BIOACCUMULATION OF CHROMIUM, COPPER AND ZINC BY FUNGI ISOLATED FROM TEXTILE EFFLUENT CONTAMINATED SITES IN KANO, NIGERIA

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JUNE, 2016

DECLARATION

I hereby declare that the work in this thesis titled **"Bioaccumulation of Chromium, Copper and Zinc by Fungi Isolated from Textile Effluent Contaminated Sites in Kano, Nigeria"** was performed by me in the Department of Microbiology, Ahmadu Bello University, Zaria, under the supervision of Dr. D. A. Machido and Prof. J. B. Ameh.

The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this work has been presented for another degree or diploma at any institution.

MOHAMMED, Hauwa Aliyu

| --- | --- | --- |
| Name of Student | Signature | Date |

CERTIFICATION

This thesis titled **"Bioaccumulation of Chromium, Copper and Zinc by Fungi Isolated from Textile Effluent Contaminated Sites in Kano, Nigeria"** by MOHAMMED, Hauwa Aliyu meets the regulations governing the award of the degree of Master of Science in Microbiology of the Ahmadu Bello University Zaria, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This dissertation is dedicated to Allah (S.W.T) for sparing my life to date, to my adorable son Abubakar Sadik and to my family.

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ABSTRACT

Physico-chemical properties and heavy metals content of textile effluent and water samples from Challawa industrial estate of Kano State, Nigeria were analyzed in this study and most of these parameters were found to be beyond the permissible standards stipulated by Nigerian Environmental Standards and Regulations Enforcement Agency (NESREA) and this call for serious concern and attention. A total of 98 fungi were isolated from textile effluent and water samples. These were dominated by Six fungal genera which comprised of three species of *Aspergillus* namely; *A.niger, A.flavus and A.versicolor* along with *Penicillium* spp., *Chrysosporium* spp., *Microsporum* spp., *Rhizopus* spp. *and Trichoderma* spp. These fungal isolates were screened for their ability to resist heavy metals (chromium, copper and Zinc) at concentrations of 20ppm and 30ppm. *Chrysosporium* sp proved to be most resistant to Zn and Cu with tolerance index of 0.445 and 0.367 respectively while *Aspergillus niger* was most resistant to Cr with tolerance index of 0.356. Fungal isolates resistant to heavy metals were there after tested for bioaccumulation ability of the heavy metals present in the textile effluent at laboratory scale level. The uptake capacity of the heavy metals per unit biomass of the fungi was highest in *Chrysosporium* sp (0.110mg/g, 0.101mg/g and 0.118mg/g in the uptake of Cr, Cu & Zn respectively), this was followed by *Aspergillus niger* (Cr=0.103mg/g, Cu=0.096mg/g and Zn=0.123mg/g), *Aspergillus flavus* (Cr=0.096mg/g, Cu=0.096mg/g, and Zn=0.092mg/g), *Penicillium* sp (Cr=0.091mg/g, Cu=0.088mg/g, and Zn=0.079mg/g), *Aspergillus versicolor* (Cr=0.090mg/g, Cu=0.071mg/g and Zn=0.072mg/g), *Trichoderma* sp (Cr=0.087mg/g, Cu=0.066mg/g, and Zn=0.066mg/g), *Rhizopus* sp (Cr=0.071mg/g, Cu=0.054mg/g, and Zn=0.059mg/g), and *Microsporon* sp (Cr=0.073mg/g, Cu=0.057mg/g, and Zn=0.053mg/g) in descending order. Also, *Chrysosporium* sp had the highest percentage removal efficiency for Cr,

Cu and Zn present in the textile effluent sample (Cr=74.67%, Cu=67.37%, and Zn=85.94%), which was followed by *Aspergillus niger* (Cr=68.21%, Cu=64.09% and Zn=62.24%), *Aspergillus flavus* (Cr=64.52%, Cu=63.85% and Zn=62.34%), *Penicillium* sp (Cr=63.37%, Cu=58.86%, and Zn=54.10%), *Aspergillus versicolor* (Cr=57.07%, Cu=51.447% and Zn=49.27%), *Trichoderma* sp (Cr=55.31%, Cu=45.24% and Zn=45.74%), *Rhizopus* sp (Cr=52.28%, Cu=35.75% and Zn=41.21%), and *Microsporum* sp (Cr=48.51%, Cu=38.19% and Zn=36.11%) in descending order. These findings further indicated that heavy metal resistant fungi are good candidate organisms for bioremediation of industrial effluent contaminated with heavy metals.

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ACRONOMYS AND ABBREVIATION

BOD: Biochemical Oxygen Demand

Cm: Centimeter

$CuSO_4.5H_2O$: Copper (II) sulphate pentahydrate.

Fig: Figure

g: Gram

HCl: Hydrochloric acid

H_2SO_4: Sulphuric acid

$K_2Cr_2O_7$: Potassium dichromate

mm: Millimeter

$MnSO_4.H_2O$: Manganese (II) sulphate monohydrate

NARICT: National Research Institute for Chemical Technology

NESREA: Nigerian Environmental Standards and Regulations Enforcement Agency

$ZnSO_4$: Zinc sulphate

CHAPTER ONE

1.0 INTRODUCTION

Rapid growth of industries during the past two hundred (200) years has resulted in a significant increase in the quantities of effluents released into the soil and aquatic environments (Sethy *et al.,* 2011).

Industrial activities among other things are a major source of environmental pollution; water ecosystem for example is mainly affected by various industrial activities such as mining, cement production, soap and detergent production and textile manufacturing (Venkatesharaju *et al.,* 2010). Developing and especially densely populated countries such as Nigeria has most of its water bodies contaminated by industrial effluents as river water is a considered means of industrial effluent disposal receptor (Kayode, 2010). Waste water treatment is not given the necessary priority it deserves, therefore, industrial waste and domestic sewage are discharged into receiving water bodies without treatment. The consequence of this is increased river pollution, loss of aquatic life and uptake of polluted water by plants and animals which in decades eventually gets into human body resulting in health related problems. The situation is compounded by the fact that the less priviledged in most of these countries does not have access to potable water and in many instances; raw river water is used as source of drinking (Suriptono and Newman, 2000).

Textile industries use a lot of water in their various manufacturing stages (i.e. scouring, bleaching, mercerizing, dyeing, printing and final finishing) and hence they generate a lot of waste water. Effluents released from these operations usually contain a significant amount of pollution load, as hundreds of dyes and auxilliary chemicals are used in the most complex stages

of wet processes (i.e. dyeing and printing) and this is usually discharged into water bodies half treated or untreated (Jamaluddin *et al.,* 2009). The major constituents of effluents discharged by dye house are the color. It is the first contaminant to be recognized in waste water and it is highly visible and undesirable (Jamaluddin *et al.,* 2009). Textile effluent have been found to contain higher amount of heavy metals especially chromium, copper, lead and cadmium as these metals are being widely used in the production of color pigments of textile dye (Ideriah *et al.,* 2012). According to Wang and Chen (2006), three categories of heavy metals are of concern and these include (I) The toxic heavy metals such as Hg, Cr, Zn, Pb, Cu, Ni, Cd, Ar and Co. (II) Precious metals such as Au, Pt, Ag. (III) Radioactive nuclides such as U, Th, Ra, Am. However the toxic metals are of special significance in relation to environmental health. This is because their concentration in the environment beyond permissible levels could cause direct toxicity to all life forms (Gadd, 1988; Nriagu and Pacyna, 1988). There are also reports on the tendency of heavy metals to bio accumulate leading to carcinogenic and teratogenic consequences (Gupta *et al.,* 2011). Metallic effluents can have ecological impacts on water bodies leading to increased nutrient load especially if they are essential metals. These metals may increase fertility of the sediment and water column and lead to eutrophication, which leads to oxygen deficiency, algal bloom and death of aquatic life (Gupta *et al.,* 2011).

Bioremediation is a process by which living organisms degrade or transform hazardous organic contaminants to less toxic compounds (Arun *et al.,* 2008). Microorganisms in the indigenous environment have been known to play key roles in the biodegradation of organic compounds (Husaini *et al.,* 2008). Unlike prokaryotes, eukaryotic fungi have shown diverse metabolic potential resulting in metabolites similar to those produced from mammalian metabolism

(Husaini *et al.,* 2008). The use of fungi as a method of bioremediation provides an option to clean up environmental pollutants. Bioremediation using fungi had drawn little attention in past two decades since most bioremediation research had focused mainly on the use of bacteria. Nevertheless, fungi have received considerable attention for their bioremediation potential which is attributed to the enzymes they produce. In addition fungi have advantages over bacteria such as fungal hyphae that can penetrate contaminated soil to reach the pollutants (Husaini *et al.,* 2008).

1.1 Statement of Research Problem

Textile and dying industries in the world pose a major environmental threat because of the large amounts of water and dyes involved in the manufacturing process (Abd El Rahim *et al.,* 2008). Large amount of chemically different dyes are employed for various industrial applications including textile dyeing (Pal and Brijmohan,1990).The waste water contains heavy metals because the water comes from the printing industries. The dye used in these industries contain synthetic chemicals, which are generally metal based. Many of the metals are harmful to human body above permissible limits (Obebiyi *et al.,* 2010).

Wastewaters released from textile industries causes serious environmental effects due to the presence of heavy metals, toxic dyes and dark coloration. Color and heavy metals from this effluent make the receiving water bodies unaesthetic affecting its water transparency and gas solubility thereby affecting aquatic life (Hussain *et al*., 2004). Moreover, the dyes without an appropriate treatment can persist in the environment for extensive periods of time and are deleterious not only for the photosynthetic processes of the aquatic plants but also for all the

living organisms since the degradation of these can lead to carcinogenic substances (Hao *et al.*,2000; Pinheiro *et al.,* 2004).

Even at low concentration heavy metals can cause toxicity to humans and other living organisms. According to World Health Organization (2004) the metals of most immediate concern are cadmium, cobalt, copper, chromium, lead, nickel, mercury and zinc.

1.2 Justification for the Study

Every production process goes on with wastes generation. Various treatment options are available for treatment of textile wastes before disposal. Traditional disposal method such as ocean dumping is now out of place following numerous incidents of severe negative impacts on the environment after years of disposal. Typical examples are the Love Canal episode of the Niagara Falls in the United States of America (Hardman *et al*., 1993) and the Mina Mata Bay experience in Japan where several tons of mercury was discharged through effluent into the bay and the inhabitants suffered the effect after over thirty years (Hardman *et al*., 1993). There are physical and chemical methods, which, in spite of costs, do not always ensure that the contaminants are completely removed (Hardman *et al*., 1993).

Also many physical and chemical methods such as adsorption, precipitation, chemical oxidation and filtration have been used for heavy metal removal from textile wastewaters. Unfortunately, high operating costs and operational problems such as development of toxic intermediates and lower removal efficiency make these approaches unattractive. Also these methods produce large quantities of sludge, which poses a special problem in its disposal. In recent years, biological processes using fungi is gradually getting momentum due to the fact that the chemical

requirement for the whole treatment process in the removal of heavy is reduced, low operating costs and eco-friendly compared to conventional techniques **(**Nutan *et al*., 2011). Consequently, this study intend to further elucidate the ability of heavy metals resistant fungi species isolated from textile effluent for their ability to bioaccumulate some selected heavy metals present in textile effluent.

1.3 Aim of the Study

To evaluate the capacity of fungi isolated from water samples and effluent from textile industry to accumulate heavy metals.

1.4 Objectives of the Study

1. To determine the physicochemical properties and heavy metals content of textile effluent and contaminated water samples.
2. To isolate and characterize the predominant fungi in the samples of the textile effluent and water.
3. To determine the capacity of the isolates to tolerate chromium, copper and zinc.
4. To determine the capacity of the isolates to bioaccumulate these heavy metals present in the effluent.

CHAPTER TWO

2.0 LITERATURE REVIEW

Kano (Lat. 11^{o} 59m 18.3s N, Long 08^{o} 32m 05.8s E) 418meters above sea level is located in Kano State and occupies a central position in Northern Nigeria. It is one of the developing industrial cities in Nigeria. The population of the city is estimated at over 10 million during the 2006 National Population Census (NPC, 2006). It is the commercial nerve center of Northern Nigeria. The high population is brought about by the much economic and industrial activities taking place in the city. Majority of the industries in the city are textiles, tanneries, chemicals and allied and iron and steel. Kano City is located on the main watershed which separates the two main river basins in the metropolis. The Jakara River basin in the North and the Kano river basin lies to the south of the water divide. The basin is being drained by two major rivers: Kano River and Challawa River. The main industrial areas of Kano – Bompai, Sharada, and Challawa – are located within these two river basins. The increasing discharge of industrial wastes in this river basin is posing serious danger to the water resources and the health of people in the area (Bichi and Anyata, 1999)

The major problem of environmental concern, facing the city, is that of wastewater discharge. The physico-chemical pollutant indicators were studied from ten textiles and tanneries in Kano and it was noted that higher levels of pH, temperature, conductivity, turbidity, color, TSS, oil and grease existed above WHO standard limit (Akan *et al*., 2009b). The indiscriminate discharge of chemical toxins especially Pb, Cd, Cr, Co etc. into the environment ensure their transfer into plants, animals and man, and it was reported that high concentration of heavy metals in irrigation waters could results in death of crops, interfere with uptake of other essential nutrients or form

objectionable deposits on fruits and render the edible portion of plants toxic to human and grazing animals (Bichi, 1999). Another study indicated a strong correlation between high cases of malaria in settlements located at Sharada, Challawa and Bompai industrial estates due to the presence of wastewater which provided a breeding ground for mosquitoes (Bichi and Anyata, 1999). Also the incidence of too much acidity and/or alkalinity in ground water in the area is attributed to contamination by industrial waste which renders the well water unpalatable (Bichi and Anyata, 1999). Residents in the area were reported to complain about the contamination of air which causes odour that is highly objectionable (Lapai, 1992). The combined effect of all these might be the reason of shorter life expectancy in this part of the world (WHO, 2003).

2.1 Textile Industry and Composition of Textile Effluent

The term textile is derived from the Latin *textilis* and the French *texere*, meaning "to weave," and it originally referred only to woven fabrics. It has, however, come to include fabrics produced by other methods. Thus, threads, cords, ropes, braids, lace, embroidery, nets, and fabrics made by weaving, knitting, bonding, felting, or tufting are textiles (Encyclopedia Britannica 2009).

Textile wastewater is characterized by strong color, high salinity, high temperature, variable pH and high chemical oxygen demand (COD) (Mantzavinos and Psillakis, 2004). The colored wastewater affects aesthetics, water transparency and gas solubility in water bodies and can be toxic to aquatic flora and fauna, and this causes severe environmental problems worldwide (Vandevivere *et al.,* 1998). Furthermore, most synthetic azo dyes and their metabolites are toxic, carcinogenic and mutagenic, posing a potential hazard to human health (Nilsson *et al.,* 1993). The treatment of textile effluent involves mainly physical and chemical methods, which are often very costly (Robinson *et al.,* 2001). It is difficult to treat dye wastewater by chemical and

physical processes because of the complex molecular structures. Furthermore, the disposal of the concentrated sludge creates another problem.

The basic raw materials in textile are cotton, flax, jute, wool, silk and synthetic polymers fibers such as nylon, polyesters, polyethylene and rayon (Dara, 1993). The cellulosic textiles are composed of pure cellulose and can be categorized as:

1. Natural cellulosic fibers, which include cotton, abaca, coir, flax hemp, henequen, jute and sisal.

2. Man- made cellulosic fibers, which are cuprammonium, polynosic and viscose.

Cotton (*Gossypium* spp) is a fiber crop, which belongs to the plant family, Malvaceae. It produces cotton lint, a white fiber used in the textile industries. Cotton fiber is a single plant cell. Its cross-section is oval, compared with the normal hexagonal plant cell. However, like small plant cells, cotton has a distinct cuticle, well-developed primary and secondary walls and lumen. The cuticle is the 'skin' of the cotton fiber. It is composed of a waxy layer (cotton wax) only a few molecules thick. The inert nature of this cotton wax protects the rest of the fiber against chemical and other degrading agents. Bleaching during cotton finishing removes much of the cuticle or wax (Gohl and Vilensky, 1987). This enables cotton to absorb moisture more quickly. Subsequently, laundering gradually removes most of the remaining cuticle. The primary cell wall, which is immediately underneath the cuticle, is composed of very fine threads of cellulose, called fibrils. The secondary cell wall forms the bulk of fiber. Concentric layers of the spiraling cellulosic fibrils make up the secondary wall. Much of the strength and stability of the cotton fiber and hence, of yarn and fabrics are attributed to these spiral fibrils. The cotton polymer is a linear cellulose polymer with repeating units of cellobiose, which consists of glucose. The

polymer consists of about 5000 cellobiose units (Gohl and Vilensky, 1987). The fibers are resistant to alkalis and relatively unaffected by normal laundering. Flax fiber is classified as a natural cellulose multi-fiber.

'Linen' is the term applied to the yarn spun from flax fibers and to the cloth or fabric woven from this yarn.

Flax polymer is chemically the same as the cotton polymer both are cellulose polymers. Viscose is a manmade natural polymer of cellulose or regenerated cellulose filament. Thus cotton, flax and viscose are all cellulosic in nature. Other textile fibers composed of natural protein include wool, silk, mohair and cashmere. Synthetic textile fibers on the other hand are composed of synthesized polymers not found in nature such as acrylic, nylon, polyester, and polyethylene (Gohl and Vilensky, 1987). The various operations involved in cotton textile mill are combing, spinning, sizing, weaving and knitting. The seeds are removed from cotton (combing) before the cotton is carded (i.e. made fluffy) and before they are spinned. The spinning wheel makes the cotton into thread (yarn) before they are dyed into different colors for weaving different patterns to make clothing, curtains, carpet and many other products. All these are dry processes except sizing. The 'grey cloth' obtained after the above operations is subjected to the various wet treatment processes such as desizing, scouring, bleaching, mercerizing, dyeing or printing and finishing. All these processes generate considerable volumes of effluents, which contain such chemical substances as dyes, alkalis, chromium, phenol, oils and waxes (Gohl and Vilensky, 1987).

