IMPACT OF TANNERY EFFLUENTS ON THE QUALITY OF ENVIRONMENTAL SAMPLES AND BIOREMEDIATION POTENTIALS OF MICROBIAL ISOLATES

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BY

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DEPARTMENT OF MICROBIOLOGY,

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NIGERIA

APRIL 2014

DECLARATION

I declare that the work in this Dissertation entitled Impact of Tannery Effluents on the Quality of Environmental Samples and Bioremediation Potentials of Microbial Isolates has been carried out by me in the Department of Microbiology. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this Dissertation was previously presented for another degree or diploma at this or any other institution.

_____	_____	_____
Name of student	Signature	Date

CERTIFICATION

This Dissertation entitled IMPACT OF TANNERY EFFLUENTS ON THE QUALITY OF ENVIRONMENTAL SAMPLES AND BIOREMEDIATION POTENTIALS OF MICROBIAL ISOLATES by Muhammad Bashir TIJJANI meets the regulations governing the award of the degree of Doctor of Philosophy of Science in Microbiology of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

To Yaya Tijjani.

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ABSTRACT

Impact of tannery effluents in the environment and bioremediation potentials of microbial isolates were evaluated using different methods. Physico–chemical properties of the effluent samples vary in each tannery with higher mean values in Tan A followed by Tan C and then Tan B, indicating higher pollution level in the order Tan A < Tan C < Tan B. Similarly, these properties in well water samples studied fluctuate. Significant differences were observed in mean TDS, DO and BOD values. Seasonal variation in the mean physico-chemical properties of well water samples using students' t-test showed significant differences, where higher TSS and BOD were recorded in the dry season while DO was considerably low in the dry season. The mean Cr (4.85mg/L) concentration was significantly higher ($P \leq 0.05$) in effluent sample compared to the well water samples. It was established that the level of chromium (Cr) were significantly higher ($P \leq 0.05$) in effluent analyzed from Tan A (5.29mg/L) then Tan B (4.91mg/L) and the level was lower in Tan C (4.36mg/L). Statistical analyses of heavy metal content in the tannery effluent from different sampling points revealed significant differences ($P \leq 0.05$) among the tanneries where it shows decrease in all metal concentrations downstream. Higher concentrations of metals were recorded in the dry compared to wet season. Bacterial contamination of the well water samples were significantly higher ($P \leq 0.05$) during the dry season compared to the wet season. The bacterial load was increasing with increasing distance from the pollution source regardless of the season. Comparisons of mean heterotrophic, total coliforms, fecal coliform and fungal counts in effluent samples from tanneries showed no significant differences ($P > 0.05$), but significant difference ($P \leq 0.05$) was prominent in the fungal counts between Tan C and the other tanneries. *Pseudomonas aeruginosa* and *Bacillus cereus* subjected to assessment for resistance to Cr were selected as the candidates for bioremediation of the effluents. In the flasks containing minimal medium with 25mg/L Cr inoculated with *P. aeruginosa* Cr concentration declined from

25 to 20mg/L after 120hr while in the aerated vessel it declined to 19.09mg/L, in the flask and aerated vessels with *B. cereus* Cr level declined to 19.15mg/L and 19.9mg/L respectively after 120hr of incubation. In flasks containing effluents inoculated with *P. aeruginosa*, *Bacillus cereus* and consortium Cr declined to 18.7mg/L, 19.81mg/L and 17mg/L respectively after 120hr indicating 20.8 – 32.1% Cr removal from the effluent. Performances of *P. aeruginosa* and consortium in the aerated vessels were significantly higher than in the flask. *Aspergillus niger, Penicillium* sp and *Trichoderma longibrachiatum* were selected as fungal candidates for bioremediation of the effluent. The fungi and their consortium performed best at pH 6 leaving behind 36 – 39.6% Cr in the media. The Cr removal in the treated effluents by *T. longibrachiatum* was higher than that by *A. niger* and *Penicillium* sp. These test strains bioadsorbed 15.7mg/L, 15.0 mg/L and 14.6 mg/L Cr respectively from the effluent after 120hr treatment in the flasks on the shaker, and Cr removal in the aerated vessels were higher. Mutant *A. niger* (MS_{UV30}) and *Penicillium* (MS_{UV10}) were more effective than their respective wild parent strains. It was observed that the weights of the biomass increases with decreasing Cr level in treated effluent with fungi indicating bioaccumulation of the Cr in the cell. In conclusion this study has established that discharge of untreated tannery effluent has negative impact on the environment and it has also established the role and efficiencies of indigenous bacterial and fungal species in the adsorption, accumulation or removal of Cr in the treated effluent. It is recommended that effluent should be properly treated to meet the discharge guideline of 0.5mg/L Cr before discharge and further studies to establish other environmental, health, economic impacts are recommended.

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CHAPTER ONE

INTRODUCTION

1.0 General Introduction

The environment is under increasing pressure from the direct discharge of effluents from industries and municipal wastewaters into soils and water bodies. This has become a growing environmental problem. This is because water is a very important element on the earth and plays a crucial role with direct impact on public health, environmental sustainability and life conservation. Water also known as blue gold, is one of the most priceless gifts of Nature. It is also regarded as the life line on Earth, because evolution of life and development of human civilization could not have been possible without water (Sinha *et al.,* 2004). All great civilizations of the world evolved around the rivers. Rivers have been life line to the Earth and supposed to be yardstick to measure the society, since a dirty river means a dirty society. Rapidly increasing population, indiscriminate urbanization and rapid industrialization along the rivers have put tremendous pressure on water resources and their quality (Sinha *et al.,* 2004; Sinha *et al.,* 2006).

Tanning industry is recognized as a serious environmental threat all over the world (Tariq *et al.,* 2005). In most developing countries voluminous tannery effluents are directly discharged to the nearby open lands where they adversely affect the quality of both soil and ground water, rendering them unsuitable for viable human use as well as the soil flora and fauna (Farooque *et al.,* 1984; Khawaja *et al.,* 1995). These effluents contain toxic chemicals such as sulfides, chromium salts and other substances, including heavy toxic trace metals that turn tannery effluents into noxious wastewaters (Ates *et al.,* 1997; Cooman *et al.,* 2003). The ground water is affected by the continued seepage of effluents (Wang *et al.,* 1990; Hongrui *et al.,* 2003), while

the dissolved chemicals infiltrate into the surrounding soil, resulting in soil and water pollution, rendering the soil unfit for cultivation (Kisku *et al.,* 1999; Barman *et al.,* 2000; Herawati *et al.,* 2000).

The tannery effluents are normally highly colored, foul smelling liquids containing both acidic and alkaline liquors (UNEP, 1994; World Bank, 1998). In addition, such water bodies are associated with deleterious health effects such as headache, stomach ache, dizziness, night blindness, dermatitis and other skin disorders (Parikh *et al.,* 1995). Tariq *et. al.* (2006) also reported that improper and unguarded use of chemicals has exposed workers and residents of the adjoining areas to health hazards of varied types. Respiratory disorders, and related diseases, diarrhea/dysentery and typhoid are common among the community population Tariq *et. al.* (2006).

A variety of chemicals are used in the tanning process along with large quantities of water which are discharged as effluents, containing a huge bulk of liquid and solid wastes, and substantial quantities of Chromium (Cr) and other heavy toxic trace metals, organic matter, lime and sulfide. In the process of tanning, chromium salt is used to convert hide to leather and the wastewater generated which contains chromium salts in excess of the maximum permissible limits is discharged into the environment (Thiagragan, 1992). This introduction of heavy metals in various forms in the environment can produce considerable modifications of the microbial communities and their activities. These metals generally exert an inhibitory action on microorganisms by blocking essential functional groups, displacing essential metal ions, or modifying the active conformation of biological molecules (Aleem *et al.,* 2003). Generally,

short-term exposure to metal contamination affects the overall activity of microbial populations (Barnhart and Vestal, 1983), whereas in long-term contaminated soils, a population shift in favor of resistant microorganisms occurs due to elimination of sensitive organisms (Tariq *et al.,* 2006).

The heavy metals pollution of the environment has led to the appearance of heavy-metal resistant microorganisms in the soil and water of industrial origins (Aleem *et al.,* 2003). Metal resistance in bacteria is frequently coded by genes that are located on plasmids and transposons and is often transferable intergenerically, interspecially and also from *in situ* microflora to indigenous microflora (Datta and Hedges, 1972; Trevors *et al.,* 1985; Malik and Jaiswal, 2000).

The metal resistance is often associated with resistance to single or multiple drugs (Foster, 1983; Malik and Ahmad, 1994). In most instances the frequency of heavy metal resistance is the same or higher than that of antibiotic resistance (Schottel, 1978; Silver and Phung, 1996).

Hexavalent chromium (Cr(VI)) compounds are being used in a wide variety of commercial processes and unregulated disposal of the chromium containing effluent in both developing and developed countries has led to the contamination of soil, sediment, surface and groundwaters (Xing and Okrent, 1993; Szulczewski *et al.,* 1997). In trace amounts, chromium is considered an essential nutrient for numerous organisms, but at elevated levels, it is toxic and mutagenic (Shen and Wang, 1993). Rajamani *et al.* (1995) reported nearly 80% of the tanneries in India are engaged in the chrome tanning process. Most of them discharge untreated wastewater into the environment. In such aqueous waste, Cr(VI) is present as either dichromate ($Cr_2O_7^{2-}$) in acidic environments or as chromate (CrO_4^{2-}) in alkaline environments. Some industrial plants treat the

Cr-rich wastewater by conventional aerobic or anaerobic treatment plants. Although, chromium concentration is lowered during these treatments, it remains at a level toxic to the flora and fauna. Wastewater with low concentrations of Cr(VI) is usually treated with ionexchange resins, but the high cost of the resin limits its usage (Kratochvil *et al.*, 1998).

Once in the environment, toxic metals derived from natural and anthropogenic activities undergo transformation into various mobile forms and/or by immobilization before ending into biomer(s) as an environmental sink. Many microbes by cellular activities and/or their products significantly contribute in these biogeochemical cycles. Biotechnological approaches to the abatement of toxic metal pollution consist of selectively using and enhancing these natural processes to treat particular wastes.

The processes by which the microorganisms interact with the toxic metals enabling their removal/and recovery are biosorption, bioaccumulation and enzymatic reduction. The potential of microbial Cr(VI) reduction has been envisaged by several authors for a long time, however, biosorption and bioaccumulation of Cr(VI) has now gained attention (Gadd and White, 1993; Fude *et al.*, 1994).

Microbial heavy metal accumulation often comprises of two phases (Gadd, 1990). An initial rapid phase involving physical adsorption or ion exchange at cell surface and by a subsequent slower phase involving active metabolism-dependent transport of metal into bacterial cells. During the bioaccumulation, many features of a living cell like intracellular sequestration followed by localization within specific organelles, metallothionein binding, particulate metal

accumulation, extracellular precipitation and complex formation can occur (Gadd, 1990). In addition to these varieties of mechanisms, cells can be genetically manipulated to alter morphological and physiological features (Langley and Beveridge, 1999; Mejare and Bulow, 2001). Recently, heavy metal accumulating strains have sought a new role as 'small factories' for production of nanoparticles (Klaus-Joerger *et al.,* 2001). Biosorption is a metabolism-independent process and thus can be performed by both living and dead cells. This adsorption is based on mechanisms such as complexation, ion exchange, coordination, adsorption, chelation and microprecipitation which may be synergistically or independently involved (Hu *et al.,* 1996). The advantages of an ideal biosorption process are, reusability of biomass, removal of metals from effluent irrespective of toxicity, short operation times (90 min) until equilibration is reached and no production of secondary compounds which might be toxic.

1.1 Statement of the Problem

Wastes and wastewaters from tanning industries are discharged into the environment without treatment. Tannery wastewater contains utilizable nutrients, but also toxic organic compounds that affect soil, surface and ground waters, and thus, might pose a threat to the local communities. Apart from organic material, which releases valuable nutrients on decomposition, tannery effluents may contain heavy metals such as chromium (Cr), and other heavy metals such as Cd, Co,Fe, Zn, Mn, Pb as well as pathogens, mainly of faecal origin (from the rumen of animals providing raw materials and domestic sewage) and toxic organic components, all of which pose a serious threat to the environment (Contreras-Ramos *et al.,* 2004). Microorganisms' resistance to antibiotics and tolerance to metals appear as the result of exposure to metal contaminated environments which cause coincidental co-selection for resistance factors for

antibiotics and heavy metals. The Tannery effluents discharged into the tributaries of Chalawa River in Kano are used for irrigation by local farmers. The solid wastes are also used as manure on farmlands. These effluents contain toxic chemicals such as sulfides, chromium salts and other substances, including toxic heavy metals that turn tannery effluents into noxious wastewaters. These waste water pollute the environment with direct impact on public health and sanitation.

1.2 Justification

Leather production is one of the major industries in Kano and makes significant contribution to the human employments and country's export in general. A major concern to the industry, regulatory bodies and the general public is the safe and long-term management of chromium and heavy metals containing waste effluents from tanneries. Although the tanning industry has been in existence for long, the problem of environmental pollution has not received serious consideration in recent years.

The Tannery effluents discharged into the tributaries of Chalawa River in Kano are used for irrigation by farmers. The solid wastes are also used as manure. These effluents contain toxic chemicals such as chromium salts, heavy metals and other toxic substances, that turn tannery effluents into noxious wastewaters. Although the application of tannery wastes to low-productive soils may have some beneficial effects due to the high nutrient content, their impact on the soil and water ecosystem also has to be considered carefully due to their high Cr and salt content. The presence of these heavy metals and their detection in the environment is increasing globally, this posses a significant threat to public health as well as the microbial population.

There is very little information on the effects of tannery wastes and wastewaters on environments humans, animals and microorganisms in Kano state. It is for this purpose that this study is significant, because in Kano (Northern Nigeria) there are many tanning industries, and preliminary survey indicated that their industrial wastes are discharged into streams drain into Challawa river. Continuous cropping and other human activities are generally observed along the streams and rivers all year round. It is therefore, important to monitor industrial effluents especially tanneries inff order to ascertain the effects of the wastes released to the environment.

The considerable damage caused to the water bodies due to pollutants from the number of tanneries in the study sites necessitated this study. The examination of water is necessary for the following reasons: to assess its quality to provide a pure and wholesome water to the public for drinking and other purposes; to find out whether water is suitable for the specific industrial purposes, (and if so, to choose the most effective treatment), to determine the extent of pollution and to suggest a possible remedy; to determine the efficiency towards natural purification when sewage and industrial wastes are discharged into water courses. Environment-friendly solution to disposal of tannery wastes and appropriate waste management options should go hand in hand for maintaining proper ecological balance. Therefore, to develop new bioremediation processes for Cr and other metals contaminated wastewater, the isolation of micro-organisms capable of tolerating and reducing high Cr concentrations is very important (Chardin *et al.*, 2002).

1.3 AIM

The aim of this research is to determine the presence of heavy metals as well as to screen bacterial and fungal isolates from tannery effluent, for bioaccumulation capacities at a selected concentration of Cr.

1.4 OBJECTIVES

The objectives of the study are to:

1. determine the physicochemical parameters of the tannery effluents and well water.
2. determine the presence of heavy metals in the tannery effluents, soil, and well waters located near the streams.
3. enumerate and isolate the predominant and Cr resistant bacterial and fungal organisms from the tannery effluents, well waters and soils located near the streams.
4. screen and assess bacterial and fungal isolates from tannery effluent for Cr and other heavy metals sorption and bioremediation potential in laboratory and
5. determine the presence of heavy metals in crops irrigated with the tannery effluents.

CHAPTER TWO

LITERATURE REVIEW

2.1 Impacts of Tannery Waste and Wastewater on the environment

Every sector of any given society generates waste: agriculture, construction, consumers, energy, industry, mining, and transportation. Waste contains pollutants which are discarded materials, process materials or chemicals. Pollution could be caused by these pollutants when they are released beyond the assimilation capacity of the environment. Industrial wastes are generated from different processes and the amount and toxicity of waste released varies with its own specific industrial processes.

Land disposal of municipal and industrial wastes and applications of fertilizers and pesticides in agriculture has contributed to a continuous accumulation of heavy metals in soils (Adriano 1986; Sarkar, 2002; Krumholz *et al.*, 2003). The fate of toxic metallic cations in the soil environment depends largely on the interactions of these metals with inorganic and organic surfaces (Mullen *et al.*, 1989). Moreover, the behavior of heavy metals in soil is governed largely by sorption and desorption reactions with different soil constituents (Singh *et al.*, 2001). Quite a few papers dealing with the adsorption or complexation of various heavy metals by soils, soil inorganic constituents and organic matter fractions have been published over the past years (Warren and Haack, 2001).

The leather tannery industry is an important activity in many developing countries, "it has been estimated that about 18 billion ft^2 of leather are made annually around the world with a trade value estimated to be approximately US$ 70 billion. This industry uses large quantities of water and, in turn, produces large quantities of liquid effluents (Abera *et al.*, 2010)

Tannery effluent is one of the hazardous pollutants of industry and the effluents are ranked as the highest pollutants among all industrial wastes (Deepali *et al.,* 2009; Belay, 2010). The tanning of hides and skins to convert them into leather has been an important activity since antiquity (Ram *et al.,* 1999; Aboulhassan *et al.,* 2008). There are two types of tanning systems which are vegetable tanning, which does not contain chromium, and chrome tanning. However, due to the high pollution load and low treatability, conventional vegetable tanning can't be considered more environmentally friendly than chrome tanning. Moreover, vegetable tanned leathers have different physical properties and specific applications, but is biodegradable (Moore and Ramamoorthy, 2001). In modern tanning industry leather processing involves conversion of putrescible hide or skin into leather. Tanning agents could help permanent stabilization of the skin matrix against biodegradation. This industry has gained a negative image in society with respect to its pollution potential and therefore is facing a severe challenge. The unit processes that cause tanneries the most difficult with regard to perceived environmental impact are unhairing and chrome tanning. Basic chromium sulfate (BCS) is a tanning agent, which is employed by 90% of the tanning industry (Belay, 2010).

Leather tanning generates many complex and high loaded effluents that require treatment before being discharged into the environment and generally leather processing is almost wholly a wet process which is characterized by a large amount of liquid and solid waste, being produced. The tanning process and the effluents generated have already been reported in several literatures (Wiegant *et al.,* 1999; Sreeram and Ramasami, 2003; Stoop, 2003; Lefebvre *et al.,* 2006).

Tannery wastewater originates from the tanning process. The waste may be classified as continuous and intermittent flow wastes. Continuous flow wastes consist of wash water after

various processes and comprise a large portion of the total waste. Spent liquors belonging to soaking, liming, bating, pickling, tanning and finishing operations are discharged intermittently. Although these are relatively small in volume, they are highly polluting and contain a variety of soluble organic and inorganic substances. The spent soak liquor contains soluble proteins of the hides, dirt and large amount of common salts when salted hides are processed. The spent liquor undergoes putrefaction very rapidly as it offers a good amount of nutrients and favourable environment for bacterial growth. The growth of pathogenic anthrax bacteria in this waste is also reported (Sreeram and Ramasami, 2003). The spent lime liquor contains dissolved and suspended lime, colloidal proteins and their degradation products, sulphides, emulsified fatty matters, and also carry a sludge composed of un-reacted lime, calcium sulphide and calcium carbonate. As such, the spent lime liquor has a high alkalinity, moderate Biochemical oxygen demand (BOD), and high ammonia-nitrogen content. The spent bate liquor contains a high amount of organic and ammonia nitrogen due to the presence of soluble skin proteins, and ammonium salts used in deliming/bating. The spent pickling and chrome tanning waste comprises a small volume, having a low BOD, and contains traces of proteinic impurities, sodium chloride, mineral acids and chromium salts. Chromium is known to be highly toxic to the living aquatic organisms in the hexavalent form and somewhat less toxic in the trivalent form. Tanning salts consist of the trivalent form. In general, tanning industry wastes are of strong colour, have high salt content, high pH, high BOD, high chemical oxygen demand (COD), high dissolved solids, and a presence of sulphides, lime and chromium.

Tanneries generate wastewater in the range of 30 - 35 L/kg skin / hide processed with variable pH and high concentrations of suspended solids, BOD, COD, tannins including chromium

(Nandy *et al.,* 1999) at about 30–35 L of water to 1 kg of processed skin (Suresh *et al.,* 2001; Sreeram and Ramasami, 2003). With the present annual global processing capacity of 9×10^9 Kg hides and skins, it is estimated that 30-40 x 10^{10} litres of liquid effluent is generated (Thanikaivelan *et al.,* 2004). This gives rise to two major problems for the leather industry: the availability of good quality water and the treatment of such large quantities of effluent because conventional chrome tanning results in wastewater containing as high as 1500-3000 ppm (parts per million) of chromium; however, the present day high-exhaust chrome tanning methods lead to a wastewater containing 500-1000 ppm of chromium (Aravindhan *et al.,* 2004)

Currently more than 90% of global leather production of 18 billion sq. ft is through chrome-tanning process (Sundar *et al.,* 2002). Chromium salts (particularly chromium sulphate) are the most widely used tanning substances today. Hides tanned with chromium salts have a good mechanical resistance, an extraordinary dyeing suitability and a better hydrothermic resistance in comparison with hides treated with vegetable substances. Unfortunately only a fraction of the chromium salts used in the tanning process react with the skins. The rest of the salts remain in the tanning exhaust bath and are subsequently sent to a depuration plant where the chromium salts end up in the sludge (Wionczyk *et al.,* 2006) and the discharge limits for trivalent chromium vary broadly ranging from 1 to 5 mg/l in the case of direct discharge into water bodies and 1 to 20 mg/l in the case of discharge into the public sewer system. Therefore, the treatment plant used by the tanning industry needs to treat the influent by 200 fold to send to water bodies, which is not practicable in most of the cases (Tadesse *et al.,* 2006).

2.2 Potential Environmental Impact of the Chemicals Used in Tannery Operations

Pollution could be caused by pollutants when they are released beyond the assimilation capacity of the environment. Industrial wastes are generated from different processes and the amount and toxicity of waste released varies with its own specific industrial processes (Shen, 1999). The direct discharge of effluents from tanneries in to water bodies has become a growing environmental problem in these days. Most of these waste waters are extremely complex mixtures containing inorganic and organic compounds that make the tanning industry potentially a pollution-intensive sector (Akan *et al.,* 2007).

Tannery effluents are ranked as the highest pollutants among all industrial wastes. They are especially large contributors of chromium pollution. The potential environmental impact of the chemicals used in tannery operations has been widely acknowledged. Cr containing effluents find their way in the environment at disposal sites where Cr undergoes oxidation reactions and forms Cr (Vl) (Bartlett and James, 1979). As Cr (VI) is readily soluble in water, it leaches down in the soil profile and could contaminate groundwater (Sumathi *et al.,* 2005). Furthermore, the discharge of such colored wastewater into the environment is not only aesthetically displeasing, but also impedes light penetration, damages the quality of the receiving streams and may be toxic to the treatment processes, to food chain organisms and to aquatic life (Mahdavi Talarposhti *et al.,* 2001).

The discharge of Chromium(VI) into aquatic ecosystems has become a matter of concern in all the tannery areas the world over in the last few decades. This pollutant is introduced into the aquatic systems significantly from the effluents of leather processing units as a result of chrome tanning of leather. Chromium's interactions with biological systems are very different and com-

plex. Toxicokinetics of Cr(VI) show higher rate of penetration into biological membranes as compared to Cr(III). Based on review of available literature, about 10% of ingested Cr(VI) is absorbed in the gastrointestinal tract of human. The carcinogenicity of Cr(VI) is well documented. Skin ulceration and allergic contact dermatitis have been reported in numerous cases (Vankar and Bajpai, 2008).

The removal of Cr(VI) from aqueous solution under different conditions using an adsorbent was investigated. This adsorbent is beech *(Fagus orientalis* L.) sawdust studied by batch techniques. Batch studies indicated that the percent adsorption decreased with increasing initial concentration of Cr(VI) (Vankar and Vajpai, 2008). The commonly used procedures for removing metal ions from aqueous streams include chemical precipitation, lime coagulation, ion exchange, reverse osmosis and solvent extraction, and the conventional treatment techniques used for removal of Cr(VI) ions from wastewaters which include chemical precipitation, oxidation reduction, ion exchange, and electrolysis. In this regard, the increasing awareness of accumulation of heavy metals in the environment has also led to a quest for new and improved "cleaner" technologies to overcome metal pollution. An innovative heavy metal removal process called bioremediation composed of phytoremediation and biosorption has gained enormous impetus. In the process of phytoremediation, certain plants have been found to remove metals from contaminated water. 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. The major advantages of

biosorption over conventional treatment methods include: low cost, good efficiency, minimization of chemical and or biological sludge, regeneration of biosorbent, and possibility of metal recovery.

The biosorption process involves a solid phase (sorbent or biosorbent; biological material) and a liquid phase (solvent, normally water) containing a dissolved species to be sorbed (sorbate, metal ions). Due to higher affinity of the sorbent for the sorbate species, the latter is attracted and bound there by different mechanisms. The process continues till equilibrium is established between the amount of solid-bound sorbate species and its portion remaining in the solution. The degree of sorbent affinity for the sorbate determines its distribution between the solid and liquid phases. Mechanisms of metal biosorption are essential to be understood first in order to successfully exploit the phenomenon and for regeneration of biosorbent materials in multiple reuse cycles. The complex nature of biosorbent materials makes this task particularly challenging. Some of the common conventional methods for the treatment of Cr(VI) contaminated wastewaters include chemical reduction followed by precipitation, ion exchange and adsorption on coal, activated carbon, alum, kaolinite and flyash and have achieved some success as well. Nevertheless, these methods have several disadvantages, such as high operating costs, the requirement of preliminary treatment steps, the difficulty of treating the solid waste subsequently generated and the requirement of large quantities of chemical adsorbents.

2.3 Tannery Pollution in Different Parts of the World

Tanning industry is recognized as a serious environmental threat all over the world (Tariq *et al.*, 2005). It is an especially large contributor of chromium pollution in India (Apte *et al.*, 2005) and Pakistan (Chaudry *et al.*, 1997; Tariq *et al.*, 2005). Among all industrial sources of

environmental contamination, the tanning activities are very widespread in North Africa and often associated with high chemical pollution levels. In India alone about 2000–3000 tone of chromium escapes into the environment annually from tannery industries, with chromium concentrations ranging between 2000 and 5000 mg/l in the aqueous effluent compared to the recommended permissible discharge limits of 2 mg/l (Altaf *et al.,* 2008).

Many industries in Bangladesh discharge their effluents directly and/or indirectly onto land or into small channels leading to the nearby rivers. Alam, (2009) in a study of four tanneries has found that almost all are violating the set effluent standards, and polluting near by rivers, agricultural land and soil. Many industries in Bangladesh have effluent discharge levels above the Ministry of Environment's (MoE) tolerance limits for discharge into inland surface water directly, or indirectly into soil and/or small channels leading to the nearby rivers. It was found out that due to a lack of compliance monitoring almost all industries are violating the standards at all times, and are polluting nearby rivers, agricultural land and soil. Similarly The chromium concentrations in sediments from river Irajà (Brazil) ranged from 210 to 70,000 mg/kg respectively before and after waste discharges from an electroplating industry (Pfeiffer *et al.,* 1982). Such metallic contaminations have also been observed in fluvial sediments in relation to tanneries in the state of Minas Gerais (Brazil) (Jordão *et al.,* 1996, 1997). Chromium concentrations in sediments were about 93.0±3.0 mg/kg upriver and 2,878.0±78.0 mg/kg downstream of the tanneries. These contaminations by tanneries have been observed previously in literature with sometimes significant pollution. It was thus reported that the concentrations of total chromium in the waste effluents of the tanneries in the Ganga river at Kampur (north of India) is over 150 mg/l (Khwaja *et al.,* 2001).

Tannery effluent is a major source of aquatic pollution in Bangladesh with high chemical and biological oxygen demand. There are nearly 300 tanneries in Hazaribagh, Dhaka. Daily discharge of wastes from these tanneries is about 18000 litres of liquid waste, 115 tones solid wastes during offpeak time. According to a recent estimate, about 60,000 tones of raw hides and skins are processed in these tanneries every year, a process which release nearly 95,000 litres of untreated effluents into the open environment daily, in addition to contamination of the water of the river Buriganga (Mohanta *et al.,* 2010).

Similarly Stuart and Milne, (2001) reported that the city of León, Mexico, is a major centre for the Latin American leather processing and shoe manufacturing industries, with more than 500 leather curing and tanning establishments. The city produces wastewater containing significant quantities of chromium that has been used untreated over a period of 40 years to irrigate agricultural land overlying an important aquifer. The city is highly dependent on these groundwater resources for public water supply. Wastewater is discharged from the sewerage system into a complex system of open distribution canals with some water being used directly for irrigation and the remaining passing through the El Mastranzo storage lagoon

Tabriz, the capital city of east Azarbaijan province in north west of Iran, has approximately 320 tanneries with an estimated wastewater discharge of 3.5–4 million L/day that are released, mostly without treatment, into the environment and cause serious environmental damages. There are similar problems in other industrial areas with large number of tanneries in Iran (Abera *et al.*, 2010). Floqi *et al*. (2007), reported that there many tanneries (some of them are working in illegal way) all over Albanian territory, from the north to the south and from the east to the west, all processing leather factories and tanneries discharge hundreds of thousands ton wastewater

effluent into water basins.

On the other hand, Tarcan *et al.* (2010) reported that more than 50 tanneries are operated in Kula, Turkey which is located on highly permeable geological units. The untreated effluents from the tanneries discharged onto the ground surface may cause pollution in surface waters, in groundwater, and in soil. Since the water resources of the region are used for drinking, agricultural, and industrial purposes, the quality assessment of groundwaters and surface waters is completed in the content of this study. Additionally, the surface soil samples were analyzed for their heavy metal content to describe the size of the pollution. The results obtained from the analysis of the water samples show that the concentrations are mostly within drinking water limits. But, concentrations are expected to increase in the next years as no effective effluent collection and treatment is present in the tanneries. Although the concentrations of K_2SO_4, Mg, Na, and Cl exceed the permissible limits for drinking water in some cases, they may not directly be caused by tannery activities. Nevertheless, they were assessed as an indicator of the beginning of groundwater pollution. Also, soil samples collected near the tannery district were extremely polluted.

2.4 Health Effect of Chromium and Tannery Effluents

Cr III is an essential nutrient for human beings (Deepali and Gangwar, 2010); at high concentrations chromium is toxic, mutagenic, carcinogenic, and teratogenic; there is no doubt that Cr (VI) compounds are both acutely and chronically toxic. The dose threshold effect for this element has not yet been determined accurately enough to allow regulations to be defined. Chromium exists in oxidation states of +2, +3, and +6. The trivalent oxidation state is the most stable form of chromium and is essential to mammals in trace concentration and relatively immobile in the aquatic system due to its low water solubility (Gupta and Sinha, 2006; Belay,

2010).

Inhalation may cause acute toxicity, irritation and ulceration of nasal septum and respiratory sensitization. Skin contact may results in systemic poisoning, damage or even severe burns, interference with the healing of cut or scrapes which, if not treated promptly, may lead to ulceration and severe chronic allergies contact. Eye exposure may cause permanent damage. In general, Cr(VI) is absorbed into cells more readily than Cr(III) (Vankar and Bajpai, 2008). Chromium can replace other metals in biological systems with toxic effects and its accumulation throughout the food chain leads to serious ecological and health problems as Cr(VI) is a known human carcinogen. In this context, most studies on prokaryotes and chromium involve reduction of Cr(VI) to less toxic and less mobile forms such as Cr(III).

However, some risk assessment analysis is currently being under-taken. Cr III is less toxic than some other elements (Hg, Cd, Pb, Ni and Zn) to mammalian and aquatic organism, probably due to the low solubility of this element in its trivalent form. Cr III compounds also have a very low mobility in soils and are thus relatively unavailable to plants.

2.5 Effect of Chromium and Tannery Effluents on Plants

Cr accumulation and toxicity were evaluated in relation to photosynthetic pigments, cysteine, and protein contents in *Valisneria spiralis* and its possible role was investigated in bioremediation of Cr rich tannery effluent through Cr accumulation (Sen *et al.,* 1987).The uptake of Cr(VI) and toxic effects of the metal on some biochemical parameters in *Pistia stratiotes* were studied. The uptake of Cr(VI) by the plants gradually increased with an increase in concentration of Cr(VI) in the culture medium. Maximum accumulation of Cr(VI) was recorded within 5 days. Maximum removal (>90%) of Cr(VI) was recorded below 5 ppm. Accumulation of Cr(VI)

occurred mainly in roots (Sen *et al.,* 1987).

In the environment Chromium (III) and (VI) are the most stable forms of metal; however, the toxicity depends on its chemical form. Chromium(VI) compounds have toxic , mutagenic, and carcinogenic nature where as the trivalent forms, which prevails in foods has a low toxicity(Gupta and Sinha, 2006). Chromium is an essential trace mineral nutrient for normal sugar and fat metabolism which functions primarily potentiating the action of insulin. Chromium as well as other metals are easily absorbed by roots and leaves of plants and then transported via the vascular system. The high levels of chromium found in water and sediment samples are worrying because this metal is known to be toxic (Leghouchi *et al.,* 2009)

Vegetables constitute essential components of the diet by contributing protein, vitamins, iron, calcium and other nutrients. They can take up very high amount of essential and toxic metals from contaminated sites (soil and water). The essential metals are required for growth of the plants in small quantities and the presence of excessive concentration may cause toxicity. Moreover, the physical and chemical forms of metals in which they are dispersed, may increase the metal availability in vegetables. The uptake of Cr from agricultural soils depends on their stability, soil pH, organic matter, plant species, fertilizer and soil type (Gupta and Sinha, 2006).

The accumulation of metals including chromium in the edible parts of the plants grown in agricultural soil contaminated with industrial wastewater has been reported by several researchers because of their ability to absorb and accumulate the metals in their tissue from the environment (Sinha *et al.,* 2001; Sinha *et al.,* 2006; Gupta and Sinha, 2006). Studies have shown that the uptake of metal mainly depends on chemical form of the metal and life form of macrophytes, submerged, rooted submerged, floating and rooted emergent. Chromium and other

metals are easily absorbed by roots and leaves of plants and then transported via the vascular system. The high levels of chromium found in water and sediment samples are worrying because this metal is known to be toxic (Jordão *et al.*, 1996; Armienta *et al.*, 1995; Chaudhry *et al.*, 1999). The hexavalent chromium is much more toxic to many plants, animals, and bacteria inhabiting aquatic environments. Tannery effluents severely affect the mitotic process and reduce seed germination in extensively cultivated pulse crops (Altaf *et al.*, 2008).

Tannery effluent is one of the most complex effluents containing a number of pollutants (Sastry and Madhavakrishna, 1984; Sujatha *et al.*, 1996) and poses great threat to ecosystems by exerting detrimental (direct toxicity, genotoxicity, mutagenicity) effects and disrupts the food chain by reduction in the structure and productivity of aquatic flora (Koteshwari and Ramnibai, 2004). Tannery effluent contains vegetable tannins in addition to soluble organic matter, suspended solids, chromium, sodium, high chloride and sulphate concentration with a very high pH, high BOD, COD and conductivity (Rao and Kumar, 1981). Some of the salts in the effluent are nutritious and some are toxic to plant growth. Tannery effluent is highly toxic to animals (Rao and Marriyappan, 1972; Saktivel, 1989), plants (Muthukumar and Mahadevan, 1981) and a variety of microorganisms (Mahadevan *et al.*, 1984). Studies on bioremediation of tannery effluent by aquatic macrophytes were carried out by Vajpaye *et al. (*1995) and Sinha *et al. (*2002) in which these plants were proved to have the ability to reduce the level of toxic metals from the polluted water. Decrease in chlorophyll content in *Cicer arietinum* L. seedlings has been observed at higher concentrations (25-100%) of tannery effluent but 5-10% effluent concentration enhanced the total chlorophyll (Rao and Kumar, 1983). Higher effluent levels delayed germination and decreased root and shoot length. Kumar and Chachan (1993) studied reduction in root and shoot number and their growth in *Allium cepa* due to effect of tannery

effluent. Madhappan (l993) observed that undiluted tannery effluent (beyond 50%) had a toxic effect on germination and growth of *Phaseolus mungo* L. and *P. aureus* L. whereas, 25% effluent concentration had a growth promotion effect. There existed a significant positive relationship between total chlorophyll content and lower effluent concentration (Lal, 2009).

Sinha *et al.* (2001) demonstrated comparison of Cr accumulating potential by the plants of *Najas indica* Cham. (Submerged), *Vallisneria spiralis* L. (rooted submerged) and *Alternanthera sessilis* R. Br. (rooted emergent) under repeated metal exposure and its effect on chlorophyll and protein concentrations. These plants were treated with different concentrations of Cr under repeated exposure in controlled laboratory conditions to assess the maximum metal accumulation potential. The plants of *V. spiralis* accumulated significantly high amount of Cr under laboratory conditions in comparison to *N. indica* and *A. sessilis*. The maximum accumulation of 1378, 458 and 201 μg g^{-1} dw Cr was found in the leaves of *V. spiralis*, *N. indica* and *A. sessilis*, respectively at 8mg/L after 9days of Cr exposure. These plants have shown a decrease in chlorophyll and protein concentrations with increase in Cr concentrations. In view of high accumulation of Cr in *V. spiralis*, the plants were treated with different concentrations of tannery effluent collected from Common Effluent Treatment Plant, Unnao (UP). The plants of *V. spiralis* treated with 100% tannery wastewater showed the maximum accumulation (57.5μg g^{-1} dw) of Cr in the roots after 10 days of exposure. The plants were found effective in removing Cr from solution and tannery effluent (Shukla *et al*., 2009).

2.6 Chrome Recovery and Recycling

Generally chrome tanning in leather production produces spent liquors containing large amounts of chromium and other organic and inorganic pollutants. From the total chromium used for tanning only 60% to 70% is utilized, while the remaining 30 to 40% is retaned in the spent

tanning liquor, which is discharged into wastewater treatment plant. This inefficient use of chromium and its release to the environment has to be compensated by designing a good recovery and recycling scheme. The recovery of chromium from spent tanning and re-tanning baths provides a significant economic advantage in terms of both its reuse and the simplification of the processing of global wastewaters (Cassano *et al.*,2007).

Several recovery techniques such as chemical precipitation, membrane processes, adsorption, redox ad-sorption, and ion exchange have been proposed for this purpose. Among these membrane process offer very interesting opportunities for the recovery and recycling of primary resources from spent liquors of unit operations such as soaking, unhairing, degreasing, pickling, dyeing, and chromium tannage. Studies showed that the application of nanofiltration (NF) and reverse osmosis (RO) in combination can provide better recovery of unreacted chromium from high concentrated spent tanning effluent. However, this technique is being challenged by the presence of considerable biological oxygen demand (BOD) and proteins, which can cause fouling and subsequent system failure, either temporarily or permanently (Cassano *et al.*,2007).

In practice, there are two ways of chrome recycling methods which are widely practiced: these are direct and indirect recycling. The direct form entails spent float being recycled direct to the chrome tanning processing for re-use. While, the indirect form entails precipitating and separating the chrome from the float containing residual chrome, and then re-dissolving it in acid for re-use. The efficiency of both methods can be very high (more than 90%); it depends on the effectiveness of the float collection process and the recycling/reusing technique. Of the two approaches, chrome recovery is more widely used than chrome precipitation. However, it is mandatory to adopt and practice the new technologies which are more efficient in recovering this chemical, which is a big challenge to tanning industry (Ludvík, 2000). Use of chemical

compounds for chrome recovery is not a new phenomenon. Ali awan *et al. (*2003), in their study identified three aqueous oxidants, namely: Hydrogen peroxide, Sodium Hypochlorite and Calcium Hypochlorite independently in oxidizing Chromium (III) containing tannery wastewaters to soluble chromate ($CrO4^{2-}$) under alkaline conditions. Among those, Hydrogen peroxide was potentially a suitable oxidant as it could recover chromate ($CrO4^{2-}$) up to 98% (from synthetic Cr^{3+} solution) and 88% (from effluent I). Despite the different experimental conditions (temperatures and oxidation time) for all the three oxidants, complete (100%) recovery could not be achieved. The recovery of chromium could help in reducing the possibility of oxidizing Cr III to Cr VI (carcinogenic) compound and helps to rescue the financial and environmental cost occurring as a result of its discharge (Ali awan *et al.,* 2003). Other kinds of methods like combined system have to be also tested since it might improve the efficiency of recovering chromium from tanning process. In fact experimental conditions like, temperature, pH, time, need to be controlled to have more efficient recovery.

2.7 Physico-Chemical Techniques Used in the Treatment of Tannery Wastewater

The major physical–chemical methods for removing Cr(VI) ions from industrial wastewater are: (1) chemical reduction to Cr(III) followed by precipitation under alkaline conditions, and/or (2) removal by reverse osmosis, ion exchange, and adsorption on activated carbon (Park *et al.,* 2004; Sumathi *et al.,* 2005; Morales-Barrera and Cristiani-Urbina, 2008). However, these methods have certain drawbacks, namely high cost, low efficiency, generation of toxic sludge or other wastes that require disposal and imply operational complexity (Şahin and Öztürk 2005). Therefore, cost-effective and more environmentally friendly techniques need to be developed to remove Cr(VI) ions from industrial wastewater. Various physico-chemical techniques have been studied for their applicability to the treatment of tannery wastewater (Orhon *et al.,* 1998;

Amokrane *et al.*, 1997). Among these are coagulation, flocculation, ozonation, reverse osmosis, ion exchange and adsorption (Arvanitoyamis *et al.,* 1989). Coagulation-flocculation is one of the important treatments given to the industrial effluent before discharging them into receiving waters to remove toxic waste. Abera *et al* (2010) reported that among the various wastewater treatment methods such as coagulation–flocculation, advanced oxidation processes, biological treatment, ozonation and activated carbon adsorption that have been applied for the treatment of tannery wastewaters, coagulation is the most widespread process in wastewater treatment. "Coagulation is usually achieved through the addition of inorganic coagulants such as aluminum or iron-based salts, and/or synthetic organic polymers commonly known as polyelectrolytes". This process forms the precipitating flocs, which adsorb the pollutants. The pollutants are removed when the flocs are precipitated. This method is used for the removal of metals, treatment of toxic wastes, removal of turbidity and suspended solids and control of color. And also it is suitable to enhance the effectiveness of subsequent treatment and to provide treatment with lower cost. This process is not always perfect and may result in small flocs when coagulation takes place at too low temperatures, or fragile flocs which break up when subjected to physical forces Flocculation is necessary to overcome these problems and to improve the coagulation process to obtain a good-quality effluent and rapid sedimentation of the flocs. So various materials, such as coagulant aids, can be used to affect coagulation or to increase flocs density and thereby improving sedimentation. Coagulant aids are different ionic, polymeric or other materials, which can enhance the flocs quality via various mechanisms. Hence, the selection of suitable coagulant aid is essential to achieve the coagulation process with high efficiency (Abera *et al.,* 2010). Demonstration of effective and efficient tannery effluent treatment using an innovative integrated and compact biological and physical treatment plant is

shown in Fig. 2.1.

Many researchers have investigated the coagulation of tannery wastewater. However, few of them attempted to fully investigate the optimisation of coagulation for color reduction or in conjunction with subsequent biological treatability of coagulated tannery wastewater. Indeed, the coagulation process is not always perfect and may result in treated wastewaters of which the characteristics did not meet the proposed effluent standards. Another major drawback with these treatment systems is sludge production, high operational cost and complicated management that require skill and expertise. This actually makes the application of these technologies to be limited only in developed countries. This motivated other researchers to try other feasible techniques that that can be cost effective to use by the majority. Research was done by preparing a new composite biosorbent which has been done by coating chitosan onto acid treated oil palm shell charcoal (Nomanbhay and Palanisamy, 2005). It is an attractive option because of its cost effective treatment system. Among other low cost absorbent identified chitosan has the highest sorption capacity for several metal ions including Chrome. Chromium adsorption was influenced by initial pH, agitation, dose of adsorbent and contact time. For instance at a pH of 5 the media managed to treat 92 % of chromium, which is significant. Further control of these environmental conditions in the laboratory may improve its treatment capacity (Nomanbhay and Palanisamy, 2005).

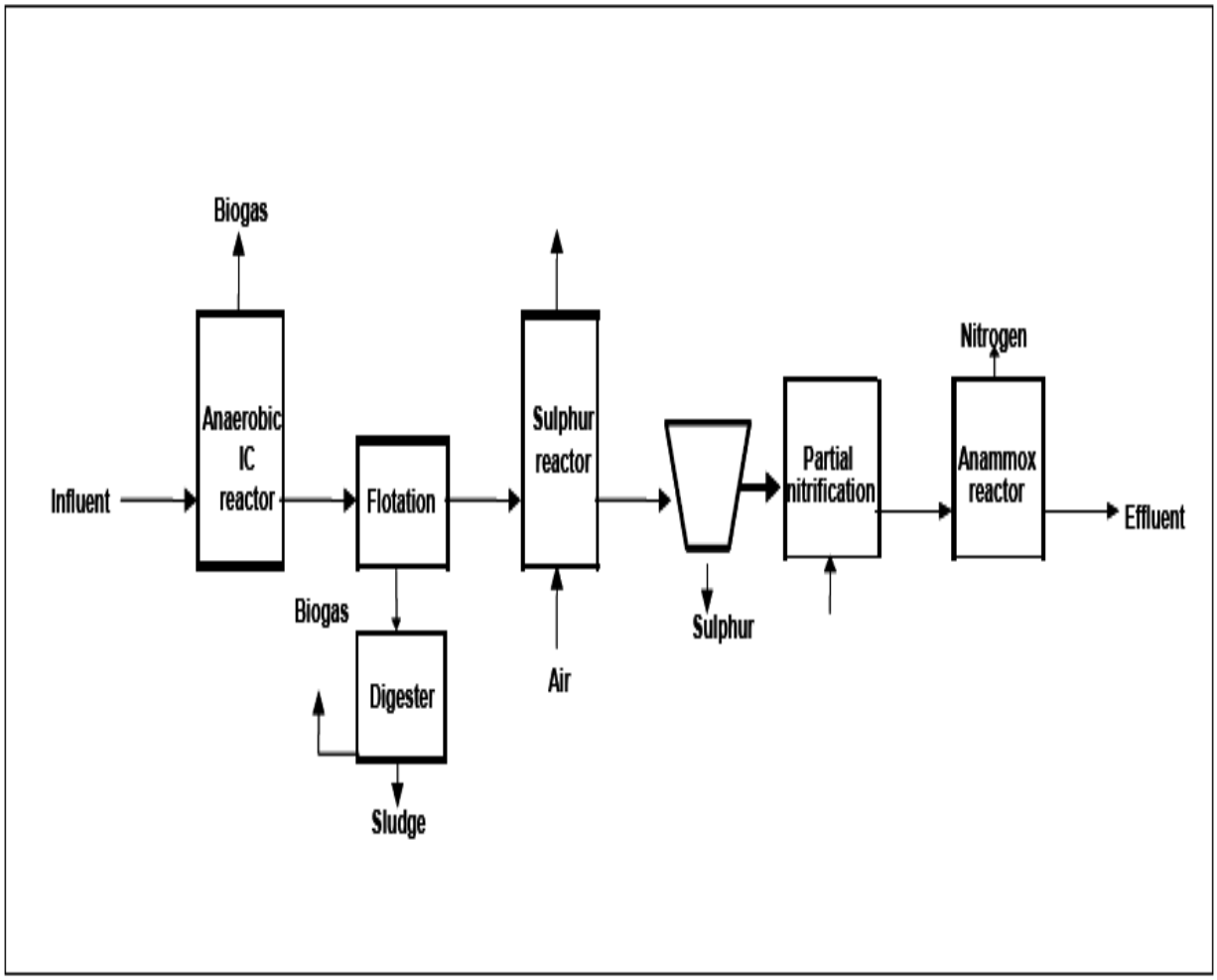


Figure 2.1: Integrated tannery effluent treatment plant

Palm shell charcoal may be cheap or readily available in some area, however, in the present study area is not available and may be costly to buy and transport to the area and consequently increase the cost of treatment or recovery.

A comparison study also conducted by leaching raw tannery effluent through mono and mixed columns (different grades) of vermiculite to evaluate their removal efficiency of chromium, the mixed column of vermiculite has the highest chromium removal (74.6%) while the mono vermiculite achieved 63.6%. This improvement in chrome removal efficiency is brought by the use of combined media which increase its adsorption capacity. It was also found out that, it could remove cat ions like Ca, Na, Mg and K, of course high cat ion exchange helps to make the system more efficient (Jayabalakrishnan, and Aselvaseelan, 2007). Recently different studies are concentrating use of combined media under the control of laboratory environment and the results seem promising. A research has been done in Ethiopia by Tadesse *et al.* (2005) to check the removal efficiency of chromium from tannery effluent in a horizontal settling tanks and subsequent Advanced Integrated Wastewater Pond System (AIWPS) reactors. The raw combined effluent from the tannery had a different pH and its removal efficiency was measured in detention time and pH to come up with the best chrome removal efficacy. After a one day detention time 58-95% of trivalent chromium has been removed in the primary settling tank when the pH is approached to 8, which is the optimum precipitation pH for trivalent chromium.

A significant amount of chromium has also removed in the secondary facultative pond and maturation pond. The presence of sulphide plays a role in the overall removal of chromium. It has some coagulating effect be-sides maintaining a conducive pH for the formation of Cr (OH)3

precipitate. However, since chromium doesn't make any stable precipitate with sulphide, it has no any effect in the chromium removal chemically. With Cr (III) concentration of 0.2–0.8 mg/l in the final treated effluent, the AIWPS preceded by horizontal settling tanks produced effluent that could easily meet most of the current Cr (III) discharge limits. This study is prominent in finding ways to achieve the maximum removal of chromium and producing an effluent that meet the standard criteria to discharge to water bodies. This technique needs get attention to be considered and applied in the conventional tannery treatment system to improve its efficiency and prevent the alleged environmental consequence due to the toxic effluent discharge (Tadesse *et al.,* 2006).

