

ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES



**HEAVY METAL ACCUMULATION BY OYSTER
MUSHROOM (*Pleurotus ostreatus*) GROWN IN
AGRICULTURAL LAND IRRIGATED BY AKAKI RIVER**

By

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**A THESIS SUBMITTED TO THE SCHOOL OF GRADUATE
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ADDIS ABABA UNIVERSITY
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This is to certify that the thesis presented by **Kuma Bedada** entitled “**Heavy metal accumulation by Oyster mushroom (*Pleurotus ostreatus*) cultivated on agricultural land irrigated by Akaki River**” submitted in partial fulfillment of the degree of masters complies with the regulations of the university and meets the accepted standards with respect to the originality and quality; signed by the examining committee.

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2.....Sign.....Date.....

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ABSTRACT

The accumulation of heavy metals in agricultural soils is of increasing concern due to the food safety issue and potential health risks. Sources of these elements in soils mainly include industries and human activities. International and national regulations on food quality have lowered the maximum permissible levels of toxic metals in the food items due to an increased awareness of the risk these metals pose to food chain contamination. Remediation of soil contaminated with heavy metals has received considerable attention in recent years. So, looking to the severity of problem due to heavy metals contamination and there need of remediation, the present study was conducted on experimental site soil situated near Aba Samuel dam build on Akaki river followed by control. The study was conducted on the effects of heavy metal contamination accumulated in selected commonly available edible mushroom (*Pleurotus ostreatus*) along with bioaccumulation potential of selected mushroom species for heavy metals (Cd, Cr, Cu, Pb, Ni & Zn). The effects of waste water on selected site soil samples were conducted by means of first and second cultivation of mushroom. Positive affect of mushroom cultivation were found on waste water irrigated soil properties due to improvement in soil pH, EC followed by reduced concentration of heavy metals. The results of soil analysis indicate that the selected mushroom species is able to bio-remediate the high heavy metals (Cr, Cu, Pb and Zn) concentration from contaminated soil. Results also indicated the need of further cultivation of mushroom to bioremediate the heavy metals (Cr, Pb & Zn) up to safe level in contaminated site soil. The highly significant negative correlations was found between pH and Cr, Pb & Zn; Significant positive correlation was between organic matter and Cu, Pb & Zn; Electrical conductivity (EC) and Cr. Bioaccumulation of heavy metals in mushroom has significantly decreased the yield parameters (color, total numbers of buds, average height, pileus diameter, stem thickness, total mass and ash content) and biochemical values (protein) in mushroom during the both first and second cultivation. Significantly higher nutrient contents (Ca, Fe, K, NO_3^- & PO_4^{2-}) were found in comparison to control indicated the presence of high nutrient content in soil due to wastewater irrigation. Cr, Cu, Pb and Zn were significantly bioaccumulated in both first and second cultivated mushroom in comparison to control indicating the better bioaccumulation potential of selected species mushrooms. Cr and Pb metal levels were found higher than maximum permissible limit (FAO/WHO, 2001) for mushroom sample cultivated on experimental site soil for both first and second cultivation indicating the possible heavy metals related health hazards upon consumption of contaminated mushroom as well as further pollution after their improper disposal. Other heavy metals will also be future health hazard at selected site if the current practice of mushroom or any agricultural crop and vegetable production will be continued in long run without proper heavy metal remediation.

Key Words: - Aba Samuel dam, Bioaccumulation, Edible mushroom, Heavy metals, Waste water irrigated soil.

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ABBREVIATIONS

μS: Micro siemens

AAWSA: Addis Ababa Water and Sewerages Authority

ANOVA: Analysis Of Variance

BPR: Business Process Re-engineering

DNA: Dioxy Ribonucleic Acid

EC: Electrical Conductivity

EU: European Commission

FAO: Food and Agricultural Organization

GDP: Gross Domestic Product

GOE: Government Of Ethiopia

OM: Organic Matter

PAHs: Polychlorinated Aromatic Hydrocarbons

PCB: Poly Chlorinated Biphenyls

RNA: Ribonucleic Acid

SG: Specific Gravity

SPSS: Statistical Package for Social Science

USD: U.S.A. Dollar

WHO: World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

1.10 BACKGROUND

Agriculture is a key driver of Ethiopia's long-term growth and food security. It directly supports 85 percent of the population, constitutes 43 percent of Gross Domestic Product (GDP), and 80 percent of export value. Ethiopia has the potential of adding up to USD 20 billion in additional GDP, establishing food security, and reaching middle-income country status by 2025. However, achieving such results will require consistent, accelerated growth in the agriculture sector and a higher productivity in the use of all inputs and resources. So, to achieve the middle-income status by 2025 and make substantial inroads against food insecurity; concerted, strategic investment and strategic choices in the agricultural sector are vital. Concentrations of food insecurity and malnutrition are endemic in rural areas, with a population of six to seven million chronically food insecure, and up to 13 million seasonally food insecure. Over 90 percent of agricultural output is driven by smallholder farmers (Ethiopia agriculture diagnostic integrated report July 2010).

To facilitate this program; adjacently preserving the environment and prevent environmental pollution is strictly needed. So the recently adopted Business Process Re-engineering (BPR) find out the five measure priority set of actions that will drive Ethiopia's overall growth strategy.

- Enhance frontline productivity through better coordinated local institutions (extension, cooperatives, traders, research, etc.) with coherent sets of interventions, driven by kebele and woreda production opportunities.
- Improve industry structure to build strong public and private actors in priority value chains – the formation of joint public or private development programs should be supported through targeted incentives and appropriate regulatory frameworks.
- Scale up sustainable irrigation development– to improve project delivery and the performance of both surface and groundwater schemes, should invest in technical

resources, ensure the financial and environmental sustainability of all of its projects, and provide extension assistance on irrigated agriculture.

- Adopt a sustainable approach to preserving and expanding cultivated land by increasing yields through an integrated soil fertility management approach and exploring land expansion systematically by developing national and regional land-use strategies that consider farming models that balance economics, sustainability, and social impact.
- Put the enabling environment in place to support the growth potential of the first four areas. The priority areas to address are: access to financing for actors along the entire value chain (including rural financial inclusion); the necessary infrastructure to give producers access to markets; innovative use of information and communication technology (e.g., radio broadcasts to support extension advice); and building the human capacity of the public sector.

These all can be assured only if continuous environmental assessment and evaluation is made properly to prevent possible potential environmental deterioration during agricultural practice and establishing productive industries. Ethiopia has been part of an international initiative to prevent environmental degradation, both at local and global level. The Ministry of Health and the Ethiopian Environmental Protection Authority are the leading institutions working on health and environment. The most obvious environmental risk factors affecting human health in urban settings are hazardous wastes from industry and transport, and poor chemical and household waste management. Indoor air pollution, food contamination and disease vectors are also risk factors. Toxic wastes are most common in Addis Ababa where industries, automobiles and hospitals are concentrated (Ministry of Health, 2008).

Pollution is one of the most important problems around the world in which thousands of millions of world inhabitants suffer health problem related to industrial pollutants. In recent years significant attention being paid to the problems of environmental contamination by a wide variety of chemical pollutants including trace metals. Heavy metals enter into our environment from both natural and anthropogenic sources. They

contaminate food source and accumulate in both agricultural products and seafood through water, air and soil pollution (Lin *et al.*, 2004).

Addition of heavy metals to soil may affect microbial proliferation and enzymatic activities, possibly leading to a decrease in the rates of the biochemical process in the soil environment. The effect of trace metals on biochemical reaction in soils may vary with pH, organic matter content, particle size distribution, vegetation and total hydrocarbon content (Esser *et al.*, 1991).

Worldwide increasing level of industrialization and urbanization has led to environmental pollution. Industries have largely been responsible for discharging effluents containing trace metals such as zinc (Zn), Copper (Cu), Manganese (Mn), Cadmium (Cd), Mercury (Hg), Nickel (Ni), lead (Pb) and Chromium (Cr) into our environments. Trace heavy metals like Fe, Cu, Zn, Mn and Ni are essential nutrients but they become harmful and toxic when their concentration exceeds the recommended standards or maximum permissible level by World Health Organization (Ibok *et al.*, 1989). Lead and Cadmium are non essential heavy metals. They are extremely toxic even in very minute amounts (Filazi *et al.*, 2003).

1.20 STATEMENT OF THE PROBLEM

Ethiopia, like all countries in the world is suffering from environmental pollution and deterioration due to hazardous wastes disposal from urbanization and industrial emission of waste chemicals. Industries are expanding at alarming rate without establishing waste water treatment plants. They use rivers and other water bodies as their waste disposal sites or reservoirs. Urbanization is increasing and expanding with technology and industrial development with no access of waste disposal sites and proper treatment. Open dumping of toxic waste from industries, house hold sewages and municipal wastes into the water bodies and rivers decreased its aesthetic value, polluted potable water, it has poisoned animals living in the water bodies like fishes and has polluted` agricultural soil (Mohamed Ali Mohamed, 2002).

Addis Ababa is the most populated city as well as industrial center in the country, but lacks adequate solid and liquid waste management facilities. Direct discharge of foreign materials from different sources to rivers and open lands and the leakage of industrial

wastes from poorly designed septic tanks to the ground water, discharge of effluents without detoxifying the waste solid or liquid from domestic and municipal, organic matter of plant and animal origin, land and surface washing and sewage effluents are among few sources of pollution that changes the water quality in the capital city. This industrial growth without care for impact on water resources eventually made much of the water across the city unfit for aesthetic value, recreational purposes, for agriculture and domestic drinking water (Frezer Eshetu, 2012).

Thus various sources have grossly polluted the Akaki River and this has been proved with several independent and interrelated studies by different researchers and institutions at different times. Under actual conditions, wastes generated from different point and non-point sources reach the rivers easily. The wastes could be hazardous and non-hazardous. These wastes, especially the solid waste could be washed away from the source to the rivers through runoff while most of the industries are in close proximity to these rivers so that they directly discharge their waste to the rivers without any treatment. In addition, the existence of poor infrastructure (sewerage system), low level of awareness, weak enforcement mechanism on pollution prevention and control, greatly contributed to the pollution of the Akaki River. Consequently, the polluted river water is causing health impact on the producer of vegetables themselves due to the fact that farmers are cultivating the vegetables bare handed and without protecting devices (Agaje Mekonnen, 2007).

1.30 SIGNIFICANCE OF THE RESEARCH

We are surrounded by agricultural and industrial waste that can be converted into fuel, medicines, and edible protein. The only thing that we should have to do is investigating our environmental-Chemical

- Biological and

- Physical deterioration by using pollution indicator aids to convert these wastes to useful byproducts and developing techniques to manage and recycle these wastes to prevent environmental deterioration and built strong chain to maintain our environmental natural balance. Fungi are opportunistic and can be trained to decompose and accumulate specific pollutants, where they can be used as pollution indicator to show

status of our environment. This research is very important to indicate current environmental pollution status of our country due to tannery and other industries that dispose their wastes directly to rivers flow out of Addis Ababa to surrounding irrigated farmlands situated around Akaki river, encourage and show how to manage waste generated from different point and non point sources and contributes to environmental management system by finding the way to solve soil pollution problem, healing the soil using biological method up to it regain to its normal condition.

1.40 LIMITATION OF THE STUDY

This study was conducted to see the current pollution status of Aba Samuel selected irrigated agricultural farm lands by comparing with another agricultural farmland around bole subcity at Ayat agricultural farm lands where industrial waste water is unavailable for irrigation. The mushroom species used for cultivation is not the species already exists at farm lands for long period of time but collected and brought to the soil samples to culture. Due to the species used to conduct the research is edible, it used only for pollution indicator and to what extent can be the toxic heavy metals are removed using bioaccumulation to remediate the fertility of the farm land around this area. Only one species used due to lack of availability and proper information about other mushroom species. Also less economic support and time duration are the major constrains for this study.

1.50 RESEARCH QUESTIONS

1. What heavy metal pollutants are present in agricultural soil irrigated by Aba Samuel dam?
2. The heavy metals pollutants present may cause what possible effects on agriculture and health with their current concentration levels?
3. Is the selected mushroom species feasible in terms of heavy metal bioaccumulation and bio indicator?
4. Can this research output may be helpful for future work on bioaccumulation and bioremediation for polluted agricultural soil?

1.60 OBJECTIVE OF THE RESEARCH

1.60a Main Objective

To study the efficiency of selected mushroom in terms of bioaccumulation of heavy metals from selected Aba Samuel dam irrigated agricultural farm land, for the improvement of soil health and management of soil pollution due to heavy metal from non point pollution sources which may be helpful in development of low cost and environmental friendly technologies.

1.60b SPECIFIC OBJECTIVES

- ❖ To know the heavy metals concentration in polluted farm land due to Akaki river irrigation.
- ❖ To know the heavy metal bioaccumulation phenomenon and its effects on selected and easily available mushroom species.
- ❖ To know correlation between the soil properties, heavy metal and mushroom productivity parameters.
- ❖ To get the possible health effects due to high concentration of heavy metals in agricultural farm land soil.
- ❖ To study the effects of heavy metals on mushroom from bioaccumulation potential and know its role as bio indicator of heavy metal pollution.
- ❖ To know the point at which edible mushrooms are safe for consumption as food sources.
- ❖ To get idea of sustainable agriculture of the area for future and ensure sustainability by using bioaccumulation phenomenon of fungi and then use some bioremediation technology like mycoremediation.

CHAPTER TWO

2.0 REVIEW OF RELATED LITRATURE

2.10 POLLUTION STATUS OF AKAKI RIVER AND EFFECTS ON POPULATION

The major implication of the pollution of the river is associated to water related health complications manifested in the pollution of Addis Ababa and the surrounding. Currently water related diseases are the major reasons for high morbidity among the population in the region. In fact in downstream locations (Oromia regional state), where a substantial percentage of the population utilizes the river for drinking purposes, the problem is a reason for major concern (Fisseha Itanna, 1998).

In addition to the high degree of pollution, the rivers have very low dissolved oxygen that seriously impedes the survival of aquatic species. From an institutional perspective at the current period, there is conducive environment for monitoring the water quality of the Akaki Rivers. Agricultural runoff is an important source of pollutants in the river. Study of the heavy metals and nutrients composition of river reveals the anthropogenic processes such as the input of pollutants into the river system from non-point sources (e.g. urban runoff) and point sources (discharges of effluents from different industries) (AAWSA, 2002).

Several institutions are implementing mitigation measures to alleviate the degree of pollution of the rivers. A number of programs undertaken by the Addis Ababa Environmental Protection Authority, the Federal Environmental Protection Authority, Ministry of Water Resources, Oromia Water Bureau, the Addis Ababa Water and sewerage Authority, and The Awash Basin Water Resources Administration Agency are only some of the activities that are ongoing. Currently the federal EPA and the clean and Addis Ababa Society have similar initiatives on the Akaki and Kebena Rivers respectively. However, there are gaps and overlaps in the mandates and activities of some of these institutions that call for the coordination of ongoing commendable efforts (Agaje Mekonnen, 2007).

2.11 DEFINITION OF HEAVY METALS

Strictly speaking, heavy metals are defined as those with higher density than 5 mg mL⁻¹ (Jarup, 2003) but the collective term now includes arsenic, cadmium, chromium, copper, lead, nickel, molybdenum, vanadium and zinc. Some interest also exists in aluminum, cobalt, strontium and other rare metals. Physiologic roles are known for iron (haemmoeties of heamoglobin and cytochromes), copper (amine oxidases, dopamine hydrolase and collagen synthesis), manganese (superoxide dismutase), zinc (protein synthesis, stabilization of DNA and RNA) with low requirements of chromium (glucose homeostasis). Other heavy metal ions are not believed to be essential to health even in trace amounts (Malkoc *etal.*, 2011).

2.12 A PARTIAL LIST DEFINITIONS IN TERMS OF DENSITY (SG=SPECIFIC GRAVITY)

Metals fall naturally into 2 groups:

- light metals (densities < 4 g/cm³) and
- Heavy metals (densities > 7 g/cm³) (Bjerrum, 1936).

Heavy metals are:-

- ❖ Metal having a SG.4 g/cm³ (Van Nostrand, 1964).
- ❖ Metal of high SG, especially a metal having a SG of 5g/cm³ or greater (Merriam, 1976).
- ❖ Metal with a density >5 g/cm³ (Brewer, 1983).
- ❖ Metal of SG > 4 g/cm³ (Grant, 1987).
- ❖ Metal with a density of 5 g/cm³ or greater (Flexner, 1987).
- ❖ Element with a density >6 g/cm³ (Thornton, 1995).

2.13 A PARTIAL LIST DEFINITIONS IN TERMS OF ATOMIC WEIGHT (MASS)

- ❖ Metal with high atomic weight (Holister, 1976).
- ❖ Metal of atomic weight > sodium (Bennet, 1986).
- ❖ Metal of atomic weight greater than sodium (Brewer, 1983) that forms soaps on reaction with fatty acids (Lewis, 1993).

- ❖ Metallic element with an atomic weight > 40 (Duffus, 2002).

Explore **key information** about the chemical elements through this periodic table

Group	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	
Period																			
1	1 H																	2 He	
2	3 Li	4 Be											5 B	6 C	7 N	8 O	9 F	10 Ne	
3	11 Na	12 Mg											13 Al	14 Si	15 P	16 S	17 Cl	18 Ar	
4	19 K	20 Ca	21 Sc	22 Ti	23 V	24 Cr	25 Mn	26 Fe	27 Co	28 Ni	29 Cu	30 Zn	31 Ga	32 Ge	33 As	34 Se	35 Br	36 Kr	
5	37 Rb	38 Sr	39 Y	40 Zr	41 Nb	42 Mo	43 Tc	44 Ru	45 Rh	46 Pd	47 Ag	48 Cd	49 In	50 Sn	51 Sb	52 Te	53 I	54 Xe	
6	55 Cs	56 Ba	* 71 Lu	72 Hf	73 Ta	74 W	75 Re	76 Os	77 Ir	78 Pt	79 Au	80 Hg	81 Tl	82 Pb	83 Bi	84 Po	85 At	86 Rn	
7	87 Fr	88 Ra	** 103 Lr	104 Rf	105 Db	106 Sg	107 Bh	108 Hs	109 Mt	110 Ds	111 Rg	112 Cn	113 Uut	114 Uuq	115 Uup	116 Uuh	117 Uus	118 Uuo	
*Lanthanoids			* 57 La	58 Ce	59 Pr	60 Nd	61 Pm	62 Sm	63 Eu	64 Gd	65 Tb	66 Dy	67 Ho	68 Er	69 Tm	70 Yb			
**Actinoids			** 89 Ac	90 Th	91 Pa	92 U	93 Np	94 Pu	95 Am	96 Cm	97 Bk	98 Cf	99 Es	100 Fm	101 Md	102 No			

Figure 1 Periodic table to illustrate position of heavy metals.