2.2 Physico Chemical Properties of Effluent and Water Bodies

The quality of any water is defined by its chemical, physical and biological contents, hence maintaining a healthy aquatic ecosystem which depends on the physicochemical properties and biological diversity, this call for a regular monitoring of water bodies with required number of parameters (Udoji *et al*., 2010). The importance of the determination of physicochemical parameter of water, effluents or sediments cannot be over emphasized as these parameters affect the concentration of heavy metals with organic matter and pH being the most important parameters controlling the accumulation and the availability of these heavy metals (Afshin and Farid, 2007). Industrial activities have been identified as a major source of pollution for water ecosystems. The production of textile, cellulose and various chemicals is usually connected with synthetic dyes usage alongside with other toxic metals and the discharge of their effluents could have a serious hazardous influence on the environment. Yusuff and Sonibare (2004) investigated effluents from five major textile industries in Kaduna, Nigeria, these effluents were tested for color intensity, chemical oxygen demand (COD), total suspended solid (TSS), NH_3, biological oxygen demand (BOD5), and S2, also metals such as Al, Mn, Zn ,Fe, Cu were also investigated. Al, Mn, Zn, Fe were found to be within limit while Cu was found to be above limit about three fold. Akan *et al.* (2009a) reported the investigations on samples collected from tanneries and textile industries from Kano industrial area, result showed that the concentrations of BOD, COD, DO, nitrate, nitrite, sulphate, phosphate, chloride and heavy metals were higher than the limits set by WHO for the discharged of tanneries and textile effluents into river. Asia *et al*, (2009) also studied the physicochemical properties and investigated some selected heavy metals in three effluent samples collected from textile factories in Kaduna, results shows that the heavy metals investigated had higher concentration than the Federal Environmental Protection Agency

(FEPA) standards for effluent discharge, physicochemical properties result indicates that the effluents may not be able to undergo up to 50% substrate biodegradation, thus biological processes may not be feasible for the treatment of these effluents.

Challawa River in Kano has also been investigated for the impact of effluent from tanneries and textile industries on it chemical characteristics. The samples collected at some selected points along the river was investigated for parameters such as biochemical oxygen demand (BOD), chemical oxygen demand (COD), total organic carbon (TOC), Dissolved oxygen (DO) total dissolved solid (TDS), anions and trace element and the result indicate that all the investigated parameters were found to be higher than the WHO limits for the protection of fish and other aquatic life (Akan *et al.,* 2009b). Awomeso *et al.*(2010) studied a river receiving effluents from textile industry in Lagos, Nigeria, dissolved oxygen at the points closest to the point of effluent discharge were found to be zero signifying that stream was heavily polluted and may not likely support aquatic lives.

2.3 Heavy Metals

Heavy metals exhibit toxic effects on soil biota and they can affect key microbial processes and decrease the number and activity of soil microorganisms (Sanranraj *et al.,* 2013). Metal contaminants are commonly found in soils, sediments and water. Metal pollutants can be produced through industrial processes such as mining, refining and electroplating. At low concentrations, metals can serve as important components in life processes, often serving important functions in enzyme productivity. Metals can become toxic to many species. Fortunately, microorganisms can affect the reactivity and mobility of metals. Microorganisms

that affect the reactivity and mobility of metals can be used to detoxify some metals and prevent further metals contamination. Thus far, tolerance mechanisms for metals such as copper, zinc, arsenic, chromium, cadmium and nickel have been identified and described in detail (Sanranraj *et al.*, 2013)

Metals such as copper and zinc are essential to biological actions, however, all metals, whether essential and nonessential will tend to show toxicity at certain levels. Their toxicity may be presented differently, depending on the isolate and its site of isolation (Asia *et al*, 2009).

2.3.1Chromium

Chromium (Cr) is a transition metal present in group VI-B of the periodic table. Although it can exist in nine valence states, from -2 to +6 (Smith *et.al*,. 2002) only trivalent chromium Cr (III) and hexavalent chromium Cr (VI) are ecologically important because these are the most stable oxidation states in the natural environment. Chromium is a chemical element which has the symbol Cr and atomic number 24. It is a steely gray, lustrous, hard metal that takes a high polish and has a high melting point. It is odorless, tasteless and malleable metal. The name of the element is derived from the Greek word ‘chroma’ meaning color, because many of its compounds are intensely colored. Chromium is an important metal due to its high corrosion, resistance and hardness. It is used extensively in manufacturing of stainless steel.

Chromium contamination of the environment is of concern because of the mobility and toxicity of Cr (VI). Trivalent and hexavalent chromium differ widely in physicochemical properties and biological reactivity. While Cr (VI) species and dichromates are extremely water-soluble and

mobile in the environment, Cr (III) is immobile. Moreover, Cr (VI) is recognized to be highly toxic, carcinogenic, mutagenic and teratogenic for mammals including humans, whereas Cr (III) is an essential trace element necessary for glucose, lipid and amino-acid metabolism as well as a popular dietary supplement (Viamajala *et al.,* 2004). Studies have revealed that Cr (VI) is approximately 100 times more toxic and 1000 times more mutagenic than Cr (III). Although, trivalent chromium (Cr (III) or Cr) is required in trace amounts for sugar and lipid metabolism in humans, however its deficiency causes disease. Hexavalent chromium (Cr (VI) or Cr) is a toxin and a carcinogen metal pollutant that tremendously affects the environment at abandoned chromium production sites. Hence its environmental cleanup is highly essential. Cr (VI) causes severe diarrhea, ulcers, eye and skin irritations, kidney dysfunction and probably lung carcinoma. It is also associated with decrease in plant growth and changes in plant morphology. Chromium is present in the environment as either Chromium (III) or Chromium (VI). Chromate [Cr (VI)] is highly soluble in bacteria, it is transported rapidly across the cell membranes *via* the sulfate pathway and reduced in the cytoplasm to trivalent (III).Trivalent chromium, which interacts with proteins and nucleic acids, however, is far less soluble than hexavalent chromium and does not pass through biological membranes (Coasta, 2003). Hexavalent chromium (Cr^{6+}) is the toxic form of chromium released during industrial processes such as leather tanning and pigment manufacture (Srivastava and Thakur, 2006).

2.3.2 Copper

Copper is a co-factor in numerous enzymatic processes and represents the third most abundant transition metal found in living organisms. (Ezzouhri *et al.,* 2009) Dissolved and particulate forms of copper are common trace contaminants in storm runoff and waste water. In the

dissolved state, copper appears in various forms, ranging from the cupric ion to numerous organic-inorganic complexes. The ionic form of copper is toxic at very low concentrations, while complexed, copper is basically nontoxic. Regulatory agencies have long known about this "Jekyll and Hyde" behavior, but the standard permitting approach has been to assume that all dissolved copper is present in the most toxic form, which is rarely accurate because the ionic form is highly reactive, readily forming nontoxic complexes (Hall *et al*., 1997).

Copper is a persistent, bio-accumulative and toxic heavy metal which does not break down in the environment, is not easily metabolized and can harm human health. Waste streams from copper electroplating industries, textile industries or washing effluents for remediation of soil contaminated with copper may contain up to 500 mg L^{-1} copper, which, according to environmental regulations worldwide must be controlled to an acceptable level before being discharged to the environment (Dermentzis *et al.,* 2009).

2.3.3 Zinc

Zinc is known as an environmentally ubiquitous heavy metal. This metal is an essential trace element needed by the normal metabolism of living organisms. However, anthropogenic inputs could cause elevated Zn concentrations in the environment due to man-induced activities (Yap *et al*., 2005). High exposure to Zn in humans can cause nephritis, anuria and extensive lesion in the kidney. It is dissolved in the aquatic ecosystems and transported by water and taken up by aquatic organisms or can be stored and transported in sediments. In Malaysia, heavy metals studies including Zn in the sediment showed that elevated levels of contamination were recorded in the aquatic areas adjacent to industrial estates (Yap *et al*., 2005).

Also Zinc as an essential trace element is not biologically redox reactive. Hence, it is not used in cellular metabolisms like respiration. However, it is structurally, a vital constituent of several cellular enzymes. Furthermore, it also forms complexes in cells for instance, zinc fingers in DNA (Nies, 1999; Spain and Alm, 2003). In addition, zinc actually, displays comparatively less toxicity to microbial cells than other heavy metals and it generally occurs in higher concentrations within microbial cells. That is why microorganisms in heavy metal polluted environment accumulate zinc by a fast but unspecific uptake mechanism (Nies, 1999). Generally, uptake of zinc ions by microbial cells is coupled with magnesium, and both ions may be transported by similar mechanism (Nies and Silver, 1995; Spain and Alm, 2003).

Zinc is essential for all organisms, although at high concentrations it can be toxic (Balsalobre *et al.*, 2003). Since Zn is persistent and non-biodegradable in the aquatic ecosystem, there is need to remove elevated Zn before it enters the complex aquatic ecosystem (Balsalobre *et al.*, 2003).

2.4 Microorganisms and Metal Tolerance

Microorganisms are of primary importance in bioremediation of contaminated soils and wastewater, essentially because of their ability to alter the chemical status of the metal ions and in turn metal ions mobility through processes such as reduction, bioaccumulation, mobilization and immobilization (Khan *et al.*, 2009). Among the microorganisms, fungi are very important for bioremediation due to their mycelial nature and well documented ability to accumulate metals of all kinds (Gadd, 1993). Fungal resistance to heavy metals results from various mechanisms, i.e., active transport of metal ions outside the cell, masking metals by chelating, enzymatic transformation of metal ions, creating vacuoles in which metal ions are gathered and immobilization in the form of polyphosphates, increased production of melanin and other pigments, and production of specific metal binding compounds inside the cell (Gonzalez-

Chavez *et al.*, 2002; Hastrup *et al.*, 2005; Balamarugan and Schaffner, 2006). The removal of metals from soil and water bodies by fungi also have industrial relevance as this process not only cleans the environment and protects its biodiversity, but it also allows the recovery of the metals and their subsequent reuse (Brierley *et al.*, 1983; Gadd, 1993).

In naturally polluted environments, the microbe's response to heavy metals toxicity depends on the concentration and the availability of metals and on the action of factors such as the type of metal, the nature of medium and microbial species (Hassen *et al.*, 1998). Fungi and yeast biomasses are known to tolerate heavy metals (Gavrilesca, 2004). They are a versatile group, as they can adapt and grow under various extreme conditions of pH, temperature and nutrient availability, as well as high metal concentrations (Anand *et al.*, 2006). They offer the advantage of having cell wall material which shows excellent metal-binding properties (Gupta *et al.*, 2000). Generally, microbial biomasses have evolved various measures to respond to heavy metals stress via processes such as transport across the cell membrane, biosorption to cell walls, entrapment in extracellular capsules, as well as precipitation and transformation of metals (Malik, 2004). Studies showed that strains isolated from contaminated sites have an excellent ability of removing significant quantities of metals both from aqueous solutions and electroplating effluents (Malik, 2004). El-Morsy (2004) studied 32 fungal species isolated from polluted water in Egypt for their resistance to metals and found that *Cunninghamella echinulata* biomass could be employed as a biosorbent of metal ions in wastewater. Vadkertiova and Slavikova (2006) have studied metal tolerance of yeasts isolated from polluted environments and found that there is an interspecific and intraspecific variation in the metal tolerance among tested strains. In the

same way, Zafar *et al.* (2007) reported promising biosorption for Cd and Cr by two filamentous fungi, *Aspergillus* sp. and *Rhizopus* sp., isolated from metal-contaminated agricultural soil.

2.5 Regulatory Limits and Necessity of Treatment

The maximum contaminant level (MCL) values as per EPA and the NESREA list of priority chemicals of some of the toxic and heavy metals are summarized in Table 2.1 (EPA, 2005). The above limits are mandatory for all the water supply systems and industrial waste water discharge to water bodies such as streams, rivers. But naturally occurring water (both surface and ground water) sometimes contain some of these heavy metals in 100 or 1000 times more in concentration than the prescribed MCL value. All water treatment facilities, therefore, are required to treat the heavy metals contaminated water to meet the regulatory requirements.

2.6 Conventional Methods Used In the Treatment of Effluent

Most of the conventional technologies used in the treatment of wastes are not designed and equipped for handling toxic waste. Metals toxicity persists even in the sludge and by product of these plants.

The following are some of the conventional methods used in the treatment of industrial effluent before being discharged including their individual advantages and disadvantages that limit their industrial application.

Table 2.1: Permissible Standard of Some Heavy Metals in Effluent and Water

Heavy Metals	EPA Maximum Concentration Limit (mg/l)	NESREA Maximum Concentration Limit (mg/l)
Arsenic (As)	0.01	0.1
Lead (Pb)	0.015	Less than 1
Mercury (Hg)	0.002	0.05
Cadmium (Cd)	0.005	Less than 1
Chromium (Cr)	0.01	1.0
Zinc (Zn)	5.0	Less than 1
Manganese (Mn)	0.05	5
Copper (Cu)	1.3	Less than 1
Selenium (Se)	0.05	Less than 1
Silver (Ag)	0.05	0.1
Iron (Fe)	0.3	20

Rank of heavy metals as per CERCLA list of priority chemicals 2005 and their regulatory limits Heavy metals Rank Maximum concentration limit (ppm) (EPA, 2005; NESREA, 2009).

2.6.1 Chemical Precipitation

Chemical precipitation is a widely used, proven technology for the removal of metals and other inorganics, suspended solids, fats, oils, greases, and some other organic substances (including organophosphates) from wastewater. Generally speaking, precipitation is a method of causing contaminants that are either dissolved or suspended in solution to settle out of solution as a solid precipitate, which can then be filtered, centrifuged, or otherwise separated from the liquid portion (EPA, 2001).Chemical precipitation can be used to remove contaminants from both municipal and industrial wastewaters. It can be used for water softening, heavy metal removal from metal plating wastes, oil and grease removal from emulsified solutions, and phosphate removal from wash-waters and other wastewater. It is an effective tool for wastewater polishing and removal of particulate matter (Li *et al*., 2004).

The precipitation process is assisted through the use of a coagulant such as polymers which combines suspended smaller particles to form larger aggregates in the solution. However, according to Li *et al*. (2004) stated that despite it is widely usage, these processes are becoming uneconomical and causes more problems than the benefits they offer as mentioned below:

Advantages of Chemical Precipitation

1. Chemical precipitation is a well-established technology with ready availability of equipment and many chemicals.
2. Some treatment chemicals, especially lime, are very inexpensive.

3. Completely enclosed systems are often conveniently self-operating and low maintenance, requiring only replenishment of the chemicals used. Often times, a sophisticated operator is not needed.

Disadvantages of Chemical Precipitation

1. The overall cost of chemical precipitation is dependent on variables such as (i) characteristics of the wastewater, (ii) chemicals and dosages to be used, (iii) volume of water to be treated, and (iv) Level of water purity desired.
2. Competing reactions, varying levels of alkalinity and other factors typically make calculation of proper chemical dosages impossible. Therefore, frequent jar tests are necessary for confirmation of optimal treatment conditions as overdosing can diminish the effectiveness of the treatment.
3. Chemical precipitation may require working with corrosive chemicals, increasing operator safety concerns.
4. The addition of treatment chemicals, especially lime, may increase the volume of waste sludge up to 50 percent.
5. Large amounts of chemicals may need to be transported to the treatment location.

2.6.2 Electrochemical Technology

Electrochemical methods oxidize and reduce pollutants in wastewater by means of electrode reactions (electrolysis). The electrodes needed are available in various shapes (bar, plate, porous and fiber) and are made of various materials. In wastewater, such oxidizable pollutants as organic compounds are oxidized at the anode surface, and such reducible pollutants as most inorganic metal cations are reduced and deposited (in most cases) at cathode surfaces. To bring

about the required reaction, as certain electropotential is applied to the anode and cathode (Jian, 1997). Electrochemical methods are employed mainly for metal ion elimination such as the recovery of copper and lead (Loomba and Pandey, 1993) and zinc (II), cobalt (II) and cadmium (Ho *et al.,* 1990). Another interesting application of electrochemical method is cyanide oxidation in wastewater. In most metal finishing and hydrometallurgical industrial wastewaters containing metal ions (such as gold, silver, chromium) and cyanides, the electrochemical method has an advantage in that simultaneously cyanide is decomposed (oxidized) at the anode and heavy metals are deposited (reduced) at the cathode without causing a sludge problem (Mylonas and Papaconstantinou, 1994).

According to Mylonas and Papaconstantinou (1994), electrochemical technology can be used for removal of wide range of metals from industrial waste water while the main limitation to this technology is that the treatment plant is highly expensive to set up.

2.6.3 Reverse Osmosis (RO)

Osmosis is a naturally occurring phenomenon and one of the most important processes in nature (Mylonas and Papaconstantinou, 1994). Whereas osmosis occurs naturally without energy required, to reverse the process of osmosis energy is applied to the more saline solution. Reverse osmosis, commonly referred to as RO, is a process where water demineralized or deionized is pushed under pressure through a semi-permeable membrane and the contaminants are not allowed through. The amount of pressure required depends on the concentration of the contaminants in the waste water. The more concentrated the waste water, the more pressure is required to overcome the osmotic pressure (Jian, 1997). A reverse osmosis semi-permeable

membrane is designed in such a way to allow free passage of water molecules but not the majority of dissolved salts, organics, bacteria and pyrogens. At the end of the whole process, two types of water are produced out of the RO system: good water and bad water. The good water that comes out of an RO system has the majority of contaminants removed and is called permeate (product water) which is the water that was pushed through the RO membrane and contains very little contaminant while the bad water is called concentrate, brine or "rejected water" (Mylonas and Papaconstantinou, 1994)**.** Below are some of the advantages and disadvantages of reverse osmosis (Jian, 1997).

Advantages of Reverse Osmosis

1. Reverse Osmosis is capable of removing up to 99% of the dissolved salts (ions), particles, colloids, and organics from the feed water.
2. It is simple and reliable process.
3. Compact design requires less space for installation.
4. Fully automatic operation with auto-start and auto-off.
5. Suitable for raw water from all types of sources like bore well, overhead storage tanks, water tankers and even municipal taps.
6. Colloidal SiO_2 can be removed by R.O. which cannot be removed by other methods.
7. The life of semi-permeable membrane is about two years and it can be easily replaced within few minutes, thereby nearly uninterrupted water supply can be provided.

Disadvantages of Reverse Osmosis

1. Require high pressure, membrane scaling and expensive to operate and maintain.
2. RO is not a reliable technology for the elimination of bacteria and virus.

