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Preface

The tanning industry is a major source of pollution worldwide, particularly in developing countries. The major public concern over tanneries has traditionally been about odours and water pollution from untreated discharges. Important pollutants associated with the tanning industry include chlorides, tannins, chromium, sulphate and sulphides as well as trace organic chemicals and, increasingly, synthetic chemicals such as pesticides, dyes and finishing agents, as well as solvents. These substances are frequently toxic and persistent, and affect both human and environmental health.

The primary focus in this book was to identify the recently developed ecotoxicological analytical trends (rapid, simple and inexpensive) related to the tanning industry on terrestrial and aquatic systems. The resultant research data reported, incorporates both field related and laboratory based techniques to address underlying environmental problems in the tanning sector. The book also includes a chapter to explore the occupational hazards in a tannery environment caused by contaminated dust. It was important to note that an optical set-up involving microscopy and digital imaging techniques was initially used to determine dust particle numbers and size distributions as a preamble to ascertaining the dust toxicity levels. To determine the toxic nature of the dust (in addition to particle size), an ecotoxicological screening of the dust samples (using a solid and liquid assay involving the response of luminescence (*lux*)-based bacterial biosensor) was conducted and which was complemented by chemical analysis to identify possible causative toxic components. Moreover, a novel technique has been discussed in this book related to the tannery effluent and associated environmental samples. This included a section describing the dissection and manipulation of samples through sparging, treatment with activated charcoal, filtration and pH adjustment.

Finally, the book succeeds in meeting its three main specific objectives at the end: characterisation of effluents, sediments and riverine samples; assessment of ecotoxicity; bioremediation potential of primary contaminants and input of environmental risk assessment through development of a quantitative and qualitative risk assessment model. Indeed this book will provide a valuable academic and referral dimension in environmental management related to the tanning industry.

Nairobi, Kenya — Dr. Mwinyikione Mwinyihija

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Contents

Biographical Notes of the Author

Dr. Mwinyikione Mwinyihija, *PhD, FCIWEM, HSC, C.Env., C.Sci., C.Biol., MSB, M.I.SoilSci., MCIWEM, Chartered Environment and Water Manager.*

Dr. Mwinyikione Mwinyihija received his PhD in Environmental Science University of Aberdeen, Scotland, UK. Current research interests are in the field of ecotoxicology especially in areas of contaminant fate, diffuse contamination, environmental management in terrestrial and aquatic ecosystems, diagnostic techniques using microbial biosensor application in environmental samples and applied bioremediation techniques. He is currently a chartered member of several peer reviewed profession institutions e.g. Council of Scientists, American Chemical Society, Institute of Biology (UK), Chartered Institute of Water and Environment, Institute of Environment and Management, Institute of Professional Soil Scientist and the esteemed Society of the Environment. He has published several papers and a reviewer, in various prestigious peer reviewed journals. He is well traveled and attends several international forums such as the International Council of Tanners, British Society of Soil Science, Institute of Biology, United Nations forum on climate change, Institute of environmental audit and management etc.

Currently Dr. Mwinyikione Mwinyihija is pursuing research interest in aspects such as: (1) Impact of salt to the environment as a pollutant of the tanning industry (a research grant for 12 months has been approved starting from August 2007 to September 2008) (2) Recovery mechanism of river sediment on pollution load from a tannery source (3) Determination of particulate matter and its ecotoxicological potency in the environment (4) Determination of diffuse contamination in terrestrial, atmospheric and aquatic ecosystems. He also has provided consultancy services to high profile organization such as OXFAM (Kenya), Malindo consultancy, UK and Office of Economic Policy and Regional Development (EPRD), Poland. Furthermore he is registered to the National Environment Management Authority, Kenya as a Lead Expert in Environmental Impact/Audit Assessment. To date he is the top Kenya government advisor in the area of value addition and environmental management of the leather subsector as the Secretary/Chief Executive Officer Leather Development Council (LDC), a technical panel member of the International standards organization and Kenya's representative to the Food and Agriculture Organization (FAO) Hides, skins and leather subsector group. In addition he has continued playing an active role in dissemination of science in various specialized and general public platforms with an aim of ensuring sustainable development in the leather sector world wide.

Abbreviations

ANOVA	Analysis of variance
ATP	Adenosine triphosphate
DC	Direct current
DHA	Dehydrogenase activity
DTPA	Diethylene triamine pentaacetic acid
DNA	Deoxyribonucleicacid
ERA	Ecological risk assessment
GPS	Global Positioning System
HMN	Human
IHL	Inhalation
IPR	Intraperitoneal
INT	*p*-Iodonitrotetrazolium
IVN	Intravenous
JPEG	Joint Photographic Experts Group
LC_{50}	Lethal concentration 50 percent kill
LCLO	Lowest published lethal concentration
LD_{50}	Lethal dose 50 percent kill
LDLO	Lowest published lethal dose
LSD	Least significant difference
MUS	Mouse
ORL	Oral
PPM	Parts per million
RAT	Rat
RBG Colour spaces	Red Blue and Green
RNA	Ribonucleicacid

TLV	Threshold limit value
TTC	Triphenyl teterazolium
TWA	Time weighted average
UNEP	United Nations Environmental Programme
USEPA	United States Environnmental Protection Agency

Chapter 1
Introduction

Abstract In this Chapter, the primary focus was to provide a holistic overview of three specific thematic areas, which equally form the primary objectives towards understanding the ecotoxicological impact of the tanning industry. These are namely; characterisation of tannery dust, effluents, sediments and riverine samples, assessment of ecotoxicity and bioremediation potential of primary contaminants, and environmental risk assessment through development of a quantitative and qualitative risk assessment model. The chapter pursues progressively pivotal issues associated with waste management. The attempt is successfully done by critically previewing the tanning industry and focusing on the main contaminants and pollutants related to the sector using novel techniques discussed in subsequent chapters of the book.

1.1 Tanning Industry Perspective

Transformation of animal hides and skins into leather, primarily involves six main phases within the production chain; slaughtering, flaying, curing to arrest putrefaction; removal of hair and flesh (for leather/leather-goods that do not require hair or fur), tanning and depending on the final leather products, finishing targeted through the application of specific chemical agents. The aim eventually is to render the raw material to be non-putrescible, impart comfort and enhance its durability. This principle imitates the morphological function of the hides and skins during the lifetime of the animal which is; to allow for thermoregulation, gaseous exchange, protection and provide cover. These same characteristic are what eventually provide the uniqueness of leather in comparison to other synthetic materials in both the clothing and shoe industry in aspect of comfort and convenience during use. The resultant effluent from the tanning industry is therefore both peculiar and complex due to various stages involved and the multiple chemical combinations that are associated with tanning.

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1.2 Socio-Economic Importance of the Tanning Industry

The global leather industry is currently valued at more than $50 billion (Dadaglio 2003). The worldwide production of leather had grown to 2.4 billion square metres by the end of 2000 (Anonymous 1996). The proportion of world leather production increased in the developing countries in comparison to the world leather production (heavy leather, 404,900 (1990) to 515,700 tonnes (1999); light leather, 13,265.6 (1990) to 15,521 million square feet (1990)) (ICT 2003). The leather and leather products industries have witnessed considerable shifts in the location of tanning and leather manufacturing to developing countries where production costs are lower and environmental regulations less stringent (Muchie 2000).

1.3 Environmental Threats

Processing of the hides and skins within the tanning industry is still an environmental concern irrespective of advances and mechanisms suggested as a way forward in adopting cleaner technologies. Researchers have in recent years (e.g. Muchie 2002) critically evaluated some of the technologies available and reported that the so called 'cleaner' technology risks being no more than old wine in new bottle. Moreover pollution from the leather processing industries which has been deemed as the largest polluter in the world (Khwaja 1998) has a negative long term impact on the economic growth potential of a country (especially those with low technological capacity) irrespective of the immediate profit accruals intended. Development of the industry in Africa for example does not match the technical know-how and capacity in protecting and predicting the environmental impacts related to the industry (Mwinyihija 2007). Cleaning up of such environmental impacts will require expenditure of funds, which could have promoted positive and sustainable development.

1.4 Critical Pollutants Identified

In Africa and most parts of Asia unlike in the developed world, leather processing still uses chromium largely as the main tanning agent. 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 is that Cr salts precipitate with NaOH followed by the dissolution of $Cr(OH)_3$ in sulphuric acid (Anonymous 2002) during leather processing. 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 remain a potentially toxic source to the environment.

Nearly 90% of all leather produced is tanned using Cr salts (Stein and Schwedt 1994) with about 8% of the basic chromium sulphate salt used for conventional tanning. Chromium binds with the collagenous protein to convert to leather. Cr^{3+} dominates from tanneries, textile (printing, dyeing) and decorative plating industry waste (Nriagu 1988). With reference to the tanning industry, Cr^{3+} in the effluents is the most expected form but with Redox reactions occurring in the sludge, an increase in the hexavalent form can occur. Under slightly acidic or neutral pH conditions in this type of wastewater, the poorly soluble $Cr(OH_3)$.aq should be the preferred form, but a high content of organic matter originated from hide/skin material processing is effective in forming soluble Cr^{3+} complexes (Stein and Schwedt 1994; Walsh and O'Haloran 1996a, b). Other related 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). Leather processing generally involves stages such as; soaking, liming, deliming, bating, pickling, tanning and retanning. However it should be noted the processing stages could be varied or combined depending on the final leather qualities intended (e.g. upholstery, shoe upper, sole or clothing 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.

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 and Asia). 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 (*Acacia mearnsii*) is extensively used as tannin. 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.

1.5 Ecotoxicology and Diagnostic Trends

Ecotoxicology is a term used to define the study of the toxic effects of natural and anthropogenic (man-made) substances on the biotic (living) and non-biotic components of the biosphere. This is an important area of specialisis in the field of Environmental science which basically is referred to as the study of environmental problems especially those created by pollution and applies the fundamentals of all scientific disciplines. The objective of ecotoxicology is to understand and predict the effects of chemicals on natural communities under realistic exposure conditions. Therefore the term toxicology (study that deals with poisons and their effects, antidotes, detection etc) emerges as the pertinent issue in this context and as such

facilitates in the focus of understanding the behaviour and characteristic of various toxicants (agents that have a harmful effect on a biological system) in the environment. At this point it is important to note that the term toxicant is not similar to a pollutant which essentially may encompasses physicochemical characteristics such as noise, deoxygenation, pH, temperature extremes, particulate size potency etc.

On the other hand the figurative meaning of diagnosis in this perspective implies a careful study of the facts about a line of enquiry to determine its essential features, faults or characteristics. Essentially environmental impact assessment (EIA) of the tanning industry entails, the determination (i.e. through physicochemical and biological techniques) of the source of the toxicant within that industry, assessing its polluting potential and where possible provides remedial measures by incorporating recent trends in ecotoxicological diagnosis.

The crux of the matter is that the use of chemical analytical methods (employs physico-chemical principles to carry out measurements of chemical species) assists in the determination of the pollutant concentration in various environmental matrices rather than determining its bioavailability potential. Bioavailability is a term used to describe the biological active form or species of substances, which can be used by organisms. Bioavailability can further be defined as a measure of the physicochemical access that a toxicant has to the biological processes of an organism. The factors that affect the bioavailability of contaminants can be categorised into physicochemical and biological ones. The physicochemical factors include the properties of the contaminant itself and the conditions of the surrounding environment; water, pore water and sediment. Biological factors are dependent on the organism, for example on its feeding habits and metabolic status.

Inferences from these definitions stated above, is that the less the bioavailability of a toxicant, the less its toxic effect on an organism. Thus unilaterally, the determination of total concentration of a contaminant alone provides information on the presence of the species detected without necessarily relating to bioavailability because the exposure of an organism may be limited by unavailable forms of the pollutant. Therefore bioavailability essentially represents the accessibility of a contaminant for possible assimilation and toxicity. However it is important to note that in metals, bioavailability is a function of the concentration of dissolved metal species in various environmental media and the resultant toxic effects it exerts on metabolic (complex physical and chemical processes occurring within the living cell or organism that are necessary for maintenance of life) or catabolic (metabolic breakdown of complex molecules to simpler molecules and mostly associated with release of energy) processes in living systems. This assertion is based on the fact that the interactions of metals with living organisms are highly dependent on the speciation of metals where changes in speciation behaviours affect the toxicity of metals. Factors that are known to affect the speciation of metals are pH, ionic strength, ageing and environmental factors. For example, pH values affect solubility of metals that associate with speciation forms of metals; ionic strength affects the availability of metals in solution; metal ageing may lead to an increase in metal bioavailability if metals are originally associated with organic compounds. Moreover bioavailability of organic contaminants on the other hand, is affected by physical and chemical properties of

the compound, its persistence and ageing. As such, the approaches depend on the fact that compounds must be bioavailable or accessible to the target organism and/or potentially biodegradable. Therefore it is with this background that both chemical (based on extraction and performances) and biological (evaluation of contaminant bioavailability) techniques will be used to explore in this book some of the recent diagnostic trends used in ecotoxicology.

1.6 Bioavailabilty, Bioaccumulation and Biomagnification

The less the bioavailability of a toxicant, the less its toxic effect on an organism. Bioavailability and sorption of heavy metals are influenced by pH, Redox potential (Fig. 1.1), exchangeable cations and biological activity (Paton et al. 1997b). It is a concept that is both specific to the individual receptor and the pollutant. Although chemical analysis of environmental samples can produce a measure of the total concentration of the chemical present in the sample, it will not indicate what is bioavailable in the sample (Steinberg et al. 1995) and hence what the likely toxic impact will be. Indeed total concentrations are not necessarily directly related to bioavailability (e.g. binding potential of metals by substrate, effectively reduces their bioavailbilty) (Tiensing 2002). Bioavailability is also important for many organic compounds, particularly when they are associated with humic and other natural complexing agents (Shaw et al. 2000). The factors that affect the bioavailability of contaminants can be categorised into physicochemical and biological ones. The physicochemical factors include the properties of the contaminant itself and the conditions of the surrounding environment; water, pore water and sediment. Biological factors are dependent on the organism, for example on its feeding habits and metabolic status (Lyytikäinen 2004).

Bioaccumulation refers to an increase in concentration of a pollutant from the environment to the first organism in a food chain while biomagnification refers to the increase in concentration of a pollutant from one link in a food chain to another (Mader 1998; Cox 1997). The concept of bioaccumulation comprises the uptake of a contaminant by an organism by means of all possible routes from any source (Spacie et al. 1995). The routes include the integument (skin), respiratory organs and intestine.

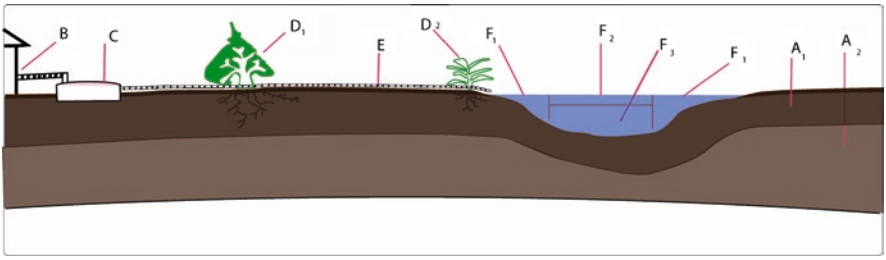

Fig. 1.1 The effect of Redox potential and pH on the speciation of chromium in the environment

At the cellular level, uptake can be passive or active (Alberts et al. 1991). The concentration gradient between different sides of the cell membrane drives the transport of uncharged molecules, whereas an electrical gradient also contributes to the uptake of charged molecules (Alberts et al. 1991). In active transport, molecules are transferred against the gradient. Contaminants are, however, mainly taken up by passive diffusion (Connell et al. 1997). Mader (1998) and Cox (1997) reported that these phenomena (biomagnification and bioaccumulation) together, meant that even small concentrations of chemicals in the environment can find their way into organisms in high enough dosages to cause problems. They further observed that in order for biomagnification to occur, the pollutant must be: long-lived; mobile; soluble in fats and biologically active.

If a pollutant is short-lived, it will be broken down before it can become dangerous. If it is not mobile, it will stay in one place and is unlikely to be taken up by organisms. If the pollutant is soluble in water it will be excreted by the organism. Pollutants that dissolve in fats, however, may be retained for a long time. It is traditional to measure the amount of pollutants in fatty tissues of organisms such as fish. In mammals, milk is tested due to the susceptibility to the offspring from toxins.

1.7 Biochemical Assays for Diagnostic Purposes

Techniques to be discussed include those that are easy to perform, rapid and economical with out compromising on their potentiality to provide quality results. For chemical analysis preference was to adopt much universal methods discussed in later chapters. However, much emphasis will be based on the use of few selected bioassay techniques that are novel and have successfully emerged as dependable and reproducible. Furthermore bioassays (e.g. Biosensors) tend to incorporate complex environmental factors such pH, Eh, CEC and biological activity in the assessment of ecotoxicity. Moreover biological methods complements chemical analysis in addressing toxicity issues arising from synergism/antagonism of contaminant mixtures; facilitate an informed choice for remediation strategies or indicate their likely success; target biological relevance and risk assessment and allow non-expert ease of interpretation. For example the development of *lux*-based biosensors has successfully provided bioluminescent microbial bioassays that are commercially (Fig. 1.2) available and used to assess the degree of toxicity as rapid, accurate, reliable and sensitive biosensors in ecotoxicological studies.

1.7.1 Biosensors

Biosensors are biological or molecular makers that can illustrate a measurable effect in biological media, tissues, cells or fluids. Biosensors combine a bioreceptor, biological component, a transducer and detection method. Biosensors provide the

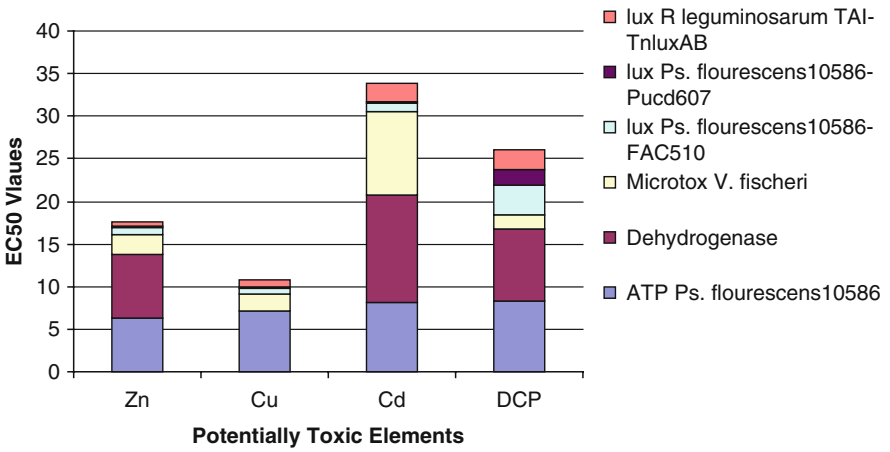

Fig. 1.2 Biosensor response of selected assays to identified four potentially toxic elements (PTE's)

technology to measure biological effects (e.g. acute and chronic physiological toxicity, genotocity, immunotoxicity, and endocrine toxicity) and the concentration of specific analytes which are difficult to detect and are important contaminants of water, waste, soil or air (e.g. surfactants, chlorinated hydrocarbons, sulphophenyls carboxyls, sulphonated dyes, fluorescent whitening agent, naphthalene, sulphonates, carboxylic acids, dioxins, pesticides and metabolites).

Microbial bioluminescence involves the activities of electron transport systems, which produce substrates for the production of light. Change in environmental conditions, which affect electron transport, should in turn have an effect on microbial bioluminescence (Steinberg et al. 1995). In nature, earthworms (*Diplocardia longa*), starfish, beetles, limpets, sea firs, shrimps and certain species of snails have been shown to luminesce (Campbell 1989), alter directly or via luminescent symbionts.

Naturally luminescence bacteria all share a common morphology as they are all gram-negative, motile rods and produce extracellular chitinase (Hastings and Nealson 1977). In bacteria, bioluminescence occurs on two operons. Operon R contains the *lux* ICDABE genes and Operon L contains the *lux* R gene (Meikle et al. 1992) (Fig. 1.3).

In the case of industrial emissions, wastes and remediation, the analytes will be dictated by the process in question, but the biosensors proposed should offer improvements over conventional analytical techniques and contribute to greater process efficiency and /or safety.

Biosensors are based on incorporation of reporter genes from eukaryotes (*luc*) and prokaryote (*lux*). *Lux* based bacterial biosensors as earlier indicated have gained increasing acceptance as rapid, relevant and reliable indicators of toxicity in a range of environmental samples such as ground water (Killham et al. 1997), soil (Paton et al. 1995), tannery wastes (Mwinyihija et al. 2005, 2006) and effluents entering rivers (Killham et al. 1996). Toxicity assessment in the environment can

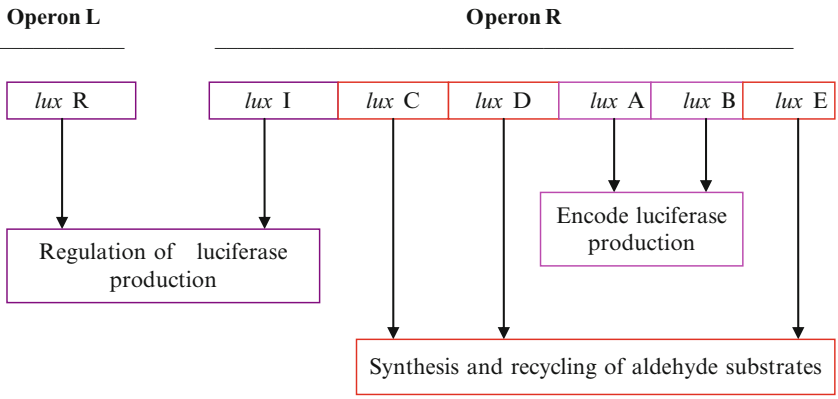

Fig. 1.3 *Lux* gene organizations on the L and R **operon** of *Vibrio fischeri*

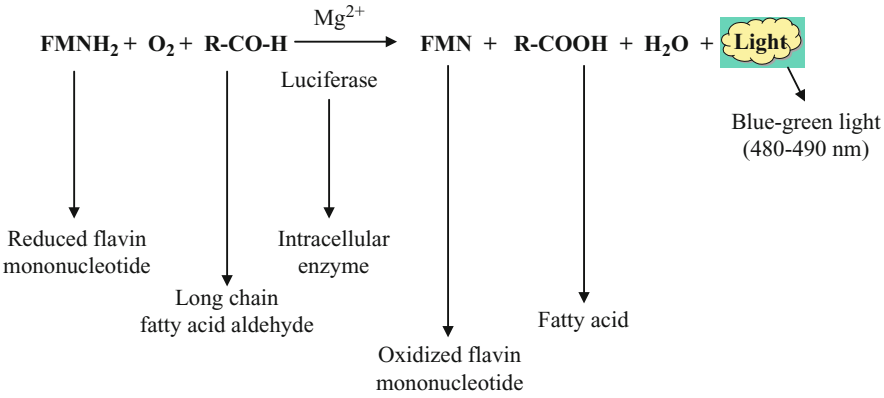

Fig. 1.4 Luciferase bioluminescence reaction in bacteria involving reduction of aldehyde to produce light

be attained by the use of *lux* reporter genes from *Vibrio fischeri* incorporated in to selected terrestrial bacteria. The production of light (bioluminescence) (Fig. 1.4) is used to determine the level of toxicity by allowing the impact of the environmental contaminant on a bacteria population to be measured (Paton et al. 1995).

A reduction in bioluminescence (visible light emission in living organisms) means an effect on metabolic activity, which has a direct effect on energy production (Steinberg et al. 1995). Bioluminescence accompanies the oxidation of organic compounds (luciferins) mediated by an enzyme catalyst (luciferase) (Meighen 1992) (Fig. 1.4). These reactions are important in biogeochemical analytical applications. The possible combinations of any bioreceptor (ensures molecular recognition) with any transducer (transforms an analytical signal) lead to a number of biosensors.

The production of light by *V. fischeri* results from the luciferase catalysed oxidation of reduced riboflavin mononucleotide ($FMNH_2$) and long chain fatty aldehyde

(RCHO). Luciferase is specific for $FMNH_2$ and a range of aldehydes varying in chain length from 8 to 14 carbons (Meighen 1993). $FMNH_2$ binds to the luciferase and reacts with the fatty aldehyde producing a highly stable enzyme-flavin-oxygen-aldehyde intermediate (Meighen 1993). The result is the emission of a blue-green light (480–490 nm), FMN, carboxylic acid and water (Meighen 1994). Hastings and Nealson (1977) showed that the natural aldehyde used by bacteria appears to be tetradecanal (Meighen 1988). However, n-tetradecylaldehyde, dodecanal, decanal or any other long chain fatty aldehydes have also been found to be effective (Prosser 1994).

Therefore the aim of a biosensor is the detection of an environmental signal, usually the presence or absence of xenobiotics. Thus in this book the use of *lux* biosensors will be based on their importance as toxicity indicators for tannery dust (solid and liquid phase) and effluent assessments. Moreover the type of biosensor selected should be able to assess the toxicity of primary tannery pollutants; chromium, sulphides, chlorinated phenolics and the presence of other organic or inorganic substances. To complement on the use of biosensor as a diagnostic tool it is prudent to include another indicator of soil/sediment health to ascertain if actually biomass activity is affected correlatively with increased/decreased toxicity.

1.8 Measurement of Biomass Activity

The measurement of enzyme activities may be used as an indirect measure of soil/sediment microbial activity (Killham and Staddon 2002). This may serve as a supplement to biomass measurements. Reliance on measurement of pollutants alone to evaluate environmental problems, without also assessing biological effects (e.g. through measurement of biomass activity), ignores potential problems (Price 1978) in various ecosystems. For example the activity of certain enzymes or cofactors such as coenzyme F_{420}, hydrogenase, dehydrogenase (DHA), and adenosine triphosphate (ATP), may also serve as indicators of these biological effects (Nybroe et al. 1992; Le Bihan and Lessard 1998; Goel et al. 1998). However for purposes of complementarity to the use of biosensor, dehydrogenase was identified as a tool of choice in measuring biomass activity of river sediments impacted by tannery effluents.

1.8.1 Dehydrogenase

Dehydrogenase is an oxidoreductase enzyme, often relatively stable, and can persist for extended periods, thereby providing a longer term perspective than measurements involving extant organisms alone (Killham and Staddon 2002). The impact of pollutants on soil and sediment health has been addressed through the measurement of enzyme activity (Killham and Staddon 2002; Mwinyihija et al. 2005).

Hongwei et al. (2002) reported that only DHA and ATP had been used successfully to monitor aerobic and anaerobic sludge activity because the methods are easy and relatively rapid. In the ATP test, maintenance of reagent activity in storage is more critical than that for the DHA test and the measurement process is more complicated than that for DHA (Hongwei et al. 2002).

The dehydrogenase assay is a simple and inexpensive test used to determine the degree of toxicity by measuring the microbial activity through the production of the enzyme dehydrogenase (Hongwei et al. 2002). Dehydrogenase activity (DHA) is a common and suitable test to quantify the impact of pollutants (e.g. heavy metals) on soil micro organisms. This is because DHA is assumed to be proportional to microbial respiration (Stevenson 1959; Thalmann 1968; Skujins 1973; Frankenberger and Dick 1983). Dehydrogenase is an enzyme, which is unspecific in its activity unlike enzymes such as urease (Killham and Staddon 2002). In other related experiments measuring microbial activity, Zn and Cu additions were found to decrease the activity of acid phosphatase, urease activity (Tyler 1976) and amylase (Ebregt and Boldewijn 1977). In principle dehydrogenase functions in living cells while phosphatase is an extracellular enzyme. Indeed adverse effects (caused by pollutants) on the metabolic functioning of the cell would result in reduction of DHA. However, reductions in phosphatase activity would not be as noticeable as those of DHA when the microbial metabolic function is inhibited. At this juncture it is important to understand the mode of action of DHA.

1.8.2 Mode of Action for DHA

As an intracellular enzyme, dehydrogenase facilitates the transfer of hydrogen and electrons from organic compounds to appropriate electron acceptors during the initial oxidation of the substrate (Skujins 1978). When electrons pass along a chain of intermediate carriers normally oxygen acts as the final electron acceptor. For example in a dehydrogenase test, tetrazolium salts (e.g. triphenyltetrazolium chloride (TTC) and 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyl tetrazolium chloride) (INT) act as terminal electron acceptors (Trevors et al. 1981). Benefield et al. (1977) suggested that in O_2 depleted conditions, aerobic as well as anaerobic dehydrogenase use TTC as an electron acceptor. In effect TTC is reduced to TPF (triphenyl formazan) a red insoluble compound. Ethanol is used to dissolve the precipitate, which is then colorimetrically measured using a spectrophotometer.

$$RH_2 \xrightarrow{DHA} R + 2H, \tag{1.1}$$

$$2H + \text{Tetrazolium salts} \rightarrow HCl + \text{Triphenylformazan} \tag{1.2}$$

(Hongwei et al. 2002)

1.8.3 Application of the DHA Test

The dehydrogenase test had previously been used to determine the effects of heavy metals present in sewage sludge where a reduction of the DHA in metal contaminated soils was observed suggesting a decline in microbial activity in polluted soils (Cenci and Morozzi 1979; Ruhling and Tyler 1973; Doleman and Haanstra 1979; Schinner et al. 1980; Brookes et al. 1984). Similarly DHA was successfully used to determine the microbial biomass of the soil (Malkommes 1988; Wilke and Keuffel 1998). In reference to this issue (microbial biomass) a correlation between the DHA and the biomass of the soil microflora was established (Beck 1984).

1.8.4 Limitation in Using DHA Test

The main limitation of dehydrogenase test (unlike in other enzymes such as urease, invertase and cellulase) is the presence of Cu, which causes a reduction in TPF (triphenylformazan) absorbance (Chander and Brookes 1991). The presence of alternative electron acceptors in the soil may cause a problem in comparing the DHA of different soil types due to a large proportion of O_2 uptake which remains unaccounted for (Burns 1978; Sommervile et al. 1978). DHA inhibition may also be caused by humic acid or soils with high inorganic nitrogen (Trevors 1984).

1.8.5 Addressing the Limitations of DHA Test

Competition of DHA with other electron acceptors in the soil/sediment may be addressed by using INT (2-p-iodophenyl-3-p-nitrophenyl-5-phenyl-tetrazolium chloride) instead of TTC (Benefield et al. 1977). This is due to the fact that INT has a rapid response, high sensitivity and competes well with O_2 for liberated electrons. Also INT as an electron acceptor is pH sensitive with optimum results at pH 4.8 (Benefield et al. 1977) although in other related studies a range of up to pH 6.49 still provided good results (Mwinyihija et al. 2006). DHA as a test may further be enhanced by shortening the incubation period from 24 to 6 h and by adding yeast extract (where a 53% increase in DHA was observed) (Rossel and Tarradellas 1991). The adding of yeast is ideal for soil samples but not for sediments (Mwinyihija et al. 2006). DHA as a test, estimates the total potential activity of the microflora rather than its total effective activity. Moreover with the necessary precautions taken for DHA test, it is also important to introduce another assay which essentially complements and strengthens deductions adduced to the biosensor and DHA as diagnostic techniques in ecotoxicology. Daphnia test using *Daphnia magna* was used for such a purpose.

1.9 Daphnia Test

Daphnia magna (a micro-crustacean) is used in the test as an invertebrate to represent the primary consumer level and identify short-term acute effects. This test is normally carried out using ten freshly bred (neonates) *Daphnia magna.* The same clone of *D. magna* is used to ensure that the same sensitivity during the testing is achieved. The test species (ten freshly bred *Daphnia magna*) are used to perform acute toxicity test according to internationally accepted Standard Methods (e.g. OECD and ISO). During this study *D. magna* was *exposed* to the impacted riverine and tannery effluent samples for 24 h.

The objective of carrying out all the mentioned bioassays (i.e Biosensor, Dehydrogenase and Daphnia assays) in this book is to provide holistic, effective, efficient and economical techniques in determining the impact of the effluents and wastes emanating from the tanning industry to the terrestrial and aquatic ecosystems.

1.10 Organisation of the Book

To comprehend the complexities associated with the tanning industry this book will attempt to evaluate the associated environmental impact by providing initially the morphology of a hide or skin, followed with a brief audit in processing, occupational risks associated, ecotoxicological diagnostic and assessment techniques used in recent times.

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Chapter 2
Main Pollutants and Environmental Impacts of the Tanning Industry

Abstract The main contaminants and pollutants related to the sector are discussed in this chapter. Indeed the characterisation of all the samples (including tannery dust, effluents, sediments and riverine) was outlined at this juncture considering the specific but traditional tanning processes. The behaviour of the principal contaminants within various ecosystems were probed and discussed. Work by other scientist related to degradation of such ecosystems was also pursued in an effort of identifying the underlying toxicity potential to both the biotic and abiotic entities. In conclusion it was demonstrated that mostly all the stages of leather processing, individually and collectively impacts negatively to the environment. However the inherent hazards and analytical techniques are discussed at depth in the next chapter.