The “periodic table” or Mendeleev’s table, organizes chemical elements according to their atomic number, electron configuration and valence numbers. This table illustrates the groups of elements. The main heavy metals are listed below, with their atomic numbers, symbols and names:

- 4 - Beryllium (Be)
- 13 - Aluminum (Al)
- 24 - Chrome (Cr)
- 25 - Manganese (Mn)
- 26 - Iron (Fe)
- 27 - Cobalt (Co)
- 28 - Nickel (Ni)
- 29 - Copper (Cu)
- 30 - Zinc (Zn)

- 33 - Arsenic (As)
- 34 - Selenium (Se)
- 42 - Molybdenum (Mo)
- 47 - Silver (Ag)
- 48 - Cadmium (Ca)
- 50 - Tin (Sn)
- 51 - Antimony (Sb)
- 56 - Barium (Ba)
- 80 - Mercury (Hg)
- 81 - Thallium (Ti)
- 82 - Lead (Plumbum, Pb)

2.14 SOURCES OF HEAVY METAL POLLUTION AND ITS EFFECTS ON SOIL, PLANTS AND VEGETABLES

2.14a SOURCES

Heavy metal pollution can arise from natural and manmade (anthropogenic) sources. Anthropogenically it often arises from metal purification processes, such as the smelting of copper and the preparation of nuclear fuels. Electroplating is the primary source of chromium and cadmium (Wong *et al.*, 2003).

Rapid and unorganized industrialization and urbanization have contributed to the elevated levels of heavy metals in the urban environment of the developing countries. Heavy metals are non-biodegradable and persistent environmental contaminants which may be deposited on the surfaces and then adsorbed into the tissues of the vegetables. Plants take up heavy metals by absorbing them from deposits on the parts of the plants exposed to the air from polluted environment as well as from contaminated soils (Sharma *et al.*, 2008).

Heavy metal pollution is one of the most serious problems of present day agriculture which negatively affects both crop yield and quality. It results by the disposal of concentrated metal wastes. When the large adverse effects of emissions of heavy metals from smelters on surrounding ecosystems were observed in the 1960s-1970s, then it was realized that how severely soil microorganisms and soil microbial processes can become

disrupted by elevated metal concentrations. Extreme metal contamination in the vicinity of smelters caused clearly visible effects such as accumulation of deep layers of organic matter on the soil surface through inhibition of the activity soil microorganisms and soil fauna (Freedman and Hutchison *et al.*, 1980).

When measures to limit the metal loading rates of soils due to the use of sewage sludge in agriculture were first introduced in many European countries during the 1970s, these limits were focused on protecting against negative effects on crop plants, on animals grazing on land to which sewage sludge had been applied. All these metal wastes have toxic potential and long term chronic effect on exposure. Monitoring of trace metals level in the soil becomes imperative, in order to prevent excessive build up of these metals in human through food chain (Yusuf *et al.*, 2003).

Appropriate measures should be put in place by the government thereby directing all industries, whose final processes involve discharges of one form or the other of waste to install plants to treat their waste effluents before their discharge into the surrounding environment, since this will control the level of trace metals in the soil. Nevertheless, the planting of food crops around industrial areas should be discouraged to avoid health hazard for consumers. In recent years there has been an increasing consumption of vegetables among the urban community. This is due to increase awareness of their nutritive value, as a result of exposure of people to proper education. Vegetables constitute an important part of the human diet since they contain carbohydrates, proteins, vitamins, minerals and fibers required for human health. They also act as neutralizing agents for acidic substances formed during digestion (Thompson and Kelly, 1990).

However, vegetables contain significance both essential elements and also toxic element that may have potential for varying degrees of contamination. Heavy metal contamination in vegetables may pose a direct threat to human health, and it is one of a range of important types of contaminants that can be found on the surface and in the tissues of fresh vegetables. The growing demand of water for irrigation has produced a marked increase in the reuse of treated and untreated waste water worldwide (Mohammed and Abdullah, 2010).

In many local urban areas, lands lying along the course of urban drainage system are used for the production of agricultural products (such as vegetable) that are in high demand by urban dwellers. Several researchers have shown that a significant proportion of a city's food requirements in developing countries are supplied from within the urban boundaries, because within those areas substantial amount of waste water (mainly from homes and industries) is available in urban drains for irrigating lands along the urban drainage course. The use of these waters therefore poses the greatest risk potential to this system of land use. Long-term use of untreated sewage water which is mainly used for the irrigation of leafy and other vegetables, has resulted in the accumulation of heavy metals in soils and their transfer to the various crops under cultivation, with levels of contamination that exceed the maximum permissible limits (Mohsen *et al.*, 2008).

Heavy metals uptake by plants grown in polluted soils has been studied to a considerable extent. Heavy metal contamination in vegetables cannot be underestimated as these food stuffs are important components of human diet. Heavy metal contamination of the food items is one of the most important aspects of food quality assurance. International and national regulations on food quality have lowered the maximum permissible levels of toxic metals in food items due to an increased awareness of the risk, these metals pose to food chain contamination (Radwan, 2006).

Heavy metals such as cadmium, lead and mercury are common air pollutants and are emitted (predominantly into the air) as a result of various industrial activities. Although the atmospheric levels are low, they contribute to the deposition and build-up in soils. Heavy metals are persistent in the environment and are subject to bioaccumulation in food-chains. Cadmium exposures are associated with kidney and bone damage. Cadmium has also been identified as a potential human carcinogen, causing lung cancer. Lead exposures have developmental and neurobehavioral effects on fetuses, infants and children and elevate blood pressure in adults (Turkekul *et al.*, 2004).

One of the consequences of the current stage of industrialization and the demand for improved quality of life has been increased exposure to air pollution coming from industrial activities, traffic and energy production (WHO, 2007).

2.14b EFFECTS ON SOIL

Other anthropogenic sources of heavy metals include the addition of manures, sewage sludge, fertilizers and pesticides which may affect the uptake of heavy metals by modifying the physico-chemical properties of the soil such as pH, organic matter and bioavailability of heavy metals in the soil found that increasing concentrations of heavy metals in soil increased the crop uptake. Cultivation areas near highways are also exposed to atmospheric pollution in the form of metal containing aerosols. These aerosols can be deposited on soil and absorbed by the vegetables or alternatively deposited on the leaves and fruits and then absorbed (Sharma *et al.*, 2008).

2.14c EFFECTS ON PLANTS AND VEGETABLES

Waste water from the industries of mining, electroplating, paint or chemical laboratories often contains high concentrations of heavy metals, including Cadmium (Cd), Copper (Cu) and lead (Pb). These elements, at concentrations exceeding the physiological demand of the plants, not only could administer toxic effect in them but also could enter food chains, get biomagnified and pose a potential threat to human health (Wittekind *et al.*, 1996). Heavy metal contamination of agricultural soils from wastewater irrigation is of serious concern since it has implications on human health. It has been established that heavy metals in soil are associated with various chemical forms that relate to their solubility which directly bear on their mobility and biological availability. Heavy metals in soluble form have high relation to their uptake by plants. Vegetables can absorb metals from soil as well as from deposits on the parts of the vegetables exposed to the air from polluted environments. In many developing countries it is a common practice to grow vegetables along banks of rivers passing through urban centres. Waters of such rivers have often been reported to be polluted by heavy metals. The extent of absorption of the elements by the plant depends on among other things, the nature of the plant, chemical constitution of the pollutant, concentration of the element in the soil, pH and the interaction with other metals (Zurera-Cosano *et al.*, 1989).

The uptake and bioaccumulation of heavy metals in vegetables is influenced by many factors such as climate, atmospheric depositions, the concentrations of heavy metals in soils, the nature of soil and the degree of maturity of the plants at the time of the harvest.

Air pollution may pose a threat to post harvest vegetables during transportation and marketing, causing elevated levels of heavy metals in the vegetables. Elevated levels of heavy metals in vegetables are reported such as long term uses of treated and untreated waste water (Singh, 1995).

2.15 TOXICITY OF HEAVY METAL AND EFFECTS ON SOIL MICROORGANISM

2.15a TOXICITY

Heavy metals are toxic because of their ionic properties. They bind to many cellular ligands and displace native essential metals from their normal binding sites. Metals also disrupt protein by binding to sulfhydryl groups and nucleic acids by binding to phosphate or hydroxyl groups. As a result, protein and DNA conformation are changed, and their function is disrupted. For example cadmium competes with cellular zinc and nonspecifically binds to DNA, inducing single strand-breaks (Alloway, 1995).

2.15b EFFECTS ON SOIL MICROORGANISMS

Toxic metals can cross membranes via diffusion or via pathways designed for other metals. For instance, Cd^{+2} transport occurs via the Mg^{+2} active transport system staphylococcus aureus. These- metal microbe interactions result in decrease microbial growth, abnormal morphological changes, and inhibition of biochemical processes in individual (Akmal *et al.*, 2005b).

2.16 HAZARDOUS EFFECTS OF HEAVY METALS ON HUMAN HEALTH

Within the European community, the 13 elements of highest concern are As, Cd, Co, Cr, Cu, Hg, Mn, Ni, Pb, Sn, and Ti, the emissions of which are regulated in waste incinerators. Some of these elements are actually necessary for humans in minute amounts (Co, Cu, Cr, Ni) while others are carcinogenic or toxic, affecting, among others, the central nervous system (Hg, Pb, As), the kidneys or liver (Hg, Pb, Cd, Cu) or skin, bones, or teeth (Ni, Cd, Cu, Cr. In medical usage, heavy metals are loosely defined and include all toxic metals irrespective of their atomic weight: "heavy metal poisoning" can possibly include excessive amounts of iron, manganese, aluminum, mercury, or beryllium (the fourth lightest element) or such a semimetal as arsenic. This definition excludes

bismuth, the heaviest of approximately stable elements, because of its low toxicity (Malkoc *et al.*, 2011).

Heavy metals are very harmful because of their non-biodegradable nature, long biological half lives and their potential to accumulate in different body parts. Most of the heavy metals are extremely toxic because of their solubility in water. Now a day's heavy metals are ubiquitous because of their excessive use in industries. Factors associated with the possible health effects of exposure to cadmium, lead and mercury have been investigated over many years in occupational settings, using experimental animals and humans exposed to environmental pollution. The types of adverse health effect are known to a great extent but, because of the very strong influence of confounding factors, it is very difficult or almost impossible to find thresholds for some outcomes such as impairment of cognitive functions in children exposed to lead or mercury. Chronic low level intakes of heavy metals have adverse effects on human beings and other animals due to the fact that there is no effective mechanism for their elimination from the body (Bahemuka and Mubofu, 1999).

Metals such as lead, mercury, cadmium and copper are cumulative poisons. These metals cause environmental hazards and are reported to be exceptionally toxic. Vegetables take up metals by absorbing them from contaminated soils, as well as from deposits on parts of the vegetables exposed to the air from polluted environments. Prolonged consumption of unsafe concentrations of heavy metals through foodstuffs may lead to the chronic accumulation of heavy metals in the kidney and liver of humans causing disruption of numerous biochemical processes, leading to cardiovascular, nervous, kidney and bone diseases (WHO, 1992). Some heavy metals such as Cu, Zn, Mn, Co and Mo act as micronutrients for the growth of animals and human beings when present in trace quantities, whereas others such as Cd, As and Cr acts as carcinogens (Freig *et al.*, 1994).

Food is the main source of cadmium exposure in the general population (representing >90% of the total intake in non-smokers). In heavily contaminated areas, dust resuspension can constitute a substantial part of the crop contamination and exposures via inhalation and digestion. Cadmium is accumulating in soils and catchments under certain environmental conditions, thus increasing the risk of future exposure through food. The

intake of heavy metal can lead to altering of humans and animals healthiness state. Thus, the carcinogenic effects generated by continuous consumption of fruits and vegetables loaded with heavy metals such as Cd, Pb or even Cu and Zn are known. This may be related to the incidence of gastrointestinal cancer and cancer of the pancreas, urinary bladder or prostate (Waalkes and Rehm, 1994).

The sad thing about the pollution of the environment with heavy metals is that they can only be transformed from one oxidation state or organic complex to another. Once the environment becomes polluted with Zinc, it begins its journey to man's body by readily absorbed by plants which are subsequently consumed by man. The transfer of cadmium from soil to the food-chain depends on a number of factors, such as the type of plant, the type and pH of the soil and the zinc and organic matter content in the soil (Ellen *et al.*, 1990). Cadmium adsorption to soil particles is greater in neutral or alkaline soils than in acidic ones and this leads to increased cadmium levels in the solution. Knowledge of Zn toxicity in humans is minimal. The most important information reported is its interference with Cu metabolism. The symptoms that an acute oral Zn dose may provoke include: Tachycardia, vascular shock, dyspeptic nausea, vomiting, diarrhea, pancreatitis and damage of hepatic parenchyma (Salgueiro *et al.*, 2000).

Lead is a toxic element that can be harmful to plants, although plants usually show ability to accumulate large amounts of lead without visible changes in their appearance or yield. Lead is a well-known neurotoxin. Impairment of neurodevelopment in children is the most critical effect. Exposure in utero, during breastfeeding and in early childhood may all be responsible for the effects. Lead accumulates in the skeleton and its mobilization from bones during pregnancy and lactation causes exposure to fetuses and breastfed infants. In many plants, Pb accumulation can exceed several hundred times the threshold of maximum level permissible for human. The introduction of Pb into the food chain may affect human health and thus, studies concerning Pb accumulation in vegetables have increasing importance (Coulter, 1992).

Exposure to potentially toxic metals from dust inhalation or soil ingestion is usually modeled simply as the concentration of a contaminant measured in the soil multiplied by the quantity of dust inhaled or soil ingested. This is a conservative approach to estimate

dose because the bio-accessibility of heavy metals adsorbed on ingested soil is not 100%. However, predicting exposure to potentially toxic metals from consumption of food crops is more complicated because uptake of metals by plants depends on soil properties and plant physiologic factors. This leads to much larger uncertainties associated with estimating potential doses through food chains compared to the uncertainties associated with other exposure pathways such as soil ingestion and dust inhalation. Heavy metals are individual metals and metal compounds that can impact human health. They are all naturally occurring substances which are often present in the environment at low levels. In larger amounts, they can be dangerous. Generally, humans are exposed to these metals by ingestion (drinking or eating) or inhalation (breathing). Working in or living near an industrial site which utilizes these metals and their compounds increases ones risk of exposure, as does living near a site where these metals have been improperly disposed. Subsistence lifestyles can also impose higher risks of exposure and health impacts because of hunting and gathering activities (McKone, 1994).

2.17 MUSHROOM AND ITS ROLE AS NUTRITIONAL VALUE

2.17a MUSHROOM

Mushroom is a fleshy, spore bearing fruiting body, a fungus, typically produced above ground on soil or on its food source. Mushroom is most often applied to fungi (Basidiomycota, Agaricomycetes, order Boletales and family Boletaceae) that have stem (stipe), a cap (Pileus) and gills (Lamellae) on the other side of the cap. Mushrooms such as the *Pleurotus* species are known to be among the largest fungi or saprophytic eukaryotes composed of hyphae filament that thrives very well in damp or moist condition (Mirko, 1985).

Mushrooms are a special group of fungi which are saprophytic in their life patterns. They lack chlorophyll and consequently cannot undergo photosynthesis. They feed on organic matter; by producing a wide range of enzymes that can breakdown complex substances after which they are able to absorb the soluble substances so formed. Mushrooms are found in all sorts of environments. They are technically confined to members of a family of fungi with gills, but in popular usage any of the larger fleshy or woody fungi. Of the thousands of species of mushrooms known throughout the world, the great

majority are tough, woody, bitter, tasteless, or of such rare occurrence that they are of no interest as food (Stamets, 2000). The mushroom species usually grown commercially attains a fleshy cap. In the young mushroom the margin of the cap is jointed to the stem by a membranous collar, which breaks at maturity to expose the gills on the undersurface of the cap. With age, the entire fruiting body changes to dark brown. Mushrooms are cultivated commercially in caves, dark cellars, or specially constructed mushroom houses in which the proper humidity and temperature are maintained. They are grown in beds consisting of a mixture of rotted manure and chemically treated straw, over which a layer of soil (casing soil) is spread. The vegetative portion of the fungus, known as the mycelium, or spawn, is used for planting, or spawning, the beds. The mycelium is grown in pure culture under laboratory conditions, thus ensuring freedom from insect and fungus contaminants. In a few weeks the spawn invests the entire bed, and the mushroom fruiting bodies, or sporophores, begin to appear (Chang and Miles, 1984).

2.17b ROLE OF MUSHROOM AS NUTRITIONAL VALUE

Mushroom cultivation serves as the most efficient and economically viable biotechnology for the conversion of lignocellulose waste materials into high quality protein food. Mushrooms are found in areas with range of temperatures 20-40⁰C and grow well in agricultural wastes. They require a moderate rainfall and pH range of 3-10 for growth (Chang, 1980 and Fernandex, 1991).

Pleurotus tuber-regium is highly nutritive and very rich in protein and also eaten for its flavour and beneficial medicinal effect. The protein content of edible mushrooms is equal to that of corn, milk and legumes. Mushrooms have been food supplement in various cultures and they are cultivated and eaten for their edibility and delicacy considered as sources of proteins, vitamins, fats, carbohydrates, amino acids and minerals. Mushrooms have been considered healthy food because they contain high quality protein which contains all the essential amino acids, vitamins B, B₂, C and D and minerals such as A, K, Zn, Na, Fe, Mg, P and low fat (Bano, 1993).