The ever increasing concern about the deterioration of the environmental condition could be a driving force to assess and remediate pollutant from the ecosystem. In this regard the use of plant species especially algae become acknowledged in indicating and managing metal pollution. Despite the fact that a large scale study and experience has been developed in different countries to use algal ponds as effective means of sewage treatment system the accumulation potential of algal species and seasonal variation of tolerance is not exhaustively addressed. Rai *et al.* (2005) conducted a study on seasonal variation of algal growth in tannery effluent and metal accumulation potential for chromium removal scheme. It has been noticed that different algal species found with accumulated chromium in their tissue, could be used in developing bioremediation strategy for pollution abatement. Of course, factors like population density, volume of effluent, the nature of mixing with effluent and optimum algal biomass should be considered and well examined before promoting for wide application (Rai *et al.,* 2005).

Wastewater characterization is an important step in designing effective treatment facilities for industrial wastewaters. This is especially true for tanneries which exhibit significant differences in their production processes that generate effluents of unique and complex nature. Characterization is also needed for assessing the performance of individual unit operations and processes. Most pollutants in wastewaters appear to exist either in particulate form or are associated with particulates. This understanding led to the wastewater treatment strategy of removing particulate and colloidal matter in the primary step using suitable coagulants. A study investigated about chemically enhanced primary treatment (CEPT) technology that uses different coagulants for enhanced pollutants removal at the primary stage of the wastewater treatment. Among those coagulants used, alum has been found to be the suitable coagulant for tannery wastewater in a dose range of 200–240 mg/L as $Al_2(SO_4)_3$ and it has removed 98.7–99.8% of chromium. The final effluent also met the national effluent quality standards for chromium and total suspended solids(Haydar, and Aziz, 2007). However other COD content needs secondary treatment for the tannery effluent. Therefore, CEPT technique offers almost complete removal of chromium and produces an effluent that will no more affect the receiving water bodies (Haydar, and Aziz, 2007).

Cleaner technologies used to reduce chromium in waste water such as high exhaustion process, direct or indirect chromium recycling cannot eliminate completely from effluent coming from post tanning section. In response to this challenge, replacement of chromium with combinations of metallic cat ions, for example titanium, magnesium, aluminium and zirconium, was tried but the results obtained at the moment are not completely satisfying for all types of leather. Synthetic organic tanning agents, alone or in combination with a metallic cat ion can be considered as a

substitute for chromium in some types of leather, provided that environmental and workers health regulations are complied with (Moore and Ramamoorthy, 2001).

2.8 Microorganisms as Biosorbent Material

Biological processes, such as bioreduction, bioaccumulation or biosorption using microbial cells, have been examined for their Cr(VI) removal capabilities (Cheung and Gu, 2003). Microorganisms such as algae, bacteria and fungi and yeasts have proved to be potential metal biosorbents (Volesky, 1986). A wide variety of microorganisms such as bacteria, yeast, algae, protozoa and fungi are found in waters receiving industrial effluents. Many of the microorganisms show adaptation to the toxic materials constantly released into their environment. They have developed strategies to resist, tolerate, metabolize, and to detoxify these toxic substances; these microorganisms have a high surface area to volume ratio and therefore provide a large contact area that can interact with metals in the surrounding environment. Microbial metal accumulation has received much attention in the last years due to the potential use of microorganisms for cleaning metal-polluted water (Huang *et al.*, 2005). The biological reduction of Cr(VI) to Cr(III) has generated growing interest among scientists and engineers because this process may not only mitigate chromium toxicity in living organisms, but may also aid by precipitating chromium at near-neutral pH in the form of $Cr(OH)_3$. This process has also been considered an economically feasible alternative for wastewater treatment (Cheung and Gu 2003). Strong biosorbent behavior waste materials of certain micro-organisms towards metallic ions are a function of the chemical make-up of the microbial cells. This type of biosorbent consists of dead and metabolically inactive cells. Some types of biosorbents would be broad range, binding and collecting the majority of heavy metals with no specific activity, while others are specific for certain metals. Several laboratories have used easily available biomass whereas

others have isolated specific strains of microorganisms. Cr(VI) reduction by bacterial consortia (Francisco *et al.,* 2002; Smith *et al.,* 2002; Cheung and Gu 2003) and pure bacterial cultures (Wang *et al.,* 1989; DeLeo and Ehrlich 1994; Fujie *et al.,* 1996; Pattanapipitpaisal *et al.,* 2001a,b; Ganguli and Tripathi 2002) has been extensively studied under aerobic and/or anaerobic conditions. Most studies report that micro-organisms are capable of reducing Cr(VI) at low concentrations, usually within the range from 0.1 to 0.5 mM (Wang *et al.,* 1989; Shen and Wang 1993; Pal 1997; McLean and Beveridge 2001; Pattanapipitpaisal *et al.,* 2001a,b; Chardin *et al.,* 2002; Humphries and Macaskie 2002; Smith *et al.,* 2002; Dmitrenko *et al.,* 2003; Konovalova *et al.,* 2003). Some microorganisms lose viability and Cr(VI)-reducing capacity in the presence of high concentrations of Cr(VI), which further complicates the biological treatment of Cr(VI)-polluted wastewater (Pattanapipitpaisal *et al.,* 2001a).

2.9 Waste Materials as Biosorbent Material

Recent biosorption experiments have focused attention on fermented substrates, which are by-products or the waste materials from large-scale industrial operations. The waste mycelia available from fermentation processes, olive mill solid residues (Kuyucak and Volesky, 1988). Kuyucak, and Volesky, (1988) carried out a preliminary study on the use of olive mill residues as heavy metal sorbent material. The results revealed that copper was maximally adsorbed in the range of 5.0 to 13.5 mg/g under different operating conditions. Another inexpensive source of biomass where it is available in copious quantities is in oceans as seaweeds, representing many different types of marine macro-algae. However most of the contributions studying the uptake of toxic metals by live marine and to a lesser extent freshwater algae focused on the toxicological aspects, metal accumulation, and pollution indicators by live, metabolically active biomass. Focus on the technological aspects of metal removal by algal biomass has been rare. Although

abundant natural materials of cellulosic nature have been suggested as biosorbents, very little work has been actually done in that respect. The mechanism of biosorption is complex, mainly comprises of ion exchange, chelations, adsorption by physical forces, entrapment in inter and intra-fibrilliar capillaries and spaces of the structural polysaccharide network as a result of the concentration gradient and diffusion through cell walls and membranes.

Relatively less attention has been paid to the role of microorganisms for metal mobility in soil and the interactions between bacteria and soil colloidal components (Guo *et al.,* 2002). Kurek *et al.* (1982) observed that live cells of *Paracoccus* sp. and *Serratia marcescens* accumulated about the same quantity of Cd^{2+} as did montmorillonite when the solid:solution ratio was the same for both bacteria and clay, whilst sorption of Cd^{2+} by dead cells was greater than that of clay. Walker *et al. (*1989) reported that the order of metal uptake by bacteria biomass and clays was *Escherichia coli* cell envelopes > *Bacillus subtilis* cell walls > smectite > kaolinite. In a multicomponent system, soil microorganisms *(Pseudomonas putida*, *Trichoderma harzianum)* could compete effectively with soil minerals and solid organic matter to accumulate Zn, Cd, and Hg (Ledin *et al.,* 1996).

Microorganisms indigenous to heavy metal-containing environments are able to survive in high concentrations of toxic metals by their intrinsic or induced detoxification mechanisms as well as by the result of environmental factors such as pH, redox (Huang *et al.,* 2005). Heavy-metal-resistant bacteria may be used for bioremeditation of metal-contaminated environments (Nies, 1999). Bioremediation, using bacteria or plants, is often regarded as a relatively inexpensive and efficient way of cleaning up wastes, sediments, or soils contaminated with toxic heavy metals (Krumholz *et al.,* 2003; Sauge-Merle *et al.,* 2003). Many efforts have been devoted to the

isolation of heavy metal resistant bacterial strains during recent years. For example, Srinath *et al.* (2002a) isolated Cr-resistant bacteria from tannery effluent capable of bioaccumulating 3 2–34 mg Cr g-1 dry weight. The non-living cells of two bacteria strains (*Bacillus* sp) isolated from metal-polluted soil by Zouboulis *et al.* (2004) had the maximum removal capacities of 142–160mg g-1 for Cd (II) and 63–73 mg g-1 for Cr(VI). The Cu(II) adsorption capacity at pH 7.0 for the marine *Bacillus* sp. Strain SG-1, which was isolated from a near-shore sediment culture, was 53 mg g-1 as dried spores (He and Tebo, 1998). Although, only scanty knowledge is available regarding the microbial diversity of biological wastewater treatment systems operating with a high salt content, Yet, it has already been shown that the diversity of a salt-tolerant ecosystem treating hypersaline industrial wastewater could be similar to the diversity of a non-salt-tolerant one (Lefebvre *et al.,* 2004). In another study Lefebvre *et al.* (2006) reported that the microbial communities of a hypersaline tannery effluent, and those of three treatment systems operating with the tannery effluent, were investigated using 16S rDNA phylogenetic markers. The comparative analysis of 377 bacterial sequences revealed the high diversity of this type of hypersaline environment, clustering within 193 phylotypes (97% similarity) and covering 14 of the 52 divisions of the bacterial domain, namely, *Proteobacteria, Bacteroidetes, Firmicutes, Actinobacteria, Chlorobi, Planctomycetes, Spirochaetes, Synergistes, Chloroflexi, Thermotogae, Verrucomicrobia.* Most of the phylotypes were related to halophilic and pollutant-degrading bacteria.

Algal and fungal biomasses have proven useful in biosorption studies for the removal of Cr from contaminated sources. Metal resistant algae reported in wastewaters and metalpolluted environments process and detoxify heavy metal ions usually through biosorption, adsorption and bioaccumulation (Verma and Singh 1995; Rehman and Shakoori, 2001). Accumulation of heavy

metals in algae, as in higher plants, induces phytochelatin synthesis (Gekeler *et al.*, 1988). Phytochelatins are low molecular mass peptides, derived from polymerization of glutathione, that bind heavy metals with high affinity, protecting cells from toxic effects. An increase in glutathione content upon exposure to mercury was reported for Chlamydomonas (Rehman and Shakoori, 2001).

Microorganisms with the ability to grow in the presence of heavy metals and with a significant metal uptake have a potential use in bioremediation of polluted waters. Multiple metal resistant algae have been reported in a number of studies (Rehman and Shakoori, 2001). Several algae have been used for elucidating mechanisms for metal accumulation (Wilde and Benemann, 1993). Several euglenoids have been reported to withstand elevated doses, as long as they have had the chance to adapt previously (Rehman and Shakoori, 2001).

Occurrence and distribution of vessicula-arbuscular-mycorrhizal (VAM) fungi in the soils polluted with tannery effluent were also reported by different researchers. Mahesh and Selveraj (2008) repoted that the rhizosphere soils and ten dominant grass species polluted with tannery effluents were tested for the occurrence and distribution of vesicular-arbuscular mycorrhizal fungi and determined the impact of the physico chemical factors in relation to the quantitative and qualitative assessment of VAM fungi in soils polluted with tannery effluent over non polluted soil. The 15 species of VAM belonging to five genera viz., *Glomus, Sclerocystis, Acaulospora, Gigaspora and Scutellospora* were recorded and identified. *Glomus aggrigatum* was noticed as the most dominant effluent tolerant strain of VAM fungi in soils polluted with effluents. In the non polluted soils, all the plant species were colonized with VAM fungi. Whereas in polluted soils, eight grass species only were colonized and the percentage of root colonization was lower.

2.9 Mechanisms of Biosorption of Microorganisms

"Biosorption" is a process in which materials of natural origin, such as micro-organisms (living or dead) are employed for sequestration of heavy metals from an aqueous environment. Metal ions in solution interact with the biosorbent and hence are sequestered. The mechanism of metal ions uptake from solution can be due to physical sorption, complexation with microbial cell surface groups or bioaccumulation. Biosorption could occur through interactions between metal ions and functional groups of the cell wall biopolymers of living and dead organisms. The major functional groups on biopolymers which act as binding sites include carboxylate (–COO−), amide ($–NH_2$), thiols (–SH), phosphate (PO_4 3−) and hydroxide (–OH) (Onyancha *et al.*, 2008)

The complex structure of microorganisms implies that there are many ways for the metal to be taken up by the microbial cell. These biosorption mechanisms are various and are not fully understood. They may be classified according to various criteria. According to the dependence on the cell's metabolism, biosorption mechanisms can be divided into: metabolism dependent and non-metabolism dependent. According to the location where the metal removed from solution is found, biosorption can be classified as

(1) Extra cellular accumulation/ precipitation.

(2) Cell surface sorption/precipitation and

(3) Intracellular accumulation.

Transport of the metal across the cell membrane yields intracellular accumulation, which is dependent on the cell's metabolism. This means that this kind of biosorption may take place only with viable cells. It is often associated with an active defense system of the microorganism, which reacts in the presence of toxic metal.

During non-metabolism dependent biosorption, metal uptake is by physico-chemical interaction between the metal and the functional groups pre-sent on the microbial cell surface. This is based on physical adsorption, ion exchange and chemical sorption, which is not dependent on the cells' metabolism. Cell walls of microbial biomass, mainly composed of polysaccharides, proteins and lipids have abundant metal binding groups such as carboxyl, sulphate, phosphate and amino groups. This type of biosorption, i.e., non-metabolism dependent is relatively rapid and can be reversible (Pagnanelli *et al.,* 2002).

2.10 Effect of Chromium and Tannery Effluents on Microorganisms

Most micro-organisms are sensitive to Cr (VI) toxicity but some groups possess resistance mechanisms to tolerate high levels. Therefore the organisms use different mechanisms to adapt.

2.10 .1 Biosorption

"Biosorption" is a process in which materials of natural origin, such as microorganisms (living or dead) are employed for sequestration of heavy metals from an aqueous environment (Preetha and Virithagiri, 2005; Romera *et al.,* 2007). Metal ions in solution interact with the biosorbent and hence are sequestered. The mechanism of metal ions uptake from solution can be due to physical sorption, complexation with microbial cell surface groups or bioaccumulation (Preetha and Virithagiri, 2005). Biosorption could occur through interactions between metal ions and functional groups of the cell wall biopolymers of living and dead organisms. The major functional groups on biopolymers which act as binding sites include carboxylate (–OO−), amide ($–NH_2$), thiols (–SH), phosphate (PO_4 3−) and hydroxide (–OH) (Ehrlich and Brierley, 1990; Naja *et al.*, 2005). Heavy metals are widespread pollutants of great concern as they are non-degradable and thus persistent. Pollution of the environment by toxic heavy metals is largely as a result of industrial activities such as mining, electroplating, leather tanning etc. The effective

removal of heavy metals from aqueous wastes is among the most important issues in the world today (Matheickal and Yu, 1999; Vilar *et al.,* 2005). A number of methods exist for the removal of heavy metal pollutants from liquid wastes when they are present in high concentrations. These methods which include chemical precipitation, evaporation, electroplating, ion-exchange and membrane processes, are otherwise suitable at high concentration but ineffective and cost prohibitive when applied to dilute solutions or lead to secondary pollution (Zhou *et al.,* 1998; Matheickal and Yu, 1999; Preetha and Virithagiri, 2005). This limitation has led to a growing interest in application of biosorption technology for the removal of toxic metal from dilute aqueous solution. Biosorption is technically feasible, economical and eco-friendly (Ehrlich and Brierley, 1990; Bishnoi *et al.,* 2007; Romera *et al.,* 2007). Several biological materials have been investigated for the removal of heavy metals and include bacteria, algae, yeast and fungi (Ehrlich and Brierley, 1990; Matheickal and Yu, 1999; Naja *et al.,* 2005). Metal binding by living or dead algae offers promise in the treatment of low-grade industrial waste streams and in remediation of environmental metal pollution. Chromium has found extensive use in tanning industry mainly because of the good quality of leather obtained. When the wastewaters containing chromium are discharged into the environment, they pose a serious problem to the quality of the latter. Removal of chromium from wastewaters is obligatory in order to avoid water pollution. Legislation by different governments, demands the concentration of Cr in discharges be less than 0.5 mg L−1 irrespective of its oxidation state (Bishnoi *et al.,* 2007). This has lead to removal of chromium from wastewaters before discharge. However the current methods being employed such as chemical precipitation are not feasible to reduce the concentration to the desired levels of 0.5 mg L−1 Cr (WHO). Using various micro-organisms as biosorbents for chromium removal, offers a potential alternative to existing methods for its recovery from industrial wastewaters and

is subject to extensive studies (Horsfall *et al.,* 2003; Hana *et al.,* 2007; Onyancha *et al.* 2008).

2.10.2 Bacterial removal of chromium

A number of bacterial species such as *Bacillus* sp., *Pseudomonas* sp., *Alcaligenes* sp., *E. coli*, and *Shewanella* alga are reported to have Cr^{6+} detoxification capability due to presence of reductases soluble in cytosol (Srinath *et al.,* 2002b). In *Pseudomonas maltophilia* and *Bacillus megaterium*, Cr^{6+} reduction is associated with membrane cell fractions (Srinath *et al.,* 2002b; Cabrera *et al.,* 2007; Shukla *et al.,* 2009). During bacterial treatment of tannery wastewater in aeration lagoons at CETP, Cr^{6+} reduction commonly occurs in two or three steps in which Cr^{6+} initially reduced to a short-lived intermediates Cr^{5+} and/ or Cr^{4+} before further reduction to the thermodynamically stable end product Cr^{3+} (Cheung and Gu, 2007). However, at present it is unclear that whether the reduction of Cr^{5+} to Cr^{4+} and Cr^{4+} to Cr^{3+} is spontaneous or enzyme mediated. The NADH, NADPH and electrons from the endogenous reservoir are implicated as electron donors in Cr6+ reduction process. During the reduction process, the enzyme Cr^{6+} reductase (ChrR) transiently reduces Cr^{6+} with a one-electron shuttle to form Cr^{5+} followed by a two-electron transfer to generate Cr^{3+} (Ackerley *et al.,* 2004). Although a proportion of the Cr^{5+} intermediate is spontaneously reoxidized to generate reactive oxygen species (ROS), its reduction through two electron transfer catalyzed by ChrR reduces the opportunity to produce harmful radicals. On the other hand, several facultative anaerobes such as *P. dechromaticans, P. chromatophila, Aeromonas dechromatica, Microbacterium* spp, *Geobacter metallireducens, Shewanella putrefaciens, Pantoea agglomerans,* and *Agrobacterium radiobacter* EPS-916 are also reported to catalyze the biotransformation of Cr^{6+} to Cr^{3+} under anoxic conditions. But, unlike to Cr^{6+} reductases isolated from aerobes, the Cr^{6+} reducing activities of anaerobes are associated with their electron transfer systems ubiquitously catalyzing the electron shuttle along

with the respiratory chains (Srinath *et al.,* 2002b; Cabrera *et al.*, 2007; Cheung and Gu, 2007). In anaerobes, both soluble and membrane-associated enzymes were found to mediate the Cr^{6+} reduction process under anaerobic conditions. Moreover, the natural metabolites of anaerobes such as H_2S produced by sulfate-reducing bacteria (SRB) are considered as an important agent for Cr^{6+} reduction under anoxic environment. In past, the anaerobic reduction of Cr^{6+} was considered as a fortuitous process that provides no energy for microbial growth. But, recently a SRB isolate was found to use the energy generated during the anaerobic Cr^{6+} reduction for its growth (Moosvi and Madamwar, 2007; Shukla *et al.,* 2009). In absence of oxygen, Cr^{6+} can also serve as a terminal electron acceptor in respiratory chain for a large array of electron donors, including carbohydrates, proteins, fats, hydrogen, NAD(P)H and endogenous electron reserves (Cheung and Gu, 2007).

2.11 Chromium toxicity and its accumulation

Chrome in the hexavalent form is very toxic. The reason for such toxicity appears to be due to its rapid permeability through biological membranes and subsequent interaction with intracellular proteins and nucleic acids (Basu *et al.,* 1997). It is quite intriguing that contaminated field by industrial effluent show a mobilization ration of less than 5 (potentially toxic) for selected plant specious. Surprisingly, the mobilization ratios for weeds become greater than 5, which have healthy morphology in the early flowering stage (Kisku *et al.,* 1999). A study done by Marchese *et al.,* 2008 about the rate of accumulation of chromium in four fresh water plant species, clams, crabs, and fishes showed that, all the four fresh water species and animals were found with high concentration of chromium which is an indication of its high accumulation potential. This clearly indicates that this problem become more serious and toxic to human beings which are found at the top of the food web due to its toxicity and bio accumulation effect.

2.12 Chromium and Tannery Effluents Contamination of Soils

The main source of chromium in natural soils is weathering of their parent materials and the average amount of chromium in various kinds of soils range from 0.02 to 58µmolg^{-1}(Richard and Bourg, 1991). An increase in total Cr concentration in agricultural soils originates from fallout and washout of atmospheric Cr containing particles as well as from chrome bearing sludge and refuse from industrial activity (Gupta and Sinha, 2006).

Worldwide chromium contamination of soils has arisen predominantly from the common practice of land-based disposal of tannery wastes under the assumption that the dominant species Cr in the tannery waste would be the thermodynamically stable Cr (III) species. However, recent detection of significant levels of toxic Cr (VI) in surface water and groundwater in different parts of the world raise critical questions relating to current disposal of Cr-containing wastes. Despite the thermodynamic stability of Cr (III), the presence of certain naturally occurring minerals, especially MnO_2 oxides, can enhance oxidation of Cr (III) to Cr (VI) in the soil environment. This factor is of public concern because at high pH, Cr (VI) is bio available, and it is this form that is highly mobile and therefore poses the greatest risk of ground-water contamination (Rai *et al.,* 2005). However some researchers reported that the total metal content in the soil does not provide an accurate guide to the amount of metal bioavailable to plants and ultimately to the food chain which necessitates investigating the plants/vegetables.

2.13 Other pollutant associated with the tanning industry

Tanning process is responsible for the generation of large quantities of chromium waste, which may also contain organic matter, and salts such as chlorides, sulfates and carbonates, as well as ammonia, detergents, emulsifiers, bactericides, fungicides, coloring agents, skin proteins, hair, fat, and other components. Besides the chromium contents, such varied and complex

composition usually characterizes this waste as a potentially toxic material, so that it should be correctly disposed of in a manner not to cause adverse effects on the surrounding land, water, and the local flora and fauna (Abreu and Toffoli, 2009). Chlorinated phenols (e.g. 3, 5-dichlorophenol) as an organic pollutant associated with the tanning industry have been found to be highly toxic and affect the cellular compounds of organisms exposed to such waste (Pasco *et al.,* 2000). Other pollutants of concern within the tanning industry include Azodyes, Cadmium compounds, Cobalt, Copper, Antimony, Barium, Lead, Selenium, Mercury, Zinc, Arsenic, Polychlorinated Biphyenls (PCB), Nickel, Formaldehyde resins and Pesticides residues.

Cadmium is a potentially hazardous pollutant in the environment. Cadmium toxicity in fish has been reported to depend on water quality criteria. Toxic effects of cadmium with different concentrations and duration of exposure are well documented in teleosts and in other animals Chemical symptoms of cadmium toxicity are well documented. Absorption and accumulation of cadmium in the digestive tract have been described in fish and in mammals (Mohanta *et al.,* 2010). However, Chromium is the second most abundant inorganic groundwater contaminant at hazardous waste sites (Abreu and Toffoli, 2009). Tanneries generate sulfate-rich wastewaters because of the use of large amounts of sulfuric acid and the use of sulfide in the unhairing process, which is oxidized to sulfate before its discharge into sewer. Some authorities have established a limit of 1500 mg L^{-1} of sulfates for the discharge into the sewer. This is a standard very difficult to meet by the tanneries that process raw hides including beamhouse operations in addition to tanning, dyeing, greasing and finishing ones. Sulfate concentration in these types of tanneries usually ranges between 2500 and 3000 mg L^{-1} (Galiana-Aleixandre *et al.,* 2011).

2.14 Tanning

The tanning of animal skins is the art of transforming them into stable and imputrescible products known as leather. In this process, the skins undergo a chemical treatment for which a large number of organic and inorganic compounds like natural and/or synthetic tannins (polyphenolic compounds), surfactants, sulfonated oils, dyes, biocides, acrylic resins, organic acids, as well as ammonium, chromium(III), chloride, and sulfide salts are employed. The transformation of skins into leather involves three main stages: (a) the beamhouse, which cleans and eliminates the substances that will not compose the leather; (b) the tanning, which is the stage when pretreated skins are transformed into leather; and (c) the finishing, which complements the main tanning and confers on leather some desired physical and mechanical properties such as uniform color, tensile strength, impermeability, softness, elasticity, as well as its definitive presentation and aspect (Costa *et al*., 2008).

Tanneries are normally classified according to the stages involved in skin processing: (a) integrated tannery, which is able to perform all the operations on going from raw leather (fresh or cured skin) to finished leather; (b) wet-blue tannery, which processes raw leather until the chrome-tanned leather called wet-blue is achieved; (c) half-finishing tannery, which uses the wet-blue leather as raw material and transforms it into half-finishing leather, also known as crust; and (d) finishing tannery, which transforms crust leather into finished leather. Tanneries that process wet-blue leather until the finished leather is achieved also belong to the latter category (Costa *et al*., 2008).

2.14.1 Tanning Processes Linked With Pollution

Figure 2.2 depicts different tanning processes associated with wastewater generation leading to water and other environmental pollution.

2.14.2 Soaking:

The first tannery operation is soaking (Fig 2.2), which is treatment of hides and skins with water. Small and thin leather (before tanning) is called skin and big, fat and strong leather (before tanning) is called hide. During curing, hides and skins lose large quantity of their physiological content of water and unless the former regains this water during soaking operation, good quality leather cannot be produced. The objectives of soaking are, therefore, to rehydrate the skin proteins, to open up the contracted fibrous structure of the skin, to remove the curing salt and to clean the surface filth. Thus, the soaking operation produces huge amount of effluent containing high BOD and COD loading (Das *et al.,* 2007)

This stage starts with washing of the soiled raw hides and skin to remove physically bound materials mostly insecticides, salts ($NaCl_2$) and other preservatives. Secondly rehydration of hides and skins occurs during soaking to replace lost moisture during the preservation phases (also referred to as curing) to allow permeation of chemicals during subsequent processes (Cassano *et al.,* 2001). At this stage very large volume of water is required about 30–40liters of water are used for the production of 1 kg leather. Soaking unit employs about 25% of total water consumed in tannery (Das *et al.,* 2007), consequently results to high discharge of effluent with high pollutant load. The current trends in the tanning industry especially in the developing world preferably use salted hides and skins rather than air dried due to their high rehydration potential. This approach of curing emit high load of salt which predisposes arid conditions to the environment (Plate 2.1. and 2.2).

2.14.3 Liming:

This involves the use of alkaline medium (e.g. lime) to condition raw hides and skins. The aim is to remove the hair, flesh and splitting up of the fibre bundles by chemical and physical means

(Ramasami and Prasad, 1991). In this process, Na_2S is added to facilitate de-hairing (Flaherty *et al.,* 1959). It is estimated that for processing 1 ton of raw skins (weight of skins before soaking), the input in a typical input audit processing (kg) of lime is 100kg with an output of 12.3kg, while Na_2S has an input of 35kg with an output of 18.3 (Thanikaivelan *et al.,* 2003).

2.14.4 De-liming, Bating and Pickling:

Weak organic acids, digestive enzymes and inorganic acids, respectively, are used to remove lime, digest and remove the non-structural proteins and eventually bring the pH to a level that will enhance the tanning process (Cassano *et al.,* 2001; Thanikaivelan *et al.,* 2003).

2.14.5 Chrome Tanning:

Chromium sulphate is used to tan the pre-treated hides or skins making them stable and imputrescible products known as leather. This involves the use of chromium sulphate at varying concentrations to treat the hides and skin, accompanied by float control to produce the desired leather usually wet-blue to crust and finally finished leather. New techniques for improving the recycling of chromium to reduce its impacts to the environment are unavailable in the developing world because of continual use of traditional methods of chrome tanning. The main assumption in such systems relies on the fact that Cr salts precipitate with NaOH followed by the dissolution of $Cr(OH)_3$ in sulphuric acid. However, the quality of the recovered solution is not always optimal due to the presence of the toxic state of the metal, lipidic substances and other impurities (Cassano *et al.,* 2001). Therefore, whatever method is used to reduce the amount of Cr salts in the final discharge, it will portend chromium as a potentially toxic source to the environment.

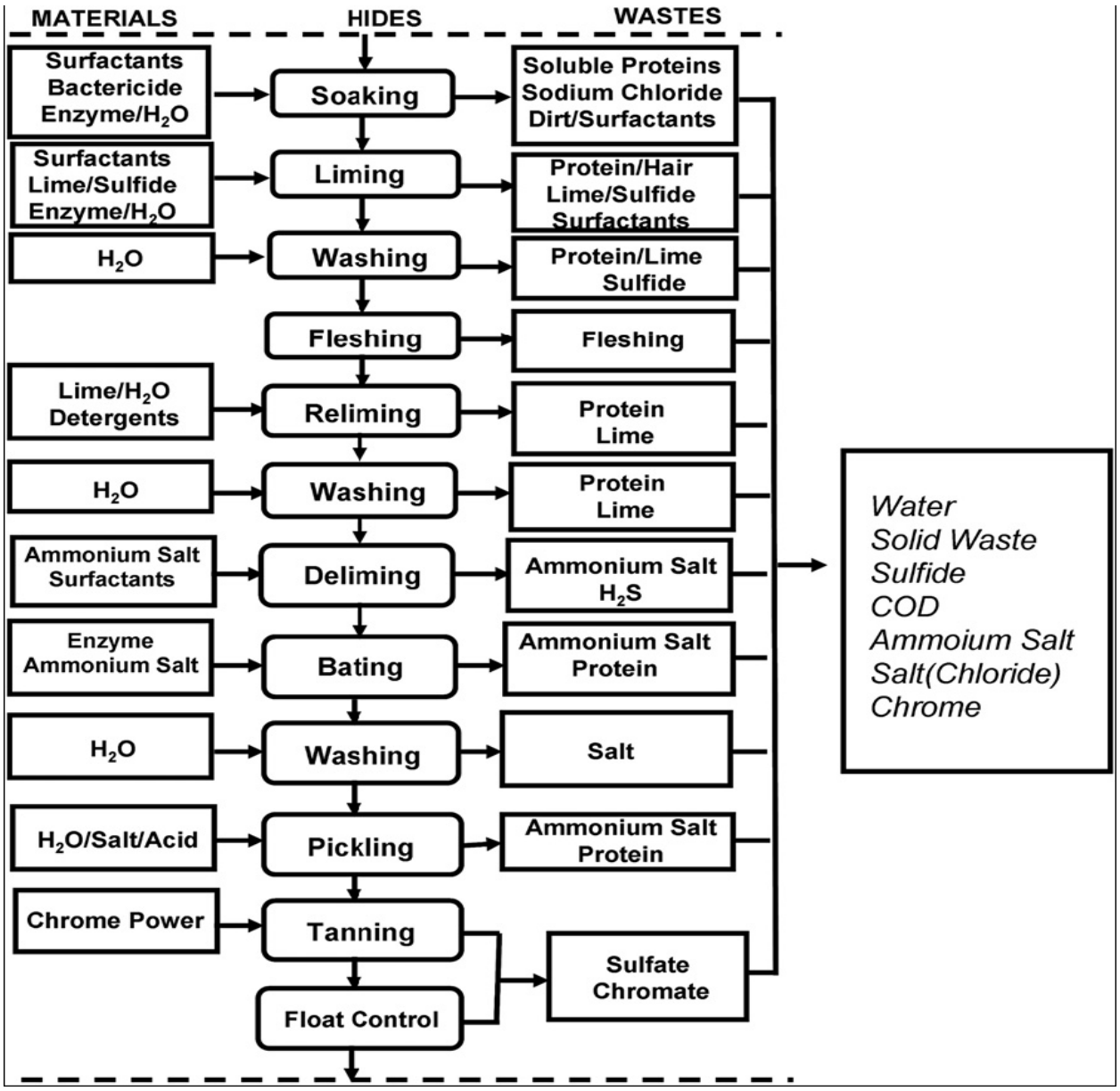


Figure 2.2: Integrated tannery effluent treatment plant (Hu *et al.*, 2010)



Plate 2.1: Effect of high load of salt on the environment



Plate 2.2: Effect of high load of salt which predisposes arid conditions to the environment

Nearly 90% of all leather produced is tanned using Cr salts (Stein and Schwedt, 1994). Generally 8% of the basic chromium sulphate salt is used for conventional tanning. It binds with the collagenous protein to convert to leather. The mentioned processes are a few of the total that are found in processing of leather but they form the vital point of intervention in pollution control. The main pollutants found during leather processing include, $NaCl_2$ and pesticides, strong alkalines and sulphides, inorganic residual compounds, dissolved matter and chromium salts. Chlorinated phenols are important compounds to be investigated due to the various mixtures used in the tanning industry and their ecotoxicity potential (UNEP, 1994).

Vegetable tannins are also used to retan leather to impart certain specific desired properties or could be used alone in producing leather especially at the rural tanning level by the use of plant material (tree barks and pods are commonly used in Africa). Mostly, the vegetable tannin materials are derived from plants and consist of condensed or hydrolysable tannins (Zywicki *et al.,* 2002). In the East African region, wattle is extensively used as a tannin material. Efforts to study the polyphenolic structures of condensed tannins have been hampered by the fact that the structure rapidly transforms during the tanning process to yet unknown products (Zywicki *et al.,* 2002). However the use of chromium salts during leather tanning is still common in most of the developing world.

2.14.6 NaCl Curing:

Salt as a curing agent in the primary level of preservation is an inorganic chemical that strongly has been identified as a pollutant. Indeed it is the inherent characteristic nature of the salt used that causes concern as an environment impact. This is due to its inertness and disruption of the soil biological activities that easily renders aridity to exposed terrestrial and aquatic ecosystems.

Salinity in tannery effluents is measured as Total Dissolved Solids (TDS) which is mainly contributed with chlorides. Furthermore impurities associated with salts such as copper and iron aggravates the situation and renders its residual effect toxic at the microorganism's level. Salinity or ionic strength can cause a small decrease in the solubility of non-polar organic compounds (naphthalene, benzene and toluene) through a process known as the salting-out effect (Pepper *et al.,* 1996). In a study in Egypt, NaCl concentration varied between 40,000 and 50,000 mg L in the effluent discharge in a tannery under study (Hafez *et al.,* 2002).

2.14.7 Organic Matter:

Organic matter associated with tannery waste includes biodegradable organic mater (e.g. proteins and carbohydrates). Their main problem is the depression of the dissolved oxygen content of stream waters caused by microbial decomposition (Balusubramanian and Pugalenthi 2000; Song *et al.,* 2000, Mwnyihija *et al.,* 2006). Their impacts are primarily the loss of dissolved oxygen, which is detrimental to aquatic organisms. In addition the depletion of dissolved oxygen encourages anaerobic activity, which leads to release of noxious gases (Pepper *et al*. 1996; Mwinyihija *et al.,* 2006).

2.14.8 Hydrogen Sulphide (H_2S):

This is a toxic gas with an offensive odour, which is associated with both natural and anthropogenic sources (Dorman *et al.,* 2000). The tanning industry is closely associated with the production of H_2S which emanates mostly in the liming yard and the anaerobic lagoons (Mwinyihija 2007). The primary biochemical effects arising from H_2S exposure are inhibition of the cytochrome oxidase and other oxidative enzymes, resulting in cellular hypoxia or anoxia (Beauchamp *et al.,* 1984; Glass 1990; Reiffenstien *et al.,* 1992; Nicholson *et al*. 1998).

Concentration dependent toxicity occurs in humans following acute exposure. Exposure to moderate levels of H_2S (50–100 mg L^{-1}) can result in Keratoconjunctivitis, respiratory tract irritation and olfactory fatigue. Prolonged exposure to 250–500 mg L-1 will result in olfactory paralysis, severe lung and eye irritation, pulmonary oedema and unconsciousness in human (Dorman *et al.,* 2000). These clinical effects are consistent with organic brain disease resulting from anoxia and thus may persist for several years after the initial exposure (Arnorld *et al.,* 1985; Tredt *et al.,* 1991; Reiffenstien *et al*. 1992; Kilburn and Warshaw 1995; Synder *et al.,* 1995).

2.15 Chemistry of Chromium:

Chromium exists in nine valence states ranging from −2 to +6. Of these states, only hexavalent chromium (Cr(VI)) and trivalent chromium (Cr(III)) are of primary environmental significance because they are the most stable oxidation forms in the environment (Smith *et al.,* 2002). Both hexavalent and trivalent chromium are found in various bodies of water and wastewaters (Shen and Wang 1994; Wang 2000). Cr(VI) typically exists in one of these two forms: chromate CrO^{2}_{4} or dichromate $Cr_2O^{2}_{7}$, depending on the pH of the solution (Shen and Wang 1994). These two divalent oxyanions are very soluble in water and poorly adsorbed by soil and organic matter, making them mobile in soil and groundwater (Smith *et al.,* 2002). Both chromate anions represent acute and chronic risks to animals and to human health, since they are severely toxic, mutagenic, carcinogenic and teratogenic (Marsh and McInerney 2001; McLean and Beveridge, 2001). In contrast to Cr (VI) forms, Cr(III) species, predominantly hydroxides, oxides or sulphates, are much less water-soluble, less mobile, 100 times less toxic (Beszedits, 1988) and 1,000 times less mutagenic (Lofroth and Ames, 1978).

The soluble fraction of Cr in the wastewater appears to be present as Cr(VI), although the remainder must be present as insoluble Cr(III) and therefore be associated with the particulate

phase (Stuart and Milne, 2008).

2.16 Chromium Salts:

Chromium basic sulphate is the most widely used tanning substance today (UNEP, 1994). The exhausted bath coming from the chromium tannage contains about 30% of the initial salt and is normally sent for cleaning up (Cassano *et al.,* 2001). Here chromium salts are entrained in the sludge creating serious problems for their disposal (Gauglhofer, 1986). Chromium is a micronutrient and Cr salts such as chromium polynicotine, chromium chloride and chromium picolinate (CrP) have been demonstrated to exhibit a significant number of health benefits in animals and humans (Anderson 2000). Hazards due to environmental contamination, depend on its oxidation state, hexavalent stage of chromium (Cr^{6+}) is more toxic than the Cr^{3+} which precipitates at higher pH.

Trivalent chromium is unable to enter into cells but Cr^{6+} enters through membrane anionic transporters. Intracellular Cr^{6+} is metabolically reduced to Cr^{3+}. Cr^{6+} does not react with macromolecules such as DNA, RNA, proteins and lipids. However both Cr^{3+} and the reductional intermediate Cr^{5+} are capable of coordinated covalent interactions with macromolecules (Shrivastava *et al.,* 2002).

2.17 Water Reuse in Tannery Processes

With the growth in environmental awareness the need to preserve natural water resources has increased. The problems of water shortage experienced nowadays have led to the need to develop strategies that enhance the development stages of the technology and management of water usage. Agenda 21, a document developed during the United Nations Conference on Environment and Development (UNCED), was drawn up through contributions from several

nations, with the goal of integrating environmental protection with economic development, mainly aimed at improved quality for human beings, thus reinforcing the concept of sustainable development. A consensus was reached on the perception of water as a limited resource of environmental and economic value (Gutterres *et al.*, 2010). Pressed by increasingly restrictive legislation, by pressure from the consumer market and the need to adapt to the globalized world, industries have invested in water reuse and recycling in their production processes, which is one way to reduce costs, increase productivity and minimize the environmental impact arising from its chaotic usage. The sequence "avoid-minimize-reuse-treat-dispose" was the agenda for discussions, including in terms of environmental legislation. The industrial activity of leather tanning was included among those that have added to environmental pollution, mainly of water bodies.

Tanneries consume large amounts of water for leather production, employing toxic substances, such as chrome and sulfide, and generating effluents with high levels of contamination. The high water consumption is due to the treatment of hide in multiple aqueous steps of the process with discontinuous discharges, making this an industrial activity which generates large amount of effluents which, in turn, require significant investment and operating costs for their treatment, to satisfy the emission standards required by environmental legislation (Gutterres *et al.*, 2010). Gutterres *et al.* (2010) reported several researchers' initiative towards water re-use in tanneries and other industries; Nazer *et al.* (2006) proposed modifications to the hair removal/liming step, in which the wastewaters from this stage are reused, decreasing the quantity of chemicals and water required. The method proposed by the authors uses fresh water in the first batch of the process and the effluent generated is reused up to four times without changing the final quality of

the leather. The proposed process reduces the economic and environmental costs. Raghava *et al.* (2003) presented possibilities for the recycling and reuse of water at each stage of leather tanning as shown in flowcharts.

According to the authors, the leather industry requires around 30-40L water per kg of skin processed, 35% is consumed by the washing and 57% in the steps of tanning and retanning. Gutterres *et al.* (2010) studied the reuse of wastewater from the retanning stage after its filtration through leather wastes from the shaving process. Leathers obtained with this water reuse showed characteristics similar to those retanned with fresh water. Vedaraman *et al.* (2003), concerned about the large amount of water used in tanning, studied the use of seawater in leather processing, and a pretreatment for the seawater was developed for its use in certain tanning steps. Two main approaches are generally used to address the issue of the systematic design of a water recovery network, namely, the graphical approach (more commonly known as the water pinch technique) and mathematical programming approach.

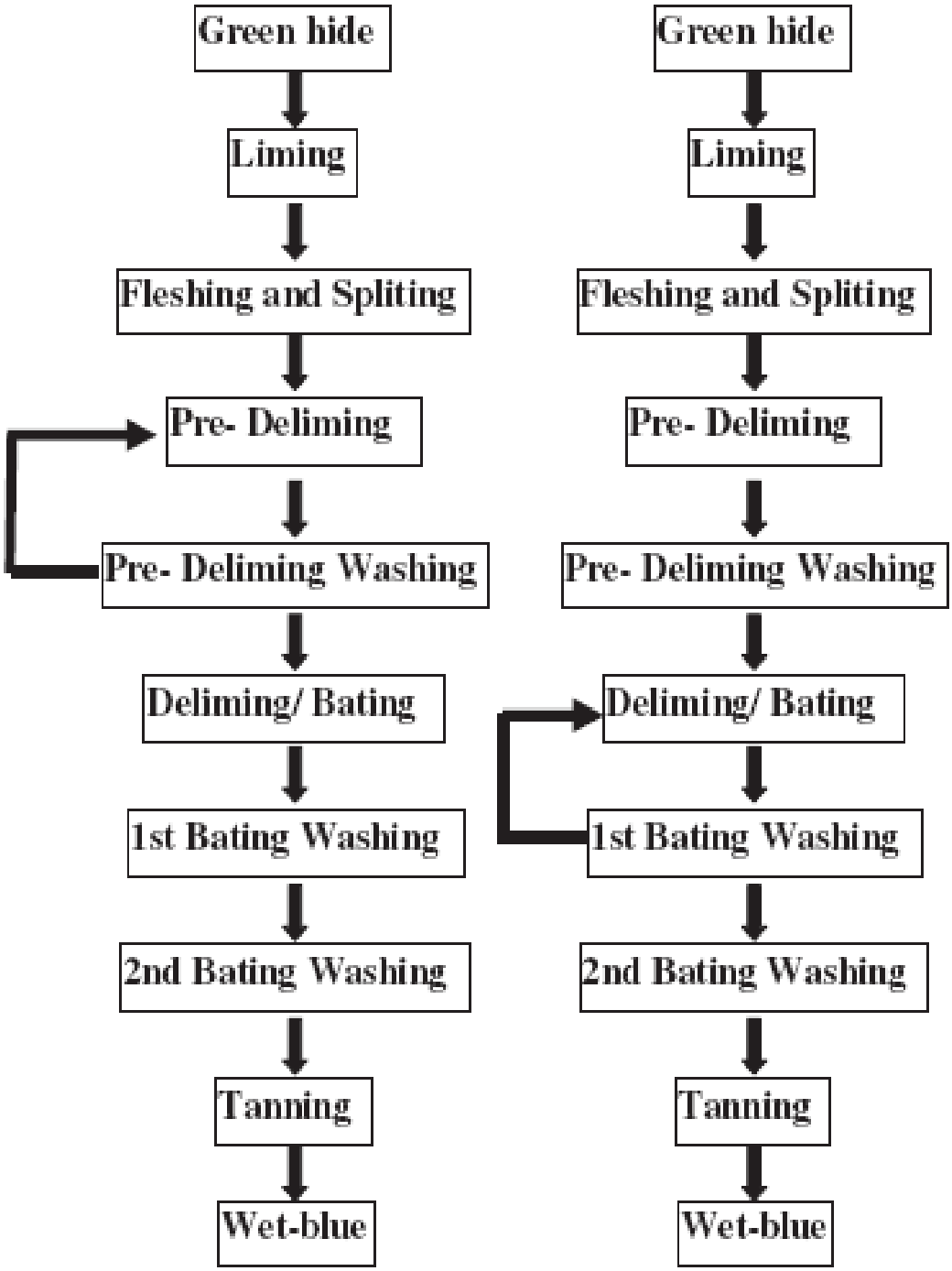


Figure 2 3: Flow charts showing pattern of water re-use in tannerrie.

Source (Gutterres *et al.*, 2010)

The first attempt to solve the water recovery network problem in a systematic way was reported by Takama *et al.* (1980) for a petroleum refinery case study. Utilizing a mathematical programming approach, these authors firstly set up a superstructure of all water using operations. This superstructure is then optimized to remove the irrelevant and uneconomical options. Wang and Smith (1994) later initiated the water pinch technique based on the more generalized problem of mass exchange network synthesis (MENS). In this two-step procedure, the limiting composite profile is introduced to locate the minimum fresh water and wastewater flow rates prior to any network design. The opportunities for regeneration-reuse and regeneration-recycling were also explored. The basic concept underlying this approach is that the water-using processes are modeled as mass transfer operations. Over the past decade, a new methodology has been developed which is capable of systematically minimizing waste and improving overall process efficiency. This approach, known as process integration, was pioneered by El-Halwagi and coworkers (e.g., El-Halwagi, 1997; Dunn and El-Halwagi, 2003; El-Halwagi *et al.*, 2005; Gabriel and El-Halwagi, 2005). It is a holistic approach to the optimal allocation, generation, and separation of streams and species within a process. Mass integration involves the optimal allocation of species (such as water, process compounds and non-process elements) throughout the process and provides an excellent framework for developing cost-effective pollution-prevention strategies. The essence of mass integration is to understand the global mass flow within a process and develop optimal strategies for the various species and streams within the process.

Ponce-Ortega *et al.* (2009) presented a new formulation and a mathematical programming model for the direct recycle and reuse of mass exchange networks considering simultaneously process and environmental constraints. The model is based on mass and property integration. The properties constrained by the sinks include composition, density, viscosity, pH, and reflectivity, whereas the environmental constraints include the composition for hazardous materials, toxicity, chemical oxygen demand, color, and odor. The results showed that even for a large size problem, the computation effort is relatively small as a result of the linearization procedure. Gomes *et al.* (2007) presented a heuristic procedure to synthesize water mass exchange networks. In addition, using mathematical programming techniques, Savelski and Bagajewicza (2000) proposed models to solve the recycle and reuse problem. Differently, Bai *et al.* (2007) employed to optimize water reuse a single-contaminant graphical method. The technology of mathematical programming is a technique in which the engineer needs less prior knowledge to understand how the units of the system are built, however, good knowledge is required to support the decision making process. Examples of the application of mathematical programs for mass integration include the studies of Savelski and Bagajewicza (2000) who reported the optimum conditions for the use of water from refineries and other plants with problems related to multicomponents.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

Kano Metropolis is located on Latitude 11^{o} 59m 18.3s N, Longitude 08^{o} 32m 05.8s E and 515m above sea level. It occupies a central position in Northern Nigeria. It is one of the industrial cities in Nigeria. Kano metropolis is the major centre of leather processing and textile manufacturing, with several leather curing and tanning establishments, mainly in Challawa, Sharada and Bompai industrial areas (Akan *et al*., 2009). The factories generate wastewater containing significant quantities of chromium and other heavy metals that has 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 Chalawa River (Latitude 11^{O}52m 41s N, Longitude 08^{O} 28m 09s E, 418m above sea level). Chalawa 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.

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. There are more tanneries than the other industries put together. 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. Satellite photo of the study area showing the vicinity of the Challawa Indusrial layout Tannery Field (Google Image 2010) is shown in Fig. 3.2

The tannery effluent samples were collected from three selected tanneries named (Tan A, Tan B and Tan C) each at Discharge point (DP), Downstream (DS) and Entry points (EP) of the Challawa River near ‘Yandanko village in Kumbotso local government and Magasawa village in Madobi local government on the other side of the River. At the downstream (DS) points there were soft drink industry, textile factory, plastic industry as well as other tanneries which also discharge their effluents into the streams.



