MICROBIAL QUALITY MANAGEMENT AND SHELF LIFE DETERMINATION OF WATER-BASED PAINTS

BY

OBIDI, OLAYIDE FOLASHADE

(B.Sc., LASU; M.Sc. IBADAN)

A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, UNIVERSITY OF LAGOS, LAGOS, NIGERIA, IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF DOCTOR OF PHILOSOPHY (Ph. D.) IN MICROBIOLOGY

MAY, 2008

SCHOOL OF POSTGRADUATE STUDIES
UNIVERSITY OF LAGOS

CERTIFICATION

This is to certify that the Thesis:

"MICROBIAL QUALITY MANAGEMENT AND SHELF LIFE DETERMINATION OF WATER- BASED PAINTS"

Submitted to the
School of Postgraduate Studies
University of Lagos

For the award of the degree of
DOCTOR OF PHILOSOPHY (Ph. D)
is a record of original research carried out

By
OBIDI, OLAYIDE FOLASHADE
in the Department of Botany and Microbiology

OBIDI OLAYIDE, F		08/05/08
AUTHOR'S NAME	SIGNATURE	DATE
Prof S.C.U. Nwachukwu		08/05/08
1ST SUPERVISOR'S NAME	SIGNATURE	DATE
Dr. O. O. Aboaba	Aboaba	08/05/08
2ND SUPERVISOR'S NAME	SIGNATURE	DATE
DR. E. O. IGBOSUAH	Igbosuah	08/05/08
1ST INTERNAL EXAMINER	SIGNATURE	DATE
Dr A. A. Adenuga	Adenuga	08/05/08
2ND INTERNAL EXAMINER	SIGNATURE	DATE
Dr T. Akpan		08/05/08
EXTERNAL EXAMINER	SIGNATURE	DATE
TOLU AJAYI		08/05/08
SPGS REPRESENTATIVE	SIGNATURE	DATE

DEDICATION

This thesis is dedicated to the Lord God Almighty, who humbleth himself to behold the things that are in heaven and on earth, from whom all blessings flow.

ACKNOWLEDGEMENTS

I am extremely grateful to my supervisors, Prof. S. C. U. Nwachukwu and Dr. (Mrs.) O. O. Aboaba for their commitment to the work, constructive criticisms, useful discussions, beneficial suggestions and advice.

I wish to thank Dr. (Mrs.) S. I. Smith, the Head of Molecular Biology and Biotechnology Division of the National Institute of Medical Research, (NIMR) Yaba, Lagos for the permission to use her laboratory, equipment, materials and reagents for some aspects of the work. I am particularly grateful to Mr. E. Omonigbehin, Mr. M. Bamidele, Mr. K. Akinside and Mrs. M. Fowora.

I would like to express my appreciation to my lecturers in persons of Prof. O. T. Ogundipe, Dr. M. A. Taiwo, Dr. E. O. Igbosuah, Dr. I. A. Adeleye, Dr. M. O. Ilori, Dr. L. O. Egwari, Dr. A. A. Adekunle, Dr. J. Olaleru, Dr. E. Esan and Dr. Adeloye for their professional assistance and encouragement.

I am grateful to Mr. B. Bamiro of the Bacteriology Research Unit and Mr. Uzo of the Biochemistry Department of the Lagos University Teaching Hospital (LUTH), Lagos for their technical assistance and the opportunity granted me to use their laboratory. I also appreciate the assistance rendered by the technical staff of Botany and Microbiology Department of this University especially Mrs. Yinka Osagie, Mr. L. Aderibigbe and Mrs. R. Dosumu. They were fantastic in their readiness to assist during the course of this work.

I acknowledge with gratitude the assistance received from the following: Prof. & Mrs. S. Ojo, for their generous help during the early stages of the project. Mr. S. Akinfenwa, for his valuable suggestions and the use of his computer.

I wish to thank also my colleagues, Miss. A. Omotayo, Dr. S. Adebusoye, Mr. G. Oyetibo, Mr. F. Obayori and Mr. S. Ajani. Their constructive criticisms and advice are highly appreciated.

Space will fail me to express the invaluable contributions of Mr. Christian Davies of the National Bureau of Statistics, Mr. & Mrs. Nojim Tairu, Mrs. Taiwo Adubi, Mr. Gbenga Adefarakan, Mr. Ike Nwando, Mr. Franklin Alexander, Mrs. Ruth Adongo, Mr. Fola Ayantunde, Mr. Okpara, Mrs. J. Onyeka, Mr. Adewara and Mr. Mejidda for their readiness to help even at short notice.

The staff of CAPL, Ikeja, especially Miss Bola Fadeiye, Miss Tope Abdulkareem, Miss Kemi Oroniran, and Mr. Jide Serrano were simply wonderful with regards to the supply of reagents, consumables and glassware. I am also highly indebted to Nouvelle Communications Limited for their support, tolerance, cooperation, technical assistance and privilege to use their office facilities at any given time.

My parents, Mr. and Mrs. David. O. Koleowo and my siblings Mrs. Fashina, Mrs. Popoola and Mrs. Adeniji have been marvelous in their care, support and understanding. I owe them a great debt of gratitude.

For their unflinching prayer support and spiritual counsel, I am highly indebted to Pastor W. F. Kumuyi and wife, Mrs. B. Kumuyi (who I fondly call mummy). Also deserving of mention in this regard are Mr. & Mrs. B. Ikhulegbu, Mr. & Mrs. P. Akeni, Mr. & Mrs. L. Nnadozie, Mr. & Mrs. E. Ukwa, Mr. Y. Olusanya and Mr. John Okafor.

Finally, I wish to thank most affectionately, my husband, Mr. Steve Obidi whose total support, commitment, financial and technical assistance has been a major contribution to the success of this work. Indeed, men like him are very rare. Not the least is the patience and understanding of our children, Esther, Eunice and Elizabeth-Elaine.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDICES

ABSTRACT

The microbial quality of materials and final products of Chemical and Allied Products Limited (CAPL), a reputable paint industry in Lagos area were analyzed. The bacterial population in the fresh paint samples monitored at two weeks intervals for a period of ten months ranged from $1.6 \times 10^1 - 4.7 \times 10^5$ cfu/ml while the fungal population ranged from $1.0 \times 10^1 - 5.5 \times 10^3$ cfu/ml. The isolated bacterial strains were identified as *Bacillus polymyxa*, *B. brevis*, *B. laterosporus*, *Proteus mirabilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Lactobacillus gasseri* and *L. brevis* based on standard cultural and biochemical techniques and isolates' phenotypic profiles using the Analytical Profile Index (API) ID 32 E test systems. The fungal isolates were *Aspergillus niger*, *A. flavus* and *Penicillium citrinum*. The physico-chemical parameters such as optical density (OD), specific gravity (SG), transmittance (TR), pH and viscosity (VIS) of freshly made paint samples were monitored every two weeks over the study period to evaluate the biodegradative activities of the indigenous microorganisms. The Optical density increased from 1.49 – 3.91, while TR, pH, SG and VIS decreased from 6.9 – 2.3, 8.5 – 5.6, 2.8658 – 1.0853, and 11.7 – 10.8 cst respectively over the period. The microbial population count and physico-chemical parameters of the spoilt paint samples which served as the control samples were also determined. The aesthetic qualities of the paint samples were observed to deteriorate with time as indicated by the measured parameters. *Pseudomonas aeruginosa*, which was repeatedly isolated in spoilt paints was observed to be the highest cellulose-utilizing organism. *Ps. aeruginosa* also harboured two plasmids with molecular weights ranging from 0.032 – 0.112 kb while the other isolated organisms had none. The cured strains of *Ps. aeruginosa* lost the existing plasmids and the initial resistance to amikacin, gentamycin and tobramycin. Therefore, the genes for cellulose utilization and paint degradation in bacterial strains from this study probably reside on the plasmid DNA. These results were further corroborated by the comparative evaluation of the biodegradative potential of the wild and the cured strains of *Ps. aeruginosa* on the physico-chemical parameters of fresh paint samples. The shelf life of water-based paints was determined to be 2 years, based on the predictive models developed in the study.

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

The monitoring, maintenance and control of paint quality are the main goals of the paint industry. The consequences of microbial contamination and related deterioration of paints have led to a drastic reduction in paint quality and its shelf life. In the liquid state, paints may be colonized by a range of Gram-negative and Gram-positive bacteria, particularly the spore formers such as *Bacillus* spp. The pH of most paints is in the range of 8 – 9.5 and this favours Pseudomonads which is the most commonly encountered group, comprising at least 75% of isolates from spoilt emulsion paints (Dey *et al.*, 2004). Spoilt emulsion paints have become a source of concern to marketers and consumers and now constitute a major problem bewildering the paint industry.

The microbial quality management of water-based paints can be achieved to a large extent by the incorporation of bio-preservatives, such as biocides into paint formulation and the practice of good quality control system. Biocides have an essential role in the control of contaminations (Tortorano *et al.*, 2004) which result in changes in viscosity, pH, colour and loss of surface adhesion properties in paints. Any one of these symptoms will render the paints unsaleable. The problem which has received most attention over the years has been loss in viscosity (Dey *et al.*, 2004). Managing the viscosity of an emulsion paint is therefore, very important as this affects its application properties. The most common thickeners added to paints to achieve high quality with very good viscosity in the paint industry are cellulose ethers, such as hyroxyethyl cellulose, and these are subject to enzymic hydrolysis in the presence of both bacterial and fungal cellulases (Saad, 1992). In addition, it has been reported that small amounts of cellulases (0.1 ppm) can cause significant (2%) decrease in paint viscosity (Dey *et al.*, 2004). Thus, the regular and continual monitoring of microbial population level and its resulting implication on viscosity and other physico-chemical properties of paints cannot be over emphasized.

Unlike antibiotics, which are generally very selective for the type of microorganism inhibited, with definite mode of actions, biocides are multi-targeted antimicrobial agents which generally "attack" most types of microorganisms (Russell, 2003). Bacteria whether Gram-positive or Gram-negative, respond differently to biocides and this disparity accounts for the variations in the efficacy of each biocide on different organisms. The underlying reasons for these varied responses are poorly understood, but the chemical composition of outer cellular layer is likely to be a factor of prime importance. Other possible contributory factors may be differences in stress responses, the presence of efflux pumps and cells

(Russell, 2003) occurring within paint biofilms. The effect of a biocide is highly concentration-dependent (Russell and Mc Donnelli, 2000). Consequently, delineating the reasons for activity against a range of organisms becomes more difficult with biocidal agents. Few data are available about the uptake of biocides by mycobacteria, fungi or other types of microorganisms (Russell, 1996; Russell and Furr, 1996). Since bacterial spores present different types of cell surfaces to biocides and microbial susceptibilities to biocides differ greatly, it could be a worthwhile investigation to evaluate and compare the efficacy of different biocides used in paint production on paint microflora.

Beginning in the mid-1920s, attention has mainly focused on improving the durability and hence, the shelf life of paints by the use of white lead which posed a significant health hazard to young children (Needleman, 1989). The lead paint industry had considerable evidence in the 1920s and 1930s that their product posed a significant health hazard to children. The question arises: when did these companies stop selling lead paint for residential use? The lead paint industry has claimed that interior lead paints were discontinued altogether by 1940 (Rabin, 1989). If indeed the paint manufacturers had ceased production of interior lead paints by 1940, one would also expect them to have taken little interest in research to improve the shelf life of paints. A further indication that the use of lead paint for improving the shelf life for interiors was still substantial in 1940 comes from its detection in high concentrations (2 mg/cm^2 or more) in about one third of the houses in Pittsburgh painted with such paints. A similar study concerning application of lead paints to houses in Washington D.C., New York, Baltimore, New Haven, Chicago, Paris, Europe, Australia, Asia and Africa yielded very similar results (Corn, 1975; Rabin, 1989; Fee, 1990; Spurgeon, 2006; Mathee *et al.*, 2007).

Incredibly, as late as 1971, there is evidence that significant amounts of lead paints were being sold for residential interior uses (Rabin, 1989). However, not until after a decade had passed did it become industry practice to place labels on paint cans, warning customers of the dangers of lead but without indication of the shelf life (Rabin, 1989). Thus, the use of lead did not solve the problem of the paint industry as the shelf life was only improved upon but remained unknown. In addition, child lead poisoning is still a public health issue of concern. Therefore, current research should focus on the estimation and indication of shelf life of paints.

A common practice of manufacturers in industries is to utilize various short cuts, e.g. bracket tables (Porterfield and Capone, 1984) and the Q-Rule (Connors *et al.*, 1973) to estimate and project product shelf life. These techniques share the advantage that decisions may be made by analyzing only a few stressed samples. However, they also have some limitations since they are based on assumptions about the product components and are valid only in so far as these assumptions are accurate. Any method adopted for determination of the validity of paint stability and shelf life should be based on analytical precision, the use of appropriate controls within the experimental design, the assumptions embodied in a mathematical model, and the measured characteristics of product components.

Over the past few decades, other methods such as microbial stability techniques (Anderson and Scott, 1991) and sensory evaluation (Trees *et al.*, 2000) have been used to determine the shelf life of other products although, they have their limitations. Microbial stability testing assessment techniques require that the test period should be long enough to allow significant product degradation under recommended storage conditions. Secondly, the testing protocol does not permit one to distinguish percent degradation from inter-assay variation. Although, data collected at an appropriate frequency is such that a trend analysis may discern instability from day-to-day imprecision. The reliability of data interpretation needs to be improved by including in each assay, a single lot of reference materials with established stability characteristics. This may help to minimize the impact of systemic drift and inter-assay imprecision.

Sensory techniques involve the use of trained laboratory panel of judges to evaluate the appearance of degradation typical of paints by use of a 5-point structured category scale. Each evaluation contains a marked reference sample that is obtained from a fresh production batch. A score of 2 on the category scale indicates 'just detectable' deterioration in sensory qualities compared to that of the marked reference which is a fresh product. A score of 3 indicates 'clearly detectable but not acceptable' deterioration, and a score of 5 indicates that the judge considers the sample unacceptable. Samples are usually evaluated twice, and means of scores are calculated over replicates for each sample (Trees *et al.*, 2000). This method is subjective and less accurate.

An alternative to direct product testing is predictive microbiology, the modeling of microbial populations, which has become an active area of research. Unfortunately, there has been no

record to date where predictive modeling has been applied to determine the shelf life of paints. Predictive models are mathematical equations which can use the information from a large database to predict inactivation or growth of microorganisms under defined conditions (Trees *et al.*, 2000). Predictive models offer considerable prospects for use in shelf life determination. Predictive microbiology has proven its value for a useful model-based description of microbial growth ever since its development (McDonald and Sun, 1999; McMkeen and Ross, 2002). Data used in building a model are usually acquired from laboratory experiments. However, the predictions agree more or less successfully with observations of the products (Walls and Scott, 1996) and validation of the model proves to be necessary in most cases. Indeed, models should be validated for prediction in the product in question, to allow for risk assessment (Pinon, 2004). Koutsoumanis (2001) reported the comparison between the observed and predicted growth of Pseudomonads on gilt-head seabream stored under dynamic temperature conditions based on the bias and accuracy factors. The time to each observed Pseudomonad count was compared to the time predicted to reach the same cell density as that observed. Predictive modeling provides an indication of the average deviation between the model predictions and observed results (Ross, 1996) and their closeness to a value of 1 or 100% is an effective and practical measure of predictive model validity.

The microbial ecology of paints is complex. Developing reliable risk assessments involving microbial growth in paints will require the skills of both microbial ecology and mathematical modeling. Simplifying assumptions will need to be made, but because of the potential for apparently small errors in growth rate to translate into very large errors in the estimate of risk, the validity of those assumptions should be carefully assessed (Russell, 2003). Thus, predictive microbiology can provide the paint industry with sufficient information about the accuracy of the model, in predicting microbial growth and shelf life of paints. It is against this background that this research was undertaken.

1.1. Paints: History and Background

The first uses of paint were entirely decorative. Thus, paint without a binder, consisting of iron oxide, was used for cave paintings about the 15th millennium BC. In Asia, several pigments made from ores, prepared mixtures, and organic compounds were known about

6000 BC. Indigo, a pigment extracted from the indigo plant, was known to the ancient Egyptians, Greeks and Romans. Gum arabic, egg white, gelatin and beeswax were the first vehicles used for these pigments. Lacquers were used to paint buildings in China about the 2nd century BC. In Europe, prospective painting began about the 12th Century AD. Linseed oil, although known as a paint vehicle by the Romans, was used by artists only from the 15th century. Water-thinned latex paint was introduced in 1949. White lead, a white pigment, became widely used in the 17th century, and paint consisting of prepared mixtures of pigments and vehicles first became commercially available in the 19th century (Barker, 1999).

1.1.1 Paints: A General Description

Paints are uniformly dispersed mixtures having a viscosity ranging from a thin liquid to a semi-solid paste, consisting of a pigment (the substance that provides colour) suspended in a liquid vehicle such as oil or water. They solidify when exposed to air (Briggs, 1980). Paint is the general term for a family of products used to protect, add colour to and beautify an object or surface by covering it with pigmented coating. Paint is applied with a brush, a roller, or a spray gun, in a thin coat to various surfaces such as wood, metal or stone. Although, its primary purpose is to protect the surface to which it is applied; from corrosion, oxidation, environmental weathering or other types of deterioration, paint also provides decorative finish (Adeleye and Adeleye, 1999). Ingredients of paint manufacture include: pigments, additives, binders and solvent (Briggs, 1980).

Paints come in different colours. Colour is a physical phenomenon of light or vision associated with the various wavelengths in the visible portion of the electromagnetic spectrum (Drasdo, 1999). Any colour sensation can be produced for paints by mixing varying quantities of red, blue and green. These colours, therefore, are known as the additive primary colours. If light of these primary colours is added together in roughly equal intensities, the sensation of white light is produced. Pairs of pure spectral colours called complementary colours also exist; if mixed additively, these will produce the same sensation as white light. Among these pairs are certain yellows and blues, and certain reds and blue-greens (Drasdo, 1999; Para *et al.*, 2007). The pigments that give colour to paints absorb certain wavelengths

of white light, and reflect or transmit others that produce the colour (Kopchick and Bormarito, 2006).

1.1.2 Chemical Composition of Paints

A modern paint formulation consists of several different categories of chemical compounds. The vehicle forms the adherent, skin-like coating; the pigment is dispersed in the vehicle and gives the final film its colour and hiding power, and the solvent, or thinner, evaporates shortly after the coating has been laid. The vehicle can be an unsaturated, or drying oil, which is an ester formed from the reaction of a long-chain carboxylic acid, such as linoleic acid, with a viscous alcohol, such as glycerine; or it can be a polymer. A filler, containing powdered components such as kaolin or barium sulphate, enhances the strength of the dried film of paint. If linseed oil is exposed to the oxygen in the air, the unsaturated ends on the hydrocarbon chain are attacked, and an oxide, or ether, is formed, thereby cross-linking one molecule to another, to yield a tough, insoluble macromolecule with high molecular weight. The drying oil is an important component of paint, it is a monomer when it is in the can and becomes a polymer after being applied to an exposed surface. If the vehicle is a synthetic polymer, it is dispersed in a suitable solvent, so that as the solvent evaporates, the individual macromolecules come into contact and become enmeshed. The solidification is improved by the presence in the solvent of a polymerization catalyst, called a drier. The types of synthetic polymer most widely employed as paint vehicles are: alkyd resins, which are polyesters of a polyhydric alcohol, such as glycerol, with a polybasic acid, such as phthalic acid, $C_6H_4(COOH)_2$; nitrocellulose, in which cellulose is depolymerized, the small molecules are nitrated, and the molecules are repolymerized; phenolic resins and linseed oil (Loor *et al.*, 2004). The concentrations of functional components in different kinds of water-based paints are shown in Table 1.1.

Table 1:1 **Presence of functional components in the most commonly occurring water-based (acrylic dispersion) and solvent-based (alkyd paint) construction paints.**

Functional Component	Acrylic dispersion	Alkyd paint
Binder	+ (polyacrylate)	+ (alkyd resin)
Pigment	+	+
Filler	+	+
Organic solvent	+(0-15%)	+(about 50%)
Ammonia	+	-
Amine	+	-
Preservative	+	-
Surfactant	+	-
Corrosion inhibitor	+	-
Thickener	+	+
Drier	-	+
Anti-skinning agent	-	+
UV absorber	+	-

(+) Present, (-) not present

Source: Faassen and Borm (1991).

1.1.2.1 Pigments

A paint pigment is a fine powder that either scatters light strongly, to yield a white effect, or absorbs certain wavelengths of light, producing a coloured effect. Typical white pigments are inorganic oxides such as titanium dioxide (TiO_2), (Hext *et al.*, 2005); antimony oxide (Sb_2O_3), and zinc oxide (ZnO) (Rabin, 1989). Other white, insoluble, inorganic compounds are also frequently used, including: zinc sulphide, (ZnS); white lead (the hydroxycarbonate, hydroxysulphate, hydroxyphosphite, or hydroxysilicate of lead); and barium sulphate, ($BaSO_4$) (Alejandre and Marquez, 2006).

The following inorganic oxides are typical coloured pigments: iron oxide, Fe_2O_3 (yellow, red or brown colours); chromium oxide, Cr_2O_3 (green), lead oxide, Pb_3O_4 (red). The chromates of lead, zinc, strontium, and nickel produce various shades of yellow and orange. A variety of organic solids are used for other colours. Paint pigments are insoluble powders usually used to provide colour and to make paint opaque, thus, protecting the substrate from the harmful effects of ultraviolet light while also increasing a paint's hiding power. They usually contain bacteria and fungi, which in an aqueous environment, may germinate and grow, leading to spoilage of the paint. Some pigments are toxic such as those used in lead paints. Paint manufacturers have replaced lead with titanium dioxide, a less toxic substitute, which can even be used to colour food (Rabin, 1989). True pigments exhibit opacity or hiding power in varying degrees, whereas, extenders are used in certain types of paints notably undercoats, primers and some low gloss finishes, to modify or control the physical properties of the paints. They make no contribution to colour unless they are very impure. Titanium white (titanium dioxide) which was first used in paints in the 19th century is the most important pigment used in paints, as it is by far the best white prime pigment available for exterior weathering performance (Ramsbothan, 2000). The titanium white used in most paints today is often coated with silicon or aluminium oxides for better durability. Some newer paints called prism paints can produce effects where the colour changes depending on the angle (orientation) at which it is viewed. These effects are produced by having pigment molecules that are long and thin and are meant to dry in a specific orientation, with different ends of the molecule being different colours (Camgna and Colinart, 2003).

1.1.2.2 Solvents

This is the liquid that makes the consistency suitable for applying the paint. The solvent evaporates and leaves the dry paint film on the surface. It plays no part in film formation and acts solely as a means of conveying the pigment/binder mixture to the surface as a thin uniform film. Solvents used are either organic compounds or water. Unfortunately, most water sources of paint industries are not treated and they also increase the level of contamination in the finished products. Classes of materials used as solvents include aliphatic hydrocarbons (white spirit), aromatic hydrocarbons (toluene, xylene and trimethyl benzene), alcohols, esters, ketones and others. Water is the solvent in water-based and emulsion paints. The solvent or thinner for drying oil paints is generally turpentine (Calnan, 1978), a mixture of cyclic hydrocarbons containing ten carbon atoms or a mixture of suitably volatile hydrocarbons derived from petroleum distillates. The solvent for most synthetic vehicles is an alcohol, a ketone or an ester (Brouwer *et al.*, 2005).

1.1.2.3 Binders

These are polymers or resins, which provide the basis of the continuous paint film by adhering the pigment particles together (Gillatt, 1992). In general, the binder or resin binds all the other paint components, mainly pigments, together into a cohesive, continuous film and provides the adhesive power for a paint to stick to a surface. The majority of binders are organic materials such as resins containing fatty acids from natural oils such as alkyds, epoxy esters, urethane oils, treated natural products (cellulose nitrate, chlorinated rubber) and completely synthetic polymers. A few inorganic binders are used in paint production, notably pre-hydrolysed ethyl silicates, quarternary ammonium silicates and alkali silicates.

1.1.2.4 Additives

These are minor components that improve the paint in different ways, e.g. dispersing agents for pigments, thickeners, defoamers, in-can biocides, in-film biocides etc. Additives in paints are used for several purposes but chiefly to facilitate dispersion of pigments and to change consistency characteristics. A typical example of a paint additive is lecithin and is widely used as a dispersing agent in paint formulation (Da Silva, 1963). It is a natural product

obtained chiefly from the processing of soybean oil. This group of chemicals comprises of a vast multitude of compounds, which are employed by paint manufacturers at low levels in coatings to perform specific functions or to counter adverse side-effects of other paint components. For example turpentine-thinned paints contain drying agents, which speed up the drying process and also contain anti-skinning agents to prevent the paints forming a tough skin-like covering in the can. Water-based paints contain anti-foamers which prevent the roller from producing a close-knit bubbling effect in the applied paint and thickeners which reduce spattering and so aid flow during painting of surfaces.

1.1.3 Types of Paints

There are three types of paints namely (i) alkyd paints (oil-based paints) that contain solvents like acetone, petroleum distillates, toluene, epoxy esters, resins, methylene chloride and aromatic hydrocarbons; (ii) Latex paints (water-based paints) which are non-flammable and offer ease of application; (iii) Specialty paints (chemical-based paints) which are used in tank linings and sewage systems. Consumers are now very much interested in paints, especially the benefits of top quality paints. Many paint vendors have fallen victim to purchasing a lesser quality product based solely on lower selling price, and ended up being dissatisfied with its performance. Studies have shown that top quality water-based paints last longer than ordinary paint since they provide better adhesion, durability and stain resistance (Brouwer *et al.*, 2005).

1.1.3.1. Latex Paints

Water-based paints otherwise known as latex paints are susceptible to more biodeterioration by microorganisms (Gillatt, 1992). They contain various organic materials which are biodegradable and therefore, act as nutrients for the microorganisms on painted surfaces and stimulate microbial growth both in-can and on the dry paint film upon application. Latex paints are non-flammable and are easily applied on metal, wood or concrete surfaces. They generally do not have a disagreeable odour and can be used on both interior and exterior surfaces. Paint brushes and other tools used for paint application on surfaces are easily cleaned up with soap and water. Latex paint wastes are not hazardous wastes and can be

disposed into most sewage treatment systems or landfills. However, depending on the location, municipal approvals may be required before final disposal of paint wastes. Over the last few decades, emphasis had shifted from solvent-based paints to aqueous latex coatings (Adeleye and Adeleye, 1999). The synthetic vehicle is emulsified, that is, suspended as very tiny droplets in the water, when the paint dries, the water evaporates and the pigment and vehicle particles bond together, forming a relatively strong film. The film is porous enough to permit the passage of moisture, thus increasing its resistance to blistering. Most latex paints are limited to interior use and are popular because they are odourless and easy to apply (Ha *et al.*, 1995). In some cases, solid-emulsion paints, or powder coatings, replace liquid paints. They are sprayed on to a metal surface, as in the production of machinery or window frames and adhere by electrostatic attraction. Heat causes the powder to flow and form a film (Brouwer *et al.*, 2001).

Recent enhancements in water-borne resin system polymers as well as the way they are formulated have produced paints with a set of barrier properties that are superior to their standard solvent-borne analogs. New generation water-borne epoxy and curing agent dispersions, and the paints formulated from them, have been introduced with changes from older generation water-bornes. These changes include (1) totally non-ionically dispersed paints in place of the older ionically dispersed types, (2) quicker coalescing, mutually soluble epoxy/amine vehicle resins rather than the former slow coalescing, highly branched epoxy/amine systems, and (3) utilization of stable, water compatible additives and fillers that complement the non-ionic epoxy/amine resin vehicles (Elmore *et al.*, 2002). Latex paints are prone to more biodeterioration than alkyd paints because of their aqueous nature. Water acts as a vehicle that transport various nutrients required by microorganisms for growth and proliferation. Nutrient molecules frequently cannot cross selectively permeable plasma membranes through passive diffusion. They must be transported by water.

1.1.3.2 Alkyd Paint

Alkyd paints contain oil and solvents like acetone, petroleum distillates, toluene, epoxy ester resins, methylene chloride and aromatic hydrocarbons (Brouwer *et al.*, 2005). Cleaning of painting equipment requires the use of solvents which have the same hazardous properties as

alkyd paint. The discolouration and decomposition of oil paintings caused by microorganisms are permanent. It is either a decomposition of the paint media or a discolouration of the substrate, the latter resulting in violet, brown or black stains. The use of a brush to apply paint to a flat surface almost inevitably means that the bristles of the brush leave behind an uneven paint surface. As the paint dries out, these non-uniformities tend to flatten out to leave a protective and aesthetically pleasing even coating. However, experiments have shown that some solvent-based high gloss alkyd paints can exhibit more unusual behaviour as they dry. The discolouration and decomposition are also of a progressive nature (Inoue and Koyano, 1991). The oils and solvents in alkyd paints and specialty coatings are toxic if released into the environment. They have the potential to contaminate drinking water supplies, ground water and can be toxic to plants and aquatic animals. Water contaminated by paints and the solvents used to clean painting tools can also contaminate drinking water supplies and other areas of the environment. Vapours released from alkyd paints are toxic to humans if inhaled over a long period of time in high enough concentrations. These vapours have the potential to start a fire if exposed to a spark or flame and support a fire once started because they are flammable, reactive or corrosive (Brouwer *et al.*, 2005). Handling and safety procedures of alkyd paints should be in accordance with the material safety data sheet (MSDS).

Alkyd paints are generally, more difficult to maintain than the water-based paints. The use of fungicides such as pentachlorophenol (PCP) and 2-(4 thiazolyl)-benezimidazal (TBZ) have been observed to be safe and temporarily effective. However, the fungicide PCP turns brown after some years, while TBZ does not completely dissolve in a solvent (Inoue and Koyano, 1991).

1.1.3.3 Specialty Paints

Specialty paints are a group of modern chemical compounds designed for protecting materials under exacting conditions such as chemical tank linings, concrete coating at sewage treatment plants and other industrial applications. Examples of specialty paints include: acrylic, asphaltic, epoxy, flexible ceramic, phenolic polyester, polyurethane and vinyl ester

paints. Specialty paints are gaining greater acceptance and becoming common in the paint industry. The hazard characteristics of specialty coatings are identified on the material safety data sheet (MSDS) provided by the manufacturer. These new generation paints are derived from chemical compositions that can withstand extreme environment and temperature conditions. Many specialty coatings are a two-component mix; a base and a hardener. Enamel paints consist of zinc oxide and lithopone in brown linseed oil and high-grade varnish (Galla *et al.*, 1981). Luminous paints contain various phosphorescent sulphides of barium, strontium and calcium. Luminous paints have been implicated in human osteogenic sarcoma (Martland and Humphries, 2007). Water-colours for artists are finished either in dry cake or moist condition. In both cases, they contain the finest pigments ground in gum arabic or dextrin.

1.1.3.3.1 Varnishes

Varnishes are transparent paint solutions that solidify into a protective coating (Gillgrass *et al.*, 2001). Opaque and coloured varnishes are called lacquers (Peters *et al.*, 2000). They are produced by heating a drying oil, resin, drier, and solvent together. If applied as a thin film, varnish gives a hard transparent coating on drying. The numerous variations in composition and preparation of varnishes make their classification difficult. So-called spirit varnish, for example, is a resin dissolved in a volatile solvent that contains no drying oil, while asphalt varnish is a solution of asphalt that gives opaque, black coatings.

1.1.3.3.2 Lacquers

Lacquers are certain natural and synthetic varnishes, particularly those obtained from the sap of the varnish tree, *Rhus verniciflua*, a Japanese sumac, containing the phenolic resin urushiol (Peters *et al.*, 2000). The sap is heated to drive off moisture, leaving a brown syrup. Pigments are added and sometimes, diluting agents. The resulting material is applied as a thin coating to wood, metal or ceramic articles; when hard, the lacquer coat is polished smooth with an abrasive, and another coat is applied over it. Often, more than 30 coats are used on a fine piece of lacquer work. Commercial lacquers used for painting metallic objects often have a pyroxylin base.

1.1.4 Microbial Contamination of Paints

As a result of the wide range of organic and inorganic molecules that are present in both paints and painted surfaces, many different types of microorganisms grow on them, provided that favourable environmental conditions (humidity, temperature, light and to a lesser extent, pH) are met (Ciferri, 1999). Microbial contamination can be separated into two categories: deterioration while the paint is still in the liquid state (Da Silva, 2003) and microbial deterioration when the paint is applied to a surface and forms a film (Woods, 1982). Paints exhibiting the effects of microbial contamination develop problems of viscosity loss, gassing, malodour, discolouration and visible surface growth (Gillatt, 1992) which reduce their shelf lives or render them unusable.

The most commonly isolated bacteria genera in paints include *Bacillus, Pseudomonas, Enterobacter, Proteus, Aerobacter, Escherichia, Micrococcus, Serratia, Aeromonas* etc (Miller, 1973; Woods 1982; Jakabowski *et al.*, 1983 and Opperman and Gull, 1984). A wide range of anaerobic bacteria including *Bacteroides, Clostridium, Desulphovibrio* and *Bifidobacterium* have also been isolated (Opperman and Gull, 1984). Studies have shown that some fungi are also associated with deterioration of paints. These fungi include *Rhizopus arrhinus, Aspergillus niger, A. ustus, Penicillium citrinum, Chaetomium globosum, Alternaria alternata* etc. (Adeleye and Adeleye, 1999). Allsopp and Seal, (1986); Gillat, (1992) and Grant *et al.* (1993) reported different fungal genera such as *Aspergillus, Fusarium, Geotricum, Penicillium, Saccharomyces, Scopulariopsis, Sporobolomyces* and *Torula* as the most commonly isolated fungi from water-based paints. *Cladosporium* is one of the major biological agents, if not the most significant agent responsible for fresco degradation (Nugari *et al., 1993).* Adeleye and Adeleye (1999) also reported the bacterial species associated with normal and deteriorated painted walls. They include *Pseudomonas, Bacillus, Micrococus* and *Staphylococcus*. The fungal genera isolated were *Rhizopus, Penicillium, Cladosporium, Aspergillus, Alternaria, Fusarium* and *Curvularia*.

Paints contain several microorganisms coexisting to effect deterioration. This deterioration is brought about by the interactions between the substrate and the microbes; the biosusceptibility of organic and inorganic constituents and the control and utilization of the

substrate by indigenous microorganisms. These microorganisms coexist as a synergistic microbial community in liquid paints (Smith *et al.*, 2003). Several reports have described various types of microbial interspecies relationships, such as antagonism, competition, commensalism, and symbiosis between two microorganisms. However, there appears to have been no report to date to demonstrate that these relationships actually operate in water-based paint communities and how they affect the structure, function and stability of paints. The evaluation of the total network of inter-species relationships would be important to understand such complex communities. The mechanisms responsible for the stable coexistence of many species of microorganisms have not yet been clarified (Kato *et al.*, 2005). One factor hampering the elucidation of these mechanisms is the difficulty of defining all members included in such complex microflora. Furthermore, it is even more difficult to clarify the roles of each member and the relationship between members of such a community. Hence, in order to facilitate our understanding of these mechanisms Kato *et al.* (2005) reproduced the stability and the function of a microflora by constructing a defined mixed culture consisting of microorganisms isolated from the microflora. Such approaches have often been applied to examine various microbial communities, especially in the field of pollutant biodegradation (Barreiros *et al.*, 2003) and in biological investigations of oral bacterial communities (Ashelfold *et al.*, 2002; Briones and Raskin, 2003).

If such a defined mixed culture were to be successfully constructed, it would facilitate the examination of general characteristics of all of the members in pure culture and the monitoring of the dynamics of the members in the community throughout a given cultivation period. Furthermore, by constructing a "knockout community" in which one of the members is eliminated from the defined mixed culture, the roles played by the eliminated member *in-situ* and its impact on the other members of the community could be evaluated. This approach is derived from the same perspective as gene disruption studies, in which the role of a gene in an organism can be evaluated by the elimination of that gene. Therefore, it seems likely that the removal of a gene from a specific spoilage organism by curing may help to evaluate the spoilage potentials of the organism.

1.1.4.1 Sources of Paint Contamination

Microbial contamination of paints can originate from a number of sources including make up and wash waters, other raw materials, the manufacturing plant itself, and the packaging materials. Water may come from a number of sources including boreholes, or even rivers. If such waters contain residual organic matter, sterilizing procedures such as chlorination may still leave residual contamination (Gillatt, 1992). Town tap water may contain some bacteria. Powdered raw materials such as fillers, extenders and pigments contain bacteria and fungi which in an aqueous environment, may germinate and grow. Liquid raw materials such as defoamers, polymer emulsions and pigment dispersions are often susceptible to microbial attack and unless protected with an adequate concentration of biocide, may also introduce contamination into the formulation (Gillatt, 1992).

1.1.4.2 The Substrates

The fact remains that there are few habitats surrounding a painted wall (indoor or outdoor) that can be considered to be completely sterile. Microorganisms interact with the substratum which is considered a mere physical support or a source of energy and nutrients. Cellulose esters used as thickening agents in paint formulations serve as carbon sources for growth of a large variety of bacteria and fungi. Microbially-produced enzymes such as cellulases can remain active in a contaminated formulation long after the causative organisms have been eliminated by a biocide. This may result in paints which appear satisfactory on filing, suffering viscosity loss and reduced shelf life after a few months in the container. This happens because of the biodegradability of the paints and the additives (glues, emulsifiers, thickeners, etc.) that facilitate drawing or application of paint layers or enhance the aesthetic quality of the finished product (Ciferri, 1999).

1.1.5 Hazards Associated with Paint Formulation

1.1.5.1 Water-based Paints

Despite the decorative advantages of paints and their preservation of surfaces against environmental weathering, paints have some adverse effects on man. Man-made chemicals

used as refrigerants, fire retardants, paints and solvents cause considerable environmental pollution and human health problems as a result of their persistence, toxicity, and transformation into hazardous metabolites. Inhalation of paint fumes has been implicated in cases of heart failure (McGee and O' Malley, 1979). Spray painters are generally exposed to aerosols containing hexavalent chromium [Cr(V1)] via inhalation of chromate-based paint sprays. Evaluating the particle size distribution of a paint spray aerosol and the variables that may affect this distribution, are necessary to determine the site and degree of respiratory deposition and the damage that results from inhaled [Cr(V1)]-containing paint particles (Sabty-Daily *et al.*, 2005).

In the 1970s, there was a substantial increase in the use of construction paints in many countries. Today, more than 90% of construction paints in Scandinavian countries are water-based (Hansen *et al.*, 1987). In Germany, some water-based constructions paints (WCP) have the image and label of "environmentally safe products", which make them popular with do-it-yourself painters. In the United States, WCP are mainly used outdoors as stains (Hansen *et al.*, 1987). In other Western countries, WCP are mainly used outdoors as alternatives to the traditional enamels. Paints of the latter type contain about 50% volatile organic solvents (mainly white spirit), that may cause chronic or acute neurotoxic effects in painters (Van Vliet, 1989; Faassen and Borm, 1991). Moreover, emitted volatile organic compounds (VOC) can interfere with the nitrogen cycle, generating oxidizing compounds, like ozone, causing acute and chronic effects on the human respiratory tract (Bruring, 1989).

In the Netherlands, construction painting is the major contributor to the VOC emission of the painting trade (Bruring, 1989). Moreover, this emission cannot easily be controlled because construction painting is a discontinuous point source. The composition and health hazards of different kinds of WCP have been studied in Denmark (Hansen *et al.*, 1987). Wall paints have been water-based for several decades already and are therefore, not alternatives for solvent-rich paints. However, it has become necessary to investigate whether or not health hazards might be expected from application of these alternative WCP. The health hazards of the WCP application were separated into occupational hazards and environmental hazards (Faassen and Borm, 1991). Occupational health hazards mainly occur during application of paints. Both professional and amateur painters are subject to these hazards, although, the

latter, less frequently. Environmental health hazards arise from human exposure to air, (drinking) water, and food which may be polluted due to application or spilling of the paints. Moreover, environmental health hazards can be caused indirectly by ecotoxic effects caused by the paint constituents.

Faassen and Borm (1991) carried out experiments to (a) track down the composition of WCP; (b) estimate the occupational and environmental exposure; and (c) estimate the health hazards due to application of WPC. Acrylic dispersion paints (ADP), being the majority of WCP reported were found to contain a number of functional components that are not present in traditional solvent-based construction paints (alkyd paints) (Table 1.1). Some examples of the hazards caused by these paints are given in Table 1.2. WCP also contain very specific organic solvents and preservatives. Organic solvents are added to ADP for several reasons. The main reason is their function in film formation. Preservatives are used for conservation of the binder and the paint during production and storage; moreover, these products contain bacteria-degradable compounds, like surfactants in an aqueous environment, and these products contain a nitrogen source as ammonia. Ammonia and volatile amines are used to stabilize the binder and the paint at a pH of 8 – 9. Less volatile amines create a longer "open time" after application. The water-soluble alkyd resin is solubilized with triethylamine.

Surfactants include antifoaming agents and emulsifiers of the binder, filler, thickener, and/or pigment. Corrosion inhibitors are needed to prevent corrosion of metallic paint cans and metallic parts of the painted material. UV absorbers are essential in paints for wood because ADP do not absorb the wood-destroying UV radiation. Table 1.2 presents information about the health hazards of some WCP constituents. The main occupational health hazards are headaches and acute chronic respiratory disorders (Hansen *et al.*, 1987). Orthoergic eczema may occur due to frequent skin contact with WCP combined with scouring and extreme climate conditions. Such an eczema ameliorates the barrier function of the skin, causing toxic compounds to penetrate more easily through the skin in to the body (Faassen and Borm, 1991). Another important occupational health hazard is sensitization of the skin caused by monomers and preservatives, which can result in allergic eczema. Suspected carcinogenic

hazards are presented by some ADP due to percutaneous or lung uptake of the suspected carcinogens, acrylonitrile and formaldehyde. The suspected tetratogens, ethylene glycol ethylether present suspected teratogenic hazards. Table 1.3 shows the result of ecotoxicity tests and the assigned toxicological hazards of constituents of WCP.

However, during and after application, ADP can present some other health hazards such as irritation of the mucous membranes of eyes and nose as well as skin irritation and sensitization. This is in accordance with the Danish study in which irritation of nose and eyes were mentioned as the main complaints of WCP users (Hansen *et al.*, 1987). In the Netherlands, 10 – 15% of the population is bronchial hyperreactive to irritating compounds (Estlander *et al.*, 1984). Furthermore, about 15% of the population is atopic, resulting in a higher risk of getting orthoergic eczema (Estlander *et al.*, 1984). Persons with orthoergic eczema and pregnant women have a higher risk of getting allergic eczema. (Menne and Christophersen, 1985). Some ADP contain compounds that should be regarded to be able to cause hematotoxicity, tetratogenicity, and carcinogenicity. Environmental health hazards from ozone due to volatilization of organic solvents from the paints are reduced strongly by using WCP. However, cleaning application materials of WCP under the tap can cause a significant burden to sewage treatment due to some slowly degradable compounds. One of them (polyacrylate) was reported also to clog the gills of fish (Faassen and Borm, 1991). When WCP are spilled to the soil, the water-soluble preservatives and slowly degradable compounds may affect soil organisms.

Table 1.2: Occupational and environmental health hazards due to application of WCP that contain compounds with toxicological properties. [a]

Compound	Compound Concentration, % weight	M	SC	Other systemic mammalian toxicity	Skin S	Skin Ir	Fish irritation	Odor	lethality
Binders and their constituents									
Polyacrylate	37	-	-	-	-	-	-	+	++
Methyl methacrylate	0.14	+	-	?	+	+	+	+	-
Acrylonitrile	0.006	+	+	?	?	+	+	+	+
Butyl benzyphthalate	1.4	-	-	?	+	?	-	-	+
Ammonia and animes									
Ammonia	0.18	-	-	Lung	-	-	+	+	+
Dimethyl ethanolamine	0.2	-	-	Nitrosable	?	-	+	+	?
Triethylamine	1.0	-	-	Eyes, lung	?	+	+	+	+
Nitrosable									
Corrosion inhibitors									
Triethanolamine	0.07	-	-	Nitrosable	+	+	-	-	?
Sodium nitrite	0.02	+	-	Nitrosable	-	-	-	-	+
Preservation									
Formaldehyde	0.1	+	+	Liver	+	+	+	+	+
(Chloro) methylisothiazolinon	(0.003)	-	-	?	++	+	-	-	+
Organic solvents									
Ethylene glycol	7.9	-	-	Kidney, tetratogenicity	-	-	-	-	+
Ethylene glycol ethyl ether	2.0	-	-	Blood cells, tetratogenicity	-	-	+	+	-
Diethylene glycol butyl ether	5.0	-	-	-	-	-	-	-	?
Surfactants									
Poly(oxyethylene)octyl phenylether	1.6	-	-	?	?	+	-	-	++
Tributylphosphate	0.6	-	-	Nervous System	?	+	-	-	+
Others Ammonia bichromate	1.0	-	-	?	++	+	-	-	+
Hydroxymethylphenylbenzotriazol	0.4	-	-	?	+	?	-	-	?

Abbreviations: (-) no health hazard; (+) health hazard not excluded; (++) health hazard expected; (?) unknown, insufficient data; (SC) suspected carcinogenicity; (S) sensitization; (Ir) irritation; (M) Mutagenility

Source: Fassen and Borm (1991)

Table 1.3: Sources of (eco) toxicity tests and assigned toxicological hazards of constituents of WCP

Test	Boundaries	Toxicological hazard
Acute oral toxicity, rat (LD_{50}, mg/kg)[a]	>15,000	Not harmful
	5,000 – 15,000	Harmful
	500 – 5,000	Toxic
	50 – 500	Very toxic
	5 – 50	Extremely toxic
	< 5 -	Very extremely toxic
Acute fish (daphinia) Toxicity (LC_{50}, mg/L)[b]	> 100	Not harmful
	10 – 100	Harmful
	10 – 10	Toxic
	< 1	Very toxic
Reproductive toxicity	Adequate studies with rat and rabbit	Toxic for reproduction
	Inadequate animal studies	Unknown reproductive Toxicity
Mutagenicity	Bacterial (+ negative S9)c and nonbacterial test positive	Mutagenic
	One of the tests positive	Not mutagenic
Carcinogenicity	Sufficient epidemiological evidence	Human carcinogen
	Sufficient animal experimental evidence	Probably human carcinogen
Skin irritation test	No irritation	No skin irritant
	Slight irritation	No skin irritant
	Intermediate irritation	Slight skin irritant
	Strong irritation	Strong skin irritant
	Corrosive	Corrosive for skin
Guinea pig maximization test	Low	Not skin allergen
	Medium	Slight skin allergen
	High	Strong skin allergen

[a]LD_{50}, does that causes 50% mortality within 14 days; [b]LC_{50}, concentration that causes 50% mortality within 24 to 96 hours; [c]S9, metabolizing supernantant of rat liver microsomes.

Source: (Fassen and Borm, 1991)

1.1.5.2 Dry Paint Spraying

One of the least studied areas of dermal exposure is exposure to paint dusts. Part of the reason for this lack of knowledge has been the lack of analytical methods to determine dusts on painted surfaces, as many dusts are insoluble and unreactive (Roff *et al.*, 2004). Evaluation of skin exposure to powder paints and liquid paints is an important part of occupational risk assessment because substances may penetrate the skin and cause harm to the human body. Dermal exposure measurement methods are now available, but generally involve expensive techniques, high skilled technicians, and elaborate chemical analyses (Fenske, 1993; Van Hemmen and Brouwer, 1995). For risk assessment purposes, models have been developed by the USA Environmental Protection Agency (Mulhausen and Damiano, 1998; Brouwer *et al.*, 2001) and by the United Kingdom Health and Safety Executive for European Union (European Chemicals Bureau, 1996) to estimate dermal exposure.

The spraying of a paint formula (Acramin F system) led to an outbreak of severe pulmonary disease (Ardystil syndrome) in the community of Valencia, Spain in 1992 among factory workers who worked in areas where textiles were air-sprayed (instead of being applied as pastes for screen printing), with dyes using the Acramin F paint system (Moya *et al.*, 1994). In order to elucidate the underlying mechanisms of the toxicity of this paint and its main polymeric components, Acramin FWR, Acramin FWN, Acrafix FHN, and Acramoll W., Hoet *et al.* (1999) undertook studies using a battery of different cell types and assessing *in vitro* cytotoxicity by measuring LDH leakage. The study showed that as in *in vitro* studies, the three polycationic paint components , Acramin FWR (a polyurea), Acramin FWN (a polyamide-amine) and Acrafix FHN (a polyamine) exhibited considerable cytotoxicity (LC_{50} generally below 100 µg/ml for an incubation of 20 – 24 h) *in vitro*, while Acramoll W, which is not a polycation, was almost non-toxic (in the concentration range tested).

The cytotoxicity was comparable in primary cultures of rat and human type 11 pneumocytes and alveolar macrophages as well as in the pulmonary cell line A549 and the hepatic cell line HepG2. In human erythrocyte, the toxicity was less pronounced. The workers reported that the multiple positive changes play an important role in the toxic mechanism. The epidemiological study showed convincingly that the outbreak was associated both in time

and location, with a formula change from Acramin FWR (a polyurea) to Acramin FWN (a polyamide-amine) in the paint system used. It was concluded that Acramin FWR and Acramin FWN have similar intrinsic toxicity and that these polymeric compounds, which have no irritant properties *or* systemic toxicity when given orally, exert a high unexpected, degree of cytotoxicity.

1.1.5.3 Occurrence of Metals in Paints

The toxic metals in hull paint leach into water during normal use, maintenance and application. They may end up in the food chains, killing marine life as they bioaccumulate, possibly contaminating sea food. Countries such as Canada and Denmark now restrict copper paints (Rabin, 1989), the kind most commonly used in recreational boats. However, other non-toxic silicone-based alternatives are available, but they are foul, harder to clean, more expensive and less durable. The challenge of an antifouling coating is to be able to address the arsenal of adhesives that marine bacteria, animals and plants use for sticking to surfaces. Hence, there is a potential conflict between protecting sea life and slowing invasive species (Fields, 2003). In the last few years, there has been a rapidly growing public concern over the need to eliminate lead-based paints due to their various health implications. Lead-based paints have been recognized as a high-dose source of lead absorption and a cause of lead poisoning in young children since the beginning of the twentieth century (Marino *et al.*, 1990).

1.1.5.4. Uses of Lead in Paint Formulation

The addition of considerable amounts of lead in paint as pigments was once widespread because of its use as pigment, dispersing agent and drying agent but mostly because it provided durability to the paint (Rabin, 1989; Fassin and Naude, 2004). Basic lead carbonate, $(PbCO_3)_2$ and $Pb(OH)_2$, called white lead, have been used for over 2,000 years as a white pigment in paint production. They are also used in ceramic glazes and in making other pigments. Red lead, or minium (Pb_3O_4), a scarlet, crystalline powder formed by oxidizing lead monoxide, is the pigment in paint used as a protective coating for structural iron work and steel work. Lead chromate, or chrome yellow ($PbCrO_4$), a crystalline powder used as a

yellow paint pigment, is prepared by the reaction of lead acetate and potassium bichromate. Chrome red, orange chrome yellow, and lemon chrome yellow are some of the pigments obtained from lead chromate. Lead (II) ethanoate ($Pb(C_2H_3O_2)_2 . 3H_2O$), a white, crystalline substance called sugar of lead because of its sweet taste, is prepared commercially by dissolving litharge in ethanoic acid. It is used as a mordant in dyeing, as a paint and varnish drier. The shelf life of paints was thus improved, yet the problem of plumbism was created. In recent years, however, because of the dangers of lead poisoning, the use of lead-based paints for interior use has largely been discontinued (Needleman, 1998).

Child-lead poisoning gained wider recognition since the mid-1920s as a common childhood disease resulting from lead paint in the home (Rabin, 1989). However, awareness that lead-based paint is a source of lead poisoning in children dates back to the first few years of the twentieth century (Berney, 1993; Fassin and Naude, 2004). Giving the continuing toll taken by child lead poisoning and the considerable resources that will be required to deal with the problem, it may be useful to consider determining the shelf life and improving the same with the use of broad spectrum biocides.

1.1.5.5. Toxicity of Lead Paints

Lead is highly toxic. Chronic lead exposure may lead to developmental delay and the effects are usually felt after it has accumulated in the body over a period of time. The symptoms of lead poisoning are anaemia, weakness, constipation, colic, palsy, and often a paralysis of the wrists and ankles (Bellinger *et al.*, 1992; Schwartz, 1994; Banks *et al.*, 1997; Satcher, 2000; Dietrich *et al.*, 2001; Fassin and Naude, 2004). Flaking lead-based paints and toys made from lead compounds are considered serious hazards for children. Children are especially at high risk from lead, even at levels once thought safe. Lead can reduce intelligence, delay motor development, impair memory and cause hearing problems and troubles in balance. In adults, one lead hazard is that of increased blood pressure. In the United States, at the beginning of the 20th century, white lead pigment in paint had been recognized as toxic and restricted since 1915. It had been banned from professional use since 1926 and from craft work since 1948. Consequently, it remained only in ancient, often dilapidated housing. Screening for lead toxicity is usually recommended for children living in houses built before 1950 (Sanborn *et al.*, 2002).

Plate 1.1 Advertisment featuring the Dutch Boy trademark on a can of white-lead paint which emphasized the durability of the product.

Source: Rabin (1989).

1.2 Paint Degradation

Increasingly, it has become evident that many materials can be degraded by the presence and/or activities of microorganisms. Paints are no exception and often contain biocides in an attempt to control such damage. The major groups of microorganisms involved in degradation of in-can paints and the dry paint films are bacteria, fungi, algae and cyanobacteria all of which are able to survive under conditions of stress such as drying (Ciferri, 1999; Da Silva, 2003). Remedial action for deteriorated water-based paints is usually unsuccessful as cellulolytic enzymes produced by the microorganisms which are relatively stable and active over a long period (Saad, 1992) will be unaffected by modern, broad spectrum biocides (Gillatt, 1992).

The microbiological degradation of water-based paints occurs because water-based paints are potentially prone to in-can attack by both bacteria and fungi. These microorganisms, needing basically light, water and organic nutrients to grow reduce drastically the shelf life of water-based surface coatings and aesthetic values of painted surfaces/walls. One of the first signs of microbial activity in in-can paints is viscosity loss caused by microorganisms which release enzymes that can digest the traditional cellulosic thickeners. The role of bacteria and their cellulases in paints have been investigated (Goll and Winters, 1974). Miller (1973) pointed out that cellulose esters used as thickening agents in paint formulations serve as carbon sources for growth of bacteria and fungi. They are liable to enzyme hydrolysis leading to decrease in viscosity of the paints (Saad, 1992; Toothill *et al.*, 1993). These enzymes which are capable of functioning independently of the cells that produce them are large proteinaceous molecules that attack the polymer chains of the thickener in water-based paints and break them down into cellobiose, which are smaller compounds that are no longer capable of functioning as a thickener. The cellobiose is then further degraded to glucose, which, bacteria and fungi ferment, producing acids and carbon dioxide (Reilly, 1991). The resulting acid formed by the degradation significantly lowers the pH, causing more problems (Alba *et al.*, 2003). As the breakdown of the thickener continues, the paint separates and the solid sediments settle to the bottom of the container, indicating loss of viscosity (Bastos *et al.*, 2003). Due to the carbon dioxide, nitrogen dioxide and hydrogen sulphide produced, it is common for contaminated paints to have foul odours, bulging cans and even lids that pop off the containers. Finally, the paints become discoloured, having a reduced shelf life.

Cellulose is the most abundant biopolymer occurring in nature and in agricultural and many industrial wastes. Cellulosic derivatives are often used as thickeners in the paint industry. The model for the complete depolymerization of crystalline cellulose by fungi which is often referred to in bacterial studies, is thought to involve synergistic action of at least two cellulase components, namely endo-1, 4 beta-glucanase and exo-1, 4-beta-glucanase, the latter often being called cellobiohydrolase (Ryu and Mandels, 1980). Cellulase(s) are relatively stable enzymes which are active over long periods. The inactivation of cellulases is very important to control paint film biodeterioration. Once a water-based paint has been contaminated by microorganisms, its physical and chemical properties change and the microorganisms start to multiply and attack organic components in the paint system. Alba *et al.* (2003) reported the tolerance and growth of the fungus, *Scopulariopsis brevicaulis* as the sole biodegradation agent of the main components of cellulose paint thinners (toluene, acetone, isopropanol and xylenes) isolated from a thinner biodegradation microbial consortium. Once a celluloid object as paint has been fabricated, it becomes subject to the chemical, physical and biological conditions of its environment. Cellulose-based formulations that have been exposed to undesirable environmental conditions by microorganisms is apt to be discoloured, warped, swollen and softened. Environmental conditions usually alter the enhancing degradation of cellulose nitrate molecules. This degradation generally leads to lower viscosities (Reilly, 1992).

Bertram and Dale (1985) reported two major ways of converting cellulose to glucose: chemical versus enzymatic. The research on both methods has for decades occupied the attention of many investigators worldwide. Due to the fact that each cellulose molecule is an unbranched polymer of one thousand to one million D-glucose units, linked together with beta- 1, 4 glycosidic bonds, cellulose from various sources are all the same at the molecular level. However, they differ in the crystalline structures and bindings by other biochemicals. It is this difference that makes possible a persistent research on cellulose. The model cellulose compounds most commonly used in today's paint industries are carboxymethyl cellulose (CMC), which has a generally amorphous structure, and Avicel, which has a highly crystalline structure conditions (Linko, 1977).

There are two types of hydrogen bonds in cellulose molecules: those that form between the C_3OH group and the oxygen in the pyranose ring within the same molecule and those that form between the C_6OH group of one molecule and the oxygen of the glucosidic bond of

another molecule. Ordinarily, the beta-l, 4 glycosidic bonds themselves are not too difficult to break. However, because of these hydrogen bonds, cellulose can form very tightly packed crystallites. These crystals are sometimes so tight that neither water nor enzyme can penetrate them; only *exoglucanase*, a subgroup of cellulase that attacks the terminal glucosidic bond, is effective in degrading it. The inability of water to penetrate cellulose also explains why crystalline cellulose is insoluble. On the other hand, amorphous cellulose allows the penetration of *endoglucanase*, another subgroup of cellulase that catalyzes the hydrolysis of internal bonds. The natural consequence of this difference in the crystalline structure is that the hydrolysis rate is much faster for amorphous cellulose than crystalline cellulose. The process of breaking the glucosidic bonds that hold the glucose basic units together to form a large cellulose molecule *is* called hydrolysis, because a water molecule must be supplied to render each broken bond inactive. In addition to crystallinity, the chemical compounds surrounding the cellulose also limit the diffusion of the enzyme into the reaction sites and play an important role in determining the rate of hydrolysis (Ghose, 1977; Bertram, 1985). The conversion of cellulose into glucose is now known to consist of two steps in the enzyme system of *Trichoderma viride*. In the first step, beta-1, 4 glucanase breaks the glucosidic linkage to *cellobiose*, which is a glucose dimer with a beta-1, 4 bond as opposed to maltose, a counterpart with an alpha-1, 4 bond. Subsequently, this beta-1, 4 glucosidic linkage is broken by beta-glucosidase.

1.2.1 Biodegradation of Applied Paints

Microbial deterioration of painted walls is a phenomenon often encountered by conservationists during the restoration of ancient buildings. Phototrophic biofilm forms act as primary colonizing layers on the internal and external surfaces of historic and cultural buildings all over the world: from the wooden huts of the first Antarctic explorers, to the stone buildings of ancient civilizations. Fungi, algae and bacteria can all grow on applied paint films and solvent and water-based coatings are both susceptible (Gaylarde *et al.*, 2003).

1.2.2. Weathered Painted Walls: Moisture, Molds and Decay Problems

The occupants of moisture-damaged buildings suffer from various respiratory and other health disorders (Borneberg *et al.*, 2004). Inhalable components or metabolites of microbes growing on moisture-damaged painted walls are considered potential inducers of these

effects (Torvinen *et al.*, 2006). The biotransformation process on painted walls attains highest levels in warm-humid climates where the environmental conditions are extremely favourable to the growth of most organisms. Painted films are exposed to weather and susceptible to influence of environmental parameters. Physical, chemical and biological factors interact with constitutive materials, inducing changes both in its compositional and structural characteristics. The biological aspect of this transformation is due to the metabolic activity connected with the growth of living organisms (Inoue and Koyano, 1991; Saad, 1992; Adeleye and Adeleye, 1999). Ogbulie (2004) reported the occurrence of the following bacteria genera: *Enterobacter, Staphylococcus, Pseudomonas, Bacillus, Micrococcus and Streptomyces* on normal and deteriorated painted walls. The fungal genera isolated included: *Rhizopus, Penicillium, Cladosporium, Aspergillus, Alternaria, Fusarium and Curvularia.* The biodegradability of painted walls by a large variety of microbial species is due mainly to their organic and inorganic constituents (Ciferri, 1999; Da Silva, 2003). This factor makes water-based paint a potential ecological niche that may be exploited by these microbial species. The microflora attacking painted walls include virtually all species of microfungi because the variety of organic components of these paints can represent a carbon source for practically all species. In addition, they show a great tolerance for the prevailing environmental conditions and can use condensation moisture as a source of water, necessary for microbial growth. The occurrence of microalgae and cyanobacteria on monuments (Ortega-Calvo *et al.*, 1991; 1993) or plaster (Danin *et al.*, 1982) has been investigated both for their ability to etch mineral components (Danin *et al.*, 1982) and to form aesthetically coloured patches and in some cases, a reddish staining of marble (Pietrini *et al.*, 1985). The presence of algae can assume great relevance, especially for monuments exposed in wooden environment (Tiano and Caneva, 1987) even if the metabolic activity and the remains of the dead cells promote the development of heterotrophic organisms and lower plants (Ortega-Calvo *et al.*, 1993).

1.2.3. The Bacterial Aetiology of Discolouration of Wall Paintings.

The microbial colonization of masonry and historical wall paintings by fungi, algae and bacteria may result in structural and/or aesthetic deterioration of painted surfaces (Ciferri, 1999). The formation of pigmented biofilms, biomineralization, degradation of organic binders and, discolouration are common deleterious effects of microbial growth. It is now

well established that the identification of the whole microbial diversity present on painted surfaces is an essential requirement for the rational design of prevention and restoration strategies. The structure and microbial aetiology of biodeteriorated monuments and painted surfaces has been investigated (Amann, 2000; Theron and Cloete, 2004; Gonzalez and Saiz-Jimenez, 2005). This is because the inventory of microorganisms responsible for biological deterioration of ancient paintings has become an integral part of restoration activities. The bacterial community from three discoloured painted walls in Italy was investigated and characterized (Imperi *et al.*, 2007). The eubacterial population was prevalently composed of Actinobacteria among which *Rubrobacter radiotolerans*- related bacteria accounted for 63 – 87% of the bacterial community in the sampled site. Archaea, with prevalence of Haloarchaea- related species, were detected in one of the three sites where they accounted for less than 0.1% of the total microbial community. The investigation provided the first evidence of a casual relationship between heavy contaminations by *Rubrobacter*-related bacterioruberin- producing bacteria and rosy discolouration of ancient wall paintings.

1.3. Biofilm Formation

The formation of biofilms on painted walls results in aesthetic discolourations. The traditional model of biofilm formation proposes development in discrete steps. These involve formation of a conditioning film, transport of microbes to the surface, and reversible initial adhesion through non-specific Van der Waals forces, followed by irreversible attachment to the painted walls and the production of extracellular polymeric substances (Busscher and Van der Mei, 2000; Hsueh *et al.*, 2006). Biosurfactants have been reported to play various roles in biofilm formation. Pellicle or surface biofilm formation in *B. subtilis* also required surfactin (Conelly *et al.*, 2004). It has been reported that the addition of about 0 – 1 mg of surfactin per ml could rescue pellicle formation by surfactin-deficient mutants of *B. subtilis*. Surfactin produced by *B. subtilis* 6051 was required for biofilm formation in microtiter plates and on *Arabidopsis* root surfaces (Bais *et al.*, 2004). In contrast, the addition of surfactin inhibited biofilm formation by *Salmonella enterica* ser ovar *typhimurium*, *Escherichia coli*, and *Proteus mirabilis* but not by *Pseudomonas aeruginosa* (Mireles *et al.*, 2001). This may account for the persistent occurrence of *Pseudomonas aeruginosa* on biodeteriorated painted

walls and on biofilms formed on spoilt paints. Rhamnolipid, a biosurfactant produced by *Ps. aeruginosa*, was reputed to be involved in the development and maintenance of biofilm architechture (Davey *et al.*, 2003; Lequette and Greenberg, 2005). On the contrary, Boles *et al.* (2005) observed that rhamnolipids mediated the detachment of *Ps. aeruginosa* from biofilms.

The cell membrane of Gram-negative bacteria allows the transport of water molecules. Weiss *et al.* (1991) showed that factors other than molecular size, such as the enzymatic hydrolysis of organic phosphates and the interaction of the substrates with the cell surface, are important for cell utilization of phosphates. *Pseudomonas* spp. show capability of utilizing organic phosphates as a phosphate source. This property could also be utilized by *Ps. aeruginosa* in adhering to biodeteriorated surfaces in addition to cell surface hydrophobicity which has been recognized as a physical, measurable characteristic of bacteria (Nikovskaya *et al.*, 1989) and this property has been commonly investigated in the adherence of bacteria to surfaces like biofilms (Rosenberg and Doyle, 1990) on painted surfaces. This also favors the indigenous microorganisms in water-based paints. Most studies related to environmental cross contamination carried out in industries showed that a daily cleaning and disinfection of the painted walls resulted in a thirty-fold reduction of bacterial numbers. Therefore concrete or masonry walls require coatings or treatments that can be efficiently cleaned, but remained impermeable to moisture, cleaning solutions, food acids, fats and other materials. Ha *et al.* (1995) also examined coating materials for walls and ceilings as potential sources of environmental cross contamination in a poultry processing plant environment. It was observed that when coating materials were inoculated daily, the chemical sanitizer used in the study effectively decreased surface contamination of uncoated and painted concrete. At many places along the production lines in a paint industry, raw materials for paint production come in close contact with surfaces of equipments, machines, tables, walls and floors. This can result in the contamination of the finished product. LeChevallier *et al.* (1988) examined the inactivation of biofilm bacteria and characterized the interaction of biocides with microbial interfaces. The research examined four disinfectants (hypochlorous acid, hypochlorite, chlorine dioxide, and monochloramine), three types of surfaces (granular activated carbon, metal coupons, and glass microscope slides), two bacterial types (HPC bacteria and coliforms) as well as several alternate biocides (copper, zinc, sodium chlorite,

and alkaline pH). The results reveal important properties of the compounds which can be exploited to improve inactivation of biofilm bacteria both in the liquid paint and on the painted surfaces. The mechanisms of reduced susceptibility to biocides of bacterial cells present within biofilms have been the subject of considerable experimentation and debate (Gupta *et al.*, 1999; Gilbert and Mc Bain, 2001). These mechanisms include:

- Reduced access in biocide molecules to bacterial cells.
- Chemical interactions between biofilm and biocides.
- Modulation of the microbial environment producing nutrient - and oxygen -limited and starved cells.
- Production of degradative enzymes that might be effective at lower biocide concentrations within the biofilm.
- Genetic exchange between cells.
- Quorum sensing.
- Presence of persisters and of pockets of surviving organisms
- Adaptation and mutation within the biofilm and
- Biocide efflux.

In nature, it is likely that paint biofilms will consist of mixed populations of different types of microorganisms. Biofilm formation is a major reason for the refractory response of many organisms to biocide (Russell, 2003). There could also be different target site affinities for biocides in different types of microorganisms although, this aspect has been less widely studied.

1.4. The Metabolic Activity of Biofilms

Biofilm cells differ phenotypically from their free-floating counterparts. Differential growth rates in biofilms are often referred to, particularly in response to limited diffusion of oxygen and nutrients. Bester *et al.* (2005) observed that the growth rates of attached *Pseudomonas* spp. strain CT07 cells were notably higher than the maximum specific growth rates measured in batch culture. The metabolic activity of paint biofilms is theoretically controlled by environmental conditions at the painted surface and the expression of specific genes induced by adhesion. Investigations into the genetic basis of biofilm formation and development

revealed several genes that are involved in biofilm formation. For example, genes encoding for flagella, type I and type IV pili, surface adhesins, homo serine lactones, and several others have been identified as being involved in the formation of biofilms under various environmental conditions (Pratt and Kotler, 1999). While numerous studies have investigated the changes in gene expression when microbes attach to surfaces like spoilt paints and biodeteriorated painted films, the process of detachment has received little attention (O'Toole *et al.*, 2000) and therefore, affords an opportunity for future research. However, once surface colonization has taken place, detachment of adherent cells is difficult and expensive, hence they leave permanent ugly marks on painted surfaces. While it is thought that a lack of internal cohesive forces or unfavorable environmental conditions may result in the detachment of parts of the biofilms on painted walls, the possible involvement of genetic determinants in the detachment process of individual cells has only recently been addressed (Bester *et al.*, 2005).

The active detachment of cells from biofilms can be mediated through the expression of extracellular enzymes that target the exopolysaccharide component of the biofilm matrix, as was shown when alginate lyase was expressed in alginate-producing *Pseudomonas aeruginosa* biofilms (Boyd and Chakrabarty, 1994). The activity of an N-acetylglucosamonidase (dispersin B) produced by *Actinobacillus actinomycetemcomitans* has been demonstrated against a component of the *Staphylococcus epidermidis* biofilm matrix. Enzymatic activity not only resulted in the detachment of existing S. *epidermidis* biofilms, but also prevented initial colonization. Gilbert *et al.* (1993) noted that since changes in bacterial physiology occur after contact with a surface, one could argue that an alteration in the properties of an attached bacterium could result in detachment. Biofilm-detached *Escherichia coli* cells were found to be significantly more hydrophilic than the attached population, which Allison *et al.* (1990) suggested could reflect the involvement of the cell division cycle in detachment from biofilms.

Sauer *et al.* (2002) showed that the development of biofilm by *Pseudomonas aeruginosa* results in the detachment of adherent bacteria. They observed motile cells exiting from within attached cell clusters, leaving behind a hollow shell of non-motile cells embedded in a matrix. Whole-cell protein profiles indicated that detached cells were more closely related to

planktonic cells than mature biofilm cells. The authors suggested that these detached bacteria are in transition from an attached to a planktonic phenotype (Sauer *et al.*, 2002). The contention that detachment is a discrete step in the process of biofilm development was further supported by the observations of Kaplan *et al.* (2003). They reported that the detachment of non-motile *A. actinomycetemcomitans* from a biofilm is dependent on the synthesis of a lipopolysaccharide component. While the attachment and development of the biofilm was not disrupted when the gene for a polysaccharide was interrupted, the detachment of single cells and/or small clusters from the biofilm was abolished (Kaplan *et al.*, 2003). This result generally suggests that biofilm formation on painted walls may or may not be plasmid-mediated.

1.5. Mechanism of Aggression and Microbial Succession

The autotrophic, nitrifying bacteria found on many different types of masonry and considered responsible for the biologically-induced deterioration of building materials (Hueck Van Der Plas, 1968) can be considered the pioneering inhabitants of many painted walls. These bacteria have been investigated for their ability to form aesthetically coloured patches and oxidize to nitrate the ammonia present in the atmosphere, thus promoting growth of heterotrophic microorganisms. The metabolic activity and the remains of the dead cells of these first colonizers promote the development of both heterotrophic organisms and lower plants which are capable of hydrolyzing bacterial cell walls (Ortega-Calvo *et al.*, 1991, 1993). The next group of organisms are the fungi, which are strictly heterotrophic organisms that need organic matter to grow. Some fungal species *(Penicillium, Cephalosporium, Trichoderma, Fusarium* and *Phoma)* have a greater biochemical decay potential than lichens (Iskandar and Syers, 1972). They chiefly utilize the polysaccharides coming from microbial cell walls, metabolic intermediates and storage materials that are available on painted surfaces. The last groups in the succession are the lower plants and weeds. These lower plants are indicators of wet conditions as they need very damp environment to complete their reproductive phase (O' Neill, 1988). The development of microflora and macroflora is heavier and more noxious on biodeteriorated painted walls in tropical climates where high rainfall and temperature greatly increase the growth of higher plants on monuments (Tiano and Caneva., 1987) than in temperate environments.

1.6. Use of Biocides in Paint Production

Increased costs of disposal of spoilt emulsion paints, allied with associated environmental difficulties and the financial loss; involved, have led to demands for improved remedial techniques. Contaminated paints can be treated with a high level of biocide, the so called "kill dose" to eliminate contamination, although the initial viscosity can not be recovered (Gillatt, 1992). Microorganisms vary in sensitivity to antimicrobial agents depending on their growth phase (greater during logarithmic phase) and the nature of the substrate in which they are found (Mirsha *et al.*, 1995). Biocidal action may result through physiochemical interaction with microbial target structures, specific reactions with biological molecules, or disturbance of selected metabolic or energetic processes. Mechanism of action studies of existing antimicrobial agents can provide direction to the development of novel biocides (Lambert *et al.*, 2001) which can be incorporated into paint formulations to prevent spoilage.

Da Silva (2003) reported the efficacy of two biocides used to protect paint films. One, a formulation containing diuron (a herbicide), carbendazin (a fungicide) and octyl-isothiazolinone (OIT- a broad spectrum biocide), and the other, a mixture of isothiazolinone and benzamidazole derivatives. The biocides were found to be effective against cyanobacterial growth and the spores of *Aureobasidium pullulans*, *Aspergillus niger* and *Penicillium* spp. The same biocide can be more or less active on different strains present in the consortium (Young *et al.*, 1995). Hence, the selected biocide should always be tested on test specimen made with the same material, possibly colonized with the same biocoenosis developing on it. More recently, some applications have been made using protective or consolidating polymers alone or mixed with biocides in order to enhance their efficacy (Grant and Bravery, 1985; Tiano *et al.*, 1995).

Normally, the results of positive biocide treatment are clearly visible on the "in situ" biological growth with the exception of lichens which can remain macroscopically unchanged for a long time even if the treatment was effective (Mirsha *et al.*, 1995). The lasting effect of a treatment in outdoor conditions ranges from 1 – 3 years depending on type of the chemical used and environmental conditions to which the treated surface is exposed. If the biocide is to be washed off after its action, or left as preventive on the surface is a matter of choice by the production team (Mirsha *et al.*, 1995).

1.7. The Relationships and Susceptibility Patterns of Microorganisms to Antibiotics and Biocides

Bacteria resistant to both the antibiotics deployed to prevent spoilage and those used to treat spoilt paints would be formidable microbes. There are many ways in which bacteria are able to resist antibiotics and biocides (Russell, 2000). The efficacy of a biocide depends on the ability to traverse the outer cell layers of the organisms. Different cell wall types exist for example in members of the group Archaea, with the peptidoglycan analogues, pseudopeptidoglycan, polysaccharide, protein, or glycoprotein being present. Thus, it is not surprising that uptake of biocides might differ greatly in a wide range of organisms in which the composition of the outer cell layers may have a limiting role, albeit for different reasons. However, Gram-negative bacteria resistant to antibiotics have been found to be susceptible to biocides (Stickler, 2002). Gram-negative bacteria, especially *Ps. aeruginosa,* show reduced susceptibility to biocide compared with the Gram-positive cocci (Russell and Chopra, 1996). The major reason for their recalcitrance to biocidal activity is the lipid-rich, waxy cell wall which limit intracellular uptake of many biocides. Bacterial spores tend to be much less susceptible to biocidal agents than non-sporulating bacteria. An obvious reason is to be found with the nature and composition of the spore coats and possibly cortex which present an effective permeability barrier to the entry of many biocides. A particular biocide may thus inactivate (or sometimes inhibit) more than one type of microorganisms (Russell, 2003).

Wolf and Riley (1965) described the fungistatic performance of 10, 10'- oxybisphenoxarsine in the exterior latex and asphalt coatings. This fungicide has been found to be outstanding in its activity against bacteria and fungi. Parallel tests with known fungistats for comparison (2,3,4,6-tetrachlorophenol and phenylmercuric acetate) have demonstrated its superior activity and persistence in an exterior acrylate paint film and in an asphalt coating. In view of its superior antimicrobial activity and its persistence, it can be used in applications in which there is no danger of ingestion while simple cleaning procedures with water-jet have been tentatively used for the eradication of biological growth (Leznicka, 1992). The ultimate approach is to increase the susceptibility of organisms to biocides prior to treatment, using product such as EDTA (Pantazidou and Theoulakis, 1997) or ionizing radiation (Tayler and May, 1994) which could lead to the use of even lower concentrations of biocides with lower

environmental impact (Pantazidou and Theoulakis, 1997). Ultraviolet rays have been used especially against bacteria, algae and fungi in the treatment of venders and plasters (Tiano, 1994). The part of the UV spectrum with germicidal activity is between 200 – 300 μm, with a maximum of activity between 230 – 275 μm. However, UV radiation can modify some materials such as cellulose, proteins and the colours (natural or dyes) of surfaces (Tiano, 1994).

Galeano *et al.* (2003) reported the antibacterial properties of a particular silver-zinc zeolite preparation, the AgION antimicrobial, applied to stainless steel sheets. The challenge organisms used were species of the genus *Bacillus*, chosen for their environmental ubiquity, their ability to form resistant spores, and their known roles as agents of food poisoning *(B.* cereus, *B. anthracis)*, food spoilage (*B. subtilis*), and biodeteriorism *(B. anthracis)*. It was observed that the silver-zinc zeolite present in AgION antimicrobial-coated stainless steel is effective at inactivating vegetative cells of the three *Bacillus* spp. tested. However, it should be stressed that the efficacy of the AgION antimicrobial was determined under laboratory conditions where parameters such as the suspension medium, ionic strength, pH, temperature and relative humidity were strictly controlled. Takai *et al.* (2002) have also demonstrated strain, species, and environment-species differences in the inactivation rates of various bacteria exposed to silver zeolites.

There is evidence to indicate that the antibacterial activity of silver zeolites results from the generation of reactive oxygen species (ROS) derived from dissolved oxygen in aerobic environments, including super oxide anions, hydrogen peroxide, hydroxyl radicals, and singlet oxygen, and that the antibacterial activity of silver zeolites can be inhibited by ROS scavengers (Inoue *et al.*, 2002). In this context, it makes sense that the different species of *Bacillus* used in the study exhibited greater or lesser sensitivity to antibacterial zeolites due to intrinsic differences in their abilities to handle oxidative stress (Herbig and Helmann, 2002). Furthermore, it is not surprising that dormant spores are resistant to silver-zinc zeolites owing to the high resistance to ROS conferred by the spore coat layers (Riesenman and Nicholson, 2000). Addressing such considerations could lead to the design and development of more effective antimicrobial coatings.

1.8. Alternate Biocides

Several reports have indicated different attempts with novel approaches to combat biofilm problems on painted walls. Copper sulfate has been applied to biofilms to inactivate coliform organisms (Geldriech and Stevens, 1986). This practice was based on the observation that copper can injure and kill coliform bacteria (Domek *et al.*, 1985). Martin *et al.* (1982) found that elevated pH helped control coliform bacteria, whereas Lowther and Moser (1984) found that application of zinc orthophosphate seemed to alleviate the problems. The exact reason for the success of zinc orthophosphate is not known. But the toxicity of zinc to microorganisms was thought to be a possible factor. LeChevallier *et al.* (1988) showed that these alternate biocides had some effectiveness on suspended bacteria but limited activity against biofilm microorganisms. The combined effect of the biocides with other disinfectants however, was not tested and could have some synergistic effect.

1.9. Predictive Modeling

Estimation of shelf life is critical to the successful marketing of a product. Variations in the production processes of paints may contribute to inconsistent product quality. Variation factors such as raw materials, production environment, packaging materials and storage conditions can influence the overall quality of a paint product as perceived by the consumer. The shelf life of any product can be predicted as the time required by the specific spoilage organisms to multiply from the initial level to a spoilage level (Dalgaard, 1995; Koutsoumanis and Nychas, 2001). Water-based paints are highly susceptible to biodeterioration (Gillat, 1992; Adeleye and Adeleye, 1999; Da Silva, 2003). Paint shelf life is influenced by a number of factors, such as initial microbiological quality, handling and packaging materials.

Several problems are related to the use of microbial, sensory, and biochemical methods of shelf life determination mainly due to time and sensitivity limitations. An alternative to these methods is predictive microbiology. Prediction of paint quality can improve significantly, its distribution and marketing (Koutsoumanis, 2001). The use of mathematical modeling of microbiological behaviour to predict and evaluate shelf life is receiving commendable

interest. Researchers are attempting to use mathematical equations that incorporate such critical growth factors as pH, a_w, and NaCl content to predict microbiological growth and/or toxin production in order to replace traditional time–intensive challenge studies. Predictive equations can be divided into probabilistic, regression, Arrhenius and square root models (Skinner *et al.*, 1994). The models can be used to predict time to reach a critical level under constant conditions within the range tested. Although, there is rapid progress in the field of chemical detection technology, little of this technology appears to have found application in the estimation of shelf life of products and early detection of spoilage (McMkeen and Ross, 2002). Predictive microbiology aims to summarize the probable behaviour of spoilage organisms and the progression of spoilage processes in different products. The quantitative knowledge generated in the field of predictive microbiology, provides a sound basis for the rational development of devices with which to monitor paint shelf life during storage, distribution and retail sale.

Applications of mathematical modeling for shelf life predictions require sufficient knowledge of the product spoilage mechanisms (Koutsoumanis and Nychas, 2001) and the specific spoilage organisms (SSO) (Gram and Russ, 1996). Koutsoumanis (2001) presented a mathematical model for the effect of temperature on the growth of the SSO (which was Pseudomonads), of aerobically stored gilt-head seabream. The data on Pseudomonad growth from 23 experiments with gilt head seabream stored at different isothermal conditions were collected and modeled as a function of temperature using a Belehradek type model. The ability of the model to predict microbial growth at non-isothermal conditions was evaluated.

The comparison between the observed and the predicted growth of Pseudomonads on gilt-head seabream under dynamic temperature models take a limited number of factors into consideration compared to the numerous factors influencing growth in paint products, this phenomenon named "completeness error" (McMkeen and Ross, 2002). Risk estimates for paint products will usually depend heavily on the numbers of microorganisms present in the paint at the time of use. As these data are seldom available directly, attention has turned to predictive microbiology as a means of inferring exposure at the time of use. The expression of microbial ecology knowledge in predictive microbiology models provides a better

understanding of shelf life using the tools of risk assessment (Ross and McMkeen, 2003). This is considered to be relevant in this study. The construction of predictive models involving equations that can use the information from a large data base to predict spoilage time was the core objective of this study.

1.10. Statement of the Problem

The paint industry suffers considerable economic losses as a result of microbial attack in the liquid state which results in spoilage and is evidenced by viscosity loss, gassing, malodour, discolouration and visible surface growth. Although, viscosity loss, a major indication of paint spoilage, can be treated with high level of broad spectrum biocide; subsequent rethickening is impossible as a result of cellulolytic enzymes produced by microorganisms which are usually unaffected by modern biocides. This seriously compromises the adhesion and durability properties of paint as well as its decorative function. The contamination occurs during production and poses greater problems when they are not detected until the paint reaches the end user, since there is no shelf life indication on the paints. In Nigeria, there are up to 100 paint manufacturing companies (Adeleye and Adeleye, 1999) but due to lack of appropriate regulatory acts, paints are produced without a shelf life indication. A number of paint industries have recorded numerous cases of foul smelling, contaminated paints being returned to them from buyers of the product and this results in significant wastage and losses. Due to the fact that paint manufacturers themselves are ignorant of the shelf life of paints, they continue to sell expired paints and paints that are near expiration to consumers. Therefore, the determination and indication of shelf life is a major challenge facing the paint industry, considering also the cost of repainting deteriorating painted walls.