2.18 KINDS OF FUNGI

Fungi can be broken down into 2 subgroups that pertain to bioremediation: brown rotters and white rotters. White rot fungi are excellent mycoremediators of toxins held together by hydrogen-carbon bonds. Enzymes secreted by white rotters include lignin peroxidases, manganese peroxidases, and laccases. Extra-cellular lignin modifying enzymes have very low substrate specificity so they are able to mineralize a wide range of highly recalcitrant organopollutants that are similar in structure to lignin. The enzymes of the lignin degradation system of white-rot fungi are extracellular, thus negating the need to internalize the pollutants and enabling the fungi to tolerate a high concentration of pollutants. White rot-fungi cannot utilize lignin as a source of energy for growth and instead require cosubstrates as a carbon source. They grow by hyphal extension and thus can reach pollutants in the soil in ways that other organisms cannot (Hamman, 2004).

2.19 OYSTER MUSHROOM

Mushroom cultivation is a worldwide practice, which utilizes almost all agricultural and agro industrial residues as substrate. Composted organic material such as plant debris and animal manure add nutrient to the soil thereby increasing the soil fertility. Poultry manure as an organic material is particularly important since it conditions and improves soil fertility and contains all macro-nutrients and most of the micro-nutrients. Mushroom cultivation serves as the most efficient and economically viable biotechnology for the conversion of lingo cellulose waste materials into high quality protein food and this will naturally open up new job opportunities especially in rural areas Mushrooms such as the *Pleurotus species* are known to be among the largest fungi or saprophytic eukaryotes composed of hyphae filament that thrives very well in damp or moist condition and reported that they are highly nutritive and very rich in protein and also eaten for its flavor and beneficial medicinal effect (Mirko, 1985).

Oyster mushroom (*Pleurotus ostreatus*) is economically highly praised for its nutritional and pharmacological value. Its fruit bodies are rich in carbohydrate, dietary fiber, proteins, essential amino acids, vitamins and minerals. Biologically active substances, especially polysaccharides and lectins, isolated from *Pleurotus ostreatus* have hypocholesterolemic, antitumor, immunomodulating, antiviral and antibacterial effects.

Their mycelia also contain amino acids like glycine, valine, threonine, serine, leucine, proline, methionine, asparagine, glutamine, lysine, arginine, histidine, cysteine and alanine (Kadiri, 1990).

Many investigations have dealt with the metal contents of mushrooms, especially edible ones. Heavy metal concentrations in mushroom are considerably higher than those in agricultural crop plants, vegetables and fruit. This led to suggestion that mushrooms possess a very effective mechanism that enables them to readily take up some heavy metals from the ecosystem. By assess the bioaccumulation of some heavy metal (Cd, Zn, Ni, Cr, and Cu) planted on metal contaminated soil, we can evaluate the level of environmental pollution and to remediate the metal polluted soil. Many studies carried out to evaluate the possible danger to human health from the ingestion of mushrooms containing heavy metals. Numerous data on metals contents in fungal fruiting bodies were published previously and the reported metal concentrations vary over a wide range within the mushrooms species, because of many factors affecting the accumulation rate (Mansur, 2003). The metals are distributed unevenly within the fruiting body, the highest concentrations have been observed in the spore-forming part, but not in the spore, a lower content in the rest of the cap and the lowest level in the stipe. Also, high level of metals concentration was observed in the vicinity of metals polluted area and metals smelter. Density and depth of the mycelium living in the soil for several months or even years influence the metals contents in the fruiting bodies. Also, the soil properties, such as pH, redox potential, organic matter content, clay mineralogy, cation exchange capacity of the soil phase, competition with other metal ions and composition of the soil solution concentrations influence metals absorption of the mushrooms. Because macro fungi are integral part of the forest ecosystems, sometimes the soil-to mycelium transfer of metals depends on relationship between mycelium and symbiotic plants species affecting element absorption and translocation (Melgar, 2003).

2.20 BIOACCUMULATION AND BIOMAGNIFICATION OF HEAVY METALS

2.21 DEFINITION OF BIOACCUMULATION

Bioaccumulation is the accumulation of a substance in a biological tissue. Organisms at any trophic level may be capable of bioaccumulation. Conservative pollutants can enter living organisms at any time and accumulate in their tissues. Such organisms are able to

build up very high levels of such pollutants e.g. oysters can concentrate DDT from 0.001 ppm in sea-water to 700ppm in their bodies. This is known as bioaccumulation (Garcia, 1998).

2.22 FACTORS AFFECTING BIOACCUMULATION

1. The concentration of pollutants in the water.
2. The water temperature - if the metabolism of the organism increases so too many its rate of uptake.
3. The age and type of an organism

2.23 SAFE LEVEL OF HEAVY METALS IN MUSHROOM (*Pleurotus ostreatus*)

2.23a CADMIUM

Cadmium might have found its way into the mushrooms as impurity in several products, including phosphate fertilizers, insecticides, fungicides, detergents and refined petroleum products introduced into the environment in which the mushrooms grows. Cadmium is also a natural element in the earth's crust. It is usually found as a mineral combined with other elements such as oxygen (cadmium oxide), chlorine (cadmium chloride), or sulphur (cadmium sulphate, cadmium sulphide). Cadmium is highly toxic element, its presence at elevated levels in soil and drinking water and it is a threat to food safety and human health. They affect biological activities and have detrimental effect on digestive, respiratory and immune system. It is known as a principal toxic metal, since excessive cadmium exposure may give rise to renal, pulmonary, hepatic, skeletal, reproductive effects, and cancer. It was reported that cadmium is accumulated mainly in kidneys, spleen, and liver, and its blood serum level increases considerably following mushroom consumption (Kalac and Svoboda, 2005).

Thus, cadmium seems to be the most deleterious among heavy metals in mushrooms. The FAO maximum permitted level for cadmium in cultivated mushrooms is 0.2 mg/kg (FAO, 2001).

2.23b CUPPER

The FAO maximum permitted level for copper in cultivated mushrooms is 73.3 mg (FAO, 2001). Copper is the third-most abundant trace element in human body, with vitamin-like impact on living systems. Small amount of copper is found in the human body (50–120 mg), but it plays a critical role in a variety of biochemical processes.

Copper forms part of at least 13 different enzymes, and its presence is needed for each if they are to function properly. It is known that copper may be toxic to both humans and animals when its concentration exceeds the safe limits (Gast *et al.*, 1988).

2.23c LEAD

Pb finds its way into the environment as constituent of pesticides and industrial waste release into the environment, such as used car batteries, alloys, solder, broken ceramics and plastics. Another source is the exhaust fumes of car using leaded fuel, though that threat is now greatly reduced as most cars use unleaded fuel. Target organs where it can be accumulate in the body are the bones, brain, blood, kidneys, and thyroid gland. Lead has no benefit to human metabolism. Gradual accumulation can lead to lead poisoning. This may lead to high blood pressure, muscular weakness, and headaches among others. The FAO maximum permitted level for lead in cultivated mushrooms is 0.3 mg/kg (FAO, 2001).

2.23d CHROMIUM

Trivalent chromium is a trace metal necessary for the normal metabolism of cholesterol, fat, and glucose. Chromium deficiencies in the diet produce elevated circulating insulin concentrations, hyperglycemia, elevated body fat, decreased sperm counts, reduced fertility, and shortened life span. The FAO maximum permitted level for chromium in cultivated mushrooms is 2.3 mg/kg (FAO, 2001).

2.23e NICKEL

Trace amounts of nickel may be beneficial as an activator of some enzyme systems, but its toxicity at higher levels is more prominent. It accumulates in the lungs and may cause bronchial hemorrhage or collapse (Demirbas, 2001).

The FAO maximum permitted level for Nickel in cultivated mushrooms is 67.9 mg/kg (FAO, 2001).

2.23f ZINC

Zinc is one of the most important mineral our body needs due to the fact that it is highly associated with protein and carbohydrate foods. Zinc is also used in medicines that treat rashes, acne, dandruff and athlete's foot. High concentration of zinc is widely used throughout the world in medicines, foods and in industries for preventing corrosion. It is an integral component of a wide variety of different enzymes in which it plays catalytic,

structural, and regulatory roles. Zinc deficiency which can result from inadequate dietary intake, impaired absorption, excessive excretion or inherited defects in zinc metabolism. The deficiency of zinc particularly in children can lead to loss of appetite, growth retardation, weakness, low spirited, stagnation in sexual growth. The FAO maximum permitted level for Zinc in cultivated mushrooms is 99.4 mg/kg (FAO, 2001).

2.24 DEFINITION OF BIOMAGNIFICATION

Biomagnification is the increasing concentration of a substance up a food chain i.e. from one trophic level to the next. Conservative pollutants are not metabolized (broken down inside an organism) and therefore when an organism containing a pollutant is eaten, the pollutants are simply passed on to the predator and accumulate in its tissues. By consuming much prey an organism may build up very high concentrations of the pollutant in its tissues. This process may continue up the food chain, leaving the top predator with very high and sometimes lethal concentrations of the pollutant (Wasser, 2003).

2.25 NUTRIENT CONTENTS OF MUSHROOM

The mineral nutrient content in mushrooms is very moderate (Fasidi, 1991) reported that potassium and phosphate are the major mineral elements in *Pleurotus species*. The results show that the cultivated mushrooms from waste water irrigated soil were rich in Calcium, Nitrate and Phosphate and were found to contain reasonable levels of Potassium and iron. This is in agreement with results of the study of some cultivated mushrooms (*Agaricus bisporus* and *Pleurotus ostreatus*) (Edeoga and Gomina, 2000).

2.25a NITRATE

Nitrate and nitrite ions are ubiquitous in the environment and occur naturally in plant foods as a part of the nitrogen cycle. Nitrate levels may vary significantly in fruit and vegetables dependent on a number of biotic and abiotic factors. Conversely, nitrite levels are generally relatively low in fresh undamaged vegetables but may increase in some nitrate rich vegetables after harvesting, particularly if stored at room temperature. Historically, there have been two main safety concerns around the presence of nitrate and nitrite in the diet. Those relate to the reaction of nitrite with hemoglobin to form methaemoglobin which can reduce oxygen transport in the blood, and a theoretical possibility of the potential for carcinogenicity through the formation of N-nitroso

compounds in foods or in humans. Significant concentrations of nitrate are found naturally in various fruits and vegetables. It has long been established that these levels are dependent upon a number of factors including; the use of fertilizers, location and soil type, carbon dioxide concentrations (in green house vegetables), seasonal light intensity and duration of light exposure and water availability (Maynard *et al.*, 1976).

Nitrate concentrations in vegetables may also vary up to orders of magnitude dependent on the vegetable species and the part of the plant sampled. High concentrations of nitrate tend to accumulate in the leaves, roots, petioles or stems of certain plants meaning that leafy vegetables including lettuce or spinach, and root crops such as beetroot, may accumulate high concentrations of nitrate. In contrast, levels of nitrate in vegetables such as carrots or onions are likely to be lower. Nitrite concentrations generally tend to be low in fresh undamaged vegetables; however levels can increase rapidly in certain nitrate rich vegetables, particularly if pureed and stored at room temperature. In addition to temperature, this increase is dependent upon nitrate reductase activity in the plant and the level of bacterial contamination. The World Health Organisation (WHO) and Australian drinking water guideline levels are 50 mg/L for nitrate (as NO_3^-) and 3mg/L for nitrite (as NO^-2) (NHMRC, 2004).

The guideline values are established to protect young infants from methaemoglobin formation, however the guideline advises that water with a nitrate concentration of up to 100 mg-nitrate/L can be used by adults and children over 3 months of age without risk of significant health effects. The WHO has also set a provisional guideline level for nitrite in drinking water of 0.2 mg/L for long term exposure (WHO, 2008).

2.25b PHOSPHATE

Phosphate is a mineral found in many foods. When you have kidney disease, phosphate may build up in your blood. This can weaken your bones over time. Phosphate works with calcium to build bone. Your body works best when these minerals are in balance. When calcium and phosphorus are out of balance you may run the risk of bone tissue forming in your heart, liver, and blood vessels. Some common symptoms of high phosphorus include bone & joint pain, weakness, itching, bone weakness and red eyes. Among the new trends in phosphorous compound technology, which are directed towards the production of fertilizing products, studies on urea phosphate have recently been

conducted. The previous study has shown that urea phosphate is potentially characterized by significant advantages as a fertilizer that would be an extremely important source of non protein nitrogen. The most recent studies have demonstrated that urea phosphate contained in the manure from the poultry also showed a strong fungicidal effect (Grata, 2008).

2.25c POTASSIUM

Mushrooms are good source of potassium. Potassium is an important electrolyte in the body and is the major cation within cells. It lessens the effect of salt on blood pressure. However, people with kidney disease should be aware that mushrooms are high in potassium, as they may need to limit their potassium intake. Potassium is a nutrient that is essential for health at the most basic level it keeps the body's cells functioning properly. Along with sodium and other compounds, potassium is an electrolyte, working to regulate the balance of body fluids. These actions affect nerve signaling, muscle contraction, and the tone of blood vessels, with far-reaching impacts on the body, including the cardiovascular system. Potassium also plays an important role to mental function as well as to physical processes. It helps to promote efficient cognitive functioning by playing a significant role in getting oxygen to the brain (Samuel *et al.*, 2010).

2.25d IRON

The EU maximum permitted level for iron in cultivated mushrooms is 0.3 mg/kg wet weight (European Commission, 2001). Iron had the highest concentration of all the heavy metals analyzed. Iron is an essential trace element for humans due to its necessity in haem proteins such as hemoglobin, myoglobin and cytochromes. Iron deficiency is common and leads to iron-deficiency, anemia. The Recommended Daily Intake of iron is 15mg/day. Consumption of these mushrooms will therefore serve as a very good source of iron supplementation particularly in low income countries where iron deficiency anemia is a serious health challenge. Reported values for iron in mushrooms are usually high. This may be due to its abundance on earth (Nuorteva *et. al.*, 1986).

2.25e CALCIUM

Calcium is of prime importance in determining the fruit nutritional value. In the tissue of many fruits, calcium is one of the mineral believed to be important factor governing fruit storage equality. It has been reported to delay ripening and senescence and to reduce storage disorder. The importance of calcium as a mineral to human health is well known. Required amount of this element must be in human diet to pursue good healthy life (San, 2009).

2.26 EFFECTS OF ENVIRONMENTAL POLLUTION ON THE METAL CONTENTS IN FRUITING BODIES OF MUSHROOM

Surprising accumulation ability in several mushroom species promoted their screening as bio-indicators. No mushroom species could be considered as a believable indicator of environmental pollution with heavy metals. However, fruiting bodies can be useful to distinguish between polluted and unpolluted areas (Wondratschek, and Roder, 1993).

High contents of the metals have been observed in mushrooms growing in heavily polluted areas, such as in close proximity to highways with heavy traffic ,landfills of sewage sludge and emission areas, including cities. Extremely high metal levels were determined in the vicinity of metal smelters. (Gast, 1988).

2.27 FACTORS AFFECTING THE HEAVY METALS CONCENTRATION IN FRUITING BODIES OF MUSHROOM

Fruiting body is visible part of a mushroom; fructification is formation of fruiting body. Fruiting body consists of cap (pileus) with spores-forming part (sporophore) and stipe (stem, stalk). Mushroom uptakes nutrients from a substrate via spacious mycelium. Contents of the metals are primarily species-dependent, while role of a genus or a family is of lower importance, as is nutritional strategy – mycorrhizal, parasitic or saprophytic. Substrate composition is an important factor; however, great differences exist in uptake of individual metals (Akmal, 2005a).

Mercury and cadmium are accumulated in fruiting bodies, while lead contents are lower in the fruiting body than in the substrate. Age and size of the fruiting body are of less importance. The proportion of the metal contents originating from atmospheric

depositions seems to be also of less importance due to the short lifetime of a fruiting body, which is usually 10-14 days. Metal contents in fruiting bodies are considerably affected by the age of mycelium and by the interval between the fructifications (Stamets, 2005).

Maximum metal contents are observed in the initial harvest wave of cultivated white mushroom (*Pleurotus ostreatus*). Probable explanation is not only in different substrate composition and contamination, but also in different age of mycelium, which may exist for several years in nature, while only for several months in a cultivation plant. Combination of all these factors causes very wide variability of the metal contents within a species, usually to one order of magnitude. Thus, ranges of the metal levels are remarkably wider than in plant materials. Contents have been usually expressed as mg kg^{-1} dry matter. There exists a consensus for recalculation to fresh matter that dry matter content of mushrooms is 10 %. For calculations, usually 300 g of fresh mushrooms per meal, is assumed. The metals are distributed unevenly within a fruiting body. Mercury transport is likely to be affected by sulphhydryl group content in a protein carrier, while cadmium transport has another mechanism (Akmal, 2005b).

2.28 CURRENT TECHNOLOGIES USED TO DECREASE DIETARY TOXICITY OF HEAVY METALS

2.29 BIOREMEDIATION

Bioremediation can be defined as any process that uses micro-organism, fungi, green plants or their enzymes to return the natural environment altered by contaminants to its original condition because of some micro-organism-based remediation techniques, such as bioremediation, show potential for their ability to degrade and detoxify certain contaminants. Although these biological systems are less amenable to environmental extremes than other traditional methods, they have the perceived advantage of being more cost-effective. Bioremediation technologies can be generally classified as in situ or ex situ. In situ bioremediation involves treating the contaminated material at the site while ex situ involves the removal of the contaminated material to be treated elsewhere (Kuforiji, 2008).

Some examples of bioremediation technologies are bio-venting, bioreactor, composting, bio-augmentation, rhizofiltration and bio-stimulation. Not all contaminants, however, are easily treated by bioremediation using microorganisms. For example, heavy metals such as cadmium and lead are not readily absorbed or captured by organisms. The assimilation of metals such as mercury into the food chain may worsen matters. Phytoremediation is useful in these circumstances because natural plants or transgenic plants are able to bioaccumulate these toxins in their above-ground parts which are then harvested for removal (Meagher, 2000).

Phytoremediation refers to the natural ability of certain plants called hyper accumulators to bioaccumulate, degrade or render harmless contaminants in soils, water or air. Phytoremediation is considered a clean, cost-effective and non-environmentally disruptive technology, to remove heavy metals from polluted lands. Green plants were proposed for in situ soil phytoremediation which has become an attractive topic of research and development. However, one major disadvantage of phytoremediation is that it requires a long-term commitment as the process is dependent on plant growth, tolerance to toxicity and bioaccumulation capacity. Biological approaches based on industrial and environmental biotechnology is focusing on the development of "clean technologies" which emphasizes on the maximum production, reduced waste generation, treatment and conversion of waste in some useful form (Kirk, 1992). Further, these clean technologies focus on the use of biological methods for the remediation of waste. One such biological method is mycoremediation which is based on the use of fungi and mushroom for the removal of waste from the environment. The mushrooms and other fungi possess enzymatic machinery for the degradation of waste or pollutants and therefore, can be applied for a wide variety of pollutants (Purnomo *et al.*, 2013).

