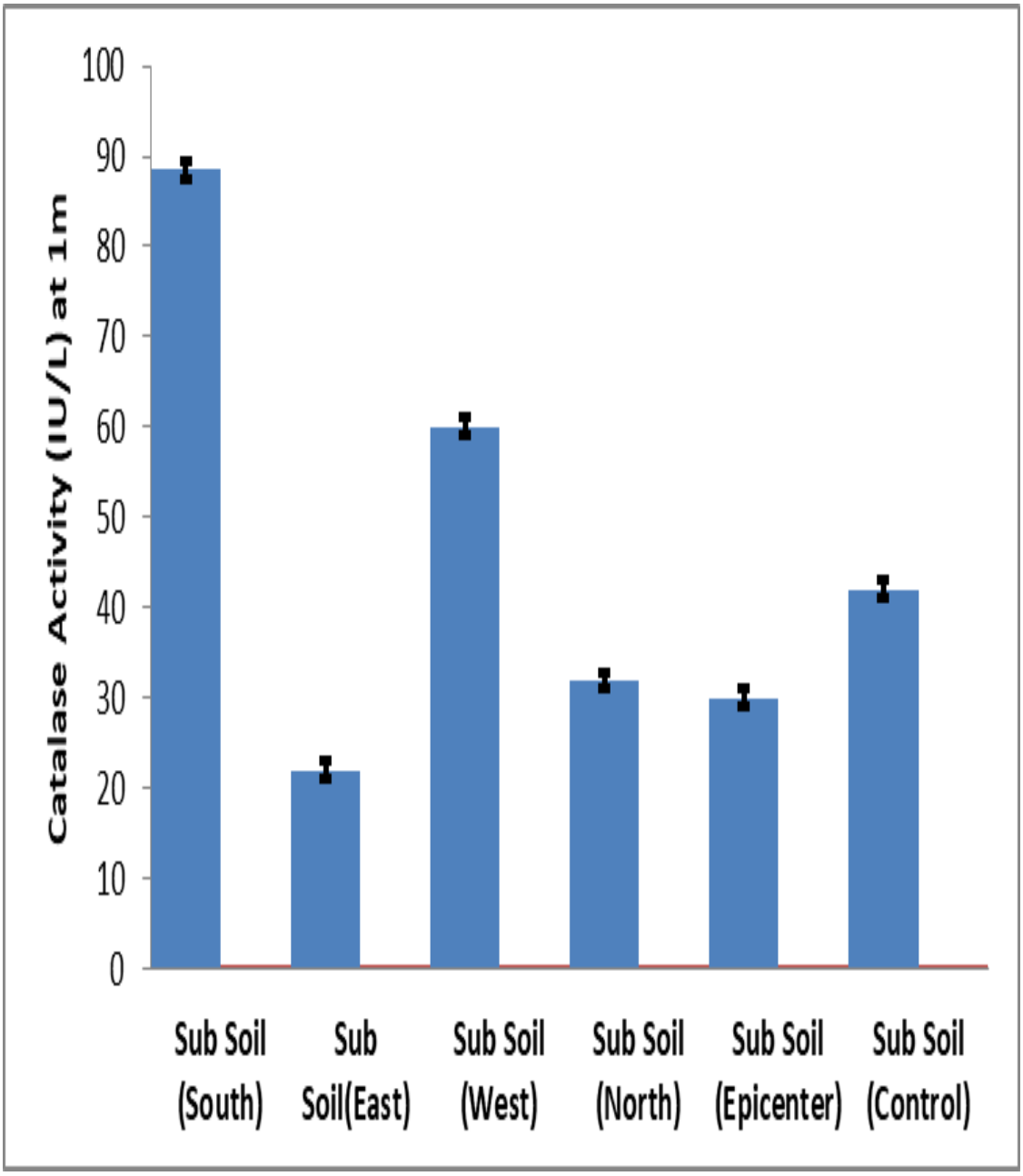


Figure 4.2: Effect of prolonged exposure to generator fume on soil catalase activity in Sub soil (1m apart).

Bars are presented as means± standard errors mean (SEM) of triplicate determinations at $p < 0.05$.

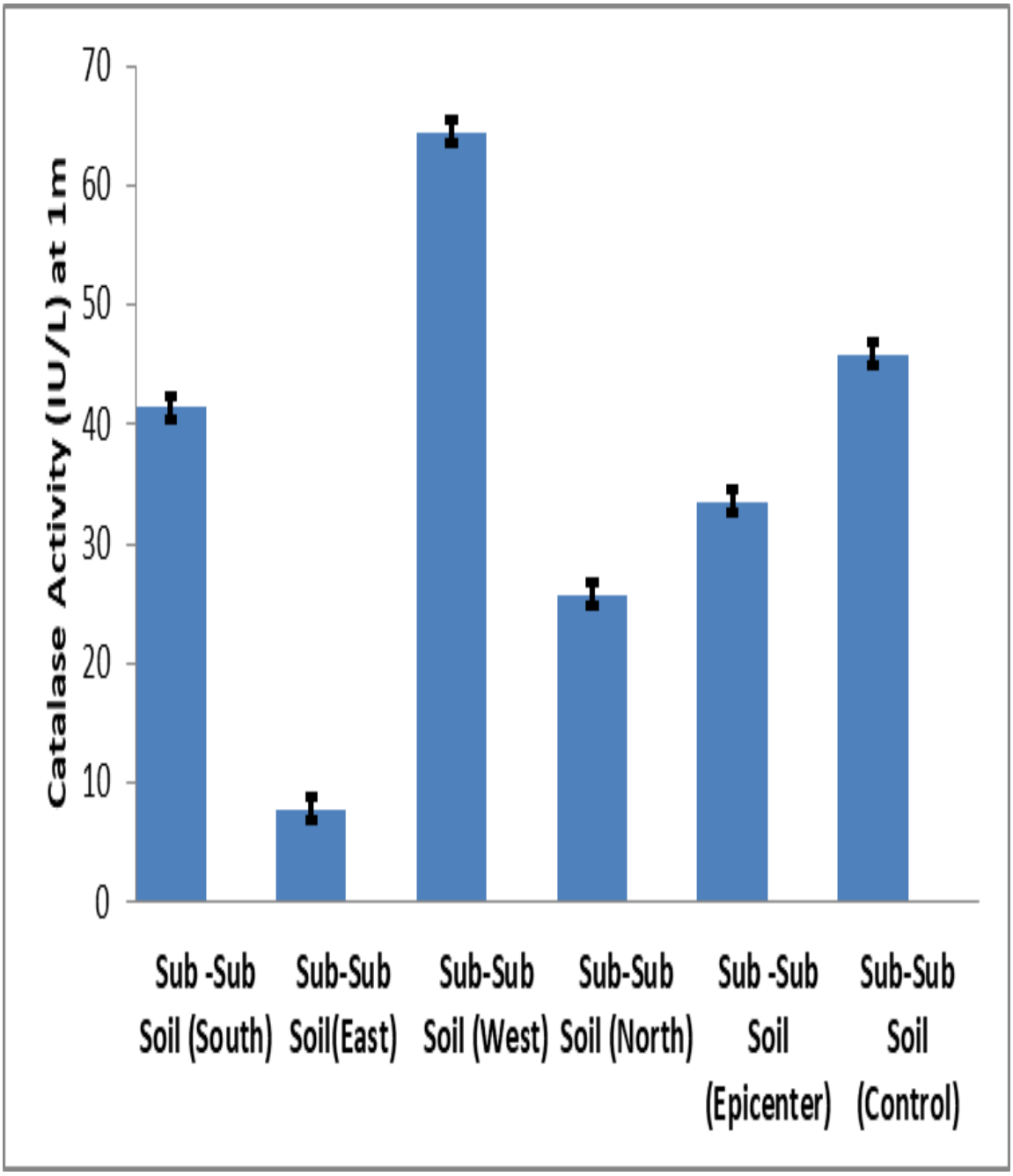


Figure 4.3: Effect of prolonged exposure to generator fume on soil catalase activity in sub-sub soil (1m apart).

Bars are presented as means± standard errors mean (SEM) of triplicate determinations at $p < 0.05$.

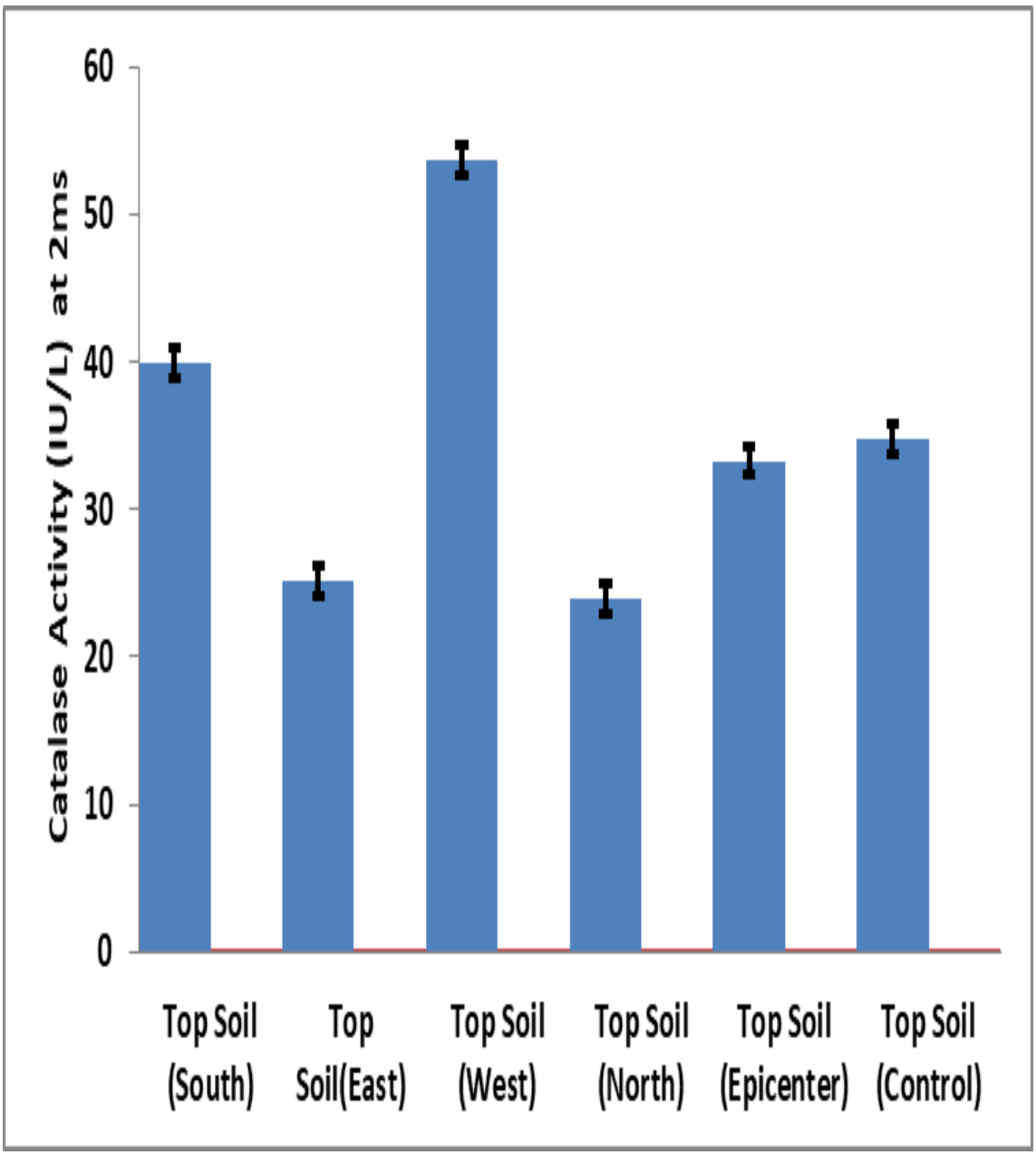


Figure 4.4: Effect of prolonged exposure to generator fume on soil catalase activity in top soil (2m apart).

Bars are presented as means± standard errors of mean (SEM) of triplicate determinations at $p< 0.05$.

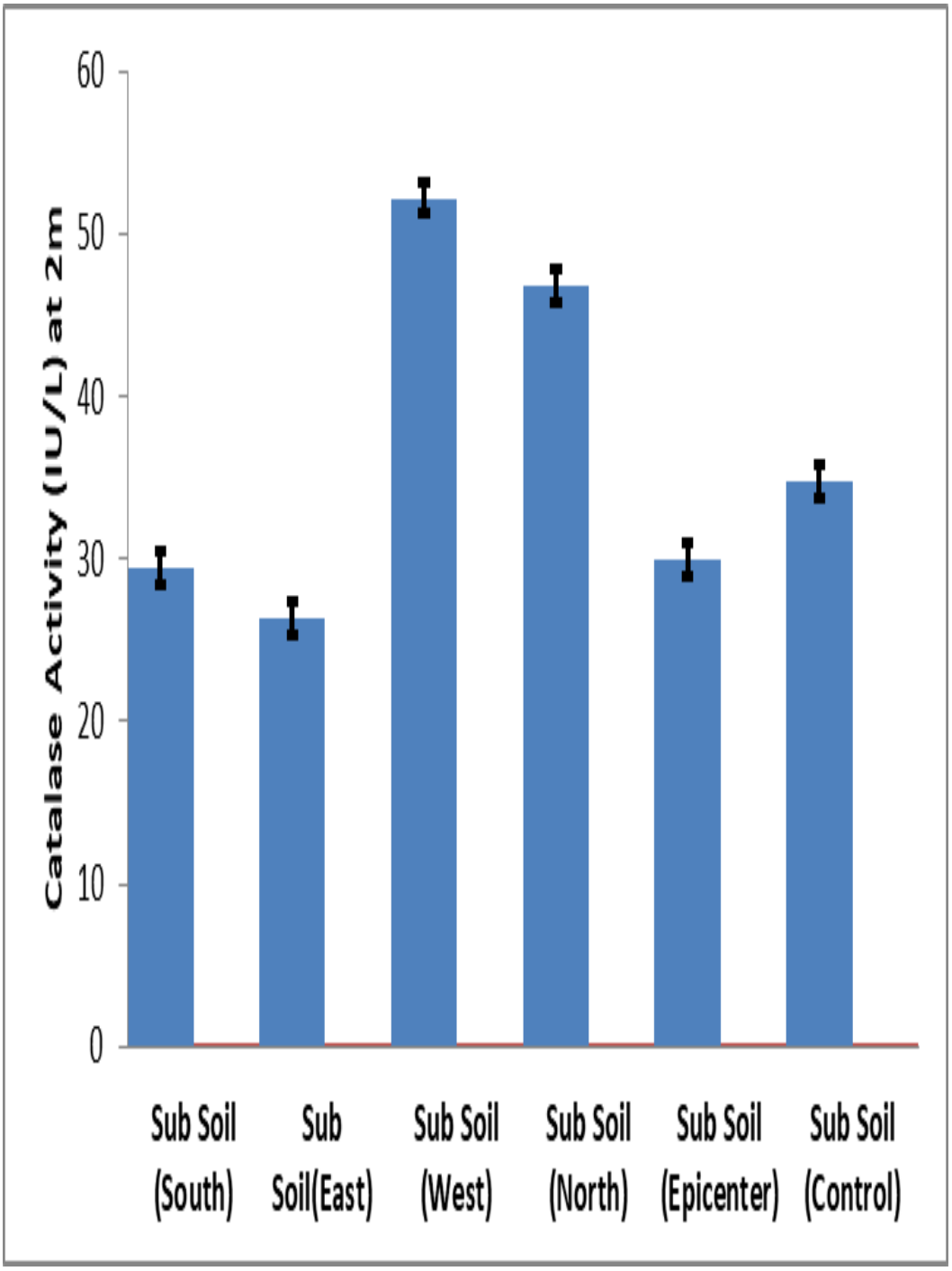


Figure 4.5: Effect of prolonged exposure to generator fume on soil catalase activity in sub soil (2m apart).

Bars are presented as means± standard errors of mean (SEM) of triplicate determinations at $p< 0.05$.

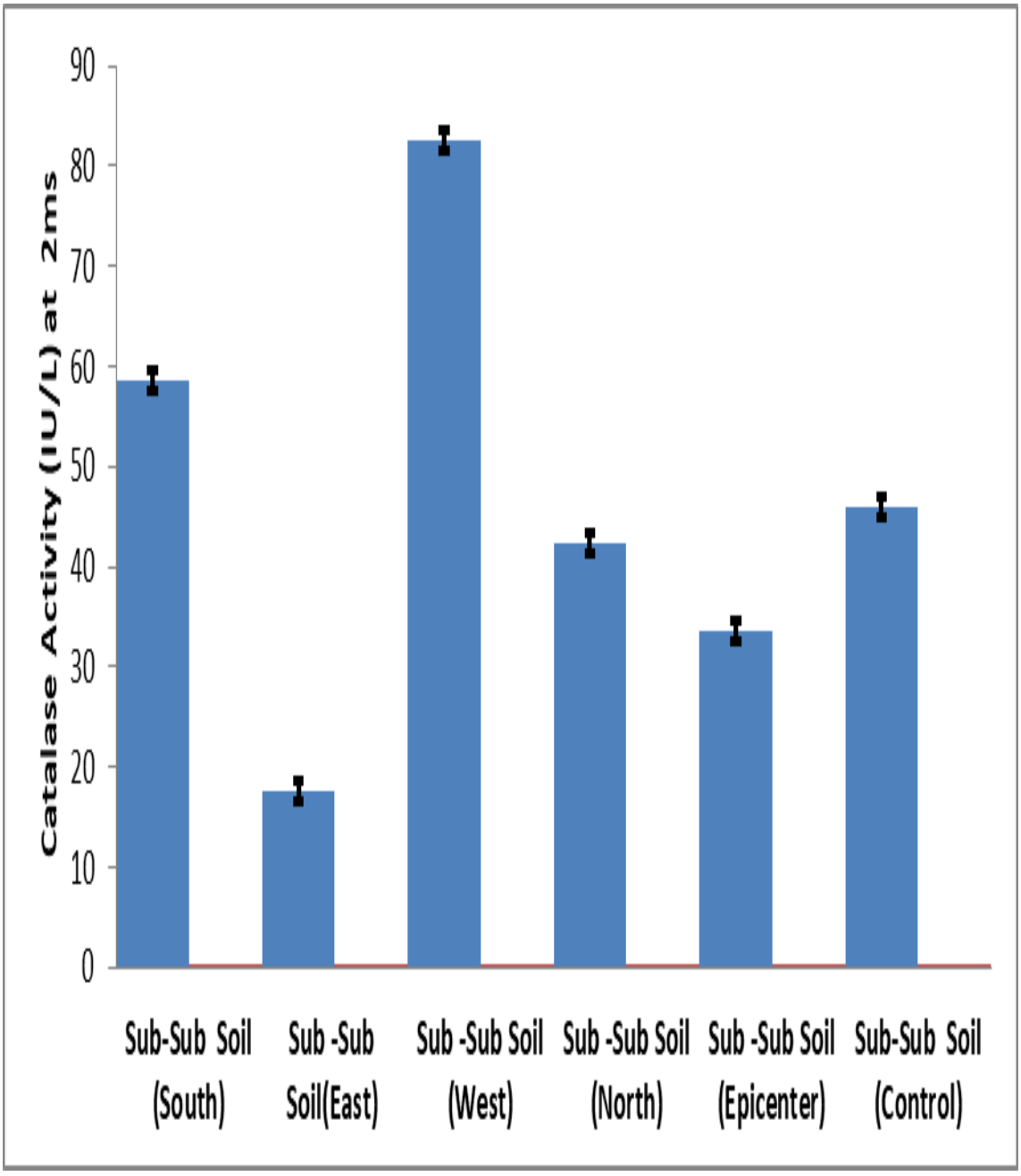


Figure 4.6: Effect of prolonged exposure to generator fume on soil catalase activity in sub-sub soil (2m apart).

Bars are presented as means± standard errors of mean (SEM) of triplicate determinations at $p < 0.05$.

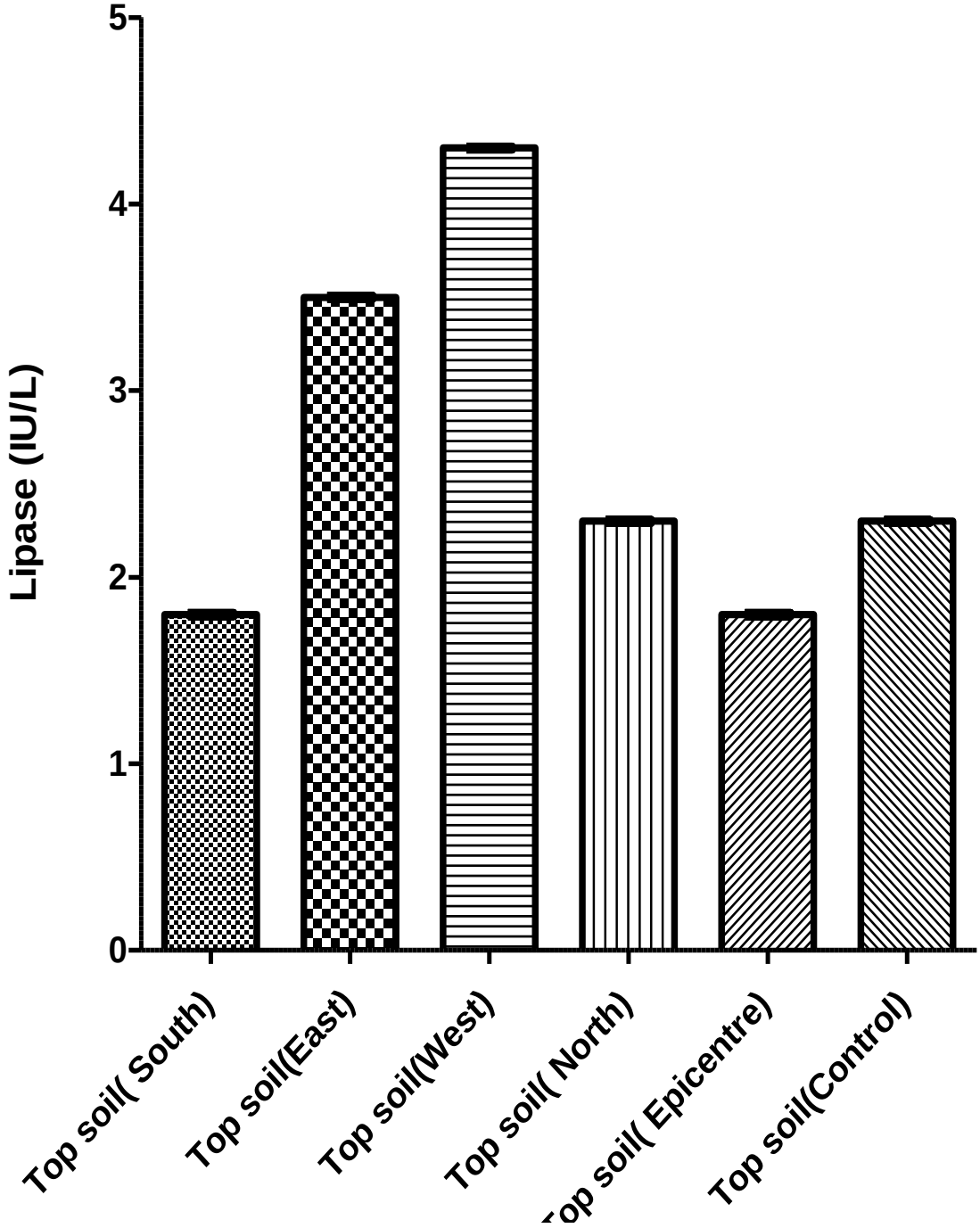


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Figure 4.7: Effect of prolonged exposure to generator fume on soil lipase activity in top soil (1m apart).

Bars are presented as means± standard errors of mean (SEM) of triplicate determinations at $p < 0.05$.

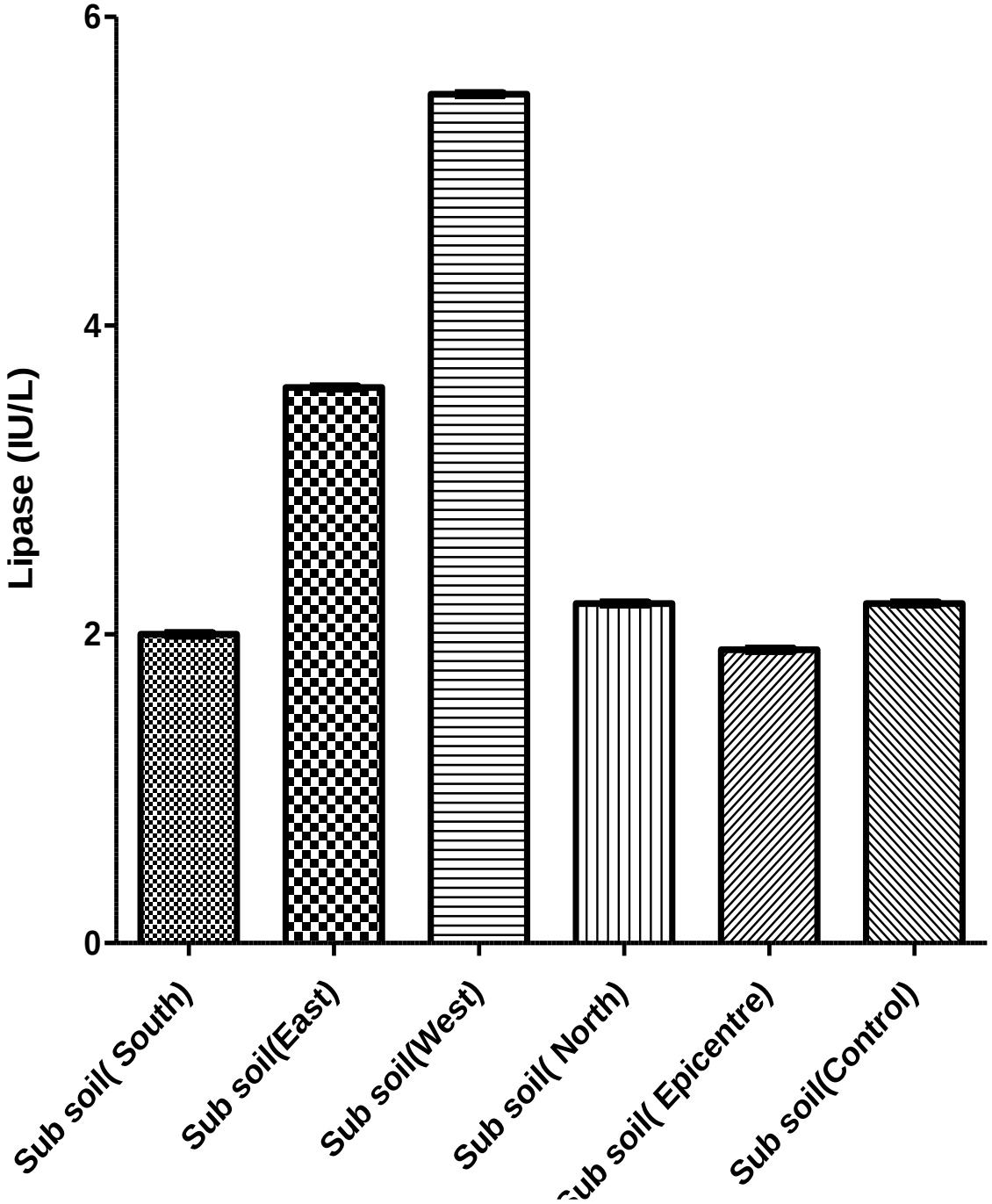

Figure 4.8: Effect of prolonged exposure to generator fume on soil lipase activity in sub soil (1m apart).

Bars are presented as means± standard errors of mean (SEM) of triplicate determinations at $p < 0.05$.

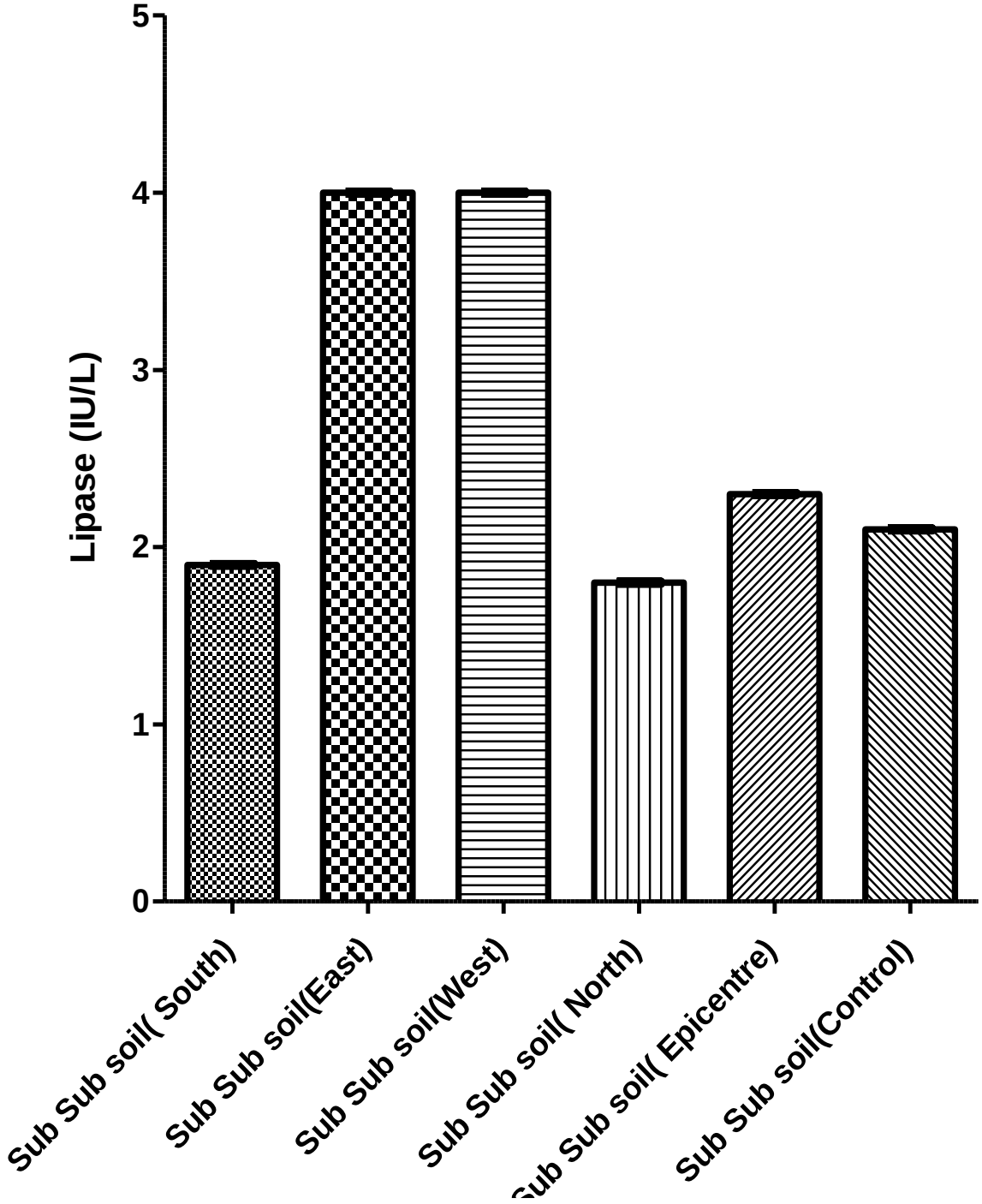

Figure 4.9: Effect of prolonged exposure to generator fume on soil lipase activity in sub-sub soil (1m apart).

Bars are presented as means ± standard errors of mean (SEM) of triplicate determinations at $p < 0.05$.