2.1 Introduction

Tanning Industry is considered to be a major source of pollution and tannery wastewater in particular, is a potential environmental concern (Ros and Ganter 1998) as discussed in this chapter (Fig. 2.1). Tanning industry wastes poses serious environmental impact on water (with its high oxygen demand, discolouration and toxic chemical constituents (Song et al. 2000), terrestrial and atmospheric systems. Tannery waste characteristically contains a complex mixture of both organic and inorganic pollutants. For example, in related studies, chlorinated phenols and chromium were found to be closely associated with the tannery waste (Reemste and Jekel 1997; Mwinyihija et al. 2005, 2006). Chromium as inorganic pollutant is a transition metal and exists in several oxidation states, with trivalent Cr^{3+} and hexavalent Cr^{6+} species being the most common forms (Kotaś and Stasicka 2000). Furthermore when the two species of chromium (trivalent and hexavalent) are compared, differences in their chemical properties are observed (Andersen 1998).

Indeed 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 (Pasco et al. 2000) exposed to such waste (Fig. 2.2).

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Fig. 2.1 Tannery operations with resultant chromium rich open disposal effluent systems

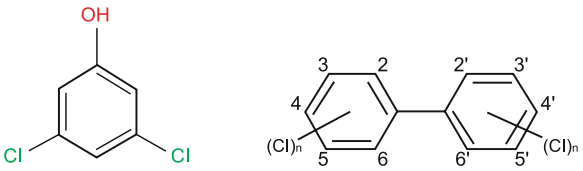

Fig. 2.2 Chlorinated phenol associated with tanning industry (e.g. 3, 5-dichlorophenol) and Polychlorinated Biphenyl's (PCB)

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.

Because tannery wastewater contains a complexity of pollutants including chromium and chlorinated phenols as indicated earlier, it is vital to dissect the toxic nature of such wastewater both to understand its environmental impacts and identify potential remediation strategies. Furthermore, there are strict regulations imposed for the environmental control of pollutants such as heavy metals and persistent organic pollutants (Tunay et al. 1994). Tannery wastewater is generally treated by various physico-chemical and biological methods and by a combination of both (Reemste and Jekel 1997). Physical and chemical processes are frequently employed to treat contaminated sites, but often do not destroy contaminants (Bouwer et al. 1994).

The tanning Industry involves the following processes linked closely with pollution (Fig. 2.3).

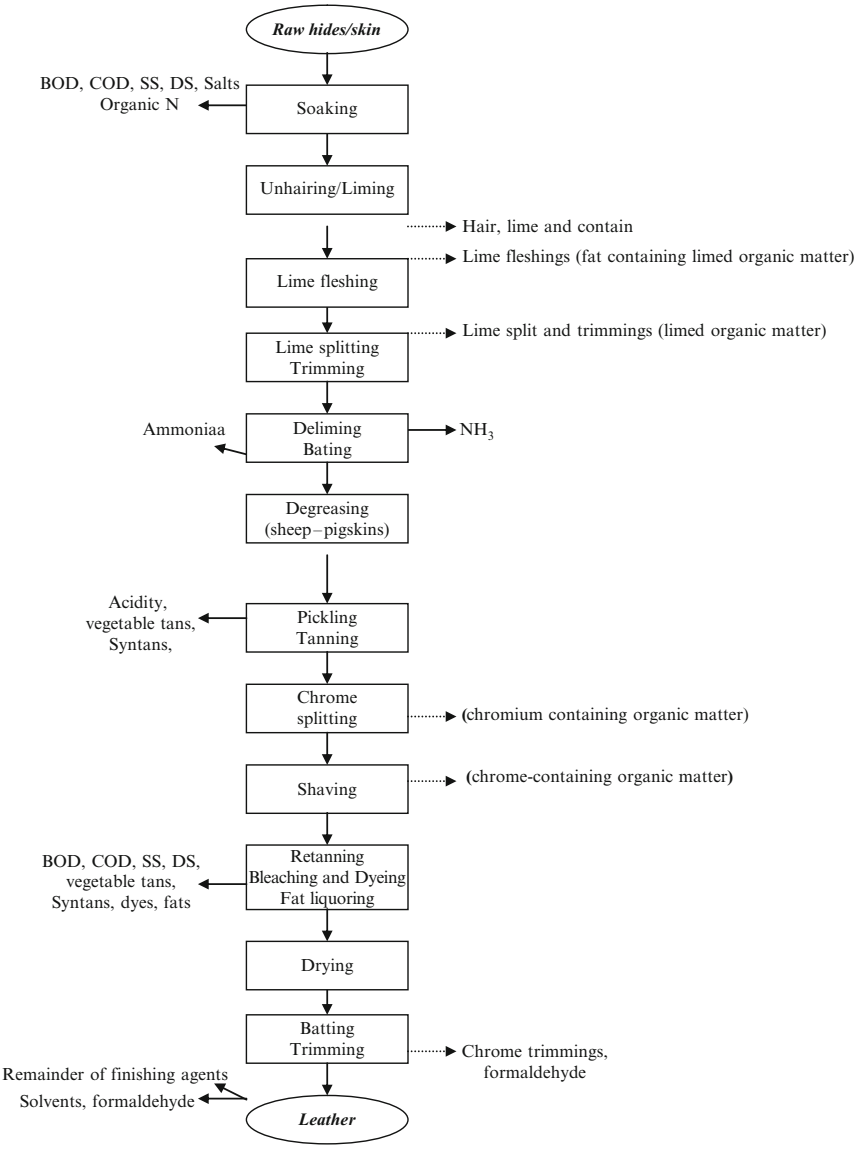

Fig. 2.3 Modified schematic diagram indicating type of pollutants during the tanning process (UNEP 1994)

2.2 Soaking

The main aim at this stage is to wash the rawstock from physically bound materials mostly insecticides, salts ($NaCl_2$) and other preservatives. Moreover rehydration of hides and skins occurs during soaking to replace lost moisture during the preservation

Fig. 2.4 (**a**, **b**) Showing the potential impact that could be caused by salt to the surrounding environment by the hides and skins curing premises at Mariakani (Pictures by M.Mwinyihija)

phases (also referred to as curing) to allow permeation of chemicals during subsequent processes (Cassano et al. 2001). It is within this stage that high volume of water used consequently results to high discharge of effluent with high pollutant load as indicated in Fig. 2.3. 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 (Fig. 2.4 a, b).

2.3 Liming

Liming 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 100 with an output of 12.3, while Na_2S has an input of 35 with an output of 18.3 (Thanikaivelan et al. 2003).

2.4 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).

2.5 Chrome Tanning

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.

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.

2.6 $NaCl_2$

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 (Fig. 2.3). 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 (e.g. naphthalene, benzene, toluene etc.) through a process known as the *salting-out effect* (Pepper et al. 1996). In a study in Egypt, $NaCl_2$ concentration varied between 40,000 and 50,000 mg L in the effluent discharge in a tannery under study (Hafez et al. 2002).

2.7 Organic Matter

Organic matter associated with tannery waste will include 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. 2006a). 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. 2006a).

2.8 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 (Nicholson et al. 1998; Reiffenstien et al. 1992; Glass 1990; Beauchamp et al. 1984). Concentration dependent toxicity occurs in humans following acute exposure. Exposure to moderate levels of H_2S (50–100 mg L^{-1}) can result in Keratoconjuntivitis, 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 (Reiffenstien et al. 1992; Arnorld et al. 1985; Kilburn and Warshaw 1995; Synder et al. 1995; Tredt et al. 1991).

2.9 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 (i.e. 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).

2.9.1 Chemistry of Chromium

Chromium can exist in several chemical forms displaying oxidation numbers from 0 to VI. Trivalent and hexavalent forms of chromium are the most stable in the environment (Shriver et al. (1994). Different chromium species also exhibit different energy levels for conversion to higher or lower oxidation states. This translitionary nature of chromium imparts certain environmental behaviour as shown earlier in Fig. 1.1.

2.9.2 Trivalent Chromium (Cr^{3+})

Cr^{3+} presence, concentration and forms depend on different chemical and physical processes such as hydrolysis, complexation, Redox reactions and adsorption (Table 2.1). In the absence of complexing agents other than H_2O or OH^-, Cr^{3+} exists as hexa-aquachromium (3+) and its hydrolysis products (Rai et al. 1989). Cr $(H_2O)_6^{3+}$ is a moderately strong acid (pK ~ 4).

Table 2.1 Summary of various Cr oxidation numbers, type and environmental behaviour

Valency	Environmental behaviour	Remarks
Cr	Unstable	
Cr^{1+}	Unstable	
Cr^{2+}	Readily oxidised to Cr_3 but stable only in the absence of any oxidant	Active under anaerobic condition
Cr^{3+}	Most stable	Considerable energy required to convert to lower or higher states
Cr^{4+}	Forms unstable intermediate reactions to trivalent and oxidation states	Exhibits this phase during oxidation and reduction
Cr^{5+}	Unstable intermediate.	Observed during oxidation and reduction
Cr^{6+}	In acidic conditions demonstrates very high positive Redox potential and unstable in the presence of electron donors.	Strongly oxidizing

$$Cr\,(H_2O)_6^{3+} + H_2O \leftrightarrow Cr\,(OH)\,(H_2O)_5^{2+} + H_3O^+ \tag{2.1}$$

$$Cr\,(OH)\,(H_2O)_5^{2+} + H_2O \leftrightarrow Cr\,(OH)_2\,(H_2O)_4^{+} + H_3O^+ \tag{2.2}$$

$$Cr\,(OH)_2\,(H_2O)_4^{+} + H_2O \leftrightarrow Cr\,(OH)_3.aq + H_3O^+ \tag{2.3}$$

The deprotonated forms are $CrO–H^{2+}$.aq, $Cr(OH)_2^+$.aq and Cr(OH).aq dominating successively within pH 4–10. Trihydroxochromium is sparingly soluble within a pH range of 5.5–12 (minimum between pH 6.5 and 11.5 (Rai et al. 1989; Saleh et al. 1989). $CrOH^{2+}$.aq and $Cr(OH)_3$.aq are expected to be the dominant forms of Cr_3 in the environment (Kotaś and Stasicka 2000). $Cr(OH)_3$.aq exhibits amphoteric behaviour and at higher pH is transformed into readily soluble tetrahydroxo complex, $Cr(OH)_4^-$ (pK = 15.4 (Rai et al. 1989) or pK = 18.3 (Bees and Mesner 1976).

$$Cr(OH)_3(s) + 2H_2O \leftrightarrow Cr(OH)_{4^-} + H_3O^+ \tag{2.4}$$

For more concentrated Cr^{3+} solutions ($C > 10^{-6}$ M) (Rai et al. 1989), the polynuclear hydrolytic products $Cr_2(OH)_2^{4+}$, $Cr_3(OH)_4^{5+}$, $Cr_4(OH)_6^{6+}$ could be expected (Kotaś and Stasicka 2000).

Cr^{3+} is a hard acid which exhibits a strong tendency to form hexacoordinate octahedral complexes with a variety of ligands such as water, ammonia, urea, ethylenediamine, and other organic ligands containing oxygen, nitrogen or sulphur donor atoms (Nakayama et al. 1981a; Saleh et al. 1989). The complexation of Cr^{3+} by ligands other than OH^- increases its solubility when the ligands are in form of discrete molecules or ions. When, however, donor atoms are bound in a macromolecular system, as humic acids than the Cr^{3+} complex is more or less mobile. If the complexation from these ligands can be neglected, under Redox and pH conditions normally found in natural systems, Cr is removed from the solutions as $Cr(OH)_3$, or in the presence of Fe^{3+}, in the form of $(Cr_xFe_{1-x})(OH)_3$., (where *x* is the

mole fraction of Cr) (Sass and Rai 1987). Mediation by Manganese oxides was found to be the effective oxidation pathway in environmental systems (Nakayama et al. 1981a; Schroeder and Lee 1975; Bartlett and James 1979). This was due to the fact that the Redox potential of the Cr^{6+}/Cr^{3+} couple is high enough, leaving only a few oxidants capable of oxidising Cr^{3+} to Cr^{6+} (Kotaś and Stasicka 2000).

2.9.3 Hexavalent Chromium (Cr^{6+})

Chromium forms several species, relative proportions of which depend on pH (e.g. between pH 1 and 6, $HCrO_4^-$ is the predominant form, until it attains the Cr^{6+} concentration 10^{-2} M when it starts to condense yielding the orange-red dichromate ion) (Cotton and Wilkinson 1980; Greenwood and Earnshaw 1984; Nieboer and Jusys 1988).

$$H_2CrO_4 \leftrightarrow H^+ + HCrO_{4^-} K = 10^{-0.75} \tag{2.5}$$

$$HCrO_{4^-} \leftrightarrow H^+ + CrO_4^{2-} K = 10^{-6.45} \tag{2.6}$$

$$2HCrO_{4^-} \leftrightarrow Cr_2O_{7^{2-}} + H_2O\ K = 10^{2.2} \tag{2.7}$$

Within the normal pH range in natural waters the CrO_4^{2-}, $HCrO_4^{2-}$ and $Cr_2O_7^{2-}$ ions are the forms expected. They constitute a lot of Cr^{6+} oxyanions, which, are readily reduced to trivalent forms by electron donors such as organic matter or reduced inorganic matter, which are ubiquitous in soil, water and atmospheric systems (Stollenwerk and Grove 1985).

2.10 Chromium in the Environment

2.10.1 Water Systems

In this system, Cr originates from weathering or rock, wet precipitation and dry fallout from the atmosphere and run off from the terrestrial systems (Kotaś and Stasicka 2000). In rivers and lakes, the Cr concentration is usually limited to 0.5–100 nM (Handa 1988; Kaczynski and Kieber 1993). In seawaters it varies from 0.1 to 16 nM (Dejong and Brinkman 1978). The tanning industry can contribute significantly to the increase in Cr concentration if located near the water systems.

In nature Cr exists in its two stable oxidation states, Cr^{3+} and Cr^{6+}. The presence and ratio between these two forms depend on various processes; chemical and photochemical, Redox transformation, precipitation/dissolution (Kotaś and Stasicka 2000). Campanella (1996) described the Cr^{3+} in oxygenated aqueous solution as predicted by thermodynamic calculations on the stable species at $pH \leq 6$ whereas

Table 2.2 Behaviour of chromium in water systems

Description	Influencing factors
Increase of Cr^{3+} in water	Nature and concentration of reducers, oxidation mediators, complexation agents, pH, and O_2 concentration (Pettine and Millero 1990; Kieber and Helz 1992), photochemical generation of Cr^{3+} (Kieber and Helz 1992). However Cr_3 is immobilised by macromolecular compounds and therefore removed from the solution, which results in decrease of Cr^{3+} mobility and bioavailabilty in water. Cr^{3+} complexes are better stabilised by ligands other than H_2O and OH^- (Kotaś and Stasicka 2000).
Reduction of Cr^{6+}	Attained via Fe^{2+} (Schroeder and Lee 1975; Rai et al. 1989; Kieber and Helz 1992; Hug et al. 1997; Sedlak and Chan 1997), hydrogen peroxide (Pettine and Millero 1990; dissolved organic matter (Schroeder and Lee 1975; Rai et al. 1989). Speciation of Cr_3 in water – result in aqua/hydroxo complexes (Kotaś and Stasicka 2000) have a tendency to be adsorbed by naturally occurring solids (Rai et al. 1989) to form other complexes with organic materials, fulvic, humic and other acids (Nakayama et al. 1981a).
Cr^{3+} oxidation in water	Occurs in presence of manganese oxide (Schroeder and Lee 1975; Bartlett and James 1979; Nakayama et al. 1981a; Pettine and Millero 1990), Cr^{6+} is weekly sorbed to inorganic surfaces. This results in high mobility in the environment.

$pH \geq 7$ the CrO_4^{2-} ion as predominate under anoxic and suboxic condition, trivalent Cr should be the only form (Kotaś and Stasicka 2000).

The nature and behaviour of various Cr forms (Table 2.2) found in wastewater can be quite different from those present in natural water because of altered physico-chemical condition of the effluents originating from various industrial sources. The presence and concentration of Cr forms in effluents depends on Cr compounds applied during processing, pH, organic and/or inorganic waste coming from the material processing (Kotaś and Stasicka 2000).

Cr^{3+} dominates from tanneries, textile (printing, dyeing) and decorative plating industry waste (Nriagu 1988). With reference to the tanning industry, Cr^{3+} in the effluents is the most expected form but with Redox reactions occurring in the sludge, an increase in the hexavalent form can occur. Under slightly acidic or neutral pH conditions in this type of wastewater, the poorly soluble $Cr(OH_3)$.aq should be the preferred form, but a high content of organic matter originated from hide/skin material processing is effective in forming soluble Cr^{3+} complexes (Stein and Schwedt 1994; Walsh and O'Haloran 1996a, b).

2.10.2 Soil Systems

An increase in local chromium concentration in soils (Table 2.3) originates from fallout and wash out of atmospheric chromium containing particles as well as from

Table 2.3 Behavior of chromium in soil systems

Description	Influencing factors
Cr^{3+} in soils	Adsorbed to soil components, which prevents Cr leaching into groundwater or its uptake by plants (Walsh and O'Haloran 1996a) and in soils, Cr is largely present as insoluble $Cr(OH)_3$.aq (Kotaś and Stasicka 2000).
Cr complexes	Under acidic conditions (pH < 4) the dominant form is $Cr(H_2O)_6^{3+}$, at pH < 5.5 the dominant hydrolysis product is $CrOH^{2+}$ (Ritchie and Sposito 1995). Both these forms are easily adsorbed by macromolecular clay compounds. Under acid conditions (<6.0) $HCrO_4^-$ is the more dominant form. The increase in pH observed could be due to increased negative charge of soil or by protonation of successive ligating groups in clay. Humic acids are among those that contain donor groups forming stable Cr^{3+} complexes, especially when they produce chelate rings (Kendorf and Schnitzer 1980).
	In neutral to alkaline soils, Cr^{6+} exists in soluble (e.g. Na_2CrO_4) to moderately to sparingly soluble chromates (e.g. $CaCrO_4$, $BaCrO_4$, $PbCrO_4$) (Bartlett and Kimble 1976).
	Under oxidation and reduction, chromium converts to Cr^{3+} to Cr^{6+} and vice versa (Bartlett and Kimble 1976). This depends on, O_2 concentration, presence of reducers and mediators (ligands or catalyst).
Cr^{6+} mobile forms	$HCrO_4^-$ and CrO_4^{2-} could be reduced by different organic reducers (e.g. Fe^{2+} or S^{2-}) (through dechromification) (James and Bartlett 1983).

the chrome bearing sludge and refuse from industrial activity (Kotaś and Stasicka 2000). Dechromification is thought of as being of vital importance because without it, theoretically all atmospheric oxygen could be a threat to life on earth (James and Bartlett 1983).

Cr^{3+} adsorption into humic acids renders it insoluble, immobile and unreactive. This process is most effective within the pH range of 2.7–4.5 (Walsh and O'Haloran 1996b). Other macromolecular ligands behave similarly. In contrast, mobile ligands such as citric acid, diethylene triamine pentaacetic acid (DTPA) and fulvic acid form soluble Cr^{3+} complexes, which mediate its relocation and oxidation to Cr^{6+} in soils (James and Bartlett 1983; James 1996).

2.10.3 Atmospheric Systems

Chromium present in the atmosphere originates from anthropogenic sources, which account for 60–70%, as well as from natural sources, which account for the remaining 30–40% (Seigneur and Constantinou 1995). Industrial activities still remain the major source of pollution to the atmospheric systems. Others could be natural sources like volcano eruptions and erosion of soil and rocks (Kotaś and Stasicka 2000). Sea salt particles and forest wild fires do not seem to be important sources of chromium (Payna and Nriagu 1988). Average atmospheric concentrations of this metal are, 1 ng/m^3 in rural to 10 ng/m^3 in polluted urban areas (Nriagu 1988). The amount of chromium at any particular time depends on the intensity of industrial

processes, proximity to the sources, the amount of chromium released and meteorological factors (Kotaś and Stasicka 2000).

Chromium forms mostly ionic compounds as illustrated earlier in Tables 2.1–2.3 depending on natural or athropogenic sources. Their vapour can be neglected and one may assume that gaseous chromium species do not exist at ambient atmospheric temperatures and chromium is present in the atmosphere in the form of particles and droplet aerosols (Seigneur and Constantinou 1995). On the other hand, chromium from sources releasing the element in lager particles (particle diameter varies: 0.2–50 μm is deposited locally and can migrate through individual, particular environmental media. The size of particles is of importance for consideration of chromium toxicity: Friess (1989) found that only the particles of diameters from 0.2 to 10 μm are respirable, and that their retention in the lung can pose carcinogenic risk.

2.10.4 Chromium Pathways in the Environment

The distance covered by a deposited metal in the environment depends on meteorological factors, topography and vegetation (Nriagu 1988). Transport within the terrestrial and water systems is greatly affected by chemical speciation; chemical forms of chromium and their affinity to chemical and photochemical Redox transformations, precipitation/dissolution and adsorption/desorption process e.g. occurring in individual compartments of the biogeochemical cycle of chromium (Kotaś and Stasicka 2000).

Cr^{6+} is known as the most mobile Cr form in soil and water and tends to dominate in these systems, whereas Cr^{3+} is generally not transported over great distances because of its low solubility and tendency to be adsorbed in the pH range typical of natural soils and water. Redox conversion of Cr^{3+} to Cr^{6+} can increase Cr dislocation from the soil into the water systems (Schroeder and Lee 1975; Bartlett and Kimble 1976; James and Bartlett 1983).

2.11 Microorganisms and Plants

Batch shake culture studies by Bai and Abraham (2001) pertaining to the assessment of the best adsorption parameters (Table 2.4) and quantitative analysis of Cr uptake by the fungus *Rhizopus nigricans* revealed the following results;

Results showed that the biomass could bind approximately 47 mg Cr/g biomass. The biomass showed 100 and 99% adsorption efficiencies at Cr concentrations of 50 and 100 $mg\,L^{-1}$, respectively. Hence *R. nigricans* exhibited the potential for application in treatment of industrial effluents containing hexavalent chromium (Bai and Abraham 2001).

Two species of bacteria, identified as *Bacillus circulans* and *Bacillus megaterium* were able to bioaccumulate 34.5 and 32.0 mg Cr/g dry weight, respectively, and

Table 2.4 Parameters at optimum conditions of adsorption by *Rhizopus nigricans* Bai and Abraham (2001)

Parameter	Value(s)
pH	2.0
Rpm	120
Cr conc. Range	50–400 mg L^{-1}
Biomass dose	0.5% (w/v)
Mycelial diameter	90 μm
Temperature	45°C
Contact time	30 min–4 h

brought the residual concentration of Cr^{6+} to the permissible limit in 24 h when the initial concentration was 50 mg Cr^{6+} L^{-1} (Bai and Abraham 2001). Biosorption of Cr^{6+} was shown by *B. megaterium* and another species, *B. coagulans* (Bai and Abraham 2001). Living and dead cells of *B. coagulans* biosorbed 23.8 and 39.9 mg Cr g^{-1} dry weight, respectively, whereas 15.7 and 30.7 mg Cr g^{-1} dry weight were biosorbed by living and dead cells of *B. megaterium,* respectively. Biosorption by the dead cells was found to be higher than the living cells. This is due to prior pH conditioning (pH 2.5 with deionised water acidified with H_2SO_4) of the dead cells (Srinath et al. 2002).

Plants grown in different chromium concentrations (50–200 μM) showed appreciable concentration of the metal accumulated in their tissue, the highest being in roots. The amounts of chromium accumulated in plant tissues resulted in significant inhibition of chlorophyll, protein contents and in vitro nitrate reductase activity in test plants (e.g. *Nelumbo nucifera* Gaertn.) (Vajpayee et al. 1999). Cr^{6+} is highly toxic to plants, and results in reduced root growth, phytomass and photosynthetic pigments: chlorosis, stunting and eventually plant death (McGrath 1982; Tripathi and Smith 1996; Gauer et al. 1994; Sharma et al. 1995).

2.12 Chlorinated Phenols

The curing and storage phase of the hides and skins utilises various types of insecticides and anti-mould chemical compounds. All these biocides including the bacteriostats used in the tanning industry are eventually discharged into the tannery effluents. Most of these compounds belong to the chlorophenols, which enter the environment through several pathways (Steiert and Crawford 1985). Lyytikäinen (2004) reported that chlorinated phenols are weakly acidic compounds, and thus pH is the most significant factor affecting their fate in the environment. In conditions where ambient pH increases above their acidity constant pKa, chlorophenols can donate protons to form phenolate ions (Schellenberg et al. 1984). The tendency increases along with increasing chlorination of the benzene ring. The phenolate form is more water-soluble than its non-ionised counterpart. For example, the solubility of PCP increases from 10.8 to 2,357 mg L^{-1} when the pH increases from 5 to 7 (Shiu et al. 1994). This obviously has a drastic effect on sorption, transport

and bioaccumulation processes of the contaminant. The tendency of phenolate ions to sorb on soil or sediment is 15–30 times lower compared to their conjugated acids (Lagas 1988).

It was reported by Escher et al. (1996) that phenolic compounds exert toxic effects on microorganisms disrupting energy transduction either by uncoupling oxidative phosphorylation or inhibiting electron transfer. Substituted phenols act by destroying proton gradients by transporting protons back across the membranes and/or inhibiting electron flow by binding to specific components of the transport chain (Escher et al. 1996).

Chlorophenols are routinely determined by chemical analysis. The disadvantage of this method is the inability to determine the bioavailable concentration of the chlorophenols present, in effect providing no information on the possible effects of the compounds on the biomass (Sinclair 1999). Furthermore, chemical analysis does not provide information in the toxicity of the compounds and factors that affect toxicity. Biological assessment can incorporate sensors that can identify the toxicity of chlorophenols in a variety of environmental matrices. It assesses the impact of any environmental factor on the toxicity of these compounds quickly and easily (Sinclair 1999; Paton et al. 1995).

2.12.1 Behaviour of Chlorophenols on Soils and Water

The uncoupling power of a phenol depends on whether it is in a neutral or charged state inside the membrane. Neutral and charged compound are not taken up at the same rate but work together to destroy the protein gradient (Escher et al. 1996). Chlorophenols can be degraded by many different pathways, which are dependent on a number of factors such as the degree of chlorination, the oxygen concentration of the medium and the organisms present (Haggblom and Valo 1995).

Microorganisms have been used in testing chlorophenol toxicity. A study by Chaudri et al. (1996) (most probabale number (MPN)) attempted to measure the toxicity of chlorophenols in soil using *Rhizobium leguminosarum* biovar *trifolii*. Biological degradation is determined mostly by the physical properties of the compound and environmental factors such as pH, temperature, soil moisture level and soil composition (Cork and Krueger 1991). Chlorophenols are co-metabolised (cometabolism is the microbial transformation of a compound which is unable to support cell replication in the presence of a transformable co-substrate that supports cell replication) by several bacteria but they have to compete with the original inducing compounds for the active sites on the enzymes (Adriaens and Vogel 1995). This infers that degradation will not occur if catabolic enzymes are not induced.

Degradation of the chlorophenols in soil by photolysis is not a major degradation pathway. The amount of light penetration depends on soil properties with the two most important factors being the presence of vegetation and the nature of the pore space at the surface (Killham 1995). The presence of other xenobiotic can inhibit degradation. Photolysis is the fastest way of degrading PCP by causing hydrolysis

and oxidation (Nielson 1990). This results in complete mineralisation to carbon dioxide and chlorine ions but only occurs in air and water (Valo et al. 1985).

Physical and chemical processes are frequently employed to treat contaminated sites, but often do not destroy contaminants (Bouwer et al. 1994). Although chemical analysis of environmental samples can produce a measure of the total concentration of the chemical present in the sample, it will not indicate what is bioavailable in the sample (Steinberg et al. 1995) and hence what the likely toxic impact will be. Bioavailability is important for many organic compounds, particularly when they are associated with humic and other natural complexing agents (Shaw et al. 2000). Bioremediation is practiced by optimising environmental conditions to stimulate the degradation of the pollutants by naturally occurring microorganisms. However, bioremediation is limited in the materials that it can treat and by conditions at the treatment site (Sousa et al. 1998).

Microorganism which carry out degradation of a specific xenobiotic may not be able to degrade if the concentration are too low to support growth if affected by toxins or predators or they use other substrates in the presence of the xenobiotics (Zaidi et al. 1988). Many abiotic factors can influence the rate of degradation in soil such as pH, Redox conditions and organic matter (Jensen 1996). In aquatic systems, chlorophenols will be available through runoff, hydrodynamic transport and direct effluent discharge.

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Chapter 3
Ecotoxicological Techniques and Assessment of Environmental Samples

Abstract In this chapter the primary aspect was to dissect and determine the toxic nature of effluent and environmental samples (river sediments and water). This also involved sample manipulation coupled to a biosensor toxicity assay for the purpose of identifying possible remediation strategies for future environmental conservancy. Traditionally, the tanning industry has been associated with odours and water pollution from partially or untreated discharges. Sparging was used to identify toxicity associated with volatile organic compounds. This type of toxicity testing technique was found to be ideal for samples collected from tannery effluent treatment pits or pans and anaerobic lagoons. For example the toxicity of contaminants removed by treatment with activated charcoal, was identified for all the sampling points (tannery effluent treatment pits, anaerobic lagoons and riverine sampling points) except for the points upstream. A similar result was observed when filtration, as a technique, was used to identify toxicity associated with suspended solids. The approach used highlighted the complex nature of the toxic pollutants in the tannery effluent. Moreover the results strongly indicated the polluting sources and also the possible remediation strategies for effluents at various stages of the tanning industry.

3.1 Introduction

The many diverse environmental impacts of tanneries have made them subject to relatively sophisticated pollution control policies in many countries. Factory sites, lagoons, storage areas and temporary waste dumps may severely contaminate the underlying soil and water systems if appropriate precautions are not taken. As the first line of environmental audit, an ecotoxicological risk assessment is taken by use of multiple criteria or multiple lines of evidence (Suter 1993; USEPA 1991). Ecotoxicological assessment entails understanding the categories of hazardous waste, hazard identification, exposure assessment, ecological effects and risk characterisation. Ecological risk assessment (ERA) is a relatively new approach to quantifying the risk of significant harm to organisms and their ecosystems, but it is already a requirement in the developed world. For example, Part IIA of the

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Environmental Protection Act 1990 and the Habitats Directive (UK) (UK Habitat 2004). Ecological risk assessment will be used during this study for a quantitative and qualitative evaluation of the tanning industry.

The need to use biological techniques for the monitoring of environmental pollution by industry and regulators alike has become a necessity (Cervantes et al. 2000; Saxena et al. 2000). Therefore the integration of both chemical and biological approaches to underpin ecotoxicity testing is essential. While chemical methods have been traditionally used to determine total concentrations of the pollutants, biologically linked measurements on the other hand have been used to assess the bioavailable fractions of the pollutants (Steinberg et al. 1995; Paton et al. 1997). In this chapter both chemical and biological approaches using *lux*-based biosensors (*Escherichia coli* HB101 pUCD607), a dehydrogenase activity test and a set of water chemistry parameters to investigate river health at the study site (Fig. 3.10) to provide a stressor (pollutants) profile is discussed. For example, whole cell microbial biosensors for monitoring environmental contamination by heavy metals (Knight et al. 1999) and organic contaminants (e.g. chlorinated phenolics) (Heitzer et al. 1992) as well as toxicity in soils and water contaminated by industrial effluents have previously been used (Brown et al. 1996; Duncan et al. 1994) (Fig. 3.1).

There is an increasing trend towards the use of biological techniques as indicated earlier for monitoring the hazards associated with environmental pollution by industry and regulators alike. This is particularly applicable to components of tannery waste when disposed in environmental systems. Chromium toxicity has been investigated using biological techniques involving microorganism (Cervantes et al. 2000; Saxena et al. 2000; Chen and Hao 1998; Chirwa and Wang 1997; Shen and Wang 1993) and plants (Prasard et al. 1991). The toxicity of chlorinated phenols has also been assessed by bioluminescence-based ecotoxicity tests (Lagido et al. 2001; Mwinyihija et al. 2005a, 2006).