The consequences of biofilms occurring on storey buildings of ancient civilization and contemporary buildings include aesthetic and structural damages on our cultural heritage. As aesthetic damage, one must consider pigment discolouration, stains and formation of paint biofilms on the painted surface, whereas as structural damage, one must consider cracking and disintegration of paint layers, formation of paint blisters and degradation of the support glues and binders resulting in the detachment of the paint layer from the support. Of course

the two types of damages are closely linked, and in the long run structural damage profoundly affects the aesthetic quality of a painted wall. Dark coloured fungi and cyanobacteria produce pigments that cause green, red, brown and black discolourations to be formed on buildings. Although, the pigments are useful to the microbial cells, protecting them against various types of stress, they add yet another weapon to the microbial arsenal against historic monuments. The removal of such deteriorated coatings results in potential health problems and high expenses (Wolf and Riley, 1964; Gaylarde *et al.*, 2003; Jacobs *et al.*, 2003). Fungal growth on a paint film normally appears as dark spots on the surface and is sometimes confused with dirt. Water supports germination, hyphal growth and sporulation of fungi. The spores produced become a source of pollution (Saad, 1992) and can be a significant health hazard to persons with respiratory allergies. Panaccione and Coyle (2005) reported the production of ergot alkaloids by *Aspergillus fumigatus*, a common airborne fungus and an opportunistic human pathogen (Prescott *et al.*, 2005) which has also been isolated from biodeteriorated painted walls (Adeleye and Adeleye, 1999).

In addition to the foregoing, the unrestricted use of lead in paint production to increase its shelf life and prevent deterioration has persisted primarily because the shelf life is not known. The problem has been traced to the fact that the shelf life of paint has been ignored by manufacturers. The importance in adhering to this strict manufacturing ethics cannot be overemphasized especially in a warm and humid environment, where deterioration is facilitated. The ingestion or inhalation of lead based paints causes irreversible damage to a child's developing brain, having a significant, long lasting impact on learning and behaviour (Bellinger *et al.*, 1992; Schwartz, 1994; Banks *et al.*, 1997; Satcher, 2000; Dietrich *et al.*, 2001).

Due to the potentially hazardous effects of lead-based paints, the federal legislation prohibited the use of lead paints in 1970 in the United States of America without industry opposition. Unfortunately, the various institutions vested with the statutory responsibility for overall determination of products' standards in Nigeria have not lived up to their expectations. It is not surprising, therefore, that today, hundreds of thousands of young

children suffer from lead levels that result in learning disabilities, hyperactivity, poor motor coordination, and other developmental deficits. It is well known that the major source of such poisoning is the lead paint applied to homes to improve their durability and shelf life (Schwartz, 1994; Lanphear *et al.*, 2000).

To date, there is no widespread existence and enforcement in African countries of legislation to control the use of lead in paints (Mathee *et al.*, 2007). Given the preventable nature of the use of lead and the serious consequences on children's health and educational attainment, there is need for greater vigilance and a more proactive approach to lead hazard prevention within African public health community, including improved surveillance and research to identify the full extent of sources and risk factors, as well as implementation of the most appropriate lead poisoning prevention mechanisms. Estimating the shelf life of paints therefore, is a critical step in evaluating new formulations. Against this background, therefore, the purpose of this study was explained and outlined.

1.11 Objectives of the Study

The broad goal of this research is to develop a predictive model for shelf life determination of water- based paints that are produced in Nigeria. The study therefore is set out to achieve the following objectives:

- To isolate and characterize microorganisms in the raw materials, stages of production, packaging materials used in paint production as well as from biodeteriorated wall scrapings.
- To identify microbial contaminants in paints, particularly the cellulose-degraders using standard cultural and biochemical methods.
- To determine the biodegradative activities of the isolates on physico-chemical parameters and chemical composition of fresh and spoilt paint samples.
- To determine the presence of lead and other heavy metals in paint samples.
- To carry out a biological assay of the biocides used in production and assess their efficacy in improving the shelf life of paints.
- To assess the growth pattern of isolates in freshly produced paint samples for spoilage potentials and determine their genetic composition for presence of plasmids with a view to ascertaining whether or not the spoilage potentials of the contaminants are plasmid-mediated.
- To determine the resistance of the cured strains to specific antibiotics in order to ascertain the effectiveness of the curing agent.
- To determine the shelf life of paints microbiologically and statistically by development of predictive models.

1.12 Operational Definition of Terms

ACRYLIC: A type of synthetic polymer used as the binder for high-performance water-based paints and sealants. Some acrylic polymers are used in "auto finishes appliance coatings" etc.

ADHESION: The ability of a dry paint film to remain attached to the surface.

AEROSOL: Container dispensing fine sprays of pressurized liquid paint etc.

ALKYD: A synthetic resin used in solvent-based paints. An alkyd resin is made by reacting a drying oil with hard synthetic material

ANTI-CORROSIVE PAINT: A paint designed to minimize rust or corrosion when applied directly to metal

ANTI-FOULING PAINT: specially formulated paint for surfaces such as boat hulls and piers. It discourages attachment and growth of marine plants and animals

ASH: A pale, powdery residue left from burning.

API: Analytical profile index

BINDER:

1. A component of a paint that "binds" the pigment particles into a uniform, continuous paint film, and makes the paint adhere to the surface. The nature and amount of binder helps determine most of the paint's performance properties – washability, toughness, adhesion, colour retention and durability.

2. In sealant, a component that "binds" the pigment particles into a homogeneous compound and makes the sealant adhere to the surface. The main performance properties of sealant - durability, adhesion and flexibility at low temperatures are determined by the binder.

BIOCIDE: A biologically active paint and sealant additive designed to keep bacteria from spoiling the paint or sealant during storage or to keep fungal and algal contamination from growing on the applied paint film.

BIOCOENOSIS

BIOFILMS: Biological growth.

COATING: A paint, stain, vanish, lacquer, or other finish that provides a protective and/or decorative layer over a substrate.

CONSORTIUM: A combination of different microorganisms

CORROSION RESISTANT: Ability of a substance to resist deterioration due to a chemical reaction with its environment. Coatings that do this usually contain a corrosion inhibitor.

DESICCA TOR: A compartment for thorough drying.

DURABILITY: The degree to which a coating or sealant can withstand the destructive effects of the environment which it is exposed. The term also refers to interior applications, including the ability to withstand scrubbing, abrasion, etc.

EDTA: Ethylene diamine triacetic acid

EMULSION: A mixture (usually milky -white) in which one liquid is dispersed (but not dissolved) in another. Water-based paint or sealant binder is often referred to as an emulsion, even though it is a dispersion of solid polymer particles in a liquid (water).

EXTENDERS: A low-hiding, inexpensive pigment that fills out and extends the high hiding and coloured pigment capabilities, provides bulk to the paint. Some common extenders are clay, calcium, carbonate and silica.

FLOW: The ability of a coating to even out upon application, so that brush and roller marks are not visible.

HIDING POWER: The ability of paint or stain to obscure the surface over which it has been applied. Hiding power is provided by the paint's pigment, and is affected by how thickly the paint tends to apply, and how well brush marks flow out.

INORGANIC MATERIALS: Matter other than that of animal or vegetable origin. For example, minerals or simple salts are inorganic materials.

LACQUER: Coating based on synthetic thermoplastic, film-forming material that is dissolved in organic solvent. Dries by solvent evaporation.

LATEX: A milky-white, fine dispersion of a solid resin in an aqueous medium. Also used to describe water-thinned paints, the principal vehicle of which is latex.

LATEX PAINT: Water-based paint made with a synthetic binder (latex), such as acrylic, vinyl acetate copolymer, or styrene acrylic latex.

LEAD: A soft malleable heavy metal. Compounds of lead are used as a white pigment and in primers to prevent deterioration.

LINSEED OIL: Drying oil obtained from flax seed. It is darker and slower drying than most other drying oils. Once widely used in coatings, it now has limited use in solvent-based house paint and oil wood finishes.

LOGARITHMIC PHASE: A phase of adaptation of microorganisms to a new environment. Growth is minimal in this phase.

MASONRY: Mineral-based building material such as cement, mortar, stone, brick and stucco.

MOISTURE: The ability of a coating to resist swelling, blistering or other damage caused by moisture.

RESISTANCE

MSDS: Material safety data sheet. An informational document provided by the manufacturer regarding the safety and handling procedure and precautions for paints.

ORGANIC MATERIALS: Refers to substance derived from living matter; the molecular structure contains carbon.

OXIDATION: A chemical reaction with oxygen. For example, the rusting of iron or steel.

PAINT: An opaque coating generally made with a binder, liquids, additives and pigments. Applied in liquid form, it dries to form a continuous film that protects and improves the appearance of a surface.

PIGMENT: A powdery substance that is one of the basic components of a paint or sealant. It provides whiteness or colour.

PLASTER: A compound used to coat or patch walls.

PHENOTYPIC PROFILES: A classification profile that groups organisms together based on the similarity of their observable characteristics.

POLYMER: A plastic-like material produced from chemical "monomers" which in turn have been produced from alcohols and petro-chemicals. Certain polymers are used as water-based paint and sealant binders. The binder's polymer particles are small and carried in water. The binder polymer particles and water mixture is blown as an emulsion or as "latex"

POLYURETHANE VARNISH: A clear coating that is based on a modified alkyd resin.

PYCNOMETER: A graduated density bottle.

RESIN: A natural or synthetic material utilized as the binder for a paint or sealant.

SHELF LIFE: The time between the production and packaging of paint and the point at which the paint becomes unacceptable under defined environmental conditions.

SOLVENT: A usually volatile liquid in which a paint's film-forming particles are dissolved or dispersed.

SPRAY: Method of application in which the paint is broken up into a fine mist and directed to the surface under pressure. Specific types of spray equipment are: aerosol, airless, and air-assisted.

SUBSTRATE: Any surface to which a coating or sealant is applied.

THINNER: A liquid that, along with the binder, forms the paint's vehicle. The thinner evaporates after the paint is applied. Water is the thinner used in water-based paint, while turpentine, mineral spirits and denatured alcohol are thinners associated with different solvent-based coatings; the liquid used to thin the coating.

TITANIUM DIOXIDE (TiO_2): An expensive, high opacity, bright white pigment that is used as a prime pigment in paints, both water and solvent-based.

TOXIC: Harmful or poisonous.

VEHICLE: The liquid portion of paint, in which the pigment is dispersed. The vehicle is composed of thinner and binder

VISCOCITY: The fluid thickness of a coating.

WATER-BASED PAINT: Paint made with acrylic, vinyl acetate copolymer or other latex resin types, and thinned with water. It dries more quickly than solvent-based paint, with relatively low odour, some water vapour permeability, and cleans up easily. The liquid component is predominantly water.

CHAPTER TWO

MATERIALS AND METHODS

2.1 Collection of Samples

Samples of raw materials for paint production, packaging materials, finished products including freshly produced paints, spoilt, foul smelling paints, from company warehouse and from different stages of production were randomly and aseptically collected from Chemical and Allied Products (CAPL) plc. a paint manufacturing industry located at Ikeja, Lagos, Nigeria. Paint samples from open markets at Mushin, Bariga and Ikeja which are major paint depots in Lagos metropolis were also randomly collected. Samples of wall scrapings from biodeteriorated painted walls were collected randomly in sterile screw-capped glass bottles. The samples were taken to the laboratory for immediate analysis.

2.2 Chemicals

Carboxymethyl cellulose (CMC), dinitrosalicylic acid (DNSA) and sodium dodecyl sulphate (SDS) were obtained from the National Institute for Medical Research (NIMR) Yaba, Lagos. All biocides coded: ZN481, ZN485, ZN489 and ZN467 were obtained from CAPL Ikeja, Lagos. Other chemicals such as HNO_3 (98% purity), $PbNO_3$, $CuSO_4$ and $MnSO_4$ were obtained from the Analytical Chemistry Laboratory, University of Lagos. Single discs (antibiotic discs) containing different antibiotics including tetracycline, gentamycin, ceftazidime, piperacillin, ofloxacin, ticarcillin, amikacin and tobramycin (Oxoid, Ltd Basingstoke Hampshire, England) were also obtained from the Biochemistry Department of the Lagos University Teaching Hospital (LUTH), Lagos. All chemicals were of analytical reagent grade.

2.3 Sterilization and Aseptic Techniques

Sterilization procedures were carried out as recommended by Pernier *et al.*, (2005). The work bench was thoroughly swabbed with absorbent cotton wool soaked in 70% (v/v) ethanol before each experiment. This was done to ensure that the working environment was free of contaminants. The incubators were periodically fumigated with phenol and 70% (v/v) ethanol to reduce contamination during incubation. The glassware such as screw-capped

glass bottles, conical flasks, test tubes etc., were washed thoroughly in detergent solution, rinsed in clean tap water and allowed to air-dry before sterilization in the oven at 170 °C for 3 h. Eppendorf tubes and pipette tips were placed in glass jars and covered appropriately while filter papers were wrapped in aluminium foil paper. They were then sterilized by autoclaving at 121 °C for 15 min. The inoculating loops and spatulas were sterilized by dipping in absolute ethanol before flaming them over a Bunsen flame until red hot. They were also allowed to cool before use. The sterilization of hockey sticks was carried out by dipping in absolute alcohol, igniting in a Bunsen flame to burn off the excess alcohol. They were subsequently allowed to cool before use.

2.4 Microbiological Studies

2.4.1 Media and Reagents

The growth media used in the study, including solid and liquid media were prepared by pouring appropriate quantities recommended by the manufacturer (Oxoid) into conical flasks, plugging the flasks with non-absorbent cotton wool and wrapping the cotton wool and the neck of the flasks with aluminium foil. They were subsequently sterilized by autoclaving at 121 °C for 15 min except otherwise indicated. Paint formulations and diluents were similarly sterilized as the media. The sugar solutions for identification tests were tyndallized by heat-steaming for 30 min daily at 100 °C for three days (Sahin *et al.*, 2002).

2.4.1.1 Liquid Media

Czapek's broth medium of Miller, (1959) was used for the growth studies of cellulose-utilizing microorganisms. Yeast extract trypticase soy (YETS) containing 0.002% SDS was used for the curing of plasmids and Tryptose soy broth was used for the standardization of inoculum as recommended by the National Committee for Clinical Laboratory standards (NCCLS, 1997). Other liquid media used in the study include nutrient broth and peptone water. The chemical compositions of these media are presented in Appendix III.

2.4.1.2 Solid Media

The solid media prepared and used during this study include nutrient agar (NA), potato dextrose agar (PDA), MacConkey agar (MCA), Yeast extract trypticase soy (YETS) agar, nutrient gelatin and starch agar. Other solid media used in the study include Czapek's agar medium for cellulose-utilizers in which carboxymethyl cellulose (CMC, 10 g/l) replaced sucrose as the sole energy and carbon source (Miller, 1959). The Mueller Hinton antimicrobial susceptibility test agar (MHA) was used for disc diffusion biocide and antibiotic susceptibility tests according to the National Committee for Clinical Laboratory Standards (NCCLS, 1997) guidelines. The chemical compositions of these media are also found in Appendix 111.

2.5 Isolation of Microorganisms from Various Samples:

2.5.1 Solid Raw Materials

The total heterotrophic microorganisms isolated from 23 solid raw materials, employed in paint production by CAPL, Ikeja, Lagos were enumerated. The raw materials were coded as MN288, MN239, MN277, MN409, MN280, MN231, MN236, Z4726, MN236X, Z4441, MN252, MN261, ZN490, RN300, RN375, ZN476, Z4740, L1140, ZN465, ZN899, ZN470, NN241 and MN286. A known quantity (10.0 g) of each raw material sample was aseptically obtained by weighing with a balance (Model: Mettler PM460 Delta Range) and placed in a universal bottle containing 90ml of sterile distilled water to give 10^{-1} dilution. The content of the bottle was mixed thoroughly by shaking vigorously to obtain a homogenous suspension. Higher dilutions up to 10^{-8} were then made from this dilution (10^{-1}). Subsequently, aliquots of 0.1 ml from both low (10^{-2}, 10^{-4}) and high dilutions (10^{-6}, 10^{-8}) of each raw material sample were plated onto nutrient agar (NA) and potato dextrose agar (PDA) for total heterotrophs. This was done in triplicates. All the NA plates were incubated aerobically at 37 ^{0}C for 1 – 2 days for isolation of bacteria and the PDA plates at room temperature (30 $\pm$ 2 ^{0}C) for 3 – 6 days for isolation of fungi (Collins and Lyne, 1976). At the end of the incubation, the plates were observed for developed colonies. They were counted and reported as colony forming units per gram (cfu/g). For the total coliform counts present in the raw

materials, aliquots (0.1 ml) from 10^{-1} – 10^{-4} dilutions were separately plated out by spread-plate technique onto MCA plates (a selective medium for coliforms) in three replicates. The plates were incubated aerobically at 37 ^{0}C for 18 – 24 h (Collins and Lyne, 1976). At the end of the incubation, the colonies that developed with characteristics typical of coliforms were enumerated.

2.5.2 Biocides

Four different biocides coded as ZN481, ZN485, ZN489 and ZN467 were analyzed for microbial population levels. Each of the biocides was serially diluted as follows: Ten ml of each biocide sample was transferred to universal bottles containing 90 ml sterile distilled water to give 10^{-1} dilution from which higher dilutions up to 10^{-3} were made. Aliquots (0.1 ml) from all the dilutions of the biocide samples were plated on NA, MCA and PDA respectively in triplicates. All the NA and MCA plates were incubated aerobically at 37 ^{0}C for 1 – 2 days for isolation of heterotrophic bacteria and coliforms. The PDA plates for isolation of fungi were incubated aerobically at room temperature (30$\pm$ 2 ^{0}C) for 3 – 6 days (Collins and Lyne, 1976). After the incubation, the developed colonies were counted, purified and identified. The relative abundance (population density) of the organisms in each sample was estimated by multiplying the plate count per ml for each sample by the dilution factor used (Nwachukwu and Ugoji, 1995).

2.5.3 Water Samples

Water samples coded as $HSDH2_0$, $MUH2_0$ and $CDH2_0$ which are sources of water inlets into the factory were analyzed for total heterotrophic microorganisms by the standard plate count technique using the spread-plate method. The NA and PDA plates were prepared and used for isolation of bacteria and fungi respectively. Aliquots (0.1 ml) from 10^{-3}, 10^{-5} and 10^{-7} dilutions of water samples were plated on the above media in triplicates, spread aseptically and incubated aerobically with NA plates at 37 ^{0}C for 1 – 3 days and PDA plates at room temperature (30 $\pm$2 ^{0}C) for 3 – 6 days. The total coliform count in the water samples, were isolated on MCA as described for NA. At the end of the incubation period, developed colonies were counted and purified.

2.5.4 Samples at Stages of Production

Paint samples were aseptically collected at different stages of production to ascertain the points at which contamination occurs. The stages include (i) dispersion, (ii) making up, (iii) texturing, (iv) tinting and (v) filling. Ten gram of the paint samples from each stage were dissolved in 90 ml sterile distilled water and shaken properly to obtain a homogenous suspension. Ten-fold serial dilutions of the paint suspensions were made. Aliquots (0.1 ml) from 10^{-2}, 10^{-4}, 10^{-6} and 10^{-8} dilutions were plated out on NA, PDA and MCA in triplicates. The plates were subsequently incubated as described above. After incubation, the developed colonies were counted and purified.

2.5.5 Packaging Materials

Six different packaging materials (plastic and metal containers) with sizes ranging from 4 – 20 liters and designated C/T 20LTS, D/W/S Plastic, C/20 LTS, D/W/S Metal, D/E 20LTS and D/E 4LTS were examined for the presence, types and numbers of microorganisms. To achieve this, sterile swab sticks were used to swab the inside of the packaging containers in triplicates Each swab was then used to inoculate NA, PDA and MCA plates respectively for enumeration of the total heterotrophic bacteria and coliforms. In addition, the containers were dipped and immersed into 150 l sterile distilled water for 5 min after which they were removed. Ten ml of this water sample was transferred aseptically to a universal bottle containing 90 ml sterile distilled water to give a 10^{-1} dilution from which higher dilutions (10^{-2} –10^{-8}) were made. Aliquots (0.1 ml) from 10^{-3}, 10^{-5} and 10^{-7} dilutions were plated out on NA, MCA and PDA plates respectively. The inoculated plates were incubated appropriately as described above. At the end of the incubation period, the colonies that developed were counted, purified and identified.

2.5.6 Paint Samples

Six paint samples (PS1 – PS6) were collected aseptically and randomly on the day of production in 4 l plastic containers. They were monitored for microbial growth at intervals of 2 wk for a 10-month study period by adopting the standard plate count technique. The

paints were stirred continuously for 3 min inside the cans for proper mixing before samples were taken. Ten gram of each paint sample was weighed into 90 ml of sterile distilled water and shaken vigorously. The paint samples dissolved readily in water. Aliquots (0.1 ml) from both low (10^{-2}, 10^{-4}) and high (10^{-6}, 10^{-8}) ten-fold serial dilutions of paint samples were plated on NA, MCA and PDA plates respectively in triplicates. The plates were incubated aerobically with NA and MCA plates at 37 ^{0}C for 1 – 3 days and PDA plates at room temperature (30±2 ^{0}C) for 3 – 6 days. The microbial population counts in spoilt paint samples (PSA, PSB, PSC, PSD and PSE) with evidence of viscosity loss, biofilm formation, malodour, discolouration, gassing and visible surface growth were enumerated as described for the fresh paint samples. Paint samples from open markets (Mushin, Bariga and Ikeja) as well as paint samples from company warehouse in storage were also investigated for total heterotrophic counts using the same method. The plates were subsequently incubated appropriately as described above.

2.5.7 Biodeteriorated Painted Films

Ten gram of wall scrapings from biodeteriorated painted walls were collected randomly and aseptically into sterile screw-capped glass bottles with the aid of a sterile spatula. They were placed in 90 ml sterile distilled water, mixed thoroughly and filtered with filter paper to obtain a homogenous solution. Ten-fold serial dilutions were made and aliquots (0.1 ml) obtained from 10^{-3}, 10^{-5} and 10^{-7} dilutions were plated onto NA plates for the isolation of bacteria and PDA plates for the isolation of fungi in triplicates. For the total coliform counts present in the samples, aliquots (0.1 ml) from 10^{-1} – 10^{-4} dilutions were separately plated out by the spread-plate technique onto MCA plates in triplicates. The inoculum was uniformly spread with a sterile hockey stick and the plates incubated aerobically as described above (Collins and Lyne, 1976). The developed colonies were counted and recorded.

2.6 Isolation of Cellulose-Utilizing Organisms

Bacteria capable of utilizing cellulose as the sole carbon and energy source were isolated on Czapek's agar medium as described by Saad (1992). The medium contained carboxymethyl cellulose (CMC, 10 g/l) which replaced sucrose as the sole source of carbon and energy. This test was carried out to identify cellulose-degraders in paints because of their role in

hydrolysing paint thickeners, leading to irreversible loss in viscosity and eventual loss of the paint. Erlenmeyer flask (250 ml) containing 250 ml Czapek's medium was sterilized by autoclaving at 121 ^{0}C for 20 min. Aliquots (0.1 ml) from both low (10^{-2}, 10^{-4}) and high (10^{-6}, 10^{-8}) ten-fold serial dilutions of the various paint samples were plated onto Czapek's medium in triplicates and incubated aerobically at 37 ^{0}C for 24 h. The isolation of cellulose-utilizing fungi was carried out in the same manner as described for bacteria. Rose Bengal reagent (0.5%) was incorporated into the medium as a bacteriostatic agent (Baggerman, 1981) prior to sterilization. The plates were incubated aerobically at room temperature (30±2 ^{0}C) for 7 days. The composition of the medium is presented in Appendix 111.

2.7 Rate of Cellulose Utilization

Pure cultures of bacteria and fungi isolated from the cultural conditions described in section 2.6 were screened for the rate of utilizing cellulose as sole carbon source in Czapek's broth medium as described by Saad (1992). At least four colonies from an overnight's culture on Czapek's agar plates were directly suspended in 4 ml Czapek's broth in tubes so that the turbidity matches the turbidity of the 0.5 Mc Farland standard. The tubes were incubated aerobically at 37 ^{0}C for 2 days for bacteria and at room temperature (30 ± 2 ^{0}C) for 7 days for fungi For the fungal cultures, 0.5% of Rose Bengal reagent was added to the broth medium to inhibit bacterial growth. The turbidity (OD) of the cultures was determined at 550nm everyday of the incubation as a function of growth. After the incubation, the culture medium was filtered and the filtrate was used as crude enzyme.

2.8 Endoglucanase Activity of the Isolates

Endoglucanase activity was assayed using carboxymethyl cellulose (CMC) as enzyme substrate according to the procedure of Saad (1992). One ml crude enzyme was added to a test tube containing 2 ml enzyme substrate (0.5% CMC in 0.05 M sodium citrate buffer, pH 5.5). The assay tubes were incubated at 50 ^{0}C for 24 h. To this enzyme-substrate mixture, 1 ml DNSA reagent was added to stop the reaction and the tubes were kept in boiling water bath for 5 min. After cooling, the volume was made up to 10 ml with sterile distilled water and centrifuged for 5 min at 5000 rpm to separate the product from the unused substrate. The extinction of supernatant was read at 620 nm with a spectrophotometer (Model: Optimal SP

3000). The concentration of the assayed tubes was then extrapolated from a 0.1% glucose standard curve to determine the concentration of glucose released. One unit of enzyme activity (IU) was defined as the amount of enzyme that produced 1 µmol glucose/min.

2.9. Identification and Biochemical Characterization of Pure Cultures

2.9.1.Fungal Isolates

For identification of fungi, a sterile inoculating needle was used to remove a small piece of mycelium free of medium. A drop of cotton blue-in-lactophenol was placed on a clean slide as a stain and mountant. The mycelium was then transferred to the slide and teased out carefully. The slide was subsequently covered with a cover slip and examined under the x 40 lens of the light microscope (Smith, 1969).

2.9.2.Bacterial Isolates

Identification of pure cultures of bacteria isolated from the various samples was carried out using standard cultural, morphological, taxonomic and biochemical characteristics (Cowan and Steele, 1970; Buchanan and Gibbons, 1984; Dalsgaard *et al.*, 1998; Kelly and Wood, 1998; Muller *et al.*, 2001; Coenye *et al.*, 2002).

2.9.2.1 Colonial morphology

The variations in the bacterial colonies that developed on agar surfaces which indicate the shape, size, colour, elevation, margin and pigment production by the bacterial isolates were observed on NA plates, which had been previously incubated aerobically for 24 – 48 h at 37 ^{0}C.

2.9.2.2 Gram Reaction

The Gram staining technique and macroscopic morphologies are the initialkey features for the identification of bacteria. Gram-positive cell walls contain large amount of

peptidoglycan and teichoic acids which consists of polymers of ribitol phosphate and /or glycerol phosphate. This factor enhances the effect of the mordant in intercalating the crystal violet on the cell which makes them retain the crystal violet more than the Gram-negative cell walls. The experiment was performed to broadly classify bacteria into 2 main groups. The Gram staining experiment was carried out with the initial preparation of a thin smear of each of the isolates from an overnight culture on clean glass slides. This was air-dried and subsequently heat-fixed by passing the slide horizontally across a Bunsen flame. The smear was then covered with crystal violet stain for sixty seconds and rinsed with slow running tap water. The smear was then flooded with Lugol's iodine for one minute to fix the crystal violet stain more firmly into the cell before washing gently under slow running tap water. The smear was then decolourized with absolute ethanol to wash off as much crystal violet as can be removed. After the decolourization, the slide was rinsed again under tap water. This was followed by counter-staining with safranin for 30 seconds and further rinsing with tap water. The slide was subsequently blotted-dry with a piece of filter paper and allowed to air-dry. Microscopic examination was then carried out under the oil immersion lens of the light microscope. The Gram-positive organisms were characterized by retention of the purple colour of the crystal violet stain while the Gram-negative organisms retained the reddish pink colour of the counter stain (Murray *et al.*, 1994).

2.9.2.3 Phenotypic Profiles of Isolates

The analytical profile index (API) identification (ID 32 E) test systems (bioMerieux Vitek, Inc. Hazelwood, MO. USA) consisting of 32 microtubes containing dehydrated substrates in a strip was used. The system, a standardized identification system for Enterobacteriaceae and other non-fastidous, Gram- negative rods was employed to determine the phenotypic profiles of the persistent and repeatedly isolated bacterial strain (OB-6) in spoilt paints and biodeteriorated wall scrapings and as a confirmatory test for the identifications obtained conventionally. Also, the regularly occurring coliform (OB-4) in all the fresh paint samples was characterized phenotypically along side (OB-5) using the API 20 E test system. The preparation of the inoculum which was used immediately was done by aseptically picking one colony from a young culture (18 – 24 h) and emulsifying in 2 ml of an 0.85% ampule of API NaCl. The bacterial suspension was prepared to match the turbidity equivalent to the 0.5 Mc Farland (10^8 cells per ml) to achieve a homogenous bacterial suspension. The strips containing the microtubes were inoculated with 55 µl of the homogenised bacterial

suspension, dispensed into each cupule of the strip using a micropipette. The strips were subsequently incubated at 37 ^{0}C for 18 – 24 h under aerobic conditions. At the end of the incubation, the dehydrated substrates were reconstituted by the bacterial suspension. During incubation, metabolism produced colour changes that were either spontaneous or revealed by the addition of reagents. The reactions obtained were coded into a numerical profile on the result sheet and used to generate identification from the API identification software (APIWEB) (Grisez *et al.*, 1991).

2.10. Determination of the Physico-chemical Parameters of Paint Samples

2.10.1 Determination of Specific Gravity

Specific gravity determinations of paint samples were carried out by pycnometry as described by Ohwoavworhua and Adelakun (2005). A pycnometer of approximately 50 ml capacity was washed, dried in the oven and placed in the desiccator to cool to room temperature before removal. It was weighed and the weight recorded as M1. Paint sample (50 g) was transferred into the pycnometer. The pycnometer with its content was weighed and the weight recorded as M2. The pycnometer containing the sample was filled with distilled water and shaken many times to allow all trapped air within the pycnometer to be expelled. Then, the pycnometer and its content were weighed and the weight recorded as (M3). The contents of the pycnometer were removed and the pycnometer was washed, cleaned and then refilled with distilled water. The outer surface of the pycnometer was dried, using a tissue paper, carefully weighed and the weight recorded as (M4.) The specific gravity of the paint sample was calculated.

$$\text{Specific Gravity (SG)} = \frac{M2-M1}{(M4-M1)(M3-M2)}$$

Where (in grams)

M1 is the mass of the pycnometer

M2 is the mass of the pycnometer and paint

M3 is the mass of the pycnometer, paint and water

M4 is the mass of the pycnometer and water

2.10.2 Determination of Optical Density

A rapid, simple method of estimating growth with fine optical instrument was adopted as described by Rieck *et al.*, (1993). This is because of its accuracy in measuring growth for physiological studies. The instrument measures the ratio of the intensity of incident light (I_0) to the intensity of the light beam leaving the cuvette (I) and the optical density (OD) of the paint sample log (I_0/I) as proportional to the cell density. The turbidimetric measurements of cell mass was done to monitor microbial growth with time in fresh and spoilt paint samples as a key factor in determining the shelf life of paints. The photoelecrtric colorimeter (Model: AE- 11C Tokyo Erma Optical works, Ltd Japan) used in the study was standardized by adjusting it to read 100% light transmittance with 5 ml of distilled water in a cuvette placed in it at 600 nm. Five milliliters each of serially diluted paint samples obtained from 10^{-3}, 10^{-5} and 10^{-7} dilutions were placed in a cuvette with a known diameter (1 cm). The optical density (OD 600_{nm}) determinations were done forthnightly for 10 months.

2.10.3 Determination of Transmittance

The transmittance (%) of the fresh paint samples and the spoilt paint samples was determined by a rapid, sensitive technique based on the fact that microbial cells scatter light striking them and the amount of scattering is directly proportional to the biomass of cells present and indirectly related to the cell number (Prescott *et al.*, 2005). As the microbial population density and turbidity increase, more light was scattered and the absorbance reading given by the colorimeter increased. The colorimeter had two scales. The bottom scale displayed the absorbance and the top scale, % transmittance. Absorbance increased as % transmittance decreased. The transmittance of the paint samples were determined to monitor the changes in paint quality with time. The instrument was standardized as described above. Five milliliters of serially diluted paint suspensions obtained from 10^{-3}, 10^{-5} and 10^{-7} dilutions were poured in 1 cm glass cuvette and placed in the photoelectric colorimeter (Model: AE-11 Tokyo Erma works Ltd, Japan) at 600 nm wavelength. The results displayed were read and recorded as explained in section 2.11.2.

2.10.4 Determination of Viscosity

The viscosity of paint samples being a rheological property involved in the resistance of the paint to flow was determined. This is because viscosity has a significant impact on the quality and shelf life of paints and is in fact, a quality control parameter for water-based paints. The viscosity of fresh and spoilt paint samples was determined according to the procedure of Rao and Yaseen (2003) using a glass capillary tubular viscometer (Model: Capirograph Toyoseikl Seisaku-Sho Ltd). The paint sample was allowed to flow through an outlet tube (measuring tube which is narrowed into a capillary tube above the outlet. Two annular reference marks on the measuring tube were used. The time it took for the sample meniscus to drop from the upper to the lower reference mark was measured manually with a stop watch. The viscosity of the paint sample was calculated by multiplying the measured time by the viscometer calibration factor at room temperature. This was done at intervals of two weeks for a 10-month study period.

2.10.5 Measurement of Mean pH

During the exponential phase of growth, microorganisms divide at a maximum, constant rate. As a result of their growth and proliferation in paint samples, acidic metabolites are produced which alter the pH of the paint samples. Therefore, the pH of the fresh paint samples was determined as a key factor in shelf life prediction at intervals of 2 weeks for a 10-month study period. The pH of spoilt paint samples was also determined with the use of a digital pH meter (Model: Jenway M50/Rev model CE 350 EU) in 1: 200 solution of the paint samples in distilled water. The pH meter was calibrated using phthalate buffer (pH, 4.0) and phosphate buffer solutions (pH, 7.0). After standardizing the pH meter, the electrode was inserted in a beaker containing 100 ml distilled water to rinse it. It was subsequently inserted in the paint suspension in a beaker and allowed to stand for 10 – 15 min before the reading was taken. The final reading was recorded.

2.10.6 Determination of Phosphate Concentration

Phosphate is an important component of most water-based paints and the concentration changes with time, which may be due to its participation in some chemical reactions going on in the paint cans or due to its utilization for growth by microbial contaminants present in paints. Consequently, the determination of phosphate concentration in the paints is an important quality parameter for shelf life determination of water-based paints and was determined spectrophotometrically as described by Chiyo *et al.* (1988). To achieve this, 5 g of the paint sample was weighed into a clean pre-weighed crucible and ashed in a furnace (Model: Carbolite Furnaces, Bamford Sheffield Eurotherm EML 5302AU, England) for 1 h 30 min at 555 ^{0}C. The sample was then removed and allowed to cool after which 10 ml of concentrated 2 M HNO_3 was added. The mixture was boiled for 30 min on a hot plate until the brown fumes of the nitric acid evaporated. The sample was then made up to 100 ml with distilled water to neutralise the effect of the acid and subsequently filtered with a filter paper into 100 ml standard flask. Ten ml of this was transferred into a beaker. One powder pillow (1 g) of HACH permachem phosphate reagent (HACH Company World Hq Loveland, C080539 USA) was added, the mixture was stirred and allowed to stand for 5 min. The concentration of phosphate in the sample was determined using the spectrophotometer (Model: Spectronic 20D+Thermoelectron Corporation) at 880 nm wavelength and subsequently calculated using the formula:

$$\frac{\text{Instrument reading}}{10} \times \frac{1000}{\text{Weight of Sample}}$$

2.10.7 Determination of Sulphate Concentration

Sulphates are of considerable concern because they are indirectly responsible for the serious problem of odour in spoilt paints. These problems result from the reduction of sulphates to hydrogen sulphide under anaerobic conditions. The total sulphate content in the fresh paint and spoilt paint samples was determined spectrophotometrically as described in section 2.10.6 (Chiyo *et al.*, 1988). The paint samples were ashed, digested and subsequently filtered. One powder pillow (1 g) of HACH permachem sulphate reagent (HACH Company World Hq Loveland, C080539 USA) was added. The mixture was stirred and allowed to stand for 5 min. The sulphate content in the paint samples were then determined

spectrophotometrically with the spectrophotometer (Model: Spectronic 20D+ Thermoelectron Corporation) at 450 nm wavelength and subsequently calculated as indicated in section 2.10.6.

2.10.8 Determination of Heavy Metals

The presence of Pb, Cu and Mn in fresh paint samples, spoilt samples and paint samples stored in company warehouse was determined using the flame atomic absorption spectrophotometer (AAS) as described by Stafilov and Zendelovska (2002). This was done to ascertain the levels of Pb and other metals in connection with the shelf life of paints. The stepwise procedure involved the digestion of 5 g paint sample with 10 ml concentrated nitric acid (98% purity), evaporation of the sample on a hot plate in a fume cupboard until the brown fumes disappeared. The remaining solution was transferred to a 100 ml standard flask, made up to mark with distilled water and filtered with a filter paper into a plastic bottle prior to analysis. Stock solutions of 1000 ppm were prepared for Pb, Cu, and Mn by weighing 1.6 g $PbNO_3$; 2.5 g $CuSO_4$ and 3.1 g $MnSO_4$ in 1l distilled water each in the 1000 ppm stock standard. Working standard solutions of 2 ppm, 4 ppm and 6 ppm were then prepared from the stock solution and were used in preparing a standard curve for instrument calibration. The absorbance of the working standard was read at 279.48 nm, 324.75 nm and 279.43 nm for Pb, Cu and Mn respectively. The concentration of the Pb, Cu and Mn in the digested paint sample was then determined by aspiration of the digested filtrate with the flame AAS (Perkins Elmer-Analyst 200), determining the absorbance value and extrapolating from the standard curve obtained from the working standards.

2.11. Bioassay of Biocides

2.11.1 Biocide Susceptibility Determination.

The test was carried out by following the protocol of Bauer (1966) to reveal the inhibition of the growth of the test organisms by varying concentrations of different biocides and hence, their varying efficacies. Three concentrations namely: 1%, 3% and 5% of each biocide were prepared in screw-capped bottles. Discs (5 – 6 mm) were punched from a sheet of filter paper, placed in a Petri dish and sterilized in the oven at 160 ^{0}C for 1 h. After cooling, 20 µl of the different concentrations was pipetted on each disc and allowed to stay for 20 min. The

discs were then dried in the incubator at 37 ^{0}C for 1 h. Subsequently, the discs were carefully placed on MHA plates previously inoculated with *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Lactobacillus gasseri* and a consortium of the three organisms. The MHA plates were poured to a depth of 4 mm (about 25 ml per plate) in 90 mm diameter sterile Petri dishes. The inocula were prepared by harvesting colonies from overnight's culture plates and suspending same in tryptose soy broth at a density of about 10^8 cells per ml. Sterile cotton wool swab was dipped into the suspension and surplus removed by rotation of the swab against the side of the tube above the suspension level. The MHA plates were then inoculated by even streaking of the swab over the surface of the plates. The plates were incubated aerobically at 37 ^{0}C for 24 h. The control plates were inoculated but they had no biocide. At the end of incubation, clear zones around the discs were observed and the photographs taken.

2.11.2 Effects of Different Concentrations of Different Biocides on Microbial Population Count of Fresh, Sterile Paints.

The antimicrobial activities of biocides differ. The test was carried out to ascertain the levels of efficacy of different biocides at different concentrations on fresh paint, in order to determine their usefulness in paint preservation. Paint samples (50 ml) without biocides were sterilized by autoclaving at 121 ^{0}C for 20 min. Each of the four biocides designated ZN481, ZN485, ZN489 and ZN467 were inoculated into the fresh sterile paints at concentrations ranging of 0.5% (v/v), 1% (v/v), 2% (v/v), and 3% (v/v) respectively. In addition, the sterile paint samples were inoculated differently with *Pseudomonas aeruginosa*, *Proteus mirabilis*, and *Aspergillus niger* at a density of 10^2 cfu/ml each. The control samples were inoculated with the organisms but not the biocides. The inoculated paint samples were then serially diluted. Aliquots (0.1 ml) from 10^{-2}, 10^{-4}, 10^{-6} and 10^{-8} dilutions were plated out on NA, MCA, and PDA. This was followed by aerobic incubation of the plates at 37 ^{0}C for 24 h for the NA plates and at room temperature (30 $\pm$2 ^{0}C) for 5 days for the PDA plates. After the incubation, the developed colonies were counted and identified.

2.11.3 Effects of Different Concentrations of Different Biocides on Physico-chemical Parameters of Fresh Sterile Paints

The test was carried out as described in section 2.11.2. However, the sterile paint samples were inoculated with *Proteus mirabilis* in addition to the different concentrations of biocides. The optical density, specific gravity, pH, transmittance and viscosity of the samples were monitored as described in section 2.10. This was carried out at 2 wk intervals during the study period.

2.12 Antibiotic Susceptibility Tests

The antibiotic sensitivity of *Pseudomonas aeruginosa* which occurred regularly and only in spoilt paints and biodeteriorated wall scrapings to 8 antibiotics was determined using the Kirby-Bauer disc diffusion technique (Bauer, 1966). The purpose of this experiment was to determine the agar disc diffusion zone diameters and hence the susceptibility of *Pseudomonas aeruginosa* to selected antibiotics. Single discs (Oxoid Ltd. Basingstokes Hampshire, England) containing tetracycline (10 µg), gentamycin (10 µg), ceftazidime (30 µg), piperacillin (100 µg), azlocillin (75 µg), ticarcillin (75 µg), amikacin (30 µg) and tobramycin (10 µg) were employed for the test. The overnight cultures were harvested from NA plates and emulsified in tryptose soy broth. The tubes were incubated aerobically for 2 – 5 h at 37 ^{0}C to produce a bacterial suspension of moderate cloudiness. The density of the suspension was standardized by dilution with tryptose soy broth to a density of about 10^{8} cells per ml. The plates were incubated within 15 – 30 min of inoculum preparation so that the density of the inoculum does not change. A sterile cotton wool swab was dipped into the suspension and the surplus removed by rotation of the swab against the sides of the tubes above the fluid level. The medium was inoculated by even streaking of the swab over the entire surface of the plate. Single discs containing different antibiotics were then placed one per plate in the center of the plates using sterile forceps. The plates were subsequently incubated aerobically for 16 – 18 h at 37 ^{0}C. The inhibition zone diameters were measured to the nearest millimeter with a millimeter rule. Classification of organisms as susceptible or resistant was based on guidelines from the National Committee for Clinical Laboratory Standards (NCCLS, 1997).

2.13. Detection of Plasmids.

Plasmids are extra chromosomal, closed, circular DNA materials capable of autonomous replication in bacterial cells. They are usually small and double stranded. The test was carried out to determine if the gene for paint degradation, antibiotic resistance and biocide resistance is plasmid-mediated or not. Two different protocols were adopted for detection of plasmid DNA from the isolated bacterial strains. The first utilized the Alkaline lysis method of Birnboim and Doly (1979). This protocol was used for the Gram-negative isolates. The stepwise protocol involved cell lysis, precipitation of plasmid DNA, agarose gel electrophoresis and viewing of the amplified plasmid DNA bands over UV (ultraviolet) transilluminator. The thicker peptidoglycan cell wall layer of the Gram-positive isolates requires the use of lystostaphine or butanolysine to lyse the strong cell wall which is not provided for in the alkaline lysis procedure hence, a second method, (TENS Mini-prep procedure) (Leeh and Brent, 1987) was adopted for plasmid DNA isolation in the Gram-positive isolates.

2.13.1 Alkaline Lysis Procedure

The alkaline lysis procedure was carried out as follows: Overnight isolates grown on NA plates and incubated at 37 ^{0}C were harvested into sterile eppendorf tubes containing 100 µl of washing buffer (50 mM glucose; 25 mM Tris HCL, pH 8.; 10 mM EDTA, pH 8.0). The contents of the tube were mixed vigorously by vortexing and subsequently stored on ice. With the aid of a micropipette, 200 µl of the lysing buffer (0.2 N NaOH, 1% Sodium dodecyl sulphate) was added to the tube. The tube was mixed gently by inverting it for about 5 times and then stored on ice. To this mixture, 150 µl of an antilysing buffer (60 ml, 5 M Potassium acetate, 11.5 ml Glacial acetic acid and 28.5 ml distilled water) was added. The mixture was vortexed for 10 seconds and the tube was stored on ice for 5 min. The tube was centrifuged at 12,000 x g for 10 min at 4 ^{0}C and the supernatant was transferred to a fresh tube. To the supernatant, 500 µl of an equal volume of phenol / chloroform (1:1) was added. The contents of the tube was mixed by vortexing and then centrifuged at 12,000 x g for 10 min at 4 ^{0}C. The supernatant was transferred to a fresh eppendorf tube. This step was repeated twice to remove further impurities. The mixture then separated into three layers in the tube. The topmost layer solution contained the DNA. the interphase solution contained

the cell impurities and the bottom layer solution contained the phenol chloroform. The supernatant was transferred to a fresh eppendorf tube, mixed gently by inverting the tube and vortexed. The mixture became cloudy. The mixture was further centrifuged at 12,000 x g for 10 min. Ice-cold absolute ethanol (500 µl) was added to precipitate the DNA out of solution. Following precipitation, the contents of the tube was mixed by vortexing and then allowed to stand for 2 min at room temperature. The tube was subsequently centrifuged at 12,000 x g for 15 min at 4 ^{0}C in a microfuge. The supernatant was removed by gentle aspiration and the tube inverted over a towel to dry. The precipitate (plasmid) was then visible. The precipitate was washed with 1 ml of 70% (v/v) ethanol at 4 ^{0}C. This was repeated twice followed by drying in gyrovap for 5 min. The nucleic acids were re-dissolved in 50 µl of TE buffer (1.21 g, 10 mM Tris, 0.37 g, 1 mM EDTA, 1000 ml Distilled water, pH, 8.0). DNA ase free RNA was vortexed and stored at -2 ^{0}C before use (Birmboim and Doly, 1979).

2.13.2 TENS–Mini Prep. Procedure

This is a modified alkaline lysis procedure which is extremely quick and reliable. The test was performed according to the procedure of Leeh and Brent (1987). The procedure involved the use of TENS solution (TE buffer containing 0.1 N NaOH and 0.5% sodium dodecyl sulphate). Overnight broth culture was spinned for 10 seconds in a microfuge to pellet cells at 1000 rpm. The supernatant was then gently removed, leaving 50 – 100 µl together with cell pellet. This was then vortexed at high speed to re-suspend the cells completely. Three hundred µl of TENS was added and the suspension was mixed for 2 – 3 min until the mixture became sticky. The tubes were constantly stored on ice after each step to prevent them from the degradation of chromosomal DNA which may be precipitated with plasmid DNA. To this mixture, 150 µl of 3.0 M sodium acetate (pH 5.2) was added, vortexed for 2 – 5 min to mix completely. The mixture was then spinned for 2 min in a microcentrifuge to pellet the cell debris and the chromosomal DNA. The supernatant was transferred to a fresh tube, mixed well with 0.9 ml of 100% (v/v) ethanol which has been pre-cooled to -20 °C. The mixture was spinned for 20 min to pellet the plasmid DNA and white pellets were observed. The supernatant was discarded and the pellet was rinsed twice with 1 ml of 70% (v/v) ethanol. The pellets were dried under vacuum for 2 – 3 min. The pellets were then re-suspended in 40 µl of TE buffer for further use.

2.14 Agarose Gel Electrophoresis

The agarose gels were run in a horizontal gel electrophoretic apparatus using TBE (Tris, Boric acid, EDTA, 0.8% agarose) buffer (Meyers *et al.*, 1976). The agarose gel in TBE buffer was boiled intermittently until the solution became clear. The solution was allowed to cool to 45 °C and 7 µl of ethidium bromide was added. The function of the ethidium bromide is to intercalate the basis of the DNA so that it fluoresces when viewed under the UV light. The gel was then poured into the gel plate with the comb in place and allowed to solidify. Subsequently, the gel tray and comb was removed and put into the tank containing the gel buffer, making sure that the buffer covered the gel completely. Two µl of the tracking dye (bromophenol blue) was mixed with 1 µl of the marker and loaded into the first well. Two µl of the bromophenol blue was then mixed with 20 µl of the samples and also loaded into the designated wells. Following the loading of the wells, the tank was covered, plugged into power and allowed to run from the negative to positive direction making sure it didn't run more than ¾ of the gel. Finally, the gel was viewed on the UV transilluminator using protective goggles. Amplified plasmid DNA appeared as sharp bands that fluoresced when excited with UV light.

2.15 Determination of Molecular Weights of Plasmids

The molecular weight of isolated plasmids was determined as described by (Ilori, 1998). The plasmids isolated from *Pseudomonas aeruginosa* were run in agarose gel alongside the plasmids that were isolated from the bacteriophage lambda (λ) DNA fragmented in a restriction digestion with Hind 111 endonuclease. The digestion reaction resulted in 8 double stranded DNA fragments. The DNA molecular weight marker provided accurate sizing of fragments over a broad range of sizes. Upon electrophoretic separations, 7 bands of known molecular weights (23130, 9416, 6557, 4361, 2322, 2027 and 564 bp respectively) were visible. The smallest fragment resulting from the Hind 111digestion (125 base pair) could not be detected on the gel due to its small size. All fragments were present in equimolar amounts therefore, the smallest band will only be visible on over-loaded gels when stained with ethidium bromide. The DNA fragment mixture showed the typical pattern with 7 bands in agarose gel electrophoresis. A standard curve was made by plotting the relative mobilities on gel against the logs of their molecular weights. The molecular weights of

plasmids isolated from the *Pseudomonas aeruginosa* were then determined by extrapolating from the standard curve.

2.16 Curing of Plasmids

The curing of plasmid detects the loss of resident plasmid DNA from a cell. The curing procedure was done as described by Sonstein and Baldwin (1972) to determine whether the plasmids isolated from *Pseudomonas aeruginosa* encoded the spoilage trait or not. To achieve this, *Pseudomonas aeruginosa* grown on yeast extract trypticase soy (YETS) (0.3% yeast extract and 1.5% agar) were harvested from the YETS plates and emulsified in normal saline at a density of about 10^5 cells per ml. One ml of this was then inoculated into 9 ml YETS broth containing 0.002% (v/v) SDS and incubated with constant shaking at 37 °C for 18 h. After incubation, 0.1 ml aliquots of the suspension were plated on NA plates. The plates were incubated aerobically at 37 °C for 24 h. Changes in the original colonial morphology was observed. The parent strains and mutants that had lost the plasmids were plated out on fresh NA plates, incubated aerobically at 37 °C for 24 h. The developed colonies were screened for loss of the plasmids by repeating the DNA extraction process. Subsequently, they were tested for antibiotic sensitivity.

2.17. Antibiotic Susceptibility Tests of Plasmid-bearing and Plasmid-cured Strains

The antibiotic sensitivity of the plasmid-bearing *Pseudomonas aeruginosa* (OB-6W) and the plasmid-cured strain (OB-6C) to 8 antibiotics was determined using the Kirby-Bauer disc diffusion technique (Bauer, 1966). The purpose of this experiment was to determine the agar disc diffusion zone diameters of the plasmid-bearing *Pseudomonas aeruginosa* strain (OB-6W) isolated from spoilt paints and biodeteriorated wall scrapings to selected antibiotics and to compare these zone diameters with those of the cured prototype. Single discs (Oxoid Ltd. Basingstoke Hampshire, England) were employed to test the sensitivity of the plasmid-bearing and plasmid-cured strains to antibiotics as described in section 2.12. The zones of inhibition produced by the test organism (plasmid-bearing strain) were compared directly with that of the control strain (plasmid-cured strain). The zones of inhibition observed around

the antibiotic discs were taken as indication of sensitivity and were interpreted based on the National Committee for Clinical Laboratory Standards (NCCLS, 1997).

2.18. Microbial Shelf life Determination of Paints

Six different paint samples (PS1-PS6) collected on the day of production were analyzed for microbial population levels during a 10-month study period (section 2.5.6). The data obtained during this period for the microbial population counts were evaluated and fitted into a suitable model to predict the time when the paint samples would reach spoilage level (3.4 x 10^{10} cfu/ml). The time it takes to reach this level (i.e N_t) was taken as the shelf life of the sample. To estimate the shelf life time of freshly produced paint samples, the model described by Dawes (1969) was used in relation to the total heterotrophic microorganisms obtained for the paint samples as given below:

$$\frac{Log_{10} N_t - Log_{10} N_o}{Log_{10} 2} = \frac{t}{T} \quad \ldots(1)$$

$$T = \frac{0.693}{K} \quad \ldots(2)$$

Where

N_t = highest cell count as colony forming units (i.e. total heterotrophic microorganisms) at the end of log. Phase;

N_o = Initial cell count as colony forming units (total heterotrophic microorganisms) at the beginning of log. Phase;

T = mean generation time (days) of (total heterotrophic microorganisms) during log. phase;

t = duration (months) taken for the population to increase exponentially from N_o to N_t

k = specific multiplying rate (day $^{-1}$) of total heterotrophic microorganisms.

Therefore, at the end of the first generation, there are 2^1 N_o cells, at the end of the second generation, there are 2^2 N_o cells and at the end of the third, there are 2^3 No cells. Thus, at the end of Y generations there will be 2^Y N_o cells in the paint sample. Assuming that the number of cells in the paint at the end of the incubation period is N_t, therefore, it follows that,

$$N_t = 2^Y N_o \quad (2.1)$$

Taking the logarithm of both sides of the model to the base of 10

$$\text{Log}_{10} N_t = \text{Log}_{10} 2^Y + \text{Log}_{10} N_o \quad (2.2)$$

$$\text{Log}_{10} N_t = Y\text{Log}_{10} 2 + \text{Log}_{10} N_o \quad (2.3)$$

$$\text{Log}_{10} N_t - \text{Log}_{10} N_o = Y\text{Log}_{10} 2 \quad (2.4)$$

$$\frac{\text{Log}_{10} N_t - \text{Log}_{10} N_o}{\text{Log}_{10} 2} = Y \quad (2.5)$$

Since T is the mean generation time, therefore the number of generations the cells go through to attain N_t was evaluated thus:

$$Y = t/T \quad (2.6)$$

Where t is the time for the exponential phase

The model equation is now:

$$\frac{\text{Log}_{10} N_t - \text{Log}_{10} N_o}{\text{Log}_{10} 2} = \frac{t}{T} = Y \quad (2.7)$$

The maximum number of cells N_t and the initial number of cells No are known. Therefore, to determine the mean generation time (T), plots of the growth of microorganisms in the paint with time was first-made (Fig 3.1), from the slope of the graph, the specific growth rate, K, was evaluated. The rate of increase of microbial population is given also by the expression (which is very useful for evaluating T with K estimated):

$$\frac{dN}{dt} = KN \quad (2.8)$$

$$\frac{dN}{N} = kdt \quad (2.9)$$

Integrating equation (2.9),

$$\frac{\ln N}{No} = kt \quad (2.10)$$

If T is equal to the mean generation time or doubling time, then,

$N = 2\ N_o$ and equation (2.10) becomes:

$$\ln \frac{2N_o}{N_o} = KT \qquad (2.11)$$

$$\ln 2 = KT$$

$$T = \frac{\ln 2}{K} \qquad (2.12)$$

But $\ln 2 = 0.693$

$$T = \frac{0.693}{K}$$

With the mean generation time or doubling time (T) evaluated, the duration of the exponential phase can be evaluated.

The slope of the line in the plot of log Nt against time during the exponential phase is equal to (K/2.303) from which K (specific growth rate) was obtained (Dawes, 1967). With K evaluated, the mean generation time (T) of the microorganisms present in the paint sample was determined from the model below:-

$\ln 2 = KT$

From equation (2-13)

$$\frac{0.693}{K} = T$$

Having known T (the mean generation time i.e the time it takes the microorganisms present in the paint sample to double their initial number (N_o) then, the model (2.7) as described by Dawes (1967) is given below:

$$\frac{\text{Log}_{10} N_t - \text{Log}_{10} N_o}{\text{Log}_{10} 2} = \frac{tsl}{T} = Y$$

This equation was applied to determine the shelf life (SL) of the paints where:

N_t = standard population density of microorganisms in spoilt paint (3.4×10^{10} cfu/ml).

N_o = Initial population density of microorganisms immediately after production of the paint.

tsl = Shelf life time during exponential phase growth of microorganisms, which can easily be calculated having known N_t, N_o and T

T = mean generation time of microorganisms present in a paint sample

Y = Mean number of generations, which the microorganisms went through before reaching the final spoilage population density (N_t).

Therefore, the total shelf-life duration (SLD) of the paint is given by the following

Model: $SLD = t_{sl} + L$

Where SLD is Shelf-life duration

L is Lag phase

2.19. Development of Statistical Predictive Models

The data obtained for the fresh paint samples and the spoilt paint samples were fitted to a multiple linear regression analysis (Neter *et al.*, 1983) to predict shelf life time for all the samples. This is because regression as a statistical analysis describes the relationship between two or more variables. The postulated model generated comprises of two equations based on the physico-chemical parameters and microbial population counts of the paint samples. The equation of the fitted model based on the physico-chemical parameters to predict shelf life is:

$$Y = \beta_0 + \beta_1 (X_1) + \beta_2(X_2) + \beta_3(X_3) + \beta_4 \ln(X_4) + \beta_5(X_5)$$

Where:

Y = Shelf life time/ response coefficient

β_0 = Intercept

β_1- $\beta 5$ = regression parameters

X_1 = specific gravity

X_2 = optical density

X_3 = transmittance

X_4 = pH

X_5 = viscosity

} regressor coefficients at the spoilt state.

While the equation for the fitted model using microbial parameters is:

$$Y = \beta_0 + B_1 (\log X_1) + \beta_2 (\log X_2) + \beta_3 (\log X_3)$$

Where Y = Shelf life time/ response coefficient

β_0 = Intercept

$\beta_1 - \beta_3$ are regression parameters

X_1 =Total bacterial count
X_2= Total coliform count
X_3= Total fungal count

} regressor coefficients at the spoilt state

The parameter estimates for the above models are presented in Appendix 11. The multiple linear regression analysis was also used to generate the confidence interval, mean, standard deviation, standard error mean, upper 95% mean and lower 95% mean. Significance limits were set at the 95% probability level.

CHAPTER THREE

RESULTS

3.1 Enumeration of Microbial Population Densities in Various Raw Materials, Packaging Materials and Stages of Paint Production

Six bacterial isolates designated OB-1, OB-2, OB-3, OB-6, OB-7 and OB-8; two coliform isolates, designated OB-4 and OB-5 and three fungal isolates designated OB-9, OB-10 and OB-11 were isolated in this study. The types and population densities of bacteria, coliforms and fungi found in 26 solid raw materials used in paint production are shown in Table 3.1. The highest population density was obtained for bacteria (9.5×10^6 cfu/g); followed by coliforms (7.05×10^4 cfu/g) and fungi (6.8×10^4 cfu/g). Sample RN375 had the highest number of bacteria, MUH_20 had the highest number of coliforms while MN252 had the highest number of fungi. Expectedly, the population density of coliforms was exceptionally high in MUH_20 and CDH_20 which are water supply inlets to the factory. The microbial population in the biocides used in paint production ranged from $1.0 \times 10^2 - 1.5 \times 10^2$, $1.0 \times 10^1 - 1.7 \times 10^1$ and $1.0 \times 10^1 - 1.5 \times 10^1$ for bacteria, coliform and fungi respectively (Table. 3.2). The data regarding the microbial population densities in the various packaging materials and stages of paint production are shown in Tables 3.3 and 3.4 respectively. The same types of organisms encountered in the raw materials were also detected in the packaging materials and the stages of paint production (Tables 3.1, 3.3 and 3.4). The population of bacteria in the packaging materials ranged from $3.45 \times 10^6 - 7.65 \times 10^6$ cfu/g. The coliform population ranged from $2.90 \times 10^4 - 4.90 \times 10^4$ cfu/g, while the fungal population ranged from $2.40 \times 10^3 - 2.80 \times 10^3$ respectively.

Table 3.1: Microbial population density of the soil samples

S/N	Sample	Total bacterial counts (x 10^6 cfu/g)	Total coliform counts (x 10^4 cfu/g)	Total fungal counts (x 10^4 cfu/g)	Fungal isolates	Bacterial isolates
1	HSD H_20	4.4	6.5	1.25	OB-10, OB-11	OB-1, OB-4, OB-5
2	CD H_20	2.7	4.5	1.50	*	OB-1, OB-4, OB-5
3	MU H_20	6.45	7.05	1.50	OB-9	OB-2, OB-4
4	MN 288	2.35	2.2	*	*	OB-3, OB-4, OB-7
5	MN 239	2.05	2.5	3.5	OB-10, OB-11	OB-1, OB-3
6	MN 277	7.0	3.5	2.25	OB-10	OB-5, OB-8
7	MN 409	7.5	3.9	3.59	OB-10, OB-11	OB-1, OB-4,
8	MN 280	7.8	1.5	2.35	OB-11	OB-2, OB-7
9	MN 231	3.75	5.6	*	*	OB-4, OB-7
10	MN 236	2.0	2.5	1.30	OB-9	OB-3
11	Z4 726	5.4	3.0	*	*	OB-3, OB-7, OB-8
12	MN 236X	2.05	2.1	*	*	OB-2, OB-4
13	Z4441	6.1	5.6	*	*	OB-3, OB-7
14	MN 252	3.0	7.0	6.8	OB-9	OB-1, OB-4,
15	MN 261	3.34	4.5	*	*	OB-3, OB-5, OB-8
16	ZN 490	7.6	3.0	*	*	OB-2, OB-3
17	RN 300	3.2	3.2	3.0	OB-11	OB-2, OB-7,
18	RN 375	9.5	3.5	*	*	OB-3, OB-4
19	Z4726	5.3	1.2	*	*	OB-1, OB-5
20	Z4740	7.30	2.95	*	*	OB-2, OB-3, OB-5
21	L1140	3.15	3.5	4.2	OB-9, OB-11	OB-1, OB-2, OB-7
22	ZN 465	1.0	2.6	1.5	OB-9	OB-4
23	Z4 899	3.5	3.0	*	*	OB-2, OB-3
24	ZN 470	5.2	1.4			OB-1, OB-8
25	MN 241	2.7	2.4	3.5	OB-11	OB-2, OB-3
26	MN 286	2.4	2.9	*	*	OB-3, OB-5, OB-7

*, not detected
Values are means of triplicate determinations.

Table 3.2. Microorganisms detected in biocides and their population densities

Biocide	Total bacterial counts (x 10^2 cfu/ml)	Total coliform counts (x 10^1 cfu/ml)	Total fungal counts (x 10^1 cfu/ml)	Fungal isolates	Bacterial isolates
ZN467	1.0	1.0	1.0	OB-9	OB-3, OB-4
ZN481	1.25	1.0	*	*	OB-2, OB-4
ZN489	1.5	1.7	1.5	OB-9, OB-10	OB-5, OB-7
ZN485	1.0	1.3	1.2	OB-10	OB-7

*, not detected
Values are means of triplicate determinations.

Table 3.3. Microorganisms and their population levels in packaging materials used in paint production

Packaging materials	Total bacterial counts (x 10^6 cfu/g)	Total coliform counts (x 10^4 cfu/g)	Total fungal counts (x 10^3 cfu/g)	Fungal isolates	Bacterial isolates
C/T 20LTS	3.45	2.9	2.4	OB-9	OB-2, OB-5, OB-7
D/W/S Plastic	4.28	4.70	*	*	OB-1, OB-5, OB-8
C/20LTS	3.82	3.42	2.8	OB-9	OB-2, OB-4, OB-7
D/W/S/Metal	4.95	4.90	*	*	OB-1, OB-3,
D/E 2OLTS	7.65	4.01	*	*	OB-2, OB-5, OB-8
D/E 4LTS	5.92	4.82	*	*	OB-1, OB-4, OB-7

C/T, caplux textured; D/W/S, dulux weather shield; C, caplux; D/E, dulux emulsion;
*, not detected. Values presented are means of triplicate samples.

Table 3.4. Microbial Types and Numbers isolated during the different stages of paint production

Paint types		After dispersion	After making up	Texture	After tinting	After filling	Fungal isolates	Bacterial isolates
D/WS	Total bacteria on NA (cfu/ml)	$1.25x10^2$	$2.25x10^2$	$5.46x10^2$	$5.73x10^2$	$6.0x10^2$		OB-1, OB-3
	Total coliform on MCA (cfu/ml)	$1.1x10^1$	$1.46x10^1$	$2.5x10^1$	$2.8x10^1$	$3.22x10^1$		OB-4, OB-5
	Total fungi on PDA	$1.0x10^1$	$1.5x10^1$	$1.9x10^1$	$2.1x10^1$	$3.3x10^1$	OB-9	
C/E	Total bacteria on NA (cfu/ml)	$2.0x10^2$	$3.2x10^2$	N/A	$3.92x10^2$	$4.4x10^2$		OB-2, OB-3, OB-7
	Total coliforms on MCA (cfu/ml)	$1.6x10^2$	$2.2x10^2$		$2.8x10^2$	$3.5x10^2$		OB-4 OB-4
	Total fungi on PDA (cfu/ml)	$1.1x10^1$	$2.3x10^1$		$2.9x10^1$	$3.1x10^1$	OB-9 OB-10	

D/W/S, dulux weather shield; C/E, caplux emulsion; N/A, not applicable
Values are means of triplicate determinations.

3.2 Populations of Microorganisms from Paint Samples.

Ten morphologically different microorganisms were isolated following initial ten-fold serial dilution and standard plate count from fresh paint samples that have been processed to meet company's regulations and specifications. In addition to these, the spoilt samples had 1 more organism designated OB-6. Isolates OB-4 and OB-7 occurred more frequently in all the fresh paint samples while OB-6 occurred only in all spoilt paint samples. They were therefore, selected for biocide and antibiotic susceptibility tests. The bacterial population in the fresh paint samples (PS-1 – PS-6) monitored every two weeks from the day of production ranged from $1.6 \times 10^1 - 4.7 \times 10^5$ cfu/ml, while the fungal population ranged from $1.0 \times 10^1 - 5.5 \times 10^3$ cfu/ml respectively over a period of 10 months. The mean changes in population density of microorganisms in fresh paint samples PS1 – PS6 are shown in Fig. 3.1. The main feature of the growth pattern of the isolated organisms as illustrated in Fig. 3.1 is an observable and definite lag period of 5 months. Subsequently, there was steady increase in the microbial population densities till the 10th month. The microbial population densities in the spoilt paint samples ranged from $2.5 \times 10^{10} - 3.4 \times 10^{10}$ cfu/ml, $1.0 \times 10^7 - 2.9 \times 10^7$ cfu/ml and $2.2 \times 10^5 - 3.2 \times 10^5$ for bacteria, coliform and fungi respectively.

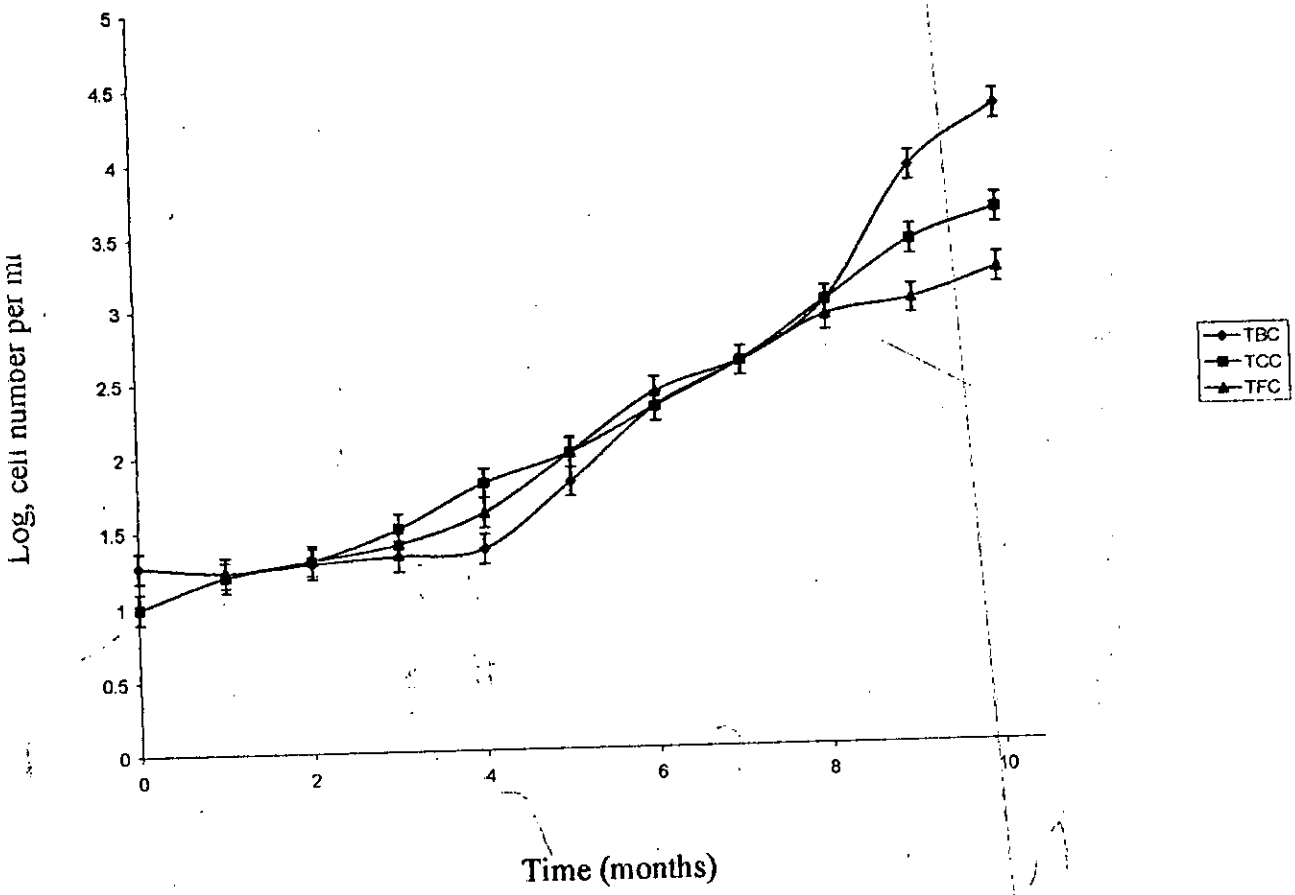


Fig. 3.1. **Mean changes in microbial population density in fresh paint samples PS1-PS6. TBC, total bacterial count; TCC, total coliform count; TFC, total fungal count. Data represent the averages of triplicate determinations.**

Table 3.5. Microbial population densities in spoilt paint samples

Paint sample	Total bacterial counts (x 10^{10} cfu/ml)	Total coliform counts (x 10^{7} cfu/ml)	Total fungal counts (x 10^{5} cfu/ml)	Fungal isolates	Bacterial isolates
PSA	2.9	1.1	2.5	OB-9	OB-2, OB-3, OB-4, OB-6, OB-7
PSB	3.4	1.1	3.2	OB-9, OB-11	OB-1 OB-6, OB-7, OB-8
PSC	3.0	1.0	2.8	OB-10, OB-11	OB-3, OB-4, OB-6, OB-7
PSD	2.5	2.9	2.5	OB-10	OB-2, OB-4, OB-6
PSE	3.1	1.1	2.2	OB-11	OB-1, OB-5, OB-6

Values presented are means of triplicate samples.

3.3 Enumeration of Microbial Population Densities in Paint Samples from Various Locations

The population levels of bacteria, coliforms and fungi isolated in paint samples from company warehouse and three different markets which are the main paint depots in Lagos are shown in Table 3.6. The bacterial population density was the highest (6.3 x 10^3 cfu/ml). This was followed by the coliform population density (5.1 x 10^2 cfu/ml) and lastly the fungal population density (3.8 x 10^1 cfu/ml) respectively. Mushin market had the highest bacterial count (6.3 x 10^3 cfu/ml) while Bariga market had the highest fungal count (3.8x10^1 cfu/ml). However, the company warehouse had the highest coliform count (5.1 x 10^2 cfu/ml) and the lowest fungal count (2.3 x 10^1 cfu/ml). Isolates OB-4 and OB-5 occurred in paint samples from all the locations.

3.4 Enumeration of Microbial Population Densities in Biodeteriorated Painted Walls.

Microbial population and types from biodeteriorated painted walls showed a high incidence of OB-6 (6.50 x 10^4 cfu/g) and OB-7 (5.26 x 10^4 cfu/g). The results are displayed in Table 3.7. Isolates OB-2 and OB-5 were the least occurring bacterial isolates. Plate 3.1 shows the points at which scrapings were collected for subsequent isolation of microorganisms from a biodeteriorated painted wall.

Table 3.6. Microbial population distribution in paints from various locations.

Sample location	Total bacterial counts (x 10^3 cfu/ml)	Total coliform counts (x 10^2 cfu/ml)	Total fungal counts(x 10^1 cfu/ml)	Isolates
Mushin	6.3	4.8	3.1	OB-2, OB-4, OB-11
Ikeja	3.9	4.2	2.9	OB-3, OB-5, OB-9
Bariga	4.7	3.8	3.8	OB-1, OB-5, 0B-11
Company Warehouse	3.7	5.1	2.3	OB-4, OB-7, OB-10

Values are means of three triplicate determinations.

Plate 3.1. Biodeteriorated painted wall showing effect of colonization by microorganisms. Arrows show scrapings collection points.

Table 3.7. Microbial population density in biodeteriorated painted walls

Bacterial count x 10^4	Fungal count x 10^2	Bacterial isolates	Fungal isolates
5.26	5.18	OB-7	OB-9
4.53	3.0	OB-4	OB-10
4.12	0.13	OB-3	OB-11
6.50		OB-6	
1.00		OB-5	
3.0		OB-2	

Data represent the averages of triplicate determinations. Microbial numbers were enumerated in colony forming units (CFU) per gram of wall scraping.

3.5 Identification of Bacterial and Fungal Isolates

Pure cultures of bacterial isolates, (OB-1 – OB-8) isolated from the raw materials, packaging materials, stages of paint production, paint samples and biodeteriorated painted walls were identified on the basis of their morphological and biochemical characteristics (Table 3.8). The substrate utilization patterns of the frequently occurring OB-6 in the spoilt paint samples and the biodeteriorated paint samples were done using Analytical Profile Index (API) identification (ID 32E) test system to obtain the phenotypic profiles of the isolate. Also, the frequently occurring coliform, OB-4 in all the fresh paints examined, were characterized phenotypically along side OB-5 using substrate utilization with the API 20 E test system (bioMerieux Vitek, Inc. Hazelwood, MO. USA). Table 3.9 show the identification profiles generated using the database code obtained from the API identification software (APIWEB). The heterotrophic bacteria isolated in the study were identified to be *Bacillus polymyxa* (OB-1), *Bacillus brevis* (OB-2), *Bacillus laterosporus* (OB-3), *Proteus mirabilis* (OB-4), *Escherichia coli* (OB-5), *Pseudomonas aeruginosa* (OB-6), *Lactobacillus gasseri* (OB-7) and *Lactobacillus brevis* (OB-8). The fungal isolates (OB-9, OB-10 and OB-11 were identified as *Aspergillus niger*, *A. flavus* and *Penicillium citrinum* respectively based on macroscopic and microscopic characteristics.

Table 3.8. Biochemical characteristics of bacterial strains

Biochemical characteristics	Isolates							
	0B-1	0B-2	0B-3	0B-4	0B-5	0B-6	0B-7	0B-8
Cellular Morphology	Large Straight rods	Short rods	Short rods	Short rods	Straight rods	Straight rods	Slender rods	Slender rods
Gram reaction	+	+	+	-	-	-	+	+
Catalase	+	+	+	*	-	+		
Oxidase	-	-	-	-	-	+	-	-
VP	+	-	+	+	-	-		
Gelatin hydrolysis	+	-	-	+	-	-		
Starch hydrolysis	+	-	-	+	-	-		
Citrate utilization	+	-	-		-	+		
Nitrate Reduction	+	-	+	+	-	+	-	-
Gas from Nitrate	*	-	-	+	-	+		
Motility	+	+	-	+	+	+	-	-
Indole	-	-	-	+	+	-		
Urease	-	-	-	+	-	-		
H_2S	*	*	*	+	-	-		
Gas from glucose				+	+		-	+
Growth in 10% NaCl	-	-	-		*	*	N/A	N/A
Growth at 15°C	*	*	*	*	*	*	-	+
Growth at 45°C	*	*	*	*	*		+	-
Growth at 50°C	-	+	-		*		*	*
Esculin hydrolysis	*	*	*	*	*	+	*	*
ONPG(ß-galactosidase)	+	-	-	-	+	-	*	*
LDC (Lysine decaboxylase	*	*	*	-	+	-	*	*
ODC (ornithine decarboxylase)	*	*	*	+	+	-	*	*
ADH (arginine dihydrolase	*	*	*	-	-	+	-	-
Glucose	+	-	+	+	+	+	-	+
Xylose	+	-	-	+	+	+	*	*
Lactose	+	*	-	-	+	-	+	+
Salicine	+	-	-	-	-	*	+	-
Sucrose	+	*	*	-	-	-	*	*
Maltose	+	*	*	-	+	-	-	-
Mannitol	+	*	*	-	+	+	-	-
Phenylalanine deaminase	*	*	*	+	-	*	*	*
Probable identity	*Bacillus polymyxa*	*B. brevis*	*B. laterosporus*	*Proteus mirabilis*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Lactobacillus gasseri*	*L. brevis*

+, positive; - negative; *, not tested; N/A- not applicable

Table 3.9. Phenotypic characterization of isolates

Biochemical characteristic	Isolates		
	OB-4	OB-5	OB-6
Gram reaction	-	+	-
ONPG (β-galáctosidase)	-	+	+
ADH (arginine dihydrolase)	-	+	-
LDC (lysine decarboxylase)	+	+	+
ODC (ornithine decarbobxylase)	-	-	*
CIT (citrate utilization)	+	-	*
H_2S	+	-	+
Urea	+	-	*
TDA (tryptophane deaminase)	-	+	-
Indole production	-	-	*
VP	+	-	*
Gelatin hydrolysis	+	+	-
Glucose	-	+	-
Mannitol	-	+	-
Inositol	-	-	-
Sorbitol	-	+	-
Rhamnose	-	+	*
Saccharose	-	-	-
Melibiose	-	-	*
Amygdalin	-	-	*
Arabinose	-	+	*
Oxidase	+	-	*
Nitrate reduction to Nitrite	-	-	*
Nitrate reduction to Nitrogen	-	+	*
Motility	+	+	*
OF-O†	+	+	*
OF-F‡	*	*	-
αMAL (α-maltosidase)	*	*	-
αGLU (α-glucosidase	*	*	-
αGAL (α-galactosidase)	*	*	-
βGUR (β-glucuronidase)	*	*	-
CEL (cellobiose)	*	*	-
LARA (L-arabinose)	*	*	-
LARL (L-arabitol)	*	*	-
βNAG (N-Acetyl-β-glucosaminidase)	*	*	-
PLE (palatinose)	*	*	+
ASPA (L-aspartic acid arylamidase)	*	*	-
5KG (5-ketogluconate)	*	*	-
TRE (trehalose)			

†, oxidative-; ‡, fermentative- utilization of glucose; +, positive; -, negative; *, not tested

3.6 Cellulolytic Activity of Isolated Strains

The growth of the isolated strains in Czapek's medium was indicated by utilization of cellulose as the sole source of carbon and energy. This was evidenced by the increase in turbidity at different rates. The optical density ($OD_{550\ nm}$) during the incubation period ranged from 0.12 – 0.20, 0.19, 0.20, 0.13, 0.14, 0.50, 0.14, 0.15, 0.22, 0.20 and 0.22 for OB-1 – OB-11 respectively. Although, experiments demonstrated the abilities of all the isolated strains to utilize cellulose as sole carbon and energy source, the enzyme activity of endoglucanase tested on carboxymethyl cellulose (CMC) proved that *Pseudomonas aeruginosa* (OB-6) utilized cellulose optimally. The results presented in Table 3.10 show that CMC induced the highest cellulolytic activity on *Pseudomonas aeruginosa* of all the eleven isolates tested. *Aspergillus niger* and *Penicillium citrinum* came in the second rank, while *Bacillus polymyxa, B. brevis, B. laterosporus* and *Aspergillus flavus* came in the third rank. *Proteus mirabilis* produced the least CMC-ase activity, while *Escherichia coli, Lactobacillus gasseri* and *L. brevis* utilized cellulose minimally.

Table 3.10. Cellulolytic activity of isolated organisms

Isolated organisms	Concentration (µg/ml)	Enzyme activity IU (µmol/min)	Relative activity (%)
	CMC -ase		
Bacillus polymyxa	179.41	0.69	40.1
B. brevis	164.71	0.64	37.2
B. laterosporus	176.47	0.68	39.5
Proteus mirabilis	88.24	0.34	19.8
Escherichia coli	95.59	0.37	21.5
Pseudomonas aeruginosa	444.59	1.72	100.0
Lactobacillus gasseri	95.59	0.37	21.5
L. brevis	122.01	0.47	27.3
Aspergillus niger	191.18	0.74	43.0
A. flavus	180.88	0.70	40.6
Penicillium citrinum	197.01	0.76	44.2

Values are means of triplicate samples.
One unit of CMC-ase activity is the amount of enzyme which produced one micromole of glucose per min.

3.7 Physico-chemical Parameters of Paint Samples

The mean changes in physico-chemical characteristics of six different freshly made paint samples (PS-1 – PS-6) monitored for a period of 10 months are summarized in Fig. 3.2. The parameters measured include optical density (OD), specific gravity (SG), transmittance (TR), pH and viscosity (VIS). The optical density (OD) at 600 nm increased with time in all the samples over the period ranging from 1.49 – 3.91. On the other hand, specific gravity, transmittance, pH and viscosity decreased over time. The decreases obtained for the parameters ranged from 2.8658 – 1.0853, 6.9 – 2.3, 8.5 – 5.6 and 11.7 cst – 10.8 cst for specific gravity, transmittance, pH and viscosity respectively. There were observable changes in the physical appearance of the paint samples during the study period as they age. The changes in the physical appearance of the paint samples over the study period indicated a steady and gradual loss of the original colour, texture and viscosity in addition to formation of biofilms on the paint surfaces. In addition to these changes, the spoilt paints had malodour and gassing. The physico-chemical parameters of the spoilt paint samples ranged from 6.82 – 7.51, 0.1058 – 0.1198, 0.15 – 0.55, 4.12 – 4.19 and 3.02 cst – 3.63 cst for optical density, specific gravity, transmittance, pH and viscosity respectively (Table 3.11).