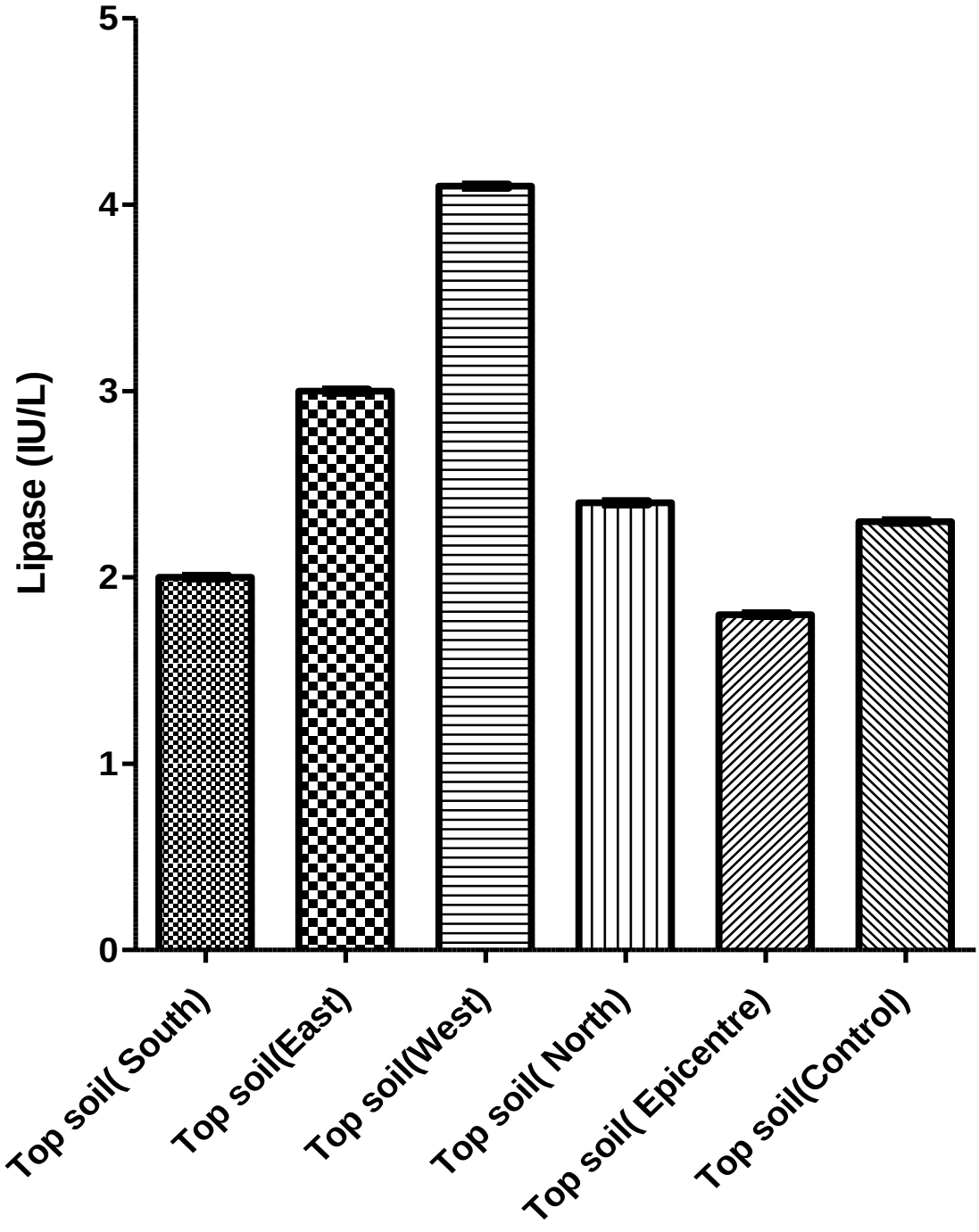

Figure 4.10: Effect of prolonged exposure to generator fume on soil lipase activity in top soil (2m apart).

Bars are presented as means± standard errors of mean (SEM) of triplicate determinations at $p< 0.05$.

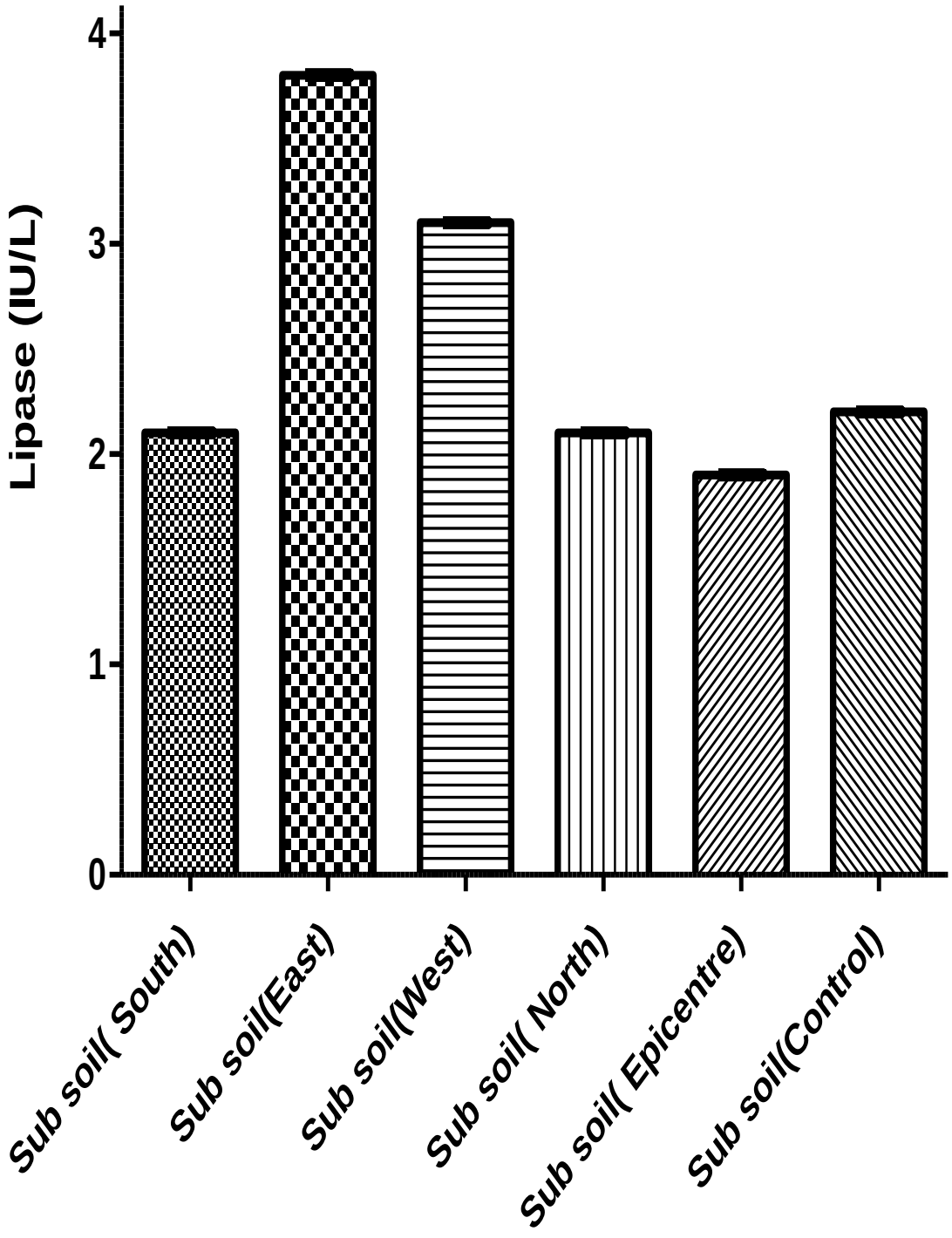

Figure 4.11: Effect of prolonged exposure to generator fume on soil lipase activity in sub soil (2m apart).

Bars are presented as means± standard errors of mean (SEM) of triplicate determinations at $p< 0.05$.

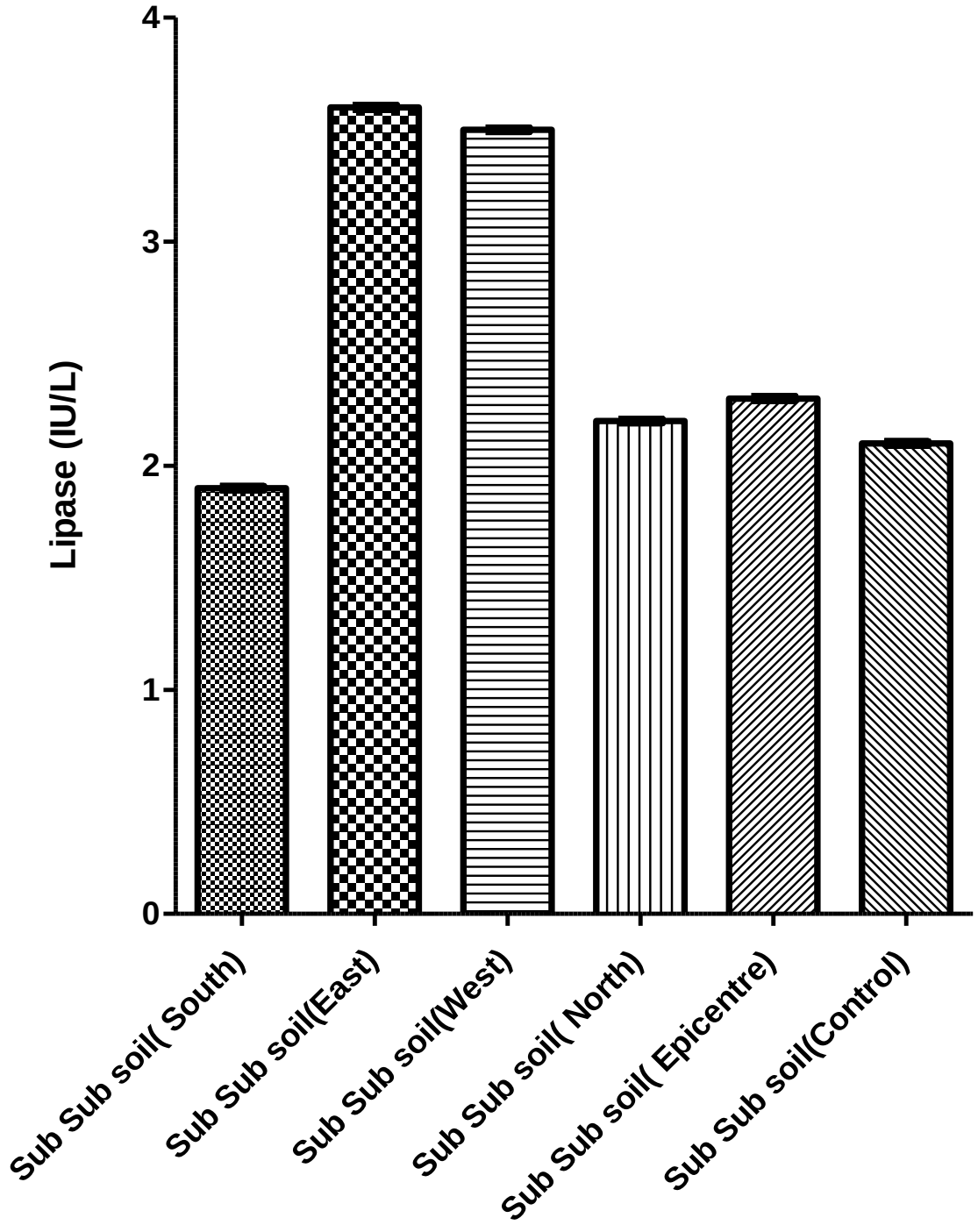

Figure 4.12: Effect of prolonged exposure to generator fume on soil lipase activity in sub-sub soil (2m apart).

Bars are presented as means± standard errors of mean (SEM) of triplicate determinations at $p < 0.05$.

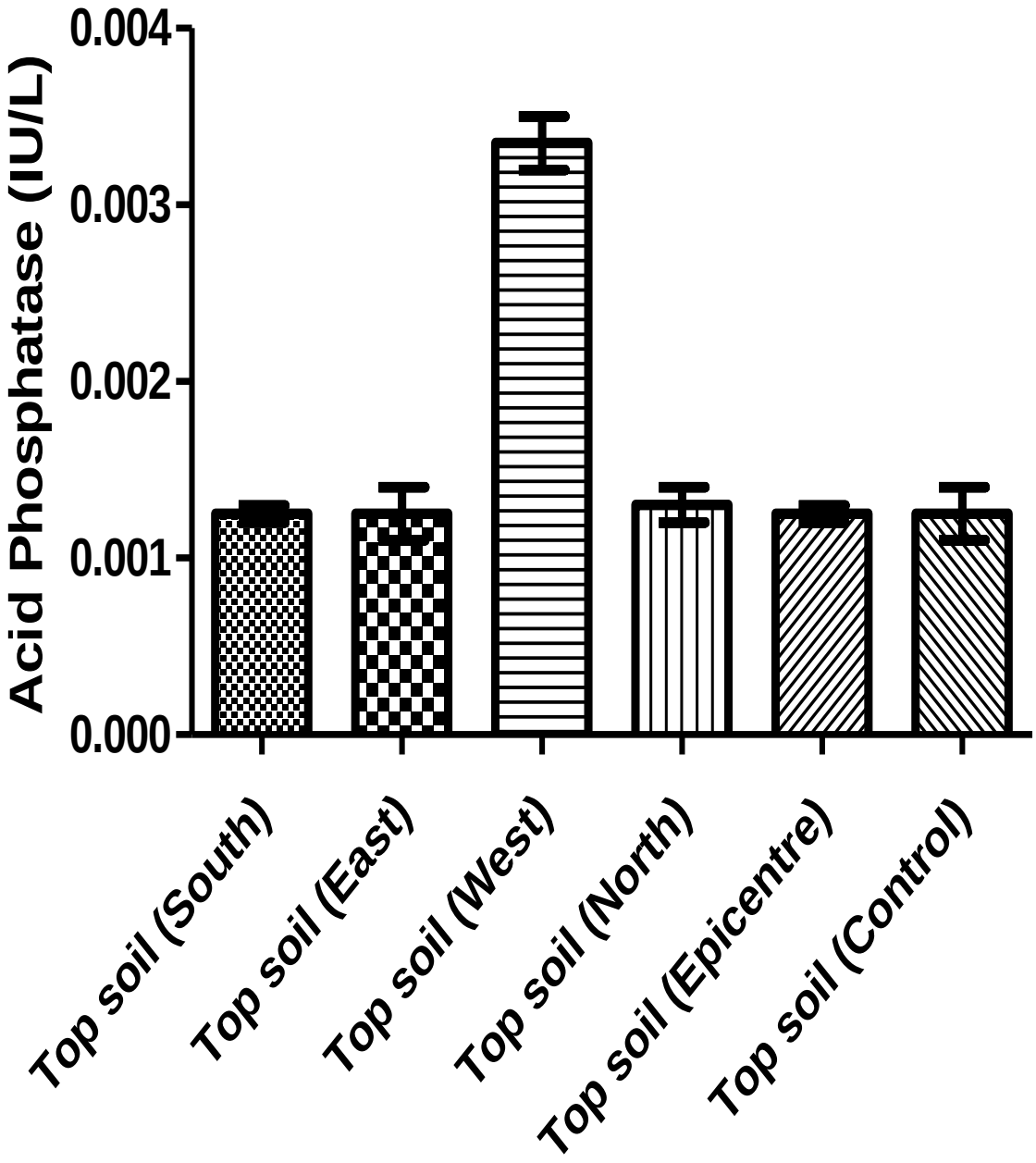

Figure 4.13: Effect of prolonged exposure to generator fume on soil acid phosphatase activity in top soil (1m apart).

Bars are presented as means± standard errors of mean (SEM) of triplicate determinations at $p < 0.05$.

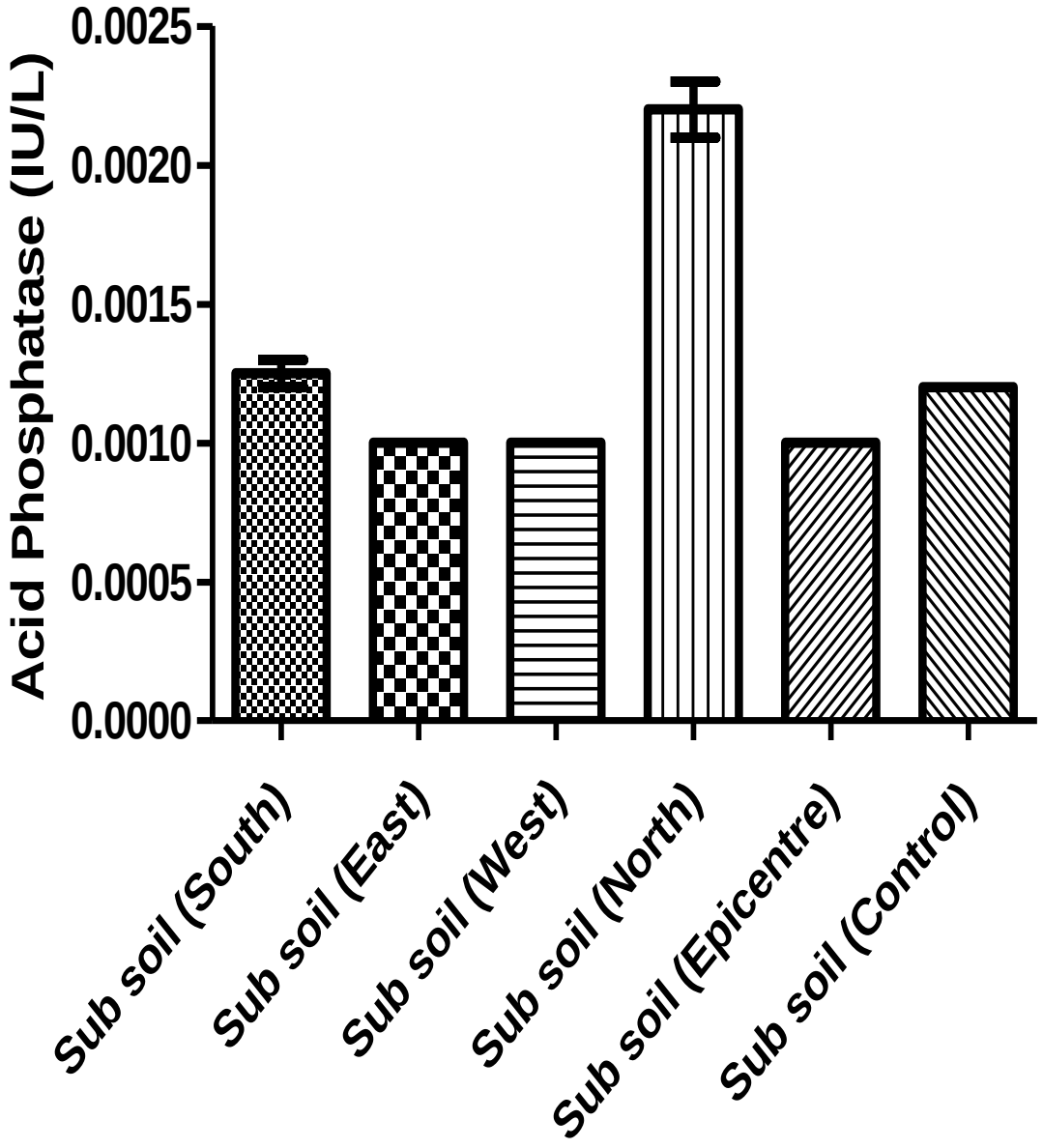

Figure 4.14: Effect of prolonged exposure to generator fume on soil acid phosphatase activity in sub soil (1m apart).

Bars are presented as means± standard errors of mean (SEM) of triplicate determinations at $p< 0.05$.

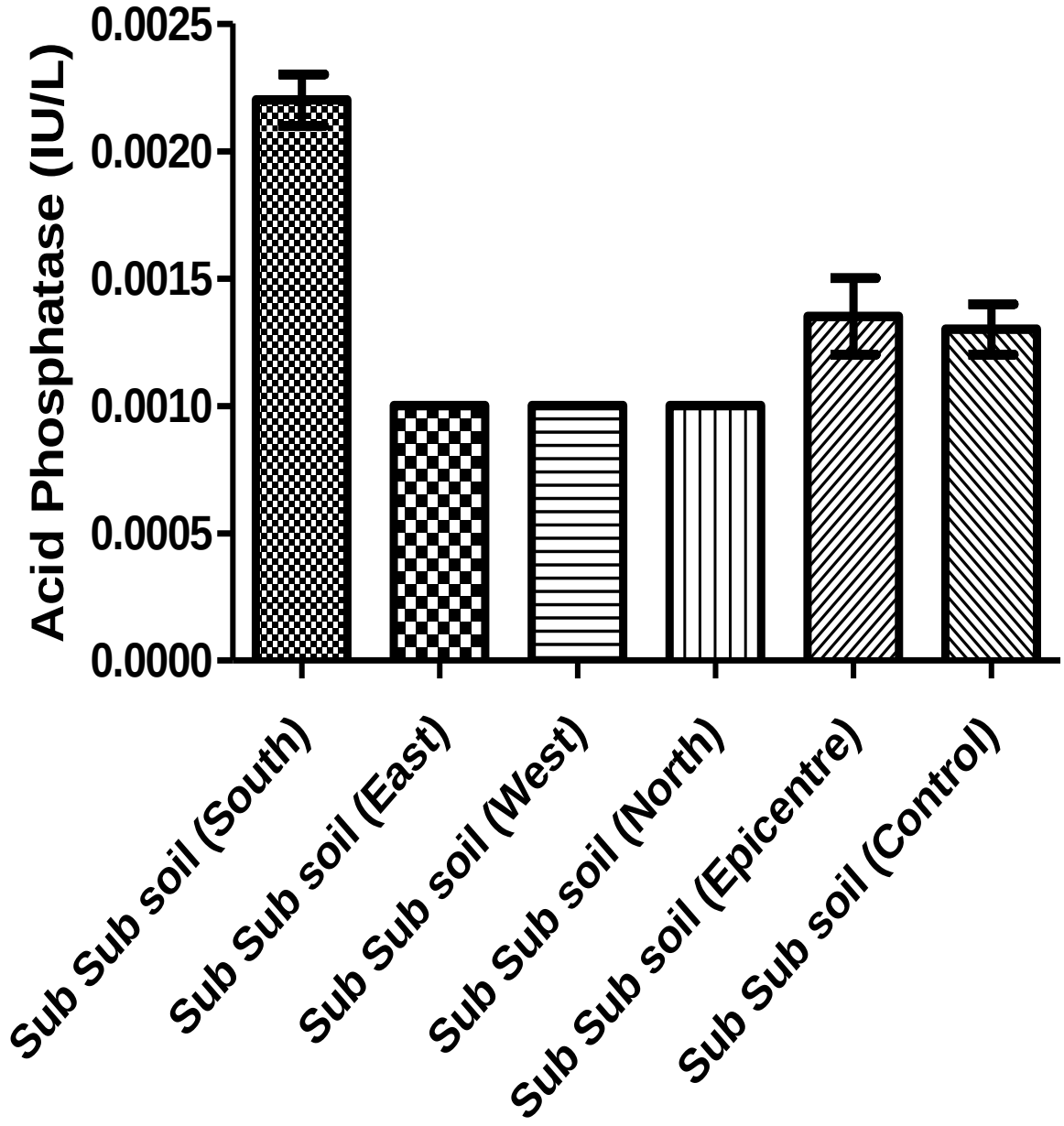

Figure 4.15: Effect of prolonged exposure to generator fume on soil acid phosphatase activity in sub-sub soil (1m apart).

Bars are presented as means± standard errors of mean (SEM) of triplicate determinations at $p < 0.05$.

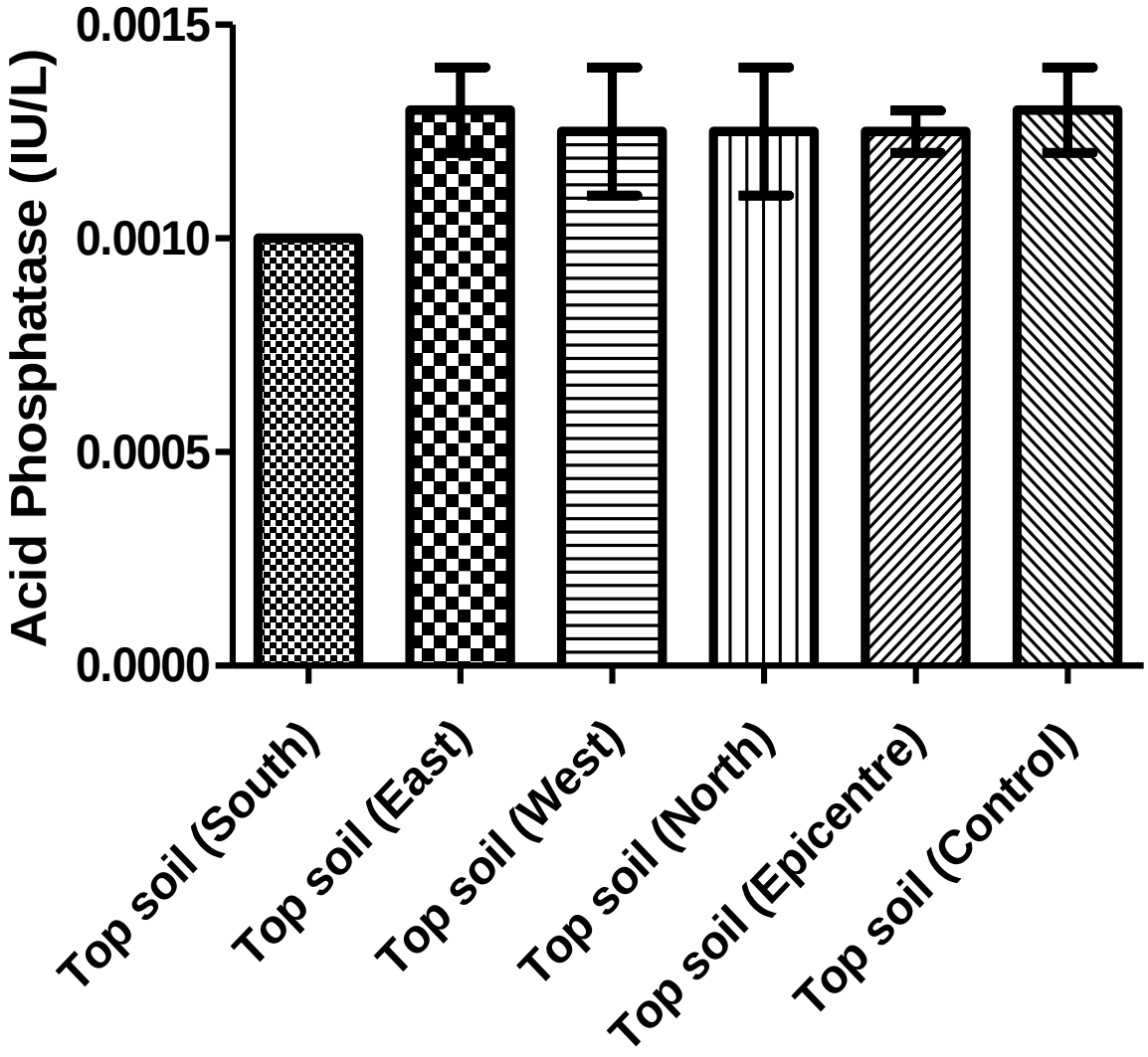

Figure 4.16: Effect of prolonged exposure to generator fume on soil acid phosphatase activity in top soil (2m apart).

Bars are presented as means± standard errors of mean (SEM) of triplicate determinations at $p< 0.05$.

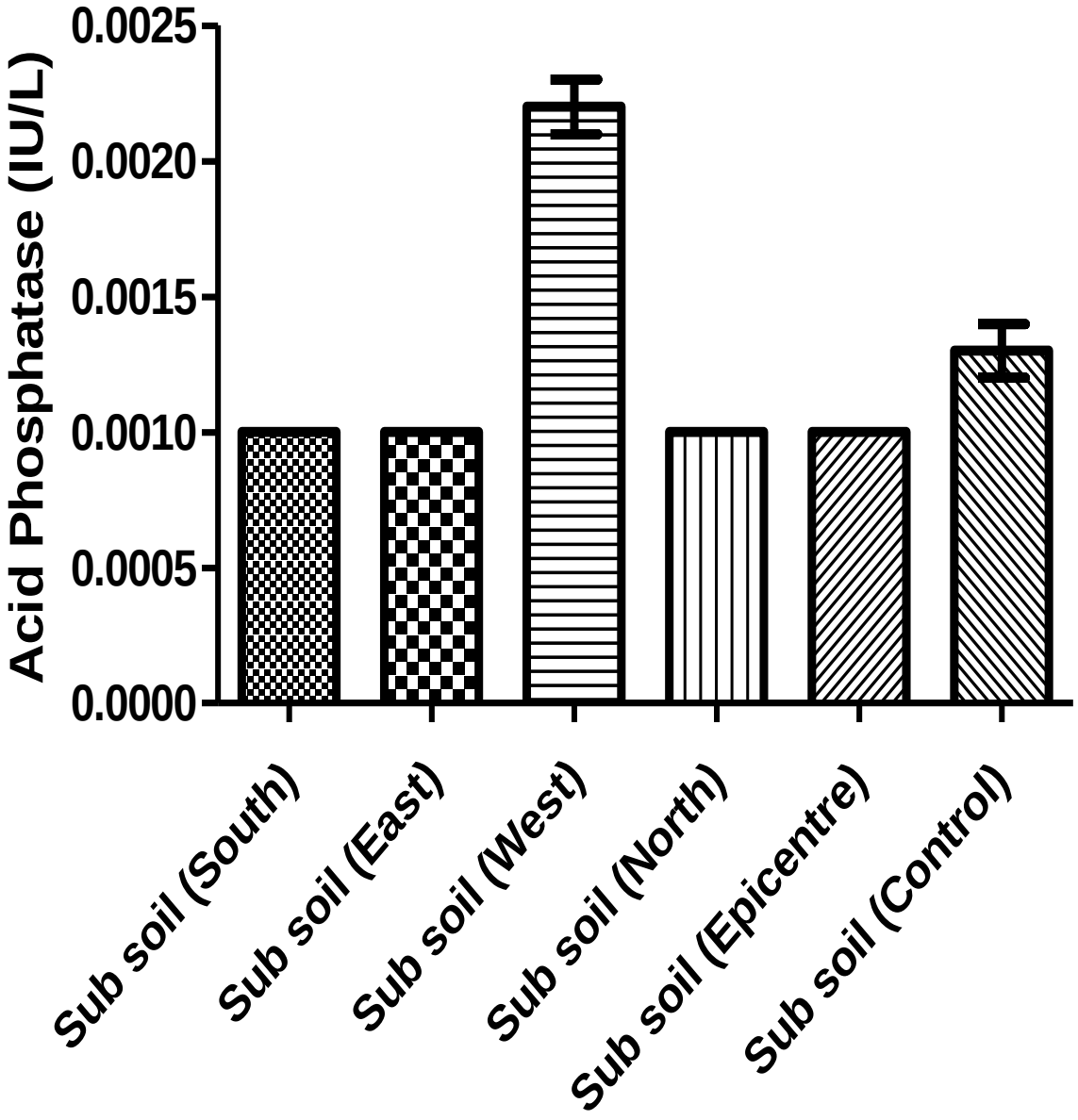

Figure 4.17: Effect of prolonged exposure to generator fume on soil acid phosphatase activity in sub soil (2m apart).

Bars are presented as means± standard errors of mean (SEM) of triplicate determinations at $p< 0.05$.