3. An RO membrane is not suitable for the removal of contaminants with molecular weight less than 200. This is because the greater the ionic charge of the contaminant, the more likely it will be unable to pass through the RO membrane. Likewise, this is why an RO system does not remove gases such as CO_2 very well because they are not highly ionized (charged) while in solution and have a very low molecular weight.
4. Due to the low molecular weight of most gasses such as CO_2, an RO system does not remove gases as a result of which the permeate water can have a slightly lower than normal pH level depending on CO_2 levels in the feed water as the CO_2 is converted to carbonic acid.

2.6.4 Evaporation

Evaporation, or distillation, is a separation process that takes advantage of the changing physical states of water, or other solvents, from liquid to vapor. It is unique from other separation processes, in that the water is removed from the contaminants rather than the pollutants being filtered from the water (Mylonas and Papaconstantinou, 1994). Evaporators are categorized by heating source, circulation method, and heat exchanger devices. The heating source for evaporation can be supplied by hot water, steam, thermal fluids, flue gases, and electrical energy either directly through resistance, or indirectly with a heat pump or mechanical vapor recompression. Circulation methods include natural circulation, forced recirculation, scraped, and thin film. Direct heat exchange techniques include immersion heaters, submerged combustion, or submerged steam. Indirect heat exchange techniques include internal or external shell and tube heat exchangers, plate heat exchangers, or heating jackets **(**Kirkelund *et al.,* 2009).

The coarse solid matters and the free oils are separated; the waste water is filtered once again and conveyed to the evaporation plant. The waste water is concentrated to approximate 30% in a falling film pre-evaporator (Jensen *et al.*, 2007). This part of the plant is heated by mechanical vapour recompression, which is an energy-saving process. High concentration takes place in a forced circulation evaporator. In most of the cases, the vapour condensate still contains oil and has to be transferred to a further treatment before being discharged to the sewage treatment plant or before it can be re-used (Mylonas and Papaconstantinou, 1994). According to Jensen *et al.* (2007) they highlighted some of the advantages and disadvantages as presented below**.**

Advantages of Evaporation

1. The treated water can be discharged as surface water or re-used as process water.
2. Treated water is clean, free of salts, solids and particles.
3. Addition of chemical substances and of auxiliary substances is not required.
4. No formation of additional sludge portions.
5. No risk of clogging and blocking of plant parts.

Disadvantage of Evaporation

1. Water evaporation and heat drying are currently expensive and require fuel consumption to remove the water.

2.6.5 Chemical Oxidation

Chemical oxidation is one of waste water treatment processes which involve the generation of hydroxyl radicals in sufficient quantity to effect water purification. The hydroxyl radical (OH) is a powerful, non-selective chemical oxidant, which acts very rapidly with most organic compounds (Brillas *et al*., 2009). During chemical oxidation process, the generated hydroxyl

radicals aggressively attack virtually all organic compounds in the waste water. Depending upon the nature of the organic species, two types of initial attack are possible: the hydroxyl radical can abstract a hydrogen atom from water, as with alkanes or alcohols, or it can add itself to the contaminant, as in the case of olefins or aromatic compounds. The attack by the OH radical, in the presence of oxygen, initiates a complex cascade of oxidative reactions leading to mineralization of the organic compound (Mouli *et al.,* 2004). The exact routes of these reactions are still not quite clear. For example, chlorinated organic compounds are oxidized first to intermediates, such as aldehydes and carboxylic acids, and finally to CO_2, H_2O, and the chloride ion (Priambodo *et al.,* 2011). Nitrogen in organic compounds is usually oxidized to nitrate or to free N_2, sulphur is oxidized to the sulphate. Cyanide is oxidized to cyanate, which is then further oxidized to CO_2 and NO_3 (Nikonenko *et al.,* 2010). Chemical oxidation systems can be used to treat organic chemicals that are normally toxic or refractory to microorganisms, in effect serving either as detoxification or refractory organic "softening" pretreatment steps for biological processes or as self-contained processes for transformation of organic or inorganic contaminants to environmentally acceptable oxidation /reduction products (Priambodo *et al.,* 2011). Large quantities of suspended or dissolved solids quickly consume chemical oxidants, so waters targeted for such treatment should have relatively low concentrations of oxidizable background material, in the range of no more than a few hundred mg/l of total organic carbon (Nikonenko *et al.,* 2010). The following are some of the advantages and disadvantages of chemical oxidation (Jensen *et al.,* 2007).

Advantage of Chemical Oxidation

1. Chemical oxidation process is not dependent on electrical energy as the source of energy there by making it suitable technology for developing countries where electricity is a major challenge.

Disadvantage of Chemical Oxidation

1. The whole process is dependent on the inputs of energy in the form of chemical oxidants, which increases operating costs.
2. Some quantity of these chemical oxidants are still disposed to the environment mostly water bodies thereby affecting aquatic life.

2.6.6 Ion Exchange

Ion exchange (IE) otherwise known as water softening is a water treatment method where one or more undesirable contaminants are removed from water by exchange with another non-objectionable, or less objectionable substance. Both the contaminant and the exchanged substance must be dissolved and have the same type (positive/ negative) of electrical charge (Dyer *et al.,* 1993). In the context of water purification, ion-exchange is a rapid and reversible process in which impurity ions present in the water are replaced by ions released by an ion-exchange resin. The impurity ions are taken up by the resin, which must be periodically regenerated to restore it to the original ionic form (an ion is an atom or group of atoms with an electrical charge) (Priambodo *et al.,* 2011). Positively-charged ions are called cations and are usually metals while negatively-charged ions are called anions and are usually non-metals. During operation there are two basic types of resin, cation-exchange and anion-exchange resins. Cation exchange resins will release hydrogen (H^+) ions or other positively charged ions in exchange for impurity cations present in the water. Anion exchange resins will release hydroxyl (OH^-) ions or other negatively charged ions in exchange for impurity anions present in the water (Dyer, *et al.,* 1993). Today's modern ion-exchange resins are prepared from synthetic polymers such as styrenedivinylbenzene copolymers which have either been sulphonated to form strongly

acidiccation- exchangers or aminated to form strongly basic or weakly basic anion-exchangers (Dyer, *et al.,* 1993).

There are three ways in which ion-exchange technology can be used in water treatment and purification: first, cation- exchange resins alone can be employed to soften water by ion exchange; secondly, anion-exchange resins alone can be used for organic scavenging or nitrate removal and thirdly, combinations of cation- exchange and anion-exchange resins can be used to remove virtually all the ionic impurities present in the feed water, a process known as deionisation. The first two technologies are forms of water treatment in which either the chemical nature of the impurities is changed (as in base-exchange softening) or certain impurities are selectively removed (as in organic scavenging or nitrate removal). By contrast, deionisation is a purification process which can produce water of exceptionally high quality (Jensen *et al.,* (2007). Some of the advantages and disadvantages of ion-exchange technology in water treatment technology include the following (Priambodo *et al.,* 2011):

Advantages of Ion Exchange

1. Ion-exchange is generally suitable for treatment of groundwater.
2. Ion-exchange plants are relatively simple to automate and are suitable for installation at groundwater abstraction sites.
3. Very little energy is required, the regenerant chemicals are cheap and if well maintained resin beds can last for many years before replacement is needed.

Disadvantages of Ion Exchange

1. Ion exchange technology is not suitable for surface waters in which the content of organics and suspended solids could compromise the performance of the resin.
2. It is relatively expensive to operate.
3. Ion-exchange increases the chloride content of the water, which will tend to make it more corrosive, especially towards brass.
4. It is not a suitable technology for the removal of organic matter from wastewater.
5. The ion exchange resins themselves can be a source of non-ionized organic contamination.

There is usually a case of bacterial contamination as a result of the inability of the resin beds to act as filters for the removal of bacteria or other microorganisms. They often tend to worsen such contamination as traces of organic matter, which invariably accumulate, constitute a nutrient source for continued growth of microorganisms.

2.7 Bioaccumulation as an Alternative in the Treatment of Waste Water

Bioaccumulation is an active process dependent upon metabolic energy of microorganisms. In other words, bioaccumulation is an energy-dependent heavy metal transport system (Gadd, 1988).

Gadd (1988) and Brierley (1990) have described the many ways in which bacteria, fungi and algae can take up toxic metal ions. Heavy-metal ions can be entrapped in the cellular structure and subsequently biosorbed onto the binding sites present in the cellular structure. This method

of uptake is independent of the biological metabolic cycle and is known as ‘‘biosorption’’ or ‘‘passive uptake’’. The heavy metal can also pass into the cell across the cell membrane through the cell metabolic cycle. This mode of metal uptake is referred to as ‘‘active uptake’’. The metal uptake by both active and passive modes can be termed as ‘‘bioaccumulation’’. Most of the studies dealing with microbial metal remediation via growing cells describe the biphasic uptake of metals, i.e., initial rapid phase of biosorption followed by slower, metabolism-dependent active uptake of metals (Garnham *et.al.*, 1992; Donmez and Aksu, 1999).

Some of the benefits of bioaccumulation as a means of bioremediation over conventional method are summarized below (EPA, 2001).

1. Works on a variety of organic and inorganic compounds
2. Can be done either on-site or off-site
3. Easy to implement and maintain
4. Low-cost compared to other treatment methods
5. Environmentally-friendly and aesthetically pleasing
6. Reduces the amount of wastes to be land filled

2.8 Mechanism of Bioaccumulation by Fungi

Although fungi are a large and diverse group of eukaryotic microorganisms, three groups of fungi have major practical importance: the molds, yeasts and mushrooms. Filamentous fungi and yeasts have been observed in many instances to bind metallic elements (Wang and Chen, 2009). Fungi are ubiquitous in natural environments and important in industrial processes. A range of morphologies are found, from unicellular yeasts to polymorphic and filamentous fungi, many of

which have complex macroscopic fruiting bodies. Their most important roles are; as decomposers of organic materials, with concomitant nutrients cycling, as pathogens and symbionts of animals and plants, and as spoilage organisms of natural and synthetic materials, e.g. wood, paint, leather, food and fabrics. They are also utilized as producers of economically important substances, e.g. ethanol, citric acid, antibiotics, polysaccharides, enzymes and vitamins (Gadd, 1993).

The process of biosorption and bioaccumulation of metals by fungi is not new. These microorganisms are known to detoxify metals by several mechanisms including ion exchange, chelation, adsorption, crystallization, valence transformation, extra and intracellular precipitation and active uptake (Gadd, 1993). In other words the accumulation of metals from solutions by fungi can be divided into three categories:

(1) Biosorption of metal ions on the surface of fungi,

(2) Intracellular uptake of metal ions, and

(3) Chemical transformation of metal ions by fungi (Sing, 2006)

Biosorption can use both living and non-living biomass in the processes as it frequently exhibits marked tolerance towards adverse conditions like heavy metals. In the case of biosorption processes for heavy metal concentration using non-living biomass, the metabolic activity needed for intracellular metal accumulation is absent, meaning that nonliving fungal biomass does not depend on growth, metabolic energy, and transport needs. The problem of toxicity of metals does not affect this type of biomass, which is seen as one major advantages of biosorption. Living fungal biomass is required in the last two categories. This process is a much more complex

bioaccumulation phenomenon based on active metabolic transport, and biosorption is based mainly on the "affinity" between the (bio-) sorbent and sorbate (Volesky, 2007). The biosorption phenomenon is a direct competitor of ion exchange in what concerns to wastewater treatment systems application for remedial processes. Therefore, the importance of the field of biosorption cannot be overestimated. Within this framework, not only can more effective engineering process design/optimization tools be developed, but also a contribution from the marketing and biomass supply sides would be most useful and very desirable for the start-up of viable commercial enterprises (Volesky, 2007). Metal sorption and accumulation depends on diverse factors, such as pH, temperature, organic matter, ionic speciation and the presence of other ions in solutions, which may be in competition, etc. (Scheuhammer, 1991).

Biosorption process using organism biomass has been studied extensively as an important preconcentration-separation method for heavy metals at trace level (Kuyucak *et al.*, 1990). Therefore, biosorption has been defined as the property of certain biomolecules (types of polymer or biomass) to bind and concentrate selected ions or molecules from aqueous solutions. Fungi under stress develop several mechanisms in order to tolerate adverse conditions. They develop adaptation through a temporary alteration in their developmental patterns or by modifications on physiological characteristics, depending on the toxicity of the metals, which in turn is influenced by the concentration and by the salt form in which the metal exists (Nazareth and Marbaniang, 2008).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Sample Area

Kano metropolis is the major centre of textile manufacturing and leather processing with several establishments, mainly in Challawa, Sharada and Bompai industrial areas (Akan *et al*., 2009a). The factories generate wastewater containing significant quantities of chromium and other heavy metals that have been used to irrigate agricultural land overlying an important aquifer ever since they were established. Inhabitants of the metropolis and surrounding villages are dependent on these surface and groundwater resources for public water supply. Wastewater is discharged from the factories' sewage system into a complex system of open canals with some of the water being used directly for irrigation; the remaining flowing into Challawa River (Latitude 11^{o} 52m 41s N, Longitude 08^{o} 28m 09s E, 418m above sea level). Challawa River originates from western parts of Kano State and some south eastern parts of Katsina State. The river flows into the Hadejia, Jama'are, (Yobe, Komadugu) River basin and finally empties into Lake Chad to the North east (Tijjani, 2014).

Challawa industrial estate is along Panshekara Road (Fig. 3.1). It is bordered to the West by Zawachiki town, to the East by Kumbotso town, to the South by Yadanko village and to the North by Panshekara town. The industrial estate houses various industries such as tanneries, pulp and paper, beverages, ceramics, water bottling and textile industries. The entire area around the canals and river is characterized by several other human activities ranging from farming, fishing, bathing, washing, and sand mining. The entire length of the river is generally characterized by this trade of sandy soil and is the recipient of direct discharge of these untreated effluents.

PLATE 1: Satellite Image of Challawa Industrial Estate of Kano State Showing Challawa River

Source: Google Image 2015 (Tijjani, 2014).

3.2 Collection and Handling of Samples

A total of 50 samples were collected for this study which comprises 20 textile effluent samples and 30 water samples (10 samples from discharge point, 10 samples from upstream and 10 samples from downstream). Textile effluents and water samples were collected in sterile containers previously cleaned by washing using detergent, rinsed with tap water, later soaked in 10% HNO_3 acid for 24 hours and finally rinsed with deionized water prior to usage (Ezike *et.al.,* 2012).

The samples were labeled at the site of collection and placed in an ice packed coolers to maintain low temperature of about 4^oC and transported immediately to microbiology laboratory, Ahmadu Bello University, Zaria for further analysis (APHA, 1992; Patrungo *et al.,* 2007).

3.3 Assessment of Physicochemical Parameters and Heavy Metals of the Textile Effluent and Water Samples

Standard methods for the assessment of heavy metals and physico-chemical contents of effluent and water samples were used during the course of this study as described in details below.

3.3.1 Temperature

Temperature (Celsius) was measured on site using procedure provided in the HACH conductivity/TDS/temperature Meter (model 44600.00) manual. The electrode of the meter was immersed into each sample for about 1minute to permit accurate and stable reading and the temperature icon on the meter was pressed. The temperature value in Celsius was displayed on the screen of the meter and the reading was recorded.

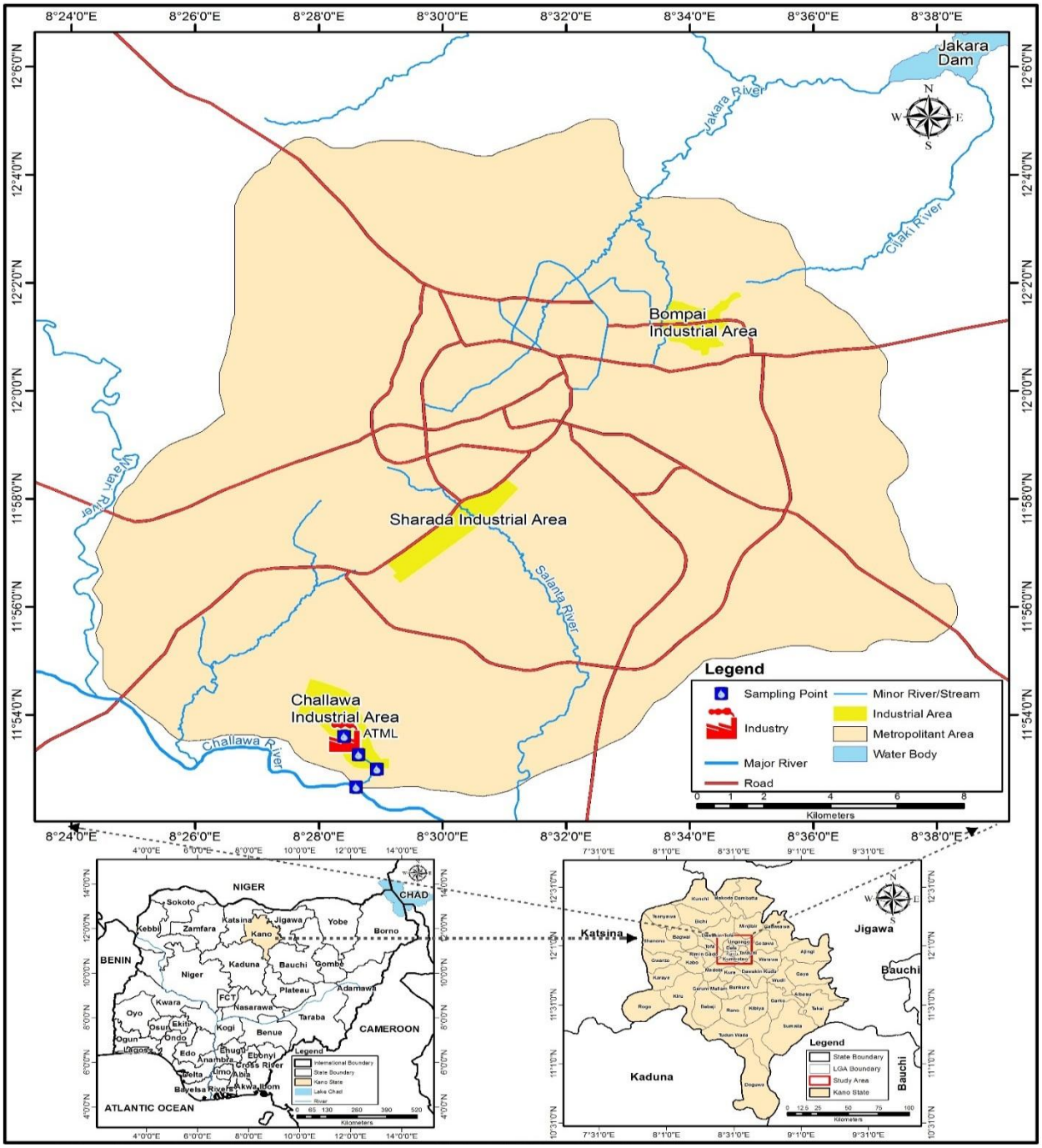

Figure 1: Map of Kano Showing textile Industries and Sampling Points in Challawa Industrial Estates of Kano Nigeria. : Adapted and modified from the administrative map of Kano.