Figure 3.1: Map of the study area showing the River

Satellite photo of the study area showing the vicinity of the Challawa Indusrial layout Tannery Field (Google Image 2010)

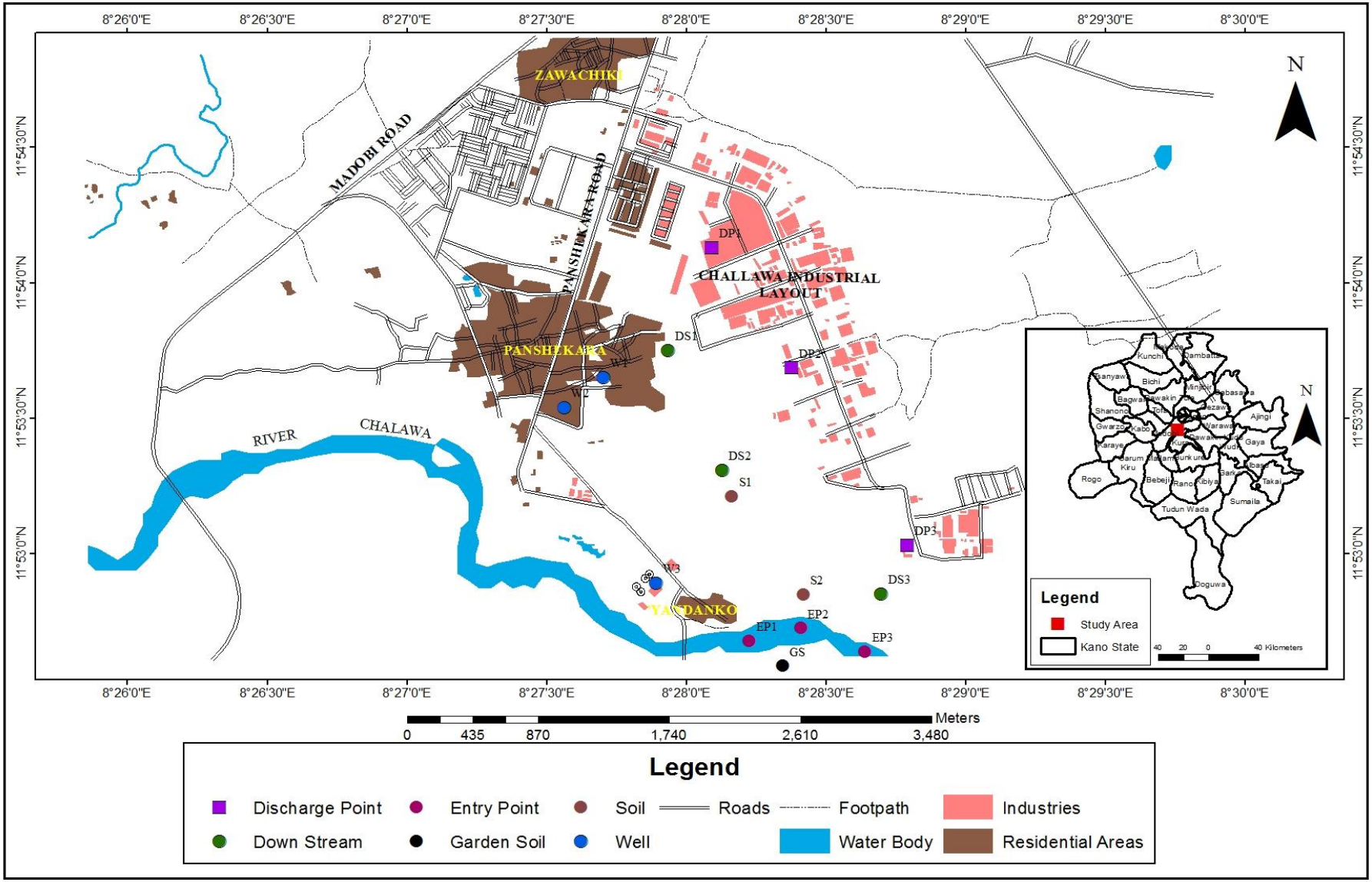


Figure 3.2: Map of the study area showing the study area, sampling sites and the River

3.2 Sampling Plan

Sampling was carried out at monthly intervals over a period of 12 months (from November, 2009 to October, 2010 to cover both dry and the wet seasons where Nov – April was the dry season while May – Oct was the wet season). The samples were collected during tanning operations from four major sites as follows:

a) The locations of the tanning industries (point of discharge, downstream (500m), entry points in streams (1000m) as effluent and upstream as control
b) The well water supply sources located 1-2km from the waste discharged sites and control well.
c) Farms where crops are grown and soils irrigated with the waste water (1000m from discharge points). The irrigated vegetables were collected during the irrigation season of six month

Three categories of tanneries were selected for sampling. In each tannery three effluent samples were collected monthly to monitor the pollution level. Control samples were collected from sites that do not have apparent waste contribution from the Tanning industry 20km upstream to establsh impact if any.

3.3.0 Sample Collection

For physico-chemical analysis, samples were collected in acid-washed plastic jars while for bacteriological analysis samples were collected aseptically in sterile glass bottles.

3.3.1 Water and Effluent

Samples of water and tannery effluents were collected aseptically in 500ml sterile bottles. Batches of 350ml effluent samples were collected by dipping the bottle with the mouth of the bottle against the current in flowing waters and by fastening a rope round the neck of sampling bottle and dipping in wells to collect the well water samples (Cheesbrough, 2004).

3.3.2 Soils

The surface soil samples from 1–15cm of top layer were collected manually using ethanol sterilized trowel (soil sampler). Samples were colllected from agricultural fields irrigated with the tannery effluents. On each occasion, five samples were collected from five different fields (about 50 – 100m apart in every direction) and a composite sample was made for the analysis. About 350g of the samples were collected from each site and placed in polythene bags and brought to the laboratory.

3.3.2.1 Preparation of soil samples for heavy metal analysis

The soil samples were air-dried over a period of one week and passed through a <2 mm sieve (Hayat *et al.,* 2002; Sethunathana *et al.,* 2005; Tariq *et al.,* 2005; Alam *et al.,* 2010) in preparation for heavy metal determination.

3.3.3 Collection of vegetable Samples

Samples of tomatoes, onions, lettuce, carrots and sugar cane, which are the predominantly planted vegetables in the area and irrigated with water containing tannery effluent were collected from the soil sampling sites. About 350g samples obtained were cut into pieces using alcohol-disnfected scissors and packed into new polythene bags (Okafor, 2003).

3.4 Transportation

All samples were transported from the sampling sites to the laboratories at (Microbiology Dept. ABU, Zaria and Soil Science Dept. Bayero University, Kano) in ice cold boxes. The samples were stored at $4^{o}C$ to arrest any biological activity and analyzed within 12 hour of collection.

3.5 Determination of Physico-chemical properties of water and effluent samples

3.5.1 pH and Temperature

The pH and temperature of the water and effluent samples were determined at the point of collection using the Hanna instrument (HANNA 3100) (www.hannainst.co.uk) by pressing the appropriate button on the instrument and then inserted into the effluent at the point of collection, then readings were therefore recorded.

3.5.2 Total Suspended and Dissolved Solid (TSS and TDS) and Electrical conductivity

Total dissolved solid and electrical conductivity were measured by pressing the appropriate button on the instrument (HANNA 3100 www.hannainst.co.uk). and then inserted into the effluent at the point of collection. TDS was recorded as mgL^{-1} while electrical conductivity was recorded as microsiemens per centimetre (µs/cm).

3.5.3 Dissolved Oxygen and Biological Oxygen Demand

The Hanna instrument (HANNA 3200) was used to determine the Dissolved Oxygen (DO) and Biological Oxygen Demand (BOD) respectively. The instrument was inserted into the effluent to determine the DO at the point of collection. While the BOD was obtained after the sample was collected, diluted with sterile distilled water a hundred folds and allowed for 5 days at temperature of $25^{o}C$. The BOD was determined by inserting the instrument into the bottle and then subtracting the initial DO from the final value to obtain BOD_5 (Radojavik and Bashkin, 1999)

3.6.0 Determination of heavy metals in samples

3.6.1 Determination of heavy metals in water and effluents

The well water sample was taken, filtered through Whatman 42 filter paper and then acidified with concentrated HNO_3 to bring down the pH to 2.0. From the acidified filtrate 100ml of sample was taken and 5ml concentrated HNO_3 was added, and then digested in a closed chamber, for 30 minutes. The concentration of the heavy metals in the digest were determined by atomic absorption spectrophotometer (V.P.C210 Bulk Scientific Model) (APHA, 1998, Campos *et al.,* 2005).

Well mixed, acid preserved effluent samples (100ml) were transferred to a beaker containing 5ml concentrated nitric acid (1:4 mixtures of $HClO_4$ and HNO_3) and a few boiling chips. The samples were heated on a water bath for complete digestion, usually indicated by light colourless, clear solution. The digest was filtered through Whatman filter paper (No. 1). The heavy metals in the digested effluent (wet digestion) were determined using (V.P.C210 Bulk Scientific Model) AAS (Vanloon and Lichwa, 1973; APHA, 1992 as sited by Verma *et al.,*. 2001; Campos *et al.,* 2005; Benazir *et al.,*2010).

3.6.2 Determination of heavy metals in soil samples

Heavy metal analyses of the soil samples were carried out as described by Aleem *et al.* (2003) and Deepali and Gangwar(2010). Soil samples were oven dried at 40^oC, ground finely (<0.01 mm), and then ashed in a crucible. One gram ash was treated with conc. HCl and HNO_3 successively in a ratio of 3:1 and heated gently until the sample was digested, indicated by the formation of a clear solution above the soil residue. The mixture was filtered through Whatman no. 42 filter paper. Doubled distilled water was added to make up the volume of the filtrate to

100ml. Digested soil samples were analyzed for metal concentrations by atomic absorption spectrophotometery (AAS).

3.6.3 Determination of heavy metals in vegetable samples

The edible portions of the vegetables were carefully rinsed with distilled water to remove surface contamination. They were then sliced using a knife and air dried on clean surfaces to eliminate excess moisture. The samples were oven dried at $60^{o}C$ to constant weight, grounded and stored in clean dry stoppered containers. After processing, these samples were digested with $HClO_4$, HNO_3 and HCl acid mixture for determination of heavy metals with AAS. Non-tannery wastewater irrigated vegetables from control sites were similarly treated.

3.7. Microbiological Analysis

3.7.0 Determination of number of heterotrophic bacteria

The water, effluent, and soil samples were serially diluted and analyzed for total aerobic heterotrophic bacteria using nutrient agar, following the standard pour plate technique methods (APHA, 1998). Plates were incubated aerobically at 37±1°C for 2days and the total number of bacteria was determined as colony forming units per milliliter (CFU/ml) of water and colony forming units per gram (CFU/g) of soil respectively.

3.7.1 Determination of coliform count

Multiple tubes tests technique was used to determine coliform and feacal coliform counts because it is suitable for the examination of turbid waters. MacConkey broth containing bromocresol purple was used to determine coliform in the water and effluent samples, 50ml, 10ml and 5ml volumes of the indictor broth were placed in the test-tubes at double and single

strength. Durham tubes were inverted in each tube and air was expelled prior to sterilization. Aseptically 50ml, 10ml and 1ml volumes of each sample were inoculated into corresponding 50ml, 10ml and 5ml volumes of the medium. The tubes were then incubated aerobically at 37^{o}C for 48hr. The production of acid indicated by colour change from purple to yellow and gas formation were considered as presumptive positive growth of coliforms. Most probable number (MPN) table was used to read the MPN of presumptive coliform bacilli in the samples.

Subcultures were made from positive presumptive tubes and incubated at 37^{o}C and 44^{o}C in Brilliant green lactose bile broth (BGLBB) for 24hr to confirm coliform. The presence of coliform was confirmed by the production of gas at 37^{o}C while *E. coli* was confirmed by the production of gas at 44^{o}C and indole from tryptophan. Isolates were purified by repeated subculture and stored for further studies.

3.7.2 Determination of faecal Streptococcal counts

Faecal streptococci were determined using tubes containing 5ml sterile glucose sodium azide broth. The tubes were placed in water bath thermostatically controlled at 44^{o}C. When the temperature was attained the tubes were then inoculated with heavy inocula from the positive presumptive tubes from 3.7.1 above and incubated at 44^{o}C for 24h (Tewari *et al.*, 2003). The production of acid indicated by colour change from purple to yellow indicating the presence of faecal streptococci and was confirmed by subculturing positive glucose azide culture on to plates of bile aesculin azide agar and incubating at 44^{o}C. The formation of brown-black colouration around the colonies after few hours of incubation is evidence of hydrolysis of aesculin and confirms the presence of faecal streptococci. Similarly from the proportion of positive tubes

which faecal streptococci was confirmed, MPN table was used to read the MPN of faecal streptococci (APHA, 1992, Tewari *et al.*, 2003).

3.7.3 Estimation and Isolation of Chromate Resistant Bacteria from Tannery effluents

The enumeration was done according to the methods described by Verma *et al.* (2001) and Basu *et al.* (1997). The chromate resistant heterotrophs and coliforms were enumerated on nutrient agar and Mac-Conkey agar plates respectively, supplemented with 12.5 and 25.0µg of Cr^{6+}/mL of Cr^{6} as $K_2Cr_2O_7$ (filter sterilized) using standard pour plate technique (APHA, 1992). Plates were incubated at 37°C and 44.5°C for 24hrs and 5 days and the total numbers of resistant bacteria were determined as colony forming units per ml (CFU/ml). Organisms isolated were stored on slants for biochemical characterization. For the isolation of resistant bacteria, positive broth samples (from 3.7.1 above) were plated in the Cr-free and Cr^{6+}- supplemented agar media. Separately sterilized chromium solution (K_2CrO_4) (AR Grade) was added to the media at a concentration of 12.5 and 25.0µg of Cr^{6+}/mL. Plates were incubated at 37°C for 3-7days. Colonies growing on Cr^{6+}- supplemented agar media differing in morphology were identified as Cr^{6+} resistant (Basu *et al.*, 1997). Individual bacterial colonies on NA plates supplemented with Cr^{6+} which varied in shape and color were picked up and purified by repeated subculturing on the same medium. Organisms isolated were stored for further studies.

3.7.4 Estimation and Isolation of Chromate Resistant Bacteria from Soil

The method of Haroun *et al.* (2007) was used to isolate and enumerate bacterial strains from agricultural soil irrigated with effluents using nutrient agar plates that are Cr-free and Cr^{6+}-supplemented agar media. Briefly, samples were analyzed for total aerobic mesophiles. A sub-sample of 10g soil was added aseptically to a 90ml sterile peptone water and shaken vigorously

for 10min and serial dilutions were prepared (10^{-1} – 10^{-6}) using sterile 0.8% NaCl solutions. An aliquot of 0.1ml of the diluent (10^{-6}) were plated on agar supplemented with Cr^{6+}- and incubated at 37^{o}C for 24h and counts were recorded. All colonies with typical characteristics (greenish pigmentation and dry powdery colonies) were streaked, purified and stored for further studies. Individual bacterial colonies on NA plates which varied in shape and color were picked up and purified by repeated subculturing on the same NA and cetrimide agar and Mannitol egg yolk polymyxin B agar (MEYP agar) and stored for further studies.

3.7.5 Biochemical Identification of chromium resistant bacterial isolates from the effluents and soils

All the stored resistant isolates from effluents and soil samples were subjected to initial staining and microscopic examinations as well as biochemical tests.

The isolates were subjected to the following biochemical tests

i. Catalase test: Two drops of 3% hydrogen peroxide were added to 18-hour-old culture of isolates on slides and was observed for gas production.

ii. Coagulase test: A smear of the isolates on slides were made with normal saline, a drop of blood plasma was added. The slides were rocked gently and observations for coagulation were made.

iii. Motility test: Each of the isolates was stabbed mid-way in the tubes containing motility medium, and was incubated at 37^{o}C for 24 hours. Motility was observed by the organisms moving down the bottom of the tubes and spreading from the stab lines.

iv. Indole test: Nine milliliters of sterile peptone water was dispensed in sterile tubes. The isolates were inoculated in peptone water and were incubated for 24 hour at 37^{o}C. The isolates in peptone water were brought out of the incubator after 24 hours and 2 drops of Kovac's reagent was added. Observation for red colour change was made to indicate either acid or alkaline.

v. Hydrogen Sulphide production: Triple Sugar Iron (TSI) agars slants was prepared and sterilized in test tubes. The TSI was allowed to gel, and a loopful of the isolate was stabbed in the TSI agar. It was incubated at 37^{o}C for 24 hours. Smell of rotten egg and a black colour production was for H_2S production.

vi. Starch hydrolysis: The isolates were streaked on the plates of Starch agar, containing 20% soluble starch and incubated at 37^{o}C for 24 hours. After incubation, the plates were flooded with Gram's iodine. The appearance of blue-black indicated the presence of starch, a negative result while clear zones indicated the complete hydrolysis of starch a positive result.

vii. Growths at different Sodium Chloride (NaCl) concentrations: Nine millilitres of peptone water containing different concentrations of NaCl (5%, 10% 15%,20% and 25%) were used. The isolates were inoculated and incubated at 37^{o}C for 24 hours.

viii. Sugar utilization: One gram each of different sugars, lactose, fructose, sucrose, mannitol and rhamnose were dissolved in 100mls peptone water into separate flasks containing inverted Durham tubes. A loop-full of the organisms were inoculated into the medium after 1.5ml of 0.2%. Bromothymol blue was added into 100ml of basal medium containing sugars, the tubes were incubated at 37^{o}C for 24hrs. Observation for gas and acid production were made.

3.7.6 Determination of Minimum Concentration of Cr(VI) inhibitory to resistant isolates

The bacterial cultures used for the study were, *P. aeruginosa* and *B. cereus* which were maintained in Cetrimide agar and Nutrient agar respecively.

The Minimal Inhibitory Concentration (MIC) of chromium at which no growth occurs was determined by agar dilution method (Luli *et al.,* 1983). The predominantly resistant bacterial isolates were selected (*P. aeruginosa* and *B. cereus*) and inoculated individually into 25ml peptone water consisting of 1.0 %(w/v) peptone and 5.0% (w/v) NaCl in 150ml conical flasks and incubated at room temperature on a rotary shaker 150rpm to achieve log phase cultures. Inocula were prepared in test tubes conntaining 9ml sterile normal saline and compared to 0.5 MacFarland standard. Nutrient agar plates containing 25, 50, 75, 100, 150 and 200mg/l Cr(VI) were inoculated aseptically with 0.1ml inoculum from the exponential growing cultures of each bacterial strain (Mondaca *et al.,* 1998). These plates were incubated at 37^{o}C for 48h. The MIC was considered to be the lowest concentration of Cr(VI) at which no growth occur. The isolates resistant to high Cr concentrations were selected, subcultured on NA slants and stored for further studies.

3.80 Isolation of Fungi

3.8.1 Isolation of Resistant Fungi from effluent

Tannery effluents from the study site were used as source of fungal isolates capable of Cr uptake under aerobic conditions. Tannery effluent was inoculated into medium containing the following, per liter of distilled water, 10g of glucose, 3g of $NH_4(SO_4)_2$, 1g of KH_2PO_4, 0.3g of $MgSO_4.7H_20$, 0.1 g of KCl, 0.1 g of yeast extract, 0.05 g of $CaCl_2$, 1mg of $FeCl_3$, and 194.2 mg of K_2CrO_4 (1 mM Cr(VI)) (Morales-Barrera and Cristiani-Urbina, 2008). The pH of the culture medium was adjusted to 6.0. For the isolation of resistant fungi, 2.5ml of the tannery effluents

was inoculated into 500ml conical flasks containing 100ml of sterile medium containing an initial Cr concentration of 1mM. The flasks were incubated on a rotary shaker for 72h at ambient temperature. All resistant isolates were stored on PDA slants for further studies (Srivastava and Thakur, 2006; Morales-Barerra and Cristiani-Urbina, 2008).

3.8.2 Isolation of fungal strains from soil

The method of Srivastava and Thakur, (2006) was used. The soil samples were serially diluted 10fold and appropriate diluted samples (0.1 ml) were inoculated on the potato dextrose agar (PDA) plate that are Cr-free and Cr^{6+}- supplemented. The plates were incubated at $30^{o}C$ for 4days. The fungal colonies on the PDA plates were isolated, purified and stored on PDA slants for further studies.

3.8.3 Identification of fungal isolates in the effluents and soils

Fungal colonies were distinguished based on their cultural characteristics such as colour, texture, and diameter of the colonies, and microscopic characteristics of the isolates using a taxonomic atlas. Important identification criteria such as macro-morphology (Colony color, Colony size), micromorphological Criteria (conidiophores and conidia) whether columnar or radiate or conidial heads: uni- or biseriate, Ascomata and Ascospores, Ascomata, ascospores, were used to identify the fungi (Raper and Thom, 1949; Davise, 2002). The fungi isolated, were further purified and subcultured on potato dextrose agar slants for further studies.

3.8.4 Preparation of stock chromium solution and effluent dilution

Stock solution of chromium was prepared by dissolving 0.38g of Potasium chromate ($K_2Cr_2O_7$) in 100ml distilled water. The stock solution was diluted with sterile distilled water to obtain

working solution (2.5 - 25mg/1) for further experiments.

3.8.5 Determination of concentration of chromium in effluent used

Effluent used was obtained immediately after chrome tanning and the concentration of chromium in the effluent was determined using AAS as described above.

3.9 Chromium sorption studies

3.9.1 Preparation of resistant bacterial isolates

The isolates identified to be *P. aeruginosa* and *B. cereus* were inoculated individually into 100ml peptone water in 500ml conical flasks and incubated on a shaker at 150rpm for 24h at 37^{o}C. The cells were grown to late exponential phase, which is deteremined by taking the absorbance using spectrophotometer until it reaches a constant, the cells are then harvested by centrifugation at 10,000rpm for 30min and washed three times with sterile normal saline. Cell suspensions for assay of biosorption potential of live bacteria were prepared by resuspending the cell pellet in sterile normal saline. Biomass concentrations in cell suspensions were determined by drying an aliquot of the cell suspension in a preweighed aluminum foil container to constant weight at 60^{o}C (Puranik and Paknikar, 1999).

3.9.2 Chromium sorption by bacteria

The pH of the working solution (100mg Cr/L) was adjusted to pH 6.0 with 0.1M sodium hydroxide and 0.1M nitric acid. Biosorption of Cr by the cultures was determined by inoculating the living cells (50mg dry weight) into triplicate 25ml volume of chromium solution in 150ml conical flasks. The flasks were shaken at 150rpm at room temperature (28±3^{0}C) for 24h. Metal-free (solution at pH 6.0 containing no Cr) and biosorbent-free solutions (solution at pH 6.0 uninoculated with test organism) were prepared as controls. The cells were harvested by

centrifuging at 10,000rpm for 10min and washed twice with sterile distilled water. The biomass were then dried at 80^{o}C in an oven and weighed, prior to digestion with the acid mixture mentioned above. The amount of chromium biosorbed was determined as mg Cr/g dry weight using AAS (Srinath *et al.*, 2002b). The isolates were coded as TNA04DP and TNA02SL2 and stored for further studies.

3.9.3 Preparation of resistant fungal isolates

The stored resistant fungal isolates obtained (sections 3.8.1 and 3.8.2) were inoculated into 200ml of basal medium containing 100ppm of streptopenicillin (to prevent bacterial contaminants) but not Cr^{6+} placed in 1000ml conical flask. The cultures were incubated aerobically at room temperature on a rotary shaker operating at 54rpm for 4days.

Mycelia of each test inoculum were harvested asceptically by filtration through sterile nylon cloth and washed in two rinses with sterile distilled water. This was then followed by further rinsing with tween 80 to remove medium components and cellular debris. The mycelia thus obtained were resuspended in small volumes (20ml) of the basal medium without Cr^{6+}. It was then homogenized to obtain mycelial suspension needed for the next stage of the study (Srivastava and Thakur, 2006; Morales-Barrera and Cristiani-Urbina, 2008).

3.9.4 Determination of Chromium removal by resistant fungal isolates

The three predominant resistant fungal isolates from effluent and soil samples identified to be *Aspergillus niger* (TN1A31) *Penicillium sp*. (TN3P09) and *Trichoderma longibrachiatum* (TN1X03) were screened for chromium removal ablity in minimal salt medium described in

section 3.8.1 (Thakur, 1995; Srivastava and Thakur, 2006; Morales-Barrera and Cristiani-Urbina, 2008). The experiments were conducted in 250ml conical flask containing 200ml culture media inoculated with *A. niger* (TN1A31), *Penicillium sp*. (TN3P09) and *T. longibrachiatum* (TN1X03) separately and in consortium.

These experiments were conducted at different pH (6, 7 and 8) and Cr concentration (10.0, 20.0 and 25mg/1) in aqueous solution. The initial biomass/inocula were 1mg dry weight/ml and 10% volume of the medium. The concentrations of Cr were 10.0, 20.0 and 25mg/1. The culture suspensions were shaken at 120rpm and ambient temperature for 120h. Samples were drawn at 24hrs intervals and filtered using Whatman and membrane filter to determine mycelial biomass and residual concentration of Cr in solution which was measured by atomic absorption spectrophotometer (V.P.C210 Bulk Scientific Model). The chromium levels were then compared to those of uninoculated controls. The most active fungi were selected based on percentage removal of chromium, for the next stage of the work.

3.10 Bioremediation studies

3.10.1 Preparation of effluent for laboratory scale bioremediation

The tannery effluent (with 25.02mg/L Cr) collected was poured into 1000ml conical flask, covered with cotton wool and tyndalized by heating to about 80^{o}C for 15 minutes and allowed to cool. This was repeated for three (3) consecutive days to kill all microorganisms including spore formers and not denature some vital components of the effluent.

3.10.2 Bioremediation using *Pseudomonas aeruginosa* and *Bacillus cereus*

The pH of the effluent was adjusted to 6.0 using NaOH and then distributed in 250 ml conical flasks (100 ml in each). Portions of 1% sucrose, 1% peptone were added and 10% of overnight grown culture of *Pseudomonas aeruginosa* (TNA04DP) and *Bacillus cereus* (TNA02SL2) were inoculated in each conical flask. The organisms were used in single, and in combination. The flasks were kept on rotary shaker at 180rpm and another set in an aerated vessel with aeration pumps at room temperature for a period of 5days. Chromium level was estimated at 24h interval to determine the chromium depletion from the effluent by the test bacteria.

3.10.3 Bioremediation using fungal isolates

Inocula of *A. niger* (TN1A31), *Penicillium sp*. (TN3P09) and *Trichoderma longibrachiatum* (TN1X03) and the fungal consortia were prepared as described above (section 3.9.3) and washed with sterile 0.2% of Tween 80 and placed into 250ml conical flask containing 100ml of tyndallized effluent, 15ml of sterilized basal medium were added into the flasks and plugged with cotton wool. Then using shake flask method similar to the experiments above (3.10.2) bioremediation using fungal isolates were also carried out with tannery effluents containing 25mg/1 of Cr obtained from the tannery (TAN1 DP) and adjusted to pH of 6 which appear to be the optimum. The same effluent samples were tested for chromium removal by the test isolates in similar manner using the vessels connected to aquarium pumps to simulate the river condition. Similarly samples (10ml) were drawn at 24hrs intervals from the conical flasks and vessels of each organism and their consortia. The drawn samples (10ml) were filtered using pre weighed Whatman filter paper and membrane filter to determine mycelial biomass and residual Cr level in solution which was measured by atomic absorption spectrophotometer (V.P.C210 Bulk Scientific

Model). Duplicates of each sample were analyzed and mean value by AAS were recorded.

To determine whether the observed Cr removal was biological or abiotic, experiments without organisms inoculated and with different concentrations of heat-killed fungal biomass were conducted at the same pH value and Cr concentration, in order to estimate Cr loss in the absence of living biomass. Heat-killed controls were autoclaved twice at 121 ℃ for 20 min. Biomass-free control was used to detect possible abiotic Cr reduction brought about by medium components.

3.11 Fungal strain improvement study by exposure to ultraviolet irradiation

Spores of the fungal isolates were washed off with 0.2% of Tween 80 into a sterlie Petri dish, 2% w/v of sterile caffeine was added into the plate. The spores were exposed to ultraviolet irradiation (UV) (254nm) for 10, 30 and 60 minutes in the dark room to effect mutation (Ado, 2004). The spores were then inoculated and grown in the dark to obtain mycelia. The mycelia were challenged with effluent (25mg/L) to test their bioremediation abilities as described above (section 3.10.3). The result of Cr removal determined as descibed above were recorded.

3.12 Determination of fungal biomass

The mycelial biomass produced by fungal isolates in bioremediation of the effluent was filtered through pre weighed 0.25um membrane filter paper. Then dried at 40^{o}c in an oven and weighed. The fungal mycelial biomass was obtained by subtracting the weight of the membrane filter from the weight of membrane filter and fungal biomass.

3.13 Data Analysis

Statistical analyses of the data were carried out. Means were compared using Analyses of variance (ANOVA) and Duncan multiple range test ($P<0.05$), as well as student t-test. The results were presented in tables, graphs and results interpreted.

CHAPTER FOUR

RESULTS

4.1 Physico-chemical properties of tannery effluent and well water samples

The pH of the tannery effluent ranged between 8.17 – 8.91 for various sites. The DO ranged between 2.21 – 2.39 mg/L, while the least TSS was 116mg/L and the BOD was as high as 198 mg/L in Tan A indicating high amount of organic matter. Analysis of variance of data has shown that, there were no significant differences ($P > 0.05$) between the pH, Temperature and DO of effluent samples from the tanneries studied. However, the mean values of TSS, TDS, EC and BOD varied significantly ($P \leq 0.05$) with the tanneries. Tan A had significantly higher values of TSS, TDS, EC and BOD compared to Tan B and C. however, Tan C had significantly ($P \leq 0.05$) higher values of TSS and TDS than Tan B. On the other hand the mean values of EC and BOD in Tan C were significantly lower than Tan B (Table 4.1).

In the well water samples the pH ranged between 6.88 and 7.14 in the studied wells, indicating near neutral pH. The TDS ranged between 10.9 – 12.6mg/L, while the control well water had TDS as low as 4.19mg/L. The BOD was as high as 5.55mg/L in Well 1 and as low as 2.30mg/L in control well water samples, indicating higher amount of organic matter in the studied wells. Significant differences ($P \leq 0.05$) exist between the physico-chemical parameters of the wells around the study area and control well where no apparent tannery or other industrial waste(s) discharge in the environment. Water from wells impacted by tannery effluents had significantly ($P \leq 0.05$) lower pH and DO compared to water from control well, which had significantly ($P \leq 0.05$) lower TSS, TDS and BOD than water from tannery impacted wells. Well 1 recorded the highest TSS, TDS and BOD compared to well 2 and well 3 but had significantly lower DO (Table 4.2).

Table 4.1: Physico-chemical properties of effluent samples from three tanneries

Properties n=36	Tanneries Mean ± SD		
	A	B	C
pH	8.91 ± 1.97^{a}	8.17 ± 1.88^{a}	8.60 ± 1.89^{a}
Temp (^{o}C)	27.48 ± 1.62^{a}	27.60 ± 2.72^{a}	26.94 ± 1.39^{a}
TSS (mg/L)	413.53 ± 282^{a}	116.89 ± 198^{c}	224.30 ± 142^{b}
TDS (mg/L)	123.47 ± 4.20^{a}	86.23 ± 41.44^{c}	93.99 ± 41.23^{b}
EC (µS/cm)	7.98 ± 5.62^{a}	5.46 ± 3.55^{b}	4.38 ± 2.87^{c}
DO (mg/L)	2.35 ± 0.7^{a}	2.39 ± 0.6^{a}	2.21 ± 0.4^{a}
BOD (mg/L)	189.00 ± 90.6^{a}	101.01 ± 39.6^{b}	63.93 ± 37.4^{c}

Means with the same letter superscript in the same row are not significantly different (P = 0.05) using ANOVA, Tan=tannery, EC= electrical conductivity (µS/cm), A, B and C = tanneries, TSS= Total suspended solids, TDS= Total disolved solids, DO= Dissolved oxygen, BOD=Biochemical oxygen demand.

Table 4.2: Physico-chemical properties of well water samples obtained from location close to tanneries

Properties	Well water Mean ± SD			
	Well 1	Well 2	Well 3	Well C
pH	7.14 ± 0.5^{b}	6.89 ± 0.5^{b}	6.88 ± 0.5^{b}	7.43 ± 0.5^{a}
Temp (^{o}C)	31.22 ± 1.4^{a}	30.9 ± 1.8^{a}	30.9 ± 1.2^{a}	27.59 ± 1.2^{a}
TSS (mg/L)	28.5 ± 9.4^{a}	27.6 ± 8.7^{a}	26.3 ± 8.0^{a}	23.9 ± 9.2^{b}
TDS (mg/L)	12.6 ± 1.8^{a}	12.1 ± 1.3^{ab}	10.9 ± 1.7^{b}	4.19 ± 1.5^{c}
EC (µS/cm)	8.6 ± 4.7^{a}	9.3 ± 4.4^{a}	8.9 ± 4.4^{a}	8.1 ± 3.7^{a}
DO (mg/L)	4.25 ± 1.8^{d}	4.70 ± 2.0^{c}	5.05 ± 2.0^{b}	5.52 ± 2.0^{a}
BOD (mg/L)	5.55 ± 2.0^{a}	4.55 ± 1.6^{b}	4.27 ± 1.5^{b}	2.30 ± 1.5^{c}

Means with the same letter superscript in the same row are not significantly different (P = 0.05) using ANOVA, Tan=tannery, EC= electrical conductivity (µS/cm), A, B and C = tanneries Key: Well C = control well sample, TSS= Total suspended solids, TDS= Total disolved solids, DO= Dissolved oxygen, BOD=Biochemical oxygen demand.

The TSS in the studied well water samples was 35.5mg/L in the dry season but much lower in wet season 19.5mg/L. Similarly TDS was higher in the dry (13.0mg/L). Conversely, DO was higher in the wet season (6.23mg/L) compared to dry season (3.11mg/L). But BOD was lower in the wet season (3.29mg/L) compared to dry season (6.31mg/L). Students t-test analysis of data on the physic-chemical properties of well water sample revealed no statistically ($P > 0.05$) significant differences in the mean pH, Temp, TDS and TS with season. However, significantly ($P \leq 0.05$) higher mean of TSS, EC, and BOD were recorded for samples collected during the dry season, while lower DO values were recorded in the dry season (Table 4.3).

Water samples from individual well had similar variation in the physicochemical parameters. The DO was increasing from well 1 (5.55mg/L) in the wet season to well 3 (6.78mg/L) in the same season. BOD in well 1 (7.25mg/L) in the dry season sharply declined to 2.90mg/L in well 3 during the wet season. ANOVA of the physico-chemical properties and the seasonal variation in individual well water samples are presented in Table 4.4. The pH and DO were significantly higher ($P \leq 0.05$) in the wet than dry season. The mean pH of the well water was tending towards neutral in the wet season and slightly alkaline; however in the dry season it tends towards slight acidic pH. The level of DO was significantly increasing from well 1 to well 3 with highest amount of DO in the wet season samples of well 3. There were no significant differences ($P > 0.05$) in the temperature in the wet and dry season samples measured on the site of collection. The mean TSS, TDS, EC and BOD were significantly higher ($P \leq 0.05$) in the dry season samples of all the wells. These mean values were decreasing from well 1 down to well 3 as shown in (Table 4.4).

Table 4.3: Seasonal variation in mean physico-chemical proprerties of water samples from wells located near tanneries studied

Properties	Season	Mean ± SD
pH	DRY	6.83 ± 0.5[a]
	WET	7.13 ± 0.4[a]
Temp	DRY	31.1 ± 1.6[a]
	WET	30.9 ± 1.3[a]
TSS	DRY	35.5 ± 3.0[a]
	WET	19.5 ± 2.4[b]
TDS	DRY	13.0 ± 1.1[a]
	WET	11.0 ± 1.8[a]
TS	DRY	48.4 ± 3.8[a]
	WET	30.4 ± 3.7[a]
EC	DRY	12.21 ± 1.7[a]
	WET	5.68 ± 3.8[b]
DO	DRY	3.11 ± 0.6[b]
	WET	6.23 ± 1.5[a]
BOD	DRY	6.31 ± 0.9[a]
	WET	3.29 ± 1.0[b]

Means with the same letter superscript in the same column under the same property are not significantly different (P = 0.05) using T-test, TSS= Total suspended solids, TDS= Total disolved solids, DO= Dissolved oxygen, BOD=Biochemical oxygen demand.

Table 4.4: Seasonal variation of mean values of Physico-chemical parameters of water samples from wells impacted by the tanneries

		Physico-chemical parameters Mean ± SD							
Wells	Season	pH	Temp (^oC)	Tss (mg/L)	Tds (mg/L)	Ts (mg/L)	Ec (µS/cm)	DO (mg/L)	BOD(mg/L)
Well 1	Dry	6.99 ± 0.64^b	31.37 ± 1.86^a	37.28 ± 1.99^a	13.80 ± 0.79^a	51.08 ± 2.58^a	12.17 ± 2.00^a	2.95 ± 1.03^b	7.25 ± 0.66^a
	Wet	7.30 ± 0.41^a	31.08 ± 1.05^a	19.63 ± 1.96^b	11.52 ± 1.98^b	31.15 ± 3.68^b	5.13 ± 4.09^b	5.55 ± 1.55^a	3.87 ± 1.27^b
Well 2	Dry	6.76 ± 0.46^b	31.02 ± 1.90^a	35.63 ± 2.77^a	13.02 ± 0.96^a	48.65 ± 3.36^a	12.46 ± 1.78^a	3.03 ± 0.24^b	6.02 ± 0.52^a
	Wet	7.05 ± 0.44^a	30.78 ± 1.78^a	19.57 ± 2.19^b	11.35 ± 1.14^b	30.92 ± 3.10^b	6.20 ± 3.96^b	6.37 ± 1.49^a	3.10 ± 0.64^b
Well 3	Dry	6.73 ± 0.51^b	30.88 ± 1.40^a	33.45 ± 3.09^a	12.05 ± 0.60^a	45.50 ± 3.53^a	12.00 ± 1.72^a	3.33 ± 0.38^b	5.65 ± 0.63^a
	Wet	7.03 ± 0.48^a	30.90 ± 1.25^a	19.17 ± 3.25^b	9.82 ± 1.91^c	28.98 ± 4.44^c	5.72 ± 4.09^b	6.78 ± 1.32^a	2.90 ± 0.76^b

Means with the same letter superscript in the same column under the same property are not significantly different (P = 0.05) using T-test.

Comparisons of mean values of physico-chemical properties of effluent from different sampling points in the tanneries area are shown in Table 4.5. Physico-chemical properties of thirty six (36) effluent samples each from the three tanneries sampling points (DP, DS and EP) selected for this study were recorded and analyzed. TSS was found to be 242.02mg/L in DP samples and declined to 120.17mg/L at the EP, also BOD was found to be 153.09mg/L in the DP but declined to 96.88mg/L at the EP. However, DO was 1.98mg/L at DP but rose to 2.74mg/L at the EP. These indicate higher microbial activities that aid tmicrobial biodegrardation of the organic matter responsible for higher BOD.

The results showed statistically significant differences ($P \leq 0.05$) among the effluent samples from three sampling points chosen for this study with regards to pH which was higher at discharge point (DP) 9.29, and less alkaline downstream (DS) and at the entry point (EP) 8.33 and 8.03 respectively. The higher pH at DP is attributed to series of tanning processes (Liming, reliming, deliming, bating etc.) that requires the use of lime which is alkaline. The TSS declined downstream with DP having 242mg/L, DS 186.9 and EP 120.2 mg/L. Similarly TDS was 162mg/L at DP, 95.6mg/L DS and 43.9mg/L EP. BOD also declined downstream with 153mg/L at DP and 98.6mg/L at EP. Conversely dissolved oxygen DO increased downstream from 1.98mg/L at DP to 2.74mg/L at EP as shown in Table 4.5.

Table 4.5: Physico-chemical properties of effluents at different points

Properties	Tan point	Range	Mean ± SD
pH	DP	3.37- 13.4	9.29 ± 2.74[a]
	DS	5.11- 10.71	8.33 ± 1.30[b]
	EP	5.38- 9.71	8.03 ± 1.03[b]
Temp	DP	22.7- 31.6	26.6 ± 1.94[a]
	DS	25.7- 34.8	28.5 ± 2.0 [b]
	EP	24.4- 31.3	26.9 ± 1.53[a]
TSS(mg/L)	DP	162.16- 298.4	242.02 ± 117.9[a]
	DS	89- 297.6	186.90 ± 162.2[b]
	EP	48.95- 160.12	120.17 ± 128.3[c]
TDS(mg/L)	DP	74.8- 302	162.30 ± 54.8[a]
	DS	48- 163	95.63 ± 25.5[b]
	EP	20- 79.5	43.92 ± 15.0[c]
EC	DP	2.23- 26.9	7.05 ± 4.84[a]
	DS	1.09- 24.8	5.08 ± 4.43[a]
	EP	1.09- 19.1	5.63 ± 3.75[a]
DO(mg/L)	DP	1.1- 2.9	1.98 ± 0.52[c]
	DS	1.12- 3.17	2.25 ± 0.52[b]
	EP	1.8- 3.5	2.74 ± 0.47[a]
BOD(mg/L)	DP	48.65- 483	153.09 ± 101[a]
	DS	29- 318	102.57 ± 66[b]
	EP	18.85- 223	96.88 ± 55[b]

Means with the same letter superscript in the same column under the same parameter are not significantly different (P = 0.05) using ANOVA, Tan=tannery, EC= electrical conductivity (µS/cm), DP= discharge point, DS=downstream, EP= entry point, TSS= Total suspended solids, TDS= Total disolved solids, DO= Dissolved oxygen, BOD=Biochemical oxygen demand.

4.2 Seasonal variation of physico-chemical properties of effluent samples

Table 4.6 presents the mean values of physico-chemical parameters in the tannery effluents which were compared and the level of significance was set at ($P \leq 0.05$). Significant difference were observed at different sampling points ($F=5.086$, $P=0.008$). Similarly, significant differences were observed in different seasons with respect to sampling points ($F=6.125$, $P=0.003$). However, these differences were not significant ($F=2.686$, $P=0.074$) between similar points of sampling regardless of the season.

There was no significant difference in the temperature readings of effluents recorded at the points of collection in different seasons. The total suspended solids and dissolved solids were significantly different ($P \leq 0.05$) from one another in different seasons and from one sampling point to another with higher TSS and TDS at discharge points (DP) followed by the downstream points (DS), the entry point (EP) had the lowest mean values (Table 4.6). Dissolved oxygen (DO) and BOD were also significantly different ($P \leq 0.05$) in different sampling points and seasons. The mean DO was significantly lower at the DP and higher at the EP. Equally DO values were significantly higher in the wet season samples than in dry season samples. Conversely mean BOD values were significantly higher at DP, lower at EP and higher in dry compared to the wet season (Table 4.6).

4.3 Heavy metal contents of water and effluent samples

Table 4.7 shows the mean concentrations of the heavy metals from all samples analyzed during the study period. Chromium (Cr) was the highest among the heavy metals in all the samples. The mean Cr (4.85mg/L) and Cd (0.21mg/L) concentrations were significantly higher ($P \leq 0.05$) in effluent samples compared to the well water samples (0.12mg/L and 0.03mg/L respectively) and control samples. The Cr level in the samples were above the standards in both well

Table 4.6: Seasonal variation of mean values of physico-chemical properties of effluent at different tannery sampling points

TAN	POINTS	SEASON	Physico-Chemical Properties Mean ± SD					
			pH	TSS	TDS	EC	DO	BOD
A	DP	DRY	9.4 ± 2.8^{b}	785.57 ± 180.8^{a}	209.71 ± 73.42^{a}	9.66 ± 8.30^{a}	1.63 ± 0.50^{e}	279.86 ± 111.4^{a}
		WET	11.2 ± 1.3^{a}	420.50 ± 103.5^{c}	221.67 ± 19.38^{a}	5.25 ± 3.11^{e}	1.95 ± 0.41^{d}	231.67 ± 83.73^{b}
	DS	DRY	8.5 ± 1.9^{c}	506.60 ± 61.2^{b}	130.40 ± 32.53^{b}	10.64 ± 8.1^{a}	2.06 ± 0.35^{c}	196.20 ± 76.42^{c}
		WET	8.4 ± 0.6^{c}	176.17 ± 123.5^{f}	93.33 ± 18.29^{c}	8.15 ± 3.52^{b}	2.55 ± 0.38^{b}	157.67 ± 57.83^{d}
	EP	DRY	8.5 ± 0.4^{c}	312.17 ± 216.6^{d}	43.00 ± 8.37^{d}	7.73 ± 5.91^{c}	2.83 ± 0.27^{b}	149.17 ± 44.01^{d}
		WET	7.2 ± 1.4^{e}	235.67 ± 424.7^{e}	29.50 ± 6.22^{e}	6.60 ± 2.51^{d}	3.15 ± 0.21^{a}	105.50 ± 20.71^{e}
B	DP	DRY	8.7 ± 2.6^{c}	427.00 ± 194.7^{c}	125.13 ± 33.95^{b}	8.39 ± 4.41^{b}	1.82 ± 0.52^{d}	103.87 ± 39.06^{e}
		WET	8.4 ± 3.2^{c}	407.2 ± 149.7^{c}	121.92 ± 10.66^{b}	4.88 ± 2.90^{e}	2.34 ± 0.50^{c}	81.03 ± 29.28^{f}
	DS	DRY	8.5 ± 1.4^{c}	442.50 ± 84.7^{c}	111.83 ± 14.74^{b}	3.91 ± 0.71^{f}	1.54 ± 0.31^{e}	106.17 ± 28.02^{e}
		WET	7.7 ± 1.2^{e}	224.83 ± 55.5^{e}	86.00 ± 13.86^{c}	1.88 ± 0.77^{g}	2.68 ± 0.46^{b}	60.33 ± 11.94^{f}
	EP	DRY	8.5 ± 0.4^{c}	312.17 ± 516.6^{d}	43.00 ± 8.37^{d}	9.35 ± 3.08^{a}	2.83 ± 0.27^{b}	149.17 ± 44.01^{d}
		WET	7.2 ± 1.4^{e}	235.67 ± 424.7^{e}	29.50 ± 6.22^{e}	4.32 ± 1.08^{e}	3.15 ± 0.21^{a}	105.50 ± 20.71^{e}
C	DP	DRY	7.2 ± 3.3^{e}	481.64 ± 92.3^{b}	145.60 ± 39.51^{b}	7.22 ± 3.86^{c}	1.90 ± 0.55^{d}	126.87 ± 35.45^{d}
		WET	10.9 ± 1.2^{a}	244.96 ± 60.2^{e}	141.87 ± 12.40^{b}	6.45 ± 3.42^{d}	2.27 ± 0.46^{c}	74.12 ± 15.94^{f}
	DS	DRY	8.3 ± 1.7^{c}	264.83 ± 50.9^{e}	88.83 ± 12.19^{c}	4.60 ± 0.84^{e}	2.02 ± 0.26^{c}	70.50 ± 18.51^{f}
		WET	8.5 ± 0.9^{c}	134.67 ± 33.2^{f}	69.17 ± 11.55^{cd}	2.22 ± 0.91^{g}	2.60 ± 0.17^{b}	40.17 ± 8.13^{g}
	EP	DRY	8.6 ± 0.6^{c}	145.66 ± 28.0^{f}	66.63 ± 9.14^{cd}	3.91 ± 0.71^{f}	1.92 ± 0.10^{d}	45.83 ± 12.03^{f}
		WET	8.0 ± 0.45^{d}	74.07 ± 18.3^{g}	51.88 ± 8.66^{d}	1.88 ± 0.77^{g}	2.57 ± 0.23^{b}	26.11 ± 5.29^{g}

Means with the same letter superscript in the same column under the same seasons are not significantly different (P = 0.05) using ANOVA, Tan=tannery, EC= electrical conductivity (µS/cm), DP= discharge point, DS= downstream, EP= entry point. TSS= Total suspended solids, TDS= Total disolved solids, DO= Dissolved oxygen, BOD=Biochemical oxygen demand

Table 4.7: Heavy metal content of water and effluent samples

Heavy metals assayed	Mean heavy metal content (mg/l) in samples of			
	Effluent samples	River water (CRW)	Impacted Well water	Control Well water (well C)
Cr	4.85 ± 3.2^{a}	0.01 ± 0.0^{c}	0.12 ± 0.1^{b}	ND
Zn	0.17 ± 0.09^{a}	0.132 ± 0.0^{a}	0.06 ± 0.08^{b}	0.008 ± 0.0^{b}
Pb	0.15 ± 0.19^{a}	ND	0.01 ± 0.01^{b}	ND
Cd	0.21 ± 0.18^{a}	ND	0.03 ± 0.08^{b}	ND
Cu	0.04 ± 0.02^{a}	0.01 ± 0.0^{c}	0.02 ± 0.01^{b}	0.010 ± 0.0^{c}
Fe	0.22 ± 0.09^{b}	0.17 ± 0.0^{c}	0.73 ± 0.4^{a}	0.141 ± 0.0^{c}
Mn	0.54 ± 0.12^{a}	0.26 ± 0.0^{b}	0.07 ± 0.05^{c}	ND

Means with the same letter superscript in the same row are not significantly different (P = 0.05) using ANOVA
Key: ND = below detection limit, CRW = control sample from river, Well C = control well sample.

(0.05mg/L) and effluent (0.5mg/L) which poses danger in the use of the well water for domestic chores or the effluent for irrigation. The Cr was below detection limit in the control samples analyzed. This indicates that the studied wells were impacted upon by the seepage and surface run-off because of high Cr content. However, Fe was found to be significantly higher ($P \leq 0.05$) in the well water samples during the study period with a mean concentration of 0.73mg/L compared to the effluent samples (0.22mg/L). No significant difference ($P > 0.05$) was observed in the mean Fe concentrations in the control samples of the well and effluent. On the other hand Pb, Cd and Mn were also below detection limits in the control samples of well water, and river water (Tan control sample collected 25Km upstream River Challawa (CRW)) samples, except three samples of Tan Control which had 0.01mg/L of Cr in each Table 4.7.

4.3 Heavy metal contents of Soil and vegetables samples from polluted sites

Table 4.8 presents the heavy metal content of soil and vegetable samples in the study area. Statistically significant differences ($P \leq 0.05$) were observed in the level of Cr in the samples. Highest mean concentration of Cr were found in soil samples (7.81mg/L) around the tannery wastes while lower Cr concentrations was found in the vegetable samples (0.35mg/L) during the study period (Table 4.8). All other heavy metals were found to be significantly higher in the soil samples around the tanneries except Pb and Cu that were not tested for in vegetables and soil samples respectively. Cr, Pb and Cd were below detection levels in control soil samples. Cu was not tested for in all soil samples including control soil samples as shown in Table 4.8.

Table 4.8: Heavy metal content of soil, contol soil and vegetables samples

Heavy metals assayed	Mean heavy metal content (mg/Kg) in samples of		
	Impacted Soil	Control Soil	Vegetables
Cr	7.81 ± 3.5^{a}	ND	0.35 ± 0.2^{b}
Zn	2.24 ± 4.15^{a}	0.099 ± 0.0^{b}	0.10 ± 0.03^{b}
Pb	0.29 ± 0.11	ND	NT
Cd	0.14 ± 0.11^{a}	ND	0.02 ± 0.006^{b}
Cu	NT	NT	0.01 ± 0.007
Fe	0.99 ± 0.4^{a}	0.18 ± 0.0^{b}	0.91 ± 0.3^{a}
Mn	0.59 ± 0.24^{a}	0.12 ± 0.0^{b}	0.47 ± 0.19^{a}

Means with the same letter superscript in the same row are not significantly different (P = 0.05) using ANOVA
Key: NT = Not tested, ND = below detection limit, Soil C = control soil sample.

The mean concentration of heavy metals in the well water samples decreases from well 1 to well 3 except Cadmium (Cd) which had highest concentration of 0.08mg/L in well 2. Analysis of variance and Duncan tests of mean concentrations of these metal pollutants between wells showed significant differences ($P \leq 0.05$) in the level of heavy metals in the well water samples, except Zn and Mn and Pb which Pb was below detection limit in all the well water samples except well 2 (Table 4.9). Fe concentration was found to be the highest in the entire samples compared to other metals. Cr, Pb, Cd and Mn were below detection limit in the control well water samples while Zn and Cu were found in trace amount in the control well water samples with significant difference ($P \leq 0.05$) compared to the other samples as shown in Table 4.9.