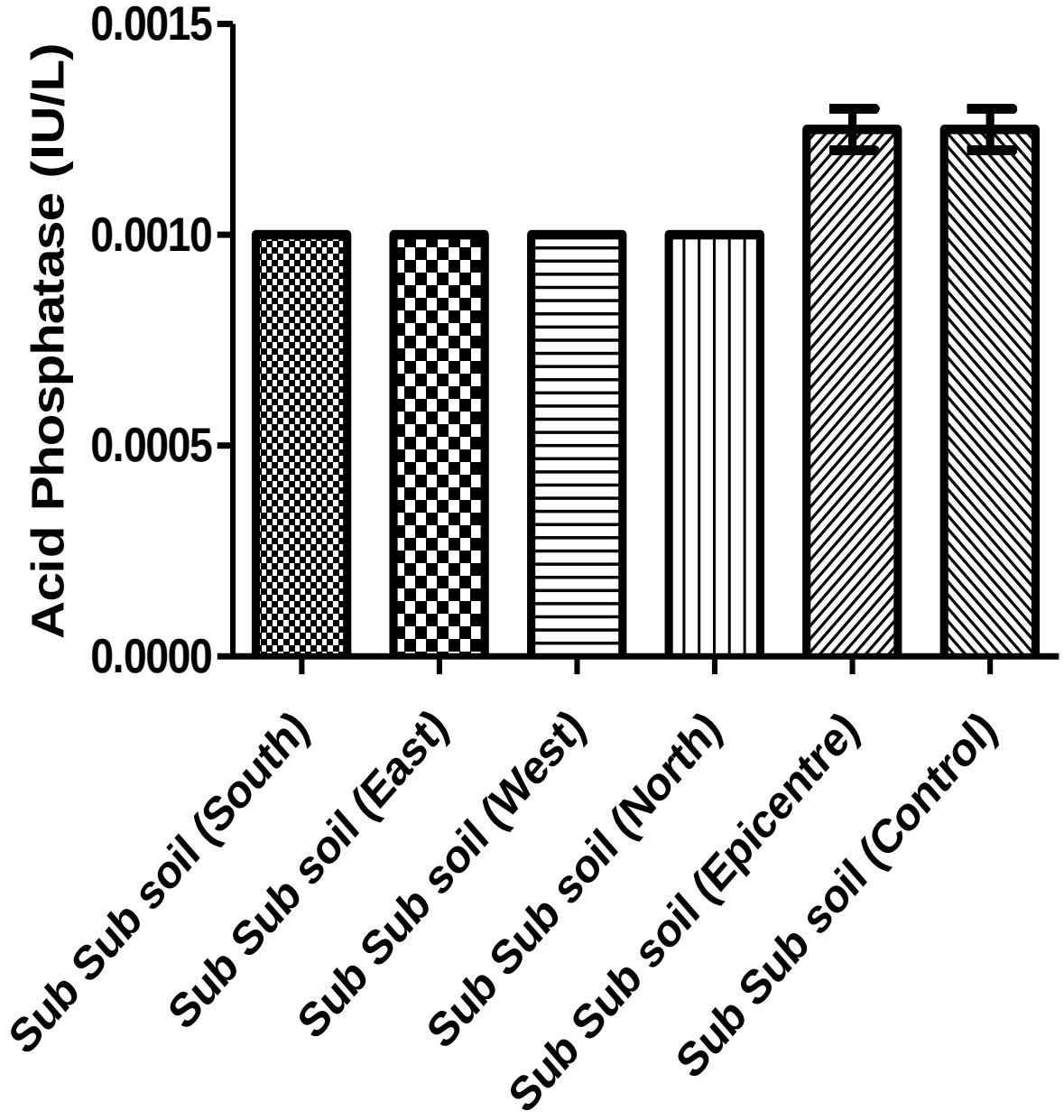

Figure 4.18: Effect of prolonged exposure to generator fume on soil acid phosphatase activity in sub-sub soil (2m apart).

Bars are presented as means± standard errors of mean (SEM) of triplicate determinations at $p < 0.05$.

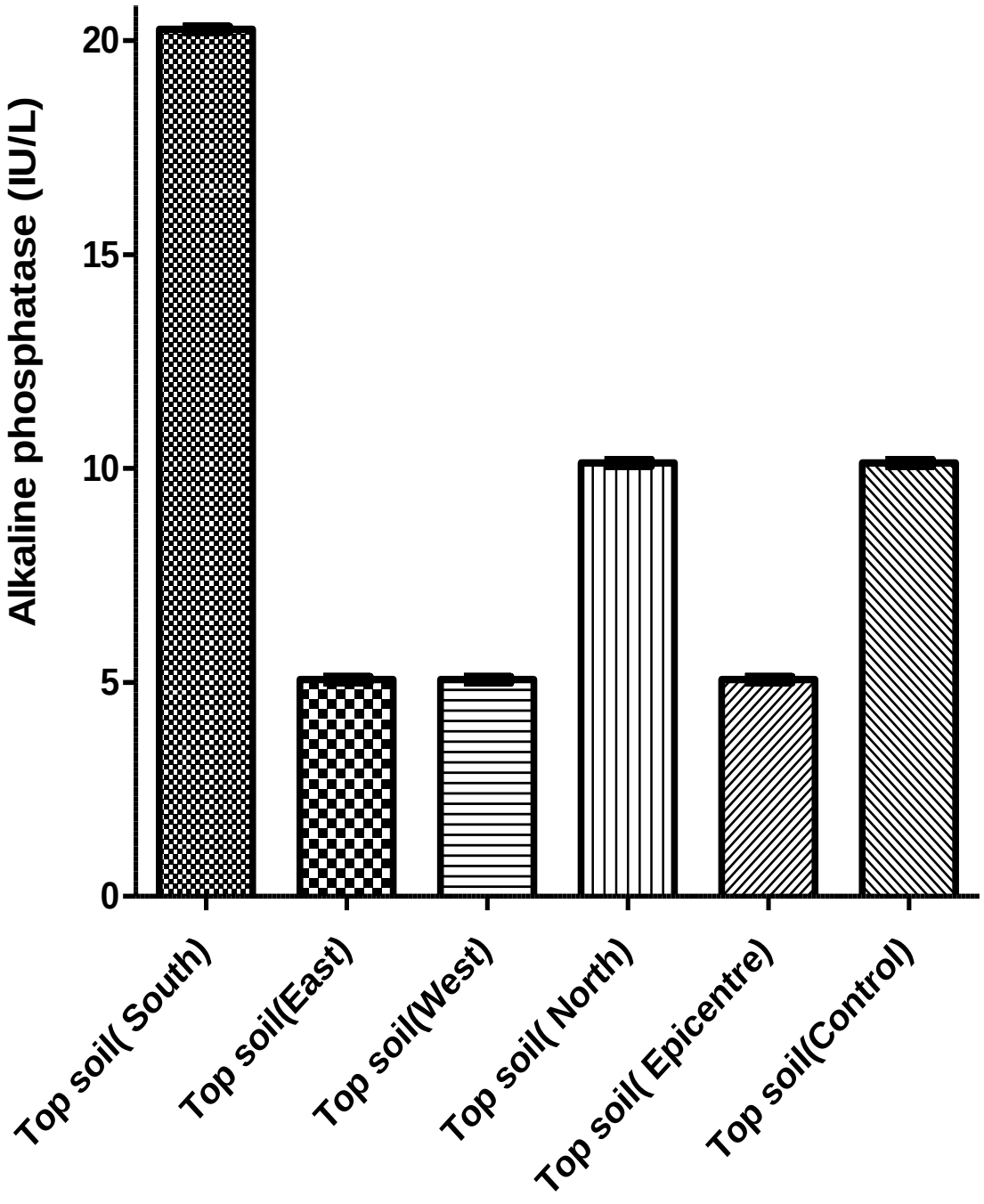

Figure 4.19: Effect of prolonged exposure to generator fume on soil alkaline phosphatase activity in top soil (1m apart).

Bars are presented as means± standard errors of mean (SEM) of triplicate determinations at $p < 0.05$.

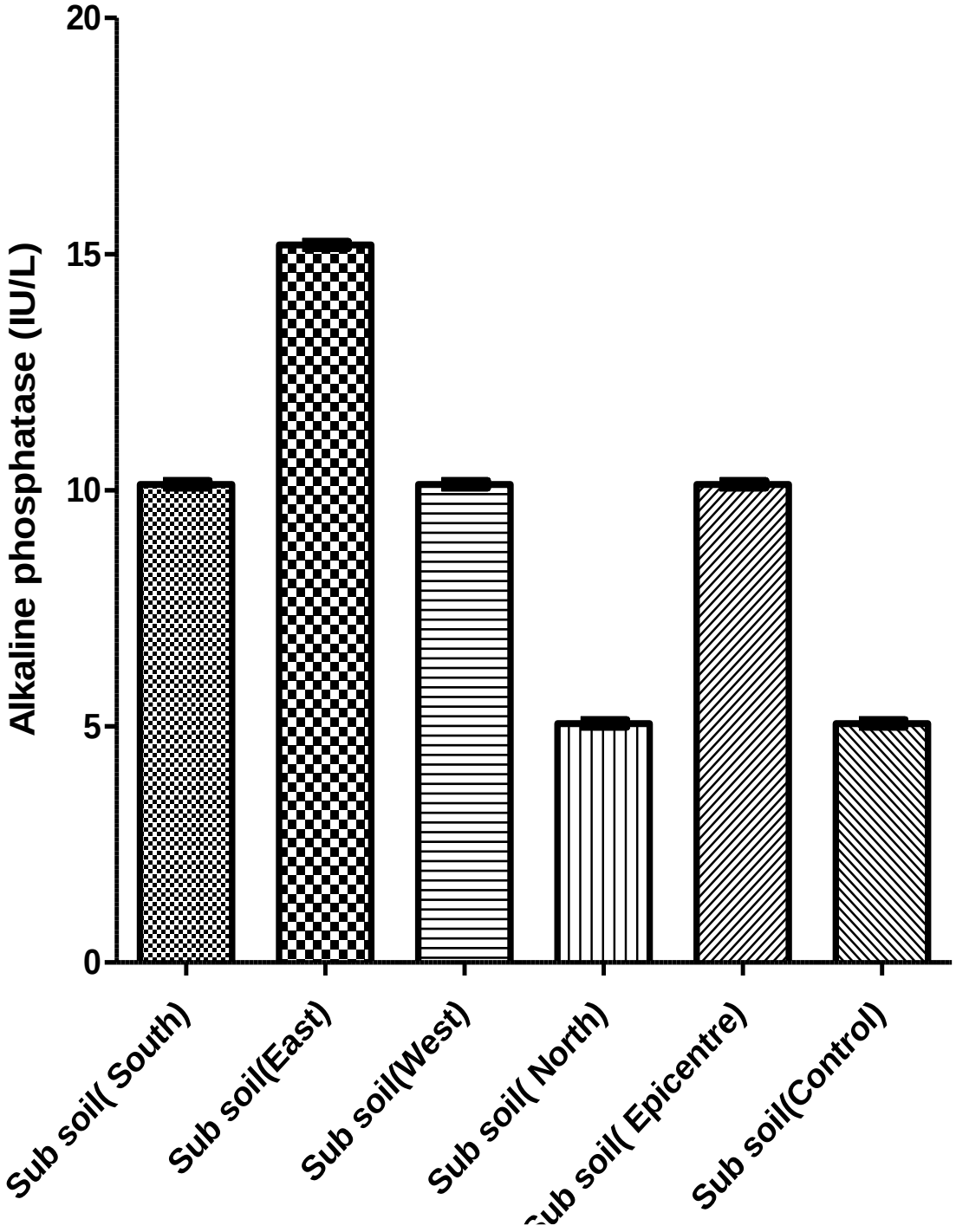

Figure 4.20: Effect of prolonged exposure to generator fume on soil alkaline phosphatase activity in sub soil (1m apart).

Bars are presented as means± standard errors of mean (SEM) of triplicate determinations at $p < 0.05$.

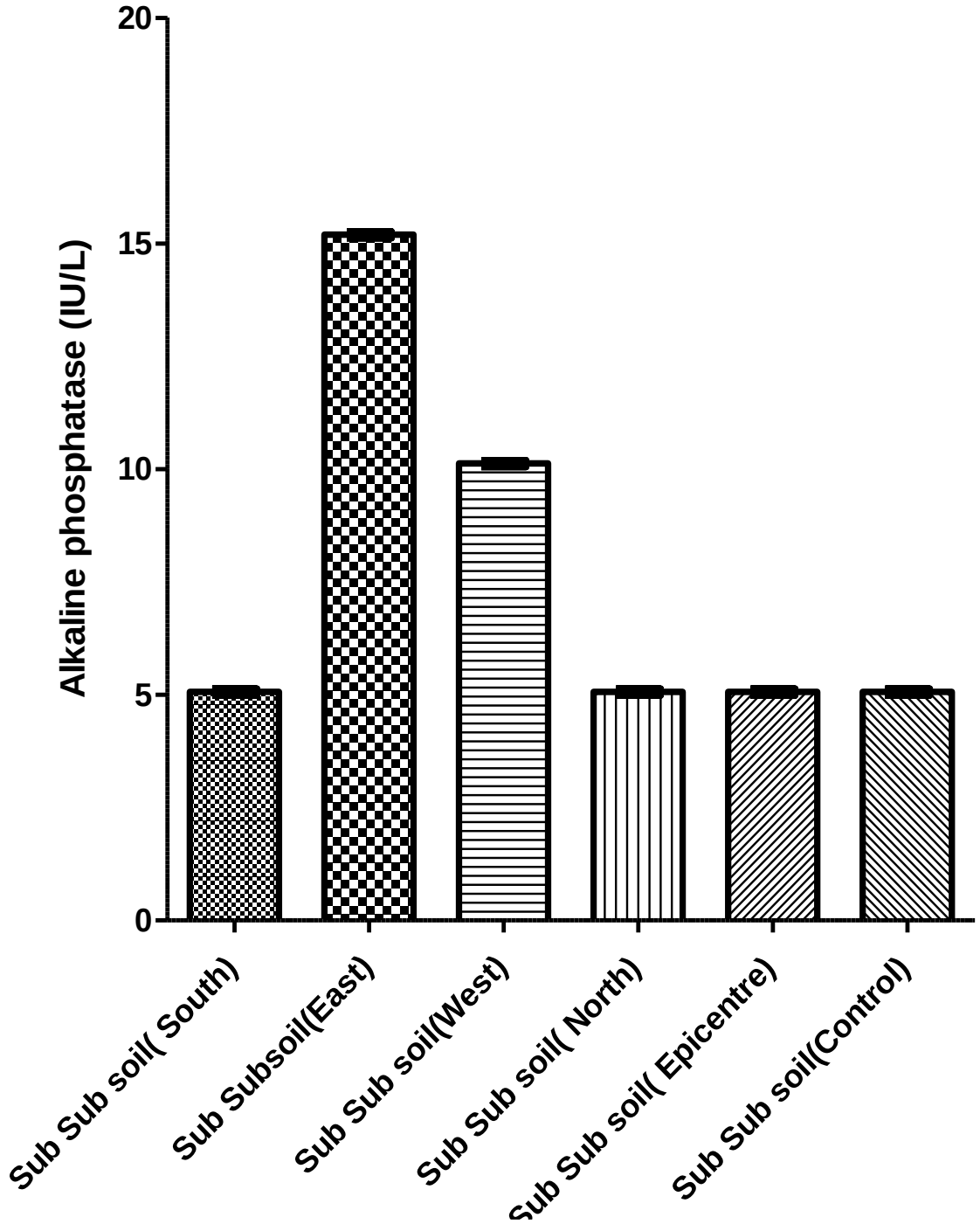

Figure 4.21: Effect of prolonged exposure to generator fume on soil alkaline phosphatase activity in sub-sub soil (1m apart).

Bars are presented as means± standard errors of mean (SEM) of triplicate determinations at $p < 0.05$.

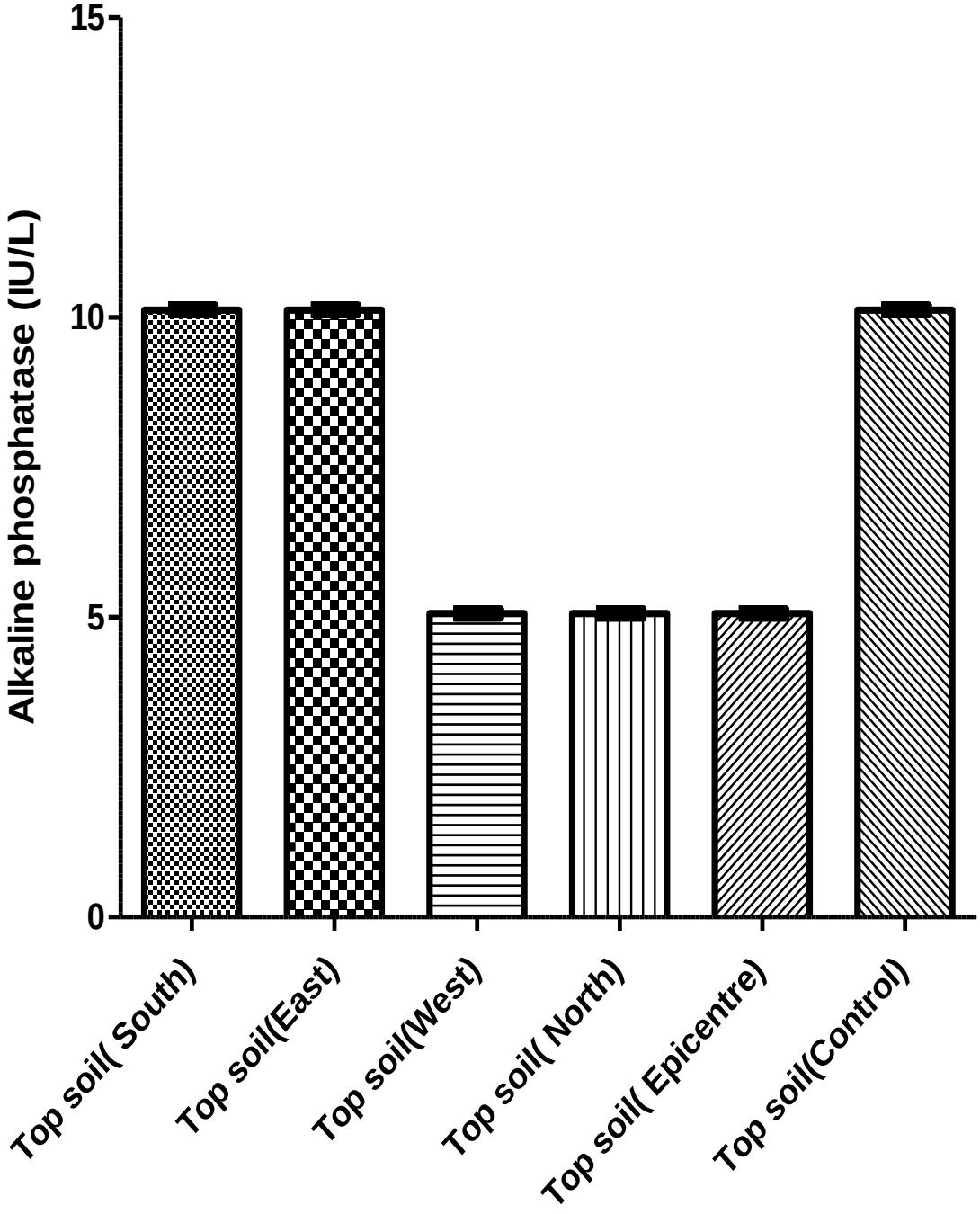

Figure 4.22: Effect of prolonged exposure to generator fume on soil alkaline phosphatase activity in top soil (2m apart).

Bars are presented as means± standard errors of mean (SEM) of triplicate determinations at $p < 0.05$.

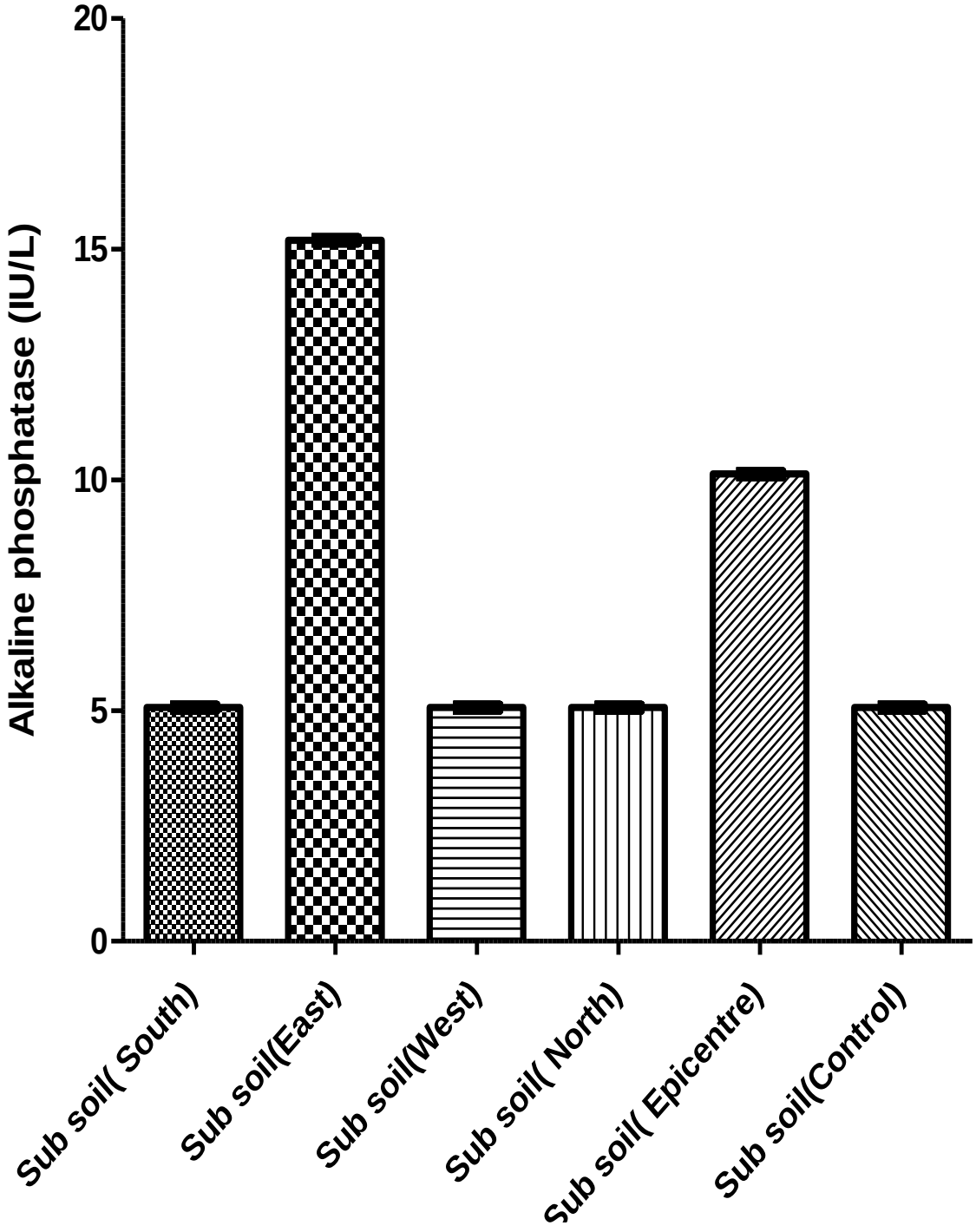

Figure 4.23: Effect of prolonged exposure to generator fume on soil alkaline phosphatase activity in sub soil (2m apart).

Bars are presented as means± standard errors of mean (SEM) of triplicate determinations at $p < 0.05$.

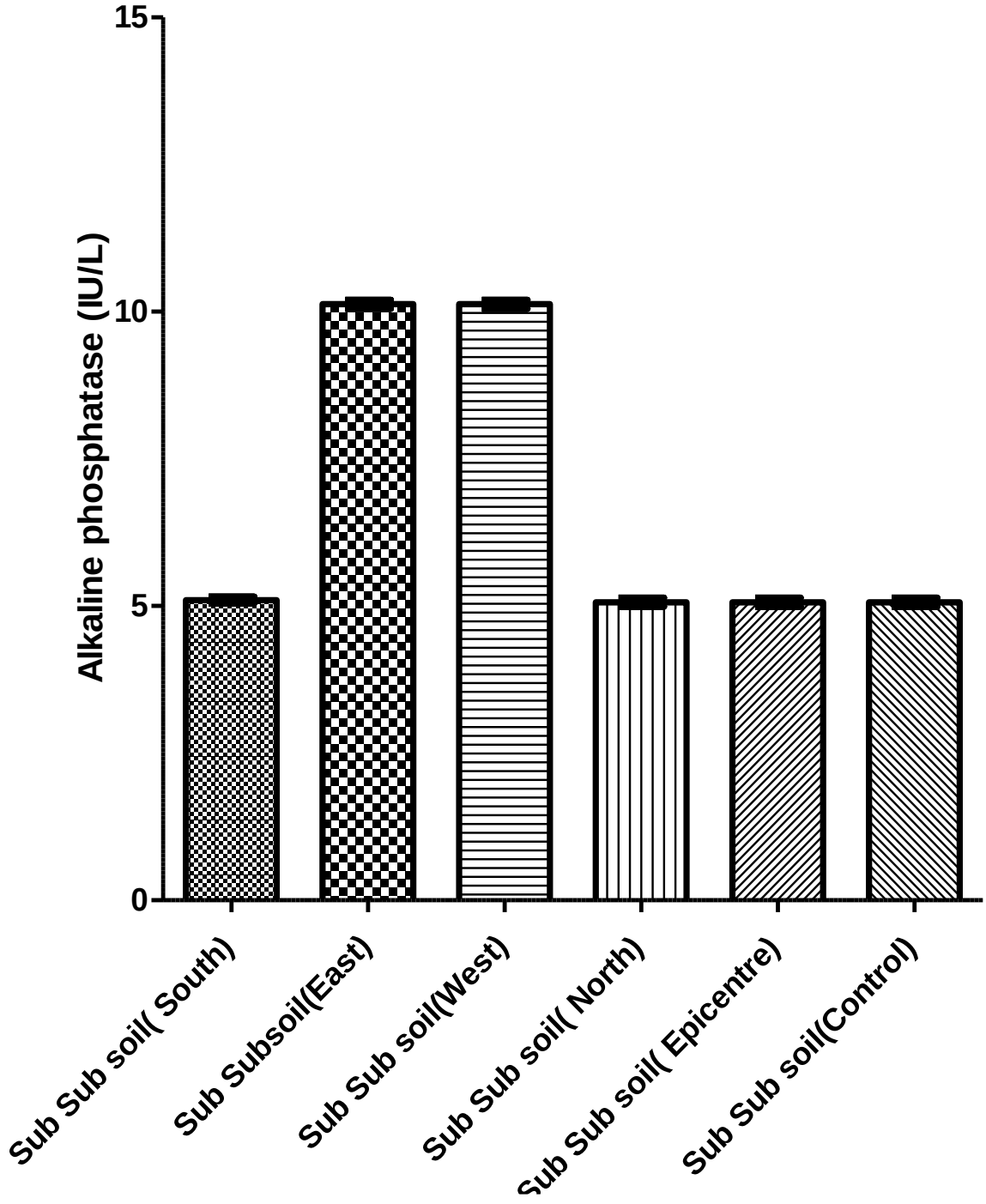

Figure 4.24: Effect of prolonged exposure to generator fume on soil alkaline phosphatase activity in sub-sub soil (2m apart).

Bars are presented as means± standard errors of mean (SEM) of triplicate determinations at $p < 0.05$.

4.1.2 MICROBIAL ACTIVITY OF THE SOIL SAMPLE

There was a significant difference at $p<0.05$ at generator impacted soil locations with highest heterotrophic bacterial counts when compared with the control site. The generator impacted soil location 1(S-2m) had the highest heterotrophic bacterial count ($5.35x10^7 \pm 3.2$ cfu/g) at top soil and sub soil ($7.45x10^7 \pm 2.58$ cfu/g), location 1(S-1m) had the highest heterotrophic bacterial count at sub-sub soil (6.13 ± 2.1 $x10^6$ cfu/g) when compared with control sites ($2.15x10^6 \pm 1.11$ cfu/g, $5.38x10^6 \pm 2.06$ cfu/g, $5.23x10^6 \pm 3.27$ cfu/g) respectively (Table 4.1). Unlike the bacterial population, there was no significant difference at $p< 0.05$ in heterotrophic fungi count of generator impacted soil when compared with the control soil (table 4.2). The spent oil degrading bacterial genera isolated in the fume impacted soil were *Enterobacter spp, Micrococci spp, Pseudomonas spp, Vibro cholera, Alcagenes spp, Bacillus spp, Klebsiella spp, Staphylococci spp, Serratia spp, Escherichia coli, Actinomyces spp, Moraxella spp, Proteus spp, Salmonella spp, Serratia spp, Streptococci spp, Shigella spp.* Also, the spent oil degrading bacterial genera isolated in control are *Escherichia coli, Micrococci spp, Pseudomonas spp, Vibro cholera, Alcagenes spp.*

Table 4.1: Total Bacteria Count for soil sample impacted with high concentration (100%) of Generator fume

Location	Depth	Top Soil (cfu/g)	Sub Soil (cfu/g)	Sub-Sub Soil (cfu/g)
Loc 1 (S)	**1m**	$2.55 \times 10^7 \pm 1.71$	$5.51 \times 10^7 \pm 3.40$	$6.13 \times 10^6 \pm 2.12$
	2m	$5.35 \times 10^7 \pm 3.21$	$7.45 \times 10^7 \pm 2.58$	$4.95 \times 10^6 \pm 2.93$
Loc 2 (E)	**1m**	$1.80 \times 10^7 \pm 0.41$	$2.65 \times 10^7 \pm 0.51$	$3.45 \times 10^6 \pm 1.42$
	2m	$2.71 \times 10^7 \pm 1.00$	$6.70 \times 10^6 \pm 3.11$	$2.62 \times 10^6 \pm 0.80$
Loc 3 (N)	**1m**	$2.10 \times 10^7 \pm 0.61$	$4.70 \times 10^6 \pm 3.71$	$0.91 \times 10^7 \pm 1.10$
	2m	$1.01 \times 10^7 \pm 1.21$	$4.45 \times 10^7 \pm 5.12$	$1.01 \times 10^7 \pm 1.22$
Loc 4 (W)	**1m**	$3.20 \times 10^7 \pm 1.24$	$1.78 \times 10^6 \pm 0.76$	$5.33 \times 10^7 \pm 3.53$
	2m	$2.68 \times 10^6 \pm 1.04$	$5.51 \times 10^6 \pm 3.51$	$2.91 \times 10^6 \pm 1.52$
Loc 5	**Epicenter**	$1.92 \times 10^6 \pm 0.72$	$3.18 \times 10^6 \pm 2.53$	$2.51 \times 10^7 \pm 1.72$
	Control	$2.15 \times 10^6 \pm 1.11$	$5.38 \times 10^6 \pm 2.06$	$5.23 \times 10^6 \pm 3.27$

Values are presented as means± standard deviation of triplicate determinations

Table 4.2: Total Fungi count for soil sample impacted with high concentration (100%) of Generator fumes

Location	Depth	Top Soil (cfu/g)	Sub Soil(cfu/g)	Sub-Sub Soil (cfu/g)
Loc 1 (S)	**1m**	$3.51 \times 10^3 \pm 0.61$	$4.25 \times 10^3 \pm 1.01$	$6.01 \times 10^3 \pm 2.95$
	2m	$3.52 \times 10^2 \pm 1.14$	$3.75 \times 10^3 \pm 1.26$	$4.52 \times 10^3 \pm 2.65$
Loc 2 (E)	**1m**	$4.25 \times 10^3 \pm 3.30$	$4.81 \times 10^4 \pm 4.93$	$4.52 \times 10^3 \pm 2.65$
	2m	$5.01 \times 10^3 \pm 1.63$	$6.25 \times 10 \pm 1.63$	$5.52 \times 10^3 \pm 1.29$
Loc 3 (N)	**1m**	$3.02 \times 10^5 \pm 1.83$	$5.25 \times 10^3 \pm 2.22$	$4.51 \times 10^3 \pm 1.73$
	2m	$3.13 \times 10^4 \pm 2.02$	$4.33 \times 10^4 \pm 2.64$	$6.25 \times 10^3 \pm 0.96$
Loc 4 (W)	**1m**	$5.51 \times 10^3 \pm 3.01$	$2.78 \times 10^4 \pm 1.21$	$4.02 \times 10^3 \pm 3.37$
	2m	$3.75 \times 10^3 \pm 3.51$	$2.78 \times 10^4 \pm 2.84$	$3.75 \times 10^3 \pm 2.06$
Loc 5	**Epicenter**	$3.25 \times 10^2 \pm 2.06$	$5.51 \times 10^3 \pm 2.65$	$2.75 \times 10^3 \pm 1.26$
	Control	$4.51 \times 10^3 \pm 2.11$	$6.33 \times 10^3 \pm 3.64$	$5.75 \times 10^4 \pm 3.40$

Values are presented as means± standard deviation of triplicate determinations

Table 4.3: Biochemical characteristics and identification of bacterial isolates from polluted and unpolluted (control) soil