Lux (reporter genes encoding marine bacterial luminescence (i.e. light production)) bacterial biosensor technology has been used to measure the toxicity of heavy metals in a number of matrices ranging from aqueous solutions of single compounds to industrial effluents (Sinclair 1999). The light emission intensity is proportional to the concentration of the toxic analyte over a certain concentration range, allowing one to perform a quantitative analysis. For example, Paton et al. (1995) used the luminescence response of a chromosomally *lux*-marked bacterium, *P. fluorescens,* to assess the toxicity of metal salts. Chaudri et al. (1999) used the luminescence response of *lux*-marked bacteria to assess the toxicity of zinc in pore water in a long-term sewage sludge field trial. Galli et al. (1994) used naturally luminescent marine bacteria (Microtox) to test the toxicity of soil from a site contaminated with various pesticides, dyes and other chemicals. Mwinyihija et al. (2005a, b, 2006) carried out the assessment of toxicity levels in various samples form the tannery effluent, dust and river sediment using *E. coli* HB101pUCD based biosensor.

The advantages associated with the use of genetically modified bacterial biosensors over other forms of ecotoxicity testing are that they are rapid, sensitive, easy to culture and maintain, flexible in terms of selecting for environmental relevance, and represent reliable tools which integrate the many factors contributing to environmental toxicity (Wild et al. 1993).

Fig. 3.1 (**a**,**b**) Tannery effluent showing the free wish blue chromium effect on the surrounding environment (**a**) along the draining canal and (**b**) within the discharge points at the river

Lux bacterial biosensor assays of toxicity can be linked to sample manipulation to assess the scope and the nature of possible remediation strategies. Sousa et al. (1998) used sample manipulation (coupled to bioassay with *lux*- marked bacteria) to examine the toxicity of a site contaminated with BTEX (benzene, toluene, ethylbenzene, xylene) compounds. The use of biosensors enabled reporting on site toxicity characteristics and contaminant bioavailability. The manipulations included sparging, filtration, muffle furnace and pH adjustment of the sediment samples. This enabled any constraints to bioremediation (such as adverse pH, heavy metals or volatile organics) to be identified (Sinclair 1999). In the case of environmental constraints, success is only likely to be attained if these constraints are identified and means are devised to alleviate them to an extent where bioremediation can effectively proceed

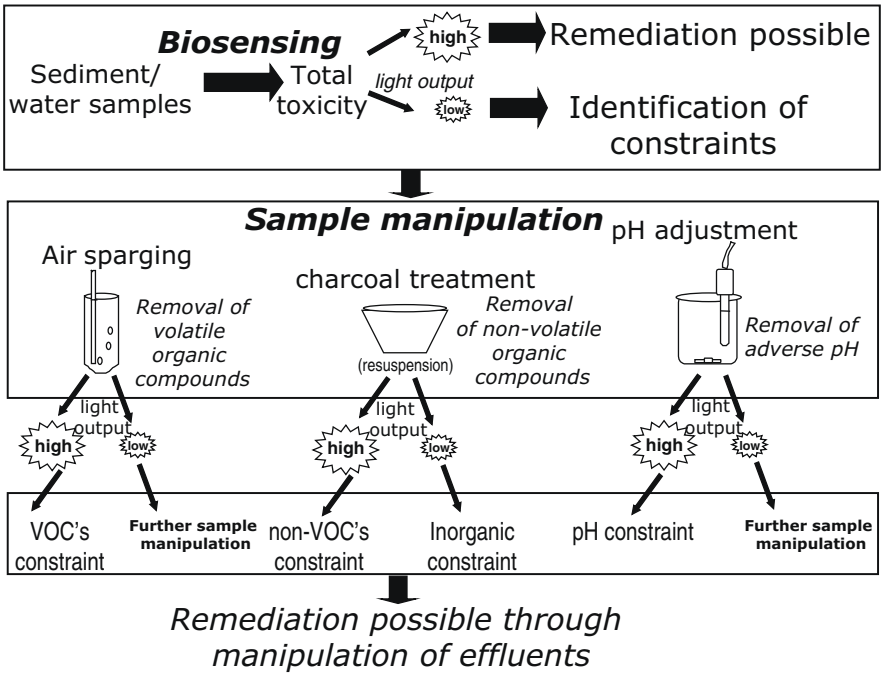

Fig. 3.2 Schematic representation of toxicity dissection VOC's (Volatile organic compounds), non-VOC's, inorganic and pH constraints in Kenya tannery effluents and riverine samples

(Allan-King et al. 1994). The use of the *lux* biosensor *E. coli* HB101 pUCD607 in relation to sample manipulation, allowing dissection and classification of sample toxicity, has not previously been applied to tannery waste (Fig. 3.2).

Triplicate 900 µl aliquots of each sample were taken using a Gilson pipette (Pipetman Model P1000) for biosensor test immediately after opening the bottles and the bioassay carried out immediately. Additional aliquots were taken for pH measurement and submitted subsequently to a series of protocols in order to identify underlying toxic constraints to remediation and assess the scope for their alleviation.

3.2 Toxicity Dissection

3.2.1 Sparging

Aliquots (900 µl) of effluent and sediment extract were pipetted into luminometer cuvettes and sparged with air (N_2) for 10 min (at 1,650 mL/min). The high flow of air allowed for rapid removal of volatiles present.

3.2.2 Activated Charcoal Treatment

Charcoal was first conditioned by placing 100 g of charcoal in a Duran bottle filled with double deionised water and allowing it to stand for 48 h, prior to multiple (×10) deionised water rinsing and recovery by filtration. Ten milliliter of the effluent or sediment extract was placed in a centrifuge tube. Charcoal (0.1 *g*) was added and shaken for 30 min. Samples were centrifuged (3,000*g*) for 20 min (MSE Coolspin 2) at 48°C and toxicity of the supernatant tested by transferring 900 μL of the sample into a luminometer cuvette (Clinicon, Petworth, West Sussex, U.K.) and adding 100 μL of resuscitated biosensor suspension.

3.2.3 Filtration

Aliquots of water samples and sediment extracts (50 mL) were filtered through a cellulose acetate membrane filter (0.22 μm pore diameter) in order to determine toxicity related to suspended solids. Triplicates of each sample were submitted to the bioassay immediately after filtration.

3.2.4 pH Adjustment

Aliquots of the water samples (pH 5.5) and effluent/sediment extracts were adjusted to pH 4.0, 6.0 and 8.0 with 0.1 M sodium hydroxide and 0.1 M hydrochloric acid. Triplicates of the samples were submitted to the bioassay immediately after pH adjustment.

3.3 Toxicity Testing Using *Escherichia coli* HB101 pUCD607

Determination of toxicity was based on the bioluminescence response of the *lux*-modified biosensor, *E. coli* HB101 pUCD607, which had previously been, marked with the *lux* CDABE genes, (isolated from *Vibrio fischeri)* using the multi-copy plasmid pUCD607 (Amin-Hanjani et al. 1993). The biosensor was stored at −20°C and resuscitated from freeze dried condition prior to bioassay. Results from the toxicity dissection is shown in Tables 3.1–3.3.

The choice of the *lux*-marked biosensor in this work offered great environmental relevance in dissecting and categorising into broad groups the toxic nature of the effluent from the tanning industry. For example Kenyan tannery samples from the effluent treatment pits (Table 3.5) and anaerobic lagoons (lagoons 1, 2, 4 and 5) (Table 3.2) were associated with this type of toxicity.

Table 3.1 Percentage maximum bioluminescence of effluent treatment pits untreated and after treating with sparging, activated charcoal, filtration and pH adjustment calculated against a blank of double-deionised water. Figures in parentheses are standard errors of the mean (SEM) (n = 9)

	No Treatment	N_2 Sparged	Activated charcoal	Filtration	pH adjustment		
Samples	Means	Means	Means	Means	4.00	6.00	8.00
Beam house	0.06 (0.04)	101.82 (8.2)	125.14 (1.06)	63.15 (0.83)	4.32 (0.72)	83.42 (1.68)	0.04 (0.04)
General sedimentation	2.40 (1.02)	141.21 (2.40)	167.53 (2.20)	105.56 (1.61)	4.72 (0.81)	202.00 (23.37)	2.40 (1.02)
Strip Chrome Tank	0.004 (0.004)	84.67 (1.40)	65.26 (0.90)	0.01 (0.003)	1.83 (1.05)	43.29 (2.20)	0.00 (0.001)
Chrome sedimentation	26.26 (12.70)	120.84 (8.3)	34.36 (1.50)	0.43 (0.02)	169.62 (33.7)	284.10 (8.10)	26.26 (12.67)
Equalisation Tank	6.83 (2.60)	46.36 (46.4)	39.14 (0.9)	5.88 (0.34)	0.03 (0.02)	36.77 (1.81)	6.83 (2.56)
Reference (Ddw)	104.85 (5.36)	94.75 (0.49)	101 (1.61)	99.97 (1.13)	102.87 (3.82)	107.29 (4.06)	114.24 (2.70)
LSD (5%)	18.27	18.23	4.39	2.61	42.71	31.80	16.66

Ddw Double deionised water

Table 3.2 Percentage maximum bioluminescence of anaerobic effluent treatment lagoons untreated and after treating with sparging, filtration and pH adjustment. Figures in parentheses are standard errors of the mean (SEM) (n=9)

	No Treatment	N_2 Sparged	Activated charcoal	Filtration	pH adjustment		
Samples	Means	Means	Means	Means	4.00	6.00	8.00
Lagoon1	27.86 (7.0)	90.89 (9.93)	70.92 (3.60)	149.32 (1.70)	2.10 (0.78)	4.86 (0.11)	115.11 (21.24)
Lagoon2	34.07 (21.2)	87.07 (8.00)	122.43 (22.55)	139.58 (6.64)	1.40 (0.36)	56.00 (1.45)	116.49 (23.14)
Lagoon3	77.08 (23.1)	89.39 (13.5)	154.07 (3.44)	145.62 (1.67)	1.02 (0.35)	4.89 (0.49)	91.76 (0.88)
Lagoon4	8.39 (0.90)	100.54 (7.70)	104.92 (20.30)	94.56 (1.50)	15.17 (0.91)	182.65 (0.70)	138.23 (0.50)
Lagoon5	1.83 (0.50)	95.59 (11.70)	69.88 (10)	104.77 (1.22)	0.62 (0.62)	3.16 (0.70)	110.74 (0.39)
Reference (Ddw)	104.85 (5.36)	94.75 (0.49)	101 (1.61)	99.97 (1.13)	102.87 (3.82)	107.29 (4.06)	114.24 (2.70)
LSD (5%)	45.37	32.72	45.52	10.29	1.85	2.43	44.29

Table 3.3 Percentage maximum bioluminescence of riverine sediments untreated and after treating with sparging, activated charcoal and pH adjustment calculated against a blank of double-deionised water. Figure in parentheses are standard error of the means (SEM)(n = 9)

	No Treatment	N_2 Sparged	Activated charcoal	Filtration	pH adjustment		
Samples	Means	Means	Means	Means	4.00	6.00	8.00
200 m upstream	97.13 (2.5)	96.33 (7.4)	82.54 (15.0)	100.67 (4.04)	86.38 (1.4)	71.83 (3.1)	49.80 (11.1)
100 m upstream	87.40 (9.3)	85.86 (1.0)	46.80 (3.0)	99.26 (3.02)	85.84 (4.6)	74.37 (8.1)	35.75 (2.6)
0 m discharge point	64.01 (6.0)	57.06 (7.3)	85.30 (8.6)	115.68 (1.81)	102.16 (3.2)	74.26 (3.9)	1.15 (0.2)
100 m downstream	90.86 (3.8)	88.93 (1.92)	65.56 (22.3)	105.07 (2.10)	68.65 (1.7)	72.73 (12.3)	24.28 (1.8)
200 m downstream	78.49 (3.6)	81.50 (4.6)	74.57 (9.0)	104.83 (8.3)	53.36 (1.3)	71.03 (1.7)	10.85 (0.4)
400 m downstream	69.91 (3.9)	87.25 (3.6)	100.13 (4.0)	103.17 (2.70)	68.67 (2.8)	68.60 (19.4)	26.17 (1.8)
800 m downstream	87.21 (9.0)	83.51 (2.7)	103.09 (6.1)	100.14 (0.70)	73.95 (2.6)	78.66 (12.9)	30.66 (2.7)
Reference (Ddw)	104.85 (5.36)	94.75 (0.49)	101 (1.61)	100.80 (4.40)	92.25 (1.97)	104.85 (1.79)	80.58 (3.14)
LSD (5%)	18.51	14.23	35.08	9.47	7.41	32.20	12.26

Table 3.4 Percentage bioluminescence of the control, pH, dehydrogenase (ugTFg^{-1} sediment 6 h^{-1}) and their correlations at different river sediment sampling points

		% Bioluminescence		**Dehydrogenase µgTFg Sed^{-1}. 6 h**			
Sampling points	Ph	Mean	SEM	Mean	SEM	**r values**	**p values**
400 m up	6.96	74.2	8.2	0.0294	0.02	0.07	**
200 m up	6.94	97.1	2.5	0.0318	0.024	–0.9	***
100 m up	6.98	87.4	9.3	0.0346	0.028	0.5	**
0 m	6.49	64	6	0.0058	0.002	0.1	**
100 m down	7.05	90.9	3.8	0.0046	0.001	0.98	***
200 m down	7.05	81.5	4.6	0.0069	0.001	–0.76	**
400 m down	7.01	87.3	3.6	0.0204	0.002	–0.84	***
600 m down	7.1	84.8	7	0.0235	0.021	–0.56	**
800 m down	7.16	87.2	9.2	0.0269	0.03	0.89	**
LSD 0.05	11.1		0.032				
0.01		15.2		0.0439			
0.001		20.7		0.0598			

SEM Standard errors of mean
Sed Sediment
*p≤0.05, ** p≤0.01, *** p≤0.001

Table 3.5 Results showing heavy metals total concentration analysed using Atomic Absorption Spectrometry (Perkin Elmer 100) and total phenol concentration (gas chromatography) within the riverine sediment sampling points

Description	Cr mg kg^{-1}	Pb mg kg^{-1}	Fe mg kg^{-1}	Cu mg kg^{-1}	Cd mg kg^{-1}	Ni mg kg^{-1}	Zn mg kg^{-1}	Chlorinated phenols (mg L^{-1})
400 m up	0.89	0.4	948	0.46	0.03	0.49	1.03	ND
200 m up	0.93	0.27	772	0.16	0.02	0.43	0.79	ND
100 m up	1.14	0.39	1303	0.46	0.04	0.72	1.63	ND
0 m	1.31	0.69	1010	0.4	0.03	0.65	1.15	30.3
100 m down	1.41	0.36	1048	0.44	0.03	0.62	1.37	17.7
200 m down	1.65	0.58	1362	0.69	0.03	0.96	1.92	11.4
400 m down	1.76	0.58	1349	0.52	0.04	1.01	2.3	5.5
600 m down	1.78	0.71	1359	0.96	0.03	1.02	2.45	ND
800 m down	1.22	0.41	1234	0.48	0.03	0.71	1.34	ND

ND None detected

The response of sparged (N_2) samples reflected the toxicity of the samples once volatile organics had been removed (untreated samples showing total toxicity). This residual toxicity would be caused by inorganic and/or non-volatile organics in the sample. However, the increase of luminescence in this study in all the effluent treatment pits and anaerobic lagoons suggested that considerable toxicity was caused by volatile organics. The river samples showed no difference between untreated and sparged (N_2), demonstrating the "self sparging effect" inherent in high flow, active rivers. Alleviation of toxicity in sparged, effluent treatment pit and anaerobic lagoon tannery samples highlights sparging as a potential remediative technique for tannery effluent, which would be based on proven technology (Boyd et al. 1998; Mwinyihija et al. 2006).

Activated charcoal treatment to the effluent treatment pit samples showed toxicity associated with organics after the removal of certain inorganics and organics (particularly chlorinated hydrocarbons). Samples from the Beam-house, general sedimentation and the anaerobic lagoon responded by showing an increased percentage luminescence (stimulation). This observation was probably related to the charcoal-mediated removal of the high total phenols load in the samples. Toxicity from chlorinated phenolics has been reported by Sinclair (1999), and most chlorinated and non chlorinated phenolics are considered to be narcotics (Cronin and Schultz 1997). Phenolic compounds are known to be toxic through a protonophoric mechanism by acting as uncouplers, and/or inhibiting electron flow in the electron transfer chain. Phenols can accumulate in the membrane and disturb membrane function, causing narcotic effects (Escher et al. 1996). The observed impact of charcoal filtration on the toxicity of key tannery and associated environmental samples suggests that it may provide an important remediative step, exploiting established and cost-effective technologies.

The removal of particulate matter and colloidal materials through filtration was critical for samples from the Beam-house, general sedimentation and all the anaerobic lagoons. In relation to this observation, studies by Thanikaivelan et al. (2003) reported that activities such as soaking, liming, reliming (including fleshing) and deliming (Beam-house activities) account for 15–20% total solids containing lime sludge, fleshing and hair. Chrome sedimentation, chrome stripping and the equalisation tank showed the lowest response to filtration, suggesting that toxicity was not bound within the particulate and colloidal content of the samples. However, as the effluent flows towards the general sedimentation tank, an effect of filtration was observed, suggesting aggregation of the effluent contents to particulate matter. Coagulation and flocculation are envisaged to be the main activities in sedimentation tanks (UNEP 1994). Filtering of chrome sedimentation samples was associated with a slight increase in bioluminescence (not necessarily indicating a decrease of toxicity) in comparison to other samples within the effluent treatment pits. This phenomenon was also observed when the river samples were filtered, with the discharge point indicating stimulation. Along with the results from charcoal treatment, the effects of filtration on sample toxicity also highlighted this treatment as likely to have an important role in the remediation of tannery effluents, again using proven technologies.

Available metals are generally in the form of soluble cations and their tendency to be present in ionic form increases with increasing acidity (Sposito 1989). Sarin (2000) reported the toxicity response of *lux*-marked *E. coli* HB101 to a range of metals. In this study, metal toxicity and bioavailability patterns were identified through pH adjustment (4.0, 6.0 and 8.0) in the tannery effluent (Table 3.1), anaerobic lagoons (Table 3.2) and riverine sampling points (Table 3.3). The increase in % luminescence (>80%) on adjustment from pH 4.0 to pH 6.0 was demonstrated for all the tannery related samples tested (Tables 3.1 and 3.2). This suggested the presence of metal toxicity and the response to pH variation on bioavailability, which is imparted by changes in speciation and portioning effects of the metals (Ritchie et al. 2001; van Leeuwen 1999; McGrath et al. 1999; Knight and McGrath 1995). The tannery treatment effluent showed increased % luminescence at pH 6.0 (representing typical environmental conditions) (Mwinyihija et al. 2006) where the majority of the metals are limited in their bioavailability (Gadd 1990). Because of the alleviation in toxicity of all tannery samples through adjustment to pH 6, pH treatment (along with charcoal treatment and filtration) offers a potentially useful remediative option for tannery effluents.

Toxicity in samples such as treatment effluents, anaerobic lagoons and downstream riverine sampling points (Tables 3.1–3.3) was attributed to high concentrations of chromium and phenols. For example, although in tannery wastewater Cr^{3+} is the most expected Cr form, the Redox reactions occurring in the sludge can increase the concentration of the hexavalent form (Kotaś and Stasicka 2000). Most metals show increased solubility with decreased pH (Artiola 1996), indicating increased bioavailability (chemical assimilation and possible toxicity) of organic/inorganic compounds (Steinberg et al. 1995; Shaw et al. 2000; Alexander 2000). Under slightly acidic or neutral pH conditions in this type of wastewater, the poorly soluble $(Cr(OH)_3$ aq should be the preferred form, but a high content of organic matter originating from the hide material processing is effective in forming soluble organic Cr^{3+} complexes (Stein and Schwedt 1994; Walsh and O'Halloran 1996a, b). Samples from the discharge points showed higher toxicity when the samples were adjusted from pH 6.0 to pH 8.0. Other related studies investigating the fractionation of chromium toxicity in water using *E. coli* HB101 pUCD607 showed that speciation of chromium at different pH levels and a synergistic effect with other metals (e.g. copper and zinc) contributed to its toxicity (Wararatananurak 2000). This observation suggested that chromium is frequently a constraint to bioremediation in contaminated environments (Killham Pers. Comm. 2004).

3.3.1 Resuscitation of Freeze Dried Cultures

Freeze dried cultures of *Escherichia coli* HB101 pUCD607 were resuscitated in 10 mL of sterile 0.1 M KCl (contained in a Universal). 1 mL of KCl was added and the culture resuspended by mixing (drawing up and down five times into a PI000

Gilson pipette). The resuspended culture was transferred back to the universal and the culture placed in a shaking (200 rpm) incubator (25°C) for 1 h.

3.3.2 Sample Addition and Luminometry Measurements

One hundred microliters of the resuscitated biosensor suspension was added to the samples at 15 s intervals, accurately timed for measurement in the Bio Orbit 1,253 luminometer (Labtech International, Uckfield, U.K). Each sample was exposed to the sensor for exactly the same time. Samples were incubated for 15 min before light output measurements were carried out at 15 s intervals. This ensured the same exposure time to the potentially toxic elements for cells in each of the cuvettes.

3.3.3 Data Analysis

The output from the luminometer resulting from each assay carried out was recorded in relative light units (RLU's) (equating to mV/10 s/mL). The light output was then converted to percentage maximum bioluminescence. This was calculated against a blank of double deionised water adjusted to pH 5.5, the optimum pH for bioluminescence.

$$\% \text{ maximum bioluminescence} = I_S / I_{C*100} \tag{3.1}$$

where I_S = RLU's emitted by the cells exposed to the sample I_C = RLU's emitted by the cells exposed to the control.

The percentage (%) maximum bioluminescence was determined for the three sample replicates. A mean of this determination was then calculated. The assay performance was monitored by reference to the response to the control, the reproducibility of the response to the three replicates and the response to a standard of trichlorophenol (TCP) (Fig. 3.3a, b). Effect of exposure time on toxicity to a range of standard solutions of Zn and Cu were prepared by dilution with double deionised water at pH 5.5 (Fig. 3.4a, b).

3.4 Dehydrogenase Assay

The method was adapted from the protocol of Packard (1971), Benefield et al. (1977) and Paton et al. (1995). A volume of 2 mg of sediment was immediately transferred to light proof, universal vials containing 2 mL of buffer solution (TES 0.5 M, Sigma-UK), 0.1% INT (p-Iodonitrotetrazolium violet, Sigma-UK) and the samples. All preparations were carried out in triplicate for all the samples and performed

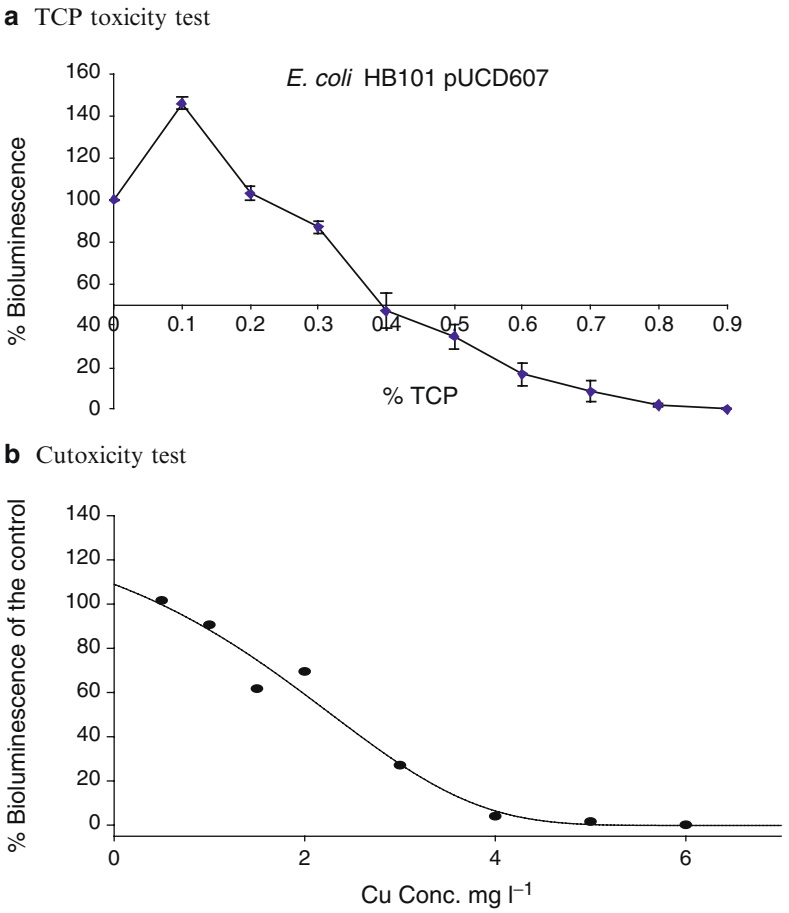

Fig. 3.3 (**a**, **b**) Quality control of *E. coli* HB101pUCD607 IMS (15 min bioassay) on TCP and Cu. **NB** (All error bars <17.6% of the largest bar show as points in the graph). All standards were prepared by dilution with double deionised water at pH 5.5. Curves were fitted using Sigma plot 9.0 and an equation and r-squared determined

under aseptic conditions. The mixture was vortexed and incubated for 6 h at 25°C, 225 rpm in an orbital shaking incubator. After 6 h of incubation, 10 mL of ethanol was added to stop bacterial activity and fix the colour. The universal vials (20 mL) were vortexed and absorbance of formazan measured at 490 nm (Fig. 3.5).

Dehydrogenase is an oxidoreductase enzyme and depends on oxygen as a terminal acceptor. The relationship between BOD and DO was therefore measured and a positive correlation was established between INT-dehydrogenase and DO ($r=0.3$, $p<0.01$) while a negative correlation was noted as expected between INT-dehydrogenase and BOD ($r=-0.6$, $p<0.001$). The BOD and DO showed a strong negative correlation ($r=-0.9$), indicating that increased oxygen demand in the breakdown of organic/inorganic matter chemically or biologically in an aquatic

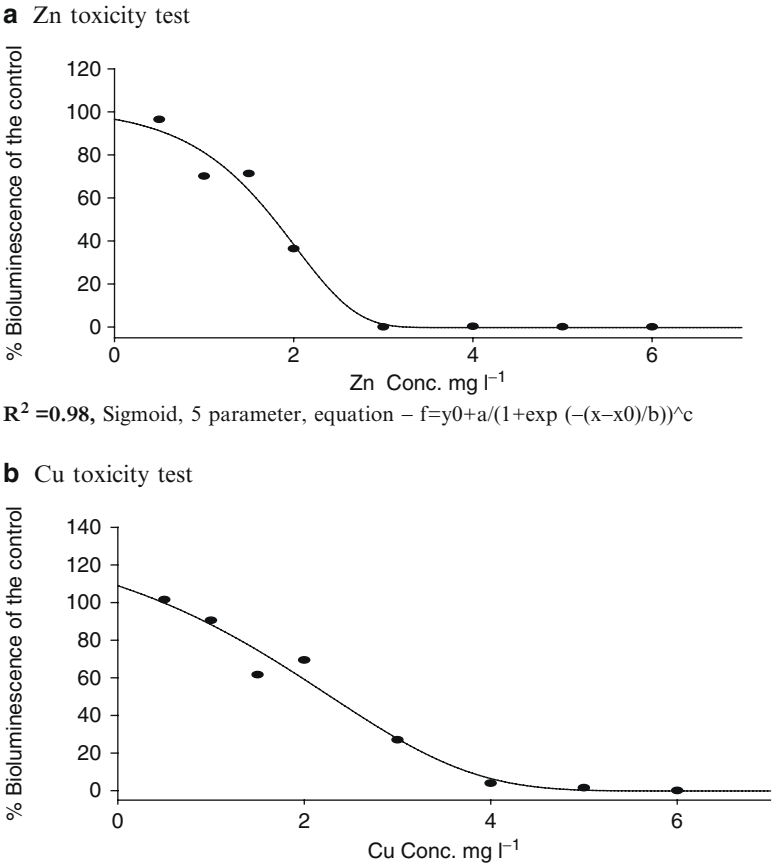

Fig. 3.4 (**a**, **b**) Quality control of *E. coli* HB101 pUCD607 IMS (15 min bioassay) on (**a**) Zn standard solutions (**b**) Cu standard solutions. **NB** (All error bars <17.6% of the largest bar show as points in the graph). All standards were prepared by dilution with double deionised water at pH 5.5. Curves were fitted using Sigma plot 9.0 and an equation and r-squared determined

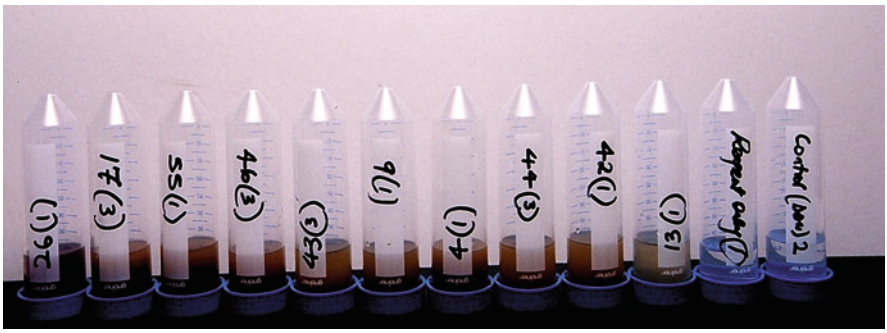

Fig. 3.5 Samples showing different colour intensity (e.g. Control samples cleaner than those from the impacted areas are progressively darker) due to the effect of INT dehydrogenase activity

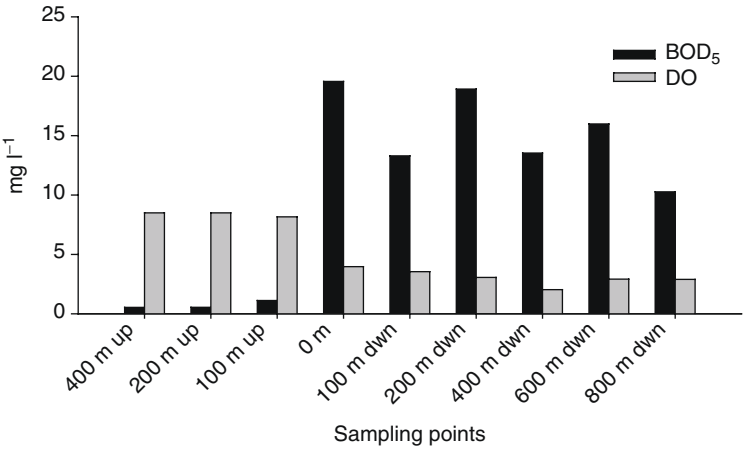

Fig. 3.6 BOD and DO levels upstream and downstream (River Sagana as impacted by the effluent from tanning industry in Kenya

system results in lowered dissolved oxygen (Fig. 3.6). This result further demonstrated that the depletion of oxygen in the river had an effect on the microbial activity. The biotic stress was highest in all cases at the discharge point rather than upstream. However, a gradual recovery was observed downstream implicating the tannery effluent as the source of contamination.

The experimental data in relation to percentage bioluminescence and dehydrogenase activity (Table 3.4) indicated that river health is impacted markedly by effluent from the tanning industry. This observation conforms to a study by Ros and Ganter (1998) who reported that tannery waste was a potential environmental pollutant. The impact of the tannery effluent was identified when the biological sensors showed a significant difference between the up and downstream data. The effectiveness of these sensors during the study was demonstrated when the biological effects (through analysis of biomass activity, bioluminescence and BOD parameters), effects of the tannery pollutants and other interacting environmental factors were predicted (Atlas and Bartha 1993; Cairns and Prat 1989; Gersberg et al. 1995, Mwinyihija et al. 2006).

Dehydrogenase activity effectively provided the status of the river sediment as a positive correlation with the dissolved oxygen level was established. This suggested that the increase in organic matter (Table 3.5) in the river resulted in depressed oxygen levels (Fig. 3.7) (due to biochemical degradation) affecting the DHA, which uses oxygen as a terminal electron acceptor (Skujins 1978). Similarly, a decrease in DHA was observed for metal contaminated soils, indicating reduced microbial activity in the soils (Cenci and Morozzi 1979; Ruhling and Tyler 1973; Doleman and Haanstra 1979; Schinner et al. 1980; Brookes et al. 1984). However in comparing DHA with oxygen status, a large proportion of O_2 uptake may not be accounted for due to the presence of alternate electron acceptors (Burns 1978; Sommervile et al. 1978) or DAH inhibition by humic acids (Pflug and Ziechman 1982),

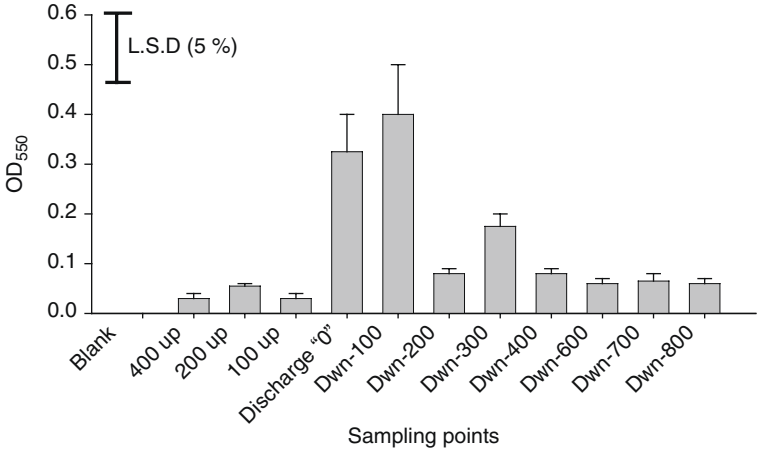

Fig. 3.7 OD550 value of water samples from (River Sagana) indicating turbidity and colour

high levels of inorganic nitrogen (Trevors et al. 1981) and reducing substances in anaerobic soils (Okazaki et al. 1983). To address this constraint, INT-DHA measurement instead of TTC-DHA was preferred due to INT competing well with O_2 liberated electrons and has a rapid and high sensitivity (Benefield et al. 1977; Hongwei et al. 2002).