Mushroom (*Pleurotus ostreatus*) has been used for consumption as product for a long time due to their flavor and richness in protein. They are also known as mycoremediation tool because of their use in remediation of different types of pollutants. Mycoremediation relies on the efficient enzymes, produced by mushroom, for the degradation of various types of substrate and pollutants. Besides waste degradation, mushroom produced a vendible product for consumption. However, sometimes they absorb the pollutant in their

mycelium (biosorption process) and cannot be consumed due to absorbed toxicants (Isildak, 2004). Edible mushrooms are highly nutritious and can be compared with eggs, milk and meat. Mushroom is a protein rich food and has been considered as the source of single cell protein. These are easily digestible and possess a high amount of amino acids but lacks cholesterol. These possess high quantities of fibers, few sugars and low calories and a high quantity of the amino acids such phenylalanine, threonine and tyrosine. As far as the nutrient profile of mushroom are concerned, these are influenced by many factors including the type of substrate on which these are cultivated. There are some differences in the nutrient content of the mushroom cultivated on different substrates (Mabrouk and Ahwany, 2008).

2.30 MYCOREMEDIATION POTENTIAL OF MUSHROOM

Mushroom (*Pleurotus ostreatus*) uses different methods to decontaminate polluted spots and stimulate the environment. These methods include –

- ❖ Biodegradation
- ❖ Biosorption
- ❖ Bioconversion

2.30a BIODEGRADATION

The term ‘Biodegradation’ is used to describe the ultimate degradation and recycling of complex molecule to its mineral constituents. It is the process which leads to complete mineralization of the starting compound to simpler ones like CO₂, H₂O, NO and other inorganic compounds by living organisms. Mushroom can produce extracellular peroxidases, ligninase (lignin peroxidase, manganese dependent peroxidases and laccase), cellulases, pectinases, xylanases and oxidases. (Nyanhongo *et al.* 2007).

These are able to oxidize recalcitrant pollutants in vitro. These enzymes are typically induced by their substrates. These enzymes have also been found to degrade non polymeric, recalcitrant pollutants such as nitrotoluenes, PAHs, organic and synthetic dyes, and pentachlorophenol under in vitro conditions. Recently, it is reported that mushroom species are able to degrade polymers such as plastics (VanAcken *et al.*, 1999). The biodegradation mechanism is very complex. The reason is the influence of other

biochemical systems and interactions of ligninolytic enzymes with cytochrome P₄₅₀ monooxygenase system, hydroxyl radicals and the level of H₂O₂ which are produced by the mushroom (Da Luz *et al.*, 2013).

2.30b BIOSORPTION

The second important process of removal of metals or pollutants from the environment by mushroom is biosorption. Biosorption is considered as an alternative to the remediation of industrial effluents as well as the recovery of metals present in effluent. Biosorption is a process based on the sorption of metallic ions (pollutants) and xenobiotics from effluent by live or dried biomass which often exhibits a marked tolerance towards metals and other adverse conditions (Gavrilescu, 2004). Biosorbents can be prepared from mushroom mycelium and spent mushroom compost. The uptake of pollutants or xenobiotics by mushrooms involves a combination of two processes: (i) bioaccumulation i.e. active metabolism-dependent processes, which includes both transport into the cell and partitioning into intracellular components; and (ii) biosorption i.e. the binding of pollutants to the biomass without requiring metabolic energy. Several chemical processes may be involved in biosorption, including adsorption, ion exchange processes and covalent binding. The polar groups of proteins, amino acids, lipids and structural polysaccharides (chitin, chitosan, and glucans) may be involved in the process of biosorption. It is reported that the biosorption capacity of dead biomass may be greater, similar to or less than that of living cells (Mar'in *et al.*, 1997). In the case of biosorption, dead biomass of mushroom offers certain advantages over living cells. Dead mushroom biomass can be obtained from industries as a waste of fermentation processes. Further, this is not sensitive to concentrations of toxicants and their toxicity effects and adverse operating conditions (pH, temperature, nutrient supply, initial metal ion concentration, and the concentration of cells etc.) unlike living mushroom biomass. The uptake of xenobiotic by living cells depends on fungal species and contact time. Biosorption techniques are now becoming very popular for the removal of pollutants. Biosorption is an effective method due to the high uptake capacity and very cost-effective source of the raw material (Heinfling *et al.* 1998).

2.30c BIOCONVERSION

Nowadays, the research on conversion of industrial or agro-industrial sludges into some other useful forms is going on. The most important bioconversion product is mushroom. Any lignocellulosic waste, generated by industries, can be used for cultivation of mushroom which can be further use as a product. Mushroom cultivation has also been successfully done on various industrial wastes. Applications of mushroom as mycoremediation tool in the bioconversion of these industrial wastes into protein rich mushroom carpophores (fruiting bodies of mushroom), on one hand provides mushroom and on the other hand helps in solving pollution problems, which their disposal may otherwise cause (Singhal *et al.*, 2005).

Fungi can remove heavy metals by channeling them into their fruit bodies. Mushroom spawn can create a network, or living "filter", on a wide range of substrates. Miles of mycelia per cubic inch of media, the cellular walls of fungi sweat a mixture of enzymatic molecules that are programmed to adapt. Fungi produce powerful enzymes that are capable of breaking down complex, toxic molecules and pathogenic organisms (Kulshreshtha *et al.*, 2011).

They grow on logs (lignicolous), animal dung (coprophylus), agricultural wastes – sawmill dust burnt grounds (phyrophylous), lawns etc. This is why they are good recyclers. Fungi are opportunistic and respond quickly to environmental disasters (Hamman, 2004).

Mushroom Mountain's spent growing media is at the perfect stage to introduce into contaminated environments. The enzymes are at their apex of production and bioremediation potential. Fungal systems can be hybridized with traditional vegetative or bacterial remediation systems to produce an ideal model for different applications. Vegetation that has hyper accumulated metals or toxins can also be removed from a contaminated system, dried, and remediated with fungi. Complex hydrocarbons and chains of toxic molecules are degraded into forms that are easily digestible for the environment, thereby improving air and water quality for future generations to enjoy. Species sequencing can also be applied to degrade a complex of contaminated media by phasing the process using fungi with unique enzymatic properties. Hence,

mycoremediation is a process of using fungi to return an environment (usually soil) contaminated by pollutants to a less contaminated state. It means using various strains of fungi to clean as radio nuclide. One of the primary roles of fungi in the ecosystem is decomposition, which is performed by the mycelium. The mycelium secretes extracellular enzymes and acids that breakdown lignin and cellulose, the two main building blocks of plant fiber (Akinyele *et al.*, 2011).

The key to mycoremediation is determining the right fungi species to target a specific pollutant. Generally, mycoremediation is the use of fungi to degrade pollutants from the environment. This practice involves mixing mushroom mycelium into contaminated soil, or placing mycelial mats over toxic sites. It is an economically and environmentally sound alternative to extracting, transporting and storing toxic waste. It restores value to depleted land. The current policy concerning toxic waste removal or clean up prescribes burning, and hauling or burying the waste. The results of these processes do not get rid of the waste or restore the ecology, but cripple it and leave it lifeless. Toxins in our food chain (including mercury, PCB's, and dioxins) become more concentrated at each step, with those at the top being contaminated by ingesting toxins consumed by those lower on the food chain. Mycelia can destroy these toxins in the soil before they enter our food supply. Enzymes are secreted by certain fungi that digest lignin and cellulose, the primary building blocks of wood. Thus fungi have the innate capability to breakdown molecules, disassembling long-chained toxins into simpler less toxic chemicals (Hattaka, 1994).

2.31 INCINERATION AND WASTE DISPOSAL

Unlike organic pollutants, heavy metals do not decay and thus pose a different kind of challenge for remediation. Through precipitation of their compounds or by ion exchange into soils and mud, heavy metal pollutants can localize and lay dormant. Currently, plants or microorganisms are tentatively used to remove some heavy metals such as mercury. Plants which exhibit hyper accumulation can be used to remove heavy metals from soils by concentrating them in their bio matter. Some treatment of mining tailings has occurred where the vegetation is then incinerated to recover the heavy metals (Duffus, 2002).

Once incinerated, the overall fate of a piece of metal depends largely on its physical and chemical properties. With respect to transfer either into the bottom ash or into the fly ash, one can distinguish four groups of metals: Iron, copper, nickel and chromium are transferred almost entirely to the bottom ash. Since these metals are prevalent in the waste in their native form, the stoking action of the grate will cause a downward segregation from the surrounding waste because of their high density. Zinc and lead are transferred to more or less equal fractions into the bottom ash and the fly ash. Most of the “native” zinc that ends up in the bottom ash is present in brass, which has a higher melting temperature than zinc itself. Much of the lead is present not as native metal but in chemical compounds such as lead crystal. Since zinc and lead melt and they engage in thermo chemical corrosion reactions, for example by chloride formation, and are hence transferred into the filter ash. Cadmium is transferred to the flue gas and either ends up in the fly ash. However some treatments are required in order to reduce their content of ferrous and non ferrous metals as well as to prevent the negative environmental impacts that a direct reuse of the raw bottom ash can cause due to the high content of heavy metal and polluting agents (Sabbas *et al.* 2003).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.10 DESCRIPTION OF THE STUDY AREA

Present study was conducted at Akaki river irrigated agricultural farm land nearby Aba Samuel dam at Dukem woreda, Leyu zone, Oromia regional state in Ethiopia. For control farm land soil, the sample was taken from Bole subcity, Ayat agricultural farm land; Addis Ababa, Ethiopia. The Akaki river catchment is located in the Western Ethiopian highlands of the Shewan plateau, and partly in the western margin of the Ethiopian Rift valley floor. The capital city, Addis Ababa and other small towns such as Akaki, Sendafa, Burayu and smaller peasant association villages are found in this catchment. The Akaki River catchment is an extensive drainage system located at the eastern edge of the Western Ethiopian plateau that slowly descends to the Main Ethiopian Rift valley. The study area, the Akaki River catchment, has an approximate surface area of around 1462 km² and boundary length of 216 km, and approximately bounded between the geographic coordinates 8⁰ 45'20'' to 9⁰ 13'17'' N latitude and 38⁰ 34' 3'' to 39⁰ 4'10'' E longitude. As Addis Ababa is found in the northern central heart of the catchment, the study area is identified at the point where little and big Akaki Rivers are joined. The location map of the study area is shown in Fig 2.

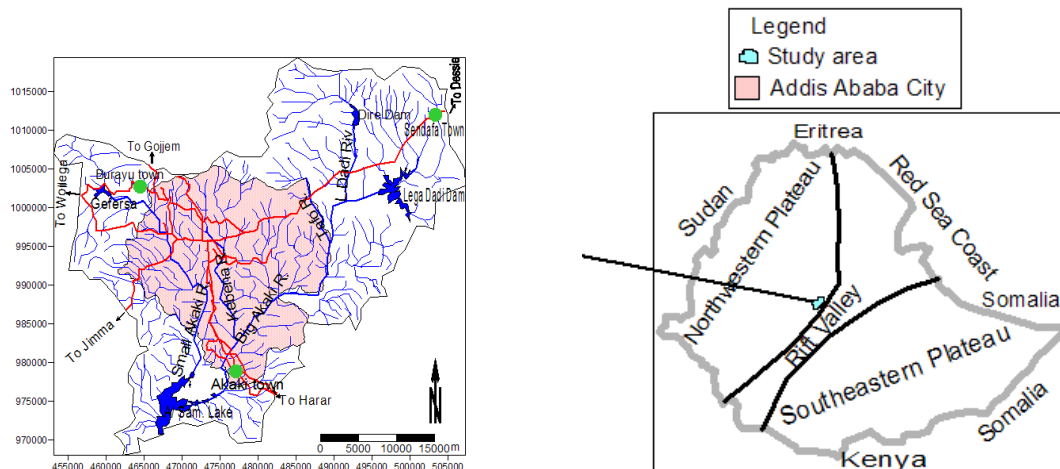


Figure 2 Map of the study area and sampling site.

The drainage system Akaki River catchment comprises of numerous small rivers. The dominant ones are the Big Akaki, which drains the eastern part of the catchment area, and the little Akaki that drains the western part of the catchment; and their respective tributaries. The two rivers form one of the biggest tributaries of the Awash River called Akaki River that enters Abba Samuel Lake, leaves the lake and passes through a gorge up to 100m deep which extends for about 8km before it joins the Awash River. Almost all the streams in the catchment originate from the northern part of the catchment. The Big Akaki suffers a total drop of about 600m in a river length of 95km from its origin to its confluence with the Awash River near Dodota, 1800m above sea level (AAWSA, 2000).

The highest and lowest mean maximum temperature over the record periods is 25⁰c in dry season (March) and 20⁰c in wet season (August), while the variation of mean monthly temperature values fall in the range of 7⁰c (December) to 12⁰c (March) throughout the year. From these values one can observe that daily variation in temperature in the area is more pronounced than the annual variation and the calculated mean annual temperature was around 16.32⁰c. In general, one can classify the climate in this area as warm temperate climate (Solomon Tale, 2000).

3.11 METHODOLOGY

3.12 EXPERIMENTAL DESIGN

Experimental practice of this thesis work was started from November 15, 2014. Green house construction, collection of Biopile trays and other culturing aid materials finished from November15, up to December 15, 2014. From December 16, 2014 up to January 1, 2015, collection of mushroom spores and excavating of soil samples for both control and waste water irrigated agricultural farm land was under taken. Analysis of soil, Culturing of mushroom and determination of mushroom yield parameters started and finished from January 15, 2015 up to July 25, 2015 according to the following orders.

- A. Excavating waste water irrigated agricultural soil from recently irrigated farm land.
 - i. Analysis of physicochemical parameters; pH, electrical conductivity, organic matter and heavy metals (January 15 up to February15, 2015).

- ii. Culturing mushroom (*Pleurotus ostreatus*). First and second cultivation of mushroom was from February 25, 2015 up to March 15, 2015 and March 26 up to April 16, 2015 respectively.
 - iii. Harvesting of mushroom for first and second cultivation from March 20 up to March 25, 2015 and April 20 up to April 25, 2015 respectively; analysis of yield parameters, protein content, organic matter, ash content, heavy metals, evaluating of bioaccumulation factors for each accumulated heavy metals and nutrient content in both first and second cultivation from March 25 up to April 10, 2015 and April 25 up to May 20, 2015 respectively.
- B. Excavating control agricultural soil from recently irrigated farm land.
- i. Analysis of physicochemical parameters; pH, electrical conductivity, organic matter and heavy metals (January 15 up to February 15, 2015).
 - ii. Culturing mushroom (*Pleurotus ostreatus*). First and second cultivation of mushroom was from February 25, 2015 up to March 15, 2015 and March 26 up to April 16, 2015 respectively.
 - iii. Harvesting of mushroom for first and second cultivation for first and second cultivation from March 20 up to March 25, 2015 and April 20 up to April 25, 2015 respectively; analysis of yield parameters, protein content, organic matter, ash content, heavy metals, evaluation of bioaccumulation factors for each accumulated heavy metals and nutrient content in both first and second cultivation from March 25 up to April 10, 2015 and April 25 up to May 20, 2015 respectively.
- C. Comparison:-Comparing and concluding the difference observed for both soil types from analyzed results of yield parameters, protein content, organic matter, ash content, heavy metals, bioaccumulation factors for each accumulated heavy metals and nutrient content in both first and second cultivation from May 20, 2015 up to June 25.
- D. Statistical data analysis for all results from June 25 up to July 25, 2015 from where thesis writing started.

3.13 SAMPLING TECHNIQUE

Waste water irrigated soil sample was collected at Dukem woreda, Leyu zone, Oromia regional state nearby Aba Samuel dam from irrigated agricultural farm land and Control farm land soil sample collected from Bole subcity, at Ayat agricultural farm land; Addis Ababa. Both soil samples have the same texture (sandy) and have been taken by the same method by isolating (30*30) cm² area, and digging out at the same depth. For waste water irrigated agricultural land soil samples, soil depth were chosen at 0-15cm, for three consecutive points of selected one farm land far from each other at distance of 15m starting from waste water entry to the other side of farm land. The same technique was used in control farm land soil sample starting at one side to other at distance of 15m from each other. Finally the samples from three sites mixed uniformly and collected to thick plastic sacks separately for both farm land soil samples. Mushroom spores (*Pleurotus ostreatus*) collected from ethio mushroom culturing center. Collected soil samples have taken to green house separately constructed for this purpose in Addis Ababa University Science faculty for culturing mushroom spores and other small soil samples to soil laboratory of environmental research laboratory, Addis Ababa University.

3.14 ANALYSIS OF SOIL SAMPLES

3.14a Measure of pH

The samples were grinded using mortar and pestle, sieved by passing them through a 1mm mesh. Samples of soil have taken in to clean plastic container, be mixed and Small amount (coffee measure) of 2 parts soil removed and added to 250ml beaker and marked to 150ml with distilled water. The soil and water mixture has been stirred or shaken vigorously. Then it allowed sitting for 1 to 2 minutes. Calibrated pH meter with buffer solutions turned on. The cap has been removed to expose the sensor, and immersed completely in the solution. The reading displayed was recorded on the meter (Ballantyne, 1980).

3.14b ELECTRICAL CONDUCTIVITY ANALYSIS

The meter was calibrated, operator manual has been followed, then the sensor immersed into the liquid of soil sample prepared and the value was displayed (Rhoades and Van Schilfgaarde, 1976).