					Tripple sugar ion agar (TSIA)														
	ISOLATE CODE	COLONY MORPHOLOGY	MICROSCOPIC CHARACTERIS	GRAM	SLANT	BUTT	GLUCOSE	LACTOSE	GAS	H_2S	INDOLE	MOTILITY	OXIDASE	CITRATE	UREASE	CATALASE	V.P	METYL RED	MOST PROBABLE BACTERIA
1	South S_1A_1	Creamy colony on NA	Straight rod	+	B	A	+	-	-	+	-	+	-	-	-	+	+	-	*Bacillus spp*
2	South S_1B_1	Creamy colony on Nutrient agar	Rod	-	B	A	+	-	+	-	+	+	-	+	-	+	-	+	*Enterobacter spp*
3	South S_1C_1	Creamy colony on nutrient agar	Rod	-	B	A	-	-	-	-	-	+	-	+	-	+	-	+	*Serratia spp*
4	South S_2A_2	Mucoidal creamy colony on Nutrient agar	Short rod	-	B	A	+	-	-	-	-	-	+	+	-	+	-	+	*Moraxella spp*
5	South S_2B_2	Creamy colony on Nutrient agar	Cocci	+	B	A	+	-	-	-	-	+	-	+	-	+	-	+	*Micrococci spp*
6	South S_2C_2	Mucoidal creamy colony on	Short rod	-	B	B	-	-	-	-	-	+	+	+	-	+	+	-	*Vibro cholera*

		Nutrient agar																	
7	**East E_1A_1**	**Shiny creamy colony on NA**	**Clustered cocci**	**+**	**A**	**A**	**+**	**+**	**-**	**-**	**-**	**-**	**-**	**+**	**+**	**+**	**+**	**-**	***Staphylococci spp***
8	**East E_1B_1**	**Creamy colony on Nutrient agar**	**Cocci**	**+**	**B**	**A**	**+**	**-**	**-**	**-**	**-**	**+**	**-**	**+**	**-**	**+**	**-**	**+**	***Micrococci spp***
9	**East E_1C_1**	**Creamy colony on Nutrient agar**	**Rod**	**-**	**B**	**A**	**+**	**-**	**+**	**-**	**+**	**+**	**-**	**+**	**-**	**+**	**-**	**+**	***Enterobacter Spp***
10	**East E_2B_2**	**Creamy colony on NA**	**Straight rod**	**+**	**B**	**A**	**+**	**-**	**-**	**+**	**-**	**+**	**-**	**-**	**-**	**+**	**+**	**-**	***Bacillus spp***
11	**East E_2A_2**	**Creamy colony on NA**	**Short rod**	**-**	**B**	**B**	**-**	**-**	**-**	**-**	**-**	**+**	**+**	**+**	**-**	**+**	**-**	**+**	***Pseudomonas spp***
12	**North N_1A_1**	**Creamy colony on NA**	**Short rod**	**-**	**B**	**B**	**-**	**-**	**-**	**-**	**-**	**+**	**+**	**+**	**-**	**+**	**-**	**+**	***Pseudomonas spp***
13	**North N_1B_1**	**Creamy colony on nutrient agar**	**Rod**	**-**	**B**	**A**	**-**	**-**	**-**	**-**	**-**	**+**	**-**	**+**	**-**	**+**	**-**	**+**	***Serratia spp***
14	**North N_1C_1**	**Creamy colony on nutrient agar**	**Rod**	**-**	**B**	**A**	**-**	**-**	**-**	**-**	**-**	**+**	**-**	**+**	**-**	**+**	**+**	**-**	***Yersinia spp***
15	**North N_1A_1**	**Creamy colony on NA**	**Short rods**	**-**	**B**	**A**	**-**	**+**	**+**	**-**	**+**	**-**	**-**	**-**	**-**	**+**	**-**	**+**	***Shigella Spp***

16	**West W_1A_1**	**Shiny creamy colony on NA**	**Clustered cocci**	**+**	**A**	**A**	**+**	**+**	**-**	**-**	**-**	**-**	**-**	**+**	**+**	**+**	**+**	**-**	***Staphylococci spp***
17	**West W_1B_1**	**Shiny creamy colony on NA**	**Clustered cocci**	**+**	**A**	**A**	**+**	**+**	**-**	**-**	**-**	**-**	**-**	**+**	**+**	**+**	**+**	**-**	***Staphylococci spp***
18	**West W_1C_1**	**Creamy colony on NA**	**Short rods**	**-**	**B**	**A**	**-**	**+**	**+**	**-**	**+**	**-**	**-**	**-**	**-**	**+**	**-**	**+**	***Shigella Spp***
19	**West W_2A_2**	**Cramy branching colony on NA**	**Rod**	**+**	**B**	**A**	**-**	**+**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**+**	**+**	**-**	***Actinomyces spp***
20	**West W_2B_2**	**Creamy colony on Nutrient agar**	**Cocci**	**+**	**B**	**A**	**+**	**-**	**-**	**-**	**-**	**+**	**-**	**+**	**-**	**+**	**-**	**+**	***Micrococci spp***
21	**West W_2C_2**	**Creamy colony on NA**	**Short rods**	**-**	**B**	**A**	**-**	**+**	**+**	**-**	**+**	**-**	**-**	**-**	**-**	**+**	**-**	**+**	***Shigella Spp***
22	**EPI A**	**Creamy colony on NA**	**Straight rod**	**+**	**B**	**A**	**+**	**-**	**-**	**+**	**-**	**+**	**-**	**-**	**-**	**+**	**+**	**-**	***Bacillus spp***
23	**EPI B**	**Creamy colony on Nutrient agar**	**Rod**	**-**	**B**	**B**	**+**	**-**	**+**	**+**	**+**	**+**	**-**	**+**	**-**	**+**	**-**	**+**	***Citrobacter spp***
24	**Control A**	**Creamy colony on Nutrient agar**	**Cocci**	**+**	**B**	**A**	**+**	**-**	**-**	**-**	**-**	**+**	**-**	**+**	**-**	**+**	**-**	**+**	***Micrococci spp***
25	**Control B**	**Creamy colony**	**Short rod**	**-**	**A**	**A**	**+**	**+**	**+**	**-**	**+**	**+**	**-**	**-**	**-**	**+**	**-**	**+**	***Escherichia coli***

Key: A = Acidic condition; B = Basic condition; + = positive; - = negative; EPI= epicentre; 1 =1m away; 2=2meters away; Isolate code A, B and C =Top , Sub and Sub- Sub soil.

Table 4.4: Biochemical characteristics and identification of spent oil degrading bacterial isolates obtained from polluted and unpolluted site.

					Tripple sugar ion agar (TSIA)														
	ISOLATE CODE	COLONY MORPHOLOGY	MICROSCOPIC CHARACTERISTI	GRAM	SLANT	BUTT	GLUCOS	LACTOS	GAS	H_2S	INDOLE	MOTILI	OXIDAS	CITRAT	UREASE	CATALA	V.P	METYL RED	MOST PROBABLE ORGANISM
1	Control A_1	Creamy colony on Nutrient agar	Rod	-	B	A	+	-	+	-	+	+	-	+	-	+	-	+	*Enterobacter Spp*
2	Control A_2	Creamy colony on Nutrient agar	Cocci	+	B	A	+	-	-	-	-	+	-	+	-	+	-	+	*Micrococci spp*
3	Control B_1	Creamy colony on NA	Short rod	-	B	B	-	-	-	-	-	+	+	+	-	+	-	+	*Pseudomonas Spp*
4	Control B_2	Mucoidal creamy colony on Nutrient agar	Short rod	-	B	B	-	-	-	-	-	+	+	+	-	+	+	-	*Vibro cholera*
5	Control C_1	Creamy colony on NA	Rod	-	B	B	-	-	-	-	-	+	+	-	-	+	-	+	*Alcagenes Spp*

6	**Control C_2**	**Creamy colony on Nutrient agar**	**Rod**	**-**	**B**	**A**	**+**	**-**	**+**	**-**	**+**	**+**	**-**	**+**	**-**	**+**	**-**	**+**	***Enterobacter spp***
7	**EP1 A_1**	**Creamy colony on NA**	**Straight rod**	**+**	**B**	**A**	**+**	**-**	**-**	**+**	**-**	**+**	**-**	**-**	**-**	**+**	**+**	**-**	***Bacillus spp***
8	**EP1 A_2**	**Creamy colony on Nutrient agar**	**Rod**	**-**	**B**	**A**	**+**	**-**	**+**	**-**	**+**	**+**	**-**	**+**	**-**	**+**	**-**	**+**	***Enterobacter spp***
9	**EP1 B_1**	**Creamy colony on NA**	**Rod**	**-**	**B**	**A**	**+**	**-**	**-**	**+**	**-**	**-**	**-**	**+**	**-**	**+**	**-**	**+**	***Klebsiella spp***
10	**EP1 B_2**	**Shiny creamy colony on NA**	**Clustered cocci**	**+**	**A**	**A**	**+**	**+**	**-**	**-**	**-**	**-**	**-**	**+**	**-**	**+**	**+**	**-**	***Staphylococci spp***
11	**EP1 C_1**	**Creamy colony on nutrient agar**	**Rod**	**-**	**B**	**A**	**-**	**-**	**-**	**-**	**-**	**+**	**-**	**+**	**-**	**+**	**-**	**+**	***Serratia spp***
12	**EP1 C_2**	**Creamy colony**	**Short rod**	**-**	**A**	**A**	**+**	**+**	**+**	**-**	**+**	**+**	**-**	**-**	**-**	**+**	**-**	**+**	***Escherichia coli***
13	**West W_1A_1**	**Creamy branching colony on NA**	**Rod**	**+**	**B**	**B**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**+**	**+**	**-**	***Actinomyces Spp***
14	**West W_1A_2**	**Creamy colony on Nutrient agar**	**Rod**	**-**	**B**	**A**	**+**	**-**	**+**	**-**	**+**	**+**	**-**	**+**	**-**	**+**	**-**	**+**	***Enterobacter Spp***
15	**West W_1B_1**	**Creamy colony on NA**	**Straight rod**	**+**	**B**	**A**	**+**	**-**	**-**	**+**	**-**	**+**	**-**	**-**	**-**	**+**	**+**	**-**	***Bacillus spp***

16	**West W_1B_2**	**Creamy colony on NA**	**Rod**	**-**	**B**	**A**	**+**	**-**	**-**	**+**	**-**	**-**	**-**	**+**	**-**	**+**	**-**	**+**	***Klebsiella spp***
17	**West W_1C_1**	**Creamy colony on nutrient agar**	**Rod**	**-**	**B**	**A**	**-**	**-**	**-**	**-**	**-**	**+**	**-**	**+**	**-**	**+**	**-**	**+**	***Serratia spp***
18	**West W_1C_2**	**Creamy colony on NA**	**Short rod**	**-**	**B**	**B**	**-**	**-**	**-**	**-**	**-**	**+**	**+**	**+**	**-**	**+**	**-**	**+**	***Pseudomonas spp***
19	**West W_2A_1**	**Creamy colony on Nutrient agar**	**Cocci**	**+**	**B**	**A**	**+**	**-**	**-**	**-**	**-**	**+**	**-**	**+**	**-**	**+**	**-**	**+**	***Micrococci spp***
20	**West W_2A_2**	**Creamy branching colony on NA**	**Rod**	**+**	**B**	**B**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**+**	**+**	**-**	***Actinomyces Spp***
21	**West W_2B_2**	**Creamy colony on nutrient agar**	**Rod**	**-**	**B**	**A**	**-**	**-**	**-**	**-**	**-**	**+**	**-**	**+**	**-**	**+**	**-**	**+**	***Serratia Spp***
22	**West W_2C_1**	**Creamy colony on NA**	**Straight rod**	**+**	**B**	**A**	**+**	**-**	**-**	**+**	**-**	**+**	**-**	**-**	**-**	**+**	**+**	**-**	***Bacillus spp***
23	**West W_2C_2**	**Creamy colony on Nutrient agar**	**Rod**	**-**	**B**	**A**	**+**	**-**	**+**	**-**	**+**	**+**	**-**	**+**	**-**	**+**	**-**	**+**	***Enterobacter Spp***
24	**North N_1A_1**	**Creamy colony on NA**	**Short rod**	**-**	**B**	**B**	**-**	**-**	**-**	**-**	**-**	**+**	**+**	**+**	**-**	**+**	**-**	**+**	***Pseudomonas spp***
25	**North N_1A_2**	**Mucoidal creamy colony on**	**Short rod**	**-**	**B**	**B**	**-**	**-**	**-**	**-**	**-**	**+**	**+**	**+**	**-**	**+**	**+**	**-**	***Vibro cholera***

		Nutrient agar																	
26	**North N_1B_1**	**Creamy branching colony on NA**	**Rod**	**+**	**B**	**B**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**+**	**+**	**-**	***Actinomyces Spp***
27	**North N_1B_2**	**Creamy colony on nutrient agar**	**Rod**	**-**	**B**	**A**	**-**	**-**	**-**	**-**	**-**	**+**	**-**	**+**	**-**	**+**	**-**	**+**	***Serratia spp***
28	**North N_1C_1**	**Creamy colony on NA**	**Rod**	**-**	**B**	**B**	**-**	**-**	**-**	**-**	**-**	**+**	**+**	**-**	**-**	**+**	**-**	**+**	***Alcagenes spp***
29	**North N_1C_2**	**Mucoidal creamy colony on Nutrient agar**	**Short rod**	**-**	**B**	**A**	**+**	**-**	**-**	**-**	**-**	**-**	**+**	**+**	**-**	**+**	**-**	**+**	***Moraxella spp***
30	**North N_2A_1**	**Creamy colony on NA**	**Straight rod**	**+**	**B**	**A**	**+**	**-**	**-**	**+**	**-**	**+**	**-**	**-**	**-**	**+**	**+**	**-**	***Bacillus spp***
31	**North N_2A_2**	**Creamy colony on NA**	**Cocci**	**+**	**B**	**A**	**+**	**-**	**-**	**+**	**-**	**+**	**-**	**-**	**-**	**-**	**+**	**-**	***Streptococci spp***
32	**North N_2B_1**	**Creamy branching colony on NA**	**Rod**	**+**	**B**	**B**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**+**	**+**	**-**	***Actinomyces Spp***
33	**North N_2B_2**	**Creamy colony on NA**	**Rod**	**-**	**B**	**A**	**+**	**-**	**-**	**+**	**-**	**-**	**-**	**+**	**-**	**+**	**-**	**+**	***Klebsiella spp***
34	**North N_2C_1**	**Mucoidal creamy colony on**	**Short rod**	**-**	**B**	**B**	**-**	**-**	**-**	**-**	**-**	**+**	**+**	**+**	**-**	**+**	**+**	**-**	***Vibro cholera***

		Nutrient agar																	
35	**North N_2C_2**	**Shiny creamy colony on NA**	**Clustered cocci**	**+**	**A**	**A**	**+**	**+**	**-**	**-**	**-**	**-**	**-**	**+**	**-**	**+**	**+**	**-**	***Staphylococci spp***
38	**South S_1A_1**	**Creamy colony on NA**	**Rod**	**-**	**B**	**B**	**-**	**-**	**-**	**-**	**-**	**+**	**+**	**-**	**-**	**+**	**-**	**+**	***Alcagenes spp***
39	**South S_1A_2**	**Creamy colony on NA**	**Rod**	**-**	**B**	**A**	**+**	**-**	**+**	**-**		**+**	**-**	**+**	**+**	**+**	**-**	**+**	***Proteus spp***
40	**South S_1B_1**	**Creamy colony on NA**	**Rod**	**-**	**B**	**A**	**+**	**-**	**-**	**+**	**-**	**-**	**-**	**+**	**-**	**+**	**-**	**+**	***Klebsiella spp***
41	**South S_1B_2**	**Creamy colony on NA**	**Cocci**	**+**	**B**	**A**	**+**	**-**	**-**	**+**	**-**	**+**	**-**	**-**	**-**	**-**	**+**	**-**	***Streptococci spp***
42	**South S_1C_1**	**Creamy colony on NA**	**Rod**	**-**	**B**	**A**	**+**	**-**	**+**	**-**	**-**	**+**	**-**	**+**	**+**	**+**	**-**	**+**	***Proteus spp***
43	**South S_1C_2**	**Creamy colony on NA**	**Rod**	**-**	**B**	**A**	**+**	**-**	**+**	**+**	**+**	**+**	**-**	**-**	**-**	**+**	**-**	**+**	***Salmonella spp***
44	**South S_2A_1**	**Shiny creamy colony on NA**	**Clustered cocci**	**+**	**A**	**A**	**+**	**+**	**-**	**-**	**-**	**-**	**-**	**+**	**+**	**+**	**+**	**-**	***Staphylococci spp***
45	**South S_2A_2**	**Creamy colony on NA**	**Cocci**	**+**	**B**	**A**	**+**	**-**	**-**	**+**	**-**	**+**	**-**	**-**	**-**	**-**	**+**	**-**	***Streptococci spp***
46	**South S_2B_1**	**Creamy colony on Nutrient agar**	**Cocci**	**+**	**B**	**A**	**+**	**-**	**-**	**-**	**-**	**+**	**-**	**+**	**-**	**+**	**-**	**+**	***Micrococci spp***

47	**South S_2B_2**	**Creamy colony on NA**	**Rod**	**-**	**B**	**A**	**+**	**-**	**-**	**+**	**-**	**-**	**-**	**+**	**-**	**+**	**-**	**+**	***Klebsiella spp***
48	**South S_2C_1**	**Creamy colony on nutrient agar**	**Rod**	**-**	**B**	**A**	**-**	**-**	**-**	**-**	**-**	**+**	**-**	**+**	**-**	**+**	**-**	**+**	***Serratia spp***
49	**South S_2C_2**	**Creamy colony on NA**	**Short rod**	**-**	**B**	**B**	**-**	**-**	**-**	**-**	**-**	**+**	**+**	**+**	**-**	**+**	**-**	**+**	***Pseudomonas spp***
50	**East E_1A_1**	**Creamy colony on NA**	**Rod**	**-**	**B**	**B**	**-**	**-**	**-**	**-**	**-**	**+**	**+**	**-**	**-**	**+**	**-**	**+**	***Alcagenes spp***
51	**East E_1A_2**	**Creamy colony on NA**	**Short rod**	**-**	**B**	**B**	**-**	**-**	**-**	**-**	**-**	**+**	**+**	**+**	**-**	**+**	**-**	**+**	***Pseudomonas spp***
52	**East E_1B_1**	**Creamy colony on Nutrient agar**	**Cocci**	**+**	**B**	**A**	**+**	**-**	**-**	**-**	**-**	**+**	**-**	**+**	**-**	**+**	**-**	**+**	***Micrococci spp***
53	**East E_1B_2**	**Creamy colony on NA**	**Straight rod**	**+**	**B**	**A**	**+**	**-**	**-**	**+**	**-**	**+**	**-**	**-**	**-**	**+**	**+**	**-**	***Bacillus spp***
54	**East E_1C_1**	**Creamy colony**	**Short rod**	**-**	**A**	**A**	**+**	**+**	**+**	**-**	**+**	**+**	**-**	**-**	**-**	**+**	**-**	**+**	***Escherichia coli***
55	**East E_1C_2**	**Creamy colony on NA**	**Rod**	**-**	**B**	**A**	**+**	**-**	**+**	**+**	**+**	**+**	**-**	**-**	**-**	**+**	**-**	**+**	***Salmonella spp***
56	**East E_2A_1**	**Creamy colony on NA**	**Short rods**	**-**	**B**	**A**	**-**	**+**	**+**	**-**	**+**	**-**	**-**	**-**	**-**	**+**	**-**	**+**	***Shigella Spp***

57	**East E_2A_2**	**Shiny creamy colony on NA**	**Clustered cocci**	**+**	**A**	**A**	**+**	**+**	**-**	**-**	**-**	**-**	**-**	**+**	**+**	**+**	**+**	**-**	***Staphylococci spp***
58	**East E_2B_1**	**Creamy colony on NA**	**Rod**	**-**	**B**	**A**	**+**	**-**	**-**	**+**	**-**	**-**	**-**	**+**	**-**	**+**	**-**	**+**	***Klebsiella spp***
59	**East E_2B_2**	**Creamy colony on NA**	**Straight rod**	**+**	**B**	**A**	**+**	**-**	**-**	**+**	**-**	**+**	**-**	**-**	**-**	**+**	**+**	**-**	***Bacillus spp***
60	**East E_2C_1**	**Creamy colony on NA**	**Rod**	**-**	**B**	**A**	**+**	**-**	**+**	**-**	**-**	**+**	**-**	**+**	**+**	**+**	**-**	**+**	***Proteus spp***
61	**East E_2C_2**	**Creamy colony on NA**	**Short rod**	**-**	**B**	**B**	**-**	**-**	**-**	**-**	**-**	**+**	**+**	**+**	**-**	**+**	**-**	**+**	***Pseudomonas spp***

Key: Isolate code A, B and C=Top, Sub andSub-Sub soil; 1=1m away; 2=2meters away; A=Acidic condition; B= Basic condition; + =positive; - =negative.

Table 4.5: Identified Fungal isolates from polluted and unpolluted soil samples and their frequency of occurrence (%)

FUNGAL ISOLATES	% FREQUENCY OF OCCURRENCE FROM POLLUTED SITE	% FREQUENCY OF OCCURRENCE FROM CONTROL
Coccidioides immitis	37.71%	24.1%
Penicillum spp	9.76%	9.20%
Fusarium spp	0.34%	-
Scopulanopsis spp	2.86%	17.24%
Aspergillus fumigates	9.09%	16.09%
Aspergillus flavus	0.84%	17.24%
Aspergillus niger	3.54%	-

Candida spp	35.19%	16.09%
Cladosporium carronii	0.67%	-

Table 4.6: Morphological characteristics of Total fungal and total spent oil degrading fungi isolated from contaminated and uncontaminated soil with its occurrence

Sample	Occurrence	Cultural	Microscopic	Most probable fungi
SOUTH 1A (Total Fungi Count)				
A.	1	Colony is blue-green in colour with powdery surface	Hyphae are hyaline and distinctly septate conidiosphore are long having a club – shape vesicle	*Asperigillius fumigatus*
	2	Purple – brown colonies which turn purple black at maturity	Hyphae are hyaline distinctly septa	*Asperigillus niger*
B.	2	Purple – brown colonies which turn purple black at maturity.	Hyphae are hyaline distinctly septa	*Asperigillus niger*
	2	Colony is blue-green in colour with powdery surface	Hyphae are hyaline and distinctly septate conidiosphore are long having a club – shape vesicle	*Asperigillius fumigatus*

SOUTH 1B (Total Fungi Count) A.	5	At early stage, the colony is whitish but at later stage, it turns to brown.	Hyphae are septate and its arthrospores are in barrel-shape.	*Coccidioides immitis*
B.	4	At early stage, the colony is whitish but at later stage, it turns to brown.	Hyphae are septate and its arthrospores are in barrel-shape.	*Coccidioides immitis*
SOUTH 1C (Total Fungi Count) A.	4	At early stage, the colony is whitish but at later stage, it turns to brown.	Hyphae are septate and its arthrospores are in barrel-shape.	*Coccidioides immitis*
B.	2	At early stage, the colony is whitish but at later stage, it turns to brown.	Hyphae are septate and its arthrospores are in barrel-shape.	*Coccidioides immitis*
SOUTH 2A (Total Fungi Count)	4	Colony is initially white and fluffy, soon turning shades of	Hyphae are hyaline and sepeate. Conidiophores give rise to	*Penicillium spp*

A.		green as pigmented spores are produced.	branching phialdes forming a brush.	
	12	Colony grows as white patches with glossy surface.	Clamydospores are numerous borne single.	*Candida spp*
B.	2	Colony is initially white and fluffy, soon turning shades of green as pigmented spores are produced.	Hyphae are hyaline and sepeate. Conidiophores give rise to branching phialdes forming a brush.	*Penicillium spp*
	2	Colony grows as white patches with glossy surface.	Clamydospores are numerous borne single.	*Candida spp*
SOUTH 2B (Total Fungi Count) A.	1	Colony is initially white and fluffy, soon turning shades of green as pigmented spores are produced.	Hyphae are hyaline and sepeate. Conidiophores give rise to branching phialdes forming a brush.	*Penicillium spp*
	3	At early stage, the colony is whitish but at later stage, it turns to brown.	Hyphae are septate and its arthrospores are in barrel-shape. Hyphae are hyaline distinctly septa	*Coccidioides immitis*

B.	1	Purple – brown colonies which turn purple black at maturity	Hyphae are septate and its arthrospores are in barrel-shape.	*Asperigillus niger*
	1	At early stage, the colony is whitish but at later stage, it turns to brown.	Hyphae are septate and its arthrospores are in barrel-shape.	*Coccidioides immitis*
SOUTH 2C (Total Fungi Count)			Hyphae are septate and its arthrospores are in barrel-shape.	
A.	3	At early stage, the colony is whitish but at later stage, it turns to brown.		*Coccidioides immitis*
B.	2	At early stage, the colony is whitish but at later stage, it turns to brown.	Hyphae are hyaline distinctly septa	*Coccidioides immitis*
			Hyphae are septate and its arthrospores are in barrel-shape.	
EAST 1A (Total Fungi Count)			Hyphae are septate and its arthrospores are in barrel-shape.	
A.	1	Purple – brown colonies which turn		*Asperigillus niger*

	3	purple black at maturity		*Coccidioides immitis*
B.	2	At early stage, the colony is whitish but at later stage, it turns to brown. At early stage, the colony is whitish but at later stage, it turns to brown.		*Coccidioides immitis*
EAST 1B (Total Fungi Count)				
A.	2	Colony is initially white and fluffy, soon turning shades of green as pigmented spores are produced.	Hyphae are hyaline and sepeate. Conidiophores give rise to branching phialdes forming a brush. Hyphae are septate and its arthrospores are in barrel-shape.	*Penicillium spp*
	1	At early stage, the colony is whitish but at later stage, it turns to brown.	Clamydospores are numerous borne single.	*Coccidioides immitis*
	12	Colony grows as white patches with glossy surface.	Clamydospores are numerous borne single.	*Candida spp*
B.	14	Colony grows as white patches with glossy surface.	Hyphae are hyaline and sepeate. Conidiophores give rise to branching phialdes forming a brush.	*Candida spp*

	3	Colony is initially white and fluffy, soon turning shades of green as pigmented spores are produced.		*Penicillium spp*
EAST 1C (Total Fungi Count) A.	7	Colony grows as white patches with glossy surface.	Clamydospores are numerous borne single.	*Candida spp*
B.	6	Colony grows as white patches with glossy surface.	Clamydospores are numerous borne single.	*Candida spp*
EAST 2A (Total Fungi Count) A.	3	Colony grows as white patches with glossy surface.	Clamydospores are numerous borne single.	*Candida spp*
	2	At early stage, the colony is whitish but at later stage, it turns to brown.	Hyphae are septate and its arthrospores are in barrel-shape.	*Coccidioides immitis*
	1	Colonies are initially white and later to	Hyphae are hyaline and septate and its microcondia is 2µ in diameter	*Fusarium spp*

		purple and red pigment observed at its edge.	Hyphae are septate and its arthrospores are in barrel-shape.	
B.	3	At early stage, the colony is whitish but at later stage, it turns to brown.		*Coccidioides immitis*
EAST 2B (Total Fungi Count)				
A.	5	At early stage, the colony is whitish but at later stage, it turns to brown.	Hyphae are septate and its arthrospores are in barrel-shape.	*Coccidioides immitis*
			Hyphae are septate and its arthrospores are in barrel-shape.	
B.	6	At early stage, the colony is whitish but at later stage, it turns to brown.		*Coccidioides immitis*
EAST 2C (Total Fungi Count)				
A.	3	At early stage, the colony is whitish but at later stage, it turns to brown.	Hyphae are septate and its arthrospores are in barrel-shape.	*Coccidioides immitis*
			Clamydospores are numerous borne single.	
	4	Colony grows as white patches with glossy surface.		*Candida spp*
			Hyphae are septate and its arthrospores are in barrel-shape.	