3.3.2 pH

The pH was measured on site using a Hanna instrument pH 210 microprocessor pH meter. The electrode of the pH meter was rinsed thoroughly with distilled water. The pH meter was standardized to a pH of 7.0 (neutral) as instructed in the manual by immersing it into a buffer solution. The pH meter was adjusted using a knob until the pH meter read 7.0. The electrode was then removed and inserted into each of the textile effluent and water samples and the pH of the samples was taken after the reading has stabilized.

3.3.3 Electrical conductivity

Electrical conductivity (in micro siemens per meter µS/m) was measured on site using procedure provided in HACH conductivity/TDS/temperature Meter (model 44600.00) manual as described below.

The textile effluent and water samples were gently shaken to mix. The electrode of the meter was rinsed thoroughly with distilled water and then immersed into each of the samples for about 1minute to permit accurate and stable reading and the electrical conductivity icon on the meter was pressed. The electrical conductivity values in micro siemens per meter (µS/m) was displayed on the screen of the meter and was recorded.

3.3.4 Turbidity

The turbidity value of the samples were analyzed on site using procedure provided in the DR/24 portable turbidity meter HACH manual as described below:

The turbidity meter was switched on by pressing the ‘on icon’ on the turbidity meter. Using a graduated cylinder, 10ml of the distilled water sample was transferred into a round sample cell bottle to serve as the blank. In order to prepare the test sample, 10mL of the textile effluent sample was transferred into a second round sample cell bottle. The blank cell bottle was cleaned using a tissue paper and inserted into the cell holder. The timer and Ok icon was pressed simultaneously and a five minutes reaction period was observed as the turbidity meter counted down to zero indicating that the turbidity meter was ready to analyze the textile effluent sample. The blank round cell bottle was removed from the cell holder and the prepared test sample containing the effluent sample was inserted into the cell holder and the read icon was pressed. Result of the turbidity value of the effluent sample was displayed on the screen of the turbidity meter in Nephelometric Turbidity Unit (NTU) and was recorded.

3.3.5 Dissolved oxygen (DO)

Dissolved oxygen is important in water systems for its effect on other chemicals in the water; it oxidizes, organic and inorganic matter altering their chemical and physical states and their capacity as a nuisance to the consumers (APHA, 1992). The type of life in a natural water depends on the amount of DO present. Most microorganisms use free DO for respiration APHA, 1992).

The level of the dissolved oxygen in the textile effluent and water samples was measured on site using procedure provided in HACH DRELL/2400 manual. The textile effluent and water samples were gently shaken to mix. The electrode of the meter was rinsed thoroughly first with distilled water and then immersed into each of the sample for about 1minute to permit accurate

and stable reading and the dissolved oxygen icon on the meter was pressed. The dissolved oxygen value in milligramme per litre (mg/l) was displayed on the screen of the meter and was recorded.

3.3.6 Biological oxygen demand (BOD)

Biological oxygen demand (BOD) is the amount of oxygen required by microorganisms to stabilize biologically decomposable organic matter in water under aerobic condition, the BOD test is widely used to determine (1) the degree of pollution in water bodies at any time and their purification capacity (2) the pollution load of waste water and (3) efficiency of water treatment plant. The BOD was determined following the method of Ademoroti (1996).

The textile effluent and water samples were pretreated with 0.5M acid (HCl) or 1M alkali (NaOH) to a pH 7. Using a volumetric flask, 60ml (20%) of the textile effluent was transferred into two separate 300ml BOD bottles and covered with a glass stopper. The BOD bottles were labeled DO_1 and DO_5 representing the initial dissolved oxygen of day 1 and final dissolved oxygen of day 5 after incubation respectively. The BOD bottles containing the samples were then filled to the brim with dilution water (procedure for the preparation of dilution water is described in Appendix XII). The BOD bottles were gently covered using a stopper without leaving any air bubble inside the bottle. The initial dissolved oxygen (DO_1) was measured and recorded on that same day using procedure provided in HACH DRELL/2400 manual as described above The second BOD (DO_5) was incubated for five days in the dark at 20^0C in a

incubator. After the incubation period, the final dissolved oxygen content was measured and recorded. The BOD of the textile effluent and water samples was calculated using the formula;

$$BOD\ (mg/L) = (DO_1 - DO_5) \times \frac{\text{volume BOD bottle used}}{\text{ml of sample used}}$$

3.3.7 Nitrate

The nitrate level in the textile effluent and water samples was analyzed using procedure provided in the DR/2400 portable spectrophotometer HACH manual as described below:

The HACH program icon on the spectrophotometer was pressed and the code for nitrate analysis (355N, Nitrate HR) was selected after which the start icon was pressed. Using a graduated cylinder, 10mL of the sample was transferred into a round sample cell bottle and one sachet of nitrate reagent powder pillow was added to the textile effluent sample and was gently swirled to mix (an amber color was observed indicating the presence of nitrate in the sample). The timer and Ok icon was pressed simultaneously and a five minutes reaction period was observed as the spectrophotometer count down to zero. A blank was prepared by measuring 10ml of the textile effluent and water sample to a second round cell. The body of the blank cell bottle was wiped and placed into the cell holder of the spectrophotometer. The zero icon on the spectrophotometer was pressed and the 0.0mg/ml NO_3^- —N was displayed on the screen of the spectrophotometer indicating that the spectrophotometer was ready to analyze the sample. The blank round cell bottle was removed from the cell holder and the prepared sample containing the nitrate reagent pillow was placed into the cell holder and the read icon was pressed. The result of the nitrate

level in the sample was displayed on the screen of the spectrophotometer in mg/ml NO_3^-—N and was recorded.

3.3.8 Phosphate

The phosphate level in the samples was analyzed using procedure provided in the DR/2400 portable spectrophotometer HACH manual as described below:

The HACH program icon on the spectrophotometer was pressed and the code for phosphate analysis (480 P) was selected after which the start icon was pressed. To prepare the blank, 25ml of deionized water was measured and transferred to a round sample cell. To prepare the sample, 25ml of the textile effluent sample was measured and transferred to a second round sample cell bottle, 1.0ml of molybdovanadate reagent was added to each sample cell and gently swirled to mix. Using a graduated cylinder, 25ml of the sample was added into the second round sample cell bottle. The timer and Ok icon was pressed simultaneously and a three minutes reaction period was observed as the spectrophotometer count down to zero. The blank (the first round cell bottle) was placed into the cell holder and the zero icon was pressed and 0.0 mg/ml PO_4^- was displayed on the spectrophotometer, the body of the prepared sample (second cell bottle) was wiped and placed into the cell holder of the spectrophotometer and the read icon was pressed. The result of the phosphate level in the sample was displayed on the screen of the spectrophotometer in mg/ml PO_4^- and was recorded.

3.3.9 Sulphate

The sulphate level in the textile effluent and water samples was analyzed using procedure provided in the DR/2400 portable spectrophotometer HACH manual as described below;

The HACH program icon on the spectrophotometer was pressed and the code for sulphate analysis (680, Sulphate) was selected after which the start icon was pressed. Using a graduated cylinder, 10ml of the sample was transferred into a round sample cell bottle and one sachet of sulphate reagent powder pillow was added to the sample and was gently swirled to mix. The timer and Ok icon was pressed simultaneously and a five minutes reaction period was observed as the spectrophotometer count down to zero. A blank was prepared by measuring 10ml of the sample to a second round cell bottle. The body of the blank cell bottle was wiped and placed into the cell holder of the spectrophotometer. The zero icon on the spectrophotometer was pressed and the 0.0mg/ml SO_4^{2-} was displayed on the screen of the spectrophotometer indicating that the spectrophotometer was ready to analyze the sample. The blank round cell bottle was removed from the cell holder and the prepared sample containing the sulphate reagent pillow was placed into the cell holder and the read icon was pressed. The result of the sulphate level in the sample was displayed on the screen of the spectrophotometer in mg/ml SO_4^{2-} and was recorded.

3.3.10 Total solids

Total solids in the samples were determined following the procedure described by APHA (1992). 100ml of the sample was filtered using 0.5 mm Whatman filter paper and placed in a pre-weighed crucible dish. The dish and the content were then dried to complete dryness in an oven at 103°C for 2 hours. After dying, the crucible dish was transferred into a desiccator and allowed

to cool for 1 hour 30 minutes. The initial weight of the empty crucible dish was subtracted from the weight of crucible after drying to give the weight of total residue. The total solids were then calculated using the formula below:

$$\text{Total solids} = \frac{\text{weight of total residue}}{\text{volume (ml) of sample}} \times 1000 \text{ (mg/l)}$$

3.3.11 Total dissolved solids

Total dissolved solids (mg/l) were measured on site using procedure provided in the HACH conductivity /TDS /temperature Meter (model 44600.00) manual. The samples were gently shaken to mix and the electrode of the meter was rinsed thoroughly with distilled water. The electrode of the meter was then immersed into each sample for about 1minute to permit accurate and stable reading and the total dissolved solids icon on the meter was pressed. The total dissolved solids value in mg/l was displayed on the screen of the meter and was recorded.

3.3.12 Total suspended solids

To calculate the total suspended solids, total dissolved solids was deducted from total solids (APHA, 1992).

3.3.13 Assessment of Cr, Cu and Zn contents of the raw effluent and water samples

Determination of chromium, copper and zinc in the samples was carried out using acid digestion method as described by Abida *et al,* (2009) and Thippeswany *et al,* (2012). For the procedure,

20ml of the effluent and water samples were heated in a beaker on a hot plate for 20 minutes, 9ml of Hydrochloric acid (HCl) and 3ml of nitric acid (HNO_3) were added into the sample in the ratio of 3:1 for 15 minutes. When brownish fumes were evident, the sample was removed from the heat and allowed to cool. The samples were then filtered using a Whatman's no. 1 filter paper and the filtrates were diluted up to 50ml with distilled water and transferred to a 50 ml plastic container. The samples were then analyzed for heavy metal content using Schemadzu atomic absorption spectrophotometer (AAS) model AA 6800.

3.4 Isolation of Fungi from Textile Effluent and Water Samples

Fungal isolation was carried out according to the method described by Ijah (1998). The textile effluent and water samples were inoculated each onto Potato dextrose broth (PDB) for pre-enrichment purpose for a period of one week. After a period of one week 1ml of the broth culture was inoculated onto Potato Carrot agar (PCA) supplemented with 100mg/100ml of chloramphenicol to suppress bacterial growth using sterile bent glass rod and incubated at room temperature for a period of four to seven days. The experiments were carried out in duplicates

3.4.1 Preservation of Fungal Isolates

After the incubation period, pure fungal colonies were obtained by subculturing distinct colonies from the culture plate onto fresh solidified Potato Carrot agar (PCA) plates and incubated for another four days after which each pure culture was sub cultured onto freshly prepared PCA slant supplemented with 100mg/100ml of chloramphenicol to suppress bacterial growth and preserved for further analysis (Liu *et al.,* 1997).

3.4.2 Preparation of Heavy Metal Stock Solution

Stock solution of 1000mg/L (equivalent to 1000ppm) of chromium, copper and zinc were prepared by dissolving 1000mg of analytical grade salts of Copper (II) sulphate pentahydrate ($CuSO_4.5H_2O$), Potassium dichromate (K_2Cr2O7) and Zinc sulphate (ZnSO4) separately in sterilized 1 liter of distilled water. The desired 20 and 30 ppm concentrations of the heavy metals solutions were prepared from the stock solution (Thippeswany *et al.,* 2012).

3.4.3 Minimum Inhibitory Concentrations (MIC) and Screening of Heavy Metal-Resistant Fungi Isolates

The minimum inhibitory concentrations of the isolates were determined as the lowest concentration of heavy metal that can inhibit visible growth of the isolates (Iram *et al*., 2012b). Selected fungal isolates were cultured in varying concentration of chromium, copper and zinc (20ppm and 30ppm) in conical flasks. The heavy metal salts were added seperately to 50ml of potato dextrose broth (PDB) medium. The conical flasks were placed on a rotary shaker for seven days at room temperature (Iram *et al*., 2012a). The presence or absence of growth was observed and recorded. Similarly, a control was set up without the heavy metal salt (Akhtar *et al*., 2013). Also the effect of each heavy metal on the growth of the fungal isolates was estimated individually by weighing (the harvested fungal biomass) against control (without heavy metal). The heavy metal tolerance index (Ti) was calculated as the ratio of the treated biomass to that of the untreated biomass as expressed in the equation below (Akhtar *et al*., 2013).

$Ti= W_t/W_u$

Where W_t= weight of the treated fungal biomass (mycelium) with heavy metals (mg)

W_u= weight of the untreated fungal biomass (mycelium) without heavy metals (mg)

3.4.4 Identification and Characterization of Heavy Metal Resistant Fungi

Isolates that were able to grow in the presence of the heavy metal salts were identified and characterized. Macromorphological characteristics such as color, texture, of the reversed side of the colony were observed and recorded. For the micromorphological characteristics, small portion of the growth were mounted on clean grease free slide with a drop of lactophenol cotton blue stain and covered with cover slip and were observed microscopically using X40 objective lens (Thippaswamy *et al.,* 2012). Characteristics of the sexual reproductive structures, presence or absence of septation, spore and chlamydospore were observed and recorded. Each fungal isolate was carefully compared and identified using appropriate taxonomic guide as described by Barnett and Hunter (1999); Larone (2002); Webster and Webber (2007); Ellis *et al.* (2007).

3.5 Bioaccumulation Experiment

The already identified screened heavy metal resistant fungi were evaluated for the uptake of chromium, copper and zinc in Potato dextrose broth containing the effluent sample previously sterilized by autoclaving at 121^{o}C under 15lb/sq for 15minutes in order to eliminate any form of microorganism present in the textile effluent sample prior to the inoculation of the selected screened heavy metal resistant fungal isolates (Dwivedi *et al.,* 2012). The broth and the effluent were mixed in the ratio of 4:1 (120ml of broth and 30ml of the sterilized effluent sample) in 250ml Erlenmeyer flask (Joshi, 2011; Dwivedi *et al.,* 2012). Each of the eight screened fungi tolerant to chromium, copper, and zinc were inoculated separately into Erlenmeyer flasks. The inoculated Erlenmeyer flasks were placed in a rotary shaker for seven days at room temperature (Iram *et al*., 2012a). A similar experiment was set up without inoculating any of the isolates to serve as control. The experiment was carried out in duplicates.

3.5.1 Analysis of Heavy Metals Content in the Textile Effluent and Water Samples after the Bioaccumulation Experiment

After incubation period of one week, the content of each Erlenmeyer flask was aseptically filtered through a pre-weighed sterile Whatman's No. 1 filter paper to separate the mycelia from the culture filtrate. The concentrations of chromium, copper, and zinc left in the filtrate after the bioaccumulation experiment were analyzed as previously described in 3.3.13 of the present study.

3.5.2 Determination of Dry Weight of Fungi Biomass after Bioaccumulation Experiment

The filter paper along with the mycelia that were harvested from the growth medium (through sieving using Whatman's No. 1 filter paper and rinsed with distilled water) was later dried in hot air oven at 70^{o}C for 18 hours (overnight) and weighed thereafter using analytical weighing balance. Differences between the weight of the filter paper bearing the mycelial mat and weight of the pre-weighed filter paper represent the fungal biomass, which was expressed in terms of dry weight of mycelial mat (mg) (Thippeswany *et al.,* 2012)

3.5.3 Estimation of Heavy Metals Uptake by the Fungal Isolates Per Unit Mycellium Biomass

The heavy metal uptake of each of the eight selected screened heavy metal resistant fungal isolates per unit mycelium biomass used in this study was calculated using the formula given below, in accordance with the methods described by Viraghawan *et al.* (1999) and Joshi *et al*. (2011)

$$Q = \frac{[V \times Ci - Cf]}{W} \times 1000$$

Where;

Q = Concentration of heavy metal uptake accumulated per unit mycelium biomass by the fungal isolates (mg/ g).

V = Volume of metal solution (textile effluent) (ml)

Ci = Initial concentration of metal in the effluent before bioaccumulation (ppm)

Cf = Final concentration of metal in the effluent after bioaccumulation (ppm)

W = Dry weight of the fungal biomass (g)

3.5.4 Estimation of Percentage Removal Efficiency of Heavy Metals by the Selected Fungal Biomass

The percentage removal efficiency of each of the selected heavy metal resistant fungi used in this study in the removal of the heavy metals present in the textile effluent were calculated using the formula given below, in accordance with the method of Kumar *et al.* (2011)

$$\text{Percentage removal efficiency \%} = \frac{\text{Con}_{\text{initial}} - \text{Con}_{\text{final}}}{\text{Con}_{\text{initial}}} \times 100$$

Where;

$\text{Con}_{\text{initial}}$ = Initial concentration of heavy metals in the effluent before bioaccumulation (ppm)

$\text{Con}_{\text{final}}$ = Final concentration of heavy metals in the effluent after bioaccumulation (ppm)

3.6.0 Statistical analysis

One way ANOVA was used to analyze the data that were obtained with the aid of statistical package for social sciences (SPSS) version 20. This is to find out if the rate of bioaccumulation of chromium, copper and zinc by the isolated fungi is statistically significant or not.

CHAPTER FOUR

4.0 RESULTS

4.1: Physico-Chemical Properties of Textile Effluents and Water from Challawa River

The physico-chemical properties of water samples from Challawa River into which the raw textile effluents are discharged were profoundly impacted at the discharge points. For instance, the pH, total solids (TS) and total dissolved solids (TDS), Nitrate, sulfate and to a lesser extent phosphate content of water samples collected at the discharge point were much higher than those of both upstream and downstream samples (Table 4.1). However, the marked increase in the total solids (TS) and total dissolved solid (TDS) as well as the nitrate and sulfate that resulted from the entry of the effluents at the discharge point were reduced in concentration downstream the river (Table 4.1).