Turbidity levels are associated with colour, colloidal and particulate matter, and, in this study, a close relationship was established with bioluminescence (positively correlated), INT-DHA and DO which were negatively correlated. The result demonstrated that high particulate matter (Fig. 3.7) increased demand of oxygen in biochemical degradation, which leads to stress in the aquatic system, eventually affecting the water quality. The impact of the tannery effluent on oxygen demand, colour and chemical compound concentration was also observed by Song et al. 2000.

3.4.1 Data Analysis

A calibration curve was prepared using standard solutions of INTF (Iodonitrotetrazolium formazan, Sigma-UK) to translate the absorbance values to concentration ($\mu gTFg^{-1}$ Sediment 6 h^{-1}).

3.5 Daphnia Test

Daphnia magna (a micro-crustacean) was used in the test as an invertebrate to represent the primary consumer level and identify short-term acute effects. This was carried out using ten freshly bred (neonates) *Daphnia magna* exposed to the

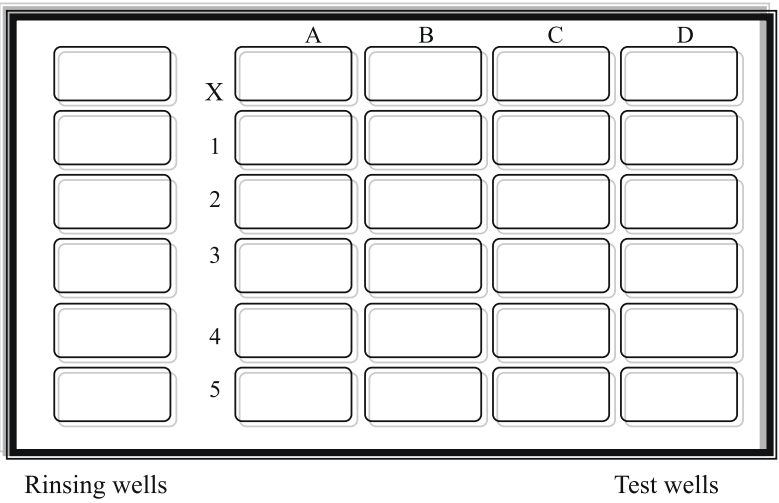

Fig. 3.8 A multiwell plate showing the rinsing well and test wells to carryout *Daphnia magna* test

riverine and tannery effluent samples for 24 h (Stuhlfauth 1995). The same clone of *D. magna* was used to ensure the same sensitivity during the testing.

The bioassays were conducted in a disposable multiwell test plates with 30 test wells (Fig. 3.8). Each plate was provided with four wells for the controls and four wells (A,B,C,D) for each toxicant concentration/sample. Additionally, the plates were provided on the left side with a column of "rinsing wells" to prevent dilution of the toxicant during the transfer of the neonates from the hatching petri dish to the test wells. Each well of the test plates was filled with 10 mL toxicant solution (or standard freshwater in the control column).

The hatching petri dish was placed on the transparent stage of a light table provided with a black strip to enhance the contrast. Ten (actively swimming) neonates were transferred with a micropipette into each rinsing cup in the sequence: row X (control), row 1 to row 5 (increasing concentrations of toxicant) (Fig. 3.8).

The data were scored on the results Sheet and calculated the % effect. The number of immobile *Daphnia magna* after 24 h was noted and expressed as a lethal dose (LD) given as a percentage.

The neonates in the multiwell plate were placed on the transparent stage of a light table provided with a black strip to enhance the contrast (Fig. 3.9) during the counting of dead and immobile *Daphnia magna*. Besides all other specific validity conditions prescribed in standard Daphnia bioassay protocols, the number of dead and immobile *D.magna* in the controls should not exceed 10%.

In order to check the correct execution of the test procedure and the sensitivity of the test animals, it is advised to perform a reference test from time to time. For example quality control tests can be performed with the reference

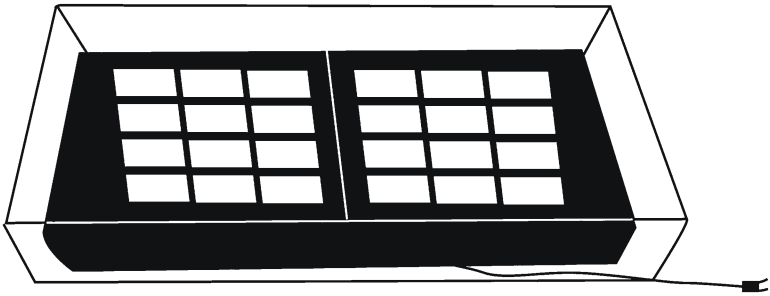

Fig. 3.9 Light table with transparent stage provided with a black strip to enhance the contrast during the counting of dead and immobile *D. magna*

Table 3.6 Lethal dose (LD) values of *Daphnia magna* on treatment pits, riverine and anaerobic lagoons of a Kenyan tannery site

Samples	LD values *D magna*
Treatment pits	
Beam-house	LD 50
General sedimentation	LD 50
Chrome stripping	LD 100
Chrome sedimentation	LD 100
Equalisation tank	LD 90
Anaerobic lagoons	
Lagoon1	LD 90
Lagoon2	LD 85
Lagoon3	LD 85
Lagoon4	LD 80
Lagoon5	LD 80
Riverine	
400 m upstream	NE
200 m upstream	NE
100 m upstream	NE
50 m upstream	NE
Discharge at 0 m	LD 85
50 m downstream	LD 80
100 m downstream	LD 80
200 m downstream	LD 80
400 m downstream	LD 60
600 m downstream	LD 60
800 m downstream	LD 50

NE No effect

toxicant potassium dichromate ($K_2Cr_2O_7$), using the following dilution series: 3.2–1.8–1–0.56–0.32 mg L^{-1}.

An example of related results (Table 3.6) obtained from a case study carried out in Kenya was as follows.

3.5.1 Invertebrate Trophic Level (Daphnia Magna)

Higher LD values were observed at the treatment pits (Chrome stripping, sedimentation and equalisation pits) (Table 3.6). The anaerobic pits showed a reduction on LD values indicating a reduction in toxicity as the effluent flows from lagoon 1 (LD 90) to lagoon 5 (LD 80). Similarly, the trend was observed in the riverine sampling points, with lower LD values noted downstream. The dilution effect of the river (reduction of LD value observed progressively) downstream and the source of toxicity at the discharge point was demonstrated. There was no effect observed upstream for *D. magna* upstream (50 m, 100 m, 200 m and 400 m upstream), indicating that the source of toxicity was from the tannery effluent at the discharge point (LD 85).

3.6 Chemical Analysis

In order to complement the bioassay tests carried out during this study, total concentration of Cr, Ni, Cu, Zn, Cd, and Fe in each tannery effluent sample, was determined (acidified with 1% HNO_3) by Atomic Absorption spectrometry (Perkins Elmer Analyst 100).

3.6.1 Sample Digestion (Sediments and Dust Samples)

Aliquots (2.5 mL) of concentrated HNO_3 (69% Analar grade) were added to 200 mg dry dust samples, which had been weighed into 75 mL digestion tubes. The mixture was then allowed to stand overnight at 15°C. The following day, the digestion tubes were placed on a heating block and the temperature was gradually raised to 100°C for 8 h. The samples were allowed to digest for 3 h, after which the volume was reduced to 3–4 mL. The digest was cooled at room temperature, and diluted to 10 mL with double deionised water in graduated tubes. Total concentrations of Pb, Cu, Zn, Fe, Ni, Cd, and Cr were determined using atomic absorption spectrometry (Perkin Elmer Analyst 100).

3.6.2 Biological Oxygen Demand (BOD)/ Dissolved Oxygen (DO) Determination

BOD and DO were determined using standard protocols (APHA 1965). Samples of water were incubated at 20°C for 5 days in a dark water bath. Every day for 5 days,

Table 3.7 Characterisation of the tannery effluent showing identified parameters and levels in three main phases (raw effluent, treated effluent and final effluent) ($n = 5$)

Parameters	Raw effluent	Treated effluent (General sedimentation)	Final effluent (Anaerobic lagoons)	LSD (5%)
pHz	7.72 (0.19)	7.1 (0.1)	7.66 (0.24)	0.58
COD	2437.84 (660.3)	5978.16 (4626.1)	1307.4 (291.4)	8329
BOD	1255 (309.9)	5738.1 (4688.7)	438.5 (194.9)	8366
Cl	1725 (495.5)	483.9 (216.4)	1693.7 (757.4)	1719
Sulphide	62.4 (14.7)	57.2 (15.1)	89.96 (26)	60
Susp. Solids	562 (121.6)	448.2 (153)	330.67 (43.3)	394
Total Cr	23.02 (18.3)	1.71 (0.4)	0.93 (0.2)	33
Oil/grease	332.3 (108.2)	273.9 (101)	94.38 (31)	267

Figures in parenthes are SEM's (Standard errors of means)

DO was determined. The difference between initial value and the value at each time ($_t$) period (i.e. Oxygen demand) was plotted as the BOD_t ($mg\,L^{-1}$).

Samples for the determination of BOD and DO were tested within 10 days of collection to avoid degradation. The protocol included adding 1.0 mL of manganous sulphate reagent followed immediately by 1.0 mL of alkaline-iodide-azide solution to the BOD bottle (300 mL). The bottle was restoppered immediately and the contents mixed by shaking vigourously for at least 20 s or until the precipitated manganous and manganic hydroxide is evenly dispersed. After 2–3 min of shaking again, the precipitate in the sample was allowed to settle for 1 h. By means of a two-way pipette and vacuum system, 100 mL of solution was transferred from the BOD bottle to a specially-painted Erlenmeyer flask containing a magnetic stirring bar. Titration was carried out immediately with thiosulphate solution until the solution turned to pale straw colour. Four drops of starch solution was added. Titration was continued until the blue colour disappeared. The dissolved oxygen was calculated using the normality and and volume of soduim thiosulphate with BOD values obtained as explained earlier. The results of the parameters mentioned are as shown in Table 3.7.

3.6.2.1 Effect of BOD/COD

Both BOD and COD levels were highest at the general sedimentation phase (BOD 5,978 $mg\,L^{-1}$, COD 5,978 $mg\,L^{-1}$) of the tannery effluent treatment pits, with the levels drastically reducing in the final effluents (BOD 438 $mg\,L^{-1}$, COD 1,307 $mg\,L^{-1}$) after the anaerobic lagoons. Beam-house operations involving soaking, liming and deliming processes generate large quantities of waste such as wastewater (up to 400% during liming and reliming process) consumed in proportion to the weight of the treated hides (Thanikaivelan et al. 2003). The discharged water is full of dissolved substances, which affect its quality. The Beam-house mainly affects the following parameters of water effluent; COD, suspended

solids, chlorides, sulphides and organic nitrogen. Conventional liming-reliming processes lead to 35–45 kg of biological oxygen demand (BOD), 100–125 kg of chemical oxygen demand (COD) and 140–160 kg of total solids (TS) for every ton of raw skins/hides processed (Aloy et al. 1976).

3.6.3 Sulphate and Chloride Determination

Appropriate standards for the determination of sulphate and chloride using ion exchange chromatography (Dionex, series 4500i – Autosampler AS40) were prepared in various concentration ranges (0, 2, 5 & 10 mg L^{-1}) and a calibration curve obtained. For sulphate determination, a stock solution of 1,000 mg L^{-1} SO_4 was prepared by dissolving 1.818 g of potassium sulphate (K_2SO_4) in 1 L of deionised water. A chloride stock standard solution of 1,000 mg L^{-1} Cl was prepared by dissolving 1.648 g of sodium chloride (NaCl) in 1 L of deionised water. The eluent was prepared by weighing out 0.95 g of Na_2CO_3 and 0.71 g of $NaHCO_3$. The preparation was then dissolved and made up to 5 L with deionised water. The regenerant was prepared by adding 3.5 mL of concentrated H_2SO_4 to approximately 300 mL of deionised water and making up to 5 L with deionised water. The following instrumentation conditions were maintained:

1. Eluent
   - Eluent flow rate : 1.2 mL min^{-1}
   - Suppressor : H_2 SO_4
   - Background conductivity : 17.3 μS
2. Analyte
   - Analyte flow rate : 1.2 mL min^{-1}
   - Temperature compensation : Selectable compensation between 0.0 and 3.0% per 1.7°C
   - Pressure : 2,900 psi
   - Limit : 5,000 psi

3.6.4 Total Phenols

A spectrophotometric determination of phenols based on a multicommuted flow system with a 100 cm optical path flow cell was used to determine total phenols (Fig. 3.10). All solutions were prepared with distilled and deionized water; and analytical grade chemicals. Phenol reference solutions within 10.0 and 100 g L^{-1} were prepared by appropriate dilutions of a 1.00 g L^{-1} stock solution. Reagent R1 was prepared by dissolving 50.0 mg of 4-aminoantipyrine (4-AAP) in 50 mL of a buffer containing 5.2 g L^{-1} $NaHCO_3$, 5.8 g L^{-1} H_3BO_3 and 6.2 g L^{-1} KOH (pH 10.0). Reagent R2 was a 0.20% *m*/*v* $K_3[Fe(CN)_6]$ solution prepared in water. Water was used as the carrier.

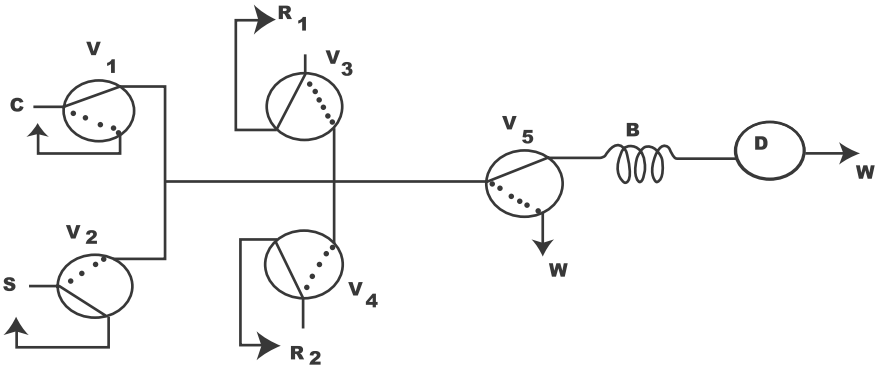

Fig. 3.10 Flow diagram of the system for determination of phenols

Where; V*i*, three-way solenoid valves; B, reaction coil (80 cm); D, long pathlength flow cell (100 cm optical path); C, water carrier (5.4 mL min^{-1}); S, sample (6.0 mL min^{-1}); R1, 0.10% (*m*/*v*) 4-AAP buffered at pH 10.0 (0.8 mL min^{-1}); R2, 0.2% (*m*/*v*) $K_3[Fe(CN)_6]$ (0.6 mL min^{-1}); W, waste. Dashed lines represent the flow paths when the valves are switched on.

Solutions containing 7.0 mol L^{-1} phenol, *m*-cresol, *p*-cresol, *p*-chlorophenol, catechol, hydroquinone, *p*-aminophenol and *p*-nitrophenol were employed for evaluation of the relative response for different phenols.

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Chapter 4
Occupational Risk in the Tanning Industry

Abstract In this chapter, the aim was to discuss and determine the inherent occupational risks and hazards (closely ascertaining the kinetic and dynamic principles of toxicity) related to the tanning industry. The study demonstrated that biosensors can be used to assess toxicity of dust in both solid and liquid phase, and to compare and complement the biosensor assay with analytical methods to identify the likely toxic agent(s) in the tannery dust. Solid phase study of the tannery dust using bacterial biosensors required close contact between the bacterial cells and the particulate matter (achieved through centrifugation), while retaining the ability to 'recover' the bacterial cells to measure the luminescence-based toxicity response. However, when using luminescent bacteria for solid phase toxicity testing certain problems can be encountered where light output (luminescence) is affected by coloured supernatant and differing numbers of cells in the sample; loss of bacteria due to adhesion to suspended sediment/dust particles and optical interference of suspended sediment particles.

4.1 Introduction

The effect of tannery pollutants on the terrestrial and aquatic ecosystem carries a positive correlation with human health as a component of the environmental matrix (UNEP 1994). Several studies in the tanning industry sector have linked its impact directly with plants and humans (Anonymous 2003; Khwaja 1998; UNEP 1994; Mwinyihija et al. 2006). As mentioned earlier in Chap. 2 certain pollutants were cited and apparently most of them pose a direct or indirect occupational risk within the tanning Industry. For example, the concentration of Azodyes can have carcinogenic properties and could even form amines, which have both carcinogenic and mutagenic properties.

Pentachlorophenols (PCP) (which are used to prevent fungal growth and decay by bacteria in leather preservation) and its salts are highly toxic and harmful to human health, aquatic systems and persist in the environment for long periods of time. Cadmium compounds are carcinogenic agents and are present in certain types

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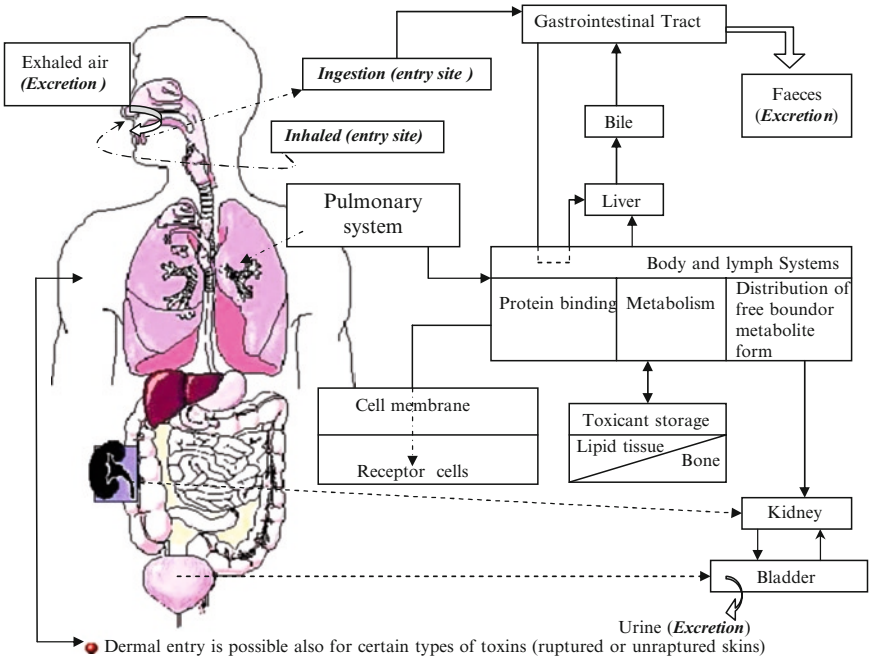

Fig. 4.1 Major routes and sites of absorption, metabolism, binding and excretion of the toxic substance in the body

of dyes or pigments. Polychlorinated Biphenyls (PCB) are found in softeners highly toxic and impacts adversely on terrestrial, aquatic and atmospheric systems. Apparently PCP was one of the substances for which the EU (European Union) was established. Formaldehyde resins (normally used as glazing agents in the finishing process) are known to irritate the mucosal membrane, allergic dermatitis and on long term exposure potentially carcinogenic. Most of the heavy metals mentioned also in Chap. 2 (e.g. Chromium, Nickel, Cadmium, Mercury etc) impact adversely to the ecosystems with some known to have mutagenic and carcinogenic properties. The major routes and sites of absorption, metabolism, binding and excretion of the toxic substances in the body is as illustrated (Fig. 4.1). Toxicants in the body are metabolised, transported and finally excreted. The process of poisoning is divided into two major phases,a kinetic phase and a dynamic phase.

4.2 Kinetic Phase

A toxicant that is absorbed may pass through the kinetic phase unchanged as active parent compound, metabolised to a detoxified metabolite that is excreted or converted to a toxic active metabolite (Fig. 4.2).

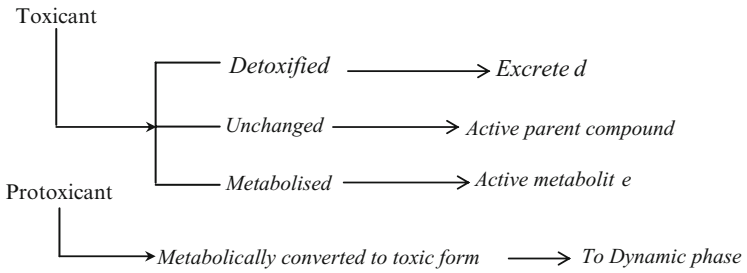

Fig. 4.2 Process involving toxicants or protoxicants in the Kinetic phase

4.3 Dynamic Phase

In the dynamic phase a toxicant or toxic metabolite interacts with cells, tissues or organs in the body to cause a toxic response. In this phase there are three major divisions Primary reactions, biochemical response and observable effects Fig. 4.3.

In this chapter, the environmental impact of the tannery dust will be discussed and also ascertain whether compliance or deficiencies in occupational standards associated with the industry exist (Fig. 4.4).

Previous international work reporting hazards from airborne dust are from non-ferrous foundries (Michaud et al. 1996), street dusts (Fergusson and Ryan 1984; Michaud et al. 1996), or wet and dry atmospheric deposits (Al-Rajhi et al. 1996). Most of the reported work has focused on characterisation of metallic, hazardous compounds of dust in order to identify the source of pollution (Linton et al. 1980; Sobanska et al. 1994). Knowledge of the number, size and chemical composition of dust particles is a valuable aid to the industrial hygienist in hazard evaluation (Sobanska et al. 1994). The effect of these particles when inhaled into the body depends on the size, shape and chemical nature of the particles (Anonymous 2001a). Futhermore, the adsorption potential of dusts associated with soils has been found to be related to their particle size distribution (Benton et al. 1995; Ringwood et al. 1995) and the presence of ligands may stongly bind dust to certain metalic compound species such as Cr^{3+} (Cohen et al. 1993; Zhitkovich et al. 1995, 1996).

Image analysis techniques can be used to determine dust particle size and distribution (Mwinyihija et al. 2006). These techniques have been applied in the pharmaceutical industry to determine occupational risk (Zingerman et al. 1992; King 1984; Niklas et al. 2002). Moreover when dust acceleration was being investgated, falling of dust particles in a tannery environment closely follow Stokes law. Since the distribution of dust particles is continuous, a stochastic model simulation (e.g. Monte Carlo) can be used (Rotariu et al. 2004, 2002a, b) for such a study. This issue will be pursued in detail later on in this chapter.

A large number of biologically active substances, including heavy metals, may have direct, indirect, primary or secondary effects on the immune system and are of interest to pathologists, immunologists and toxicologists. Heavy metals (e.g. chromium from

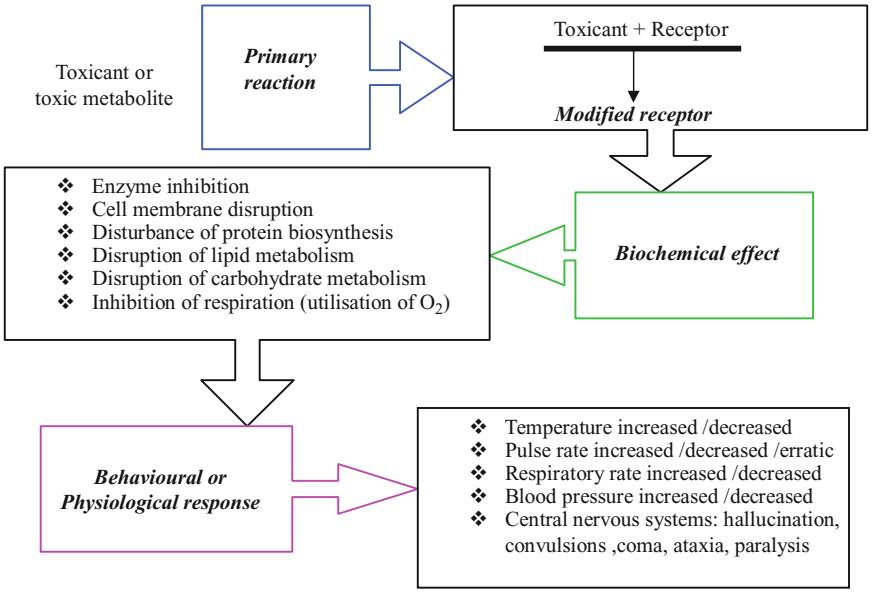

Fig. 4.3 Dynamic phase of toxicant action

Fig. 4.4 A tannery worker predisposed to occupational hazards at a weighing in area

the tanning industry) are of significant importance in altering the immune response via immunostimulatory or immunosuppressive mechanisms (Shrivastava et al. 2002).

For example, in a study by Cerulli et al. (1998), it was reported that while Cr^{3+} was inactive in entering the cell (thus its use in dietary supplementation as Cr picolinate),

high doses in excess of the recommended levels resulted in some levels of toxicity. It is therefore interesting to note that recent studies have shown that soluble metal ions can activate pre-existing signalling pathways in the cell that can cause the cell to respond to what it thinks are physiological signals (Ye and Shi 2001). In results reported earlier, the issue of heterogeneity of the pollutants was raised. In a related observation by Costa (2003), it was found out that in many cases with a toxic metal ion, these signals are not physiological and may be multicomponent.

The hexavalent form is considered to be a group 'A' human carcinogen because of its mutagenic and carcinogenic properties (Murti 1989). Deleterious effects have been reported on tannery workers who were exposed to chromium as an occupational hazard. Some of the effects observed were related to iron metabolism which are inherent and possibly associated with chromium accumulation (Kornhauser et al. 2002). The hexavalent form is 500 times more toxic than the trivalent type (Kowalski 1994). The high risk factor based on this metal compound is due to its high concentration in the effluent. The tannery waste can have between 40 and 50,000 mg L^{-1} of total chromium (Hafez et al. 2002).

In terms of human health, chromium is a major risk factor in the environment (Hertel 1986). Chromium poisoning includes toxicosis and puerperal haemorrhages during pregnancy and childbirth, especially observed in a case study of women working in a dichromate factory (Shmitova 1978, 1980). There are also effect on the immune system (Shrivastava et al. 2002), and apoptosis (cell death of human lymphocytes) in the presence of both Cr^{5+} and Cr^{6+} (Vasant et al. 2001) (Fig. 4.5). The impacts and remedial measure of ecotoxicity of this metal in the environment has been established where chromium is a contaminant of surface and ground water,

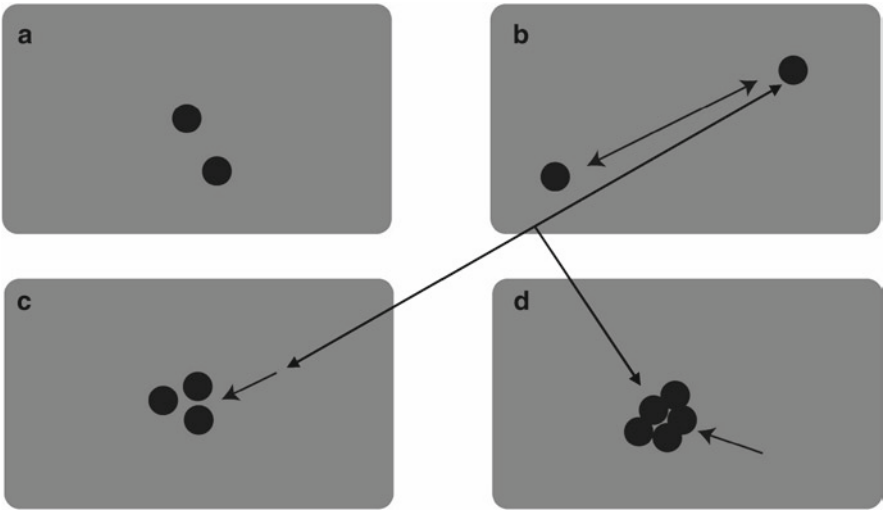

Fig. 4.5 Photomicrographs of Giemsa stained lymphocyte cell (approximately 7.5 μm in diameter) demonstrating chromium induced abnormalities resulting from incubation for 48 h with Cr^{3+} as 1×10^{-4}M and for 8 h with Cr^{6+} at 1×10^{-5}M during cell proliferation. (**a**) Normal cells unexposed to Cr; (**b**) Normal and apoptotic cells from Cr(salen)$(H_2O)_2^+$; (**c**) Apoptotic cells from Cr (salprn) $(H_2O)_2^+$; and (**d**) K_2Cr_2O, treatment Apoptotic cells are indicated by *arrowheads*

agricultural land and aquatic life (Permuter and Lieber 1970; Handa et al. 1985). Therefore, the determination of trace levels of Cr in environmental samples is of great importance due to its toxicity (Kendorf and Schnitzer 1980).

In relation to occupational risk chlorophenols can enter the environment through accidental spills, illegal release of industrial and municipal wastewater and excessive use of pesticides (Park et al. 1999). Chlorophenols are also used as preservatives for a number of materials such as wood, textiles and leather. Chlorophenols are well known for their biocidal activities and have been found to be toxic, possibly mutagenic to terrestrial biota (Jensen 1996).

4.4 Techniques Used in Determining Risks and Hazards

Dust within the tanning processing units is potentially hazardous to the workforce because of the presence of toxic contaminants (Cr, Zn, Pb etc) and their associated particulate characteristics. The effect of these particles when inhaled into the body depends on the size, shape and chemical nature of the particles. A large number of biologically active substances, including heavy metals, may have direct, indirect, primary or secondary effects on the immune system and are of interest to pathologists, immunologists and toxicologists. Heavy metals (e.g. chromium from the tanning industry) are of significant importance in altering the immune response via immunostimulatory or immunosuppressive mechanisms (Shrivastava et al. 2002).

4.5 Characterisation of Tannery Dust and Image Analysis

Dust within the leather processing units is a potential occupational hazard to the workers at the tanning sites. This is due to the chemical nature of the compounds used as well as the characteristic nature of the particulate matter generated. Therefore the importance of conducting a study which rapidly and efficiently characterises, hazards from dusts within the individual processing units of the tanning industry, using microscopy and image processing technique is essential.

4.6 Rapid and Efficient Technique to Characterise Hazards from Dusts

4.6.1 Dust Sampling Technique

Dust samples were collected in triplicate from seven points within a Kenyan tannery site (Chemical handling, Dyeing, Shaving & trimming, Buffing, Weighing, Rawstock handling, Splitting & fleshing (Fig. 4.6). Dust samples (Fig. 4.6, Table 4.1) were collected within the tannery after 24 h from a 1 m² area. These points were identified

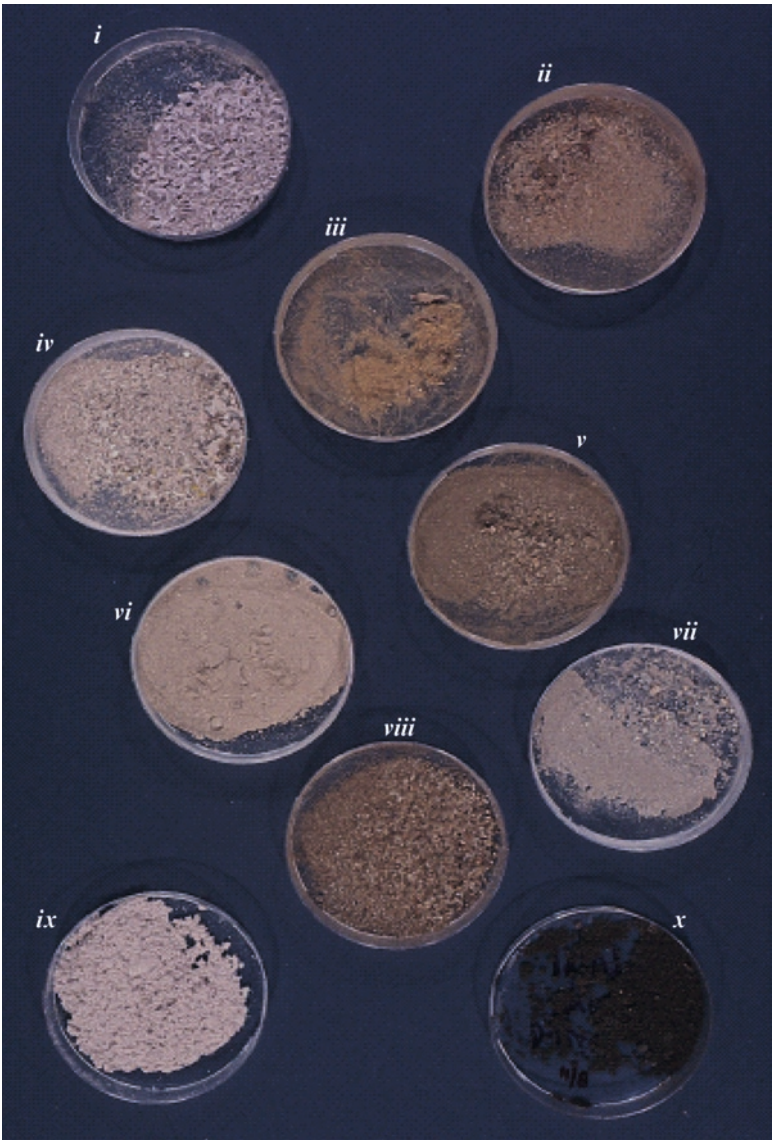

Fig. 4.6 Different type of dust samples photographed in 9 cm petri dishes and collected from various points of the Kenyan tannery workstations (Splitting & fleshing (i), Rawstock handling (ii, iii, viii), Shaving & trimming (iv), Weighing (v), Chemical handling (vi), Buffing (ix) and Dyeing (x))

as high risk due to their dust emission potential directly associated with the operations. It was eventually observed that the greatest ($p \leq 0.05$) amount of dust (11.8 g) (Table 4.1) was collected from the chemical handling area. This was concidently expected due to storage and handling of chemicals in this particular area.