B.	5	At early stage, the colony is whitish but at later stage, it turns to brown.		*Coccidioides immitis*
NORTH 1A (Total Fungi Count) A. B.	7 3 4	Purple – brown colonies which turn purple black at maturity. Colony is initially white and fluffy, soon turning shades of green as pigmented spores are produced. Purple – brown colonies which turn purple black at maturity.	Hyphae are hyaline distinctly septa Hyphae are hyaline and sepeate. Conidiophores give rise to branching phialdes forming a brush. Hyphae are hyaline distinctly septa	*Asperigillus niger* *Penicillium spp* *Asperigillus niger*
NORTH 1B (Total Fungi Count) A. 6	2	At early stage, the colony is whitish but at later stage, it turns to brown.	Hyphae are septate and its arthrospores are in barrel-shape. Clamydospores are numerous borne single.	*Coccidioides immitis*

		Colony grows as white patches with glossy surface.	Clamydospores are numerous borne single.	*Candida spp*
B.	3	Colony grows as white patches with glossy surface.	Hyphae are hyaline and sepeate. Conidiophores give rise to branching phialdes forming a brush.	*Candida spp*
	1	Colony is initially white and fluffy, soon turning shades of green as pigmented spores are produced.		*Penicillium spp*
NORTH 1C (Total Fungi Count)				
A.	2	At early stage, the colony is whitish but at later stage, it turns to brown.	Hyphae are septate and its arthrospores are in barrel-shape.	*Coccidioides immitis*
	5	Colony grows as white patches with glossy surface.	Clamydospores are numerous borne single. Hyphae are septate and its arthrospores are in barrel-shape.	*Candida spp*
B.	4	At early stage, the colony is whitish but at later stage, it turns to brown.		*Coccidioides immitis*

NORTH 2A (Total Fungi Count) A.	10	At early stage, the colony is whitish but at later stage, it turns to brown.	Hyphae are septate and its arthrospores are in barrel-shape. Clamydospores are numerous borne single.	*Coccidioides immitis*
	5	Colony grows as white patches with glossy surface.		*Candida spp*
B.	6	At early stage, the colony is whitish but at later stage, it turns to brown.	Hyphae are septate and its arthrospores are in barrel-shape.	*Coccidioides immitis*
NORTH 2B (Total Fungi Count) A.	12	Colony grows as white patches with glossy surface.	Clamydospores are numerous borne single.	*Candida spp*
	1	At early stage, the colony is whitish but at later stage, it turns to brown.	Hyphae are septate and its arthrospores are in barrel-shape. Clamydospores are numerous borne single.	*Coccidioides immitis*
B.	7	Colony grows as white patches with glossy surface.		*Candida spp*

NORTH 2B (Total Spent Oil Degrading Fungi Count) A. B.	3 5 1	At early stage, the colony is whitish but at later stage, it turns to brown. At early stage, the colony is whitish but at later stage, it turns to brown Colony grows as white patches with glossy surface.	Hyphae are septate and its arthrospores are in barrel-shape Hyphae are septate and its arthrospores are in barrel-shape. Clamydospores are numerous borne single.	*Coccidioides immitis* *Coccidioides immitis* *Candida spp*
NORTH 2C (Total Fungi Count) A.	2 2	Colonies are powdery, buff to brown in colour	Hyphae are hyaline and septate. Conidiophores branches and with Conidia are borne in chains. Clamydospores are numerous borne single.	*Scopulanopsis spp* *Candida spp*

	3	Colony grows as white patches with glossy surface. Colony is blue-green in colour with powdery surface	Hyphae are hyaline and distinctly septate conidiosphore are long having a club – shape vesicle Clamydospores are numerous borne single.	*Asperigillius fumigatus*
B.	4	Colony grows as white patches with glossy surface.	Hyphae are hyaline and septate. Conidiophores branches and with Conidia are borne in chains.	*Candida spp*
	2	Colonies are powdery, buff to brown in colour	Hyphae are hyaline and sepeate. Conidiophores give rise to branching phialdes forming a brush	*Scopulanopsis spp*
NORTH 2C (Total Spent Oil Degrading Fungi Count) A.	5	Colony is initially white and fluffy, soon turning shades of green as pigmented spores are produced.	Hyphae are hyaline and sepeate. Conidiophores give rise to branching phialdes forming a brush	*Penicillium spp*

B	7	Colony is initially white and fluffy, soon turning shades of green as pigmented spores are produced.		*Penicillium spp*
WEST 1A (Total Fungi Count) A.	1	Colony is blue-green in colour with powdery surface	Hyphae are hyaline and distinctly septate conidiosphore are long having a club – shape vesicle	*Asperigillius fumigatus*
	1	Colony is initially white and fluffy, soon turning shades of green as pigmented spores are produced.	Hyphae are hyaline and sepeate. Conidiophores give rise to branching phialdes forming a brush.	*Penicillium spp*
B.	3	Colony is blue-green in colour with powdery surface	Hyphae are hyaline and distinctly septate conidiosphore are long having a club – shape vesicle Clamydospores are numerous borne single.	*Asperigillius fumigatus*
	1	Colony grows as white patches with glossy surface.	Hyphae are hyaline and distinctly septate conidiosphore	*Candida spp*

WEST 1A (Total Spent Oil Degrading Fungi Count) A.	2	Colony is blue-green in colour with powdery surface	are long having a club – shape vesicle Clamydospores are numerous borne single.	*Asperigillius fumigatus*
	6	Colony grows as white patches with glossy surface.	Clamydospores are numerous borne single	*Candida spp*
B	8	Colony grows as white patches with glossy surface.		*Candida spp*
WEST 1B (Total Fungi Count) A.	2	Colony is blue-green in colour with powdery surface	Hyphae are hyaline and distinctly septate conidiosphore are long having a club – shape vesicle	*Asperigillius fumigatus*
	1	Colonies are initially white and later to purple and red pigment observed at its edge.	Hyphae are hyaline and septate and its microcondia is 2µ in diameter Clamydospores are numerous borne single.	*Fusarium spp*
	8			

		Colony grows as white patches with glossy surface.	Hyphae are hyaline and distinctly septate conidiosphore are long having a club – shape vesicle	*Candida spp*
B.	4	Colony is blue-green in colour with powdery surface	Hyphae are hyaline and sepeate. Conidiophores give rise to branching phialdes forming a brush.	*Asperigillius fumigatus*
WEST 1B (Total Spent Oil Degrading Fungi Count)	1	Colony is initially white and fluffy, soon turning shades of green as pigmented spores are produced.	Clamydospores are numerous borne single.	*Penicillium spp*
A.			Hyphae are hyaline and distinctly septate conidiosphore are long having a club – shape vesicle	
	2	Colony grows as white patches with glossy surface.	Hyphae are hyaline and sepeate. Conidiophores give rise to branching phialdes forming a brush	*Candida spp*
B	2	Colony is blue-green in colour with powdery surface		*Asperigillius fumigatus*

	1	Colony is initially white and fluffy, soon turning shades of green as pigmented spores are produced		*Penicillium spp*
WEST 1C (Total Fungi Count) A.	1	Colony is blue-green in colour with powdery surface	Hyphae are hyaline and distinctly septate conidiosphore are long having a club – shape vesicle	*Asperigillius fumigates*
	2	Colony grows as white patches with glossy surface.	Clamydospores are numerous borne single.	*Candida spp*
	5	At early stage, the colony is whitish but at later stage, it turns to brown.	Hyphae are septate and its arthrospores are in barrel-shape. Clamydospores are numerous borne single.	*Coccidioides immitis*
B.	3	Colony grows as white patches with glossy surface.	Clamydospores are numerous borne single.	*Candida spp*
WEST 1C	5	Colony grows as white patches with glossy surface.	Hyphae are hyaline and distinctly septate conidiosphore are long having a club – shape vesicle	*Candida spp*

(Total Spent Oil Degrading Fungi Count) A. B	4 3	Colony is blue-green in colour with powdery surface Colony grows as white patches with glossy surface	Clamydospores are numerous borne single	*Asperigillius fumigates* *Candida spp*
WEST 2A (Total Fungi Count) A. B. WEST 2A (Total Spent Oil Degrading Fungi Count) A.	8 5 2	Colony grows as white patches with glossy surface Colony grows as white patches with glossy surface Colony grows as white patches with glossy surface	Clamydospores are numerous borne single Clamydospores are numerous borne single Clamydospores are numerous borne single	*Candida spp* *Candida spp* *Candida spp*

B	NO GROWTH			
WEST 2B (Total Fungi Count) A.	1	Colony is blue-green in colour with powdery surface	Hyphae are hyaline and distinctly septate conidiosphore are long having a club – shape vesicle Clamydospores are numerous borne single	*Asperigillius fumigatus*
	11	Colony grows as white patches with glossy surface		*Candida spp*
B.	7	Colony grows as white patches with glossy surface	Clamydospores are numerous borne single	*Candida spp*
WEST 2B (Total Spent Oil Degrading Fungi Count) A.	17	Colony grows as white patches with glossy surface	Clamydospores are numerous borne single	*Candida spp*
	2	Colony is blue-green in colour with powdery surface	Hyphae are hyaline and distinctly septate conidiosphore are long having a club – shape vesicle Clamydospores are numerous borne single	*Asperigillius fumigatus*

B	6 4	Colony grows as white patches with glossy surface Colony is blue-green in colour with powdery surface	Hyphae are hyaline and distinctly septate conidiosphore are long having a club – shape vesicle	*Candida spp* *Asperigillius fumigates*
WEST 2C (Total Fungi Count) A. B. WEST 2C	2 3 6	Colony grows as white patches with glossy surface. At early stage, the colony is whitish but at later stage, it turns to brown. Colony grows as white patches with glossy surface.	Clamydospores are numerous borne single. Hyphae are septate and its arthrospores are in barrel-shape. Clamydospores are numerous borne single. Hyphae are hyaline and distinctly septate conidiosphore	*Candida spp* *Coccidioides immitis* *Candida spp*

(Total Spent Oil Degrading Fungi Count) A. B	1 1 2	Colony is blue-green in colour with powdery surface Well developed white fluccy mycelium at the early stage which turn yellow brown colour at mature stage. Colony is blue-green in colour with powdery surface	are long having a club – shape vesicle Hyphae are hyaline and distinctly septate with spores with spores. 3µm in short chains. Hyphae are hyaline and distinctly septate conidiosphore are long having a club – shape vesicle	*Asperigillius fumigatus* *Aspergillus flavus* *Asperigillius fumigatus*
EPI A (Total Fungi Count) A.	 3 2	 Colony is initially white and fluffy, soon turning shades of green as pigmented spores are produced Colony is initially white and fluffy, soon turning shades of	 Hyphae are hyaline and sepeate. Conidiophores give rise to branching phialdes forming a brush Hyphae are hyaline and sepeate. Conidiophores give rise to branching phialdes forming a brush	 *Penicillium spp* *Penicillium spp*

		green as pigmented spores are produced	Hyphae are hyaline and sepeate. Conidiophores give rise to branching phialdes forming a brush	
B.	1	Colony is initially white and fluffy, soon turning shades of green as pigmented spores are produced	Hyphae are septate and its arthrospores are in barrel-shape.	*Penicillium spp*
	4	At early stage, the colony is whitish but at later stage, it turns to brown.		*Coccidioides immitis*
EPI A (Total Spent Oil Degrading Fungi Count) A.	1	At early stage, the colony is whitish but at later stage, it turns to brown.	At early stage, the colony is whitish but at later stage, it turns to brown.	*Coccidioides immitis*
B.	2	At early stage, the colony is whitish but at later stage, it turns to brown.	At early stage, the colony is whitish but at later stage, it turns to brown.	*Coccidioides immitis*
EPI B (Total Fungi Count) A.	4			*Penicillium spp*

		Colony is initially white and fluffy, soon turning shades of green as pigmented spores are produced	Hyphae are hyaline and sepeate. Conidiophores give rise to branching phialdes forming a brush	
	1	Colonies are initially white and later to purple and red pigment observed at its edge.	Hyphae are hyaline and septate and its microcondia is 2µ in diameter Hyphae are hyaline and septate. Conidiophores branches and with Conidia are borne in chains	*Fusarium spp*
	1	Colonies are powdery, buff to brown in colour	Hyphae are hyaline and sepeate. Conidiophores give rise to branching phialdes forming a brush	*Scopulanopsis spp*
B.	3	Colony is initially white and fluffy, soon turning shades of green as pigmented spores are produced	Hyphae are hyaline and septate. Conidiophores branches and with Conidia are borne in chains	*Penicillium spp*
	1	Colonies are powdery, buff to brown in colour	Hyphae are septate and its arthrospores are in barrel-shape Hyphae are hyaline and distinctly septate conidiosphore	*Scopulanopsis spp*

			are long having a club – shape vesicle	
EPI B (Total Spent Oil Degrading Fungi Count) A.	2	At early stage, the colony is whitish but at later stage, it turns to brown	Hyphae are hyaline and distinctly septate conidiosphore are long having a club – shape vesicle Hyphae are septate and its arthrospores are in barrel-shape	*Coccidioides immitis*
	7	Colony is blue-green in colour with powdery surface		*Asperigillius fumigates*
B	2	Colony is blue-green in colour with powdery surface		*Asperigillius fumigates*
	1	At early stage, the colony is whitish but at later stage, it turns to brown		*Coccidioides immitis*

CONTROL A (Total Fungi Count)				
A.	3	At early stage, the colony is whitish but at later stage, it turns to brown.	Hyphae are septate and its arthrospores are in barrel-shape. Clamydospores are numerous borne single	*Coccidioides immitis*
	4	Colony grows as white patches with glossy surface.	Hyphae are hyaline and distinctly septate with spores with spores. 3µm in short chains	*Coccidioides immitis*
B.	3	Well developed white fluccy mycelium at the early stage which turn yellow brown colour at mature stage	Hyphae are septate and its arthrospores are in barrel-shape	*Penicillium spp*
	1	At early stage, the colony is whitish but at later stage, it turns to brown	Hyphae are hyaline and septate. Conidiophores branches and with Conidia are borne in chains	*Coccidioides immitis*
CONTROL A (Total Spent Oil Degrading Fungi Count)				
A.	5	Colonies are powdery, buff to brown in colour	Hyphae are hyaline and septate. Conidiophores branches and with Conidia are borne in chains	

B	2	Colonies are powdery, buff to brown in colour		
CONTROL B (Total Fungi Count) A.	5	At early stage, the colony is whitish but at later stage, it turns to brown.	Hyphae are septate and its arthrospores are in barrel-shape.	
	4	Well developed white fluccy mycelium at the early stage which turn yellow brown colour at mature stage	Hyphae are hyaline and distinctly septate with spores with spores. 3µm in short chains	
		At early stage, the colony is whitish but at later stage, it turns to brown.	Hyphae are septate and its arthrospores are in barrel-shape.	
			Clamydospores are numerous borne single	
B.	3	Colony grows as white patches with glossy surface.	Hyphae are hyaline and distinctly septate with spores with spores. 3µm in short chains	
	7	Well developed white fluccy mycelium at the early stage which turn		

	3	yellow brown colour at mature stage		
CONTROL B (Total Spent Oil Degrading Fungi Count) A.	8	Colony is initially white and fluffy, soon turning shades of green as pigmented spores are produced	Hyphae are hyaline and sepeate. Conidiophores give rise to branching phialdes forming a brush Hyphae are hyaline and septate. Conidiophores branches and with Conidia are borne in chains	
	1	Colonies are powdery, buff to brown in colour	Hyphae are hyaline and septate. Conidiophores branches and with Conidia are borne in chains	
B	6	Colonies are powdery, buff to brown in colour		

CONTROL C (Total Fungi Count) A. \ B.	5 2 3 4 3	At early stage, the colony is whitish but at later stage, it turns to brown. Well developed white fluccy mycelium at the early stage which turn yellow brown colour at mature stage. Colony grows as white patches with glossy surface. At early stage, the colony is whitish but at later stage, it turns to brown. Well developed white fluccy mycelium at the	Hyphae are septate and its arthrospores are in barrel-shape. Hyphae are hyaline and distinctly septate with spores with spores. 3µm in short chains Clamydospores are numerous borne single Hyphae are septate and its arthrospores are in barrel-shape. Hyphae are hyaline and distinctly septate with spores with spores. 3µm in short chains	

		early stage which turn yellow brown colour at mature stage		
CONTROL C (Total Spent Oil Degrading Fungi Count) A.	9	Colony is blue-green in colour with powdery surface	Hyphae are hyaline and distinctly septate conidiosphore are long having a club – shape vesicle	
B	5	Colony is blue-green in colour with powdery surface	Hyphae are hyaline and distinctly septate conidiosphore are long having a club – shape vesicle	
	1	Colonies are powdery, buff to brown in colour	Hyphae are hyaline and septate. Conidiophores branches and with Conidia are borne in chains	

Table 4.7: Concentration of air Pollutants within the four sampled locations during the morning and evening Periods

Location	Time of the Day	CO (ppm)	NO_2 (ppm)	SO_2 (ppm)	H_2S (ppm)	NH_3 (ppm)	SPM (ppm)
Public Procurement Office (PPO)	**Morning (M)**	10.12±0.81	0.40±0.01	0.96±0.01	0.65±0.01	0.18±0.01	1.36±0.21
	Evening (E)	4.24±0.21	0.25±0.01	0.72±0.01	0.36±0.01	0.04±0.00	1.43±0.38
BJ Business Centre (BJ)	**Morning (M)**	20.26±1.01	0.55±0.01	1.22±0.12	5.09±0.32	0.44±0.01	2.71±0.12
	Evening (E)	19.35±1.02	0.43±0.01	1.04±0.01	0.96±0.01	0.08±0.00	2.30±0.32
Mr Umunnakwe Business Centre (UB)	**Morning (M)**	24.74±1.75	0.46±0.01	1.00±0.01	3.26±0.27	0.42±0.01	2.58±0.21
	Evening (E)	16.64±1.23	0.38±0.01	1.00±0.01	0.74±0.01	0.12±0.01	2.91±0.30
Old Registry Business Centre (ORBC)	**Morning (M)**	18.56±2.08	0.36±0.01	1.00±0.01	0.73±0.01	0.28±0.01	2.55±0.28
	Evening (E)	15.20±0.96	0.34±0.01	1.00±0.01	1.26±0.10	0.81±0.03	2.92±0.22

Values are presented as Mean ± standard deviation with n = 5

Legend: CO = Carbon (II) Oxide, NO_2 = Nitrogen (IV) Oxide, SO_2 = Sulphur (IV) Oxide, H_2S = Hydrogen Sulphide, NH_3 = Ammonia, SPM = Suspended Particulate Matter.

Table 4.8: Air Quality Index of Air Pollutants Analysed

AQI >Category	AQI Rating	NO_2 (ppm)	SO_2 (ppm)	H_2S (ppm)	CO (ppm)	NH_3 (ppm)	SPM (ppm)	FLAME (ppm)
Very Good (0-15)	A	0.00-0.02	0.00-0.02	NA	0-2.0	0-50	0-50	NA
Good (16-31)	B	0.00-0.03	0.00-0.03	NA	21-40	51-100	51-75	NA
Moderate (32-49)	C	0.03-0.04	0.03-0.04	NA	41-60	101-200	76-100	NA
Poor (50-99)	D	0.04-0.06	0.03-0.04	NA	61-90	201-300	101-150	NA
Very Poor (100 or over)	E	>0.06	>0.06	NA	>90	300-500	>150	NA

Legend: AQI = Air Quality Index, CO = Carbon (II) Oxide, NO_2 = Nitrogen (IV) Oxide, SO_2 = Sulphur (IV) Oxide, H_2S = Hydrogen Sulphide, NH_3 = Ammonia, SPM = Suspended Particulate Matter, NA = Not Available

4.2 DISCUSSION

4.2.1 Soil Microbial Enzymes

Effects of soil pollution on enzyme activities are complex. The response of different enzymes to the same pollutant may vary greatly and the same enzyme may respond differently to different pollutants. Various contaminants such as generator fumes and heavy metals have been found to alter soil biochemical equilibrium and energy balance. Their presence causes alteration in soil microbial properties, pH, oxygen and nutrient availability (Odjegba and Sadiq, 2002) and their exudates (pH, enzymes). Microorganisms are known to release extracellular enzymes to mineralize organic compounds to elemental minerals (Nannipieri *et al*., 2020). It is important to underline that these enzymes attached at the outer microbial cells initiate the hydrolysis, and oxidation of high molecular weight substrates such as hydrocarbons to mineral elements (Nannipieri *et al*., 2020).

Catalase (EC. 1.11.1.6) is a common enzyme found in nearly all living organisms that are exposed to oxygen, where it catalyzes the decomposition of hydrogen peroxide to water and oxygen (Nelson and Cox, 2000). Generally speaking, there was hydrogen peroxide of high concentration in soil and the activity of catalase decreased with corresponding increase in the concentration of the generator fumes (Yu, 2008).

The prolonged exposure to generator fumes (pollutant) altered soil catalase activity significantly at $p< 0.05$. In figure 4.1 (top soil-1m away), there was significant difference (increase) at $p< 0.05$ on the level of catalase enzyme activity on polluted soils (loc W (west) when compared with control (unpolluted) site. Also, there was a significant reduction at $p<0.05$ at loc S(south), E(east) and no significant difference at $p < 0.05$ at loc N(north) and Epi(epicenter) when compared with

control (unpolluted) site. In figure 4.4 (top soil-2m away), there was significant increase at $p< 0.05$ at loc W, S, and significant reduction at $p<0.05$ at loc E and N when compared with control (unpolluted soil). No significant difference at loc epi.

In figure 4.2 (sub soil-1m), there was significant difference (increase) at $p< 0.05$ at loc S, W and significant reduction at $p< 0.05$ at loc E, N and epi when compared with the control. In figure 4.5 (sub soil -2ms), there was a significant difference (increase) at $p<0.05$ at W, N and significant reduction at $p<0.05$ at loc S, E and epi when compared with the control (unpolluted) soil.

In figure 4.3 (sub-sub soil -1m apart), there was a significant difference (increase) at $p<0.05$ at loc W and a significant reduction at $p<0.05$ at loc S, E, N, epi when compared with the unpolluted soil (control). In figure 4.6 (sub-sub soil-2meters apart), there was a significant increase at $p<0.05$ at loc W, S and a significant reduction at $p<0.05$ at loc E, N, and epi when compared with the control (unpolluted) soil. As shown in figures above at top soil loc S, E, at 1m and E and N at 2ms, Sub soil at loc E, N, and epi at 1m, E and N at 2ms, Sub-sub soil at loc S, E, N and epi at 1m and E, N,and epi at 2ms the catalase activities were decreased which indicate that petroleum hydrocarbon inhibited the catalase activity, that is the toxic action during petroleum hydrocarbon degradation which led to decrease the catalase activity.

This finding corroborates that of Achuba and Okoh (2014), which found that there was altered catalase activity as a result of the petroleum hydrocarbon. However, its activity increased as a result of the increased microbial activity towards biodegradation of available petroleum hydrocarbon. Achuba and Peretiemo-Clarke (2008) also asserted that the initial reduction at the top soil of catalase activity could be because being an enzyme; its activity is altered by unfavorable conditions, such as hypoxia, unavailability of nutrient and changes in pH. This finding puts

catalase as a useful biomarker for indicating the onset of biodegradation process in the soil (Ajao *et al.,* 2011).

The comparatively high level of sub soil catalase activity from polluted soil appeared to suggest the presence of a high quantity of biodegradable substrates in this soil type (Tejada *et al.*, 2007). Additionally, Yu (2008) has proposed that soil catalase activity increased with increase in concentrations of hydrocarbon pollutants. In an *in vivo* study, Airaodion *et al*., (2019), reported that feeding Wistar rats with a hydrocarbon contaminated diet significantly increased the activity of catalase. This result is also in accordance with the report of Nwaogu *et al.,* 2012. The prolonged exposure to generator fumes to these sites brought changes in its soil condition such as pH, hypoxia as well as reduction in the number and activities of soil micro organisms (Maila and Cloete, 2005). These changes also caused the fertile soil to lose its productive potential (Maila and Cloete, 2005). Catalase have been found only to be useful for indicating the onset of the biodegradation process as it exhibits decrease in its activities after the rate of biodegradation of petroleum has decreased as seen in sub-sub soil (Frankenberger and Bingham, 2012).

Lipase is secreted to the environment by plant roots and microorganisms. It is an important hydrolytic enzyme which catalyzes hydrolysis of oils and fats under physiological conditions (Nelson and Cox, 2004). Soil lipase can be used in monitoring bioremediation of hydrocarbons (Margesin *et al*., 2000). An indicator of lipase may be attributed to the appearance of products released from oil degradation which may be substrate for lipase.

In figure 4.7 (top soil (1m apart), there was significant difference at $p< 0.05$ when control (unpolluted soil) was compared with loc S(South), E(East), W(West) and epi(epicenter) but no significant difference at $P<0.05$ when compared with loc N(North). There was significant increase

at p<0.05 at loc E, W, S and epi. In figure 4.10 (2m apart -top soil), there was significant increase at p <0.05at loc E, W, significant reduction at p< 0.05 at loc epi and no significant difference at p > 0.05 at loc S, N when compared with control (unpolluted soil).

In figure 4.8 (Sub soil -1m apart), there was significant difference (increase) at p< 0.05 at loc E, W, epi when compared with control (unpolluted soil). There was a significant reduction at p < 0.05 at loc epi and no significant difference at p> 0.05 at loc N and S when compared with control (unpolluted soil). In figure 4.11 (2 meters apart- sub soil, there was significant difference (increase) at p< 0.05 at loc E, W when compared with control (unpolluted soil). There was also a significant reduction at p < 0.05 at loc epi.

In figure 4.9 (Sub-Sub soil -1m apart), there was significant difference (increase) at p <0.05 at loc E, W, Epi when compared with control (unpolluted soil). Also, there was a significant reduction at p < 0.05 at loc S, N. In figure 4.12 (sub-sub soil-2 meters apart, there was a significant increase at p < 0.05 at E, W, Epi, N and significant reduction at p < 0.05 at loc S when compared with the control (unpolluted soil).

The lipase activity increased at p< 0.05 as the volume of the contaminant (fumes components) increased, however the increment was more pronounced in loc W and E of the top soil, sub and sub-sub soil. This finding corroborates studies by Frankenberger and Bingham, (2012), Margesin *et al.,* (2000) were it was found that increasing contaminant concentration increased microbial extracellular lipase activity, thus fronting lipase as a good option for study of contaminated soil bioremediation. The increase also at the sub, sub-sub soil was as a result of no colloidal suspension (unrecovered oil) that prevents the triacylglycerol and other substrate of lipase from getting to the sub soil or sub-sub soil. This increase of lipase activity in this hydrocarbon polluted locations

might be the consequence of stimulation of the growth of microorganisms brought about by oily substances contained in petroleum contaminants. It was also observed that there was significant ($P < 0.05$) reduction in the concentration of lipase at top soil loc S, Epi at 1meter and 2meters apart, Sub soil at Epi at 1m and2ms apart, Sub-sub soil at loc Epi at 1m and S at 2ms apart. A similar study by Lin *et al.,* (2005) recorded that it was observed that lipase activity in a petroleum polluted site decreased after bioremediation, other works (Margesin *et al*., 2000 and Riffaldi *et al*., 2006) have shown that lipase is closely related with the organic pollutants present in the soil.

Lipase activity has also been reported to be the reason behind the drastic reduction of total hydrocarbon from contaminated soil and its activity has been found to be a very useful indicator parameter for testing hydrocarbon degradation in soil (Margesin and Schinner, 2000). Lipase degrades lipids and other lipid-like compounds derived from a large variety of microorganisms, animals, plants. Lipids catalyze various reactions such as hydrolysis, interesterification, esterification, alcoholysis, aminolysis of organic pollutants laying credence to their avowed role in bioremediation.

The prolonged exposure to generator fumes (pollutant) altered the lipase activity. This could arise from unfavorable conditions such as hypoxia and a reduction in pH which occurred in the oil polluted environment indicating that oil biodegradation by microorganisms and metabolic enzymes could lead to production of organic acids. It could also imply that the amino acids at the active sites of soil lipase (such as serine, histidine and aspartate) are irritable to hypoxic conditions and pH decrease, and any condition that creates oxygen tension with a rise in acidic environment adversely affected the activity. This finding is in consonant with the report of Margesin and Schinner (2000) on the inhibition of the activity of soil lipase following an insult of soil ecosystem with spent oil.

Phosphatases (P) are inducible enzymes regulated by end-product inhibition. Plant roots and microbes will increase the excretion of phosphatases into soils when available phosphate (P) does not meet their demands. Soil acid phosphatase plays a vital role in controlling P mineralization, and its activity reflects the capacity of organic P mineralization potential in soils.

In figure 4.13 (top soil -1m apart), there was significant difference (increase) at $P< 0.05$ when control (unpolluted soil) was compared with sample loc W but no significant difference at $P<0.05$ at loc S, E, N and epi. In fig 4.16 (top soil-2ms apart, there was a significant reduction at $p<0.05$ when sample location S was compared with control (unpolluted soil) but no significant difference at $p> 0.05$ at sample loc E, W, N and epi.

In figure 4.14 (sub soil -1m apart), there was significant difference (increase) at $P< 0.05$ with sample location N and a significant decrease at $p< 0.05$ with sample location E, W, epi when compared with the control (unpolluted soil). No significant difference at $p> 0.05$ with sample loc S when compared with the control. In figure 4.17 (sub soil-2ms apart, there was a significant increase at $p<0.05$ at loc W and a significant reduction at $p<0.05$ at loc S, E, N, epi.

In figure 4.15 (sub -sub soil -1m apart), there was a significant difference (increase) at $p<0.05$ at loc S, a significant reduction at $p<0.05$ at loc E, W, N and no significant difference at $P<0.05$ at loc epi when compared with the control (unpolluted soil). In figure 4.18 (sub sub-2ms apart, there was a significant difference (reduction) at $p<0.05$ at loc S, E, W, N, when compared with control (unpolluted soil). No significant difference at $p> 0.05$ at loc epi when compared with control.

Results showed that driven by seasonal variation, changes in soil acid phosphatase activities coincided with the seasonal climate pattern. Since this research was conducted in the month of June (rainy season), it is expected that soil acid phosphatase activity would be clearly greater in

the wet season than the dry season, a seasonal pattern observed by Grierson and Adams (2000). Actually, in the wet season, plants grow fast, and microbial biomass is always high. This increasing enzyme activity would respond to meet the increasing P demand by plant and microbe growth in the wet season. Since heavy rain in the wet season often leads to nutrient loss, low available P detected in the wet season further intensifies the completion for P in ecosystems in the growing season.

Moreover, soil available P was relatively high to meet the biological demands because of the accumulation of nutrients released from litter decomposition and low soil nutrient diffusion (Xia *et al.,* 1997).Soil acid phosphatase activity is more dependent on soil available P in the growing season than in the least biologically active season (dry season).

The decrease in the soil acid phosphatase activity could be produced by drought which might result in a reduction of P supply to plants and further aggravate the pressure of P limitation in this study area in the long term. Apart from the decrease in soil moisture, the increasing acidity of soil accompanied by prolonged drought conditions during the time of research might be one of the mechanisms involved in the reduction of soil acid phosphatase activity. Also, reduction of the soil acid phosphatase activity could be as a result of doubled rainfall which brought about more available N input into the ecosystems because excessive available N input would result in a nutrient imbalance which is deleterious to microbial growth that requires balanced nutrient proportions (Kramer and Green, 2000). The prolonged exposure to generator fumes which lowers the soil pH, would inhibit plant root growth and nutrient absorption. This factor would go against acid phosphatase since it is closely bound up with microbe, root growth and plant demand for P.

The effect of prolonged exposure to generator fumes on soil alkaline phosphatase activity was significantly ($p<0.05$) different. In figure 4.19 (top soil -1m apart), there was a significant difference (increase) at $p<0.05$ at sample loc S and a significant reduction at $p<0.05$ at loc E, W and epi when compared with control (polluted soil). No significant difference at $P<0.05$ at loc N. In figure 4.22 (top soil-2ms apart, there was a significant difference (reduction) at $p<0.05$ at loc W, N and epi when compared with control (unpolluted soil). Also, no significant difference at $p< 0.05$ at loc S and E when compared with the control.

In figure 4.20 (sub soil -1m apart), there was a significant difference (increase) at $p< 0.05$ at sample loc E, a significant reduction at $p<0.05$ at sample loc N when compared with the control. No significant difference at $p< 0.05$ at loc S, N and epi when compared with the control (unpolluted soil). In figure 4.23 (sub soil-2ms apart, there was a significant difference (reduction) at $p<0.05$ at sample loc W, N and epi, also no significant difference at $p<0.05$ at sample loc S and E when compared with the control (unpolluted) site.

In figure 4.21 (sub-sub soil -1m apart), there was a significant difference (increase) at $p< 0.05$ at loc E and W when compared with control. No significant difference at $p<0.05$ at sample loc S, N and epi. In figure 4.24 (sub -sub soil - 2ms apart, there was a significant difference (increase) at $p<0.05$ at sample loc E and W when compared with control. No significant difference at $p<0.05$ at sample loc S, N and epi when compared with the control.

4.2.2 Soil Microbiological Activities

The heterotrophic bacterial and fungi counts from the generator fume impacted soils are shown in Tables 4.1 and 4.2. There was a significant difference at $p<0.05$ at generator impacted soil location with highest heterotrophic bacterial counts when compared with the control site (table 4.1). The generator impacted soil location 1(S-2m) had the highest heterotrophic bacterial count ($5.35x10^7 \pm 3.2$ cfu/g) at top soil and sub soil ($7.45x10^7 \pm 2.58$ cfu/g), location 1(S-1m) had the highest heterotrophic bacterial count at sub-sub soil (6.13 ± 2.1 $x10^6$ cfu/g) when compared with control sites ($2.15x10^6 \pm 1.11$ cfu/g, $5.38x10^6 \pm 2.06$ cfu/g, $5.23x10^6 \pm 3.27$cfu/g) respectively. This trend is collaborated by the higher organic compounds of diesel exhausts (hydrocarbons) in comparison to the control soil. This might be as a result that the generator impacted soil harbours microbial flora capable of utilizing the hydrocarbon as energy and carbon source, also due to the presence of residual hydrocarbons in the polluted soil which boost the carbon supply in the soil, hence favours the growth of bacteria (hydrocarbon -utilizing bacteria) which corroborates with the report of Ijah and Abioye (2003).

Unlike the bacterial population, there was no significant difference at $p< 0.05$ in heterotrophic fungi count of generator impacted soil when compared with control soil (table 4.2). This is as a result of the slow growth rate exhibited by the fungi species. The viable heterotrophic fungi count obtained from the generator impacted soils could be reflective of the adaptive abilities of those fungal isolates to thrive even in the event of anthropogenic intermittent discharges of various types and quantities of petroleum products on these soil surfaces over periods of time (Husaini *et al*., 2008).

In sub soil (table 4.2), the control site had the highest heterotrophic fungi count ($6.33x\ 10^3 \pm 3.64$) when compared with sub soil generator impacted soil. This could be as a result of complex

substrates of plant origin on control site and reduction in the mineralization process of organic matter in exhaust fume impacted soil.

4.2.3 Microbial Species

In table 4.3, the bacterial genera isolated in the fume impacted soil were *Bacillus spp, Enterobacter spp, Serratia spp, Moraxella spp,Micococci spp,Vibro cholera, Staphylococci spp,Pseudomonas spp,Yersinia spp,Shigella spp,Actinomyces spp,Citrobacter spp* as compared with the bacteria isolated in control which are *Escherichia coli* and *Micrococci spp.* The fungal genera on the other hand were *Asperigillius fumigatus, Asperigillus niger, , Coccidioides immitis, Candida spp, Penicillium spp, Fusarium spp, Scopulanopsis spp, Aspergillus flavus.*

The spent oil degrading bacterial genera isolated in the fume impacted soil were *Enterobacter spp, Micrococci spp, Pseudomonas spp, Vibro cholera, Alcagenes spp, Bacillus spp, Klebsiella spp, Staphylococci spp, Serratia spp, Escherichia coli, Actinomyces spp, Moraxella spp, Proteus spp, Salmonella spp, Serratia spp, Streptococci spp, Shigella spp* (table 4.4). Also, the spent oil degrading bacterial genera isolated in control are *Escherichia coli, Micrococci spp, Pseudomonas spp, Vibro cholera, Alcagenes spp.*

In table 4.5, the result indicated the presence of nine genera of fungi namely *Coccidioides immitis, Penicillum spp, Fusarium spp, Scopulanopsis spp, Aspergillus fumigates, Aspergillus flavus, Aspergillus niger, Candida spp, Cladosporium carronii.* In table 4.6, the spent oil degrading fungi genera on the other hand were *Scopulanopsis spp, Coccidioides immitis, Penicillum spp, Cladosporium carronii, Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger.* Also, the spent oil degrading fungi genera isolated in control are *Coccidioides immitis, Penicillum spp.*

Coccidioides immitis was the most prevalent fungal isolate occurring at all the soil samples with a percentage frequency of 37.71% (polluted) and 24.1% (control). *Fusarium spp* was the least fungal isolate occurring only at the polluted soil sample with a percentage frequency of 0.34%.