4.4 Heavy metal pollution of effluent samples

The level of Mn in the effluents was highest after Cr followed by Fe < Zn < Cd < Pb and then Cu had the least concentration in the effluent analyzed with no significant difference ($P > 0.05$) among the tanneries. No statistical significant differences ($P > 0.05$) existed among the three tanneries in this study with respect to heavy metal pollution in the effluent sample analyzed, except the level of chromium (Cr) where it was established that the Cr level (4.36mg/L – 5.29mg/L) were significantly higher ($P \leq 0.05$) in effluents analyzed from Tan A (5.29mg/L) then followed by Tan B (4.91mg/L) and the level was lower in Tan C(4.36mg/L) (Table 4.10).

Analyses of heavy metal content in the tannery effluent from different sampling points revealed significant differences ($P \leq 0.05$) among the three tanneries in this study where it shows decrease in all metal concentrations from DP to DS and then EP, except the level of Cadmium (Cd) which was significantly higher ($P \leq 0.05$) in DS (0.25mg/L) effluents.

Table 4.9: Mean concentration of heavy metals in well water samples

Heavy metal assayed	Mean heavy metal content (mg/l) in samples of			
	Well 1	Well 2	Well 3	Well C
Cr	0.16 ± 0.05^{a}	0.11 ± 0.07^{ab}	0.08 ± 0.04^{b}	ND
Zn	0.10 ± 0.11^{a}	0.05 ± 0.05^{a}	0.04 ± 0.04^{a}	0.008^{b}
Pb	ND	0.03 ± 0.01	ND	ND
Cd	0.02 ± 0.001^{a}	0.08 ± 0.006^{b}	0.02 ± 0.001^{a}	ND
Cu	0.03 ± 0.004^{a}	0.02 ± 0.004^{b}	0.01 ± 0.005^{c}	0.01^{c}
Fe	1.22 ± 0.2^{a}	0.63 ± 0.1^{b}	$0.33\ \ 0.05^{c}$	0.141d
Mn	0.09 ± 0.07^{a}	0.06 ± 0.04^{a}	0.06 ± 0.04^{a}	ND

Means with the same letter superscript in the same column under the same metal are not significantly different (P = 0.05) using ANOVA Key: NT = Not tested, ND = below detection limit.

Table 4.10: Comparison of mean heavy metal contents of effluent from different Tanneries studied

Properties	Mean heavy metal content (mg/l) in samples of		
	Tan A	Tan B	Tan C
Cr	5.29 ± 3.75 [a]	4.91 ± 3.16 [b]	4.36 ± 2.79 [c]
Zn	0.15 ± 0.09 [a]	0.17 ± 0.10 [a]	0.20 ± 0.08 [a]
Cd	0.13 ± 0.19 [a]	0.18 ± 0.22 [a]	0.14 ± 0.17 [a]
Pb	0.11 ± 0.01 [a]	0.11 ± 0.01 [a]	0.11 ± 0.00 [a]
Cu	0.05 ± 0.01 [a]	0.04 ± 0.02 [a]	0.03 ± 0.01 [a]
Fe	0.26 ± 0.13 [a]	0.20 ± 0.10 [a]	0.20 ± 0.04 [a]
Mn	0.67 ± 0.09 [a]	0.53 ± 0.08 [a]	0.43 ± 0.09 [a]

Means with the same letter superscript in the same row (metal) are not significantly different (P = 0.05) using ANOVA, Tan=tannery, EC= electrical conductivity (µS/cm), A, B and C = tanneries

However no significant difference ($P > 0.05$) in the levels of Cd at DP and EP, levels of Mn at DS and EP and the levels of Cu at DP and DS (Table 4.11).

4.5 Seasonal variation of heavy metal contamination of well water samples

Cr content in the well water sample was higher in the dry season (0.16mg/L) than in the wet season (0.07m/L). Similarly, Zn (0.11mg/L) and Mn (0.12mg/L) were also higher in the dry season than the wet season. However, equal mean concentrations of Cu (0.02mg/L) were found in the samples during the wet and dry seasons. Cd content was silightly higher in the dry season (0.03) compared to the wet season (0.02) samples Table 4.12. Analyses of the seasonal variation in the heavy metal pollution of well water samples using students t-test is presented in Table 4.12. Significant differences ($P \leq 0.05$) were observed in the concentrations of Cr, Zn, and Mn in the samples during the dry and wet seasons. Higher concentrations of metals were recorded in the dry compared to wet season, although the mean value were much higher than the permissible limit of 0.05mg/L. These metal concentrations were in the order Cr > Fe > Mn > Zn. However, no statistically significant differences were observed in the level of Pb, Cd, Cu and Fe in the two seasons (Table 4.12).

The mean heavy metal pollutant levels in the well water samples were significantly higher ($P \leq 0.05$) in the dry than wet season samples of all the wells. The level of these metals in the well water samples analyzed was in this pattern in Fe > Cr > Zn > Mn > Cd > Cu. There were significant differences ($P \leq 0.05$) in the levels of the metals analyzed in different season. Significant difference was observed in the Cr level in the samples. Cr was higher in well 1 during the dry season which decreases in well 2 of the same season, however, Cr level in well was comparable to that of dry season samples in well 2 (Table 4.13). However, there was no

Table 4.11: Heavy metal content in effluent samples from different points in tanneries

Heavy Metal	Tan Location	Range (mg/l)	Mean heavy metal content (mg/l)
Cr	DP	2.83 - 19.1	7.81 ± 3.32^{a}
	DS	1.43 - 11.3	4.44 ± 1.70^{b}
	EP	0.47 - 6.1	2.21 ± 1.20^{c}
Zn	DP	0.11 - 0.41	0.22 ± 0.10^{a}
	DS	0.01 - 0.37	0.17 ± 0.09^{b}
	EP	0.04 - 0.27	0.13 ± 0.06^{c}
Pb	DP	0.042 - 0.92	0.23 ± 0.28^{a}
	DS	0.02 - 0.63	0.14 ± 0.14^{b}
	EP	0.02 - 0.25	0.09 ± 0.05^{c}
Cd	DP	0.001 - 0.16	0.11 ± 0.01^{b}
	DS	0.001 - 0.27	0.25 ± 0.01^{a}
	EP	0.0021 - 0.36	0.11 ± 0.01^{b}
Cu	DP	0.0167 - 0.058	0.03 ± 0.01^{b}
	DS	0.013 - 0.065	0.04 ± 0.01^{b}
	EP	0.014 - 0.075	0.05 ± 0.02^{a}
Fe	DP	0.167 - 0.45	0.27 ± 0.06^{a}
	DS	0.12 - 0.38	0.24 ± 0.07^{b}
	EP	0.013 - 0.36	0.15 ± 0.11^{c}
Mn	DP	0.442 - 0.82	0.60 ± 0.11^{a}
	DS	0.28 - 0.84	0.53 ± 0.15^{b}
	EP	0.22- 0.6	0.50 ± 0.11^{b}

Means with the same letter superscript in the same column under the same point of a metal are not significantly different (P = 0.05) using ANOVA, Tan=tannery, DP= discharge point, DS=downstream, EP= entry point.

Table 4.12: Seasonal variation of heavy metal content in well water samples

Metal	SEASON	Mean heavy metal content (mg/l) in samples
Cr	DRY	0.16 ± 0.05^{a}
	WET	0.07 ± 0.04^{b}
Zn	DRY	0.11 ± 0.09^{a}
	WET	0.02 ± 0.01^{b}
Pb	DRY	0.03 ± 0.01^{a}
	WET	0.01 ± 0.01^{b}
Cd	DRY	0.03 ± 0.00^{a}
	WET	0.02 ± 0.01^{a}
Cu	DRY	0.02 ± 0.01^{a}
	WET	0.02 ± 0.01^{a}
Fe	DRY	0.80 ± 0.43^{a}
	WET	0.66 ± 0.36^{b}
Mn	DRY	0.12 ± 0.04^{a}
	WET	0.03 ± 0.04^{b}

Means with the same letter superscript in the same column under the same seasons are not significantly different (P = 0.05) using T-test.

Table 4.13: Seasonal variation of mean values of heavy metals in well water samples

Heavy metal assayed	Mean heavy metal content (mg/l) in samples of					
	Well 1		Well 2		Well 3	
Season	Dry	Wet	Dry	Wet	Dry	Wet
Cr	0.21 ± 0.03^{a}	0.11 ± 0.03^{c}	0.16 ± 0.04^{b}	0.06 ± 0.05^{d}	0.11 ± 0.03^{c}	0.05 ± 0.03^{d}
Zn	0.18 ± 0.12^{a}	0.02 ± 0.01^{c}	0.08 ± 0.06^{b}	0.02 ± 0.01^{c}	0.07 ± 0.05^{b}	0.02 ± 0.01^{c}
Cd	0.04 ± 0.01^{a}	0.02 ± 0.01^{b}	0.03 ± 0.01^{a}	0.01 ± 0.01^{c}	0.02 ± 0.00^{b}	0.01 ± 0.01^{c}
Pb	ND	ND	0.01 ± 0.00	0.01 ± 0.01	ND	ND
Cu	0.04 ± 0.00^{a}	0.03 ± 0.00^{b}	0.02 ± 0.00^{c}	0.02 ± 0.01^{c}	0.01 ± 0.00^{d}	0.01 ± 0.01^{d}
Fe	1.33 ± 0.24^{a}	1.11 ± 0.09^{b}	0.71 ± 0.08^{c}	0.55 ± 0.05^{d}	0.37 ± 0.04^{e}	0.30 ± 0.03^{e}
Mn	0.15 ± 0.04^{a}	0.04 ± 0.05^{c}	0.10 ± 0.03^{b}	0.02 ± 0.03^{d}	0.10 ± 0.03^{b}	0.02 ± 0.03^{d}

Means with the same letter superscript in the same column under the same metal are not significantly different (P = 0.05) using ANOVA, ND= not detected

significant difference observed in its level in well 2 and 3 during the wet season sampling also the mean values of metals were much higher than the permissible limit of 0.05mg/L. Similar pattern was observed in the concentration of Zn in the same samples. Like other pollution parameters heavy metal concentration was decreasing from well 1 to well 3 (Table 4.13).

4.6 Seasonal variation of heavy metal pollution of effluent samples

The Cr concentrations in the effluents were much higher than the permissible limit of 0.5mg/L. The level of Chromium in all effluents samples analyzed was significantly higher ($P \leq 0.05$) at each sampling points compared to other metals. Also the levels of all metals in the effluent samples were higher ($P \leq 0.05$) at DP and decline downstream to the EP where concentrations were lowest. However, the levels of cadmium Cd were much higher at the middle point (DS) of sample collection during the dry season. The level of these metals generally were in the order Cr > Cd > Mn > Zn > Fe > Pb except in some few cases the order varies at certain points of sampling (Table 4.14).

The highest mean level of Cr was 9.71mg/L in the DP of Tan A during the dry season and no significant differences ($P > 0.05$) were observed in other DPs of Tan B (7.6mg/L) and Tan C (8.68mg/L). Cr levels were relatively low in effluents analyzed from the EP of the tanneries (Tan A 1.26mg/L, Tan B 1.26mg/L and Tan C 1.83 mg/L), also no significant differences ($P > 0.05$) among the EP Cr levels in all the tanneries. Significant differences ($P \leq 0.05$) in the seasonal Cr level was very clear with dry season having higher concentrations than wet, except at the DP of Tan B where no significant differences ($P > 0.05$) was observed in the Cr level in the two seasons.

Table 4.14: Seasonal variation of mean values of Heavy metals contents in effluent at tanneries sampling points

Tan	Points	Season	Heavy metals (mg/L) Mean ± SD						
			Cr	Zn	Pb	Cd	Cu	Fe	Mn
A	DP	DRY	9.71 ± 4.52^{a}	0.27 ± 0.11^{a}	0.13 ± 0.04^{b}	0.91 ± 0.31^{c}	0.04 ± 0.01^{d}	0.36 ± 0.04^{a}	0.75 ± 0.04^{a}
		WET	6.74 ± 1.79^{b}	0.16 ± 0.05^{b}	0.34 ± 0.42^{a}	0.48 ± 0.16^{d}	0.03 ± 0.01^{e}	0.30 ± 0.03^{b}	0.68 ± 0.03^{b}
	DS	DRY	6.71 ± 2.88^{b}	0.17 ± 0.07^{b}	0.11 ± 0.02^{b}	2.20 ± 0.44^{a}	0.06 ± 0.00^{b}	0.36 ± 0.01^{a}	0.77 ± 0.05^{a}
		WET	3.69 ± 1.17^{d}	0.06 ± 0.05^{c}	0.05 ± 0.02^{c}	0.80 ± 0.41^{c}	0.05 ± 0.00^{c}	0.28 ± 0.05^{b}	0.66 ± 0.05^{b}
	EP	DRY	3.12 ± 1.54^{d}	0.13 ± 0.03^{b}	0.12 ± 0.04^{b}	2.77 ± 0.50^{a}	0.07 ± 0.00^{a}	0.23 ± 0.10^{b}	0.58 ± 0.01^{c}
		WET	1.26 ± 0.42^{e}	0.07 ± 0.03^{c}	0.05 ± 0.02^{c}	1.47 ± 0.49^{b}	0.06 ± 0.00^{b}	0.02 ± 0.01^{d}	0.55 ± 0.02^{c}
B	DP	DRY	7.60 ± 1.56^{a}	0.17 ± 0.06^{b}	0.14 ± 0.03^{b}	0.75 ± 0.16^{c}	0.04 ± 0.01^{d}	0.29 ± 0.04^{b}	0.61 ± 0.03^{b}
		WET	8.40 ± 4.37^{a}	0.26 ± 0.12^{a}	0.33 ± 0.41^{a}	0.24 ± 0.37^{e}	0.03 ± 0.01^{e}	0.24 ± 0.03^{b}	0.55 ± 0.02^{c}
	DS	DRY	5.36 ± 0.71^{c}	0.27 ± 0.08^{a}	0.35 ± 0.21^{a}	1.00 ± 0.01^{b}	0.03 ± 0.00^{e}	0.25 ± 0.04^{b}	0.51 ± 0.06^{c}
		WET	3.74 ± 0.45^{d}	0.12 ± 0.02^{b}	0.09 ± 0.09^{b}	0.33 ± 0.52	0.02 ± 0.01^{f}	0.19 ± 0.02^{c}	0.37 ± 0.03^{d}
	EP	DRY	3.12 ± 1.54^{d}	0.13 ± 0.03^{b}	0.12 ± 0.04^{b}	2.77 ± 0.50^{a}	0.07 ± 0.00^{a}	0.23 ± 0.10^{b}	0.58 ± 0.01^{c}
		WET	1.26 ± 0.42^{e}	0.07 ± 0.03^{c}	0.05 ± 0.02^{c}	1.47 ± 0.49^{b}	0.06 ± 0.00^{b}	0.02 ± 0.01^{d}	0.55 ± 0.02^{c}
C	DP	DRY	8.68 ± 3.71^{a}	0.21 ± 0.10^{a}	0.12 ± 0.03^{b}	0.77 ± 0.12^{c}	0.04 ± 0.01^{d}	0.24 ± 0.03^{b}	0.52 ± 0.03^{c}
		WET	5.46 ± 1.45^{c}	0.23 ± 0.11^{a}	0.30 ± 0.37^{a}	0.52 ± 0.20^{d}	0.03 ± 0.01^{e}	0.19 ± 0.02^{c}	0.46 ± 0.02^{c}
	DS	DRY	4.46 ± 0.9^{cd}	0.26 ± 0.04^{a}	0.20 ± 0.10^{a}	0.80 ± 0.09^{c}	0.03 ± 0.01^{e}	0.24 ± 0.03^{b}	0.52 ± 0.03^{c}
		WET	3.05 ± 0.69^{d}	0.14 ± 0.02^{b}	0.06 ± 0.02^{c}	0.48 ± 0.16^{d}	0.03 ± 0.01^{e}	0.17 ± 0.03^{c}	0.37 ± 0.06^{d}
	EP	DRY	2.68 ± 0.52^{d}	0.23 ± 0.04^{a}	0.14 ± 0.07^{b}	0.56 ± 0.06^{d}	0.02 ± 0.00^{f}	0.23 ± 0.02^{b}	0.41 ± 0.02^{c}
		WET	1.83 ± 0.41^{e}	0.12 ± 0.02^{b}	0.04 ± 0.01^{c}	0.34 ± 0.11^{e}	0.02 ± 0.00^{f}	0.16 ± 0.03^{c}	0.30 ± 0.04^{d}

Means with the same letter superscript in the same column under the same seasons are not significantly different (P = 0.05) using ANOVA, Tan=tannery, DP= discharge point, DS= downstream, EP= entry point

Cadmium (Cd) level in the effluents were significantly higher ($P \leq 0.05$) at DS and EP points in the three tanneries studied (Table 4.14) with highest levels in Tan A and B EPs (2.77mg/L each). Seasonal levels of Cd in effluents were significantly higher ($P \leq 0.05$) at the same location during the dry season while the least concentration of Cd was found in the wet season samples of DP of Tan A and EP of Tan C. Fe and Mn had similar pattern of pollution in the effluents, significant differences ($P \leq 0.05$) were observed between dry (Fe, 0.36mg/L and Mn, 0.75mg/L) and wet season. Like Cr and Zn levels Fe and Mn levels were reducing downstream and lowest levels were found in EP in the wet season where Fe was 0.02mg/L and Mn 0.30mg/L in Tan C Table 4.14.

4.7 Heavy metal pollution levels in soil samples

Table 4.15 presents the seasonal variations of heavy metal pollution of the soil samples around the tanneries. Heavy metal pollution of the soil from gardens close to tanneries were significantly lower ($P \leq 0.05$) in the wet than dry seasons. Higher levels were recorded during the dry season in all the three locations. Cr levels were higher than other metals analyzed and decreases in Cr > Fe > Mn > Zn > Cd > Pb. Cr concentrations in the dry season of soil 1 and 2 (10.2mg/Kg and 10.7mg/Kg) are not statistically different ($P > 0.05$) but significantly different from wet season Cr level in the same site. Similarly overall level Cr in soil 1 and 2 are not significantly different ($P=0.915$) in the wet season. However these levels (in soil 1 and 2) in both seasons are significantly different from the Cr levels in farm soils where vegetables are irrigated downstream. The levels of Zn, Cd, Fe and Mn are not significantly different ($P > 0.05$) in soil1 and 2 of the same season. But all heavy metal concentrations in the dry season samples were higher than in wet season samples (Table 4.15).

Table 4.15: Seasonal variation of mean values of heavy metals contents in soil sampling points

Soil	Season	Heavy metals (mg/Kg) Mean ± SD					
		Cr	Zn	Cd	Pb	Fe	Mn
Site_1	Dry	10.33 ± 1.28^{a}	0.60 ± 0.10^{a}	0.39 ± 0.04^{a}	0.04 ± 0.01^{c}	1.16 ± 0.23^{a}	0.71 ± 0.22^{a}
	Wet	6.86 ± 2.31^{b}	0.45 ± 0.13^{b}	0.22 ± 0.10^{b}	0.04 ± 0.07^{c}	0.62 ± 0.28^{b}	0.41 ± 0.11^{b}
Site_2	Dry	10.66 ± 1.40^{a}	6.35 ± 6.12^{a}	0.35 ± 0.04^{a}	0.04 ± 0.01^{c}	1.27 ± 0.23^{a}	0.66 ± 0.20^{a}
	Wet	6.18 ± 2.10^{b}	2.46 ± 3.82^{b}	0.20 ± 0.09^{b}	0.02 ± 0.01^{d}	0.52 ± 0.31^{b}	0.41 ± 0.13^{b}
Garden	Dry	2.84 ± 0.88^{c}	0.13 ± 0.03	0.23 ± 0.02^{a}	0.07 ± 0.01^{a}	1.37 ± 0.34^{a}	0.81 ± 0.27^{a}
	Wet	1.61 ± 0.45^{d}	0.13 ± 0.36	0.19 ± 0.02^{b}	0.06 ± 0.01^{b}	0.87 ± 0.41^{b}	0.41 ± 0.23^{b}

Means with the same letter superscript in the same column under the same site are not significantly different (P = 0.05) using ANOVA.

4.8 Heavy metal contents in vegetable samples

Vegetables grown on the farms irrigated along the sampling points tested for the uptake of heavy metals had varying concentrations of these metals indicating a negative impact on the vegetables. Fe was found tob higher in all the vegetables with highest concentration of carrots (1.19mg/Kg) followed by onions (1.12mg/Kg) and sugar cane (0.98 mg/Kg). Similarly, Cr content was highest in onions (0.53mg/Kg) and carrot (0.52mg/Kg) while tomatoes had the least concentration of Cr (0.06mg/Kg). ANOVA of the level of these revealed significance difference ($P \leq 0.05$) among the vegetables. Cr uptake in onions (0.53mg/kg) and carrots (0.52mg/kg) were higher than lettuce and sugar cane (0.28mg/kg) and the least concentration was found in tomatoes (0.06mg/kg). No significant difference in the level of Zn found in carrots, onions, lettuce and sugar cane that range between 0.10 - 0.13mg/kg and tomatoes had 0.06mg/kg of Zn. Like Cr, Cd levels in carrots and onions were not significantly different (0.30mg/kg) and higher Cd levels found in lettuce (0.20mg/kg), sugar cane (0.15mg/kg) and tomatoes (0.1mg/kg). Cu was below detection limit in tomatoes samples. Fe levels in the vegetables were higher in carrots (1.19mg/kg) and onions (1.12mg/kg) and lowest in tomatoes (0.44mg/kg). Mn levels in the samples were significantly different ($P \leq 0.05$) from one another with the highest level in onions (0.61mg/kg) and least in tomatoes (0.26mg/kg) as shown in (Table 4.16).

Table 4.16: Comparison of heavy metals contents in vegetables irrigated at the study site

Vegetable	Heavy metal (mg/Kg) Mean ± SD					
	Cr	Zn	Cd	Cu	Fe	Mn
Tomatoes	0.06 ± 0.01^{c}	0.05 ± 0.01^{b}	0.10 ± 0.00^{c}	ND	0.44 ± 0.11^{d}	0.26 ± 0.09^{e}
Carrots	0.52 ± 0.10^{a}	0.13 ± 0.02^{a}	0.30 ± 0.00^{a}	0.02 ± 0.00^{a}	1.19 ± 0.29^{a}	0.58 ± 0.20^{b}
Onions	0.53 ± 0.11^{a}	0.12 ± 0.02^{a}	0.30 ± 0.00^{a}	0.02 ± 0.00^{a}	1.12 ± 0.28^{a}	0.61 ± 0.21^{a}
Lettuce	0.28 ± 0.09^{b}	0.10 ± 0.02^{a}	0.20 ± 0.00^{b}	0.01 ± 0.00^{a}	0.71 ± 0.18^{c}	0.42 ± 0.14^{d}
Sugar cane	0.28 ± 0.04^{b}	0.11 ± 0.02^{a}	0.15 ± 0.00^{c}	0.02 ± 0.00^{a}	0.98 ± 0.24^{b}	0.53 ± 0.18^{c}

Pb was found to be beyond detection limits on all vegetables. Means with the same letter superscript in the same column are not significantly different (P = 0.05) using ANOVA.

4.9 Bacteriological quality of well water samples

The heterotrophic counts in water samples from well 1 (2.90cfu/ml) increased to 3.50cfu/ml in samples from well 3 although is lower than that of the control well water samples (4.27cfu/ml). Similarly all other counts followed the same pattern increasing from well 1 down to well 3. The overall bacterial counts Table 4.17 in the well water samples from tannery sites were generally lower compared to the control well (well C) where significant differences ($P \leq 0.05$) were clearly observed in comparison to the well water samples around the tanneries. Analyses of variance (ANOVA) of the mean bacteriological counts of well water samples in the study sites revealed variations in faecal streptococci and *E. coli* counts in the well water samples (Table 4.17). There were no significant differences ($P > 0.05$) in the total heterotrophic and total coliform counts in well 1 and well 2, but significant difference ($P \leq 0.05$) was observed in well 3. However, faecal streptococcal count showed significant difference ($P \leq 0.05$) among the wells. *E. coli* counts in well 2 and 3 were similar but there was variation in the counts of samples from well 1.

Seasonal variation of the bacteriological quality of the well water samples showed that in the dry season, heterotrophs had an average counts of $\log_{10}$ 3.15 cfu/ml, while in the wet season the count was $\log_{10}$ 2.80cfu/ml. for all the organisms, the trend was similar that is, higher in dry season than wet season. Bacterial load in the well water samples were significantly higher ($P \leq 0.05$) in the dry season than wet season (Table 4.18).

Table 4.17: Bacteriological quality of water samples from wells around tanneries

Samples	Viable count (Log_{10} cfu/ml) Mean ± SD			
	Total heterotrophs	Total coliforms	Faecal streptococci	*E. coli*
Well 1	2.92 ± 0.3^{c}	2.10 ± 0.4^{c}	1.56 ± 0.7^{d}	1.23 ± 0.001^{c}
Well 2	2.95 ± 0.3^{c}	2.14 ± 0.3^{c}	1.73 ± 0.5^{c}	1.35 ± 0.006^{b}
Well 3	3.50 ± 0.2^{b}	2.25 ± 0.2^{b}	1.85 ± 0.3^{b}	1.37 ± 0.001^{b}
Well C	4.27 ± 0.2^{a}	2.70 ± 0.2^{a}	1.93 ± 0.2^{a}	1.45 ± 0.2^{a}

Means with the same letter superscript in the same column under the same seasons are not significantly different (P = 0.05) using ANOVA.
Key: NT = Not tested, ND = below detection limit.

Table 4.18: Seasonal variation of bacteriological profile of well water samples

Bacterial counts	Season	Log_{10} CFU/ml Mean ± SD*
Heterotrophs	Dry	3.15 ± 0.06^{a}
	Wet	2.80 ± 0.33^{b}
Total Coliforms	Dry	2.38 ± 0.06^{a}
	Wet	1.95 ± 0.30^{b}
Fecal coliforms	Dry	2.04 ± 0.09^{a}
	Wet	1.39 ± 0.59^{b}
Escherichia coli	Dry	1.47 ± 0.10^{a}
	Wet	1.16 ± 0.18^{b}

Means with the same letter superscript in the same column in the same well are not significantly different (P = 0.05) using T-test, *All water collected from studied wells.

Coliform count was found to be $\log_{10}$ 2.37cfu/ml in well 1 and 2 in the dry season but increased slightly to $\log_{10}$ 2.41cfu/ml in samples from well 3 in the same season. Faecal streptococcal and *E. coli* counts followed the same trend of slight increase with distance from pollution source (Table 4.19). Microbial levels of the well water samples were also significantly higher ($P \leq 0.05$) during the dry season compared to the wet season. Unlike the heavy metal concentrations that were declining from well 1 to well 3, the bacterial load was increasing with increasing distance from the pollution source regardless of the season, although was more clear in the wet season. This indicated that the bacterial counts were inversely proportional to the concentrations of the heavy metals. Faecal streptococcal and *E. coli* count of well 1 in the wet season were significantly lower than counts in well 2 and 3 of the same season (Table 4.19).

4.10 Microbiological load in effluent samples

TAN A effluent samples had a mean heterotrophic count of $\log_{10}$ 3.80cfu/ml, TAN B ($\log_{10}$ 3.84cfu/ml) and TAN C ($\log_{10}$ 4.02cfu/ml). Other bacterial counts in the same samples were similar in the different tanneries with no clear cut pattern. The mean fungal counts were $\log_{10}$ 2.51, 2.48 and $\log_{10}$ 1.80cfu/ml in TAN A, B, and C respectively. Comparisons of mean counts in effluent samples from three different tanneries in the study area using ANOVA showed no significant differences ($P > 0.05$) among the effluent samples from three tanneries chosen for this study with regards to heterotrophic, total coliforms, and fecal coliform counts. In the same way no significant difference ($p > 0.05$) in the fungal counts between Tan A and Tan B but significant difference ($P \leq 0.05$) was obtained in the fungal counts between Tan C and the other Tanneries. Fungal counts were found to be significantly lower in effluent samples from Tan C compared to Tan A and B (Table 4.20).

Table 4.19: Seasonal variation in the bacteriological profile of well water samples

Well	Season	Log_{10} of Mean values (CFU/ml) ± SD			
		Heterotrophs	Total Coliform	Faecal streptococci	*E. coli*
Well 1	Dry	3.13 ± 0.05^{a}	2.37 ± 0.05^{a}	1.99 ± 0.11^{a}	1.40 ± 0.09^{a}
	Wet	2.71 ± 0.37^{b}	1.84 ± 0.36^{b}	1.15 ± 0.76^{c}	1.06 ± 0.18^{c}
Well 2	Dry	3.15 ± 0.06^{a}	2.37 ± 0.05^{a}	2.04 ± 0.08^{a}	1.48 ± 0.10^{a}
	Wet	2.76 ± 0.38^{b}	1.93 ± 0.33^{b}	1.42 ± 0.54^{b}	1.22 ± 0.13^{b}
Well 3	Dry	3.18 ± 0.06^{a}	2.41 ± 0.06^{a}	2.09 ± 0.05^{a}	1.54 ± 0.08^{a}
	Wet	2.93 ± 0.24^{b}	2.09 ± 0.19^{b}	1.62 ± 0.43^{b}	1.22 ± 0.21^{b}

Means with the same letter superscript in the same column in the same well are not significantly different (P = 0.05) using T-test.

Table 4.20: Microbiological profile of effluent samples from the tanneries

Microbial counts	Tan	Log_{10} of Mean values (CFU/ml) ± SD
Heterotrophs	A	3.80 ± 0.39^{a}
	B	3.84 ± 0.37^{a}
	C	4.02 ± 0.23^{a}
Total Coliforms	A	2.52 ± 0.33^{a}
	B	2.46 ± 0.25^{a}
	C	2.55 ± 0.40^{a}
Fecal coliforms	A	1.44 ± 0.32^{a}
	B	1.44 ± 0.29^{a}
	C	1.38 ± 0.42^{a}
Fungi	A	2.51 ± 1.10^{a}
	B	2.48 ± 1.06^{a}
	C	1.80 ± 1.14^{b}

Means with the same letter superscript in the same column are not significantly different (P = 0.05) using ANOVA, Tan=tannery, A, B and C = tanneries

Comparisons of mean microbial levels in effluent from different sampling points in the tanneries area are shown in Table 4.21. ANOVA and Duncan ($P \leq 0.05$) showed statistically significant differences ($P \leq 0.05$) among the effluent samples from three sampling points chosen for this study with regards to Heterotrophic bacterial counts. DP (log_{10} 3.53cfu/ml) had significantly low counts compared to DS and EP, but no significant difference ($P > 0.05$) between counts in DS (log_{10} 4.06cfu/ml) and EP (log_{10} 4.09cfu/ml) samples. Total coliforms were significantly ($P \leq 0.05$) higher in DS (log_{10} 2.82cfu/ml) effluents compared to those in DP and EP. Similarly, faecal streptococci count were significantly ($P \leq 0.05$) higher in DS (log_{10}1.73 cfu/ml) effluent samples compared to counts in DP and EP. Conversely significant difference ($P \leq 0.05$) in the fungal counts was found in the different points were decreasing from the DP (log_{10} 2.73 cfu/ml) to EP (log_{10} 1.73cfu/ml) (Table 4.21).

Although ANOVA and t-test of the seasonal of heterotrophic plate counts in the effluent samples analyzed showed no statistically significant difference ($P \leq 0.05$), there were significant differences ($P \leq 0.05$) in the sampling points, the DP had the lowest mean count of Log_{10} 3.36CFU/ml of the heterotrophs while the EP had highest mean count of Log_{10} 4.08CFU/ml that was not significantly different ($P > 0.05$) from counts obtained in the DS samples. Tan A and B total plate counts were significantly different from counts obtained in Tan C (Table 4.22). The mean coliform counts of the effluent samples in the tanneries showed significant difference ($P=0.003$) in the two seasons. The coliform counts were increasing downstream from the DP to the EP. The counts in the three points were significantly different ($P \leq 0.05$) from one another. The mean feacal coliform counts also followed the same pattern as total coliforms in the effluent samples, although with much lower values and no significant ($P=0.198$) seasonal variations in the mean counts at the discharge points of all the tanneries (Table 4.22).

Table 4.21: Microbiological profile of effluent samples from different points in the tanneries

Microbial counts	Location	Range	Log_{10} of Mean values (CFU/ml) ± SD
Heterotrophs	DP	2.95- 4.02	3.53 ± 0.30^{b}
	DS	3.64- 4.24	4.06 ± 0.18^{a}
	EP	3.35- 4.31	4.09 ± 0.20^{a}
Total Coliforms	DP	1.85- 2.79	2.21 ± 0.16^{c}
	DS	2.36- 3.06	2.82 ± 0.19^{a}
	EP	1.60- 2.96	2.51 ± 0.29^{b}
Fecal_coliforms	DP	0.60- 1.73	1.07 ± 0.16^{c}
	DS	1.28- 1.91	1.73 ± 0.13^{a}
	EP	0.10- 1.90	1.48 ± 0.31^{b}
Fungi	DP	0.12- 3.62	2.70 ± 1.13^{a}
	DS	0.6- 3.46	2.35 ± 0.95^{b}
	EP	0.08- 3.06	1.73 ± 1.13^{c}

Means with the same letter superscript in the same column under the same point of are not significantly different (P = 0.05) using ANOVA, Tan=tannery, EC= electrical conductivity (µS/cm), DP= discharge point, DS=downstream, EP= entry point

Table 4.22: Seasonal variation in microbial profile of effluent from tanneries sampling points

Tan	Points	Season	Log_{10} of Mean values (CFU/ml) ± SD			
			Heterotrophs	Coliforms	Faecal streptococci	Fungi
A	DP	Dry	3.32 ± 0.31^{a}	2.29 ± 0.26^{a}	1.11 ± 0.28^{a}	3.44 ± 0.13^{a}
		Wet	3.42 ± 0.13^{a}	2.14 ± 0.08^{b}	1.07 ± 0.04^{a}	1.97 ± 1.17^{b}
	DS	Dry	3.94 ± 0.24^{a}	2.84 ± 0.15^{b}	1.71 ± 0.10^{a}	3.38 ± 0.08^{a}
		Wet	4.10 ± 0.13^{a}	2.95 ± 0.05^{a}	1.78 ± 0.05^{a}	1.87 ± 1.16^{b}
	EP	Dry	4.16 ± 0.04^{a}	2.62 ± 0.07^{a}	1.61 ± 0.04^{a}	2.96 ± 0.09^{a}
		Wet	3.99 ± 0.20^{a}	2.38 ± 0.18^{b}	1.43 ± 0.19^{b}	1.44 ± 1.13^{b}
B	DP	Dry	3.36 ± 0.16^{a}	2.22 ± 0.08^{a}	1.08 ± 0.05^{a}	3.43 ± 0.09^{a}
		Wet	3.39 ± 0.19^{a}	2.19 ± 0.14^{b}	1.07 ± 0.08^{a}	2.03 ± 1.04^{b}
	DS	Dry	3.94 ± 0.13^{b}	2.51 ± 0.10^{b}	1.62 ± 0.10^{b}	3.26 ± 0.09^{a}
		Wet	4.21 ± 0.02^{a}	2.81 ± 0.11^{a}	1.81 ± 0.03^{a}	1.75 ± 1.13^{b}
	EP	Dry	4.16 ± 0.04^{a}	2.62 ± 0.07^{a}	1.61 ± 0.04^{a}	2.96 ± 0.09^{a}
		Wet	3.99 ± 0.20^{a}	2.38 ± 0.18^{b}	1.43 ± 0.19^{b}	1.44 ± 1.13^{b}
C	DP	Dry	3.76 ± 0.20^{b}	2.15 ± 0.17^{b}	0.95 ± 0.20^{a}	3.52 ± 0.12^{a}
		Wet	3.96 ± 0.05^{a}	2.29 ± 0.05^{a}	1.09 ± 0.09^{a}	1.70 ± 1.43^{b}
	DS	Dry	3.94 ± 0.15^{b}	2.83 ± 0.14^{b}	1.62 ± 0.18^{b}	2.25 ± 0.10^{a}
		Wet	4.22 ± 0.02^{a}	3.02 ± 0.04^{a}	1.84 ± 0.04^{a}	1.75 ± 0.57^{b}
	EP	Dry	4.24 ± 0.04^{a}	2.85 ± 0.10^{a}	1.71 ± 0.11^{a}	1.04 ± 0.09^{a}
		Wet	3.99 ± 0.34^{b}	2.19 ± 0.42^{b}	1.09 ± 0.55^{b}	0.53 ± 0.42^{b}

Means with the same letter superscript in the same column under the same point of are not significantly different (P = 0.05) using ANOVA, Key: DP= discharge point, DS=downstream, EP= entry point, A.B and C =Tanneries

The mean fungal counts in the effluents followed a different pattern from the bacterial counts. The fungal counts were decreasing downstream from the DP to EP, where Tan C discharge point samples had the highest mean count of $\log_{10}$ 3.2cfu/ml in the dry season and the least count of $\log_{10}$ 0.53cfu/ml at the entry point (EP) of the same tannery effluent in the wet season. The mean fungal counts of effluent samples collected from the discharge points of Tan A ($\log_{10}$ 3.44cfu/ml) and B ($\log_{10}$ 3.43cfu/ml) where found to be similar during the dry season. ANOVA and Duncan showed significant ($P \leq 0.05$) seasonal differences in the counts as well as in the tannery points (P=0.018), but when similar point from different tanneries were compared no significant difference (P=0.219) between Tan A EP and Tan B EP or Tan C EP (Table 4.22).

Heterotrophic bacterial count was significantly increasing downstream where the highest counts were recorded in the EP samples of all the sampling sites where ANOVA of the heterotrophic bacterial counts of the effluents analyzed showed statistically significant difference ($P \leq 0.05$). There were also significant differences ($P \leq 0.05$) in the sampling points, the DP had the lowest mean count of $\log_{10}$ 3.36, 3.37 and $\log_{10}$ 3.86cfu/ml of the heterotrophs in DP of Tan A, B and C respectively while the EP had highest mean count of $\log_{10}$ 4.08cfu/ml that was not significantly different ($P > 0.05$) from counts obtained in the DS samples. Tan A and B total plate counts were significantly different from counts obtained in Tan C (Table 4.23). Similarly coliform counts were higher in DS samples with no significant difference among the three tanneries. The coliform counts were lower in the DP of all the tanneries. The mean fungal counts in the effluents followed a different pattern from the bacterial counts. The fungal counts were decreasing downstream from the DP to EP with the lowest mean count recorded in samples from EP of Tan C ($\log_{10}$ 0.78cfu/ml) as shown in Table 4.23.

Table 4.23: Microbial counts of effluent samples in the studied tanneries sampling points

Tan	Points	Log_{10}cfu/ml Mean ± SD			
		Heterotrophs	Coliforms	Faecal streptococci	Fungi
A	DP	$3.36 \pm 0.24^{*}$	$2.22 \pm 0.20^{*}$	$1.09 \pm 0.20^{*}$	$2.76 \pm 1.08^{***}$
	DS	$4.03 \pm 0.20^{**}$	$2.90 \pm 0.12^{***}$	$1.75 \pm 0.08^{***}$	$2.55 \pm 1.14^{**}$
	EP	$4.08 \pm 0.16^{**}$	$2.50 \pm 0.18^{**}$	$1.52 \pm 0.16^{**}$	$2.20 \pm 1.10^{*}$
B	DP	$3.37 \pm 0.17^{*}$	$2.21 \pm 0.11^{*}$	$1.08 \pm 0.07^{*}$	$2.73 \pm 1.02^{***}$
	DS	$4.07 \pm 0.17^{**}$	$2.66 \pm 0.19^{**}$	$1.71 \pm 0.12^{***}$	$2.50 \pm 1.10^{**}$
	EP	$4.08 \pm 0.16^{**}$	$2.50 \pm 0.18^{**}$	$1.52 \pm 0.16^{**}$	$2.20 \pm 1.10^{*}$
C	DP	$3.86 \pm 0.18^{*}$	$2.22 \pm 0.14^{*}$	$1.02 \pm 0.17^{*}$	$2.61 \pm 1.36^{***}$
	DS	$4.08 \pm 0.18^{**}$	$2.92 \pm 0.14^{***}$	$1.73 \pm 0.17^{***}$	$2.00 \pm 0.47^{**}$
	EP	$4.11 \pm .26^{**}$	$2.52 \pm 0.45^{**}$	$1.40 \pm 0.50^{**}$	$0.78 \pm 0.39^{*}$

Means with the same number of asterix superscript in the same column under the same point are not significantly different (P = 0.05) using ANOVA, Key: DP= discharge point, DS=downstream, EP= entry point, A.B and C =Tanneries

4.10. 1 Principal component analysis

Principal component analysis Fig. 4.1 comparing parameters in the tannery effluent showed different relationship among the parameters analyzed in the samples. Factor 1 shows significant positive relationship between the heavy metals Cr, Cd, Cu, and Pb concentrations and the tannery effluent samples, the metals were closely related to one another but with negative relationship to BOD, TSS, TDS, Mn, Fe, EC and Fungal count which were also closely related to one another. Heterotrophs, coliforms and feacal coliforms were also closely related to one another and the heavy metals Cr, Cd, Cu, and Pb concentrations had negative impact on the Heterotrophs, coliforms and feacal coliforms. Similarly the BOD, TSS, TDS and EC had negative impact on DO regardless of the season or even months. The pH, temp and month of collection made no significant contribution to the principal component analyses as shown in Fig. 4.1

Fig. 4.2 showed the principal component analysis comparing physic-chemical properties and microbial counts in the well water samples which showed different relationship among the parameters analyzed in the samples. Factor 1 show significant positive relationship between Heterotrophs, coliforms and feacal coliforms counts and the well water samples; on the other hand factor 2 show the heavy metals were closely related to one another but with negative impact on Heterotrophic, coliform and feacal coliform counts which were also closely related to wells as shown in Fig. 4.2. Similarly the BOD, TSS, TDS and EC had negative impact on DO change with the season. The pH also made no significant contribution to the principal component analyses as shown in Fig. 4.2

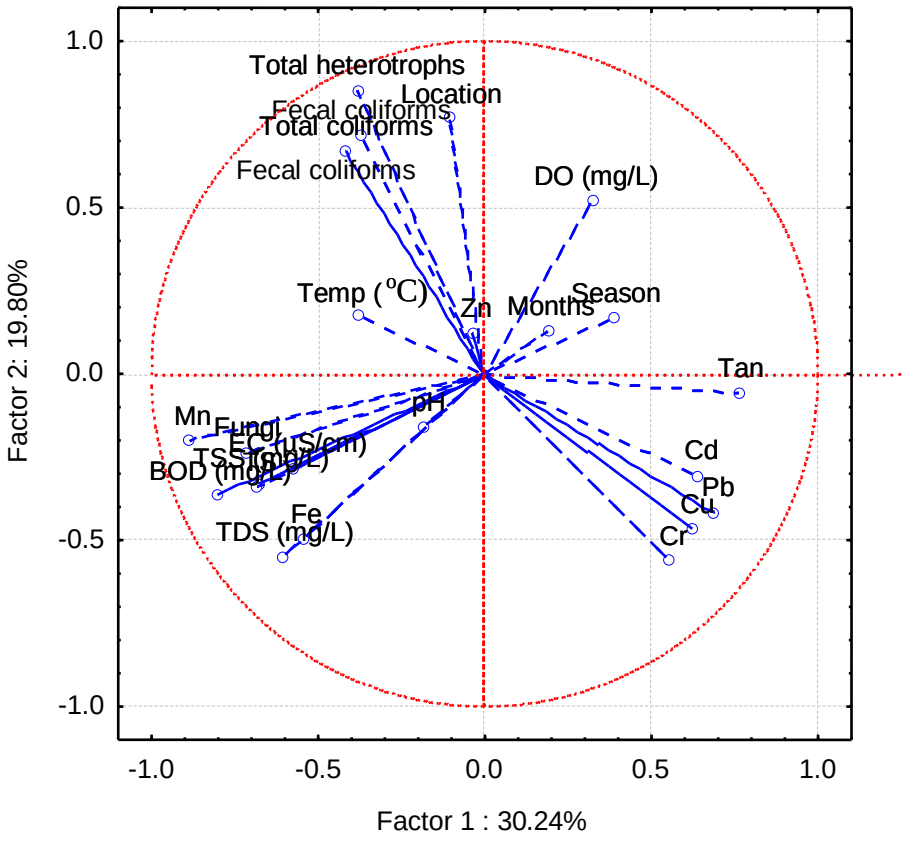


Figure 4.1: Principal component analysis comparing parameters of the tannery effluent samples

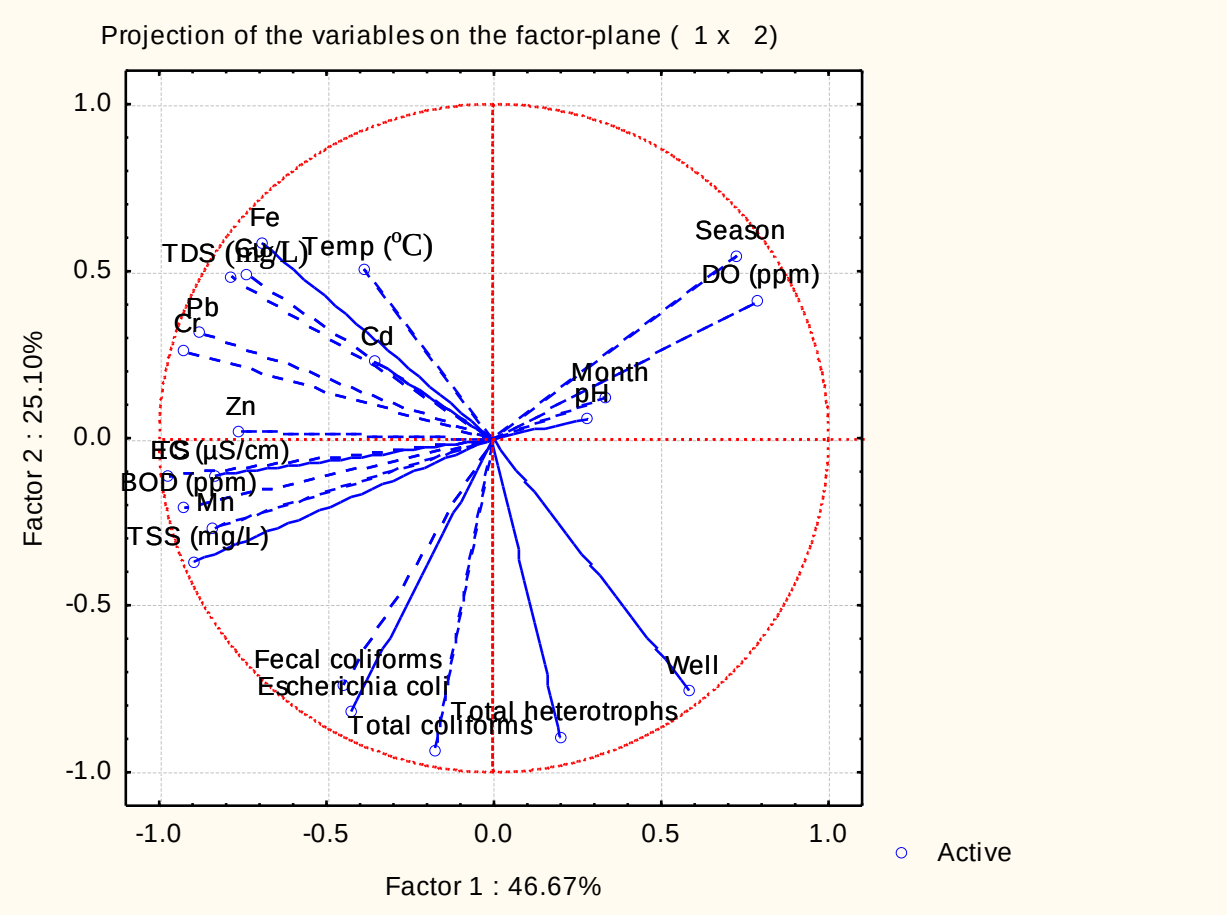


Figure 4.2: Principal component analysis comparing parameters in the well water samples

4.11 Microbiological load of effluent contaminated soil

The heterotrophic count in the soil samples was found to increase with distance to the pollution source at site 1 it was $\log_{10}$ 4.09cfu/g, site 2 was $\log_{10}$ 4.19cfu/g and $\log_{10}$4.30cfu/g in the farm soil durin the dry season. Similarly other bacterial counts followed the same trend. The total plate counts, coliform and faecal streptococcal counts were significantly higher ($P \leq 0.05$) during the wet season in the soil samples. Comparison between seasons of bacterial counts showed significant differences but no differences between samples of soil1 and 2 of the same season (Table 4.24). The mean fungal counts in the soil samples were significantly different in the wet and dry seasons, unlike the bacterial counts; the mean fungal counts were higher in dry season than wet season. No significant difference ($P > 0.05$) among the soil samples analyzed (Table 4.24).

Fig. 4.3 is the principal component analysis comparing parameters in the soil samples which shows different relationship among the parameters analyzed in the samples. Factor 1 shows significant positive relationship between heterotrophs, coliforms and feacal coliforms counts and the soil samples; on the other hand factor 2 shows the heavy metals were closely related to one another but with negative impact on Heterotrophic, coliform and feacal coliform counts, but the heavy metals had no negative impact on the fungal counts based on Factor 2 as shown in Fig. 4.3. The concentration of the heavy metals in the soil was affected by month except Cd which appeared not to be affected base on factor 1. But the concentration of the heavy metals varies with location base on factor 1.