It was also observed that, the Chromium (Cr), Copper (Cu) and Zinc (Zn) contents of water from the effluents recipient river were greatly increased to levels much higher than are considered safe to the environment and human health (Table 4.1). On the other hand, the temperature, electrical conductivity, dissolved oxygen (DO) and biological oxygen demand (BOD) of water from the recipient river was not drastically altered by the entry of the effluent even at the discharge point (Table 4.1). However, the dissolved oxygen (DO) level of water from the recipient river was much lower than is expected of water source meant for domestic uses.

4.1: Physico-Chemical Properties of Textile Effluent and Water Samples from Challawa River

Parameters Assessed	Samples					
	Effluent		Water			
	Raw samples(A)	Partially treated effluent (B)	Discharge point(C)	Upstream (D)	Downstream (E)	Permissible limit (NESREA)
pH	11.81	11.65	11.14	7.31	7.15	6.0-9.0
Temperature (^{0}C)	26.20	26.30	26.35	26.25	26.40	<40
Electrical conductivity (µs/cm)	2.72	3.07	0.78	0.12	0.08	2.0
Turbidity (NTU)	6.35	8.80	5.35	8.25	9.15	5*
Total dissolved solids (mg/l)	1360	1520	500	110	40	500
Total solids (mg/l)	2000	1530	520	120	170	2000
Total soluble solids (mg/l)	1.28	1.22	0.35	0.08	0.15	30
Biochemical oxygen demand (mg/l)	31.9	28.45	39.25	29.45	35.85	30
Dissolved oxygen (mg/l)	1.85	3.40	3.05	1.95	2.50	10
Nitrate (mg/l)	24.19	18.55	22.15	9.25	4.35	20
Sulphate (mg/l)	73.50	74.00	43.50	15.50	13.00	500
Phosphate (mg/l)	43.5	40.8	32.0	23.3	37.7	5.0
Chromium (ppm)	1.71	0.52	0.25	0.08	0.74	0.50
Copper (ppm)	2.30	0.56	0.49	0.16	0.43	0.10
Zinc (ppm)	6.34	5.61	5.91	6.24	6.33	<1

NTU= Nephelometric Turbidity Unit. µs/cm = Microsiemens per centimeter. mg/l= milligram per liter. ppm= parts per million. *= World health organization (WHO) Permissible standard NESREA= Nigerian Environmental Standards and Regulatory Enforcement Agency

4.2 Composition of Fungal Flora of the Effluent and Water Samples from Challawa River

Results of mycological analysis have shown that, the fungal flora of effluents and water samples from three points along the Challawa river was composed mainly of three species of Aspergillus namely; *A. niger, A. flavus and A. versicolor* along with *Penicillium* sp, *Chrysosporium* sp, *Microsporum* sp, *Rhizopus* sp *and Trichoderma* sp (Table 4.2). Of the six genera detected, all but *Trichoderma* sp were found to occur in all the samples of raw effluent, treated effluent and water samples collected at the point of effluent discharge into the recipient river (Table 4.2). Similarly, only *Penicillium* sp was not detected in water samples collected downstream of the discharge point (Table 4.2). On the other hand, water samples collected upstream of the discharge point were found to contain *Aspergillus* sp, *Rhizopus* sp and *Trichoderma* sp while *Penicillium* sp, *Microsporum* sp and *Chrysosporium* sp were not detected (Table 4.2).

However, it was observed that, the frequency with which member genera were detected in the various samples varied greatly (Table 4.3). For instance, of the 98 Isolates obtained in this study, 39 (40%), 27 (28%) and 21 (21%) were obtained from samples of raw effluents, partially treated effluents and water samples from effluent discharge point respectively (Table 4.3). Water samples collected from upstream and downstream of effluent discharge point on the other hand only yielded 5(5%) and 6(6%) of the total isolates obtained respectively (Table 4.3). Furthermore, it was noted that, the mycoflora of both the raw and treated effluents is dominated by members of the genera *Aspergillus* sp, *Chrysosporium* sp, *Penicillium* sp, *Microsporum* sp *Rhizopus* sp and *Trichoderma* sp. Of these, three species of *Aspergillus* namely; *A.niger, A.flavus* and *A.versicolor* as well as *Chrysosporium sp* were especially dominant (Table 4.3).

Table 4.2: The Fungal Flora of Textile Effluents and Water from Effluent Recipient River Challawa

Sites Sampled	Genera of Fungi Detected
Raw Textile Effluent	*Aspergillus, Penicillium, Chrysosporium, Microsporum* and *Rhizopus.*
Partially treated Textile Effluent	*Aspergillus, Penicillium, Chrysosporium, Microsporum, Rhizopus* and *Trichoderma*
Water sample from Discharge point	*Aspergillus, Penicillium, Chrysosporium, Microsporum, Rhizopus* and *Trichoderma*
Water samples from upstream	*Aspergillus, Trichoderma* and *Rhizopus.*
Water samples from Downstream	*Aspergillus, Penicillium, Trichoderma, Microsporum* and *Rhizopus.*

Table 4.3: Frequency of Occurrence of Fungal Isolates in the Textile effluent and Water Samples

Fungal Isolates detected	Raw effluent	Partially treated effluent	Effluent Discharge Point	Upstream Water Sample	Downstream Water sample	Total
Aspergillus niger	10	7	3	2	1	23
Aspergillus flavus	6	2	2	1	0	11
Aspergillus versicolor	4	5	4	0	0	13
Penicillium sp	5	2	1	0	1	9
Trichoderma sp	0	2	3	1	1	7
Chrysosporium sp	9	6	2	0	0	17
Microsporum sp	3	1	3	0	1	8
Rhizopus sp	2	2	3	1	2	10
Total	39	27	21	5	6	98

Together, the three species of *Aspergillus* accounted for 51 and 52 % of the total mycoflora of the raw and treated effluents respectively. This was followed by *Chrysosporium* sp and the three species of *Aspergillus* accounted for 51 and 52 % of the total mycoflora of the raw and treated effluents respectively. This was followed by *Chrysosporium* sp and *Penicillium* sp which accounted for 23 and 13% of the mycoflora of the raw effluent samples respectively (Table 4.3).

Members of the genus *Trichoderma* sp were conspicuously absent in the raw effluents and accounted for only 7% of the mycoflora of the treated effluents. Similarly, water samples collected from the point of discharge of effluents into Challawa River revealed the presence of same spectrum of genera of fungi found in the effluents with members of the genus *Aspergillus* sp accounting for 43% of the total mycoflora (Table 4.3). On the other hand, water samples collected upstream of the discharge point were found to contain fewer numbers of fungal genera than were found in the effluents and water samples collected at the point of discharge of the effluent into the river (Table 4.3). In these samples, only *A.niger, A. flavus, Rhizopus* sp and *Trichoderma* sp were detected at very low frequency. *Penicillium* sp, *Chrysosporium* sp and *Microsporum* sp were conspicuously absent. On the contrary, all but *Aspergillus flavus*, *Aspergillus versicolor* and *Chrysosporium* sp were detected in water samples collected from downstream of the discharge point but with much lower frequency (Table 4.3).

4.3: Resistance of Fungal Isolates to Cr, Cu and Zn *in vitro*

The resistance of each fungus isolated from the study sites to Cr^{2+}, Cu^{2+} and Zn^{2+} ions *invitro*, was assessed based on its capacity to grow in Potato Dextrose Broth charged with 20 and 30ppm of the metal ions.

Isolates that were able to grow with a yield of 80mg of mycelial biomass or more were considered to be resistant. Results obtained based on such *invitro* studies revealed that, fungal genera varied greatly in their resistance to the tested doses of Cr, Cu and Zn invitro. It was observed that, resistance to these metal ions was more wide spread among strains of *Aspergillus* sp followed by strains of *Chrysosporium* sp (Appendix XIII-XX). The least preponderance of resistance to Cr, Cu and Zn was recorded among isolates of *Trichoderma s*p. It was also observed that species of the same genus varied greatly in their resistance to the test metal ions. Of the 18 resistant strains of *Aspergillus* isolated, 10 (56%) were *A. niger* while *A. flavus* and *A. versicolor* accounted for 4 (22%) each (Appendix XIII-XX).

The frequency of resistant strains among the six genera isolated was also found to be strongly related to the source of the isolates. For instance, higher frequencies of resistant strains were observed among genera isolated from samples of the raw effluents (Table 4.4a), followed by those isolated from the treated effluents (Table 4.4b). On the other hand, only a few resistant strains were isolated from water samples collected at the effluent discharge point into the recipient river (Table 4.4c) and non from water samples collected upstream and downstream of entry of effluent into the river (Table 4.4d). This observation tends to suggest that the growth environment plays an important role in the development of resistance to the metal ions among members of mycoflora.

An assessment of the tolerance indices of the various strains tested to the three metal ions exhibited similar trends with strains able to bioaccumulate significantly higher quantities of the heavy metal ions exhibiting statistically higher tolerance indices (Table 4.5).

Table 4.4a: Frequency of Strains Resistant to Cr, Cu and Zn in vitro among Fungi Isolated from Raw Textile Effluent

Fungal Isolates Tested	Number of Isolates tested	Number of Strains Resistant** to 20 and 30ppm		
		Cr	Cu	Zn
Aspergillus niger	10	4	4	6
Aspergillus flavus	6	1	0	1
Aspergillus versicolor	4	2	3	3
Penicillium sp	5	3	2	2
Chrysosporium sp	9	5	3	3
Microsporum sp	3	2	2	2
Rhizopus sp	2	0	0	0
Total	39	17	14	15

** = Isolates which yielded > 80mg of mycelial biomass when grown in potato dextrose broth supplemented with 20 and 30ppm of Cr, Cu and Zn

Cr- Chromium

Cu- Copper

Zn- Zinc

Table 4.4b: Frequency of Strains Resistant to Cr, Cu and Zn in vitro among Fungi Isolated from Partially Treated Textile Effluents

Fungal Isolates Tested	Number of Isolates tested	Number of Strains Resistant** to 20 and 30ppm		
		Cr	Cu	Zn
Aspergillus niger	7	3	1	2
Aspergillus flavus	2	1	1	1
Aspergillus versicolor	5	0	0	0
Penicillium sp	2	0	0	1
Chrysosporium sp	6	2	1	2
Microsporum sp	1	1	1	1
Rhizopus sp	2	2	1	2
Trichoderma sp	2	0	0	0
Total	27	8	4	9

** = Isolates which yielded > 80mg of mycelial biomass when grown in potato dextrose broth supplemented with 20 and 30ppm of Cr, Cu and Zn.

Cr- Chromium

Cu- Copper

Zn- Zinc

Table 4.4c: Frequency of Strains Resistant To Cr, Cu and Zn Invitro among Fungi Isolated From Water Samples Collected At Point of Effluent Discharge into Challawa River

Fungal Isolates Tested	Number of Isolates tested	Number of Strains Resistant** to 20 and 30ppm		
		Cr	Cu	Zn
Aspergillus niger	3	0	0	0
Aspergillus flavus	2	0	0	2
Aspergillus versicolor	4	0	1	1
Penicillium sp	1	0	0	0
Chrysosporium sp	2	0	0	0
Microsporum sp	3	0	0	0
Rhizopus sp	3	0	0	0
Trichoderma sp	3	1	1	0
Total	21	1	2	3

** = Isolates which yielded > 80mg of mycelial biomass when grown in potato dextrose broth supplemented with 20 and 30ppm of Cr, Cu and Zn.

Cr- Chromium

Cu- Copper

Zn- Zinc

Table 4.4d: Frequency of Strains Resistant to Cr, Cu and Zn Invitro among Fungi Isolated from Water Samples Collected Upstream and Downstream of Effluent Discharge into Challawa River

Fungal isolates tested	Number of isolates tested		Number of Strains Resistant** to 20 and 30ppm					
			Cr		Cu		Zn	
	US	DS	US	DS	US	DS	US	DS
Aspergillus niger	2	1	0	0	0	0	0	0
Aspergillus flavus	1	ND	0	Nd	0	Nd	0	Nd
Penicillium sp	ND	1	Nd	0	Nd	0	Nd	0
Microsporum sp	ND	1	Nd	0	Nd	0	Nd	0
Rhizopus sp	1	2	0	0	0	0	0	0
Trichoderma sp	1	1	0	0	0	0	0	0
Total	5	6	0	0	0	0	0	0

** = Isolates which yielded > 80mg of mycelial biomass when grown in potato dextrose broth supplemented with 20 and 30ppm of Cr, Cu and Zn.
US = Upstream, DS = Down Stream
ND = Not Detected, Nd = not determined
Cr- Chromium

Cu- Copper

Zn- Zinc

Table 4.5: Mean Tolerance Indices of the Resistant Fungal Isolates to Cr, Cu and Zn

Fungal isolates tested	Tolerance index		
	Cr	Cu	Zn
Chrysosporium sp	0.340^{ab}	0.367^{a}	0.445^{a}
Aspergillus niger	0.356^{a}	0.328^{b}	0.334^{c}
Aspergillus flavus	0.207^{d}	0.088^{g}	0.306^{ed}
Penicillium sp	0.327^{b}	0.260^{c}	0.357^{b}
Aspergillus versicolor	0.134^{e}	0.178^{d}	0.311^{d}
Trichoderma sp	0.192^{d}	0.164^{e}	0.153^{g}
Rhizopus sp	0.212^{d}	0.055^{h}	0.183^{f}
Microsporum sp	0.276^{c}	0.104^{f}	0.303^{e}

Means with different superscripts across the rows and down the columns are statistically different ($P \leq 0.05$).

Cr- Chromium

Cu- Copper

Zn- Zinc

4.4: Capacity of Resistant Fungal Isolates To Remove Cr^{2+}, Cu^{2+} and Zn^{2+} Ions From Raw Textile Effluents

Results obtained indicate that, the six genera of fungi tested had the capacity to remove Cr^{2+}, Cu^{2+} and Zn^{2+} ions from broth cultures charged with raw textile effluents (Table 4.6). It was observed that, from 48.51to 78.64% of Cr, 38.19 to 67.37% of Cu and 36.11 to 85.94% of Zn were removed by the test strains. However, strains of *Chrysosporium* sp, *A. niger, A. flavus* and *Penicillium* sp proved to be the most efficient in the removal of Cr with the capacity to remove 78.64%, 68.21%, 64.52% and 63.37% respectively (Table 4.6). These were followed by *Aspergillus versicolor, Trichoderma* sp and *Microsporum* sp which removed 57.07%, 57.07% and 52.28% of Cr respectively. *Microsporum* sp was found to be the least efficient in the removal of Cr (Table 4.6). On the other hand, only *Chrysosporium* sp, *A. niger* and *A. flavus* proved to be efficient in the removal of Cu with performance rates of 67.37%, 64.09% and 63.85% respectively. These were followed by *Penicillium* sp, *Trichoderma* sp, *Rhizopus* sp and *Microsporum* sp in that order of decreasing efficiency (Table 4.6). Similar trend was observed in the efficiency of strains with regards to removal of Zn (Table 4.6). Thus, strain of *Chrysosporium* sp was found to remove 78.64% of Cr, 67.37% of Cu but same strain was found to remove up to 85.94% of Zn from the raw textile effluent (Table 4.6). Similar variations were observed for strains of the other genera of fungi tested.

Table 4.6: Capacity to Fungal Isolates to Remove Cr, Cu and Zn from Raw Textile Effluent

Fungal isolates tested	Capacity to remove (%)		
	Cr	Cu	Zn
Chrysosporium sp	78.64[a]	67.37[a]	85.94[a]
Aspergillus niger	68.21[b]	64.09[b]	62.24[b]
Aspergillus flavus	64.52[c]	63.85[b]	62.34[c]
Penicillium sp	63.37[d]	58.86[c]	54.10[d]
Aspergillus versicolor	57.07[e]	51.44[d]	49.27[e]
Trichoderma sp	55.31[f]	45.24[e]	45.74[f]
Rhizopus sp	52.28[g]	35.75[g]	41.21[g]
Microsporum sp	48.51[h]	38.19[f]	36.11[h]

Means with different superscripts across the rows and down the columns are statistically different ($P \leq 0.05$).

Cr- Chromium

Cu- Copper

Zn- Zinc

4.5: Capacity of Resistant Fungal Isolates to Bioaccumulate Cr^{2+}, Cu^{2+} and Zn^{2+} Ions from Raw Textile Effluents

The test fungi were tested for their capacity to take up and bioaccumulate Cr^{2+}, Cu^{2+} and Zn^{2+} ions from the raw effluent. Data obtained revealed that strain of *Chrysosporium* sp had statistically ($P \leq 0.05$) high potential for bioaccumulation of Cr^{2+} ions from the raw textile effluent (Table 4.7). This was followed by strains of *A. niger, A. flavus, A. versicolor* and *Penicillium* sp, *Trichoderma* sp, *Microsporum* sp and *Rhizopus* sp in that descending order of efficiency. Similarly, strain of *Chrysosporium* sp was found to be most efficient in the bioaccumulation of Cu^{2+} and Zn^{2+}ions followed closely by strain of *A. niger* (Table 4.7). But while strains of *A. niger* and *A. flavus* were able to bioaccumulate statistically comparable amounts of Cu^{2+} ions, the former was found to bioaccumulate significantly higher quantity of Zn^{2+} ions than the latter (Table 4.7). On the other hand, strains of *Trichoderma* sp, *Rhizopus* sp and *Microsporum* sp consistently exhibited lower capacity to take up and bioaccumulate the three heavy metal ions from broth culture (Table 4.7).

Table 4.7: Amount of Cr, Cu and Zn Bioaccumulated by Six Genera of Fungi Isolated from Raw Textile Effluents

Fungal isolates tested	Amount bioaccumulation (mg/g)		
	Cr	Cu	Zn
Chrysosporium sp	0.110^{a}	0.101^{a}	0.118^{a}
Aspergillus niger	0.103^{b}	0.096^{b}	0.123^{a}
Aspergillus flavus	0.096^{c}	0.096^{b}	0.092^{b}
Penicillium sp	0.091^{d}	0.088^{c}	0.079^{c}
Aspergillus versicolor	0.090^{d}	0.071^{d}	0.072^{cd}
Trichoderma sp	0.087^{e}	0.066^{e}	0.066^{de}
Rhizopus sp	0.071^{g}	0.054^{f}	0.059^{ef}
Microsporum sp	0.073^{f}	0.057^{g}	0.053^{f}

Means with different superscripts across the rows and down the columns are statistically different ($P \leq 0.05$).