4.2.4 Air Sampling Analysis

Disparate 5-day mean levels of air pollution indicators, CO (Carbon II Oxide), NO_2 (Nitrogen (IV) Oxide), H_2S (Hydrogen Sulphide), SO_2 (Sulphur (IV) Oxide), SPM_{10} (Suspended Particulate Matter) and NH_3 (Ammonia) were undertaken in the morning and evening hours from the four sampled locations; Public Procurement Office, BJ Business Centre, Mr Umunnakwe Business Centre and old Registry Business Centre. These variations are presented in table 4.7.

Generally, levels of CO recorded from the four locations (Public Procurement Office, BJ Business Centre, Mr Umunnakwe Business Centre and old Registry Business Centre) varied between 4.24 ppm and 24.74 ppm which is lower than 30-70 ppm reported by Adelagun *et al*. (2012). The mean concentration of CO obtained in this study (10.12 ± 0.81 ppm, 20.26 ± 1.01 and 19.35±1.02 ppm, 24.74±1.75 and 16.64±1.23 ppm, 18.56±2.08 ppm and 15.20±0.96 ppm) from the four locations is higher than 10ppm recommended as limit in the atmosphere by FEPA (1991) except the mean CO concentration obtained in Public Procurement Office in the evening hour (4.24±0.21 ppm).

At the Public Procurement centre, there was significant difference with mean concentrations of CO at $P<0.05$ in the morning (10.12± 0.81 ppm) when compared with the evening hours (4.24±0.21 ppm) but no significant difference when compared with FEPA limit (10 ppm). There was significant decrease with mean concentrations of CO at $P<0.05$ in the evening (4.24±0.21 ppm) hours when compared with FEPA limit (10 ppm). At the BJ Business centre, there was no significant difference with mean concentrations of CO at $P<0.05$ in the morning (20.26±1.01ppm)

and in the evening hours (19.35±1.02ppm) and however, both were significantly higher when compared with FEPA limit (10 ppm).

At the Mr Umunnakwe Business centre, there was significant difference with mean concentrations of CO at $P<0.05$ in the morning (24.74±1.75 ppm) and in the evening hours (16.64±1.23 ppm) and when compared with FEPA limit (10 ppm). At the Old Registry Business centre, there was significant difference with mean concentrations of CO at $P<0.05$ in the morning (18.56±2.08 ppm) and in the evening hours (15.20±0.96 ppm) as well as when compared with FEPA limit (10 ppm).

CO showed significant temporal and spatial variations and higher mean CO concentrations was obtained in the morning hours than in the afternoon. Prolonged exposure to this elevated level of CO in the sampled locations may result to death as the gas combine readily with haemoglobin in red blood displacing oxygen and forming insoluble carboxyhaemoglobin (CoHb) which has been reported to be toxic to health (Beeby, 1993). Studies have shown that CO is asphyxiate, it can cause tissue damage after prolonged exposure and its affinity for haemoglobin is almost 220 times greater than that of oxygen. The high CO concentrations reported in this study may be attributed to high number of vehicles and traffic congestion experienced in the area.

The concentrations of nitrogen (IV) oxide (NO_2) obtained in this study varied between 0.25 ppm and 0.55 ppm (table 4.7). Like CO, there were higher mean concentrations of NO_2 during the morning hours than in the evening hours. The mean concentration of NO_2 recorded in this research (0.40±0.01 ppm and 0.25±0.01 ppm, 0.55±0.01 and 0.43±0.01 ppm, 0.46±0.01 and 0.38±0.01 ppm, 0.364 ± 0.01 and 0.336 ± 0.01 ppm) from the four locations is much higher than the 0.06 ppm limit by FEPA (1991) for the gas in the atmosphere. At the Public Procurement centre, there was significant difference with mean concentrations of NO_2 at $P<0.05$ in the morning

(0.40±0.01ppm) and in the evening hours (0.25±0.01ppm) as well as when compared with FEPA limit (0.06 ppm).

At the BJ Business centre, there was significant difference with mean concentrations of NO_2 at $P<0.05$ in the morning (0.55±0.01 ppm) and in the evening hours (0.43±0.01 ppm) and when compared with FEPA limit (0.06 ppm). At the Mr Umunnakwe Business centre, there was no significant difference with mean concentrations of NO_2 at $P<0.05$ in the morning (0.46±0.01ppm) and in the evening hours (0.38±0.01ppm) but there was significant difference with both mean NO_2 concentration when compared with FEPA limit (0.06 ppm). At the Old Registry Business centre, there was no significant difference with mean concentrations of NO_2 at $P<0.05$ in the morning (0.36±0.01 ppm) when compared in the evening hours (0.34±0.01 ppm) but there was significant difference with mean NO_2 concentrations in the morning and evening hours when compared with FEPA limit (0.06 ppm). Thus, a prolonged inhalation of air from these locations may affect the lungs and throat of human that are exposed to the gases. The high NO_2 levels in these study areas can also elevate morbidity and mortality in young children, those suffering from asthma and chronic bronchitis. The high concentrations of NO_2 in the sampled locations may be attributed to the extensive emissions from vehicle exhaust systems, power generating systems, and other combustion sources.

In this study, the concentrations of sulphur (IV) oxide (SO_2) recorded from the four sampled locations ranges between 0.7 and 1.22 ppm (table 4.7). This range is higher than the 0.03-0.09 ppm reported by Okunola *et al*., (2012). Similarly, the mean concentrations recorded in this study (0.96 ± 0.01 ppm and 0.7±0.01 ppm, 1.22±0.12 and 1.04 ± 0.01 ppm, 1.00 ± 0.01 and 1.00 ± 0.01 ppm, 1.00 ± 0.01 and 1.00 ± 0.01ppm) are higher than the 0.10 ppm recommended by FEPA (1991).

Thus, inhalations of air from these locations for a long period by humans may cause respiratory problems and severe headache (Tse and Oguama, 2014).

At the Public Procurement centre, there was significant difference with mean concentrations of SO_2 at $P<0.05$ in the morning (0.96± 0.01 ppm) and in the evening hours (0.7± 0.01 ppm) when compared with FEPA limit (0.10 ppm). At the BJ Business centre, there was significant difference with mean concentrations of SO_2 at $P<0.05$ in the morning (1.22± 0.12 ppm) and in the evening hours (1.04± 0.01 ppm) when compared with FEPA limit (0.10). At the Mr Umunnakwe Business centre, there was no significant difference with mean concentrations of SO_2 at $P<0.05$ in the morning (1.00± 0.01 ppm) and in the evening hours (1.00± 0.01 ppm) but there was significant difference at $P<0.05$ in the morning and evening hours when compared with FEPA limit (0.10 ppm). At the Old Registry Business centre, there was no significant difference with mean concentrations of SO_2 at $P<0.05$ in the morning (1.00± 0.01ppm) and in the evening hours (1.00± 0.01ppm) but there was significant difference at $P> 0.05$in the morning and evening hours when compared with FEPA limit (0.10 ppm). Elevated levels of SO_2 reported in this work may be attributed to high numbers of vehicles using these sampled locations as Ukemenam, 2014 documented that vehicular emission is the major source of SO_2 in the air.

The concentrations of hydrogen sulphide (H_2S) obtained in this study ranged between 0.36 and 5.09 ppm (table 4.7). The distributions of the mean H_2S concentrations were higher also in the morning hours than in the evening hours. However, the mean concentrations recorded in this study (0.65± 0.01 ppm and 0.36± 0.01 ppm, 5.09 ± 0.32 and 0.96 ± 0.01 ppm, 3.26 ± 0.27 and 0.74 ± 0.01 ppm ,0.73 ± 0.01 and 1.26 ± 0.01 ppm) is higher than 0.10 ppm recommended by FEPA (1991).

At the Public Procurement centre, there was significant difference with mean concentrations of H_2S at $P<0.05$ in the morning (0.65± 0.01 ppm) and in the evening hours (0.36± 0.01 ppm) and when compared with FEPA limit (0.10 ppm). At the BJ Business centre, there was significant difference with mean concentrations of H_2S at $P<0.05$ in the morning (5.09± 0.32 ppm) and in the evening hours (0.96± 0.01 ppm) and when compared with FEPA limit (0.10 ppm). At the Mr Umunnakwe Business centre, there was significant difference with mean concentrations of H_2S at $P<0.05$ in the morning (3.26± 0.27 ppm) and in the evening hours (0.74± 0.01 ppm) and when compared with FEPA limit (0.10 ppm). At the Old Registry Business centre, there was significant difference with mean concentrations of H_2S at $P<0.05$ in the morning (0.73± 0.01 ppm) and in the evening hours (1.26± 0.01 ppm) when compared with FEPA limit (0.10 ppm). The high concentrations of hydrogen sulphide in sampled locations may be attributed to the heavy emissions from generators and vehicles, petroleum and petrochemical processes (Asuoha and Osu, 2015; Utang and Peterside, 2011).

In this study, the mean concentrations of ammonia determined were between the range of 0.04 and 0.44 ppm during the morning and evening hours (table 4.7). There was significant difference with mean of NH_3 concentrations at $P<0.05$ in the morning hours when compared with FEPA limit (0.30 ppm) and with mean concentrations in the evening hours. At the Public Procurement centre, there was significant difference with mean concentrations of NH_3 at $P<0.05$ in the morning (0.18± 0.01 ppm) and in the evening hours (0.04± 0.00 ppm) when compared with FEPA limit (0.30 ppm).

At the BJ Business centre, there was significant difference with mean concentrations of NH_3 at $P<0.05$ in the morning (0.44± 0.01 ppm) and in the evening hours (0.08± 0.00 ppm) when compared with FEPA limit (0.30 ppm). At the Mr Umunnakwe Business centre, there was significant difference with mean concentrations of NH_3 at $P<0.05$ in the morning (0.42± 0.01 ppm)

and in the evening hours (0.1± 0.01 ppm) when compared with FEPA limit (0.30 ppm). At the Old Registry Business centre, there was significant difference with mean concentrations of NH_3 at $P<0.05$ in the morning (0.28± 0.01 ppm) and in the evening hours (0.81± 0.03 ppm) when compared with FEPA limit (0.30 ppm). Consequently, the high concentrations of ammonia recorded may be attributed to agricultural processes, vehicular emission and volatilization from soils as reported by Behera *et al*., (2013)

Suspended particulate matter (SPM) is a term used to indicate very small solid and liquid particles in the air. SPM observed in this present study varied between 1.36 and 2.90 ppm (table 4.7). The mean concentration of SPM recorded in this research (1.36 ± 0.21ppm and 1.43± 0.38 ppm, 2.71 ± 0.12 and 2.30 ± 0.32ppm, 2.58 ± 0.21 and 2.91 ± 0.30ppm, 2.55 ± 0.28 and 2.90 ± 0.22 ppm) from the four locations is much lower than 15.00 ppm recommended limit by FEPA (1991) for the gas in the atmosphere. At the Public Procurement centre, there was no significant difference when the mean concentration of SPM at $P<0.05$ in the morning (1.36± 0.21 ppm) was compared with that of the evening hours (1.43± 0.38 ppm) but both were significantly lower than the 15.00 ppm recommended by FEPA. This is also applicable to the results obtained from BJ Business centre, Mr Umunnakwe Business centre as well as Old Registry Business centre.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 CONCLUSION

It is imperative to understand the possible roles of soil microbial enzymes in the maintenance of soil fertility and management of the ecosystem. Alterations in soil enzyme activity is one of the numerous strategies researchers use to evaluate the progress of bioremediation.

Overall, increases in soil enzyme activities were indications of corresponding impact of pollutant on the soil ecosystems. However, the findings of this study showed that prolonged exposure to generator fume and spent oil on soil microbial enzymes caused a shift in equilibrium and energy imbalance in the affected ecosystem. It altered the entire soil biochemistry and the microbial biomass. Microbial activities and their exudates are veritable indices of polluted soil. The enzymatic activities of catalase, lipase, acid and alkaline phosphatases in the affected ecosystem provides information on the activities of hydrocarbonclastic organisms.

Lipase, catalase, acid and alkaline phosphatases activities have been shown from this study to vary with fume pollution relative to soil depth. These selected microbial enzymes were effective in degrading the fume components as seen in the different polluted soil samples. Nevertheless, overall variability in soil enzyme activities of soil strata from different polluted sites, for the most part, defined the pattern of soil contamination, which could serve as biomarkers for ascertaining level of soil pollution as well monitoring indices for bioremediation.

The results of generator impacted soil have shown that microbial biomass has been adversely affected. Result also revealed that polluted soil was good source of hydrocarbonclastic fungi such

as *Aspergillus niger, Aspergillus fumigatus* etc. It was also observed that oil degrading fungi and bacteria could also be isolated as they are not directly contaminated with generator fume or similar pollutants. These microbial isolates can be effectively utilized in bioaugmentation aimed at removal of hydrocarbon pollutants from contaminated environments.

In conclusion, **the** impact of generator fumes through petroleum processing plants from industrial activities and homes today has deleterious effects on soil microbial enzymes. This ranges from immobilizing the available soil nutrients to unconducive life conditions in the soil, thus limiting vegetation and affecting land for agricultural activities with poor crop yield. This has therefore, necessitated the call to improve power supply in the country inorder to reduce the excessive use of generating sets, as this will not only reduce the risk of inhaling exhaust fumes but will save our ecosystem from pollution and enhance our crop production.

5.2 RECOMMENDATIONS

Sequel to the findings of this study, it is recommended that:

1. Stable power supply should be priortized to reduce the use of generator.
2. There should be strict regulation on the use of generators and other machines that causes this pollution.
3. Humans as well as animals should avoid prolonged exposure to polluted environments.
4. Biostimulation and bioaugmentation should be adopted as bioremedial processes in the polluted soil to enhance the degradation of the pollutants (i.e. generator fumes components).

5.3 **CONTRIBUTION TO KNOWLEDGE**

The study revealed different responses of these selected soil microbial enzymes (Catalase, Lipase, Acid & Alkaline phosphatase) to generator fume and spent oil exposure with regards to different soil depth hence supporting the introduction of bioaugmentation as a remedial process to the polluted environment.

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APPENDIX

LIPASE (1m)

Sub soil(South)	Sub soil(East)	Sub soil(West)	Sub soil(North)	Sub soil(Epicentre)	Sub soil(Control)
2.01	3.61	5.51	2.21	1.91	2.21
2.00	3.60	5.50	2.20	1.90	2.20
1.99	3.59	5.49	2.19	1.89	2.19
Sub Sub soil(South)	Sub Sub soil(East)	Sub Sub soil(West)	Sub Sub soil(North)	Sub Sub soil(Epicentre)	Sub Sub soil(Control)
1.91	4.01	4.01	1.81	2.31	2.11
1.90	4.00	4.00	1.80	2.30	2.10
1.89	3.99	3.99	1.79	2.29	2.09

LIPASE (2m)

Top soil(South)	Top soil(East)	Top soil(West)	Top soil(North)	Top soil(Epicentre)	Top soil(Control)
2.01	3.01	4.11	2.41	1.81	2.31
2.00	3.00	4.10	2.40	1.80	2.30
1.99	2.99	4.09	2.39	1.79	2.29

Sub soil(South)	Sub soil(East)	Sub soil(West)	Sub soil(North)	Sub soil(Epicentre)	Sub soil(Control)
2.11	3.81	3.11	2.11	1.91	2.21
2.10	3.80	3.10	2.10	1.90	2.20
2.09	3.79	3.09	2.09	1.89	2.19

Sub Sub soil(South)	Sub Sub soil(East)	Sub Sub soil(West)	Sub Sub soil(North)	Sub Sub soil(Epicentre)	Sub Sub soil(Control)
1.91	3.61	3.51	2.21	2.31	2.11
1.90	3.60	3.50	2.20	2.30	2.10
1.89	3.59	3.49	2.19	2.29	2.09

ALKALINE PHOSPHATASE (1m)

Top soil(South)	**Top soil(East)**	**Top soil(West)**	**Top soil(North)**	**Top soil(Epicentre)**	**Top soil(Control)**
20.33	**5.14**	**5.14**	**10.20**	**5.14**	**10.20**
20.26	**5.06**	**5.06**	**10.13**	**5.06**	**10.13**
20.19	**4.99**	**4.99**	**10.05**	**4.99**	**10.05**

Sub soil(South)	Sub soil(East)	Sub soil(West)	Sub soil(North)	Sub soil(Epicentre)	Sub soil(Control)
10.20	15.27	10.20	5.14	10.20	5.14
10.13	15.19	10.13	5.06	10.13	5.06
10.05	15.12	10.05	4.99	10.05	4.99

Sub Sub soil(South)	Sub Subsoil(East)	Sub Sub soil(West)	Sub Sub soil(North)	Sub Sub soil(Epicentre)	Sub Sub soil(Control)
5.14	15.27	10.20	5.14	5.14	5.14
5.06	15.19	10.13	5.06	5.06	5.06
4.99	15.12	10.05	4.99	4.99	4.99

ALKALINE PHOSPHATASE (2m)

Top soil(South)	Top soil(East)	Top soil(West)	Top soil(North)	Top soil(Epicentre)	Top soil(Control)
10.20	10.20	5.14	5.14	5.14	10.20
10.13	10.13	5.06	5.06	5.06	10.13
10.05	10.05	4.99	4.99	4.99	10.05
Sub soil(South)	Sub soil(East)	Sub soil(West)	Sub soil(North)	Sub soil(Epicentre)	Sub soil(Control)
5.14	15.27	5.14	5.14	10.20	5.14
5.06	15.19	5.06	5.06	10.13	5.06
4.99	15.12	4.99	4.99	10.05	4.99
Sub Sub soil(South)	Sub Subsoil(East)	Sub Sub soil(West)	Sub Sub soil(North)	Sub Sub soil(Epicentre)	Sub Sub soil(Control)
5.14	10.20	10.20	5.14	5.14	5.14
5.06	10.13	10.13	5.06	5.06	5.06
	10.05	10.05	4.99	4.99	4.99

Parameter					
Table Analyzed	Lipase(Top soil) 1m apart				
One-way analysis of variance					
P value	< 0.0001				
P value summary	***				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	6				
F	30800				
R square	0.9999				
ANOVA Table	SS	df	MS		
Treatment (between columns)	15.40	5	3.080		
Residual (within columns)	0.001200	12	10.000e-005		
Total	15.40	17			
Tukey's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
Top soil(South) vs Top soil(East)	-1.700	294.4	Yes	***	-1.727 to -1.673
Top soil(South) vs Top soil(West)	-2.500	433.0	Yes	***	-2.527 to -2.473
Top soil(South) vs Top soil(North)	-0.5000	86.60	Yes	***	-0.5274 to -0.4726
Top soil(South) vs Top soil(Epicentre)	0.0	0.0	No	ns	-0.02743 to 0.02743
Top soil(South) vs Top soil(Control)	-0.5000	86.60	Yes	***	-0.5274 to -0.4726
Top soil(East) vs Top soil(West)	-0.8000	138.6	Yes	***	-0.8274 to -0.7726
Top soil(East) vs Top soil(North)	1.200	207.8	Yes	***	1.173 to 1.227
Top soil(East) vs Top soil(Epicentre)	1.700	294.4	Yes	***	1.673 to 1.727
Top soil(East) vs Top soil(Control)	1.200	207.8	Yes	***	1.173 to 1.227
Top soil(West) vs Top soil(North)	2.000	346.4	Yes	***	1.973 to 2.027
Top soil(West) vs Top soil(Epicentre)	2.500	433.0	Yes	***	2.473 to 2.527
Top soil(West) vs Top soil(Control)	2.000	346.4	Yes	***	1.973 to 2.027
Top soil(North) vs Top soil(Epicentre)	0.5000	86.60	Yes	***	0.4726 to 0.5274

Top soil(North) vs Top soil(Control)	0.0	0.0	No	ns	-0.02743 to 0.02743
Top soil(Epicentre) vs Top soil(Control)	-0.5000	86.60	Yes	***	-0.5274 to -0.4726

	Top soil(South)	Top soil(East)	Top soil(West)	Top soil(North)	Top soil(Epicentre)	Top soil(Control)
Number of values	3	3	3	3	3	3
Minimum	1.790	3.490	4.290	2.290	1.790	2.290
25% Percentile	1.790	3.490	4.290	2.290	1.790	2.290
Median	1.800	3.500	4.300	2.300	1.800	2.300
75% Percentile	1.810	3.510	4.310	2.310	1.810	2.310
Maximum	1.810	3.510	4.310	2.310	1.810	2.310
Mean	1.800	3.500	4.300	2.300	1.800	2.300
Std. Deviation	0.01000	0.01000	0.01000	0.01000	0.01000	0.01000
Std. Error	0.005773	0.005773	0.005773	0.005773	0.005773	0.005773
Lower 95% CI	1.775	3.475	4.275	2.275	1.775	2.275
Upper 95% CI	1.825	3.525	4.325	2.325	1.825	2.325

Parameter	
Table Analyzed	Lipase(Sub soil) 1m apart
One-way analysis of variance	
P value	< 0.0001
P value summary	***
Are means signif. different? (P < 0.05)	Yes
Number of groups	6
F	60240
R square	1.000

ANOVA Table	SS	df	MS
Treatment (between columns)	30.12	5	6.024
Residual (within columns)	0.001200	12	0.0001000

Total	30.12	17			
Tukey's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
Sub soil(South) vs Sub soil(East)	-1.600	277.1	Yes	***	-1.627 to -1.573
Sub soil(South) vs Sub soil(West)	-3.500	606.2	Yes	***	-3.527 to -3.473
Sub soil(South) vs Sub soil(North)	-0.2000	34.64	Yes	***	-0.2274 to -0.1726
Sub soil(South) vs Sub soil(Epicentre)	0.1000	17.32	Yes	***	0.07257 to 0.1274
Sub soil(South) vs Sub soil(Control)	-0.2000	34.64	Yes	***	-0.2274 to -0.1726
Sub soil(East) vs Sub soil(West)	-1.900	329.1	Yes	***	-1.927 to -1.873
Sub soil(East) vs Sub soil(North)	1.400	242.5	Yes	***	1.373 to 1.427
Sub soil(East) vs Sub soil(Epicentre)	1.700	294.4	Yes	***	1.673 to 1.727
Sub soil(East) vs Sub soil(Control)	1.400	242.5	Yes	***	1.373 to 1.427
Sub soil(West) vs Sub soil(North)	3.300	571.6	Yes	***	3.273 to 3.327
Sub soil(West) vs Sub soil(Epicentre)	3.600	623.5	Yes	***	3.573 to 3.627
Sub soil(West) vs Sub soil(Control)	3.300	571.6	Yes	***	3.273 to 3.327
Sub soil(North) vs Sub soil(Epicentre)	0.3000	51.96	Yes	***	0.2726 to 0.3274
Sub soil(North) vs Sub soil(Control)	0.0	0.0	No	ns	-0.02743 to 0.02743
Sub soil(Epicentre) vs Sub soil(Control)	-0.3000	51.96	Yes	***	-0.3274 to -0.2726

	Sub soil(South)	Sub soil(East)	Sub soil(West)	Sub soil(North)	Sub soil(Epicentre)	Sub soil(Control)
Number of values	3	3	3	3	3	3
Minimum	1.990	3.590	5.490	2.190	1.890	2.190
25% Percentile	1.990	3.590	5.490	2.190	1.890	2.190
Median	2.000	3.600	5.500	2.200	1.900	2.200
75% Percentile	2.010	3.610	5.510	2.210	1.910	2.210
Maximum	2.010	3.610	5.510	2.210	1.910	2.210

Mean	2.000	3.600	5.500	2.200	1.900	2.200
Std. Deviation	0.01000	0.01000	0.01000	0.01000	0.01000	0.01000
Std. Error	0.005773	0.005773	0.005774	0.005773	0.005773	0.005773
Lower 95% CI	1.975	3.575	5.475	2.175	1.875	2.175
Upper 95% CI	2.025	3.625	5.525	2.225	1.925	2.225

Parameter	
Table Analyzed	Lipase (Sub Sub soil) 1m apart
One-way analysis of variance	
P value	< 0.0001
P value summary	***
Are means signif. different? (P < 0.05)	Yes
Number of groups	6
F	32090
R square	0.9999

ANOVA Table	SS	Df	MS
Treatment (between columns)	16.05	5	3.209
Residual (within columns)	0.001200	12	0.0001000
Total	16.05	17	

Tukey's Multiple Comparison Test	Mean Diff.	Q	Significant? P < 0.05?	Summary	95% CI of diff
Sub Sub soil(South) vs Sub Sub soil(East)	-2.100	363.7	Yes	***	-2.127 to -2.073
Sub Sub soil(South) vs Sub Sub soil(West)	-2.100	363.7	Yes	***	-2.127 to -2.073
Sub Sub soil(South) vs Sub Sub soil(North)	0.1000	17.32	Yes	***	0.07257 to 0.1274
Sub Sub soil(South) vs Sub Sub soil(Epicentre)	-0.4000	69.28	Yes	***	-0.4274 to -0.3726
Sub Sub soil(South) vs Sub Sub soil(Control)	-0.2000	34.64	Yes	***	-0.2274 to -0.1726
Sub Sub soil(East) vs Sub Sub soil(West)	0.0	0.0	No	ns	-0.02743 to 0.02743
Sub Sub soil(East) vs Sub Sub soil(North)	2.200	381.1	Yes	***	2.173 to 2.227
Sub Sub soil(East) vs Sub Sub soil(Epicentre)	1.700	294.4	Yes	***	1.673 to 1.727
Sub Sub soil(East) vs Sub Sub soil(Control)	1.900	329.1	Yes	***	1.873 to 1.927
Sub Sub soil(West) vs Sub Sub soil(North)	2.200	381.1	Yes	***	2.173 to 2.227

Sub Sub soil(West) vs Sub Sub soil(Epicentre)	1.700	294.4	Yes	***	1.673 to 1.727
Sub Sub soil(West) vs Sub Sub soil(Control)	1.900	329.1	Yes	***	1.873 to 1.927
Sub Sub soil(North) vs Sub Sub soil(Epicentre)	-0.5000	86.60	Yes	***	-0.5274 to -0.4726
Sub Sub soil(North) vs Sub Sub soil(Control)	-0.3000	51.96	Yes	***	-0.3274 to -0.2726
Sub Sub soil(Epicentre) vs Sub Sub soil(Control)	0.2000	34.64	Yes	***	0.1726 to 0.2274

	Sub Sub soil(South)	Sub Sub soil(East)	Sub Sub soil(West)	Sub Sub soil(North)	Sub Sub soil(Epicentre)
Number of values	3	3	3	3	3
Minimum	1.890	3.990	3.990	1.790	2.290
25% Percentile	1.890	3.990	3.990	1.790	2.290
Median	1.900	4.000	4.000	1.800	2.300
75% Percentile	1.910	4.010	4.010	1.810	2.310
Maximum	1.910	4.010	4.010	1.810	2.310
Mean	1.900	4.000	4.000	1.800	2.300
Std. Deviation	0.01000	0.01000	0.01000	0.01000	0.01000
Std. Error	0.005773	0.005774	0.005774	0.005773	0.005773
Lower 95% CI	1.875	3.975	3.975	1.775	2.275
Upper 95% CI	1.925	4.025	4.025	1.825	2.325

Parameter	
Table Analyzed	Lipase(Top soil) 2m apart
One-way analysis of variance	
P value	< 0.0001
P value summary	***
Are means signif. different? (P < 0.05)	Yes
Number of groups	6
F	21240
R square	0.9999

ANOVA Table	SS	df	MS
Treatment (between columns)	10.62	5	2.124
Residual (within columns)	0.001200	12	10.000e-005
Total	10.62	17	

Tukey's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
Top soil(South) vs Top soil(East)	-1.000	173.2	Yes	***	-1.027 to -0.9726
Top soil(South) vs Top soil(West)	-2.100	363.7	Yes	***	-2.127 to -2.073
Top soil(South) vs Top soil(North)	-0.4000	69.28	Yes	***	-0.4274 to -0.3726
Top soil(South) vs Top soil(Epicentre)	0.2000	34.64	Yes	***	0.1726 to 0.2274
Top soil(South) vs Top soil(Control)	-0.3000	51.96	Yes	***	-0.3274 to -0.2726
Top soil(East) vs Top soil(West)	-1.100	190.5	Yes	***	-1.127 to -1.073
Top soil(East) vs Top soil(North)	0.6000	103.9	Yes	***	0.5726 to 0.6274
Top soil(East) vs Top soil(Epicentre)	1.200	207.8	Yes	***	1.173 to 1.227
Top soil(East) vs Top soil(Control)	0.7000	121.2	Yes	***	0.6726 to 0.7274
Top soil(West) vs Top soil(North)	1.700	294.4	Yes	***	1.673 to 1.727
Top soil(West) vs Top soil(Epicentre)	2.300	398.4	Yes	***	2.273 to 2.327
Top soil(West) vs Top soil(Control)	1.800	311.8	Yes	***	1.773 to 1.827
Top soil(North) vs Top soil(Epicentre)	0.6000	103.9	Yes	***	0.5726 to 0.6274
Top soil(North) vs Top soil(Control)	0.1000	17.32	Yes	***	0.07257 to 0.1274
Top soil(Epicentre) vs Top soil(Control)	-0.5000	86.60	Yes	***	-0.5274 to -0.4726

	Top soil(South)	Top soil(East)	Top soil(West)	Top soil(North)	Top soil(Epicentre)	Top soil(Control)
Number of values	3	3	3	3	3	3
Minimum	1.990	2.990	4.090	2.390	1.790	2.290
25% Percentile	1.990	2.990	4.090	2.390	1.790	2.290
Median	2.000	3.000	4.100	2.400	1.800	2.300
75% Percentile	2.010	3.010	4.110	2.410	1.810	2.310
Maximum	2.010	3.010	4.110	2.410	1.810	2.310
Mean	2.000	3.000	4.100	2.400	1.800	2.300
Std. Deviation	0.01000	0.01000	0.01000	0.01000	0.01000	0.01000
Std. Error	0.005773	0.005773	0.005773	0.005773	0.005773	0.005773

Lower 95% CI	1.975	2.975	4.075	2.375	1.775	2.275
Upper 95% CI	2.025	3.025	4.125	2.425	1.825	2.325

Parameter	
Table Analyzed	Lipase(Sub soil) 2m apart
One-way analysis of variance	
P value	< 0.0001
P value summary	***
Are means signif. different? (P < 0.05)	Yes
Number of groups	6
F	16880
R square	0.9999

ANOVA Table	SS	df	MS
Treatment (between columns)	8.440	5	1.688
Residual (within columns)	0.001200	12	10.000e-005
Total	8.441	17	

Tukey's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
Sub soil(South) vs Sub soil(East)	-1.700	294.4	Yes	***	-1.727 to -1.673
Sub soil(South) vs Sub soil(West)	-1.000	173.2	Yes	***	-1.027 to -0.9726
Sub soil(South) vs Sub soil(North)	0.0	0.0	No	ns	-0.02743 to 0.02743
Sub soil(South) vs Sub soil(Epicentre)	0.2000	34.64	Yes	***	0.1726 to 0.2274
Sub soil(South) vs Sub soil(Control)	-0.1000	17.32	Yes	***	-0.1274 to -0.07257
Sub soil(East) vs Sub soil(West)	0.7000	121.2	Yes	***	0.6726 to 0.7274
Sub soil(East) vs Sub soil(North)	1.700	294.4	Yes	***	1.673 to 1.727
Sub soil(East) vs Sub soil(Epicentre)	1.900	329.1	Yes	***	1.873 to 1.927
Sub soil(East) vs Sub soil(Control)	1.600	277.1	Yes	***	1.573 to 1.627
Sub soil(West) vs Sub soil(North)	1.000	173.2	Yes	***	0.9726 to 1.027
Sub soil(West) vs Sub soil(Epicentre)	1.200	207.8	Yes	***	1.173 to 1.227
Sub soil(West) vs Sub soil(Control)	0.9000	155.9	Yes	***	0.8726 to 0.9274
Sub soil(North) vs Sub soil(Epicentre)	0.2000	34.64	Yes	***	0.1726 to 0.2274

Sub soil(North) vs Sub soil(Control)	-0.1000	17.32	Yes	***	-0.1274 to -0.07257
Sub soil(Epicentre) vs Sub soil(Control)	-0.3000	51.96	Yes	***	-0.3274 to -0.2726