Table 4.1 Weight of dust sample collected from 1 m^2 floor area at the identified sites on different days after 24 h

Selected sites within the processing area	Mean wt (g)
Rawstock handling	4.4 (±1.1)
Splitting and fleshing	5.6 (±1.1)
Shaving and trimming area	4.2 (±0.3)
Buffing area	2.0 (±0.2)
Dyeing area	3.6 (±0.2)
Chemical handling	11.8 (±1.7)
Weighing area	1.7 (±0.3)
LSD	1.4 ($P<0.05$)

4.6.2 Image Analysis

From each dust sample, a 0.06 g sub-sample was spread onto a microscope slide measuring 48 by 28 mm. The microscope slides were placed on a light microscope set with a mounted colour video camera (JVC – TK 1085E) attached to a Personal Computer and a video display (Fig. 4.7). The microscopy illumination system was connected to a variable 12 V DC power supply unit. The microscope (×82 magnification) and the condenser were set to enable high quality images of the dust particles to be taken. Images obtained from the Camera were digitised using an image processing system (SM Camera II version 1.04 (93)) and then stored on a PC. The images were processed using Scion Image software program Beta 4.0.2 (National Institute of Health, USA). The 24 bit RBG images were stored in JPG format with a resolution of 736 (width) by 560 (height) pixels (equivalent size 2,355 × 1,792 μm).

4.6.3 Data Analysis

Five random images were collected from each dust-coated slide. The images were threshold to distinguish the particles from the background. The number of particles in each image was automatically counted, and particle size and shape measured (i.e. area, length, ellipse major axis and minor axis). These data were then collated using Microsoft Excel.

Statistical analysis involved two way analysis of variance (ANOVA) and treatment effects determined by least significant difference (LSD) with graphs generated by an appropriate software e.g. Sigma plot.

Examples of images obtained from dust samples are shown in Fig. 4.6. Image analysis distinguished individual particles and the mean particle diameters and distribution as shown in Fig. 4.8. The chemical handling (15.0 ± 0.6 μm) and weighing areas (17 ± 1.3 μm) were associated with the smallest mean particle sizes, suggesting greatest proportion of thoracic dust (i.e. 5–10 μm) ($p<0.05$). The dyeing area (58 ± 15 μm) and rawstock area (51 ± 3 μm) were associated with the largest mean particle sizes ($P<0.05$), less than the total inhalable dust particle level (100 μm).

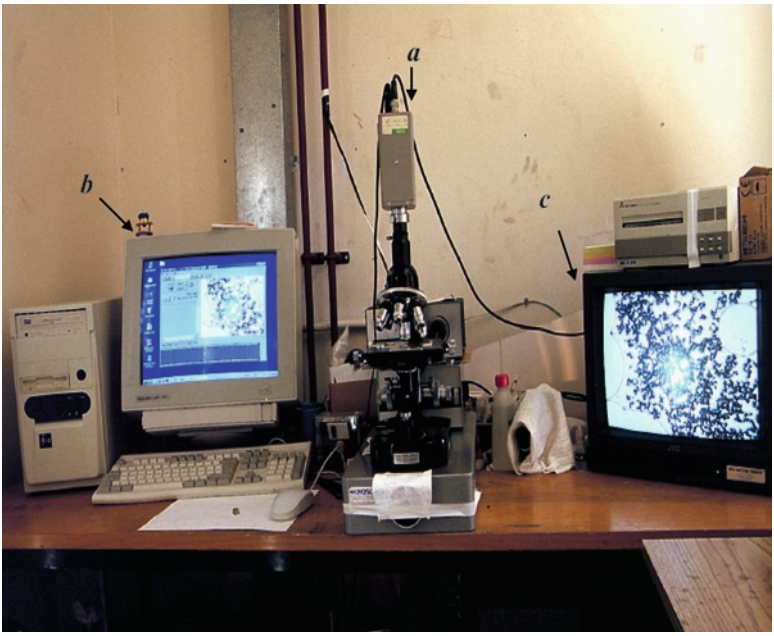

Fig. 4.7 A light microscope set with a mounted colour video camera (JVC – TK 1085E) (**a**) attached to a Personal Computer (**b**) and a video display (**c**) to enable high quality images of the dust particles

When all the leather processing units were compared, they generally showed a potential health hazard due to particle sizes generated being predominantly <100 μm in diameter. For example Table 4.2 demonstrates that all three types of dust (total inhalable dust, thoracic dust and respirable dust) were present within the processing units of the tannery. However, considering only thoracic dust (regarded as second most hazardous after respirable) the buffing, dyeing and raw-stock were associated with significantly lower numbers of dust particles per unit area (levels) than the chemical handling, weighing area, splitting and fleshing ($p \leq 0.05$). This result suggests that the chemical, weighing and splitting areas are potentially the most hazardous to the workforce and in contributing pollution of the environment. Buffing is highly mechanised and results in elevated production of dust during processing, with the presence of protective filters on the machinery having minimal effect.

The dyeing area, raw stock handling and splitting/fleshing sample areas were associated with the highest dust terminal velocities and minimum settling times. This indicate that for these sampling areas each dust particle spent less time (on average) in the air. The greater the dust settling time (e.g. Shaving/trimming, chemical handling, weighing and buffing area) the more likely the substance is to be inhaled. The results demonstrate that most of the dust in these areas took more than 60 s in the air before eventually settling. This period of settling is only sustained within the

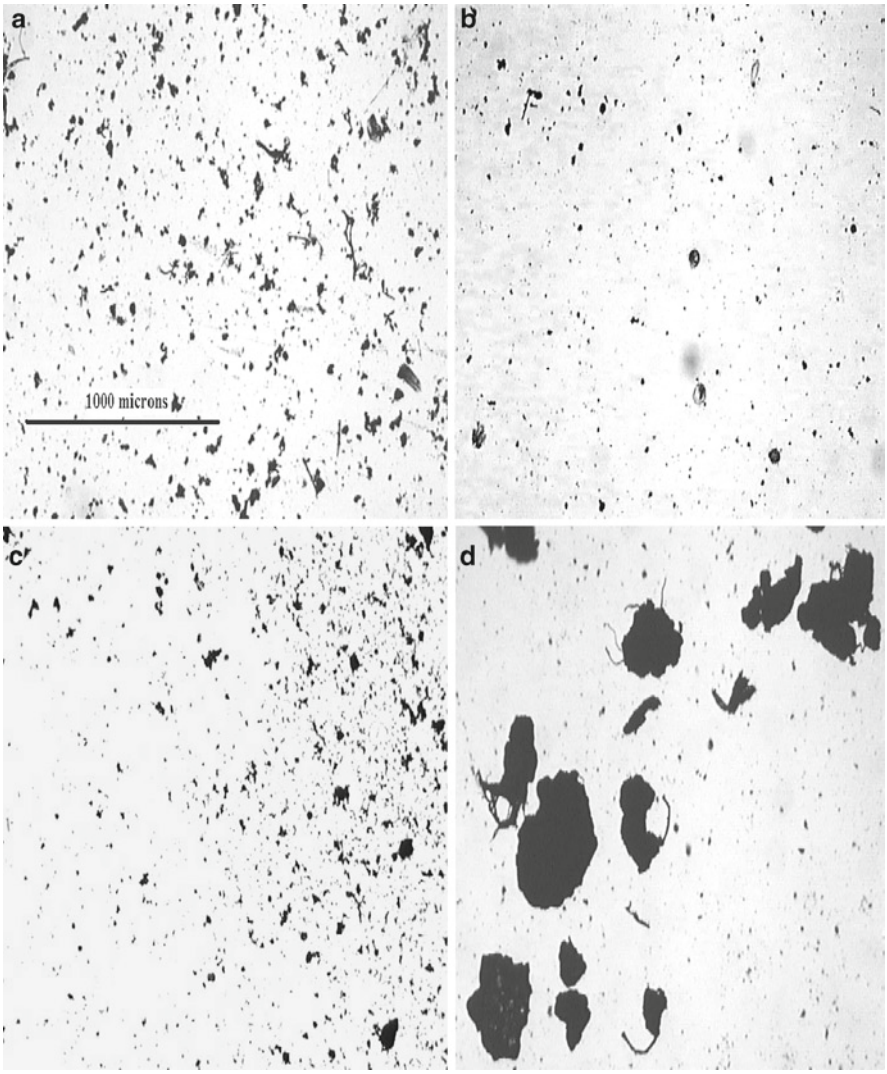

Fig. 4.8 Photographs (both are of the same scale) of dust from identified processing points, obtained by the image processing system and used to determine particle distribution. Examples from (**a**) Buffing area (**b**) Weighing area in the tannery (**c**) Chemical handling and (**d**) Splitting and Shaving area (×*82 magnification*)

8 h working period so long as the dusting activity continues. This was the reason for collecting the dust every 24 h to facilitate the collection of long settling particles within the dust producing area (1 m^2). The dust particle distributions from the study site were expected to be multimodal because of the various types of chemical and activities that take place in the tanning industry (Fig. 4.9).

Table 4.2 Mean number of dust particles categorised by diameter (μm) categorised for each processing points

	Respirables (≤5)	Thoracic (6≤10)	Total Inhal. (11≤100)	>100	% PM
Shaving and trimming	55 (±23)	62 (±26)	77 (±22)	2 (±0.95)	59
Chemical handling	154 (±10)	226 (±23)	315 (±28)	0.5 (±.6)	54.6
Weighing area	102 (±28)	128 (±9)	158 (±30)	0.75 (±1.0)	59.2
Buffing area	19 (±21)	29 (±27)	33 (±29)	2.3 (±2)	57.4
Dyeing area	24 (±42)	48 (±87)	74 (±23)	2.8 (±2)	48
Rawstock handling	29 (±16)	34 (±10)	175 (±20)	14 (±1.3)	24.8
Splitting and fleshing	96 (±48)	123 (±63)	167 (±121)	5 (±4.1)	28
LSD	LSD 74.1 ($P<0.05$)				

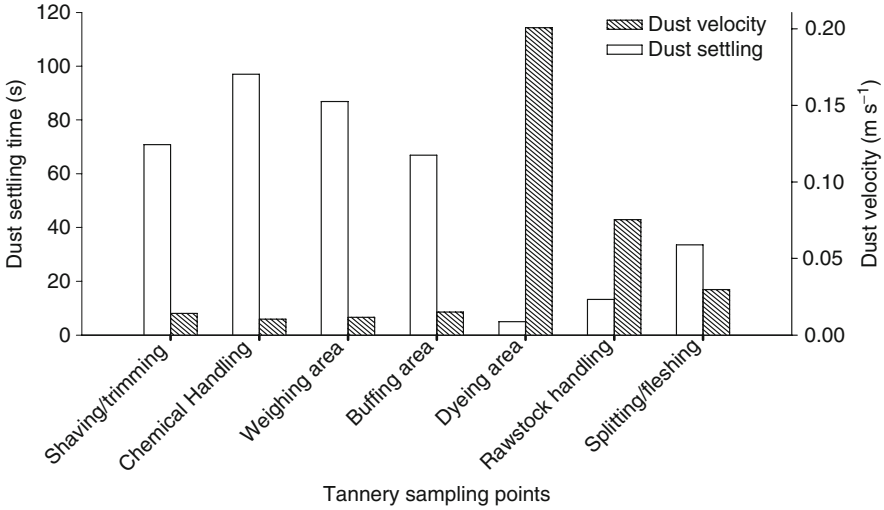

Fig. 4.9 Mean dust terminal velocities and settling times for each sampling point

International concern over particulate matter or dust has led to both the National Research Council of the National Academy of Sciences (Anonymous 1979) and the United Nations (United Nations 1979) publishing studies detailing the dangers of small particles to humans. In the UK, the statutory instruments (2002) defining health and safety have been put into law. The control of substances hazardous to health regulations (2002) on occupational exposure standards state that a concentration in air equal to or greater than 10 mg m^{-3}, as a time-weighted average over an 8-h period, of inhalable dust, or 4 mg m^{-3}, as a time-weighted average over an 8-h

Table 4.3 Risk identification of dust and chemical hazards in the Kenyan tanning Industry

Process/Sources: sampling areas	Raw materials/chemicals	Qualitative assessment of dust hazard
Hides and skins handling: *Rawstock handling*	Preservatives (bacteriostats/ fungicides and Substrate Dust)	High (air dried/preserved)
Curing	Sodium chloride	Medium (depending on curing method)
Soaking	Sodium carbonate, emulsifiers, Surfactants	Medium High for open vats
Liming	Calcium hydroxide, Sodium sulphide, Sodium sulphydrate	High
Deliming: *Fleshing & splitting*	Sulphuric acid, hydrochloric acid formic acid, boric acid, ammonium. Sulphate, ammonium chloride, sodium bisulphite	High for salts
Bating	Bacterial proteases, pancreatic enzyme	Unknown
Pickling	Acetic acid, hydrochloric acid, formic acids and sodium chloride	Medium for salts
Degreasing	White spirits, perchloroethene, surfactants, sodium chloride	Medium
Tanning/Retanning, Bleaching: *Shaving/ trimming and Buffing*	Chromium salts, syntans, resins Sodium sulphide, oxalic acid Sodium bicarbonate, magnesium oxide Sodium formate (masking agent)	High for all salts
Dyeing: *Dyeing*	Formic acid, dyestuffs	High for powdered dyes
Finishing	Butyl acetate, formic acid, methyl ethyl ketones, xylenes	Low
General sources: *Chemical handling and weighing*	Include all indirect contact with the primary sources e.g. Mixing, decanting, cleaning, maintenance, transportation, storage & weighing	High

period, of respirable dust can be referred to as a substance hazardous to health (Statutory Instrument 2002).

It is with such a perspective that investigation within the tanning industry on dust particulate matter and its charaterisation became crucial. Indeed very little work has so far been carried out on assessing dust pollution and its potential impact within leather processing sector. A summarized qualitative assessment of the risks associated with dust and chemical hazards in a selected site of a Kenyan tanning industry is presented in Table 4.3.

Previous international work reporting hazards from airborne dust elsewhere are from nonferrous foundries (Michaud et al. 1996), street dusts (Fergusson and Ryan 1984; Michaud et al. 1996), or wet and dry atmospheric deposits (Al-Rajhi et al. 1996). This work focused on characterisation of metallic, hazardous compounds of dust in order to identify the source of pollution (Linton et al. 1980; Sobanska et al. 1994).

4.6.4 *Estimating the Concentration of Dust in the Tannery Air Environment*

The mean density (ρ_ρ) of the particles for each high risk area was estimated by measuring the mass of the particles in the 1 m² sample area (optimum vertical and horizontal dust contact area from source) and dividing by the volume. It was assumed that the particles were circular and subsequently the diameters of the particles calculated by the image analysis were used to estimate their size distribution. Having determined the mean density, the total number (N) of particles in each dust sample could be estimated.

It was assumed that all of the particles from each sample were dropped randomly from a height of 1 m during the 8 h working period and that the particles fell according to Stoke's law (Kane and Sternheim 1978). A Monte Carlo method was used to simulate this process during a working day in the tannery.

Briefly, the Monte Carlo routine was as follows:

1. $\frac{N}{x}$ particles were randomly selected from the size distribution of the particles during each 10 s increase, where N is the total number of particles that have fallen in the 8 h time period and $x = 2{,}880$ is the number of 10 s increments.
2. The settling times for each particle were calculated from:

$$t_s = \frac{18\eta_{air} \cdot h}{\rho_p \cdot d_p{}^2 g} \tag{4.1}$$

where

$\eta_{air} = 1.72 \cdot 10^{-5}\, kg \cdot m^{-1} \cdot s^{-1}$ – viscosity of air
$h = 1$ m height of dropping particles
ρ_p = density of particle (variable with the type of dust)
d_p = diameter of particle
$g = 9.81\ kg\,m^{-2}$ (gravity constant)

3. The time for each particle to reach the ground: was calculated as;

$$t_{fin} = t_{in} + t_{in} \tag{4.2}$$

where
t_{fin} is the final settling time to the ground
t_{in} is the initial time for the dust
t_i is the settling time

4. If $t_{in} < t_i < t_{fin}$, the particles were counted and the mass of the particles updated at the t_i moment;
5. This was repeated for each particle
6. The mean concentration of particles during an 8 h working day was calculated;

Image analysis techniques can be used to determine dust particle size and distribution. These techniques have been applied in the pharmaceutical industry to determine occupational risk (Zingermann et al. 1992; King 1984; Niklas et al. 2002). Falling of dust particles in a tannery approximately follow stokes law. Since the distribution of dust particles is continuous, a stochastic model simulation (e.g. Monte Carlo) can be used (Rotariu et al. 2004, 2002a, b). The main processing points within the tannery, which were identified as potential areas of dusting were subjected to the simulation. This approach will show the areas predisposing workers to health risk based on the UK occupational exposure standard.

The Monte Carlo simulation method determined the airborne dust concentration for all of the sampled areas within the tannery (Fig. 4.10). The simulation used a 1 m^3 value as a representative volume in obtaining the particulate concentration. The results obtained provided the mean concentration of particles during an 8-h working day.

Higher concentration of the dust particle within the workplace creates a risk to health due to elevated occupational exposure. The highest mean concentration was found in the chemical handling area (12 $mg\,m^{-3}$) with the shaving area the lowest (168.7 $\mu g\,m^{-3}$) (Fig. 4.10).

The methods used in this study demonstrated that image analysis coupled with a Monte Carlo simulation can categorise dust particles rapidly and efficiently for the purpose of risk analysis and impact assessment in the tanning industry. Further work was carried out to quantify the biochemical/toxicological risks associated with dust in the tanning industry using luminescent based bacterial biosensors.

4.7 Screening of Tannery Dust Using a Luminescent Based Bacterial Biosensor

Environmental toxicity screening requires rapid, reliable and sensitive monitoring procedures. The knowledge of dust composition and its toxicity is crucial for occupational health risks to be assessed by the industrial hygienist (Sobanska et al. 1999). The knowledge of dust composition and its toxicity is crucial for occupational health risks to be assessed by the industrial hygienist (Sobanska et al. 1999). The integration of both chemical and biological approaches to underpin

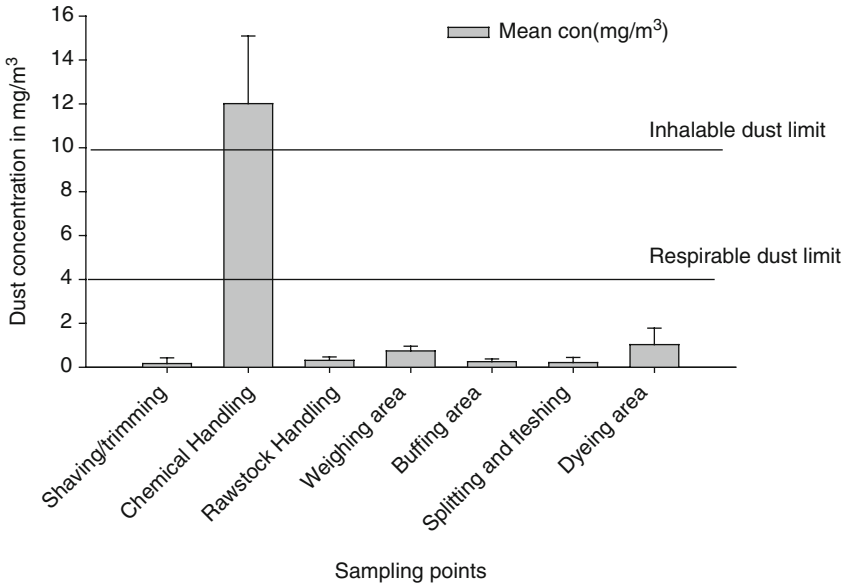

Fig. 4.10 Mean concentration of airborne dust particles per second calculated by Monte Carlo technique (error bars are ± standard deviation)

ecotoxicity testing is essential. Chemical methods have been traditionally used to determine total concentrations of pollutants (Stuhlfauth 1995) and biologically linked measurements have been used to assess the bioavailable fractions of pollutants (Steinberg et al. 1995; Paton et al. 1997b). Many analytical methods determine the total concentrations of the contaminants. However, total concentrations are not necessarily directly related to bioavailability e.g. due to the binding of metals by substrate, which reduces their bioavailbilty (Tiensing 2002). Bioavailability refers to the fraction of a chemical that can be taken up or transformed by living organisms (Semple et al. 2003). It is a concept that is both specific to the individual receptor and the pollutant.

Bioavailability and sorption of heavy metals are influenced by pH, Redox potential, exchangeable cations and biological activity (Paton et al. 1997b). Matrix pH further influences metal concentration in solution through ionisation and subsequent charge in the matrix. Thus the pH of samples is critical in determining mobility, bioavailability and sorption of chemicals (and species distribution) in environmental and cellular matrices.

Biosensors offer an ecotoxicological tool, with extensive dose-response databases, for rapidly and reliably screening environmental samples for their inherent toxicity. Dose-response relationships provide the basis for assessment of hazards and risk presented by environmental chemicals (Walker et al. 1996). Different toxicity curves can be obtained from different types of either chemicals or tested organisms. The different types of dose response curves can be characterised into four types (Welp and Brümmer 1997). These are divided into type I “No effect

range followed by inhibition"; Type II, "Stimulation followed by inhibition"; type III "stimulation"; and type IV, "no change".

Lux-based bacterial biosensor assays have been developed for toxicity testing of industrial effluents (Brown et al. 1996; Paton et al. 1995), contaminated groundwater (Boyd et al. 1997) and sites undergoing bioremediation (Sousa et al. 1998). Bacterial tests have been shown to be cost effective in toxicity screening (Ross and Henebry 1989; Ross 1993; Mwinyihija et al. 2006), and are also valuable in adding another trophic level to multi-trophic level test batteries (Burton et al. 1989; Giesy and Hoke 1990; Ross and Henebry 1989; Ross 1993; Mwinyihija et al. 2005).

Bacterial biosensor cultures have also been used effectively in soil ecotoxicity testing where centrifugation is the method of extraction. Centrifugation enables the solution to be isolated from the matrix (soil/sediment or even dust), allowing rapid and reliable solid phase testing (Vedy and Brucket 1992; Thibault and Sheppard 1982; Kittrick 1983; Elkhatib et al. 1986, 1987; Ross and Bartlett 1990; Mwinyihija et al. 2006). Detection of contaminants, sorbed to dust particles, using bacterial biosensors requires close contact between the bacterial cells and the particulate matter, while retaining the ability to recover the bacterial cells to measure the toxicity response but was first attempted by Mwinyihija et al. 2006. Previous studies in the use of solid phase techniques have been applied in the determination of toxicity of contaminated soils and sediments (Benton et al. 1995; Dombroski et al. 1996; Goicolea et al. 1998; Ringwood et al. 1997; Rönnpagel et al. 1995).

Contaminant toxicity in dust will be affected by the type of dust, while quenching of light due to the colour of the sample solution and the presence of suspended particles may affect bioluminescence (Benton et al. 1995; Brower et al. 1990). The recovery of bacteria once added to the suspension is extremely variable, depending on degree of adsorption of the cells to the dust particles. The adsorption will also be dependent on the particle size distribution as observed in soils (Benton et al. 1995; Ringwood et al. 1995), affecting the availability of the contaminants and their toxicity (Benton et al. 1995).

Therefore it was necessary to demonstrate that biosensors can be used to assess toxicity of tannery dust (which could entail chemicals from several uses in the tanning Industry (Table 4.4) in both solid and liquid phase, and to compare and complement the biosensor assay with analytical chemical methods to identify the likely toxic agent(s) in the tannery dust.

4.7.1 Solid Phase Ecotoxicity Testing

A disposable micro centrifuge tube (Whatman, polypropylene, Vectaspin-micro) containing a 0.45 μm filter was introduced into a luminometer cuvette (clinicon). The dust was prepared in triplicate for each sample. A 300 μL aliquot of each dust suspension was placed in the microcentrifuge tube filter and 100 μL of the reconstituted

Table 4.4 The mainstream tanning chemicals in Kenya and their identified potential biohazards

Chemical	Uses	Toxicology
Pyrase 1000	Enzymes for bating	Low hazard – Principle of precautionary measure required
DIA Soak/Biosoak	Soaking agent	Low hazard – Principle of precautionary measure required
Diamol FN	Bactericide	Hazardous Principle of precautionary measure required
Soda ash	Basification/neutralisation	Hazardous (ORL-RAT LD50 7,340 $mg\,kg^{-1}$ ORL-MUS LD50 7,300 $mg\,kg^{-1}$
Lime	Used in liming process	low Hazard TLV/TWA 10 $mg\,m^{-3}$[a]
Sodium Sulphide	Used in deliming	Hazardous-ORL-HMN LDLO 50 $mg\,kg^{-1}$
Ammonium Sulphate	Used in deliming	Hazardous (ORL-RAT LD50 3,000 $mg\,kg^{-1}$ IPR-MUS LD50 610 $mg\,kg^{-1}$
Sodium Bi-sulphide	Used in deliming	Hazardous -IVN-RAT ACUTE 0.013 $mg\,kg^{-1}$
Salt	Used in Pickling	Low hazard (ORL-RAT LD50 3,000 $mg\,kg^{-1}$ IPR-MUS LD50 2,602 $mg\,kg^{-1}$
Indofil Oil	Fat liquors	Low hazards
Formic acid	Acid used in Pickling/ fatliqouring	Hazardous (ORL-RAT LD50 1,100 $mg\,kg^{-1}$ IPR-MUS LD50 940 $mg\,kg^{-1}$
Dye powder/liquid	Used during Dyeing	Respirable (powder) Hazardous
Sulphuric acid	Acid used in Pickling	Hazardous (ORL-RAT LD50 2,140 $mg\,kg^{-1}$ (25% solution) IHL-MUS LC50 320 $mg\,m^{-3}$ /2 h^{-}
[a]Chrome sulphate, Chrometan powder 33%, Chromitan B & FM	Used during tanning	Principle of precautionary measure required – ORL-RAT LD50 >3,530 $mg\,kg^{-1}$
Indofil LP 30	Leather preservative	Hazardous – Principle of precautionary measure required
Wattle powder	Used in retanning	Respirable hazard – Principle of precautionary measure required

[a]Appears in the market under different brand names

(after resuscitation of the bacterial cells) biosensor cell suspension was added to the dust slurry (Fig. 4.11). The tubes were vortexed and then centrifuged at 7,500 *g* for 10 min in a microcentaur centrifuge. The small filter containers were then removed and placed into luminometer cuvettes and bioluminescence measured on a portable luminometer (Jade, labtech International), at 15 s intervals. A thin dust layer was formed on top of the filter and the contact between cells and the dust particles was established through centrifugation. The total contact time between cell addition and measurement of light output from each dust sample was 15 min.

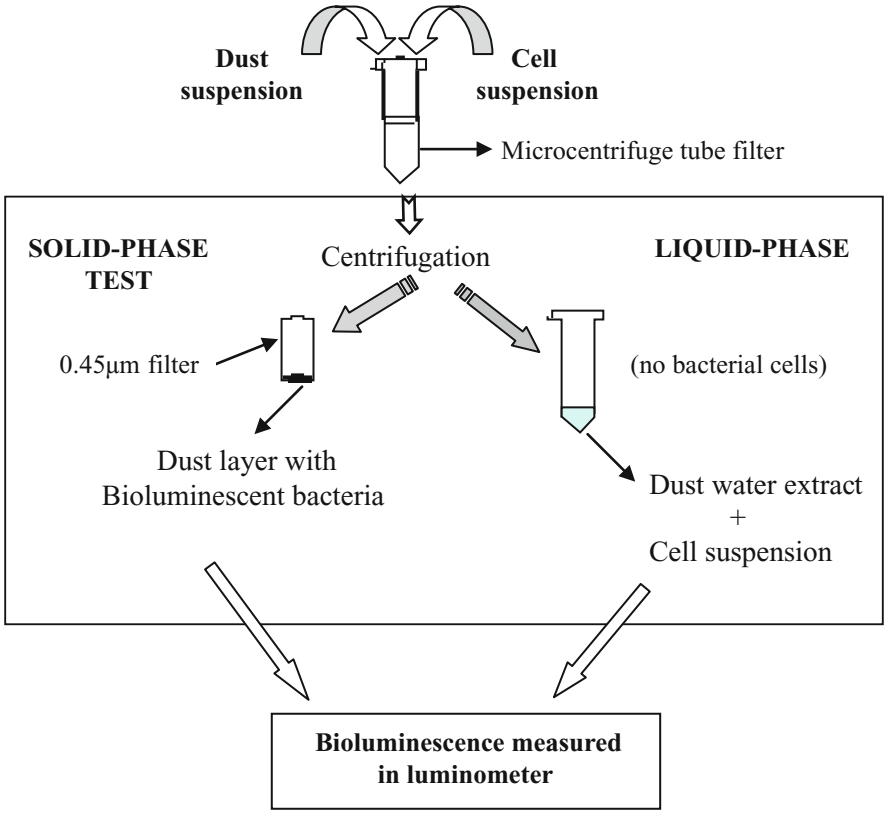

Fig. 4.11 Ecotoxicity testing (solid and liquid phase) using resuscitated freeze dried cultures of bacteria biosensors and tannery dust samples

4.7.2 Liquid Phase (Aqueous) Ecotoxicity Testing

The filtrates were collected (in triplicate) and background bioluminescence measured to confirm that no bacterial cells had passed through the 0.45 μm filter before carrying out the liquid phase assay. The liquid phase bioassay was performed by adding 10 μL of reconstituted cell suspension to 90 μL of each dust suspension sample. Biolumine scence was measured in a portable luminometer (Jade, labtech International) after a 15 min exposure time. A control of deionised water was also measured. The toxicity results were expressed as percentages of the maximum bioluminescence, calculated against a control dust (taken from outside the tannery environment, previously analysed and found to be free from contamination) (Table 4.5).