Table 4.24: Seasonal variation of microbial counts at soil sampling points

Location	Season	Microbial counts (log_{10} CFU/g) Mean ± SD			
		Heterotrophs	Coliforms	Faecal streptococci	Fungi
Site _1	Dry	4.09 ± 0.19^{b}	1.72 ± 0.17^{b}	0.66 ± 0.59^{b}	6.10 ± 0.06^{a}
	Wet	4.34 ± 0.11^{a}	1.93 ± 0.12^{a}	1.53 ± 0.18^{a}	4.54 ± 0.79^{b}
Site _2	Dry	4.19 ± 0.20^{b}	1.81 ± 0.11^{b}	0.93 ± 0.58^{b}	6.09 ± 0.05^{a}
	Wet	4.37 ± 0.08^{a}	2.00 ± 0.11^{a}	1.62 ± 0.23^{a}	4.55 ± 0.77^{b}
FARM	Dry	4.30 ± 0.09^{a}	1.90 ± 0.08^{a}	1.23 ± 0.16^{b}	6.12 ± 0.01^{a}
	Wet	4.29 ± 0.24^{b}	1.78 ± 0.19^{b}	1.34 ± 0.36^{a}	5.09 ± 1.35^{b}

Means with the same letter superscript in the same column under the same point of are not significantly different (P = 0.05) using ANOVA

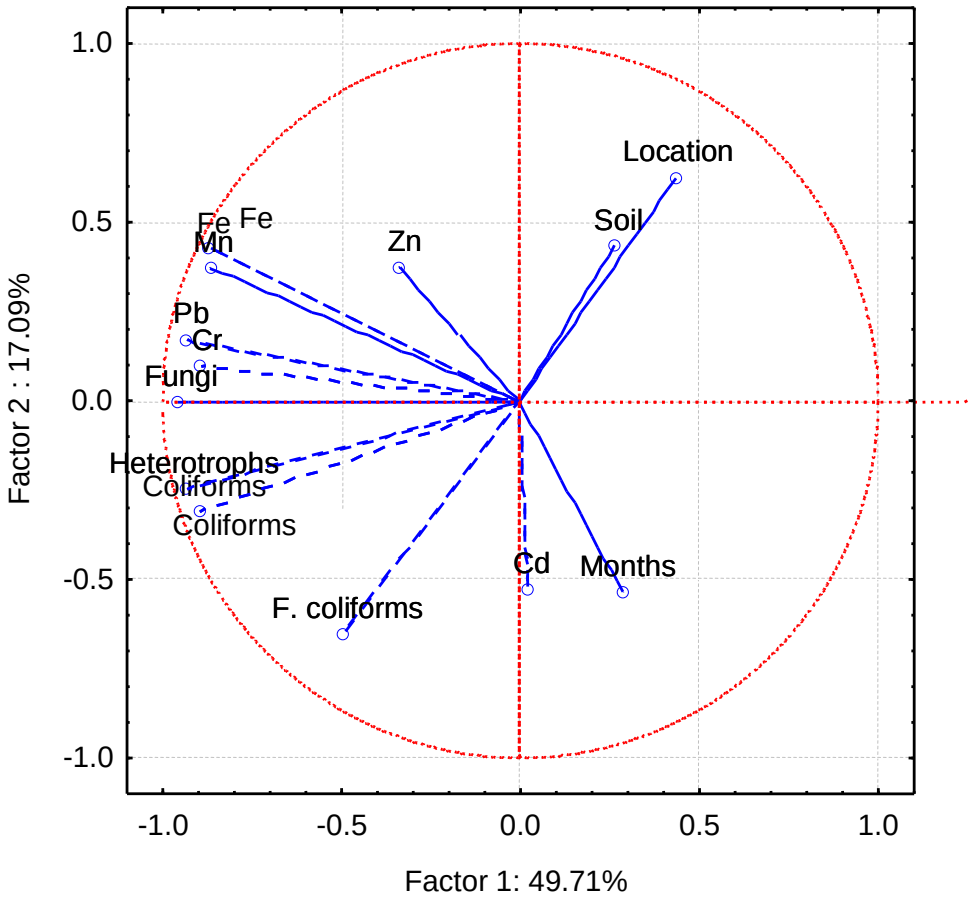


Figure 4.3: Principal component analysis comparing parameters of the soil samples

4.12 Minimum Inhibitory Concentration (MIC) of chromium to the bacterial isolates

Table 4.25 showed *Pseudomonas* spp, *Bacillus* spp, *Escherichia coli*, *Klebsiella* spp, and *Proteus* spp which were found to be predominant. These were isolated from the tannery effluents and soils under direct influence of the effluents (wastewater) and characterized. *Escherichia coli* had the lowest MIC of 50mg/L of Cr, followed by *Klebsiella* spp, and *Proteus* spp which had MIC of 75mg/L of Cr. Among these isolates *Pseudomonas aeruginosa* (TNA04DP) and *Bacillus cereus* (TNA02SL2) were found to be the most resistant bacteria to different concentrations of the Cr with MIC of 100mg/L (Table 4.25).

Pseudomonas aeruginosa and *Bacillus cereus* showed best performance (ability to grow in high Cr concentrations) for bioremediation of the tannery effluents. The metal (Cr) sorption ability of the selected bacterial strains is presented in Table 4.26. *Pseudomonas aeruginosa* adsorb 2.78mg/g dry weight in 24h which rose to 7.31mg/g of Cr after 48h while *Bacillus cereus* take up 2.33mg/g dry weight in 24h which also rose to 6.55mg/g Cr dry weight after 48h incubation. The Cr free culture yielded no detectable amount of the Cr in cultures after similar incubation (Table 4.26).

4.13 Predominant fungal isolates in the effluents and soils

Table 4.27 shows frequency of occurrence of fungal species in the study site. A total of 119 isolates were obtained of which *Aspergillus niger* had highest number of occurrence with 23(19.3%), followed by Penicillium sp with 15(12.6%). *Alternaria* sp, *Rhizopus* sp and White rot fungi had the same occurrence of 9(7.6%) each. Yeast had 12(10.1%) frequency of occurrence and others had 24(20.2%) in the samples analyzed.

Table 4. 25: Minimum Inhibitory Concentration (MIC) of chromium to the bacterial isolates

Test organism	Cr concentration in mg/L					
	25	50	75	100	150	200
Escherichia coli	+	+	-	-	-	-
Klebsiella spp	++	++	+	-	-	-
Proteus spp	++	+	+	-	-	-
Pseudomonas aeruginosa	++	++	+	+	-	-
Bacillus cereus	++	++	+	+	-	-

Key: + = growth, - = no growth

Table 4.26: Metal (Cr) sorption by the bacterial isolates

Test organism	Cr mg/g			Cr free culture		
	12h	24h	48h	12h	24h	48h
Pseudomonas aeruginosa	2.78	5.42	7.31	ND	ND	ND
Bacillus cereus	2.33	4.11	6.55	ND	ND	ND

Key: ND = not detected, Cr= $K_2Cr_2O_7$ analytical grade (William and Hopkins UK)

Table 4.27: Frequency of occurrence of fungal spp isolated from the study sites

Fungi	Sampling points No. (%)					
	CTRL SOIL	DP	DS	EP	SOIL	FARM
Others	27.3	18.5	15.7	20.0	25.0	15.0
Aspergillus niger	27.3	22.2	15.7	13.3	12.5	20.0
Penicillum spp	13.6	14.8	15.7	6.6	12.5	10.0
Yeast	4.5	11.1	5.3	13.3	18.8	10.0
Fusarium spp	4.5	11.1	10.5	13.3	6.3	5.0
Alternaria spp	4.5	7.4	10.5	20.0	6.3	0.0
Rhizopus spp	4.5	3.7	10.5	6.6	6.3	15.0
White rot fungi	9.1	7.4	5.3	0.0	6.3	15.0
Rhodotorula	4.5	3.7	10.5	6.6	6.3	10.0

Key: CTRL SOIL= Control soil sample, DP= Discharge points, DS= Down stream, EP= Entry point

4.14 Laboratory Scale treatment of effluent

Effluent collected from the discharge point of TAN A during chrome tanning was used for the study.

4.14.1 Chromium removal from effluent using bacteria

Pseudomonas aeruginosa (TNA04DP) and *Bacillus cereus* (TNA02SL2) isolated previously from the samples were used for these experiments using both orbital shaker and aerated vessel. In the minimal medium containing 25mg/L Cr the bacterial isolates were found to be effective in the removal of Cr. Fig. 4.4 shows the removal of Cr in minimal media inoculated with bacterial cultures in conical flasks on orbital shaker. In the flask containing minimal medium inoculated with TNA04DP Cr concentration declined from 25 to 23.36mg/L after 72hr incubation on orbital shaker and finally to 20mg/L after 120hr. The flask inoculated with TNA02SL2 Cr level declined to 24.65mg/L in 24hr and kept declining to 19.15mg/L after 120hr of incubation and shaking. The bacterial consortium was more effective than individual organism Cr declined to 24.02mg/L in 24hr, then steadily declined to 18mg/L after 120hr. There was also a slight decrease in Cr concentration to 24.35mg/L after 120hr of shaking in the control flask that was not inoculated with any organism as shown in Fig. 4.4.

The performances of TNA04DP (*Pseudomonas aeruginosa*), TNA02SL2 (*Bacillus* sp) and the consortium in the aerated vessel were slightly different from their performance in the shake flask methods. In aerated vessel containing medium inoculated with TNA04DP the Cr concentration declined to 24.39mg/L in 24hr and finally declined to 19.09mg/L after120hr of incubation under constant aeration in the vessel. Similarly Cr uptake in TNA02SL2 cultures under constant aeration declined from 25mg/L at 0hr to 19.9mg/L after 120hr while the flask containing medium inoculated with bacterial consortia showed a constant decline of Cr level

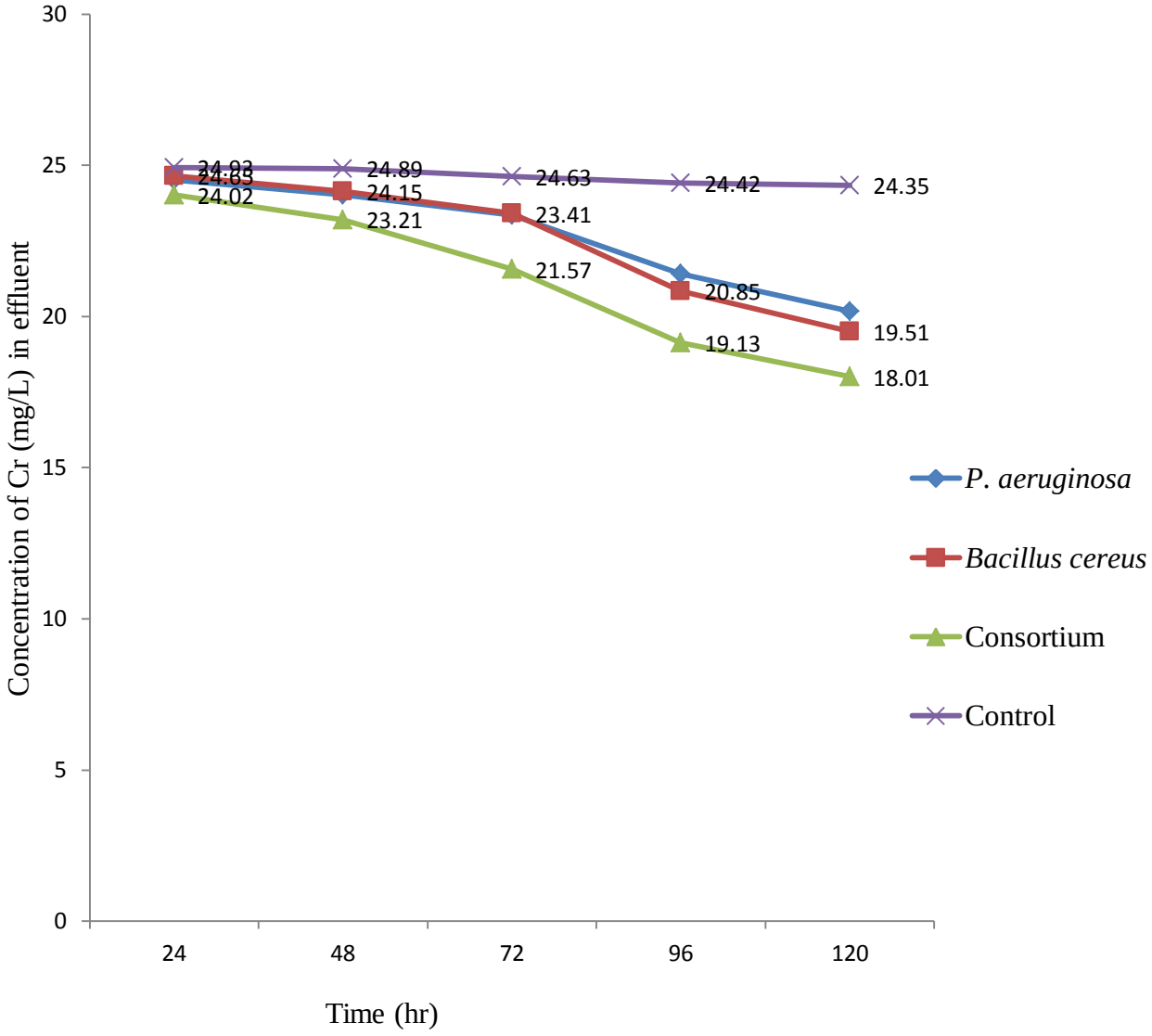


Figure 4.4: Removal of Cr in minimal media inoculated with bacterial cultures using shake flask method

in the culture to 18mg/L after 120hr a shown in Fig. 4.5. Control aerated vessel containing uninoculated medium also had a slight reduction in Cr level to 24.2mg/L.

4.14.2 Bacterial effluent treatment

The same bacterial isolates were used in the laboratory scale treatment of the prepared effluent containing 25.02mg/L Cr. The concentration of Cr in effluent samples inoculated with overnight culture of *Pseudomonas aeruginosa* (TNA04DP) declined to 22.8mg/L after 48hr, 19.3mg/L in 96hr and finally stabilized to18.7mg/L after 120hr of effluent treatment on the orbital shaker indicating 25.3% removal of Cr from the effluent. In flasks containing effluent samples inoculated with *Bacillus cereus* (TNA02SL2) the residual Cr after 120hr treatment was found to be 19.81mg/L also indicating about 20.8% Cr uptake by the organism.

There was no significant difference ($P > 0.05$) comparing use of equipment in Cr removal by *B. cereus* and bacterial consortium, but significant difference ($P \leq 0.05$) was observed with *P. aeruginosa* where the aerated vessel proved to be more efficient equipment in the treatment. *P. aeruginosa* and *B. cereus* effectively reduced the Cr with significant difference ($P \leq 0.05$) from the effluent than in the media. But no significant difference ($P > 0.05$) in the Cr removal from the two medium by the consortia (Appendix II)

The bacterial consortium culture at the initial stage was less effective in Cr removal from the effluent than individual organism. Initially, 24.2mg/L Cr was found in the effluent after 48hr treatment in the shake culture which was higher than concentrations in other flasks after similar treatment. However, it stabilized after 72hr treatment and finally 17.0mg/L Cr was left in the effluent after 120hr treatment, indicating 32.1% Cr removal from the effluent as shown in Fig. 4.6.

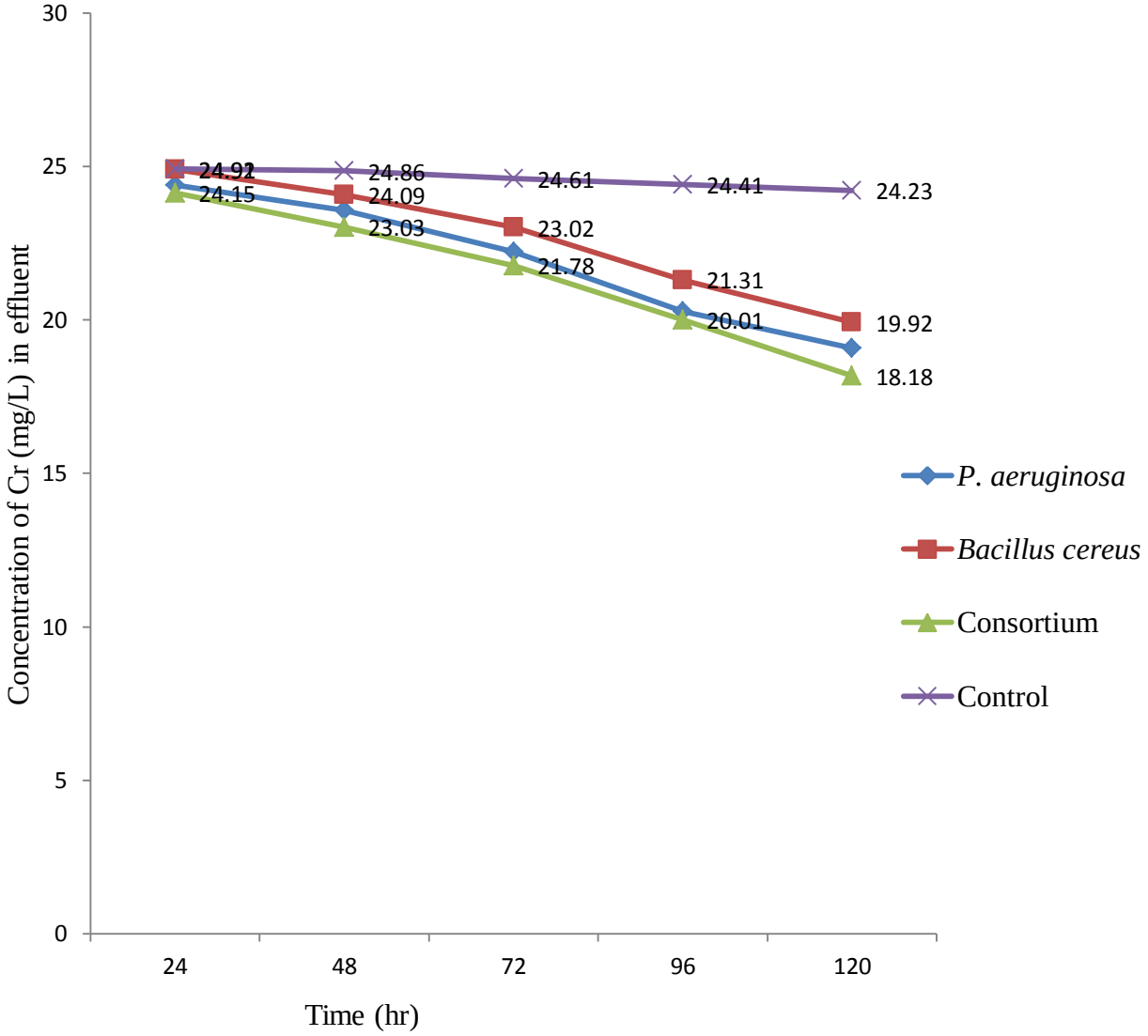


Figure 4.5: Removal of Cr in minimal media inoculated with bacterial cultures using aerated vessels

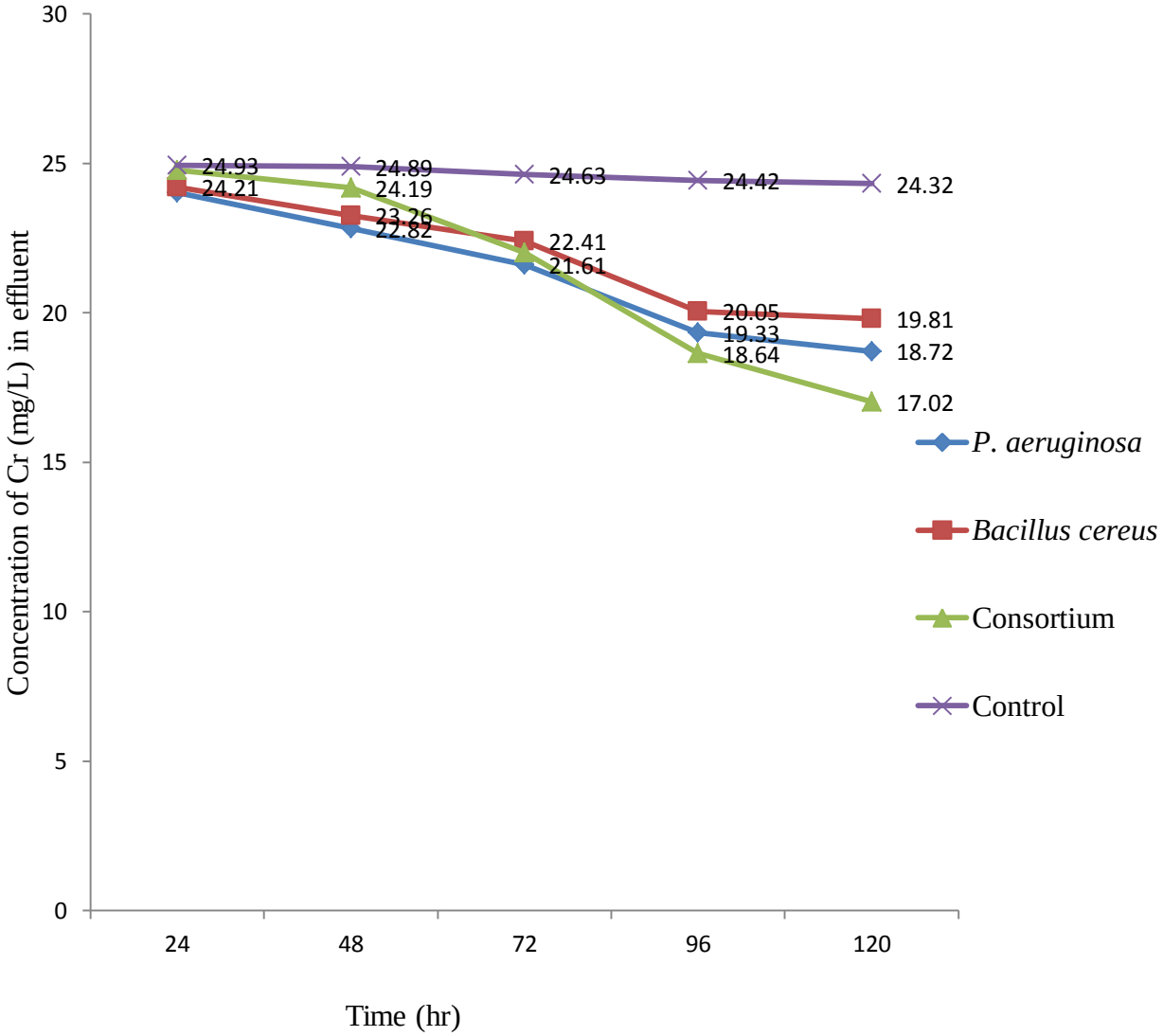


Figure 4.6: Chromium level in treated effluent using conical flasks on orbital shaker

Fig. 4.7 shows the Chromium level in effluent treated using aerated vessels. Performances of *Pseudomonas aeruginosa* (TNA04DP), *Bacillus cereus* (TNA02SL2) and the consortium in the aerated vessels were significantly different from their performance in the shake flask method. TNA04DP reduced the Cr to 16.4mg/L removing 34.6%, the consortium reduced the Cr to 15.3mg/L also removing 38.8% of the total Cr in the effluent, however, TNA02SL2 performance in the aerated vessel was not different from the shake flask where only 24.0% Cr was effectively removed from the effluent.

4.15 Chromium removal from effluent using fungi

4.15.1 Chromium removal from media using *Aspergillus niger*

The removal of Cr from the medium inoculated with *Aspergillus niger* (TN1A31) was observed to be within similar range in all pH at the initial stage 0-24hr Fig. 4.8. But after 48hr of incubation with shaking and agitation the removal of Cr increased rapidly between 48-72hr and then declined 96-120hr of shaking. At concentrations of 10mg/L the percentage (%) removal was more efficient after 24-48hr incubation with 81.1% residual Cr in the medium. The Cr removal then attained average after 72hr treatment in all the concentrations (60% residual Cr) at all pHs, while between 96-120hr of the experiments, medium containing Cr concentrations of 20 and 25mg/L the removal peaked leaving behind 39.6% at pH 6 as shown in Fig. 4.8. However, at pH 7 removal of Cr from the medium was more conspicuous 24h after incubation in medium containing 25mg/L. The removal proceeded in a similar pattern in all the media inoculated with *Aspergillus niger* (TN1A31) at pH 7 and finally leaving behind 40 – 43% residual Cr in the media as shown in Fig. 4.9. At pH 8 the treatment was also similar but less efficient than at the other pH after 120h incubation with agitation 41 – 45% residual Cr was left in the media as shown in Fig. 4.10. It was also observed that pH 6 was the optimum for the removal of Cr by *Aspergillus niger*.

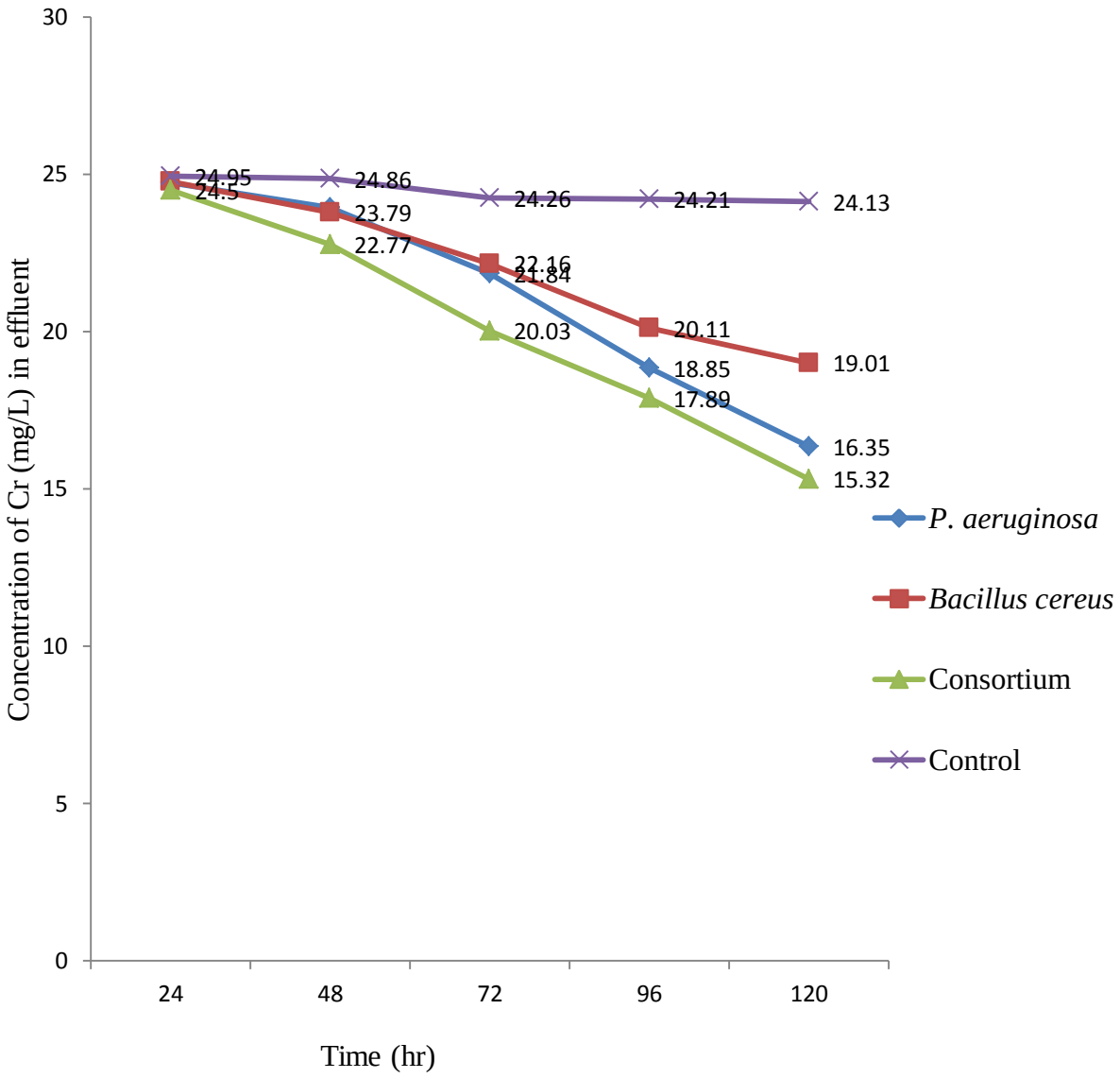


Figure 4.7: Chromium level in treated effluent using aerated vessels

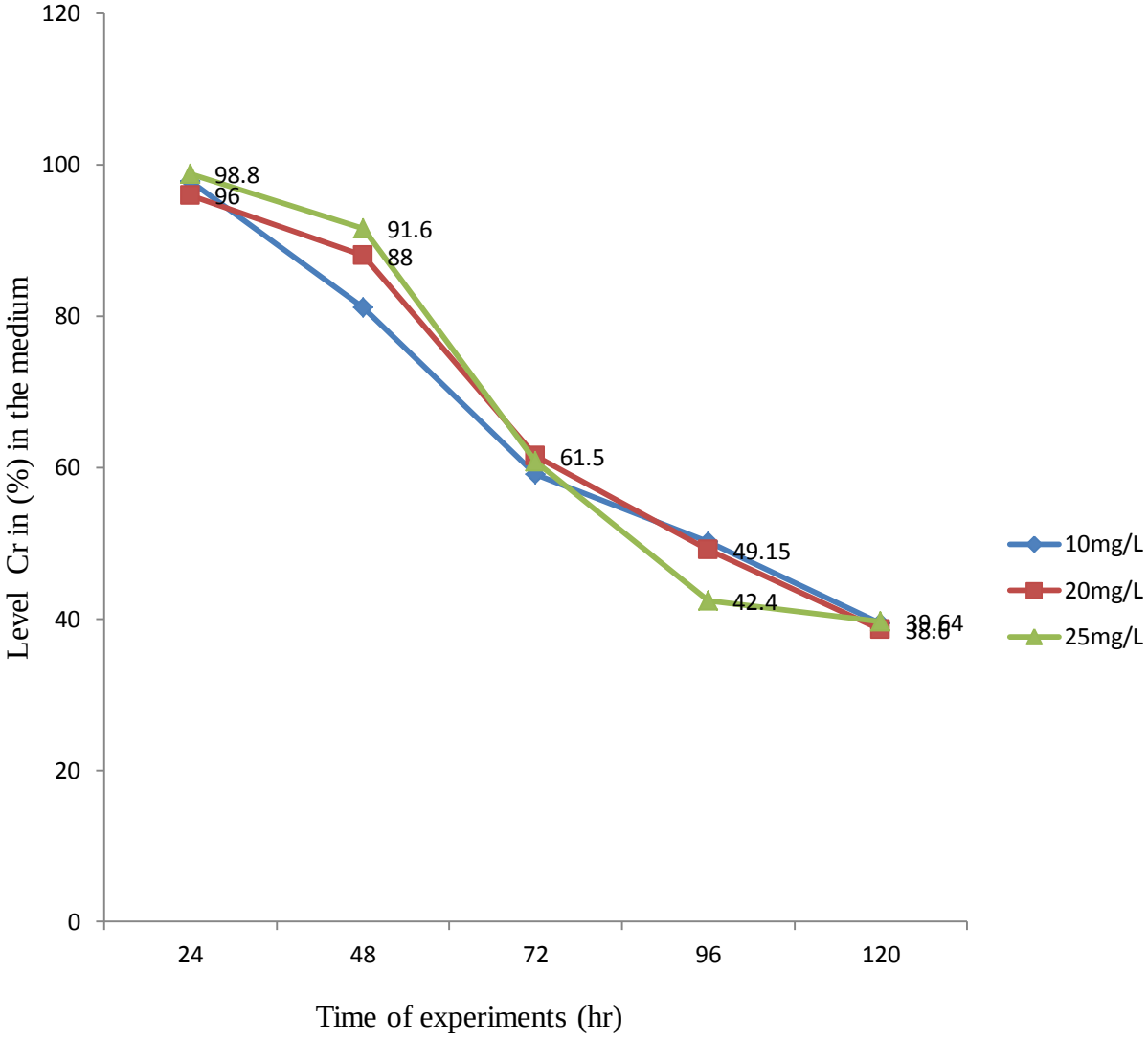


Figure 4.8: Removal of Cr in culture inoculated with *Aspergillus niger* at pH 6

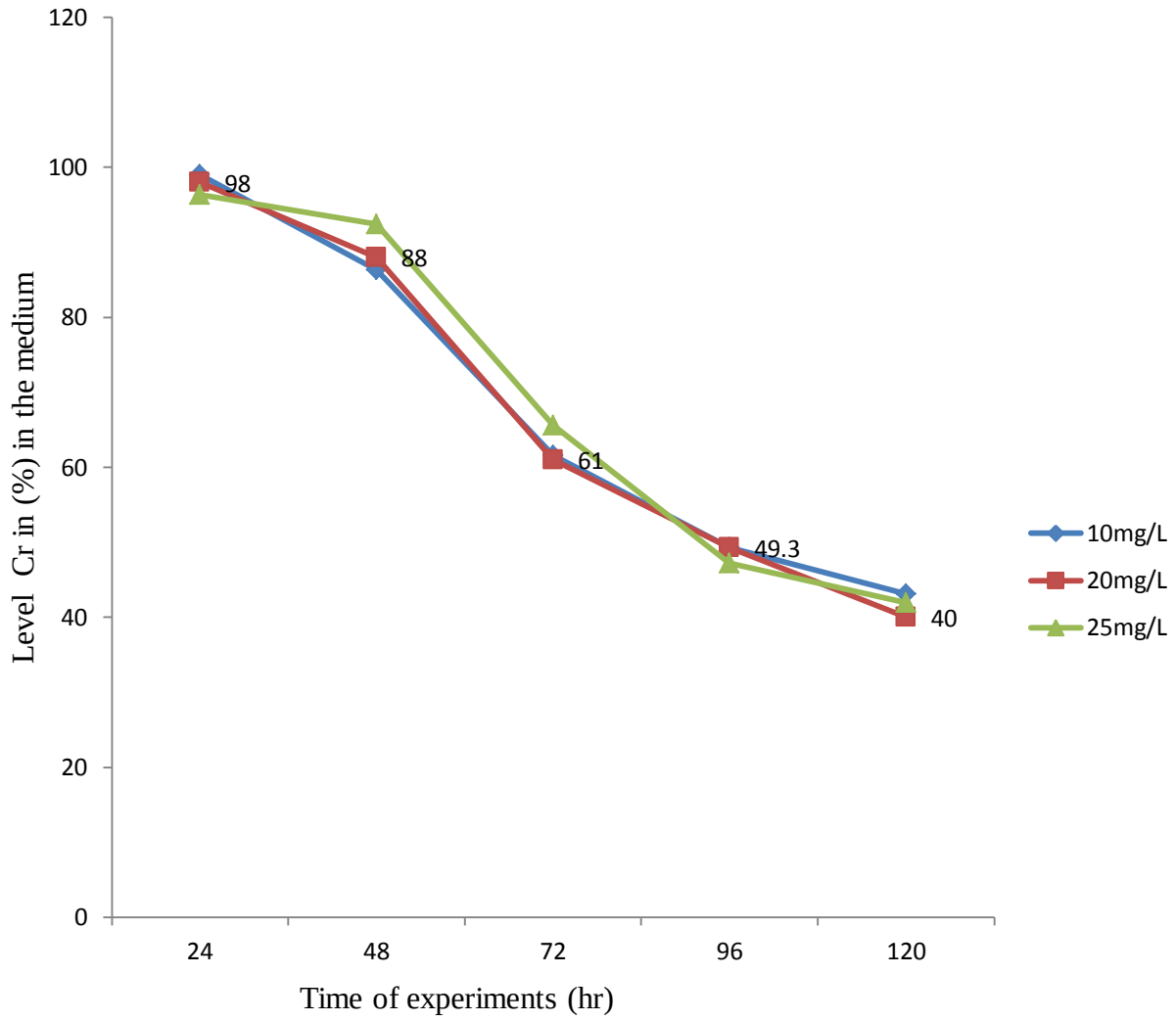


Figure 4.9: Removal of Cr in culture inoculated with *Aspergillus niger* at pH 7

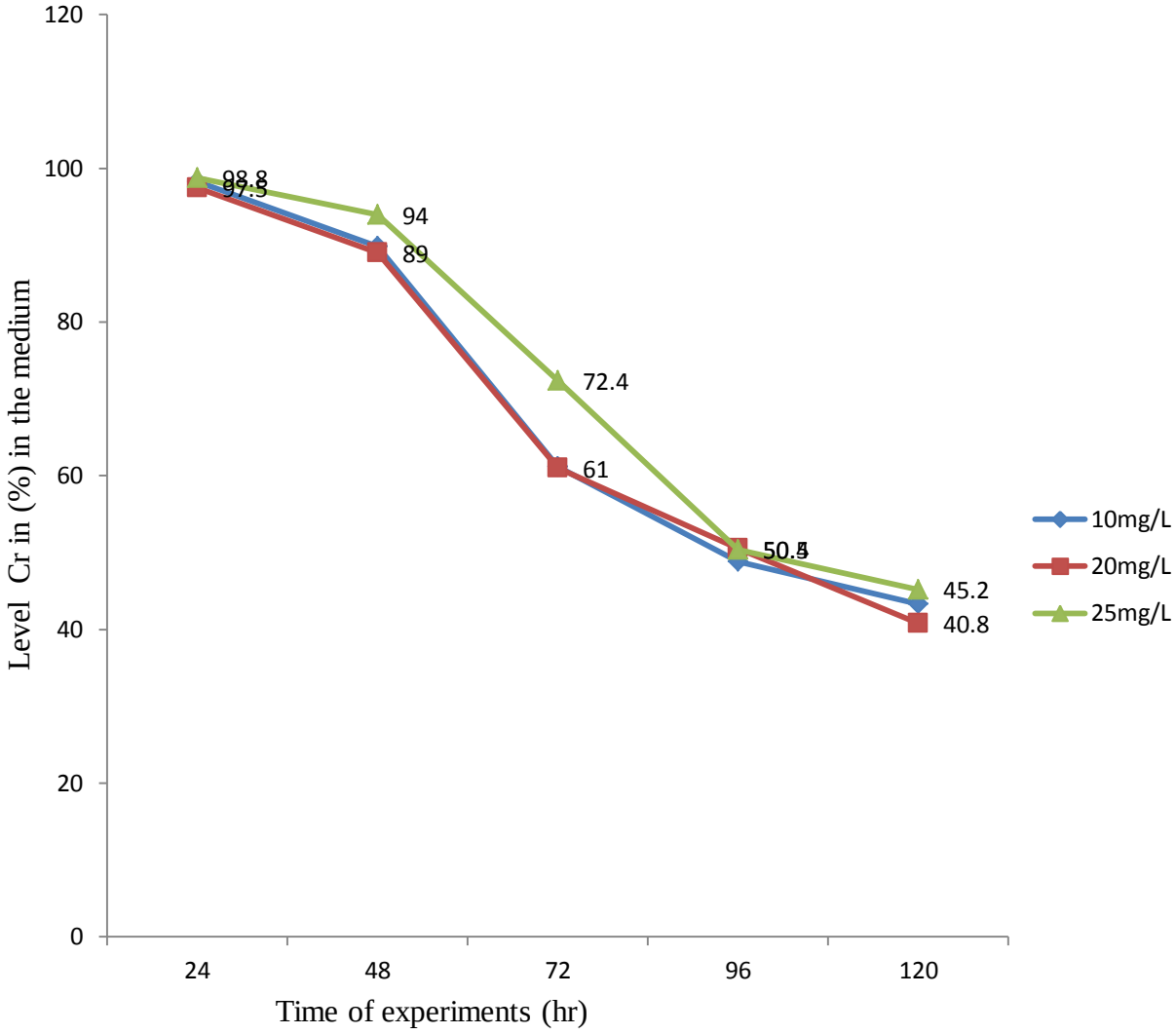


Figure 4.10: Removal of Cr in culture inoculated with *Aspergillus niger* at pH 8

4.15.2 Chromium removal from media using *Penicillium* species

Similar pattern was observed in the removal of Cr by *Penicillium* species (TN3P09) (*Pen*) (Fig. 4.11) which was more efficient at pH 6; however the metal was more efficiently removed from the media containing 10 and 20mg/L of Cr after 120hr incubation with 36.2% and 39.0% residual Cr in the media Fig. 4.11. There was also no remarkable reduction in Cr level at the initial stage of the experiment, nevertheless after 48hr of incubation with shaking and agitation the removal of Cr increased rapidly between 48-72hr Fig. 4.11.

Similarly no remarkable reduction in Cr level at the initial stage of the experiment at pH 7 and 8 but after 72h of treatment about 40% of Cr was removed and at the end of incubation 40 – 42% of residual Cr was left in the media as shown in Fig. 4.12. In media with pH 8 inoculated with TN3P09 (*Pen*) about 50% removal were recorded after 96h incubation with agitation, and 41 – 45% Cr were left in media at pH 8 as shown in Fig. 4.13. It was also observed that pH 6 was the optimum for the removal of Cr by *Penicillium* species (TN3P09) (*Pen*) and the removal efficiency were decreasing with increasing pH.

4.15.3 Removal of chromium by *Trichoderma longibrachiatum* TN1X03 (TN3)

The removal of Cr by *T. longibrachiatum* TN1X03 (TN3) fungal organism appeared more efficient than TN1A31 and TN3P09 because after 48hr incubation with constant agitation about 13 – 23% of the Cr in the medium was removed (in flasks with medium at pH 6). At the initial stage the Cr removal was more efficient in flask containing 10mg/L, followed by that in flask with 20mg/L and more than 50% of the Cr was removed by *T. longibrachiatum* after 96h of incubation with agitation. Barely 37% of the Cr was left in the flasks containing different concentrations of the Cr at pH 6 after 120h of the experiment as shown in Fig. 4.14.

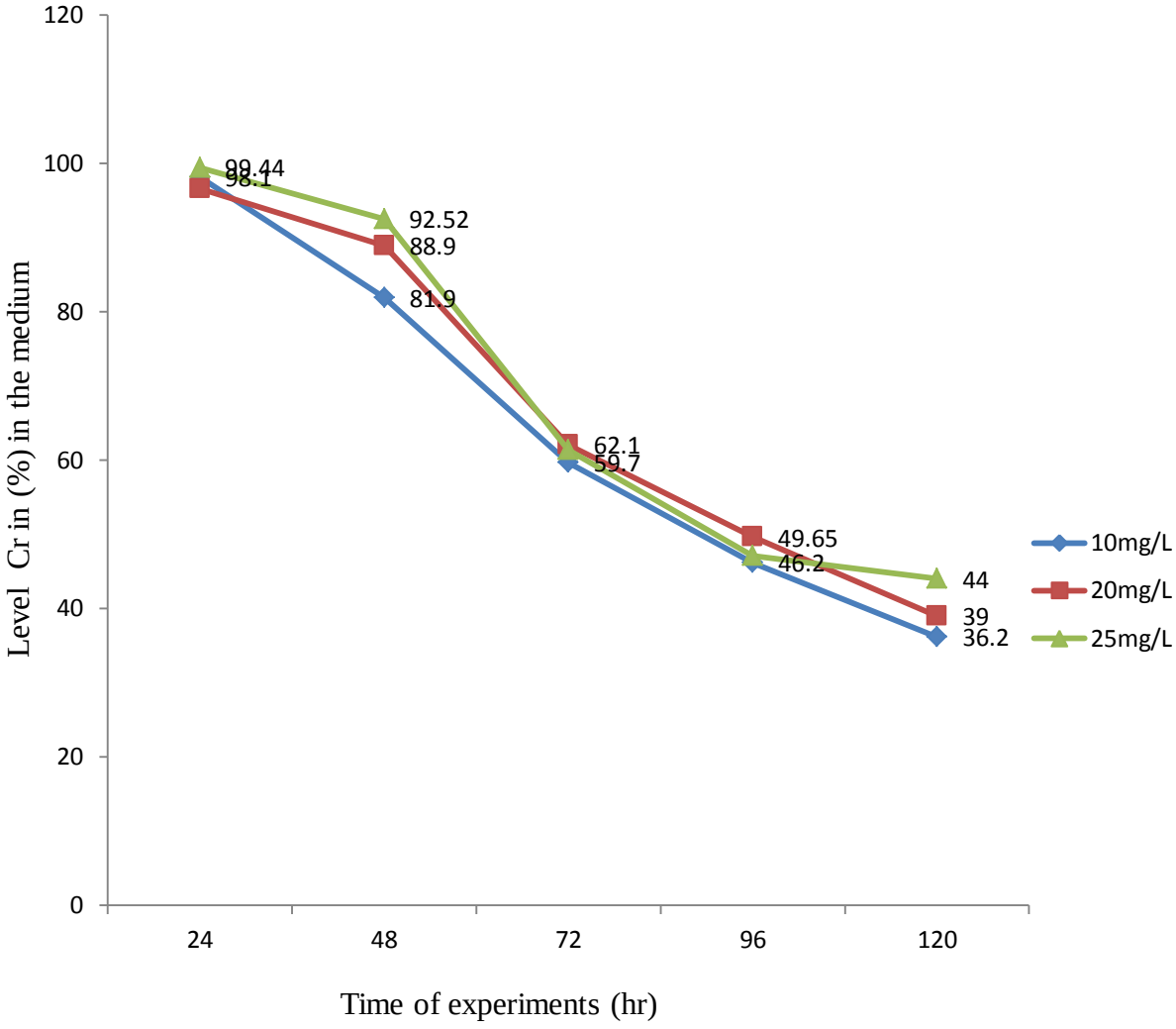


Figure 4.11: Removal of Cr in culture inoculated with *Penicillium* sp at pH 6

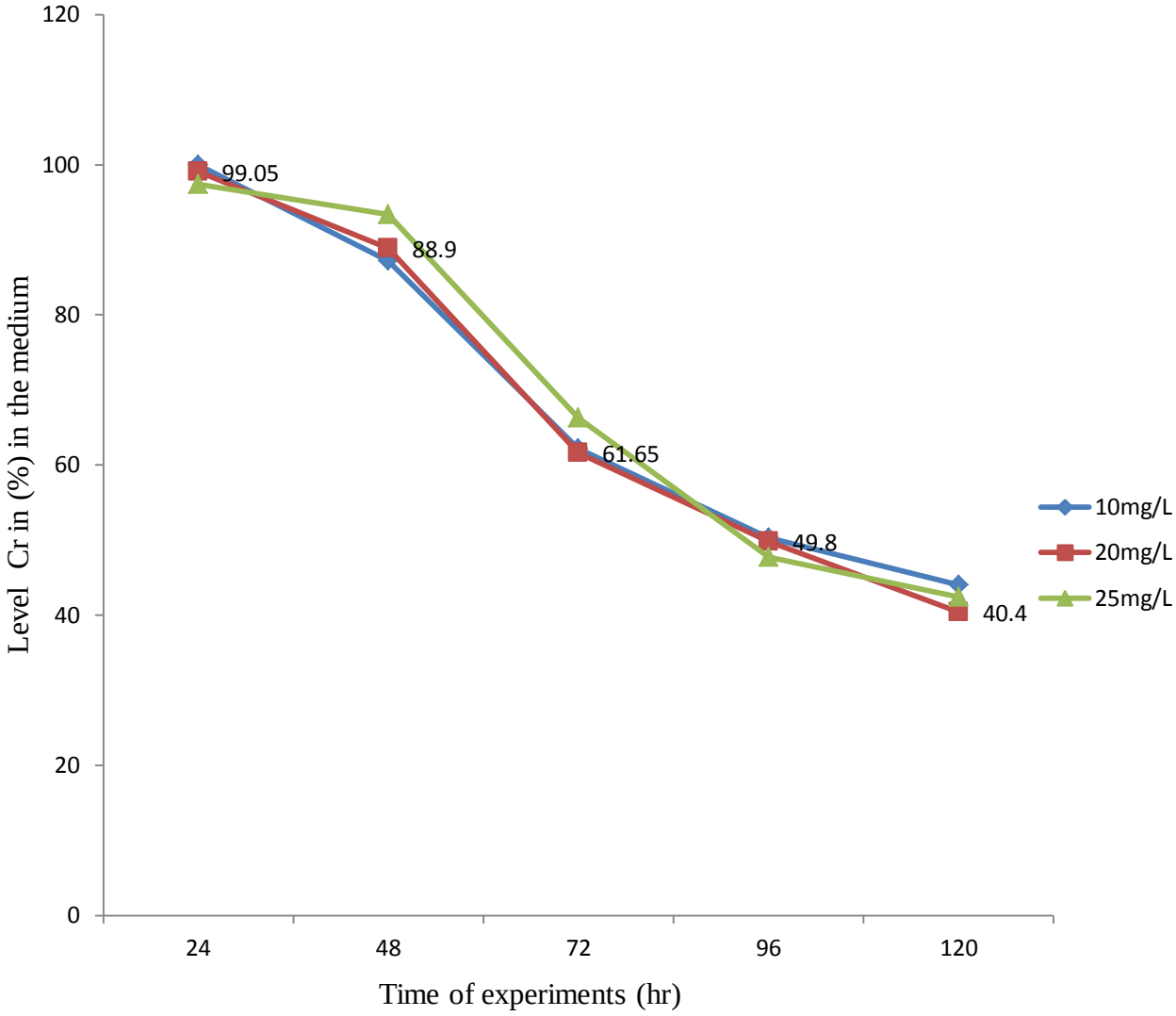


Figure 4.12: Removal of Cr in culture inoculated with *Penicillium* sp at pH 7

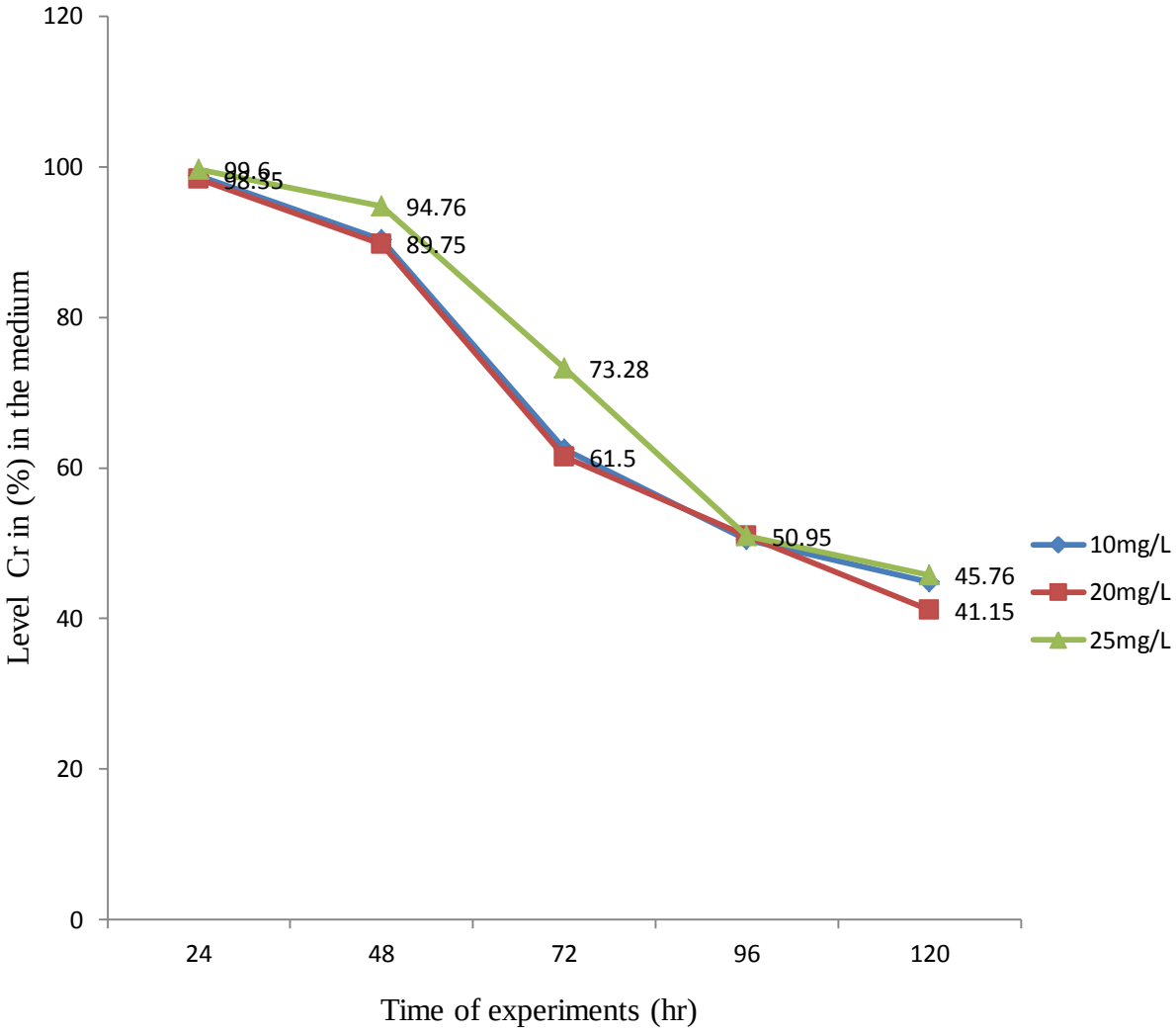


Figure 4.13: Removal of Cr in culture inoculated with *Penicillium* sp at pH 8

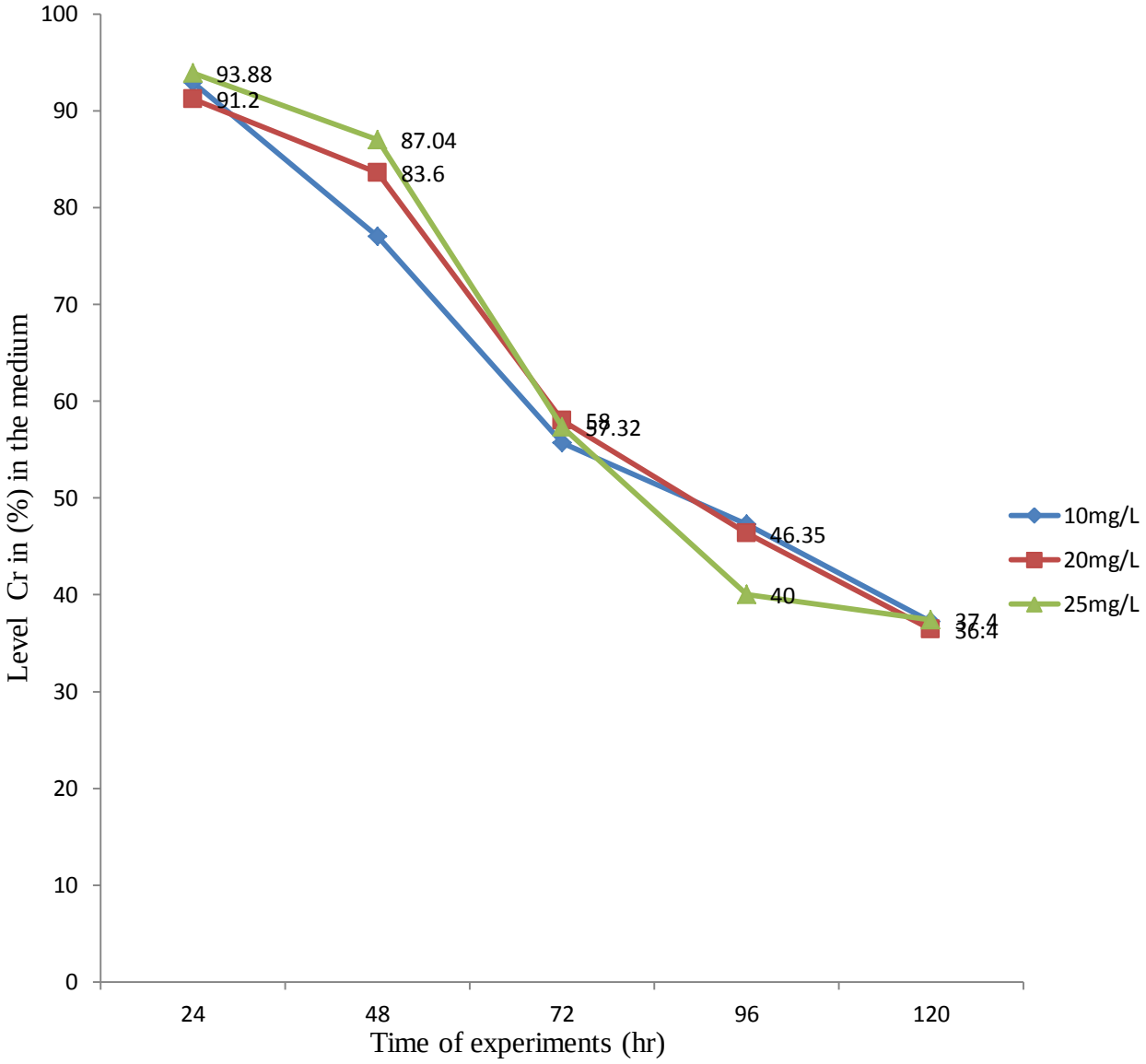


Figure 4.14: Removal of Cr in culture inoculated with *T. longibrachiatum* TN3 at pH 6

Similar pattern of Cr removal was observed in the flask containing medium at pH 7 inoculated with *T. longibrachiatum* TN1X03 where at the initial stage the Cr removal was more efficient in the flask containing 10mg/L, followed by that in the flask with 20mg/L between 24 – 72h of incubation but stabilized after 96h and 39 – 42% Cr was left at the end of the experiment as shown in Fig. 4.15.