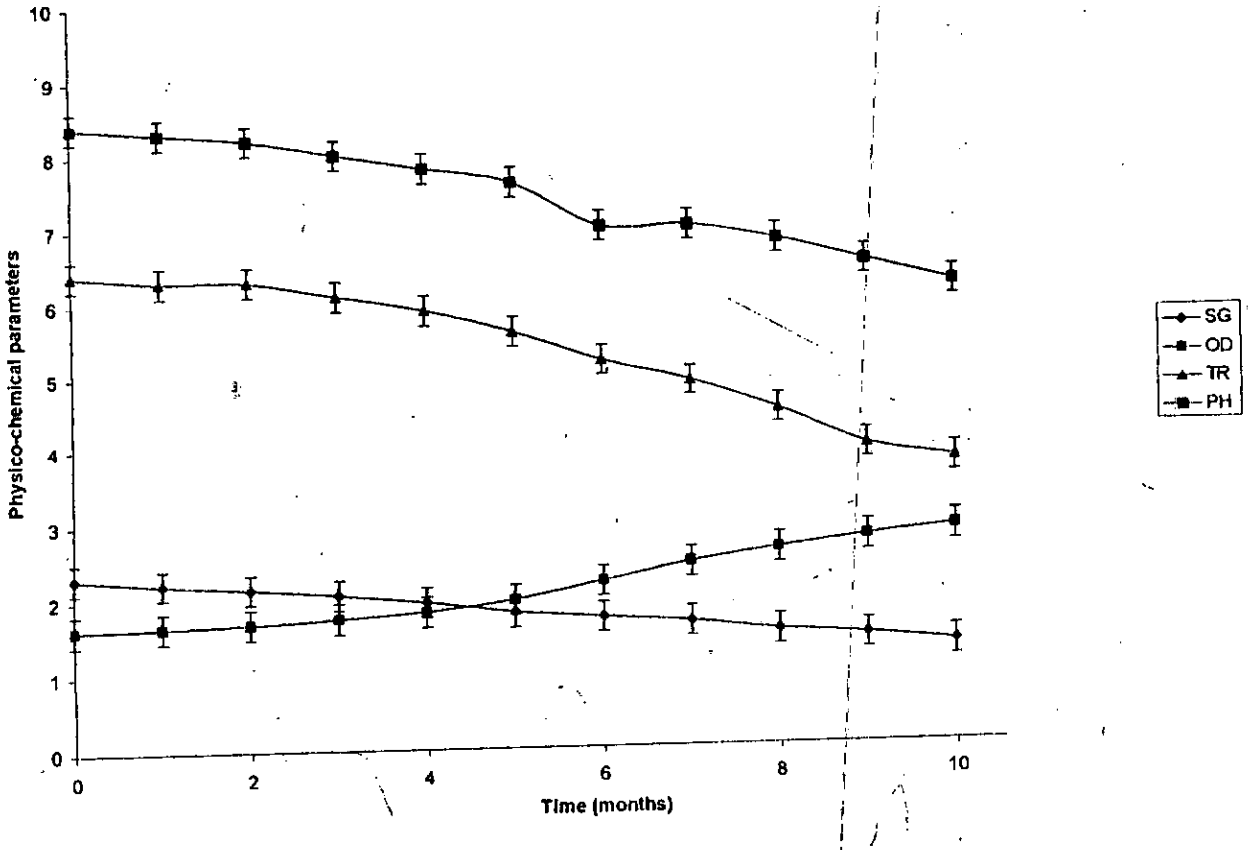


Fig. 3.2 Mean changes in the physico-chemical parameters in fresh paint samples PS1 - PS6. SG, specific gravity; OD, $_{600\,nm}$; TR, transmittance. Data represent the averages of triplicate determinations.

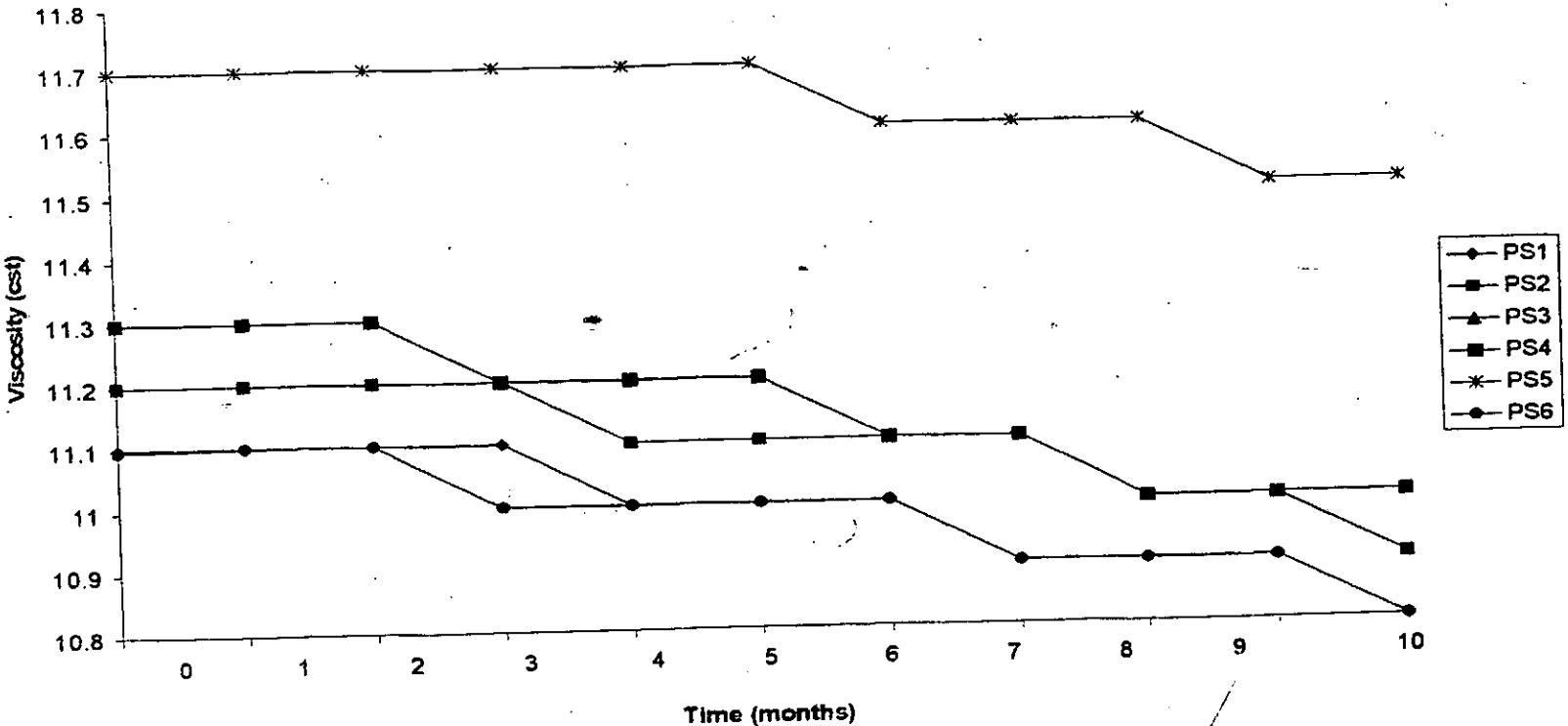


Fig. 3.3 **Mean changes in viscosity of fresh paint samples PS1 - PS6 monitored for ten months after production**

Table 3.11. Physico-chemical parameters of spoilt paint samples

Paint samples	SG	OD	TR	pH	VIS (cst)
PSA	0.1175	6.98	0.52	4.14	3.64
PSB	0.1198	7.26	0.46	4.12	3.02
PSC	0.1179	6.95	0.15	4.15	3.68
PSD	0.1166	6.82	0.54	4.19	3.69
PSE	0.1058	7.51	0.55	4.16	3.63

SG, specific gravity, $OD_{600\ nm}$; TR, transmittance; VIS, viscosity (cst).
Values presented are means of triplicate samples.

3.8 Concentration of Phosphates, Sulphates and Heavy Metals

The results of the PO_4^{3-} and SO_4^{2-} content in fresh paint samples, spoilt paint samples and paint samples stored in the company warehouse shown in Figs. 3.4 – 3.10. indicate higher concentrations of these inorganic nutrients in the fresh paint samples than in the spoilt paint samples. The results showed a steady decline of PO_4^{3-} from 15.5 – 2.4 mg/kg and SO_4^{2-} from 12.8 – 2.0 mg/kg in the fresh samples over the study period. In contrast, the spoilt paint samples had lower levels of PO_4^{3-} which decreased from 2.6 – 0.1 mg/kg and SO_4^{2-} which decreased from 1.2 – 0.0 mg/kg respectively. This is most likely the reason for the observed odour in the spoilt samples as SO_4^{2-} is being reduced to hydrogen sulphide under anaerobic conditions. Table 3.12 shows the availability of heavy metals in fresh, spoilt and stored paint samples in the company warehouse. The concentrations of Pb, Cu and Mn were generally lower in all fresh samples than in the warehouse samples. Generally, the spoilt paints had higher concentrations of all the metals which ranged from 2.0 – 5.3, 2.5 – 5.8 and 3.0 – 5.0 mg/kg for Pb, Cu and Mn respectively.

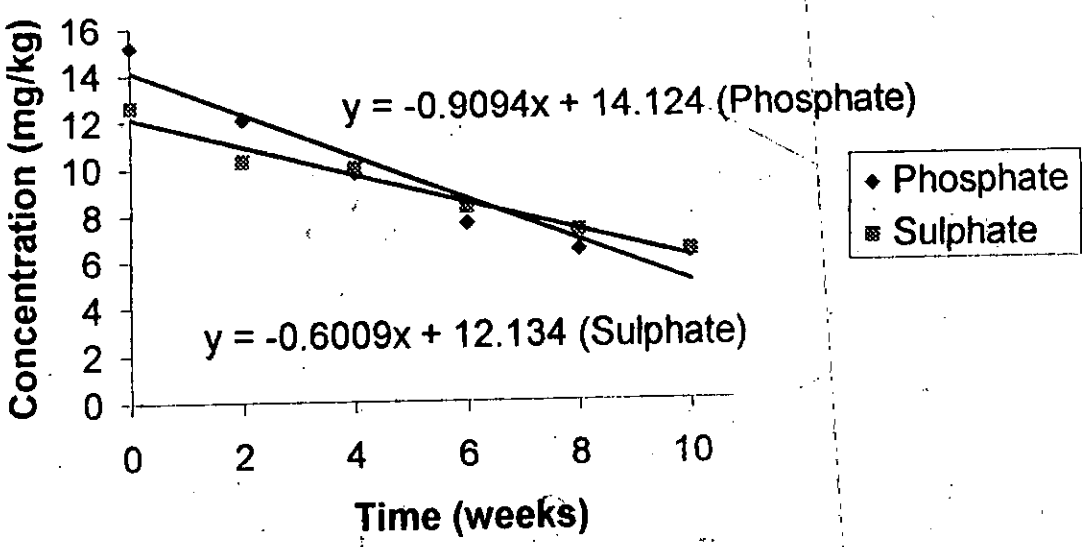


Fig 3.4 **Concentration of phosphates and sulphates in paint sample PS-1. Data represent the means of triplicate samples.**

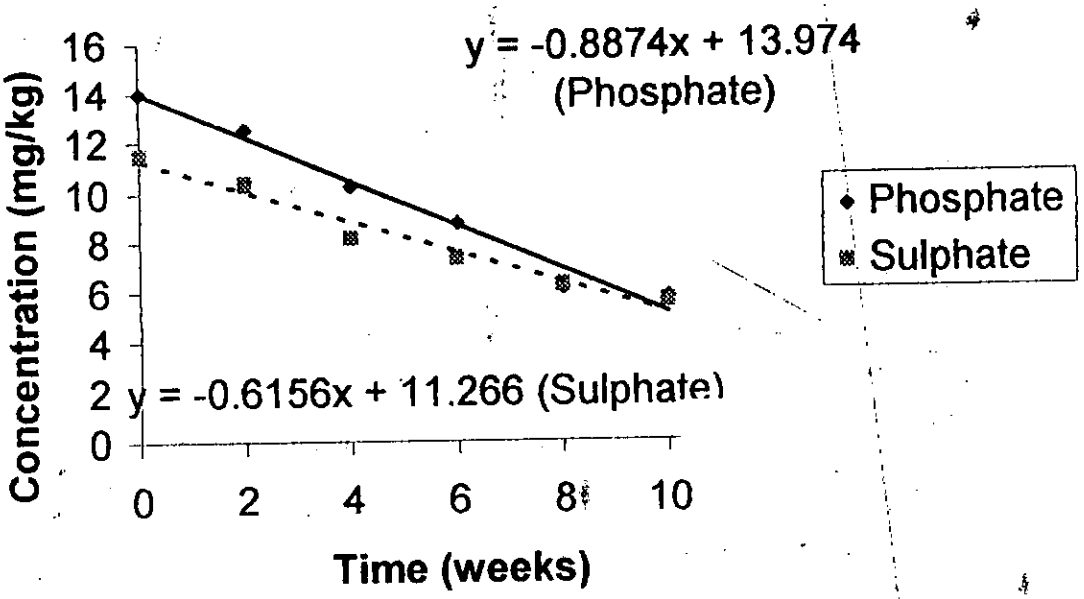


Fig. 3.5 Concentration of phosphates and sulphates in paint sample PS-2. Data represent the means of triplicate samples.

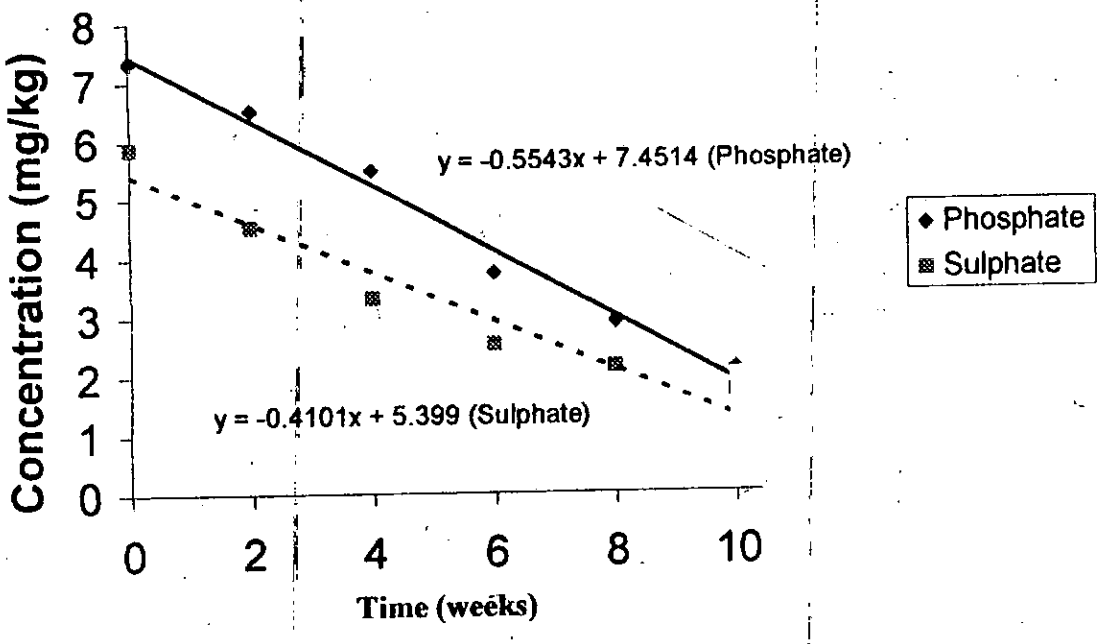


Fig. 3.6 Concentration of phosphates and sulphates in paint sample PS-3. Data represent the means of triplicate determinations.

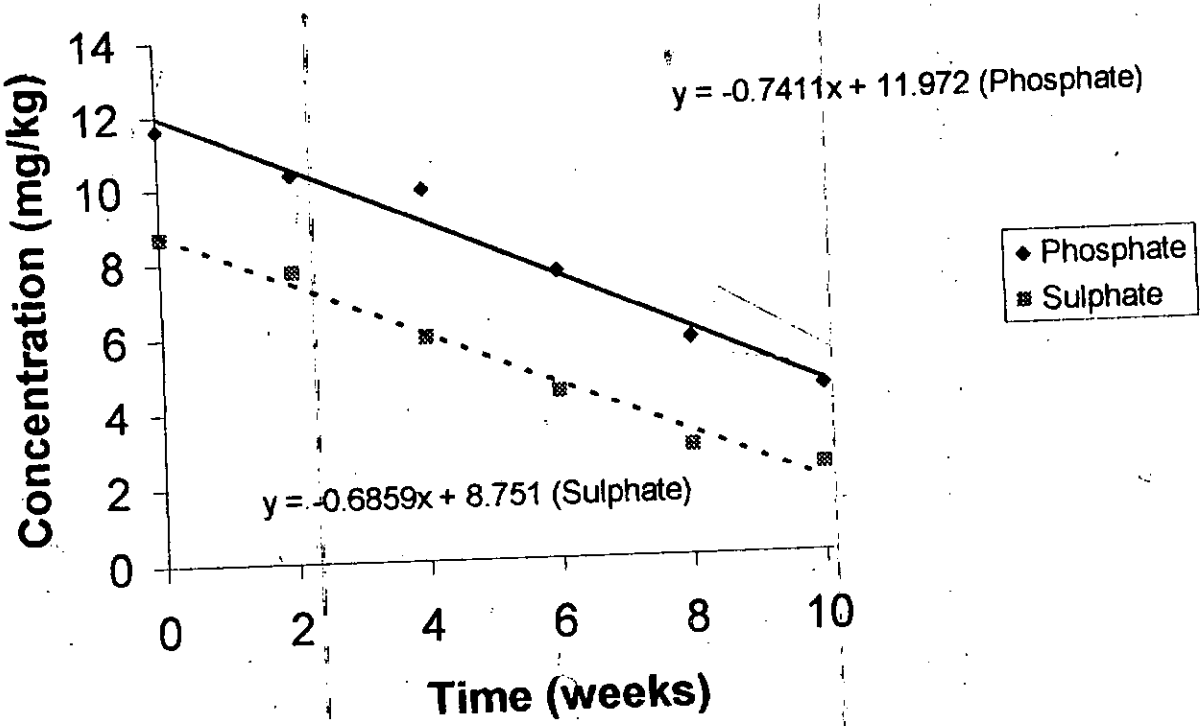


Fig. 3.7 Concentration of phosphates and sulphates in paint sample PS-4. Data represent the means of triplicate determinations.

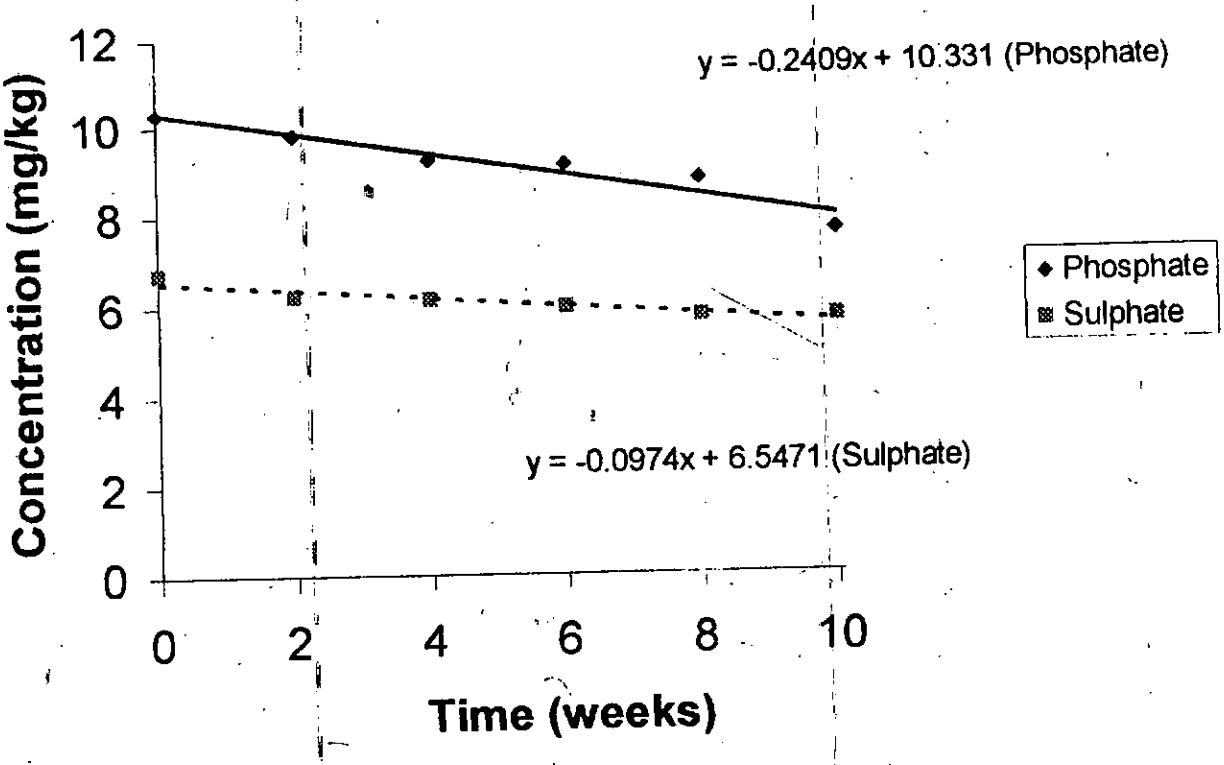


Fig. 3.8 Concentration of phosphates and sulphates in paint sample PS-5. Data represent the means of triplicate determinations.

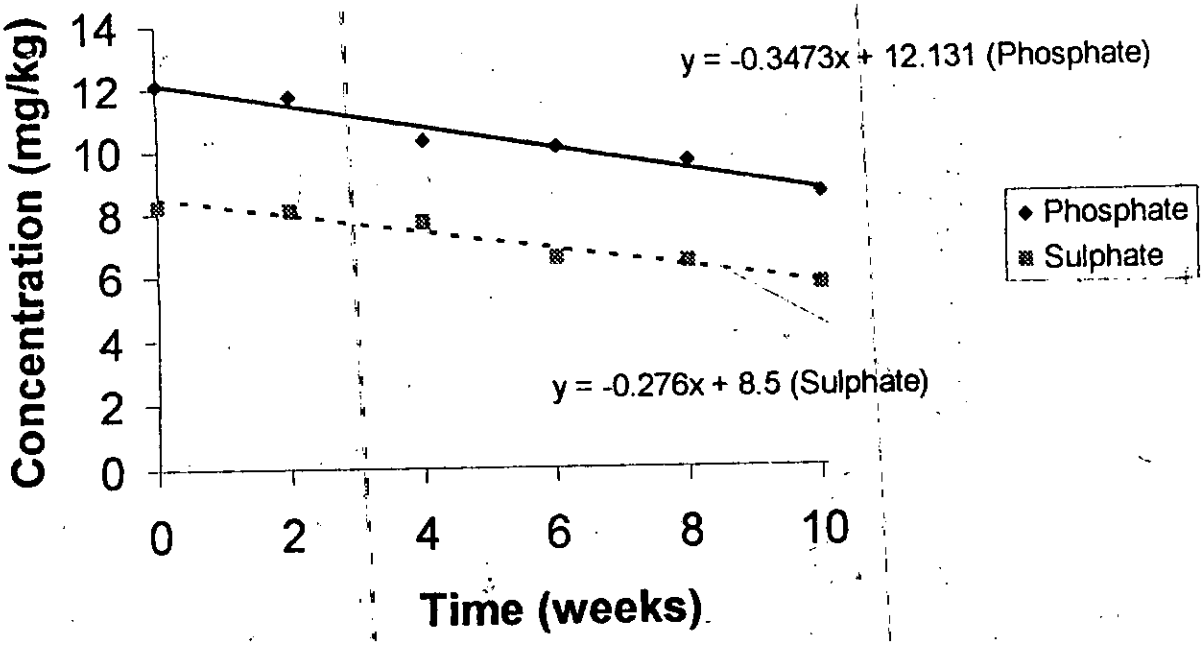


Fig. 3.9 Concentration of phosphates and sulphates in paint sample PS-6. Data represent the means of triplicate determinations.

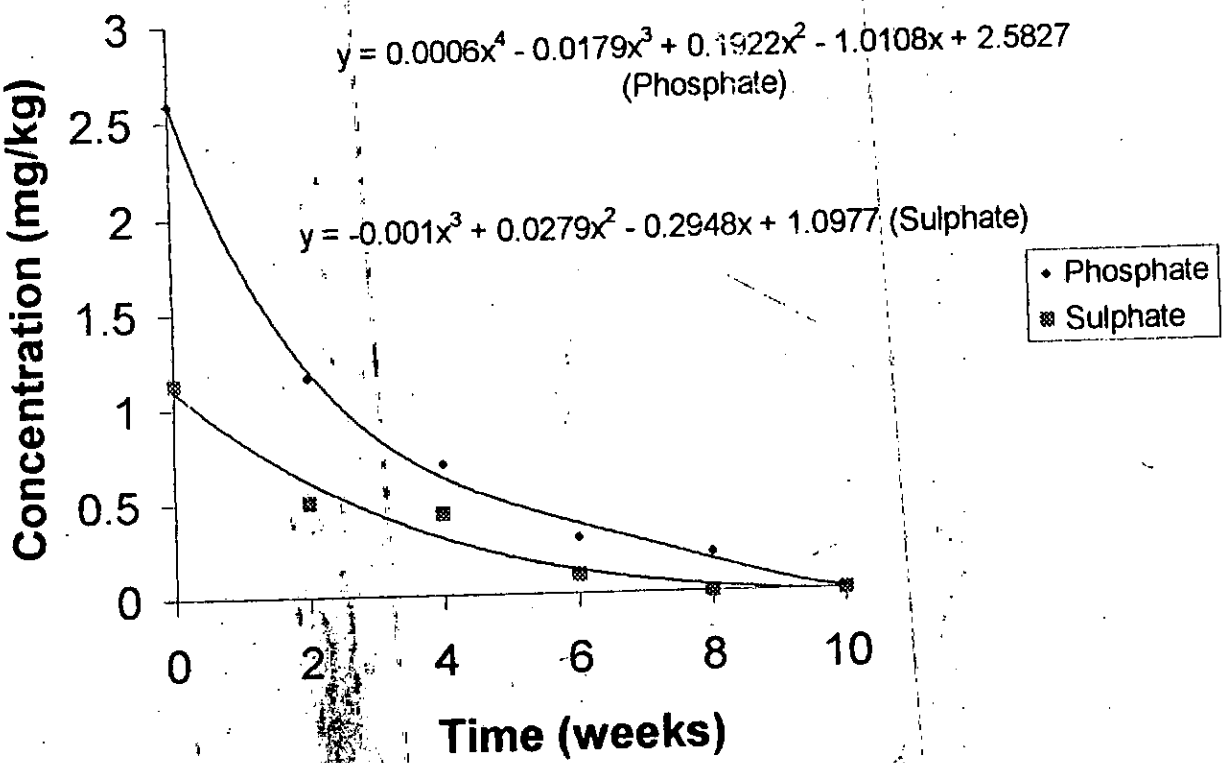


Fig 3.10 Concentration of phosphates and sulphates in spoilt paint samples. Data represent the means of triplicate determinations.

Table 3.12 Atomic absorption spectrophotometric analysis of heavy metals in various paint samples.

Paint sample	Status	Mn (mg/kg)	Cu (mg/kg)	Pb (mg/kg)
D/E chocolate	SS	3.0	5.8	3.0
D/E Summer Blue	SS	5.0	3.7	2.0
Shell	SS	5.0	4.3	2.0
Estrucian Red	SS	4.5	2.5	5.3
Brilliant White	WS	2.4	1.5	2.6
D/W/S	WS	4.1	3.1	3.0
Summer Blue	WS	3.8	3.2	3.3
Magnolia	WS	3.9	3.6	2.0
Shell	FS	3.1	1.0	2.5
Summer Blue	FS	3.0	1.8	2.0
Magnolia	FS	2.0	3.0	2.4
Brilliant White	FS	2.6	2.0	2.1

SS, spoilt paint samples; WS, warehouse samples in storage; FS, fresh samples.
Values are means of triplicate determinations.

3.9 Antimicrobial Patterns of Biocides

The disc diffusion technique revealed that all the four biocides (ZN467, ZN481, ZN485, and ZN489) were effective only at 3% (v/v) concentration. However, optimal results were obtained at 5% (v/v) (Plates 3.2 – 3.11). The co-contaminant effect on biocidal activity toward individual test organisms was studied with the test organisms in mixed suspension. The results further demonstrated that the biocides were more effective on individual organisms than on consortium of organisms (Plates 3.9, 3.10 & 3.11). As in the pure suspension, ZN467 was observed to be most effective against the consortium of organisms compared to the other biocides. Biocide ZN489 performed poorly against individual organisms and a consortium of organisms (Plates 3.7, 3.8 & 3.11). Biocides ZN481 and ZN485 demonstrated moderate activity against *Proteus mirabilis* (Plates 3.4 & 3.6). Biocide ZN467 had the highest activity against individual organisms. This was followed by ZN481, ZN485 and lastly ZN489. As expected, *Pseudomonas aeruginosa*, having a lipid-rich waxy cell wall demonstrated substantial resistance to ZN489 (Plate 3.8) and the least resistance to ZN467 (Plate 3.3). *Pseudomonas aeruginosa* had the least inhibition from all the biocides tested.

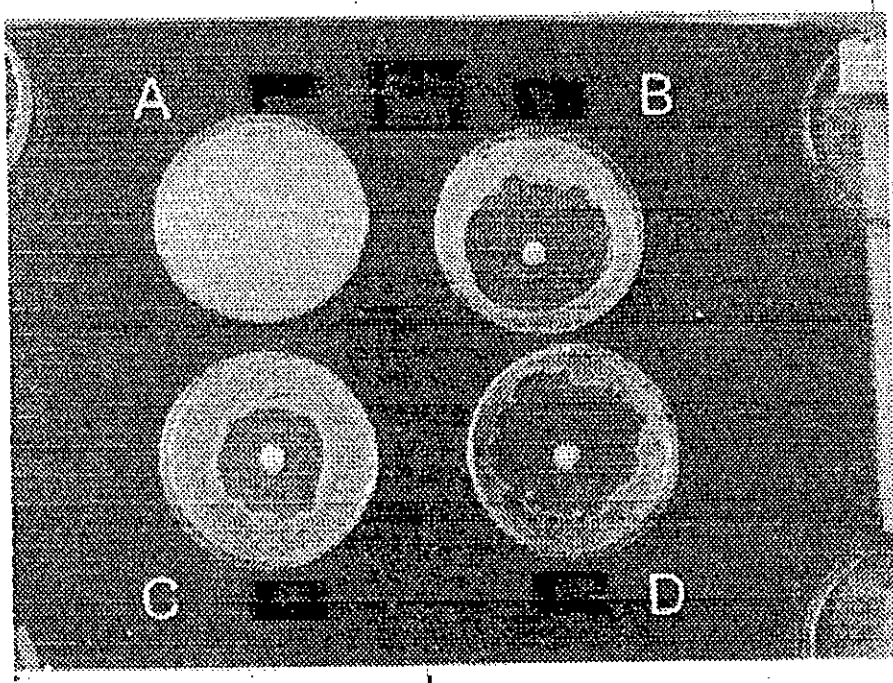


Plate 3.2 Effect of biocide ZN467 on *Lactobacillus gasseri*
A, control; B, 1% v/v; C, 3% v/v; D, 5% v/v

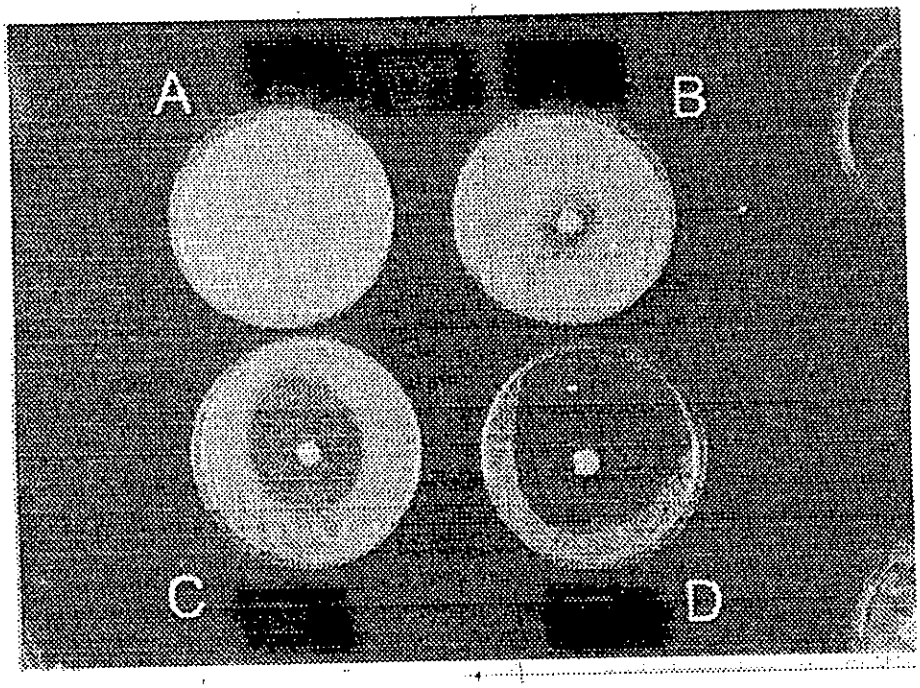


Plate 3.3 Effect of biocide ZN467 on *Pseudomonas aeruginosa*
A, control; B, 1% v/v; C, 3% v/v; D, 5% v/v

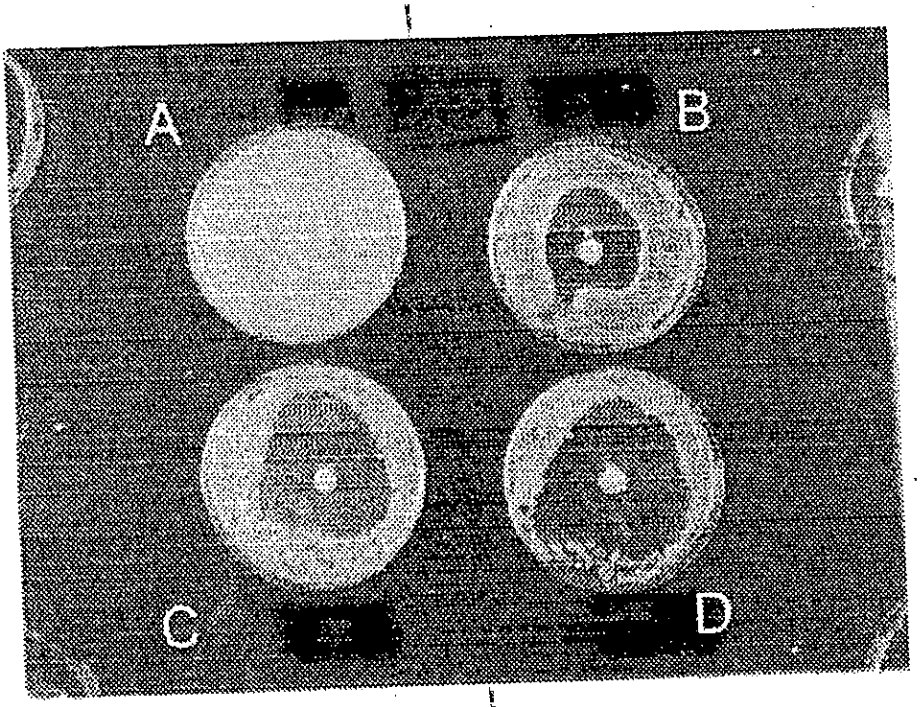


Plate 3.4 Effect of biocide ZN481 on *Proteus mirabilis*
A, control; B, 1% v/v; C, 3% v/v; D, 5% v/v

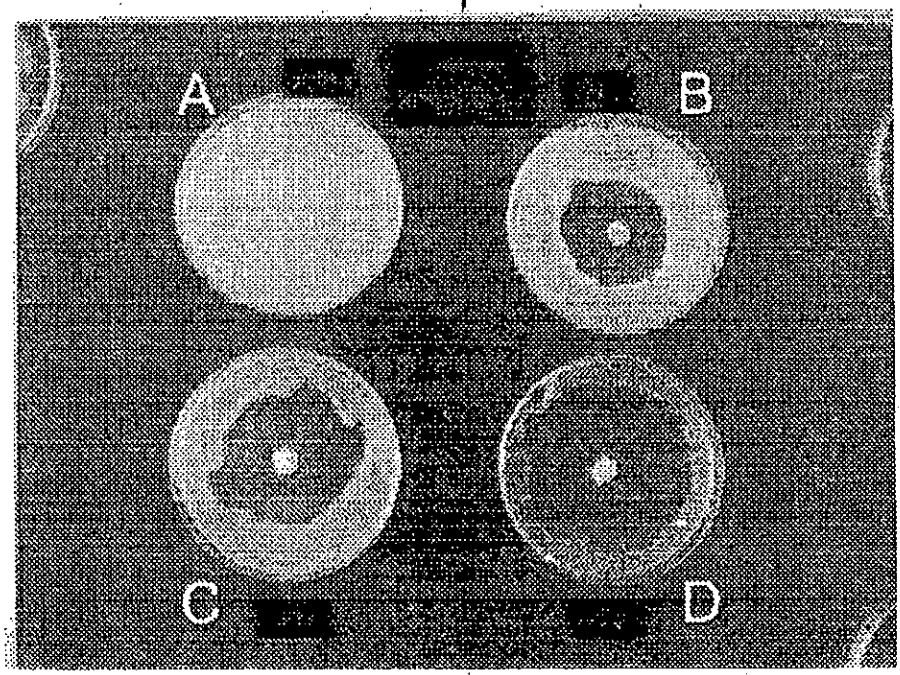


Plate 3.5 Effect of biocide ZN481 on *Lactobacillus gasseri*
A, control; B, 1% v/v; C, 3% v/v; D, 5% v/v

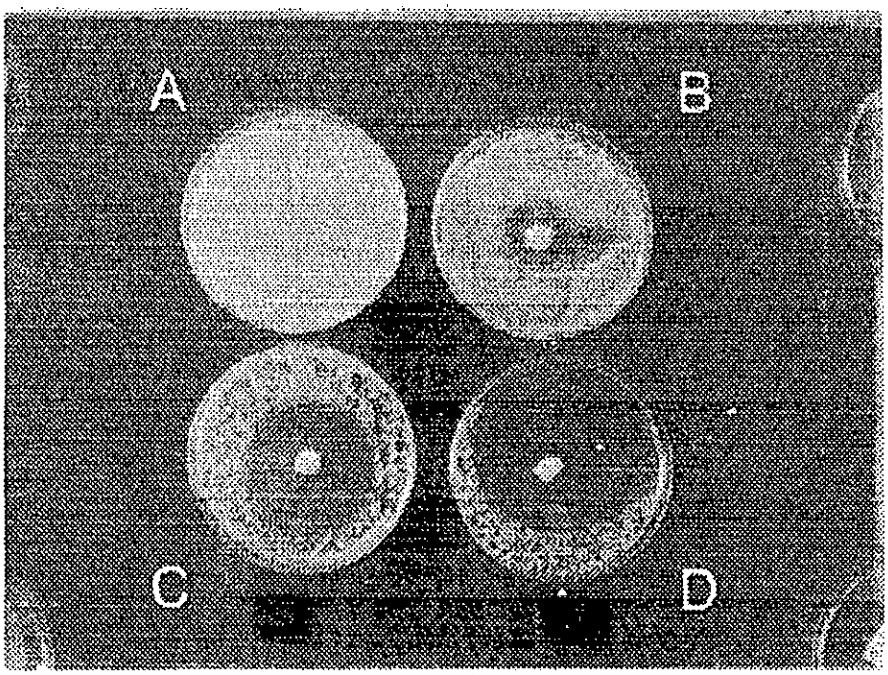


Plate 3.6 Effect of biocide ZN485 on *Proteus mirabilis*
A, control; B, 1% v/v; C, 3% v/v; D, 5% v/v

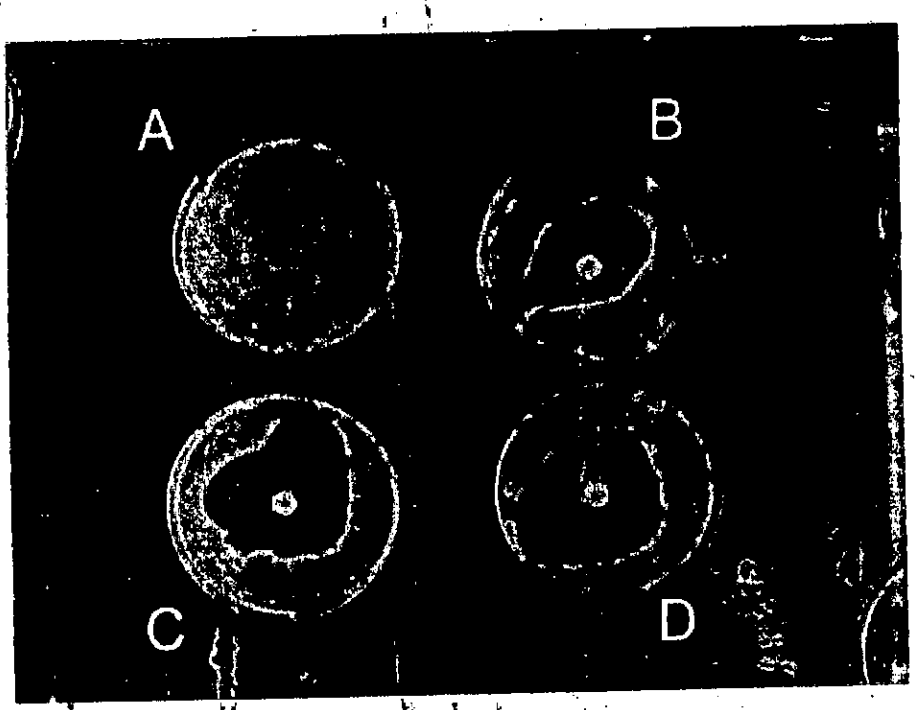


Plate 3.7 Effect of biocide ZN489 on *Lactobacillus gasseri*
A, control; B, 1% v/v; C, 3% v/v; D, 5% v/v

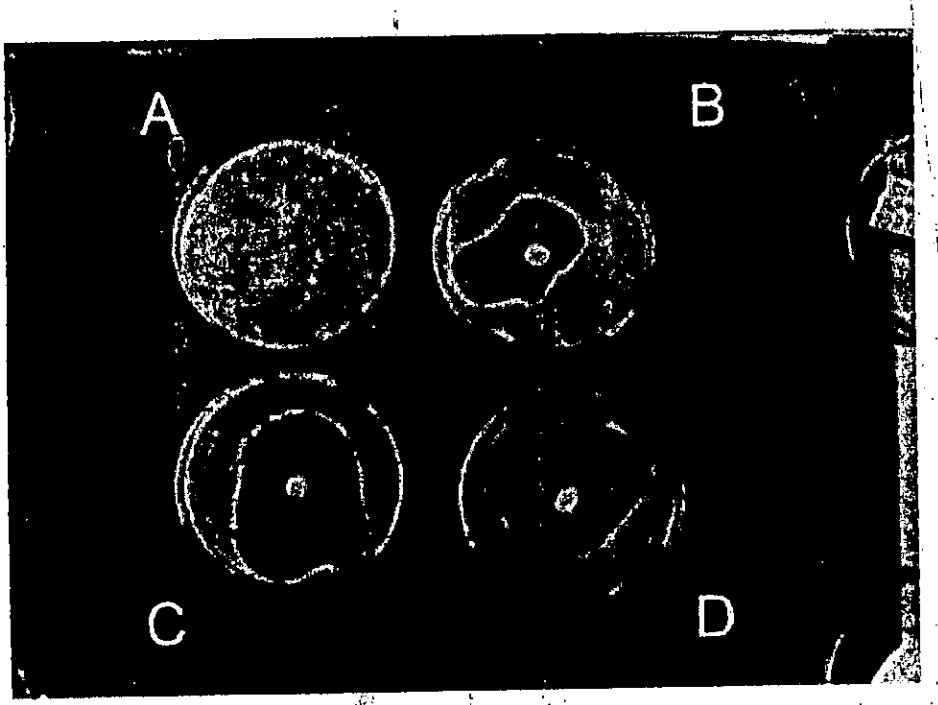


Plate 3.8 Effect of biocide ZN489 on *Pseudomonas aeruginosa*
A, control; B, 1% v/v; C, 3% v/v; D, 5% v/v

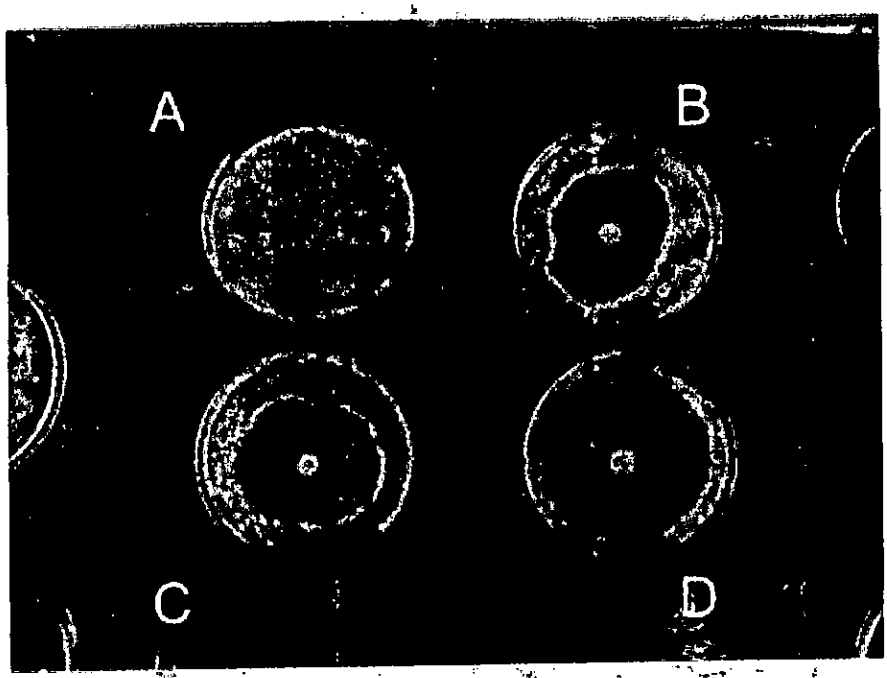


Plate 3.9 Effect of biocide ZN467 on a consortium of organisms A, control; B, 1% v/v; C, 3% v/v; D, 5% v/v

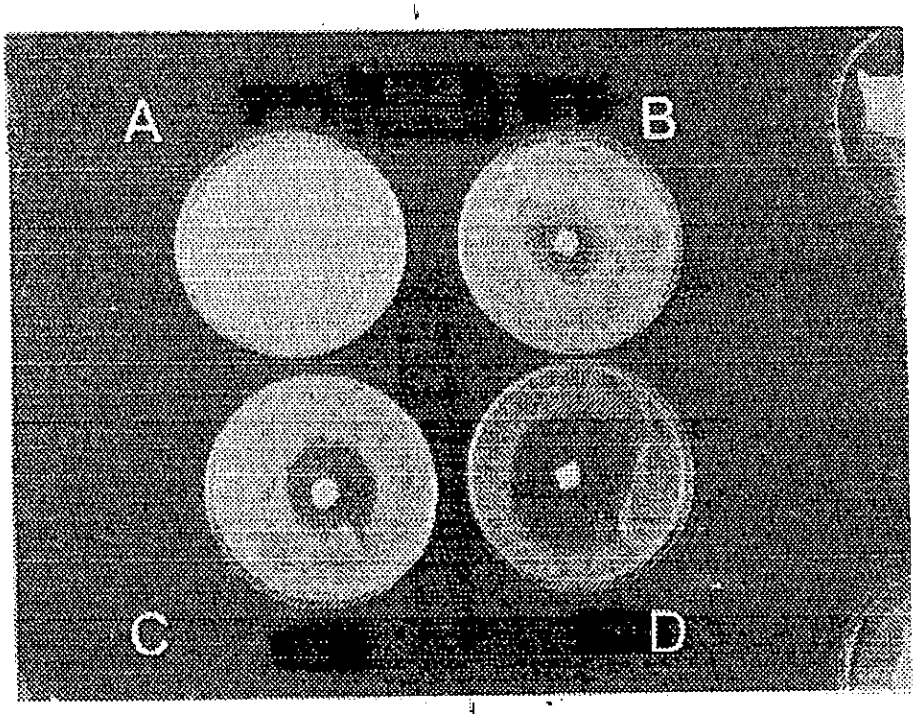


Plate 3.10 Effect of biocide ZN485 on a consortium of organisms
A, control; B, 1% v/v; C, 3% v/v; D, 5% v/v

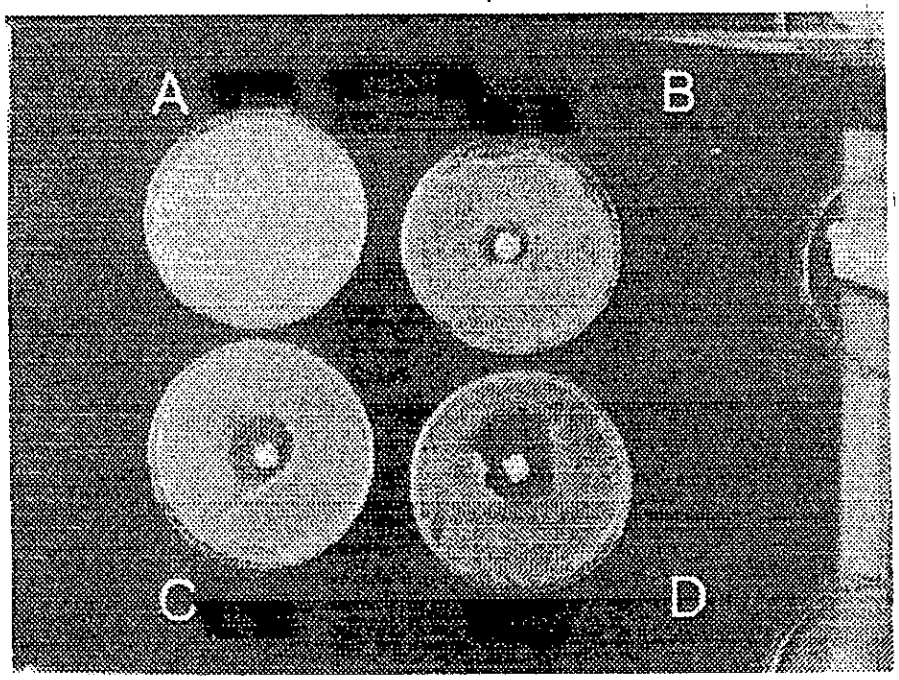


Plate 3.11 Effect of biocide ZN489 on a consortium of organisms
A, control; B, 1% v/v; C, 3% v/v; D, 5% v/v

3.10 Effects of Different Concentrations of Different Biocides on Microbial Population Densities of Fresh, Sterile Paints

The results of the different concentrations of four biocides used in paint production showed that 0.5% (v/v) of each of the biocides had minimal effect on the microbial population densities of the paint samples. The four biocides used in paint production designated ZN467, ZN481, ZN485 and ZN489 all inhibited microbial growth in fresh sterile paints at different rates (Figs. 3.11 – 3.22). At concentrations of 0.5% – 3% (v/v) of all the biocides, there was no bacterial and fungal growth at week 0 (Figs. 3.11 – 3.22). The samples with 3% (v/v) ZN467 had bacterial growth (1.0×10^1 cfu/ml) only at week 8 (Fig. 3.14) and fungal growth at week 8 (Fig. 3.22). However, bacterial growth (1.0×10^1 cfu/ml) and fungal growth (1.0×10^1 cfu/ml) was observed in samples with 3% ZN489 by week 4 (Fig. 3. 22). Coliform growth (1.2×10^1 cfu/ml) was observed in samples with 0.5% (v/v) of ZN481 and ZN485 by week 4. In contrast, coliform growth was observed from week 2 in samples with 0.5% (v/v) ZN489 (Fig.3.15). Only 3% (v/v) ZN467 inhibited coliform growth till the 8th week (Fig. 3.18). In contrast, there was coliform growth (1.0×10^1cfu/ml) in samples with 3% (v/v) ZN489 from the 4th week and coliform growth (1.0×10^1cfu/ml) in samples with 3% (v/v) ZN481 and ZN485 from the 6th week (Fig. 3.18).

3.11 Effects of Different Concentrations of Different Biocides on Physico-chemical Parameters of Fresh, Sterile Paints

The results represented in Figs.3.23– 3.42 summarize the differences in the antimicrobial activities of the biocides on physico-chemical parameters of fresh sterile paint samples. Biocide ZN467 was observed to be effective at lower concentrations compared to the other test biocides. The efficacy of ZN467 was particularly more pronounced from 1% (v/v) compared to others. The SG of samples with 1% (v/v) ZN467 decreased from 2.8658 – 2.4683 from week 0 – week 10. However, at the same concentration, samples with ZN489 had decrease in SG from the initial 2.8658 – 1.9251 by week 10. The SG started decreasing by week 2 in samples with 2% ZN489 (Fig. 3.25). In contrast, samples with 2% (v/v) of other biocides had decrease in SG from week 6. At week 10, samples with 3% (v/v) ZN489 had maximum increase in optical density from the initial 1.63 – 1.84. On the other hand, samples with 3% (v/v) ZN467 had OD inreased from an initial value of 1.63 – 1.67 by week 10 (Fig.3.30).The rate of reduction in viscosity was the same for all the samples from week 0

– week 4 at 3% (v/v) concentration. On the 6th week, samples with 3% (v/v) ZN481 and ZN485 had reduced viscosity from the initial 11.2 –11.1 cst. It is noteworthy to observe that samples with 3% (v/v) ZN467 had viscosity of 11.2 cst from week 0 till the 10th week (Fig.3.42). This further confirms the superior efficacy of biocide ZN467 over the other test biocides.

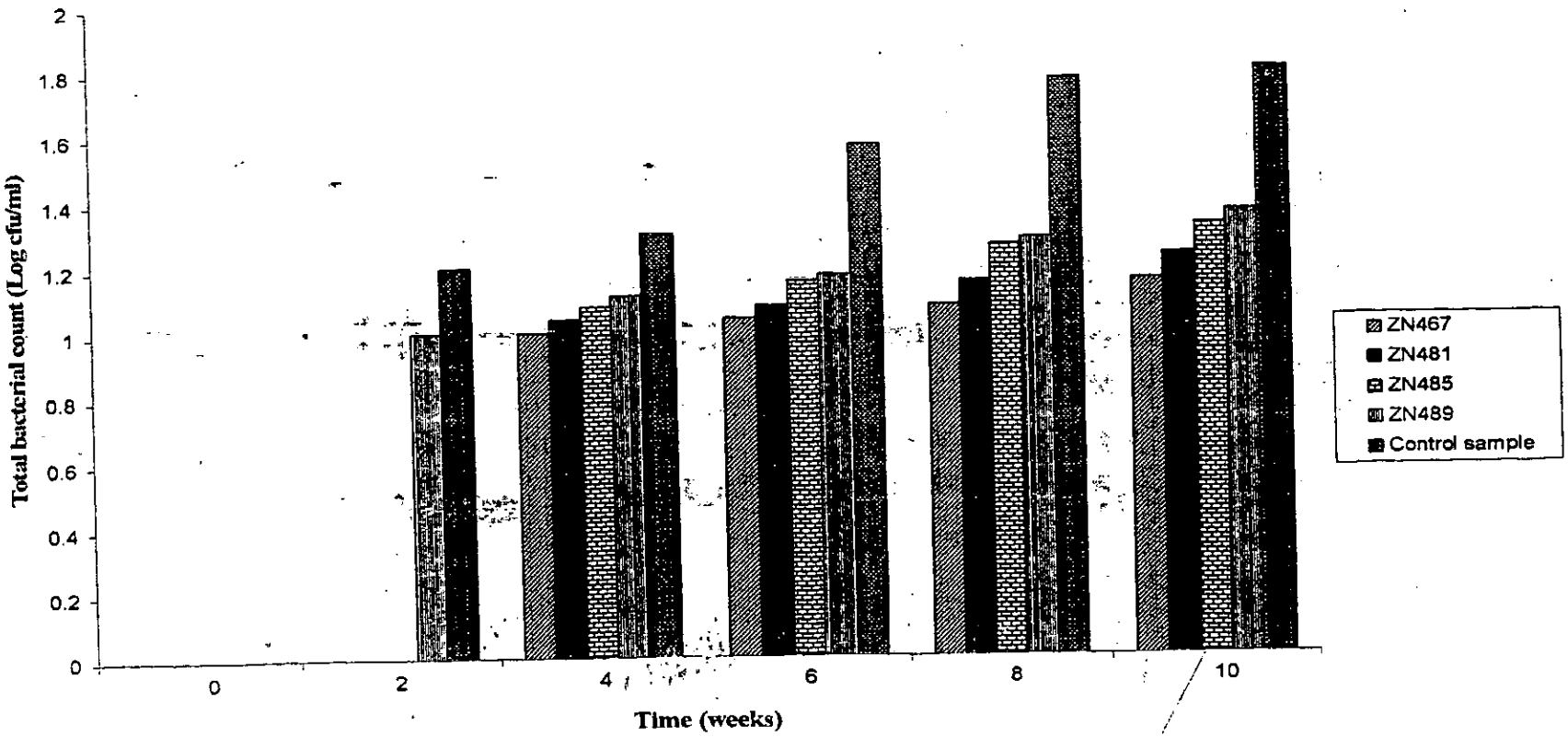


Fig. 3.11 **Effect of 0.5% v/v of biocides on total bacterial count of fresh, sterile paint samples. The control samples had no biocides.**

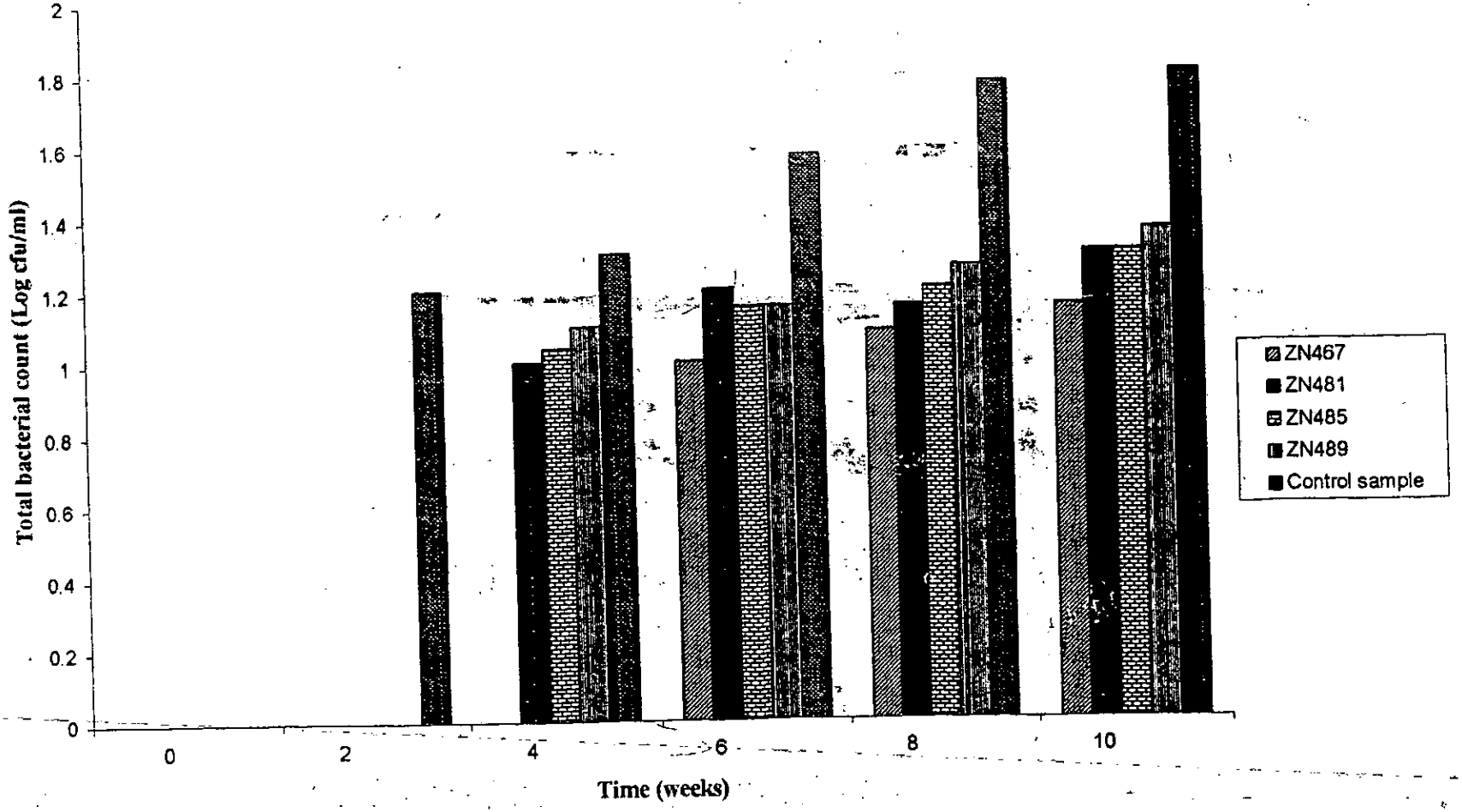


Fig. 3.12 **Effect of 1% v/v of biocides on total bacterial count of fresh, sterile paint samples. The control samples had no biocides.**

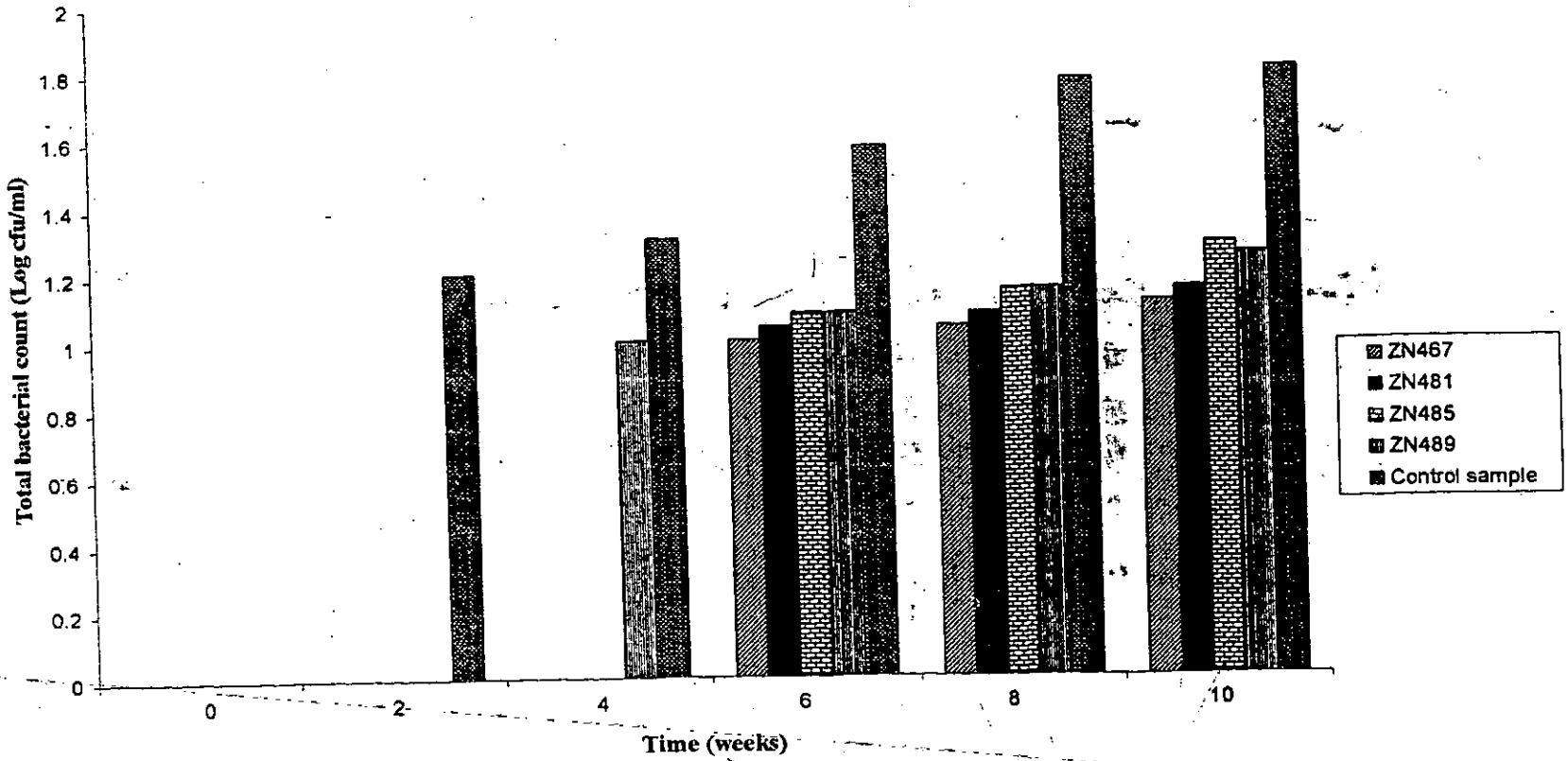


Fig. 3.13 Effect of 2% v/v of biocides on total bacterial count of fresh, sterile paint samples. The control samples had no biocides.

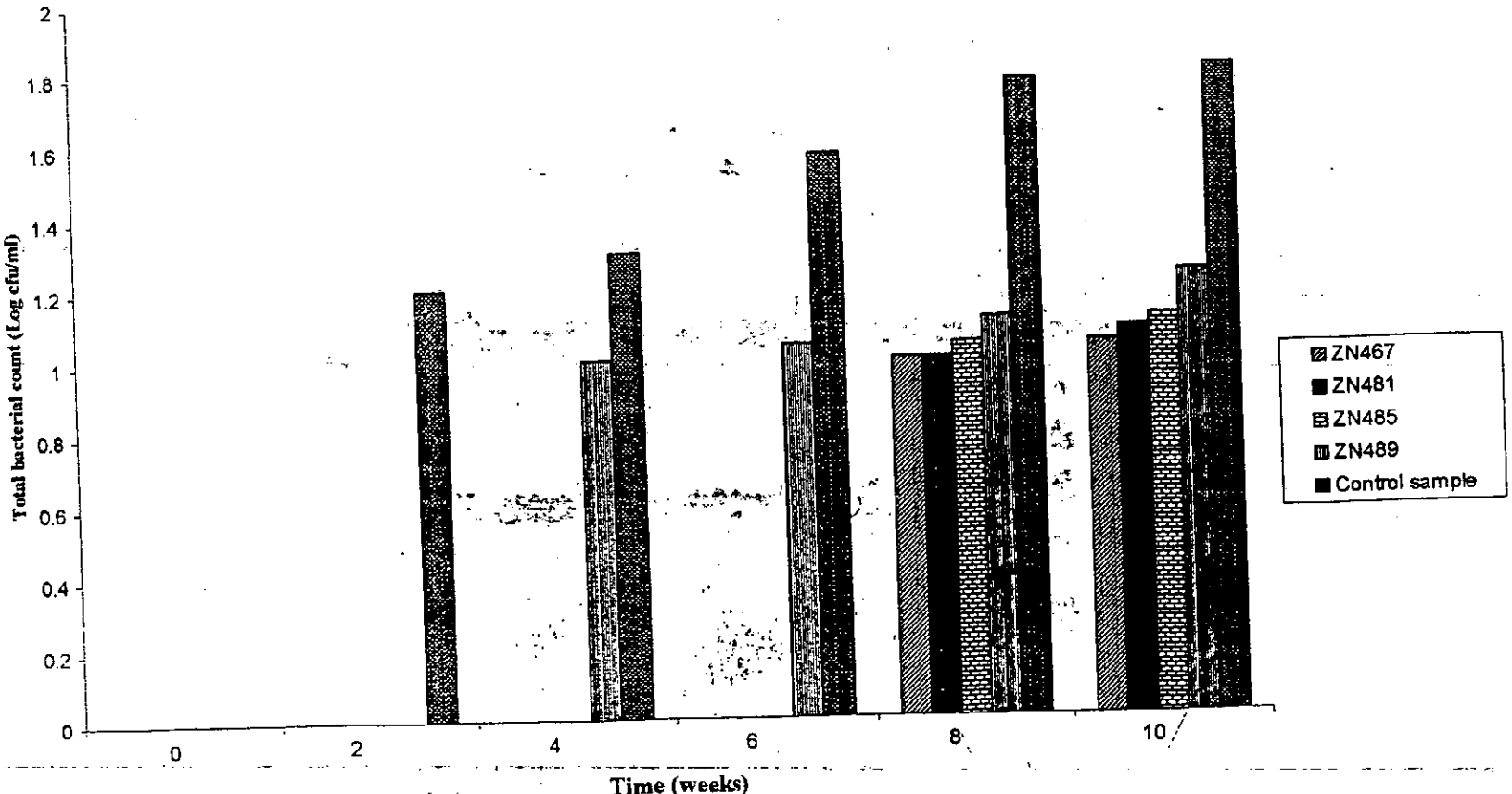


Fig. 3.14 **Effect of 3% v/v of biocides on total bacterial count of fresh, sterile paint samples. The control samples had no biocides.**

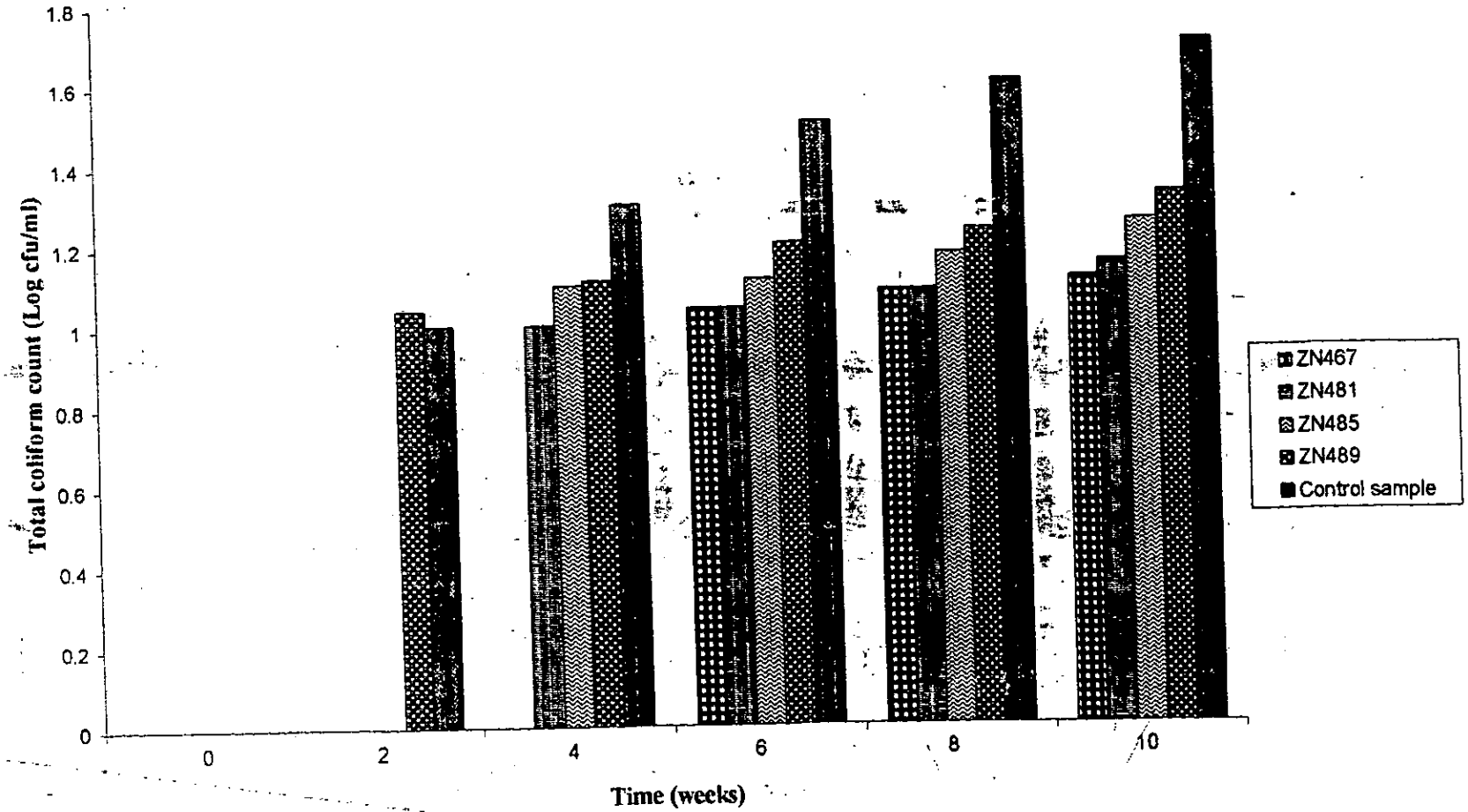


Fig. 3.15 **Effect of 0.5% v/v of biocides on total coliform count of fresh, sterile paint samples. The control samples had no biocides.**

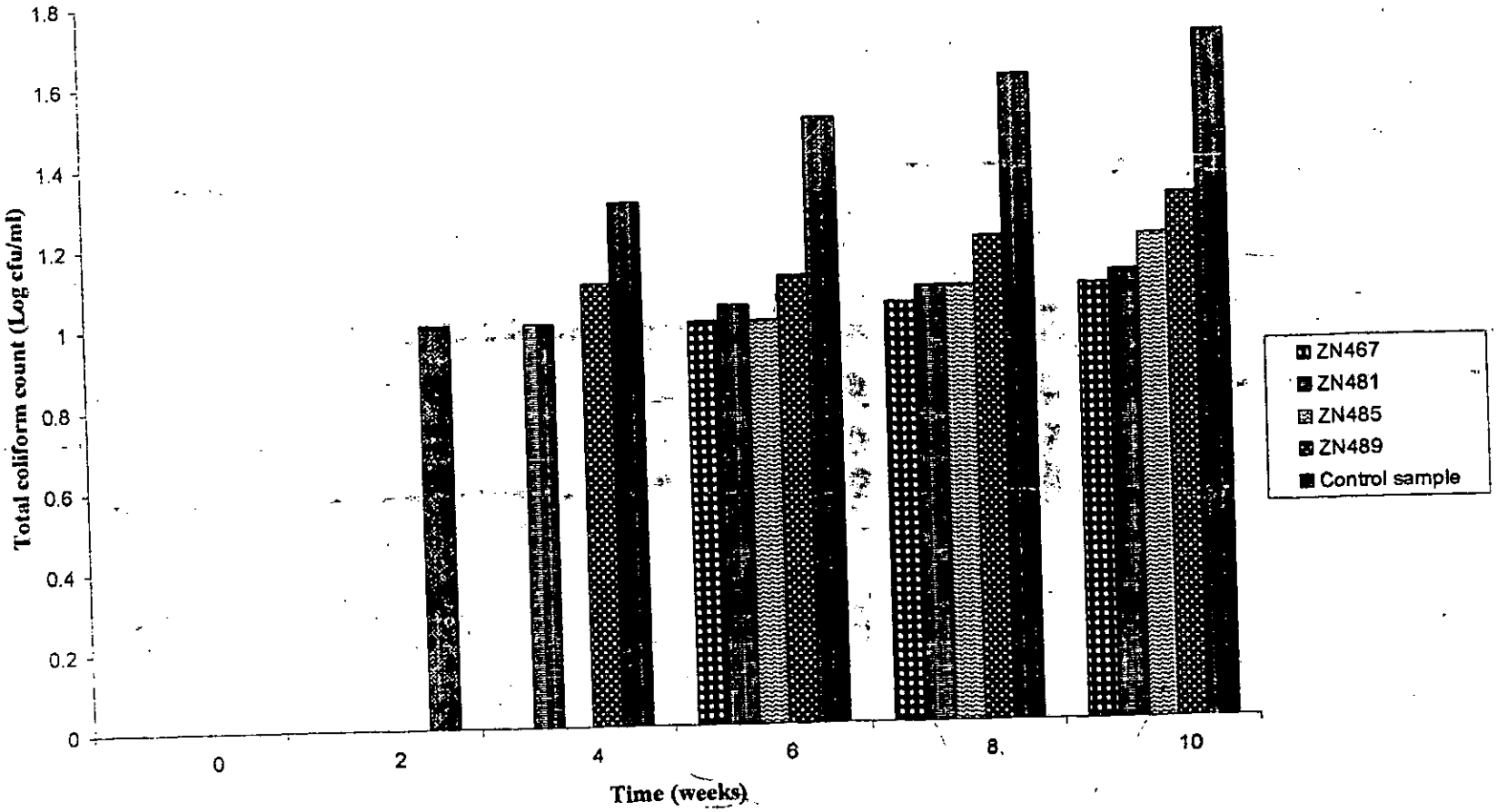


Fig. 3.16 **Effect of 1% v/v of biocides on total coliform count of fresh, sterile paint samples. The control samples had no biocides.**

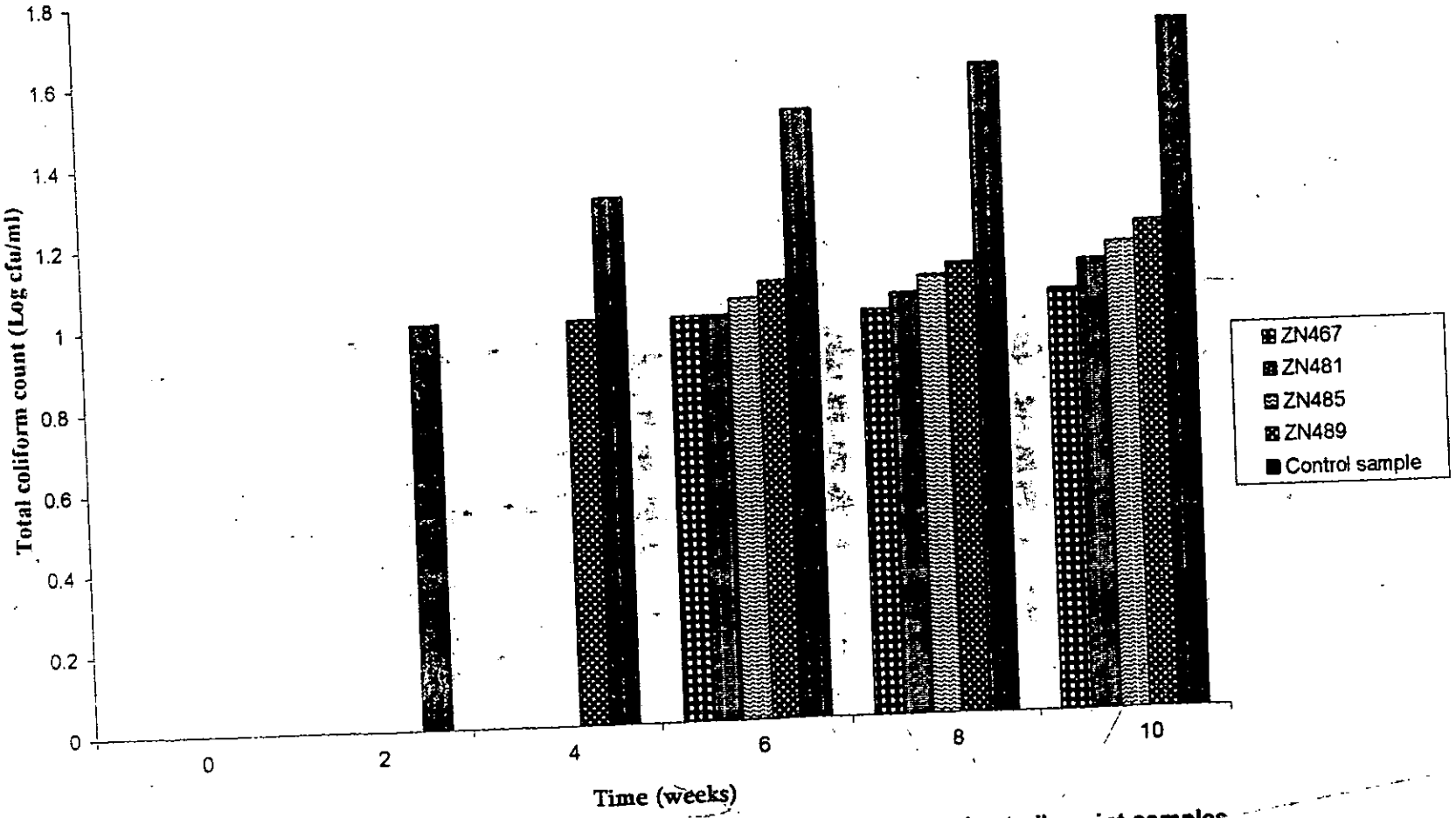


Fig. 3.17 **Effect of 2% v/v of biocides on total coliform count of fresh, sterile paint samples. The control samples had no biocides.**

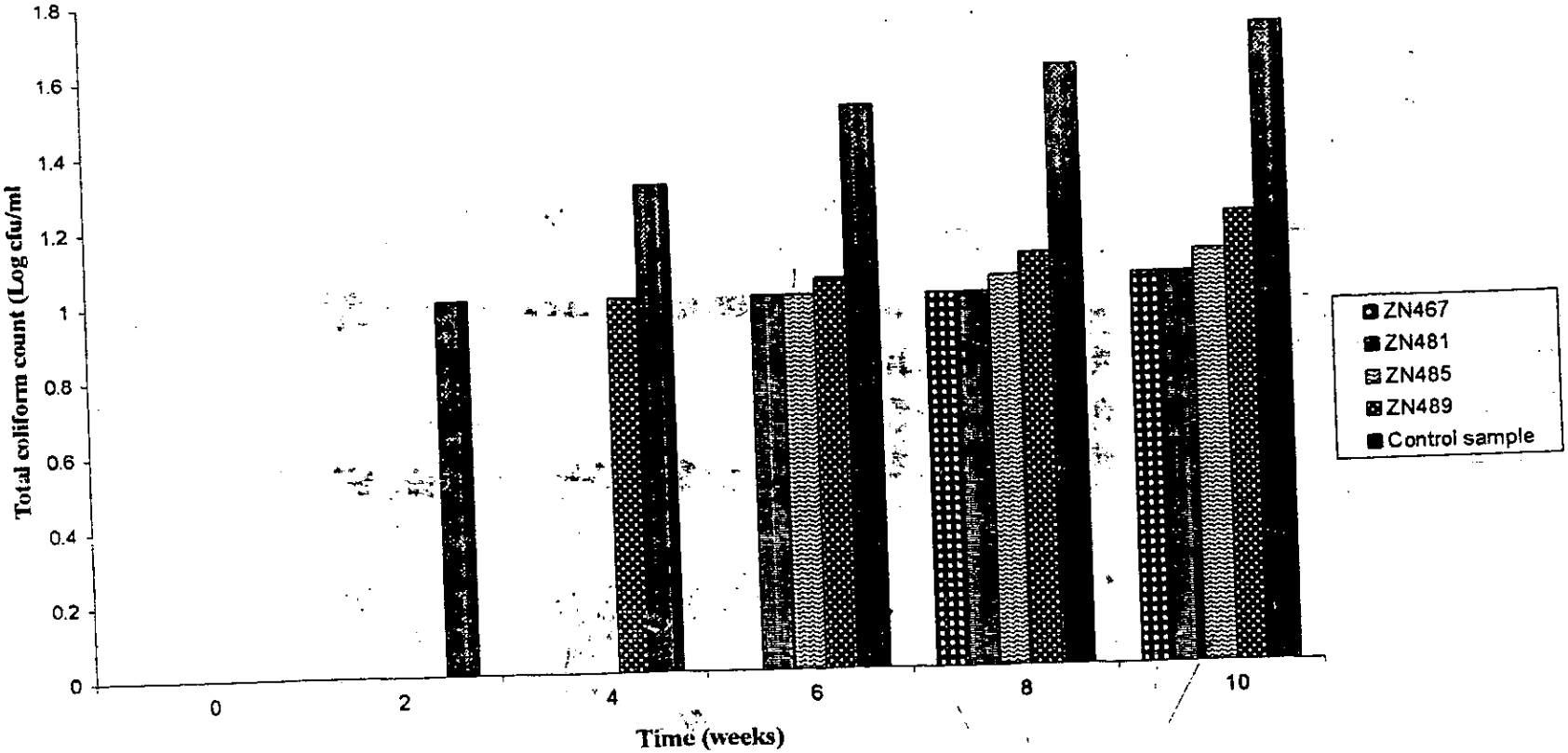


Fig. 3.18 Effect of 3% v/v of biocides on total coliform count of fresh, sterile paint samples. The control samples had no biocides.