	Sub soil(South)	Sub soil(East)	Sub soil(West)	Sub soil(North)	Sub soil(Epicentre)	Sub soil(Control)
Number of values	3	3	3	3	3	3
Minimum	2.090	3.790	3.090	2.090	1.890	2.190
25% Percentile	2.090	3.790	3.090	2.090	1.890	2.190
Median	2.100	3.800	3.100	2.100	1.900	2.200
75% Percentile	2.110	3.810	3.110	2.110	1.910	2.210
Maximum	2.110	3.810	3.110	2.110	1.910	2.210
Mean	2.100	3.800	3.100	2.100	1.900	2.200
Std. Deviation	0.01000	0.01000	0.01000	0.01000	0.01000	0.01000
Std. Error	0.005773	0.005773	0.005773	0.005773	0.005773	0.005773
Lower 95% CI	2.075	3.775	3.075	2.075	1.875	2.175
Upper 95% CI	2.125	3.825	3.125	2.125	1.925	2.225

Parameter	
Table Analyzed	Lipase (Sub Sub soil) 2m apart
One-way analysis of variance	
P value	< 0.0001
P value summary	***
Are means signif. different? (P < 0.05)	Yes
Number of groups	6
F	16800
R square	0.9999

ANOVA Table	SS	df	MS
Treatment (between columns)	8.400	5	1.680
Residual (within columns)	0.001200	12	10.000e-005

Total	8.401	17			

Tukey's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
Sub Sub soil(South) vs Sub Sub soil(East)	-1.700	294.4	Yes	***	-1.727 to -1.673
Sub Sub soil(South) vs Sub Sub soil(West)	-1.600	277.1	Yes	***	-1.627 to -1.573
Sub Sub soil(South) vs Sub Sub soil(North)	-0.3000	51.96	Yes	***	-0.3274 to -0.2726
Sub Sub soil(South) vs Sub Sub soil(Epicentre)	-0.4000	69.28	Yes	***	-0.4274 to -0.3726
Sub Sub soil(South) vs Sub Sub soil(Control)	-0.2000	34.64	Yes	***	-0.2274 to -0.1726
Sub Sub soil(East) vs Sub Sub soil(West)	0.1000	17.32	Yes	***	0.07257 to 0.1274
Sub Sub soil(East) vs Sub Sub soil(North)	1.400	242.5	Yes	***	1.373 to 1.427
Sub Sub soil(East) vs Sub Sub soil(Epicentre)	1.300	225.2	Yes	***	1.273 to 1.327
Sub Sub soil(East) vs Sub Sub soil(Control)	1.500	259.8	Yes	***	1.473 to 1.527
Sub Sub soil(West) vs Sub Sub soil(North)	1.300	225.2	Yes	***	1.273 to 1.327
Sub Sub soil(West) vs Sub Sub soil(Epicentre)	1.200	207.8	Yes	***	1.173 to 1.227
Sub Sub soil(West) vs Sub Sub soil(Control)	1.400	242.5	Yes	***	1.373 to 1.427
Sub Sub soil(North) vs Sub Sub soil(Epicentre)	-0.1000	17.32	Yes	***	-0.1274 to -0.07257
Sub Sub soil(North) vs Sub Sub soil(Control)	0.1000	17.32	Yes	***	0.07257 to 0.1274
Sub Sub soil(Epicentre) vs Sub Sub soil(Control)	0.2000	34.64	Yes	***	0.1726 to 0.2274

	Sub Sub soil(South)	Sub Sub soil(East)	Sub Sub soil(West)	Sub Sub soil(North)	Sub Sub soil(Epicentre)
Number of values	3	3	3	3	3
Minimum	1.890	3.590	3.490	2.190	2.290
25% Percentile	1.890	3.590	3.490	2.190	2.290
Median	1.900	3.600	3.500	2.200	2.300
75% Percentile	1.910	3.610	3.510	2.210	2.310
Maximum	1.910	3.610	3.510	2.210	2.310
Mean	1.900	3.600	3.500	2.200	2.300
Std. Deviation	0.01000	0.01000	0.01000	0.01000	0.01000
Std. Error	0.005773	0.005773	0.005773	0.005773	0.005773
Lower 95% CI	1.875	3.575	3.475	2.175	2.275
Upper 95% CI	1.925	3.625	3.525	2.225	2.325

ALKALINE PHOSPHATSE (1m)

Parameter	
Table Analyzed	Alkaline phosphatase(Top soil)1m apart
One-way analysis of variance	
P value	< 0.0001
P value summary	***
Are means signif. different? (P < 0.05)	Yes
Number of groups	6
F	19089
R square	0.9999

ANOVA Table	SS	df	MS
Treatment (between columns)	526.0	5	105.2
Residual (within columns)	0.06613	12	0.005511
Total	526.1	17	

Tukey's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
Top soil(South) vs Top soil(East)	15.20	354.6	Yes	***	14.99 to 15.40
Top soil(South) vs Top soil(West)	15.20	354.6	Yes	***	14.99 to 15.40
Top soil(South) vs Top soil(North)	10.13	236.4	Yes	***	9.930 to 10.34
Top soil(South) vs Top soil(Epicentre)	15.20	354.6	Yes	***	14.99 to 15.40
Top soil(South) vs Top soil(Control)	10.13	236.4	Yes	***	9.930 to 10.34
Top soil(East) vs Top soil(West)	0.0	0.0	No	ns	-0.2036 to 0.2036
Top soil(East) vs Top soil(North)	-5.063	118.1	Yes	***	-5.267 to -4.860
Top soil(East) vs Top soil(Epicentre)	0.0	0.0	No	ns	-0.2036 to 0.2036
Top soil(East) vs Top soil(Control)	-5.063	118.1	Yes	***	-5.267 to -4.860
Top soil(West) vs Top soil(North)	-5.063	118.1	Yes	***	-5.267 to -4.860
Top soil(West) vs Top soil(Epicentre)	0.0	0.0	No	ns	-0.2036 to 0.2036
Top soil(West) vs Top soil(Control)	-5.063	118.1	Yes	***	-5.267 to -4.860
Top soil(North) vs Top soil(Epicentre)	5.063	118.1	Yes	***	4.860 to 5.267
Top soil(North) vs Top soil(Control)	0.0	0.0	No	ns	-0.2036 to 0.2036
Top soil(Epicentre) vs Top soil(Control)	-5.063	118.1	Yes	***	-5.267 to -4.860

	Top soil(South)	Top soil(East)	Top soil(West)	Top soil(North)	Top soil(Epicentre)	Top soil(Control)
Number of values	3	3	3	3	3	3
Minimum	20.19	4.990	4.990	10.05	4.990	10.05
25% Percentile	20.19	4.990	4.990	10.05	4.990	10.05
Median	20.26	5.060	5.060	10.13	5.060	10.13
75% Percentile	20.33	5.140	5.140	10.20	5.140	10.20
Maximum	20.33	5.140	5.140	10.20	5.140	10.20
Mean	20.26	5.063	5.063	10.13	5.063	10.13
Std. Deviation	0.07000	0.07506	0.07506	0.07506	0.07506	0.07506
Std. Error	0.04041	0.04333	0.04333	0.04333	0.04333	0.04333
Lower 95% CI	20.09	4.877	4.877	9.940	4.877	9.940
Upper 95% CI	20.43	5.250	5.250	10.31	5.250	10.31

Parameter	
Table Analyzed	Alkaline phosphatase(Sub soil) 1m apart
One-way analysis of variance	
P value	< 0.0001
P value summary	***
Are means signif. different? (P < 0.05)	Yes
Number of groups	6
F	7741
R square	0.9997

ANOVA Table	SS	df	MS
Treatment (between columns)	218.0	5	43.61
Residual (within columns)	0.06760	12	0.005633
Total	218.1	17	

Tukey's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff

Sub soil(South) vs Sub soil(East)	-5.067	116.9	Yes	***	-5.273 to -4.861
Sub soil(South) vs Sub soil(West)	0.0	0.0	No	ns	-0.2059 to 0.2059
Sub soil(South) vs Sub soil(North)	5.063	116.8	Yes	***	4.857 to 5.269
Sub soil(South) vs Sub soil(Epicentre)	0.0	0.0	No	ns	-0.2059 to 0.2059
Sub soil(South) vs Sub soil(Control)	5.063	116.8	Yes	***	4.857 to 5.269
Sub soil(East) vs Sub soil(West)	5.067	116.9	Yes	***	4.861 to 5.273
Sub soil(East) vs Sub soil(North)	10.13	233.8	Yes	***	9.924 to 10.34
Sub soil(East) vs Sub soil(Epicentre)	5.067	116.9	Yes	***	4.861 to 5.273
Sub soil(East) vs Sub soil(Control)	10.13	233.8	Yes	***	9.924 to 10.34
Sub soil(West) vs Sub soil(North)	5.063	116.8	Yes	***	4.857 to 5.269
Sub soil(West) vs Sub soil(Epicentre)	0.0	0.0	No	ns	-0.2059 to 0.2059
Sub soil(West) vs Sub soil(Control)	5.063	116.8	Yes	***	4.857 to 5.269
Sub soil(North) vs Sub soil(Epicentre)	-5.063	116.8	Yes	***	-5.269 to -4.857
Sub soil(North) vs Sub soil(Control)	0.0	0.0	No	ns	-0.2059 to 0.2059
Sub soil(Epicentre) vs Sub soil(Control)	5.063	116.8	Yes	***	4.857 to 5.269

	Sub soil(South)	Sub soil(East)	Sub soil(West)	Sub soil(North)	Sub soil(Epicentre)	Sub soil(Control)
Number of values	3	3	3	3	3	3
Minimum	10.05	15.12	10.05	4.990	10.05	4.990
25% Percentile	10.05	15.12	10.05	4.990	10.05	4.990
Median	10.13	15.19	10.13	5.060	10.13	5.060
75% Percentile	10.20	15.27	10.20	5.140	10.20	5.140
Maximum	10.20	15.27	10.20	5.140	10.20	5.140
Mean	10.13	15.19	10.13	5.063	10.13	5.063
Std. Deviation	0.07506	0.07506	0.07506	0.07506	0.07506	0.07506
Std. Error	0.04333	0.04333	0.04333	0.04333	0.04333	0.04333
Lower 95% CI	9.940	15.01	9.940	4.877	9.940	4.877
Upper 95% CI	10.31	15.38	10.31	5.250	10.31	5.250

Parameter

Table Analyzed	Alkaline phosphatase(Sub Sub soil) 1m apart
One-way analysis of variance	
P value	< 0.0001
P value summary	***
Are means signif. different? (P < 0.05)	Yes
Number of groups	6
F	9563
R square	0.9997

ANOVA Table	SS	df	MS
Treatment (between columns)	269.3	5	53.87
Residual (within columns)	0.06760	12	0.005633
Total	269.4	17	

Tukey's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
Sub Sub soil(South) vs Sub Subsoil(East)	-10.13	233.8	Yes	***	-10.34 to -9.924
Sub Sub soil(South) vs Sub Sub soil(West)	-5.063	116.8	Yes	***	-5.269 to -4.857
Sub Sub soil(South) vs Sub Sub soil(North)	0.0	0.0	No	ns	-0.2059 to 0.2059
Sub Sub soil(South) vs Sub Sub soil(Epicentre)	0.0	0.0	No	ns	-0.2059 to 0.2059
Sub Sub soil(South) vs Sub Sub soil(Control)	0.0	0.0	No	ns	-0.2059 to 0.2059
Sub Subsoil(East) vs Sub Sub soil(West)	5.067	116.9	Yes	***	4.861 to 5.273
Sub Subsoil(East) vs Sub Sub soil(North)	10.13	233.8	Yes	***	9.924 to 10.34
Sub Subsoil(East) vs Sub Sub soil(Epicentre)	10.13	233.8	Yes	***	9.924 to 10.34

Sub Subsoil(East) vs Sub Sub soil(Control)	10.13	233.8	Yes	***	9.924 to 10.34
Sub Sub soil(West) vs Sub Sub soil(North)	5.063	116.8	Yes	***	4.857 to 5.269
Sub Sub soil(West) vs Sub Sub soil(Epicentre)	5.063	116.8	Yes	***	4.857 to 5.269
Sub Sub soil(West) vs Sub Sub soil(Control)	5.063	116.8	Yes	***	4.857 to 5.269
Sub Sub soil(North) vs Sub Sub soil(Epicentre)	0.0	0.0	No	ns	-0.2059 to 0.2059
Sub Sub soil(North) vs Sub Sub soil(Control)	0.0	0.0	No	ns	-0.2059 to 0.2059
Sub Sub soil(Epicentre) vs Sub Sub soil(Control)	0.0	0.0	No	ns	-0.2059 to 0.2059

	Sub Sub soil(South)	Sub Subsoil(East)	Sub Sub soil(West)	Sub Sub soil(North)	Sub Sub soil(Epicentre)	S
Number of values	3	3	3	3	3	3
Minimum	4.990	15.12	10.05	4.990	4.990	4
25% Percentile	4.990	15.12	10.05	4.990	4.990	4
Median	5.060	15.19	10.13	5.060	5.060	5
75% Percentile	5.140	15.27	10.20	5.140	5.140	5
Maximum	5.140	15.27	10.20	5.140	5.140	5
Mean	5.063	15.19	10.13	5.063	5.063	5
Std. Deviation	0.07506	0.07506	0.07506	0.07506	0.07506	0
Std. Error	0.04333	0.04333	0.04333	0.04333	0.04333	0
Lower 95% CI	4.877	15.01	9.940	4.877	4.877	4
Upper 95% CI	5.250	15.38	10.31	5.250	5.250	5

ALKALINE PHOSPHATASE (2m)

Parameter

Table Analyzed	**Alkaline phosphatase(Top soil) 2m apart**				
One-way analysis of variance					
P value	**< 0.0001**				
P value summary	*******				
Are means signif. different? (P < 0.05)	**Yes**				
Number of groups	**6**				
F	**4096**				
R square	**0.9994**				
ANOVA Table	**SS**	**df**	**MS**		
Treatment (between columns)	**115.4**	**5**	**23.07**		
Residual (within columns)	**0.06760**	**12**	**0.005633**		
Total	**115.4**	**17**			
Tukey's Multiple Comparison Test	**Mean Diff.**	**q**	**Significant? P < 0.05?**	**Summary**	**95% CI of diff**
Top soil(South) vs Top soil(East)	**0.0**	**0.0**	**No**	**ns**	**-0.2059 to 0.2059**
Top soil(South) vs Top soil(West)	**5.063**	**116.8**	**Yes**	*******	**4.857 to 5.269**
Top soil(South) vs Top soil(North)	**5.063**	**116.8**	**Yes**	*******	**4.857 to 5.269**
Top soil(South) vs Top soil(Epicentre)	**5.063**	**116.8**	**Yes**	*******	**4.857 to 5.269**
Top soil(South) vs Top soil(Control)	**0.0**	**0.0**	**No**	**ns**	**-0.2059 to 0.2059**
Top soil(East) vs Top soil(West)	**5.063**	**116.8**	**Yes**	*******	**4.857 to 5.269**
Top soil(East) vs Top soil(North)	**5.063**	**116.8**	**Yes**	*******	**4.857 to 5.269**
Top soil(East) vs Top soil(Epicentre)	**5.063**	**116.8**	**Yes**	*******	**4.857 to 5.269**
Top soil(East) vs Top soil(Control)	**0.0**	**0.0**	**No**	**ns**	**-0.2059 to 0.2059**
Top soil(West) vs Top soil(North)	**0.0**	**0.0**	**No**	**ns**	**-0.2059 to 0.2059**
Top soil(West) vs Top soil(Epicentre)	**0.0**	**0.0**	**No**	**ns**	**-0.2059 to 0.2059**

Top soil(West) vs Top soil(Control)	**-5.063**	**116.8**	**Yes**	*******	**-5.269 to -4.857**
Top soil(North) vs Top soil(Epicentre)	**0.0**	**0.0**	**No**	**ns**	**-0.2059 to 0.2059**
Top soil(North) vs Top soil(Control)	**-5.063**	**116.8**	**Yes**	*******	**-5.269 to -4.857**
Top soil(Epicentre) vs Top soil(Control)	**-5.063**	**116.8**	**Yes**	*******	**-5.269 to -4.857**

	Top soil(South)	Top soil(East)	Top soil(West)	Top soil(North)	Top soil(Epicentre)	Top soil(Control)
Number of values	**3**	**3**	**3**	**3**	**3**	**3**
Minimum	**10.05**	**10.05**	**4.990**	**4.990**	**4.990**	**10.05**
25% Percentile	**10.05**	**10.05**	**4.990**	**4.990**	**4.990**	**10.05**
Median	**10.13**	**10.13**	**5.060**	**5.060**	**5.060**	**10.13**
75% Percentile	**10.20**	**10.20**	**5.140**	**5.140**	**5.140**	**10.20**
Maximum	**10.20**	**10.20**	**5.140**	**5.140**	**5.140**	**10.20**
Mean	**10.13**	**10.13**	**5.063**	**5.063**	**5.063**	**10.13**
Std. Deviation	**0.07506**	**0.07506**	**0.07506**	**0.07506**	**0.07506**	**0.07506**
Std. Error	**0.04333**	**0.04333**	**0.04333**	**0.04333**	**0.04333**	**0.04333**
Lower 95% CI	**9.940**	**9.940**	**4.877**	**4.877**	**4.877**	**9.940**
Upper 95% CI	**10.31**	**10.31**	**5.250**	**5.250**	**5.250**	**10.31**

Parameter	
Table Analyzed	Alkaline phosphatase(Sub soil) 2m apart
One-way analysis of variance	
P value	< 0.0001
P value summary	***
Are means signif. different? (P < 0.05)	Yes
Number of groups	6
F	9563
R square	0.9997

ANOVA Table	SS	df	MS
Treatment (between columns)	269.3	5	53.87
Residual (within columns)	0.06760	12	0.005633
Total	269.4	17	

Tukey's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
Sub soil(South) vs Sub soil(East)	-10.13	233.8	Yes	***	-10.34 to -9.924
Sub soil(South) vs Sub soil(West)	0.0	0.0	No	ns	-0.2059 to 0.2059
Sub soil(South) vs Sub soil(North)	0.0	0.0	No	ns	-0.2059 to 0.2059
Sub soil(South) vs Sub soil(Epicentre)	-5.063	116.8	Yes	***	-5.269 to -4.857
Sub soil(South) vs Sub soil(Control)	0.0	0.0	No	ns	-0.2059 to 0.2059
Sub soil(East) vs Sub soil(West)	10.13	233.8	Yes	***	9.924 to 10.34
Sub soil(East) vs Sub soil(North)	10.13	233.8	Yes	***	9.924 to 10.34
Sub soil(East) vs Sub soil(Epicentre)	5.067	116.9	Yes	***	4.861 to 5.273
Sub soil(East) vs Sub soil(Control)	10.13	233.8	Yes	***	9.924 to 10.34
Sub soil(West) vs Sub soil(North)	0.0	0.0	No	ns	-0.2059 to 0.2059
Sub soil(West) vs Sub soil(Epicentre)	-5.063	116.8	Yes	***	-5.269 to -4.857
Sub soil(West) vs Sub soil(Control)	0.0	0.0	No	ns	-0.2059 to 0.2059
Sub soil(North) vs Sub soil(Epicentre)	-5.063	116.8	Yes	***	-5.269 to -4.857
Sub soil(North) vs Sub soil(Control)	0.0	0.0	No	ns	-0.2059 to 0.2059
Sub soil(Epicentre) vs Sub soil(Control)	5.063	116.8	Yes	***	4.857 to 5.269

	Sub soil(South)	Sub soil(East)	Sub soil(West)	Sub soil(North)	Sub soil(Epicentre)	Sub soil(Control)
Number of values	3	3	3	3	3	3
Minimum	4.990	15.12	4.990	4.990	10.05	4.990
25% Percentile	4.990	15.12	4.990	4.990	10.05	4.990
Median	5.060	15.19	5.060	5.060	10.13	5.060
75% Percentile	5.140	15.27	5.140	5.140	10.20	5.140
Maximum	5.140	15.27	5.140	5.140	10.20	5.140
Mean	5.063	15.19	5.063	5.063	10.13	5.063
Std. Deviation	0.07506	0.07506	0.07506	0.07506	0.07506	0.07506
Std. Error	0.04333	0.04333	0.04333	0.04333	0.04333	0.04333

Lower 95% CI	4.877	15.01	4.877	4.877	9.940	4.877
Upper 95% CI	5.250	15.38	5.250	5.250	10.31	5.250

Parameter	
Table Analyzed	Alkaline phosphatase(Sub Sub soil) 2m apart
One-way analysis of variance	
P value	< 0.0001
P value summary	***
Are means signif. different? (P < 0.05)	Yes
Number of groups	6
F	3669
R square	0.9994

ANOVA Table	SS	df	MS
Treatment (between columns)	99.27	5	19.85
Residual (within columns)	0.05953	11	0.005412
Total	99.33	16	

Tukey's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
Sub Sub soil(South) vs Sub Subsoil(East)	-5.027	105.9	Yes	***	-5.256 to -4.798
Sub Sub soil(South) vs Sub Sub soil(West)	-5.027	105.9	Yes	***	-5.256 to -4.798
Sub Sub soil(South) vs Sub Sub soil(North)	0.03667	0.772 1	No	ns	-0.1924 to 0.2657
Sub Sub soil(South) vs Sub Sub soil(Epicentre)	0.03667	0.772 1	No	ns	-0.1924 to 0.2657
Sub Sub soil(South) vs Sub Sub soil(Control)	0.03667	0.772 1	No	ns	-0.1924 to 0.2657
Sub Subsoil(East) vs Sub Sub soil(West)	0.0	0.0	No	ns	-0.2049 to 0.2049
Sub Subsoil(East) vs Sub Sub soil(North)	5.063	119.2	Yes	***	4.858 to 5.268

Sub Subsoil(East) vs Sub Sub soil(Epicentre)	5.063	119.2	Yes	***	4.858 to 5.268
Sub Subsoil(East) vs Sub Sub soil(Control)	5.063	119.2	Yes	***	4.858 to 5.268
Sub Sub soil(West) vs Sub Sub soil(North)	5.063	119.2	Yes	***	4.858 to 5.268
Sub Sub soil(West) vs Sub Sub soil(Epicentre)	5.063	119.2	Yes	***	4.858 to 5.268
Sub Sub soil(West) vs Sub Sub soil(Control)	5.063	119.2	Yes	***	4.858 to 5.268
Sub Sub soil(North) vs Sub Sub soil(Epicentre)	0.0	0.0	No	ns	-0.2049 to 0.2049
Sub Sub soil(North) vs Sub Sub soil(Control)	0.0	0.0	No	ns	-0.2049 to 0.2049
Sub Sub soil(Epicentre) vs Sub Sub soil(Control)	0.0	0.0	No	ns	-0.2049 to 0.2049

	Sub Sub soil(South)	Sub Subsoil(East)	Sub Sub soil(West)	Sub Sub soil(North)	Sub Sub soil(Epicentre)	Sub Sub soil(Control)
Number of values	2	3	3	3	3	3
Minimum	5.060	10.05	10.05	4.990	4.990	4.990
25% Percentile	5.060	10.05	10.05	4.990	4.990	4.990
Median	5.100	10.13	10.13	5.060	5.060	5.060
75% Percentile	5.140	10.20	10.20	5.140	5.140	5.140
Maximum	5.140	10.20	10.20	5.140	5.140	5.140
Mean	5.100	10.13	10.13	5.063	5.063	5.063
Std. Deviation	0.05657	0.07506	0.07506	0.07506	0.07506	0.07506
Std. Error	0.04000	0.04333	0.04333	0.04333	0.04333	0.04333
Lower 95% CI	4.592	9.940	9.940	4.877	4.877	4.877
Upper 95% CI	5.608	10.31	10.31	5.250	5.250	5.250

CATALASE

Top soil(South)	Top soil(East)	Top soil(West)	Top soil(North)	Top soil(Epicentre)	Top soil(Control)
7.51	13.81	40.21	36.31	33.31	34.81
7.50	13.80	40.20	36.30	33.30	34.80
7.49	13.79	40.19	36.29	33.29	34.79

Sub soil(South)	Sub soil(East)	Sub soil(West)	Sub soil(North)	Sub soil(Epicentre)	Sub soil(Control)
88.51	21.91	60.01	31.90	30.00	42.01
88.50	21.90	60.00	31.89	29.99	42.00
88.49	21.89	59.99	31.88	29.98	41.99

Sub Sub soil(South)	Sub Sub soil(East)	Sub Sub soil(West)	Sub Sub soil(North)	Sub Sub soil(Epicentre)	Sub Sub soil(Control)
41.41	7.81	64.51	25.81	33.61	45.91
41.40	7.80	64.50	25.80	33.60	45.90
41.39	7.79	64.49	25.79	33.59	45.89

Top soil(South)	Top soil(East)	Top soil(West)	Top soil(North)	Top soil(Epicentre)	Top soil(Control)
39.91	25.21	53.71	24.01	33.31	34.81
39.90	25.20	53.70	24.00	33.30	34.80
39.89	25.19	53.69	23.99	33.29	34.79

Sub soil(South)	Sub soil(East)	Sub soil(West)	Sub soil(North)	Sub soil(Epicentre)	Sub soil(Control)
29.41	26.41	52.21	46.81	30.00	42.01
29.40	26.40	52.20	46.80	29.99	42.00
29.39	26.39	52.19	46.79	29.98	41.99

Sub Sub soil(South)	Sub Sub soil(East)	Sub Sub soil(West)	Sub Sub soil(North)	Sub Sub soil(Epicentre)	Sub Sub soil(Control)
58.51	17.71	82.51	42.31	33.61	45.91
58.50	17.70	82.50	42.30	33.60	45.90
58.49	17.69	82.49	42.29	33.59	45.89

ACID PHOSPHATASE

Top soil(South)	Top soil(East)	Top soil(West)	Top soil(North)	Top soil(Epicentre)	Top soil(Control)
0.001	0.001	0.002	0.001	0.001	0.001
0.002	0.002	0.005	0.002	0.002	0.002
Sub soil(South)	Sub soil(East)	Sub soil(West)	Sub soil(North)	Sub soil(Epicentre)	Sub soil(Control)
0.001	0.001	0.001	0.001	0.001	0.001
0.002	0.001	0.001	0.003	0.001	0.002

Sub Sub soil(South)	Sub Sub soil(East)	Sub Sub soil(West)	Sub Sub soil(North)	Sub Sub soil(Epicentre)	Sub Sub soil(Control)
0.001	0.001	0.001	0.001	0.001	0.001
0.003	0.001	0.001	0.001	0.002	0.002
Top soil(South)	Top soil(East)	Top soil(West)	Top soil(North)	Top soil(Epicentre)	Top soil(Control)
0.001	0.001	0.001	0.001	0.001	0.001
0.001	0.002	0.002	0.002	0.002	0.002

Sub soil(South)	Sub soil(East)	Sub soil(West)	Sub soil(North)	Sub soil(Epicentre)	Sub soil(Control)
0.001	0.001	0.001	0.001	0.001	0.001
0.001	0.001	0.003	0.001	0.001	0.002
Sub Sub soil(South)	Sub Sub soil(East)	Sub Sub soil(West)	Sub Sub soil(North)	Sub Sub soil(Epicentre)	Sub Sub soil(Control)
0.001	0.001	0.001	0.001	0.001	0.001
0.001	0.001	0.001	0.001	0.002	0.002

Parameter	
Table Analyzed	Catalase (Top soil) 1m apart
One-way analysis of variance	
P value	< 0.0001
P value summary	***
Are means signif. different? (P < 0.05)	Yes
Number of groups	6
F	5.479e+006
R square	1.000

ANOVA Table	SS	df	MS
Treatment (between columns)	2740	5	547.9
Residual (within columns)	0.001200	12	0.0001000
Total	2740	17	

Tukey's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
Top soil(South) vs Top soil(East)	-6.300	1091	Yes	***	-6.327 to -6.273
Top soil(South) vs Top soil(West)	-32.70	5664	Yes	***	-32.73 to -32.67
Top soil(South) vs Top soil(North)	-28.80	4988	Yes	***	-28.83 to -28.77

Top soil(South) vs Top soil(Epicentre)	-25.80	4469	Yes	***	-25.83 to -25.77
Top soil(South) vs Top soil(Control)	-27.30	4728	Yes	***	-27.33 to -27.27
Top soil(East) vs Top soil(West)	-26.40	4573	Yes	***	-26.43 to -26.37
Top soil(East) vs Top soil(North)	-22.50	3897	Yes	***	-22.53 to -22.47
Top soil(East) vs Top soil(Epicentre)	-19.50	3377	Yes	***	-19.53 to -19.47
Top soil(East) vs Top soil(Control)	-21.00	3637	Yes	***	-21.03 to -20.97
Top soil(West) vs Top soil(North)	3.900	675.5	Yes	***	3.873 to 3.927
Top soil(West) vs Top soil(Epicentre)	6.900	1195	Yes	***	6.873 to 6.927
Top soil(West) vs Top soil(Control)	5.400	935.3	Yes	***	5.373 to 5.427
Top soil(North) vs Top soil(Epicentre)	3.000	519.6	Yes	***	2.973 to 3.027
Top soil(North) vs Top soil(Control)	1.500	259.8	Yes	***	1.473 to 1.527
Top soil(Epicentre) vs Top soil(Control)	-1.500	259.8	Yes	***	-1.527 to -1.473

	Top soil(South)	Top soil(East)	Top soil(West)	Top soil(North)	Top soil(Epicentre)	Top soil(Control)
Number of values	3	3	3	3	3	3
Minimum	7.490	13.79	40.19	36.29	33.29	34.79
25% Percentile	7.490	13.79	40.19	36.29	33.29	34.79
Median	7.500	13.80	40.20	36.30	33.30	34.80
75% Percentile	7.510	13.81	40.21	36.31	33.31	34.81
Maximum	7.510	13.81	40.21	36.31	33.31	34.81
Mean	7.500	13.80	40.20	36.30	33.30	34.80
Std. Deviation	0.01000	0.01000	0.01000	0.01000	0.01000	0.01000
Std. Error	0.005774	0.005774	0.005774	0.005774	0.005774	0.005774
Lower 95% CI	7.475	13.78	40.18	36.28	33.28	34.78
Upper 95% CI	7.525	13.82	40.22	36.32	33.32	34.82

Parameter	
Table Analyzed	Catalase (Sub soil) 1m apart
One-way analysis of variance	
P value	< 0.0001
P value summary	***

Are means signif. different? (P < 0.05)	Yes
Number of groups	6
F	1.832e+007
R square	1.000

ANOVA Table	SS	df	MS
Treatment (between columns)	9162	5	1832
Residual (within columns)	0.001200	12	10.000e-005
Total	9162	17	

Tukey's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
Sub soil(South) vs Sub soil(East)	66.60	11536	Yes	***	66.57 to 66.63
Sub soil(South) vs Sub soil(West)	28.50	4936	Yes	***	28.47 to 28.53
Sub soil(South) vs Sub soil(North)	56.61	9805	Yes	***	56.58 to 56.64
Sub soil(South) vs Sub soil(Epicentre)	58.51	10134	Yes	***	58.48 to 58.54
Sub soil(South) vs Sub soil(Control)	46.50	8054	Yes	***	46.47 to 46.53
Sub soil(East) vs Sub soil(West)	-38.10	6599	Yes	***	-38.13 to -38.07
Sub soil(East) vs Sub soil(North)	-9.990	1730	Yes	***	-10.02 to -9.963
Sub soil(East) vs Sub soil(Epicentre)	-8.090	1401	Yes	***	-8.117 to -8.063
Sub soil(East) vs Sub soil(Control)	-20.10	3481	Yes	***	-20.13 to -20.07
Sub soil(West) vs Sub soil(North)	28.11	4869	Yes	***	28.08 to 28.14
Sub soil(West) vs Sub soil(Epicentre)	30.01	5198	Yes	***	29.98 to 30.04
Sub soil(West) vs Sub soil(Control)	18.00	3118	Yes	***	17.97 to 18.03
Sub soil(North) vs Sub soil(Epicentre)	1.900	329.1	Yes	***	1.873 to 1.927
Sub soil(North) vs Sub soil(Control)	-10.11	1751	Yes	***	-10.14 to -10.08
Sub soil(Epicentre) vs Sub soil(Control)	-12.01	2080	Yes	***	-12.04 to -11.98

	Sub soil(South)	Sub soil(East)	Sub soil(West)	Sub soil(North)	Sub soil(Epicentre)	Sub soil(Control)
Number of values	3	3	3	3	3	3
Minimum	88.49	21.89	59.99	31.88	29.98	41.99
25% Percentile	88.49	21.89	59.99	31.88	29.98	41.99
Median	88.50	21.90	60.00	31.89	29.99	42.00
75% Percentile	88.51	21.91	60.01	31.90	30.00	42.01