Fig. 4.16 shows the Cr removal by *T. longibrachiatum* (TN1X03) at pH 8 which was slightly less efficient than at pH 7 in the same medium. Removal of Cr in culture media inoculated with TN3 also showed that pH 6 as the optimum pH for bioadsorption of Cr. Similarly the metal was more efficiently removed from the media containing 10, and 20mg/L than 25mg/L of Cr after 120hr incubation even at the different pH. Statistical analyses (ANOVA) comparing Cr removal by fungal sp at varying pH showed significant differences ($P \leq 0.05$) with higher efficiency at pH 6 in media inoculated with the fungal sp.

4.15.4 Removal of chromium by Fungal consortium

Like the bacterial consortium, fungal consortium used in this study was found to be more promising. The bioaccumulation potential of the consortium is shown in Figs. 4.17 – 4.19. The mean uptake of Cr by the organisms in the media at pH 6 was remarkable after 72h incubation where 44% of the metal was removed and only 36% was left at the end of the entire experiment as shown in Fig. 4.17. Remarkable decrease in Cr concentrations were observed in flasks containing media inoculated with the fungal consortium at pH 7, 24h after incubation and these proceeds until 72h. At the of 120h end only 38 – 41% Cr was left in the media as shown in Fig. 4.18.

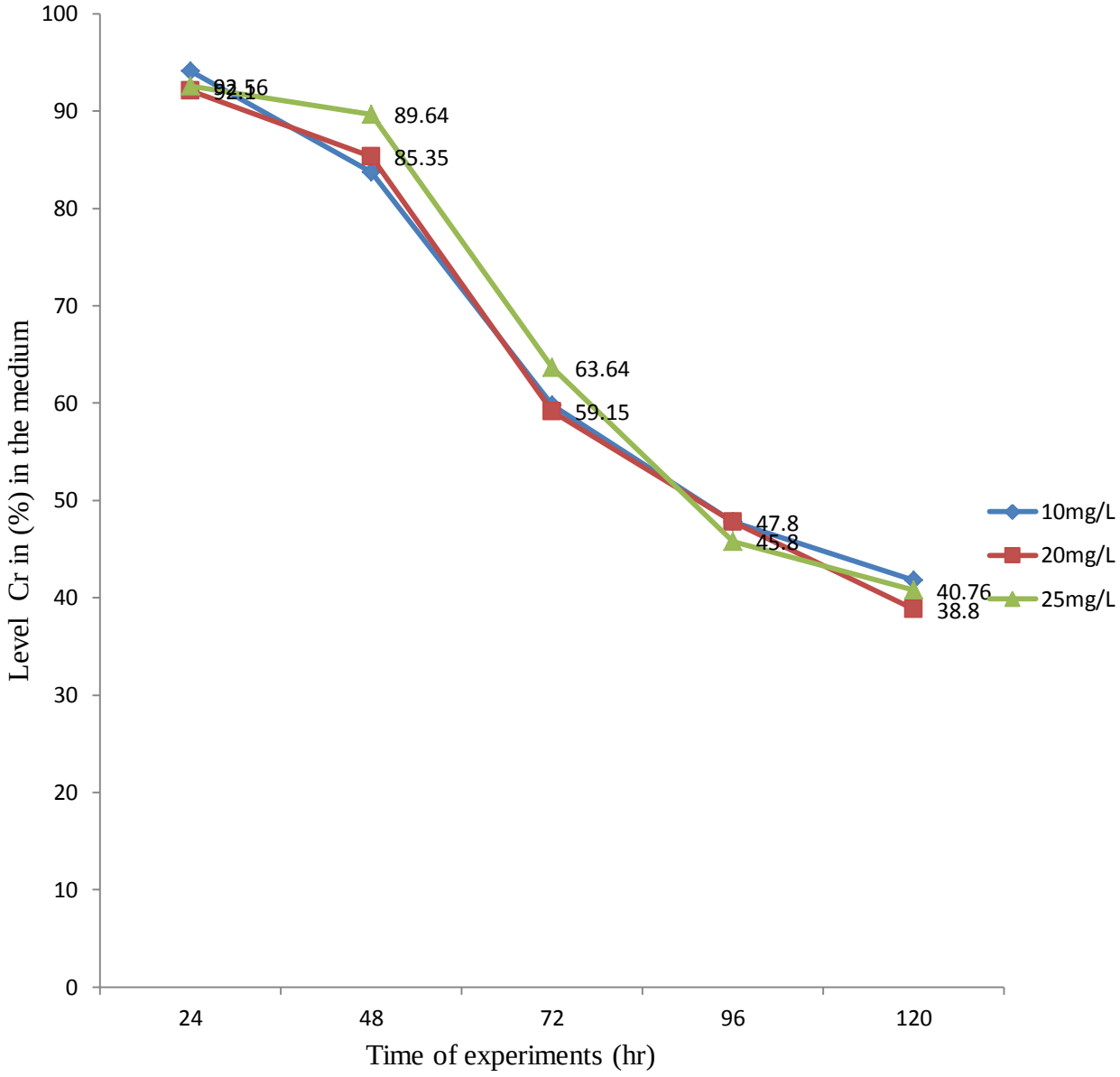


Figure 4.15: Removal of Cr in culture inoculated with *T. longibrachiatum* TN3 at pH 7

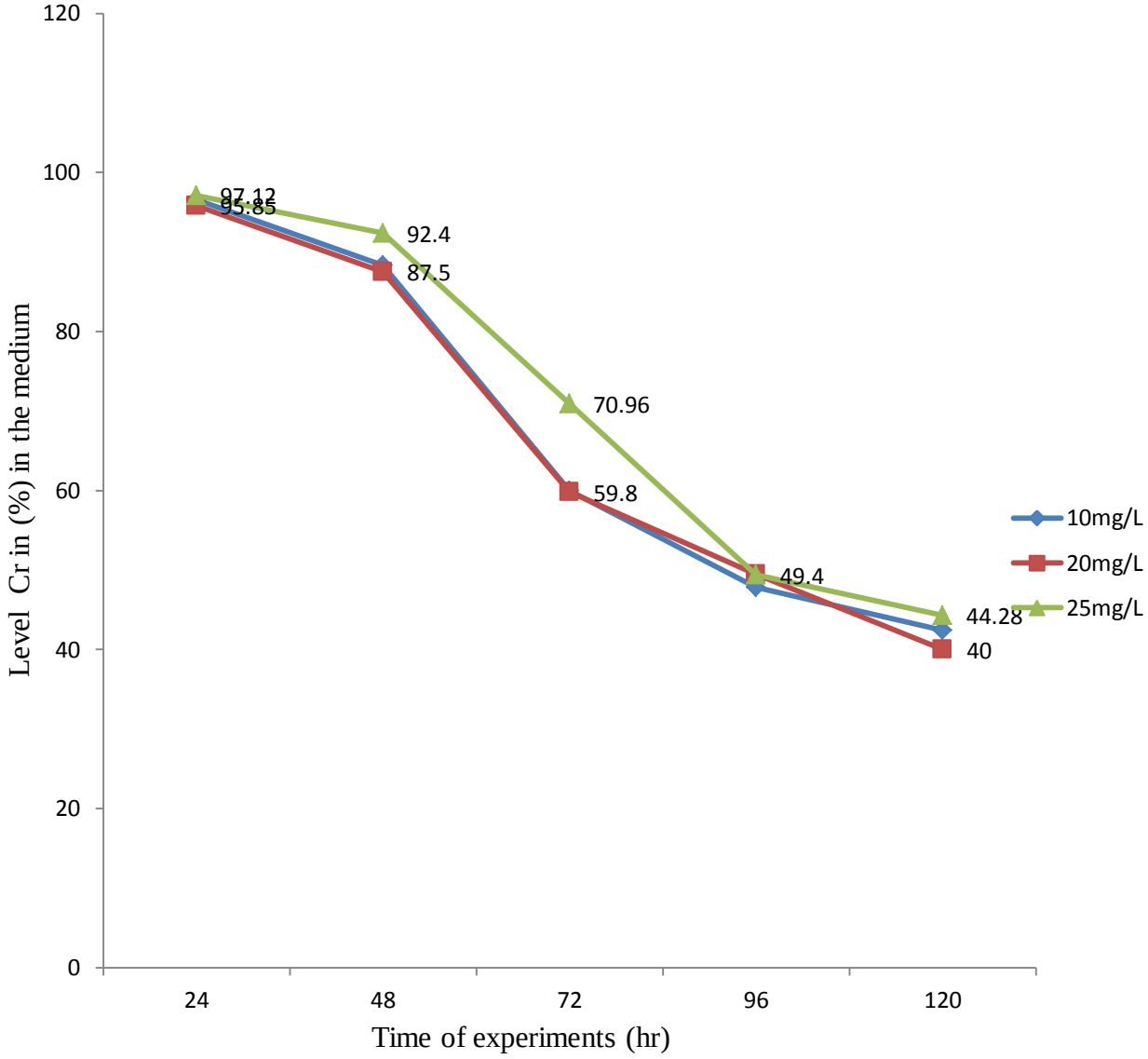


Figure 4.16: Removal of Cr in culture inoculated with *T. longibrachiatum* TN3 at pH 8

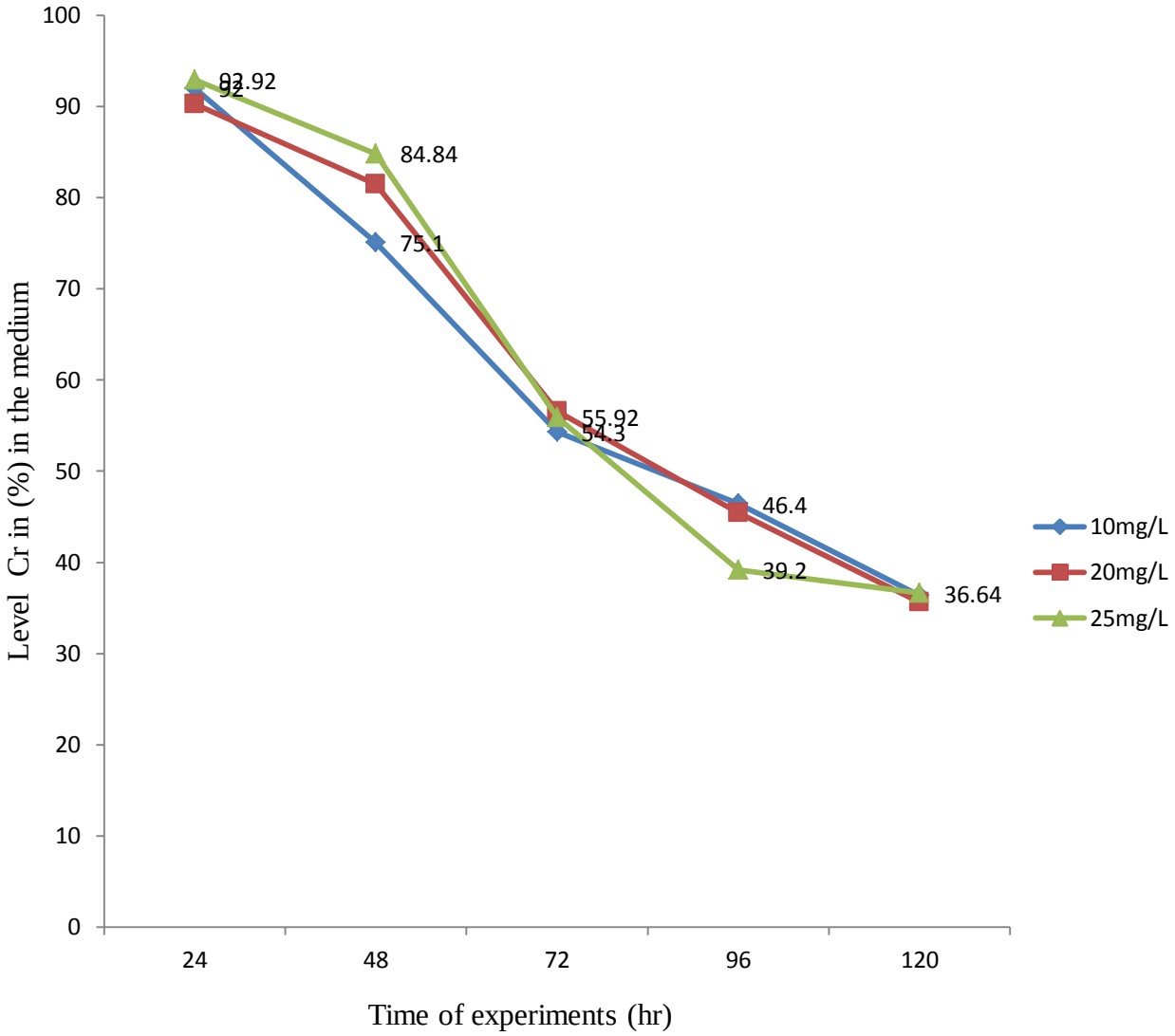


Figure 4.17: Removal of Cr in culture inoculated with fungal consortium at pH 6

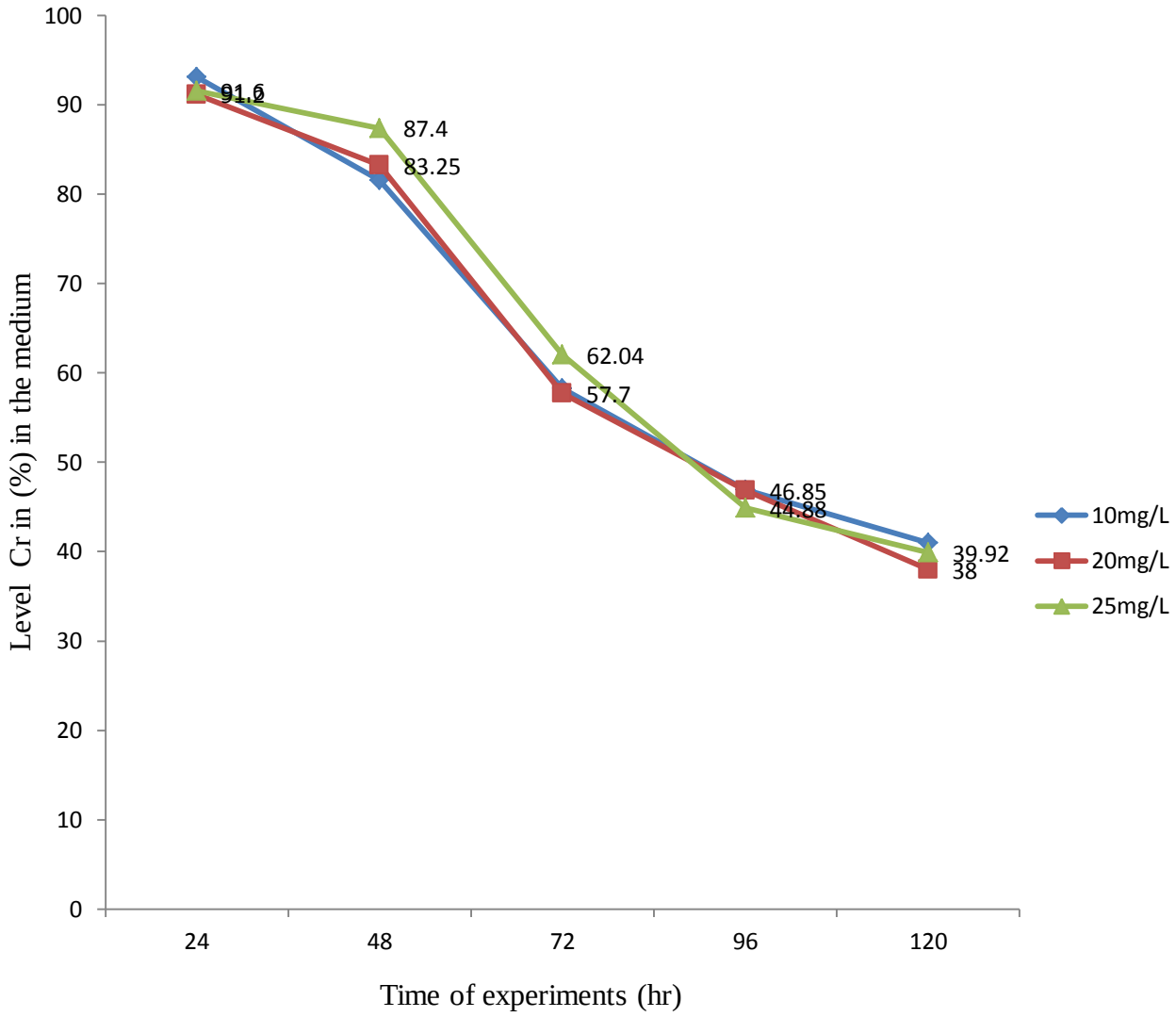


Figure 4.18: Removal of Cr in culture inoculated with Fungal Consortium at pH 7

The same pattern of Cr removal was observed in flasks with medium at pH 8 where the greater removal was attained after 72h of incubation Fig. 4.19. The metal was more efficiently removed from the media containing 10 and 20mg/L than 25mg/L of Cr after 120hr incubation. The fungal consortium exhibit synergistic effect in the Cr removal from the media (Fig 4.19).

Fig. 4.20 reveals the mean potential of Cr removal in cultures inoculated with different fungal species. The Cr removal ability of the test organisms in the cultures generally increased from 48 – 72hr. About 40% of the initial Cr concentration in the media were effectively adsorbed or removed by the test organisms after 72hr incubation.

The mean Cr bioadsorbtion among these test organisms and in consortia at pH 6 showed that *Aspergillus niger* (TN1A31) and *Penicillium species* (TN3P09) removed a total of 59% of the initial Cr from the media in 120hr. TN3 and the consortia left an average of 38.8% and 38.1% of the total Cr in the media after 120hr.

The fungal consortium was found to be more efficient than individual organism, while *T. longibrachiatum* TN1X03 (TN3) was more efficient than *Aspergillus niger* (TN1A31) and *Penicillium species* (TN3P09). However 38% of the total Cr concentrations were still left in the medium. The consortia appear to be the most suitable candidate for bioremediation of Cr containing effluent Fig. 4.20.

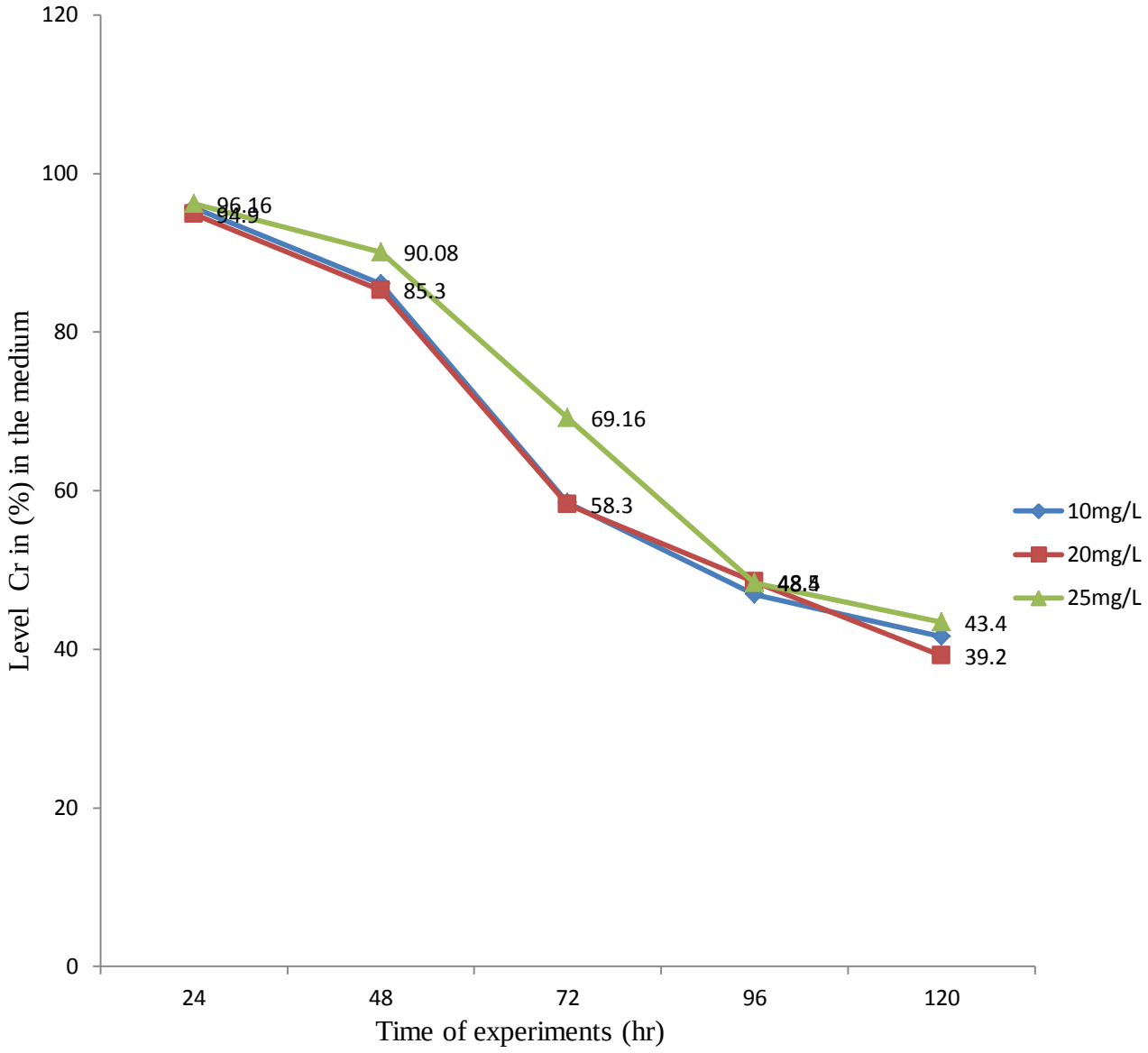


Figure 4.19: Removal of Cr in culture inoculated with fungal Consortium at pH 8

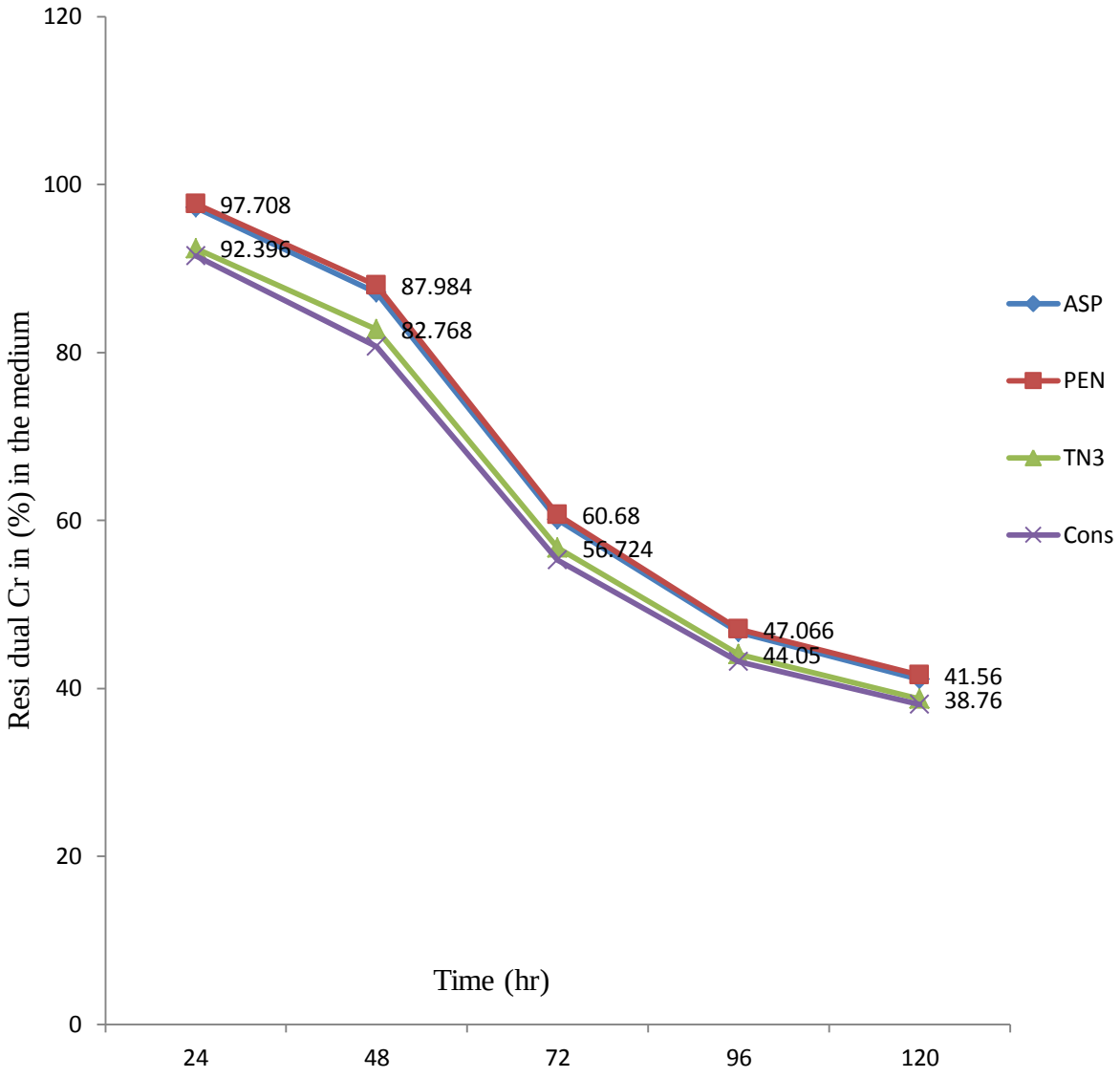


Figure 4. 20: Mean removal of Cr in cultures inoculated with different fungal species

4.16 Effluent treatment using fungi

Orbital shakers and aerated vessels were used to determine the ablity of the test organisms to remove Cr from the tannery effluent containing 25mg/L Cr at pH of 6. The pH 6 and 25mg/L concentration were chosen for the effluent treatment study.

The level of residual Cr in treated effluents using orbital shaker as shown in Fig. 4.21 About 95% of the initial Cr was still found in the effluent after 24hr treatment with the test organisms. Comparison of adsorption capacities among the fungal strains and consortia in this study showed that the consortia had higher removal or adsorption capacity than individual organism followed by *T. longibrachiatum* TN1X03 (TN3). After 48hr of effluent treatment in the conical flasks on the orbital shaker the consortia adsorbed 4.8mg/L Cr from the effluent and the adsorption rose to 11.9mg/L and 15.8mg/L after 72 and 96hr respectively.

Finally 16.2mg/L of the Cr was effectively removed from the effluent by the consortia (Fig. 4.21). The Cr removal efficiency in the treated effluents by TN3 was higher than that in *Aspergillus niger* (TN1A31) and *Penicillium species* (TN3P09) (*Pen*). These test strains adsorbed 15.0 mg/L (ASP), 14.6 mg/L (PEN) and 15.7mg/L Cr from the effluent after 120hr treatment in the conical flasks on the shaker. The uninoculated control flask had (24.3mg/L Cr) about 97% of the initial concentration of Cr still after 120hr treatment period (Fig. 4.21).

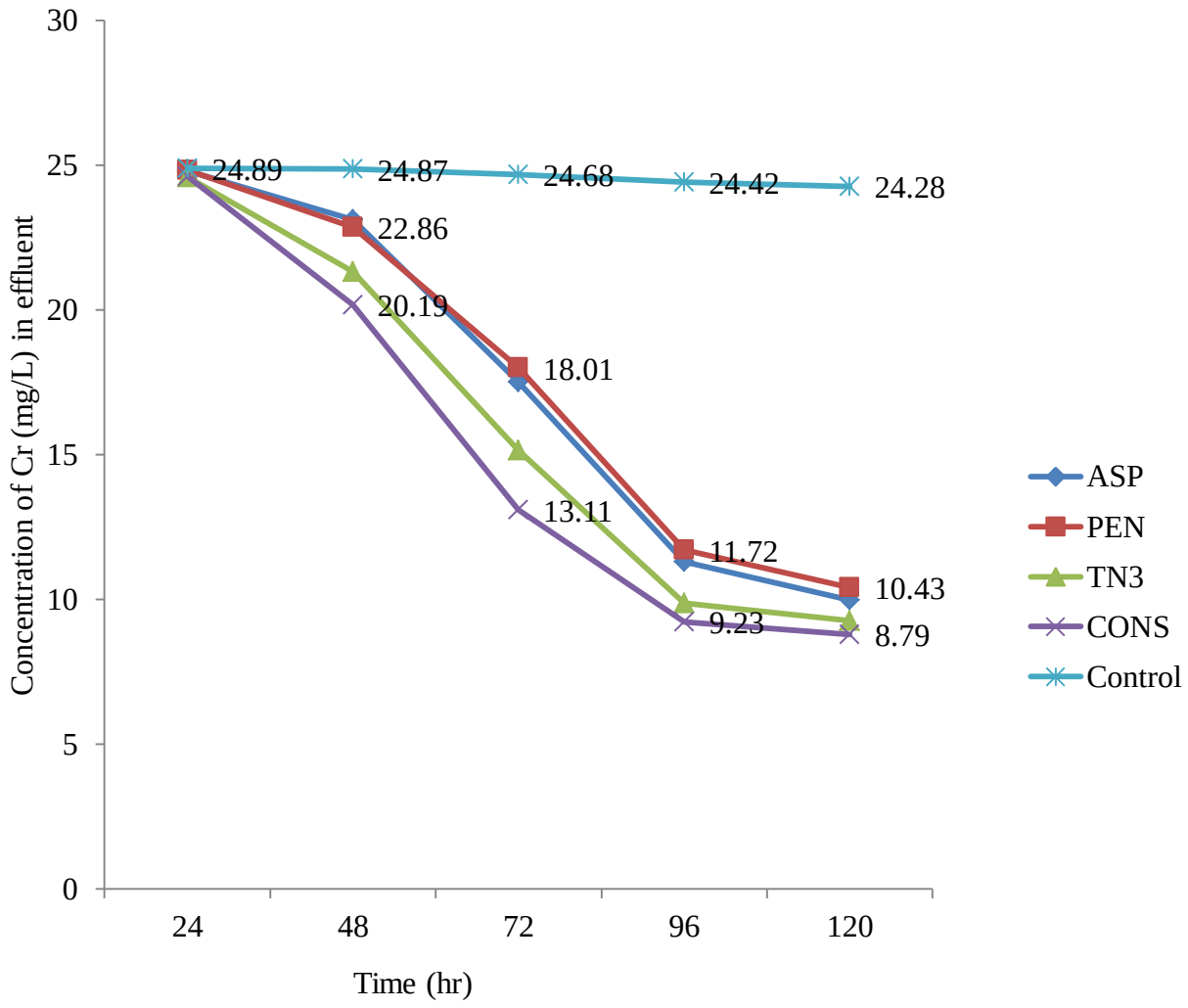


Figure 4.21: Chromium level in treated effluent using conical flask on orbital shaker

Key: ASP= *A. niger*, PEN= *Penicillium sp*, TN3= *T. longibrachiatum* CONS= Cosortium

The efficiencies of effluent treatments by the test strains in the aerated vessels were slightly higher in comparison with the shake flask methods Fig. 4.22. TN1A31 (*Asp*) adsorbed 15.7mg/L compared to 15.0mg/L Cr in shake flasks; TN3P09 (*Pen*) adsorbed 14.9mg/L Cr compared to 14.6mg/L Cr. No significant variation was observed in the Cr uptake by TN1X03 (TN3) in both shaker and vessels (15.7mg/L and 15.5mg/L) were removed respectively. The major difference was observed in the effluent treated with the fungal consortium were in the vessel 17.1mg/L Cr was removed after 120hr of treatment while only 16.2mg/L was removed from effluent treated in the flask (Fig. 4.21 and 4.22).

4.17 Effluent treatment using UV Mutant fungi

The efficient test organisms (*Aspergillus niger, Penicillium species* and *Trichoderma longibrachiatum*) were subjected to Ultraviolet light (UV) rays at different time intervals to improve their Cr removal capabilities as a result of probable mutation in the cells. Some of the mutant strains actually performed better than than the wild parent strains for instance MS_{UV10} *Penicillium species* removed 17.2mg/L of Cr from the effluent sample leaving behind 7.82mg/L in the treated effeluent. *Aspergillus niger* and *Trichoderma longibrachiatum* exposed to UV for 10min (MS_{UV10}) left about 9.11mg/L in the effluent which was similar to the performances of their wild parent strains respectively Fig. 4.23. *Aspergillus niger* exposed for 30min (MS_{UV30} (*Asp*)) performed better than its wild parent strains by removing 16.93mg/L of Cr from the effluent leaving behind 8.09mg/L in the treated effluent as shown in Fig. 4.24. All the fungal starins exposed to UV for 60min (MS_{UV60}) had less efficient Cr removal ability than their respective wild parent strains as shown in Fig. 4.25.

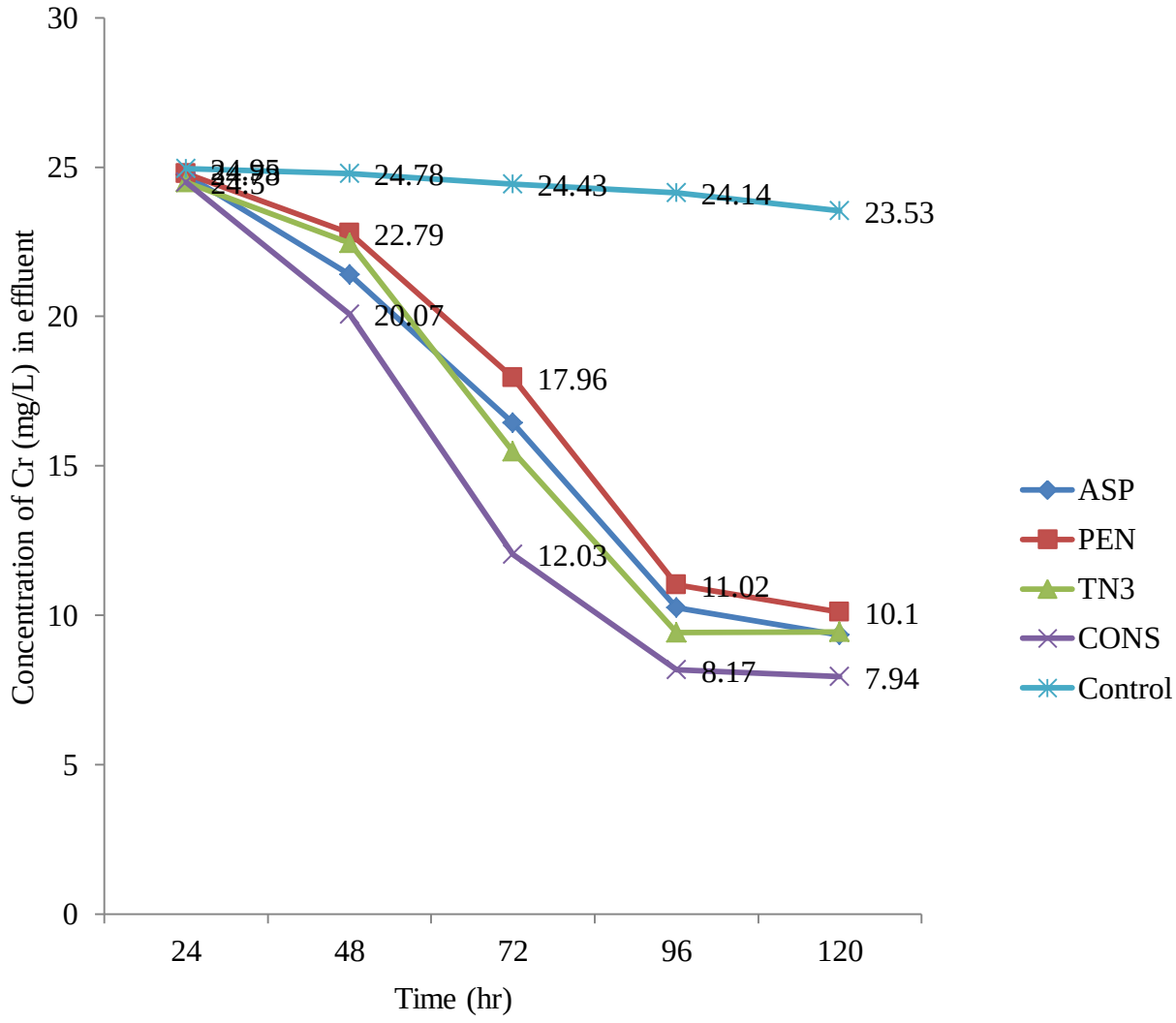


Figure 4.22: Chromium level in treated effluent using aerated vessels

Key: ASP= *A. niger*, PEN= *Penicillium sp*, TN3= *T. longibrachiatum* CONS= Cosortium

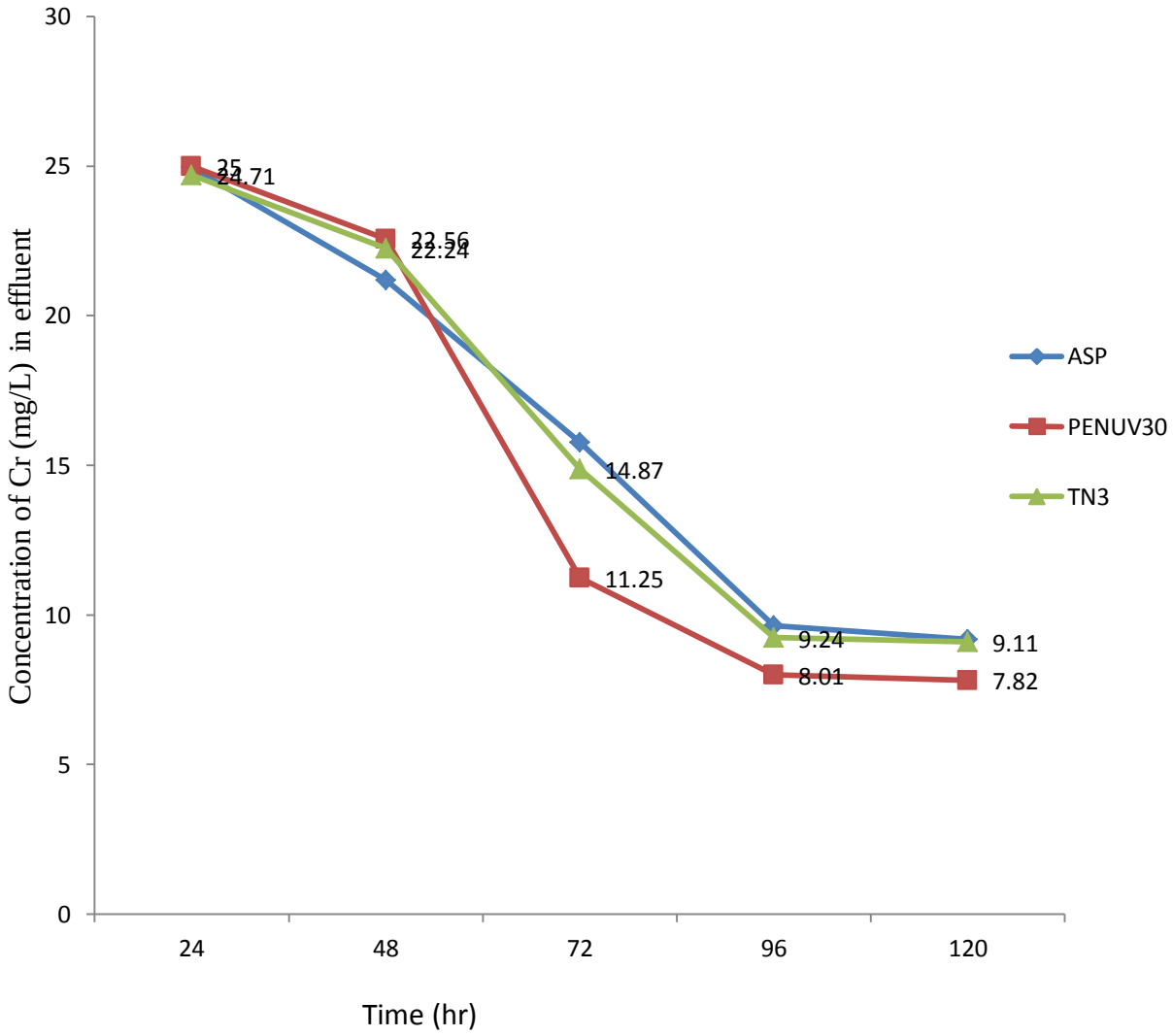


Figure 4.23: Chromium level in treated effluent with mutant strains ($MSUV_{30}$) in vessels

Key: ASP= *A. niger*, PEN= *Penicillium sp*, TN3= *T. longibrachiatum*

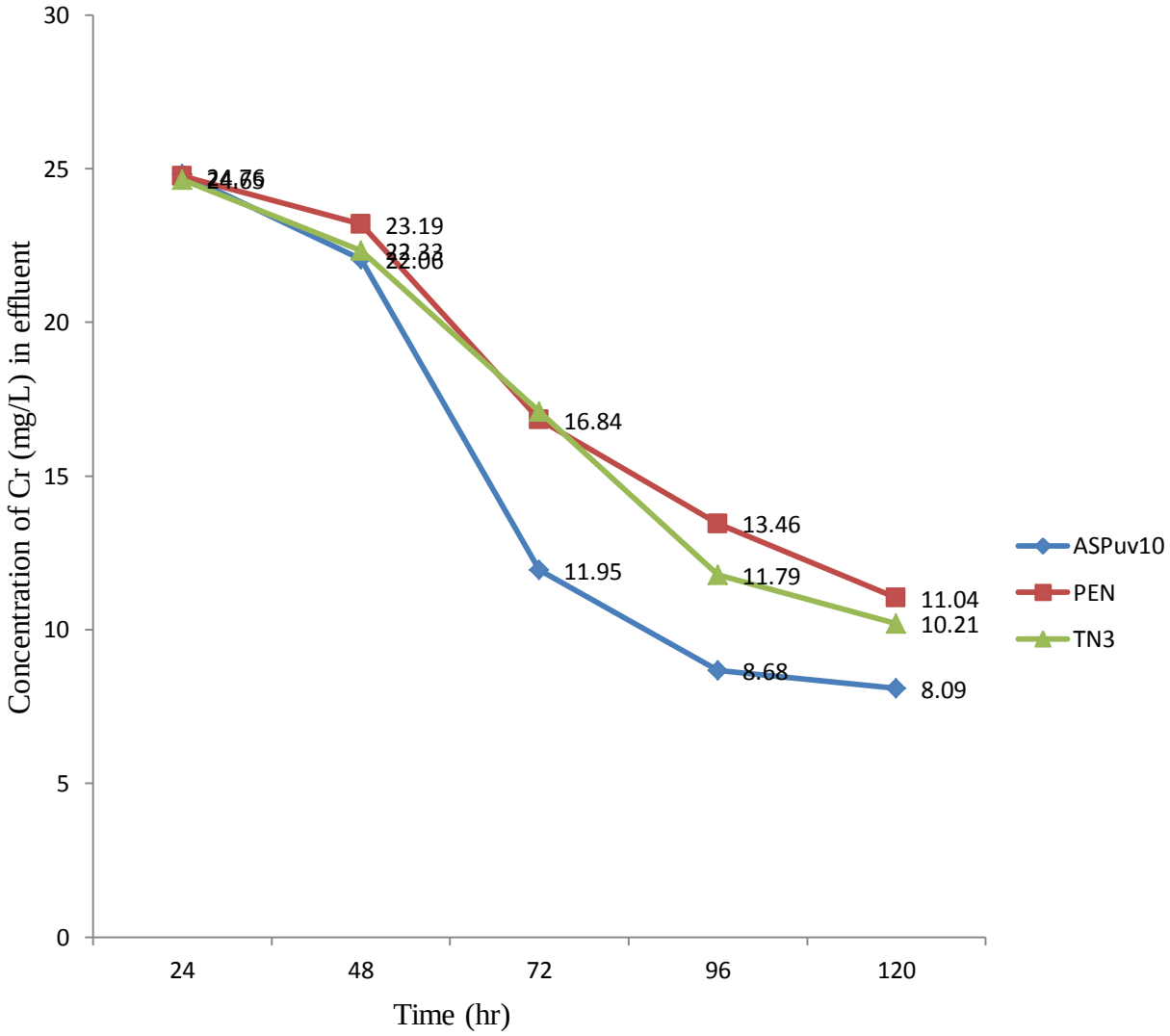


Figure 4.24: Chromium level in treated effluent with mutant strains ($MSUV_{10}$) in vessels

Key: ASP= *A. niger*, PEN= *Penicillium sp*, TN3= *T. longibrachiatum*

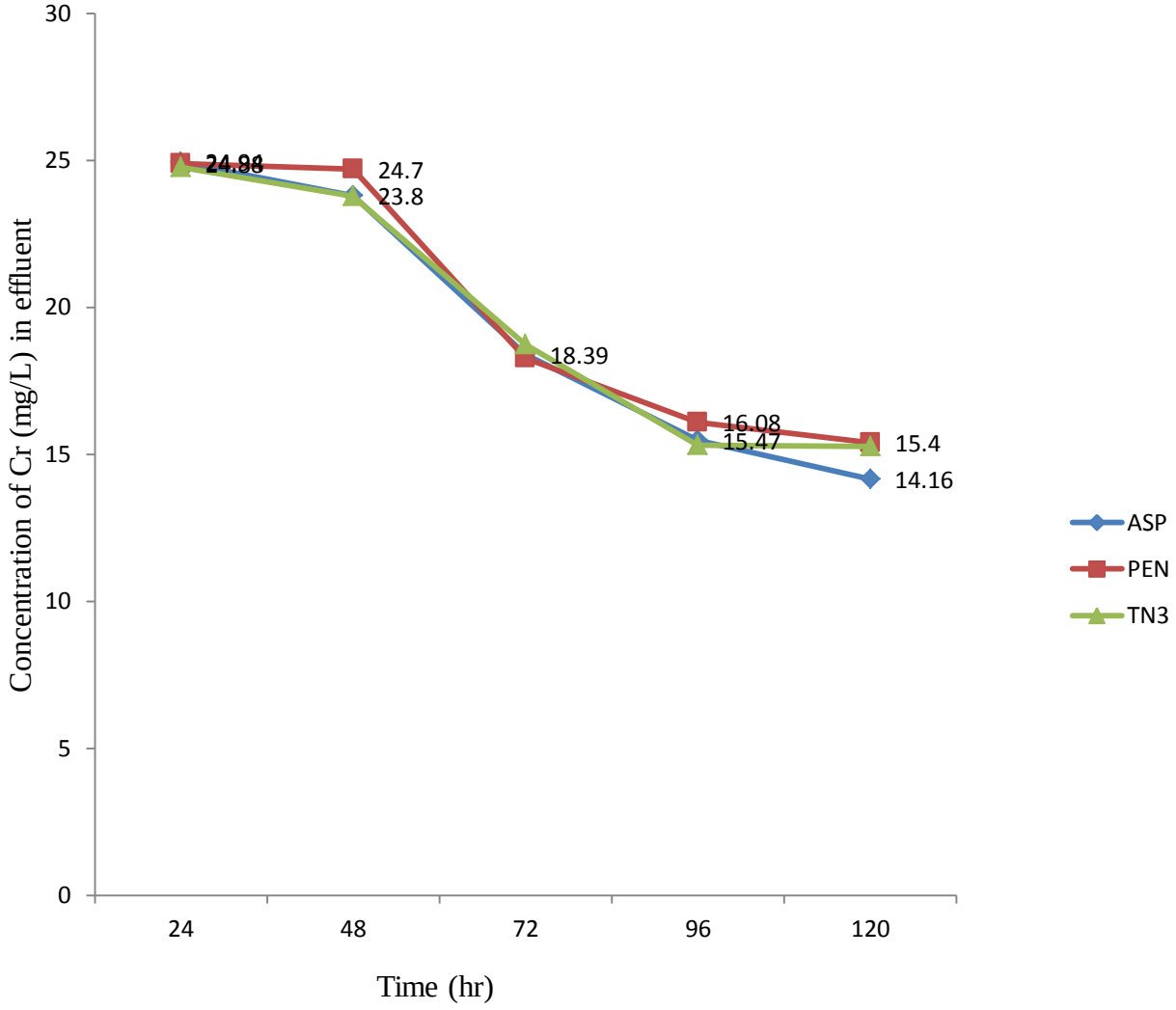


Figure 4.25: Chromium level in treated effluent with mutant strains ($MSUV_{60}$) in vessels

Key: ASP= *A. niger*, PEN= *Penicillium sp*, TN3= *T. longibrachiatum*

4.18 Fungal biomass production in treated effluent

The growths of fungal isolates were determined by measuring the dry biomass at the same time intervals the residual Cr in the effluent were analyzed. It was observed that the weights of the biomass were increasing with decreasing Cr level in the treated effluent using both methods. Fig. 4.26 illustrates the fungal biomass produced in treated effluent using shake flasks inoculated with wild parent strains. At 24hr, *Aspergillus niger* (TN1A31) produced net 2.3mg/L of the fungal biomass. Then there was remarkable increase in the biomass to 7.4mg/L after 120hr, while *Penicillium species* (TN3P09) at the beginning of the treatment produced 2.5mg/L of the biomass, before rising slightly after 48hr of treatment and finally producing 7.1mg/L in the whole process. *T. longibrachiatum* TN1X03 (TN3) and the consortia produced near equal biomass in the treatment process where the consortium produced 7.8mg/L which was slightly higher than that produced by *T. longibrachiatum* TN1X03 as shown in Fig. 4.26.