The pollutant concentration measured by absorption spectrometry was converted to pollutant concentration in the air (within the tannery environment), using the mean concentration of the dust present (in air), which was calculated by numerical analysis (Mwinyihija et al. 2005). The conversion formula is

Table 4.5 Concentration (μg g^{-1} of dust) of heavy metals related to the dust from a tanning industry determined by atomic absorbance spectrometry (Perkin Elmer Analyst 100)

Samples (dust)	pH	Cr μg g^{-1} of dust	Pb μg g^{-1} of dust	Fe μg g^{-1} of dust	Cu μg g^{-1} of dust	Cd μg g^{-1} of dust	Ni μg g^{-1} of dust	Zn μg g^{-1} of dust
Chemical Handling	5.92	2092.4	17.8	4.1	5.5	0.6	13.9	319
Dyeing area	8.1	716.8	36.2	8.29	13.4	0.05	5.6	63
Shaving/trimming	4.5	1383.6	2.7	4	5	0.3	3.9	22.1
Buffing area	4.57	1669.6	1.3	2.5	10.9	0.5	5.6	63
Weighing area	8.55	249.6	25.2	4.23	15.3	0.8	18.7	106.1
Rawstock Handling	9.5	77.4	6.3	3.55	2.5	3	5.2	17.8
Splitting & fleshing	4.76	1159	17.8	4.85	5.1	0.8	13.9	31.9
Control dust	7.13	0.0027	0.0004	0.287	0.0012	0.0002	0.0017	0.0029
Ref material	ND	0.0067	0.0439	6	0.0332	0.0002	0.0102	0.0113
LOD		0.092	0.248	0.207	0.021	0.016	0.079	0.023

LOD limit of detection (The limit of detection (LOD) was determined on the basis of five blank samples at average blank signal plus three and ten times the standard deviation
ND not determined

$$C_{\text{pol air}}(\text{mg}/\text{m}^3) = C_{\text{pol/g dust}}(\text{mg}/\text{g}) \times C_{\text{dust air}}(\text{g}/\text{m}^3) \quad (4.3)$$

Where $C_{\text{pol air}}$ is the concentration of the pollutant contained within the air (expressed as mg m^{-3} of air), $C_{\text{pol/g dust}}$ is the concentration of the pollutant within the dust (expressed as mg/g of dust and measured by absorption spectrometry) and $C_{\text{dust air}}$ is the concentration of the dust suspended within the air (expressed as g m^{-3} of air and calculated by numerical analysis using Stokes law and Monte Carlo simulation of dust concentration in 8 h working period). These data were compared to the National Institute for Occupational Safety and Health (NIOSH 2002) regulatory limits.

The comparison between the solid and liquid phase showed a highly significant ($p<0.001$), strong, positive correlation coefficient within the chemical ($r=0.99$), buffing ($r=0.95$) and dyeing area ($r=0.90$). However, weighing (–0.85) as well as splitting and fleshing (–0.83) showed a significantly ($p<0.01$) strong, negative correlation (Table 4.6). In both cases, the toxicity effect of the chemicals on the biosensor was demonstrated through inhibition (low luminescence values) or stimulation (luminescence values above 100%), indicating a strong relationship between the solid and liquid phase results.

Biosensors represent a rapidly developing tool in ecotoxicological work. In this study, the *lux*-modified bacterial biosensor, *Escherichia coli* HB101 pUCD607 was used to investigate the toxicity of tannery dust both in the liquid and solid-phase. The areas investigated within the tannery were areas prone to high dust production. The approach included the use of chemical analysis (determination of heavy metals in the dust) for confirmation of biosensor-based toxicity results obtained from the tannery dust samples. The biosensor provided a means of assessing bioavailability by measuring the impact of the dust pollutants on the metabolic activity of the *lux*-marked microorganism. Since luminescence is linked to the electron transport chain of the organism, it is, therefore, a measure of the metabolic activity of the cell (Isenberg 1993). Similarly, a decrease in metabolic health, in the presence of a toxicant, leads to a commensurate decrease in bioluminescence. It is critical to note though that bioavailability of contaminants is organism and species specific (Reid et al. 2000). The main principle in both toxicology and ecotoxicology is the relationship between the dose of a chemical to which an organism or an ecosystem is exposed and the resultant response (i.e. degree of subsequent toxic effects).

The solid phase analysis of the samples collected from the chemical handling location showed a response characterised by stimulation of light output (348.8% luminescence) with a decline (18.1% luminescence) in the liquid phase. The stimulation was attributed to the low EC_{50} of the pollutant in the solid phase (Sinclair 1999) unlike the liquid phase, which is highly concentrated after centrifugation. The presence of bating (digestive enzymes) chemicals (containing 1–5% pancreatic enzymes) (UNEP 1994) in the chemical store (Table 4.5) may also provide ligands to complex the heavy metals, rendering them unavailable and hence causing stimulation (increase in luminescence) to the biosensor. The ligands act as substrates for the microorganisms and on entry into the cells, the contaminants cause toxicity. However solid phase as a technique can have several disadvantages which include extracts that expose the bacteria (biosensor) to more contaminant than would generally

Table 4.6 Percentage bioluminescence of control (dust – solid phase) and (double deionised water – liquid phase) measuring toxicity at various sampling points at the Kenyan tannery site

	% Bioluminescence of the control					
	Solid phase (Sp)		Liquid phase (Lp)			
Samples (dust)	Mean (Sp)	SEM	Mean (Lp)	SEM	r values	p values
Chemical Handling	348.80	26.00	18.07	0.06	0.99	**
Dyeing area	58.56	2.00	15.61	0.90	0.90	***
Shaving & trimming	68.17	0.40	12.32	0.40	0.57	***
Buffing area	130.59	3.00	10.59	0.40	0.95	***
Weighing area	0.38	0.03	0.01	0.00	−0.84	**
Rawstock	73.53	0.70	7.36	0.40	0.72	***
Splitting & fleshing	47.60	0.50	15.39	0.60	−0.83	**
LSD (5%)	45.00		2.21			

No significant difference between control dust and double deionised water
SEM standard error of the mean (n = 9)
***p ≤ 0.001, **p ≤ 0.01, * p ≤ 0.05

be in the environment. In essence therefore producing an overestimated toxicity; uneven force exerted on the sensor and stress to the biosensors. Thus it is worth mentioning that there is no particular universally acceptable method in solid phase analysis (apart from the one hitherto discussed in this chapter) and the lack of an appropriate method of calibrating spiked solid phase samples with standard toxins.

In comparison to the solid phase, liquid phase mostly demonstrated low luminescence and this could possibly be linked with pollutants associated with exchangeable fraction of the dust or particles easily brought into solution. The chemical analysis of the tannery dust samples indicated that chromium concentration was higher than rest of the heavy metals. The chromium source found in the tannery originates from chromium salt ($Cr_2(SO_4)_3$) which is a basic tri-valent chromium (Anonymous 2002), poorly soluble in water (Cr^{3+}) at neutral pH (Costa 2003) and which readily precipitates in solution (UNEP 1994). This could be the reason why, irrespective of the high amount of Cr found in the chemical handling area, stimulation in luminescence was observed in the solid phase test. In the presence of ligands (available from bating chemicals in the tannery) Cr^{3+} is able to form very strong bonds (Cohen et al. 1993; Zhitkovich et al. 1995, 1996). However the toxic concentrations in the liquid phase after centrifugation reflected increased solubility of the pollutants.

Costa (2003) reported that, in solution, the dominant species is Cr^{6+} rather than Cr^{3+}, which is considered highly toxic. This observation is highly likely to be the case in the weighing area (second lowest Cr concentration) due to the high pH. Eventually, all the Cr^{6+} that enters any living system will be converted to Cr^{3+}. If this occurs outside the cell, the toxicological consequences are probably negligible. However if it occurs inside the cell Cr^{3+} can react with protein and DNA to form very stable bonds (Cohen et al. 1993; Zhitkovich et al. 1995) resulting in serious toxicological effects, including cancer, activation of apoptosis and cell death (Costa 2003; Ramasami et al. 1995).

The tanning chemicals (e.g. Chrome salts - Table 4.4) mainly contain approximately 25% (minimum) Cr_2O_3 (UNEP 1994). In a study by Cerulli et al. (1998), it was reported that while Cr^{3+} was inactive in entering the cell (thus its use in dietary supplementation as Cr picolinate), high doses in excess of the recommended concentrations resulted in toxicity. It is therefore interesting to note that recent studies have shown that soluble metal ions can activate pre-existing signalling pathways in the cell that can cause the cell to respond to what it thinks are physiological signals (Ye and Shi 2001). However, these signals may not be physiological since they may be multi-component (Costa 2003). For example, Cadmium is thought to induce metallothionein by signalling on the cell surface without ever entering a cell (Adams et al. 2002; Huang et al. 2001). Thus Cr^{3+} not entering the cell might also activate some signalling pathways that are as yet are not uncovered (Costa 2003).

The behaviour of Chromium therefore elucidates the need to explore on another very important parameter (i.e. pH) and its effect on the potency/impotency of this complex trace metal. For instance the pH variations observed in this study (Table 4.5) suggest that different Chromium species are prevalent in various samples (Kotaś and Stasicka 2000). The pH at the surface of many soil-based dusts is lower

than the pH of water surrounding the dust particles; thus an acid-catalysed hydrolysis reaction is favoured (Brusseau and Bohn 1996), when the pollutant is associated (sorbed) with the dust. At the lower pH values, the favoured species of Cr^{3+} are: $Cr(H_2O)_6^{3+}$ and $CrOH^{2+}$, Cr^{6+} occurs mainly as CrO_4^- and $Cr_2O_7^{2-}$. The latter dominates at increased Cr^{6+} concentrations, especially in aerosols $^+$ (Kotaś and Stasicka 2000). In aerosol droplets with high pH values, $Cr(OH)_3$ precipitates; when iron compounds are also present, mixed hydroxides such as $(Cr, Fe)(OH)_3$ are formed.

In the context of occupational risk, a study of human lymphoblast cell lines showed that, exposure to 1–2 μM Cr^{6+} as K_2CrO_4 revealed a 50–70% reduction in cell division after 4–7 days of exposure (Zhang et al. 2002). The sizes of the dust particles are important also when Cr toxic effects are considered: it was indicated in a related study that respirable particles (from 0.2 to 10 μm) could be retained in the lungs and pose a potential carcinogenic risk (Friess 1989). Substantive scientific evidence points to hexavalent Cr as a human carcinogen by ingestion, although this is probably a less potent route than the inhalation route (Costa 1997; IARC 1989), which is of concern because of occupational exposure within the tannery.

Schedule 1 of the Controls of substances hazardous to health (COSHH) regulations developed by HSE indicates 0.05 mg m^3 (8 h TWA reference period) to be the limit of Cr^{6+} exposure (Anonymous 2002). However, the Health and Safety Executive (HSE), UK and American Conference of Governmental Industrial Hygienist (ACGIH) and National Institute for Occupational Safety and Health (NIOSH), USA stipulate an occupational exposure requirement of 0.5 mg Cr m^{-3} (8 h TWA) for total chromium (NIOSH 2002). Non-occupational sources of exposure to chromium include food, air and water, but the concentrations are usually several orders of magnitude lower than those typically encountered in occupational situations. Safely enough legal limits (for homogenous contaminant levels) for all the tannery pollutants identified were not exceeded in this study. However any toxic exposure (Table 4.7) to the tannery workers could most likely be associated with heterogeneity (multi-elemental and synergistic effects) in airborne dust.

In this study, the chemical composition of the dust, biosensor kinetics and the toxicity of the samples to bacteria (not necessarily the same to humans) were established. Further, the dust particle size distribution (of the same tannery dust samples in the current study) for all sampling points had a greater proportion of respirable dust. This, in conjunction with the toxic nature of the dust, as observed in most samples is a possible cause of concern (Statutory Instrument 2002).

In relation to the use of *lux*-marked *E. coli* HB101 pUCD607 as an appropriate test organism, it suffices that the genetically modified bacteria showed a toxic response to both solid and liquid phase assays of the tannery dust. This observation indubitably demonstrated the use of the biosensor to be very effective in testing contaminated tannery dust samples. The biosensor based toxicity assessments were reproducible and consistent and the use of solid and liquid phase assays offers a new technique to explore the bioavailability of pollutants in dust.

However, the full ecotoxicological assessment of contaminants in dust requires further investigation incorporating eukaryotic cellular matrices to extend the findings in the present study, which primarily focused on prokaryotic receptors (*E. coli* HB101 pUCD607).

Table 4.7 Mean pollutants total concentration (mg m^{-3} in air) in a tannery environment compared to the recommended exposure limit (NIOSH)

Sampling points	Mean conc. (mg m^{-3} air)[a]	mg Cr m^{-3} air	mg Pb m^{-3} air	mg Cu m^{-3} air	mg Cd m^{-3} air	mg Ni m^{-3} air	mg Zn m^{-3} air
Chemical handling	12.014 (1.03)	2.51E-02	2.14E-04	6.61E-05	7.21E-06	1.67E-04	3.83E-03
Dyeing area	0.214 (0.08)	1.54E-04	7.76E-06	2.87E-06	1.07E-08	1.20E-06	1.35E-05
Shaving/trimming	0.169 (0.09)	2.33E-04	4.55E-07	8.43E-07	5.06E-08	6.58E-07	3.73E-06
Weighing area	0.743 (0.07)	1.85E-04	1.87E-05	1.14E-05	5.94E-07	1.39E-05	7.88E-05
Rawstock handling	0.312 (0.05)	2.42E-05	1.97E-06	7.80E-07	9.36E-07	1.62E-06	5.56E-06
Buffing area	0.252 (0.04)	4.20E-04	3.27E-07	2.74E-06	1.26E-07	1.41E-06	1.58E-05
Splitting/fleshing	1.032 (0.25)	1.16E+00	1.78E-02	5.10E-03	8.00E-04	1.39E-02	3.19E-02
Legal limit[b] (NIOSH REL, TWA 8 h mg m^3)		0.50	0.050	1.000	0.005	0.015	5.000

Mean concentration ± 6 SEM (standard error of the mean) in parentheses
REL recommended exposure limit
TWA time weighted average
[a]Mean concentration obtained from the numerical analysis
[b]Legal limits (NIOSH 2002) of total concentration of identified contaminants

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Chapter 5
Ecological Risk Assessment (ERA) of a Tanning Industry

Abstract The complex nature of the pollutants (posing risks and hazards within the working tannery environment) demonstrated in this study and explained in the previous chapters, led to the development of an ecological risk assessment (ERA) associated with the tanning industry. This approach acts both as a diagnostic and remediative tool pertinent to the tanning industry. This chapter attempted to capture the impact of the tanning industry in a much more holistic manner and encompassing experiences learnt in the earlier chapters. This resulted to the development of an ecological risk assessment (ERA) to provide possible mitigating factors geared towards the tanning industry. Ecological risk assessment utilised various techniques to evaluate the probability that adverse ecological effects will occur as a result of exposure to one or more stressors. Ecological risk assessment determined and documented actual or potential effects and impacts of contaminants on ecological receptors and habitats as a basis for evaluating remedial alternatives. Therefore the main aim of this chapter was to integrate principal issues such as identifying stressors/hazards, application of biological (Bioassays) and chemical assays (to determine heavy metals, COD, BOD, and Total Phenols).

5.1 Introduction

Industry is by far the largest source of hazardous wastes worldwide. There are no reliable estimates of the quantity and types of hazardous waste generated in most developing countries. Approximately 10–15% of the wastes produced by industry overall are likely to be hazardous, increasing at a rate of 2–5% per year (Chaaban 2001). Khwaja (1998), identified leather tanning and finishing, as one of the main industries producing hazardous wastes and pollution. This is an aspect the current study has attested to by dissecting the contaminants biological and chemical characteristic at atmospheric (e.g. determination of dust inhalation potential), terrestrial and aquatic (e.g. river health status) phases. Indeed, there is very little information on hazardous waste production, waste disposal and management practices in most developing countries. The major public concern over tanneries has traditionally been about odours and water pollution from untreated discharges. Important

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pollutants associated with the tanning industry include chlorides, tannins, chromium, sulphate and sulphides as addition to trace organic chemicals and increasing use of synthetic chemicals such as pesticides, dyes and finishing agents, as well as from the application of newer processing chemical solvents (Anonymous 2003). These substances are frequently toxic and persistent, and affect both human health and the environment (UNEP 1994).

The many diverse environmental impacts of tanneries have made them subject to relatively sophisticated pollution control policies in many countries (Mwinyihija et al. 2006). Factory sites, lagoons, storage areas and temporary waste dumps may severely contaminate the underlying soil if appropriate precautions are not taken. This contamination can interfere with subsequent land use (agriculture, building) as well as potentially contribute to groundwater pollution. Groundwater contamination occurs when wastewater and chemicals seep through the soil from unlined ponds, pipes and drains, or from dumps and spills. Groundwater may take a long time to cleanse itself because it moves slowly and is out of contact with air (Wierenga 1996).

In addition to deposition of solids, raw unsettled tannery wastewaters can cause encrustation (of calcium carbonate) and serious corrosion of metals as well as concrete sewers (due to H_2S biological oxidation to H_2SO_4 (Balusubramanian and Pugalenthi 2000). High pollutant loads, involving chromium, can interfere with key biological processes used in sewage treatment plants. These pollutants may also damage the ecology of the receiving waters in the vicinity of the discharge points.

Biological decomposition of organic matter from industrial waste, as well as sulphide emissions from wastewaters (also due to bacterial reduction of sulphate) (Balusubramanian and Pugalenthi 2000), are responsible for the characteristic objectionable odours from tanneries in the form of H_2S (Dorman et al. 2000). Potential sources of odour include sulphide emissions from dehairing and waste treatment, ammonia emissions from dehairing and delime liquors and fleshings.

Direct contact with some industrial chemicals can cause disability, illness (toxigenic/carcinogenic) and death in humans (Murti 1989). Minor exposures when frequent can cause the build-up of toxic levels within humans. Solvents from degreasing and finishing are a source of exposure through vapours. Human health can also be affected by toxic hazards through the unskilled and unprotected handling of pesticides, tanning chemicals and treated hides and skins. This was demonstated in details in Chap. 4 (Mwinyihija et al. 2005) as an occupational hazard. Also visual impacts, excessive noise and air emissions are known to be associated with the tanning industry.

Ecological risk assessment of the tanning industry entails understanding the categories of hazardous waste, its identification, exposure assessment, ecological effects and risk characterisation. To identify the toxic nature of the effluent, bioassays (to evaluate responses to stressors) and chemical analysis (to provide information on the concentration and identification of the stressor) were used.

5.2 Categories of Hazardous Waste

Hazardous wastes do not have a universally accepted definition, but similar definitions are used in many countries and the United Nations Organisations (Chaaban 1996). In the United Nations Environment Programme (UNEP) the definition is:

"Waste other than radioactive wastes which, by reason of their chemical reactivity or toxic, explosive, corrosive or other characteristics causing danger or likely to cause danger to health or the environment, whether alone or coming into contact with other wastes, are legally defined hazardous in the state in which they are generated or in which they are disposed of or through which they are transported"

In the USA, generally the hazardous waste is defined as:

"One that may cause or significantly contribute to serious illness or death or that poses a substantial threat to human health or the environment when improperly managed".

However in the United States, Environmental Protection Agency (US EPA) particularly stratifies the influencing conditions and defines any waste as hazardous which meets one of the following:

1. Ignitability. Waste that poses a fire hazard during routine management. Fires not only present immediate dangers of heat and smoke, but also can spread harmful particles over wide areas.
2. Corrosivity. Wastes requiring special containers or segregation from other wastes because of their ability to dissolve toxic contaminants.
3. Reactivity. Wastes that tend to react spontaneously, to react vigorously with air or water, to be unstable to shock or heat, to generate gases or to explode.
4. Toxicity. Wastes that, when improperly managed may release toxicants in sufficient quantities to pose a substantial hazard to human health or the environment.

Tannery effluent, because of its complexity, is associated with the four main characteristics mentioned above (ignitability, corrosivity, reactivity and toxicity). This will therefore form the basis of assessment of a site in Kenya depicting the production tannery effluent as shown in Fig. 5.1.

5.3 Ecological Risk Assessment

The impact of the tanning industry will be evaluated through ecological risk assessment, which is a process that evaluates the probability that adverse ecological effects will occur as the result of exposure to one or more stressors. The objective of an ecological risk assessment is to determine and document actual or potential effects of contaminants on ecological receptors and habitats as a basis for evaluating remedial alternatives. Ecological risk may be expressed as a probabilistic (Ecological Quantitative Risk Assessment (EQRA)) estimate of adverse effects (as in human health risk assessment), or may be expressed in a more qualitative manner (Gerba 1996).

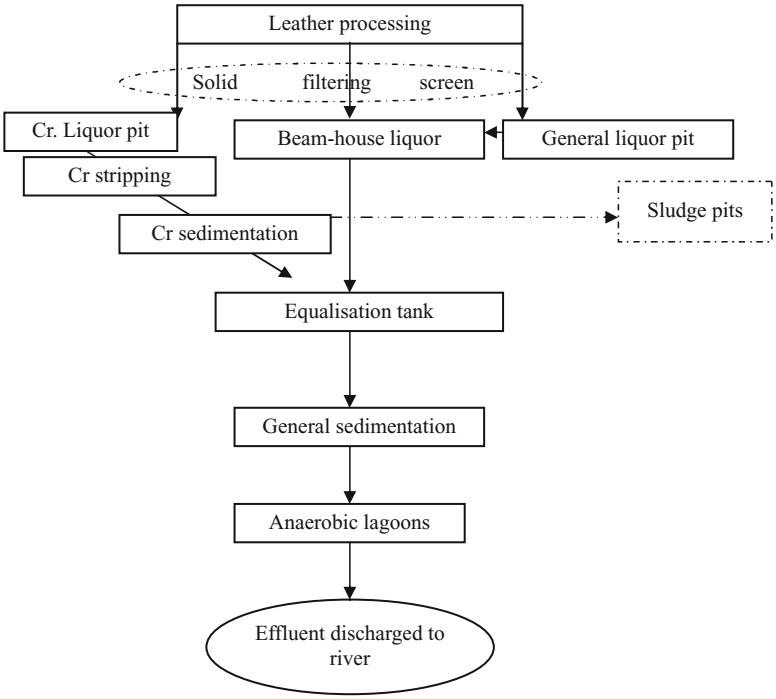

Fig. 5.1 Flow diagram alleviating effluent treatment in a typical leather processing site in Kenya

Quantitative risk assessment (QRA) is a technique, which can be used to estimate the likelihood and severity of an adverse event (Cassin et al. 1998). When performed in conjunction with Monte Carlo simulation, QRA offers precise explanation of the uncertainty and variability associated with the risk (Vose 2000). In this study, QRA was applied to the ecosystem (i.e. to determine the risks to the ecosystem). The ecosystem can be defined as a grouping of organisms (microorganisms, plants, animal) interacting together, with and through their physical and chemical environment, to form a functional entity (Duffus 1993; WHO 1978).

The terminology in risk assessment is not yet fixed but after an initial statement of purpose (Codex Alimentarius Commission, CAC 1998; Vosey and Brown 2000), the process involves four primary stages described below in relation to this current study (Gerba 1996).

1. Hazard identification – identifies the toxic chemical of concern and whether it is actually a hazard in the context that it is being studied. The problem-formulation process will involve the evaluation of the stressor (a substance that has the inherent ability to impose adverse effects upon a biological system) characteristics, the

ecosystem at risk, and the likely ecological effects. Assessment end-points (environmental values to be protected such as the decline of fish in a river due to effluent discharge) and measurement end-points (quantitatively or qualitatively measurable factors such as laboratory results showing mortality of test organism due to effluent discharge) will be selected to develop management strategies.
2. Exposure assessment – aims to determine the environmental concentration range of a particular stressor and estimating its rate of intake in target organisms. This measurement will combine quantitative parameters describing the frequency and magnitude of contact. Mathematical models will be used to predict the fate and resultant exposure to a stressor and to determine the outcome of a variety of scenarios.
3. Ecological effects which integrates toxicity assessments, aims to identify and quantify the adverse effects elicited by a stressor and, to the extent possible, determine cause-and-effect relationships. In this phase, acute toxicity data will be used. The combination of the exposure-analysis data with the ecological-effect data results in a stressor-response profile. This profile represents an attempt to match ecosystem impacts to the levels of stressor concentration under study.
4. Risk characterisation – consists of comparing the exposure and stressor-response profiles to estimate the probability of effects, given the distribution of the stressor in the systems.

5.4 Bioassays and Chemical Analysis in Context of ERA

Bioassays involve studying biotics (living organism) in responses towards xenobiotics in the environment (e.g. Phytotoxicity, bioluminescence response etc.). Bioassays have larger variability than most chemical analysis due to biological variation. The major advantage of bioassays is that the total toxicity of wastewater can be assessed by taking into account bioavailability and synergistic or antagonistic effects. Also, transforming information on concentration to information on biological response is useful for risk assessment. Chemical analysis provides information on the concentration of a substance in a sample and may help to identify that substance (Stuhlfauth 1995). However, this does not give direct information relating to the bioavailability and impact of environmental pollutants.

The requirements for a bioassay as a test to effluents must meet the following criteria: the test should be representative; reproducible; simple to use, inexpensive (Stuhlfauth 1995).

5.4.1 Toxicity Test

Considering the criteria for the selection of appropriate bioassay in environmental toxicity assessment, a toxicity test (rather than ecosystem or sub-organismic test)

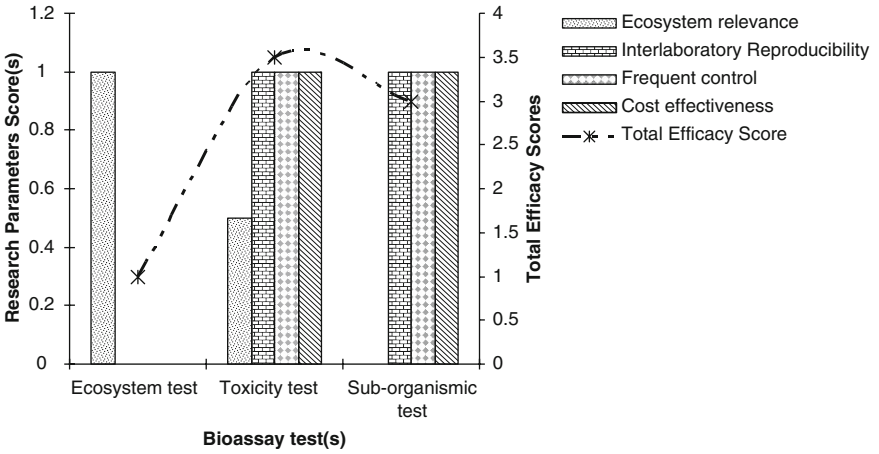

Fig. 5.2 Comparison between individual test parameters and their total efficacy scored on 0 to 1 value depicting low to excellent score respectively (Modified from Stuhlfauth1995)

was identified to be ideal (with the highest total efficacy score of 3.5) in this study due to its ecosystem relevance, inter-laboratory reproducibility and cost effectiveness as per the efficacy test scores depicted in Fig. 5.2

Several bioassay system hierarchies that might be considered for the purpose include;

1. Ecosystem tests (e.g. Mesocosm, Microcosm or Biocenosis (EWOFFT 1992, Scholz and Müller 1992))
2. Toxicity tests with single species (e.g. fish, daphnia, algae); or
3. Sub-organismic test systems (e.g. fish cell tests (Hansen et al. 1989))

5.4.2 Evaluation of Biomass Activity in Sediments

Monitoring of the biomass activity (e.g. intracellular enzyme activity) at the river sediment level can provide vital information on stressor-response and ecological–effects. The activity of certain enzymes and cofactors such as $F_{420,}$ hydrogenase, dehydrogenase (DHA), and adenosine triphosphate (ATP), may serve as indicators of these biological effects (Nybroe et al. 1992; Le Bihan and Lessard 1998; Goel et al. 1998). DHA and ATP have been used successfully to monitor biomass activity (e.g. in aerobic and anaerobic sludge activity) because the methods of determining them are easy and relatively rapid. Dehydrogenase is an oxidoreductase soil enzyme and is intracellular, often relatively stable, and can persist for extended periods, thereby providing a longer term perspective than measurements involving extant organisms alone. The impact of pollutants on soil health has been addressed through the measurement of enzyme activity (Killham and Staddon 2002). DHA is

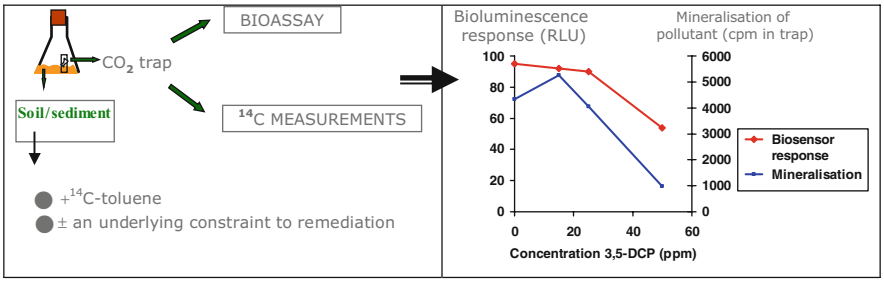

Fig. 5.3 Correlation to mineralization of natural pollutant substrate and biosensor response

measured generally by adding a tetrazolium salt, such as triphenyltetrazolium chloride (TTC) or 2-(*p*-iodophenyl)-3-(*p* nitrophenyl)-5-phenyl tetrazolium chloride (INT), to a biological system (Hongwei et al. 2002). Knowledge of the reduction in sediment capacity to act as a fully functioning mineralisation medium for natural pollutant substrates (Fig. 5.3) is critical in overall river health assessment (Killham and Staddon 2002).

5.5 Weight of Evidence in Ecological Risk Assessment

The use of multiple criteria or multiple lines of evidence (refered to as weight of evidence) in this study has been previously applied in the study of ecological systems elsewhere (e.g. Woodman and Cowling 1987; Suter 1993) and is recommended for use in ecological risk assessment (USEPA 1991; 1993a, b; 1997; 1998). Seven causal criteria (*Strength of association, Consistency of association, Specificity of association, Time order or temporality, Biological gradient, Experimental evidence, Biological plausibility*) were chosen for application during this study because they have already been established in this area (Adams 2003), and are readily applicable to environmental samples sourced from the Kenyan tanning industry. A linkage will be developed using the three levels and seven tiers reported by Hewitt et al. (2003) involved in a casual identification, including identification of effects, development of correlative relationships and confirmation that specific chemicals are responsible for the observed effects.

5.6 Risk Questions

Two questions were developed: (1) has the water quality of the river been affected by the discharged tannery effluent? and (2) has the river sediment health been affected by the deposition of the contaminants?

5.7 Objectives

The overall objective of this chapter is to discuss the ecological risk caused by the tannery industry by responding to the risk questions raised. To achieve this objective the following specific criterions were pursued:

1. Identify stressors/hazards.
2. Apply biological (Bioassay) and chemical assays (to determine heavy metals, COD, BOD, Total Phenols) to quantify the stressors and their effects on the ecosystem.
3. Determine the probability that the identified stressors (toxic chemicals from the tanning industry) caused a significant impact to the environment (analogous to the statement of purpose of the risk assessment).
4. Determine the effectiveness of risk mitigation strategies to reduce the risk of the ecological contamination (e.g. by reducing contaminants in the raw effluent, sedimentary, final effluent and combination of all the strategies involved).

5.8 Methodology

Risk assessments using bioassays were performed under a series of different scenarios, which include duration of exposure of the organism to the hazard, toxicity levels at different sampling points and variation in percentage bioluminescence over the control by the biosensor. These parameters were used to run the deterministic model. Chemical, deterministic models and USEPA protocols defining causes of acute toxicity in industrial discharges (USEPA 1991, 1993a, b, 1997) were used in determining ecological risk. Samples were collected from a riverine site and tannery effluent lagoons as described Chap. 3.

The study design entailed the effluent treatment phase (raw effluent, treated effluent (general sedimentation) and final effluent (anaerobic lagoons)), upstream and downstream sampling points. Random selection of the sampling was carried out on eight occasions during the sixteen week period. Observations on visual (water colour, vertebrates, plants etc) and odorous peculiarities were noted. Biological investigations were performed in conjunction with chemical analysis to identify the class or causative chemicals involved.

5.8.1 Assessment and Measurement End-Points

In accordance with the needs of this study, the assessment end-point selected was the protection of the aquatic receptors (surface and sedimentary levels) of the river. To achieve this assessment end-point, the mortality of primary decomposers

(*Escherichia coli* HB101 pUCD607) and primary consumers (*Daphnia magna*) as surrogate (trophic levels) species was measured (measurement end-points). Based on knowledge of the fate and transport of tannery effluent contaminants in aquatic systems, and the ecotoxicity of the contaminants to aquatic organisms, a conceptual model was developed (Fig. 5.4). Tannery effluent contaminants deposited in the sediments can be released to the water column during re-suspension and redistribution of the sediments. The benthic community would be an initial receptor for the contaminants in the sediments. The ecological risk assessment indicated the assessment end-point for the study site would be the protection of surface river water (using *Daphnia magna*) and sediments (using *Escherichia coli* HB101 pUCD607) (aquatic receptors) from toxicity caused by tannery effluent contaminants that concentrate or pre-concentrate in aquatic receptors. A measure of biomass activity (dehydrogenase) will further provide the river sediment health status (comparing upstream and downstream sampling points) in relation to the stressor impact.

An appropriate diagram was developed for the conceptual model to identify the exposure pathways by which the aquatic receptors could be exposed to tannery effluent contaminants originating from the tannery site. Based on this information, two assessment-end-points were identified: (1) maintaining river surface water quality (2) protecting river sediment health.

The diagram identifies the primary, secondary and tertiary sources of the tannery effluent contaminants at the study site, as well as end-point aquatic receptors that could be exposed.