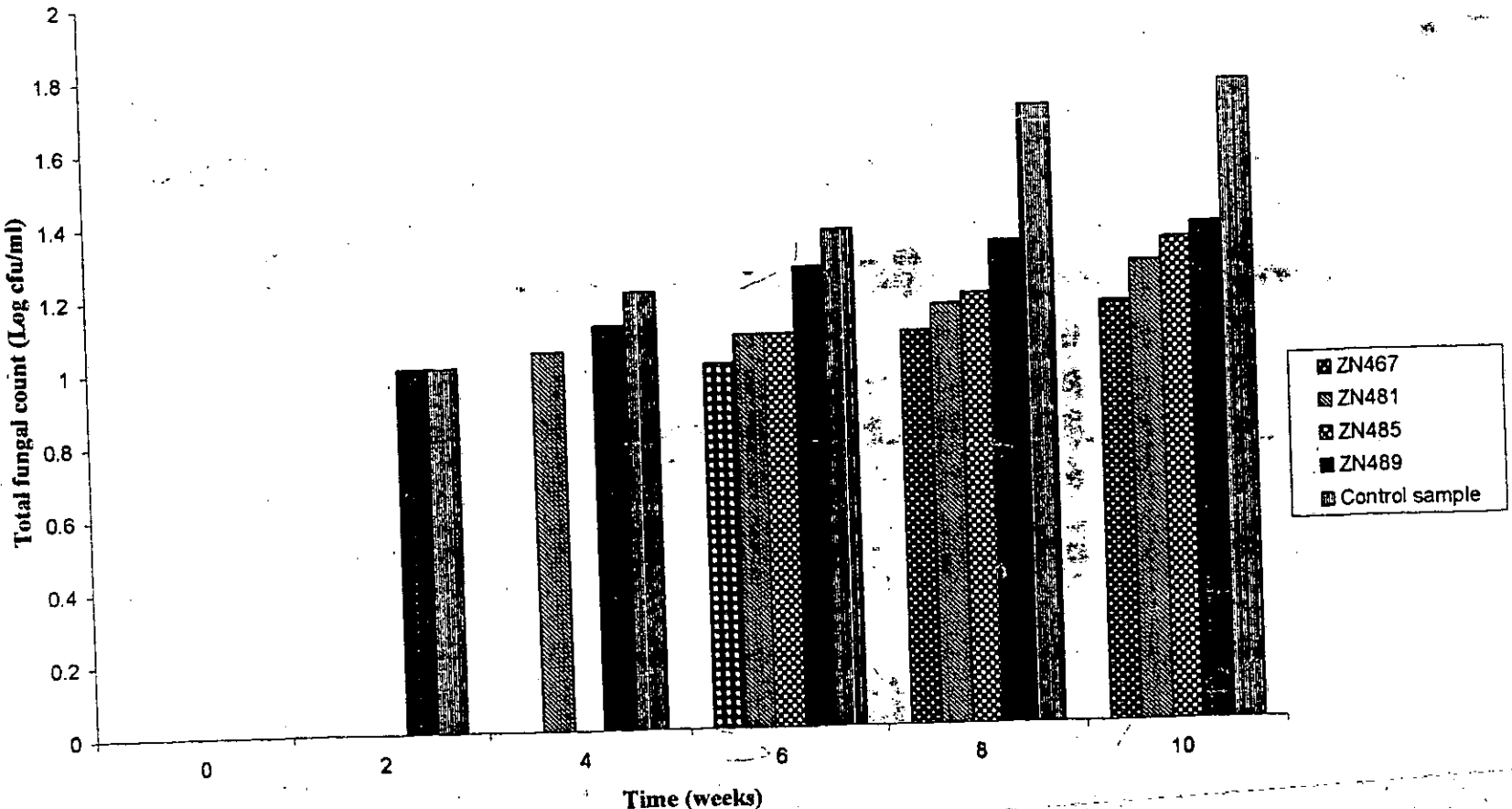


Fig. 3.19 Effect of 0.5% v/v of biocides on total fungal count of fresh, sterile paint samples. The control samples had no biocides.

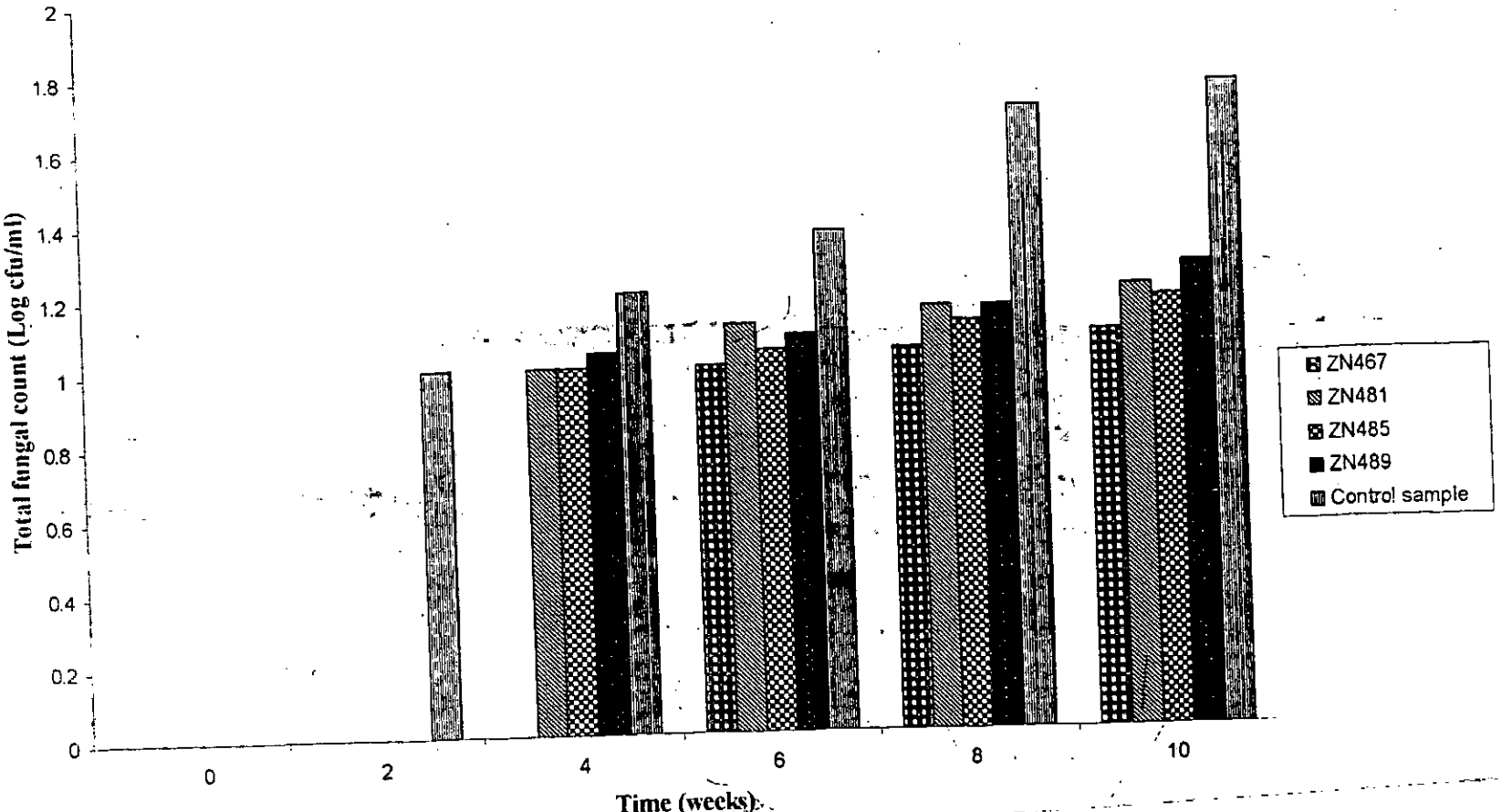


Fig. 3.20 **Effect of 1% v/v of biocides on total fungal count of fresh, sterile paint samples. The control samples had no biocides.**

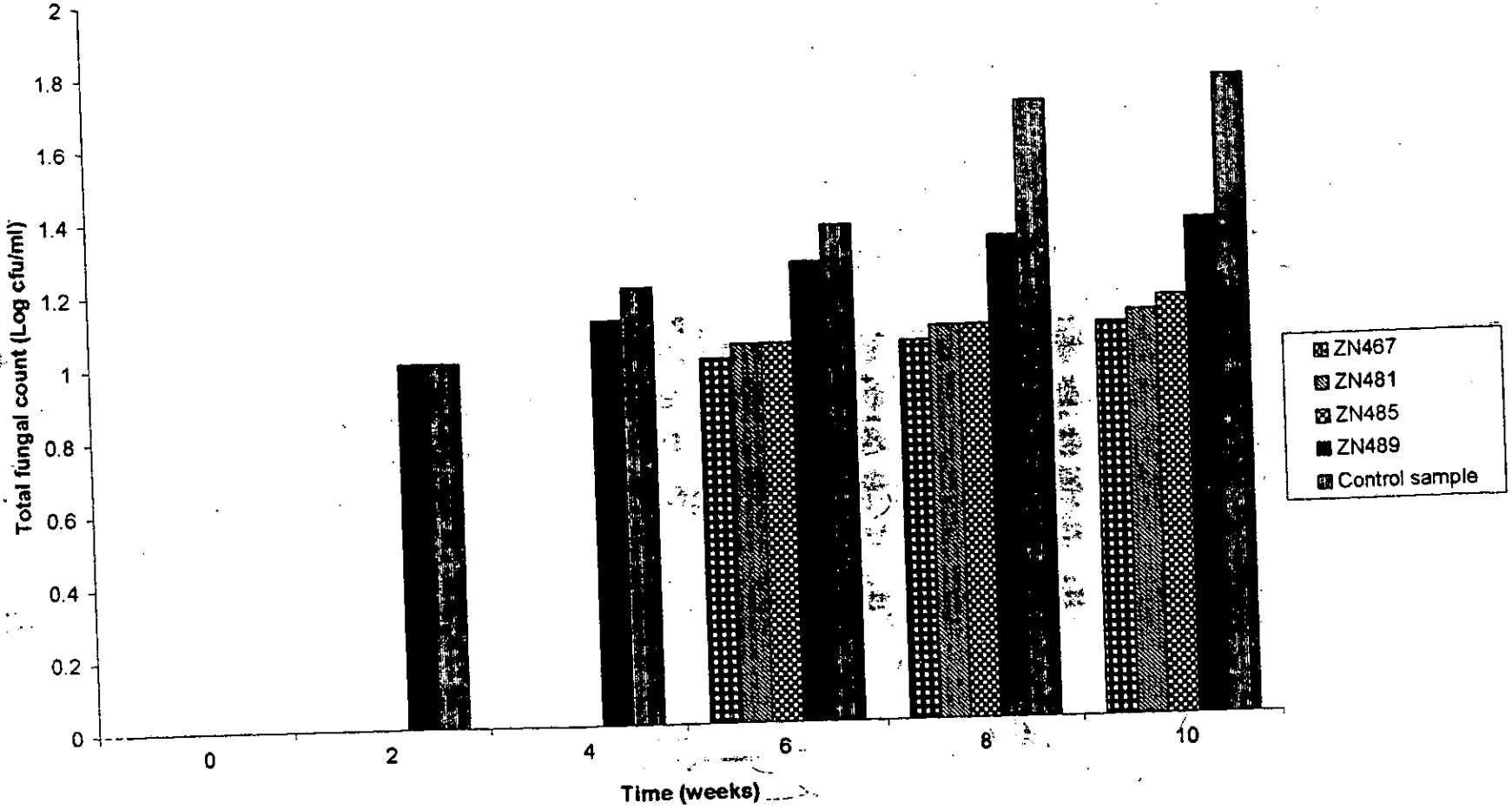


Fig. 3.21 **Effect of 2% v/v of biocides on total fungal count of fresh, sterile paint samples. The control samples had no biocides.**

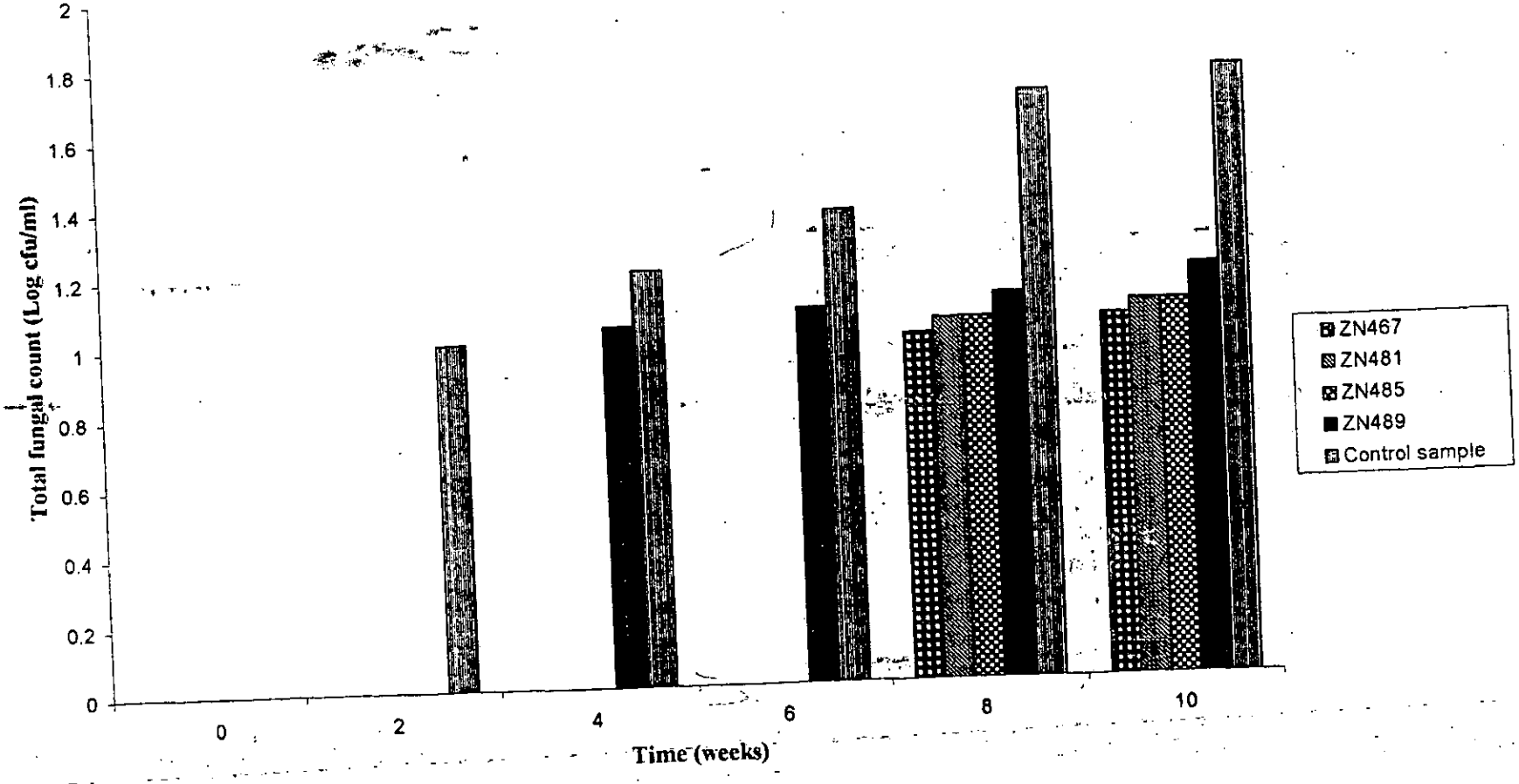


Fig. 3.22 **Effect of 3% v/v of biocides on total fungal count of fresh, sterile paint samples. The control samples had no biocides.**

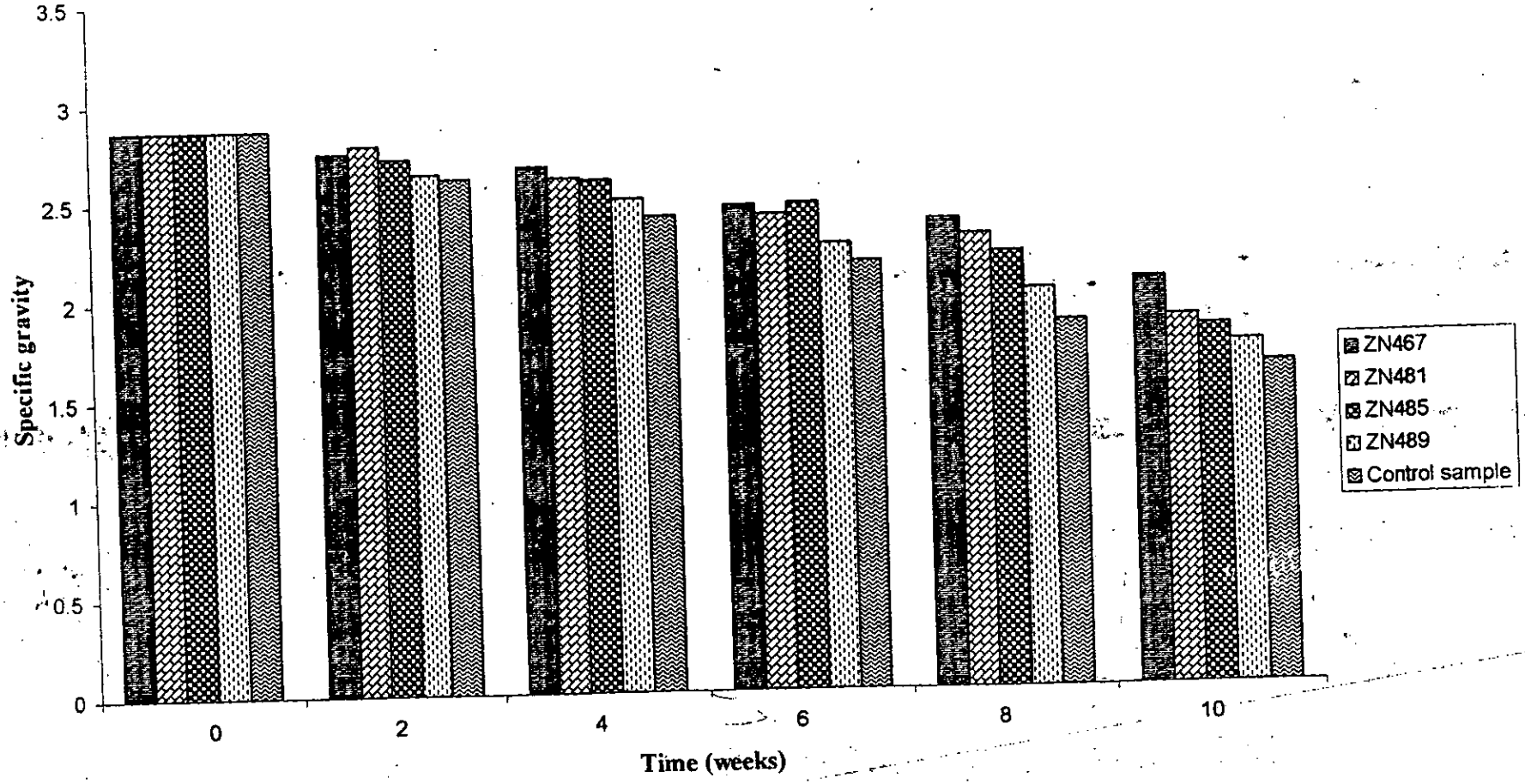


Fig. 3.23 **Effect of 0.5% v/v of biocides on specific gravity of fresh, sterile paint samples. The control samples had no biocides.**

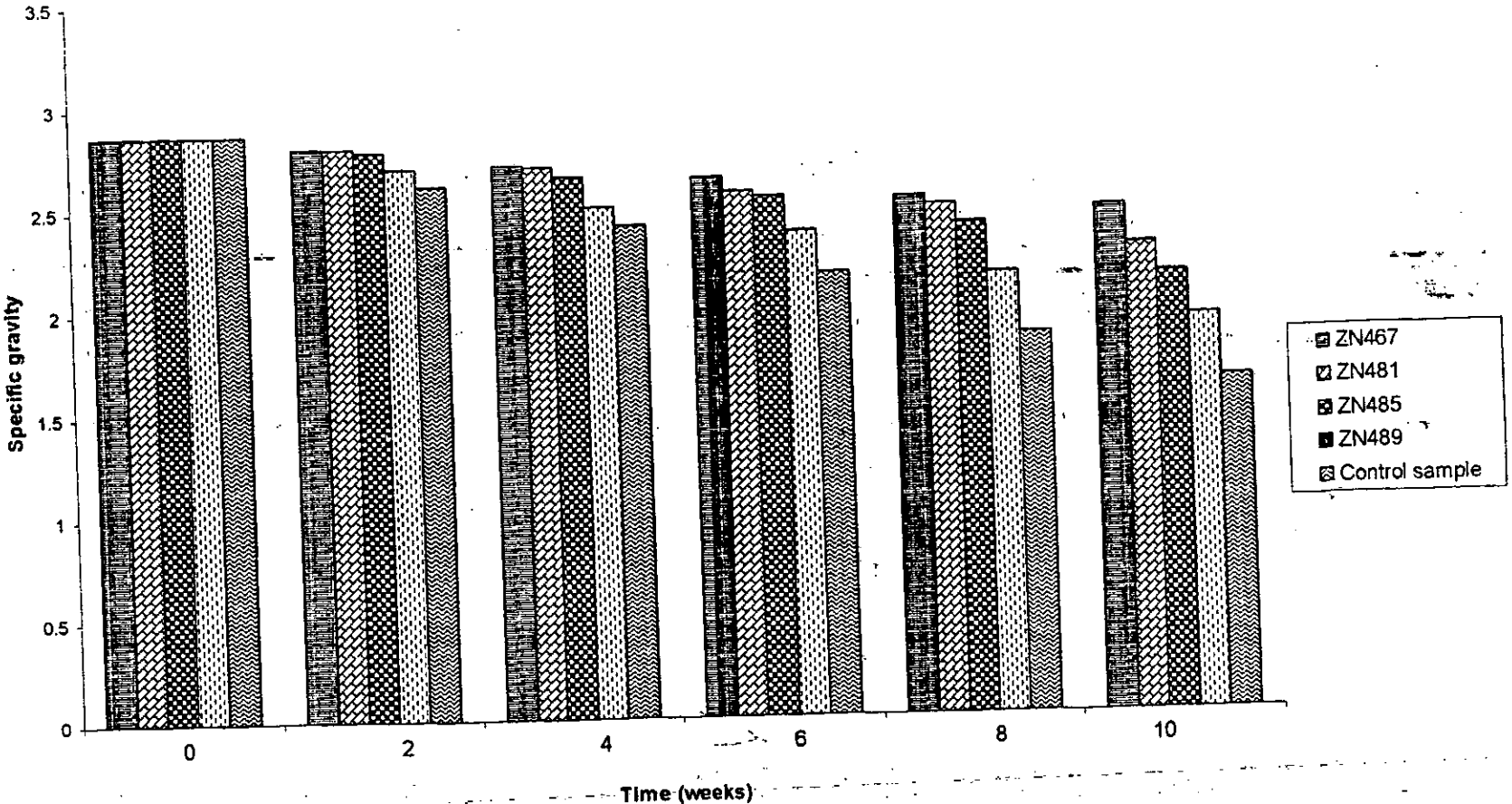


Fig. 3.24 Effect of 1% v/v of biocides on specific gravity of fresh, sterile paint samples. The control samples had no biocides.

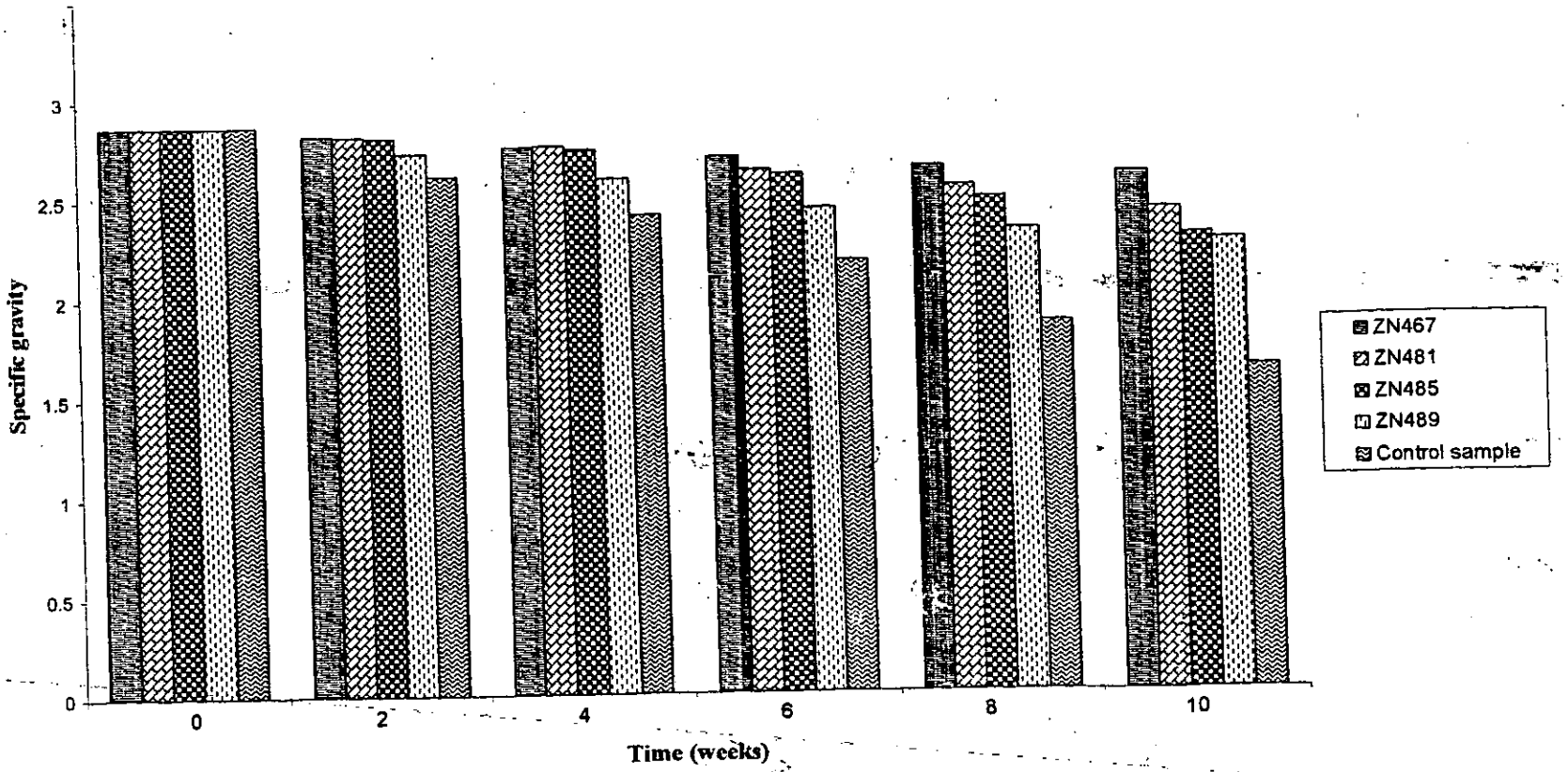


Fig. 3.25 **Effect of 2% v/v of biocides on specific gravity of fresh, sterile paint samples. The control samples had no biocides.**

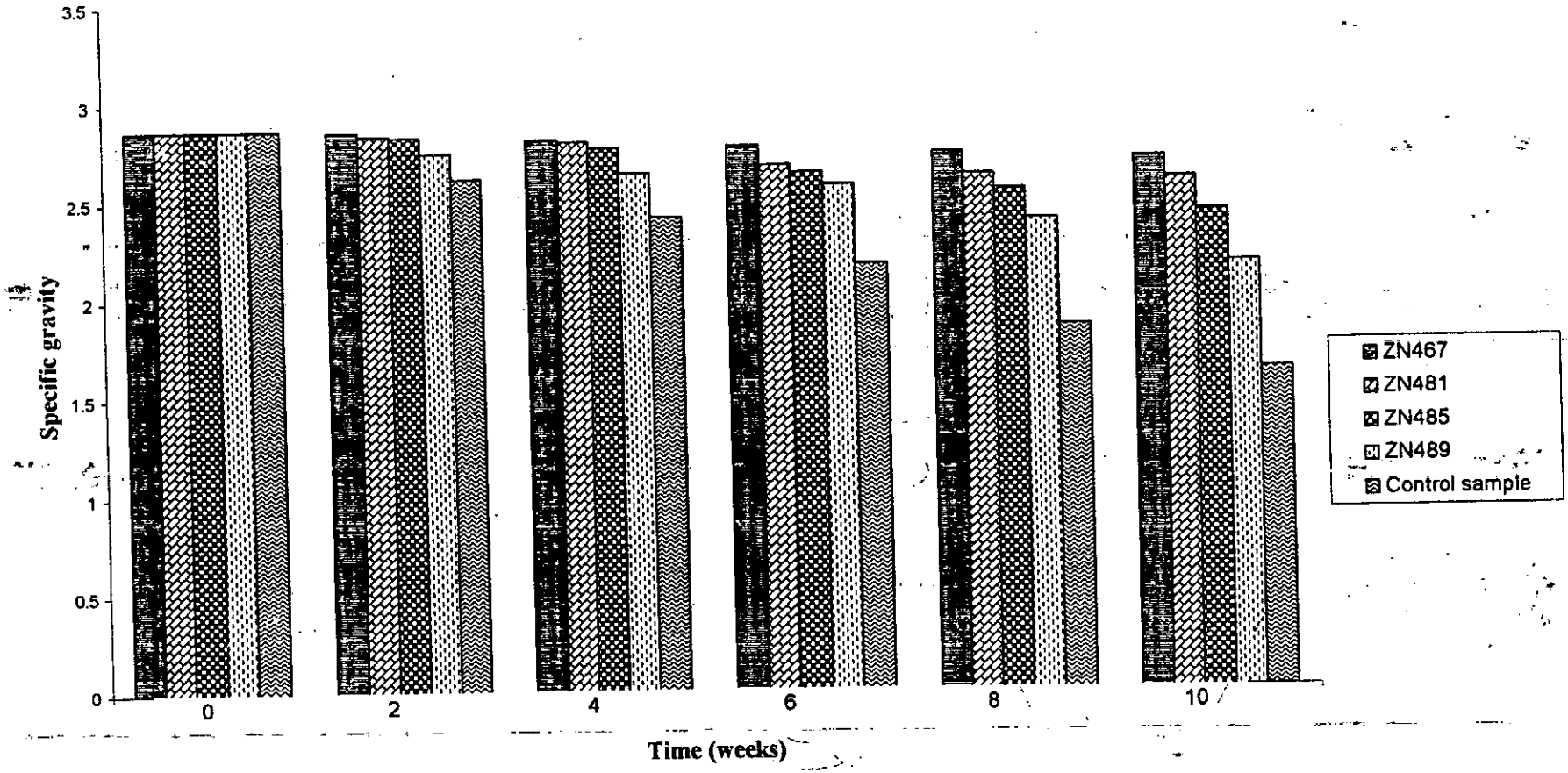


Fig. 3.26 Effect of 3% v/v of biocides on specific gravity of fresh, sterile paint samples. The control samples had no biocides.

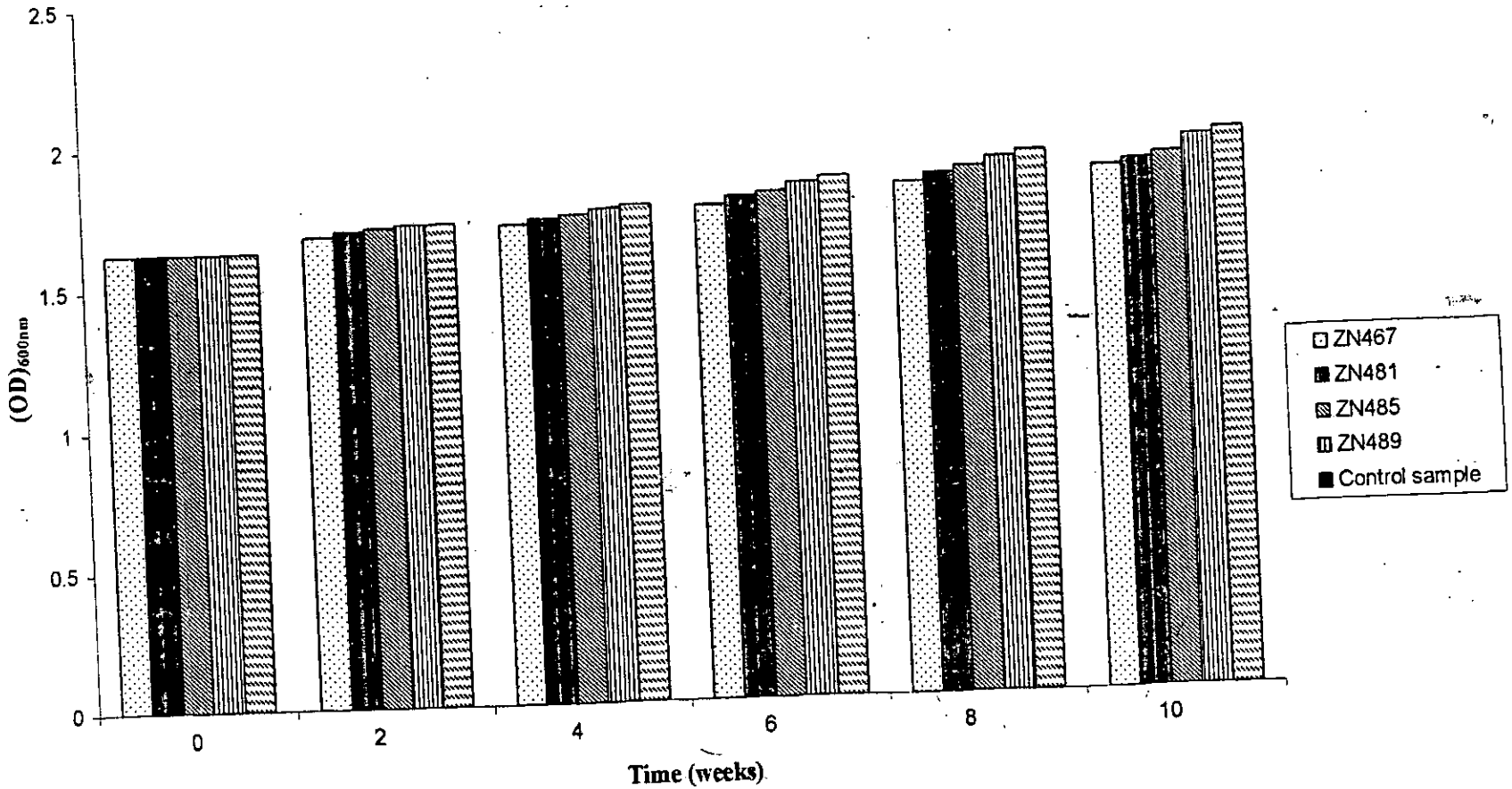


Fig. 3.28 Effect of 0.5% v/v of biocides on $OD_{600\,nm}$ of fresh, sterile paint samples. The control samples had no biocides.

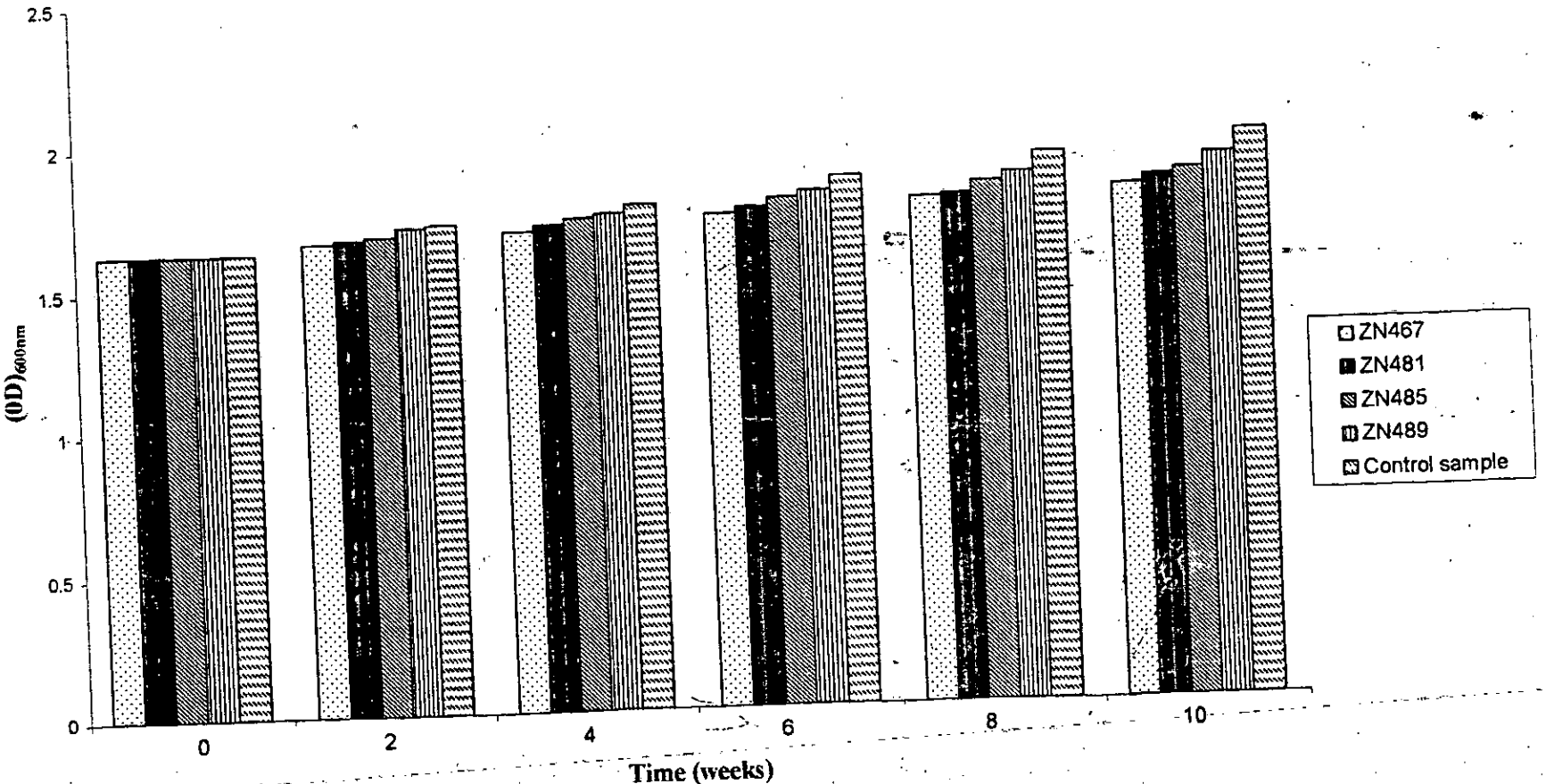


Fig. 3.28 Effect of 1% v/v of biocides on $OD._{600\ nm}$ of fresh, sterile paint samples. The control samples had no biocides.

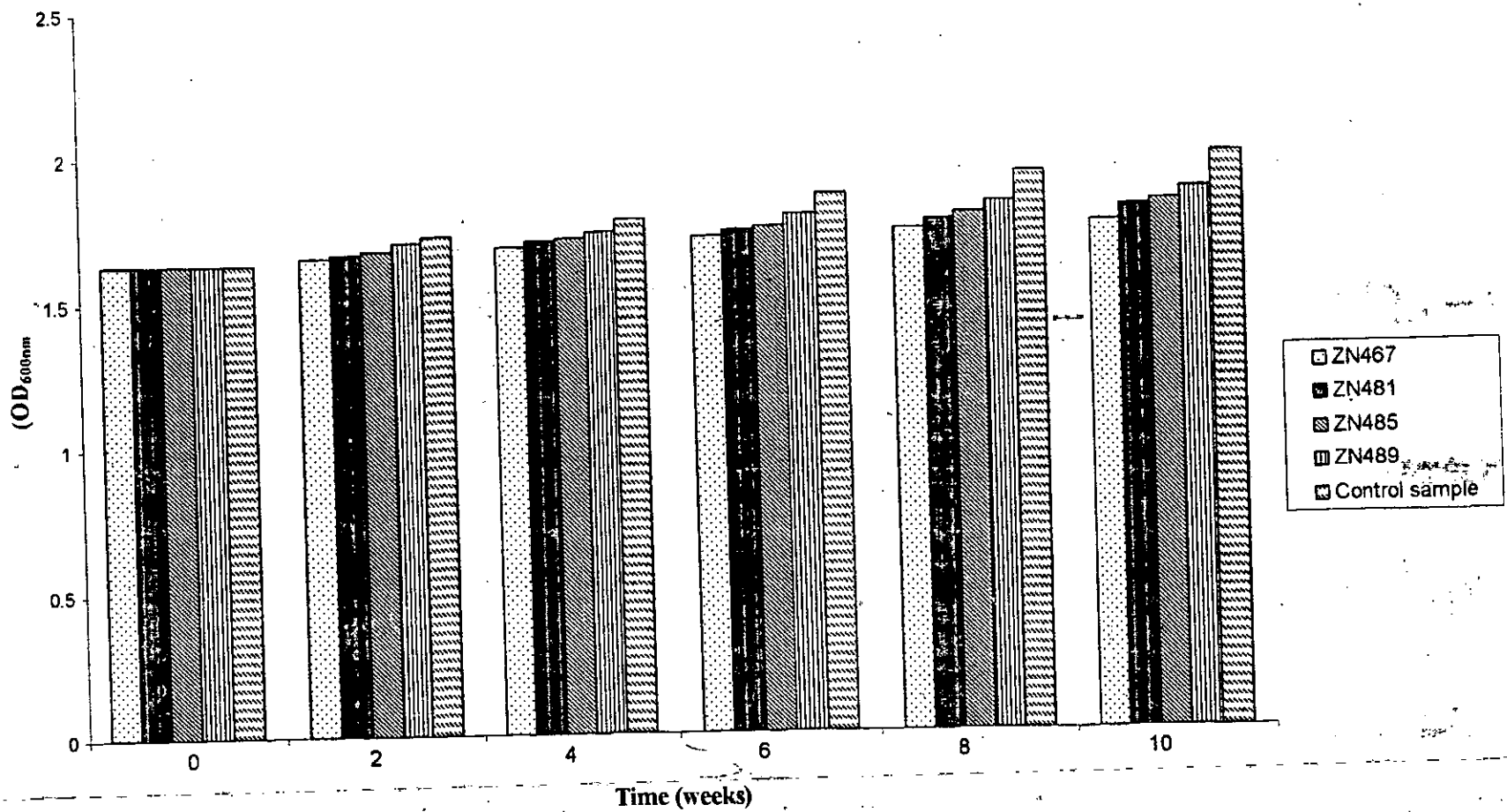


Fig. 3.29 Effect of 2% v/v of biocides on $OD_{,600\ nm}$ of fresh, sterile paint samples. The control samples had no biocides.

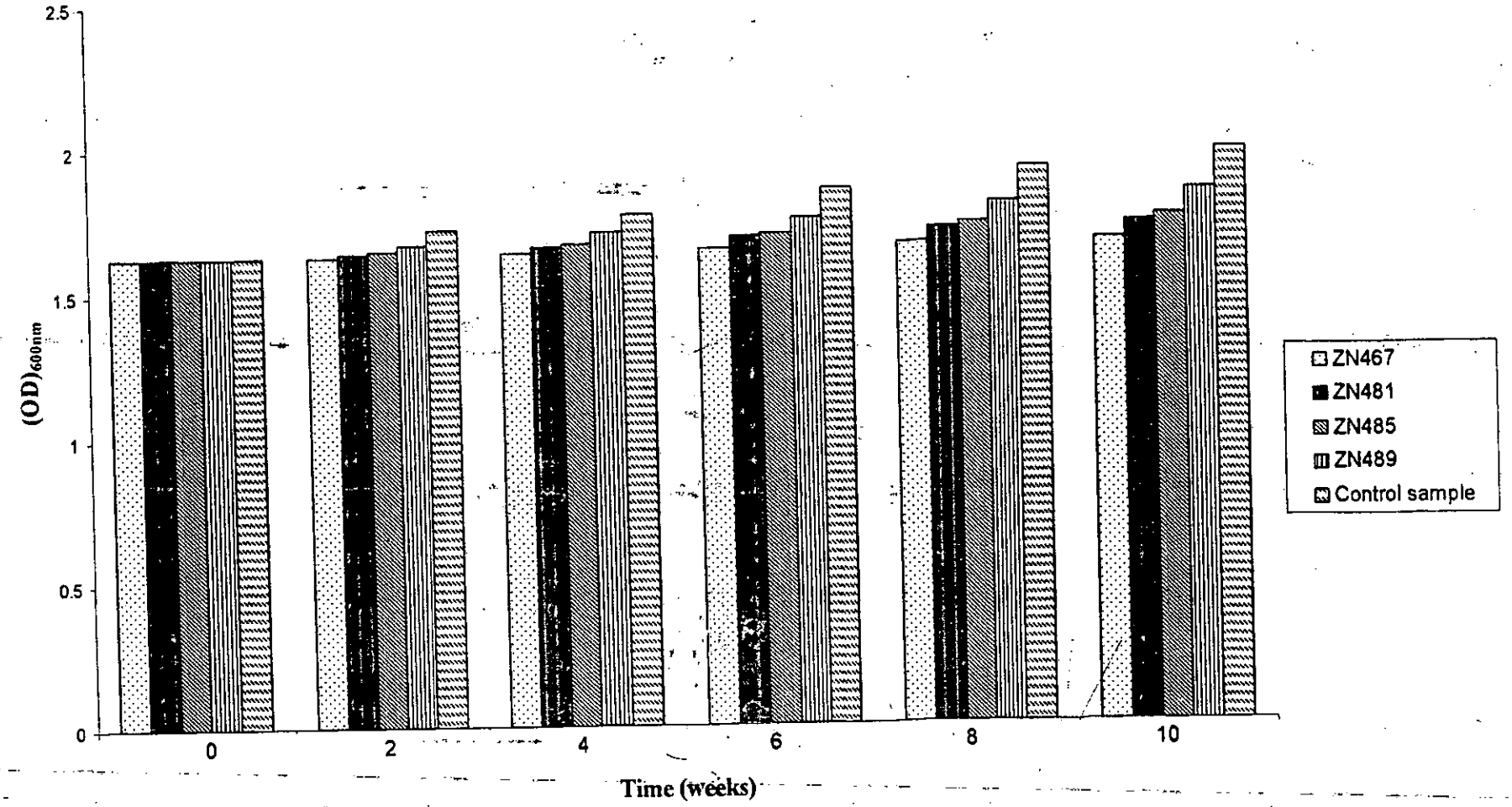


Fig. 3.30 Effect of 3% v/v of biocides on $OD_{600\ nm}$ of fresh, sterile paint samples. The control samples had no biocides.

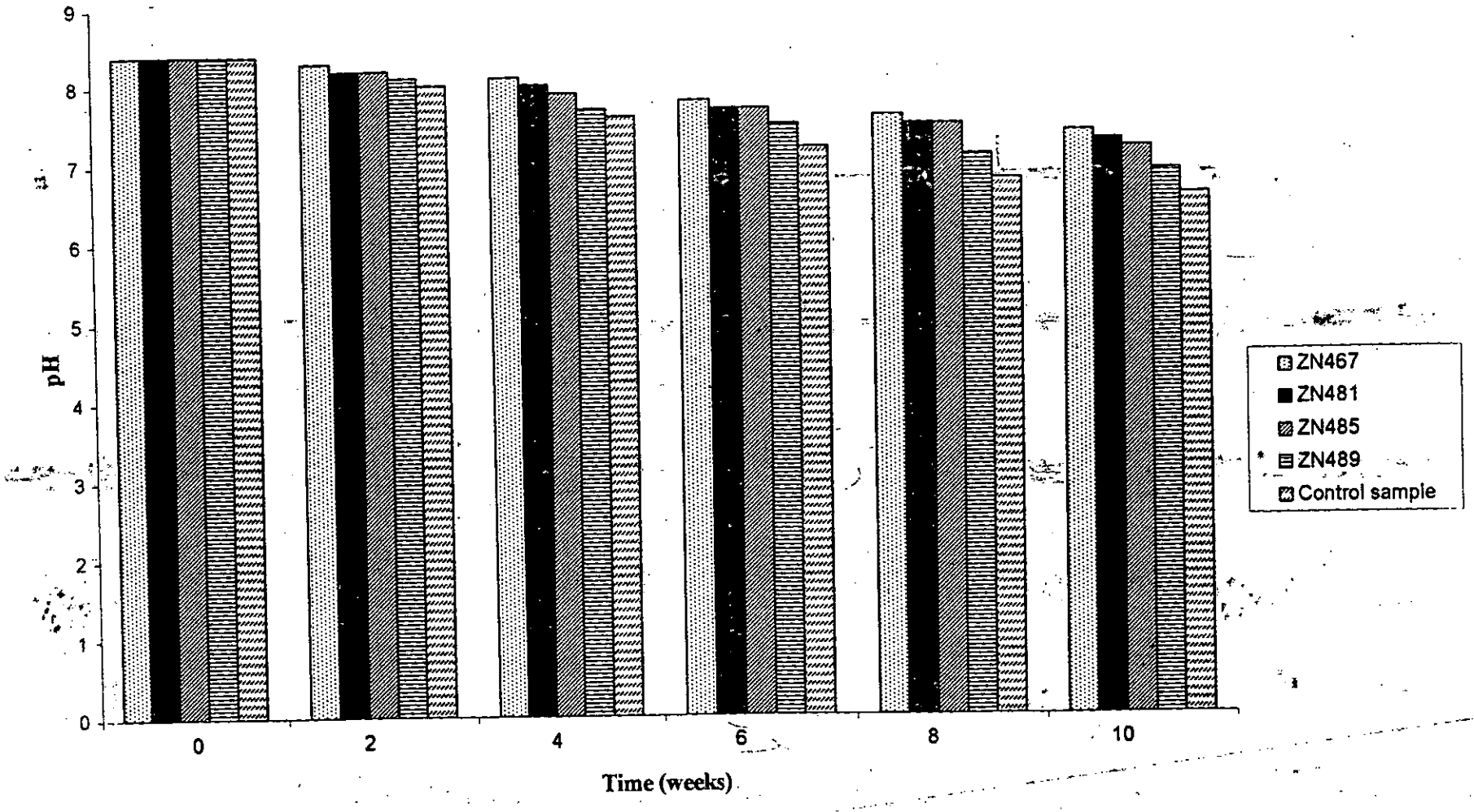


Fig. 3.31 **Effect of 0.5% v/v of biocides on pH of fresh, sterile paint samples. The control samples had no biocides.**

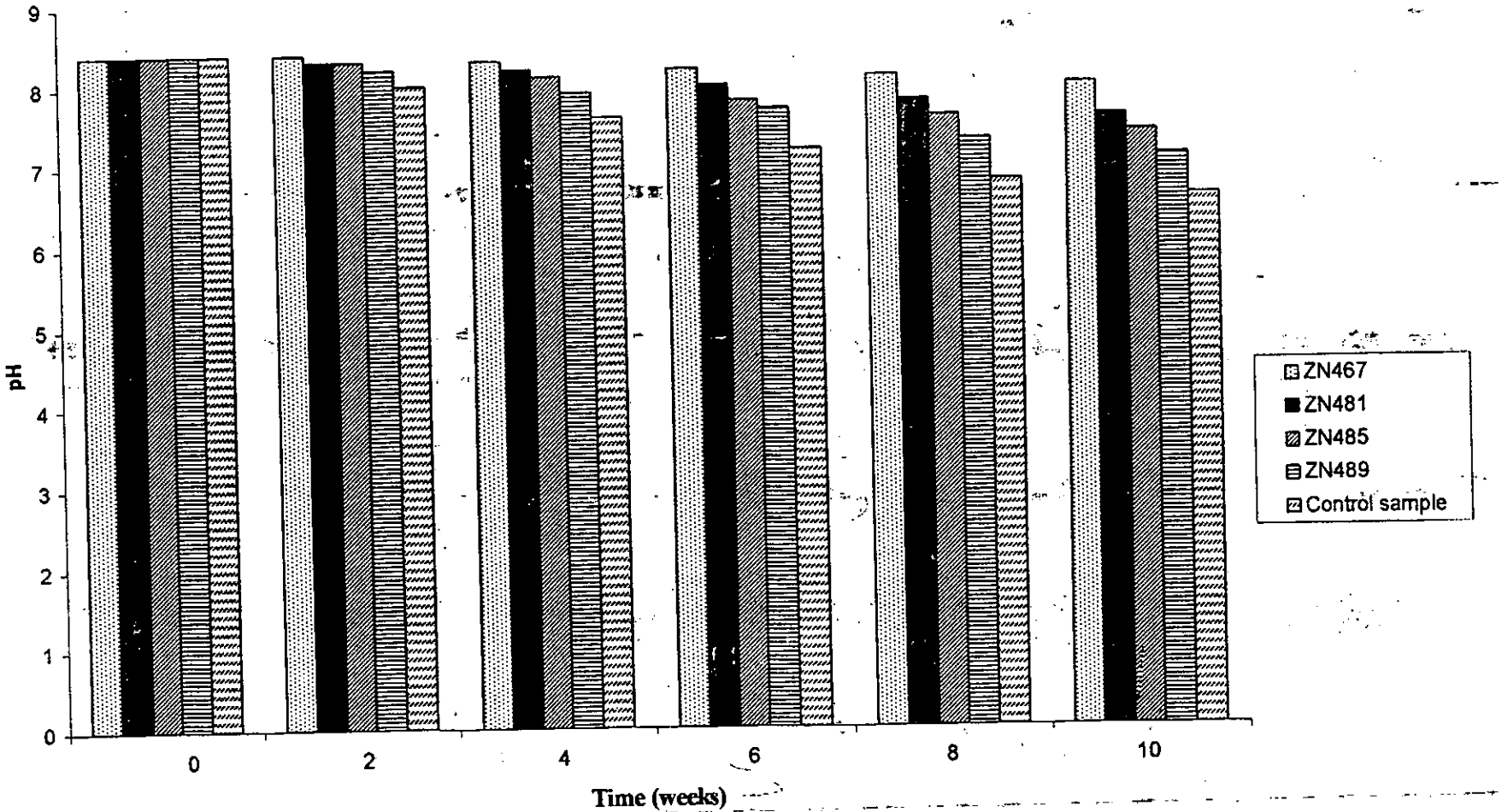


Fig. 3.32 **Effect of 1% v/v of biocides on pH of fresh, sterile paint samples. The control samples had no biocides.**

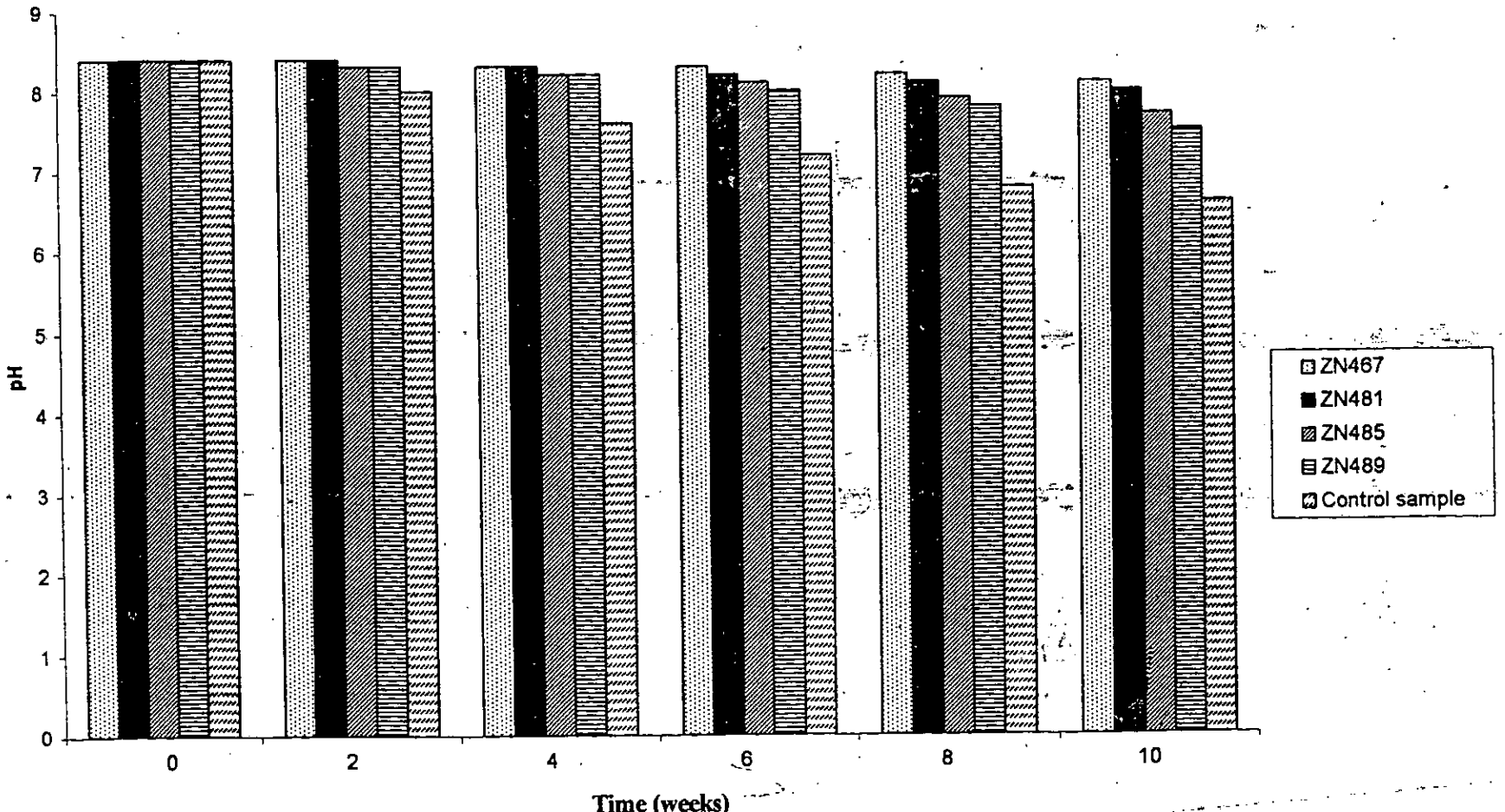


Fig. 3.33 **Effect of 2% v/v biocides on pH of fresh, sterile paint samples. The control samples had no biocides.**

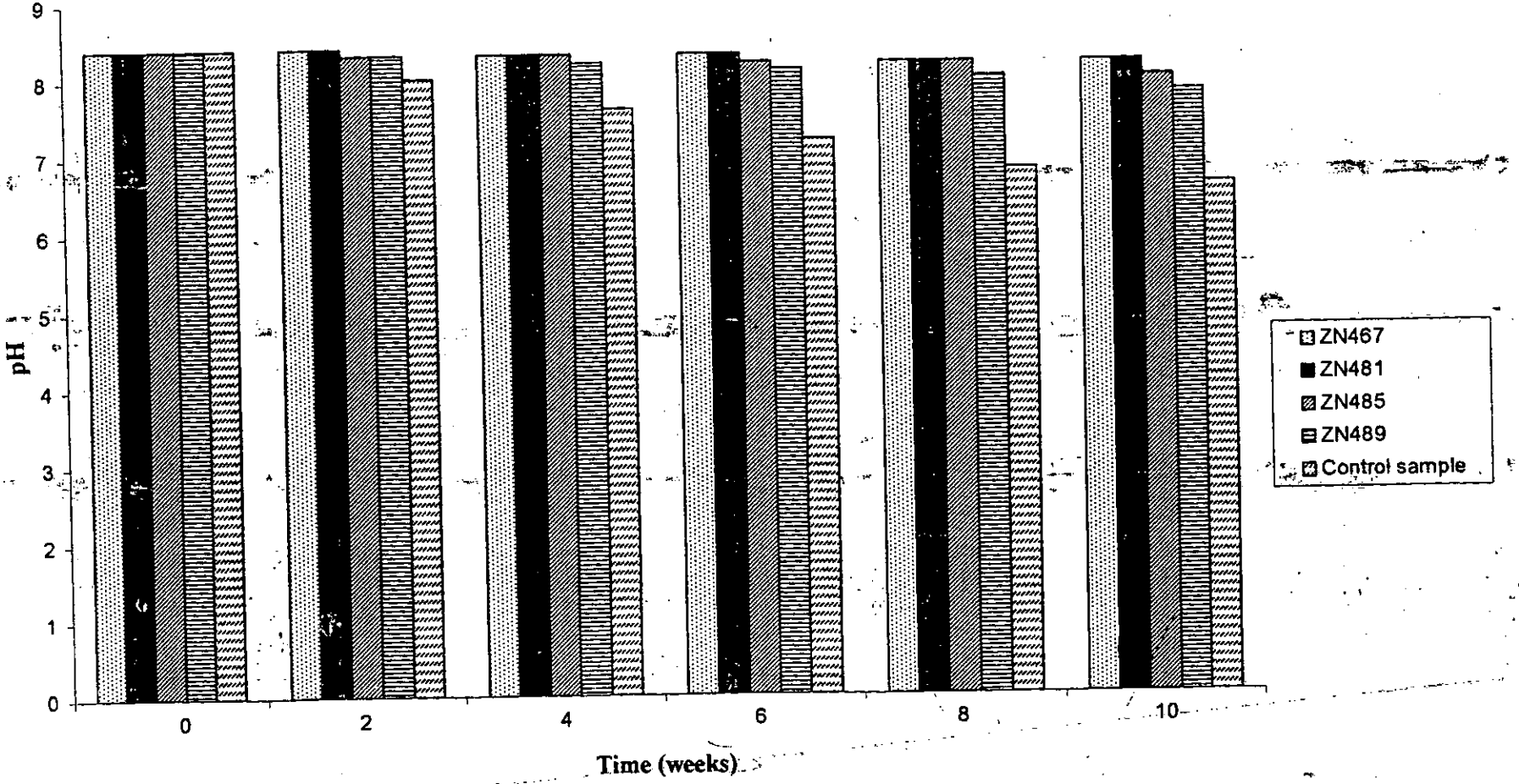


Fig. 3.34 **Effect of 3% v/v of biocides on pH of fresh, sterile paint samples. The control samples had no biocides.**

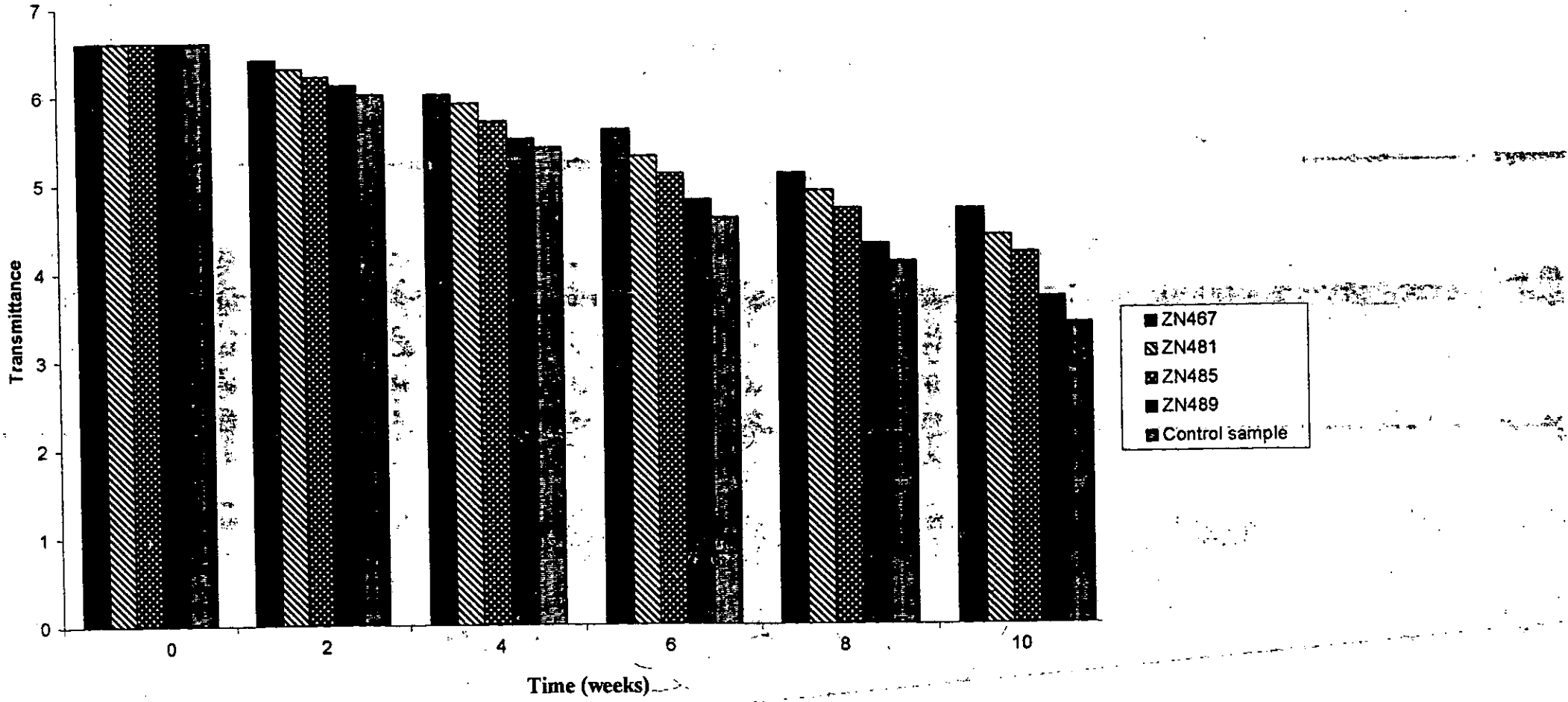


Fig. 3.35 **Effect of 0.5% v/v of biocides on transmittance of fresh, sterile paint samples. The control samples had no biocides.**

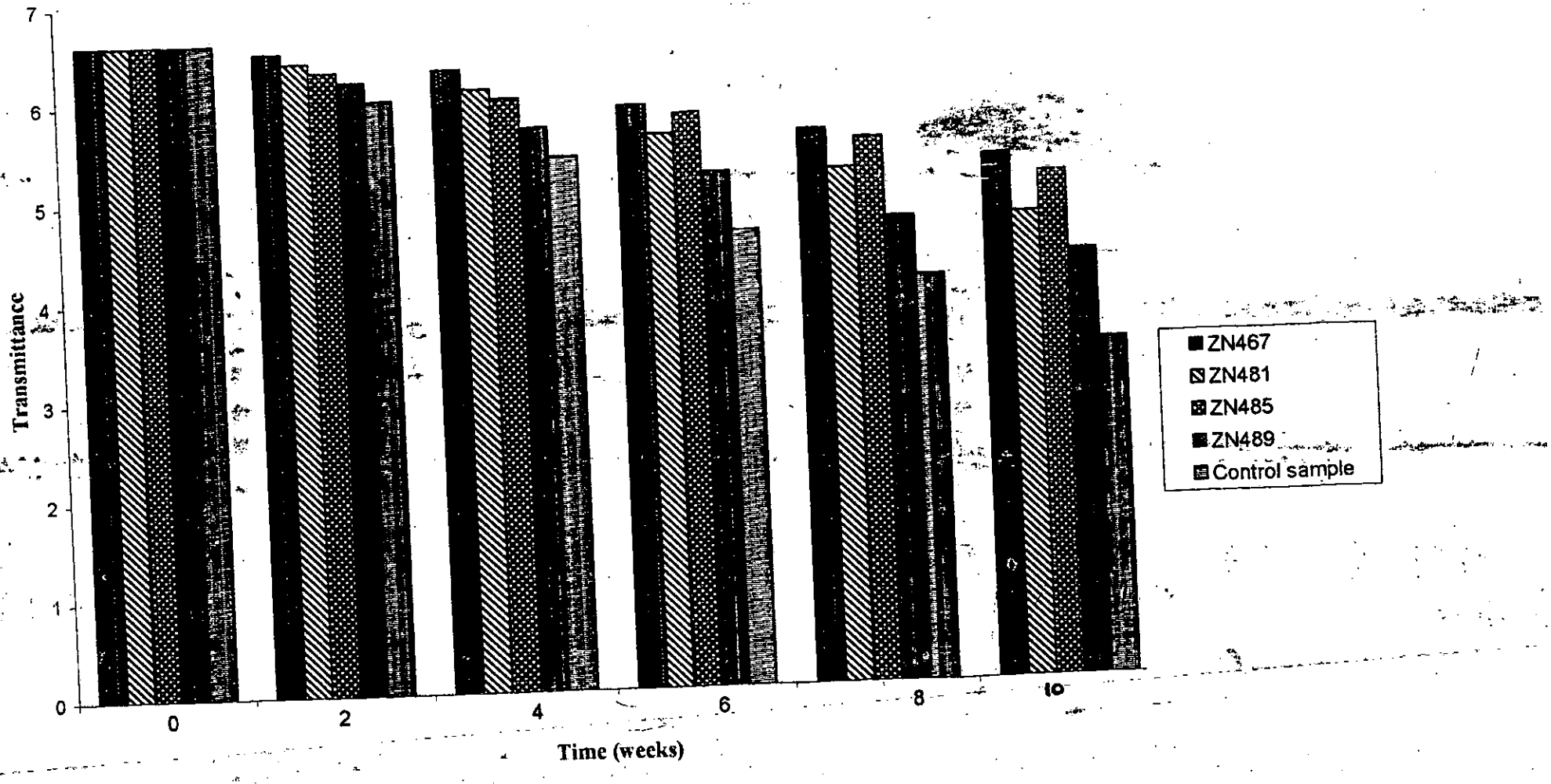


Fig. 3.36 Effect of 1% v/v of biocides on transmittance of fresh, sterile paint samples. The control samples had no biocides.