Fungal biomass produced in treated effluent in aerated vessels inoculated with wild parent strains were higher than those produced in the shake flasks. *Aspergillus niger* (TN1A31) biomass rises from 2.6mg/L in 24hr to 7.1mg/L in 96hr and finally to 8.0mg/L when 120hr treatment lapsed. *Penicillium species* (TN3P09) also rose from 2.5mg/L to produce only 7.3mg/L as the final biomass in the treated effluent. *T. longibrachiatum* TN1X03 (TN3) produced biomass as much as that produced by *Aspergillus niger* (TN1A31). While the fungal consortium of the three organisms produced 2.9mg/L of biomass in 24hr and then increased at a similar phase producing 7.8mg/L at the completion of the treatment as shown in Fig. 4.27.

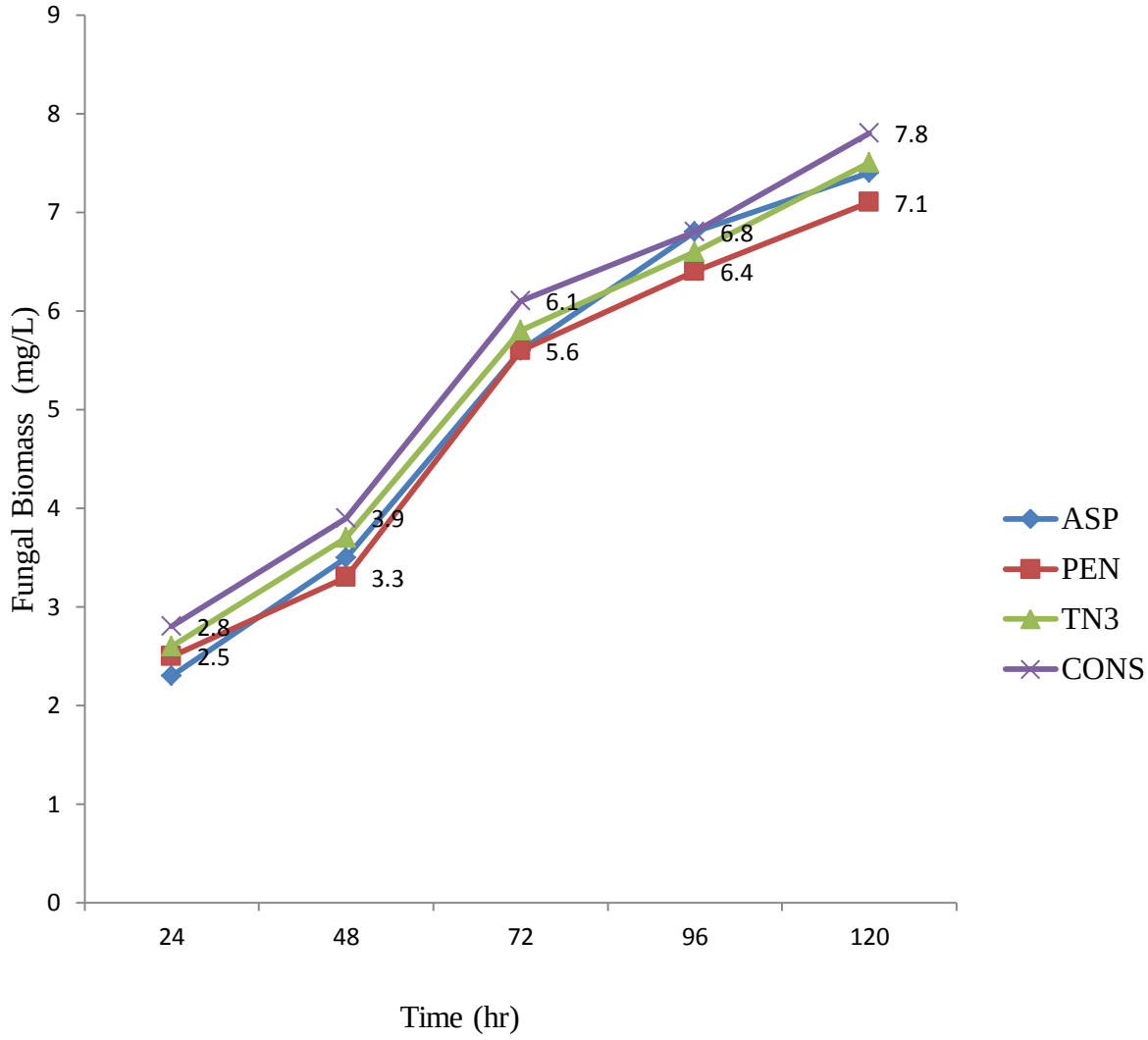


Figure 4.26: Fungal biomass produced in treated effluent using shake flasks

Key: ASP= *A. niger*, PEN= *Penicillium sp*, TN3= *T. longibrachiatum* CONS= Cosortium

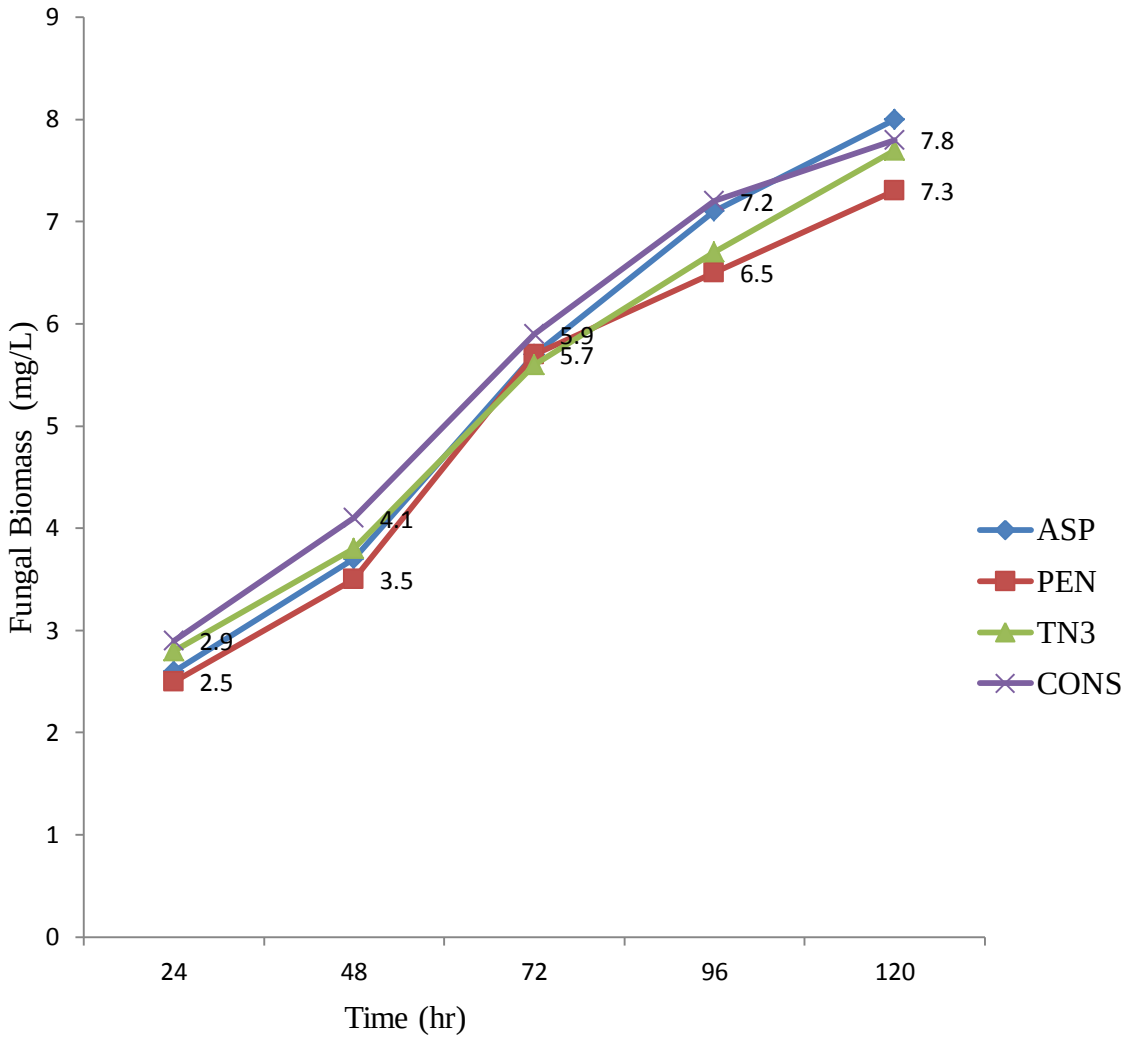


Figure 4.27: Fungal biomass produced in treated effluent using aerated vessels

Key: ASP= *A. niger*, PEN= *Penicillium sp*, TN3= *T. longibrachiatum* CONS= Cosortium

4.18.1 Effect of UV irradiation on biomass production by test strains

Fig. 4.28 shows the fungal biomass produced in treated effluent using UV exposed strains (MS_{UV30}) in the aerated vessel. Like the Cr removal efficiency TN3P09 (*Pen*) produced larger biomass of 2.8mg/L in 24hr than TN1A31 (*Asp*) and TN1X03 (TN3). The biomass however increased in effluent inoculated with TN3P09 (*Pen*) and TN1A31 (*Asp*) to 6.3mg/L after 72hr of treatment. Then TN3P09 (*Pen*) biomass rose higher than TN1A31 (*Asp*) after 96hr to 7.7mg/L and finally produced 8.3mg/L. TN1A31 (*Asp*) and TN1X03 (TN3) produced final biomass of 7.9mg/L and 7.1mg/L respectively.

In Fig. 4.29 strains exposed for 10min (MS_{UV10}) also produced different fungal biomass after 24hr treatment TN1A31 (*Asp*) produced 2.7mg/L of fungal biomass, TN3P09 (*Pen*) had 2.4mg/L while TN1X03 (TN3) produced 2.3mg/L of the fungal biomass. This pattern was maintained and at the end of the treatment TN1A31 (*Asp*) produced 8.5mg/L, TN3P09 (*Pen*) produced 7.7mg/L and TN1X03 (TN3) produced 7.3mg/L each of the biomass.

The strains exposed for 60min (MS_{UV60}) produced the least amount of biomass which was lower than those produced by their wild parent strains. At 24hr the all fungi produced 1.8 - 2.1mg/L biomass. TN1A31 (*Asp*) then accelerated over the entire treatment period to produce only 5.5mg/L in 120hr, while TN3P09 (*Pen*) and TN1X03 (TN3) 5.2mg/L and 5.3mg/L respectively as shown in Fig. 4.30.

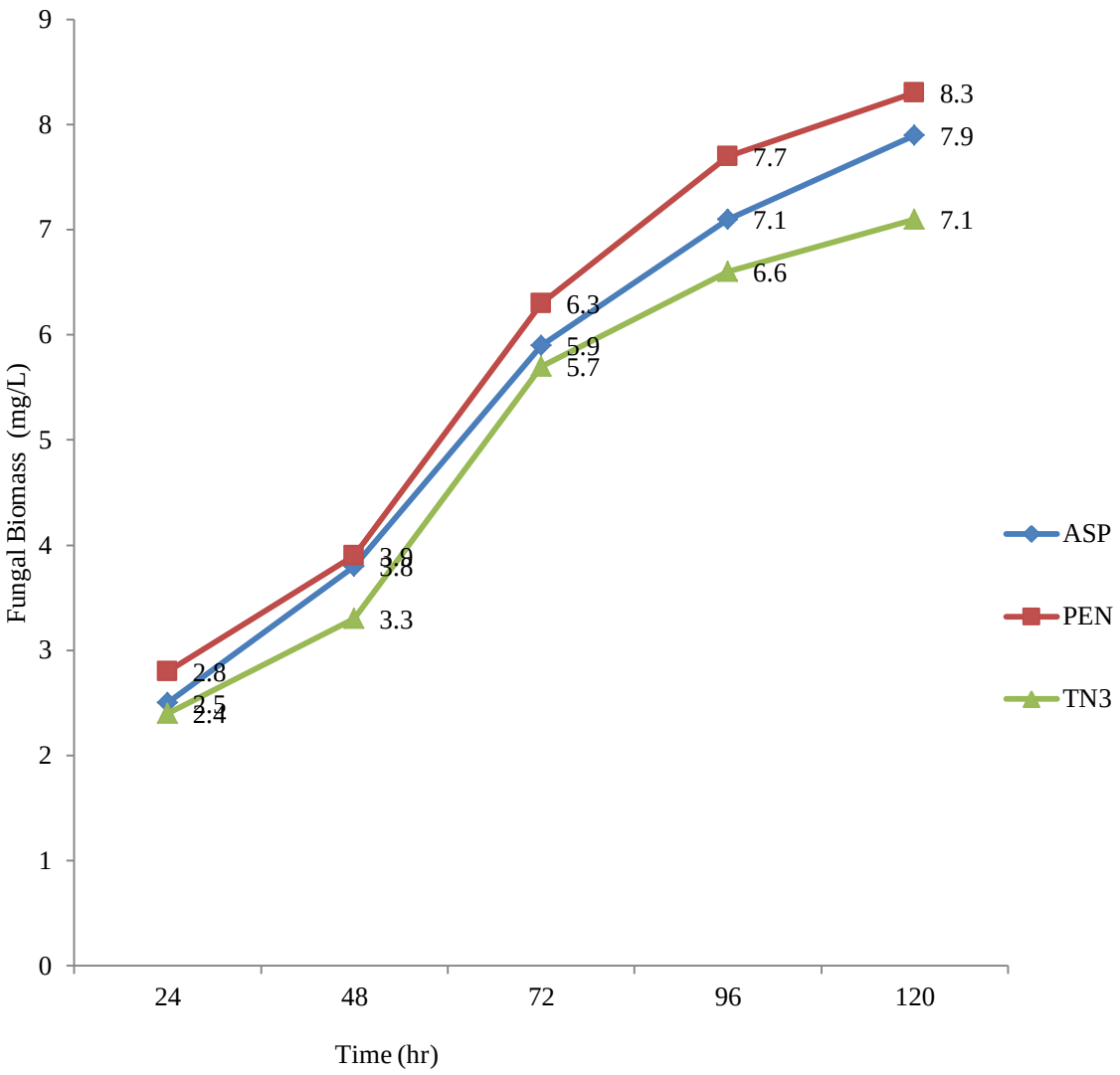


Figure 4.28: Fungal biomass produced in treated effluent using UV exposed strain UV_{30} in vessels

Key: ASP= *A. niger*, PEN= *Penicillium sp*, TN3= *T. longibrachiatum* CONS= Cosortium

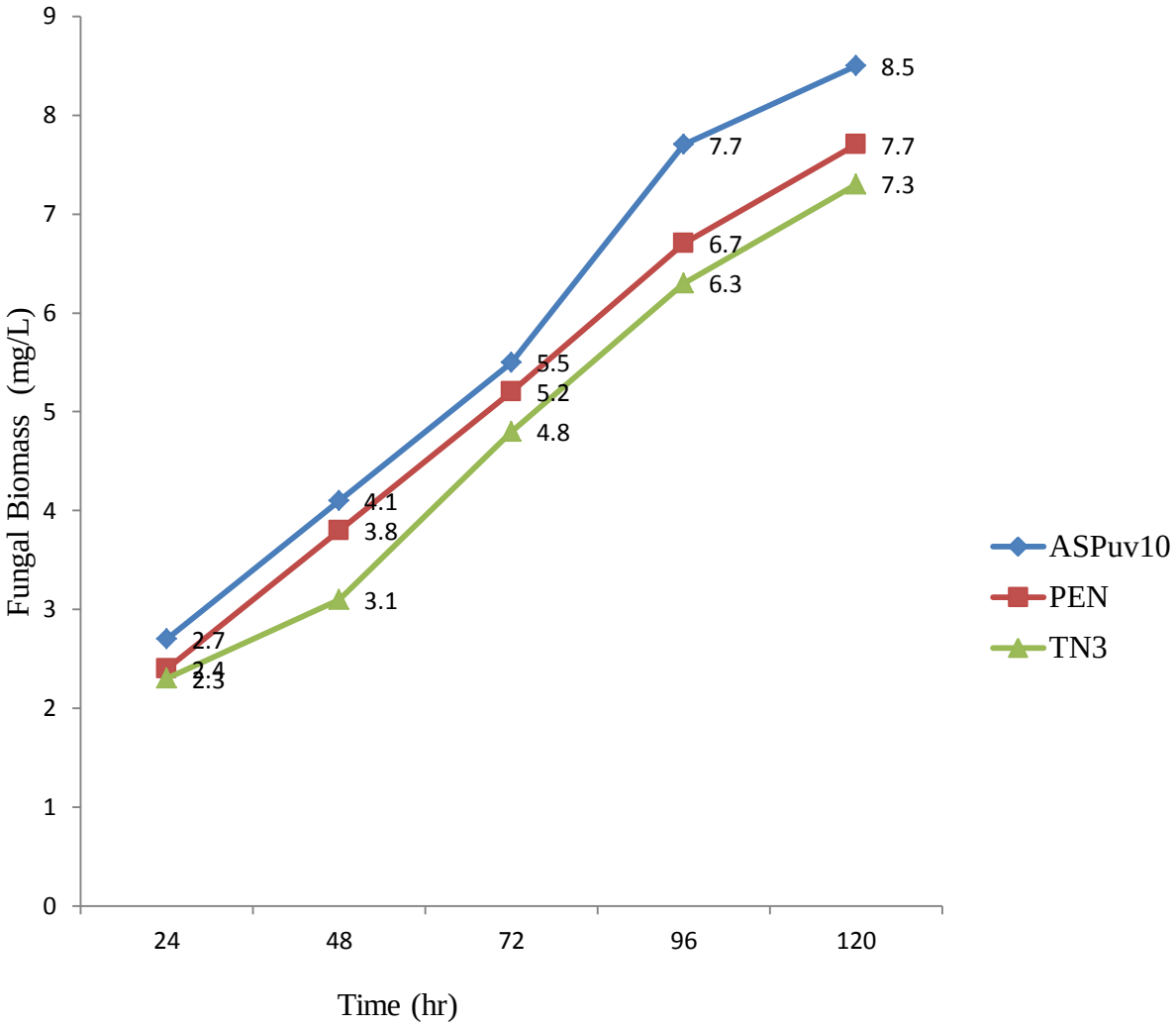


Figure 4.29: Fungal biomass produced in treated effluent using UV exposed strain UV_{10} in vessels

Key: ASP= *A. niger*, PEN= *Penicillium sp*, TN3= *T. longibrachiatum* CONS= Cosortium

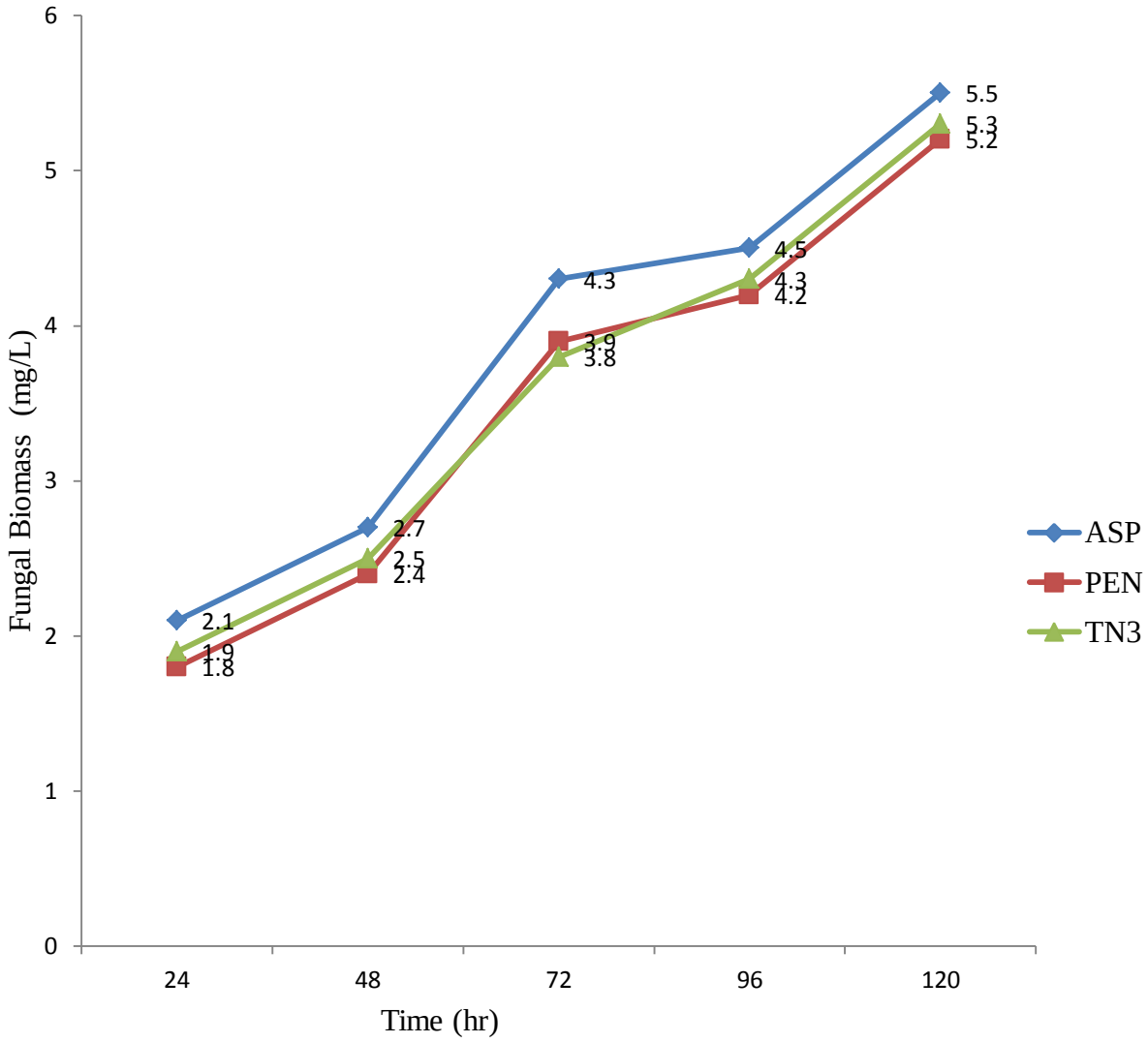


Figure 4.30: Fungal biomass produced in treated effluent using UV exposed strain UV_{60} in vessels

Key: ASP= *A. niger*, PEN= *Penicillium sp*, TN3= *T. longibrachiatum* CONS= Cosortium

CHAPTER FIVE

DISCUSSION

5.1 Physico-chemical properties of tannery effluent and well water samples

The tanning industries located in the Challawa industrial estate, near residential areas produce tannery wastes that are characterized by high BOD, high suspended and dissolved solids bad odour and strong colour. These wastes when discharged deplete the dissolved oxygen of the stream rapidly, due to chemical and biological oxidation of sulphur and other organic compounds. A secondary pollution of the stream occurs due to the deposition of solids near the discharge points and its subsequent putrefaction. The gas evolved during this process has a typical foul odour.

The odour and colour of the effluent sample were the first pollution to be observed in the effluents that impacts on the aesthetics, water transparency, odorless, tasteless and gas solubility of water bodies. All the tannery effluent samples analyzed were highly coloured and had a highly offensive odour from the putrefaction of leather, skin and hair by microorganisms present in the effluent. Indra and Mycin, (2009) reported that the odour is as a result of reactions of several chemicals such as lime, H_2SO_4, hydrogen sulphide, chromium sulphate etc. that were used in the tanning processes.

The effluents discharged into the environment caused high variability of the physical and chemical characteristics due to different quantities of processed hides and different batches at different days. Surita *et al*. (2007) attributed this type of variability in physico-chemical characteristics of effluents to different quantities of processed hides and different batches at different days. Pollution level was increasing in these areas due to effluents from tanneries. Improper drainage system and dumping of leather waste materials in open ground was also

responsible for groundwater contamination and related hazards. Mondal *et al* (2005) in their study reported similar observation in India.

5.1.1 Physico-chemical parameters in discharged effluent samples

The effluent samples were highly coloured ranging from reddish, yellow, brown, bluish-black, brown and very dark brown to black colours and therefore not fulfilling the criteria (odourless and colourless) of EPA(2002) effluent discharge standard. These various colours arise from the different stages of leather processing from soaking, liming, bating to finishing. All the tannery effluent samples analyzed had a highly offensive odour from the putrefaction of leather, skin and hair as well as flesh present in the effluent (Indra and Mycin, 2009; EPA, 2002).

The discharge of effluent into the environment cause wide range of impact ranging from the rise to fall of pH affecting the size and activities of each microbial population there in. Majority of the samples analyzed were found to be alkaline (pH 8). However, a few were found to be acidic as pH 3 ranging from sampling points although this acidic pH were found only at the discharge point (DP) in all the tanneries. No significant difference was observed in the mean pH of the effluents in all the three tanneries which was found to be alkaline (8.17-8.91). These similarities can be attributed to the fact that the same activities are employed by these tanneries in leather processing and the use of similar chemical reagents, raw materials and other consumables. The little variations might have arisen from the volume of leather processed (work), chemical dosing and the volume of water used in processing which acts as diluents as well as sanitary conditions in different tannery. Babyshakila, (2009) reported that chemicals such as sodium carbonate, sodium bicarbonate, sodium chloride based in tanning cause alkalization of water and soil resulting in the increase pH of the aquatic and soil

environment. Other researchers however reported acidic pH (Dikshit and Shukla 1989; Pathe *et al.,* 1995; Deepali *et al.,* 2009). The findings by Indra and Mycin, (2009) contradict the finding in this study. However, Sakthivel and Sampath (1990); Shukla and Shuklaz, (1994); Shukla *et al*., (2007); Akan *et al*., (2009); Ramteke *et al*., (2010) reported alkaline pH in tannery wastewater and is in agreement with findings in this study. Gomes *et al.,* (2010) reported that pH 6.0 - 9.0 were in the range of wastewater discharge permissible limit". The pH values of effluent analyzed in this study also agrees with the findings of Akan *et al* (2009) who conducted physio-chemical analysis of industrial effluents in the Challawa industrial area in Kano, Nigeria. Akan *et al* (2009) stated that the mean pH values were above the stipulated Nigeria FEPA (Federal Environmental Protection Agency) pH tolerance limit of 6.0- 9.0 for effluent discharged from tanneries. The pHs were however dropping from alkaline downstream the initial sampling point DP from a mean pH of 9.29 ± 2.74 - 8.03 at the entry point into river Challawa. This drop in pH down steam can be attributed to the increase in volume of wastewater from different activities in other factories within the layouts, fertilizer application and run-off from farms as well as several other chemical and biological activities down the drain.

The mean TSS values which were significantly different ($P \leq 0.05$) with higher mean values in Tan A (413.53mg/l) followed by Tan C (224.30mg/l) and then Tan B (116.89mg/l), similarly TDS, values in Tan A (123.47mg/l) were significantly higher than mean value in Tan C (93.99mg/l) and Tan B (86.23mg/l), Surita *et al*. (2007), attributed these variability in TSS of effluents to different quantities of processed hides and different batches at different tanneries in different days**.** The mean electrical conductivity (EC) values vary also in different order with values in Tan A (7.98µS/cm) followed by Tan B (5.46µS/cm) and then Tan C (4.38µS/cm) and BOD values were significantly different ($P \leq 0.05$) with higher mean

values in Tan A (189.0mg/l) followed by Tan C (101.01mg/l) and then Tan B (63.93mg/l) indicating higher pollution level in the order Tan A > Tan C >Tan B. These variations might have arise from the volume of leather processed (work), chemical dosing and the volume of water used in processing, tanning procedures as well as the method used by each tannery in removing solid wastes eg use of screen (mesh) at discharge points (Indra and Mycin 2009).

5.1.2 Physico-chemical parameters in well water samples

Physico-chemical parameters of the well water samples around the tanneries showed high fluctuations, although no statistically significant differences ($P > 0.05$) were observed in mean pH, Temperature, TSS, and Electrical conductivity, significant differences ($P \leq 0.05$) were observed in mean TDS, DO and BOD values. Mean TDS and BOD values were significantly higher in well 1 and well 2 while mean DO was higher in well 3. The mean BOD values reduced from well 1 down to well 3 and could be attributed to relative distance from point source as well as diminishing effect of the pollutants (Yusuff and Sonibare, 2004; Deepali *et al*., 2009). Significant differences ($P \leq 0.05$) exist between the physico-chemical parameters of the wells around the study area and control well where no apparent tannery or other industrial waste(s) discharge in the environment which validates the negative impact of the wastewater discharge from these industries. All the physic-chemical properties were above the minimum discharge limits with attending impact on the environment.

5.1.3 Seasonal variations of physico-chemical parameters in well water samples

Analyses of the seasonal variation in the mean physico-chemical parameters of well water samples showed no significant differences ($P > 0.05$) in pH, Temp, TDS and TS in the samples analyzed throughout the study period. However, significant differences ($P \leq 0.05$) observed in TSS, DO and BOD, can be attributed to the amount of rainfall that washes in the wells as well as the water level in the well during the seasons (Yusuff and Sonibare, 2004;

Deepali *et al*., 2009). Higher TSS and BOD were recorded in the dry season while DO was considerably low in the dry season than wet season. These may be due to lower volume of water in wells during the dry season that concentrates the pollutant. The mean pH of the well water was tending towards neutral in the wet season and slightly alkaline; this can be attributed to the impact of fertilizers used in the vicinity that is washed up which tend to neutralizes the acidic pH, however, in the dry season it tends towards slight acidic pH. The level of DO was significantly increasing from well 1 to well 3 with highest amount of DO in the wet season samples of well 3. The mean Tss, Tds, Ec and BOD were significantly higher ($P \leq 0.05$) in the dry season samples of all the wells. These mean values were decreasing from well 1 down to well 3 indicating a deminshing impact of the pollution with distance.

5.1.4 Variations of physico-chemical parameters in effluent samples from sampling points

The comparative analysis of the TSS and TDS of effluent from the three sampling points (DP, DS and EP) ANOVA showed significant differences ($P \leq 0.05$). The mean values where significantly higher in the DP and lowest at the entry point EP. This as well can be due to the increase in the volume of discharged water from domestic sewage and other factories located in challawa with lesser amount of TSS and TDS, as well as to the deposits of TSS and TDS in channels down the drain. These suspended and dissolved impurities cause turbidity, low DO and higher BOD to the receiving streams and rivers with attending severe impacts to the water and other environmental qualities. Many researchers reported even much high TSS, TDS and TS.

The electrical conductivity (EC) of the samples analyzed varied significantly ($P \leq 0.05$) from tannery with TAN A having the highest mean EC and TAN C had the least. Similarly there were significant differences ($P \leq 0.05$) in the mean EC values from DP sampling point to the

EP in all the tanneries. The EC values were decreasing from DP to EP indicating decreasing amount of ions downstream. Decrease in EC values indicates the presence of low concentrations of ions in the effluent. Shukla *et al.,* (2007) and Deepali *et al.,* (2009) also reported much higher EC in tannery effluents analyzed. Elevated conductivities in surface and shallow polluted waters are mainly due to sodium chloride (NaCl) residues from skins preserved in salt prior to tanning (Stuart and Milne, 2008).

The amount of DO in the effluent sample in this study ranges between 1.98mg/l and 2.74mg/l with significant differences ($P \leq 0.05$) in the sampling points. The DO increased significantly down the drain which may be attributed to the decrease in the TSS, TDS and TS and the increase in the water volume containing less organic matter from other factories. Dissolved oxygen was found to be very low in this study. The amount of DO in the effluent sample in this study ranges between 2.21mg/l and 2.35mg/l with no significant differences ($P>0.05$) in the tanneries. Ramteke *et al.,* (2010) reported absolute zero DO in untreated effluent and 1.9mg/l in the treated effluent from CETP (Common Effluent Treatment Plant). Gomes *et al* (2010) suggested that minimum value of DO in effluent should be 6mg/l which is ideal. They observed that a small concentration of DO in streams can cause harmful effects depending on the aquatic species. Fish and other species cannot survive, without oxygen. The decomposition of organic matter becomes anaerobic, with methane and carbon dioxide formation (Kapdan and Oztekin, 2003).

The high BOD values are indications of the pollution strength of the effluents. The mean BOD values of diluted effluent samples in this study were very high which ranges between 126 – 279.8mg/l in the discharge points of all three different tanneries during the dry season and 74 – 231mg/l in the wet season at the same sampling points.

The mean BOD values were however sharply decreasing down the drain in the tanneries (i.e. DP > DS > EP) especially during the wet season were the EP had mean BOD range from 26.11 – 105mg/l. However, these values are very high and are indicators of strong organic pollution of the water bodies with very high organic matter load. Several researchers reported even much higher BOD values in tannery wastewaters, Deepali, (2009) reported mean BOD range of 1425 – 1500mg/l and Kaushik,(1994) reported 600mg/l. Khawaja *et al* (1999); Koteswari and Ramanibai, (2003) also reported similar high BOD values in tannery effluent. Khawaja *et al* (1999) however reported lower BOD values in summer and winter of similar study in Ganga River, India where the BOD values range between 1.0 to 5.6mg/l.

The impact here is that high BOD and low DO content of tannery effluent will limit aerobic microbial activities such as redox reaction by organisms using O_2 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. Yusuff and Sonibare (2004) suggested that high BOD values would affect survival and proliferation of gill breathing animals of the receiving water body. Ramteke *et al.* (2010) in a similar study reported much higher BOD values of 1,520mg/L than those found in this study.

5.1.5 Seasonal variations of physico-chemical parameters in effluent samples

The seasonal variation of the pH in effluents analyzed showed that the pH were generally higher (more alkaline) in the dry season and dropping slightly in the wet season, this pattern was observed in all the samples analyzed and sampling points. The drop may be due to the increased volume of rain storm that washes and drain these wastes faster in addition to fertilizers washed during wet season which may be acidic and the rain is also acidic. Akan *et al. (*2009) did not observe any seasonal variation in pH.

Although there were slight variations in the temperature reading of samples collected and analyzed at the site of collection, no statistically significant differences ($P > 0.05$) from site to site and from tannery to tannery. These slight variations were attributed to the prevailing weather conditions, season, stage of activities in the tanneries, and the time (morning/ afternoon) of sample collection. Previous study in the same Challawa however, reported much high temperature than in this study ranging from 27.0 to 74.0^{o} C in both tanneries and textile industries (Akan, 2009) which contradicts the finding in this study where the temperature recorded range of 25.5^{o} to 31^{o}c. The temperatures recorded in this study were however within the permissible limit of WHO and USEPA of 40^{o}c for discharge of tannery effluent into surface water (USEPA, 1999). However the temperatures recorded in this study agrees with that reported by Kotaswari and Ramanibai, (2003).

The mean values of Total Suspended Solids (TSS), Total Dissolved Solids (TDS) were having ranges of 224.30 – 413.53mg/l and 93.99 – 123.47mg/l respectively. While the mean value of total solid range between 318.29 – 536.96mg/l in samples from the three tanneries studied. The amount of solids (TSS, TDS and TS) in the samples analyzed showed statistically significant differences ($P \leq 0.05$) in the two seasons with higher mean values in dry season. These variations in the amount of solids in the samples can be attributed to the amount and nature of raw materials processed, stages of processing in the tanneries at the time of sample collection and most importantly the amount of rain water during the dry season which dilutes out the solids. The findings in this study are similar to the reports of Ramteke *et al.,* (2010) that had 700mg/l TSS in untreated effluents. Other researchers also reported similar fluctuations in the amount of total solids in tannery effluents ranging from 8000 – 76,500mg/l (Dishkit and Shukla 1989; Shukla and Shukla, 1994; Shukla *et al*., 2007; Akan *et al*., 2009; Deepali *et al*., 2009) these researchers also stated that the total solids (TSS,

TDS, and TS) may increase salinity of the water and thus render it unfit for drinking, irrigation and other domestic purposes. Consumption of water with high concentrations of total dissolved solids (TDS) has been reported to cause disorders of gastro intestine track, respiratory system, coronary systems, causing miscarriage and cancer (Deepali *et al*., 2009). The low TSS, reported in this study may be attributed to the use of screen (wire mesh) in some industries to collect as much solid waste which were use as manure in some farms far away from the study site.

The DO increased significantly down the drain which has been attributed to the decrease in the TSS, TDS and TS as well as the increase in the water volume containing less organic matter from other factories and residential areas. Dissolved oxygen (DO) is the most important parameter of water quality. Dissolved oxygen was found to be very low in this study. The amount of DO in the effluent sample in this study has no significant differences ($P>0.05$) in the tanneries, but significantly varies ($P \leq 0.05$) with the sampling points and the season where wet season had higher DO in all sampling points than dry. The higher DO is also attributed to increase in the water volume and aeration caused by the turbulence of water current. BOD is the direct reverse of the DO which was found to significantly vary ($P \leq 0.05$) with the sampling points and the season where wet season had lower BOD in all sampling points than dry.

5.2 Heavy metal analyses in samples

5.2.1 Heavy metal contents of water and effluent samples

In the well water samples analyzed in this study significantly ($P \leq 0.05$) lower concentrations of the metals were detected compared to the effluents. This can be attributed to the relative distances of the wells from the pollution point source, mobility of the pollutants and other geological factors. Unlike in the effluents samples where Cr had higher mean values followed

by Cd, in the well water samples the concentrations was in the order Fe > Cr > Mn > Zn > Pb > Cu > Cd with values ranging from 0.01 - 0.73mg/L. Deepali and Gangwar, (2010) reported higher values of Cr in similar ground water of 0.93mg/L while Kannan *et al. (*2005) reported a much lower mean value 0.05ppm in ground water contaminated with effluent of dyeing unit. All these values are however, above the permissible limit of Cr in drinking water which is 0.05mg/L (De, 2002). Similarly the concentration of iron (Fe) in the well water (0.73mg/L) was within the desirable limit of 0.3 – 1.0mg/L. This finding agrees with that of Tariq *et al.*, (2006) that reported 0.003 – 1.097mg/L in ground water at tannery sites in Pakistan. Mn, Zn, Pb, Cu and Cd values were also within the prescribed permissible limits (Gomes *et al.*, 2010).

5.2.2 Heavy metal contents of Soil and Vegetables samples

In this study the soil samples had significantly ($P \leq 0.05$) higher concentrations of Cr compared to all other samples analyzed this may be attributed to continuous flow of the effluent and deposited solid wastes all year round. Highest mean concentration of Cr were found in soil samples (7.81mg/L) around the tannery wastes while lower Cr concentrations was found in the vegetable samples (0.35mg/L) during the study period. The soil serves more or less as the reservoir of these metals that accompany the effluents. All other heavy metals were found to be significantly higher in the soil samples around the tanneries except Pb and Cu that were not tested for in vegetables and soil samples respectively. Cr, Pb and Cd were below detection levels in control soil samples which indicate the negative impact of the tannery effluent in the study area.

5.2.3 Heavy metal contents of well water

The concentrations of metals decreased down the sampling points from well 1 – 3, except in the case of Cadmium that was higher in well 2 and then decreased in well 3. This may be attributed to the existence of a textile industry around the area. Textile industries are well known for discharge of higher concentrations of Cd (Gomes *et al.*, 2010). Higher values than

the prescribed limits of the metals impart taste, appearance (discoloration) and causes corrosion. The use of such bodies of polluted water can be deleterious to health and skin contact can cause skin ulcers, allergic reactions and swelling of the skin (Martin and Grisworld, 2009; Deepali and Gangwar, 2010). Pb was beyond detection limit in all wells except well 2. Similarly all the metals were beyond detection limit in control well except Zn and Fe that were found in trace amounts which points to the fact that the tanning activities impacts negatively on the well water around the study area.

5.2.4 Heavy metal pollution in effluent samples

The heavy metal concentrations in the effluent samples vary with the tanneries, sampling points (DP, DS and EP) and seasons. These variations can be accounted for by different activities, chemical dosing, and stages of operations in the tanneries, other factories and other environmental conditions in the study site. Analytical results revealed average of 4.36mg/L, 4.91mg/L and 5.29mg/L of Cr in the effluent samples of Tan C, Tan B and Tan A respectively. The significant difference observed between Tan A on one hand and the other tanneries on the other may be attributed to the nature of work, amount of raw materials processed (hides and skin), stage of operation and the chemical dosing at the time sampling. The volume of raw water used in the leather tanning and processing can also account for the variation. Deepali and Gangwar (2010) reported similar mean values of Cr level in tannery effluent (7.2mg/L) and lower mean value in textile effluent (2.38mg/L) studied in India. However, chromium level was found to be much lower (0.255mg/L) in textile effluent in Lagos Nigeria (Ugoji and Aboaba, 2004). Koteswari and Ramanibai (2003) reported higher amount of Cr in a similar study in India. In the environment Cr (III) and Cr (VI) are the most common and stable forms of the metal, however, their toxicity depends on the chemical form. Cr (VI) compounds have a toxic, mutagenic, and carcinogenic nature, whereas the trivalent forms, which prevail in foods, have low toxicity (Gupta and Sinha, 2006). Although, it is an

essential mineral nutrient for normal sugar and fat metabolism which functions primarily by potentiating the action of insulin, it can be very toxic at high concentration. Leghouchi *et al.* (2009) in an evaluation study of Cr contamination of river Mouttas in Jijel, Algeria also reported lower values than those found in this study and attributed the contamination of the river water, sediments and plants on the inefficiency of the wastewater treatment plants in the area. The authors' further stated that the level of Cr upstream before the point of intake was within the Algerian permissible limit of 0.5mg/L but downstream of the tannery, Cr concentrations were always higher (1.17mg/L) despite dilution. Khawaja *et al.* (1999) suggested that a times Chromium levels may be apparently lower because of the alkaline conditions prevailing in the effluents which can result in the precipitation of hydroxides of Chromium.

Samples from these tanneries were found to contain the average of 0.15-0.20mg/L of Zn which is also above the EPA standard guideline value of 0.01mg/L. Cadmium was found to be 0.13-0.18mg/L, these mean values are very much higher than those reported by (Dubey *et al*., 2003) (0.02mg/L); (Deepali and Gangwar, 2010) (0.018mg/L) and (Tariq *et al*., 2006) who reported 0.076mg/L in Pakistan. These concentrations also are above the level of the discharge limits although apparently low. Yet the long term exposure to lower levels of Cadmium (Cd) in air, food or water leads to buildup of Cd in the kidneys and possible Kidney disease (Deepali And Gangwar, 2010). Other potential long term effects are lung damage and fragile bones.

The analyzed tannery samples were found to contain between 0.20 – 0.26mg/L of iron. These values were found to be much lower than the values in other similar studies. Yusuff and Sonibare (2004) reported 0.45-2.14mg/L in textile effluents in Kaduna Nigeria, Tariq *et al.*

(2006) reported 0.351mg/L in tannery effluents in Peshwar Pakistan while Deepali and Gangwar (2010) reported 1.70mg/L and 0.75mg/L in textile and tannery effluents respectively. Iron (Fe) pollution of water bodies produces undesirable effects such as discolouration, turbidity, deposits, taste impairment and development and growth of iron reducing bacteria in pipes affecting the acceptability of water for domestic and other use (Das and Borah, 1983; Deepali and Gangwar, 2010)

Manganese (Mn) was found to be the second highest polluting heavy metal in the effluents with mean value of 0.46 – 0.67mg/L. These values were similar to that reported by Deepali and Gangwar, (2010) (0.57mg/L) but much higher values were reported in Nigeria of 1.67mg/L (Yussuff and Sonibare, 2004). USEPA (1988) listed Mn as the least toxic of the heavy metals analyzed in this study, but if inhaled can enter the brain in two ways; by olfactory (nasal way) which provide a direct path to brain tissue and by lung uptake that could provide a source of containing exposure and bioaccumulation (Weiss, 2006).

Pb (lead) and Cu (copper) had the least concentrations in the effluent. Pb had a mean value of 0.1mg/L in the samples. This value is lower than the 0.06mg/L reported by Deepali and Gangwar, (2010) in tannery effluent but it was below detection limit in textile effluent. Pb values in this study was also lower than what Tariq *et al.*, (2006) reported (0.646mg/L) and 0.6mg/L reported by Deepali and Gangwar, (2010) in tannery effluents. Kaushik, (2003) also reported higher levels than these; Excess Pb affects central nervous system (CNS), particularly in children and also damages kidneys and immune system (Deepali and Gangwar, 2010). In the area children can be seen all year round swimming, playing, hawking or doing other activities in the study sites. The recent incidence of illegal mining of Pb in Zamfara state is still fresh in the minds of relatives, residents and the country at large. Cu was

the least polluting metal in the samples analyzed ranging from 0.03 – 0.05mg/L. Like other metals several researchers have reported varying degree of concentration Cu (Yusuff and Sonibare, 2004; Deepali and Gangwar, 2009; 2010). Cu is an essential element in mammalian nutrition as a component of metallo-enzymes in which it act as electron donor or acceptor. However, exposure to high levels of Cu can lead to adverse health effects.