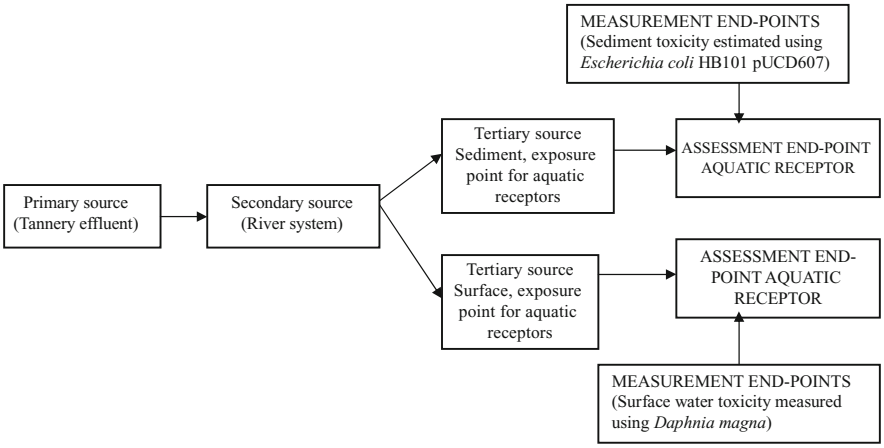

Fig. 5.4 Conceptual model for the Tannery site showing the primary source of pollution and the measurement end-points for sediment river health (*Escherichia coli* HB101 pUCD607) and surface water (*Daphnia magna*)

5.8.2 Bioassays

The determination of toxicity was based on the bioluminescence response as described in Sect. 3.3 Chap. 3.

5.8.3 Sample Addition and Luminometry Measurements

Sample addition and luminometry measurement was as described in Sect. 3.3.2.

5.8.4 Daphnia Test

Daphnia magna (micro-crustacean) was used in the test as an invertebrate to represent the primary consumer level and identify short-term acute effects. This was carried out using the protocol described in Sect. 3.5.

5.8.5 Dehydrogenase Activity Test

This method was as described in Sect. 3.4 and data analysed as indicated therein.

5.8.6 Chemical Analysis

Total concentrations of Cr, Ni, Cu, Zn, Cd, and Fe in each effluent sample were determined as indicated in Sect. 3.6. Chloride and sulphate were determined as described in Sect. 3.6.3. While total phenols were determined as shown in Sect. 3.6.4, BOD and DO were determined as described in Sect. 3.6.2.

5.8.7 Data Analysis

The different scenarios under the risk assessment model were run under the deterministic model as shown in Table 5.1.

The input data for the model were obtained from the scientific literature, ongoing studies into tannery effluents in Kenya and expert opinion (Table 5.2). Importance analysis (quantitative ranking measures for components) was performed using deterministic model to determine which parameters (stressors) were correlated with contamination, to the riverine ecosystems.

Table 5.1 The different scenarios under which the risk assessment was run using a deterministic model

Scenario No	Scenario	Base Scenario value
1	Amount of contaminants in the effluents before treatment (C_{re});	
	(a) COD	2437.84
	(b) BOD	1,255
	(c) Cl	1,725
	(d) Sulphide	62
	(e) SS	562
	(f) Total Cr	23
	(g) Grease/oil	332
2	Amount of contaminants in the effluent after sedimentation C_{sed})	
	(a) COD	1579.30
	(b) BOD	5,738
	(c) Cl	1,875
	(d) Sulphide	57
	(e) SS	448
	(f) Total Cr	1.71
	(g) Grease/oil	273
3	Amount of contaminants in the final effluent C_{fin});	
	(a) COD	1355.93
	(b) BOD	438
	(c) Cl	1,926
	(d) Sulphide	89
	(e) SS	330
	(f) Total Cr	0.9
	(g) Grease/oil	94
4	Effect of introducing efficiency (percentage) during effluent treatment:	
	(a) Raw effluent	10
	(b) Sedimentation	30
	(c) Final effluent	20

5.9 Risk Assessment and Identification

5.9.1 Hazard Identification and Problem Formulation

In the tanning process, the chemical and gaseous contaminants are identified as the main pollutants in Table 5.3.

1. Soaking activities

This process aims at cleaning, conditioning and ensuring the correct moisture content of the hide or skin is attained prior to Beam-house operations (liming,

Table 5.2 Model variables

Variable	Description	Model formulae/units
T_{re}	Toxicity measurements of raw effluent	%
T_s	Toxicity measurement of effluent after sedimentation	%
T_{fe}	Toxicity measurements at final effluent	%
RE_v	Raw effluent values	$mg\,L^{-1}$
RE_{COD}	COD level in raw effluent	$mg\,L^{-1}$
RE_{BOD}	BOD level in raw effluents	$mg\,L^{-1}$
RE_S	Sulphide levels in raw effluents	$mg\,L^{-1}$
RE_{Cr}	Total Cr levels in raw effluent	$mg\,L^{-1}$
RE_{Ph}	Total phenols in raw effluents	$mg\,L^{-1}$
S_v	Effluent contaminant values after general sedimentation	$mg\,L^{-1}$
S_{efc}	Effluent treatment efficiency after general sedimentation	$S_{efc} = (RE_v - S_v) \times 100/RE_v$
S_{COD}	COD level in effluent after sedimentation	$mg\,L^{-1}$
S_{BOD}	BOD level in effluent after sedimentation	$mg\,L^{-1}$
S_S	Sulphide levels in effluent after sedimentation	$mg\,L^{-1}$
S_{Cr}	Total Cr levels in effluent after sedimentation	$mg\,L^{-1}$
S_{Ph}	Total phenols in effluent after sedimentation	$mg\,L^{-1}$
FE_v	Final effluent values after anaerobic lagoons	$mg\,L^{-1}$
FE_{efc}	Final effluent treatment efficiency after anaerobic lagoons	$FE_{efc} = (S_v - FE_v) \times 100/S_v$
FE_{COD}	COD level in the final effluent after anaerobic lagoons	$mg\,L^{-1}$
FE_{BOD}	BOD level in the final effluent after anaerobic lagoons	$mg\,L^{-1}$
FE_S	Sulphide levels in the final effluent after anaerobic lagoons	$mg\,L^{-1}$
FE_{COD}	Total Cr levels in the final effluent after anaerobic lagoons	$mg\,L^{-1}$
FE_{Ph}	Total phenols in the final effluent after anaerobic lagoons	$mg\,L^{-1}$
P_{MCL}	Maximum contaminant levels (target) for identified pollutants	$mg\,L^{-1}$

Table 5.3 Potential chemical and gaseous contaminants produced at different stages of leather processing

Leather processing stage	Water pollutants	Air pollutants
Soaking/Liming	BOD, COD, SS, DS, Sulphides	H_2S
Deliming & Bating	BOD, COD, SS	NH_3
Degreasing	BOD, COD, DS	
Pickling/Tanning	BOD, COD, DS, Acids, Salts	Acidic fumes
Retanning/Bleaching/ Dyeing	Acids, Salts, Chrome, Chlorinated phenols	Volatilised, chlorinated phenolics

deliming and pickling). However, this results in production of salts previously used for preservation, free ammonia, unpleasant odours, dirt, blood, dung and other related chemicals (e.g. wetting agents, surfactants, emulsifiers etc) which, are released in the effluents at this stage.

2. Beam-house activities

This stage involves the preparation of the hide/skin (known as pelts) for the tanning process. During this stage, large quantities of water are consumed in proportion to the weight of the pelts leading to high dissolved substances, COD, suspended solids, chlorides, sulphides, organic nitrogen and high pH. Aloy et al. (1976) reported that, conventional liming-reliming processes lead to 35–45 kg of BOD, 100–125 kg of COD and 140–160 kg of total solids (TS) for every ton of raw skins/hides processed. Comparatively BOD and COD loads contribute 50–70% of the total load from a tannery wastewater while TS load accounts for 15–20% (solid waste containing lime sludge, fleshings and hair (Ramasami and Prasad 1991). Beam-house activities contribute immensely to the total pollutant load of the tannery effluent. Sulphides lead to production of the toxic and foul smelling hydrogen sulphide gas while ammonical nitrogen (ammonium) results in high oxygen demand (toxic to aquatic life) and stimulates eutrophication. Conventional cleaning-up treatments are generally not able to reduce chlorides and sulphates of the exhausted pickling solutions to meet the limits required by regulations; for example if chloride content in the region of 9 $g L^{-1}$ way above most legal requirements could represent a considerable problem for biological plants (Cassano et al. 2001).

3. Tan yard activities

Tanning operations consume high quantities of water based on the pelt weights. The effluent is characterised with high levels of COD, surfactants, chlorides, sulphates, ammonium-N and chromium (mostly Cr^{3+}). A third of the chrome applied in tanning goes into useful leather with the rest (if not re-used) discharged into the effluent. This, in addition to the leather fibres in the chromium liquor discharged, results in tannery effluent with high chromium concentration. These liquors also contain proteinuos material, neutral salts including sodium sulphate and chloride.

4. Post tanning activities

Post tanning activities compose mechanical operations (e.g. sammying, splitting, shaving and trimming), wet work, drying and finishing. These operations could yield a combination of solid wastes, squeezed-out water and unfixed tanning chemicals with the finishing process producing mostly air emission of solvents (UNEP 1994).

5.9.2 Effluent Production

The amount of effluent discharged per month at the study site varied depending on the demand of the export market. For example Fig. 5.5 depicts data from a tannery

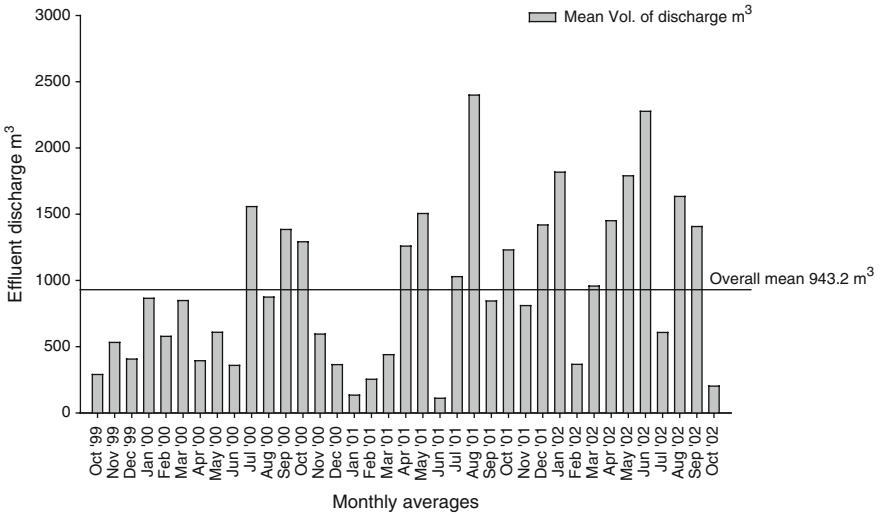

Fig. 5.5 Tannery effluent mean, monthly discharge 1999–2002 at a Kenyan site

Table 5.4 Characterisation of the Kenyan tannery effluent showing identified parameters and levels in three main phases (raw effluent, treated effluent and final effluent) ($n=5$)

Parameters	Raw effluent	Treated effluent (General sedimentation)	Final effluent (Anaerobic lagoons)	LSD (5%)
pH	7.72 (0.19)	7.1 (0.1)	7.66 (0.24)	0.58
COD	2437.84 (660.3)	5978.16 (4626.1)	1307.4 (291.4)	8329
BOD	1255 (309.9)	5738.1 (4688.7)	438.5 (194.9)	8366
Cl	1725 (495.5)	483.9 (216.4)	1693.7 (757.4)	1719
Sulphide	62.4 (14.7)	57.2 (15.1)	89.96 (26)	60
Susp. Solids	562 (121.6)	448.2 (153)	330.67 (43.3)	394
Total Cr	23.02 (18.3)	1.71 (0.4)	0.93 (0.2)	33
Oil/grease	332.3 (108.2)	273.9 (101)	94.38 (31)	267

Figures in parenthesis are SEM's (Standard errors of means)

site covering the period January 1999 to end of 2002. A mean discharge rate of 943.2 m^3 was observed over time.

Different parameters of the discharges from the study site in Kenya were measured (Table 5.4) and then compared to other countries (in relation to the effluent standards). Countries with the highest (maximum) contaminant permissable levels in tannery waste discharge standards were noted and used to determine the trends in the Kenyan tannery site. High levels of BOD (480%), COD (344.8%), suspended solids (440%), sulphides (157.5×10^3%), total chrome (130%), chloride (105%) and oil/grease (350%) were observed beyond the maximum tolerable levels of the countries noted.

5.9.3 *Effluent Composition and Resulting Stressor Characteristics*

The evaluation of the stressor characteristics (tannery effluents), the ecosystem at risk (upstream and downstream riverine points), and the likely ecological effects were analysed. The parameters investigated during the characterisation of the effluents included: pH, COD, BOD, Cl, sulphide, suspended solids, total chromium and oil and grease (Table 5.4). These parameters were then compared over three main phases (raw effluent, sedimentary and final effluent). During an initial analysis the total chromium observed in the final effluent before discharge was 0.93 mg L^{-1} (Table 5.5). The determination of total chromium is critical in obtaining the non-labile Cr^{3+} fraction (Johnson 1990, Beaublen et al. 1994). The two primary oxidation states of chromium in natural waters Cr^{3+} and Cr^{6+} differ significantly in biological, geochemical, and toxicological properties (Florence 1989; Krull et al. 1982; Fendorf and Zasoski 1992; Eary and Rai 1988).

1. Effect of Chromium

The toxicity and mutagenity of hexavalent chromium in the environment is well established. As an oxyanion, Cr^{6+} is highly mobile in soil and water environments. Trivalent chromium, on the other hand, is a cationic species and is rather immobile due to its low solubility, high adsorption and complexation. Due to its toxicity and mobility, Cr^{6+} as a contaminant in the environment has often been considered more problematic than Cr^{3+}. Once introduced into the environment, the speciation of chromium can change as a result of several environmental factors or persist unchanged for a long time, depending upon environmental conditions (Pantsar-Kallio et al. 2001).

2. Effect of pH

The pH at discharge point was observed to vary between 7.72 (Raw effluent) and 7.66 (Final effluent). Most of the biological processes operate at optimum levels near neutral pH (6.0–7.5). The presences of carbonates and bicarbonates exert a buffering effect in water systems and could maintain the pH to tolerable range of (~6–8) following acid rain events for a long time (in years) (Artiola 1996).

This is because the water solubility of most metals increases as pH decreases. Thus one management strategy to reduce metal mobility and toxicity in plants is to lime soils to a neutral or alkaline pH (Artiola 1996). Metal toxicity (imparted by changes in speciation and partitioning effects of the metals) (Ritchie et al. 2001; van Leeuwen 1999; McGrath et al. 1999) and other pH dependent toxicity within the tannery effluents were observed.

3. Effect of Chloride

The high chloride content in the effluent (1693.7 ± 757.4 mg L^{-1}) can affect aquatic plants and certain species of animals. Each species adapted to low salinities have a certain range of salinities within which they can survive. However, animals not affected by salinity may be indirectly affected by habitat modification and altered

Table 5.5 Total Phenols, pH, and metal concentrations of tannery effluent treatment pits (mg L^{-1}), anaerobic lagoons (mg L^{-1}) and riverine sediments (mg kg^{-1})

Samples	pH	Cr mg L^{-1}	Pb mg L^{-1}	Fe mg L^{-1}	Cu mg L^{-1}	Cd mg L^{-1}	Zn mg L^{-1}	Ni mg L^{-1}	Total Phenol (mg L^{-1})
Effluent treatment pits (mg L^{-1})									
Beam-house	12	0.07	0	1.75	0.02	0.01	0.07	0.05	ND
General sedimentation	8.34	0.31	0	0.15	0.01	0.01	0.07	0	72
Chrome stripping	9.6	22.58	0.06	1.39	0.03	0.01	0	0.15	ND
Chrome sedimentation	8.25	191.47	0	7.42	0.06	0.01	0.71	0.53	52.9
Equalisation tank	8.05	0.3	0	0.5	0	0.01	0	0.02	36.8
Anaerobic lagoons (mg L^{-1})									
Lagoon 1	7.8	0.1	0	0.17	0	0.01	0.02	0.03	30
Lagoon 2	7.92	0.06	0	0.09	0.01	0.01	0	0.04	NA
Lagoon 3	8.3	0.07	0.01	0.03	0	0.01	0	0.04	48.1
Lagoon 4	7.82	0.13	0.04	0.06	0.01	0.01	0.05	0.01	24.5
Lagoon 5	8.40	0.03	0	0	0	0.01	0.01	0.06	16.6
Riverine sediment sample (mg kg^{-1})									
200 m upstream	7.02	0.91	0.55	1139	0.51	0.04	1.94	0.75	ND
100 m upstream	7.06	1.14	0.27	772	0.16	0.02	0.79	0.43	ND
0 m discharge point	8.01	1.41	0.39	4237	0.57	0.02	1.63	0.72	30
100 m downstream	7.31	1.31	0.36	1048	0.44	0.03	1.14	0.62	17.7
200 m downstream	7.30	1.65	0.58	1362	0.69	0.03	1.15	1.01	11.4
400 m downstream	7.4	1.76	0.58	1349	0.52	0.04	1.37	1.02	5.5

LOD The limit of detection (LOD) was determined on the basis of five blank samples at average blank signal plus three and ten times the standard deviation.
NA Not available
ND None detected

food supply. Aquatic invertebrates, macrophages and plants are generally affected (e.g. in plants, a reduction in growth rates, leaf production and die-back of the growth tips is observed) at salinities over 1,000 mg L^{-1}, while more than 4,000 mg L^{-1} affects the survival of some common macrophytes (Anonymous 2001b). Cassano et al. (2001) also reported that 9 g L^{-1} of chloride could represent a considerable problem for biological plants.

4. Effect of BOD/COD

Both BOD and COD levels were highest at the general sedimentation phase (BOD 5,978 mg L^{-1}, COD 5,978 mg L^{-1}) of the tannery effluent treatment pits, with the levels drastically reducing in the final effluents (BOD 438 mg L^{-1}, COD 1,307 mg L^{-1}) after the anaerobic lagoons. Beam-house operations involving soaking, liming and deliming processes generate large quantities of waste such as wastewater (up to 400% during liming and reliming process) consumed in proportion to the weight of the treated hides (Thanikaivelan et al. 2003). The discharged water is full of dissolved substances, which affect its quality. The Beam-house mainly affects the following parameters of water effluent; COD, suspended solids, chlorides, sulphides and organic nitrogen. Conventional liming-reliming processes lead to 35–45 kg of biological oxygen demand (BOD), 100–125 kg of chemical oxygen demand and 140–160 kg of total solids (TS) for every ton of raw skins/hides processed (Aloy et al. 1976).

5. Effect of Sulphide

Sulphide levels increased during the treatment phases when raw effluent (62.4 mg L^{-1}) was compared to the final effluent (anaerobic lagoons) (89.96 mg L^{-1}). The increase in sulphide content in the final effluent lagoons was due to the increasing anaerobic conditions that cause a reversion of sulphates to sulphides. Sulphate and sulphide combinations have a variety of potential health (sulphide forming obnoxious and toxic H_2S gas) and environmental impacts and cause damage to structures (Sulphates accelerates corrosion of concrete sewers) (UNEP 1994; Balusubramanian and Pugalenthi 2000). Sulphide is not only toxic for higher organisms, it is also known as an inhibiting substance in anaerobic microbial processes (Wiemann et al. 1998).

6. Effect of Phenols

Chlorophenols are the most predominant phenolic compounds in the tanning industry. Chlorophenols can enter the environment through accidental spills, illegal release of industrial and municipal wastewater and excessive use of pesticides (Park et al. 1999). Chlorophenols are used as preservatives for a number of materials such as wood, textiles and leather. Chlorophenols are well known for their biocidal activities and have been found to be toxic, possibly mutagenic to terrestrial biota (Jensen 1996). Studies on toxicity of chlorophenols have been conducted involving plants (e.g. *Lemna gibba)* (Hulzebos et al. 1993), Fish (e.g. *Pimphales promelas, Cyprinodon variegates, Poecilia reticulata)* (Smith et al. 1994), earthworms (e.g. *Lumbricus terrestris, Eisenia foetida*) (Giggleman et al. 1998), Protozoa (e.g. *Tetrahymena pyriformis*) (Bryant and Schultz 1994) and micro-organisms (e.g. *Rhodococcus chlorophenolicus)* (Apajalahti and Salkinoja-Salonen 1986) (e.g. *Pseudomonas putida* MT-2, *Pseudomonas putida* 50026, *Rhodococcus erythropolis*

A177) (van Beelen and Fleuren-Kemila 1997) and (*Rhizobium leguminosarum* bioavar *trifolii) (*Chaudri et al. 1996).

7. Overall summary of tanning and post tanning operations

Tanning operations and post tanning operations (contribute to the remaining 40% of the total pollution) consume quantities of water in proportion to the weight of the hides washed , and produce pollution in the effluent water, consisting of COD, surfactants, chlorides, sulphates, ammonium-N and Cr^{3+}. The effluent from the dyeing operations, which, per unit of product processed is smaller than, that from previous phases is discharged, at modified values of temperature, COD, ammonia nitrogen, phenolics compounds and fats. Finishing operations, and in particular the surface application of the products affect the quality of emissions into the atmosphere as regards particulate dust and volatile organic substances (VOS) (UNEP 1994).

5.10 Exposure Assessment

5.10.1 End-Points and Conceptual Model

Based on the screening-level risk assessment, the ecotoxicity literature review, and the complete exposure pathways, development of a conceptual model for the study site were initiated (Fig. 5.4). Two main contaminants of the effluent (Chromium and Phenols) were identified which could acutely or chronically be toxic to organism within an aquatic community. Direct exposure of the effluent (untreated) to the river could cause acute or chronic toxicity in fish and /or benthic invertebrates, and in aquatic plants. The current status of the effluent after treatment was determined and evaluated for contents toxicity. The exposure pathways were therefore evaluated through direct contact with contaminated sediments and water. *Daphnia magna* (primary consumer) and *Escherichia coli* HB101 pUCD607 (Primary decomposers) were used to evaluate the community structure.

To complement the bioassay, river sediments were analysed for heavy metals (Table 5.5). The speed and the depth of the river at the discharge point (1.17 $m\,s^{-1}$ (high water levels – rainy seasons) to 0.513 $m\,s^{-1}$ (reduced water flow period low rainy seasons)) were observed. This was to understand the river dynamics and dilution factor of the river.

Total chromium levels were highest at the chrome stripping (22.58 $mg\,L^{-1}$) and chrome sedimentation tank (191.47 $mg\,L^{-1}$). The riverine system showed higher levels of chromium in the sediments at 200 m (1.65 $mg\,L^{-1}$) and 400 m (1.76 $mg\,L^{-1}$) downstream. Similarly, this trend was observed for all the metals analysed (Table 5.5). This suggested a settling out of solution of the metals to the sediment as the speed of the river slowed downstream (between 0.68 and 0.40 $m\,s^{-1}$) in comparison to the discharge point (between 1.17 and 0.51 $m\,s^{-1}$). Further analysis for total phenols showed none detected at any of the points upstream, but 30 $mg\,L^{-1}$ was observed at the discharge point (Table 5.5), progressively diluting downstream.

1. Toxicity assessment of the trophic levels

The exposure assessment used the concentration ranges of the effluent (stressor) within all three phases (raw effluent, treated effluent (general sedimentation), final effluent (anaerobic)) (Table 5.5) to predict the fate and resultant exposure to the stressor using a mathematical (deterministic) model. Toxicity assessment on trophic levels involved actual downstream biotic (LD) values for daphnia (Table 5.6) and percentage bioluminescence for treatment pits, anaerobic lagoons and riverine sampling points (Figs. 5.6–5.8).

(a) Invertebrate trophic level (Daphnia magna)

Higher LD values were observed at the treatment pits (Chrome stripping, sedimentation and equalisation pits) (Table 5.6). The anaerobic pits showed a reduction on LD values indicating a reduction in toxicity as the effluent flows from lagoon 1 (LD 90) to lagoon 5 (LD 80). Similarly, the trend was observed in the riverine sampling points, with lower LD values noted downstream. The dilution effect of the river (reduction of LD value observed progressively) downstream

Table 5.6 Lethal dose (LD) values of *Daphnia magna* on treatment pits, riverine and anaerobic lagoons of a Kenyan tannery site

Samples	LD values *D magna*
Treatment pits	
Beam-house	LD 50
General sedimentation	LD 50
Chrome stripping	LD 100
Chrome sedimentation	LD 100
Equalisation tank	LD 90
Anaerobic Lagoons	
Lagoon1	LD 90
Lagoon2	LD 85
Lagoon3	LD 85
Lagoon4	LD 80
Lagoon5	LD 80
Riverine	
400 m upstream	NE
200 m upstream	NE
100 m upstream	NE
50 m upstream	NE
Discharge at 0 m	LD 85
50 m downstream	LD 80
100 m downstream	LD 80
200 m downstream	LD 80
400 m downstream	LD 60
600 m downstream	LD 60
800 m downstream	LD 50

NE No effect

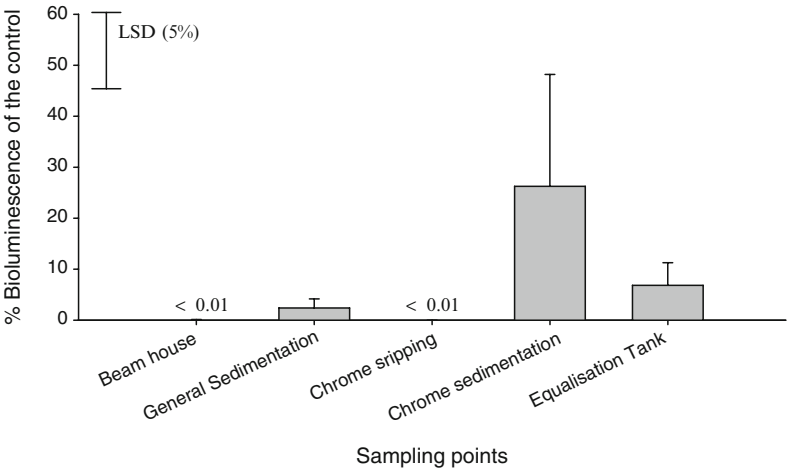

Fig. 5.6 Toxicity (percentage bioluminescence) of different effluent treatment pits

and the source of toxicity at the discharge point was demonstrated. There was no effect observed upstream for *D. magna* upstream (50 m, 100 m, 200 m and 400 m upstream), indicating that the source of toxicity was from the tannery effluent at the discharge point (LD 85).

(b) *Primary decomposers trophic level* (*Escherichia coli* HB101 pUCD607)

Bioluminescence was generally low in all the treatment pits with extreme values observed within the Beam-house, general sedimentation and chrome-stripping tank (Fig. 5.6). During chrome sedimentation, a slight reduction in toxicity is observed (with increased luminescence values) indicating effluent treatment effect at that stage.

The anaerobic lagoons showed stimulation (Except lagoon 3) of the bioluminescence (values >100%) (Fig. 5.7), possibly, due to the presence of organic compounds. Anaerobic lagoons cause sedimentation of suspended solids, partial conversion of COD to methane gas and conversion of sulphide into sulphates and production of biological sludge (Balakrishnan et al. 2002). Sulphur occurs organically in different compounds in highly loaded wastewaters, as organic or inorganic sulphur of various oxidation numbers (Wiemann et al. 1998). Study of luminescence stimulation by chlorophenols found that there is a relationship between higher stimulation of light output and higher EC50 values with *lux*-marked biosensors (Sinclair 1999; Boyd et al. 1998; Sousa et al. 1998). A mechanism involving release of fatty acids provided an increase in substrate for the luminescence reaction and therefore an increase in light output (Heitzer et al. 1998). At the discharge point, values for both *D. magna* (LD85) and percentage bioluminescence (64%) (Fig. 5.8) indicated higher levels of stress. The recovery of the river downstream was observed.

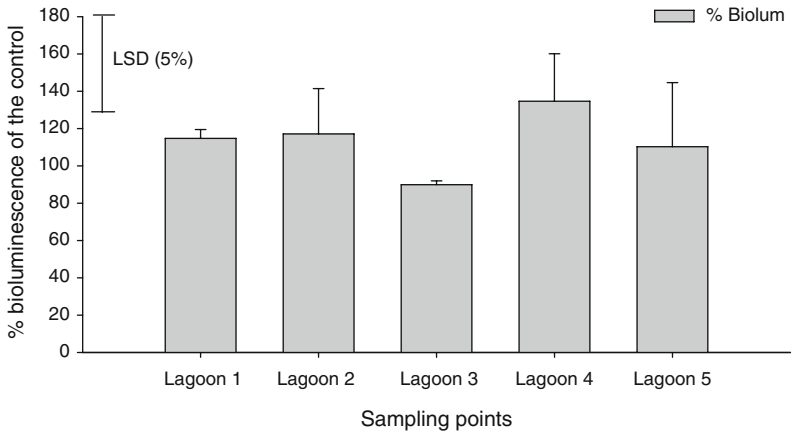

Fig. 5.7 Toxicity (% bioluminescence) of interconnected anaerobic lagoons

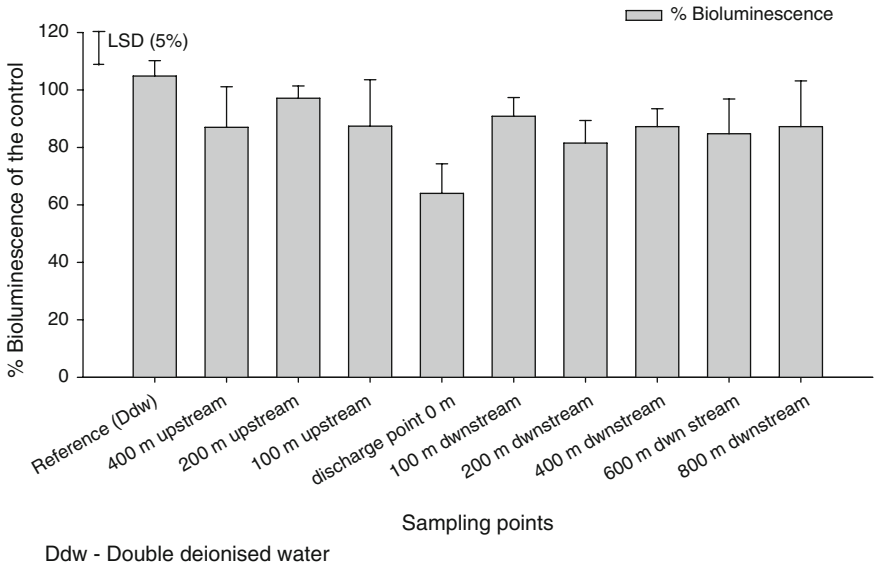

Fig. 5.8 Toxicity (% bioluminescence) of various upstream and downstream sampling points in a river ecosystem

Comparatively, the anaerobic lagoons showed no marked differences when values for both *D. magna* and percentage bioluminescence were compared (Table 5.6, Fig. 5.7). However low LD (LD 50) and higher bioluminescence (>80%) values noted at 800 m demonstrated the recovery of the river downstream.

5.11 Ecological Effects/Toxicity Assessments

Field observation and controlled laboratory work provided added information during this phase. The cause and effect-relationship results showed that, at different point's, toxicity varied and was influenced by different groups of contaminants (heavy metals, suspended solids/colloidal materials, and phenols). The analyses at all the stages from raw effluent to the final sampling point in the river were carried out as earlier indicated. The biosensor (*Escherichia coli* HB101 pUCD607) provided acute toxicity data for the stressor during the effluent treatment phase and flow towards the aquatic receptors showing areas that were high in toxicity (effluent discharge point). Similarly, biomass activity demonstrated the impact of the stressor on the river sediment by showing low values ($\mu gTFg^{-1}$ Sediment 6 h^{-1}.) within the discharge point areas in comparison to areas upstream (200 m, 100 m) and further downstream (600 m, 800 m) (Fig. 5.8). The combination of the exposure-analysis data with the ecological-effects data resulted in a stressor-response profile. This profile represented an attempt to match ecosystem impacts to the levels of stressor concentration under study.

5.11.1 Contamination Levels for Identified Pollutants

The stressor profile was compared to the maximum contaminant levels (MCLs) of identified pollutants (parameters investigated) permitted in water for domestic use (Table 5.7). This stretch is impacted by a tannery effluent discharge and serves as the main water source (domestic and agricultural use) for the local population (estimated at 3,500 in 2004). The levels analysed in the current study exceeded the stipulated levels of all the parameters. This demonstrated the ecological effects and the potential human risk the tannery effluent posed.