3.14c ORGANIC MATTER ANALYSIS

Soil moisture was removed before the base weight of the sample is taken by air drying. Muffle furnace capable of being heated to 400°C was used. Apparatus and materials were as follows-

- ✓ Beakers, 20 ml.
- ✓ Crucible rack, stainless steel
- ✓ Balance accurate to 0.001 g in a draft free, low humidity environment
- ✓ Soil scoop calibrated to hold 5 g of light-colored silt loam soil
- ✓ Drying oven, 105°C
- ✓ 5 g scoop of soil was placed into a tarred 20-ml beaker
- ✓ It was dried for 2 hours at 105°C
- ✓ Record weight to 0.001 g was used
- ✓ Oven was brought to 360°C.
- ✓ Samples were then remained at 360°C for two hours.
- ✓ It has been cooled to < 150°C
- ✓ It has weighed to 0.001 g, in a draft-free environment

Organic % matter was estimated by the following equation.

$$\% \text{OM} = \frac{(\text{wt. at } 105^{\circ}\text{C}) - (\text{wt. at } 360^{\circ}\text{C})}{\text{Weigh at } 105^{\circ}\text{C}} \times 100 \dots\dots\dots 1$$

(Schulte and Hopkins, 1996).

3.14d HEAVY METAL ANALYSIS

For both control and waste water irrigated soil samples, 2g soil samples have been transferred to a Teflon beaker and 25ml of distilled water and 2ml of concentrated HNO₃ were added and allowed to dryness. This followed by addition of three drop of concentrated H₂SO₄ and 10ml of HF. The samples were then been placed on stove while

the temperature was slowly raised to 200°C and were allowed to evaporate to dryness. This was followed by the addition of 15ml of concentrated HNO₃, 2ml of H₂SO₄ and 5ml of HClO₄. Heating was continued until strong fumes of SO₃ were produced. The Teflon containers have been cooled and the solutions have transferred quantitatively to a 50ml volumetric flask by adding distilled water (Baker and Amaker, 1982). Then the prepared sample solution was analyzed using AAS, analytical jena (nouVAA).

3.15 MUSHROOM CULTURING USING CONTROL AND WASTE WATER IRRIGATED AGRICULTURAL FARM LAND SOIL SAMPLES.

Culturing aid materials (Large tray 10cm deep, Heavy flat object, Water spay, News papers to seal after soil and spore mixed) were prepared. Edible mushroom spores for culturing (*Pleurotus ostreatus*) were collected from ethiomushroom culturing center.

3.16 CULTURING TECHNIQUES

Soil samples were watered and then any excess moisture squeezed out with hands to make it moist with no excess water. The tray lined with the soil and filled nearly to the top. The mushroom spores were taken and injected several times into the tray of soil and mixed it thoroughly with the soil by hands. Then the tray sealed with magazines and heavy flat object laid on the sealed tray to keep moist and regulate room temperature. The mycelium of *Pleurotus ostreatus* grew optimally at a temperature of 25°C and pH of 6.5 thus the ability of the mycelia to growth at this temperature and pH range enabled them to flourish in agro wastes (Fasidi, 2008).

The heavy objects lied down top of the soil (9kg) with spore mix and left to press down and set for a few hours, preferably overnight or longer. The heavy object taken off; moved and the trays kept in dark and warm place. The trays watered every day for two weeks to keep its moisture but not soaking. The temperature kept from 45-60 degrees Fahrenheit. Once a white coating began to appear on the surface of the soil biopiles, they have been kept moist with the water spraying. The newspaper used to cover the biopiles (70*50*30) cm³ volume of length width and height respectively to create a darker environment in which the mushrooms can spawn. The newspaper sealed the Biopile removed after a week and the mushrooms kept moist. They sprouted and have been ready to pick in around 3-4 weeks (Bruhn and Mihail, 2009).

3.17 ANALYSIS OF HARVESTED MUSHROOM (*Pleurotus ostreatus*).

3.17a IDENTIFICATION OF MORPHOLOGICAL CHARACTERISTICS (during growth period) Height, Diameter of mycelial mat and Thickness).

Yield obtained during culturing process is strictly depending on environmental factors such as pH, and available minerals. Many researchers have studied the effect of temperature, pH and carbon sources on the mycelial growth of fungi. There is limited information on the nutritional requirements and the cultivation conditions to improve mycelial growth and the feasibility of mushroom production. The mycelial growth depends on some factors such as culture media, pH, temperature and nutrient elements. These factors greatly affect the formation and growth of mushroom both in the field and laboratory conditions. For this reason, it is very important to evaluate the factors for the optimum mycelia growth of mushroom (Lilleskov *et al.*, 2002).

3.17b DETERMINATION OF HEAVY METALS (Cd, Cr, Cu, Ni, Pb and Zn)

Dried fungus samples (500 mg) were introduced into the digestion vessels; then 3 ml nitric acid and 5 ml hydrogen peroxide added. After digestion time (40 min) the vessels cooled to room temperature (about 30 min). The clear solution volume marked up to 50 ml for each sample using deionised water. Prepared samples analyzed by an AAS, analytical jena (nou VAA) an instrument of for metals analysis. Determination of elemental concentrations in samples of mushrooms and their substrates performed using the method of calibration curve according to the absorber concentration. Several solutions of different known concentrations will be prepared and the elemental concentration in unknown sample was determined by extrapolation from the calibration curve. All samples concentrations were reported as mg/kg dry weight of material (Kalac and Svoboda, 2005).

3.17c DETERMINATION OF BIOACCUMULATION FACTORS

Bioaccumulation factor is a number that describes bioaccumulation as the ratio of the concentration of a chemical inside an organism to the concentration in the surrounding environment.

The bioaccumulation factor of heavy metals was calculated using relation:

BAF=Cm/Cs,.....2

Where: **Cm** is the concentration of heavy metal in mushroom (Mg/Kg) and

Cs is the concentration of heavy metal in soil (mg/Kg) (Fogel *et al.*, 1976).

3.17d ANALYSIS OF YIELD PARAMETERS,

A. PRODUCTIVITY PARAMETERS.

- Total weight of production
- Total number of mushroom buds for both, waste water irrigated and control soil samples.

B. QUALITY PARAMETERS

- Color change and
- Biochemical parameter (analysis of protein content),

The mushrooms harvested from the different substrates were oven dried at 100°C for 24 hours before they were analyzed for their protein content using Micro- Kjeldahl’s method. Oven dried samples of 0.5 g from each substrate was separately put into 30 ml Kjeldahl flask. Ten gram Na₂SO₄, 20 ml concentrated H₂SO₄ and 1g CuSO₄ were later added. The mixture was cautiously heated in a fume hood until a bluish green clear solution appeared. The digest was allowed to cool and solidify for 24 hours while the color changed to white. Distilled water (20ml) was added to the solidified sample and allowed to cool in a refrigerator. Sixty milliliter (60 ml) of 40% NaOH was added to the sample and two pieces of zinc metal connected to the mixture in a distillation column. In the set up, 100 ml of 4% boric acid was added to a conical flask with two drops of screen methyl red indicator. A light pink color appeared when boric acid and screen methyl red indicator came in contact. The distillation was stopped and collected in a receiver flask when it reached 200 ml. This was titrated with 0.1M H₂SO₄ until a pink color emerged.

Calculation Formula= $\frac{100 \times \text{titre value} \times 0.0014 \times 6.25}{\dots}$ 3

Weight of sample used

Where,

100 = conversion to %

0.0014 = constant which means that 0.0014 is liberated by 1ml of 0.1M H₂SO₄

6.25 = protein constant according to Kjeldahl's method.

$$\text{Percentage Protein} = \frac{100 \times \text{titre value} \times 0.0014 \times 6.25}{0.5}$$

(Association of Official Analytical Chemists; AOAC, 1995).

3.17f ASH CONTENT ANALYSIS

The total ash was done using the furnace incineration gravimetric method and the weight of ash obtained in percentage as follows:

$$\% \text{ Ash} = \frac{W_2 - W_3}{\text{Wt of Sample}} \times 100$$

W2 -wt of crucible + sample4
W3-wt of crucible+sample ash (A.O.A.C, 1995)

3.18 DETERMINATION OF NUTRIENT CONTENTS

The mushroom (*pleurotus ostreatus*) was known to contain calcium, potassium, phosphorus as phosphate and nitrogen as nitrate and these elements are very important in human nutrition. They are required in repairing worn-out cells, strong bone and teeth, building blood cells and maintaining osmotic balance (WHO, 1996).

3.18a DETERMINATION OF CALCIUM, POTASSIUM AND IRON

An amount of 5-15 g of the homogenized sample was dried in an air oven at 105°C for 3Hrs. The dried sample was next charred until it ceased to smoke. The charred sample was then ashed in a muffle furnace at 550°C until a whitish or greyish ash was obtained. The ash was treated with concentrated hydrochloric acid, transferred to a volumetric flask and made up to 50 ml. Per sample studied, single ash solution was prepared, and analysis was carried out. An aliquot of each ash solution was used for the determination of calcium, potassium and iron by the AAS method, analytical jena (nou VAA) (Nivozamsky *et al.*, 1983).

3.18b DETERMINATION OF NITRATE

The concentration of nitrate analyzed in both of the control and waste water irrigated cultivation of soil samples were carried out using smart Spectrophotometer. Vegetable samples solutions were prepared by chopping each sample into smaller sizes. A known

amount (1g) of the chopped sample was transferred into 100ml flask and soaked with 50ml of distilled water. The flask was capped and shaken for 30minutes, then filtered into another 100ml volumetric flask and the volume made to the mark with distilled water. Nitrate was determined spectrophotometrically using standard cadmium reduction method (Radojevic, 1999).

3.18c DETERMINATION OF PHOSPHATE

The mushroom sample was chopped into small pieces. The chopped sample was then air dried. The air-dried sample was ground and sieved with a sieve of mesh 1mm. A known amount (1g) of each of the ground and sieved sample was weighed into acid-washed porcelain crucibles. The crucible was labeled and 5ml of 20% (w/v) magnesium acetate was added and evaporated to dryness. The crucible was then transferred into the furnace and the temperature was raised to 500⁰C. The sample was ashed at this temperature for four (4) hours, removed and cooled in desiccators. Ten (10) ml of 6 M HCl was then added to each of the crucible and covered, then heated on a steam bath for fifteen minutes. The content of crucible was completely transferred into different evaporating basins and 1ml of concentrated HNO₃⁻ was added. The heating was continued for 1Hr. 1ml of 6M HCl was then added, swirled and then followed by the addition of 10ml distilled water and again heated on the steam bath to complete dissolution. The contents of the evaporating basins was cooled and then filtered through a What man no.1 filter paper into 50ml volumetric flasks and the volumes made up to the marks with distilled water. Phosphate was determined using Spectrophotometer. The molybdenum blue phosphorous method in conjunction with UV-Visible spectrophotometer is a very sensitive method for the determination of phosphorus at 830 nm. Orthophosphate and molybdate ions condense in an acidic solution to form phosphomolybdic acid, upon selective reduction (*e.g.* with hydrazinium sulphate) a blue color is produced. The intensity of the blue color is proportional to the amount of phosphate initially incorporated into the acid. If the acidity at the time of reduction is 0.5M in sulphuric acid and hydrazinium sulphate is the reductant then the resulting blue complex exhibits a maximum absorbance at 820-830 nm. Intensity of the color solution was normally

measured on a visible spectrophotometer using shimadzu uv-1700 spectrophotometer at 830nm (Bassett, 1979).

3.19 INCINERATION AND DISPOSAL OF MUSHROOM AFTER CULTIVATION

The incineration residue of mushroom (*Pleurotus ostreatus*) may consist essentially of inorganic materials and metals. Thus, the complex chemical reactions of organic compounds, e.g. acids and chelating agents, with metals are prevented. In contrast, the inorganic chemistry of mineralized incineration residues is well understood and can be fairly easily controlled.

- The incinerator hole was prepared.
- The waste or heavy metal bioaccumulated mushroom was introduced into the incinerator hole.
- Within the incineration hole, the mushroom burnt off.
- The mineralized non-combustible residue mushroom from the incineration is called ash and is essentially a mix of minerals and metals.
- Disposed as landfills (Duffus, 2002).

3.20 STATISTICAL DATA ANALYSIS

All the samples were in triplicate to get accuracy by using mean and standard deviation, correlation and regression with the help of SPSS version 20 statistical soft ware package.

The data obtained after chemical analysis will statistically analyzed by using analysis of variance (ANOVA).

Data analysis was computed using SPSS soft ware version 20. Descriptive statistics like arithmetic mean and SD were used to express the levels of each metal, organic matters and proteins content in soil samples and cultivated mushrooms. ANOVA was used to identify interaction effect of soil pH, electrical conductivity organic matter and heavy metals concentration. The levels of significance was compared with the keys at $p < 0.005$. Figures and tables were drawn using micro soft excel 2007.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.10 SOIL PHYSICO-CHEMICAL PARAMETERS RESULT

4.10a pH

The data of physico-chemical properties of waste water irrigated soil samples and control revealed that the pH value were found 5.49 in before first cultivation and 5.93 after first cultivation in waste water irrigated soil which are below control values (table 1). So the result indicated that there was an increase in soil pH from before cultivation to after cultivation in both sites may be due to decrease in availability of organic matter. The increment is significantly more in waste water irrigated farm land soil samples in comparison to control which may be due to decrease in concentration of organic matters by mushroom after first cultivation from waste water irrigated soil. Organic matter usually considered to lower soil pH by releasing ions that were associated with anions (Porter *et al.*, 1980).

Table 1: pH of soil collected from selected sites during first cultivation of mushroom (*Pleurotus ostreatus*).

Soil sample	Before first cultivation	After first cultivation	Net increment
	pH	pH	
Control	6.212±0.001	6.513±0.0042	0.302
Waste water irrigated	5.494±0.001	5.935±0.001	0.441

The values given above in table 1 summarized below in bar graph (Figure 3). So more difference was found in pH level between waste water irrigated soil sample and control soil sample due to more availability of waste organic matters which may also increase soil pH by mineralization of organic matters CO₂ and H₂O thereby removing H⁺ in waste water irrigated agricultural farm land soil (Helyar, 1976). High organic matter in waste

water irrigated soil may be due to industrial and municipal waste water having high BOD and COD values (Furedy, 1999).

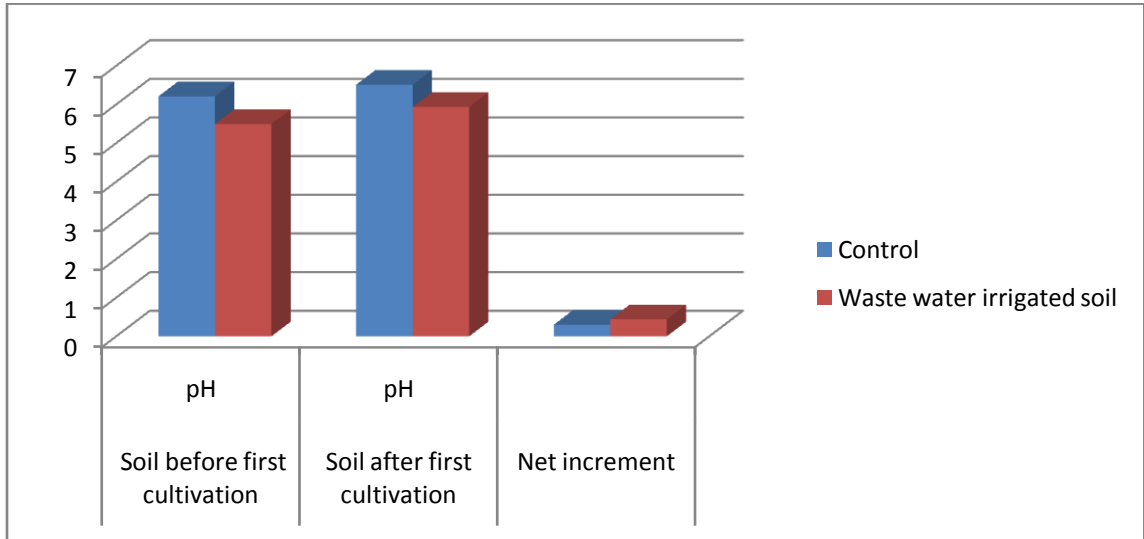


Figure 3: pH of soil from the selected sites during first cultivation of mushroom (*Pleurotus ostreatus*)

4.10b ORGANIC MATTER (% OM)

From data analyzed for soil organic matter percentage (OM %) (table 2), the values were found 58.52 % in before first cultivation and 53.95 % after first cultivation in waste water irrigated soil which were above control values. The levels of organic matter decreased significantly from first cultivation to second cultivation in both cases because most of organic matters consumed during first cultivation. The significant decrement was higher for control farm land soil sample; this may be due to availability of less organic matters. The value of organic matter for waste water irrigated farm land soil was significantly higher than control farm land soil in both before and after first cultivation due to use of Akaki River water from dam for irrigation practice which may contain waste matter rich in organic matter load i.e. BOD and COD (Alemtsehaye Biru, 2002). Continuous application of sewage sludge and municipal waste water may influence the soil physicochemical properties (Willett *et al.*, 1984).

Table 2: Organic matter of soil collected from selected sites during first cultivation of mushroom (*Pleurotus ostreatus*).

Soil sample	Before first cultivation	After first cultivation	% Change
	OM%	OM%	
Control	43.54±0.001	37.11±0.001	6.43
Waste water irrigated	58.52±0.001	53.95±0.001	4.57

From before first cultivation to after cultivation most of organic substrate consumed and the values decreased in second cultivation. So graphically the values are given to summarize results more clearly (Figure 4).

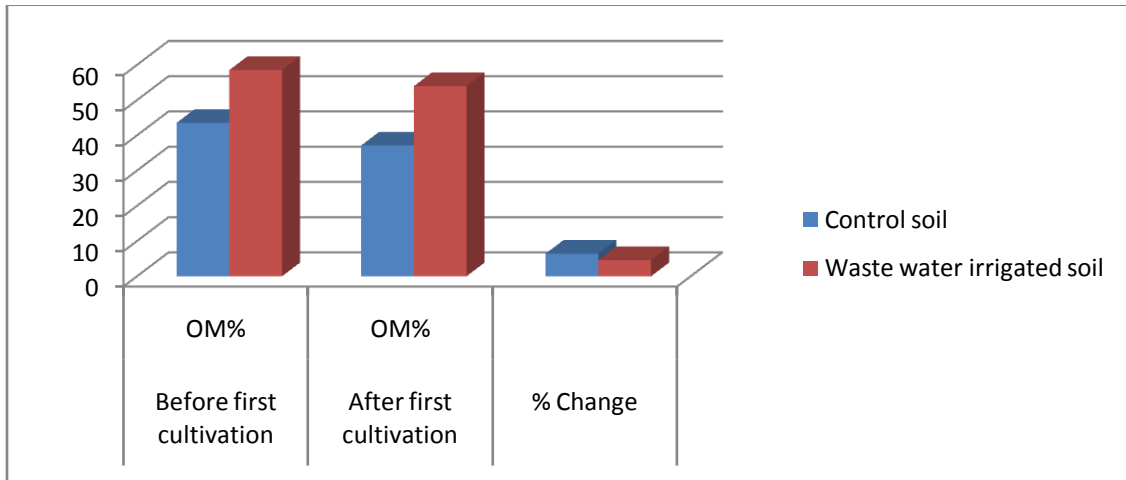


Figure 4: Organic matter of soil from the selected sites during first cultivation of mushroom (*Pleurotus ostreatus*).