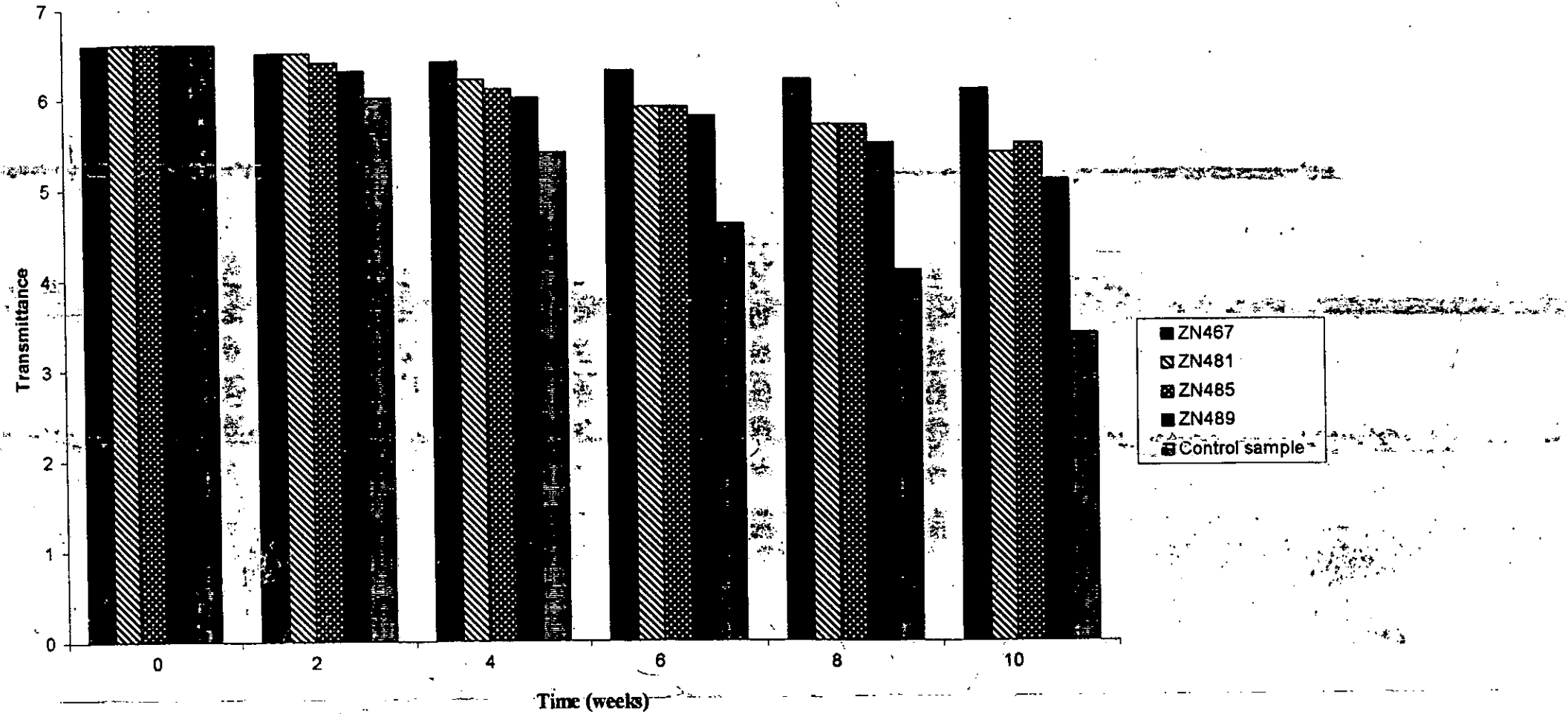


Fig. 3.37 **Effect of 2% v/v of biocides on transmittance of fresh, sterile paint samples. The control samples had no biocides.**

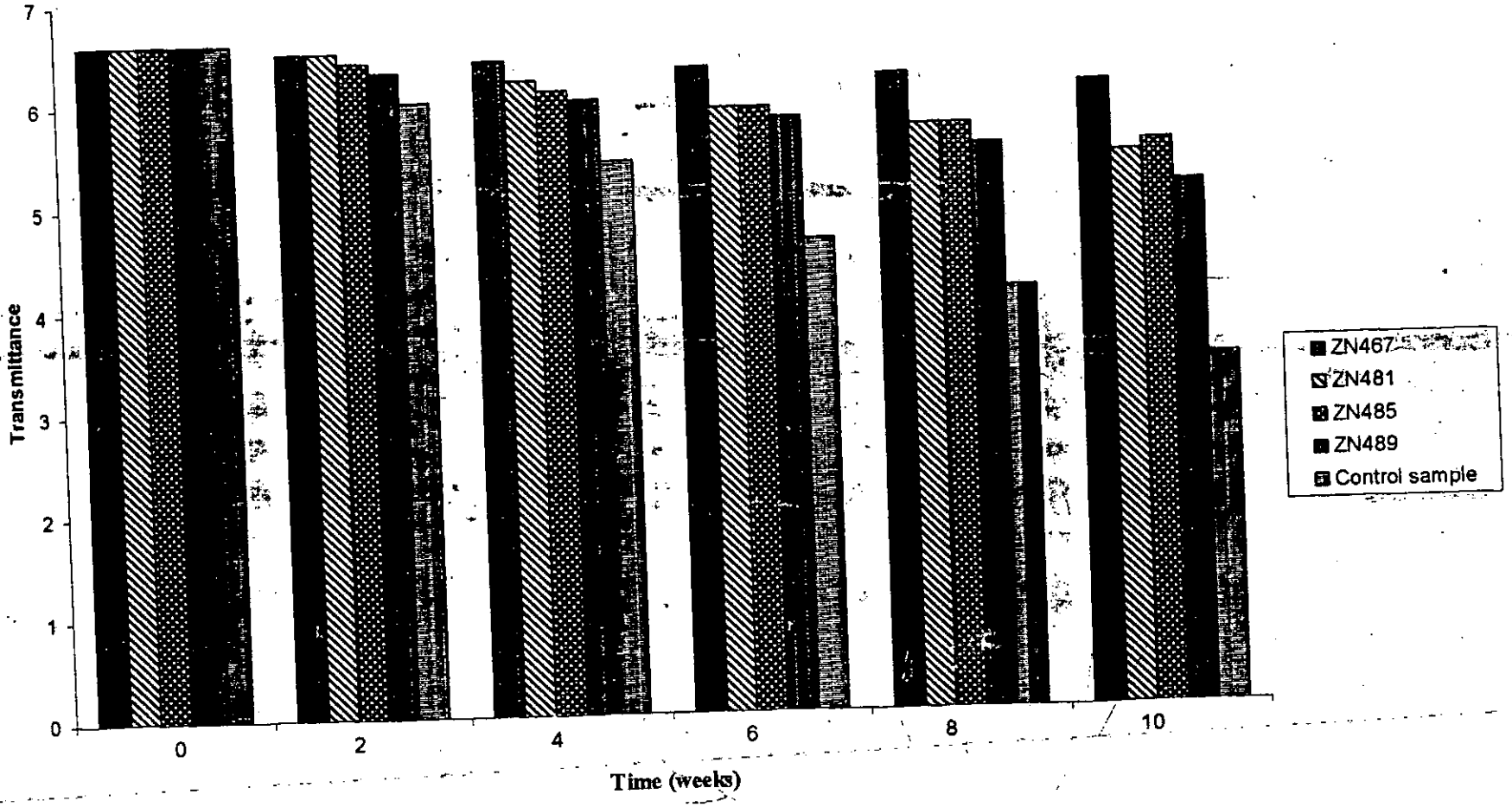


Fig. 3.37 **Effect of 2% v/v of biocides on transmittance of fresh, sterile paint samples. The control samples had no biocides.**

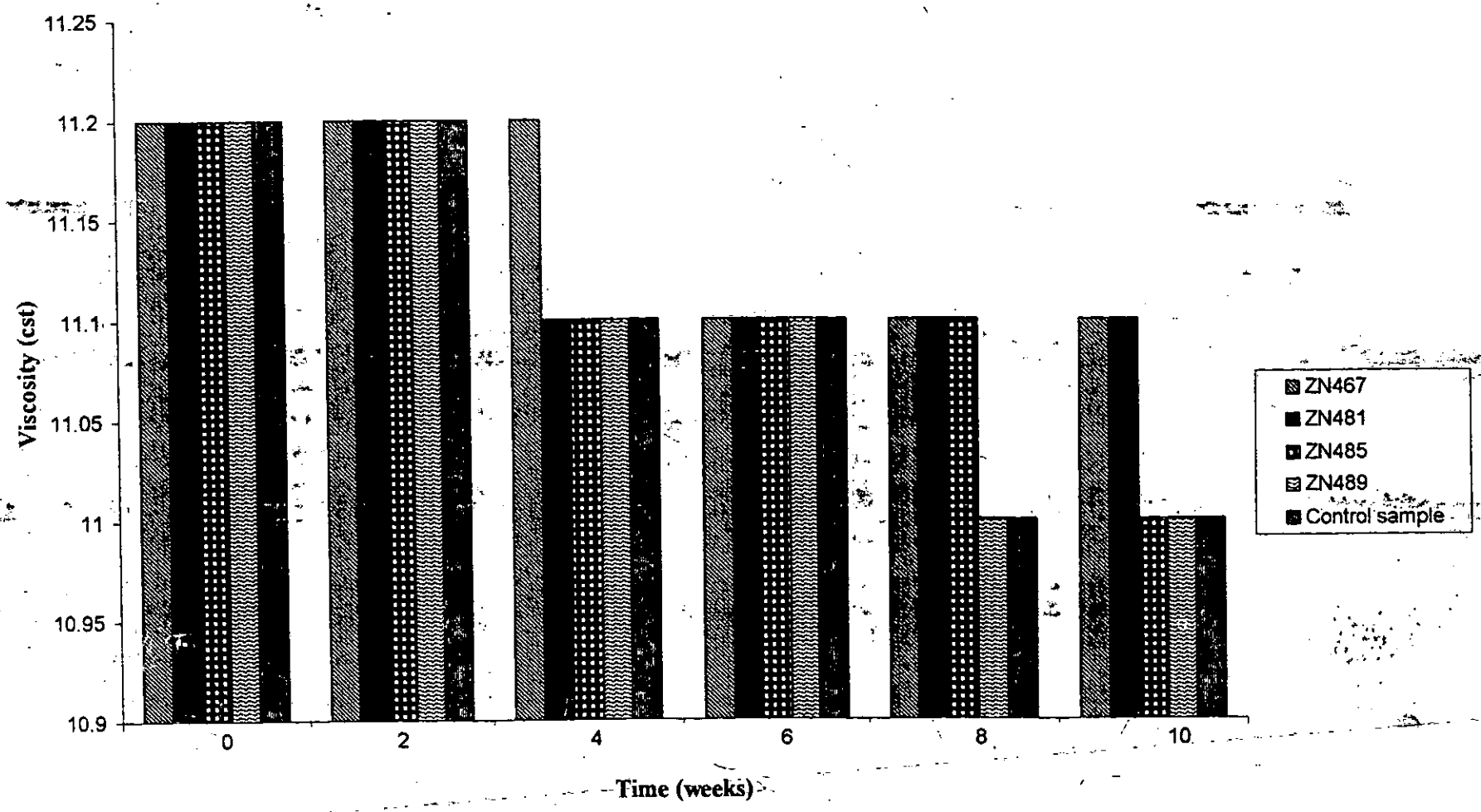


Fig. 3.39 **Effect of 0.5% v/v of biocides on viscosity of fresh, sterile paint samples. The control samples had no biocides.**

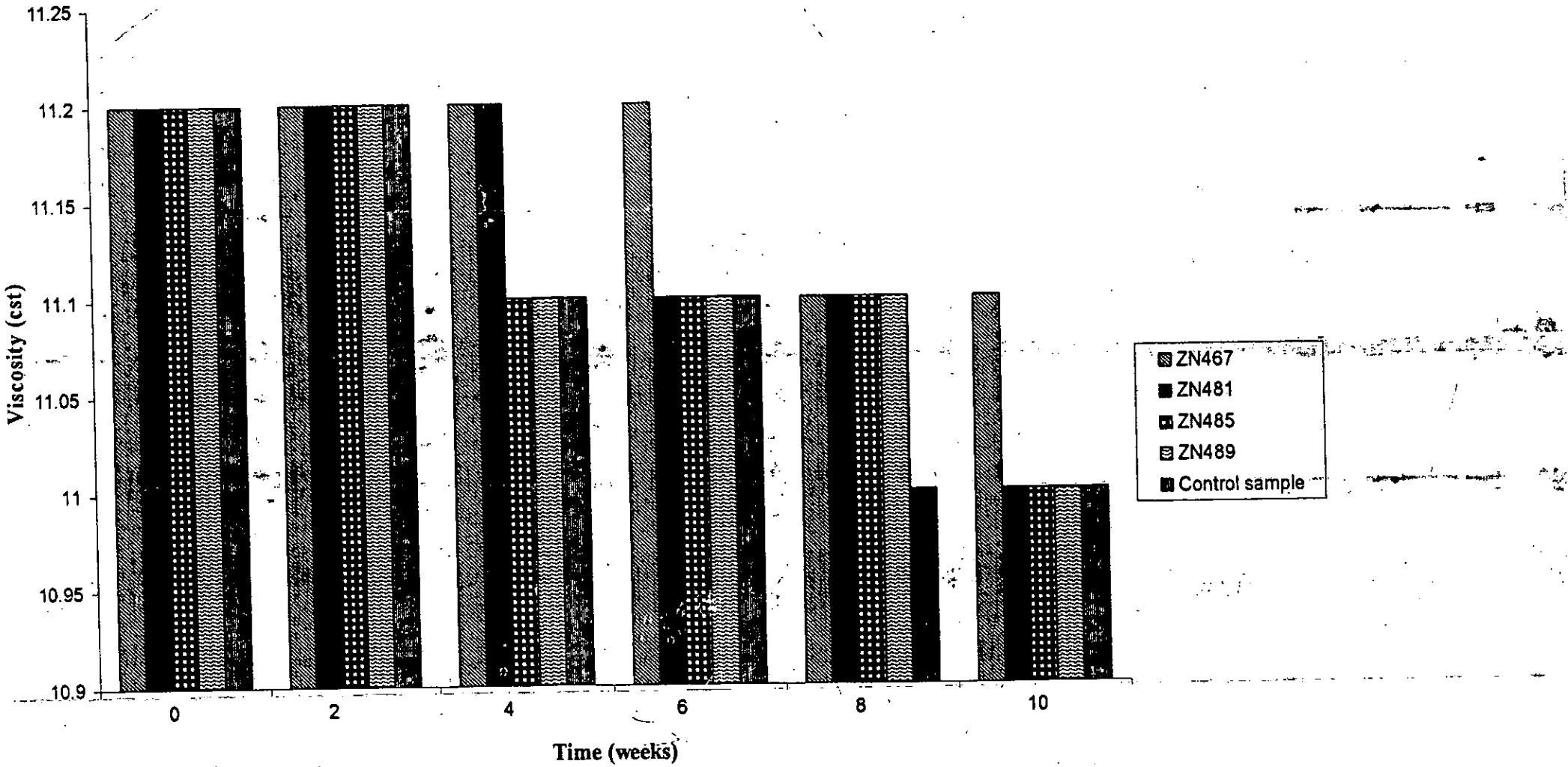


Fig. 3.40 **Effect of 1% of biocides on viscosity of fresh, sterile paint samples. The control samples had no biocides.**

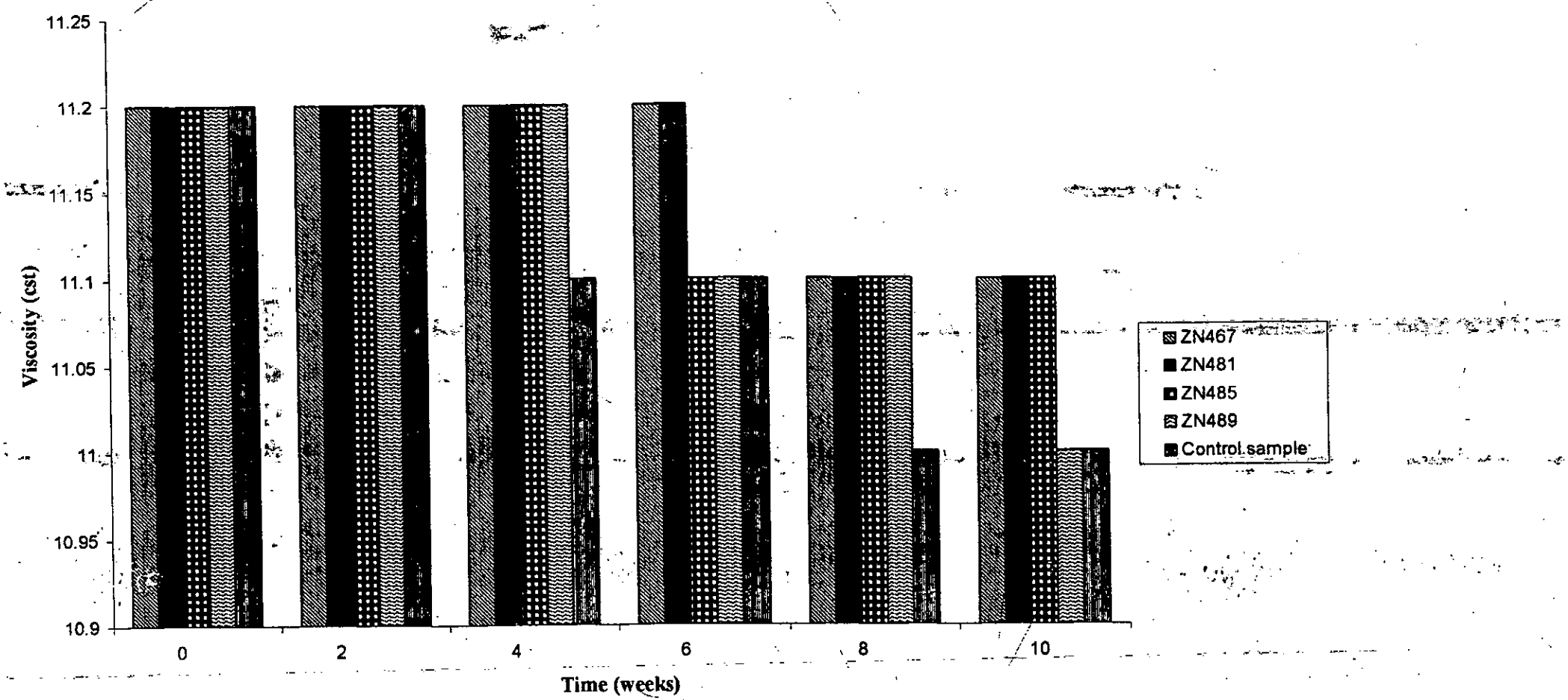


Fig. 3.41 **Effect of 2% v/v of biocides on viscosity of fresh, sterile paint samples. The control samples had no biocides.**

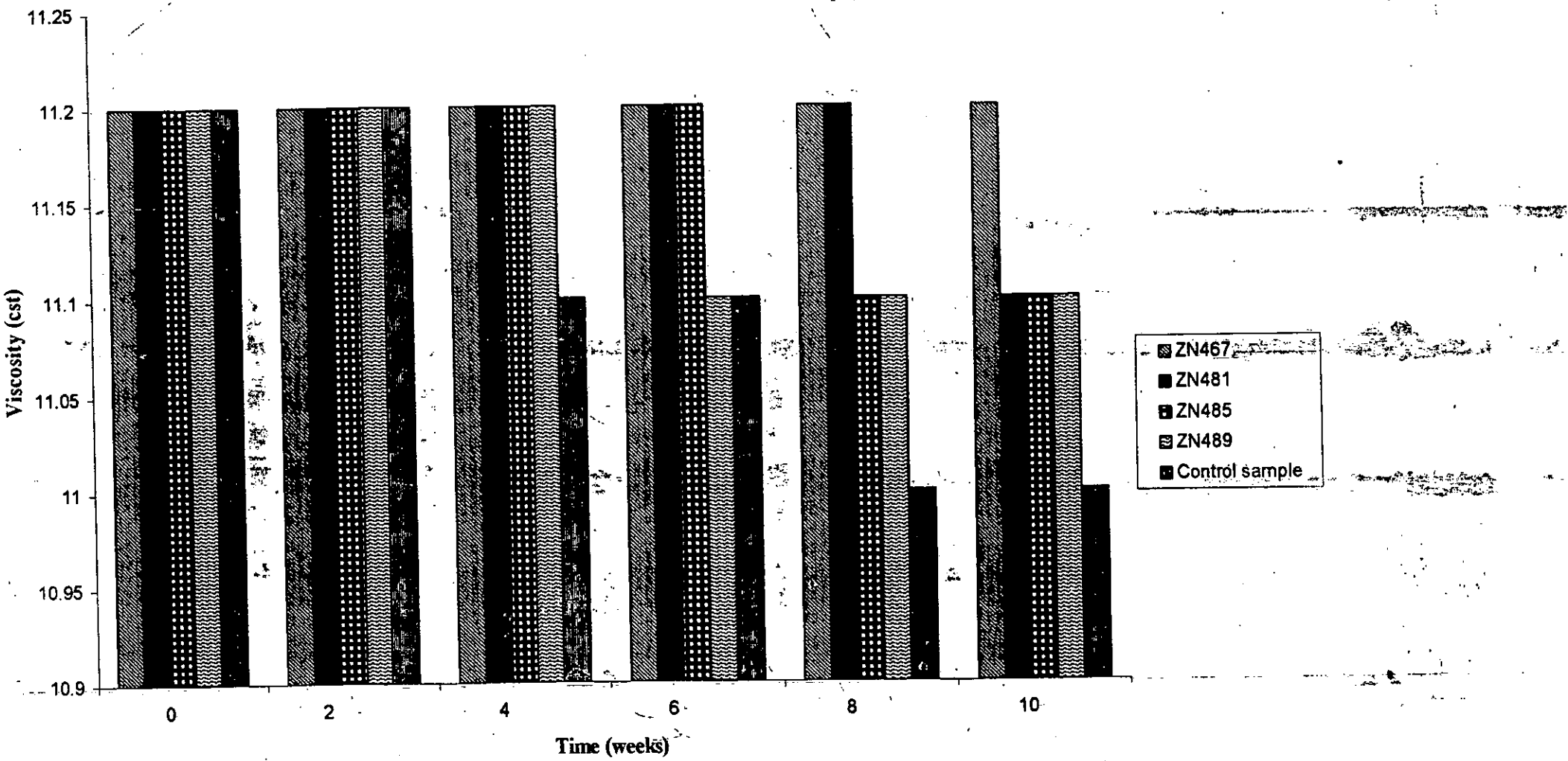


Fig. 3.42 **Effect of 3% v/v of biocides on viscosity of fresh, sterile paint samples. The control samples had no biocides.**

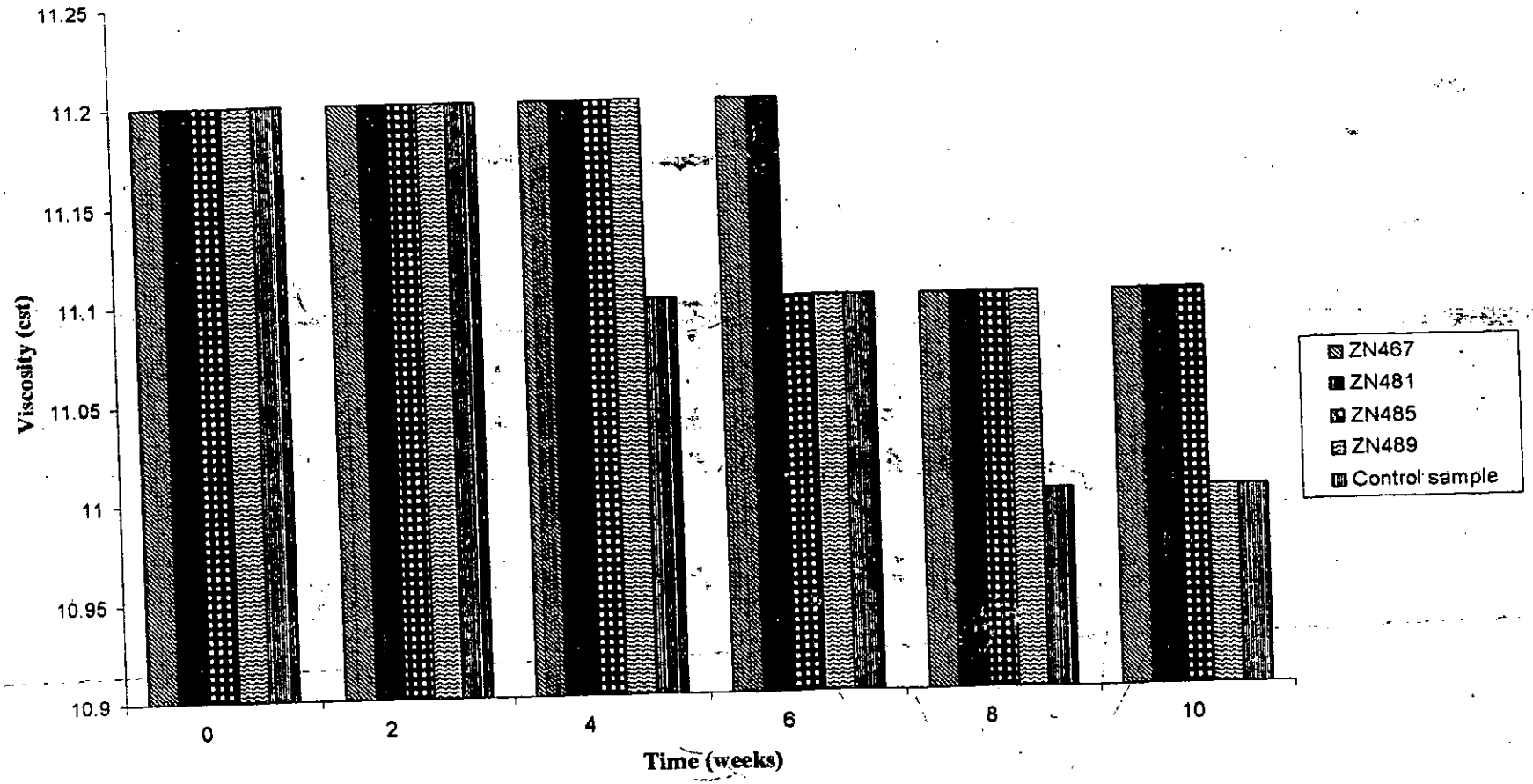


Fig. 3.41 **Effect of 2% v/v of biocides on viscosity of fresh, sterile paint samples. The control samples had no biocides.**

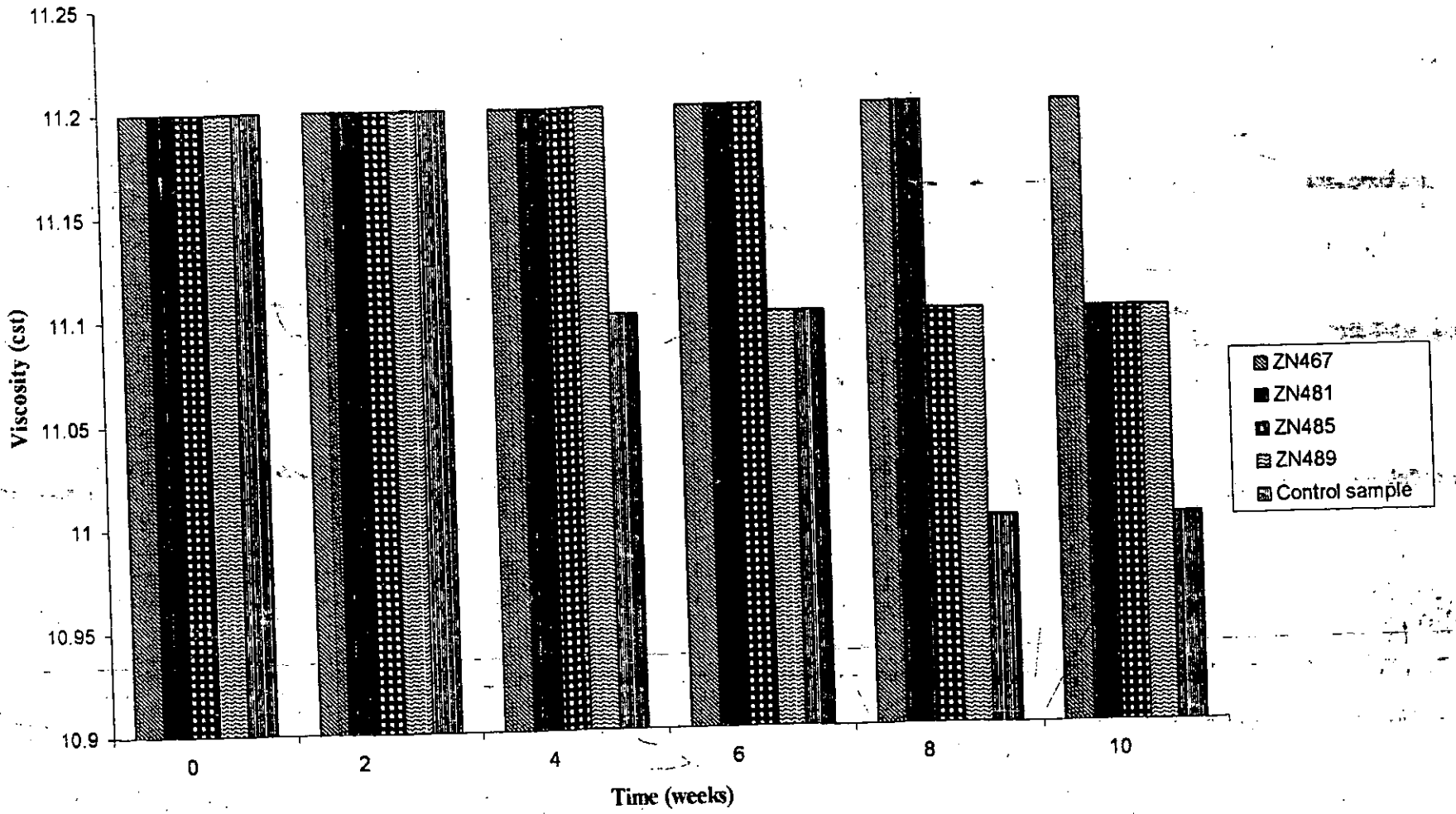


Fig. 3.42 **Effect of 3% v/v of biocides on viscosity of fresh, sterile paint samples. The control samples had no biocides.**

3.12. Detection of Plasmids

The eight isolated bacterial strains were screened for the presence of plasmid DNA. The plasmid isolation techniques revealed that only *Pseudomonas aeruginosa* haboured 2 plasmids of different molecular weights (Plates 3.12 and 3.13). The other organisms did not show the presence of any plasmid DNA. The result of molecular weights of the isolated plasmids determined revealed that the molecular weights of the plasmids contained in *Pseudomonas aeruginosa* ranged from 0.030 to 0.112 Kb (Table 3.13).

Table 3.13 Molecular weights of plasmid DNA detected in *Pseudomonas aeruginosa*

Organism	Plasmid number	Molecular weight (kb)
Pseudomonas aeruginosa	1	0.112
Pseudomonas aeruginosa	2	0.030

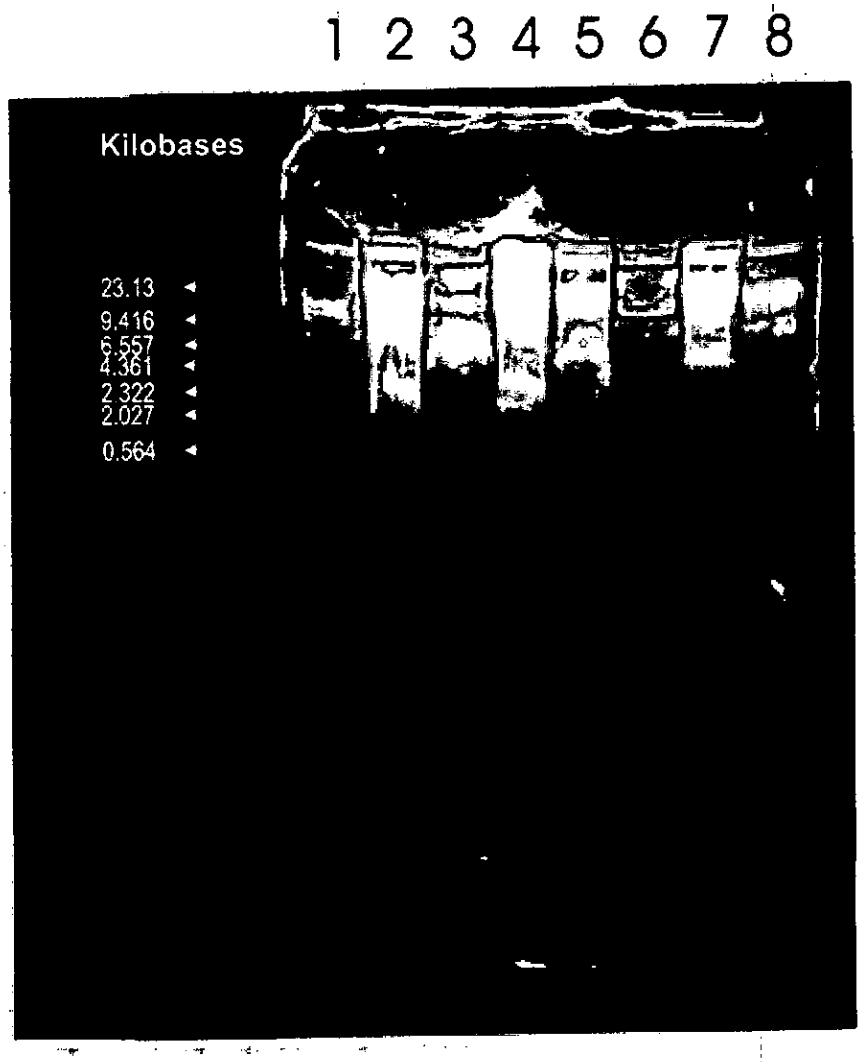


Plate 3.12 **Agarose gel electrophoresis plate showing no detectable plasmid from bacterial strains. Organisms were screened following the TENS – Mini Prep. Protocol of Leeh and Brent (1987). Lanes: 1, λ DNA. Hind 111 digested (marker); 2, OB-1; 3, OB-1; 4, OB-2; 5, OB-2; 6, OB-3; 7, OB-7; 8, OB-8.**

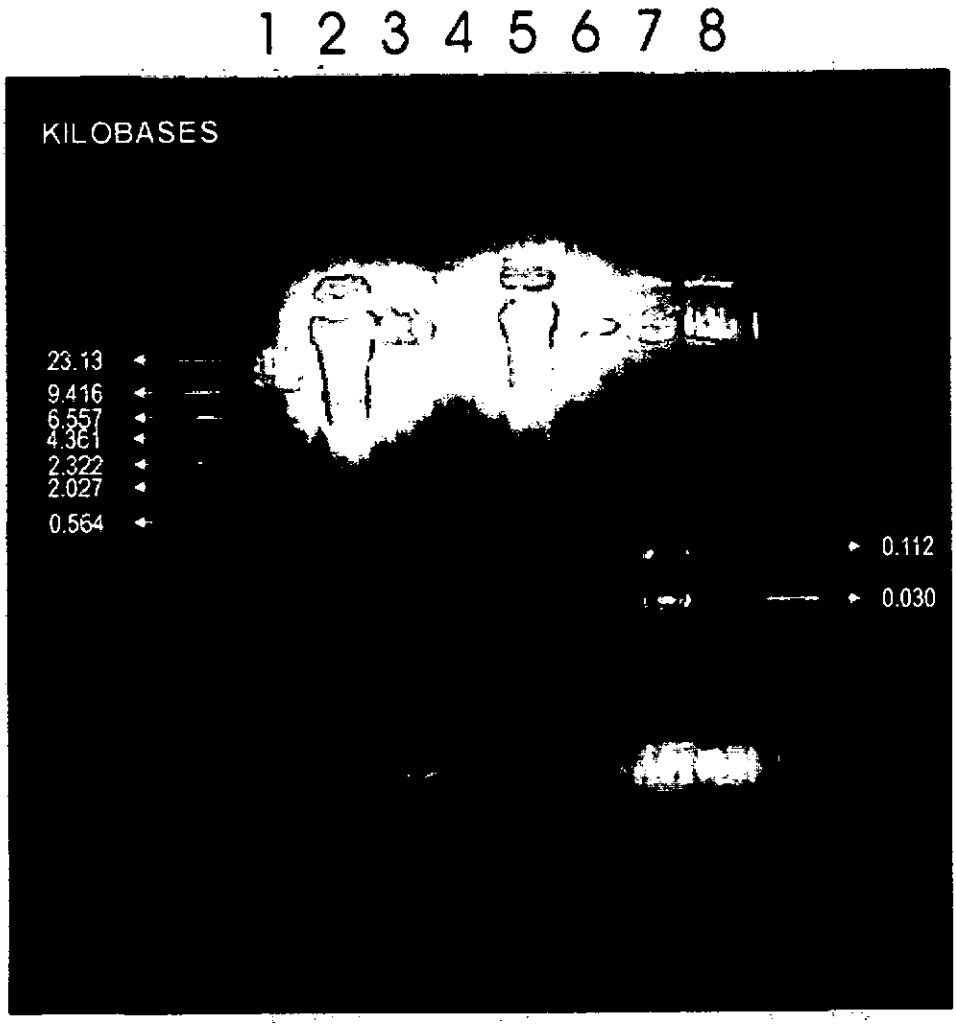


Plate 3.13 **Agarose gel electrophoresis plate showing plasmid DNA isolated from OB-6 using the protocol of Birnboim and Doly (1979). Lanes: 1, λ DNA Hind 111 digested (marker); 2, OB-4; 3, OB-4; 4, OB-5; 5, OB-5; 6, OB-5; 7, 0B-6; 8, OB-4.**

3.13 Plasmid Curing

Prior to the curing experiments, it was observed that *Pseudomonas aeruginosa* had 2 plasmids of molecular weight ranging from 0.030 – 0.112 kb (Table 3.13). Howver, after the curing experiments, *Pseudomonas aeruginosa* was found to have lost the 2 plasmids that it possessed earlier on (Plate 3.14). Therefore, growth in YETS broth containing 0.002% v/v SDS resulted in the complete elimination of the plasmids. This result suggests that SDS is an effective curing agent. The comparative evaluation of the spoilage potentials of the plasmid-bearing and the plasmid-cured strains on the physico-chemical parameters of fresh, sterile paints further proves that the plasmid-bearing strain had higher degradative potentials (Figs. 3.44 – 3.49). It was therefore, thought that the genes for cellulose degradation may be enclosed in the plasmid.

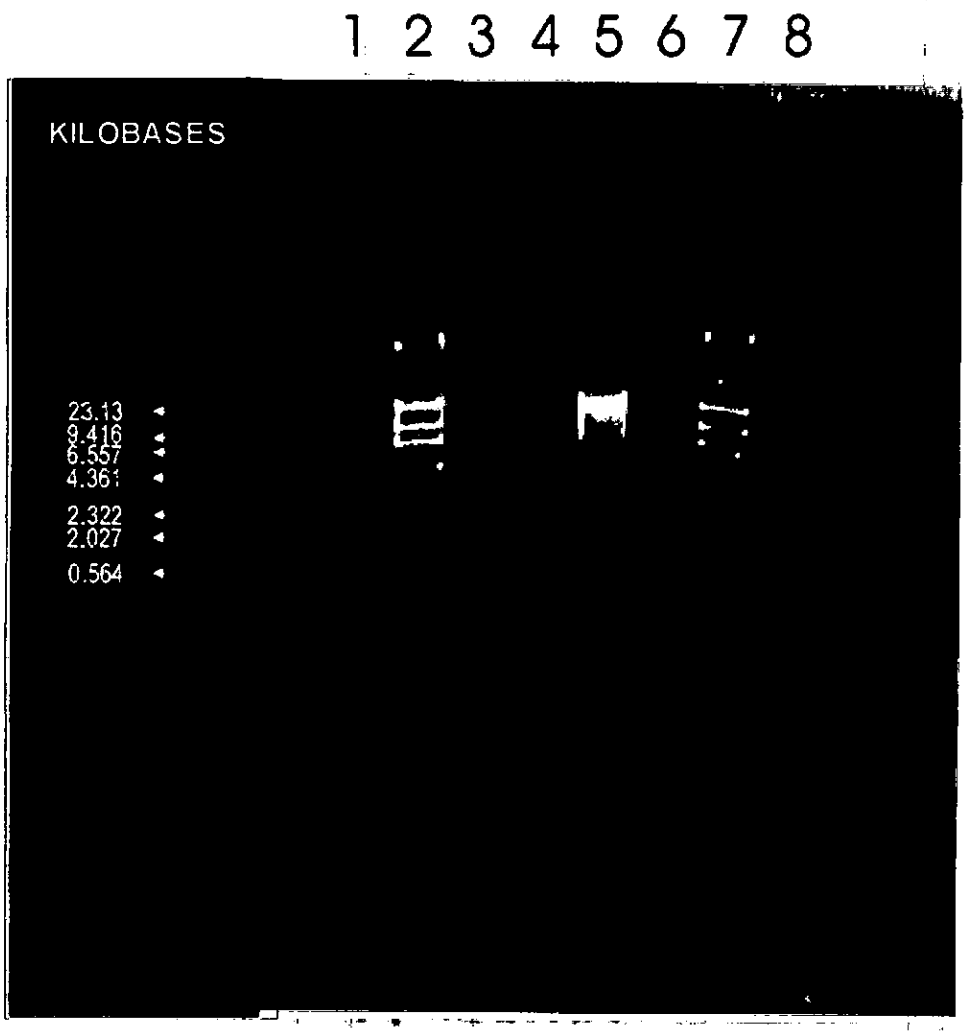


Plate 3.14 **Agarose gel electrophresis plate of cured plasmids of *Pseudomonas aeruginosa* following the protocol of Birnboim and Doly (1979). Lanes: 2, DNA Hind 111 digested (marker); 5, OB-6; 7, λ DNA Hind 111 digested (marker).**

3.14 Antibiotic Sensitivity Pattern of Plasmid-bearing and Plasmid-cured strains of *Pseudomonas aeruginosa*

The sensitivity and resistance patterns of the plasmid-bearing *Pseudomonas aeruginosa* and the cured strain to selected antibiotics are presented in Fig. 3.43. The susceptibility classification of the strains was based on the zone-size interpretative chart for disc diffusion susceptibility testing of the National Committee for Clinical Laboratory Standards (NCCLS). The plasmid-bearing strain OB-6 (W) was resistant to ceftazidime, amikacin, tobramycin, gentamycin and tetracycline. However after the curing experiments, it was observed to be sensitive to tobramycin, gentamycin and amikacin. The plasmid-cured strain OB-6(C) was sensitive to all antimicrobials tested except tetracycline and ceftazidime. It demonstrated intermediate susceptibility to piperacillin.

3.15. Determination of Spoilage potentials of Plasmid-bearing and Plasmid-cured strains of *Pseudomonas aeruginosa*

The ability of each of the plasmid-bearing and plasmid-cured strain of *pseudomonas aeruginosa* to cause spoilage was evaluated mainly by the increase in microbial population density, changes in physico-chemical parameters and macroscopic observation of the paint samples as compared with the controls. The results as summarized in Figs. 3.44 – 3.49 showed that the wild strain had higher degradative impact on the sterile paints than the cured strain.

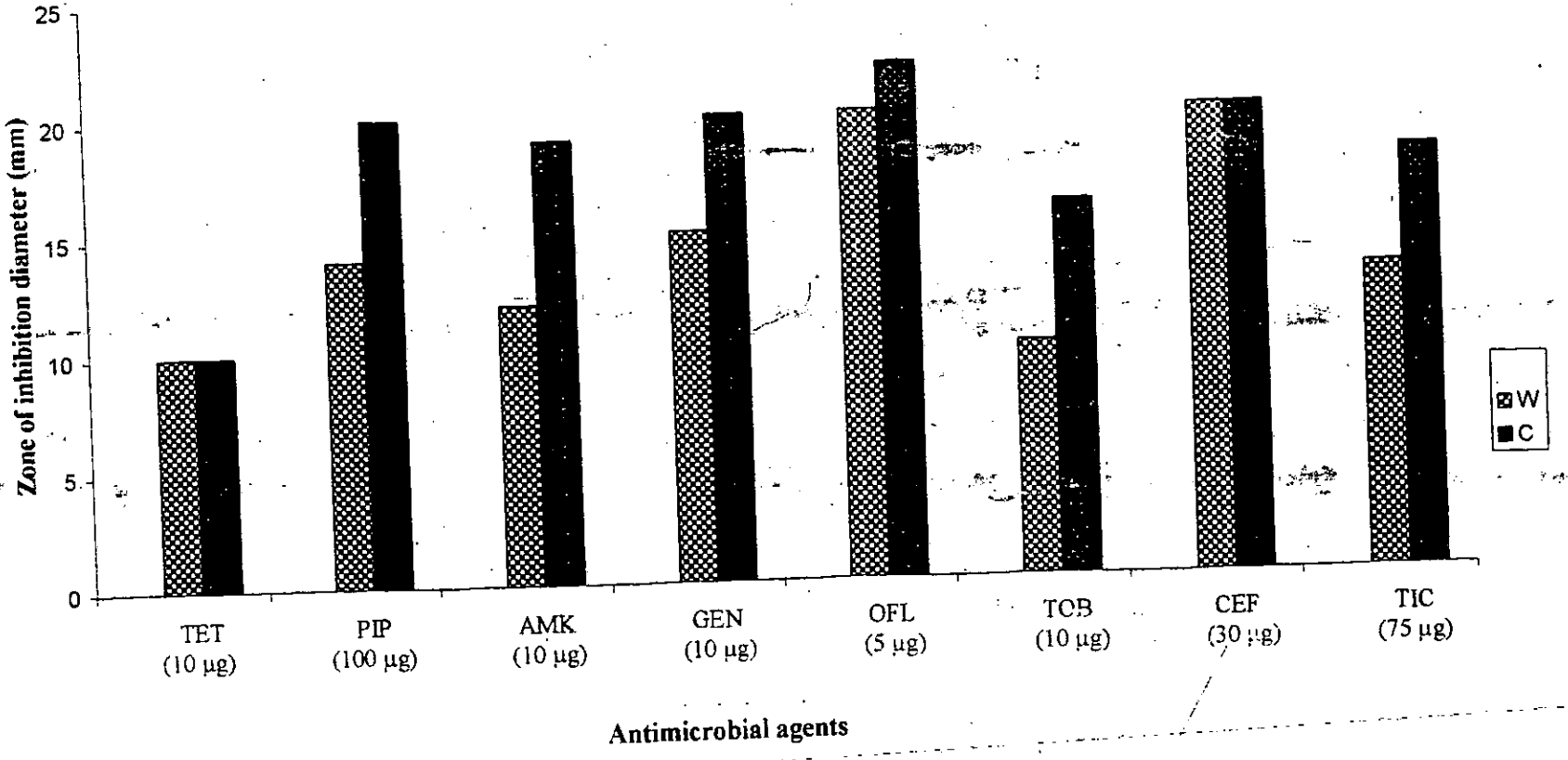


Fig. 3.43 Antimicrobial sensitivity patterns of plasmid-bearing and plasmid-cured strains of *Pseudomonas aeruginosa*

TET, tetracycline; PIP, piperacillin; AMK, amikacin; GEN, gentamycin; OFL, ofloxacin; TOB, tobramycin; CEF, ceftazidime; TIC, ticarcillin; W, plasmid-bearing; C, plasmid-cured strain. The disc potency is indicated in parentheses.

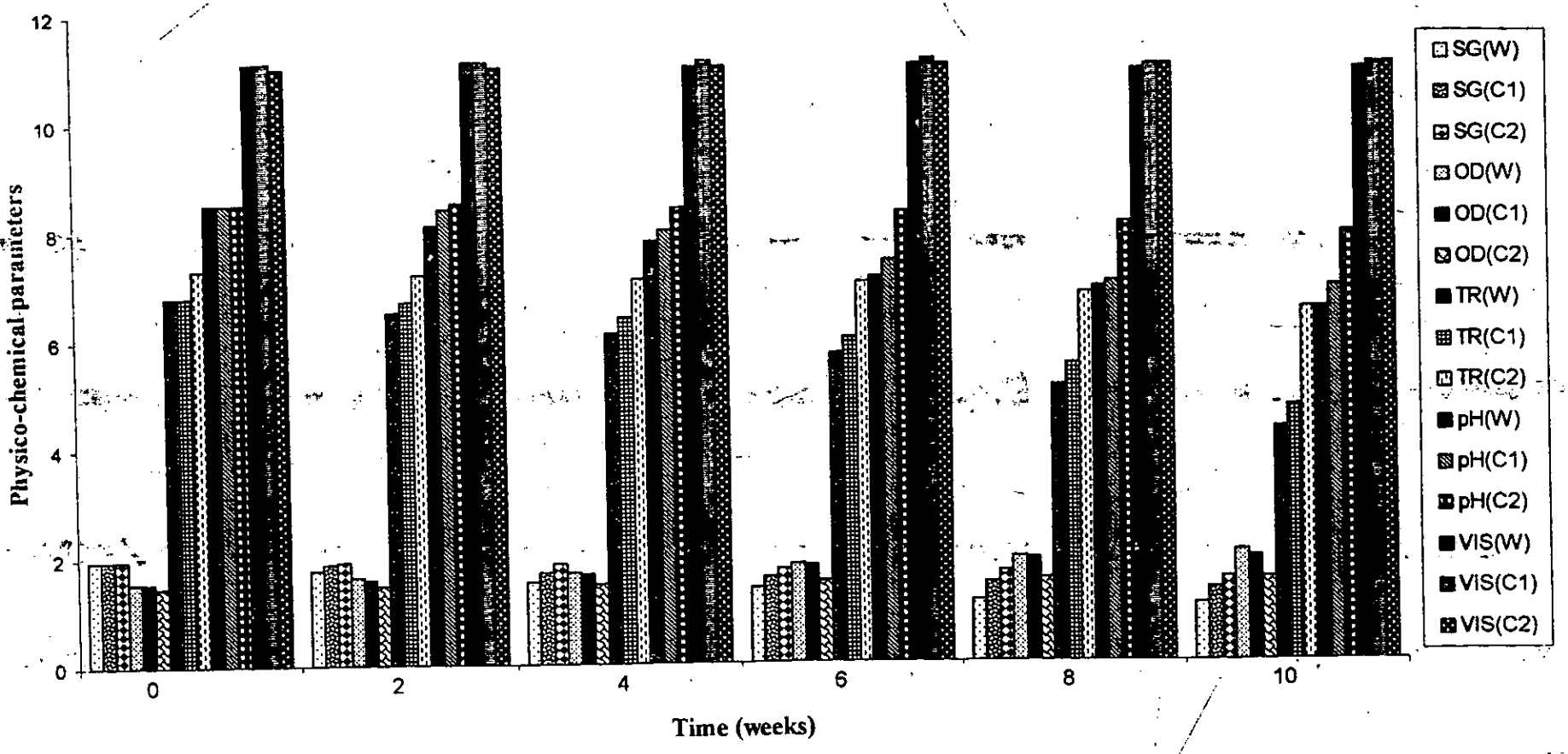


Fig. 3.44 Spoilage potential of the plasmid-bearing and plasmid-cured strains of *Pseudomonas aeruginosa* on the physico-chemical parameters of paint samples PS-1. VIS, viscosity (cst); TR, transmittance; OD_{600}; SG, specific gravity; (C2), control sample; (C1), plasmid-cured sample; (W), plasmid-bearing sample. Data represent the means of triplicate samples.

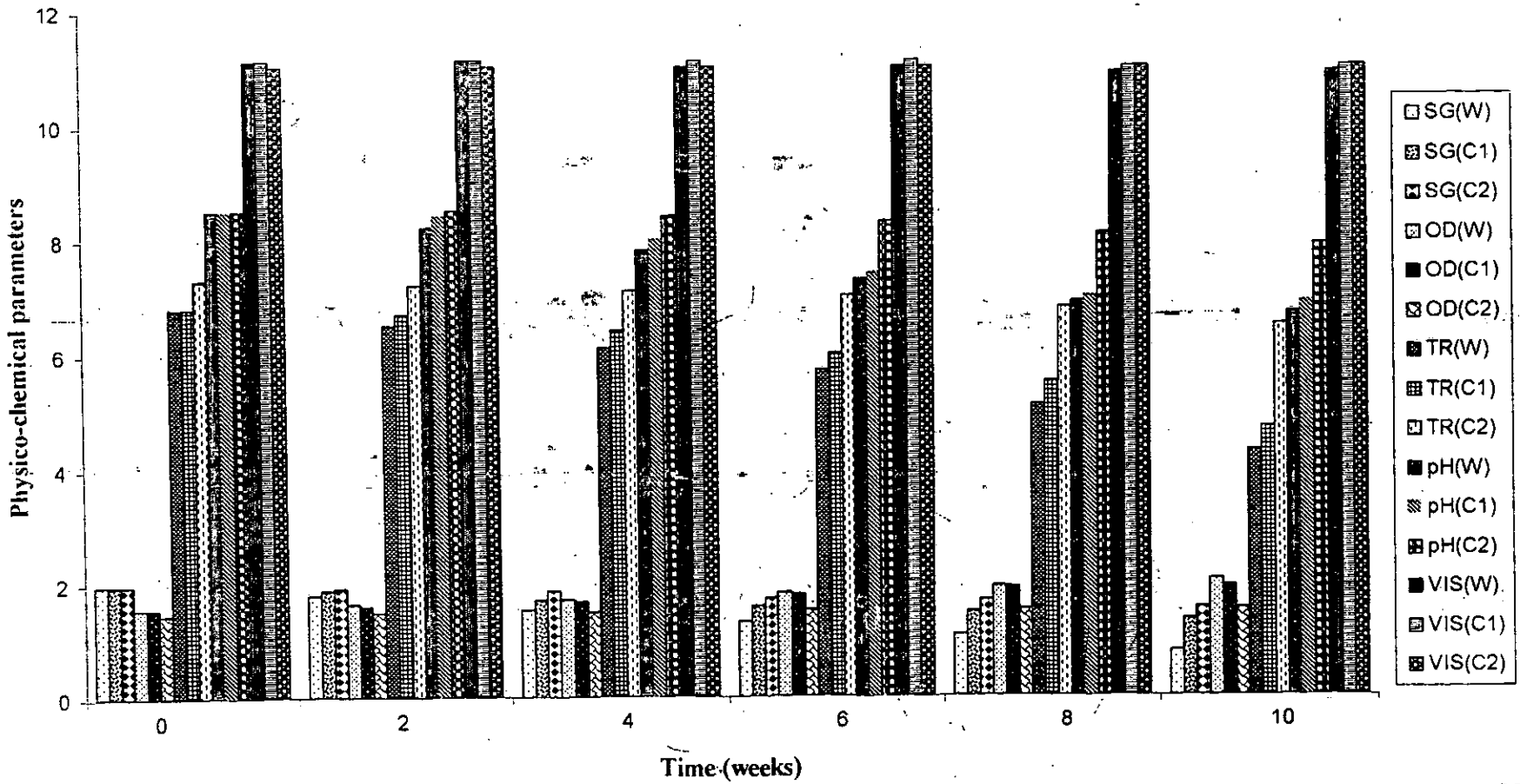


Fig. 3.45 Spoilage potential of the plasmid-bearing and plasmid-cured strains of *Pseudomonas aeruginosa* on the physico-chemical parameters of paint samples PS-2. VIS, viscosity (cst); TR, transmittance; OD_{600}; SG, specific gravity; (C2), control sample; (C1), plasmid-cured sample; (W), plasmid-bearing sample. Data represent the means of triplicate samples.

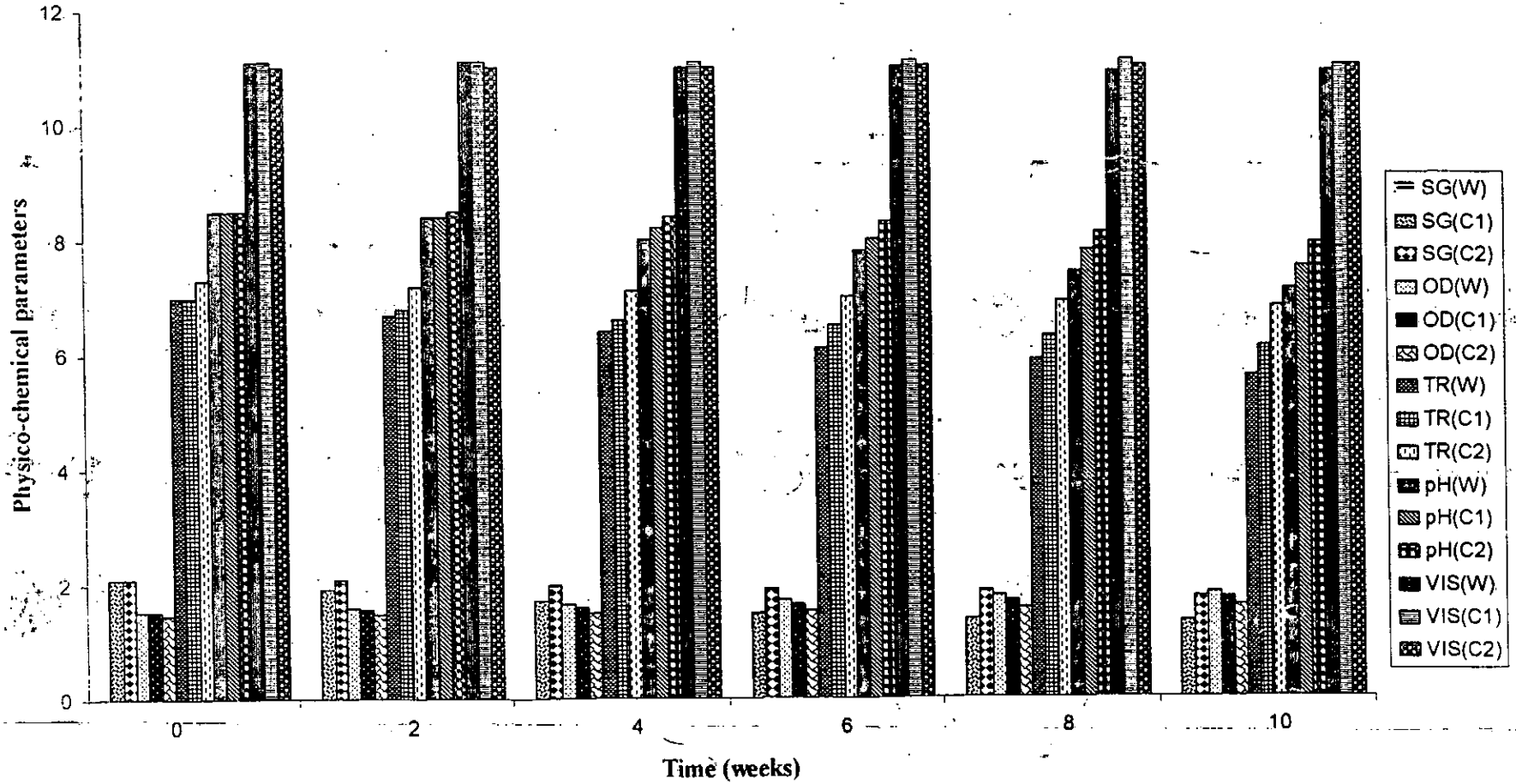


Fig. 3.46 Spoilage potential of the plasmid-bearing and plasmid-cured strains of *Pseudomonas aeruginosa* on the physico-chemical parameters of paint samples PS-3. VIS, viscosity (cst); TR, transmittance; OD_{600}; SG, specific gravity; (C2), control sample; (C1), plasmid-cured sample; (W), plasmid-bearing sample. Data represent the means of triplicate samples.

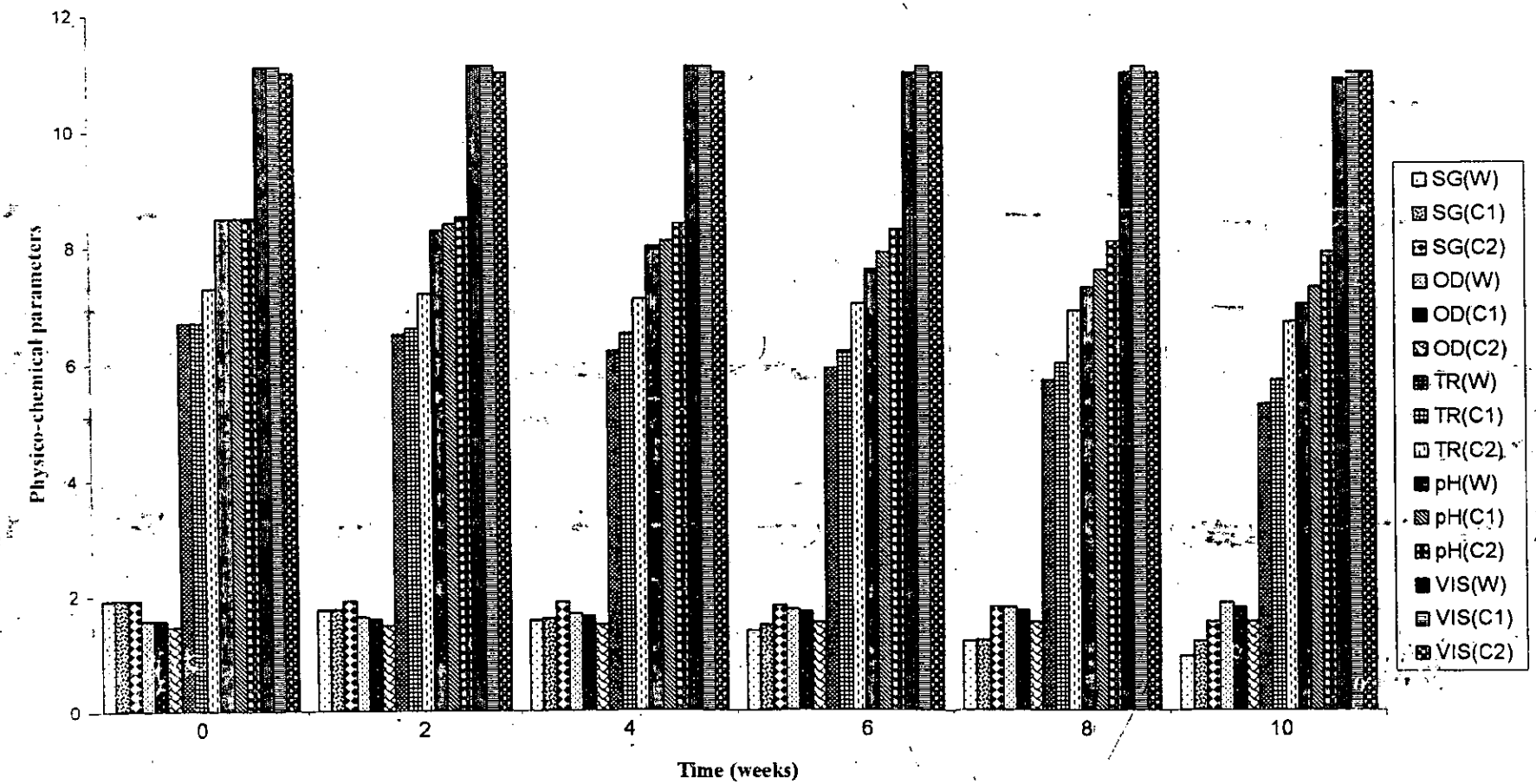


Fig. 3.47 Spoilage potential of the plasmid-bearing and plasmid-cured strains of *Pseudomonas aeruginosa* on the physico-chemical parameters of paint samples PS-4. VIS, viscosity (cst); TR, transmittance; OD_{600}; SG, specific gravity; (C2), control sample; (C1), plasmid-cured sample; (W), plasmid-bearing sample. Data represent the means of triplicate samples.

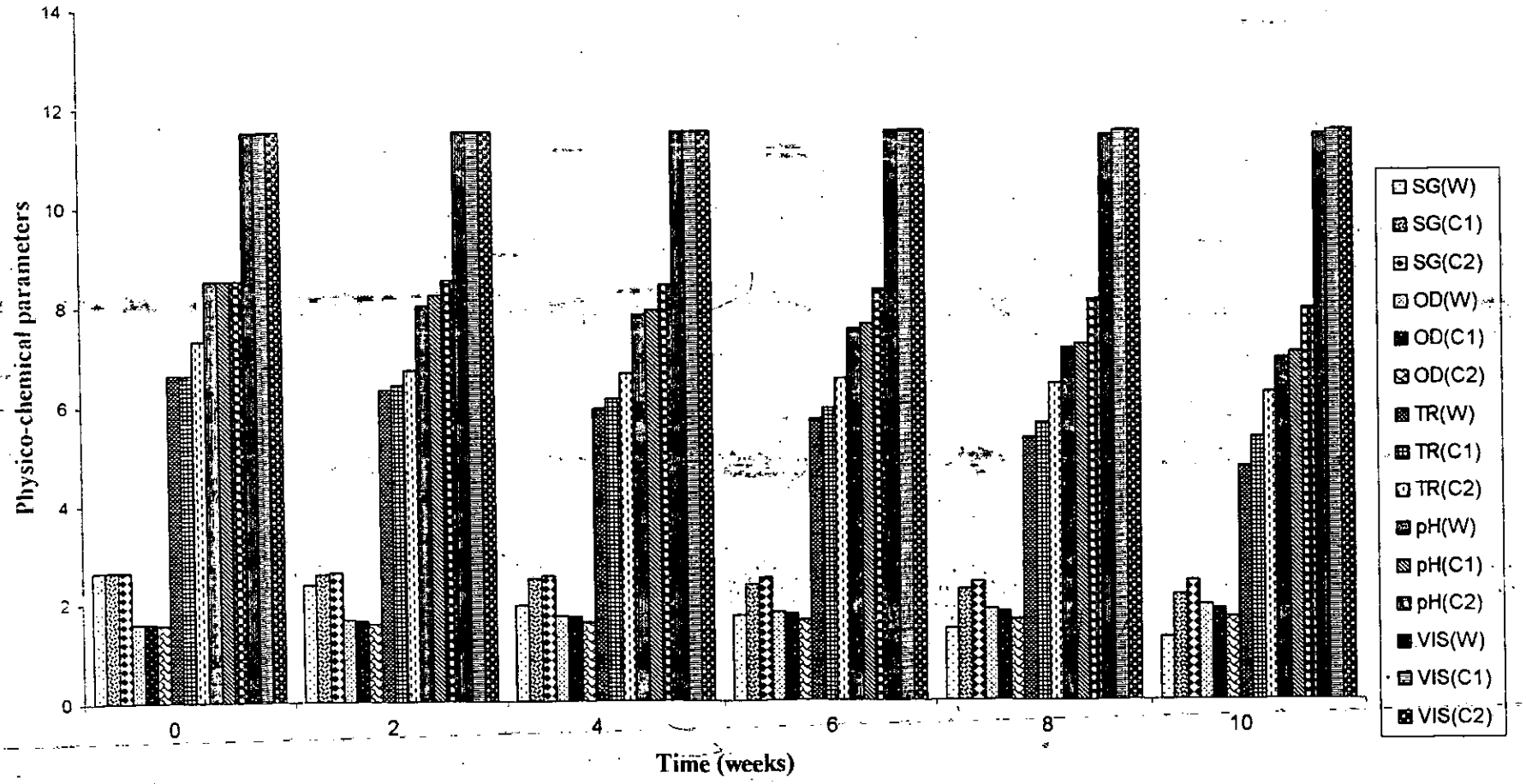


Fig. 3.48 Spoilage potential of the plasmid-bearing and plasmid-cured strains of *Pseudomonas aeruginosa* on the physico-chemical parameters of paint samples PS-5. VIS, viscosity (cst); TR, transmittance; OD_{600}; SG, specific gravity; (C2), control sample; (C1), plasmid-cured sample; (W), plasmid-bearing sample. Data represent the means of triplicate samples.

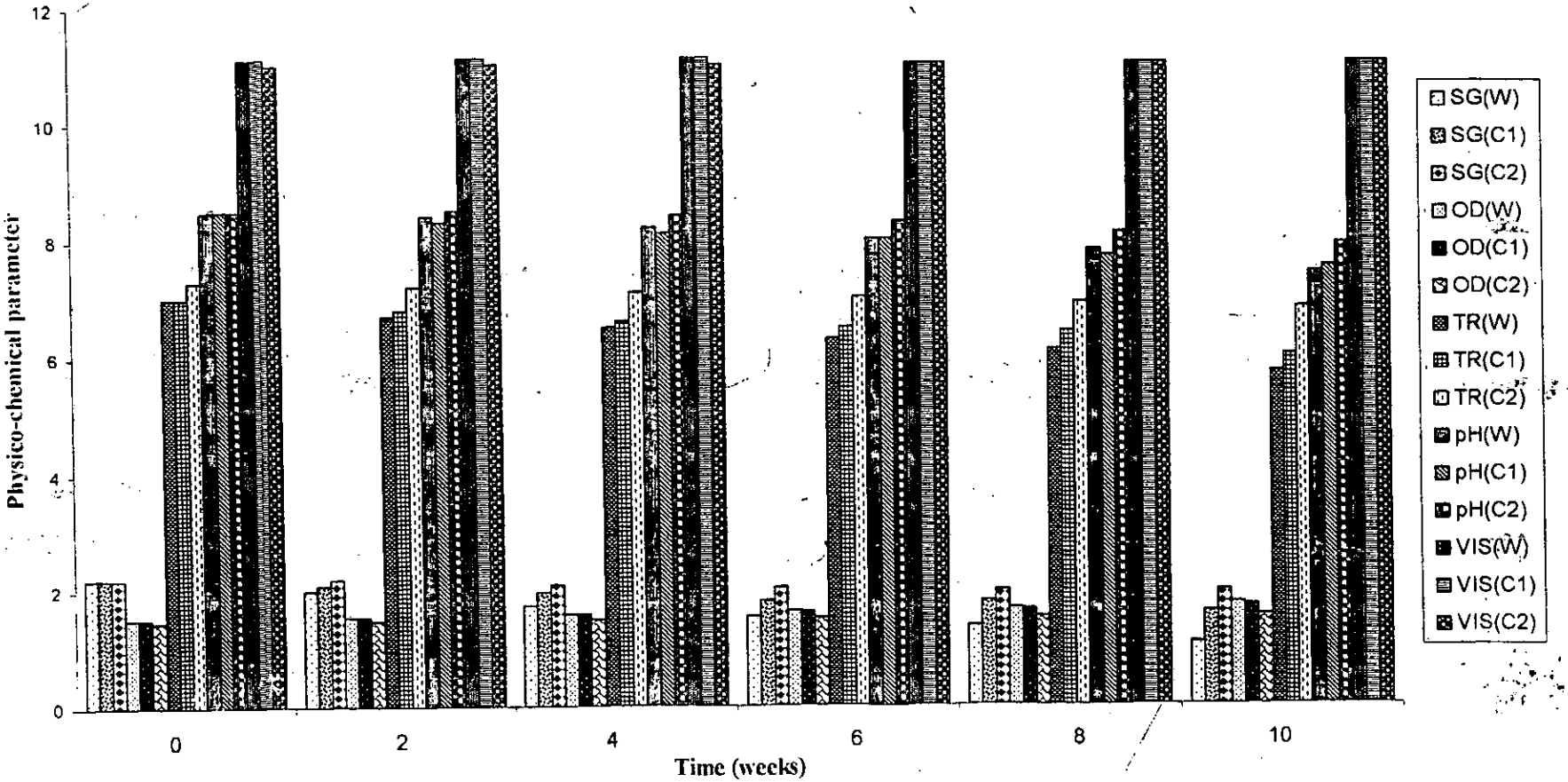


Fig. 3.49 Spoilage potential of the plasmid-bearing and plasmid-cured strains of *Pseudomonas aeruginosa* on the physico-chemical parameters of paint samples PS-6. VIS, viscosity (cst); TR, transmittance; OD_{600}; SG, specific gravity; (C2), control sample; (C1), plasmid-cured sample; (W), plasmid-bearing sample. Data represent the means of triplicate samples.

3.16. Microbiological Shelf Life Determination.

A summary of the mean changes in microbial population density of fresh paint samples monitored at two weeks intervals is given in Fig. 3.1. The results show that there was a time interval which elapsed before the exponential growth of the organisms. This time interval, known as the lag phase (L) varied from 4 – 5 months in the paint samples tested. This is probably because of differences in the types and concentrations of biocides incorporated during production. Generally, the microbial growth profiles showed a steady increase after the lag phase in all the paint samples ranging from $1.6 \times 10^1 - 4.7 \times 10^5$ cfu/ml during the study period. The spoilt paint samples had a microbial population density of 3.4×10^{10} cfu/ml (Table 3.5). When the data obtained for the fresh paint and the spoilt paint samples were fitted into population model dynamics, it was observed that the time it would take the fresh paint samples to get spoilt in terms of the microbial population density is 27, 22, 30, 36, 22 and 23 months for paint samples PS-1 – PS-6 respectively. This suggests that the average shelf life duration (SLD) of the paints determined microbiologically is 2 years.

3.17. Statistical Shelf Life Determination Based on Paint Physico-chemical Parameters

The mean changes in physico-chemical parameters of fresh paint samples that were also monitored over a period of 10 months at two weeks intervals are illustrated in Fig. 3.2. The optical density increased in all the samples from 1.49 – 3.91. The pH profiles showed a decreasing trend throughout the period in all the samples. The initial pH value was 8.5 but this decreased to 5.6. The spoilt paint sample had pH ranging from 4.12 – 4.19 (Table 3.11). The trends in the specific gravity were similar in all the fresh paint samples; it was highest at month zero 2.8658 and dcreased to 1.0853 at the 10^{th} month. In like manner, the other parameters of the fresh paint samples showed decreasing trends from 6.9 – 2.3 and 11.7 to 10.8 cst for transmittance and viscosity respectively. When the data obtained for both fresh and spoilt paints were fitted into a statistical model, the quantitative knowledge generated, predicted the time the paints will reach a critical spoilage level under constant conditions within the range tested. The shelf lives of the paints were thus, determined to be 19, 21, 23, 22, 37 and 22 months for PS-1 – PS-6 respectively.

3.18. Statistical Shelf Life Determination Based on Paint Microbial Population Count

The statistical model fitted using the data from microbial population counts of fresh paint samples monitored every two weeks for a period of 10 months and applied to paint samples PS-1, PS-2, PS-3, PS-4, PS-5 and PS-6 predicted the shelf lives of the samples. The shelf lives obtained were 21, 18, 21, 19, $19^{1}/_{2}$ and 30 months for paint sample PS-1 – PS-6 respectively.

CHAPTER FOUR

DISCUSSION

The high microbial population counts detected in the raw materials suggested that these materials are potential sources of microorganisms for paint deterioration. In the present study, the raw materials were observed to be highly contaminated with bacteria ranging from 1.0×10^6 to 9.5×10^6. Similarly, the raw materials were also contaminated with fungi ranging from 1.25×10^4 to 6.8×10^4 (Table 3.1). The result of extensive microbial analysis carried out to examine all the stages involved in paint production, packaging materials, fresh paints and spoilt paints samples with a view to exploring and identifying the sources of contamination also revealed gross contamination (Tables 3.3, 3.4, and 3.5). These contaminations can result in rapid paint spoilage and reduction of shelf life. The biocides contained low levels of microorganisms ranging from 1.0×10^2 to 1.5×10^2 cfu/ml of bacteria; 1.0×10^1 to 1.7×10^1 cfu/ml of coliforms and 1.0×10^1 to 1.5×10^1 cfu/ml of fungi respectively (Table 3.2).

The random microbial increases and/or decreases in number, particularly of bacteria during the stages of paint production (Table 3.4) could be attributed to production or manufacturing details. Paint production involves the use of several powdery raw materials such as fillers, extenders and pigments (Gillatt, 1992). These raw materials contain bacteria and fungi which in an aqueous environment will germinate and grow. Liquid raw materials such as defoamers and polymer emulsions are often susceptible to bacterial and fungal attack. The addition of these raw materials at different stages of production to achieve the specified quality characteristics could introduce contamination, cause fluctuations and variations in numbers and microbial types. For example, some raw materials added to achieve dispersion, texturing or tinting in the paint industry, when subjected to extensive agitation and heat may act as abrasives, inflicting injury and hence cell death of the resident microorganisms. These factors may be responsible for the different types and numbers of the microbial contaminants observed during the processing. This also reflects the observation of Gillatt (1992) that microbial contamination can originate from paint processing units and the manufacturing plant itself. He also reported that plant hygiene and manufacturing process do have a profound effect on the microbial quality of the paint produced. The microbial contamination observed in

the fresh paints (Fig. 3.1) could therefore, be attributed to the addition of these contaminated raw materials at different stages of production.

More bacteria were present in the spoilt paints samples than in the fresh paint samples (Table 3.5). Similar observation was also made by Adeleye and Adeleye (1999). The high counts of bacteria observed in the fresh paints (Fig. 3.1) further suggests that the shelf life of the paints would be rather short. The results obtained in this study demonstrate that microorganisms utilize the paints as a source of nutrients and that the constituents of paints are conducive to increased cell multiplication and population buildups in the paint. Therefore, a can of water-based paint is highly susceptible to deterioration.

Work done by Da Silva (2003) proved that the various organic constituents of paints such as pigments, additives, binders etc, which act as nutrients for microorganisms, help to stimulate microbial growth. The factory water inlets, HSD H_20, CD H_20, and MU H_20 had high levels of coliform bacteria ($6.5 \times 10^4 - 7.05 \times 10^4$ cfu/ml). The high incidence of coliforms in samples MU H_20, (7.05×10^4 cfu/ml), CD H_20 (7.0×10^4 cfu/ml and HSD H_20 (6.5×10^4 cfu/ml) (Table 3.1) indicate faecal contamination. In Nigeria, most industrial waters are not properly treated and often contain residual organic matter (Onabanjo, 1977). Town tap waters have also been found to contain appreciable number of bacteria (Azuonye, 1990). All these may have been responsible for the contamination found in the paint samples.

The mean changes in the microbial population densities in the fresh paint samples (PS1-PS6) during a study period of 10 months (Fig. 3.1.) revealed a protracted lag phase of 4 to 5 months before exponential growth of the organisms. The observed protracted lag phases are indicative of the gradual reduction in the usefulness and impact of the biocides incorporated in paints during production (Russell and Mc Donelli, 2000; Gilbert and Mc Bain, 2001; Stickler, 2002; Russell, 2003; Petersen *et al.*, 2004). In addition, the observed consistent increase in the microbial population counts in the fresh paint samples after the lag phase from the initial 1.6×10^1 cfu/ml at day zero to the levels of 4.7×10^5 cfu/ml at the 10^{th} month suggests the exhaustion and limitation of the incorporated biocides or

resistance developed against them by the indigenous contaminants. The microbial increases also revealed the role of microorganisms in the deterioration of water-based paints and reduction of their shelf lives. Microbial counts have been used by many investigators to establish the deterioration of paints (Miller, 1973; Jakabowski *et al.*, 1983; Saad, 1992; Adeleye and Adeleye, 1999; Da Silva, 2003 and Ogbulie, 2004). In this study, the paints immediately after production were found to have a total bacterial count of approximately 1.6×10^1 cfu/ml and total fungal count of 1.0×10^1 cfu/ml. The bacterial count increased to approximately 3.4×10^{10} cfu/ml at the time of spoilage, while the fungal count increased to approximately 2.8×10^5 cfu/ml (Table 3.5). The estimated microbial population count in the study therefore, appeared to be a good and direct index for shelf life prediction.

The subsequent identification of individual isolates revealed by standard cultural and biochemical tests coupled with the phenotypic profiling of frequently occurring isolates showed that the same organisms were repeatedly encountered in various samples. *Bacillus polymyxa* (OB-1), *B. brevis* (OB-2), *B. laterosporus* (OB-3), *Proteus mirabilis (OB-4), Escherichia coli* (OB-5), *Lactobacillus gasseri* (OB-7), *L. brevis* (OB-8), *Aspergillus niger* (OB-9), *A. flavus* (OB-10), *and Penicillium citrinum* (OB-11), were isolated from the raw materials, finished products and packaging materials. *Pseudomonas aeruginosa* (OB-6) was isolated repeatedly only in the spoilt paints and biodeteriorated painted walls. This is consistent with earlier publications that *Pseudomonas aeruginosa* constitutes 75% of isolates in spoilt paints (Dey *et al.*, 2004) and occurs mostly in biodeteriorated painted walls (Ogbulie, 2004). Fungi are aerobic microorganisms and hence were not encountered in large numbers in packaged paints (Table 3.3). They occurred mostly in the biodeteriorated painted walls (Adeleye and Adeleye, 1999). *Bacillus* spp. were encountered more in the fresh samples than in the biodeteriorated wall scrapings (Table 3.7). Previous work by Miller (1975) had shown that *Bacillus* spp. were the most frequently isolated bacteria found in paints. *Bacillus* spp. are contaminants found freely in nature and are known to show reasonable resistance to heat, ultra violet radiation and light (Prescott *et al.*, 2005). In the present study, three different fungal species were isolated from all the samples tested. Two of the three fungal species isolated belong to the

genus *Aspergillus*. *Aspergillus* spp. were the most regularly occurring fungal isolates in all the samples examined (Tables 3.2, 3.3 and 3.4). This genus has been reported as one of the most abundant fungi isolated from biodeteriorated paint films in Egypt (Saad, 1992). This agrees with the results obtained by Inuoe and Koyano (1991) from contaminated paints in Japan. Similar observation was also made by Jakabowski *et al.* (1983); Gillat, (1992). The third fungal spp. *Penicillium citrinum* was observed mainly in the raw materials, biodeteriorated painted walls and spoilt paint samples (Tables 3.1, 3.5 and 3.7).

Several workers have also reported the occurrence of *Bacillus*, *Pseudomonas*, *Enterobacter*, *Proteus*, *Aerobacter*, *Escherichia*, *Micrococcus*, *Serratia*, *Aeromonas* etc. in paints and painted walls (Miller, 1973; Woods, 1982; Jakabowski *et al.* 1983 and Opperman and Gull, 1984). Anaerobic bacteria including *Bacteroides*, *Clostridium*, *Desulphovibrio* and *Bifidobacterium* have also been isolated (Opperman and Gull, 1984). Allsopp and Soal (1980) also reported some fungi associated with the deterioration of paints. These fungi include *Rhizopus arrhinus*, *A. niger*, *A. ustus*, *P. citrinum*, *Chaetomium globosum* and *Alternaria alternata*. Similar observation was made by Nugari (1993). The ability of these organisms to reduce paint shelf life is thus evident, but not documented. The estimation of shelf life of paints made in Nigeria has therefore, received no attention over the years.

To a large extent, quality changes in the fresh paint samples detected by monitoring physico-chemical changes during the 10-month study period were evident (Fig. 3.2). The observed decreases in pH values from 8.5 – 5.6 in the fresh samples analyzed during the study period could be attributed to the presence of microbial contaminants in the paint samples. During their metabolism, microorganisms produce acidic metabolites which change the original pH values (Prescott, *et al.*, 2005) of the fresh paint samples to an acidic range. Therefore, pH, as a key quality parameter of paint could serve as an index in estimating paint spoilage. The optical density (OD) which is a function of microbial population density was found to increase during the study from 1.49 – 3.91 (Fig. 3.2) The drop in the viscosity of fresh paint samples over the study period from 11.7 to 10.8 cst (Fig. 3.3) could be attributed to the cellulolytic activities of the indigenous organisms

which utilized cellulose by hydrolyzing the celluloytic derivatives used as thickeners in paint, thereby reducing the paint viscosity (Gillatt, 1992; Saad, 1992; Toothill *et al.*, 1993). These observations emphasize the degradative role of microorganisms on the physico-chemical parameters of water-based paints.

The purpose of investigating a wide range of physico-chemical parameters with different paint samples was to assess their roles in paint stability. However, all parameters showed a considerable decline in quality parameter assessment. Therefore, physico-chemical parameters appear to provide a sensitive and reliable measure of the paint deterioration. Shelf life determination must therefore, depend on such criteria which include the rate of deterioration of quality parameters. Due to the fact that the stability of these physico-chemical parameters infer quality, their stability was regarded as a key factor or determinant of shelf life of paints in the present study.

In this study, particular emphasis was given to endoglucanase activity of isolates because of the role of cellulolytic enzymes in drastically reducing the viscosity of paints. Once the viscosity of paint is lost, the paint cannot be recovered, not even with high concentration of broad spectrum biocides (Gillat, 1992). The selection of cellulose degraders in the study was therefore, not arbitrary but was motivated by the fact that the isolated microorganisms could utilize cellulose as sole sources of carbon and energy to reduce the paint viscosity leading to the eventual loss of the paint. In the last two decades, much effort has gone into the study of enzymes with cellulolytic activity as a potential means of obtaining energy, chemicals and single-cell proteins from the abundant renewable resource, cellulose. Bacterially or fungally-produced cellulolytic enzymes have been detected in water-based paints that have been exposed to microbiological contamination (Toothill *et al.*, 1993). This was demonstrated by surveying the cellulolytic activity of the various isolates. These enzymes degrade the various water-soluble hydroxyethyl cellulose and carboxymethyl cellulose used for thickening the paint. Biocides can kill bacteria or fungi but they have no effect on the cellulolytic enzymes (Gillatt, 1992). Therefore, the production of the enzyme causes degradation of the cellulosic polymer, even though the microorganisms have been controlled with a biocide. The endoglucanases initiate the degradation process, creating

new chain ends from which the exoglucanases release cellobiose or glucose, depending upon the types of exoglucanase concerned. The cellobiose released is further hydrolysed to glucose by the third component of the cellulase system, the cellobiase (ß - glucosidase) Lee *et al.* (1985).

It was found in this study that maximum cellulolytic, activity was produced by *Pseudomonas aeruginosa*. However, the endoglucanase activities of the other isolates were much lower (Table 3.10). It is noteworthy to observe that *Ps. aeruginosa* (OB-6) occurred only in the spoilt paint samples and in the biodeteriorated wall scrapings (Table 3.5 and 3.7). This is most likely possible because the Pseudomonads can degrade an exceptionally wide variety of organic molecules. Thus, they are very important in the mineralization process. Recent work by Sauer *et al.* (2002) also showed that biofilm development by *Pseudomonas aeruginosa* proceeds through five physiologically distinct phases, the last phase involving the detachment of adherent bacteria. This may account for the persistence of *Pseudomonas aeruginosa* on biodeteriorated painted films and spoilt paints.

Interestingly too, the molecular studies of the isolated organisms showed that of all the microorganisms isolated, *Ps. aeruginosa* was the only organism that harboured plasmids. It is not unlikely therefore, that the degradation of cellulose, which is the main thickening agent in water-based paints may be plasmid-mediated (Standal *et al.*, 1993). It was clear that the enzymic hydrolysis of the celluloses which are thickening agents in paints by these isolates as sole carbon and energy sources resulted in reduction of paint viscosity. Similar observation was reported by Saad (1992).

The consistent decrease in PO_4^{3-} and SO_4^{2-} in both fresh and spoilt paint samples may be due to their high demand by microorganisms for sugar phosphorylation, as a component of cell membranes (phospholipids), and nucleic acids (nucleotides) (Andrews and Jackson, 1996). Microorganisms require inorganic nutrient sources (mineral elements) for growth. If any of the required inorganic nutrients is lacking or becomes limiting, particularly those needed in relatively larger amounts known as P, S, C, N_2, the microorganisms will not

grow even if other factors such as carbon and energy sources required for growth are available (Andrews and Jackson, 1996). Therefore, the increase in the mean changes in microbial population count observed for the fresh paint samples may be the reason for the drop in the concentrations of PO_4^{3-} and SO_4^{2-}. It is clear from the results on available PO_4^{3-} and SO_4^{2-} that initial levels of $PO_4^{3-}$and SO_4^{2-} decrease remarkably as the microbial population densities increase in fresh paint samples. This obviously is attributed to the utilization of these PO_4^{3-} and SO_4^{2-} by microorganisms to meet their protein and nucleic acid requirements (Rosenberg and Doyle, 1990). The reduction of sulphates to hydrogen sulphide under anaerobic conditions can be linked to the odour observed in the spoilt paint samples.

Out of the eight bacterial strains isolated in the study, five (*Bacillus polymyxa*, *Bacillus brevis*, *Bacillus laterosporus*, *Lactobacillus gasseri* and *Lactobacillus brevis* were Gram-positive and three (*Proteus mirabilis*, *Escherichia coli*, and *Pseudomonas aeruginosa* were Gram –negative. The alkaline lysis method of Birmboim and Doly (1979) was readily applied to the Gram-negative organisms. However, because the thicker peptidoglycan cell wall layer of the Gram-positive isolates require the use of lystostaphine or butanolysine to lyse the strong cell wall, which is not provided for in the alkaline lysis procedure, the TENS-Mini Prep. Procedure (Leeh and Brent, 1987) involving the use of TENS solution (TE buffer containing 0.1N NaOH and 0.5% sodium dodecyl sulphate) was adopted.

Subsequently, curing experiment was carried out on *Ps. aeruginosa* which had two plasmids. Sodium dodecyl sulphate (SDS), an effective plasmid curing agent with rates of curing far surpassing rates obtained with ethidium bromide and elevated temperatures (Sonstein and Baldwin, 1972) was used to eliminate the two plasmids detected in *Pseudomonas aeruginosa*. Various methods of eliminating plasmids from microorganisms have been reported (Kulkarni and Kanekar, 1998). These methods effect curing either by taking advantage of differences in the rates of plasmid and chromosomal DNA replication during growth at elevated temperatures (Ghosh *et al.*, 2000) or by the use of agents such as the acridine dyes (Tomas and Kay, 1984) and ethidium bromide (Hein *et al.*, 2006)

which are believed to inhibit the synthesis of DNA. It appears that SDS may be a useful agent in the study of extra chromosomal genetic elements and their relationships to the cell membrane because of its ability to disrupt biological materials and cause a permanent, heritable alteration in genetic materials.

The antimicrobial sensitivity test was done to select effective antimicrobial agents against *Pseudomonas aeruginosa*, before and after the curing experiments. Quality control procedures currently recommended for analysis of disk diffusion antibiotic susceptibility tests are capable of measuring both accuracy and precision (Gavan *et al.*, 1981). A major defect of present quality control procedures, however, is the inability to provide guidelines for correct setting of breakpoints when the accuracy has been determined. This leads to wide variability in inhibition zone diameter interpretation. The quality control limits set by the National Committee for Clinical Laboratory Standard are therefore quite generous (Knowles and Moore, 1979; Gavan *et al.*, 1981).

To establish a link between the ability to cause spoilage with the presence of the plasmid in the wild strain, the cured and the wild strains of *Ps. aeruginosa* were tested for degradative ability on the physico-chemical properties of fresh sterile paints and subsequently, subjected to antibiotic sensitivity. Agarose gel electrophoresis of the plasmid extract from the cured strain of *Ps. aeruginosa* showed the absence of the plasmids found in the wild strains. It was observed that the cured strains of *Ps. aeruginosa* had lost the two plasmids it earlier had (Plate 3.14), it had also lost its degradative ability. Further proof of this was indicated by the results of the comparative evaluation of the spoilage potentials of the wild and cured strains of *Ps. aeruginosa* exhibited in the physico-chemical parameters of fresh and sterile paint samples (Figs. 3.44 – 3.49). The wild strain showed higher degradative ability than the cured strain. It also had higher resistance to antibiotics than the cured strain (Fig. 3.43). The cured strain was found to have lost its initial resistance to tobramycin, gentamycin and amikacin. This further confirmed that the gene for degradation of paint may be plasmid-borne. Sodium dodecyl sulphate, probably affected the genes for degradation and spoilage. Since SDS is known to cause disruption of biological materials (Sonstein and Baldwin, 1972), the possibility

exists that SDS as a curing agent cures the organism of its plasmid by disrupting the membrane sites of plasmid attachment. The present sensitivity of the cured strain to tobramycin, gentamycin and amikacin was thought to be due to the loss of these plasmids. *Pseudomonas aeruginosa* might have acquired the antimicrobial resistance plasmid to survive in the spoilt paints and biodeteriorated painted walls since many bacteria are known to produce antibiotics.

The results of concentration of heavy metals in the fresh, spoilt and stored paint samples in the company warehouse indicated that higher concentrations of these heavy metals were observed in the spoilt samples. However, the highest concentrations observed, which are 5.3 mg/kg of Pb; 5.8 mg/kg of Cu and 5.0 mg/kg of Mn were very low when compared to the 500 mg/kg of Pb reported by Dumont (2000) as the acceptable level of Pb in paints. This suggests that the concentrations of Pb, Cu and Mn detected in the study had no impact on the shelf life of the paints examined and as such, were not considered in the models developed. The use of Pb concentrations as high as 2.6% – 10.8% rather than the acceptable limit of 0.06% to improve the durability and shelf life of paints has however been previously reported (Rabin, 1989). This, however, has been found to be hazardous and leads to high blood lead levels (Marino *et al.*, 1990). The persistence of *Ps. aeruginosa* observed in the spoilt paints could be linked to its ability to resist the high concentrations of heavy metals observed in the spoilt paints. This finding also reflects the observation of Silver and Misra (1988) that the development of resistance to heavy metals by microorganisms is plasmid-mediated.

The results obtained from the antibiotic resistance patterns revealed that the antibiotic resistant *Pseudomonas aeruginosa* was also resistant to ZN489 to a large extent. This organism poses particular problems in spoilt paints and has been found in this study to possess plasmids. Studies have shown that the same microorganism can evolve its own resistance genes to protect itself from its own products (Novick, 1980). It is also possible that *Ps. aeruginosa* has acquired these antimicrobial resistance genes from other bacterial species. Sharp *et al.* (1973) reported that transfer of resistance determinants between bacterial strains suggested that some of these genes can hop or transpose from one

replicon to another. A drug resistance gene can hop from a drug resistance plasmid to the chromosome, to another plasmid or to the genome of a temperate bacteriophage. This process occurs in both Gram-negative and Gram-positive bacteria and is known to involve determinants for the resistance to at least seven different antibiotics.

During the studies on the efficacy of biocides, it was observed that the persistent *Pseudomonas aeruginosa* growing on biodeteriorated walls and spoilt paint samples, were particularly more resistant to biocides ZN481, ZN485 and ZN489. Subsequently, it was demonstrated that 1% v/v concentrations of ZN467 which were effectively bactericidal against *Lactobacillus gasseri* had less effect against *Pseudomonas aeruginosa* (Plates 3.2 and 3.3). It seems therefore, that once a biofilm forms on spoilt paints or deteriorating walls, they become biocide resistant. This may be the reason for the persistence of *Pseudomonas aeruginosa* on spoilt paints and not in the fresh paint samples. An interesting observation made by Spoering and Lewis (2001) was that *Pseudomonas aeruginosa* biofilms have the ability to resist killing by antimicrobial agents. They attribute this ability to survive antimicrobial treatment to slow growth and the presence of persister cells. It is now recognized that an intrinsic property of mature biofilms is their increased resistance to antibiotics and biocides (Costerton *et al.*, 1994; Stickler, 2002). Biocides are biologically active paint and sealant additives designed to keep bacteria from spoiling the paint or sealant during storage; or to keep fungal/algal contamination from growing on the applied paint film. Most known biocides are preservative in nature (Russell, 2003). Paints exhibiting the effect of microbiological contamination especially viscosity loss can be treated with a high level of biocide, a so-called "kill dose" to eliminate the microorganisms.