5.2.5 Variations of heavy metal pollution in soil and vegetable samples

Heavy metal pollution of the tannery effluents contaminated soils were significantly different ($P \leq 0.05$) in the wet and dry seasons. Higher levels were recorded during the dry season in all the three locations. The lower levels in the wet season can be attributed to the fact that the rain washes significant amount of the metals downstream away from the study site, leaching can also be a very important factor during the season. Cr levels were higher than other metals analyzed and decreases in Cr > Fe > Mn > Zn > Cd > Pb. Cr concentrations in the dry season of soil 1 and 2 (10.2mg/Kg and 10.7mg/Kg) are not statistically different ($P > 0.05$) but significantly different from wet season Cr level in the same site. Similarly overall level Cr in soil 1 and 2 that were not significantly different ($P > 0.05$) in the wet season may be attributed to the water logged nature of the terrain in the same season. However these levels (in soil 1 and 2) in both seasons are significantly different from the Cr levels in farm soils where vegetables are irrigated downstream this is because the level of metals in the effluent was reducing downstream. The levels of Zn, Cd, Fe and Mn are not significantly different ($P > 0.05$) in soil1 and 2 of the same season. But all heavy metal concentrations in the dry season samples were higher than in wet season samples.

Vegetables constitute essential component of the diet by contributing protein, vitamins, iron, calcium and other nutrients. Vegetables can take up high amount of essential and toxic metals from contaminated soils and irrigation water. The essential metals are required for the growth

of the plants in small quantity and excess concentrations may cause toxicity (Gupta and Sinha, 2006). In this study varying concentrations of these metals were found in the vegetables examined from farms irrigated with mixtures of effluents.

In this study tomatoes were found to contain lowest concentration of all the metals and Cu was completely below the detection limit in tomatoes examined. Gupta and Sinha, (2006) reported higher value of Cr (32.5mg) in tomatoes and lowest value in potatoes. Onions and carrots had highest level of Cr and Mn uptake. The mean Cr levels were 0.53mg/kg and 0.52mg/kg respectively, while Mn levels in these vegetables were found to be 0.61mg/kg and 0.58mg/kg respectively. The mean Cd levels were found to be similar in onions and carrots (0.30mg/kg). The higher uptake of these heavy metals by onions and carrots can be attributed to their direct contact with soil. As Sen *et al*. (1987) reported that accumulation of Cr in plants mainly occur in the roots.

Iron (Fe) and Manganese (Mn) were found to be higher than Cr and Mn in lettuce and sugar cane samples analyzed. Lettuce had 0.28mg/kg Cr, 0.20mg/kg Cd, 0.71mg/kg Fe and 0.42mg/kg Mn. The sugar cane also had 0.28mg/kg Cr, 0.15mg/kg Cd, and 0.98mg/kg Fe and 0.53mg/kg Mn. Lead (Pb) was found beyond detection limits in all vegetable samples examined.

Barman *et al*. (2000) studied the accumulation of metals in vegetables irrigated with tannery wastewater after treatment and reported much higher values of Cr accumulation than those found in this study. Similarly Sinha *et al*. (2006) reported higher values in a similar study. However, Santos *et al*. (2004) had lower values in samples collected from different sources in Rio dejaneiro, Brazil. Cereals and vegetables were also reported to accumulate Cr up to 0.007

to 0.46µg/g fresh weight (Lendinez *et al*., 2001). Higher Cr bioaccumulation in vegetables grown around tannery wastewater were also reported in India than those found in this study where tomatoes had 32.5-108mg/kg, cabbage 40mg/kg, spinach 30.4mg/kg, cucumber 17.6mg/kg and so on. The finding in this study also contradicts the much higher values reported by Leghouchi *et al.* (2009) in *Agropynum repens* growing on the river bank in Jijel Algeria. The bioaccumulation of metals in vegetables grown in soil contaminated or irrigated with industrial waste has been well documented (Sinha *et al*., 1996; 2005, 2006; Barman *et al*., 2000; Lendinez *et al*., 2001; Gupta and Sinha, 2006; Leghouchi *et al*., 2009)

Akan *et al* (2009) reported that though there were several tanneries in Kano, Nigeria, only two had functional primary treatment plant (PTP). They also reported that the current practice is the discharge of effluent in the streams or canals after the retention for some hours in the stabilization ponds without secondary or tertiary treatments. In this study we cross checked these and found that as at August 2010 even those two PTP had developed technical faults and were not functional. Hence the trend earlier reported by (Akan *et al*., 2009) is simply the discharge of these effluents into the environment without treatment, in some tanneries without even the retention in the stabilization ponds. Instead of having a common effluent treatment plant(s) as obtainable in some cities the world over, the Challawa had common effluent collection ponds that are always full (around the clock) overflowing into the river and its tributaries (Plate 2.1). Bishnoil *et al.* (1991) reported that diluted effluents could be use as a liquid fertilizer not regarding the inherent danger of bioaccumulation of the heavy metals in vegetable. Barman *et al.* (2000) studied the accumulation of metal in the economically important crops and vegetables irrigated with tannery wastewater and reported that the accumulation of metal from soil /water to plants does not follow any particular pattern rather varried with respects to metal, species of plant and parts. However,

Babyshakila and Usha **(**2009) recommended that tannery effluent should be used for irrigation at a lower concentration.

5.3 Microbiological analyses of samples

5.3.1 Bacteriological quality of well water samples

Bacteriological counts of well water samples in the study sites revealed variations in heterotrophic counts which was increasing with distance from the pollution source and dcreasing concentrations of the metals. Similarly, coliform and *E. coli* counts in the well water samples followed the same trend. This increase in counts with distance from pollution source can be attributed to the impact of the metal toxicity and other pollution impacts on the survival and proliferation of alloctonous and authoctonous bacteria in the environment. No significant differences in the total heterotrophic and total coliform counts in well 1 and well 2 may be due to the similar concentrations of the metals in the well water samples. But significant difference was observed in well 3 can be attributed to other environmental conditions and human activities at the site. Faecal streptococcal count showed significant difference among the wells; while *E. coli* count in well 2 and 3 are similar there is variation in the counts of samples from well 1. The overall bacterial counts in these wells where generally higher in control well where significant differences ($P \leq 0.05$) were clearly indicated in comparison to the well water samples around the tanneries the lower counts in the polluted wells may be attributable to the selective pressure on the bacterial organisms because of metal toxicity and growth inhibition.

Bacterial load in the well water samples were significantly higher in the dry season than wet season. This may be due to lower level or volume of water in the wells which may concentrate the organisms. There may also be lower level of inflow of the pollutants into the wells during the dry season. Unlike the heavy metal concentrations that were declining from

well 1 to well 3, the bacterial load was increasing with increasing distance from the pollution source regardless of the season, although was more clear in the wet season. Faecal streptococci and *E. coli* count of well 1 in the wet season were significantly lower than counts in well 2 and 3 of the same season. This may also be attributable to the selective pressure on the bacterial organisms because of metal toxicity and growth inhibition which is declining with distance and diminishing levels of the metals.

5.3.2 Bacteriological contaminations of effluent samples

Bacteria are biological indicators of pollution, the total and fecal coliform are the most widely used. Bacteria of fecal origin can indicate the presence of pathogens characteristic of warm blooded animals (Gouses *et al.*, 2010). The presence of high heterotrophic aerobic plate count is also an indication of the presence of different organic carbon source in the samples that can be degraded by the organisms.

Coliform organism released by human and animal were estimated to be about 100-400 billions per day. They are discharged into wastepipe, wastewater, natural waters and soils that receive recent faecal contamination. The waters for irrigation of vegetables and fruits that are grown in this vicinity and are consumed raw must not be polluted (CONAWA, 2005) as sited by Gomes *et al.* (2010).

Water will be considered satisfactory when 80% of samples collected in five weeks in the same place, present a maximum value of 1,000 faecal coliforms (thermotolerant) or 800cfu/100ml of *E. coli* or enterococcus 100cfu/100ml enterococcus, Gomes *et al.* (2010). Comparing the result obtained in this study indicates that all the effluent samples analyzed exceed the permissible limit of bacteriological contamination of the wastewater. However,

farmers within the study area use this water for irrigation of all vegetables in the dry season, despite the fact that the water is not suitable for irrigation and recreational activities.

The heterotrophic bacterial counts, total coliform and fecal coliform counts increased with decrease in the metal pollution level in the samples especially Cr concentration. This may have been due to the inability of sensitive organisms to grow on higher level of Cr and other heavy metal concentrations. Similar declines in bacteria populations (CFU) in Cr contaminated samples were reported by Luli *et al* (1983); Losi and Frankkenberge (1993) and Basu *et al*. (1997). Basu *et al* (1997) attributed Cr toxicity to its rapid permeability through biological membranes and subsequent interaction with intracellular proteins and nucleic acids.

In the present study, no attempt was made to determine the mechanism of heavy metal tolerance in these microorganisms. However, chromate and other heavy metal tolerance in bacteria have been reported to include reduction, methylation, precipitation at the cell surface, cellular uptake by altering uptake pathway and removal from the cytoplasm by efflux pumps (Lovely and Philips, 1994; Basu *et al.*, 1997). At high levels, heavy metals like chromium damage cell membranes, alter enzymes specificity, disrupt cellular functions and damage structure of DNA (Bruins *et al*., 2000). Basu *et al*. (1997) also reported that the tannery sediments provide an enriched Cr-contaminated environment where Cr-resistant bacteria could be isolated suggesting that if mechanisms of chromate-resistance are worked out in detail can be used as a possible tool of bioremediation.

In a study by Ramteke *et al.* (2010) to estimate the bacteriological contamination of tannery effluents in common effluent treatment plant (CETP), Bacterial indicators such as heterotrophs and total coliforms were significantly reduced to less than 90% from the initial counts in treated effluent. Perhaps this may be another reason for the decline in bacterial load

downstream found in this study because of self purification, dilution factor and organic matter degradation as well as nutrient depletion. Ramteke *et al.* (2010) reported complete elimination of fecal streptococci and fecal coliforms in the treated effluents. This contradict the finding in this study because fecal coliforms were detected in all effluent samples collected and agreed with the findings of Verma *et al.* (2001) who also detected heavy metal tolerant heterotrophs and coliforms in tannery effluents.

Researchers have documented the detection of pathogenic bacteria in raw and treated effluents and the presence of heavy–metal-resistant pathogenic strains in the environment particularly in water bodies is a situation that requires the highest attention (Verma *et al.*, 2002a; 2002b; Gomes *et al.*, 2010; Ramteke *et al.*, 2010). It was observed that chromium resistant strains of *E. coli* transfer their plasmids in wastewater under wide range of conditions (Verma *et al.*, 2002). As such the environmental spread of such pathogens may have serious effect on public health therefore, proper disinfection of treated effluents is necessary before its safe discharge in the environment (Ramteke *et al.*, 2010).

5.3.3 Principal component analysis

Principal component analysis comparing parameters in the tannery effluent showed different relationship among the parameters analyzed in the samples. Factor 1 showed significant positive relationship between the heavy metals Cr, Cd, Cu, and Pb concentrations and the tannery effluent samples. These showed that the effluent is the major source of these polluting metals, the metals were closely related to one another but with negative relationship to BOD, TSS, TDS, Mn, Fe, EC and Fungal count which were also closely related to one another. Heterotrophs, coliforms and feacal coliforms were also closely related to one another. The heavy metals Cr, Cd, Cu, and Pb concentrations had negative impact on the Heterotrophs, coliforms and feacal coliforms. Similarly the BOD, TSS, TDS and EC had

negative impact on DO regardless of the season or even months. The pH, temp and month of collection made no significant contribution to the principal component analyses. The same analysis comparing parameters in the well water samples showed different relationship among the parameters analyzed in the samples. Factor 1 showed significant positive relationship between Heterotrophs, coliforms and feacal coliforms counts and the well water samples; on the other hand factor 2 showed the heavy metals were closely related to one another but with negative impact on Heterotrophic, coliform and feacal coliform counts which were also closely related to wells. Similarly the BOD, TSS, TDS and EC had negative impact on DO change with the season. The pH also made no significant contribution to the principal component analyses.

5.3.4 Microbiological quality of effluent contaminated soil

The bacterial counts (total plate counts, coliform and faecal streptococci counts) were significantly higher during the wet season in the soil samples which is attributed to lower level of metals, high moisture content and other favourable growth conditions. The mean fungal counts in the soil samples were significantly different in the wet and dry seasons, unlike the bacterial counts; the mean fungal counts were higher in dry season than wet season. No significant difference among the soil samples analyzed. Principal component analysis comparing parameters in the soil samples shows different relationship among the parameters analyzed in the samples. Factor 1 showed significant positive relationship between Heterotrophs, coliforms and feacal coliforms counts and the soil samples; on the other hand factor 2 showed the heavy metals were closely related to one another but with negative impact on Heterotrophic, coliform and feacal coliform counts.

5.4 Laboratory Scale treatment of effluent

Chemical processes are commonly used to remove heavy metals from wastes. Such methods have several disadvantages, for instance, unpredictable metal ion removal, high reagent requirements, and the generation of toxic sludges (Ciba *et al*., 1999). Significant proportion of soluble matter in the effluent may not be removed by such physico-chemical treatment. There is always the need for further treatments prior to discharge in order to achieve higher quality treated water (Aboulhassan *et al*., 2008). Recently, microbial bioremediation has emerged as an alternative technique to such traditional chemical treatments (Brierley, 1990; Compos *et al*., 2008; Benazir *et al.,* 2010). Microorganisms such as bacteria, fungi, algae and actinomycetes have effectively sequestered heavy metals (Wong and So, 1993). These have been used to remove metals from polluted industrial and domestic effluents on a large scale. Microbiological detoxification of polluted water is economical, safe, and sustainable (Rehman *et al*., 2006).

As earlier stated the tanneries in the study site had neither functional treatment plant nor common effluent treatment plant. Thus, there is need for a simple and inexpensive treatment process to reduce the level of environmental pollution. Aboulhassan *et al* (2008) suggested that biological treatment, as a cheap process is always needed to be incorporated for maximum treatment.

In this study, bacterial and fungal isolates were found to be metal tolerant and can be used to treat or reduce Cr and other heavy metal contaminating effluent by biosorption and bioaccumulation (Srinath *et al.,* 2002a; Turpeinen *et al.,* 2002; Teitze and Mathew, 2003), or by transformation of heavy metals, entrapment in extra cellular capsules, protein DNA adduct formation, induction of stress, transformation of components by oxidation, reduction, methylation and demethylations and by binding cystolic molecules (Ksheminska *et al*., 2003;

Compos *et al*., 2005; Benazir *et al*., 2010; Ramteke *et al*., 2010). Biosorption appears to be an economically feasible means for the removal and/or recovery of heavy metals from industrial wastewaters. The low cost of biosorbents is a tangible advantage over other technologies, such as ion exchange and reverse osmosis (Sharma *et al*., 2009). Therefore, bacterial and fungal isolates as well as their respective consortia were used in a laboratory scale to treat the effluent.

5.4.1 Bacterial effluent treatment

The bacterial isolates *Pseudomonas aeruginosa* and *Bacillus cereus* reduced the level of Cr in the effluent under aerobic conditions. This may be attributed to the presence of chromium reductases. *P. aeruginosa* treatment using aerated vessels with aeration pumps appeared to be more efficient ($P \leq 0.05$) laboratory scale treatment with overall Cr level reduction rate of 34.6% using single bacterial organism. The bacterial consortium culture at the initial stage was less effective in Cr removal from the effluent than individual organism using aerated vessels, where 22.7mg/L Cr was found in the effluent after 48hr treatment in the shake culture which was higher than concentrations in other flasks after similar treatment. However, it stabilized after 72hr treatment and finally 15.3mg/L Cr was left in the effluent after 120hr treatment, indicating 38.7% Cr removal from the effluent, making the consortia more efficient than individual organism. Generally the use of the aerated vessels appeared more efficient in these treatments except where *Bacillus cereus* was used for the treatment in conical flask- shaker method and removed the Cr more effectively than in the vessels. James (2002) reported chromium VI reduction by *Bacillus coagulans*, while Srinath *et al.* (2002a) also demonstrated the accumulation of Chromium VI by *B. circulans.* Removal of Chromium by *P. aeruginosa* was reported by several researchers (Meller *et al*., 1996; Jin, 2000). Wang *et al.* (1990) attributed these reduction to the presence of chromium reductase gene. Campos

et al. (2005) reported that *Pseudomonas* sp reductase enzyme acts extracellularly when Cr(IV) reduce to Cr(III) and when taken up intracellularly cannot be removed from cells as long as their membranes remained intact. Other researchers also reported that the formation of insoluble Cr III on the cell surface was thought to offer protection against Cr(VI) toxicity (Wang *et al*., 1990; Shen and Wang 1993). Ohtake (1987) reported that although bacterial strains were able to reduce and tolerate chromate, these two properties may be unrelated because it is still not very clear whether the chromate reduction ability found in several bacteria species confered resistance to chromium. This ability to reduce chromate may be a secondary activity of a soluble reductase enzyme with a different physiological role (Compos *et al*., 2005). However, the accumulation of other heavy metals such as uranium by *Pseudomonas aeruginosa* in its cell can also be attributed to its tendency to remediate chromium. There are also reports on biosorption that mainly involve cell specific complexation, ion exchange and microprecipitations (Figveria *et al.,* 1999; Benazir *et al.,* 2010). Several other bacterial species were reported to have the potential of remediating Cr and other heavy metal polluted wastewater such as *Serratia marcesens* (Campos *et al*., 2005).

Ramteke *et al.* (2010) in a bioremediation study noted that 10.8% of microbial isolates from effluent showed the ability to reduce chromate by greater than 90% and less than 10% of the isolate were found with no bioremediation potential. Srinath *et al.* (2002b) also reported similar result and noted that biological reduction of Cr (VI) usually occur in a neutral pH range. This offers a potential cost-effecctive remediation strategy. Gram positive, chromium resistant bacteria strains (ATCC 700729) were isolated by Shakoori *et al.* (2000) who checked their potential dichromate reduction potentials and reported more than 87% reduction of dichromate within 72hours. The feasibility of the use of different bacterial strains for bioremediation of polluted effluents cannot be over emphasized.

5.4.2 Fungal experiment and effluent treatment

In the experimental study the fungi *Aspergillus niger* (TN1A31), *Penicillium* sp (TN3P09) and *T. longibrachiatum* TN1X03 (TN3) as well as the consortia showed different abilities in Cr removal from the minimal medium at different Cr concentrations and pH (6, 7 and 8). The removal of Cr from the medium inoculated with *Aspergillus niger* (TN1A31) (*Asp*) was observed to be within similar range in all pH at the initial stage 0-24hr. But after 48hr of incubation with shaking and agitation the removal of Cr increased rapidly between 48-72hr and then declined 96-120hr of shaking. It was also observed that pH 6 was the optimum for the removal of Cr as the time progressed. The Cr removal then attained average after 72hr treatment in all the concentrations (65% residual Cr) at all pHs, while between 96-120hr of the experiments, medium containing Cr concentrations of 20 and 25mg/L the removal peaked leaving behind 42.4% and 39.5% respectively at pH 6.

Similar pattern was observed in the removal of Cr by *Penicillium* sp (TN3P09) (*Pen*) which was more efficient at pH 6; however the metal was more efficiently removed from the media containing 10 and 20mg/L of Cr after 120hr incubation with 36.2% and 39.0% residual Cr in the media. There was also no remarkable reduction in Cr level at the initial stage of the experiment, nevertheless after 48hr of incubation with shaking and agitation the removal of Cr increased rapidly between 48-72hr.

The removal of Cr by *Trichoderma longibrachiatum* TN1X03 (TN3) appeared more efficient than *A. niger* TN1A31 and *Penicillium* sp TN3P09 because after 48hr incubation with constant agitation about 37-40% of the Cr in the medium was removed (in flask with pH 6 and 7 and Cr concentrations 10 – 25mg/L). Removal of Cr in culture inoculated with TN3 also showed that pH 6 as the optimum pH for bioadsorption of Cr. The metal removal efficiencies were decreasing with increasing pH. Similarly the metal was more efficiently

removed from the media containing 10, 20 and 25mg/L of Cr after 120hr incubation with 37.2%, 36.4% and 37.4.0% residual Cr in the media respectively.

Like the bacterial consortium, fungal consortium used in this study was found to be much more promising. The bioaccumulation potential of the consortiaor the mean uptake of Cr in the media with pH 6, 7 and 8 after 72hr were 44.4%, 40.7% and 38.0% respectively. The metal was more efficiently removed from the media containing 10, 20 and 25mg/L of Cr after 120hr incubation with 63.6%, 64.3% and 63.3% Cr removed from the media respectively. The fungal consortium exhibited synergistic effect in the Cr removal from the effluent.

The Cr removal ability of the test organisms in the cultures generally increase from 48 – 72hr. Statistical analyses (ANOVA) comparing Cr removal by fungal sp at varying pH showed significant differences ($P \leq 0.05$) with higher efficiency at pH 6 in most media inoculated with the fungal sp.

The fungal consortium was found to be more efficient than individual organism, while TN1X03 (TN3) was more efficient than *Aspergillus niger* (TN1A31) (*Asp*) and *Penicillium* sp (TN3P09) (*Pen*). However 38% of the total Cr concentrations were still left in the medium. As earlier mentioned the consortia appear to be the most suitable candidate for bioremediation of Cr containing effluent.

It is documented that pH is an important parameter affecting biosorption of heavy metals (Onyancha *et al.*, 2008; Sharma *et al*., 2009). The effect of pH on Cr biosorption was studied in the range 6–8. This pH range was chosen based on studies reported in the literature (Morales-Barrera *et al.,* 2008; Onyancha *et al*., 2008; Pramod *et al*., 2008; Sharma *et al*., 2009) and the mean pH of the effluents collected in this study.

The Cr-removal from the experimental cultures at the initial stage (0 – 24hr) was similar in all pH ranges and with different test organisms. However, the removals of Cr in the cultures were clearly more efficient at pH 6. Low pH favoured Cr reduction by organic matter as well as adsorption by microorganisms (Morales-Barrera *et al.*, 2008). In this study as the time increased the efficiencies of Cr removal became more prominent at pH 6 compared to 7 and 8. It was also observed that Cr concentrations decreased as increase in cell numbers progressed. Different concentrations of Cr were also detected in the biomass produced in these experiments indicating adsorption and bioaccumulation of the metal by these test organisms. However, the concentrations were inconsistent while the inability of 100% detection of the concentrations of Cr in treated effluent and biomass may be attributed to the organisms ability to reduce the most stable forms of Cr (Cr IV and Cr III) to less toxic forms of lower valence (Moralles-Barerra, *et al*., 2008). The Cr-removal was more efficient using stream/river simulated aerated vessels connected with aquarium pumps constantly pumping air into the effluent treatment setup to improve the DO. However, the difference in removal of the metal was observed after 72hr of inoculation and aeration of the effluent in the vessels. Similarly microbial consortia was more efficient in the effluent treatment with only 7.94mg/L of Cr left after 120hr followed by *Aspergillus niger* (TNA31) (*ASP*) and *Trichoderma longibrachiatum* TN3. The reduction in Cr level in the control uninoculated experiments showed higher reduction in level in the aerated vessels than in flasks.

The results in this study indicates that the decrease in Cr concentration in experimental cultures with viable strain can be attributed to different factors including adsorption to fungal cell mycelia, reduction by medium components, pH changes that occur during fungal growth and biological activity of growing cells that enhances Cr reduction. Other researchers reported the capabilities of *Aspergillus* sp *and Penicillium* sp in the biological reduction of Cr

in different medium (Gouda, 2000; Acevedo-Aguilla *et al.*, 2006). Total chromium levels compared to those of uninoculated controls were found to contain between 22.0-23.5mg/L (More than 94%) of the chromium initially added into the experimental laboratory media while the uninoculated tannery effluent had between 23.5-24.3mg/L (97%) of the initial Cr concentration. In the Heat-killed (autoclaved) control, the loss in Cr content was attributed to Cr reduction by medium components and adsorption on fungal biomass. This result however, contradicts the findings Morales-Barrera *et al.* (2008) who reported that no measurable changes in Cr concentration were detected after 200hr of incubation of *Tricoderma inhamatum* killed and cell free controls.

Chromium ions were found to bind more strongly as the pH was increased from 3 to 5 and decreased from 9 to 6. The pH dependence occurs when metal ions and protons compete for the same active metal binding sites such as carboxylate or amine groups on the cell surface (Onyancha *et al*., 2008; Vankar *et al*., 2008). The functional groups act as binding sites for the metal ions. The experimental Cr removal or adsorption as well as effluent treatment studies indicated various results and capabilities of the test fungi under different conditions.

5.4.3 Fungal effluent treatment

The fungal isolates performed more efficiently in the removal of Cr than the bacterial isolates. Similarly the mutant fungal strains had an improved performance in Cr removal as well as biomass production. Gupta and Ahuja (2002) reported that the chromium remediation by fungi and yeast particularly might be due to the excellent potential of metal biosorption. Total chromium levels compared to those of uninoculated controls were found to contain between 22.0-23.5mg/L (More than 94%) of the chromium initially added into the experimental laboratory media while the uninoculated tannery effluent had between 23.5-24.3mg/L (97%) of the initial Cr concentration. In the Heat-killed (autoclaved) control, the

loss in Cr content was attributed to Cr reduction by medium components and adsorption on fungal biomass.

Orbital shakers and aerated vessels were used to determine the ablity of the test organisms to treat the tannery effluent containing 25mg/L Cr at a pH of 6. The pH 6 and 25mg/L concentration chosen for the effluent treatment study was also based on the initial experiments and the literature as stated earlier. About 95% of the initial Cr was still found in the effluent after 24hr treatment with the test organisms. Comparison of adsorption/removal capacities among the fungal strains and consortia in this study shows that consortia have higher removal or adsorption capacity than individual organism followed by *Trichoderma longibrachiatum* (TN1X03) (TN3). After 48hr of effluent treatment in the conical flasks on the orbital shaker the consortia adsorbed 4.8mg/L Cr from the effluent and the adsorption rises to 11.9mg/L and 15.8mg/L after 72 and 96hr respectively. Finally 16.2mg/L of the Cr was effectively removed from the effluent by the consortia. The Cr removal efficiency in the treated effluents by TN3 was higher than that in *Aspergillus niger* (TN1A31) (*Asp*) and *Penicillium* sp (TN3P09) (*Pen*). These test strains adsorbed 15.0 mg/L (ASP), 14.6 mg/L (PEN) and 15.7mg/L Cr from the effluent after 120hr treatment in the conical flasks on the shaker. The uninoculated control flask had (24.3mg/L Cr) about 97% of the initial concentration of Cr still after 120hr treatment.

The efficiencies of effluent treatments by the test strains in the aerated vessels were slightly higher in comparison to the shake flask methods. *Aspergillus niger* (TN1A31) (*Asp*) adsorbed 15.7mg/L compared to 15.0mg/L Cr in shake flasks; *Penicillium* sp (TN3P09) (*Pen*) adsorbed 14.9mg/L Cr compared to 14.6mg/L Cr. No significant variation was observed in the Cr uptake by *Trichoderma longibrachiatum* (TN1X03) (TN3) in both shaker and vessels (15.7mg/L and 15.5mg/L) were removed respectively. The major difference was observed in

the effluent treated with the fungal consortium were in the vessels 17.1mg/L Cr was removed after 120hr of treatment while only 16.2mg/L was removed from effluent treated in the flask.

As described in chapter three, efficient test organisms were subjected to mutation by Ultraviolet light (UV) (250nm) at different time intervals of 10, 30, and 60, to improve their Cr removal capabilities. Some mutant strains (MS_{UV30} (*Aspergillus niger*) and MS_{UV10} (*Penicillium* sp) performed better than their respective wild parent strains while mutant MS_{UV60} strains did not performed as efficient as the wild parent strains this may attributed to deleterious mutations in the mutant strains. Similarly the concentration of Cr in the effluent treated with mutant strains MS_{UV10} (*Penicillium* sp) (TN3P09) for 30min was lower than those treated with (MS_{UV30} (*Aspergillus niger*) (TN1A31) and MS_{UV30} *Trichoderma longibrachiatum* (TN1X03) respectively. This difference was more conspicuous after 48hr treatment. MS_{UV10} (*Aspergillus niger*) (TN1A31) performed better in the treatment compared to MS_{UV10} (*Pen*) and MS_{UV10} (*Trichoderma longibrachiatum*). These organisms lost Cr removal ability after being exposed to UV for 60min because less than 40% of the Cr was removed by the mutant MS_{UV60} from the treated effluent which shows that the ability to remove Cr has started degenerating. Sekar *et al.* (2009) reported that UV mutation supports and retards the growth of pure strains depending on the nature of the strain. But the finding here contradicts that of Sekar and coworkers who found in their study that UV mutation has no effect on consortia of halotolerant bacterial isolates in the degradation of tannery soak liquor. However, they concluded that nitrous acid mutation aids the growth and adaptation of halotolerant bacterial strains of *Pseudomonas sp* compared with UV-mutated strains.

5.4.4 Fungal effluent treatment and biomass production

It was generally observed in course of effluent treatment that the growths (weight) of fungal isolates and the consortia was observed, the weights of the biomass were increasing with

decreasing Cr level in the treated effluent using both methods. The results showed that decreased Cr level in the filtrate from the residue was dependent on time and can be attributed to bioaccumulation and adsorption of Cr by increasing fungal mycelia over time. The finding in this study agrees with the finding of Srivastava and Thakur, (2006). These authors reported that the bioaccumulation of heavy metal ions in fungal biomass is affected by specific surface properties of the adsorption medium as well as metal ion concentration, temperature, pH and the amount of biosorbents. The fungal cell wall is mainly composed of polysaccharides, some of which may have associated protein, lipid and melanin. These biomolecules on the fungal cell wall components have several functional groups such as amino, carboxyl, thiol, sulfhydryl and phosphates. "Biosorption" is a process in which materials of natural origin, such as micro-organisms (living or dead) are employed for sequestration of heavy metals from an aqueous environment. Metal ions in solution interact with the biosorbent and hence are sequestered. The mechanism of metal ions uptake from solution can be due to physical sorption, complexation with microbial cell surface groups or bioaccumulation. This biosorption could occur through interactions between metal ions and functional groups of the cell wall biopolymers of living and dead organisms. The major functional groups on biopolymers which act as binding sites include carboxylate (–COO−), amide ($–NH_2$), thiols (–SH), phosphate (PO_4 3−) and hydroxide (–OH) (Onyancha *et al.*, 2008). Moralles-Barerra *et al.* (2008) in a similar study with viable *Trichoderma inhamatum* also reported that pH favour Cr reduction by organic matter and adsorption by microorganism. They attributed the reduction of Cr by *T. inhamatum* to adsorption and biological activity of the growing cells. The ability of filamentous fungi to reduce Cr concentration has been reported in other genera such as *Phanerochaeta* (Pal, 1997); *Rhizopus nigricans* (Kodgej and Pavco, 2001) *Streptomyces sp* (Sharma and Goyal, 2009) *Aspergillus*

sp and *Penicillium* (Gouda, 2000; Acevedo-Aguillar *et al.,* 2006) *Trichoderma sp* (Srivastava and Thakur, 2006; Moralles-Barerra *et al.,* 2008; Vankar *et al*., 2008).

The fungal biomass produced in treated effluent using shake flasks inoculated with wild parent strains at 24hr, *Aspergillus niger* (TN1A31) (*Asp*) produced net 2.3mg/L of the fungal biomass. Then there was remarkable increase in the biomass to 7.4mg/L after 120hr, while *Penicillium* sp (TN3P09) (*Pen*) at the beginning of the treatment produced 2.5mg/L of the biomass, before rising slightly 48hr of treatment and finally producing 7.1mg/L in the whole process. *Trichoderma longibrachiatum* (TN1X03) (TN3) and the consortia produced near equal biomass of 7.5 and 7.8mg/L in the treatment process respectively. Fungal biomass produced in treated effluent in aerated vessels using wild parent strains were higher than those produced in the shake flasks. *Aspergillus niger* (TN1A31) (*Asp*) biomass rises from 2.6mg/L in 24hr to 7.1mg/L in 96hr and finally to 8.0mg/L when 120hr treatment lapsed. *Penicillium* sp (TN3P09) (*Pen*) also rose from 2.5mg/L to produce only 7.3mg/L as the final biomass in the treated effluent. While the fungal consortium of the three organisms produced 2.9mg/L of biomass in 24hr and then increased at the phase with *Trichoderma longibrachiatum* (TN1X03) (TN3) producing 7.8mg/L at the completion of the treatment. TN1X03 (TN3) produced biomass as much as that produced by the consortia.

The mutant strains (MS_{UV30}) in the aerated vessels like the Cr removal efficiency *Penicillium* sp (TN3P09) (*Pen*) produced larger biomass of 2.2mg/L in 24hr than *Aspergillus niger* (TN1A31) (*Asp*) and *Trichoderma longibrachiatum* (TN1X03) (TN3). The biomass however increased in effluent inoculated with *Penicillium* sp (TN3P09) (*Pen*) and *Aspergillus niger* (TN1A31) (*Asp*) to 6.3mg/L after 72hr of treatment.

It was also observed in this study that Cr removal in the treated effluent decline after certain amount had been adsorbed. This may attributed to Cr toxicity and set in of the stationary and death phase in the culture. The stationary and death phase have effect on microbial enzymatic activity and growth (biomass production) which also declined. This pattern may be an indication of a finite Cr removal capacity per cell (Wand and Shen, 1997). The finite Cr reduction capacity is defined as the amount of Cr a unit biomass can reduce after a particular incubation time when reduction approaches zero.

In this study Cr removal was observed to be highly dependent on the biomass (fungal growth), time of incubation and organisms used in the treatment with the consortia having higher potential of Cr removal than individual organism

5.5 Conclusion

The tanning industry is one of the major consumers of water and most of it is discharged as wastewaters, which contains high amounts of Cr and other heavy metals along with high biochemical oxygen demand BOD, TSS, TDS and very low DO. Based on the finding of this study it can be concluded that the effluent discharged from the tanneries is contaminated and had significant contributions in the pollution of the streams, rivers and other water bodies since large amounts of effluents are discharged without any treatment, with high pH, BOD, TSS, TDS and very low DO as well as high heavy metal contents all exceeding the values of the standard FEPA guidelines. The presence of chromium and other heavy metals in industrial wastewater poses serious problems when discharged into water bodies due to its constant deposition, which affect surface and ground water quality through seepage as well as their accumulation in vegetables grown in the studied site. The Challawa River receives waste from these tanneries, however, tanneries are not the only industries responsible for pollution of the environment, because Cadmium was found to be higher downstream (DS)

points and was attributed to textile effluent discharged in nearby factories. The high BOD, TSS, TDS, high total coliforms, faecal coliforms etc were also contributed from other industrial discharges, urban and municipal wastewater, other human activities and agricultural run-offs from farming communities along its course.

Removal of chromium from wastewaters is obligatory in order to prevent or reduce water pollution. Legislation by different governments, demands the concentration of Cr in discharges be less than 0.5 mg L^{-1} regardless of its oxidation state. This has lead to the need for removal of chromium from wastewaters before discharge. However, the direct discharge of untreated effluent or the current methods being employed such as chemical precipitation are not feasible to reduce the concentration to the desired levels of 0.5 mg/L Cr. Using various micro-organisms as biosorbents for chromium removal, offers a potential alternative to existing methods for its recovery from industrial wastewaters and is subject to extensive studies.

This study has established the role and different efficiencies of indigenous bacterial and fungal species in the adsorption, accumulation or removal of Cr in the treated effluent, showing that biological reduction of chromate is good candidate technology for low cost bioremediation, especially in developing countries, where discharge of industrial wastes poses environmental threats. The study also provides a foundation for the implementation of indigenous technology for rapid and effective removal of Cr from polluted water as well as bioremediation with biostimulation and bioaugumentation for wastewater treatment.

5.6 Recommendations

1. There is the need for proper treatment of effluent to meet the standard guidelines for wastewater discharge with regards to the heavy metals and other physicochemical

parameters such as BOD, DO, TSS, TDS, in order to avoid water and environmental pollution

2. The direct discharge of untreated effluent or the current methods being employed such as chemical precipitation are not feasible to reduce the concentration to the desired levels of 0.5 mg/L Cr thus the need to be augmented with biological techniques
3. The tanneries in the area should provide/construct a Common Effluent Treatment Plant (CEPT) as is obtained elsewhere to minimize cost of treatment and meet the standard discharge guidelines.
4. Legislation by different governments, demands the concentration of Cr in discharges be less than 0.5 mg/L regardless of its oxidation state. This should be strictly followed and offenders should be sanctioned or punished as appropriate
5. There is the need for further studies to establish more environmental, health, economic and other impacts of these wastes to the receiving communities.
6. It is also recommended that the levels of these heavy metals be determined in human blood and urine samples from the population group associated with the study site to determine risk and impact on human (this include employees of the tanneries, farmers working downstream, men fetching and selling sandy soil on the river bed and inhabitants of the adjacent communities consuming water and vegetables produced in the area.
7. The river serves as fishing, farming and water supplies for the communities in the area and beyond (up and downstream). Hence, there is the need for further studies to establish more environmental, health, economic and other impacts of these wastes to the receiving communities.
8. Further studies from laboratory scale should be carriedout on the experimental/industrial scale treatment of the effluent using these organisms (isolated from the study sites), starting from pilot to industrial scale.

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Appendix I

Appendix I provide a comparison between the legal discharge limits for Cr adopted in several countries (Tahir and Nassem, 2007).

Appendix I: Total chromium legal discharge limits in sewers and surface waters in some countries

Country	Total chromium legal discharge limits (mg/l)	
	Surface waters	Sewers
Austria	4.0	15
Brazil	0.5	0.5
Denmark	2.0a	2.0a
Spain	2.0	4.0
France	1.0	–
Greece	0.5	0.5
The Netherlands	0.05a	2.0a
Italy	2.0	4.0
New Zealand	–	5–50
Pakistan*	1.0	-
Poland	–	0.5
Portugal	l 2.0	2.0
United Kingdom	2.0	5–10
Switzerland	2.0a	2.0a

a Limits refer to trivalent chromium only. (Source: Tahir and Nassem, 2007), *Haydar and Anwar, 2009

Appendix II

ANOVA Comparing Cr removal by bacterial in media and effluent

		Intercept	Time (h)	Equipment	Culture medium	Error	Total
Degr. of freedom		1	4	1	1	33	39
P. aeruginosa	SS	18942.08	191.57	2.25	11.67	17.45	222.94
P. aeruginosa	MS	18942.08	47.89	2.25	11.67	0.53	
P. aeruginosa	F	35826.82	90.58	4.26	22.08		
P. aeruginosa	p value	0.000000	0.000000	0.046993	0.000045		
B. cereus	SS	19835.00	147.36	0.06	3.88	5.32	156.61
B. cereus	MS	19835.00	36.84	0.06	3.88	0.16	
B. cereus	F	123145.5	228.7	0.4	24.1		
B. cereus	p value	0.000000	0.000000	0.535354	0.000024		
Consortium	SS	17734.63	300.69	1.56	2.51	21.91	326.66
Consortium	MS	17734.63	75.17	1.56	2.51	0.66	
Consortium	F	26712.06	113.23	2.34	3.77		
Consortium	p value	0.000000	0.000000	0.135288	0.060659		
Control	SS	24184.76	2.93	0.09	0.04	0.31	3.38
Control	MS	24184.76	0.73	0.09	0.04	0.01	
Control	F	2537629	77	10	4		
Control	p value	0.000000	0.000000	0.003844	0.046044		

Appendix III

ANOVA Comparing Cr removal in mnimal media at different pH

		Intercept	Time	pH	Error	Total
Degr. of freedom		1	4	2	8	14
ASP 10	SS	677.7793	69.3607	0.2212	0.3120	69.8938
ASP 10	MS	677.7793	17.3402	0.1106	0.0390	
ASP 10	F	17380.44	444.66	2.84		
ASP 10	p value	0.000000	0.000000	0.117254		
ASP 20	SS	2711.386	292.774	0.124	0.139	293.037
ASP 20	MS	2711.386	73.193	0.062	0.017	
ASP 20	F	155618.1	4200.9	3.5		
ASP 20	p value	0.000000	0.000000	0.078897		
ASP 25	SS	4486.237	491.468	4.845	2.829	499.142
ASP 25	MS	4486.237	122.867	2.422	0.354	
ASP 25	F	12687.68	347.48	6.85		
ASP 25	p value	0.000000	0.000000	0.018468		
PEN 10	SS	683.7075	71.8769	0.7279	0.2680	72.8728
PEN 10	MS	683.7075	17.9692	0.3639	0.0335	
PEN 10	F	20411.21	536.45	10.87		
PEN 10	p value	0.000000	0.000000	0.005243		
PEN 20	SS	2762.174	297.680	0.122	0.168	297.970
PEN 20	MS	2762.174	74.420	0.061	0.021	
PEN 20	F	131720.2	3548.9	2.9		
PEN 20	p value	0.000000	0.000000	0.111677		
PEN25	SS	4646.400	473.250	2.929	2.778	478.958
PEN25	MS	4646.400	118.313	1.465	0.347	
PEN25	F	13379.15	340.68	4.22		
PEN25	p value	0.000000	0.000000	0.056146		
TN3 10	SS	630.3745	64.2541	0.6532	0.3452	65.2525
TN3 10	MS	630.3745	16.0635	0.3266	0.0431	
TN3 10	F	14609.48	372.29	7.57		
TN3 10	p value	0.000000	0.000000	0.014289		
TN3 20	SS	2516.315	269.139	1.174	0.152	270.465
TN3 20	MS	2516.315	67.285	0.587	0.019	
TN3 20	F	132402.8	3540.4	30.9		
TN3 20	p value	0.000000	0.000000	0.000173		
TN3 25	SS	4185.020	459.026	9.326	2.376	470.728
TN3 25	MS	4185.020	114.756	4.663	0.297	
TN3 25	F	14090.42	386.37	15.70		

TN3 25	p value	0.000000	0.000000	0.001700		
CONS 10	SS	606.6168	63.0256	0.6310	0.3287	63.9853
CONS 10	MS	606.6168	15.7564	0.3155	0.0411	
CONS 10	F	14764.32	383.49	7.68		
CONS 10	p value	0.000000	0.000000	0.013762		
CONS 20	SS	2419.858	263.357	1.132	0.149	264.639
CONS 20	MS	2419.858	65.839	0.566	0.019	
CONS 20	F	129843.9	3532.8	30.4		
CONS 20	p value	0.000000	0.000000	0.000183		
CONS 25	SS	4022.601	447.155	8.927	2.230	458.312
CONS 25	MS	4022.601	111.789	4.463	0.279	
CONS 25	F	14428.44	400.97	16.01		
CONS 25	p value	0.000000	0.000000	0.001597		

Appendix IV

ANOVA Comparing Cr removal fungal effluent treatment

		Intercept	Time (h)	Equipment	Error	Total
Degr. Of freedom		1	4	1	14	19
ASP	SS	5702.402	722.977	4.168	5.645	732.790
ASP	MS	5702.402	180.744	4.168	0.403	
ASP	F	14141.41	448.23	10.34		
ASP	p value	0.000000	0.000000	0.006233		
PEN	SS	6081.328	690.141	0.330	0.546	691.017
PEN	MS	6081.328	172.535	0.330	0.039	
PEN	F	155922.9	4423.7	8.5		
PEN	p value	0.000000	0.000000	0.011417		
TN3	SS	5144.349	744.275	0.995	4.602	749.872
TN3	MS	5144.349	186.069	0.995	0.329	
TN3	F	15649.16	566.02	3.03		
TN3	p value	0.000000	0.000000	0.103892		
CONS	SS	4419.959	830.397	2.022	1.530	833.950
CONS	MS	4419.959	207.599	2.022	0.109	
CONS	F	40435.61	1899.20	18.50		
CONS	p value	0.000000	0.000000	0.000731		
Control	SS	11997.65	2.74	0.35	0.39	3.48
Control	MS	11997.65	0.69	0.35	0.03	
Control	F	429495.5	24.6	12.4		
Control	p value	0.000000	0.000003	0.003407		

Appendix V

ANOVA Comparing Cr removal fungal effluent treatment

		Intercept	Time (hr)	Error	Total
Degr. of freedom		1	4	5	9
P. aeruginosa	SS	4469.419	98.815	0.691	99.505
P. aeruginosa	MS	4469.419	24.704	0.138	
P. aeruginosa	F	32351.93	178.82		
P. aeruginosa	p value	0.000000	0.000014		
B. cereus	SS	4825.930	46.872	0.354	47.226
B. cereus	MS	4825.930	11.718	0.071	
B. cereus	F	68105.14	165.37		
B. cereus	p value	0.000000	0.000017		
Consortium	SS	4116.841	126.456	0.271	126.727
Consortium	MS	4116.841	31.614	0.054	
Consortium	F	75928.46	583.07		
Consortium	p value	0.000000	0.000001		
Control	SS	5992.704	1.231	0.049	1.280
Control	MS	5992.704	0.308	0.010	
Control	F	610255.0	31.3		
Control	p value	0.000000	0.000981		
ASP	SS	2699.120	364.794	3.581	368.375
ASP	MS	2699.120	91.199	0.716	
ASP	F	3768.511	127.331		
ASP	p value	0.000000	0.000032		
PEN	SS	2996.015	358.200	0.085	358.285
PEN	MS	2996.015	89.550	0.017	
PEN	F	175925.7	5258.4		
PEN	p value	0.000000	0.000000		
TN3	SS	2644.201	399.429	0.019	399.448
TN3	MS	2644.201	99.857	0.004	
TN3	F	697678.4	26347.6		
TN3	p value	0.000000	0.000000		
CONS	SS	2116.443	441.725	0.498	442.223
CONS	MS	2116.443	110.431	0.100	
CONS	F	21228.11	1107.63		
CONS	p value	0.000000	0.000000		
ASPUV30	SS	2605.319	386.950	0.508	387.459
ASPUV30	MS	2605.319	96.738	0.102	
ASPUV30	F	25625.25	951.49		

ASPUV30	p value	0.000000	0.000000		
PENUV30	SS	2226.064	542.553	0.125	542.678
PENUV30	MS	2226.064	135.638	0.025	
PENUV30	F	89042.56	5425.53		
PENUV30	p value	0.000000	0.000000		
TN3UV30	SS	2570.250	418.187	0.120	418.307
TN3UV30	MS	2570.250	104.547	0.024	
TN3UV30	F	107183.1	4359.8		
TN3UV30	p value	0.000000	0.000000		
ASPuv10	SS	2285.842	486.266	0.181	486.447
ASPuv10	MS	2285.842	121.566	0.036	
ASPuv10	F	63092.51	3355.41		
ASPuv10	p value	0.000000	0.000000		
PENuv10	SS	3187.653	285.843	1.529	287.372
PENuv10	MS	3187.653	71.461	0.306	
PENuv10	F	10426.71	233.75		
PENuv10	p value	0.000000	0.000007		
TN3uv10	SS	2963.218	319.768	0.115	319.883
TN3uv10	MS	2963.218	79.942	0.023	
TN3uv10	F	129285.3	3487.9		
TN3uv10	p value	0.000000	0.000000		
ASPuv60	SS	3742.677	187.670	0.815	188.485
ASPuv60	MS	3742.677	46.918	0.163	
ASPuv60	F	22955.58	287.77		
ASPuv60	p value	0.000000	0.000004		
PENuv60	SS	3945.388	170.560	0.164	170.724
PENuv60	MS	3945.388	42.640	0.033	
PENuv60	F	120617.2	1303.6		
PENuv60	p value	0.000000	0.000000		
TN3uv60	SS	3829.458	164.440	0.135	164.575
TN3uv60	MS	3829.458	41.110	0.027	
TN3uv60	F	141779.3	1522.0		
TN3uv60	p value	0.000000	0.000000		

Appendix VI

Classification of surface water quality based on BOD values

Degree of pollution	BOD_5 (MgO_2L^{-1}
Very clean	< 1.0
Clean	1.1 – 1.9
Moderately polluted	2.0 – 2.9
Polluted	3.0 – 3.9
Very polluted	4.0 – 4.9
Extremely polluted	> 10

BOD_5 values for wastewaters

Type of wastewater	BOD_5 (MgO_2L^{-1})
Very well treated effluent	3 – 5
Standard affluent (after primary and secondary treatment)	10 – 30
Badly treated sewage	40 – 80
Strong sewage	400 – 600
Trade effluents (animal and vegetable waste)	> 1000

Source: Rodjevic and Bashkin (1999).

Appendix VII

Characterization of bacterial isolates from effluent and soil samples

Test	Isolates A	Isolates B
Growth on Nutrient Agar	Green colored colonies with increasing intensity on incubation at room temp	Gray dry opaque colonies
Growth on MacConkey Agar	Pale colored colonies	NT
Growth on Blood Agar	Greenish colored colonies	NT
Gram reaction	-	+
Motility	+	+
Catalase	+	+
Coagulase test	NT	+
Aerobic growth	+	+
Anaerobic growth	-	+
Fluorescence under UV light at 254nm	+	NT
Citrate utilization	NT	+
Indole test	+	+
Starch hydrolysis	NT	+
Growth on 7% Nacl	NT	+
Growth at 42^{o}C	+	NT
Growth at 4^{o}C	+	NT
Growth at 50^{o}C	NT	-
Glucose fermentation	+	+
Nitrate utilization	NT	+
Maltose fermentation	-	NT
Acid from mannitol	+	NT
Spore formation	NT	+
MYP Medium	NT	Pink, greyish colonies, lecithinase hydrol (clear zone)
Inference	*Pseudomonas aeruginosa*	*Bacillus cereus*

Key + = positive; - negative; NT= not tested



Plate 1: Discharge point (DP) of a tannery showing the out flow of the effluent



Plate 2: Entry point (EP) of tannery the effluent into the receiving water body



Plate 3: Downstream point (DS) of a tannery showing the effluent before entry into the river



Plate 4: Entry point (EP) of tannery effluent during wet season



Plate 5: Downstream point (DS) showing the over flow of the effluent in wet season



Plate 6: Downstream and entry points of the effluents