Cr- Chromium

Cu- Copper

Zn- Zinc

CHAPTER FIVE

5.0 DISCUSSION

5.1: Physico-Chemical Analysis of Textile Effluent and Challawa River Samples

Textile wastewater is a mixture of colorants (dyes and pigments) and various organic compounds used as cleaning solvents, plasticizers, etc. It also contains high concentrations of heavy metals, total dissolved solids, and has high chemical and biological oxygen demand. The major metal pollutants such as copper, zinc, chromium, etc. come mainly from the metal complex dyes and chromium salts used in wool dyeing or as oxidizing agents in sulfur dyeing (Chavan, 2001).

5.1.1 pH

The pH of the textile effluent samples were highly alkaline (11.14-11.81), which is above the permissible limit, this might be due to excessive use of carbonate, bicarbonate, H_2O_2 and NaOH during bleaching process. pH is the most important parameter used in determining the corrosive nature of water. The lower the pH value the higher is the corrosive nature of the water (Gupta *et al*., 2000). Any change in pH of water bodies as a result of influx of effluent, can cause serious change in water chemistry, which can affect resources especially around the coastal areas. These effects on water bodies can be very significant. While the water samples (upstream and downstream) had a pH within limits (7.15-7.31) which is due to dilution effect that is, even as the effluent is directly discharged into the river, the regular flowing of the river and discharge from other sources dilutes the water.

Most aquatic animals prefer a pH range of 6.5 - 8.0. Lawson (2011) reported that aquatic organisms require optimum pH range of 6.8 - 8.7 for maximum growth and reproduction. At adverse pH level, they may die, stop reproducing, or move away from the region. Low pH can also allow toxic compounds to become more available to aquatic plants and animals (Lawson, 2011).

5.1.2 Temperature

The temperature levels of the effluents analyzed from different sampling points were found to be within the permissible limit stipulated by Nigerian Environmental Standards and Regulations Enforcement Agency (NESREA) ($\leq 40^{o}C$). This can be as a result of the fact that warm water was not discharged from the factory and also due to the fact that the samples were collected at the early hours of the day. However in a study conducted by Krishnamurthy (1990) reported high temperature in the textile effluent samples that were analyzed and attributed the high temperature to the direct discharge of warm effluent after production and also to the weather condition at the time of collection of the samples. High level of temperature in water bodies such as river can result to death of aquatic life. It is also known to affect the amount of oxygen water can hold. Cold water holds more oxygen than warm water, and all aquatic animals need oxygen to survive. Temperature also affects the rate of photosynthesis by aquatic plants, reproduction and metabolic activities (Lawson, 2011) and the sensitivity of organisms to toxic wastes, parasites and diseases. Murhekar (2011), reported that warm water discharged from factories and the removal of trees and vegetation that shade streams, and water that runs off city streets can cause temperature changes that may threaten the balance of aquatic systems. Water temperatures usually show a characteristic annual cycle, with higher values during the (dry season) summer and lower values in the (rainy season) winter (Arain *et al.*, 2008).

5.1.3 Electrical conductivity (EC)

Conductivity is the ability of a substance to conduct electricity and the electrical conductivity of water is a more-or-less linear function of the concentration of dissolved ions in water (Deepali *et al.*, 2009). Conductivity is measured in terms of conductivity per unit length and meters, it uses the unit microsiemens/centimeter (µs/cm). The values recorded for the textile effluent was found to be beyond the permissible limit of 2µs/cm stipulated by NESREA. Conductivity is a proxy indicator of

total dissolved solids, and therefore an indicator of the taste or salinity of the water. Its acts as a good indicator of water quality problems, particularly when it changes with time. High conductivity water, for example, can cause excessive scaling in water pipes, heaters, boilers and household appliances (WHO, 2004). Increase in EC values indicates the presence of high concentration of ions in the textile effluent (Deepali *et al.*, 2009). Because of the fact that it is easily measured, it can serve as an indicator of possible contamination of water bodies and if the conductivity of a stream suddenly increases, it indicates that there is a source of dissolved ions in the vicinity (Barnes *et al.*, 1998).

5.1.4 Turbidity

The turbidity values recorded in this study were found to be higher than WHO permissible standards of 5 NTU (WHO, 2004). The highest turbidity value of 9.15 NTU was recorded at downstream which is due to the presence of pollutants being discharged by industries surrounding Challawa River. This had been reported to have adverse effect on aquatic plant as a result of decrease in the rate of photosynthesis due to the high level of the turbidity in the river which precludes deep penetration of sunlight into the river (Muoghalu and Omocho, 2000). In a similar study by Surita *et al.* (2007) and Ezike *et al.* (2012), they all attributed high level of turbidity in effluent to the production processes which involves the use of different chemicals at each stage of production that consequently increases the turbidity of the waste water produced after production. Also excessive turbidity in water can cause problem for water purification processes such as flocculation and filtration which may increase treatment cost and is often associated with microbial contamination (Department of Water Affairs and Forestry, DWAF, 1998).

5.1.5 Dissolved oxygen

Dissolved oxygen is one of the most important parameters used to assess the level of pollution in contaminated water. Its correlation with water body gives direct and indirect information e.g. bacterial activity, photosynthesis, availability of nutrients, stratification, etc (Premlata, 2009).The amount of DO in this study was found to be below the minimum permissible standard of 10mg/l stipulated by Nigerian Environmental Standards and Regulations Enforcement Agency (NESREA) in all the samples, this might be due to the presence of decomposed organic matter in the samples. In a similar study carried out by Gomes *et al*. (2010) it was reported that small amount of DO in streams can cause harmful effects on the aquatic life. Fish and other aquatic life cannot survive without oxygen and the decomposition of organic matter creates an anaerobic environment with high level of methane and carbondioxide formation (Basavaraddi *et al.,* 2012).

In most non polluted waters, oxygen is usually maintained at the saturation level and does not either under saturate or super saturate because of factors responsible for its addition and removal and remains at equilibrium (Gomes *et al*., 2010). However, the addition of organic matter disturbs this equilibrium because of the excessive supply of readily available food (organic matter) which increases the respiratory demand for oxygen by aerobic microorganisms beyond the level that can be replenished. As a result, the level of oxygen drops and water becomes totally anoxic (Kapdan and Oztekin, 2003). The discharge of domestic and industrial wastes and agriculture runoff percolates in ground water and pollutes both ground water and surface water bodies by surface runoff (Gomes *et al.,* 2010). Changes in levels of dissolved oxygen in the aquatic system have a detrimental effect on aquatic biota in the system (Basavaraddi, *et al.,* 2012). Also, low level of dissolved oxygen below the permissible standard can serve as an indicator of pollution of water bodies. Hence, analysis of dissolved oxygen plays an important role in water pollution control and

waste water treatment process (Basavaraddi, *et al.*, 2012). The oxygen may diminish to levels that are lethal for most fish and other aquatic life (Gomes *et al*., 2010).

5.1.6 Biological Oxygen Demand

Biological oxygen demand (BOD) is a chemical procedure for determining the amount of dissolved oxygen needed by aerobic organisms in a body of water to break down organic material present in a given water sample at certain temperature over a specific period of time (Suthar *et al*., 2012). The BOD_5 test is a standardized test that provides information regarding the organic strength of wastewater (Basavaraddi, *et al*., 2012). The amount of oxygen consumed in a sample within a five day period is measured under carefully controlled and standardized condition (Suthar *et al.*, 2012). The degradation of organic matter in water is facilitated by different microorganisms that stabilize the organic matter by forming the end products usually immune to further degradation such as carbondioxide, water, ammonia, hydrogen sulphide etc (Khawaja *et al*., 1995).

The BOD values recorded in this study were found to be above the permissible standard of 30 mg/l stipulated by Nigerian Environmental Standards and Regulations Enforcement Agency (NESREA) The high level of BOD recorded across the sampling points is a further indication of possible pollution of Challawa river by other factories located in the same area that discharge their waste water into Challawa river. Khawaja *et al*. (1999); Koteswari and Ramanibai, (2003) all reported that high value of BOD in water body is an indication of high organic pollution load of the effluent. When BOD levels are high, dissolved oxygen (DO) levels decrease because the oxygen that is available in the water is being consumed by microorganisms (Sullivan *et al*., 2010). The impact here is that high BOD and low DO content of the textile effluent will limit aerobic microbial

activities such as redox reaction by organisms using oxygen as their final electron acceptor there by affecting oxidation of organic matter content of the effluent and self-purification. This has an attending consequence of eutrophication in some water bodies and finally anoxic conditions that may not be redeemable as a result of which fish and other aquatic organisms may not survive (Yusuf and Sonibare, 2004).

5.1.7 Nitrate, Phosphate and Sulphate

Effluents contribute a lot of organic loads into the river, which depletes downstream due to microbial activities. In this present investigation, the highest nitrate value of 24.19 mg/l was recorded in the raw effluent samples while the lowest was found in downstream of Challawa river (4.35mg/l). The high nitrate values recorded above the permissible standard across the sampling points could be attributed to the activities from the textile and also other possible secondary sources such as the addition of nitrogenous fertilizers on plants by farmers near the Challawa River, decay of dead plants and animals, animal urines feces, etc. They are all oxidized to nitrate by natural process and hence nitrogen is present in the form of nitrate in water (Sullivan *et al*., 2010). The increases in one or all the above factors are responsible for the increase of nitrate content in effluent and water bodies (Igbinosa and Oko, 2009).

The high level of phosphate observed in this study was found to be above the permissible limit of 5mg/l stipulated by Nigerian Environmental Standards and Regulations Enforcement Agency (NESREA). Igbinosa and Oko (2009) ; Kugali *et al.* (2013) all reported that both phosphate and nitrate are essential nutrient in plant but when found in excess quantities through pollution of water bodies can cause plant life and algae to grow quickly and when this plant die they are decomposed

by bacteria there by increasing the organic load of the water body. This will lead to high BOD levels which consequently will lead to decrease in the level of dissolved oxygen and affect aquatic life such as fishes as a result of limited amount of dissolved oxygen. Other possible sources of high level of phosphate downstream (Challawa river) are improper discharge of domestic sewage, detergents, the use of fertilizers by farmers and industrial waste water from other factories located in Challawa that channeled their waste downstream as well (Challawa river) (Akan *et al*., 2009a).

5.1.8 Total Dissolved Solids, Total Solids and Total Suspended Solids

The total dissolved solids and total solids were higher than the permissible limits by NESREA in the textile effluent samples but within range as the samples move downstream while total soluble solids of the samples were within permissible range. This is because of the presence of lots of impurities and sludge in the raw effluent samples but as the effluent flows along the channels and mix with water, the level of impurities reduces due to dilution effect. Islam *et al.*, (2014) reported that these solid impurities cause turbidity in the receiving streams and the composition of solids present in effluent mainly depends upon the nature and quality of raw materials used for production processes. These suspended and dissolved impurities have also been reported to cause low DO and higher BOD to the receiving streams and rivers with severe impacts to the water and other environmental qualities such as poor photosynthetic activity in the aquatic system and clogging of gills and respiratory surfaces of fishes (Goel, 2000).

5.2 Heavy Metals Content in Textile Effluent and Challawa River

High levels of chromium, copper and zinc beyond the permissible limit set by the regulatory agency (NESREA) of effluent being discharged directly from the textile industry is due to the direct use of the salts of these metals during production processes such as dying. Asia *et al.* (2009) also studied the physicochemical properties and investigated some selected heavy metals in three effluent samples collected from textile factories in Kaduna, Nigeria, the results showed that the heavy metals investigated had higher concentration than the Federal Environmental Protection Agency, FEPA (Nigeria) standards for effluent discharge. Rattan *et al*. (2002) and Marshall *et al.* (2003) also reported that heavy metals as environmental contaminants are not a new phenomenon. They are essential part of all living organisms and are present in trace amount in soil naturally. The man made sources of metal contamination are mainly associated with certain industrial activities, agricultural practices, automobile emissions, coal fired power generation plants, municipal incinerators (Marks and DeLeo, 1997). The source of metal in the effluent being the dyes used in textile industry, as about 10-15% of dyes are lost into the waste water during the dyeing process (Correia *et al.,* 1994; McMullan *et al.,* 1995; Sabour *et al.,* 2001).

5.3 Identification, Characterization and Frequency of Occurrence of Fungi Isolated From Study Sites

As revealed by the findings made in this study, a good number of fungal genera have the capacity to grow in the raw or partially treated textile effluent and in the river that serve as recipient of both. This tends to suggest that such fungal genera have the capacity to withstand the harsh environment both within the textile effluent and the water contaminated with the effluent (Edward and White, 1999). The presence of these fungi species can be attributed to the fact that fungi are natural inhabitants of soil and waste water disposal environment. Due their ability to adapt, they also have

great potential for remediation by virtue of their aggressive growth; greater biomass production and extensive hyphal reach in soil. They are a versatile group, as they can adapt and grow under various extreme conditions of pH, temperature and nutrient availability, as well as high metal concentrations (Anand *et al.,* 2006; Machido *et al.,* 2014). They offer the advantage of having cell wall material which shows excellent metal-binding properties (Gupta *et al*., 2000). Describing the ability to grow at high metal concentrations, Malik (2004) distinguished fungi tolerant to heavy metals. Generally, the contaminated sites are the sources of metal resistant micro-organism (Gadd, 1993). The ability of fungi to secrete a wide range of extracellular enzymes into their growth environments has been advanced as an explanation of their capacity to grow on a wide range of carbon sources (Kari *et.al.,* 2003). On the other hand, resistance to high levels of toxic heavy metals has been attributed to the capacity of fungi to bioconvert (David and Jay, 2009), bioabsorb (Shankar *et al.,* 2007; Nilanjana *et al.,* 2008; Ashok *et al.,* 2010) or bioaccumulate (David and Jay, 2009; Martins *et al.,* 2010) the metal ions.

Chrysosporium sp*, Aspergillus niger, Aspergillus flavus, Aspergillus versicolor*, *Penicillium* sp*, Trichoderma* sp*, Microsporon sp and Rhizopus* sp. have also been isolated from effluent and water samples by Niu, *et al.,* (1993); Say *et al.,* (2003); Zafar, *et al.,* (2007) and Machido *et al.,* (2014).

5.4 Resistance to Cr, Cu and Zn, and Tolerance Indices of the Fungal Isolates

The results found in this work suggest that the resistance level against individual metal was dependent on the isolates. The variation in the metal tolerance might be due to the presence of one or more types of tolerance strategies or resistance mechanisms exhibited by different fungi.

Similar study was reported by Price *et al.* (2001) who showed that *Aspergillus niger* was better to grow or exhibit high level of tolerance to heavy metals as compared to other fungi such as *Penicillium* sp., and *Fusarium* sp. The occurrence of various fungi such as *Aspergillus, Rhizopus, Penicillium, Fusarium, Chaetomium, Geomyces* and *Paecilomyces* species in the soil polluted by heavy metals (Cu, Cd, Pd, As and Zn) has also been reported by other workers (Babich and Stotzky, 1985; Gadd, 1993). Tolerance to toxic metals is based on ionic species associating with the cell surface or extra cellular polysacharides, proteins and chitins (Volesky, 1990).

The results of the present study depicted that all test isolates showed different tolerance behavior for different metals. Some isolates were sensitive, moderately tolerant and tolerant. Most of the isolates were sensitive and very few were tolerant. Among all test isolates, *Chrysosporium* sp, was the most tolerant species. As Baldrian and Gabriel (2002) reported that various genera and also isolates of the same genus did not necessarily have the same heavy metal tolerance. It is apparent that these fungal strains exhibit considerable tolerance towards Zn and Cu and can become dominant microorganisms in some polluted habitats. The resistance of the fungi species isolated in this study further suggests that they can be exploited for the remediation of heavy metal in contaminated sites as previously reported by McGrath. (2002).

The screening test revealed heterogeneity in the heavy metal tolerance of the isolates. Similar results were reported by other researchers (Verma *et al.,* 2001; Zafar *et al.,* 2007). The resistance against individual metals was much more dependent on the isolate than on the sites of its isolation. Brown and Wilkins (1985) suggested no relation between zinc tolerance of *Amanita muscaria hooker* and *Paxillusinvolutus*, and its concentration in the medium. Jones and Hutchinson (1988) demonstrated also that whatever the concentration in the medium, comparable tolerance rates were observed for isolates originating from metal contaminated and uncontaminated sites. Isolates of the

same genus could present a marked difference in the levels of metal resistance. Major differences in Cu, Cr and Zn tolerance have been found among the isolates obtained in this study. The variation in the metal tolerance may be due to the presence of different types of tolerance processes or resistance mechanisms exhibited by different isolates. Sun and Shao (2007) reported that both intracellular bioaccumulation and extracellular biosorption contributed to the high resistance of *Penicillium* sp to lead. Sintuprapa *et al.* (2000) found that ion exchange and intracellular accumulation in the form of polyphosphate precipitation are mechanism of Zn^{2+} uptake by living cells of *Penicillium sp.*

The results obtained also affirmed that the response of the isolates to heavy metals depended on the metal tested, its concentration in the medium and on the isolate considered. The results obtained were comparable with those reported by Badar *et al.,* (2000); Verma *et al.,* (2001); Bai and Abraham (2003), Malik (2004); Zouboulis *et al.,* (2004); Yoshida *et al.,* (2006) and Zafar *et al.,* (2007).

5.5 Bioaccumulation of Heavy Metals in Textile Effluent Using Heavy Metal Resistant Fungal Isolates

The fungi species isolated in this study from heavy metals contaminated sites and used for the bioaccumulation experiment showed high level of heavy metals removal from the textile effluent most especially by *Chrysosporium* sp. The differences in terms of the ability of the fungi species could be attributed to the intrinsic ability of the organisms and chemical composition of cell wall leading various types of interaction of metals with fungi (Gadd, 1993). Fungi had previously been recognized for their superior aptitudes for the treatment of different waste water and heavy metal removal from the contaminated samples (Galun *et al*., 1983; Say *et al*., 2003; Leitao, 2009). Also

filamentous fungi such as *Aspergillus*, *Rhizopus* and *Penicillium* species are frequently used in bioremediation processes due to their metal uptake variation (Gomes *et al*., 1998; Saxena And Bhattacharyya, 2006; Bajwa *et al.*, 2010).