4.10c ELECTRICAL CONDUCTIVITY (EC)

The data of electrical conductivity estimation of waste water irrigated soil samples followed by control revealed that the Electrical conductivity values were found 666.33 $\mu\text{s}/\text{cm}$ before first cultivation and 591.32 $\mu\text{s}/\text{cm}$ after first cultivation in waste water irrigated soil which were found above control values (table 3). This may be due to high salt deposition due to wastewater irrigation at experimental site (Doerge *et al.*, 1999).

Results showed the decrease in soil electrical conductivity from before first cultivation to after first cultivation in both experimental and control analyzed sites soil samples. The decrement was significantly more in waste water irrigated farm land soil samples in comparison to control which may be due to decrease in concentration of ionic substances and heavy metals by mushroom after first cultivation from soil.

Table 3: Electrical conductivity of soil collected from selected sites during first cultivation of mushroom (*Pleurotus ostreatus*).

Soil sample	Before first cultivation	After first cultivation	Net change
	EC ($\mu\text{s/cm}$)	EC ($\mu\text{s/cm}$)	EC ($\mu\text{s/cm}$)
Control	99.896 \pm 0.0061	75.980 \pm 0.0053	23.92
Waste water irrigated	666.337 \pm 0.0046	591.320 \pm 0.0086	75.02

Thus more difference was found in electrical conductivity value between waste water irrigated soil sample and control soil sample due to more availability of large amount of ionic substances or heavy metals that have resulted in increased value of Electrical conductivity in the industrial effluents irrigated soil samples in comparison to control (Rhoades and Van Schilfgaarde, 1976).

When the values of electrical conductivity increase it may leads toxic to the plants. Soil electrical is an important indicator of soil health. It affects crop yields, crop suitability, plant nutrient availability, and activity of soil microorganisms which influence key soil processes including the emission of greenhouse gases such as nitrogen oxides, methane, and carbon dioxide. Excess ions hinder plant growth by affecting the soil-water balance. Ionic substance can increase as a result of cropping, irrigation, and land management (Zhang *et al.*, 1996).

The values given above in table 3 is summarized below in bar graph (figure 5) which showed the mushroom better efficiency in reducing the high EC from wastewater irrigated farmland soil sample after first cultivation of mushroom.

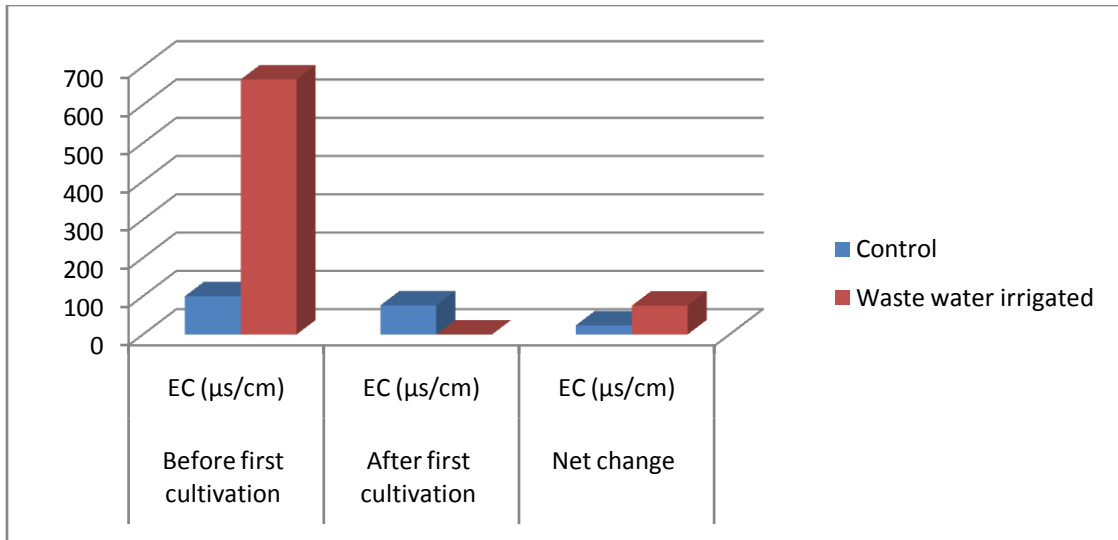


Figure 5: Electrical conductivity of soil from the selected sites during first cultivation of mushroom (*Pleurotus ostreatus*).

4.10d HEAVY METALS

Results of soil analysis (table 4) for heavy metals concentration revealed that the concentration of Cr, Cu, Ni, Pb and Zn were significantly higher in experimental soil samples than control during both before and after first cultivation mushroom which indicates the higher pollution at experimental site due to wastewater. Heavy metals concentration in terms of Cr, Cu, Pb and Zn were significantly decreased in wastewater irrigated farmland soil samples after first cultivation of mushroom in comparison to wastewater irrigated soil samples from before first cultivation of mushroom which indicates the better heavy metal removal efficiency of selected species of mushroom. Organic matter also may play an important role in metal binding and influences the metal leaching (Sebastien sauve, 1998). Heavy metals may be influenced in their solubility due to the presence of organic matter in wastewater affecting experimental soil by increasing mobility of heavy metals by lowering soil pH resulting into more heavy metals bioavailability to mushroom for getting bio-accumulated in fruiting body of mushroom. So due to low pH and high organic matter the heavy metals concentration also decreased significantly in experimental soil samples from after first cultivation of mushroom in comparison to before first cultivation. The decrements of heavy metals concentration

were found significantly higher in experimental soil samples than control. The result (table 4) showed that the concentration of Cr, Cu, Pb and Zn were significantly higher than standard maximum recommended level (WHO /FAO, 2001) of heavy metals indicating the need of second cultivation of mushroom for the selected sites soil samples.

Table 4: Heavy metals of soil collected from selected sites during first cultivation of mushroom (*Pleurotus ostreatus*).

Soil sample	Before first cultivation		After first cultivation	Maximum permissible limit (WHO /FAO, 2001) (mg/Kg)
	Parameter	(mg/Kg)	(mg/Kg)	
Control	Cd	ND	ND	0.03
	Cr	0.459±0.001	0.394±0.001	0.1
	Cu	0.548±0.002	0.385±0.001	0.1
	Ni	0.384±0.001	0.383±0.001	0.5
	Pb	0.319±0.001	0.288±0.002	0.1
	Zn	2.010±0.002	1.678±0.001	0.3
	Waste water irrigate	Cd	ND	ND
Cr		20.500±0.002	11.058±0.003	0.1
Cu		10.154±0.0036	7.257±0.0025	0.1
Ni		0.482±0.001	0.4811±0.001	0.5
Pb		1.180±0.001	0.884±0.0036	0.1
Zn		8.827±0.002	5.667±0.001	0.3

The values given above in table is summarized below in bar graph (figure 6) which showed that the selected mushroom species heavy metal removal efficiency from polluted experimental site in the sequence of Cr > Cu > Zn > Pb. Nickel was not accumulated in cultivation. This may be due to extent of absorption and competition with other heavy metals like Cu which may cause a significant decrease in Ni sorption (Harter 1992).

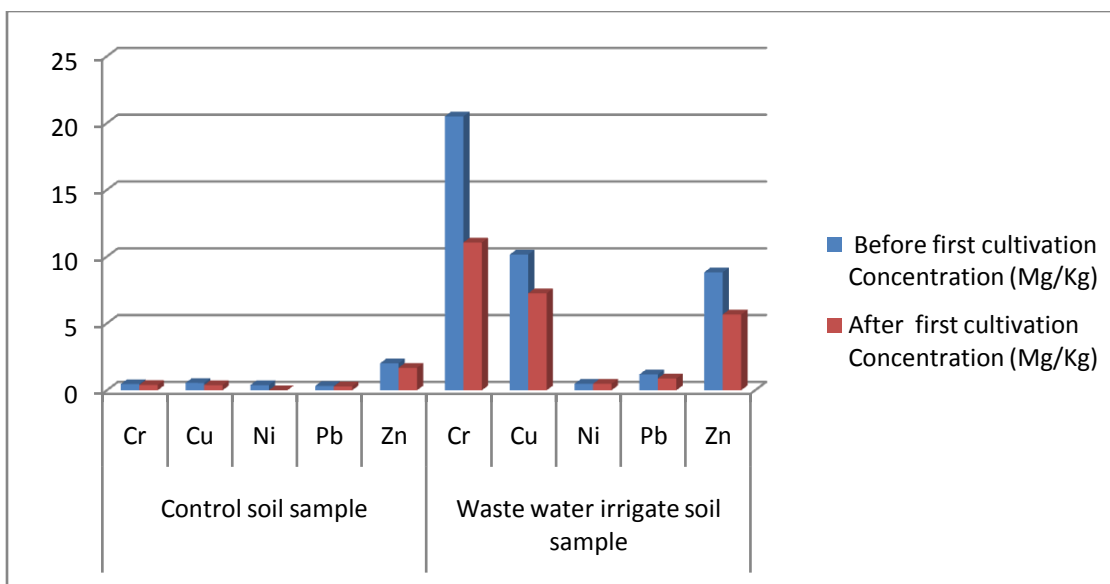


Figure 6: Heavy metal of soil from the selected sites during first cultivation of mushroom (*Pleurotus ostreatus*).

The relationship between different physico-chemical properties and heavy metals concentration were analyzed by Pearson's correlation coefficient. The correlation analysis is a bivariate method which is applied to describe the relation between two different parameters. The high correlation coefficient (near +1 or -1) means a good relation between two variables, and its concentration around zero means no relationship between them at a significant level of 0.05% level, it can be strongly correlated, if $r > 0.7$, whereas r values between 0.5 to 0.7 shows moderate correlation between two different parameters. Table 5 showed the significant negative correlation between pH and chromium (Cr), lead (Pb) & zinc (Zn). Whereas, significant positive correlation were found between OM and Copper (Cu), Pb, & Zn.

Table 5: Pearson's correlation between analyzed heavy metals and physico-chemical properties for selected sites soil sample.

	pH	OM (%)	EC	Cr	Cu	Ni	Pb	Zn
pH	1	-0.972 0.028	-0.909 0.091	-0.959 0.041	-0.943 0.057	-0.869 0.131	-0.953 0.047	-0.968 0.032
OM (%)	-0.972 0.028	1	0.966 0.034	0.943 0.057	0.966 0.034	0.947 0.053	0.967 0.033	0.955 0.045
EC	-0.909 0.091	0.966 0.034	1	0.951 0.049	0.989 0.011	0.996** 0.004	0.983 0.017	0.957 0.043
Cr	-0.959 0.041	0.943 0.057	0.951 0.049	1	0.986 0.014	0.92 0.08	0.991 0.009	0.999 0.001
Cu	-0.943 0.057	0.966 0.034	0.989 0.011	0.986 0.014	1	0.972 0.028	0.999 0.001	0.989 0.011
Ni	-0.869 0.131	0.947 0.053	0.996 0.004	0.92 0.08	0.972 0.028	1	0.963 0.037	0.926 0.074
Pb	-0.953 0.047	0.967 0.033	0.983 0.017	0.991 0.009	0.999 0.001	0.963 0.037	1	0.994 0.006
Zn	-0.968 0.032	0.955 0.045	0.957 0.043	0.999 0.001	0.989 0.011	0.926 0.074	0.994 0.006	1

*Correlation is significant at the 0.05 level (2-tailed).

**Correlation is significant at the 0.01 level (2-tailed).

4.11 ANALYSIS RESULTS OF HARVESTED MUSHROOM

4.11a YIELD PARAMETERS

Results (table 6) showed that total number of budding, average diameter, total mass and height were increased significantly in selected mushroom species after second cultivation in comparison to mushroom of first cultivation from both experimental farm lands soil and control. All identified yield parameters were found significantly lower for mushrooms cultivated on waste water irrigated farm land soil than control in both first and second time cultivated mushroom. The highly significant difference was found in terms total number of buds in mushroom of first and second cultivation in comparison to control indicating towards the presence of soil pollutants may be like heavy metals and high EC. The color of the cultivated mushroom samples also revealed that mushrooms those cultivated on control soil were white, while those cultivated on experimental soil

changed to slight yellowish color. This may be due to industrial effluent pollution effect on experiment sites soil which may decreased the yield parameters of mushroom significantly in comparison to control (Zukowska and Biziuk, 2008).

Table 6: Yield parameters of mushroom (*Pleurotus ostreatus*) cultivated on selected sites soil sample after first and second cultivation.

Mushroom Sample	Parameter	Time interval	First cultivation	Second cultivation
Control	Color	0-7 days	White cream	White cream
		7-14 days	Dense White cream	Dense white cream
		14-21 days	White buds	yellowish buds
		21-28 days	white stock with hyphae	yellowish stock with hyphae
	Total number of buds		21	25
	Average height (cm)		10.575	12.13
	Average pileus diameter (cm)		7.645	7.951
	Average stem thickness (cm)		2.565	2.654
	Total mass (Kg)		1.19	1.25
Waste water irrigated	Color	0-7 days	Yellowish cream	Yellowish cream
		7-14 days	Dense yellowish cream	Dense yellowish cream
		14-21days	yellowish buds	yellowish buds
		21-28 days	yellowish stock with hyphae	yellowish stock with hyphae
	Total number of buds		9	15
	Average height (cm)		8.43	9.25
	Average pileus diameter (cm)		6.35	6.69
	Average stem thickness(cm)		1.51	1.91
	Total mass (Kg)		0.77	0.95

4.11b BIOCHEMICAL (PROTEIN)

The results of protein content analysis in selected mushroom (*Pleurotus ostreatus*) from selected sites (table 7) revealed that the increase of protein content (26.27g/100g) in mushroom cultivated on waste water irrigated soil after second cultivation in comparison to first cultivation (24.69g/100g) which were also significantly lower than control. So the results indicated that there is increase in mushroom protein value from first cultivation to second cultivation in both sites even though the increment is significantly less in waste water irrigated farm land soil samples in comparison to control. That may be due to higher bioavailability of heavy metals to which mushroom mycelium still exposed to more concentrated trace of heavy metals in experimental site soil during both first and second cultivation. So, heavy metals may cause metabolic disorders and growth inhibition in plants, vegetables and funguses (Sinha, 2005).

Vegetables contribute protein, vitamins, iron, calcium and other nutrients to the human diet. But heavy metals are toxic to vegetables and other edible parts and greatly affect vegetables and crop quality (Thompson, and Kelly, 1990). Metals also disrupt protein by binding to sulfhydryl groups and nucleic acids by binding to phosphate or hydroxyl groups. As a result, protein and DNA conformation are changed, and their function get disrupted (Bruce *et al.*, 2003).

Table 7: Protein contents of mushrooms (*Pleurotus ostreatus*) cultivated on selected sites soil sample.

Mushroom sample (<i>Pleurotus ostreatus</i>)	First cultivation	Second cultivation	Net change
	Protein (g/100g)	Protein (g/100g)	
Control	29.44±0.459	36.550±0.560	7.11
Waste water irrigated	24.69±0.321	26.270±0.287	1.58

The non-significant increment of protein value after second cultivation in mushroom cultivated from waste water irrigated farmland soil sample in comparison to control were may be due to less removal of heavy metals after first cultivation of mushroom at

experimental polluted site and interruption of toxic heavy metals in protein synthesis by mushroom exposed to waste water irrigated agricultural farm land soil (figure 7).

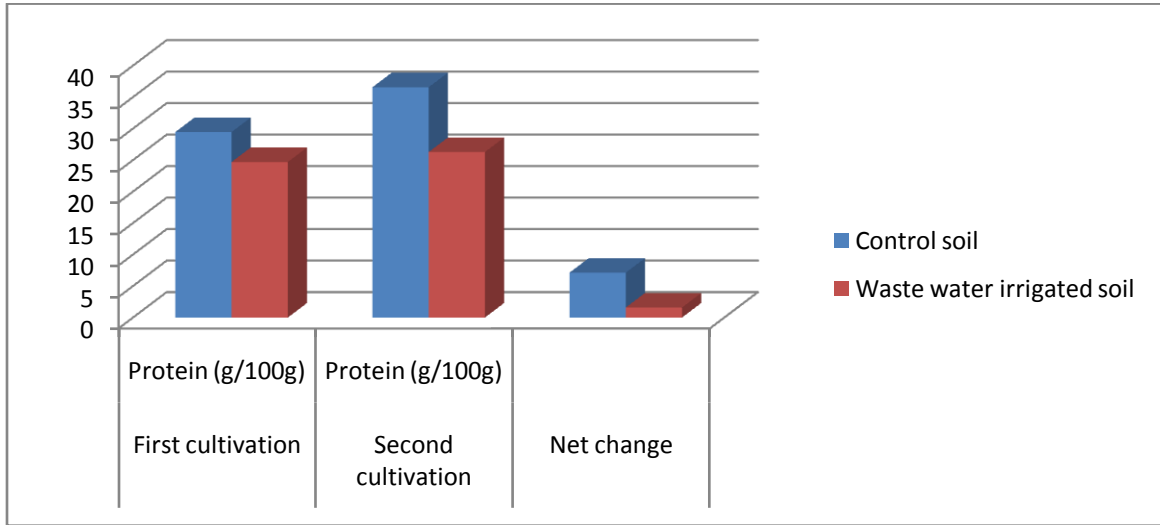


Figure 7: Protein contents of mushrooms (*Pleurotus ostreatus*) cultivated on selected sites soil samples.