Initially maximum tolerable levels of tannery effluent were discussed in comparison to the study site to determine trends worldwide of tannery waste standards. However, when the Kenyan tannery contaminants on final effluent

Table 5.7 Maximum contaminant levels (MCLs) for identified pollutants in drinking water used as the target for final effluent tannery discharge

Parameter	MCLs (mg/L)	Reference
COD	200 (Germany)	UNEP IE/PAC 1994
BOD	100 (UK)	UNEP IE/PAC, 1994
[a]Cl	250 (USA)	EPA/810/K-92-001, 1992
Sulphide	2.0 (India/France)	UNEP IE/PAC 1994
SS	60 (UK)	UNEP IE/PAC 1994
Cr	0.1(USA)	EPA/810/K-92-001, 1992
Grease/oil	20 (Switzerland)	UNEP IE/PAC 1994

[a]Recommended but none enforceable guideline

(Table 5.4) were specifically compared to permitted levels (Table 5.7), all the parameters exhibited exceedances.

5.12 Ecological Risk Characterisation

This phase comprised the comparison between exposure and stressor-response profiles to estimate the probability of effects, using the distribution or magnitude of the stressor in the sampling points. During this study the link to identify cause and effects, correlative relationship and specific chemicals responsible for the observed stressor effects was established (Hewitt et al. 2003) (Table 5.8). The decrease in percentage bioluminescence (64%), dehydrogenase activity (0.0058 $\mu gTFg^{-1}$ sediment 6 h^{-1}) and daphnia count (LD 85) values at the discharge point, and immediately thereafter downstream indicated the impact of the stressor to the riverine ecosystems (Table 5.8, Figs. 5.8 and 5.9). However, no effect was observed upstream, demonstrating the possible source of contamination emanated from the tannery discharge point. Similarly, chemical analysis exhibited an increase of the

Table 5.8 Key questions and evidence for causal determination to a riverine ecosystem at a site in Kenya

Tier	Key questions	Evidence
I	Do similar discharges cause the effect at other sites?	Yes – studies of a tannery (Bulleys) in another town (Thika) showed similar effects (with current site).
II	Is the effluent at particular site causing the changes?	Yes – refer to upstream, discharge point and downstream results (Table 5.10, Figs. 5.7 and 5.8).
III	Is the response pattern characteristic of a response type?	Yes – as areas of low toxicity (upstream) behave different to exposed areas downstream
IV	Where in the production process is the effect originating?	Mostly the beam-house and tan yard (Fig. 5.1) and reported in related studies elsewhere (Thanikaivelan et al. 2003; Cassano et al. 2001)
V	Can we characterize the responsible chemicals by (1) behaviour and response characteristics and (2) exposure characteristics?	Yes, through use of bioassays (different trophic levels), biomass activity (river sediment) and chemical analysis from effluent production to ecosystem receptors (Tables 5.5, 5.6 and 5.8; Figs. 5.5, 5.7 and 5.8).
VI	Can we isolate or eliminate chemical classes that may be responsible?	Yes through use of clean technology and remediative options on tannery effluents treatments (e.g. charcoal, sparging, filtration and pH adjustment)
VII	What specific compounds are responsible for the effects?	Chemical analysis results (Table 5.7)

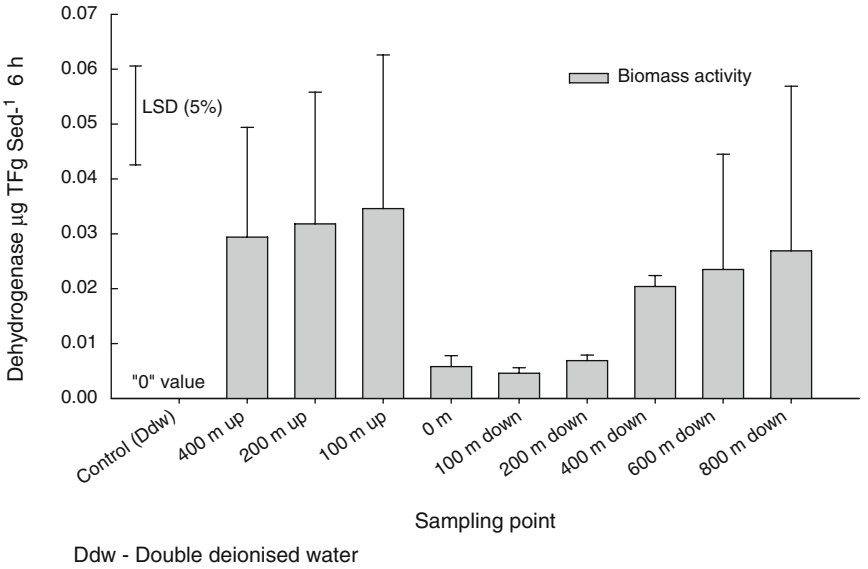

Fig. 5.9 Measurement of dehydrogenase (µgTFg^{-1} Sediment 6 h^{-1}) both upstream and downstream as a measure of biomass activity in the river sediment (n = 9)

stressor at the discharge point when compared to areas upstream and extreme sampling points downstream (Table 5.5).

5.13 Ecological Risk Mitigation

Leather processing generally involves a combination of single and multi-step processes that employ as well as expel several biological, organic and inorganic materials (Germann 1999). During this study, base values were obtained and model variables identified (Table 5.2) after the tannery effluents were characterised (Table 5.4). This was necessitated by the requirement to reveal in-plant methods of reducing the quantity and quality of the wastewater. Such a reduction will reduce the pollution load of the tannery, and improve on water, energy and chemical consumption (UNEP 1994). Hence, appropriate mitigation strategies that could address such complexicity were performed through an importance analysis using a deterministic model. The different scenarios (Table 5.1) are now included as probable mitigation strategies (Table 5.10). The reduction of contaminants and or increase in treatment efficiencies is manipulated to meet target requirements in the effluent composition (Table 5.7).

5.13.1 Mitigation Strategies

The deterministic model can be amended to take into account a single input parameter change or alternately a change in one of the assumptions upon which the model is based. This is important when considering hypothetical risk mitigation strategies (Table 5.9). These strategies can be implemented in the model and the change in the output (i.e. to meet for domestic use the maximum contaminant levels guideline (Table 5.7) for identified tannery effluent pollutants) can be calculated to determine whether it is feasible or not. The following strategies were considered;

Strategy 1: Reducing contaminant levels in the raw effluents (mg L^{-1}). The base result assumed that reduction of the contaminant was directed to the raw effluent (i.e. intervene at the processing stages rather than end of the pipe) with no further treatment of the effluent at the treatment pits and the anaerobic lagoons (Fig. 5.2). Reduction of waste (water conservation and chemical use), reuse and recycling of process liquors were recommended to meet this mitigation strategy.

Strategy 2: Reducing contaminant levels at the treatment pits (chrome stripping, sedimentation and equalisation tank) (%), focuses on placing interventions at the treatment pits without changing the processing techniques, and

Strategy 3: Reducing contaminant levels (%) in the final anaerobic lagoons. Simple technology involving use of physical-chemical treatment allows removal of 95% of suspended solids and around 70% of BOD (Cassano et al. 2001; UNEP 1994). In addition, tertiary treatment (filtration, stripping, Redox processes) facilitate in reduction of waste load in the tannery effluent (Pezzo et al. 1980; Peila 1981; UNEP 1994). However, the practicality of reducing the total effluent contaminants across the board seems unattainable. The assumption is that no clean technologies are employed (therefore no change in raw effluent composition) and in addition no further efficiencies are instituted either in the treatment pits or the final anaerobic lagoons.

Strategy 4: Combination of strategies 1, 2 and 3. By introducing management plans that could reduce contaminant load at all the stages of production, improve efficiencies at both treatment pits and anaerobic lagoons (Table 5.9).

The reductions of contaminants and or increase in treatment efficiencies are manipulated to meet target requirement (Table 5.7) in the effluent composition to avoid stress in the aquatic ecosystem related to base scenario values (Table 5.1).

5.13.2 Mitigation Calculation

The effectiveness of risk mitigation strategies (Table 5.9) was attained after performing a deterministic model for the scenarios detailed in Table 5.1 and variables in Table 5.2. The effectiveness of each individual mitigation strategy used a calculation approach shown in Table 5.10.

Table 5.9 Effectiveness of risk mitigation strategies by increasing efficiency of effluent treatment at various levels

Strategy		Amount of reduction expected in raw effluent (mg L^{-1} or %)	Increased (%) efficiency at treatment pits	Increased efficiency (%) at anaerobic lagoons treatment	References and Recommendations to achieve targets (Table 9).
(1) Reducing contaminant levels in the raw effluents (mg/l)	– COD	639.9	Remain unchanged	Remain unchanged	– Water conservation – Reduction of waste, reuse and recycling of process liquors (Thanikaivelan et al. 2003; Cassano et al. 2001).
	– BOD	968.5			
	– Cl	829.4			
	– Sulphide	58.6			
	– SS	391.7			
	– Cr	10.2			
	– Grease/oil	296.7			
(2) Reducing contaminant levels at treatment pits (%)	– COD	Remain unchanged	52.2	Remain unchanged	Simple technology use of physical-chemical treatment allows removal of 95% of suspended solids and around 70% of BOD (Cassano et al. 2001; UNEP 1994).
	– BOD		4.4		
	– Cl		43.6		
	– Sulphide		94.8		
	– SS		75.8		
	– Cr		95.9		
	– Grease/oil		91.3		
(3) Reducing contaminant levels in the final effluents (%)	– COD	Remain unchanged	Remain unchanged	36.7	Tertiary treatment (filtration, stripping, Redox processes (Pezzo et al. 1980; Peila 1981; UNEP 1994).
	– BOD			98.3	
	– Cl			46.7	
	– Sulphide			91.2	
	– SS			77.7	
	– Cr			70.8	
	– Grease/oil			96.3	

(4) Combination of strateg y 1, 2 and 3 (%)	– COD	79	47	26	Clean technology and rationalisation (recovery and reuse of primary resources) of the operating steps in leather manufacture (Thanikaivelan et al. 2003; Cassano et al. 2001; UNEP 1994) and increased efficiencies at the treatment pits and lagoons.
	– BOD	80	47	25	
	– Cl	65	47	22	
	– Sulphide	80	47	68.5	
	– SS	74	47	22	
	– Cr	90	90	50	
	– Grease/oil	80	60	25	

NB The mitigation strategies in this study were related and modified from the definition provided for the microbial risk mitigation strategies reported by Vosey and Brown 2000

Table 5.10 Mitigation calculations for each strategy, which ensures that the acceptable maximum contaminant level (MCL's) is attained

Description	Mitigation calculations
Strategy 1: reduce stressors at raw effluent Levels (Processing)	$\alpha = RE_v - (P_{MCL}/((1 - S_{efc}) \times (1 - FE_{efc}/100)))$
Strategy 2: Improve efficiency of effluent Treatment pits (After general sedimentation)	$\alpha_2 = 100 \times (1 - (P_{MCL}/(RE_v \times (1 - FE_{efc}/100))))$
Strategy 3: Improve efficiency of Final effluent treatment (After anaerobic lagoons)	$\alpha_3 = 100 \times (1 - (P_{MCL}/(RE_v \times (1 - S_{efc}/100))))$
Strategy 4: Combination of strategy 1,2 and 3	$\alpha + \alpha_2 + \alpha_3 = RE_v \times (1 - {}^aRE_v/100) \times (1 - {}^*S_{efc}/100) \times (1 - {}^aFE_{efc}/100)$

[a]Depicts predicted values to meet target MCL's value

For example using *strategy 1;* $\alpha = RE_v - (P_{MCL}/((1 - S_{efc}) \times (1 - FE_{efc}/100)))$. To attain the MCL's target for COD which is 1988.4 mg L^{-1} (mg L^{-1}) corresponding to alpha = 100%.

where

RE_v = 2,437.8 mg L^{-1}

S_{efc} = 35.22 (%)

FE_{efc} = 14.14 (%)

P_{MCL} = 250 mg L^{-1}

5.13.3 Achieving the Targets Towards Mitigation Strategies

Tangible and potential criterion that are possible to achieving the targets towards mitigating strategies are two folds i.e. operational and waste management factors (Table 5.11). These two factors cover three main domains (i.e. rationalisation during leather processing (raw effluent), increasing efficiencies during effluent treatment (chrome stripping, effluent equalisation/sedimentation) and final effluent treatment (aerobic or anaerobic lagoons)).

Effectiveness of risk mitigation strategies by increasing efficiency of effluent treatment at various levels was crucial to reduce the stressor levels identified (at the study site) (Table 5.4) as demonstrated using the deterministic model. Similarly, the model showed that the area requiring major intervention was within the processing stages (approx. >70% efficiency needed (Table 5.9)) followed by effluent treatment phase (Thanikaivelan et al. 2003; Cassano et al. 2001). However, the model showed that, for sulphide reduction to be achieved there was a need to have optimal efficiencies within the processing stages and the anaerobic lagoons. Studies by Thanikaivelan et al. (2003) recommended the re-use of sodium sulphide in an effort to reduce its impact and reduction of enhanced aerobic conditions in the lagoons (UNEP 1994). Furthermore the same studies agreed

Table 5.11 Twofold factors (operational and wastewater management) towards achieving targets in risk mitigating strategies

Factors	Interventions
(a) Operational	(i). Use of chemicals having low toxicity or less environmental impact.
	(ii). Near 100% utilisation of chemicals.
	(iii). Out-of stock or surplus chemicals should be returned to the supplier or stabilized and repackaged before disposal.
	(iv). Product innovation.
	(v). Integration of processes.
(b) Wastewater management	(i). Continuous pH monitoring during the critical phases of oxidation.
	(ii). Emission monitoring for hydrogen sulphide.
	(iii). Enhanced biological oxidation.
	(iv). Nitrification and denitrification (improvement in sedimentation efficiency).
	(v). Chlorination of final effluent.
	(vi). Normal sewage type human waste and ordinary waste from office activity, building and repair should never be mixed with the tannery effluent (could complicate biogeochemistry of the effluent in rivers if discharged).

Modified from Thanikaivelan et al. (2003), Cassano et al. (2001), UNEP (1994)

with the current results, requiring an initial reduction of waste during processing so as to eventually manage pollution loads at the end of pipe. Moreover, 60% or more of the total pollution in the tannery effluent was associated with Beam-house operation (Thanikaivelan et al. 2003). Any intervention within the beam-house operations will have substantial mitigating factor to the final effluent.

5.14 Conclusion

Ecological risk assessment (ERA) is a relatively new approach to quantifying the risk of significant harm to organisms and their ecosystems, but it is already a requirement of a number of regulatory regimes, such as Part IIA of the Environmental Protection Act 1990 and the Habitats Directive (UK Habitat directives 2004). During this study, the maximum contaminant levels (MCL's) involving pH, COD, BOD, chlorides, sulphides, suspended solids, total chromium and oil/grease were identified with regard to the stressors. In addition, the use of bioassays and biomass activity was complemented with chemical analysis, which quantified the stressors and their effects in the ecosystems. The significant impact of the stressors to the riverine ecosystem was demonstrated when the upstream, discharge point and downstream sampling points were compared. A deterministic model was used as a tool to assess the effectiveness of mitigation risk strategies and predicting the probability of reducing contamination for different scenarios. These parameters

answered the risk questions initially raised and concluded that firstly, the river water was affected by the tannery effluent discharge, and secondly, the river sediment health was affected by the deposition of the contaminants as demonstrated in this study. A possibility in mitigating the problem of pollution is possible (Tables 5.9 and 5.11) although the cost effectiveness of the strategies recommended need to be investigated further. It is necessary in future studies, to integrate life cycle assessment and include other trophic levels (producers and secondary consumers) to provide a wider perspective in the ERA. The policy of *polluter pays* needs to be tied to effective ecological risk management strategies to ensure compliance.

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Chapter 6
General Discussion

Abstract This is a concluding chapter and served the purpose of addressing the three key specific objectives mentioned at the beginning chapters of this book. Indeed the studies reported in this book, indicated that a multifaceted approach was important and thus adopted, to investigate the impact of the tanning industry to various ecosystems. Such approaches included novel techniques entailing biological, chemical and physico-chemical assays. Finally the study highlighted the value of a true, multidisciplinary approach to resolve the environmental impact of anthropogenic activities associated with the tanning industry and to identify strategies for environmental and waste remediation. However, the cost effectiveness of the strategies recommended needs to be investigated further. The policy of the *polluter pays* needs to be tied to effective ecological risk management strategies to ensure compliance and portend the sector's role as an economic driver for development rather than a threat to environmental sustainability.

6.1 Introduction

This book focused on three specific objectives to explore on current trends associated with ecotoxicological diagnosis; characterisation of tannery dust, effluents, sediments and riverine samples, assessment of ecotoxicity, bioremediation potential of primary contaminants, and subsequently the environmental risk assessment through development of a quantitative and qualitative risk assessment model.

The first objective, characterisation of all the samples (tannery dust, effluents, sediments and riverine) was achieved and detailed in this book. The study demonstrated that image analysis coupled with a Monte Carlo simulation could categorise dust particles rapidly and efficiently for the purpose of risk analysis and impact assessment in the tanning industry. In addition, ecotoxicological characterisation using a *lux*-marked *E. coli* HB101 pUCD607 biosensor showed the toxicity response to both solid and liquid phase assays to be very effective in testing contaminated tannery dust samples. While chemical analysis identified individual heavy metal contaminants being below the legal limits (except for chromium),

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the bioassay indicated the presence of biological effects (toxic inhibition and stimulation). This result suggested that workers in the study tannery site are likely to be affected by multi-elemental and synergistic effects identified through the use of the solid phase biosensor based technique. The ecotoxicological approach reported can contribute the key information necessary to review existing legal occupational exposure limits. The second objective, assessment of ecotoxicity and bioremediation potential of primary contaminants was successfully achieved by use of *lux*-marked biosensors to determine toxicity of the tannery effluent.

Furthermore, through toxicity dissection involving sample manipulation coupled to biosensor assay, potential remediative strategies have been identified. In relation to river sediment health, the bioassay was performed in conjunction with a dehydrogenase test to confirm toxicity effects on sediments. Results highlighted the toxicity of the tannery effluent to the sediments at the point of discharge. The third objective, environmental risk assessment through development of quantitative and qualitative risk assessment used a deterministic model as a tool to assess the effectiveness of mitigation risk strategies and predicting the probability of reducing contamination for different scenarios.

These parameters answered the risk questions initially raised and concluded that the river water was affected by the tannery effluent discharge and, eventually, the river sediment health was affected by the deposition of the contaminants. A possibility in mitigating the problem of pollution through the adoption of the recommended strategies is possible through rationalisation during leather processing, increasing efficiencies in effluent treatment (either the aerated or anaerobic lagoons). However all techniques have advantages and disadvantages as was illustrated during the discussion.

Quantitative and qualitative assessments of the risk of dust and chemical hazards in the tanning industry were undertaken in this study as detailed in Chap. 4. The experimental approach in this chapter successfully demonstrated and identified through the use of particle size analysis and Monte Carlo simulation which were the high-risk points in the processing line (tanning Industry). Due to the inadequate policy and legislative regulatory instruments in Kenya where the study site is located, the UK regulatory limits (Statutory Instrument 2002) were considered. All three types of dust were observed (total inhalable, thoracic and respirable dust) in the respective sampling points which included areas such as shaving and trimming, chemical handling, weighing, dyeing, raw stock handling and splitting and fleshing. However, areas which store or handle chemicals more frequently showed the highest occupational risk by being most associated with dust particles of diameter <10 μm (also referred to as PM10). Exceedances of 4.0 mg m^{-3} (respirable dust limit) and 10 mg m^{-3} (inhalable dust limit) were observed particularly in chemical handling area where dust concentration level was 12 mg m^{-3}.

The limitation to the microscopy method was the fact that during the spread of dust on the slides, spillage or uneven spread of the particles could easily contribute to errors in size particle distribution results. This constraint could be addressed by use of a real-time aerosol monitor which carries a filter and sampling pump providing data simultaneously on dust settling and concentration potential. However, low

levels of dust concentration (irrespective of the method of determination) will be considered safe if the toxic nature of dust has been biochemically analysed.

Furthermore in Chap. 4, the aim was to demonstrate that biosensors can be used to assess toxicity of dust in both solid and liquid phase, and to compare and complement the biosensor assay with analytical methods to identify the likely toxic agent(s) in the tannery dust. Solid phase study of the tannery dust using bacterial biosensors required close contact between the bacterial cells and the particulate matter (achieved through centrifugation), while retaining the ability to 'recover' the bacterial cells to measure the luminescence-based toxicity response. Previous studies in the use of solid phase techniques had been applied to the determination of toxicity of contaminated soils and sediments (Benton *et al*. 1995; Dombroski *et al*. 1996; Goicolea *et al*. 1998; Ringwood *et al*. 1997; Rönnpagel *et al*. 1995).

The UK Health and Safety Executive (HSE), and American Conference of Governmental Industrial Hygienist (ACGIH) and National Institute for Occupational Safety and Health (NIOSH), USA stipulate an occupational exposure limit of 0.5 mg Cr m^{-3} (8 h TWA) for total chromium. However, schedule 1 of the controls of substances hazardous to health (COSHH) regulations indicates 0.05 mg m^{-3} to be the limit for Cr^{6+} exposure. The exposure limit for individual contaminants (homogeneity) was not exceeded, but potential impact of heterogeneity on toxicity requires application of the precautionary principle. However, when using luminescent bacteria for solid phase toxicity testing certain problems can be encountered where light output (luminescence) is affected by coloured supernatant and differing numbers in sample supernatant (Brower et al. 1990); loss of bacteria due to adhesion to suspended sediment/dust particles and optical interference of suspended sediment particles (Benton *et al*. 1995).

False positives (due to inducer substrates e.g. salicylate or anthranilate common in the environment) or false negatives (significant quantities of metabolic inhibitors such as naturally occurring compounds including antibiotics or contaminants such as cyanides and chlorinated aromatics) may be encountered. In addition, the extracts after centrifugation may expose to the biosensor organism more contaminant than would actually occur in the environment, producing overestimated toxicity values. Further issues in the development of an effective solid phase toxicity test are highlighted in the discussion therein.

The fourth chapter provided more details about dissection of the toxic nature of effluent and environmental samples (river sediments and water), sample manipulation was used, coupled to a biosensor toxicity assay, to identify possible remediation strategies for future environmental protection. Traditionally, the tanning industry has been associated with odours and water pollution from partially or untreated discharges. Untreated samples (before any manipulations) showed a strong toxic effect at the discharge point in comparison to other upstream and downstream sampling points and all treatment pits. Sparging was used to identify toxicity associated with volatile organic compounds, with this type of toxicity being found for the effluent treatment and anaerobic lagoons. The toxicity of contaminants, removed by treatment with activated charcoal, was identified for all the sampling points (tannery effluent treatment pits, anaerobic lagoons and riverine sampling points)

except for the points upstream. A similar result was observed when membrane (0.22 μm) filtration was used to identify toxicity associated with suspended solids. The greatest reduction in toxicity after filtration was demonstrated for the chromium stripping (0.01% luminescence), chromium sedimentation (0.43% luminescence) and equalisation tank (5.88% luminescence) areas of the tannery. Changes in availability of toxic contaminants due to pH adjustment of samples were also identified with the adjustment to extreme pH (4.0 and 8.0) showing high toxicity for all sampling points (including the discharge point), except for the upstream and downstream riverine samples. The approach used highlighted the complex nature of the toxic pollutants in effluent from the tanning industry, and the dissection of toxicity pointed to possible remediation strategies for effluents at various stages of the tanning industry.

These substances in the tannery effluent are frequently toxic and persistent, and affect both human health and the environment (UNEP 1994). However due to the main aspect in this chapter being toxicity dissection using *E.coli* HB101 pUCD607, environmental relevance within the tannery would have been enhanced if a resident organism could be identified and used in the bioassay. This is because bioavailability (used to describe the biologically active form or fraction of a chemical that is available for uptake or transformation by living organisms) in relation to ecotoxicity assessment of pollutants is species dependent, with the rate of transfer of the compound from solution, uptake and metabolism (Bosma *et al*. 1997).

The characterisation of the tannery effluent in Chap. 4 also led to the investigation of its impact on the river sediment health. In this chapter, the concern was on the fate of the tannery effluent that could cause an impact to the riverine ecosystem. To evaluate the river health status therefore, river sediment was analysed. Subsequently, tannery effluent was found to cause a serious environmental impact due to the effluent's high oxygen demand, colour and toxic chemical constituents. Furthermore, physico-chemical analysis was used to determine pH, Biological Oxygen Demand (BOD_5), Dissolved Oxygen (DO) values, heavy metals (Cr, Fe, Pb, Cd, Ni, Cu and Zn), total phenols, and turbidity (OD_{550}). Bioluminescence results in this chapter showed lower values at the discharge points, indicating toxicity to the biosensor.

Similarly, the INT-dehydrogenase activity test indicated the impact on river health by demonstrating varied microbial activity based on toxicity levels in the river, with the lowest DHA values observed at the discharge point. This result showed the effect of the tannery effluent on microbial activity in the sediments of the river. The BOD and DO results indicated a similar pattern to bioluminescence and INT-dehydrogenase activity. However other site-based methods for determining BOD using electronic based sensors (e.g. TOC UV/Heated persulphate or High temperature combustion (STAR™) would have reduced possible errors due to delays between sampling and analysis. While other studies in monitoring general river pollution using multiband truth radiometer (which uses spectral reflectance data) have been used in India (Tripathi and Smith 1996), the methods are limited in providing the bioavailability status of the pollutants.

Indeed, studies investigating the riverine ecosystem (which represents a very complex medium) require advanced techniques and tools to address heterogeneous

nature of the pollutants and their interaction through chemical-chemical, toxicokinetic and toxicodynamic mechanisms (Steevens and Henson 2001) to cause the toxicity. However, the importance of the results obtained in this study demonstrated that tannery effluents contained a number of toxic pollutants. Moreover when discharged into the river systems, they caused severe environmental impact as such the importance of integrating river sediment health to water quality assessment is necessary. Clean water as a resource has been the main agenda for all world public organisation (e.g. WHO, UNICEF etc.) and governments. To capture the impact of the tanning industry in a much more holistic manner, experiences learnt in earlier chapters were used. This led also to the development of an ecological risk assessment (ERA) to provide possible mitigating factors in Chap. 5.

The main aim of Chap. 5 was to determine the ecological risk caused by the tanning industry. Ecological risk assessment utilised various techniques to evaluate the probability that adverse ecological effects will occur as a result of exposure to one or more stressors. Ecological risk assessment determines and documents actual or potential effects of contaminants on ecological receptors and habitats as a basis for evaluating remedial alternatives (Gerba 1996). The main aim of this chapter integrated principal issues such as identifying stressors/hazards, application of biological (Bioassays) and chemical assays (to determine heavy metals, COD, BOD, and Total Phenols). To quantify the stressors and their effects on the ecosystem, it was necessary to determine the probability that the identified stressor (toxic chemicals from the tanning industry) caused a significant impact on the environment (analogous to the statement of purpose of the risk assessment), and determine the effectiveness of risk mitigation strategies to reduce the risk of the ecological contamination (e.g. by reducing contaminants in the raw effluent, sediments and final effluent as well as a combination of these strategies).

Chapter 5 then addressed questions on water quality and contaminant deposition on river sediment. The exposure pathway was further evaluated through direct contact with contaminated sediments and water using *Daphnia magna* (primary consumer) and *Escherichia coli* HB101 pUCD607 (primary decomposers) to demonstrate the effect on community structure in the riverine ecosystem. The cause and effect relationship results suggested that, at different points, toxicity varied and was influenced by different groups of contaminants. The stressor profile was subsequently compared to the maximum contaminant levels (MCL's) of identified pollutants permitted in water for domestic use. The levels analysed in the current study exceeded the stipulated levels. However, the main challenge in using ecotoxicological assessment as a technique is that it is still in early stages of development. Various techniques have therefore been recommended to provide all inclusive and reliable ecotoxicological assessment. To be able to meet this aspiration, the ecotoxicological risk assessment in this study adopted the eco-epidemiological criteria (also referred to as forensic toxicology) reported by Woodman and Cowling, 1987, Suter, 1993 and further developed by US EPA (1991, 1993a, b, 1997, and 1998). The main advantage in this study, while adapting eco-epidemiological criteria, was the actual data obtained during processing and effluent treatment stages. The disadvantage on the other hand was the incomplete trophic levels that should have also

incorporated secondary and tertiary consumers (e.g. water spiders, fish etc.) and producers (e.g. Plants) (Stuhlfauth 1995).

In conclusion, this book, in addressing the three specific objectives mentioned at the beginning of Chap. 6 has taken a multifaceted approach to investigate the impact of the tanning industry. This was essential because different experimental approaches detailed in each chapter necessitated different techniques (i.e. biological, chemical and physico-chemical). Finally the study has highlighted the value of a true, multidisciplinary approach to resolve the environmental impact of anthropogenic activities associated with the tanning industry and to identify strategies for environmental and waste remediation. The disciplines brought to bear comprised analytical chemistry, environmental biology/toxicology (biosensors and bioindicators), biogeochemistry, applied physics, mathematical modelling, statistics and socioeconomics. It is proposed that it is only through this kind of multidisciplinary approach that complex environmental problems such as those associated with the tanning industry can be understood and resolved in a sustainable manner. However, the cost effectiveness of the strategies recommended needs to be investigated further. The policy of the *polluter pays* needs to be tied to effective ecological risk management strategies to ensure compliance and the economic driver for development of appropriate technologies.

6.2 Suggestion for Future Work

During the ecotoxicological screening of dust sampled throughout a tannery, a luminescence (*lux)*-based bacterial biosensor for solid and liquid assay was used offering a new technique to explore the bioavailability of pollutants in dust. However, the full ecotoxicological assessment of contaminants in dust requires further investigation incorporating eukaryotic receptors to extend the findings in the present studies, which mainly focused on prokaryotic receptors (*E. coli* HB101 pUCD607). This would strengthen the ecotoxicological approach reported in this study (Chap. 4), progressing towards predictions of human health issues, and contribute key information necessary to understand the mode of toxicity and review the existing legal occupational exposure limits. Other Studies by Bondarenko et al. 2008 using bacteria sensor was used to assess soils, where water-extracted bioavailable (in particle free soil water extracts) and total bioavailable (in soil-water suspensions) fractions of metal were analysed (e.g. Hg, Cd and Zn). However results from such studies, indicated that bioavailability of different heavy metals demonstrated diverse response to different bacterial groups. This essentially suggests the need for better characterization techniques of the bacterial sensors to optimise their performance.

Biosensor based toxicity dissection of tannery and associated environmental samples highlighted the complexicity of toxic pollutants in effluent from the tanning industry and identified possible remediation strategies. However, the future use of indigenous organisms as biosensors should enable exploration of adaptability and

acquired resistance over time to pollutants in such areas. In addition, during the field survey related to this study, a certain type of plant (*Ricinus communis)* was observed to grow in a tannery effluent disposal pit (Fig. 6.1 a, b). This suggested a potential plant-based, decontamination strategy (i.e. phytoremediation). This plant, also commonly known as Castor oil, belongs to the Family Euphorbiaceae and normally grows to an average of 10–15 feet. The plant is very variable in habit and appearance with the known varieties being numerous in Africa. The plant is characterised by being drought tolerant, highly toxic if any part of the plant is ingested, grows in soils

Fig. 6.1 (**a**, **b**) Thriving Castor oil plant (*Ricinus communis)* in a Kenyan tannery effluent disposal pit (Pictures by Mwinyihija, M)

which are mildly acidic (pH 6.1 to 6.5) to neutral (pH 6.6 to 7.5) and it has been observed that moles, voles and other pests are driven away by the plant. It is proposed that a range of studies be carried out to assess the phytoremediative potential of *R. communis* in relation to the tannery effluent. The studies should include both laboratory investigations to explore phtoremediation mechanisms as well as field studies to quantify phytoremediative performance under conditions encountered in the vicinity of impacted site.

During the study, which assessed the impact of tanning industry effluent on river health (Chap. 4), biological sensors complemented the power of analytical methods to identify the pollutants. However, future integration of higher trophic levels will further characterise these biosensor tests and develop more efficient monitoring techniques for aquatic systems.

Finally, the ecological risk assessment of the tanning industry in Kenya (Chap. 5) entailed understanding the categories of hazardous waste. It also involved identification, exposure assessment of these components as well as assessment of ecological effects and risk characterisation involving primary consumers (*Daphnia magna*) and primary decomposers (*Escherichia coli* HB101 pUCD607), complementing chemical analysis. However, to fully identify the constraints of the toxic nature of the tannery effluent to the ecosystem, wider representation of the trophic levels to include producers (e.g. Algae) and secondary consumers (e.g. Fish) to evaluate responses to stressors is necessary. Further, life cycle assessment (LCA) as a technique for evaluating the environmental impacts associated with leather processing, is essential to understand the *toxico-dynamics* of the pollutants in the environment. Life cycle assessment will further provide insight into the overall environmental load of the contaminant and facilitate improvement in the sustainability of the product and the underpinning processing techniques in the tanning industry.

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Index