Several workers have also reported that most microbial surfaces are negatively charged because of the ionization of functional groups, thus contributing to the metal binding (Huang *et al.*, 1988; Hughes and Poole, 1989; Rosen, 2002; Valavanidis and Vlachioganni, 2010; Ezeonuegbu *et al*., 2014).

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

1. Physico-chemical and heavy metal contents of textile effluent samples and water samples from challawa river analysed revealed high level of the pollutants beyond the permissible limit stipulated by NESREA

2. A total of 98 fungi were isolated from textile effluent and water samples comprising six (6) fungal genera (*Chrysosporium, Aspergillus, Penicillium, Trichoderma, Microsporum and Rhizopus*).

3. *Chrysosporium* sp proved to be most resistant to Zn and Cu with tolerance index of 0.445 and 0.367 respectively while *Aspergillus niger* was most resistant to Cr with tolerance index of 0.356

4. The most resistant of the fungi were selected for the bioaccumulation experiment, *Chrysosporium* sp had the highest capacity to remove the heavy metal present in the effluent sample (Cr=74.67%, Cu=67.37%, & Zn=85.94%), which was followed by *Aspergillus niger* (Cr=68.21%, Cu=64.09% and Zn=62.24%), *Aspergillus flavus* (Cr=64.52%, Cu=63.85% and Zn=62.34%), *Penicillium* sp (Cr=63.37%, Cu=58.86% and Zn=54.10%), *Aspergillus versicolor* (Cr=57.07%, Cu=51.447% and Zn=49.27%), *Trichoderma* sp (Cr=55.31%, Cu=45.24% and Zn=45.74%), *Rhizopus* sp (Cr=52.28%, Cu=35.75% and Zn=41.21%), and *Microsporum* sp (Cr=48.51%, Cu=38.19% and Zn=36.11%) in descending order.

5. *Chrysosporium* sp was observed to have the highest capacity to bioaccumulate the metals (chromium, copper and Zinc) present in the textile effluent (0.110mg/g, 0.101mg/g and 0.118mg/g), this was followed by *Aspergillus niger* (Cr=0.103mg/g, Cu=0.096mg/g and

Zn=0.123mg/g), *Aspergillus flavus* (Cr=0.096mg/g, Cu=0.096mg/g and Zn=0.092mg/g), *Penicillium* sp (Cr=0.091mg/g, Cu=0.088mg/g and Zn=0.079mg/g), *Aspergillus versicolor* (Cr=0.090mg/g, Cu=0.071mg/g and Zn=0.072mg/g), *Trichoderma* sp (Cr=0.087mg/g, Cu=0.066mg/g and Zn=0.066mg/g), *Rhizopus* sp (Cr=0.071mg/g, Cu=0.054mg/g and Zn=0.059mg/g), and *Microsporum* sp (Cr=0.073mg/g, Cu=0.057mg/g and Zn=0.053mg/g) in descending order

In general, all the test isolates proved to be capable of bioaccummulating Cr, Cu and Zn.

6.3 Recommendations

It is recommended that:

1. Effluent emanating from the textile industries should be treated before discharge into the river to avoid pollution of water sources.
2. *Chrysosporium* sp, *Aspergillus* sp. and *Penicillium* sp should be employed in the treatment of the textile effluent.
3. Further studies should be carried out with the aim of improving the capacity of the promising fungal genera for use as tools in the treatment of industrial effluent.
4. It is necessary to carry out more detailed studies to optimize the conditions for maximum bioadsorption of heavy metals from multi -metal solution and diluted wastewater.
5. Further investigations are required using multi-metal systems for the assessment of fungal growth and metal uptake in different wastewaters. Also there is the need to assess the suitability of the biomass in industrial scale processes.

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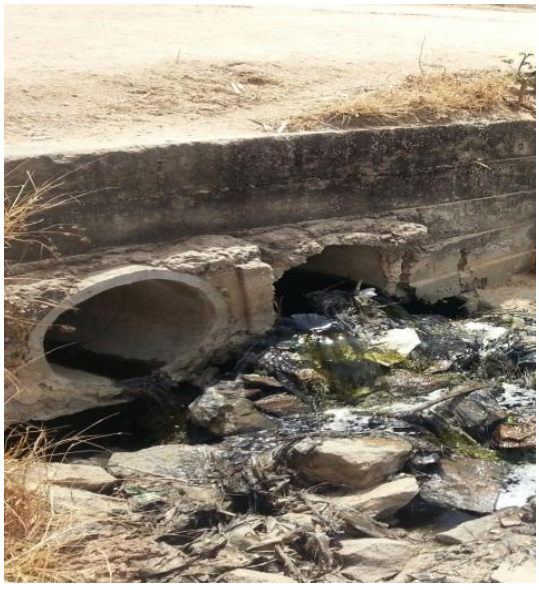
APPENDIX I

African Textile Manufacturers Ltd and the Discharge Outlet of the Textile Effluent



APPENDIX II

Outlet of the Textile Effluent and Discharge Point into Challawa River

APPENDIX III

Sample Collection and the Collected Samples

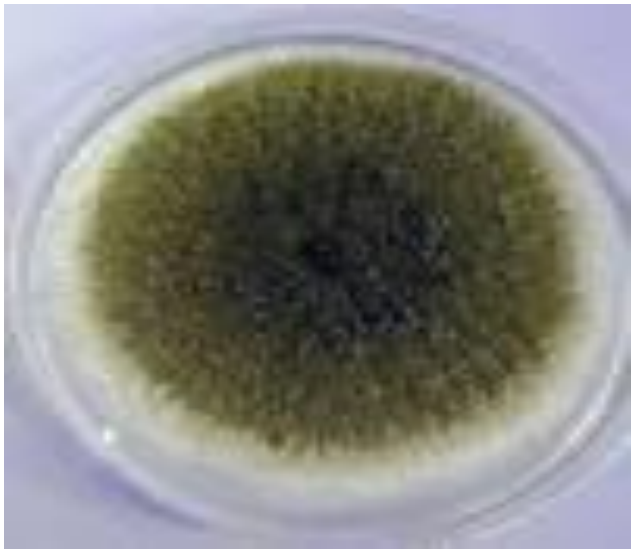
APPENDIX IV

Characterization of Fungal Isolates

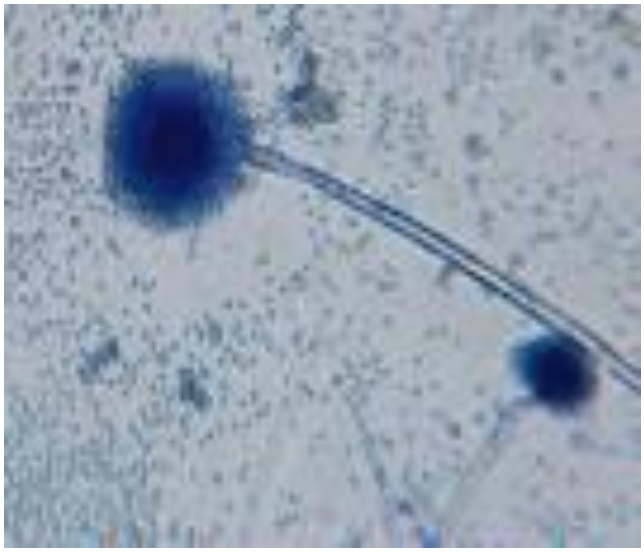
Cultural\Microscopic Characteristics	Microscopic Characteristics	Inferences
Wooly colonies appeared white at first and later turned black	Long and smooth conidiophore, the conidial heads were biserate (covering the entire vesicle), large, globose and dark brown.	*Aspergillus niger*
Colonies appeared to have granular and flat surface,first appearing to be yellow and later turning to green with a brown reverse colouration on PDA.	Mature vessicle bearing phialides over the entire surface with hyaline and coarsely rough conidiosphores	*Aspergillus flavus*
Velvety colonies,whitish at first but later turned to yellowish-green.	Long and smoooth conidiophore. The conidial heads were biserate (phialides\sterigmata) and it covers most part of vesicle.	*Aspergillus versicolor*
Colonies appeared flat and very granular with the colour changing from white to cream.	Septate hyphae,conidia were numerous, single-celled round apex and broad flatenned base. Which are mostly directly on the hyphae with some on the conidiophores.	*Chrysosporium* sp
Bluish-green colonies with cottony surface and white boarder.	Septate hyphae and flasked shaped phialides with unbranched chains of smooth and round conidia.	*Penicillium* sp
White cottony colonies with green patches at the centre which later spread to the margin	Septate hyphae,short conididophore flasked shaped phialides with single celled round and clustered together conidia at the end of each conidia.	*Trichoderma* sp
White cottony colonies at first, later turned gray	The hyphae appeared broad non septate with numerous stolons that runs along the mycelium with root like hyphae (rhizoid).	*Rhizopus* sp
White cottony colonies with cream reverse colouration	Septate hyhae which were branched and clubbed shaped, and fragmented. They had intercalary chlamydoconidia-like cells.	*Microsporum* sp

APPENDIX V

Pictorial Presentation of the Macroscopic and Microscopic Feature of *Aspergillus flavus and Chrysosporium* sp

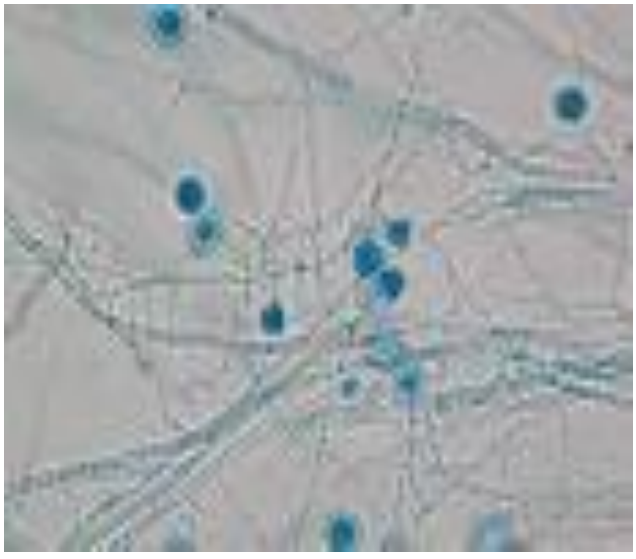


Aspergillus flavus culture plate



Microscopic view of *Aspergillus flavus.*

Chrysosporium sp culture plate



Microscopic view of *Chrysosporium* sp

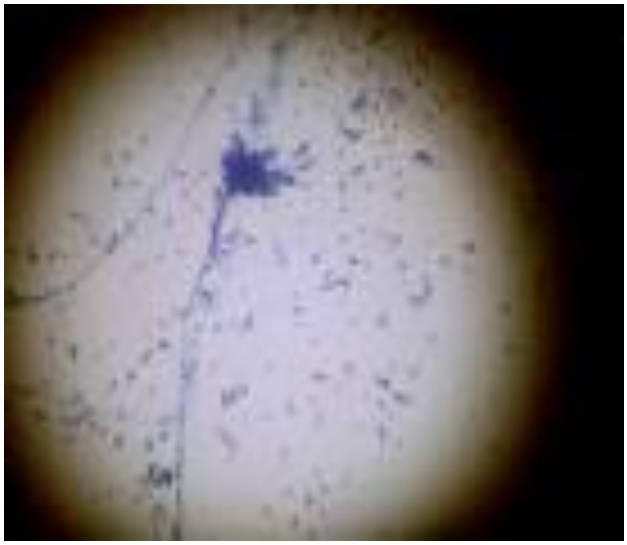
APPENDIX VI

Pictorial Presentation of the Macroscopic and Microscopic Feature of *Aspergillus niger and Aspergillus versicolor*

Aspergillus niger culture plate

Microscopic view of *Aspergillus niger*

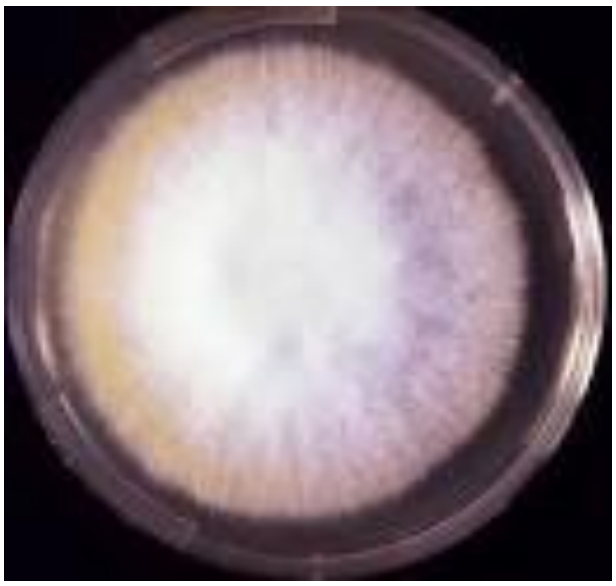
Aspergillus versicolor culture plate



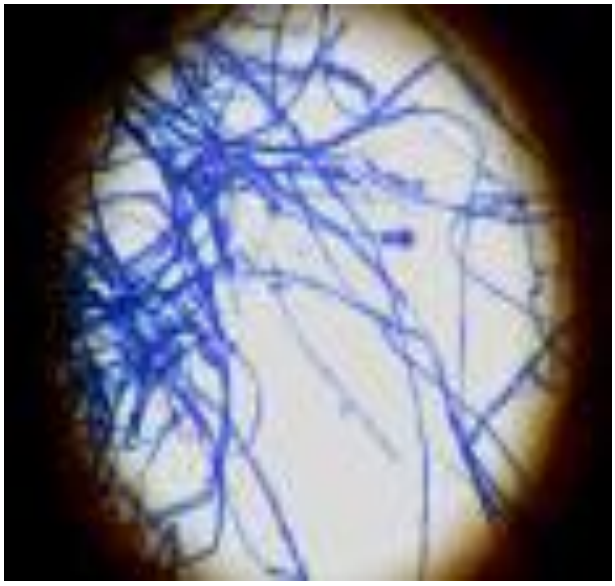
Microscopic view of *Aspergillus versicolor*

APPENDIX VII

Pictorial Presentation of the Macroscopic and Microscopic Feature of *Microsporum* sp *and* *Penicillium* sp

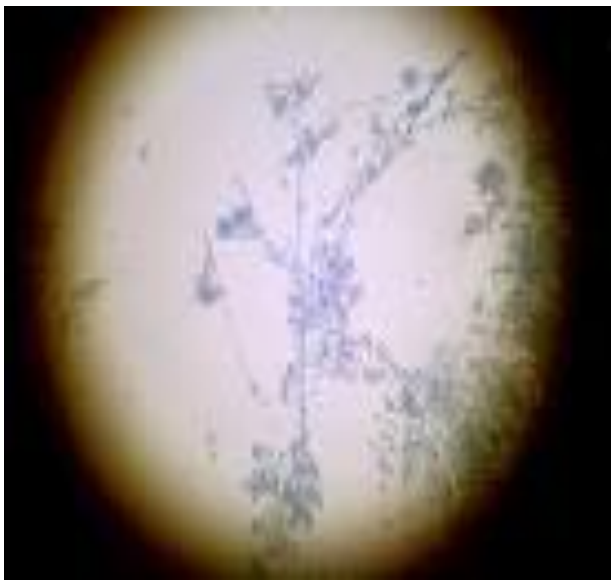


Microsporum sp cultural plate



Microscopic view of *Microsporum* sp

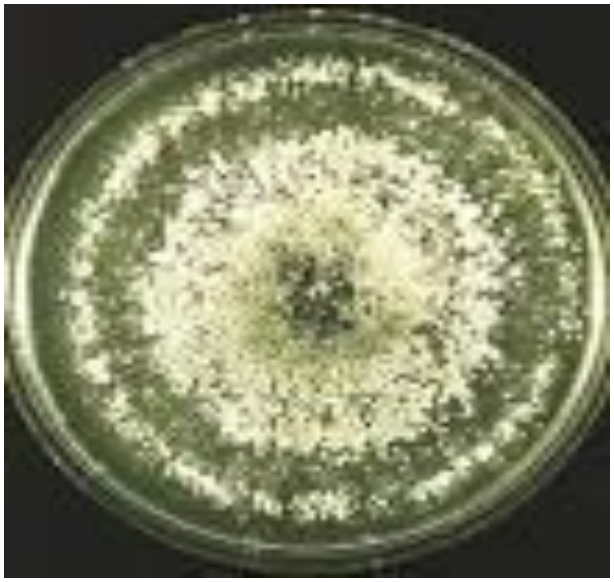
Penicillium sp cultural plate



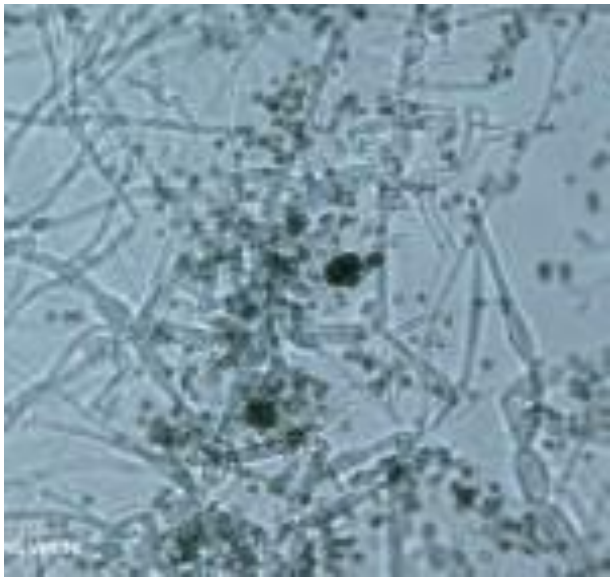
Microscopic view of *Penicillium* sp

APPENDIX VIII

Pictorial Presentation of the Macroscopic and Microscopic Features of *Trichoderma* sp *and* *Rhizopus* sp