A comparative study of the biocides in this study provided a perspective for evaluating the effectiveness of the biocides on bacterial films. By comparing biocides on the basis of activity, it was possible to evaluate the potency of each biocide. The result obtained in this study is similar to those published by other investigators (Russell, 2003). The efficacy of the four biocides used in paint production was compared at concentrations ranging from 1 – 5%. The results presented in plates 3.2 – 3.11 indicate that although the biocides were effective at 3% v/v, 5% v/v was required for optimal inhibition. At 5% v/v,

the biocides were almost, but not totally inhibitory to the resident microorganisms, suggesting growth of tolerant opportunistic species (Petersen *et al.* 2004). Previous studies have also reported the differences in biocidal activities against different organisms (Russell, 2003). The co-contaminant effect of biocidal activity on a consortium of organisms indicated that the organisms were more resistant to the biocide in mixed suspension than in pure suspension. As a result, the actual concentration of biocide contacting each organism in the mixed suspension experiments was less than in the pure suspension experiments thereby, leading to greater survival or protection from killing. Biocide ZN467 showed an increased biocidal activity against a mixed suspension. All the organisms have the ability to tolerate the biocides albeit to a varying extent. However, *Pseudomonas aeruginosa* was more tolerant to the tested biocides. These observations point to the limited usefulness of commercial biocides commonly applied for microbial control in paint formulation. The observations in this study emphasize the importance of evaluating each commercial biocide against appropriate problem strains of bacteria commonly prevalent in paints and the effect of co-contaminants for such evaluation. The present study also showed that high concentrations (5% v/v) of biocides are required for effective prevention of contamination. In like manner, the effect of a biocide was found to be highly concentration-dependent. Similar observations were made by Russell and Mc Donnelli (2000).

In contrast to this view, Blekinsopp *et al.* (1992) suggested that where high biocide concentrations are impractical, uneconomic or environmentaly harzadous and prohibited by law, the application of industrial biocides within a low-strength electric field (± 12V/cm) with a low current density (+2.1m A/cm ^{2}) exhibits enhanced killing action against *Pseudomonas aeruginosa* biofilms. They also demonstrated that the enhancement of biocidal efficacy was non-dependent on the conditions (presence or absence of an EF-CD) under which the biofilm was established. If this bioelectric effect holds true for a wide variety of biofilms, biocides and surfaces, this technology may have great promise in circumstances where high biocide concentrations are impractical.

Despite active research on predictive modeling over the last few decades, several studies that have been published (Fu *et al.*, 1991; Fu and Labuza, 1993; Ross, 1996; Taoukis *et al.*, 1999; Koutsoumanis and Nychas, 2001), show that the emphasis of predictive microbiology has been on perishable and processed foods. Predictive food microbiology, the modeling of microbial populations particularly those of food–borne pathogens, has become an active field of research. It is noteworthy therefore, that predictive models have been used in the present study to determine and predict the shelf life of paints based on microbial growth kinetics and statistical regression analysis. To our knowledge, evidence to date indicates that the predictive models used in the present study have not been previously used with respect to paints. The good agreement of predicted and observed results in the study allows for reproducibility.

Time (Y) had a negative effect on all the parameters tested to a 99% significant level. All the independent variables ($X_1 - X_5$) of the physico-chemical parameters and ($X_1 - X_3$) of the microbial parameters contributed positively to deterioration of the paints at different rates. Specific gravity and total bacterial count was found to contribute most significantly and viscosity contributed least significantly based on the regression parameter estimate value confirmed by the t-test. The regression model and microbiological model applied in this study indicated shelf life of paint to be between 19months and 35months. The regression (ANOVA) is highly significant at P value of 0.0001, indicating that the regression is very useful. Therefore, the physico-chemical parameters and microbial population counts can be used to predict shelf life. The regression model developed in this study to determine the shelf life of paint samples proved adequate based on the coefficient of multiple determination ($R^2 > 0.95$) indicating that the model as fitted, can predict shelf life up to 95%. Hence, prediction is highly accurate, considering also the percentage of the residual error (Koutsoumanis, 2001) for all samples which ranged from 0.001 to 0.5%.

Conclusion

The results of the extensive analysis of freshly made paint samples monitored over a period of 10 months, showed the characterization and documentation of the microorganisms associated with spoilage of water based paints made in Nigeria. Based on the data presented in this work, it is clear that the increasing levels of deterioration which resulted from contaminated raw materials, factory processing units and packaging materials all have significant impact on the physico-chemical properties and hence aesthetic qualities of water-based paints. These have also contributed to the gradual reduction of the shelf life of paint to 2 years. Biocidal treatment continues to be an important tool used by paint manufacturers for the prevention of paint contamination. To provide the best tools for the prevention of paint spoilage, biocides currently being developed for the paint industry should include interpretative criteria that have been established with specific paint microflora. The study has also highlighted the efficacy of individual biocides at different concentrations on pure and mixed populations. The results showed that ZN467 was most effective in controlling the contamination, being able to resist individual organisms and a consortium of organisms at low concentrations.

Furthermore, *Pseudomonas aeruginosa*, suspected to be the specific spoilage organism (SSO) (Koutsoumanis, 2001), occurred only in the contaminated paint samples and the biodeteriorated wall samples regularly in highest frequencies. It also had the highest cellulolytic activity. In addition, the presence of plasmids only in this organism amongst others, suggests that the gene for cellulolytic activity and degradation may be plasmid-borne. Interestingly, subsequent curing of these plasmids with SDS as described by Sonstein and Baldwin (1972) resulted in the loss of the existing plasmids, loss of initial resistance to tobramycin, gentamycin and amikacin. Furthermore, the comparative evaluation of the degradative effects of the wild and cured strains of this organism on the physico-chemical parameters of fresh, sterile paints proved further that the gene for paint deterioration may be plasmid-borne.

Most importantly, microbiological and statistical modeling has been successfully used to predict or forecast the shelf life of water-based paints made in Nigeria. The adequacy of the developed model determined by the correlation coefficient which was greater than 0.90 indicate a good fit.

Contributions to Knowledge

- The biodegradative effects of the microorganisms associated with spoilage of water-based paints have been determined.
- The efficacy of biocides used in paint production has been ascertained in relation to paint microflora.
- The study has proved that the postulated model as fitted, determined the shelf life of emulsion paints produced locally to be 2 years. This was confirmed with high accuracy from the results of the t-test conducted, the coefficient of multiple determinations and the percentage residual error.
- The molecular studies of the organisms associated with spoilage revealed that the genes for degradation of paints may be plasmid-borne.
- Having developed a useful model for prediction of shelf life and having studied the incidence of deterioration, causes and implications, the shelf life of paints could be improved by treatment with SDS without introduction of lead and other toxic metals.
- For the paint industry, expiry dates can now be indicated on new paint formulations made in Nigeria, which in the past had not been done, thus reducing waste and consumer trust.

Suggestions for Future Work

The use of plant extracts in place of biocides to control contamination in liquid paint may be studied.

REFERENCES

Adeleye, I. A. and Adeleye, O. A. (1999). Isolation and identification of microbes associated with paints and weathered painted walls. *Journal of Science and Research Development* **4:** 71-76.

Alba, J., Conde, E. and Guevara Perez, F. (2003). Degradation of the main components of cellulose-paint thinner by the mould *Scopulariopsis brevicaulis* cultured on rice hulls. *Letters in Applied Microbiology* **37** (1): 7-11.

Alejandre, F. J. and Marquez, G. (2006). Copper-zinc hydroxychlorides. *European Journal of Mineralogy* **18** (3): 403-409.

Allison, D. G., Evans, D. J., Brown, M. R. W. and Gilbert, P. (1990). Possible involvement of the division cycle in dispersal of *Escherichia coli* from biofilms. *Journal of Bacteriology* **172:** 1667-1669.

Allsopp, C. and Allsopp, D. (1983). An updated survey of commercial products used to protect materials against biodeterioraton. *Biodeterioration Bulletin* **19:** 99-146.

Allsopp, D. and Seal, K. J. (1986). Introduction to biodeterioration. In: *13th International Biodeterioration and Biodegradation Symposium.* London: Edward Arnold, Inc. pp. 13-16.

Amann, R. (2000). Who is out there ? Microbial aspects of biodiversity. *Systematic Applied Microbiology* **23:** 1-8.

Anderson, G. and Scott, M. (1991). Determination of product shelf life and activation energy for five drugs of abuse. *Clinical Chemistry* **37** (3): 398-402.

Andrews, R. W. J. and Jackson, J. M. (1996). Pollution and waste management. In: Environmental Science: The natural environment and human impact. Longman, Singapore Publishers (Pte) Ltd., pp. 281-297.

Armstrong, J. L., Calomiris, J. J. and Seideler, R. J. (1982). Selection of antibiotic-resistant standard plate count bacteria during water treatment. *Applied and Environmental Microbiology* **44:** 308-316.

Ashelfold, K. E., Weightman, A. J. and Fry, J. C. (2002). PRIMROSE: A computer program for generating and estimating the phylogenetic range of 16S rRNA oligonucleotide probes and primers in conjunction with the RDP-11 database. *Nucleic Acids Research* **30**: 3481-3489.

Azuonye, H. I. (1990). Bacteria in chlorinated pipe-borne water in Akoka, Lagos. B.Sc. Thesis. Department of Botany and Microbiology, University of Lagos, Akoka, Lagos.

Baggerman, W. (1981). A modified Rose Bengal medium for the enumeration of yeast and moulds from foods. *Applied Microbiology and Biotechnology* **12** (4): 242-247.

Bais, H. P., Fall, R. and Vivanco, J. M. (2004). Biocontrol of *Bacillus subtilis* against infection of *Arabidopsis* roots by *Pseudomonas syringae* is facilitated by biofilm formation and surfactin production. *Plant Physiology* **134:** 307-319.

Banks, E. C., Ferretti, C. E. and Shucard, D. W. (1997). Effects of low level lead exposure on cognitive function in children: A review of behavioural, neuropsychological and biological evidence. *Neurotoxicology* **18** (1): 237-282.

Barker, E. (1999). History painting/ painting history. *Art History* **22** (5): 760-769.

Barreiros, L., Nogales, B., Manaia, C. M., Ferreira, A. C. S., Pieper, D. H., Reis, M. A. and Nunes, O. C. (2003). A novel pathway for mineralization of the thiocarbamate herbicide molinate by a defined mixed culture. *Environmental Microbiology* **5:** 944-953.

Barrow, G. I. and Feltham, R. K. A. (1995). Cowan and Steel's Manual for Identification of Medical Bacteria, 3rd Ed. Cambridge: Cambridge University Press, pp. 94-128.

Bastos, F. S. C., Castro, P. M. L. and Jorge, R. F. (2003). Biological treatment of a contaminated gaseous emission from a paint and varnish plant- from laboratory studies to pilot scale operation. *Journal Chemical Technology and Biotechnology* **78** (11): 1201-1207.

Bauer, A. W. (1966). Antibiotic susceptibility testing by a standardized single disk Method. *American Journal of Clinical Pathology* **45:** 493-496.

Bellinger, D. C., Stiles, K. M. and Needleman, H. L. (1992). Low level lead exposure, intelligence and academic achievement: A long term follow-up study. *Pediatrics* **90** (6): 855-861.

Berkely, R.C.W., Logan, N. A., Shute, L. A. and Capey, A.G. (1984). Identification of *Bacillus* species. In: *Methods in Microbiology*. Bergan, T. (ed.). London: Academic press, pp. 291-328.

Berney, B. (1993). 'Round and round it goes: The epidemiology of childhood lead poisoning'. *Milbank Memorial Fund Quarterly* **71**: 3-39.

Bertram, M. S. and Dale, B. E. (1985). Enzymatic hydrolysis and recrystallization behaviour of initially amorphous cellulose. *Biotechnology and Bioengineering* **27:** 177.

Bester, E., Wolfaarrdt, G., Joubert, L., Garny, K. and Saftic, S. (2005). Planktonic-cell yield of a Pseudomonad biofilm. *Applied and Environmental Microbiology* **71** (12): 7792-7798.

Birnboim, A. C. and Doly, J. (1979). A rapid alkaline extraction procedure for screening recombinant plasmid DNA. *Nucliec Acids Research* **1:**1513-1523.

Blenkinsopp, S. A., Khoury, A. E. and Costerton, J. W. (1992). Electrical enhancement of biocide efficacy against *Pseudomonas aeruginosa* biofilms. *Applied and Environmental Microbiology* **58** (11): 3770-3773.

Bock, E., Sand, W., Meinicke, M., Wolters, B., Ahlers, S., Meyer, C. and Sameluck, E. (1988). Biologically induced corrosion of natural stones and strong contamination of monuments with nitrifying organisms. In: *Biodeterioration*. Houghton, D. R., Smith, R. N. and Eggins, H. O. W. (eds.). New York: Elsevier Applied Science. pp. 436-440.

Bock, E. and Sand, W. (1993). The microbiology of masonry biodeterioration. *Journal of Applied Bacteriology* **74:** 503-514.

Boles, B. R., Thoendel, M. and Singh, P. K. (2005). Rhamnolipids mediate detachment of *Pseudomonas aeruginosa* from biofilms. *Molecular Microbiology* **57:** 1210-1223.

Borneberg, C. G., Sundell, J., Bonini, S., Custovic, A., Malmberg, P., Skerfring, T., Sisguard, T. and Verhoeff, A. (2004). Dampness in buildings as a risk factor for health effects. Euro Expo: A multidisciplinary review of the literature (1998-2000) on dampness and mite exposure in buildings and health effects. *Indoor Air* **14:** 243-257.

Boyd, A. and Chakrabarty, A. M. (1994). Role of alginate lyase in cell detachment of *Pseudomonas aeruginosa*. *Applied and Environmental Microbiology* **60:** 2355-2359.

Briggs, M. A. (1980). Emulsion paint preservation: Factory practice and hygiene. *Paint Research Association Technical Report* TR/8/78 Teddington, UK.

Briones, A. and Raskin, L. (2003). Diversity and dynamics of microbial communities in engineered environments and their implications for process stability. *Current Opinions in Biotechnology* **14:** 270-276.

Brouwer, D. H., Semple, S., Marquart, J. and Cherries, J. W. (2001). A dermal model for spray painters, Part 1: Subjective exposure modeling of spray paint deposition. *Annals of Occupational Hygiene* **45** (1): 15-23.

Brouwer, D. H., Nettie, A., De Parter, J., Zomer, C., Marc, W., Lurunk, M. and Vantemmen, J. J. (2005). An experimental study to investigate the feasibility to classify paints according to neurotoxicological risks: Occupational air requirement (OAR) and indoor use of alkyd paints. *The Annals of Occupational Hygiene* **49:** 443-251.

Bruring, W. J. (1989). A strategy for the reduction of VOC-emissions . In: *Man and His Ecosystem. Proceedings of the World Clean Air Congress.* Brasser, L. J. and Mulder, W. C. (eds.). pp. 171-176.

Buchanan, R. E. and Gibbons, N. E. (1974).Bergey's Manual of Determinative Bacteriology. The Williams and Wilkins Company Baltimore., pp. 1246-1249.

Burns, J. M., Baghurst, P. A., Sawyer, M. G., McMichael, A. J. and Tongs, S. L. (1999). Life time low-level exposure to environmental lead and children's emotional and behavioural development at ages 11-13 years: The Port Pirie cohort study *American Journal of Epidemiology* **149** (8): 740-749.

Busscher, H. J. and Van der Mei, H. C. (2000). Initial microbial adhesion events: mechanisms and implications. Community structure and co-operation in biofilms. Cambridge University Press., Cambridge, United Kingdom.

Calnan, C. D. (1978). Turpentine in paint brush cleaner. *Contact Dermatitis* **4** (1): 57-58.

Camgna, S. P. and Colinart, S. (2003). The Egyptian green pigment: Its manufacturing process and links to Egyptian blue. *Archeometry* **45** (4): 637-658.

Caneva, G., D'urbano, M. S. and Salvadori, O. (1993). Test methods for comparative evaluation of biocide treatments. In: *Proceedings of the international Congress on "The conservation of stone and other materials."* Thiel, M. J. (ed.). Paris: Spon publishers, London., pp. 565-572.

Canfield, R. L., Henderson, C. R., Slechta, D. A., Cox, C., Jusko, T. A. and Lanphear, B. (2003). Intellactual impairment in children with blood lead concentration below 10 microgram per deciliter. *New England Journal of Medicine* **348:** 1517-26.

Castillejo-Rodriguez, A. M., Gimeno, R. M. G., Cosana, G. Z., Aleala, F. B. and Perez, M. R. R. (2002). Assessment of mathematical model for predicting *Staphylococcus aureus* growth in cooked meat products. *Journal of Food Protection* **65:** 659-665.

Chenoweth, M. B. and De Vrieze, J. D. (1980). Methyl-cellulose paint as a possible cause of heart failure. *British Medical Journal* **280** (62): 569.

Chepesiuk, R. (2001). This old house: Comparative hazards of paint removal techniques. *Environmental Health Perspectives* **109 (9):** A434.

Chiyo, M., Masashi, F. and Kiyoyo, T. (1988). Simple spectrophotometric determination of phosphate on the surface of vegetables on the market: Pre-concentration of molybdophosphate-malachite green aggregates on the membrane filter. *Japanese Journal of Toxicology and Environmental Health* **34** (2): 123-127.

Ciferri, O. (1999). Microbial degradation of paintings. *Applied and Environmental Microbiology* **65** (3): 879-885.

Clinical Laboratory Standards Institute. (2006). Performance standards for antimicrobial susceptibility testing. 16th Informational Supplement (M100-S16), Wayne (PA).

Coenye, T; Goris, J; Spiker, T; Vandamme, P. and Lipuma, J.J. (2002). Characterization of unusual bacteria isolated from respiratory secretions of cystic fibrosis patients and descriptions of *Inquilinas limosus*. *Journal of Clinical Microbiology* **40** (6): 2062-2069.

Collins, C. H. and Lyne, P. M. (1976). Microbiological methods, 4th Ed. Butterworths and Co. Ltd., pp. 520-525.

Conelly, M. B., Young, G. M. and Sloma, A. (2004). Extracellular proteolytic activity plays a central role in swarming motility in *Bacillus subtilis*. *Journal of Bacteriology* **186**: 4159-4167.

Connors, K. A., Amidon, G. L. and Kennon, L. (1973). Chemical stability of pharmaceuticals. In: *A handbook for pharmacists*. New York: John Wiley and Sons, Inc., pp. 8-119.

Corn, J. K. (1975). Historical perspective to a current controversy on the clinical spectrum of plumbism. *Mibank Memorial Fund Quarterly* **53:** 93-114.

Costerton, J. W., Lewandowski, Z., De Beer, D., Caldwell, D., Korber, D. and James, G. (1994). Biofilms, the customized microniche. *Journal of Bacteriology* **176:** 2137-2142.

Cowan, S. T. and Steele, K. J. (1970). Manual for the identification of medical bacteria. Cambridge: Cambridge University Press. pp. 50-171.

Dalgaard, I., Gudmundsdottir, B. K., Helgason, S., Hoie, S., Thoresen, O. F., Wichardt, U. P. and Wiklund, T. (1998). Identification of a typical *Aeromonas salmonicida*: inter-laboratory evaluation and harmonization of methods. *Journal of Applied Microbiology* **84**: 999-1006.

Dalgaard, P. (1995). Modelling of microbial activity and prediction of shelf life of packed fresh fish. *International Journal of Food Microbiology* **19:** 305-317.

Da Silva, R. H. (1963). The problem of the binding medium particularly in wall painting. *Archaeometry* **6** (1): 54-56.

Da Silva, V. Q. (2003). Microbial deterioration of paints. *Microbiologist* **4** (1): 43.

Danin, A., Gerson, R., Marton, K. and Garty, J. (1982). Patterns of limestone and dolomite weathering by lichens and blue-green algae and their paleoclimatic significance. *Paleogeographical Paleoclimatology* **37:** 221-23.

Davey, M. E., Caiazza, N. C. and O' Toole, G. A. (2003). Rhamnolipid surfactant production affects biofilm architecture in *Pseudomonas aeruginosa* PAO1. *Journal of Bacteriology* **185**: 1027-1036.

Dawes, E. A. (1969). Quantitative problems in biochemistry. 4th Edn. E. and S Livingstone Ltd., Edinburg and London., pp. 220-239.

De Angelis, M., Corsetti, A., Totsi, N., Rossi, J., Corbo, M. R. and Gobetti, M. (2001). Characterization of non-starter Lactic acid bacteria from Italian ewe cheeses on phenotypic, genotypic and cell wall protein analyses. *Applied and Environmental Microbiology* **67** (5): 2011-2020.

Denning, D. W. (1998). Invasive aspergillosis. *Clinical Infectous Diseases* **26:** 781-805.

Denyer, S. P. (1990). Mechanism of action of biocides. *International Biodeterioration* **26** (2): 4.

De Souza, A. and Gaylarde, C. C. (2002). Biodeterioration of varnished wood with and without biocide: Implications for standard test methods. *International Biodeterioration and Biodegradation* **49** (1): 21-25.

Dey, B. K., Hashim, M. A., Hassan, S. and Gupta, B. S. (2004). Microfilteration of water-based paint effluents. *Advances in Environmental Research* **8** (3): 455-466.

Dhawan, S. and Agrawal, O. P. (1986). Fungal flora of miniature paintings and lithographs. *International Biodeterioration Bulletin* **22** (2): 95-99.

Dietrich, K.N., Ris, M. D., Succop, P. A., Berger, O. G. and Bornschein, R.L. (2001). Early exposure to lead and juvenile delinquency *Neurotoxicology and Teratology* **23** (6): 511-518.

Domek, M. J., LeChevallier, M. W., Cameron, S. C. and McFeters, G. A. (1985). Evidence for the role of copper in the injury process of coliforms in drinking water. *Applied and Envioronmental Microbiology* **48**: 289-293.

Drasdo, N. (1999). Vision research: A practical guide to laboratory methods. *Journal of Neurology* **122** (10): 2000-2002.

Delgado, P., Porcel, J., Abril, I., Torres, N., Teran, A. and Zugasti, A. (2004). Potential dermal exposure during the painting process in car body repair shops. *Annals of Occupational Hygiene* **48** (3):229-236.

Dumont, M. P. (2000). Lead, mental health and social action: A view from the bridge. *Public Health Reports* **115:** 505-510.

Elmore, J. D., Kincaid, D. S., Komar, P. C. and Nielsen, J. E. (2002). Water-borne epoxy protective coating for metals. *Journal of Coatings Technology* **74** (931): 63-72.

Eguia, E. and Trueba, A. (2007). Application of marine biotechnology in the production of natural biocides for testing on environmentally innocuous antifouling coatings. *Journal of Coatings Technology and Research* **4** (2): 191-202.

Estlander, T., Raniemi, R. and Jolanki, R. (1984). Hand dermatitis in dental technicians. *Contact Dermatitis* **10:** 201-205.

European Chemicals Bureau (1996). Technical guidance document in support of the commission directive 93/67/EEC on risk assessment for new notified substances and the commission regulation (EC) 1488/94 on risk assessment for existing chemicals . European Chemicals Bureau, Ispra, Italy.

Faassen, A. V. and Borm, P. J. A. (1991). Composition and Health hazards of water-based construction paints: Results from a survey in the Netherlands. *Environmental Health Perspectives* **92:** 147-154.

Fassin, D. and Naude, A. J. (2004). Plumbism reinvented, childhood lead poisoning in France, 1985-1990. Public health then and now. *American Journal of Public Health* **94** (11): 1854-1863.

Fenske, R. (1993). Dermal exposure assessment techniques. *Annals of Occupational Hygiene* **37:** 687-706.

Fee, E. (1990). "Public health in practice: An early confrontation with the 'silent epidemic'of childhood lead paint poisoning". *Journal of the History of Medicine and Allied Sciences* **45:** 570-606.

Fields, S. (2003). New paint: no harm, no foul? *Environmental Health Perspectives* **111** (9): A457.

Fu, B., Taoukis, T. S. and Labuza, T. P. (1991). Predictive microbiology for monitoring spoilage of dairy products with time-temperature integrators. *Journal of Food Science* **56:** 1209-1215.

Fu, B. and Labuza, T. P. (1993). Shelf life prediction: theory and application. *Food Control* **4:** 125-133.

Galeano, B., Korff, E. and Nicholson, W. L. (2003). Inactivation of vegetative cells, but not spores, of *Bacillus anthracis, B. cereus,* and *B. subtilis* on stainless steel surfaces coated with an antimicrobial silver- and zinc-containing zeolite formulation. *Applied and Environmental Microbiology* **69** (7): 4329-4331.

Galla, E. A., Mascioli, R. C. and Bechara, R. S. (1981). Rim catalysis and coating interaction. *Journal of Elastomers and Plastics* **13** (4): 205-223.

Gavan, T. L., James, R. N., Berry, A. L., Fuchs, P. C., Gerlach, E. H., Matsem, J. M., Reller, L.B., Thornsberry, C. and Thrup, L. D. (1981). Quality control limits for ampicillin, carbecillin, mezlocillin and piperacillin disc diffusion susceptibility tests: A collaborative study. *Journal of Clinical Microbiology* **14:** 67-72.

Gaylarde, C., Silva, R. M. and Warsheid, T. (2003). Microbial impact on building materials. *Materials and Constructions* **36**: 342-352.

Gaylarde, C. and Gaylarde, P. M. (2005). A comparative study of the major microbial biomass of biofilms on exteriors of buildings in Europe and Latin America. *International Biodeterioration and Biodegradation* **55:** 131-139.

Geldreich, E. E. and Stevens, A. A. (1986). Workshop on coliform non-compliance nightmares : scenarios and action plans, In: *Proceedings of the American Water Works Association Water Quality Technology Conference. American Water Works Association, Denver.*, pp. 979-999.

Ghose, T. K. (1977). Cellulase biosynthesis and hydrolysis of cellulosic substances. *Advances in Biochemical Engineering* **6:** 25.

Ghosh, S., Mahapatra, N. R., Ramamurthy, T. and Banerjee, P. C. (2000). Plasmid curing from an acidophilic bacterium of the genus *Acidocella*. *FEMS Microbiology Letters* **183** (2): 271-274.

Gilbert, P., Evans, D. J. and Brown, M. R. W. (1993). Formation and dispersal of bacterial biofilms *in-vivo* and *in-situ*. *Journal of Applied Bacteriology Symposium Supplement* **74:** 67S-78S.

Gilbert and Mc Bain, A. J. (2001). Biofilms: their impact upon health and their recalcitrance towards biocides. *American Journal of Infection Control* **29:** 252-255.

Gillatt, A. C. (1992). Bacterial and fungal spoilage of water-borne formulations. *Additives* **10:** 387-393.

Gillgrass, T. J., Creanor, S. L., Foye, R. H. and Millett, D. F. (2001). Varnish or polymeric coatings for the prevention demineralization? An *ex-vivo* study. *Journal of Orthodontics* **28** (4): 291-295.

Goll, M. and Winters, H. (1974). Pseudomonads and their cellulases, mechanism of action and mode of detection. *Journal of Paint Technology* 46: 49-52.

Gonzalez, J. M. and Saiz- Jimenez, C. (2005). Application of molecular nucleic acid-based techniques for the study of microbial communities in monuments and art works. *International Microbiology* **8:** 189-194.

Gram, L. and Huss, H. (1996). Microbiological spoilage of fish and fish products. *International Journal of Food Microbiology* **33:** 121-137.

Grant, C. and Bravery, A. F. (1985). Laboratory evaluation of algicidal biocides for use on constructional materials and use of vermiculite bed technique to evaluate toxic washes, surface coatings and surface treatments. *International Biodeterioration* **21** (4): 285-293.

Grant, C., Wright, I. C., Springle, W. R. and Greenhalgh, M. (1993). Collaborative investigation of laboratory test methods for evaluation of the growth of pink yeasts on paint films. *International Biodeterioration and Biodegradation* **32:** 279-288.

Greenberg, G. (2001). Hazards associated with lead paints. *The New England Journal of Medicine* **344** (19): 1421-1426.

Greene, L. A. (2000). Healthy indoor painting practices. *Environmental Health Perspectives* **108** (12): A552.

Grisez, L., Ceusters, R. and Ollevier, F. (1991). The use of API 20E for the identification of *Vibrio anguillarum* and *V. ordalii*. *Journal of Fish Diseases* **14** (3): 359-365.

Gupta, A., Matsui, K. and Lo, J. F. (1999). Molecular basis for resistance to silver cations in *Salmonella*. *Nature Medicine* **5:** 183-188.

Ha, S. D., Ricke, S. C. and Carey, J. B. (1995). Comparisons of interior coatings to control surface microbiological contamination. *Journal of Applied Poultry Research* **4**: 7-12.

Hansen, M. K., Larsen, M. and Cohr, K. H. (1987). Water-borne paints. A review of their chemistry and toxicology and the results of determinations made during their use. *Scandinavian Journal of Work and Environmental Health* **13:** 473-485.

Harmon, S. A. and Baldwin, J. N. (1964). Nature of the determinant controlling penicillinase products in *Staphylococcus aureus*. *Journal of Bacteriology* **87**: 593-597.

Hein, I., Flekna, G., Wagner, M., Nockler, A. and Camper, A. K. (2006). Possible errors in the interpretation of ethidium bromide and picogreen DNA staining results fro from ethidium monazide-treated DNA. *Applied and Environmental Microbiology* **72** (10): 6860-6862.

Herbig, A. F. and Helmann, J. D. (2002). Metal ion uptake and oxidative stress. In: *Bacillus subtilis and its closest relatives: from genes to cells*. Sonnenshein, A. L., Hoda, J. A. and Lorsick, R. (eds.). Washington, D.C: ASM press, pp. 405-414.

Hext, P. M., Tomenson, J. A. and Thompson, P. (2005). Titanium dioxide: Inhalation, toxicology and epidemiology. *Annals of Occupational Hygiene* **49** (6): 461-472.

Hill, E. C. (1990). Biocide for the future. *International Biodeterioration* **26:** 281-285.

Hoet, P. H. M., Gilissen, L. P., Leyva, M. and Nemery, B. L. (1999). *In vitro* cytotoxicity of textile paint components linked to the 'Ardystil' Syndrom'. *Toxicological Sciences* **52:** 209-216.

Horder, J. (1989). Why I paint. *British Medical Journal* **229** (6715): 1577-1578.

Horvath, R. S., Brent, M. M. and Cropper, D. G. (1976). Paint deterioration as a result of the growth of *Aureobasidium pollulans* on wood. *Applied and Environmental Microbiology* **32** (4): 506-507.

Hsueh, Y., Somers, E. B., Lereclus, D. and Wong, A. C. L. (2006). Biofilm formation by *Bacillus cereus* is influenced by PlcR, a pleiotropic regulator. *Journal of Applied and Environmental Microbiology* **72** (7): 5089-5092.

Hueck Van Der Plas, H. E. (1968). The microbiological deterioration of porous building materials. *International Biodeterioration Bulletin* **4** (1): 11-28.

Ilori, M. O. (1998). Determination of molecular weights of plasmids. Ph.D Thesis. Department of Botany and Microbiology, University of Lagos, Lagos, Akoka.

Imperi, F., Caneva, G., Cancelliieri, L., Ricci, M. A., Sodo, A. and Visca, P. (2007).The bacterial aetiology of rosy discolouration of ancient wall paintings. *Journal of Environmental Microbiology* **11:** 146-292.

Inoue, M. and Koyano, M. (1991). Fungal contamination of oil paintings in Japan. *International Biodeterioration* **28:** 23-35.

Inoue, Y., Hoshino, M., Takahashi, H., Noguchi, T., Murata,Y., Kanzaki, H., Hamashima, A. and Sasatsu, M. (2002). Bactericidal activity of Ag zeolite mediated by reactive oxygen species under aerated conditions . *Journal of Inorganic Biochemistry* **92:** 37-42.

Iskandar, I. K. and Syers, J. K. (1972). Metal-complex formation by lichen compounds. *Journal of Soil Science* **23:** 255-265.

Jacobs, D. E., Mielke, H. and Pavur, N. (2003). The high cost of improper removal of lead-based paint from housing: a case report. *Environmental Health Perspectives* **111**(2): 185-186.

Jakabowski, J. A., Gyuris, J. and Simpson, S. L. (1983). Microbiology of modern coating system. *Journal of Coating Technology* **58** (707): 49-57.

Jefffries, P. (1986). Growth of *Beauvaria alba* on mural paintings in Canterbury Cathedral. *International Biodeterioration* **22** (1): 11-13.

Kaplan, E. and Shaull, R. S. (1961). Determination of lead in paint scrapings as an aid in the control of lead paint poisoning in young children. *American Journal of Public Health* **5** (1): 65-69.

Kaplan, J. B., Meyenhoefer, M. F. and Fine, D. H. (2003). Biofilm growth and detarchment of *Actinobacillus actinomycetemcomitans*. *Journal of Bacteriology* **185:** 1399-1404.

Kato, S., Haruta, S., Cui, Z. J., Masaharu, I. and Igarashi, Y. (2005). Stable coexistence of five bacterial strains as a cellulose-degrading community. *Journal of Applied and Environmental Microbiology* **71** (11): 7099-7106.

Katsuyama, A. M. and Strachan, J. P. (1980). Sanitary construction of buildings and equipment. In: *Principles of food processing sanitation*. Katsuyama, K. M. and Strachan, J. P. (eds.). Washington, D. C.: pp. 91-127.

Kelly, D. P. and Wood, A. P. (1998). Microbes of the sulphur cycle. In: *Techniques in microbial ecology*. Burlage, R. S., Atlas, R., Stahl, D., Geisey, G. and Sayler, G. (eds.). New York, USA: Oxford University Press, pp. 31-57.

Knowles, R. C. and Moore, T. D. (1979). Quality control of agar diffusion susceptibility tests. Data from the quality assurance service Microbiology program of the College of American Pathologists. *American Journal of Clinical Pathology* **72:** 365-371.

Kopchick, K. A. and Bormarito, R. (2006). Colour analysis of apparently achromatic automotive paints by visible microspectrophotometry. *Journal of Forensic Science* **51** (2): 340-343.

Koutsoumanis, K. (2001). Predictive modeling of the shelf life of fish under non-isothermal conditions. *Journal of Applied and Environmental Microbiology* **67** (4): 1821-1829.

Koutsoumais, K. and Nychas, G. J. E. (2001). Application of a systematic experimental procedure to develop a microbial model for rapid fish shelf life prediction. *International Journal of Food Microbiology* **60**: 174-184.

Kulkarni, R. S. and Kanekar, P. P. C. (1998). Effects of some curing agents on phenotypic stability in *Pseudomonas putida* degrading E-Capro lactam. *World Journal of Microbiology and Biotechnology* **14** (2): 255-257.

Lambert, R. J. W., Joynson, J. and Forbes, B. (2001). The relationships and susceptibilities of some industrial laboratory and clinical isolates of *Pseudomonas aeruginosa* to some antibiotics and biocides. *Journal of Applied Microbiology* **91** (6): 972-976.

Landrigan, P. J. (2000). Pediatric lead poisoning: Is there a threshold? *Public Health Reports* **115** (6): 530-531.

Lanphear, B. P., Dietrich, K. Auinger, P. and Cox, C. (2000). Cognitive deficits associated with blood lead concentrations 10 micrograms/dL in U.S. children and adolescents. *Public Health Reports* **115** (6): 521-529.

Latge, J. P. (1999). *Aspergillus fumigatus* and aspergillosis. *Clinical Microbiology Reviews* **12:** 310-350

LeChevallier, M. W., Cawthon, C. D. and Lee, R. G. (1988). Inactivation of biofilm bacteria. *Journal of Applied and Environmental Microbiology* **54** (10): 2492-2499.

Lee, S. F., Forseberg, C. W. and Gibbins, L. N. (1985). Cellulolytic activity of *Clostridium acetobutylicum*. *Applied and Environmental Microbiology* **50** (2): 220-228.

Leeh, K. and Brent, R. (1987). Minipreps of plasmid DNA. In: *Current Protocols in Molecular Biology*. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D. Seidman, J. G., Smith, J. A. and Struhl (eds.). New York: John Wiley and Sons, Inc., pp. 161-164.

Lewendon, G., Kiinra, S., Nelder, R. and Cronin, T. (2001). Should children with developmental and behavioural problems be routinely screened for lead? *Archives of Disease in Childhood* **85** (4): 286-288.

Lequette, Y. and Greenberg, E. P. (2005). Timing and localization rhamnolipid synthesis gene expression in *Pseudomonas aeruginosa* biofilms. *Journal of Bacteriology*. **187:** 37-44.

Leznicka, S. (1992). Antimicrobial protection of stone monuments with P-hydroxybenzoic acid esters and silicone resins. In: *Proceedings of the 7th International Congress on Deterioration and Conservation of Stone*. L. D. Rodriguez, L. D., Henri, J. and Telmo, F. J. (eds.). pp. 481-490.

Linko, M. (1977). An evaluation of enzymatic hydrolysis of cellulosic substances. *Advances in Biochemical Engineering* **5:** 39.

Loor, J. J., Ude, K., Ferlay, T., Chilliard and Doreau, M. (2004). Bihydrogenation, duodenal flow and intestinal digestibility of trans fatty acids and conjugated linoleic acids in response to dietary forage: Concentrate ratio and linseed oil in dairy cows. *Journal of Dairy Science* **87:** 2472-2485.

Lowther, E. D. and Moser, R. H. (1984). Detecting and eliminating coliform regrowth. In: *Proceedings of the American Water Works Association. Water Quality Technology Conference*. American Water Works Association, Denver., pp. 323-336.

Maillard, J. Y. (2000). Bacterial target sites for biocide action. *Journal of Applied Microbiology* **92:** 16S-27S.

Marino, P. E., Landrigan, P. J., Graef, J., Nussbaum, A., Bayan, G. and Boch, S. (1990). A case report of lead paint poisoning during renovation of a victorian farmhouse. *American Journal of Public Health* **80** (10): 1183-1185.

Martin, R. S., Gates, W. H., Tobin, R. S., Grantham, D., Wolfe, P. and Forestall, P. (1982). Factors affecting coliform bacteria growth in distribution systems. *Journal of the American Water Works Association* **74:** 34-39.

Martland, H. S. and Humphries, R. E. (2007). Osteogenic sarcoma in dial painters using luminous paints. *Cancer Journal for Clinicians*

Mathee, A., Rollin, H., Levin, J. and Naik, I. (2007). Lead in paints: Three decades later and still a hazard for African Children. *Environmental Health Perspectives* **1151**(31): 321-322.

May, J. W., Houghton, R. H. and Perret, C. T. (1964). The effect of growth at elevated temperatures on some heritable properties of *Staphylococcus aureus*. *Journal of General Microbiology* **37:** 157- 169.

McDonald, K. and Sun, D. W. (1999). Predictive food microbiology for the meat industry: a review. *International Journal of Food Microbiology* **52:** 1-27.

McGee, J. O. and O'Malley, B. P. (1979). Methyl-cellulose paint possibly causing heart failure. *British Medical Journal* **3:** 1113-1114.

McMkeen, T. A. and Ross, T. (2002). Predictive microbiology: Providing a knowledge-based framework for change management. *International Journal of Food Microbiology* **78:** 133-153.

Menne, T. and Christophersen, J. (1985). Epidemiology of allergic contact sensitization. *Current Problems in Dermatology* **14:** 1-30.

Meyers, J. A., Sanchez, D., Elwell, L. and Pand-Falkow, S. (1976). Simple agarose gel electrophoresis of plasmid DNA. *Journal of Bacteriology* **127:** 1529-1537.

Miller, G. L. (1959). Use of Dinitrosalicylic acid reagent for determination of reducing sugars. *Journal of Analytical Chemistry* **31:** 426-428.

Miller, W.G. (1973). Incidence of microbial contamination of emulsion paints during the manufacturing process. *JOCCA*. **56** (7): 307-310.

Mireles, J. R., Toguchi, A. and Hashey, R. M. (2001). *Salmonella enterica ser ovar typhimurium* swarming mutants with altered biofilm-forming abilities. *Journal of Bacteriology* **183:** 5848-5854.

Mirsha,A. K., Kamal, J. K. and Garg, K. L. (1995). Role of higher plants in deterioration of historic buildings. *The Science of the Total Environment.* **167**: 375-392.

Moya, C., Anto, J. M. and Newman Tailor, A. J. (1994). Outbreak of organizing pneumonia in textile printing sprayers. *Lancet* **344:** 498-502.

Mulhausen, J. R. and Damiano, J. (1998). A strategy for assessing and managing occupational exposures. Appendix 11: Dermal exposure assessments. *American Industrial Hygiene Association, Fairfax, VA.*

Muller, T., Urlich, A., Oh, E. M. and Muller, M. (2001). Identification of plant-associated *enterococci. Journal of Applied Microbiology* **91** (2): 268-278.

Murray, R. G. E., Doetsch, R. N. and Robinow, C. F. (1994). Determinative and cytological light microscopy. *In: Methods for General and Molecular Bacteria*. Phillip, G., Murray, R. G. E., Wood, W. A. and Krieg, N. R. (eds.). Washington, D.C: ASM press., pp. 22-41.

NCCLS (1997). Methods for dilution in antimicrobial susceptibility tests for bacteria that grow aerobically. Document No. M7-A4. NCCLS, Wayne, Pa.

Needleman, H. L. (1989). The persistent threat of Lead: A singular opportunity. *American Journal of Public Health* **79:** 643-645.

Needleman, H. L. (1998). Childhood lead poisoning: The promise and abandonment of primary prevention. *American Journal of Public Health* **88:** 1871-1877.

Neter, J., Wasserman, W. and Kutner, M. H. (1983). General multiple regressions. In: *Applied Linear Regression Models*. Richard, D. (ed.). Homewood, Illinois: Irwin, Inc., pp. 226-294.

Nikovskaya, G. N., Gordienko, A. S. and Globa, L. I. (1989). Hydrophilic-hydrophobic properties of microorganisms under different growth conditions. *Mikrobiologiya* **58:** 448-457.

Novick, R. P. (1980). Plasmids. *Scientific American* **243** (16): 77-90.

Nugari, M. P., Reacini M. and Roccardi, C. (1993). Contamination of mural paintings by indoor airborne fungal spores. *Aerobiologia* **9:** 131-139.

Nwachukwu, S. C. U. and Akpata, T. V. I. (2003). Isolation of microorganisms by spread plate technique. In: *Principles of Quantitative Microbiology*. University of Lagos Press. pp. 3-6.

Nwachukwu, S. C. U. and Ugoji, E. O. (1995). Impacts of crude petroleum spills on microbial communities of tropical soils. *International Journal of Ecology and Environmental Science* **21:** 169-176.

Ogbulie, J. N. (2004). Microbial deterioration of surface paint coatings. *Global Journal of Pure and Applied Sciences* **10** (4): 485-490.

Ohwoavworhua, F. O. and Adelakun, T. A. (2005). Phosphoric acid-mediated depolymerization and decrystallization of cellulose obtained from corn cob: preparation of low crystallinity cellulose and some physicochemical properties. *Tropical Journal of Pharmaceutical Research* **4** (2): 509-516.

O'May, G. A., Alisson, D. G. and Gilbert, A. (2004). A rapid method for the evaluation of both extrinsic and intrinsic contamination and resulting spoilage of water-in-oil-emulsions. *Journal of Applied Microbiology* **96** (5): 1124.

Onabanjo, A. A. (1977). Bacteriological quality of pipe-borne water at Agbara Housing Estate, Ogun State. M.Sc. Thesis. Department of Botany and Microbiology, University of Lagos.

O' Neill, T. B. (1988). Succession and interrelationships of microorganisms on painted surfaces. *Journal of Coating Technology* **58**: 51-56.

Opperman, A. A. and Gull, M. (1984). Presence and effects of anaerobic bacteria in water-based paints. *Journal of Coating Technology* **56:** 51-57.

Ortega-Calvo, J. J., Hermandez- Marine, M., Saiz-Jimenez, C. (1991). Biodeterioration of building materials by cyanobacteria and algae. *International Biodeterioration* **2** (28): 165-185.

Ortega-Calvo, J. J., Hermandez-Marine, M., Saiz-Jimenez, C. (1993). Cyanobacteria and algae on historic buildings and monuments. In: *Biodeterioration of cultural heritage*. Garg, K. L., Garg, N. and Mukerji, K. G. (eds.). Calcutta: Naya prokash publ., pp. 173-203.

O'Toole, G. A. and Kotler, R. (1998). The initiation of biofilm formation in *Pseudomonas fluorescens* WCS365 proceeds via multiple, convergent signaling pathways: a genetic analysis. *Molecular Microbiology* **28:** 449-461.

O'Toole, G. A., Kaplan, H. B. and Kotler, R. (2000). Biofilm formation as microbial developments. *Annual Review of Microbiology* **54:** 49-79

Panaccione, D. G. and Coyle, C. M. (2005). Abundant respirable ergot alkaloids from the common air-borne fungus. *Aspergillus fumigatus. Applied and Environmental Microbiology* **71** (6): 3106-3111.

Pantazidou, A. and Theoulakis, P. (1997). Cyanophytes and associated flora at the neoclassical place of St. George and Michael in Corfu (Greece). Aspect of cleaning procedures. In: *Proceedings of IVth international symposium on the conservation of monuments in the Mediterranean basin, Rhodes*. pp. 35-36.

Para, J., Da Silva, F. H., Stroink, H. and Kalitzen, S. (2007). Is colour modulation an independent factor in human visual photosensitivity? *A Journal of Neurology* **130** (6): 1679-1689.

Pernier, C., Grosgogeat, B., Ponsonnet, L., Benay, G. and Lissac, M. (2005). Influence of autoclave sterilization on the surface parameters and mechanical properties of six orthodontic wires. *European Journal of Orthodontics* **27:** 72-81.

Peters, W. C., Harris, G., Miller, G. and Petro, J. (2000). Tailoring thin–film/lacquer coatings for space applications. *High performance polymers* **12:**105-112.

Petersen, G. D., Dahlllof, J. and Nielsen, L. P. (2004). Effects of zinc pyrithione and copper pyrithione on microbial community function and structure in sediments. *Environmental Toxicological Chemistry* **23** (4): 921-928.

Petushkova, J. P., Lyalikova, N. N. and Nichiporov, F. G. (1988). Effect of ionizing radiation on monument deteriorating organisms. *Journal of Radioanalytical and Nuclear Chemistry* **125** (2): 367-371.

Pietrini, A. M., Ricci, S., Bartolini, M. and Giuliani, M. R. A. (1985). A reddish colour alteration caused by algae on stone works preliminary study. In: *Proceedings of VIth international congress on deterioration and conservation of stone*. Lausanne: Press polythechniques, pp. 65-66.

Pinon, A., Zwietering, M., Perrier, L., Membre, S., Leporq, B., Metter, E., Thault, D., Coroller, L., Straw, V. and Vialette, M. (2004). Determination and validation of experimental protocols for use of cardinal models for prediction of microorganisms growth in food products. *Applied and Environmental Microbiology* **70** (2): 1081-1087.

Porterfield, R. I. and Capone, J. J. (1984). Application of kinetic models and Arrhenius methods to product stability evaluation. *Medical Device and Diagnostic Industry* **2:** 45-50.

Pratt, L. A. and Kotler, R. (1999). Genetic analysis of bacterial biofilm formation. Current *Opinions in Microbiology* **2:** 598-603.

Prescott, L.M., Harley, J. P. and Klein, D. A. (2005). Microbial nutrition, growth and control. In: *Microbiology*. 6th Ed. New York, U.S.A: McGraw-Hill publication., pp. 109-133.

Rabin, R. (1989). Warnings unheeded: A history of child lead poisoning. *American Journal of Public Health* **79** (12): 1668-1674.

Rammohan, R. M. V. and Yassen, M. (2003). Determination of intrinsic viscosity by single specific viscosity measurements. *Journal of Applied Polymer Science* **31** (8): 2501-2508.

Ramsbothan, O. (2000). Reflection on UN Post-setlement peace building. *Peace Keeping and Conflict Resolution*. **17** (1): 169-189.

Reilly, J. A. (1991). Celluloid objects: Their chemistry and preservation. *Journal of the American Institute for Conservation* **30** (2): 145-162.

Rieck, P., Peters, D., Hartmann, C. and Coutois, Y. (1993). A new rapid colorimetric assay for quantitative determination of cellular proliferation, growth inhibition and viability. *Methods in Soil Science* **15** (1): 37-41.

Ris, M. D., Dietrich, K. N., Succop, P. and Hill, T. (2000). Early low lead exposure and antisocial behaviour in adolescence. *Journal of International Neuropsychological Society* **6:** 385.

Riesenman, P. J. and Nicholson, W. L. (2000). Role of the spore coat layers in *Bacillus subtilis* spore resistance to hydrogen peroxide, artificial UV-C, UV-B, and solar UV radiation. *Applied and Environmental Microbiology* **66:** 620- 626.

Ryu, D. D. Y. and Mandels, M. (1980). Cellulases: biosynthesis and applications. *Enzyme Microbiology and Technology* **2:** 91-102.

Roff, M., Bagon, D. A., Chambers, H., Dilworth, E. M. and Warren, N. (2004). Dermal exposure to dry powder spray paints using PXRF and the method of dirichlet tessellations. *Annals of Occupational Hygiene* **48** (3): 257-265.

Rosenberg, M. and Doyle, R. J. (1990). Microbial cell surface hydrophobicity: History, measurement and significance. In: *Microbial Cell Surface Hydrophobicity*. Doyle, R. J. and Rosenberg, M. (ed.). Washington D.C. American Society for Microbiology, pp. 1-3.

Ross, T. (1996). Indices for performance evaluation of predictive models in food microbiology. *Journal of Applied Microbiology* **81:** 501-508.

Ross, T. and McMkeen, T. A. (2003). Modelling microbial growth within food safety risk assessments. *Risk Analysis* **23** (1): 179-197.

Russell, A. D. (1996). Activity of biocides against mycobacteria. *Journal of Applied Bacteriology* **81**: 875-891.

Russell, A. D. (2000). Do biocides select for antibiotic resistance? *Journal of Pharmacy and Pharmacology* **52**: 227-233.

Russell, A. D. (2003). Similarities and differences in the response of microorganisms to biocides. *Journal of Antimicrobial Chemotherapy* **52** (5): 750-763.

Russell, A. D. and Chopra, I. (1996). Understanding antibacterial action and resistance. 2nd Ed. Ellis Horwood, Chichester, UK. pp. 226-237.

Russell, A. D. and Furr, J. R. (1996). Biocides: mechanism of antifungal action and fungal resistance. *Science Progress* **79:** 27-48.

Russell, A. D. and Mc Donelli, G. (2000). Concentration: a major factor in studying biocidal action. *Journal of Hospital Infection* **44**: 1-3.

Saad, R. R. (1992). Fungi of biodeteriorated paint films and their cellulolytic activity. *Zentralbart fur Mikrobiologie* **147:** 427-430.

Sabty-Daily, R. A., Hinds, W. C. and Froines, J. R. (2005). Size distribution of chromate paint aerosol generated in a bench-scale spray booth. *Annals of Occupational Hygiene* **49** (1): 33-45.

Sahin, N., Gokler, I. and Abdurrahman, U. T. (2002). Isolation, characterization and numerical taxonomy of novel oxalate-oxidizing bacteria. *The Journal of Microbiology* **(4)** 2: 109-118.

Salter, M. A., Ratkowsky, D., Ross, T. and McMckeen, F. A. (2000). Modelling the combined temperature and salt (NaCl) limits for growth of a pathogenic *Escherichia coli* strains using non-linear logistic regression. *International Journal of Food Microbiology* **61:** 159-167.

Salvadori, O. and Nugari, M. P. (1988). The effect of microbial growth on synthetic polymers used in works of art. In: *Biodeterioration*. Houghton, D. R., Smith, R. N. and Eggins, H. O. W. (eds.). London: Elsevier Applied Science. pp. 424-427.

Sanborn, M. D., Abelsohn, A., Campbell, M., Weir, E. (2002). Identifying and managing adverse environmental health effects. *Canadian Medical Association Journal* **166** (11): 1287-1292.

Satcher, D. S. (2000). The surgeon general on the continuing tragedy of childhood lead poisoning. *Public Health Reports* **115** (6): 579-580.

Sauer, K., Camper, A. K., Ehrlich, G. D., Costerton, J. W. and Davies, D. G. (2002). *Pseudomonas aeruginosa* displays multiple phenotypes during development as a biofilm. *Journal of Bacteriology* **184:** 1140-1154.

Schlecht, P. C., Groff, J. H., Feng, A. and Ruiguang, S. (1996). Laboratory and analytical method performance of lead measurements in paint chips, soils and dusts. *American Industrial Hygiene Association Journal* **59:** 1035-1043.

Schmidt, K. and Bouma, J. (1992). Estimating shelf-life of cottage cheese using hazard analysis. *Journal of Dairy Science* **75:** 2922-2927.

Schwartz, J. (1994). Low-level lead exposure and children's IQ: A meta-analysis and search for a threshold. *Environmental Research* **65** (1): 42-55.

Shadomy, S. and Kirchoff, C. (1972). *In vitro* susceptibility testing with tobramycin. *Antimicrobial Agents and Chemotherapy* **1** (15): 412-416.

Sharp, P. A., Cohen, S. N. and Davidson, N. (1973). Electron microscopy heteroduplex studies of sequence relations among plasmids of *Escherichia coli* 11. Structure of Drug Resistance(R) Factors and F factors. *Journal of Molecular Biology* **73:** 235-236.

Silver, S. and Misra, T. K. (1988). Plasmid-mediated heavy metal resistance. *Annual Review of Microbiology* **42:** 717-743.

Skinner, G. E., Larkin, J. W. and Rhodehamel, R. J. (1994). Mathematical modeling of microbial growth: a review. *Journal of Food safety* **14** (3): 175-217.

Smith, G. (1969). Classification and identification of fungi. In: *Introduction to industrial mycology*, 6th Ed. Edward Arnold, London., pp: 56-64.

Smith, R. D. (1986). Background, use and benefits of blast freezers in the prevention and experimentation of insects. In: *Biodeterioration*. Liewellyn, C. C. and O'Rear, C. E. (eds.). Cab International Mycological Institute, England: pp. 374-379.

Smith, N. R., Yu, Z. and Mohn, W. W. (2003). Stability of the bacterial community in a pulp mill effluent treatment system during normal operation and a system shutdown. *Water Research* **37:** 4873-4884.

Sonstein, S. A. and Baldwin, J. A. (1972). Loss of the penicillinase plasmid after treatment of *Staphylococcus aureus* with sodium dodecyl sulphate. *Journal of Bacteriology* **109** (1): 262-265.

Spoering, A. L. and Lewis, K. (2001). Biofilms and planktonic cells of *Pseudomonas aeruginosa* have similar resistance to killing by antimicrobials. *Journal of Bacteriology* **183**: 6746-6751.

Spurgeon, A. (2006). Watching paints dry: Organic solvent syndrome in late twentieth-century Britain. *Medical History* **50:** 167-188.

Stafilov, T. and Zendelovska, D. (2002). Determination of trace elements in iron minerals by Atomic Absorption Spectrophotometry. *Turkish Journal of Chemistry* **26**: 271-280.

Standal, R., Iversen, T. G., Coucheron, D. H., Fjaerink, E., Blatny, J. M. and Valla, S. (1993). A new gene required for cellulose production and a gene encoding cellulolytic activity in *Acetobacter xylinum*. *Journal of Bacteriology* **176** (3): 665-672.

Stickler, D. J. (2002). Susceptibility of antibiotic-resistant Gram-negative bacteria to biocides: a perspective from the study of catheter biofilms. *Journal of Applied Microbiology Symposium Supplement* **92:** 163S-170S.

Strzelcyzk, A. B. (1981). Paintings and sculptures. In: *Microbial Biodegradation.* Rose, A. H. (ed.). London: Academic press. pp. 203-234.

Takai, K., Ohtsuka, T., Senda, Y., Nakao, M., Yamamoto, K., Matsuoka-Junji, J. and Hirai, Y. (2002). Antibacterial properties of antimicrobial-finished textile products. *Microbial Immunology* **46:** 75-81.

Tang, F., Kowalke, G., Meyer, A. E., Bright, F. V., Callow, M. E., Wendt, D. and Delty, M. R. (2005). Hybrid xergel films as novel coatings for antifouling and fouling release. *Biofouling* **21** (1): 59-71.

Taoukis, P. S., Koutsoumanis, K. and Nychas, G. J. E. (1999). Use of time-temperature integrators and predictive modeling for shelf life control of chilled fish under dynamic storage conditions. *International Journal of Food Microbiology* **53**: 21-31.

Tayler, S. and May, E. (1994). The investigation of biocides against bacteria isolated from stone and their effectiveness against *in situ* population. *Material Und Organismes* **28:** 265-278.

Tellsman, S., Cvitkovic, P., Jurasvoic, J., Pizent, A., Gavella, M. and Rocic, B. (2000). Semen quality and reproductive endocrine function in relation to biomarkers of lead, cadmium, zinc and copper in men. *Enviromental Health Perspectives* **108** (1): 45-53.

Thayer, D. W. (1978). Carboxymethyl cellulase produced by facultative bacteria from the hind gut of the termite *Reticulitermes hesperus*. *Journal of General Microbiology* **106**: 13-18.

Theron, J. and Cloete, T. E. (2004). Molecular techniques for determining microbial diversity and community structure in natural environments. *Critical Review of Microbiology* **26**: 37-57.

Tiano, P. (1994). Biodeterioration of stone monuments. In: *Biodeterioration of Cultural Heritage*. Garg, K. L., Garg, N. and Mukerfi, K. G. (eds.). Calcutta: Naya Prokash Publ. pp. 301-321.

Tiano, P., Acolla, P. and Tomaselli, L. (1995). The effectiveness of some biocides against algal biodeterioration. In: *Proceedings of the International Congress on "The conservation of stone and other materials". Paris.* Thiel, M. J. (ed.). London: Spon. Publ. pp. 573-580.

Tiano, P. and Caneva, G. (1987). Procedure for the elimination of vegetal biodeteriogens from stone monuments. In: *Preprints of 8th Triennial meeting ICOM, Sydney.* pp. 1201-1205.

Tomas, J. M. and Kay, W. W. (1984). A simple and rapid method for the elimination of plasmids from enteric bacteria. *Current Microbiology* **11** (3): 155-157.

Toothill, I. E., Best, D. J. and Seal, K. J. (1993). Detection of cellulolytic enzymes in water-borne paint. *International Biodeterioration and Biodegradation* **31:** 115-128.

Tortorano, A. M., Viviani, M. A., Biraghi, E., Rigoni, A. L., Prigitano, A. and Grillot, R. (2004). *In vitro* testing of fungicidal activity of biocides against *Aspergillus fumigatus*. *Journal of Medical Microbiology* **54:** 955-957.

Torvinen, E., Meklin, T., Torkko, P., Suomulainen, S., Reiman, M. Katila, M. L., Paulin, L. and Nevalainen, A. (2006). Mycobacteria and fungi in moisture-damaged building materials. *Applied and Environmental Microbiology* **27** (10): 6822-6824.

Trees, E. H., Sky, H., Mokkila, M., Kinunen, A., Lindstrom, M., Lahtenmaki, L., Ahrencinen, R. and Korkeala, H. (2000). Safety evaluation of sous vide-processed products with respect to non proteolytic *Clostridium botulinum* by use of challenge studies and predictive microbiological models. *Applied and Environmental Microbiology* **66** (1): 223-229.

Van Hemmen, J. J. and Brouwer, D. H. (1995). Assessment of dermal exposure to chemicals. *Science of the Total Environment* **168:** 131-141.

Van Vliet, C. (1989). Organic solvent exposure and neuropsychiatric disorders. Results from an epidemiological study among Dutch painters and construction workers. Thesis, State University, Limburg, Maastricht, The Netherlands.

Wakefield, J. (2002). The lead effect. *Environmental Health Perspectives* **110:** 1-3.

Walls, L. and Scott, V. N. (1996). Validation of predictive mathematical models describing the growth of *Escherichia coli* 0157: H7 in raw ground beef. *Journal of Food Protection* **59:** 1331-1335.

Weiss, M. S., Abele, U., Weckesser, J., Welte, W., Schiltz, E. and Schulz, G. E. (1991). Molecular architechture and electrostatic properties of a bacterial porin. *Science* **254:** 1627-1630.

Weissberg, P. L. and Green, I. D. (1979). Methyl-cellulose paint possibly causing heart failure. *British Medical Journal* **2 (189):** 1113-1114.

Whelan, A. and Regan, F. (2006). Antifouling strategies for marine and riverine sensors. *Journal of Environmental Monitoring* **8:** 880-886.

Winters, H., Isquith, I. R. and Goll, M. (1975). A study of the ecological succession in Biodeterioration of vinyl acrylic paint film. *Developments in Industrial Microbiology* **17:** 167-71.

Wolf, P. A. and Riley, W. H. (1965). Fungistatic performance of 10, 10'-oxybisphenoxarsine in exterior latex and asphalt coatings. *Applied Microbiology* **13** (1): 28-33.

Woods, W. B. (1982). Prevention of microbial spoilage of latex paints. *Journal of Waterborne Coatings* **2:** 20-27.

Young, M. E., Wakefield, R, Urquhart., D. M. C., Nicholson, K. and Tonge, K. (1995). Assessment in a field setting of the efficacy of various biocides on sandstones. In: *International Colloquim on methods of evaluating products for the conservation of porous building materials in monuments, Rome* ICCROM. pp. 93-99.

APPENDIX 1

Table 1: Mean changes in microbial population density of fresh paint sample PS-1

Time (Month)	Total Bacterial Count		Total Coliform Count		Total Fungal Count	
	Cfu/ml	Log No	Cfu/ml	Log No	Cfu/ml	Log No
0	1.8×10^1	1.27	1.0×10^1	1.0	1.0×10^1	1.0
1	1.7×10^1	1.23	1.5×10^1	1.2	1.5×10^1	1.2
2	1.9×10^1	1.28	1.8×10^1	1.3	2.1×10^1	1.3
3	2.1×10^1	1.32	3.0×10^1	1.5	2.7×10^1	1.4
4	2.3×10^1	1.36	6.5×10^1	1.8	3.7×10^1	1.6
5	6.3×10^1	1.8	8.9×10^1	2.0	4.0×10^1	2.0
6	2.0×10^2	2.3	1.8×10^2	2.3	2.5×10^2	2.4
7	3.6×10^2	2.6	3.6×10^2	2.6	3.6×10^2	2.6
8	5.2×10^2	3.0	1.1×10^3	3.0	6.5×10^2	2.9
9	8.2×10^3	3.9	2.5×10^3	3.4	1.0×10^3	3.0
10	2.1x 104	4.3	3.6×10^3	3.6	1.8×10^3	3.2

Table 2: Mean changes in microbial population density of fresh paint sample PS-2

Time (Month)	Total Bacterial Count		Total Coliform Count		Total Fungal Count	
	Cfu/ml	Log No	Cfu/ml	Log No	Cfu/ml	Log No
0	1.6×10^1	1.20	1.0×10^1	1.0	1.0×10^1	1.0
1	1.7×10^1	1.23	1.2×10^1	1.1	1.2×10^1	1.1
2	1.5×10^1	1.18	1.5×10^1	1.2	1.5×10^1	1.2
3	2.3×10^1	1.36	3.7×10^1	1.6	2.3×10^1	1.4
4	3.2×10^1	1.51	6.5×10^1	1.8	4.2×10^1	1.6
5	6.7×10^1	1.83	1.6×10^2	2.2	8.1×10^1	1.9
6	1.8×10^2	2.31	3.6×10^2	2.6	1.4×10^2	2.1
7	1.1×10^3	3.0	1.0×10^3	3.0	2.5×10^2	2.4
8	4.9×10^3	3.7	2.3×10^3	3.4	3.6×10^2	2.6
9	2.2×10^4	4.3	3.6×10^3	3.5	1.0×10^3	3.0
10	3.0×10^4	4.5	3.9×10^3	36	1.8×10^3	3.2

Table 3: Mean changes in microbial population density of fresh paint sample PS-3

Times (Month)	Total Bacterial Count		Total Coliform Count		Total Fungal Count	
	Cfu/ml	Log No	Cfu/ml	Log No	Cfu/ml	Log No
0	2.4×10^1	1.38	1.2×10^1	1.11	1.0×10^1	1.0
1	2.3×10^1	1.41	2.0×10^1	1.3	1.2×10^1	1.1
2	2.3×10^1	1.40	2.3×10^1	1.4	2.0×10^1	1.3
3	2.5×10^1	1.42	4.0×10^1	1.7	2.3×10^1	1.4
4	2.7×10^1	1.44	1.4×10^2	2.1	4.5×10^1	1.7
5	3.7×10^1	1.63	2.5×10^2	2.4	4.0×10^1	2.0
6	9.0×10^1	1.95	3.6×10^2	2.6	1.6×10^2	2.2
7	2.5×10^2	2.42	1.0×10^3	3.0	3.4×10^2	2.5
8	1.1×10^3	3.0	1.8×10^3	3.2	6.5×10^2	2.9
9	4.9×10^3	3.7	3.5×10^3	3.5	1.3×10^3	3.1
10	2.2×10^4	4.3	4.9×10^3	3.7	2.1×10^3	3.3

Table 4: Mean changes in microbial population density of fresh paint sample PS-4

Time (Month)	Total Bacterial Count		Total Coliform Count		Total Fungal Count	
	Cfu/ml	Log No	Cfu/ml	Log No	Cfu/ml	Log No
0	2.6×10^1	1.45	1.2×10^1	1.1	1.0×10^1	1.0
1	3.0×10^1	1.47	1.5×10^1	1.2	1.5×10^1	1.2
2	3.1×10^1	1.49	2.3×10^1	1.4	2.3×10^1	1.4
3	3.2×10^1	1.50	3.8×10^1	1.6	3.7×10^1	1.6
4	3.2×10^1	1.50	8.2×10^1	1.9	4.0×10^1	2.0
5	5.5×10^1	1.74	1.4×10^2	2.1	1.8×10^2	2.3
6	8.7×10^1	1.94	2.4×10^2	2.4	3.6×10^2	2.6
7	2.1×10^2	2.32	6.2×10^2	2.8	1.0×10^3	3.0
8	6.3×10^2	2.79	1.0×10^3	3.0	1.8×10^3	3.2
9	1.8×10^3	3.20	2.4×10^3	3.4	2.3×10^3	3.4
10	1.5×10^4	4.17	3.9×10^3	3.6	4.9×10^3	3.7

Table 5: Mean changes in microbial population density of fresh paint sample PS-5

Time (Month)	Total Bacterial Count		Total Coliform Count		Total Fungal Count	
	Cfu/ml	Log No	Cfu/ml	Log No	Cfu/ml	Log No
0	3.2×10^1	1.51	1.2×10^1	1.1	1.0×10^1	1.0
1	3.1×10^1	1.50	1.5×10^1	1.2	1.5×10^1	1.2
2	3.6×10^1	1.55	2.1×10^1	1.3	2.1×10^1	1.3
3	3.5×10^1	1.54	3.0×10^1	1.5	4.2×10^1	1.6
4	3.7×10^1	1.56	6.5×10^1	1.8	8.1×10^1	1.9
5	8.1×10^1	1.94	1.6×10^2	2.2	1.4×10^2	2.1
6	1.8 x 102	2.30	2.9×10^2	2.5	2.9×10^2	2.5
7	3.1×10^3	3.49	6.3×10^2	2.8	3.6×10^2	2.6
8	1.6×10^4	4.20	1.8×10^3	3.2	1.0×10^3	3.0
9	8.9×10^4	4.95	3.7×10^3	3.6	1.8×10^3	3.2
10	2.3×10^5	5.36	5.7×10^3	3.8	2.1×10^3	3.3

Table 6: Mean changes in microbial population density of fresh paint sample PS-6

Time (Month)	Total Bacterial Count		Total Coliform Count		Total Fungal Count	
	Cfu/ml	Log No	Cfu/ml	Log No	Cfu/ml	Log No
0	1.7×10^1	1.23	1.2×10^1	1.1	1.0×10^1	1.0
1	1.6×10^1	1.20	1.5×10^1	1.2	1.5×10^1	1.2
2	1.7×10^1	1.23	2.3×10^1	1.4	2.8×10^1	1.4
3	1.9×10^1	1.28	3.3×10^1	1.5	3.0×10^1	1.5
4	2.5×10^1	1.40	4.5×10^1	1.7	6.5×10^1	1.8
5	6.4×10^1	1.81	8.1×10^1	1.9	4.0×10^1	2.0
6	1.6×10^2	2.21	1.4×10^2	2.1	1.8×10^2	2.3
7	7.9×10^2	2.90	2.6×10^2	2.4	2.9×10^2	2.5
8	1.5×10^3	3.17	3.9×10^2	2.6	6.3×10^2	2.8
9	2.4×10^3	4.38	7.1×10^2	2.9	1.0×10^3	3.0
10	1.2×10^5	5.07	1.8×10^3	3.2	1.8×10^3	3.2

Table 7: Mean changes in physico-chemical parameters of paint sample PS-1

Time (Mth)	S.G	$O.D_{600nm}$	T.R	P.H	VIS
0	2.1772	1.49	6.6	8.5	11.1
1	2.0918	1.5	6.5	8.4	11.1
2	1.9637	1.51	6.5	8.4	11.1
3	1.8357	1.55	6.3	8.2	11.1
4	1.7076	1.6	6	8	11
5	1.4942	1.73	5.8	7.9	11
6	1.3234	1.84	5.3	7.4	11
7	1.2954	1.91	4.7	7.2	10.9
8	1.1926	1.99	4.1	7	10.9
9	1.1526	2.1	3.8	6.6	10.9
10	1.0896	2.32	3.2	6.1	10.8

Table 8: Mean changes in physico-chemical parameters of paint sample PS-2

TIME	SG	OD_{600nm}	TR	PH	VIS
0	2.1469	1.52	6.5	8.4	11.2
1	2.1345	1.59	6.4	8.3	11.2
2	2.0948	1.63	6.4	8.1	11.2
3	1.9211	1.71	6.2	8	11.2
4	1.7076	1.79	5.9	7.8	11.1
5	1.5319	1.91	5.5	7.7	11.1
6	1.4028	2.02	5.2	7.5	11.1
7	1.3661	2.23	4.8	7.2	11.1
8	1.1998	2.39	4.3	6.8	11
9	1.1631	2.45	3.7	6.5	11
10	1.0853	2.59	3.5	6.2	10.9

Table 9: Mean changes in physico-chemical parameters of paint sample PS-3

TIME	SG	OD_{600nm}	TR	PH	VIS
0	2.0919	1.63	6.6	8.4	11.2
1	1.9635	1.69	6.5	8.4	11.2
2	1.8786	1.77	6.5	8.3	11.2
3	1.7503	1.82	6.4	8.1	11.2
4	1.5938	1.89	6.2	7.9	11.1
5	1.4298	2.28	5.9	7.6	11.1
6	1.3976	2.62	5.3	7.3	11.1
7	1.2983	2.96	5.1	7.1	11.1
8	1.1839	3.3	4.8	6.9	11
9	1.1651	3.59	4.2	6.6	11
10	1.0931	3.74	3.9	6.3	10.9

Table 10: Mean changes in physico-chemical parameters of paint sample PS-4

TIME	SG	OD_{600nm}	TR	PH	VIS
0	2.1931	1.57	6.6	8.2	11.3
1	2.0969	1.59	6.5	8.1	11.3
2	1.9726	1.61	6.4	8	11.3
3	1.9183	1.74	6.3	7.8	11.2
4	1.8576	1.8	6.1	7.5	11.2
5	1.7861	1.88	5.8	7.1	11.2
6	1.6984	1.93	5.4	6.8	11.1
7	1.5318	2.06	5	6.6	11.1
8	1.3773	2.19	4.7	6.4	11
9	1.292	2.36	4.3	6.3	11
10	1.1093	2.49	4.1	6.1	11

Table 11: Mean changes in physico-chemical parameters of paint sample PS-5

TIME	SG	OD_{600nm}	TR	pH	VIS
0	2.8658	1.63	6.9	8.3	11.7
1	2.7395	1.65	6.8	8.3	11.7
2	2.7163	1.68	6.8	8.1	11.7
3	2.6549	1.71	6.7	8.1	11.7
4	2.5587	1.75	6.6	8	11.7
5	2.4583	1.82	6.4	7.9	11.7
6	2.4129	1.89	6.1	7.7	11.6
7	2.3234	1.93	5.9	7.5	11.6
8	2.2178	2.02	5.7	7.2	11.6
9	2.1796	2.18	5.6	6.9	11.5
10	2.1056	2.25	5.5	6.8	11.5

Table 12: Mean changes in physico-chemical parameters of paint sample PS-6

TIME	SG	OD_{600nm}	TR	PH	VIS
0	2.3614	1.79	5.2	8.4	11.1
1	2.2898	1.82	5.1	8.3	11.1
2	2.2293	1.89	5	8.1	11.1
3	2.2573	1.92	4.7	7.8	11
4	2.2851	2.09	4.5	7.3	11
5	2.1485	2.22	4.3	7.1	11
6	2.1039	2.98	3.9	7	11
7	2.0948	3.52	3.6	6.7	10.9
8	1.9765	3.8	3.1	6.4	10.9
9	1.7532	3.86	2.6	6	10.9
10	1.5487	3.91	2.3	5.6	10.8

Table 13: Concentration of Phosphate and Sulphate in Paint samples PS-1

Time (weeks)	Phosphate (mg/kg)	Sulphate (mg/kg)
0	15.5	12.8
2	12.2	10.4
4	10.0	10.6
6	8.0	8.8
8	6.3	8.2
10	5.5	7.8

Table 14: Concentration of Phosphate and Sulphate in paint sample PS-2

Time (weeks)	Phosphate (mg/kg)	Sulphate (mg/kg)
0	14.0	11.8
2	13.0	10.7
4	10.4	8.8
6	9.2	7.9
8	6.9	6.7
10	6.0	6.1

Table 15: Concentration of Phosphate and Sulphate in paint sample PS-3

Time (weeks)	Phosphate (mg/kg)	Sulphate (mg/kg)
0	7.4	5.9
2	6.5	4.6
4	5.7	3.5
6	4.0	2.8
8	3.2	2.3
10	2.4	2.0

Table 16 Concentration of Phosphate and Sulphate in paint sample PS-4

Time (weeks)	Phosphate (mg/kg)	Sulphate (mg/kg)
0	11.9	8.6
2	10.6	8.0
4	10.0	6.2
6	7.9	5.0
8	6.3	3.6
10	5.0	2.8

Table 17: Concentration of Phosphate and Sulphate in paint sample PS-5

Time (weeks)	Phosphate (mg/kg)	Sulphate (mg/kg)
0	10.3	6.7
2	9.9	6.5
4	9.3	6.3
6	9.2	6.2
8	9.1	6.1
10	8.0	6.0

Table 18: Concentration of Phosphate and Sulphate in paint sample PS-6

Time (weeks)	Phosphate (mg/kg)	Sulphate (mg/kg)
0	12.2	8.3
2	11.9	8.2
4	10.8	8.0
6	10.3	6.9
8	9.9	6.6
10	9.0	6.0

Table 19 Concentration of Phosphate and Sulphate in spoilt paint sample

Time (weeks)	Phosphate (mg/kg)	Sulphate (mg/kg)
0	2.6	1.2
2	1.2	0.6
4	0.7	0.4
6	0.4	0.3
8	0.2	0.2
10	0.1	0.0

Table 20 Effect of 0.5% v/v of biocides on total bacterial count of fresh, sterile paint samples

Time (weeks)	ZN467	ZN481	ZN485	ZN489	Control sample
0				1	1.2
2	1	1.04	1.08	1.11	1.3
4	1.04	1.08	1.15	1.17	1.57
6	1.08	1.15	1.26	1.28	1.77
8	1.15	1.23	1.32	1.36	1.8
10					

Table 21 Effect of 1% v/v of biocides on total bacterial count of fresh, sterile paint samples

Time (weeks)	ZN467	ZN481	ZN485	ZN489	Control sample
0					1.2
2		1	1.04	1.1	1.3
4	1	1.2	1.15	1.15	1.57
6	1.08	1.15	1.2	1.26	1.77
8	1.15	1.3	1.3	1.36	1.8
10					

Table 22 Effect of 2% v/v of biocides on total bacterial count of fresh, sterile paint samples

Time (weeks)	ZN467	ZN481	ZN485	ZN489	Control sample
0					1.2
2				1	1.3
4	1	1.04	1.08	1.08	1.57
6	1.04	1.08	1.15	1.15	1.77
8	1.11	1.15	1.28	1.25	1.8
10					

Table 23 Effect of 3% v/v of biocides on bacterial population density of fresh sterile paint Samples

Time (weeks)	ZN467	ZN481	ZN485	ZN489	Control sample
0					1.2
2				1	1.3
4				1.04	1.57
6	1	1	1.04	1.11	1.77
8	1.04	1.08	1.11	1.23	1.8
10					

Table 24 Effect of 0.5% v/v of biocides on total coliform count of fresh sterile paint samples

Time (weeks)	ZN467	ZN481	ZN485	ZN489	Control sample
0				1.04	1
2		1	1.1	1.11	1.3
4	1.04	1.04	1.11	1.2	1.5
6	1.08	1.08	1.17	1.23	1.6
8	1.11	1.15	1.25	1.32	1.7
10					

Table 25 Effect of 1% v/v of biocides on total coliform count of fresh sterile paint samples

Time (weeks)	ZN467	ZN481	ZN485	ZN489	Control sample
0					1
2				1.1	1.3
4		1		1.11	1.5
6	1	1.04	1	1.2	1.6
8	1.04	1.08	1.08	1.3	1.7
10	1.08	1.11	1.2		

Table 26 Effect of 2% v/v of biocides on total coliform count of fresh sterile Paint samples

Time (weeks)	ZN467	ZN481	ZN485	ZN489	Control sample
0					1
2				1	1.3
4	1	1	1.04	1.08	1.5
6	1	1.04	1.08	1.11	1.6
8	1.04	1.11	1.15	1.2	1.7
10					

Table 27 Effect of 3% v/v of biocides on total coliform count of fresh sterile paint samples

Time (weeks)	ZN467	ZN481	ZN485	ZN489	Control sample
0					1
2				1	1.3
4		1	1	1.04	1.5
6	1	1	1.04	1.1	1.6
8	1.04	1.04	1.1	1.2	1.7
10					

Table 28 Effect of 0.5% v/v of biocides on total fungal count of fresh sterile paint samples

Time (weeks)	ZN467	ZN481	ZN485	ZN489	Control sample
0					
2				1	1
4		1.04		1.11	1.2
6	1	1.08	1.08	1.26	1.36
8	1.08	1.15	1.18	1.32	1.69
10	1.15	1.26	1.32	1.36	1.75

Table 29 Effect of 1% v/v of biocides on total fungal count of fresh sterile paint samples

Time (weeks)	ZN467	ZN481	ZN485	ZN489	Control sample
0					
2					1
4		1	1	1.04	1.2
6	1	1.11	1.04	1.08	1.36
8	1.04	1.15	1.11	1.15	1.69
10	1.08	1.2	1.17	1.26	1.75

Table 30 Effect of 2% v/v of biocides on total fungal count of fresh sterile paint samples

Time (weeks)	ZN467	ZN481	ZN485	ZN489	Control sample
0					
2				1	1
4				1.11	1.2
6	1	1.04	1.04	1.26	1.36
8	1.04	1.08	1.08	1.32	1.69
10	1.08	1.11	1.15	1.36	1.75

Table 31 Effect of 3% v/v of biocides on total fungal count of fresh sterile paint samples

Time (weeks)	ZN467	ZN481	ZN485	ZN489	Control sample
0					1
2				1.04	1.2
4				1.08	1.36
6				1.11	1.69
8	1	1.04	1.04	1.18	1.75
10	1.04	1.08	1.08		

Table 32 Effect of 0.5% of biocides on specific gravity of fresh sterile paint samples

Time (months)	ZN467	ZN481	ZN485	ZN489	Control sample
0	2.8659	2.8659	2.8659	2.8659	2.8659
2	2.7448	2.7826	2.711	2.6348	2.611
4	2.6633	2.6094	2.5986	2.4962	2.4058
6	2.4516	2.4072	2.4625	2.2538	2.1639
8	2.373	2.2936	2.2015	2.0165	1.8517
10	2.059	1.8653	1.8132	1.7319	1.6218

Table 33 Effect of 1% of biocides on specific gravity of fresh sterile paint samples

Time (weeks)	ZN467	ZN481	ZN485	ZN489	Control sample
0	2.8658	2.8658	2.8658	2.8659	2.8659
2	2.7985	2.7952	2.7785	2.6978	2.6110
4	2.7059	2.6983	2.6509	2.5019	2.4058
6	2.6348	2.5642	2.5378	2.3676	2.1639
8	2.5271	2.4867	2.3951	2.1479	1.8517
10	2.4683	2.2798	2.1358	1.9251	1.6218

Table 34 **Effect of 2% of biocides on specific gravity of fresh sterile paint samples**

Time (weeks)	ZN467	ZN481	ZN485	ZN489	Control sample
0	2.8659	2.8659	2.8659	2.8659	2.8659
2	2.8146	2.8124	2.8069	2.7265	2.6110
4	2.7535	2.7586	2.7382	2.5938	2.4058
6	2.6958	2.6257	2.6051	2.4283	2.1639
8	2.6362	2.5384	2.4763	2.3152	1.8517
10	2.6012	2.4165	2.2846	2.2579	1.6218

Table 35 **Effect of 3% of biocides on specific gravity of fresh sterile paint samples**

Time (weeks)	ZN467	ZN481	ZN485	ZN489	Control sample
0	2.8657	2.8657	2.8657	2.8659	2.8659
2	2.8542	2.8327	2.8259	2.7397	2.6110
4	2.8068	2.7935	2.7617	2.6351	2.4058
6	2.7656	2.6683	2.6285	2.5688	2.1639
8	2.7281	2.6176	2.5446	2.3917	1.8517
10	2.7055	2.5939	2.4269	2.1634	1.6218

Table 36 **Effect of 0.5% v/v of biocides on OD of fresh sterile paint samples**

Time (weeks)	ZN467	ZN481	ZN485	ZN489	Control sample
0	1.63	1.63	1.63	1.63	1.63
2	1.68	1.70	1.71	1.72	1.72
4	1.71	1.73	1.74	1.76	1.77
6	1.76	1.79	1.80	1.83	1.85
8	1.82	1.85	1.87	1.90	1.92
10	1.86	1.88	1.90	1.96	1.98

Table 37 Effect of 1% v/v of biocides on OD of fresh sterile paint samples

Time (weeks)	ZN467	ZN481	ZN485	ZN489	Control sample
0	1.63	1.63	1.63	1.63	1.63
2	1.66	1.67	1.68	1.71	1.72
4	1.69	1.71	1.73	1.74	1.77
6	1.73	1.75	1.78	1.80	1.85
8	1.77	1.78	1.82	1.85	1.92
10	1.80	1.83	1.85	1.90	1.98

Table 38 Effect of 2% v/v of biocides on OD of fresh sterile paint samples

Time (weeks)	ZN467	ZN481	ZN485	ZN489	Control sample
0	1.63	1.63	1.63	1.63	1.63
2	1.65	1.66	1.67	1.7	1.72
4	1.68	1.70	1.71	1.73	1.77
6	1.71	1.73	1.74	1.78	1.85
8	1.73	1.76	1.78	1.82	1.92
10	1.75	1.80	1.82	1.86	1.98