4.11c ASH CONTENT

Results of analyzed values of ash content in mushroom (*Pleurotus ostreatus*) (table 8) cultivated on waste water irrigated agricultural farm land soil samples were found 4.93% and 5.91% respectively during first and second cultivation of mushroom which were also found significantly lower than control. So the results indicated that there was increase in ash content from first cultivation to second cultivation at both sites even though the increment was less for waste water irrigated agricultural soil sample. The increment in ash content was may be due to decrease in concentration of heavy metals after first cultivation in experimental site soil during mushroom cultivation and bioaccumulation of heavy metals by mushroom in that process. The decrease ash content value of mushroom at experimental site in comparison to control indicates the possible growth inhibition by heavy metal by causing metabolic disorders (Masarovicova, 2007).

Table 8: Ash content of mushroom cultivated on selected sites soil sample.

Mushroom sample (<i>pleurotus ostreatus</i>)	First cultivation	Second cultivation	% increase
	Ash (%)	Ash (%)	
Control	6.79±0.002	9.15±0.003	2.36
Waste water irrigated	4.93±0.004	5.91±0.003	0.98

The values given in table 8 are summarized below in bar graph (figure 8). So, the analysis indicated that results of analyzed ash content were found significantly lower for wastewater irrigated agricultural farm land soil sample than control due to possible presence of heavy metals after the first mushroom cultivation which affects the nutrients availability in soil for mushroom growth (Bano, 1993).

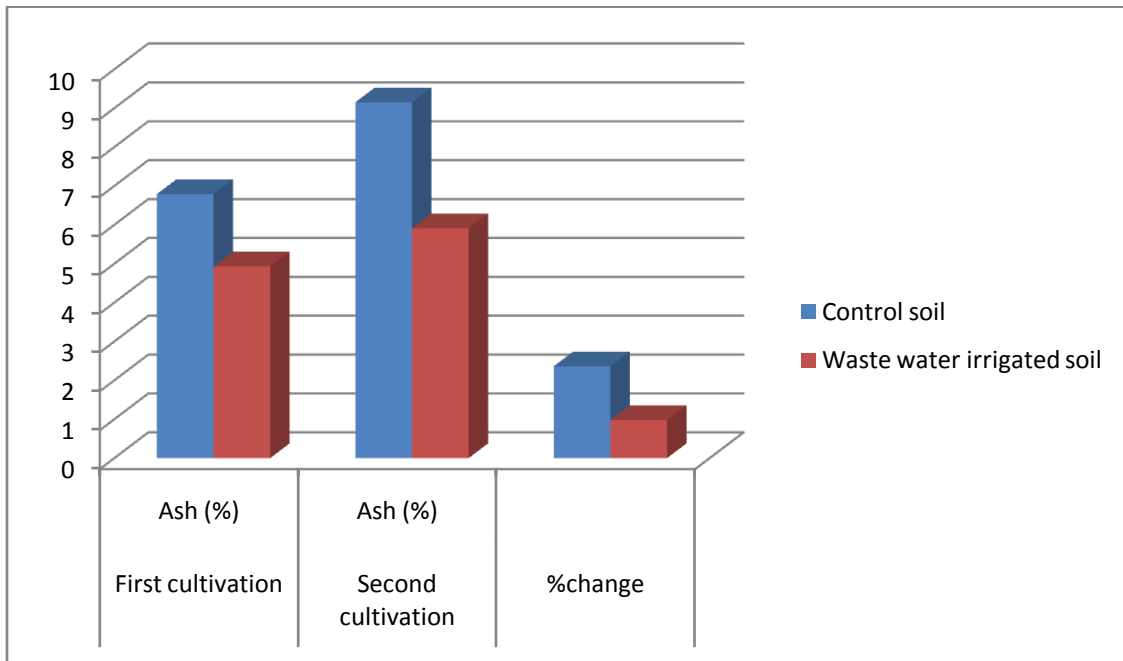


Figure 8: Ash content of mushroom (*Pleurotus ostreatus*) cultivated on selected sites soil sample.

4.11d ORGANIC MATTER

Results of the organic matter analysis in mushroom (*Pleurotus ostreatus*) cultivated on selected site for first and second cultivation are presented in table 9. Organic matter contents for mushroom samples cultivated on wastewater irrigated agricultural land soil samples (48.023 %) after first cultivation and 68.84% after second cultivation) were found significantly lower in comparison to control. So the results indicated that there was highly significant increase inorganic matter of mushroom cultivated on wastewater irrigated soil after second cultivation in comparison to first cultivation as well as control, which may be due to decrease in concentration of toxic heavy metals by mushroom after first cultivation from waste water irrigated soil. As the excess amount of heavy metals inhibit a large number of enzymes and interfere with several aspects with mushroom biochemistry including protein and organic matter synthesis (Masarovicova, 2011).

Table 9: Organic matter of mushroom (*Pleurotus ostreatus*) cultivated on selected sites soil sample.

Mushroom Samples	First cultivation	Second cultivation	Net change
	OM (%)	OM (%)	
Control	66.980±0.001	71.950±0.001	4.97
Waste water irrigated	48.023±0.001	68.841±0.001	20.82

The values given above in table are summarized below in bar graph (figure 9). So there was significant net change of organic matter from first to second cultivation for mushroom harvested from waste water irrigated agricultural farm land soil sample indicating the positive effect of mushroom cultivation on polluted site.

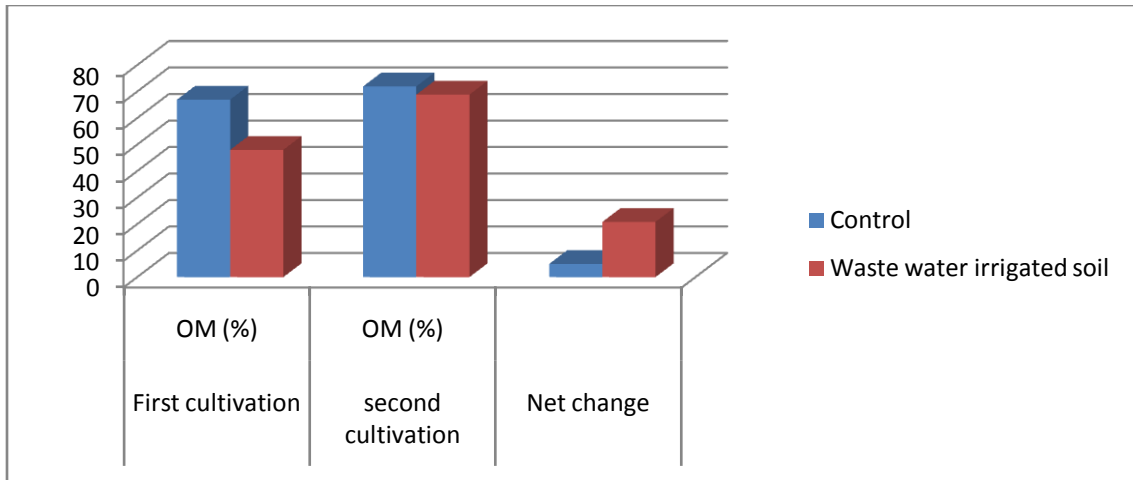


Figure 9: Organic matter of mushroom (*Pleurotus ostreatus*) cultivated on selected sites soil sample.

4.11e HEAVY METALS BIOACCUMULATED IN MUSHROOM

The result of heavy metals content analysis in mushroom (table 10) showed that mushroom from wastewater irrigated site followed by control have better heavy metals bioaccumulation and removal efficiency in terms Cr, Cu, Pd and Zn metals. As the accumulated heavy metals values were significantly higher in both first and second cultivated mushroom from waste water irrigated agricultural farm land soil in comparison with control soil that may be due to higher availability of heavy metals in waste water irrigated agricultural farm land soil. Similar findings were reported by Zhu and Qu (2010) as they also found that mushrooms possess a very effective mechanism that enables them to readily take up some heavy metals from the ecosystem.

Metals accumulated in mushroom from experimental site in both after first and second cultivation of mushroom were found above the maximum permissible limit (FAO/WHO, 2001) and so unfit for human consumption. A number of serious health problems can develop due to uptake of heavy metals contaminated food as a result of excessive uptake of heavy metals by mushroom, vegetables and crops from polluted sites (Alam, 2003). The consumption of heavy metal-contaminated food can seriously deplete essential nutrients in the body causing, or contributing to a number of diseases (Arora *et al.*, 2008).

Table 10: Heavy metals bioaccumulation of mushroom (*Pleurotus ostreatus*) cultivated on selected sites soil sample.

Mushroom Sample	Parameter	First cultivation (mg/Kg)	Second cultivation (mg/Kg)	Maximum permissible limit (FAO/WHO,200) (mg/Kg)
Control	Cd	ND	ND	0.2
	Cr	0.245±0.001	0.197±0.001	2.3
	Cu	0.139±0.002	0.088±0.001	73.3
	Ni	ND	ND	67.9
	Pb	0.203±0.003	0.115±0.0025	0.3
	Zn	0.556±0.003	0.388±0.001	99.4
Waste water irrigated	Cd	ND	ND	0.2
	Cr	10.684±0.003	5.06±0.0025	2.3
	Cu	4.306±0.003	1.029±0.001	73.3
	Ni	ND	ND	67.9
	Pb	0.651±0.003	0.448±0.003	0.3
	Zn	5.404±0.0015	2.265±0.0026	99.4

4.11f BIOACCUMULATION FACTOR

The results of heavy metals bioaccumulation factor for mushroom are presented in table 11. It revealed that selected mushroom (*Pleurotus ostreatus*) species is good bio-accumulator of Cr, Cu, Pb and Zn metals. The trace element concentrations in mushrooms are generally species-dependent and were hardly affected by the pH or organic matter content of the soil (Falandysz *et al.*, 2008). The bioaccumulation factor for heavy metals in selected mushroom species were found higher after first cultivation may be due to more availability of heavy metals in soil samples during first cultivation of mushroom in comparison to after first cultivation.

Table-11: Bioaccumulation factors of mushroom (*Pleurotus ostreatus*) cultivated on selected sites soil sample.

Mushroom sample	Parameter	First cultivation	Second cultivation
Control		Bioaccumulation factor	Bioaccumulation factor
	Cd	0	0
	Cr	0.533769	0.5
	Cu	0.25364964	0.228571
	Ni	0	0
	Pb	0.63636	0.399305
	Zn	0.2766192	0.23122765
Waste water irrigated	Cd	0	0
	Cr	0.52117073	0.45758727
	Cu	0.42406933	0.14179413
	Ni	0	0
	Pb	0.55169	0.50678733
	Zn	0.61221253	0.39968237

The values tabulated above (table 11) are summarized below (figure 10) to understand briefly and more clearly the bioaccumulation factor for each analyzed heavy metals.

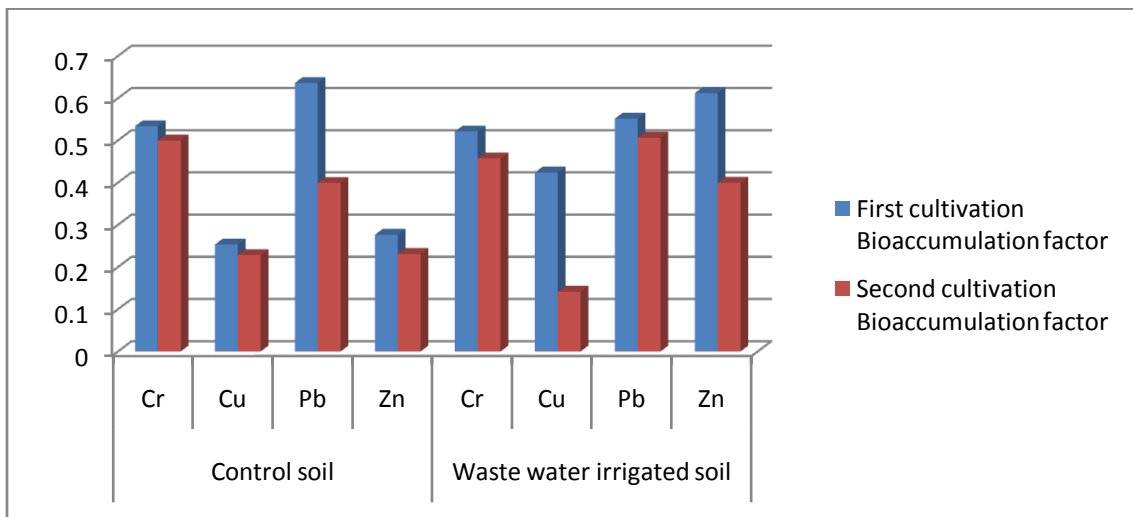


Figure 10: Bioaccumulation factors of mushroom (*Pleurotus ostreatus*) cultivated on selected sites soil sample.

4.11g NUTRIENT CONTENTS OF MUSHROOM

Result from table 12 showed that the values of nutrients i.e. Ca, Fe, K, NO_3^- and PO_4^{2-} in waste water irrigated soil samples were significantly higher both in first and second harvested mushroom sample in comparison to control. Among the analyzed nutrients Ca, nitrate and phosphate were significantly highest in analyzed second cultivated mushroom harvested from waste water irrigated agricultural farm land soil sample than first cultivated mushroom which may be due to heavy metals removal and improvement in soil by mushroom after the first cultivation of mushroom. Similar findings were also reported by Rhodes (2013).

Table 12: Nutrient contents of mushroom (*Pleurotus ostreatus*) cultivated on selected sites soil sample.

Mushroom Sample	First cultivation		Second cultivation
	Parameter	(mg/Kg)	Concentration (mg/Kg)
Control	Ca	11.018±0.001	14.189±0.004
	Fe	3.274±0.0045	5.675±0.003
	K	14.535±0.056	17.336±0.05
	NO_3^-	80.226±0.002	85.100±0.002
	PO_4^{2-}	61.760±0.06	65.877±0.06
Waste water irrigated	Ca	28.323±0.002	35.912±0.003
	Fe	6.027±0.002	9.393±0.0035
	K	22.747±0.003	26.952±0.001
	NO_3^-	280.437±0.001	330.715±0.001
	PO_4^{2-}	234.403±0.003	250.957±0.003

The figure 11 showed that mushroom contains significantly higher amount of nutrient in term of NO_3^- & PO_4^{2-} at experimental site than control. Edible mushrooms have high nutritional attributes and potential applications in decomposing and changing waste organic matter released from industries (Moore, 2005).

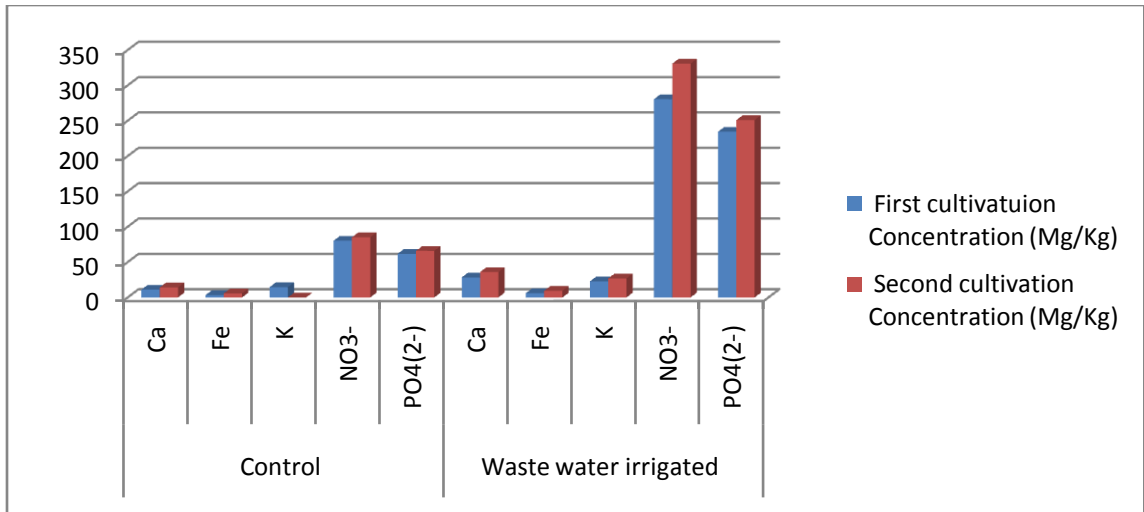


Figure 11: Nutrient contents of mushroom (*Pleurotus ostreatus*) cultivated on the selected sites soil sample.

Result of table 13 showed that there was significant negative correlation between Cr and Fe; significant positive correlation between Fe and K as well as between K and PO_4^{2-} .

Table 13: Pearson's correlation between analyzed heavy metals and nutrient contents of mushroom (*Pleurotus ostreatus*) cultivated on selected sites soil sample during first cultivation.

	Cr	Cu	Pb	Zn	Ca	Fe	K	NO ₃ ⁻	PO ₄ ²⁻
Cr	1	0.686 0.314	0.516 0.484	0.033 0.967	-0.707 0.293	-0.938 0.062	-0.768 0.232	-0.6 0.4	-0.534 0.466
Cu	0.686 0.314	1	0.27 0.73	0.686 0.314	-0.061 0.939	-0.403 0.597	-0.119 0.881	0.056 0.944	0.147 0.853
Pb	0.516 0.484	0.27 0.73	1	0.259 0.741	-0.094 0.906	-0.435 0.565	-0.199 0.801	0.021 0.979	0.038 0.962
Zn	0.033 0.967	0.686 0.314	0.259 0.741	1	0.672 0.328	0.313 0.687	0.612 0.388	0.761 0.239	0.817 0.183
Ca	-0.707 0.293	-0.061 0.939	-0.094 0.906	0.672 0.328	1	0.898 0.102	0.994** 0.006	0.989 0.011	0.975 0.025
Fe	-0.938 0.062	-0.403 0.597	-0.435 0.565	0.313 0.687	0.898 0.102	1	0.938 0.062	0.825 0.175	0.782 0.218
K	-0.768 0.232	-0.119 0.881	-0.199 0.801	0.612 0.388	0.994** 0.006	0.938 0.062	1	0.968 0.032	0.95 0.05
NO ₃ ⁻	-0.6 0.4	0.056 0.944	0.021 0.979	0.761 0.239	0.989* 0.011	0.825 0.175	0.968 0.032	1	0.996** 0.004
PO ₄ ²⁻	-0.534 0.466	0.147 0.853	0.038 0.962	0.817 0.183	0.975 0.025	0.782 0.218	0.95 0.05	0.996** 0.004	1

** . Correlation is significant at the 0.01 level (2-tailed).

* . Correlation is significant at the 0.05 level (2-tailed).