Trichoderma sp cultural plate



Microscopic view of *Trichoderma* sp

Rhizopus sp cultural plate

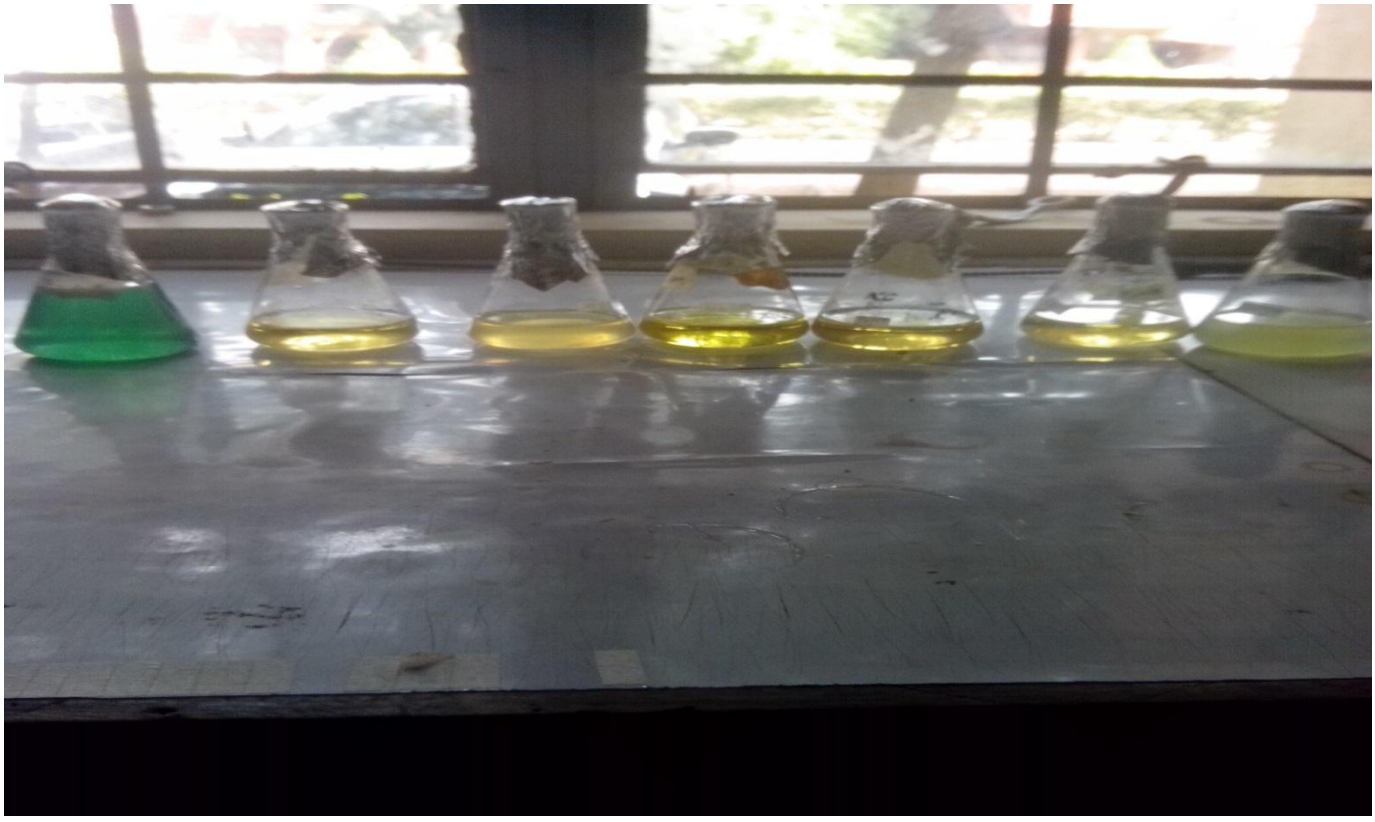
Microscopic plate of *Rhizopus* sp

APPENDIX IX

Screening of Fungal Isolates for Heavy Metal Resistance and Dried Fungal Biomass

APPENDIX X

Bioaccumulation Experiment and the Filtrate after Bioaccumulation



APPENDIX XI

Chemical composition of the Chemicals used for the Analysis

A. Chromium

Compound: Potassium dichromate

Molecular formula: K_2Cr2O7

Molecular weight: 294.16 g/mol

Appearance: Red-orange crystalline solid

B. Copper

Compound: Copper (II) sulphate pentahydrate

Molecular formula: $CuSO_4.5H_2OM$

Molecular weight: 249.69 g/mol

Appearance: Blue

C. Zinc

Compound: Zinc sulphate

Molecular formular: $ZnS0_4$

Molecular weight: 161.47 g/mol

Appearance: white powder

Preparation of dilution water for BOD

Reagents needed include:

A: Phosphate buffer solution

Dissolve 8.5g KH_2PO_4, 21.75K_2HPO_4, 33.4g $NaHPO_4.7H_2O$ and 1.7g NH_4Cl in about 500ml distilled water and then dilute to 1 liter. Labeled and store in the refrigerator at 20^0C.

B: Magnesium sulphate solution.

Dissolve 22.5g $MgSO_4.7H_2O$ in distilled water, make it up to 1 liter.

C: Calcium chloride solution

Dissolve 27.5g anhydrous $CaCl_2$ in distilled water, make it up to 1 liter.

D: Iron III chloride solution

Dissolve 0.25g $FeCl_3.6H_2O$ in distilled water, make it up to 1 liter.

APPENDIX XII

Procedure for preparing 1 liter dilution water

The dilution water was prepared using the method described by Ademoroti, (1996)

1. 1ml each of the above mentioned reagents was added into a conical flask containing 1 litre of distilled water.
2. The mixture was shaken gentle and stored in the refrigerator at 20^{o}

APPENDIX XIII

Capacity of *Aspergillus niger* from Study Sites to Resist and Grow in Potato Dextrose Broth Containing 20 And 30ppm Of Cr^{2+}, Cu^{2+} and Zn^{2+} ions

Strains tested	Sources of isolate	Yield of dry mycelial biomass (mg)					
		Cr (ppm)		Cu (ppm)		Zn (ppm)	
		20	**30**	**20**	**30**	**20**	**30**
*Aspergillus niger***	A	118.3	87.6	111.3	72.5	**132.3**	**90.0**
*Aspergillus niger***	A	143.2	109.5	140.7	99.2	**150.0**	**96.7**
*Aspergillus niger***	A	98.9	57.6	85.3	41.6	**126.4**	**91.2**
*Aspergillus niger***	A	253.5	203.5	202.0	164.8	**199.1**	**145.6**
Aspergillus niger+	A	39.0	16.3	36.3	14.2	**86.0**	47.9
Aspergillus niger+	A	51.2	23.0	47.8	19.4	**82.1**	49.6
Aspergillus niger	A	29.7	11.2	27.8	15.6	60.2	46.4
Aspergillus niger	A	40.8	18.9	43.4	19.1	49.6	30.4
Aspergillus niger	A	43.8	19.1	51.2	34.1	76.3	35.0
Aspergillus niger	A	15.7	-	21.6	11.8	39.6	17.4
*Aspergillus niger***	B	116.7	89.6	109.1	86.5	**121.4**	**98.7**
Aspergillus niger+	B	26.0	9.8	22.1	12.6	**199.1**	**145.6**
Aspergillus niger+	B	110.5	69.0	54.6	20.5	39.4	18.8
*Aspergillus niger***	B	104.9	89.1	98.6	60.1	**119.4**	**88.4**
Aspergillus niger	B	15.8	-	17.1	8.9	22.8	8.9
Aspergillus niger	B	23.5	7.9	29.8	14.3	24.9	11.6
*Aspergillus niger**	B	94.6	39.9	118.1	91.0	35.4	15.0
Aspergillus niger	C	42.0	24.7	59.2	43.4	82.6	51.7
Aspergillus niger+	C	40.9	18.1	48.9	23.4	**198.6**	**152.7**
Aspergillus niger	D	48.6	23.5	39.9	17.6	52.8	30.8
Aspergillus niger	D	32.1	17.8	40.0	19.9	51.2	34.5
Aspergillus niger	D	56.6	24.8	58.9	25.4	56.9	11.8
Aspergillus niger	E	41.9	18.6	52.7	22.1	39.9	-

A=Raw effluent, B= Partially treated effluent , C=Upstream water sample, D=Water samples from discharge point, E=Downstream water samples

** = Resistant to all metal ions, * = Resistant to two metal ions, + = Resistant to one metal ion.

APPENDIX XIV

Capacity of *Aspergillus versicolor* from Study Sites to Resist and Grow in Potato Dextrose Broth Containing 20 And 30ppm Of Cr^{2+}, Cu^{2+} and Zn^{2+} ions

Strains tested	Sources of isolate	Yield of dry mycelial biomass (mg)					
		Cr (ppm)		Cu (ppm)		Zn (ppm)	
		20	**30**	**20**	**30**	**20**	**30**
*Aspergillus versicolor***	A	99.8	41.1	116.8	97.6	97.0	46.6
*Aspergillus versicolor***	A	137.6	103.0	146.6	110.9	150.9	98.1
Aspergillus versicolor	A	47.2	23.9	46.1	31.7	66.5	42.7
*Aspergillus versicolor**	A	76.5	41.9	96.5	47.0	99.9	54.6
Aspergillus versicolor	B	41.7	20.2	43.3	42.8	76.8	32.7
Aspergillus versicolor	B	51.3	33.8	56.0	28.7	39.0	18.6
Aspergillus versicolor	B	16.4	-	25.2	11.1	34.7	21.2
Aspergillus versicolor	B	42.1	20.8	43.3	19.5	51.1	28.9
Aspergillus versicolor	B	26.9	9.5	30.9	16.7	51.3	36.1
Aspergillus versicolor	D	37.9	22.0	45.6	22.7	56.4	32.7
Aspergillus versicolor	D	53.4	29.1	31.4	17.5	65.1	39.6
*Aspergillus versicolor**	D	39.8	17.0	173.8	146.0	198.6	152.7
Aspergillus versicolor	D	39.9	18.2	41.2	19.8	51.8	25.3

A=Raw effluent, B=Effluent from flow channel, D=Discharge point, ** = Resistant to all metal ions, * = Resistant to two metal ions, + = Resistant to one metal ion, - = no growth

APPENDIX XV

Capacity of *Aspergillus flavus* from Study Sites to Resist and Grow in Potato Dextrose Broth Containing 20 And 30ppm Of Cr^{2+}, Cu^{2+} and Zn^{2+} ions

Strains tested	Sources of isolate	Yield of dry mycelial biomass (mg)					
		Cr (ppm)		**Cu** (ppm)		**Zn** (ppm)	
		20	30	20	30	20	30
Aspergillus flavus	A	39.9	22.0	35.5	14.8	60.1	38.3
Aspergillus flavus+	A	54.3	26.7	54.6	20.6	88.1	52.6
Aspergillus flavus+	A	88.1	19.6	48.2	30.0	47.3	25.8
Aspergillus flavus	A	59.1	37.5	41.6	18.1	52.1	25.7
Aspergillus flavus	A	37.5	19.1	13.9	8.2	17.3	9.5
Aspergillus flavus	A	39.9	17.6	48.4	27.1	33.2	16.8
Aspergillus flavus	B	41.0	19.2	43.3	19.1	54.3	28.4
*Aspergillus flavus***	B	179.8	132.3	115.6	81.0	199.7	150.1
Aspergillus flavus	C	17.6	_	16.5	8.3	31.2	12.6
Aspergillus flavus+	D	14.7	_	16.4	_	199.7	150.1
Aspergillus flavus	D	74.7	36.8	47.0	22.3	82.5	57.3

A=Raw effluent, B=Effluent from flow channel, C=Upstream, D=Discharge point. ** = Resistant to all metal ions, * = Resistant to two metal ions,

+ = Resistant to one metal ion, -- = no growth

APPENDIX XVI

Capacity of *Penicillium* sp from Study Sites to Resist and Grow in Potato Dextrose Broth Containing 20 And 30ppm Of Cr^{2+}, Cu^{2+} and Zn^{2+} ions

Strains tested	Sources of isolate	Yield of dry mycelial biomass (mg)					
		Cr (ppm)		Cu (ppm)		Zn (ppm)	
		20	30	20	30	20	30
Penicillium sp	A	46.0	31.6	67.5	42.0	48.3	24.0
Penicillium sp	A	15.9	-	15.8	6.1	48.8	25.7
Penicillium sp**	A	99.8	41.1	97.0	46.6	116.8	97.6
Penicillium sp+	A	89.6	51.1	47.2	25.4	59.9	31.8
Penicillium sp**	A	201.0	163.4	199.0	155.1	107.5	134.8
Penicillium sp+	B	-	-	-	-	247.6	218.0
Penicillium sp	B	36.3	17.4	33.9	14.7	42.7	25.3
Penicillium sp	D	24.5	8.9	47.9	23.6	36.0	14.2
Penicillium sp	E	28.6	16.0	51.8	28.7	43.1	19.6

A=Raw effluent, B=Effluent from flow channel, D=Discharge point, E=Downstream, ** = Resistant to all metal ions, * = Resistant to two metal ions, + = Resistant to one metal ion, -- = no growth

APPENDIX XVII

Capacity of *Trichoderma* sp from Study Sites to Resist and Grow in Potato Dextrose Broth Containing 20 And 30ppm Of Cr^{2+}, Cu^{2+} and Zn^{2+} ions

Strains tested	Sources of isolate	Yield of dry mycelial biomass (mg)					
		Cr (ppm)		Cu (ppm)		Zn (ppm)	
		20	30	20	30	20	30
Trichoderma sp	B	21.8	8.3	23.8	12.1	-	-
Trichoderma sp	B	15.9	-	17.6	-	31.2	12.6
Trichoderma sp	C	33.6	14.1	24.6	9.8	34.7	21.2
Trichoderma sp	D	28.6	16.9	16.4	-	26.9	9.7
Trichoderma sp	D	39.6	17.0	37.4	18.2	39.4	18.8
Trichoderma sp*	D	139.8	111.6	148.9	101.8	22.1	12.6
Trichoderma sp	E	60.7	38.5	54.9	32.6	33.2	17.6

B=Effluent from flow channel, C=Upstream, D=Discharge point, E=Downstream, * = Resistant to two metal ions, -- = no growth

APPENDIX XVIII

Capacity of *Rhizopus* sp from Study Sites to Resist and Grow in Potato Dextrose Broth Containing 20 And 30ppm Of Cr^{2+}, Cu^{2+} and Zn^{2+} ions

Strains tested	Sources of isolate	Yield of dry mycelial biomass (mg)					
		Cr (ppm)		Cu (ppm)		Zn (ppm)	
		20	30	20	30	20	30
Rhizopus sp	A	24.8	10.1	31.2	15.9	17.5	8.6
Rhizopus sp	A	71.4	39.9	78.8	40.2	49.9	23.8
Rhizopus sp*	B	175.1	128.7	76.0	35.4	113.2	99.7
Rhizopus sp**	B	97.1	40.9	148.9	101.8	99.6	50.1
Rhizopus sp	C	27.8	17.9	31.0	16.5	35.6	14.5
Rhizopus sp	D	44.1	28.9	28.9	11.3	33.1	16.0
Rhizopus sp	D	41.0	22.5	36.3	15.8	39.1	18.6
Rhizopus sp	D	24.2	10.9	28.0	12.1	33.6	17.0
Rhizopus sp	E	5.0	14.8	27.1	17.9	54.7	28.5
Rhizopus sp	E	31.9	16.3	51.7	24.3	33.1	16.7

A=Raw effluent, B=Effluent from flow channel, C=Upstream, D=Discharge point, E=Downstream, ** = Resistant to all metal ions, * = Resistant to two metal ions,

APPENDIX XIX

Capacity of *Microsporum* sp from Study Sites to Resist and Grow in Potato Dextrose Broth Containing 20 And 30ppm Of Cr^{2+}, Cu^{2+} and Zn^{2+} ions

Strains tested	Sources of isolate	Yield of dry mycelial biomass (mg)					
		Cr(ppm)		Cu(ppm)		Zn(ppm)	
		20	30	20	30	20	30
Microsporum sp	A	41.0	18.7	31.4	17.5	65.1	39.6
Microsporum sp*	A	53.6	28.1	114.6	75.3	111.7	84.3
Microsporum sp**	A	163.0	115.6	138.2	99.9	150.6	121.0
Microsporum sp**	B	103.5	78.4	97.4	49.7	123.1	99.6
Microsporum sp	D	44.6	20.1	25.8	11.1	33.5	16.9
Microsporum sp	D	36.8	18.3	48.0	27.9	51.7	26.5
Microsporum sp	D	37.9	22.0	45.6	22.0	56.4	32.7
Microsporum sp	E	27.4	16.1	39.9	18.4	28.8	18.1

A=Raw effluent, B=Effluent from flow channel, D=Discharge point, E=Downstream, ** = Resistant to all metal ions, * = Resistant to two metal ions,

APPENDIX XX

Capacity of *Chrysosporium* sp from Study Sites to Resist and Grow in Potato Dextrose Broth Containing 20 And 30ppm Of Cr^{2+}, Cu^{2+} and Zn^{2+} ions

Strains tested	Sources of isolate	Yield of dry mycelial biomass (mg)					
		Cr (ppm)		Cu (ppm)		Zn (ppm)	
		20	30	20	30	20	30
Chrysosporium sp**	A	199.8	146.5	163.2	118.6	99.5	41.3
Chrysosporium sp+	A	119.8	87.4	61.6	33.5	38.3	16.4
Chrysosporium sp	A	50.9	21.6	49.8	26.4	32.6	14.3
Chrysosporium sp**	A	201.0	165.4	199.0	155.1	176.5	134.8
Chrysosporium sp+	A	102.3	81.5	58.3	29.8	45.7	20.1
Chrysosporium sp	A	24.0	10.9	16.8	8.5	33.8	18.4
Chrysosporium sp	A	53.3	29.6	38.9	18.0	38.6	19.4
Chrysosporium sp	A	50.1	27.7	51.0	23.6	52.7	24.3
Chrysosporium sp**	A	219.3	160.1	251.8	210.4	247.6	218.0
Chrysosporium sp	B	55.8	26.1	29.9	15.7	49.8	20.4
Chrysosporium sp**	B	186.5	148.9	193.8	152.5	87.8	41.3
Chrysosporium sp	B	46.2	21.3	56.0	26.8	54.5	29.7
Chrysosporium sp	B	51.8	23.5	39.8	19.1	46.8	23.9
Chrysosporium sp**	B	98.7	54.1	85.0	51.6	124.9	99.7
Chrysosporium sp	B	20.9	9.2	36.1	17.8	19.3	6.6
Chrysosporium sp	D	51.1	19.7	31.8	17.4	41.4	18.3
Chrysosporium sp	D	59.7	27.3	41.8	18.9	50.9	21.6

A=Raw effluent, B=Effluent from flow channel, D=Discharge point, ** = Resistant to all metal ions, * = Resistant to two metal ions, + = Resistant to one metal ion.