Table 39 Effect of 3% v/v of biocides on OD of fresh sterile paint samples

Time (weeks)	ZN467	ZN481	ZN485	ZN489	Control sample
0	1.63	1.63	1.63	1.63	1.63
2	1.63	1.64	1.65	1.67	1.72
4	1.64	1.66	1.67	1.71	1.77
6	1.65	1.69	1.70	1.75	1.85
8	1.66	1.71	1.73	1.80	1.92
10	1.67	1.73	1.75	1.84	1.98

Table 40 **Effect of 0.5% v/v of biocides on transmittance of fresh sterile paint samples**

Time (weeks)	ZN467	ZN481	ZN485	ZN489	Control sample
0	6.6	6.6	6.6	6.6	6.6
2	6.4	6.3	6.2	6.1	6.0
4	6.0	5.9	5.7	5.5	5.4
6	5.6	5.3	5.1	4.8	4.6
8	5.1	4.9	4.7	4.3	4.1
10	4.7	4.4	4.2	3.7	3.4

Table 41 **Effect of 1% v/v of biocides on transmittance of fresh sterile paint samples**

Time (weeks)	ZN467	ZN481	ZN485	ZN489	Control sample
0	6.6	6.6	6.6	6.6	6.6
2	6.5	6.4	6.3	6.2	6.0
4	6.3	6.1	6.0	5.7	5.4
6	5.9	5.6	5.8	5.2	4.6
8	5.6	5.2	5.5	4.7	4.1
10	5.3	4.7	5.1	4.3	3.4

Table 42 **Effect of 2% v/v of biocides on transmittance of fresh sterile paint samples**

Time (weeks)	ZN467	ZN481	ZN485	ZN489	Control sample
0	6.6	6.6	6.6	6.6	6.6
2	6.5	6.5	6.4	6.3	6.0
4	6.4	6.2	6.1	6.0	5.4
6	6.3	5.9	5.9	5.8	4.6
8	6.2	5.7	5.7	5.5	4.1
10	6.1	5.4	5.5	5.1	3.4

Table 43 Effect of 3% v/v of biocides on transmittance of fresh sterile paint samples

Time (weeks)	ZN467	ZN481	ZN485	ZN489	Control sample
0	6.6	6.6	6.6	6.6	6.6
2	6.6	6.5	6.4	6.3	6.0
4	6.5	6.3	6.2	6.0	5.4
6	6.5	6.2	6.1	5.8	4.6
8	6.4	6.1	5.9	5.6	4.1

Table 44 Effect of 0.5% v/v of biocides on pH of fresh sterile paint samples

Time (weeks)	ZN467	ZN481	ZN485	ZN489	Control sample
0	2	4	6	8	10
8.4	8.3	8.1	7.8	7.6	7.4
8.4	8.2	8.0	7.7	7.5	7.3
8.4	8.2	7.9	7.7	7.5	7.2
8.4	8.1	7.7	7.5	7.1	6.9
8.4	8.0	7.6	7.2	6.8	6.6

Table 45 Effect of 1% v/v of biocides on pH of fresh sterile paint samples

Time (weeks)	ZN467	ZN481	ZN485	ZN489	Control sample
0	2	4	6	8	10
8.4	8.4	8.3	8.2	8.1	8.0
8.4	8.3	8.2	8.0	7.8	7.6
8.4	8.3	8.1	7.8	7.6	7.4
8.4	8.2	7.9	7.7	7.3	7.1
8.4	8.0	7.6	7.2	6.8	6.6

Table 46 Effect of 2% v/v of biocides on pH of fresh sterile paint samples

Time (weeks)	ZN467	ZN481	ZN485	ZN489	Control sample
0	2	4	6	8	10
8.4	8.4	8.3	8.3	8.2	8.1
8.4	8.4	8.3	8.2	8.1	8.0
8.4	8.3	8.2	8.1	7.9	7.7
8.4	8.3	8.2	8.0	7.8	7.5
8.4	8.0	7.6	7.2	6.8	6.6

Table 47 Effect of 3% v/v of biocides on pH of fresh sterile paint samples

Time (weeks)	ZN467	ZN481	ZN485	ZN489	Control sample
0	8.4	8.3	8.3	8.3	8.2
8.4	2	8.0	8.2	8.2	8.2
8.4	8.4	4	7.6	8.1	8.2
8.4	8.4	8.3	6	7.2	8.0
8.4	8.3	8.3	8.3	8	6.8
10	8.2	8.2	8.0	7.8	6.6

Table 48 Effect of 0.5% v/v of biocides on viscosity of fresh sterile paint samples

Time (weeks)	ZN467	ZN481	ZN485	ZN489	Control sample
0	2	4	6	8	10
11.2	11.2	11.2	11.1	11.1	11.1
11.2	11.2	11.1	11.1	11.1	11.1
11.2	11.2	11.1	11.1	11.1	11.0
11.2	11.2	11.1	11.1	11.0	11.0
11.2	11.2	11.1	11.1	11.0	11.0

Table 49 Effect of 1% v/v of biocides on viscosity of fresh sterile paint samples

Time (weeks)	ZN467	ZN481	ZN485	ZN489	Control sample
0	2	4	6	8	10
11.2	11.2	11.2	11.2	11.1	11.1
11.2	11.2	11.2	11.1	11.1	11.0
11.2	11.2	11.1	11.1	11.1	11.0
11.2	11.2	11.1	11.1	11.1	11.0
11.2	11.2	11.1	11.1	11.0	11.0

Table 50 Effect of 2% v/v of biocides on viscosity of fresh sterile paint samples

Time (weeks)	ZN467	ZN481	ZN485	ZN489	Control sample
0	2	4	6	8	10
11.2	11.2	11.2	11.2	11.1	11.1
11.2	11.2	11.2	11.2	11.1	11.1
11.2	11.2	11.2	11.1	11.1	11.1
11.2	11.2	11.2	11.1	11.1	11.0
11.2	11.2	11.1	11.1	11.0	11.0

Table 51 Effect of 3% v/v of biocides on viscosity of fresh sterile paint samples

Time (weeks)	ZN467	ZN481	ZN485	ZN489	Control sample
0	2	4	6	8	10
11.2	11.2	11.2	11.2	11.2	11.2
11.2	11.2	11.2	11.2	11.2	11.1
11.2	11.2	11.2	11.2	11.1	11.1
11.2	11.2	11.2	11.1	11.1	11.1
11.2	11.2	11.1	11.1	11.0	11.0

Table 55 Spoilage potential of wild and cured strains of OB-6 on physico-chemical parameters of fresh paint sample PS-4

TIME(MTH)	SG(W)	SG(C1)	SG(C2)	OD(W)	OD(C1)	OD(C2)	TR(W)	TR(C1)	TR(C2)	pH(W)	pH(C1)	pH(C2)	VIS(W)	VIS(C1)	VIS(C2)
0	1.9211	1.9211	1.9205	1.57	1.57	1.46	6.7	6.7	7.3	8.5	8.5	8.5	11.1	11.1	11
2	1.7494	1.7549	1.9038	1.63	1.6	1.48	6.5	6.6	7.2	8.3	8.4	8.5	11.1	11.1	11
4	1.5673	1.5982	1.8763	1.68	1.63	1.49	6.2	6.5	7.1	8	8.1	8.4	11.1	11.1	11
6	1.3853	1.4678	1.8162	1.74	1.69	1.51	5.9	6.2	7	7.6	7.9	8.3	11	11.1	11
8	1.1954	1.2238	1.7942	1.79	1.73	1.53	5.7	6	6.9	7.3	7.6	8.1	11	11.1	11
10	0.9378	1.1989	1.5389	1.86	1.79	1.54	5.3	5.7	6.7	7	7.3	7.9	10.9	11	11

Table 56 Spoilage potential of wild and cured strains of OB-6 on physico-chemical parameters of fresh paint sample PS-5

TIME(MTH)	SG(W)	SG(C1)	SG(C2)	OD(W)	OD(C1)	OD(C2)	TR(W)	TR(C1)	TR(C2)	pH(W)	pH(C1)	pH(C2)	VIS(W)	VIS(C1)	VIS(C2)
0	2.6391	2.6391	2.6385	1.6	1.6	1.56	6.6	6.6	7.3	8.5	8.5	8.5	11.5	11.5	11.5
2	2.3665	2.5842	2.6179	1.66	1.64	1.58	6.3	6.4	6.7	8	8.2	8.5	11.5	11.5	11.5
4	1.9365	2.4765	2.5354	1.73	1.7	1.6	5.9	6.1	6.6	7.8	7.9	8.4	11.5	11.5	11.5
6	1.7328	2.3544	2.4869	1.79	1.76	1.62	5.7	5.9	6.5	7.5	7.6	8.3	11.5	11.5	11.5
8	1.4586	2.2489	2.4038	1.85	1.8	1.64	5.3	5.6	6.4	7.1	7.2	8.1	11.4	11.5	11.5
10	1.2627	2.1192	2.3965	1.91	1.83	1.67	4.7	5.3	6.2	6.9	7	7.9	11.4	11.5	11.5

Table 57 Spoilage potential of wild and cured strains of OB-6 on physico-chemical parameters of fresh paint sample PS-6

TIME(MTH)	SG(W)	SG(C1)	SG(C2)	OD(W)	OD(C1)	OD(C2)	TR(W)	TR(C1)	TR(C2)	pH(W)	pH(C1)	pH(C2)	VIS(W)	VIS(C1)	VIS(C2)
0	2.1983	2.1983	2.1977	1.51	1.51	1.46	7	7	7.3	8.5	8.5	8.5	11.1	11.1	11
2	1.9873	2.0911	2.1936	1.54	1.54	1.48	6.7	6.8	7.2	8.4	8.3	8.5	11.1	11.1	11
4	1.7382	1.9637	2.0938	1.59	1.59	1.49	6.5	6.6	7.1	8.2	8.1	8.4	11.1	11.1	11
6	1.5329	1.8075	2.0269	1.63	1.62	1.51	6.3	6.5	7	8	8	8.3	11	11	11
8	1.3606	1.7949	1.9785	1.67	1.65	1.53	6.1	6.4	6.9	7.8	7.7	8.1	11	11	11
10	1.0673	1.6038	1.9677	1.75	1.71	1.54	5.7	6	6.8	7.4	7.5	7.9	11	11	11

Table 52 Spoilage potential of wild and cured strains of OB-6 on physico-chemical parameters of fresh paint sample PS1

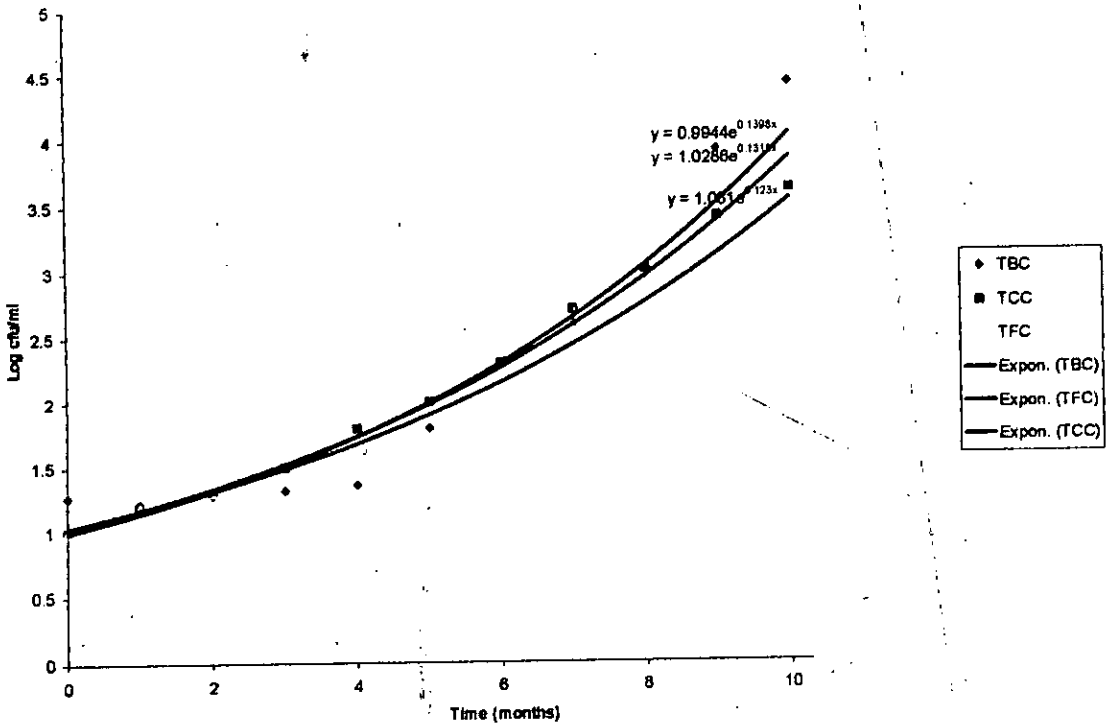
TIME(MTH)	SG(W)	SG(C1)	SG(C2)	OD(W)	OD(C1)	OD(C2)	TR(W)	TR(C1)	TR(C2)	pH(W)	pH(C1)	pH(C2)	VIS(W)	VIS(C1)	VIS(C2)
0	1.9637	1.9637	1.9631	1.55	1.55	1.46	6.8	6.8	7.3	8.5	8.5	8.5	11.1	11.1	11
2	1.7588	1.8754	1.9148	1.63	1.59	1.48	6.5	6.7	7.2	8.1	8.4	8.5	11.1	11.1	11
4	1.5295	1.6988	1.8763	1.71	1.68	1.49	6.1	6.4	7.1	7.8	8	8.4	11	11.1	11
6	1.3963	1.5891	1.7271	1.82	1.79	1.51	5.7	6	7	7.1	7.4	8.3	11	11.1	11
8	1.1397	1.4672	1.6794	1.93	1.91	1.53	5.1	5.5	6.8	6.9	7	8.1	10.9	11	11
10	1.0584	1.3551	1.5532	2.05	1.93	1.54	4.3	4.7	6.5	6.5	6.9	7.9	10.9	11	11

Table 53 Spoilage potential of wild and cured strains of OB-6 on physico-chemical parameters of fresh paint sample PS-2

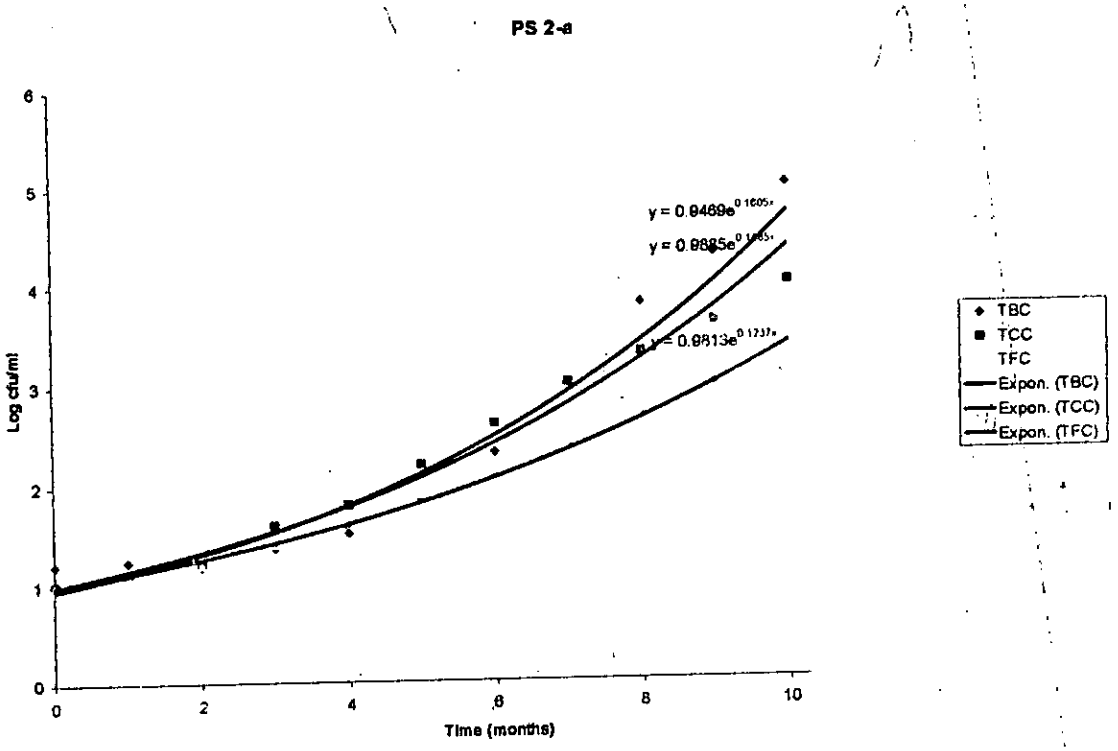
TIME(MTH)	SG(W)	SG(C1)	SG(C2)	OD(W)	OD(C1)	OD(C2)	TR(W)	TR(C1)	TR(C2)	pH(W)	pH(C1)	pH(C2)	VIS(W)	VIS(C1)	VIS(C2)
0	1.9637	1.9637	1.9631	1.55	1.55	1.46	6.8	6.8	7.3	8.5	8.5	8.5	11.1	11.1	11
2	1.7938	1.8754	1.9148	1.63	1.59	1.48	6.5	6.7	7.2	8.2	8.4	8.5	11.1	11.1	11
4	1.5377	1.6988	1.8763	1.71	1.68	1.49	6.1	6.4	7.1	7.8	8	8.4	11	11.1	11
6	1.3286	1.5891	1.7271	1.82	1.79	1.51	5.7	6	7	7.3	7.4	8.3	11	11.1	11
8	1.0844	1.4672	1.6794	1.93	1.91	1.53	5.1	5.5	6.8	6.9	7	8.1	10.9	11	11
10	0.7829	1.3551	1.5532	2.05	1.93	1.54	4.3	4.7	6.5	6.7	6.9	7.9	10.9	11	11

Table 54 Spoilage potential of wild and cured strains of OB-6 on physico-chemical parameters of fresh paint sample PS-3

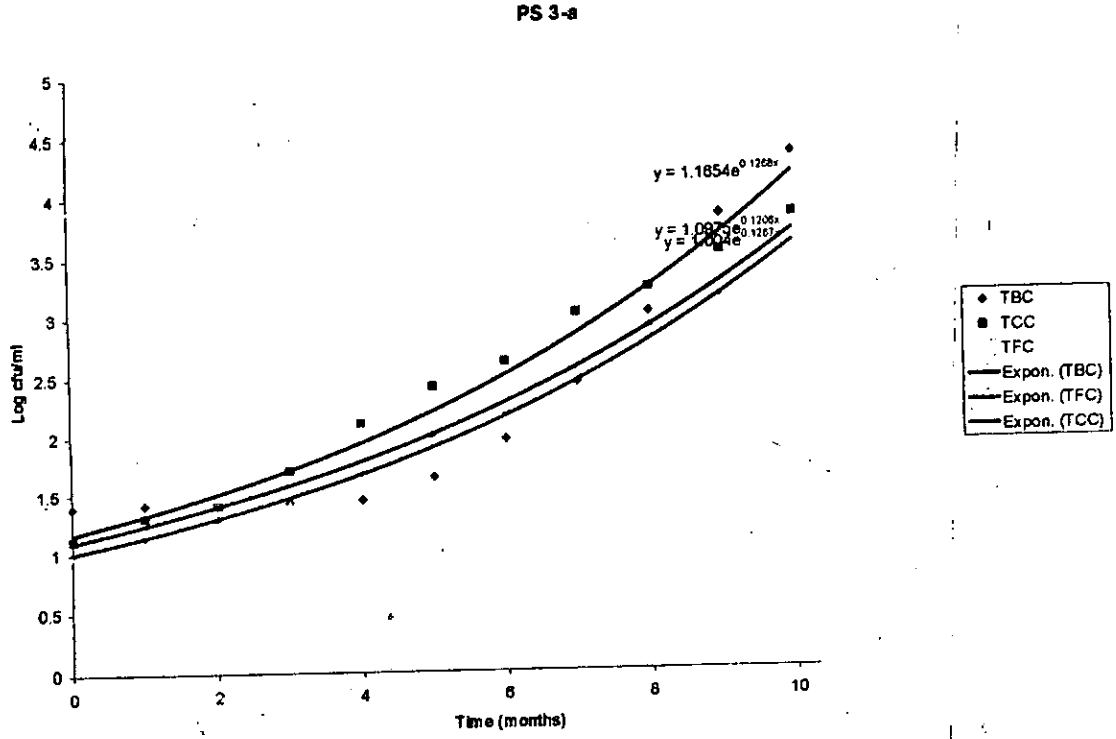
TIME(MTH)	SG(W)	SG(C1)	SG(C2)	OD(W)	OD(C1)	OD(C2)	TR(W)	TR(C1)	TR(C2)	pH(W)	pH(C1)	pH(C2)	VIS(W)	VIS(C1)	VIS(C2)
0	2.0919	2.0919	2.0911	1.52	1.52	1.46	7	7	7.3	8.5	8.5	8.5	11.1	11.1	11
2	1.8357	1.9211	2.0863	1.59	1.56	1.48	6.7	6.8	7.2	8.4	8.4	8.5	11.1	11.1	11
4	1.6295	1.7076	1.9837	1.65	1.6	1.5	6.4	6.6	7.1	8	8.2	8.4	11	11.1	11
6	1.4589	1.4943	1.9118	1.72	1.64	1.53	6.1	6.5	7	7.8	8	8.3	11	11.1	11
8	1.2837	1.3661	1.8673	1.77	1.69	1.56	5.9	6.3	6.9	7.4	7.8	8.1	10.9	11.1	11
10	1.0475	1.3234	1.7637	1.83	1.73	1.6	5.6	6.1	6.8	7.1	7.5	7.9	10.9	11	11



Appendix Fig 1: Graph of microbial growth pattern and fitted curve of paint sample PS-1



Appendix Fig 2: Graph of microbial growth pattern and fitted curve of paint sample PS-2



Appendix Fig 3: Graph of microbial growth pattern and fitted curve of paint sample PS-3

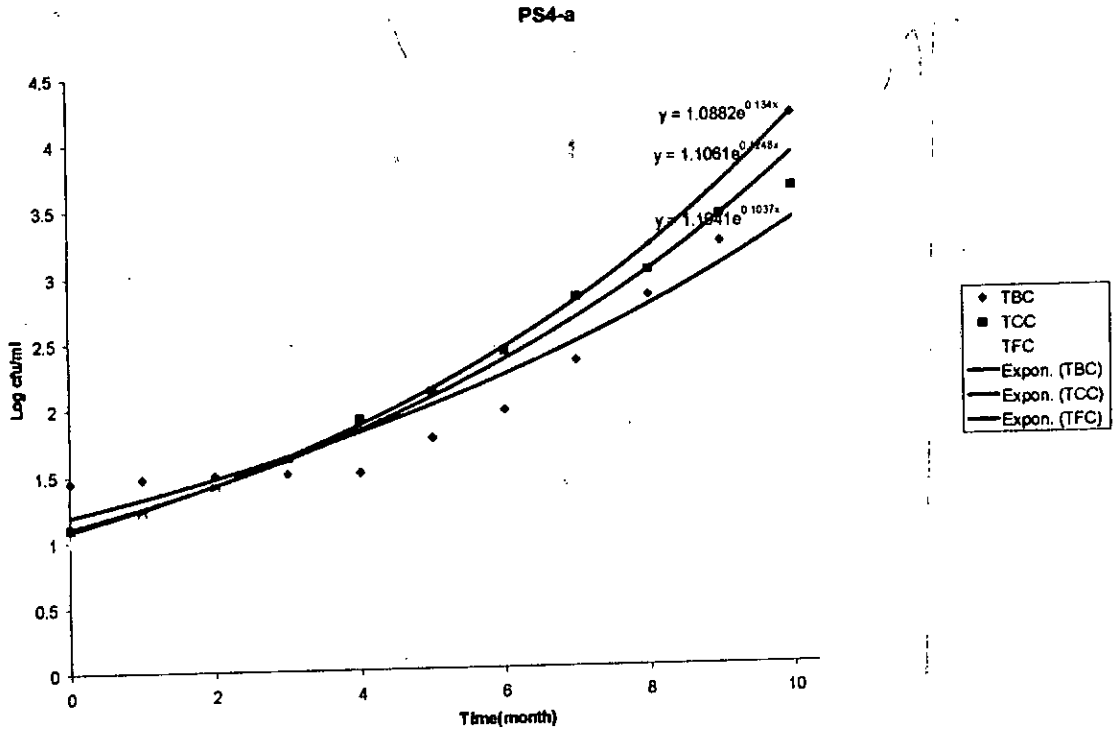
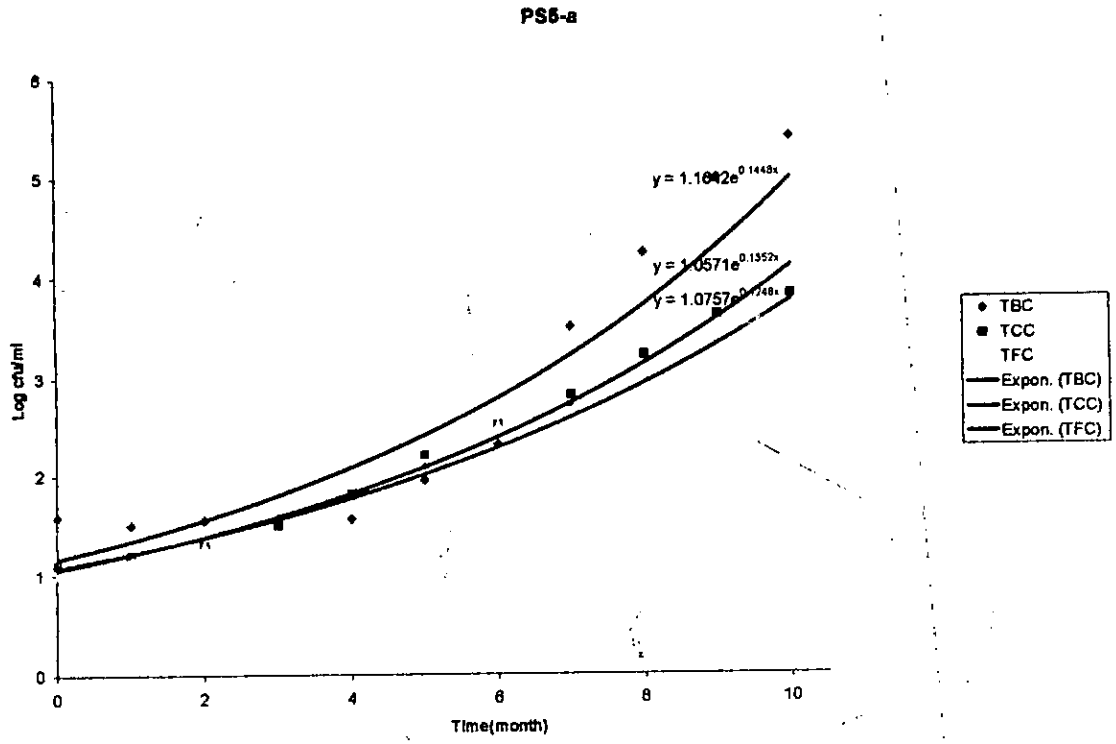
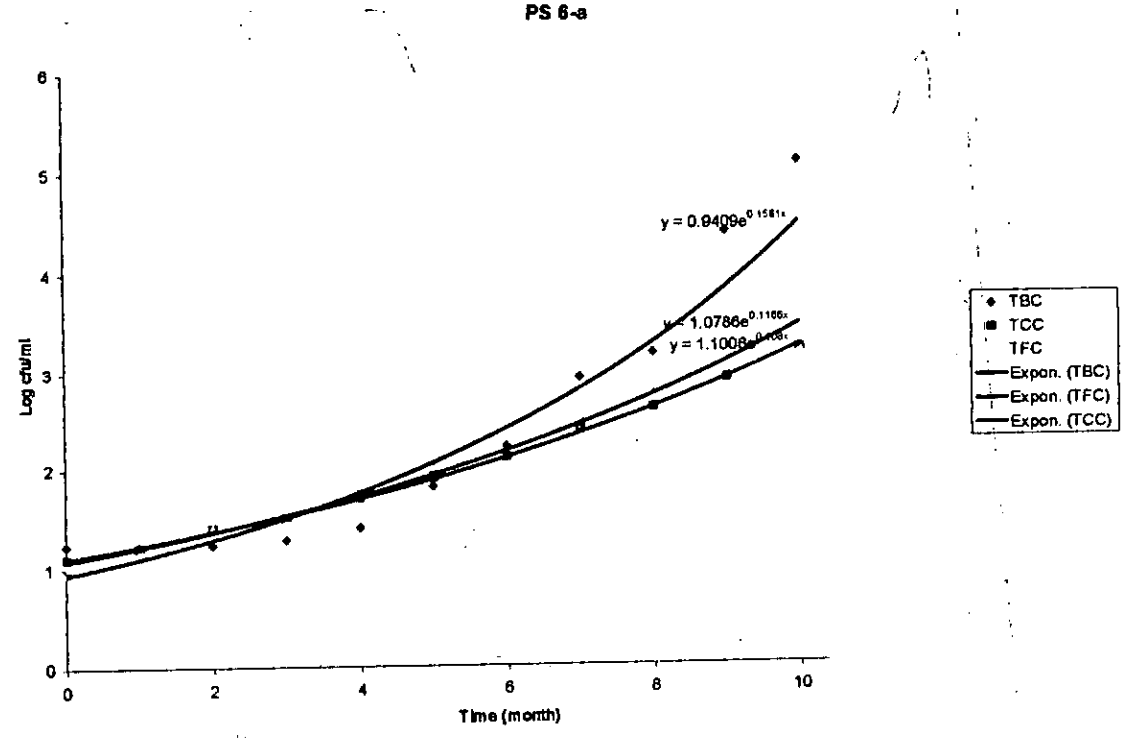


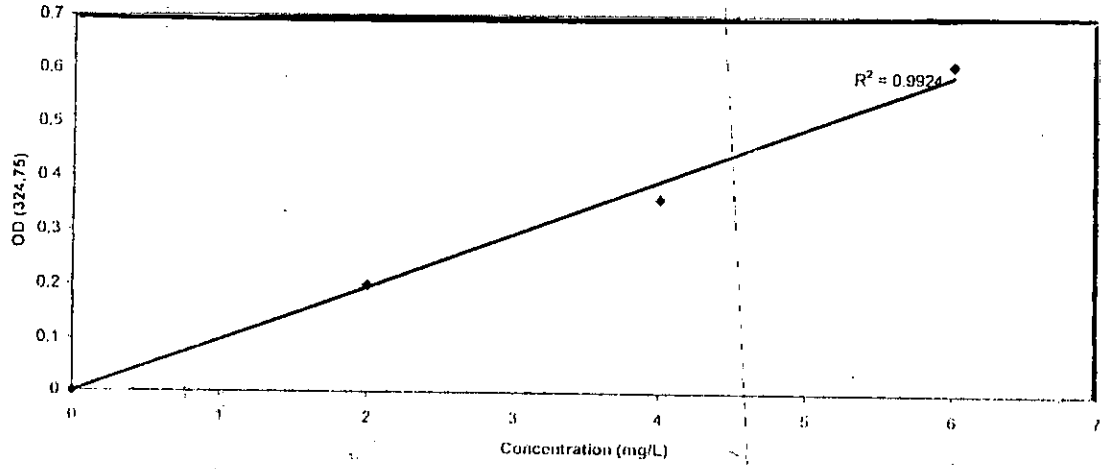
Fig Appendix Fig.4: Graph of microbial growth pattern and fitted curve of paint sample PS-4



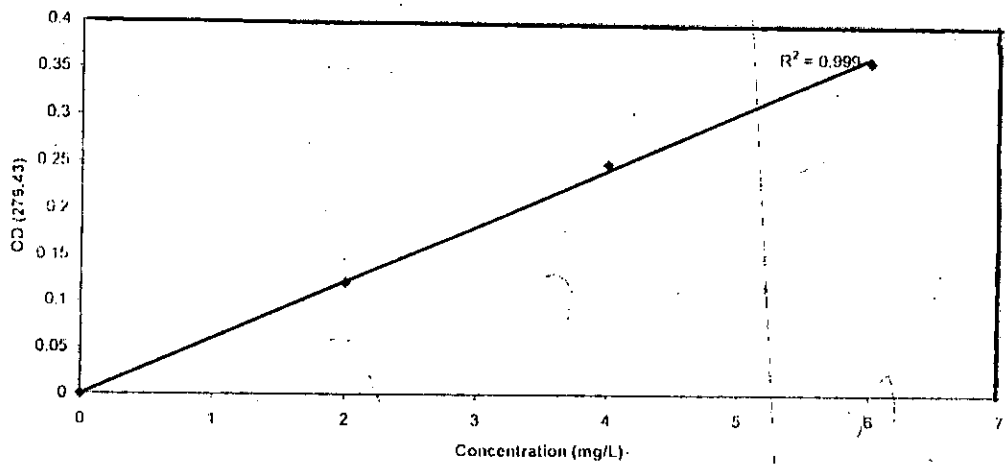
Appendix Fig 5: Graph of microbial growth pattern and fitted curve of paint sample PS-5



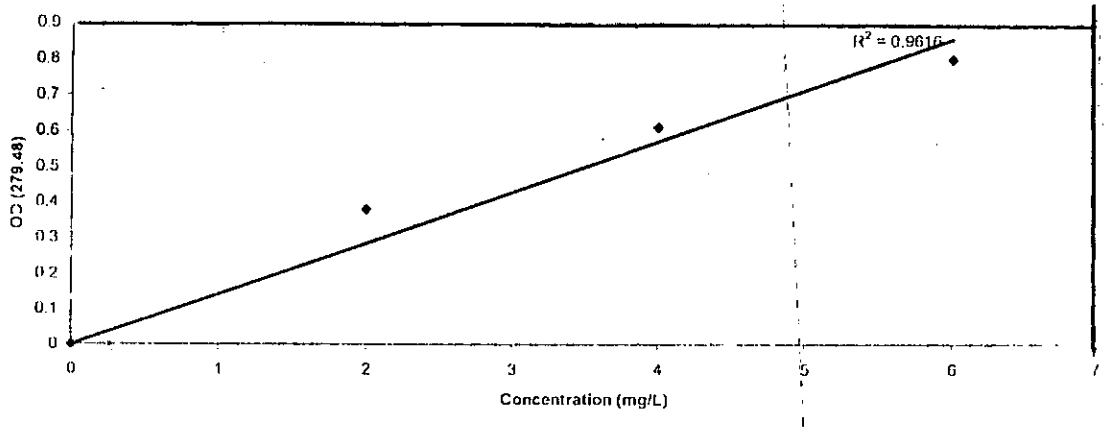
Appendix Fig 6: Graph of microbial growth pattern and fitted curve of paint SamplePS-6



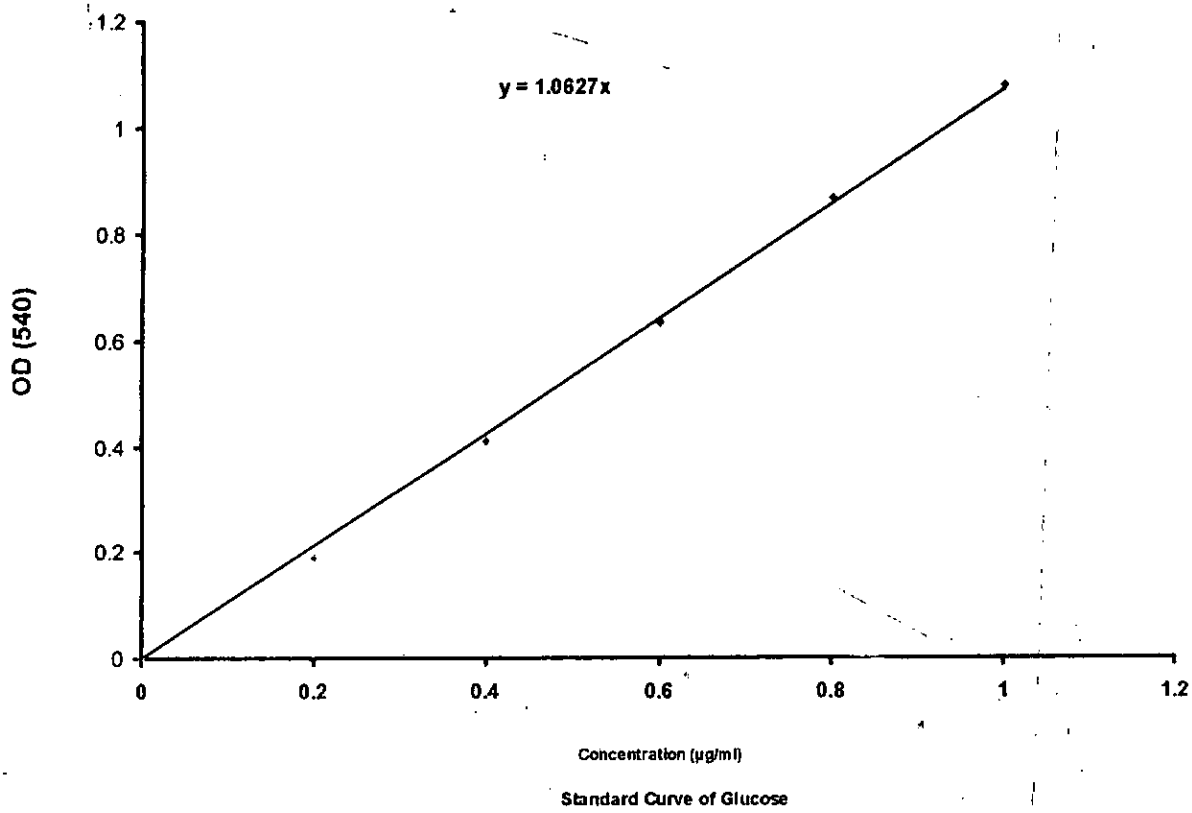
Standard Curve of Cu



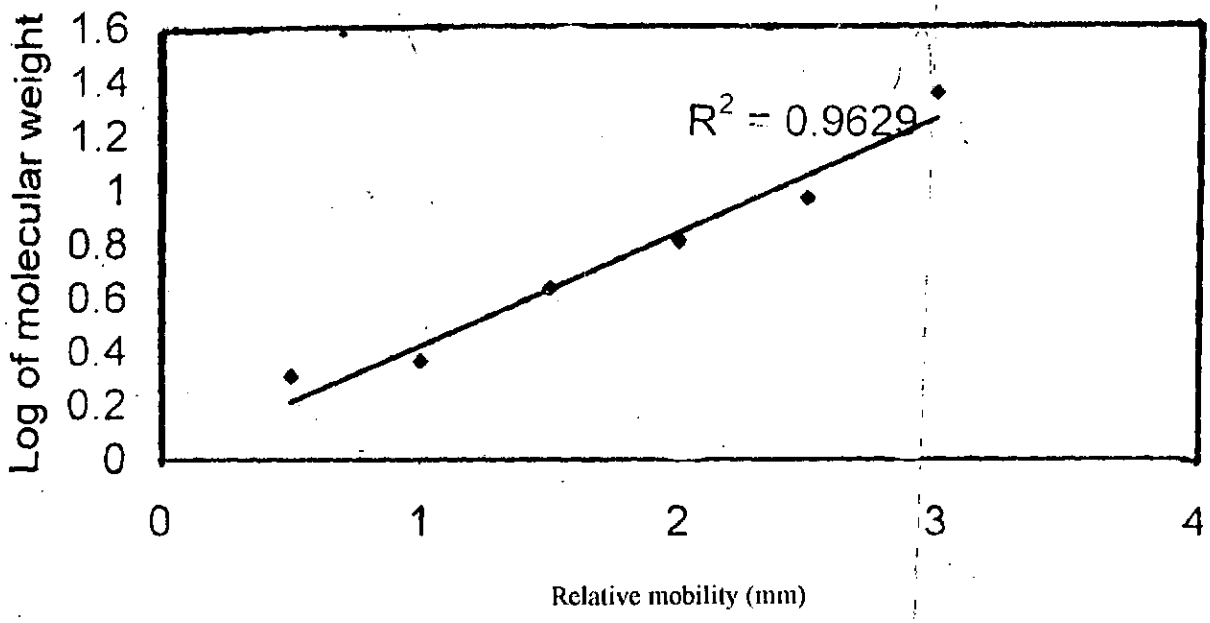
Standard Curve of Mn



Standard Curve of Pb



Standard Curve of Glucose



Standard curve of λ DNA hind III digested plasmids

Microbial Shelf Life Calculations

SLD = tsl + L (6.1)

Where SLD = Shelf –life duration

L= lag phase

6.13.1Therefore, applying the model to sample PS1

Slope of the exponential phase = 0.425

But, slope = $\frac{K}{2.303}$

K = slope x 2.303

K = 0.425 x 2.303

= 0.9788

$T = \frac{0.693}{K}$

$T = \frac{0.693}{0.9788}$

= 0.7080

$\frac{\text{Log } N_+ - \text{Log No}}{\text{Log 2}} = \frac{t}{T}$

$\frac{\text{Log } 3.4 \times 10^{10} - \text{Log } 18}{\text{Log 2}} = \frac{t}{0.7080}$

t = 22 months

Total time = exponential period + Lag period

Total time = (22 +5) months

= 27 months

6.13.2 Microbial shelf life determination of sample PS2

Slope of the experiential phase = 0.549

But slope = $\frac{K}{2.303}$

K = slope x 2.303

= 0.549 X 2.303

$= \frac{1.2643}{K}$

$T = \frac{0.693}{K}$

$T = \frac{0.693}{1.2643}$

$= 0.5481$

$\frac{\text{Log } N^t - \text{Log No}}{\text{Log 2}} = \frac{t}{T}$

$\frac{\text{Log } 3.4 \times 10^{10} - \text{Log16}}{\text{Log 2}} = \frac{t}{0.5481}$

t = 17 months

Total time= exponential period + Lag period

Total time = (17+5) months

22 months

6.13.3 Microbial Shelf life determination of sample PS3

Slope of the exponential phase = 0.347

But slope = $\frac{K}{2.303}$

K = slope X 2.303

= 0.347 X 2.303

= 0.7991

$T = \frac{0.693}{K}$

$T = \frac{0.693}{0.7991}$

= 0.8672

$\frac{\text{Log } N_t - \text{Log } N_0}{\text{Log 2}} = \frac{t}{T}$

$\frac{\text{Log } 3.4 \times 10^{10} - \text{Log 24}}{\text{Log 2}} = \frac{t}{0.8672}$

t = 30 months.

6.13.4 Microbial Shelf life determination of PS4

Slope of the exponential phase = 0.284

But slope = $\frac{K}{2.303}$

K= slope x 2.303

= 0.284 x 2.303

= 0.6541

$T = \frac{0.693}{K}$

$T = \frac{0.693}{0.6541} = 1.0595$

$\frac{\text{Log } N_t - \text{Log } N_o}{\text{Log } 2} = \frac{t}{T}$

$\frac{\text{Log } 3.4 \times 10^{10} - \text{Log } 26}{\text{Log } 2} = \frac{t}{T}$

t = 36 months

6.13.5. Microbial shelf-life determination of paint sample PS5

Slope of the exponential phase 0.537

But slope = $\frac{K}{2.303}$

K = Slope x 2.303

= 0.537 x 2.303

= 1.2367

$T = \frac{0.693}{K}$

$T = \frac{0.693}{1.2367}$

= 0.5603

$\frac{\text{Log } N_t - \text{Log } N_0}{\text{Log } 2} = \frac{t}{T}$

$$\frac{\text{Log } 3.4 \times 10^{10} - \text{Log } 32}{\text{Log } 2} = \frac{t}{T}$$

t = 17 months

Total time = exponential period + Lag period

Total time = (17 + 5) months

= 22 months

6.13.6 Microbial shelf life determination of paint sample PS6

Slope of the exponential phase = 0.512

But slope = $\frac{K}{2.303}$

K = slope X 2.303

= 0.512 X 2.303

= 1.1791

$T = \frac{0.693}{K}$

$T = \frac{0.693}{1.1791}$

= 0.5877

$$\frac{\text{Log } N_t - \text{Log } N_o}{\text{Log } 2} = \frac{t}{T}$$

$$\frac{\text{Log } 3.4 \times 10^{10} - \text{Log } 17}{\text{Log } 2} = \frac{t}{T}$$

t = 18 months

Total time = exponential period + lag period

Total time = (18 +5) months = 23 months

DETERMINATION OF THE SLOPES OF THE EXPONENTIAL PHASES

PS-1

Total Bacterial Count (TBC) : $Y = 0.9944e^{0.1398x}$

$$\frac{dy}{dx} = 0.9944\,(0.1398)\,e^{0.1398x}$$

$$\therefore \text{ slope} = 0.139e^{0.1398x}$$

at x = 8 months,

$$\text{slope} = 0.139e^{0.1398(8)} = 0.1398^{1.1184}$$

$$= 0.139\,(3.06) = 0.425$$

PS-2

Total Bacterial Count (TBC) : $Y = 0.9469e^{0.1605x}$

$$\frac{dy}{dx} = 0.9469\,(0.1605)\,e^{0.1605x}$$

$$\therefore \text{ slope} = 0.152e^{0.1605x}$$

at x = 8 months,

$$\text{slope} = 0.152e^{0.1605(8)}$$

$$= 0.152e^{1.284}$$

$$= 0.152\,(3.611) = 0.549$$

PS-3

Total Bacterial Count (TBC) : $Y = 1.0975e^{0.1206x}$

$$\frac{dy}{dx} = 1.0975\,(0.1206)\,e^{0.1206x}$$

$$\therefore \text{slope} = 0.1324e^{0.1206x}$$

at x = 8 months,

$$\text{slope} = 0.1324e^{0.1206(8)} = 0.1324e^{0.9648}$$

$$= 0.1324\,(2.6243) = 0.347$$

PS-4

Total Bacterial Count (TBC) : $Y = 1.1941e^{0.1037x}$

$$\frac{dy}{dx} = 1.1941\,(0.1037)e^{0.1037x}$$

$$\therefore \text{slope} = 0.124e^{0.1037x}$$

at x = 8 months,

$$\text{slope} = 0.124e^{0.1037(8)} = 0.124e^{0.8296}$$

$$= 0.124\,(2.2924) = 0.284$$

PS-5

Total Bacterial Count (TBC) : $Y = 1.1642e^{0.1448x}$

$$\frac{dy}{dx} = 1.1642\,(0.1448)e^{0.1448x}$$

$$\therefore \text{slope} = 0.1686e^{0.1448x} = 0.1686e^{0.1448x}$$

at x = 8 months,

slope = $0.1686e^{0.1448(8)} = 0.1686e^{1.1584}$

$= 0.1686\ (3.1848) = 0.537$

PS-6

Total Bacterial Count (TBC) : $Y = 0.9409e^{0.1561x}$

$$\frac{dy}{dx} = 0.9409\ (0.1561)e^{0.1561x}$$

$$\therefore \text{slope} = 0.1469e^{0.1561x}$$

at x = 8 months,

slope = $0.1469e^{0.1561\ (8)} = 0.1469e^{1.2488}$

$= 0.1469\ (3.486) = 0.512$

DETERMINATION OF RESIDUAL ERRORS (PHYSICO-CHEMICAL PARAMETER ESTIMATES)

PS-1

$$Y = \beta_0 + \beta_1 (X_1) + \beta_2(X_2) + \beta_3(X_3) + \beta_4 \ln(X_4) + \beta_5(X_5) + e$$

Where Y = 19 months

$\therefore$ 19 = 55.5769 + (-5.0878 x0.1058) +(-3.8218x7.51) +(-0.4877x0.15)+(-2.0445xln4.13)+ (-1.6022x3) + e

e =19-55.5769 +0.5382 + 28.7017 +0.0731 + 2.8996 +4.8066

e = 0.4423

Substituting the value of error in the equation to get Y

Y =55.5769-0.5382-28.7017-0.0731-2.8996-4.8066 +0.4423

Y = 19

PS-2

$$Y = \beta_0 + \beta_1 (X_1) + \beta_2(X_2) + \beta_3(X_3) + \beta_4 \ln(X_4) + \beta_5(X_5) + e$$

Where Y = 21

21 = 22.56-(-2.87x0.11) –(0.09x7.51)-(-0.37x0.15)-(-2.43x1.42)-(0.60x3.0)

e = 21-22.56-(-0.3157)-0.6534-(-0.0555)-(-3.4506)-1.8

e = 21-22.56+0.3157-0.6759+0.0555+3.4506-1.8

e = -0.2587

Y = 22.56+ 0.3157+ 0.6534-0.0555-3.446+1.8-0.2587

Y = 21.56

PS-3

$$Y = \beta_0 + \beta_1 (X_1) + \beta_2(X_2) + \beta_3(X_3) + \beta_4 \ln(X_4) + \beta_5(X_5) + e$$

Where Y = 23

23 = 5+ (-0.3654)+(-4.6039)+(-0.3635)+(0.4451)+0.00528+ e

e = 19-5+0.3654+ 4.6039 + 0.3638-0.4451-0.00528

e = 22.8827

Y = 5-0.3654-4.6039-0.3635+0.4451+0.00528+22.8827

Y = 23.00028

PS-4

$$Y = \beta_0 + \beta_1 (X_1) + \beta_2(X_2) + \beta_3(X_3) + \beta_4 \ln(X_4) + \beta_5(X_5) + e$$

Where Y = 22 months

Y = -2.4655+(--3.8391x0.11)+(2.5345x7.51)+(-0.4055x0.15)+(-0.64270xln4.13)+ (1.3394x3.0) +e

e =22 +2.4655+0.4223-19.0340+0.06082+0.9115-4.0018

e = -2.824

Y = (-2.4655-0.4223+ 19.0340-0.06082-0.9115+4.0018-2.82432

Y =21.28

PS-5

$Y = \beta_0 + \beta_1 (X_1) + \beta_2(X_2) + \beta_3(X_3) + \beta_4 \ln(X_4) + \beta_5(X_5) + e$

Where Y = 37 months

37 = 0.4004+ (-10.5699x0.11)+ (3.9713x7.51)+ (0.6006x0.15)+(-0.7441xln4.13)+(2.1956x3.0) + e

e = 37 -0.4004+1.1626-29.8222-0.0900+1.0553-6.5868

e = 2.3185

Y = 0.4004-1.1626+29.8222+0.0900-1.0553+6.5868+2.3185

Y = 37

PS-6

$Y = \beta_0 + \beta_1 (X_1) + \beta_2(X_2) + \beta_3(X_3) + \beta_4 \ln(X_4) + \beta_5(X_5) + e$

Where Y= 22 months

22= 17.3469 + (-0.3066x0.11) +(1.0694x7.51)+(1.1678x0.15)+(-3.7947xln4.13)+(0.7143x3.0) +e

e = 22-17.3469+0.0337-8.0311-0.1751+5.3819-2.1429

e = -0. 2797

Y = 17.3469-0.0337+8.0311+0.1751-5.3819+2.1429-0.2797

Y =22.0007

DETERMINATION OF RESIDUAL ERRORS (MICROBIAL POPULATION PARAMETER ESTIMATES)

PS-1

$$\dot{Y} = \beta_0 + \beta_1 (\log X_1) + \beta_2(\log X_2) + \beta_3(\log X_3) + e$$

Where Y = 21 months

$e = Y - B_0 - B_1X_1 - B_2X_2 - B_3X_3$

$e = 21 - (-3.4065) - (-1.1967 \times 10.531) - (4.3871 \times 7.462) - (0.7663 \times 5.505)$

$e = 21 + 3.4065 + 12.6024 - 32.7365 - 4.2184$

$e = -0.6679$

Substituting the value of error in the original equation

$Y = -3.4065 - 12.6024 + 32.7365 + 4.2184 - 0.946$

$Y = 21$

PS-2

$$Y = \beta_0 + \beta_1 (\log X_1) + \beta_2(\log X_2) + \beta_3(\log X_3) + e$$

Where Y = 18 months

$e = Y - B_0 - B_1X_1 - B_2X_2 - B_3X_3$

$e = 18 - (-4.1313) - (-1.1274 \times 10.531) - (0.6973 \times 7.462) - (5.2258 \times 5.505)$

$e = 18 + 4.1313 + 11.8726 - 5.2032 - 28.7680$

$e = 0.0327$

Substituting the value of error in the equation above

$Y = -4.1313 - 11.8726 + 5.2032 + 28.7680$

$Y = 17.96$

PS-3

$Y = \beta_0 + \beta_1 (\log X_1) + \beta_2(\log X_2) + \beta_3(\log X_3) + e$

Where Y = 21 months

$e = Y - \beta_0 - \beta_1 (\log X_1) - \beta_2(\log X_2) - \beta_3(\log X_3)$

e = 21-(-3.4495)-(0.276x10.531)-(2.6303x7.462)-(1.3757x5.505)

e = 21+3.4495-2.9139-19.6272-7.5732

e = -5.664

Substituting the value of e in the equation above

Y = -3.4495+2.9139+19.6272+7.5732-5.664

Y = 21

PS-4

$Y = \beta_0 + \beta_1 (\log X_1) + \beta_2(\log X_2) + \beta_3(\log X_3) + e$

Y = 19 months

$e = Y - \beta_0 - \beta_1 (\log X_1) - \beta_2(\log X_2) - \beta_3(\log X_3)$

e = 19-(-3.2160)-(-0.1650x10.531)-(1.6862x7.462)-(2.0767x5.505)

e = 19+3.2160+1.7376+12.5824-11.4322

e = 25.1038

Substituting for Y

Y= -3.2160-1.7376-12.5824+11.4322+25.1038

Y = 19

PS-5

$Y = \beta_0 + \beta_1 (\log X_1) + \beta_2(\log X_2) + \beta_3(\log X_3) + e$

$Y = 19.5$

$e = Y - \beta_0 - \beta_1 (\log X_1) - \beta_2(\log X_2) - \beta_3(\log X_3)$

$e = 19.5-(-3.4631)-(-0.2216x10.531)-(1.3058x7.462)-(2.8189x5.505)$

$e = 19.5+3.4631+2.3336—9.7438-15.5180$

$e = 0.0349$

Substituting the error from the equation above,

$Y = -3.4631-2.3336+9.7438+15.5180$

$Y = 19.46$

PS-6

$Y = \beta_0 + \beta_1 (\log X_1) + \beta_2(\log X_2) + \beta_3(\log X_3) + e$

$Y = 30$ months

$e = Y - \beta_0 - \beta_1 (\log X_1) - \beta_2(\log X_2) - \beta_3(\log X_3)$

$e = 30-(-5.62024)-(-0.95373x10.531)-(5.3652x7.462)-(1.0368x5.505)$

$e = 30+5.62024+10.0437-40.0351-5.7075$

$e = 0.0786$

Substituting the value of error in the equation

$Y = -5.62024-10.0437+40.0351+5.7075-0.0786$

$Y = 30$

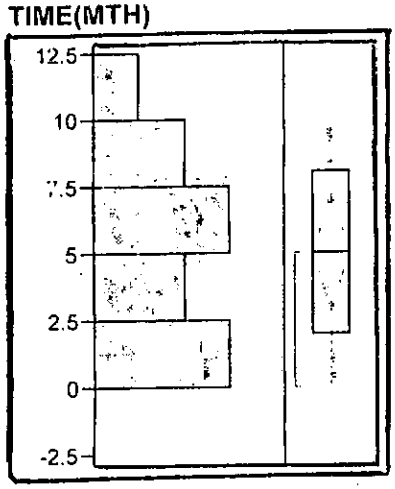
APPENDIX II

Regression analysis of microbial population model parameter estimates

DataTable=PS 1

Distributions

TIME(MTH)



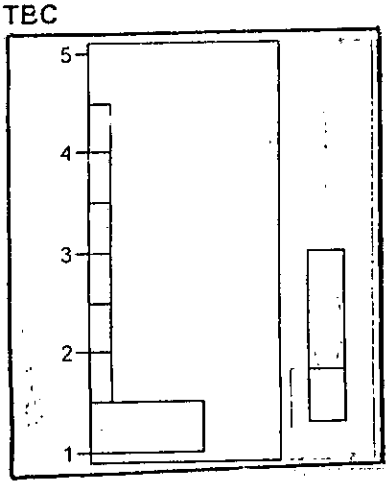
Quantiles

100.0%	maximum	10.000
99.5%		10.000
97.5%		10.000
90.0%		9.800
75.0%	quartile	8.000
50.0%	median	5.000
25.0%	quartile	2.000
10.0%		0.200
2.5%		0.000
0.5%		0.000
0.0%	minimum	0.000

Moments

Mean	5
Std Dev	3.3166248
Std Err Mean	1
upper 95% Mean	7.2281389
lower 95% Mean	2.7718611
N	11

TEC



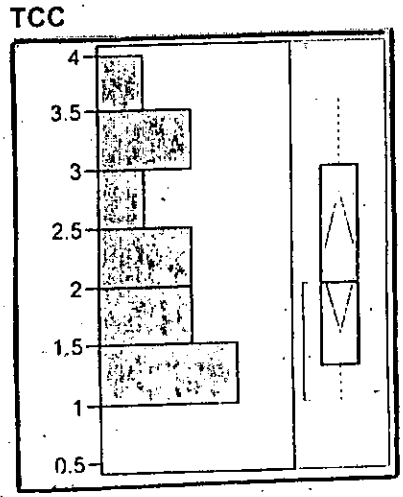
Quantiles

100.0%	maximum	4.3000
99.5%		4.3000
97.5%		4.3000
90.0%		4.2200
75.0%	quartile	3.0000
50.0%	median	1.8000
25.0%	quartile	1.2800
10.0%		1.2300
2.5%		1.2200
0.5%		1.2200
0.0%	minimum	1.2200

Moments

Mean	2.2227273
Std Dev	1.1183031
Std Err Mean	0.3371811
upper 95% Mean	2.9740135
lower 95% Mean	1.471441
N	11

TCC



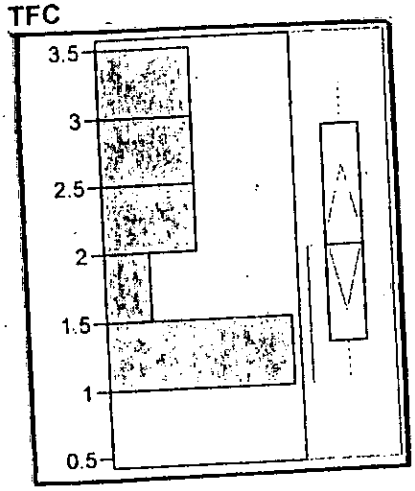
Quantiles

100.0%	maximum	3.6000
99.5%		3.6000
97.5%		3.6000
90.0%		3.5600
75.0%	quartile	3.0000
50.0%	median	2.0000
25.0%	quartile	1.3000
10.0%		1.0400
2.5%		1.0000
0.5%		1.0000
0.0%	minimum	1.0000

Moments

Mean	2.1636364
Std Dev	0.9069429
Std Err Mean	0.2734536
upper 95% Mean	2.7729289
lower 95% Mean	1.5543438
N	11

TFC



Quantiles

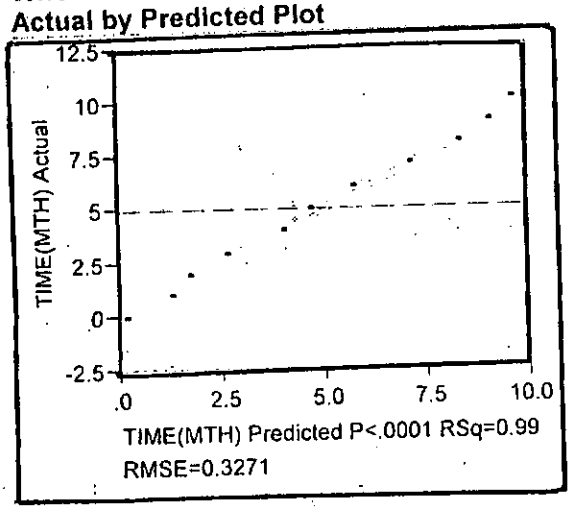
100.0%	maximum	3.2000
99.5%		3.2000
97.5%		3.2000
90.0%		3.1600
75.0%	quartile	2.9000
50.0%	median	2.0000
25.0%	quartile	1.3000
10.0%		1.0400
2.5%		1.0000
0.5%		1.0000
0.0%	minimum	1.0000

Moments

Mean	2.0545455
Std Dev	0.7992041
Std Err Mean	0.2409691
upper 95% Mean	2.5914581
lower 95% Mean	1.5176328
N	11

DataTable=PS 1

Response TIME(MTH)
Whole Model
Actual by Predicted Plot



Summary of Fit

R-square is the portion of variation attributed to the model, between 0 and 1. Root Mean Squared Error "RMSE" estimates the standard deviation of the residual.

RSquare	0.993192
RSquare Adj	0.990275
Root Mean Square Error	0.327072
Mean of Response	5
Observations (or Sum Wgts)	11

Analysis of Variance

The test that the whole model fits better than a simple mean, i.e. testing that all the parameters are zero except the intercept

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	3	109.25117	36.4171	340.4227
Error	7	0.74883	0.1070	Prob > F
C. Total	10	110.00000		<.0001

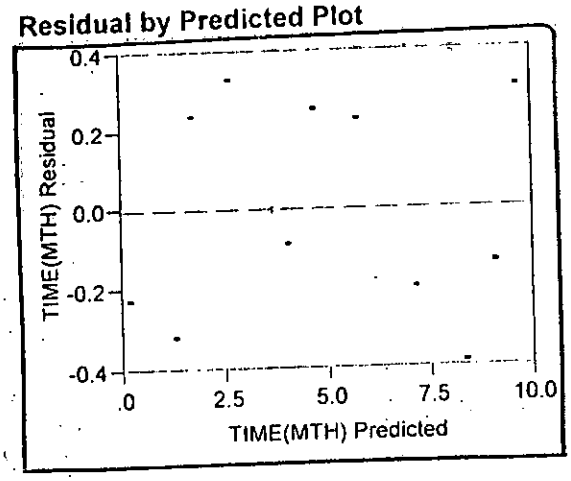
Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	-3.406559	0.321125	-10.61	<.0001
TBC	-1.196751	0.404378	-2.96	0.0211
TCC	4.387154	1.077085	4.07	0.0047
TFC	0.7663026	0.938454	0.82	0.4411

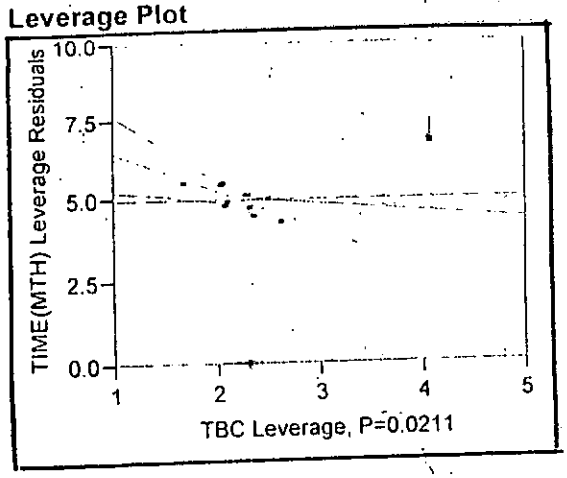
Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
TBC	1	1	0.9369545	8.7585	0.0211
TCC	1	1	1.7748106	16.5907	0.0047
TFC	1	1	0.0713281	0.6668	0.4411

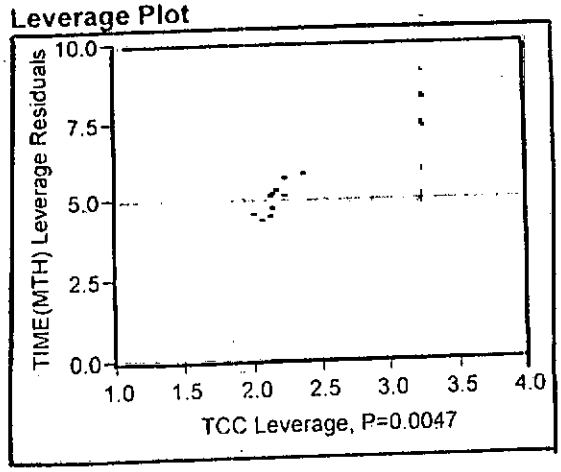
Residual by Predicted Plot



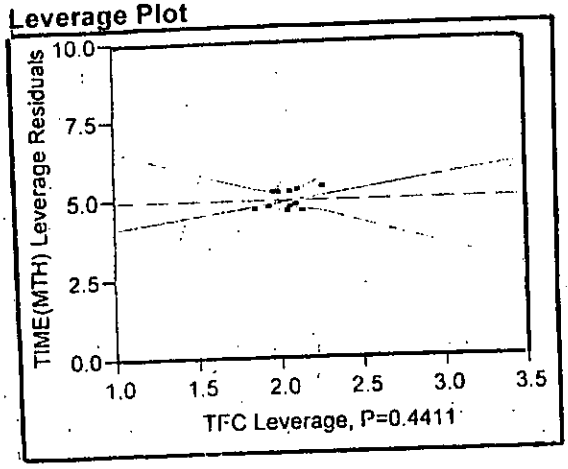
TBC
Leverage Plot



TCC
Leverage Plot

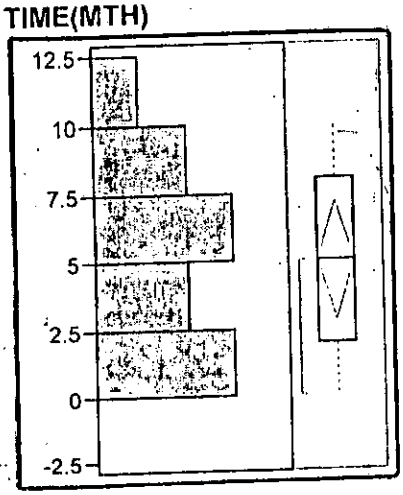


TFC
Leverage Plot



DataTable=PS 2

Distributions
TIME(MTH)



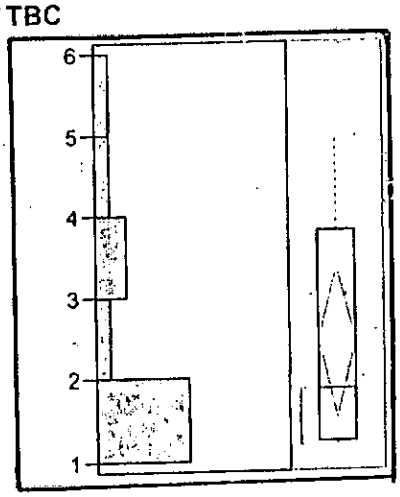
Quantiles

100.0%	maximum	10.000
99.5%		10.000
97.5%		10.000
90.0%		9.800
75.0%	quartile	8.000
50.0%	median	5.000
25.0%	quartile	2.000
10.0%		0.200
2.5%		0.000
0.5%		0.000
0.0%	minimum	0.000

Moments

Mean	5
Std Dev	3.3166248
Std Err Mean	1
upper 95% Mean	7.2281389
lower 95% Mean	2.7718611
N	11

TBC



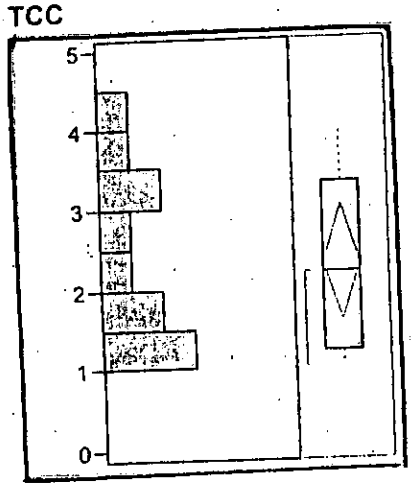
Quantiles

100.0%	maximum	5.0000
99.5%		5.0000
97.5%		5.0000
90.0%		4.8600
75.0%	quartile	3.8000
50.0%	median	1.8500
25.0%	quartile	1.2300
10.0%		1.1840
2.5%		1.1800
0.5%		1.1800
0.0%	minimum	1.1800

Moments

Mean	2.4309091
Std Dev	1.3842359
Std Err Mean	0.4173628
upper 95% Mean	3.3608514
lower 95% Mean	1.5009667
N	11

TCC



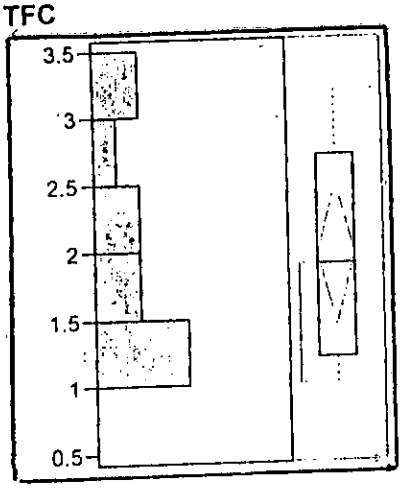
Quantiles

100.0%	maximum	4.0000
99.5%		4.0000
97.5%		4.0000
90.0%		3.9200
75.0%	quartile	3.3000
50.0%	median	2.2000
25.0%	quartile	1.2000
10.0%		1.0200
2.5%		1.0000
0.5%		1.0000
0.0%	minimum	1.0000

Moments

Mean	2.3090909
Std Dev	1.0606173
Std Err Mean	0.3197882
upper 95% Mean	3.0216233
lower 95% Mean	1.5965585
N	11

TFC



Quantiles

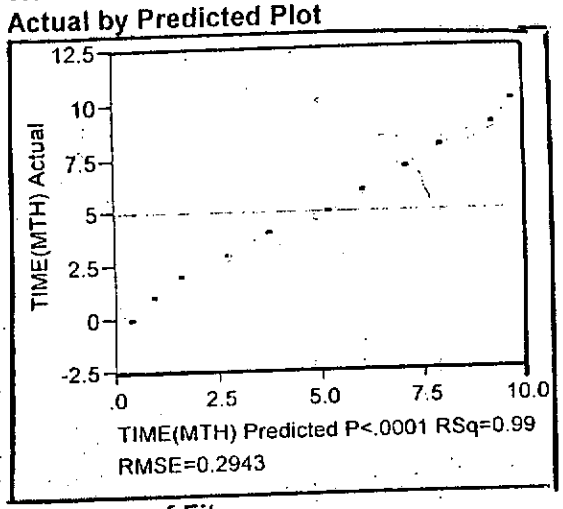
100.0%	maximum	3.2000
99.5%		3.2000
97.5%		3.2000
90.0%		3.1600
75.0%	quartile	2.7000
50.0%	median	1.9000
25.0%	quartile	1.2000
10.0%		1.0200
2.5%		1.0000
0.5%		1.0000
0.0%	minimum	1.0000

Moments

Mean	1.9636364
Std Dev	0.7788103
Std Err Mean	0.2348201
upper 95% Mean	2.4868482
lower 95% Mean	1.4404245
N	11

DataTable=PS2

Response TIME(MTH)
Whole Model
Actual by Predicted Plot



Summary of Fit

R-square is the portion of variation attributed to the model, between 0 and 1. Root Mean Squared Error "RMSE" estimates the standard deviation of the residual.

RSquare	0.994488
RSquare Adj	0.992126
Root Mean Square Error	0.294307
Mean of Response	5
Observations (or Sum Wgts)	11

Analysis of Variance

The test that the whole model fits better than a simple mean, i.e. testing that all the parameters are zero except the intercept

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	3	109.39368	36.4646	420.9875
Error	7	0.60632	0.0866	Prob > F
C. Total	10	110.00000		<.0001

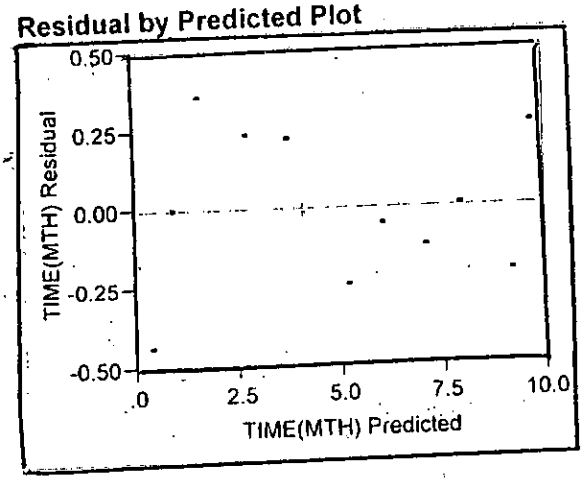
Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	-4.131364	0.73449	-5.62	0.0008
TBC	-1.127415	0.317378	-3.55	0.0093
TCC	0.6973637	1.464073	0.48	0.6484
TFC	5.2258815	2.231368	2.34	0.0517

Effect Tests

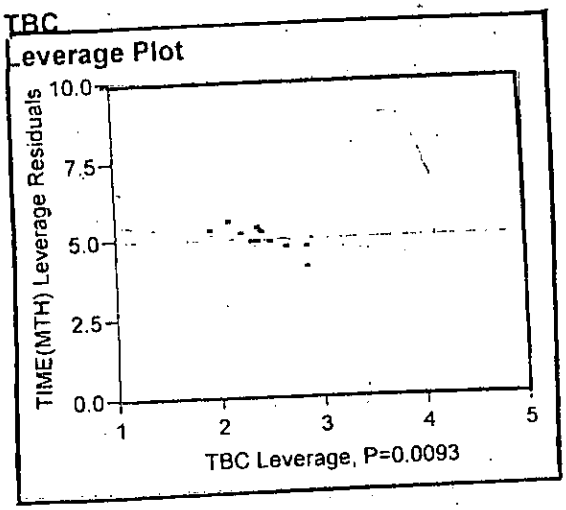
Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
TBC	1	1	1.0929911	12.6187	0.0093
TCC	1	1	0.0196515	0.2269	0.6484
TFC	1	1	0.4750927	5.4850	0.0517

Residual by Predicted Plot

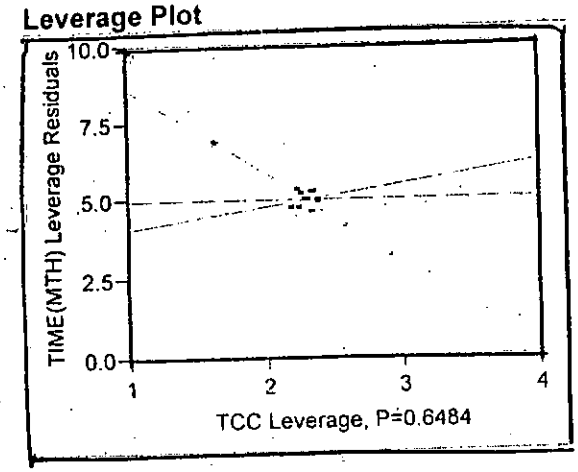


TBC

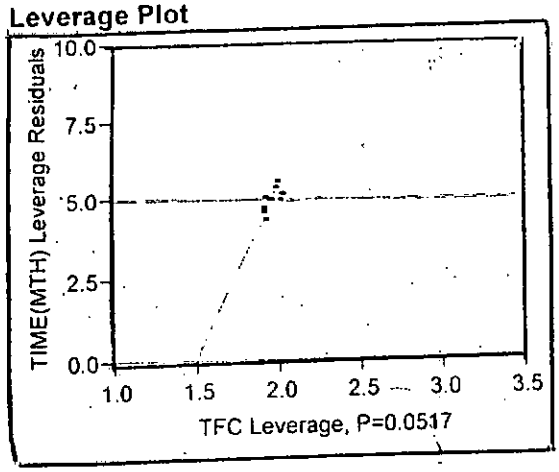
Leverage Plot



TCC
Leverage Plot

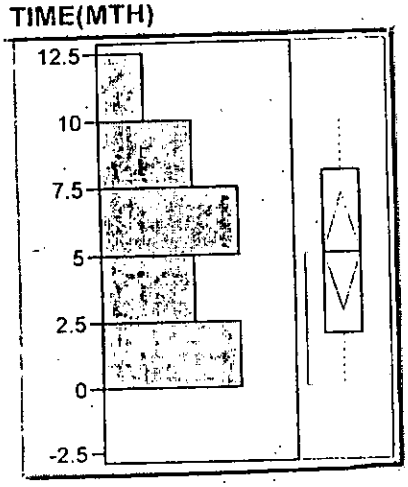


TFC
Leverage Plot



DataTable=PS 3

Distributions
TIME(MTH)



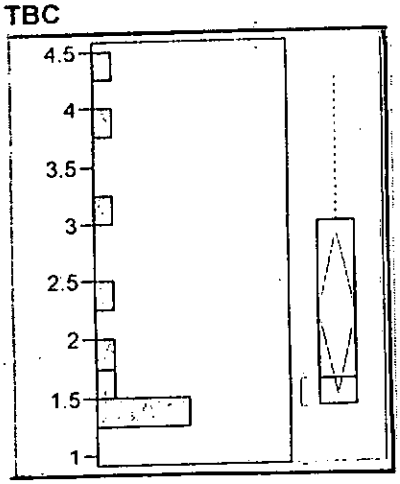
Quantiles

100.0%	maximum	10.000
99.5%		10.000
97.5%		10.000
90.0%		9.800
75.0%	quartile	8.000
50.0%	median	5.000
25.0%	quartile	2.000
10.0%		0.200
2.5%		0.000
0.5%		0.000
0.0%	minimum	0.000

Moments

Mean	5
Std Dev	3.3166248
Std Err Mean	1
upper 95% Mean	7.2281389
lower 95% Mean	2.7718611
N	11

TBC



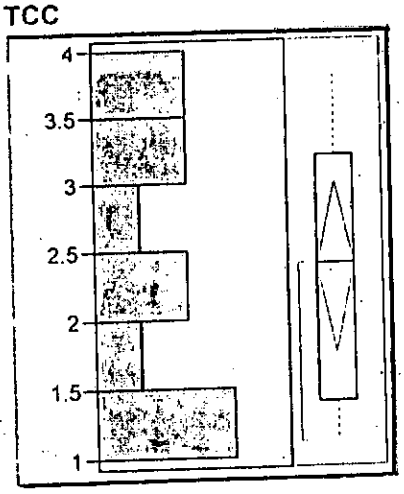
Quantiles

100.0%	maximum	4.3000
99.5%		4.3000
97.5%		4.3000
90.0%		4.2000
75.0%	quartile	3.0000
50.0%	median	1.6300
25.0%	quartile	1.4100
10.0%		1.3920
2.5%		1.3900
0.5%		1.3900
0.0%	minimum	1.3900

Moments

Mean	2.1963636
Std Dev	1.0560234
Std Err Mean	0.318403
upper 95% Mean	2.9058098
lower 95% Mean	1.4869175
N	11

TCC



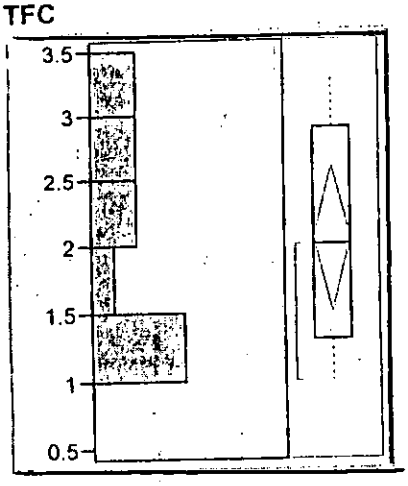
Quantiles

100.0%	maximum	3.8000
99.5%		3.8000
97.5%		3.8000
90.0%		3.7400
75.0%	quartile	3.2000
50.0%	median	2.4000
25.0%	quartile	1.4000
10.0%		1.1480
2.5%		1.1100
0.5%		1.1100
0.0%	minimum	1.1100

Moments

Mean	2.3736364
Std Dev	0.9304007
Std Err Mean	0.2805264
upper 95% Mean	2.9986881
lower 95% Mean	1.7485847
N	11

TFC



Quantiles

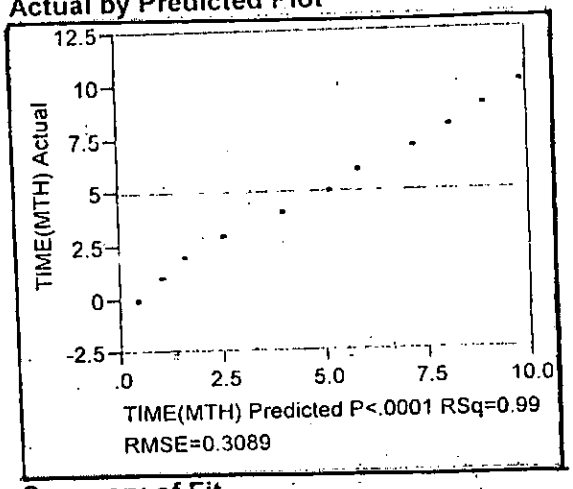
100.0%	maximum	3.3000
99.5%		3.3000
97.5%		3.3000
90.0%		3.2600
75.0%	quartile	2.9000
50.0%	median	2.0000
25.0%	quartile	1.3000
10.0%		1.0200
2.5%		1.0000
0.5%		1.0000
0.0%	minimum	1.0000

Moments

Mean	2.0454545
Std Dev	0.8201995
Std Err Mean	0.2472995
upper 95% Mean	2.5964721
lower 95% Mean	1.494437
N	11

DataTable=PS 3

Response TIME(MTH)
Whole Model
Actual by Predicted Plot



Summary of Fit

R-square is the portion of variation attributed to the model, between 0 and 1. Root Mean Squared Error "RMSE" estimates the standard deviation of the residual.