From the above presented correlation matrix, we can observe no any correlation was noticed between nutrient contents and trace heavy metals.

4.12 SOIL ANALYSIS RESULTS AFTER SECOND CULTIVATION

4.12a pH

Result of table 14 showed that there was significant increase in soil pH of waste water irrigated agricultural farm land soil as well as control from before second cultivation to after second cultivation of mushroom. The reduction in organic matter may be resulted into significant change in soil pH at both sites. Organic matter content was found higher at initial before first cultivation and it may rapidly decreased as cultivation went from first cultivation of mushroom to second cultivation. The importance of H⁺ release by organic matter may have been estimated as mechanism of soil acidification (Hoyt and Turner, 1975).

Table 14: pH of soil collected from selected sites during second cultivation of mushroom (*Pleurotus ostreatus*).

Soil sample	Before second cultivation	After second cultivation	Net increment
	pH	PH	
Control	6.513±0.0042	6.807±0.0045	0.294
Waste water irrigated	5.935±0.001	6.175±0.004	0.240

Not only organic matter, also all ionic species and cationic elements like heavy metals were may be depleted in soils from before second cultivation to after second cultivation for both waste water irrigated farm lands and control, which may also, affected the pH value of soil (figure 12).

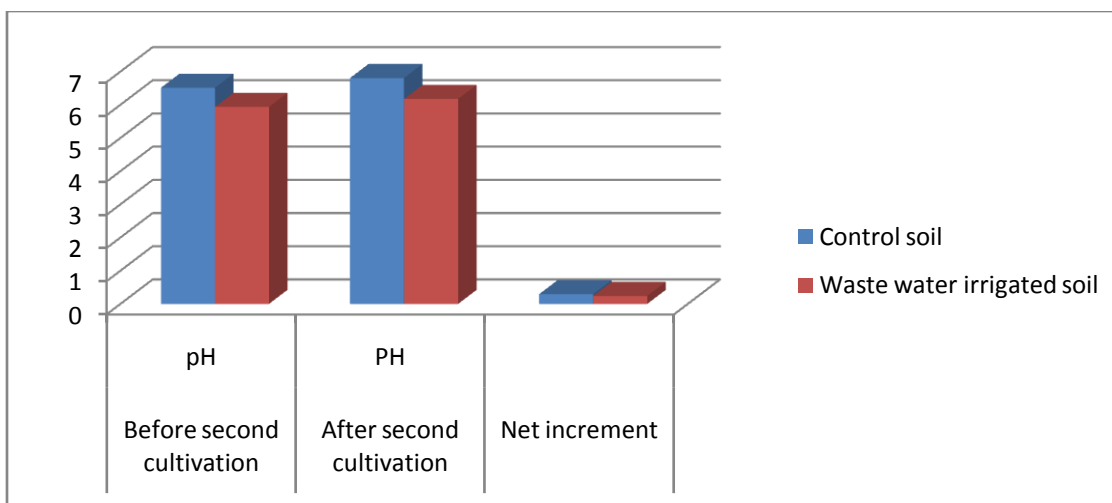


Figure 12: pH of soil collected from selected sites during second cultivation of mushroom (*Pleurotus ostreatus*).

4.12b ORGANIC MATTER

The soil organic matter percentage (OM %) (table 15), the values were found 53.95 % in before second cultivation and 40.22 % after second cultivation in waste water irrigated soil which were significantly greater than the control values. As, fungi are proficient molecular disassembles due to mycelial enzymes which break down the toxins and complex organic compound into simpler less toxic chemicals which may maintained bioavailability of organic matter from wastewater irrigated site soil, rich in organic matter may be coming from high BOD & COD containing wastewater (Adenipekun and Lawal, 2012).

Table 15: Organic matter of soil collected from selected sites during second cultivation of mushroom (*Pleurotus ostreatus*).

Soil sample	Before second cultivation	After second cultivation	% Change
	OM%	OM%	
Control	37.11±0.001	22.19±0.001	14.92
Waste water irrigated	53.95±0.001	40.22±0.001	13.73

As may be due to significant reduction of heavy metals concentration by mushroom bioaccumulation phenomenon during the first cultivation resulted into improvement in the bio-absorption of bioavailable organic matter by mushroom during the second cultivation from wastewater irrigated site soil significantly (figure 13).

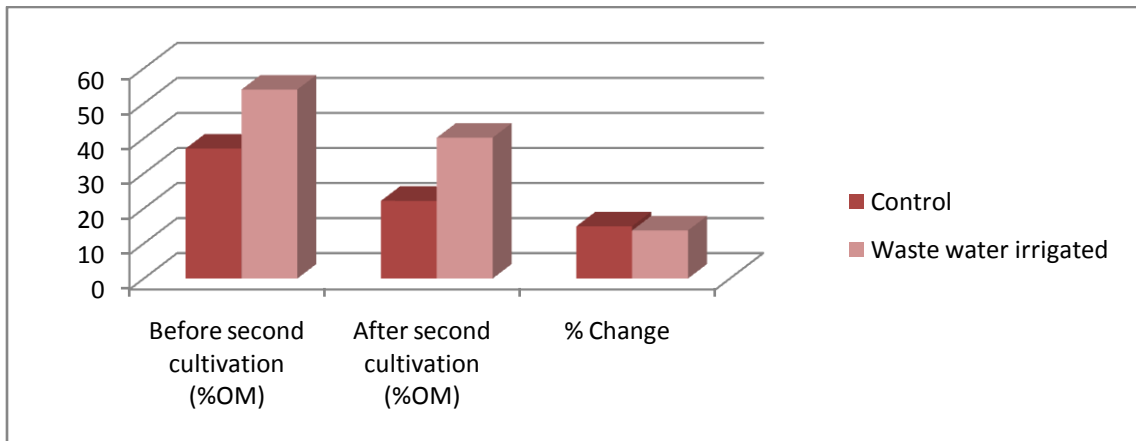


Figure 13: Organic matter of soil collected from selected sites during second cultivation of mushroom (*Pleurotus ostreatus*).

4.12c ELECTRICAL CONDUCTIVITY

The value of Electrical conductivity of waste water irrigated farm land soil sample was found significantly decreased in comparison to control (table 16). Electrical conductivity reduced after first and second cultivation of mushroom at experimental site highly significantly due to reduction in heavy metals concentration in soil samples due to metal bioaccumulation by cultivated mushroom and as well as may be due to adsorption excess salt from soil samples by mushroom during the cultivation.

Table 16: Electrical conductivity of soil collected from selected sites during second cultivation of mushroom (*Pleurotus ostreatus*).

Soil sample	Before second cultivation	After second cultivation	Net change
	Electrical Conductivity ($\mu\text{s}/\text{cm}$)	Electrical Conductivity ($\mu\text{s}/\text{cm}$)	
Control	75.980 \pm 0.0053	51.261 \pm 0.001	24.72 \pm 0.0043
Waste water irrigated	591.32 \pm 0.008	490.362 \pm 0.002	100.956 \pm 0.006

The value of electrical conductivity was found proportional to the available ionic species of heavy metals and may be salts also. The value of electrical conductivity more significantly decreased in waste water irrigated agricultural soil in comparison to control after second cultivation of mushroom (figure 13).

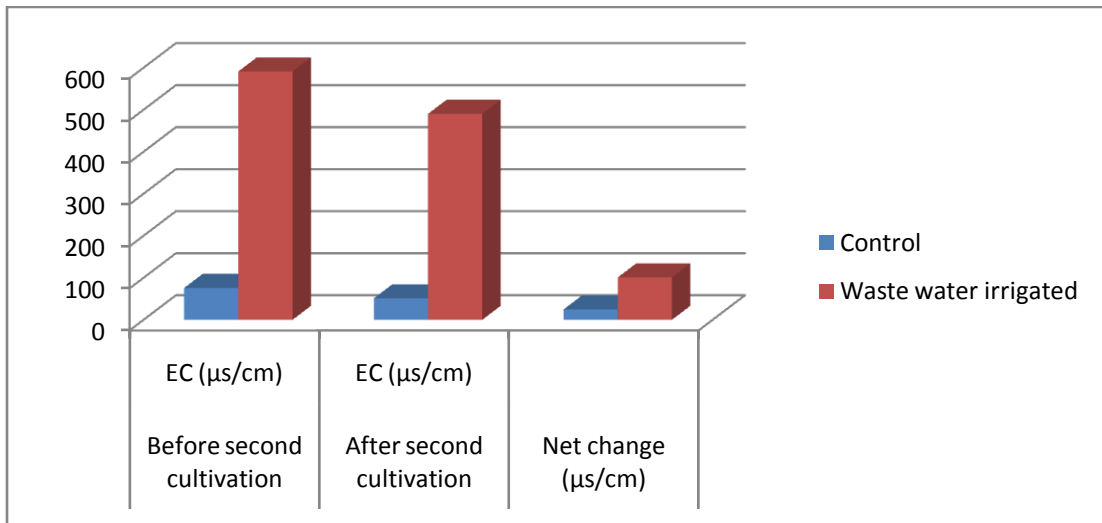


Figure 14: Electrical conductivity of soil collected from selected sites during second cultivation of mushroom (*Pleurotus ostreatus*).

4.12d HEAVY METALS

Results of table 17 and figure 15 showed the significant reduction in concentration of Cr, Cu and Zn in waste water irrigated agricultural farm land soil than comparison to control during the second cultivation of mushroom. So, as a result of consecutive first and second cultivation of mushroom at selected site soil samples, lot of bio-available heavy metals were bio accumulated in mushroom and which may cause also reduction in soil. The second cultivation of mushroom at selected site soil samples reclaimed the sites soil up to safe level (WHO, 2001) in terms of Cu, Ni and Pb concentration in soil. Mushroom removes heavy metal from the soil by channeling them to the fruiting bodies (Okhuoya, 2011).

Table 17: Heavy metals of soil collected from selected sites during second cultivation of mushroom (*Pleurotus ostreatus*).

Soil sample	Parameter	Before second cultivation	After second cultivation	Maximum permissible limit (WHO /FAO, 2001)
		(mg/Kg)	(mg/Kg)	(mg/Kg)
Control	Cd	ND	ND	0.03
	Cr	0.394±0.001	0.099±0.001	0.1
	Cu	0.385±0.002	0.039±0.001	0.1
	Ni	0.383±0.001	0.380±0.002	0.5
	Pb	0.288±0.001	0.081±0.003	0.1
	Zn	1.678±0.002	0.101±0.001	0.3
	Waste water irrigated	Cd	ND	ND
Cr		11.058±0.002	3.13±0.0025	0.1
Cu		7.257±0.0036	0.092±0.003	0.1
Ni		0.4811±0.001	0.478±0.001	0.5
Pb		0.884±0.001	0.210±0.002	0.1
Zn		5.667±0.002	1.11±0.0025	0.3

Figure 15 showed that there was significant removal of Chromium, Copper, Lead and Zinc from polluted wastewater irrigated soil by mushroom in comparison to control during the second cultivation of mushroom due to their effective bio-accumulation in selected species mushroom.

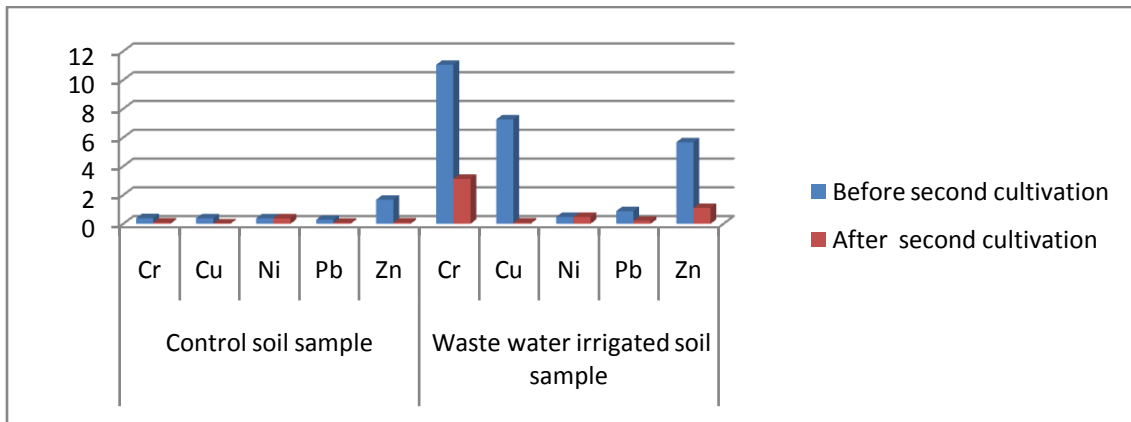


Figure 15: Heavy metals of soil collected from selected sites during second cultivation of mushroom (*Pleurotus ostreatus*).

4.13 HEAVY METALS BIOACCUMULATED MUSHROOM INCINERATION AND DISPOSAL

The cultivated mushroom (*Pleurotus ostreatus*) collected from waste water irrigated farm land were found unfit for consumption because of accumulated chromium and lead which were above maximum permissible limit (FAO, 2001) and direct disposal on the open land surface will be unsafe as it may cause clinical problem due to accumulated heavy metals.

So, the harvested contaminated mushroom was disposed out by carried in following steps:

- Mushroom collected after both first and second cultivation,
- Hole was prepared by digging in to depth and
- Ashed manually the collected mushroom in ground and buried (Duffus, 2002).

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.10 CONCLUSION

As it was observed from determination of soil physico-chemical properties, there are significant differences between control and waste water irrigated agricultural farm land in terms of soil physico-chemical properties i.e. concentration of heavy metals, electrical conductivity and pH values. Significantly high electrical conductivity and low pH indicate higher available metal pollutants. Almost all the research documents reviewed and study made in this research revealed that both the Little and Great Akaki rivers were highly polluted due to discharges from different anthropogenic activities that include specially industrial discharges followed by municipal and sewage waste discharges directly into the river. So, the result of soil analysis from experimental site clearly indicates the high level of pollutants to use of water for irrigation from Aba Samuel dam polluted due to Little and Great Akaki Rivers as the heavy metals Cu, Cr, Pb & Zn were found higher at experimental site than control as well as higher than safe values recommended for agricultural by FAO/WHO (2001).

The result indicates the positive effect of (*Pleurotus ostreatus*) exposure on the polluted soil of experimental site as the concentration of heavy metals (Cu, Cr, Pb and Zn) were removed significantly followed by increase in soil pH and decrease in soil EC due to subsequent two times mushroom cultivation. The concentrations of Cr, Pb and Zn were found still above maximum permissible limit (FAO/WHO, 2001).

Mushroom cultivation yield parameters indicate the negative impact of waste water irrigated farmland soil on mushroom in terms of color, total number of buds, average height, average diameter of cap, average thickness of stalk and total mass in comparison to control. So, on the basis of yield parameters selected mushroom species indicated the effect of pollution at experimental site and were bio-indicator of pollution. Ash content, organic matter, protein and heavy metals concentration in mushroom were indicating the negative impact of soil pollution specially due to higher heavy metals concentration in experimental soil and there bioaccumulation in cultivated mushroom. Results of bioaccumulation potential of mushroom (*Pleurotus ostreatus*) revealed that *Pleurotus*

ostreatus is good bio-accumulator of heavy metals especially Pb, Cr, and Zn. The cultivated mushroom (*Pleurotus ostreatus*) after the two times cultivation were found unfit for consumption because of higher level of these heavy metals (Cr and Pb) higher than maximum permissible limit (FAO, 2001).

The nutrient content Ca, Fe, K, NO_3^- and PO_4^{2-} in selected mushroom species were found higher in experimental site in comparison to control indicates the presence of high concentration of nutrient in experimental soil samples may be due to nutrient rich waste water irrigation.

Therefore higher level of these heavy metals might place the population with in the vicinity of the area at health risk at current level due to heavy metal contamination in soil of experimental site and may aggravate upon after long term use of the polluted wastewater on the selected site.

5.20. RECOMMENDATIONS

- ❖ This experiment has to extend for third and fourth cultivation for effective heavy metal removal from experimental site.
- ❖ There is also need of comparative study on different locally available non-edible mushroom species isolated from polluted sites at different variables like pH, EC, OM and higher concentration of different heavy metals etc.
- ❖ The selected species of edible mushroom can be recommended for bioaccumulation and bioremediation of Cu, Cr, Pb and Zn from contaminated sites along with its safe disposal after the incineration as an alternative species in absence of or along with non-edible species by following earlier studies.
- ❖ Selected mushroom species will be beneficial for soil fertility by improving soil pH and EC.
- ❖ Proper monitoring of waste water discharge into Akaki River to control water and soil pollution.
- ❖ Need of in-situ bioremediation of polluted site for getting safe consumable agri-products and sustainable agriculture.

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APPENDICES

Appendix-1

Irrigated Waste water and soil sampling from the farm land



Figure1a Aba Samuel dam

Figure1b collection of solid wastes in the dam



Figure 1c Waste water for irrigation

Figure 1d Soil sampling from irrigated farm land

Appendix-2

Soil Analysis



Figure 2a grinding and meshing the soil the soil. Figure 2b Digesting the soil.



Figure 2c AAS for soil sample analysis

Appendix-3

Green house construction at Addis Ababa University and sample preparation



Figure 3a green house



Figure 3b Sample preparation.

APPENDIX-4

Culturing mushroom



Figure 4a mixing spores with soil samples for culturing.

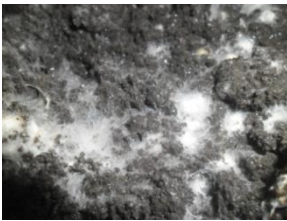


Figure 4b culturing mushroom on
(Control soil)



Figure 4c culturing mushroom on
(Waste water irrigated soil)

Appendix-5

Harvesting mushroom for analysis



Figure 5a from control farm land soil Figure 5b from waste water irrigated farm land soil

Appendix-6

Analysis of harvested mushroom



Figure 6a grinding and meshing mushroom Figure 6b digesting and sample preparation



Figure 6c AAS for sample preparation

Appendix 7 Incineration and disposal of mushroom



Figure 7a manually burning mushroom samples

Figure 7b Burying ashed mushroom