Maximum	88.51	21.91	60.01	31.90	30.00	42.01
Mean	88.50	21.90	60.00	31.89	29.99	42.00
Std. Deviation	0.01000	0.01000	0.009998	0.01000	0.01000	0.009998
Std. Error	0.005775	0.005774	0.005773	0.005774	0.005774	0.005773
Lower 95% CI	88.48	21.88	59.98	31.87	29.97	41.98
Upper 95% CI	88.52	21.92	60.02	31.91	30.01	42.02

Parameter	
Table Analyzed	Catalase (Sub Sub soil) 1m apart
One-way analysis of variance	
P value	< 0.0001
P value summary	***
Are means signif. different? (P < 0.05)	Yes
Number of groups	6
F	1.106e+007
R square	1.000

ANOVA Table	SS	df	MS
Treatment (between columns)	5529	5	1106
Residual (within columns)	0.001200	12	0.0001000
Total	5529	17	

Tukey's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
Sub Sub soil(South) vs Sub Sub soil(East)	33.60	5819	Yes	***	33.57 to 33.63
Sub Sub soil(South) vs Sub Sub soil(West)	-23.10	4001	Yes	***	-23.13 to -23.07
Sub Sub soil(South) vs Sub Sub soil(North)	15.60	2702	Yes	***	15.57 to 15.63
Sub Sub soil(South) vs Sub Sub soil(Epicentre)	7.800	1351	Yes	***	7.773 to 7.827
Sub Sub soil(South) vs Sub Sub soil(Control)	-4.500	779.4	Yes	***	-4.527 to -4.473
Sub Sub soil(East) vs Sub Sub soil(West)	-56.70	9820	Yes	***	-56.73 to -56.67
Sub Sub soil(East) vs Sub Sub soil(North)	-18.00	3118	Yes	***	-18.03 to -17.97
Sub Sub soil(East) vs Sub Sub soil(Epicentre)	-25.80	4469	Yes	***	-25.83 to -25.77

Sub Sub soil(East) vs Sub Sub soil(Control)	-38.10	6599	Yes	***	-38.13 to -38.07
Sub Sub soil(West) vs Sub Sub soil(North)	38.70	6703	Yes	***	38.67 to 38.73
Sub Sub soil(West) vs Sub Sub soil(Epicentre)	30.90	5352	Yes	***	30.87 to 30.93
Sub Sub soil(West) vs Sub Sub soil(Control)	18.60	3222	Yes	***	18.57 to 18.63
Sub Sub soil(North) vs Sub Sub soil(Epicentre)	-7.800	1351	Yes	***	-7.827 to -7.773
Sub Sub soil(North) vs Sub Sub soil(Control)	-20.10	3481	Yes	***	-20.13 to -20.07
Sub Sub soil(Epicentre) vs Sub Sub soil(Control)	-12.30	2130	Yes	***	-12.33 to -12.27

	Sub Sub soil(South)	Sub Sub soil(East)	Sub Sub soil(West)	Sub Sub soil(North)	Sub Sub soil(Epicentre)
Number of values	3	3	3	3	3
Minimum	41.39	7.790	64.49	25.79	33.59
25% Percentile	41.39	7.790	64.49	25.79	33.59
Median	41.40	7.800	64.50	25.80	33.60
75% Percentile	41.41	7.810	64.51	25.81	33.61
Maximum	41.41	7.810	64.51	25.81	33.61
Mean	41.40	7.800	64.50	25.80	33.60
Std. Deviation	0.01000	0.01000	0.01000	0.009999	0.01000
Std. Error	0.005774	0.005773	0.005775	0.005773	0.005774
Lower 95% CI	41.38	7.775	64.48	25.78	33.58
Upper 95% CI	41.42	7.825	64.52	25.82	33.62

Parameter	
Table Analyzed	Catalase (Top soil) 2m apart
One-way analysis of variance	
P value	< 0.0001
P value summary	***
Are means signif. different? (P < 0.05)	Yes
Number of groups	6
F	3.561e+006
R square	1.000

ANOVA Table	SS	df	MS
Treatment (between columns)	1781	5	356.1
Residual (within columns)	0.001200	12	0.0001000
Total	1781	17	

Tukey's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
Top soil(South) vs Top soil(East)	14.70	2546	Yes	***	14.67 to 14.73
Top soil(South) vs Top soil(West)	-13.80	2390	Yes	***	-13.83 to -13.77
Top soil(South) vs Top soil(North)	15.90	2754	Yes	***	15.87 to 15.93
Top soil(South) vs Top soil(Epicentre)	6.600	1143	Yes	***	6.573 to 6.627
Top soil(South) vs Top soil(Control)	5.100	883.3	Yes	***	5.073 to 5.127
Top soil(East) vs Top soil(West)	-28.50	4936	Yes	***	-28.53 to -28.47
Top soil(East) vs Top soil(North)	1.200	207.8	Yes	***	1.173 to 1.227
Top soil(East) vs Top soil(Epicentre)	-8.100	1403	Yes	***	-8.127 to -8.073
Top soil(East) vs Top soil(Control)	-9.600	1663	Yes	***	-9.627 to -9.573
Top soil(West) vs Top soil(North)	29.70	5144	Yes	***	29.67 to 29.73
Top soil(West) vs Top soil(Epicentre)	20.40	3533	Yes	***	20.37 to 20.43
Top soil(West) vs Top soil(Control)	18.90	3274	Yes	***	18.87 to 18.93
Top soil(North) vs Top soil(Epicentre)	-9.300	1611	Yes	***	-9.327 to -9.273
Top soil(North) vs Top soil(Control)	-10.80	1871	Yes	***	-10.83 to -10.77
Top soil(Epicentre) vs Top soil(Control)	-1.500	259.8	Yes	***	-1.527 to -1.473

	Top soil(South)	Top soil(East)	Top soil(West)	Top soil(North)	Top soil(Epicentre)	Top soil(Control)
Number of values	3	3	3	3	3	3
Minimum	39.89	25.19	53.69	23.99	33.29	34.79
25% Percentile	39.89	25.19	53.69	23.99	33.29	34.79
Median	39.90	25.20	53.70	24.00	33.30	34.80
75% Percentile	39.91	25.21	53.71	24.01	33.31	34.81
Maximum	39.91	25.21	53.71	24.01	33.31	34.81
Mean	39.90	25.20	53.70	24.00	33.30	34.80
Std. Deviation	0.01000	0.009999	0.01000	0.01000	0.01000	0.01000

Std. Error	0.005774	0.005773	0.005774	0.005774	0.005774	0.005774
Lower 95% CI	39.88	25.18	53.68	23.98	33.28	34.78
Upper 95% CI	39.92	25.22	53.72	24.02	33.32	34.82

Parameter	
Table Analyzed	Catalase(Sub soil) 2m apart
One-way analysis of variance	
P value	< 0.0001
P value summary	***
Are means signif. different? (P < 0.05)	Yes
Number of groups	6
F	3.405e+006
R square	1.000

ANOVA Table	SS	df	MS
Treatment (between columns)	1703	5	340.5
Residual (within columns)	0.001200	12	10.000e-005
Total	1703	17	

Tukey's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
Sub soil(South) vs Sub soil(East)	3.000	519.6	Yes	***	2.973 to 3.027
Sub soil(South) vs Sub soil(West)	-22.80	3949	Yes	***	-22.83 to -22.77
Sub soil(South) vs Sub soil(North)	-17.40	3014	Yes	***	-17.43 to -17.37
Sub soil(South) vs Sub soil(Epicentre)	-0.5900	102.2	Yes	***	-0.6174 to -0.5626
Sub soil(South) vs Sub soil(Control)	-12.60	2182	Yes	***	-12.63 to -12.57
Sub soil(East) vs Sub soil(West)	-25.80	4469	Yes	***	-25.83 to -25.77
Sub soil(East) vs Sub soil(North)	-20.40	3533	Yes	***	-20.43 to -20.37
Sub soil(East) vs Sub soil(Epicentre)	-3.590	621.8	Yes	***	-3.617 to -3.563
Sub soil(East) vs Sub soil(Control)	-15.60	2702	Yes	***	-15.63 to -15.57
Sub soil(West) vs Sub soil(North)	5.400	935.3	Yes	***	5.373 to 5.427
Sub soil(West) vs Sub soil(Epicentre)	22.21	3847	Yes	***	22.18 to 22.24
Sub soil(West) vs Sub soil(Control)	10.20	1767	Yes	***	10.17 to 10.23

Sub soil(North) vs Sub soil(Epicentre)	16.81	2912	Yes	***	16.78 to 16.84
Sub soil(North) vs Sub soil(Control)	4.800	831.4	Yes	***	4.773 to 4.827
Sub soil(Epicentre) vs Sub soil(Control)	-12.01	2080	Yes	***	-12.04 to -11.98

	Sub soil(South)	Sub soil(East)	Sub soil(West)	Sub soil(North)	Sub soil(Epicentre)	Sub soil(Control)
Number of values	3	3	3	3	3	3
Minimum	29.39	26.39	52.19	46.79	29.98	41.99
25% Percentile	29.39	26.39	52.19	46.79	29.98	41.99
Median	29.40	26.40	52.20	46.80	29.99	42.00
75% Percentile	29.41	26.41	52.21	46.81	30.00	42.01
Maximum	29.41	26.41	52.21	46.81	30.00	42.01
Mean	29.40	26.40	52.20	46.80	29.99	42.00
Std. Deviation	0.01000	0.01000	0.01000	0.01000	0.01000	0.009998
Std. Error	0.005774	0.005774	0.005774	0.005774	0.005774	0.005773
Lower 95% CI	29.38	26.38	52.18	46.78	29.97	41.98
Upper 95% CI	29.42	26.42	52.22	46.82	30.01	42.02

Parameter	
Table Analyzed	Catalase (Sub Sub soil) 2m apart
One-way analysis of variance	
P value	< 0.0001
P value summary	***
Are means signif. different? (P < 0.05)	Yes
Number of groups	6
F	1.472e+007
R square	1.000

ANOVA Table	SS	df	MS
Treatment (between columns)	7360	5	1472
Residual (within columns)	0.001200	12	0.0001000
Total	7360	17	

Tukey's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
Sub Sub soil(South) vs Sub Sub soil(East)	40.80	7067	Yes	***	40.77 to 40.83
Sub Sub soil(South) vs Sub Sub soil(West)	-24.00	4157	Yes	***	-24.03 to -23.97
Sub Sub soil(South) vs Sub Sub soil(North)	16.20	2806	Yes	***	16.17 to 16.23
Sub Sub soil(South) vs Sub Sub soil(Epicentre)	24.90	4313	Yes	***	24.87 to 24.93
Sub Sub soil(South) vs Sub Sub soil(Control)	12.60	2182	Yes	***	12.57 to 12.63
Sub Sub soil(East) vs Sub Sub soil(West)	-64.80	11224	Yes	***	-64.83 to -64.77
Sub Sub soil(East) vs Sub Sub soil(North)	-24.60	4261	Yes	***	-24.63 to -24.57
Sub Sub soil(East) vs Sub Sub soil(Epicentre)	-15.90	2754	Yes	***	-15.93 to -15.87
Sub Sub soil(East) vs Sub Sub soil(Control)	-28.20	4884	Yes	***	-28.23 to -28.17
Sub Sub soil(West) vs Sub Sub soil(North)	40.20	6963	Yes	***	40.17 to 40.23
Sub Sub soil(West) vs Sub Sub soil(Epicentre)	48.90	8470	Yes	***	48.87 to 48.93
Sub Sub soil(West) vs Sub Sub soil(Control)	36.60	6339	Yes	***	36.57 to 36.63
Sub Sub soil(North) vs Sub Sub soil(Epicentre)	8.700	1507	Yes	***	8.673 to 8.727
Sub Sub soil(North) vs Sub Sub soil(Control)	-3.600	623.5	Yes	***	-3.627 to -3.573
Sub Sub soil(Epicentre) vs Sub Sub soil(Control)	-12.30	2130	Yes	***	-12.33 to -12.27

	Sub Sub soil(South)	Sub Sub soil(East)	Sub Sub soil(West)	Sub Sub soil(North)	Sub Sub soil(Epicentre)
Number of values	3	3	3	3	3
Minimum	58.49	17.69	82.49	42.29	33.59
25% Percentile	58.49	17.69	82.49	42.29	33.59
Median	58.50	17.70	82.50	42.30	33.60
75% Percentile	58.51	17.71	82.51	42.31	33.61
Maximum	58.51	17.71	82.51	42.31	33.61
Mean	58.50	17.70	82.50	42.30	33.60
Std. Deviation	0.009998	0.009999	0.01000	0.01000	0.01000
Std. Error	0.005773	0.005773	0.005775	0.005774	0.005774
Lower 95% CI	58.48	17.68	82.48	42.28	33.58
Upper 95% CI	58.52	17.72	82.52	42.32	33.62

Parameter

Table Analyzed	Acid phosphatase(Top soil) 1m apart
One-way analysis of variance	
P value	0.4302
P value summary	ns
Are means signif. different? (P < 0.05)	No
Number of groups	6
F	1.143
R square	0.4878

ANOVA Table	SS	df	MS
Treatment (between columns)	6.667e-006	5	1.333e-006
Residual (within columns)	7.000e-006	6	1.167e-006
Total	1.367e-005	11	

Tukey's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
Top soil(South) vs Top soil(East)	0.0	0.0	No	ns	-0.004298 to 0.004298
Top soil(South) vs Top soil(West)	-0.0020	2.619	No	ns	-0.006298 to 0.002298
Top soil(South) vs Top soil(North)	0.0	0.0	No	ns	-0.004298 to 0.004298
Top soil(South) vs Top soil(Epicentre)	0.0	0.0	No	ns	-0.004298 to 0.004298
Top soil(South) vs Top soil(Control)	0.0	0.0	No	ns	-0.004298 to 0.004298
Top soil(East) vs Top soil(West)	-0.0020	2.619	No	ns	-0.006298 to 0.002298
Top soil(East) vs Top soil(North)	0.0	0.0	No	ns	-0.004298 to 0.004298
Top soil(East) vs Top soil(Epicentre)	0.0	0.0	No	ns	-0.004298 to 0.004298
Top soil(East) vs Top soil(Control)	0.0	0.0	No	ns	-0.004298 to 0.004298
Top soil(West) vs Top soil(North)	0.0020	2.619	No	ns	-0.002298 to 0.006298
Top soil(West) vs Top soil(Epicentre)	0.0020	2.619	No	ns	-0.002298 to 0.006298
Top soil(West) vs Top soil(Control)	0.0020	2.619	No	ns	-0.002298 to 0.006298
Top soil(North) vs Top soil(Epicentre)	0.0	0.0	No	ns	-0.004298 to 0.004298
Top soil(North) vs Top soil(Control)	0.0	0.0	No	ns	-0.004298 to 0.004298
Top soil(Epicentre) vs Top soil(Control)	0.0	0.0	No	ns	-0.004298 to 0.004298

	Top soil(South)	Top soil(East)	Top soil(West)	Top soil(North)	Top soil(Epicentre)	Top soil(Control)
Number of values	2	2	2	2	2	2

Minimum	0.0010	0.0010	0.0020	0.0010	0.0010	0.0010
25% Percentile	0.0010	0.0010	0.0020	0.0010	0.0010	0.0010
Median	0.0015	0.0015	0.0035	0.0015	0.0015	0.0015
75% Percentile	0.0020	0.0020	0.0050	0.0020	0.0020	0.0020
Maximum	0.0020	0.0020	0.0050	0.0020	0.0020	0.0020
Mean	0.0015	0.0015	0.0035	0.0015	0.0015	0.0015
Std. Deviation	0.0007071	0.0007071	0.002121	0.0007071	0.0007071	0.0007071
Std. Error	0.0005	0.0005	0.0015	0.0005	0.0005	0.0005
Lower 95% CI	-0.004853	-0.004853	-0.01556	-0.004853	-0.004853	-0.004853
Upper 95% CI	0.007853	0.007853	0.02256	0.007853	0.007853	0.007853

Parameter	
Table Analyzed	Acid phosphatase(Sub soil) 1m apart
One-way analysis of variance	
P value	0.6634
P value summary	ns
Are means signif. different? (P < 0.05)	No
Number of groups	6
F	0.6667
R square	0.3571

ANOVA Table	SS	df	MS
Treatment (between columns)	1.667e-006	5	3.333e-007
Residual (within columns)	3.000e-006	6	5.000e-007
Total	4.667e-006	11	

Tukey's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
Sub soil(South) vs Sub soil(East)	0.0005	1.000	No	ns	-0.002314 to 0.003314
Sub soil(South) vs Sub soil(West)	0.0005	1.000	No	ns	-0.002314 to 0.003314
Sub soil(South) vs Sub soil(North)	-0.0005000	1.000	No	ns	-0.003314 to 0.002314

Sub soil(South) vs Sub soil(Epicentre)	0.0005	1.000	No	ns	-0.002314 to 0.003314
Sub soil(South) vs Sub soil(Control)	0.0	0.0	No	ns	-0.002814 to 0.002814
Sub soil(East) vs Sub soil(West)	0.0	0.0	No	ns	-0.002814 to 0.002814
Sub soil(East) vs Sub soil(North)	-0.0010	2.000	No	ns	-0.003814 to 0.001814
Sub soil(East) vs Sub soil(Epicentre)	0.0	0.0	No	ns	-0.002814 to 0.002814
Sub soil(East) vs Sub soil(Control)	-0.0005	1.000	No	ns	-0.003314 to 0.002314
Sub soil(West) vs Sub soil(North)	-0.0010	2.000	No	ns	-0.003814 to 0.001814
Sub soil(West) vs Sub soil(Epicentre)	0.0	0.0	No	ns	-0.002814 to 0.002814
Sub soil(West) vs Sub soil(Control)	-0.0005	1.000	No	ns	-0.003314 to 0.002314
Sub soil(North) vs Sub soil(Epicentre)	0.0010	2.000	No	ns	-0.001814 to 0.003814
Sub soil(North) vs Sub soil(Control)	0.0005000	1.000	No	ns	-0.002314 to 0.003314
Sub soil(Epicentre) vs Sub soil(Control)	-0.0005	1.000	No	ns	-0.003314 to 0.002314

	Sub soil(South)	Sub soil(East)	Sub soil(West)	Sub soil(North)	Sub soil(Epicentre)	Sub soil(Control)
Number of values	2	2	2	2	2	2
Minimum	0.0010	0.0010	0.0010	0.0010	0.0010	0.0010
25% Percentile	0.0010	0.0010	0.0010	0.0010	0.0010	0.0010
Median	0.0015	0.0010	0.0010	0.0020	0.0010	0.0015
75% Percentile	0.0020	0.0010	0.0010	0.0030	0.0010	0.0020
Maximum	0.0020	0.0010	0.0010	0.0030	0.0010	0.0020
Mean	0.0015	0.0010	0.0010	0.0020	0.0010	0.0015
Std. Deviation	0.0007071	0.0	0.0	0.001414	0.0	0.0007071
Std. Error	0.0005	0.0	0.0	0.0010	0.0	0.0005
Lower 95% CI	-0.004853	0.0010	0.0010	-0.01071	0.0010	-0.004853
Upper 95% CI	0.007853	0.0010	0.0010	0.01471	0.0010	0.007853

Parameter	
Table Analyzed	Acid phosphatase(Sub Sub soil) 1m apart
One-way analysis of variance	
P value	0.6634

P value summary	ns
Are means signif. different? (P < 0.05)	No
Number of groups	6
F	0.6667
R square	0.3571

ANOVA Table	SS	df	MS
Treatment (between columns)	1.667e-006	5	3.333e-007
Residual (within columns)	3.000e-006	6	5.000e-007
Total	4.667e-006	11	

Tukey's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
Sub Sub soil(South) vs Sub Sub soil(East)	0.0010	2.000	No	ns	-0.001814 to 0.003814
Sub Sub soil(South) vs Sub Sub soil(West)	0.0010	2.000	No	ns	-0.001814 to 0.003814
Sub Sub soil(South) vs Sub Sub soil(North)	0.0010	2.000	No	ns	-0.001814 to 0.003814
Sub Sub soil(South) vs Sub Sub soil(Epicentre)	0.0005000	1.000	No	ns	-0.002314 to 0.003314
Sub Sub soil(South) vs Sub Sub soil(Control)	0.0005000	1.000	No	ns	-0.002314 to 0.003314
Sub Sub soil(East) vs Sub Sub soil(West)	0.0	0.0	No	ns	-0.002814 to 0.002814
Sub Sub soil(East) vs Sub Sub soil(North)	0.0	0.0	No	ns	-0.002814 to 0.002814
Sub Sub soil(East) vs Sub Sub soil(Epicentre)	-0.0005	1.000	No	ns	-0.003314 to 0.002314
Sub Sub soil(East) vs Sub Sub soil(Control)	-0.0005	1.000	No	ns	-0.003314 to 0.002314
Sub Sub soil(West) vs Sub Sub soil(North)	0.0	0.0	No	ns	-0.002814 to 0.002814
Sub Sub soil(West) vs Sub Sub soil(Epicentre)	-0.0005	1.000	No	ns	-0.003314 to 0.002314

Sub Sub soil(West) vs Sub Sub soil(Control)	-0.0005	1.000	No	ns	-0.003314 to 0.002314
Sub Sub soil(North) vs Sub Sub soil(Epicentre)	-0.0005	1.000	No	ns	-0.003314 to 0.002314
Sub Sub soil(North) vs Sub Sub soil(Control)	-0.0005	1.000	No	ns	-0.003314 to 0.002314
Sub Sub soil(Epicentre) vs Sub Sub soil(Control)	0.0	0.0	No	ns	-0.002814 to 0.002814

	Sub Sub soil(South)	Sub Sub soil(East)	Sub Sub soil(West)	Sub Sub soil(North)	Sub Sub soil(Epicentre)
Number of values	2	2	2	2	2
Minimum	0.0010	0.0010	0.0010	0.0010	0.0010
25% Percentile	0.0010	0.0010	0.0010	0.0010	0.0010
Median	0.0020	0.0010	0.0010	0.0010	0.0015
75% Percentile	0.0030	0.0010	0.0010	0.0010	0.0020
Maximum	0.0030	0.0010	0.0010	0.0010	0.0020
Mean	0.0020	0.0010	0.0010	0.0010	0.0015
Std. Deviation	0.001414	0.0	0.0	0.0	0.0007071
Std. Error	0.0010	0.0	0.0	0.0	0.0005
Lower 95% CI	-0.01071	0.0010	0.0010	0.0010	-0.004853
Upper 95% CI	0.01471	0.0010	0.0010	0.0010	0.007853

Parameter	
Table Analyzed	Acid phosphatase(Top soil) 2m apart
One-way analysis of variance	
P value	0.9510
P value summary	ns
Are means signif. different? (P < 0.05)	No
Number of groups	6
F	0.2000

R square	0.1429

ANOVA Table	SS	df	MS
Treatment (between columns)	4.167e-007	5	8.333e-008
Residual (within columns)	2.500e-006	6	4.167e-007
Total	2.917e-006	11	

Tukey's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
Top soil(South) vs Top soil(East)	-0.0005	1.095	No	ns	-0.003069 to 0.002069
Top soil(South) vs Top soil(West)	-0.0005	1.095	No	ns	-0.003069 to 0.002069
Top soil(South) vs Top soil(North)	-0.0005	1.095	No	ns	-0.003069 to 0.002069
Top soil(South) vs Top soil(Epicentre)	-0.0005	1.095	No	ns	-0.003069 to 0.002069
Top soil(South) vs Top soil(Control)	-0.0005	1.095	No	ns	-0.003069 to 0.002069
Top soil(East) vs Top soil(West)	0.0	0.0	No	ns	-0.002569 to 0.002569
Top soil(East) vs Top soil(North)	0.0	0.0	No	ns	-0.002569 to 0.002569
Top soil(East) vs Top soil(Epicentre)	0.0	0.0	No	ns	-0.002569 to 0.002569
Top soil(East) vs Top soil(Control)	0.0	0.0	No	ns	-0.002569 to 0.002569
Top soil(West) vs Top soil(North)	0.0	0.0	No	ns	-0.002569 to 0.002569
Top soil(West) vs Top soil(Epicentre)	0.0	0.0	No	ns	-0.002569 to 0.002569
Top soil(West) vs Top soil(Control)	0.0	0.0	No	ns	-0.002569 to 0.002569
Top soil(North) vs Top soil(Epicentre)	0.0	0.0	No	ns	-0.002569 to 0.002569
Top soil(North) vs Top soil(Control)	0.0	0.0	No	ns	-0.002569 to 0.002569
Top soil(Epicentre) vs Top soil(Control)	0.0	0.0	No	ns	-0.002569 to 0.002569

	Top soil(South)	Top soil(East)	Top soil(West)	Top soil(North)	Top soil(Epicentre)	Top soil(Control)
Number of values	2	2	2	2	2	2
Minimum	0.0010	0.0010	0.0010	0.0010	0.0010	0.0010
25% Percentile	0.0010	0.0010	0.0010	0.0010	0.0010	0.0010
Median	0.0010	0.0015	0.0015	0.0015	0.0015	0.0015
75% Percentile	0.0010	0.0020	0.0020	0.0020	0.0020	0.0020
Maximum	0.0010	0.0020	0.0020	0.0020	0.0020	0.0020
Mean	0.0010	0.0015	0.0015	0.0015	0.0015	0.0015

Std. Deviation	0.0	0.0007071	0.0007071	0.0007071	0.0007071	0.0007071
Std. Error	0.0	0.0005	0.0005	0.0005	0.0005	0.0005
Lower 95% CI	0.0010	-0.004853	-0.004853	-0.004853	-0.004853	-0.004853
Upper 95% CI	0.0010	0.007853	0.007853	0.007853	0.007853	0.007853

Parameter	
Table Analyzed	Acid phosphatse(Sub soil) 2m
One-way analysis of variance	
P value	0.5665
P value summary	ns
Are means signif. different? (P < 0.05)	No
Number of groups	6
F	0.8400
R square	0.4118

ANOVA Table	SS	df	MS
Treatment (between columns)	1.750e-006	5	3.500e-007
Residual (within columns)	2.500e-006	6	4.167e-007
Total	4.250e-006	11	

Tukey's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
Sub soil(South) vs Sub soil(East)	0.0	0.0	No	ns	-0.002569 to 0.002569
Sub soil(South) vs Sub soil(West)	-0.0010	2.191	No	ns	-0.003569 to 0.001569
Sub soil(South) vs Sub soil(North)	0.0	0.0	No	ns	-0.002569 to 0.002569
Sub soil(South) vs Sub soil(Epicentre)	0.0	0.0	No	ns	-0.002569 to 0.002569
Sub soil(South) vs Sub soil(Control)	-0.0005	1.095	No	ns	-0.003069 to 0.002069
Sub soil(East) vs Sub soil(West)	-0.0010	2.191	No	ns	-0.003569 to 0.001569
Sub soil(East) vs Sub soil(North)	0.0	0.0	No	ns	-0.002569 to 0.002569
Sub soil(East) vs Sub soil(Epicentre)	0.0	0.0	No	ns	-0.002569 to 0.002569
Sub soil(East) vs Sub soil(Control)	-0.0005	1.095	No	ns	-0.003069 to 0.002069
Sub soil(West) vs Sub soil(North)	0.0010	2.191	No	ns	-0.001569 to 0.003569
Sub soil(West) vs Sub soil(Epicentre)	0.0010	2.191	No	ns	-0.001569 to 0.003569

Sub soil(West) vs Sub soil(Control)	0.0005000	1.095	No	ns	-0.002069 to 0.003069
Sub soil(North) vs Sub soil(Epicentre)	0.0	0.0	No	ns	-0.002569 to 0.002569
Sub soil(North) vs Sub soil(Control)	-0.0005	1.095	No	ns	-0.003069 to 0.002069
Sub soil(Epicentre) vs Sub soil(Control)	-0.0005	1.095	No	ns	-0.003069 to 0.002069

	Sub soil(South)	Sub soil(East)	Sub soil(West)	Sub soil(North)	Sub soil(Epicentre)	Sub soil(Control)
Number of values	2	2	2	2	2	2
Minimum	0.0010	0.0010	0.0010	0.0010	0.0010	0.0010
25% Percentile	0.0010	0.0010	0.0010	0.0010	0.0010	0.0010
Median	0.0010	0.0010	0.0020	0.0010	0.0010	0.0015
75% Percentile	0.0010	0.0010	0.0030	0.0010	0.0010	0.0020
Maximum	0.0010	0.0010	0.0030	0.0010	0.0010	0.0020
Mean	0.0010	0.0010	0.0020	0.0010	0.0010	0.0015
Std. Deviation	0.0	0.0	0.001414	0.0	0.0	0.0007071
Std. Error	0.0	0.0	0.0010	0.0	0.0	0.0005
Lower 95% CI	0.0010	0.0010	-0.01071	0.0010	0.0010	-0.004853
Upper 95% CI	0.0010	0.0010	0.01471	0.0010	0.0010	0.007853

Parameter	
Table Analyzed	Acid phosphatase(Sub Sub soil) 2m apart
One-way analysis of variance	
P value	0.5876
P value summary	ns
Are means signif. different? (P < 0.05)	No
Number of groups	6
F	0.8000
R square	0.4000

ANOVA Table	SS	df	MS
Treatment (between columns)	6.667e-007	5	1.333e-007
Residual (within columns)	1.000e-006	6	1.667e-007
Total	1.667e-006	11	

Tukey's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
Sub Sub soil(South) vs Sub Sub soil(East)	0.0	0.0	No	ns	-0.001625 to 0.001625
Sub Sub soil(South) vs Sub Sub soil(West)	0.0	0.0	No	ns	-0.001625 to 0.001625
Sub Sub soil(South) vs Sub Sub soil(North)	0.0	0.0	No	ns	-0.001625 to 0.001625
Sub Sub soil(South) vs Sub Sub soil(Epicentre)	-0.0005	1.732	No	ns	-0.002125 to 0.001125
Sub Sub soil(South) vs Sub Sub soil(Control)	-0.0005	1.732	No	ns	-0.002125 to 0.001125
Sub Sub soil(East) vs Sub Sub soil(West)	0.0	0.0	No	ns	-0.001625 to 0.001625
Sub Sub soil(East) vs Sub Sub soil(North)	0.0	0.0	No	ns	-0.001625 to 0.001625
Sub Sub soil(East) vs Sub Sub soil(Epicentre)	-0.0005	1.732	No	ns	-0.002125 to 0.001125
Sub Sub soil(East) vs Sub Sub soil(Control)	-0.0005	1.732	No	ns	-0.002125 to 0.001125
Sub Sub soil(West) vs Sub Sub soil(North)	0.0	0.0	No	ns	-0.001625 to 0.001625
Sub Sub soil(West) vs Sub Sub soil(Epicentre)	-0.0005	1.732	No	ns	-0.002125 to 0.001125
Sub Sub soil(West) vs Sub Sub soil(Control)	-0.0005	1.732	No	ns	-0.002125 to 0.001125
Sub Sub soil(North) vs Sub Sub soil(Epicentre)	-0.0005	1.732	No	ns	-0.002125 to 0.001125
Sub Sub soil(North) vs Sub Sub soil(Control)	-0.0005	1.732	No	ns	-0.002125 to 0.001125

Sub Sub soil(Epicentre) vs Sub Sub soil(Control)	0.0	0.0	No	ns	-0.001625 to 0.001625

	Sub Sub soil(South)	Sub Sub soil(East)	Sub Sub soil(West)	Sub Sub soil(North)	Sub Sub soil(Epicentre)
Number of values	2	2	2	2	2
Minimum	0.0010	0.0010	0.0010	0.0010	0.0010
25% Percentile	0.0010	0.0010	0.0010	0.0010	0.0010
Median	0.0010	0.0010	0.0010	0.0010	0.0015
75% Percentile	0.0010	0.0010	0.0010	0.0010	0.0020
Maximum	0.0010	0.0010	0.0010	0.0010	0.0020
Mean	0.0010	0.0010	0.0010	0.0010	0.0015
Std. Deviation	0.0	0.0	0.0	0.0	0.0007071
Std. Error	0.0	0.0	0.0	0.0	0.0005
Lower 95% CI	0.0010	0.0010	0.0010	0.0010	-0.004853
Upper 95% CI	0.0010	0.0010	0.0010	0.0010	0.007853