RSquare	0.993927
RSquare Adj	0.991324
Root Mean Square Error	0.308927
Mean of Response	5
Observations (or Sum Wgts)	11

Analysis of Variance

The test that the whole model fits better than a simple mean, i.e. testing that all the parameters are zero except the intercept

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	3	109.33195	36.4440	381.8683
Error	7	0.66805	0.0954	Prob > F
C. Total	10	110.00000		<.0001

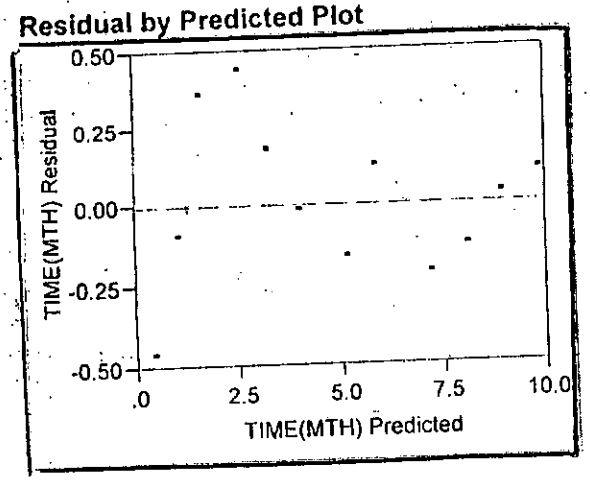
Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	-3.449597	0.271759	-12.69	<.0001
TBC	-0.276725	0.286664	-0.97	0.3665
TCC	2.6303107	1.206558	2.18	0.0656
TFC	1.3757261	1.562422	0.88	0.4078

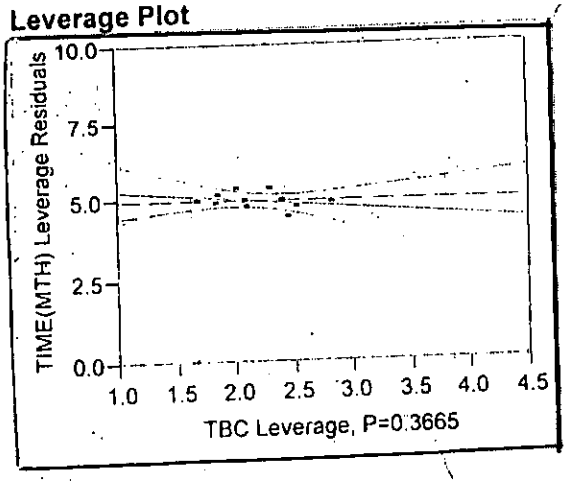
Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
TBC	1	1	0.08893331	0.9319	0.3665
TCC	1	1	0.45355493	4.7525	0.0656
TFC	1	1	0.07399106	0.7753	0.4078

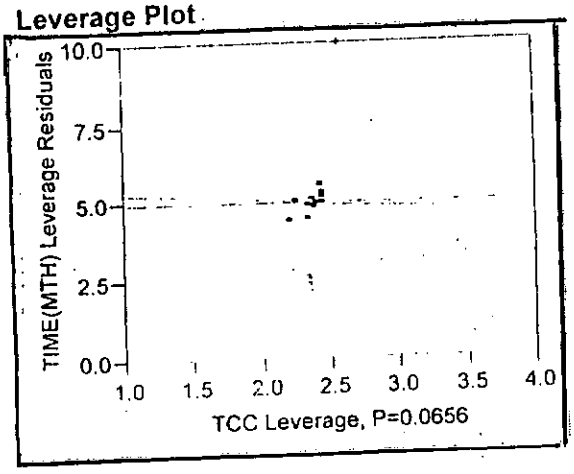
Residual by Predicted Plot



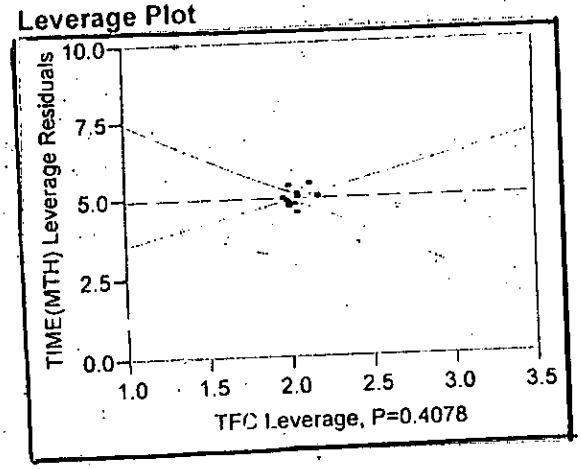
TBC
Leverage Plot



TCC
Leverage Plot

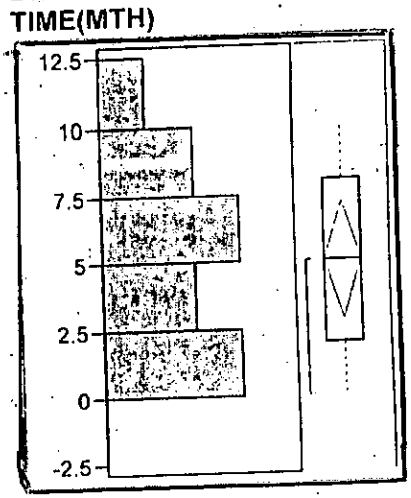


TFC
Leverage Plot



DataTable=PS4

Distributions
TIME(MTH)



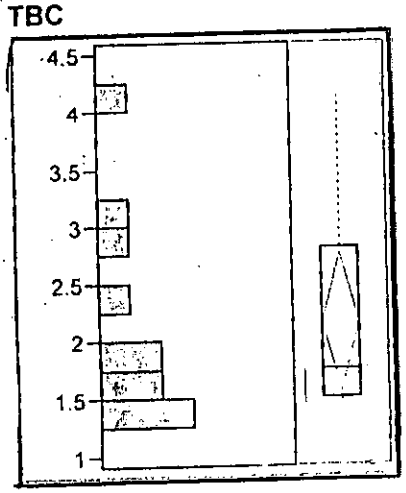
Quantiles

100.0%	maximum	10.000
99.5%		10.000
97.5%		10.000
90.0%		9.800
75.0%	quartile	8.000
50.0%	median	5.000
25.0%	quartile	2.000
10.0%		0.200
2.5%		0.000
0.5%		0.000
0.0%	minimum	0.000

Moments

Mean	5
Std Dev	3.3166248
Std Err Mean	1
upper 95% Mean	7.2281389
lower 95% Mean	2.7718611
N	11

TBC



Quantiles

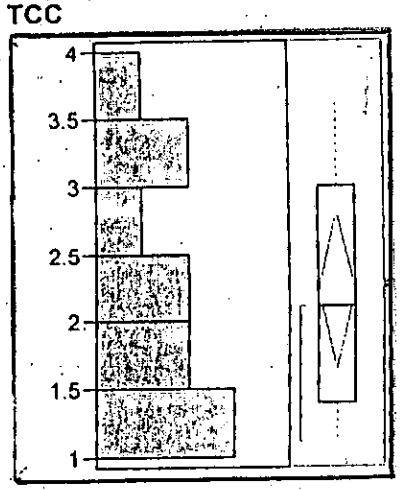
100.0%	maximum	4.1500
99.5%		4.1500
97.5%		4.1500
90.0%		3.9600
75.0%	quartile	2.8100
50.0%	median	1.7500
25.0%	quartile	1.4900
10.0%		1.4540

2.5%		1.4500
0.5%		1.4500
0.0%	minimum	1.4500

Moments

Mean	2.1445455
Std Dev	0.8944089
Std Err Mean	0.2696744
upper 95% Mean	2.7454175
lower 95% Mean	1.5436734
N	11

TCC



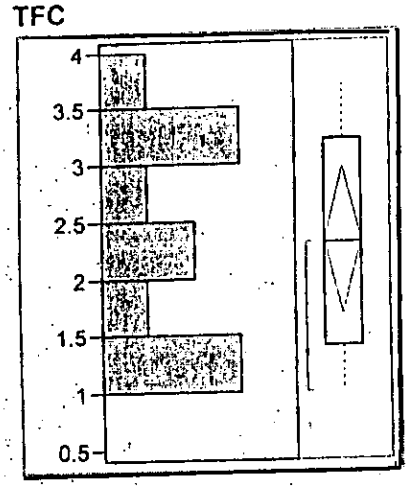
Quantiles

100.0%	maximum	3.6000
99.5%		3.6000
97.5%		3.6000
90.0%		3.5600
75.0%	quartile	3.0000
50.0%	median	2.1000
25.0%	quartile	1.4000
10.0%		1.1200
2.5%		1.1000
0.5%		1.1000
0.0%	minimum	1.1000

Moments

Mean	2.2272727
Std Dev	0.879876
Std Err Mean	0.2652926
upper 95% Mean	2.8183815
lower 95% Mean	1.636164
N	11

TFC



Quantiles

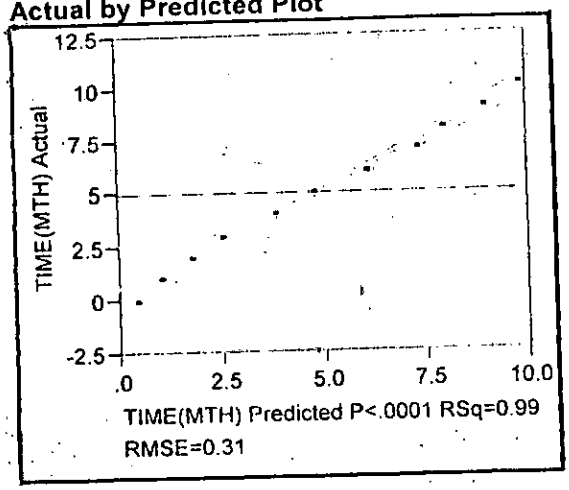
100.0%	maximum	3.7000
99.5%		3.7000
97.5%		3.7000
90.0%		3.6400
75.0%	quartile	3.2000
50.0%	median	2.3000
25.0%	quartile	1.4000
10.0%		1.0400
2.5%		1.0000
0.5%		1.0000
0.0%	minimum	1.0000

Moments

Mean	2.3181818
Std Dev	0.944265
Std Err Mean	0.2847066
upper 95% Mean	2.9525477
lower 95% Mean	1.683816
N	11

DataTable=PS 4

Response TIME(MTH)
Whole Model
Actual by Predicted Plot



Summary of Fit

R-square is the portion of variation attributed to the model, between 0 and 1. Root Mean Squared Error "RMSE" estimates the standard deviation of the residual.

RSquare	0.993885
RSquare Adj	0.991265
Root Mean Square Error	0.309978
Mean of Response	5
Observations (or Sum Wgts)	11

Analysis of Variance

The test that the whole model fits better than a simple mean, i.e. testing that all the parameters are zero except the intercept

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	3	109.32739	36.4425	379.2666
Error	7	0.67261	0.0961	Prob > F
C. Total	10	110.00000		<.0001

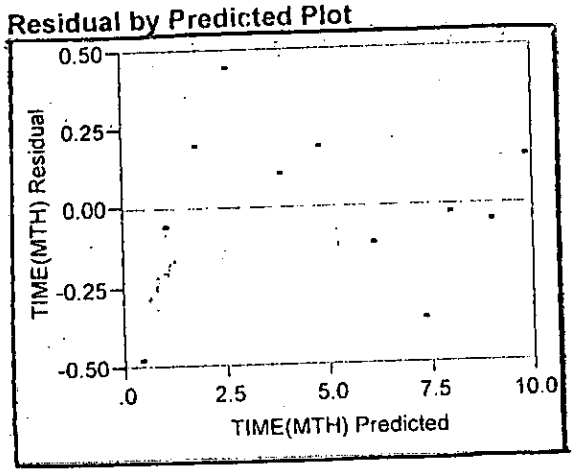
Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	-3.216096	0.271159	-11.86	<.0001
TBC	-0.165058	0.374687	-0.44	0.6728
TCC	1.6862682	1.547861	1.09	0.3120
TFC	2.0767531	1.21043	1.72	0.1299

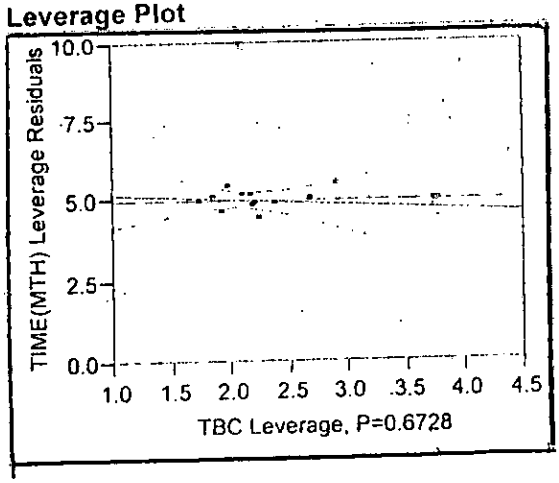
Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
TBC	1	1	0.01864659	0.1941	0.6728
TCC	1	1	0.11403879	1.1868	0.3120
TFC	1	1	0.28284848	2.943?	0.1299

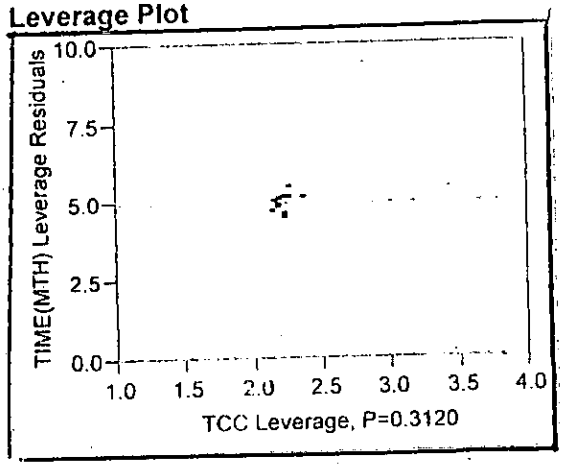
Residual by Predicted Plot



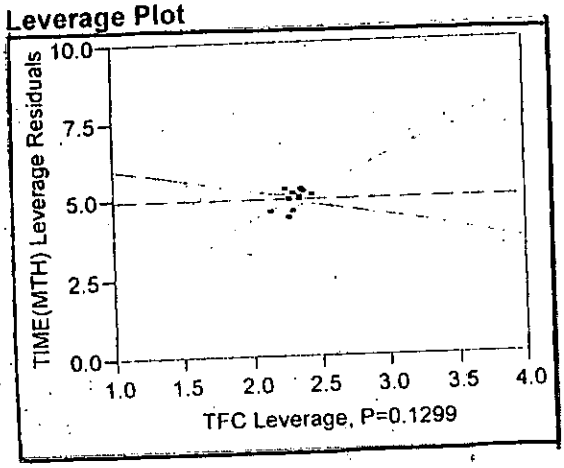
TBC
Leverage Plot



TCC
Leverage Plot

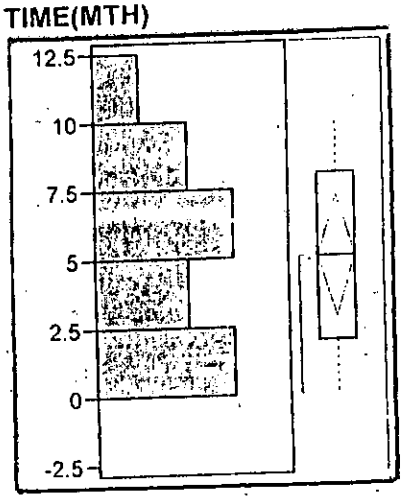


TFC
Leverage Plot



DataTable=PS 5

Distributions
TIME(MTH)



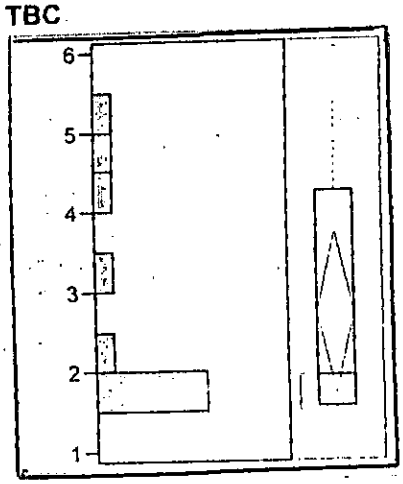
Quantiles

100.0%	maximum	10.000
99.5%		10.000
97.5%		10.000
90.0%		9.800
75.0%	quartile	8.000
50.0%	median	5.000
25.0%	quartile	2.000
10.0%		0.200
2.5%		0.000
0.5%		0.000
0.0%	minimum	0.000

Moments

Mean	5
Std Dev	3.3166248
Std Err Mean	1
upper 95% Mean	7.2281389
lower 95% Mean	2.7718611
N	11

TBC



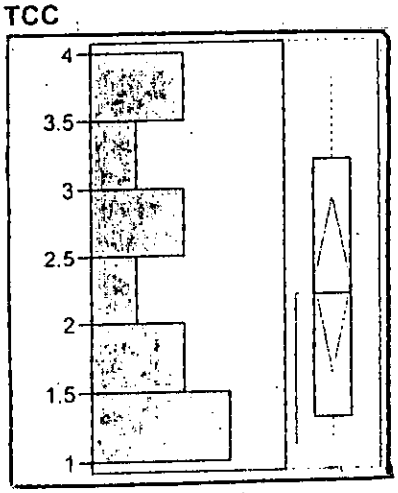
Quantiles

100.0%	maximum	5.3600
99.5%		5.3600
97.5%		5.3600
90.0%		5.2780
75.0%	quartile	4.2200
50.0%	median	1.9400
25.0%	quartile	1.5500
10.0%		1.5060
2.5%		1.5000
0.5%		1.5000
0.0%	minimum	1.5000

Moments

Mean	2.7254545
Std Dev	1.4996157
Std Err Mean	0.4521511
upper 95% Mean	3.7329101
lower 95% Mean	1.717999
N	11

TCC



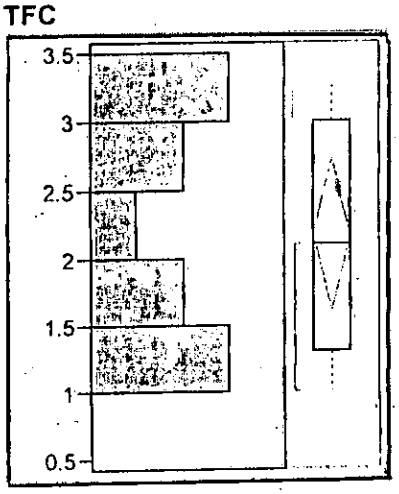
Quantiles

100.0%	maximum	3.8000
99.5%		3.8000
97.5%		3.8000
90.0%		3.7600
75.0%	quartile	3.2000
50.0%	median	2.2000
25.0%	quartile	1.3000
10.0%		1.1200
2.5%		1.1000
0.5%		1.1000
0.0%	minimum	1.1000

Moments

Mean	2.2727273
Std Dev	0.9788676
Std Err Mean	0.2951397
upper 95% Mean	2.9303395
lower 95% Mean	1.6151151
N	11

TFC



Quantiles

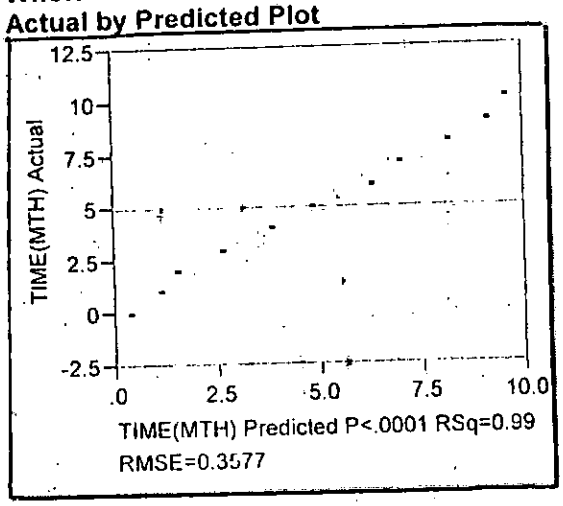
100.0%	maximum	3.3000
99.5%		3.3000
97.5%		3.3000
90.0%		3.2800
75.0%	quartile	3.0000
50.0%	median	2.1000
25.0%	quartile	1.3000
10.0%		1.0400
2.5%		1.0000
0.5%		1.0000
0.0%	minimum	1.0000

Moments

Mean	2.1636364
Std Dev	0.8297864
Std Err Mean	0.25019
upper 95% Mean	2.7210944
lower 95% Mean	1.6061783
N	11

DataTable=PS 5

Response TIME(MTH)
Whole Model
Actual by Predicted Plot



Summary of Fit

R-square is the portion of variation attributed to the model, between 0 and 1. Root Mean Squared Error "RMSE" estimates the standard deviation of the residual.

RSquare	0.991857
RSquare Adj	0.988367
Root Mean Square Error	0.357724
Mean of Response	5
Observations (or Sum Wgts)	11

Analysis of Variance

The test that the whole model fits better than a simple mean, i.e. testing that all the parameters are zero except the intercept

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	3	109.10424	36.3681	284.2006
Error	7	0.89576	0.1280	Prob > F
C. Total	10	110.00000		<.0001

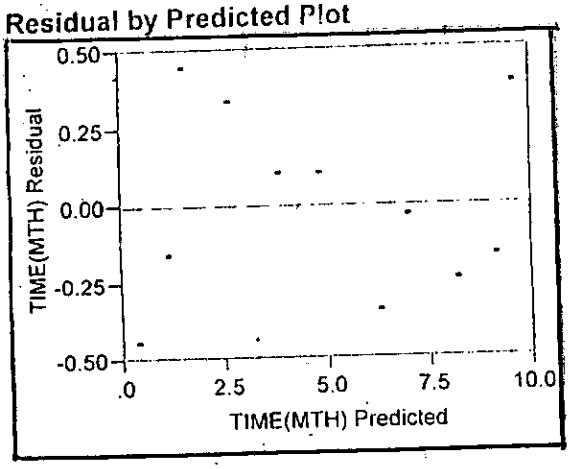
Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	-3.463151	0.391731	-8.84	<.0001
TBC	-0.221601	0.393228	-0.56	0.5907
TCC	1.3058815	1.819701	0.72	0.4962
TFC	2.8189592	1.590928	1.77	0.1197

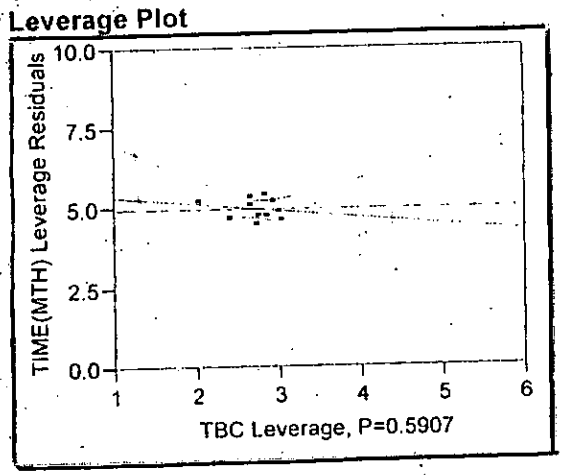
Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
TBC	1	1	0.04063972	0.3176	0.5907
TCC	1	1	0.06590263	0.5150	0.4962
TFC	1	1	0.40176470	3.1396	0.1197

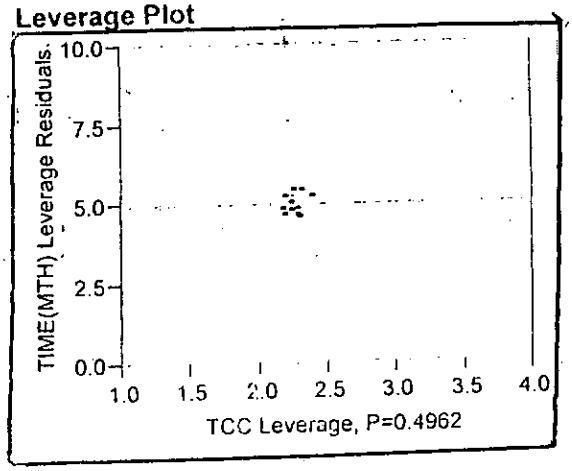
Residual by Predicted Plot



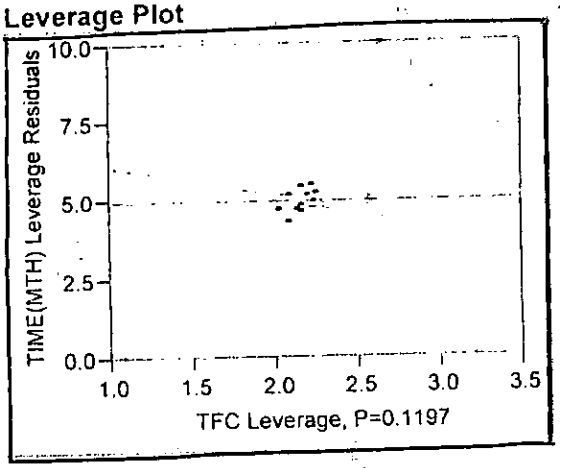
TBC
Leverage Plot



TCC
Leverage Plot

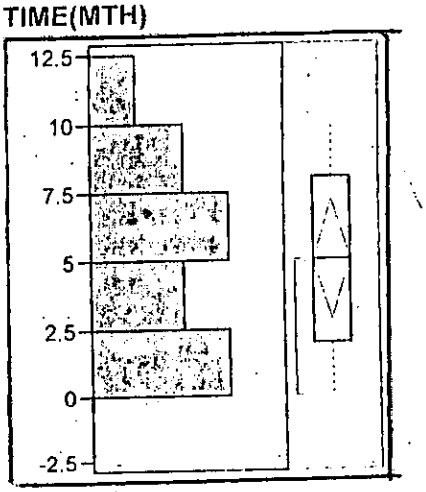


TFC
Leverage Plot



DataTable=PS 6

Distributions
TIME(MTH)



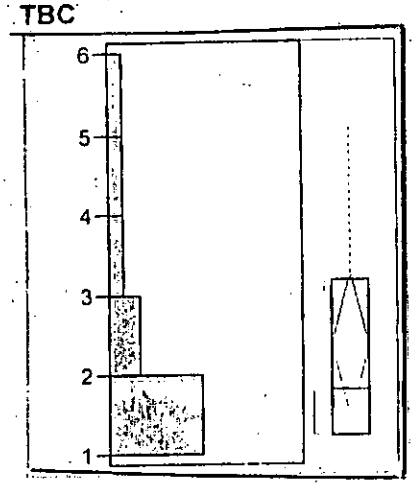
Quantiles

100.0%	maximum	10.000
99.5%		10.000
97.5%		10.000
90.0%		9.800
75.0%	quartile	8.000
50.0%	median	5.000
25.0%	quartile	2.000
10.0%		0.200
2.5%		0.000
0.5%		0.000
0.0%	minimum	0.000

Moments

Mean	5
Std Dev	3.3166248
Std Err Mean	1
upper 95% Mean	7.2281389
lower 95% Mean	2.7718611
N	11

TBC



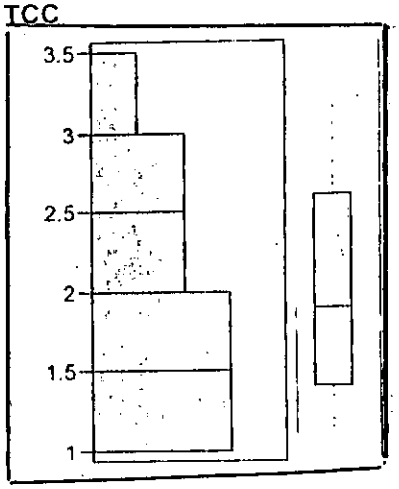
Quantiles

100.0%	maximum	5.0900
99.5%		5.0900
97.5%		5.0900
90.0%		4.9500
75.0%	quartile	3.1700
50.0%	median	1.8100
25.0%	quartile	1.2300
10.0%		1.2220
2.5%		1.2200
0.5%		1.2200
0.0%	minimum	1.2200

Moments

Mean	2.3590909
Std Dev	1.3714916
Std Err Mean	0.4135203
upper 95% Mean	3.2804715
lower 95% Mean	1.4377103
N	11

TCC



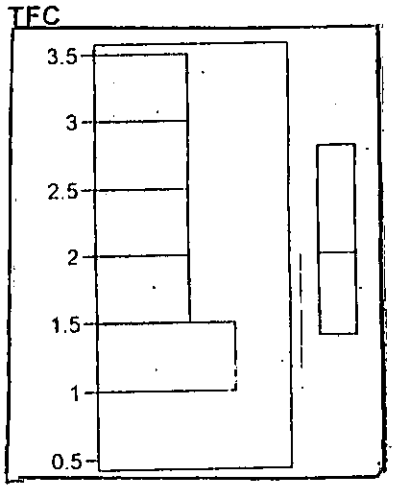
Quantiles

100.0%	maximum	3.2000
99.5%		3.2000
97.5%		3.2000
90.0%		3.1400
75.0%	quartile	2.6000
50.0%	median	1.9000
25.0%	quartile	1.4000
10.0%		1.1200
2.5%		1.1000
0.5%		1.1000
0.0%	minimum	1.1000

Moments

Mean	2
Std Dev	0.7028513
Std Err Mean	0.2119177
upper 95% Mean	2.472182
lower 95% Mean	1.527818
N	11

TFC



Quantiles

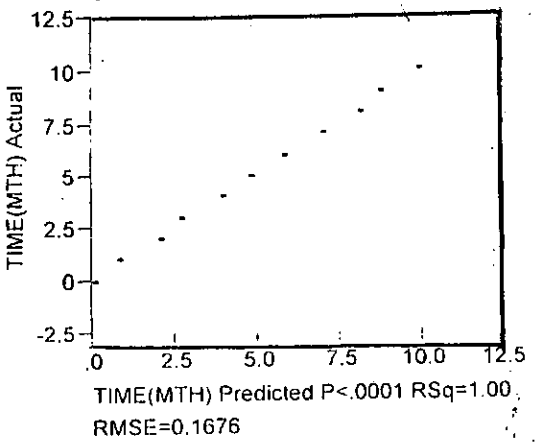
100.0%	maximum	3.2000
99.5%		3.2000
97.5%		3.2000
90.0%		3.1600
75.0%	quartile	2.8000
50.0%	median	2.0000
25.0%	quartile	1.4000
10.0%		1.0400
2.5%		1.0000
0.5%		1.0000
0.0%	minimum	1.0000

Moments

Mean	2.0636364
Std Dev	0.7526921
Std Err Mean	0.2269452
upper 95% Mean	2.5693018
lower 95% Mean	1.5579709
N	11

DataTable=PS 6

Response TIME(MTH)
Whole Model
Actual by Predicted Plot



Summary of Fit

R-square is the portion of variation attributed to the model, between 0 and 1. Root Mean Squared Error "RMSE" estimates the standard deviation of the residual.

RSquare	0.998213
RSquare Adj	0.997447
Root Mean Square Error	0.167582
Mean of Response	5
Observations (or Sum Wgts)	11

Analysis of Variance

The test that the whole model fits better than a simple mean, i.e. testing that all the parameters are zero except the intercept

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	3	109.80341	36.6011	1303.281
Error	7	0.19659	0.0281	Prob > F
C. Total	10	110.00000		<.0001

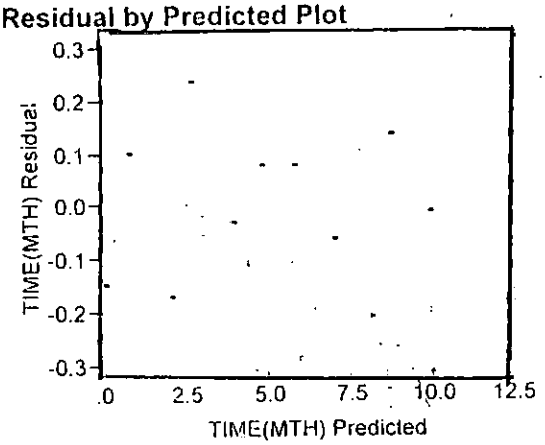
Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	-5.62024	0.469951	-11.96	<.0001
TBC	-0.953703	0.266103	-3.58	0.0089
TCC	5.365221	1.735815	3.09	0.0175
TFC	1.0368447	1.213908	0.85	0.4213

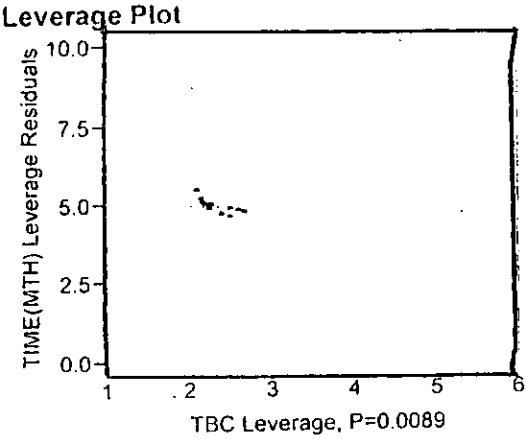
Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
TBC	1	1	0.36073146	12.8448	0.0089
TCC	1	1	0.26830263	9.5536	0.0175
TFC	1	1	0.02048859	0.7296	0.4213

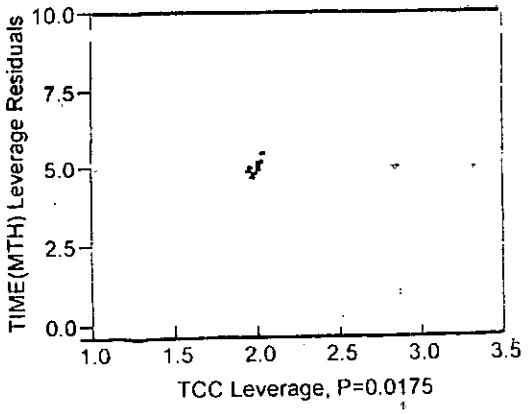
Residual by Predicted Plot



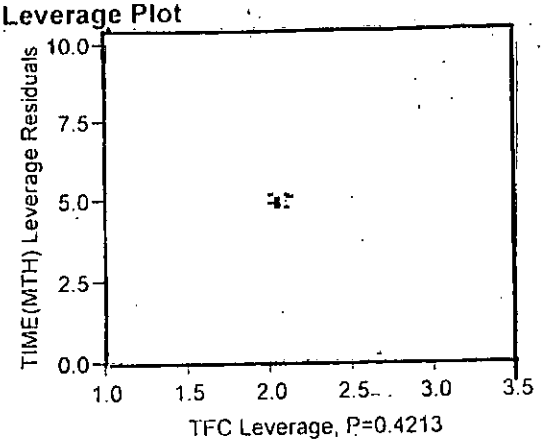
TBC
Leverage Plot



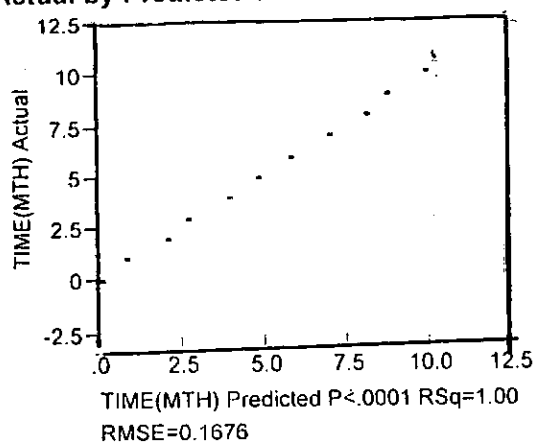
TCC
Leverage Plot



TFC
Leverage Plot



Response TIME(MTH)
Whole Model
Actual by Predicted Plot



Summary of Fit

R-square is the portion of variation attributed to the model, between 0 and 1. Root Mean Squared Error "RMSE" estimates the standard deviation of the residual.

RSquare	0.998213
RSquare Adj	0.997447
Root Mean Square Error	0.167582
Mean of Response	5
Observations (or Sum Wgts)	11

Analysis of Variance

The test that the whole model fits better than a simple mean, i.e. testing that all the parameters are zero except the intercept

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	3	109.80341	36.6011	1303.281
Error	7	0.19659	0.0281	Prob > F
C. Total	10	110.00000		<.0001

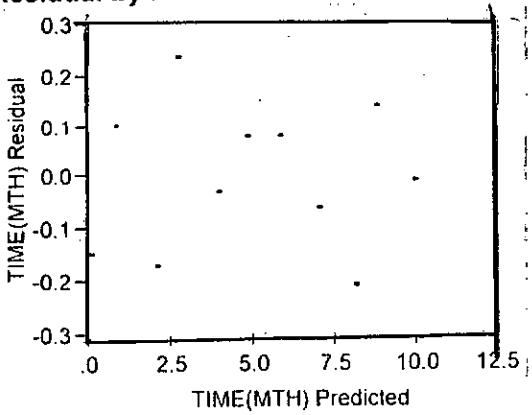
Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	-5.62024	0.469951	-11.96	<.0001
TBC	-0.953703	0.266103	-3.58	0.0089
TCC	5.365221	1.735815	3.09	0.0175
TFC	1.0368447	1.213908	0.85	0.4213

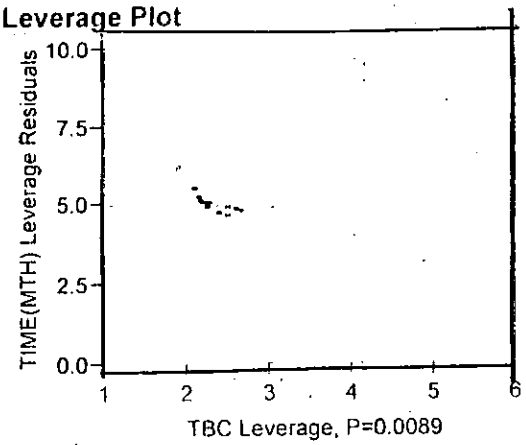
Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
TBC	1	1	0.36073146	12.8448	0.0089
TCC	1	1	0.26830263	9.5536	0.0175
TFC	1	1	0.02048859	0.7296	0.4213

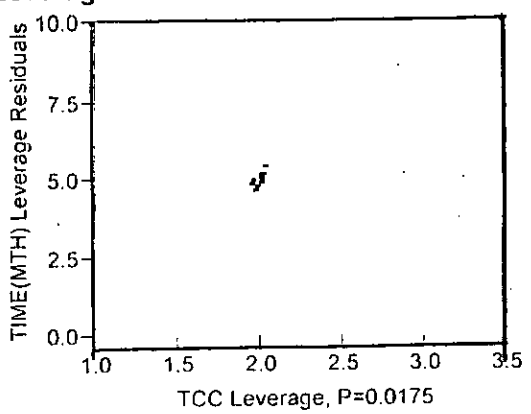
Residual by Predicted Plot

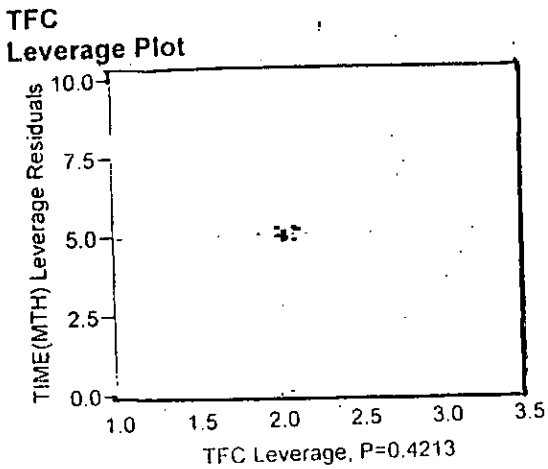


TBC
Leverage Plot



TCC
Leverage Plot



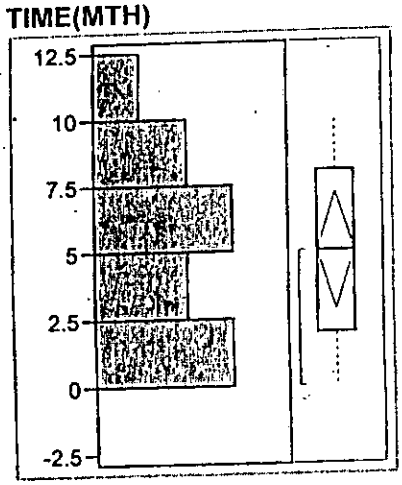

TFC
Leverage Plot
TIME(MTH) Leverage Residuals
10.0
7.5
5.0
2.5
0.0
1.0
1.5
2.0
2.5
3.0
3.5
TFC Leverage, P=0.4213

Regression analysis of physico-chemical model parameter estimate

DataTable=PS 1

Distributions

TIME(MTH)



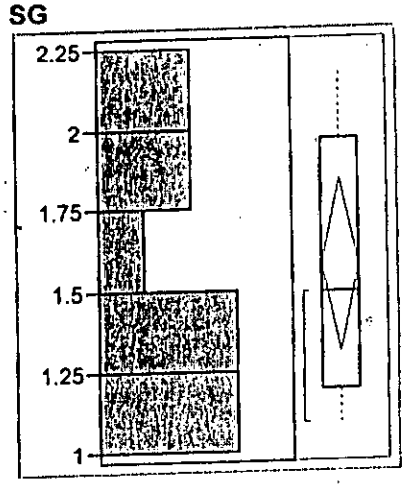
Quantiles

100.0%	maximum	10.000
99.5%		10.000
97.5%		10.000
90.0%		9.800
75.0%	quartile	8.000
50.0%	median	5.000
25.0%	quartile	2.000
10.0%		0.200
2.5%		0.000
0.5%		0.000
0.0%	minimum	0.000

Moments

Mean	5
Std Dev	3.3166248
Std Err Mean	1
upper 95% Mean	7.2281389
lower 95% Mean	2.7718611
N	11

SG



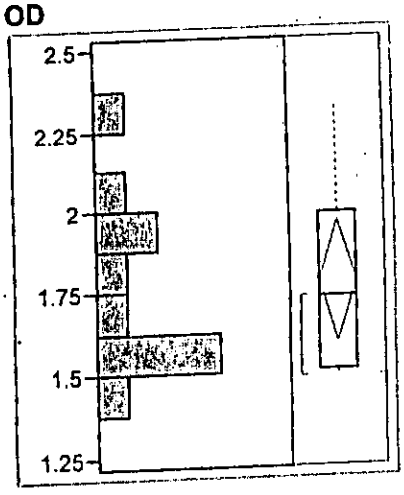
Quantiles

100.0%	maximum	2.1772
99.5%		2.1772
97.5%		2.1772
90.0%		2.1601
75.0%	quartile	1.9637
50.0%	median	1.4942
25.0%	quartile	1.1926
10.0%		1.1022
2.5%		1.0896
0.5%		1.0896
0.0%	minimum	1.0896

Moments

Mean	1.5748909
Std Dev	0.3968166
Std Err Mean	0.1196447
upper 95% Mean	1.8414759
lower 95% Mean	1.3083059
N	11

OD



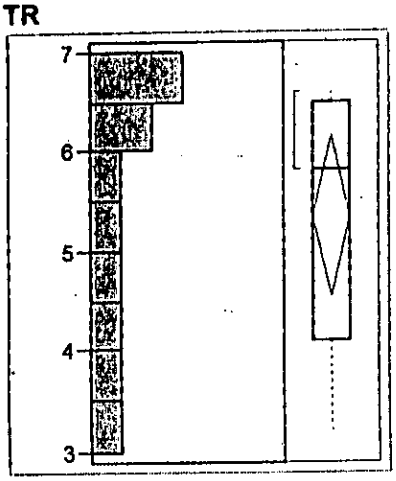
Quantiles

100.0%	maximum	2.3200
99.5%		2.3200
97.5%		2.3200
90.0%		2.2760
75.0%	quartile	1.9900
50.0%	median	1.7300
25.0%	quartile	1.5100
10.0%		1.4920
2.5%		1.4900
0.5%		1.4900
0.0%	minimum	1.4900

Moments

Mean	1.7763636
Std Dev	0.2795809
Std Err Mean	0.0842968
upper 95% Mean	1.9641886
lower 95% Mean	1.5885387
N	11

TR



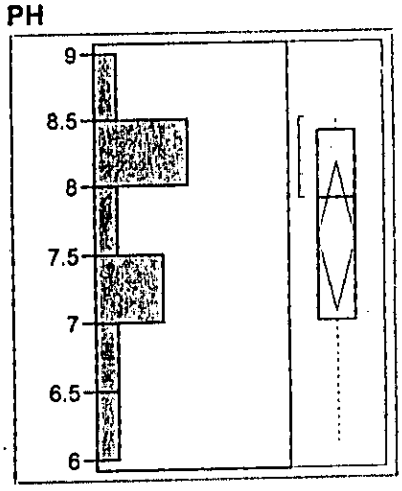
Quantiles

100.0%	maximum	6.6000
99.5%		6.6000
97.5%		6.6000
90.0%		6.5800
75.0%	quartile	6.5000
50.0%	median	5.8000
25.0%	quartile	4.1000
10.0%		3.3200
2.5%		3.2000
0.5%		3.2000
0.0%	minimum	3.2000

Moments

Mean	5.3454545
Std Dev	1.2143835
Std Err Mean	0.3661504
upper 95% Mean	6.1612885
lower 95% Mean	4.5296206
N	11

PH



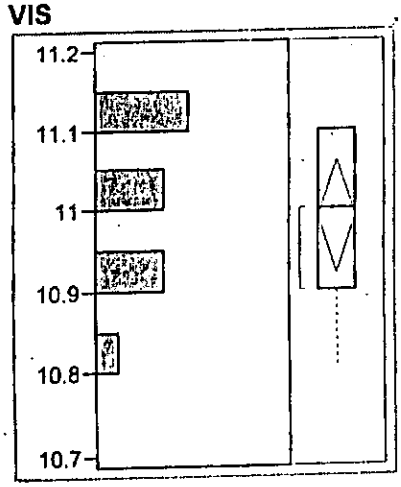
Quantiles

100.0%	maximum	8.5000
99.5%		8.5000
97.5%		8.5000
90.0%		8.4800
75.0%	quartile	8.4000
50.0%	median	7.9000
25.0%	quartile	7.0000
10.0%		6.2000
2.5%		6.1000
0.5%		6.1000
0.0%	minimum	6.1000

Moments

Mean	7.6090909
Std Dev	0.8067894
Std Err Mean	0.2432561
upper 95% Mean	8.1510994
lower 95% Mean	7.0670824
N	11

VIS



Quantiles

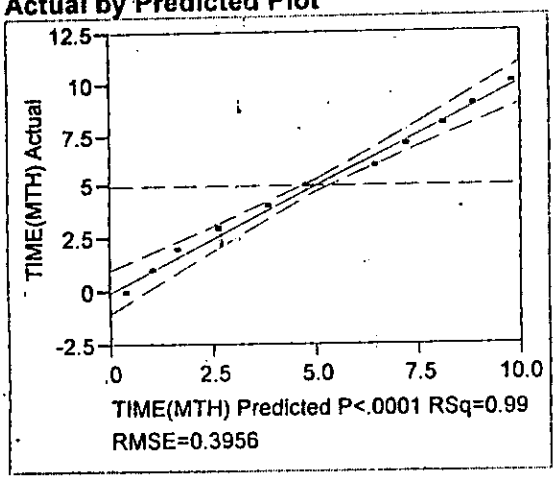
100.0%	maximum	11.100
99.5%		11.100
97.5%		11.100
90.0%		11.100
75.0%	quartile	11.100
50.0%	median	11.000
25.0%	quartile	10.900
10.0%		10.820
2.5%		10.800
0.5%		10.800
0.0%	minimum	10.800

Moments

Mean	10.990909
Std Dev	0.1044466
Std Err Mean	0.0314918
upper 95% Mean	11.061077
lower 95% Mean	10.920741
N	11

DataTable=PS 1

Response TIME(MTH)
Actual by Predicted Plot



Summary of Fit

R-square is the portion of variation attributed to the model, between 0 and 1. Root Mean Squared Error "RMSE" estimates the standard deviation of the residual.

RSquare	0.992886
RSquare Adj	0.985772
Root Mean Square Error	0.395617
Mean of Response	5
Observations (or Sum Wgts)	11

Analysis of Variance

The test that the whole model fits better than a simple mean, i.e. testing that all the parameters are zero except the Intercept

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	5	109.21744	21.8435	139.5637
Error	5	0.78256	0.1565	Prob > F
C. Total	10	110.00000		<.0001

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	55.576943	59.28496	0.94	0.3916
SG	-5.087866	0.924425	-5.50	0.0027
OD	-3.821861	4.886011	-0.78	0.4695
TR	-0.487706	0.904919	-0.54	0.6130
PH	-2.044592	2.033798	-1.01	0.3609
VIS	-1.602288	4.891654	-0.33	0.7565

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
SG	1	1	4.7410833	30.2920	0.0027
OD	1	1	0.0957614	0.6118	0.4695
TR	1	1	0.0454618	0.2905	0.6130
PH	1	1	0.1581784	1.0106	0.3609
VIS	1	1	0.0167926	0.1073	0.7565

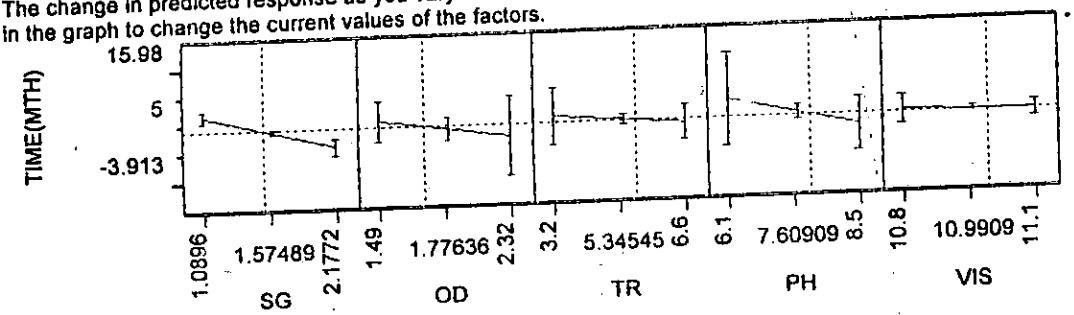
Scaled Estimates

Continuous factors centered by mean, scaled by range/2

Term	Scaled Estimate	Plot Estimate	Std Error	t Ratio	Prob>|t|
Intercept	5		0.119283	41.92	<.0001
SG	-2.766782		0.502702	-5.50	0.0027
OD	-1.586072		2.027695	-0.78	0.4695
TR	-0.829101		1.538363	-0.54	0.6130
PH	-2.45351		2.440557	-1.01	0.3609
VIS	-0.240343		0.733748	-0.33	0.7565

Prediction Profiler

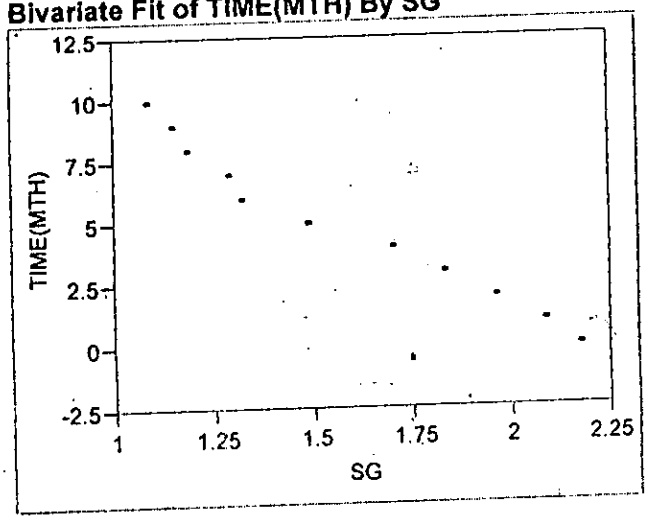
The change in predicted response as you vary one factor at a time, holding the other factors at their current values. Click in the graph to change the current values of the factors.



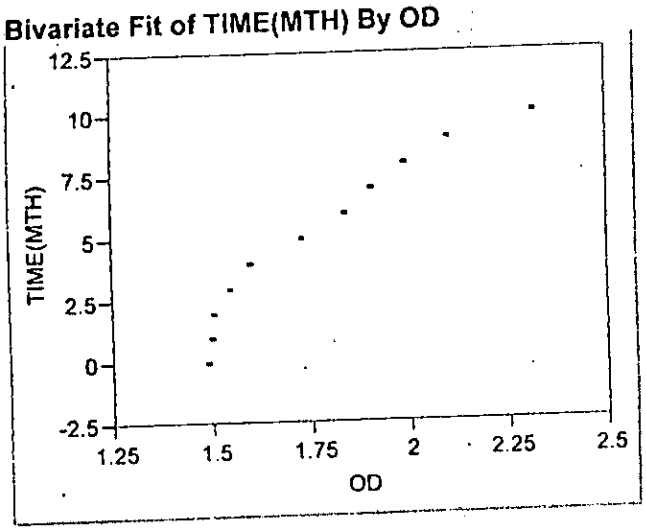
DataTable=PS 1

Fit Y by X Group

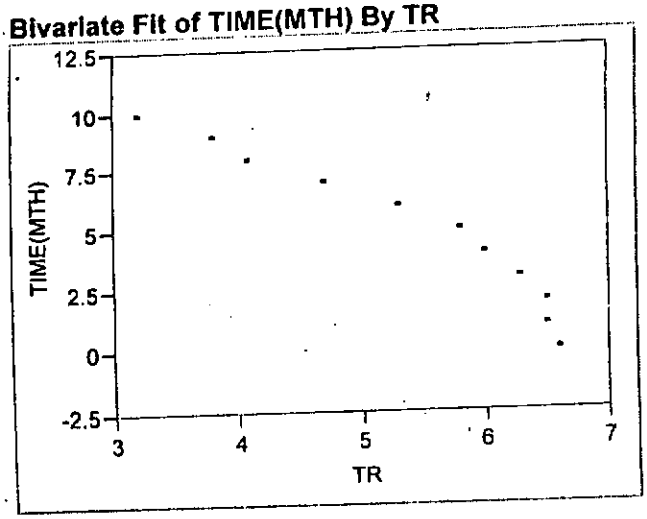
Bivariate Fit of TIME(MTH) By SG



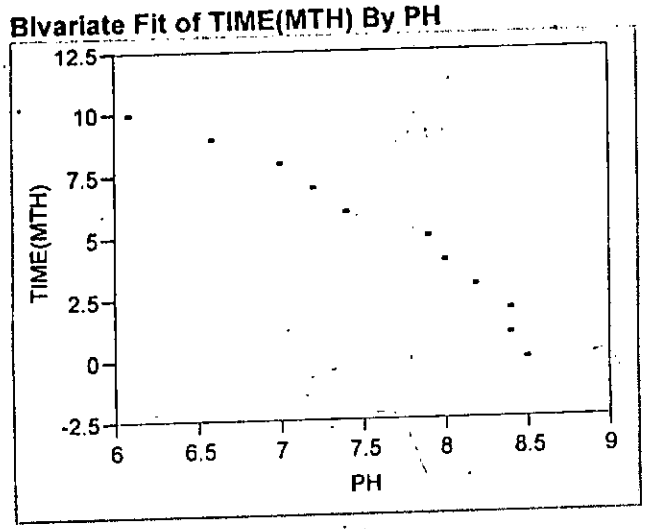
Bivariate Fit of TIME(MTH) By OD



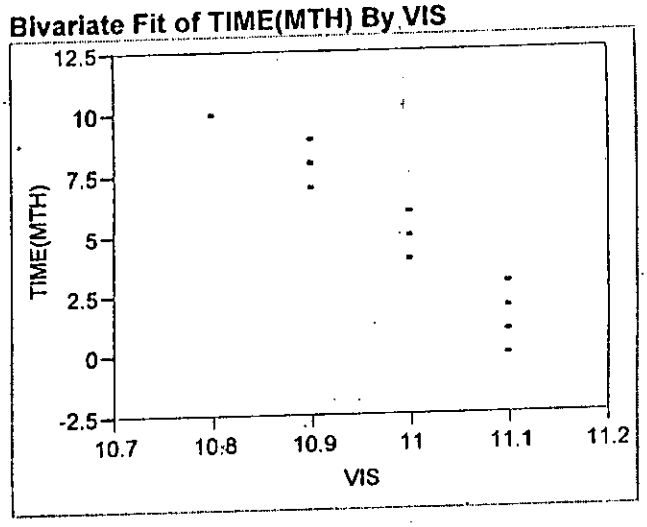
Bivariate Fit of TIME(MTH) By TR



Bivariate Fit of TIME(MTH) By PH



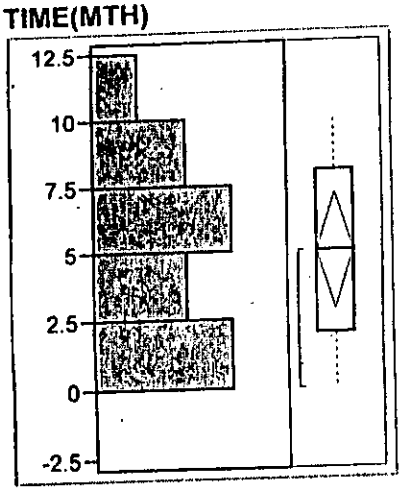
Bivariate Fit of TIME(MTH) By VIS



DataTable=PS 2

Distributions

TIME(MTH)



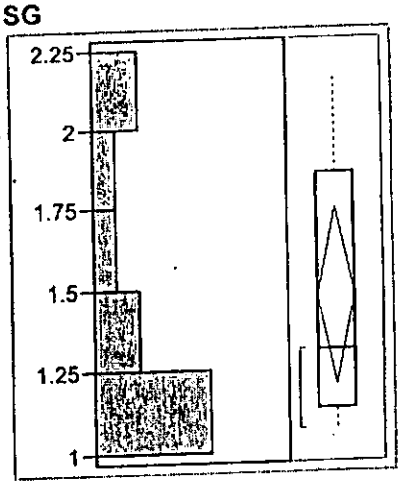
Quantiles

100.0%	maximum	10.000
99.5%		10.000
97.5%		10.000
90.0%		9.800
75.0%	quartile	8.000
50.0%	median	5.000
25.0%	quartile	2.000
10.0%		0.200
2.5%		0.000
0.5%		0.000
0.0%	minimum	0.000

Moments

Mean	5
Std Dev	3.3166248
Std Err Mean	1
upper 95% Mean	7.2281389
lower 95% Mean	2.7718611
N	11

SG



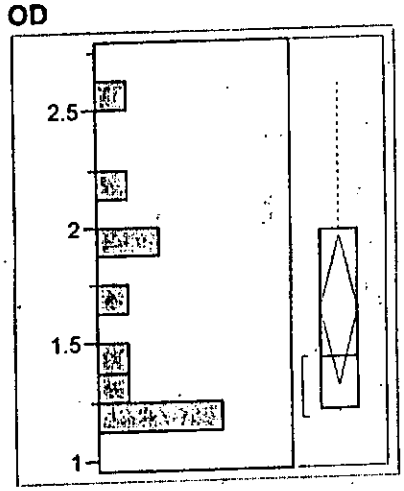
Quantiles

100.0%	maximum	2.1663
99.5%		2.1663
97.5%		2.1663
90.0%		2.1455
75.0%	quartile	1.8511
50.0%	median	1.3103
25.0%	quartile	1.1337
10.0%		1.0771
2.5%		1.0693
0.5%		1.0693
0.0%	minimum	1.0693

Moments

Mean	1.4733545
Std Dev	0.4032144
Std Err Mean	0.1215737
upper 95% Mean	1.7442376
lower 95% Mean	1.2024714
N	11

OD



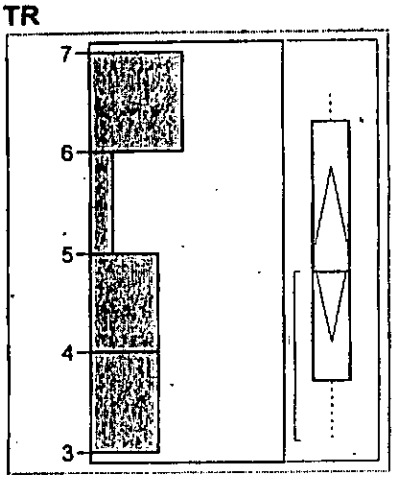
Quantiles

100.0%	maximum	2.6000
99.5%		2.6000
97.5%		2.6000
90.0%		2.5200
75.0%	quartile	1.9700
50.0%	median	1.4200
25.0%	quartile	1.2000
10.0%		1.1740
2.5%		1.1700
0.5%		1.1700
0.0%	minimum	1.1700

Moments

Mean	1.6245455
Std Dev	0.4804032
Std Err Mean	0.144847
upper 95% Mean	1.9472847
lower 95% Mean	1.3018062
N	11

TR



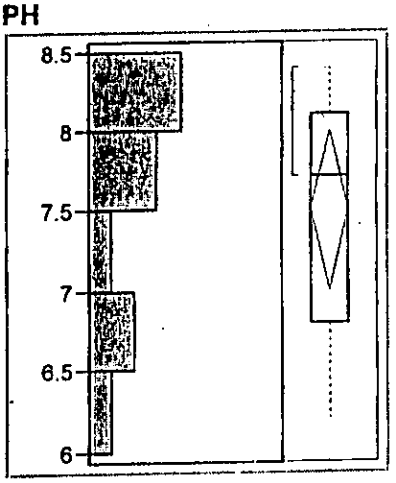
Quantiles

100.0%	maximum	6.6000
99.5%		6.6000
97.5%		6.6000
90.0%		6.5800
75.0%	quartile	6.3000
50.0%	median	4.8000
25.0%	quartile	3.7000
10.0%		3.1600
2.5%		3.1000
0.5%		3.1000
0.0%	minimum	3.1000

Moments

Mean	4.9636364
Std Dev	1.3086426
Std Err Mean	0.3945706
upper 95% Mean	5.8427944
lower 95% Mean	4.0844783
N	11

PH



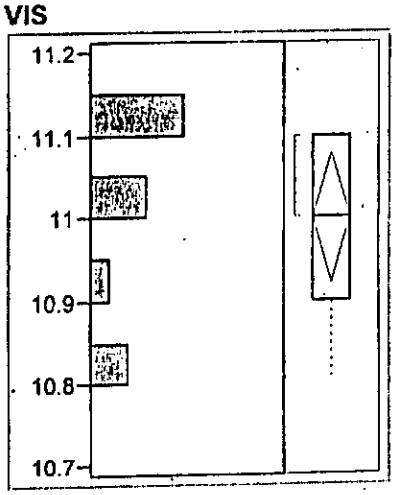
Quantiles

100.0%	maximum	8.4000
99.5%		8.4000
97.5%		8.4000
90.0%		8.3800
75.0%	quartile	8.1000
50.0%	median	7.7000
25.0%	quartile	6.8000
10.0%		6.2600
2.5%		6.2000
0.5%		6.2000
0.0%	minimum	6.2000

Moments

Mean	7.5
Std Dev	0.7389181
Std Err Mean	0.2227922
upper 95% Mean	7.996412
lower 95% Mean	7.003588
N	11

VIS



Quantiles

100.0%	maximum	11.100
99.5%		11.100
97.5%		11.100
90.0%		11.100
75.0%	quartile	11.100
50.0%	median	11.000
25.0%	quartile	10.900
10.0%		10.800
2.5%		10.800
0.5%		10.800
0.0%	minimum	10.800

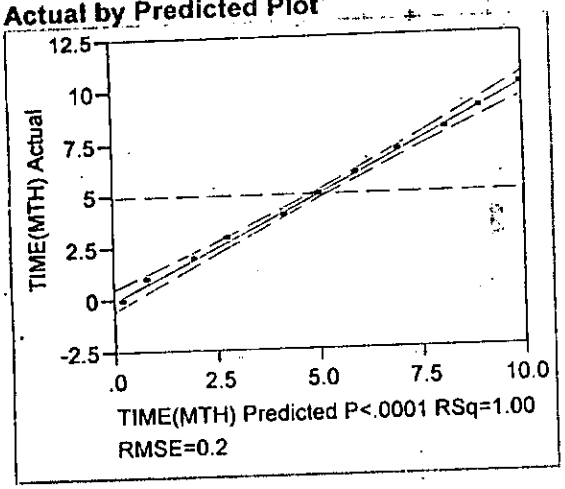
Moments

Mean	11
Std Dev	0.1183216
Std Err Mean	0.0356753
upper 95% Mean	11.07949
lower 95% Mean	10.92051
N	11

DataTable=PS 2

Response TIME(MTH)

Actual by Predicted Plot



Summary of Fit

R-square is the portion of variation attributed to the model, between 0 and 1. Root Mean Squared Error "RMSE" estimates the standard deviation of the residual.

RSquare	0.998182
RSquare Adj	0.996363
Root Mean Square Error	0.200017
Mean of Response	5
Observations (or Sum Wgts)	11

Analysis of Variance

The test that the whole model fits better than a simple mean, i.e. testing that all the parameters are zero except the Intercept

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	5	109.79997	21.9600	548.9054
Error	5	0.20003	0.0400	Prob > F
C. Total	10	110.00000		<.0001

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	22.557599	27.68806	0.81	0.4523
SG	-2.874986	0.961134	-2.99	0.0304
OD	0.087203	1.074567	0.08	0.9385
TR	-0.366431	0.441682	-0.83	0.4445
PH	-2.428843	0.913722	-2.66	0.0450
VIS	0.5974329	3.00871	0.20	0.8504

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
SG	1	1	0.35796278	8.9475	0.0304
OD	1	1	0.00026347	0.0066	0.9385
TR	1	1	0.02753594	0.6883	0.4445
PH	1	1	0.28268689	7.0660	0.0450
VIS	1	1	0.00157744	0.0394	0.8504

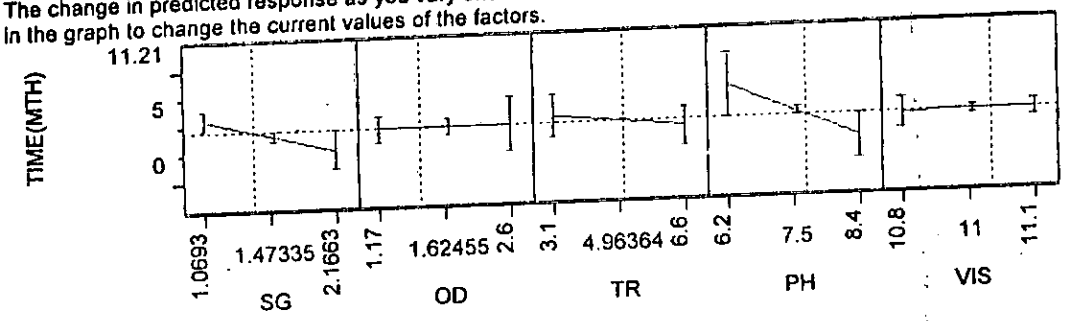
Scaled Estimates

Continuous factors centered by mean, scaled by range/2

Term	Scaled Estimate	Plot Estimate	Std Error	t Ratio	Prob>|t|
Intercept	5		0.060307	82.91	<.0001
SG	-1.57693		0.527182	-2.99	0.0304
OD	0.0623502		0.768316	0.08	0.9385
TR	-0.641255		0.772944	-0.83	0.4445
PH	-2.671727		1.005094	-2.66	0.0450
VIS	0.0896149		0.451306	0.20	0.8504

Prediction Profiler

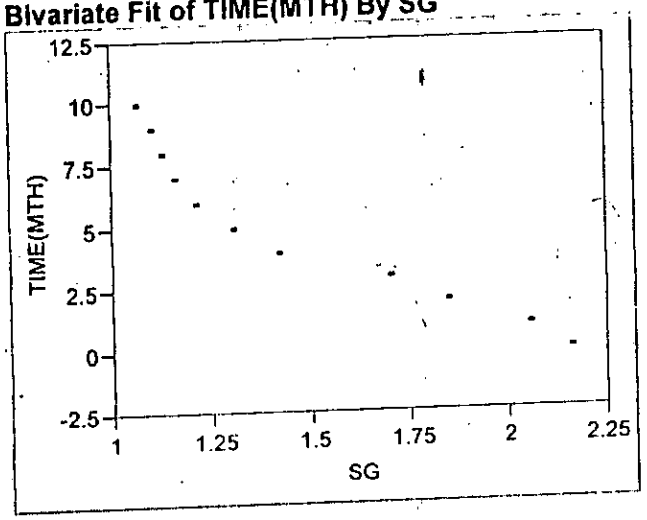
The change in predicted response as you vary one factor at a time, holding the other factors at their current values. Click in the graph to change the current values of the factors.



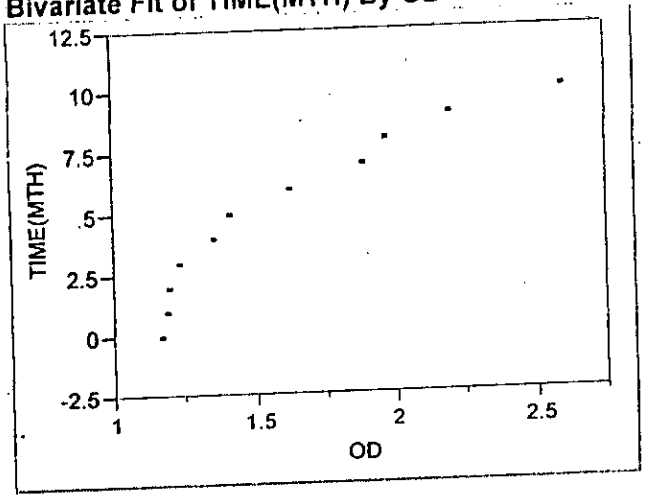
DataTable=PS 2

Fit Y by X Group

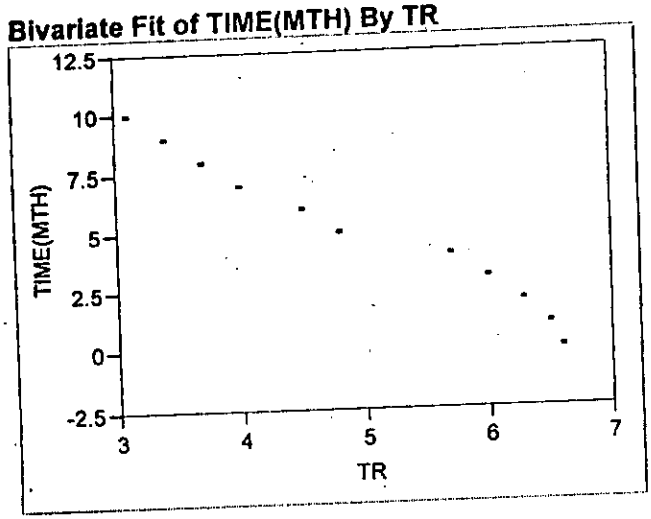
Bivariate Fit of TIME(MTH) By SG



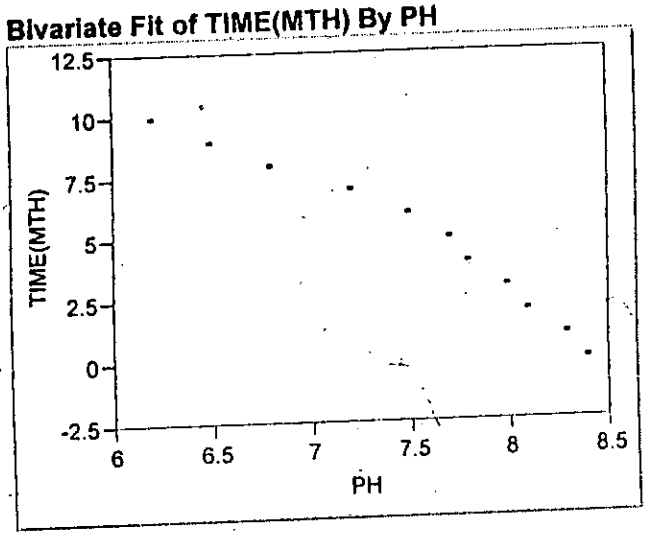
Bivariate Fit of TIME(MTH) By OD



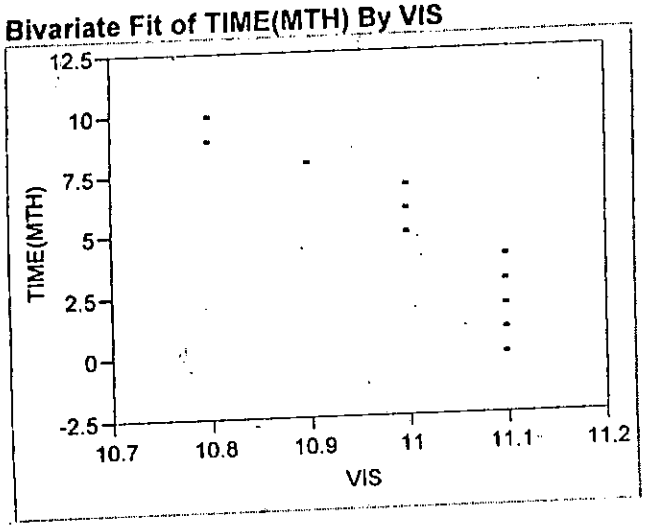
Bivariate Fit of TIME(MTH) By TR



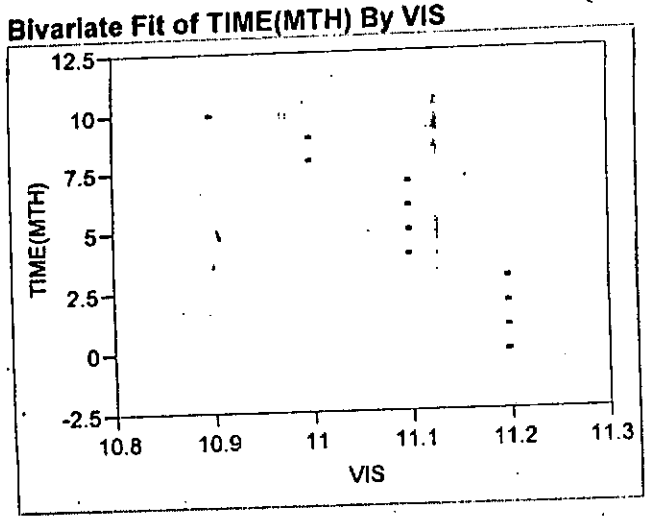
Bivariate Fit of TIME(MTH) By PH



Bivariate Fit of TIME(MTH) By VIS

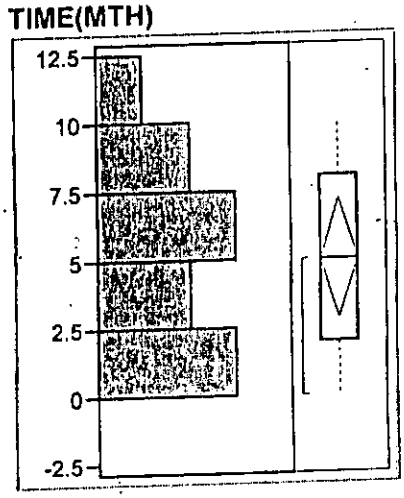


Bivariate Fit of TIME(MTH) By VIS



DataTable=PS 3

Distributions
TIME(MTH)



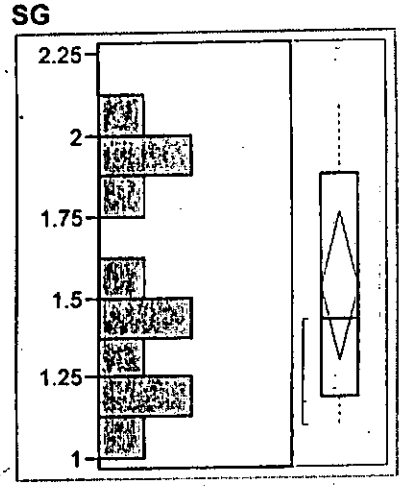
Quantiles

100.0%	maximum	10.000
99.5%		10.000
97.5%		10.000
90.0%		9.800
75.0%	quartile	8.000
50.0%	median	5.000
25.0%	quartile	2.000
10.0%		0.200
2.5%		0.000
0.5%		0.000
0.0%	minimum	0.000

Moments

Mean	5
Std Dev	3.3166248
Std Err Mean	1
upper 95% Mean	7.2281389
lower 95% Mean	2.7718611
N	11

SG



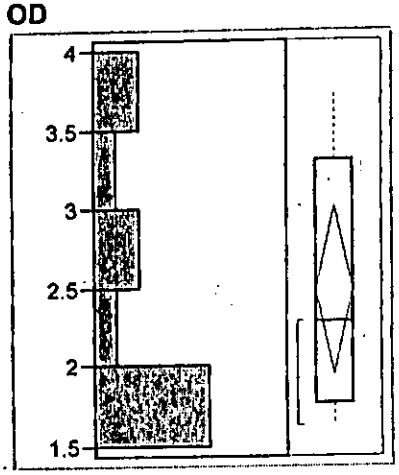
Quantiles

100.0%	maximum	2.0919
99.5%		2.0919
97.5%		2.0919
90.0%		2.0662
75.0%	quartile	1.8786
50.0%	median	1.4298
25.0%	quartile	1.1839
10.0%		1.1075
2.5%		1.0931
0.5%		1.0931
0.0%	minimum	1.0931

Moments

Mean	1.5314455
Std Dev	0.3468182
Std Err Mean	0.1045696
upper 95% Mean	1.7644411
lower 95% Mean	1.2984498
N	11

OD



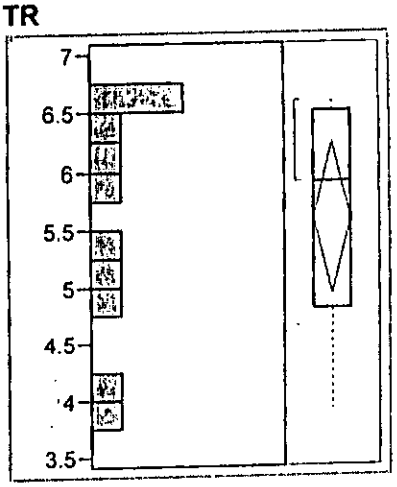
Quantiles

100.0%	maximum	3.7400
99.5%		3.7400
97.5%		3.7400
90.0%		3.7100
75.0%	quartile	3.3000
50.0%	median	2.2800
25.0%	quartile	1.7700
10.0%		1.6420
2.5%		1.6300
0.5%		1.6300
0.0%	minimum	1.6300

Moments

Mean	2.4809091
Std Dev	0.80103
Std Err Mean	0.2415196
upper 95% Mean	3.0190484
lower 95% Mean	1.9427698
N	11

TR



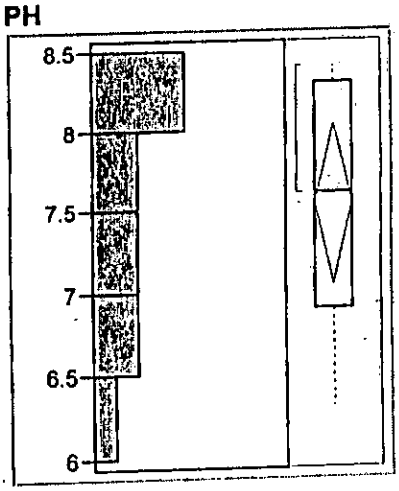
Quantiles

100.0%	maximum	6.6000
99.5%		6.6000
97.5%		6.6000
90.0%		6.5800
75.0%	quartile	6.5000
50.0%	median	5.9000
25.0%	quartile	4.8000
10.0%		3.9600
2.5%		3.9000
0.5%		3.9000
0.0%	minimum	3.9000

Moments

Mean	5.5818182
Std Dev	0.9765431
Std Err Mean	0.2944388
upper 95% Mean	6.2378687
lower 95% Mean	4.9257676
N	11

PH



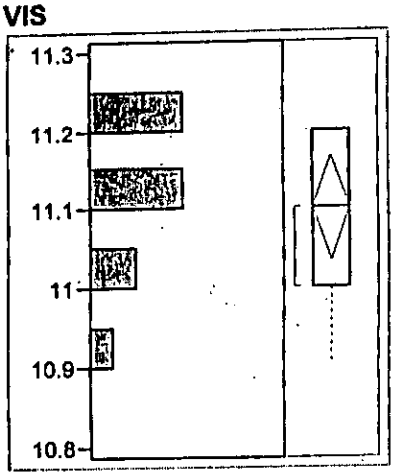
Quantiles

100.0%	maximum	8.4000
99.5%		8.4000
97.5%		8.4000
90.0%		8.4000
75.0%	quartile	8.3000
50.0%	median	7.6000
25.0%	quartile	6.9000
10.0%		6.3600
2.5%		6.3000
0.5%		6.3000
0.0%	minimum	6.3000

Moments

Mean	7.5363636
Std Dev	0.747359
Std Err Mean	0.2253372
upper 95% Mean	8.0384462
lower 95% Mean	7.034281
N	11

VIS



Quantiles

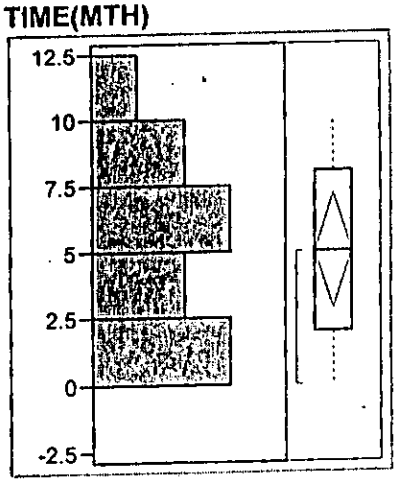
100.0%	maximum	11.200
99.5%		11.200
97.5%		11.200
90.0%		11.200
75.0%	quartile	11.200
50.0%	median	11.100
25.0%	quartile	11.000
10.0%		10.920
2.5%		10.900
0.5%		10.900
0.0%	minimum	10.900

Moments

Mean	11.1
Std Dev	0.1
Std Err Mean	0.0301511
upper 95% Mean	11.167181
lower 95% Mean	11.032819
N	11

DataTable=PS 3

Distributions
TIME(MTH)



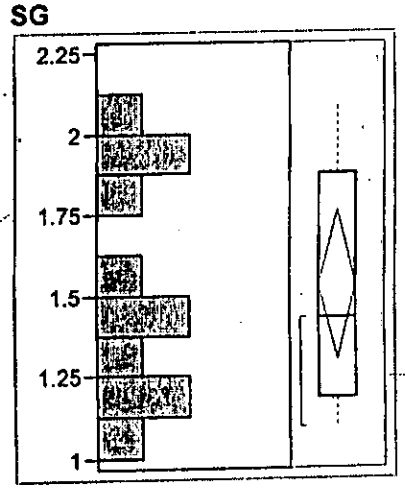
Quantiles

100.0%	maximum	10.000
99.5%		10.000
97.5%		10.000
90.0%		9.800
75.0%	quartile	8.000
50.0%	median	5.000
25.0%	quartile	2.000
10.0%		0.200
2.5%		0.000
0.5%		0.000
0.0%	minimum	0.000

Moments

Mean	5
Std Dev	3.3166248
Std Err Mean	1
upper 95% Mean	7.2281389
lower 95% Mean	2.7718611
N	11

SG



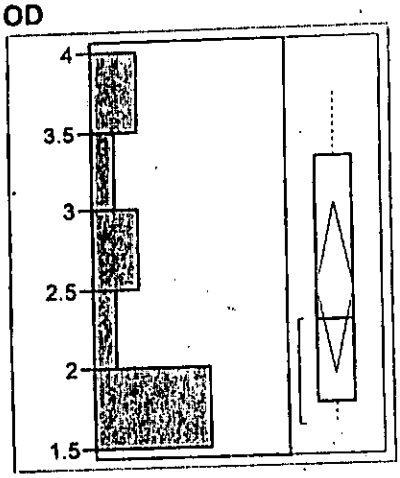
Quantiles

100.0%	maximum	2.0919
99.5%		2.0919
97.5%		2.0919
90.0%		2.0662
75.0%	quartile	1.8786
50.0%	median	1.4298
25.0%	quartile	1.1839
10.0%		1.1075
2.5%		1.0931
0.5%		1.0931
0.0%	minimum	1.0931

Moments

Mean	1.5314455
Std Dev	0.3468182
Std Err Mean	0.1045696
upper 95% Mean	1.7644411
lower 95% Mean	1.2984498
N	11

OD



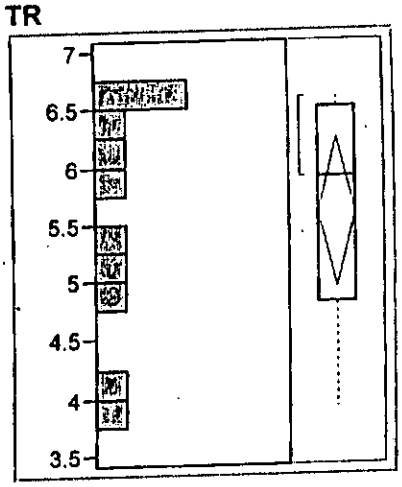
Quantiles

100.0%	maximum	3.7400
99.5%		3.7400
97.5%		3.7400
90.0%		3.7100
75.0%	quartile	3.3000
50.0%	median	2.2800
25.0%	quartile	1.7700
10.0%		1.6420
2.5%		1.6300
0.5%		1.6300
0.0%	minimum	1.6300

Moments

Mean	2.4809091
Std Dev	0.80103
Std Err Mean	0.2415196
upper 95% Mean	3.0190484
lower 95% Mean	1.9427698
N	11

TR



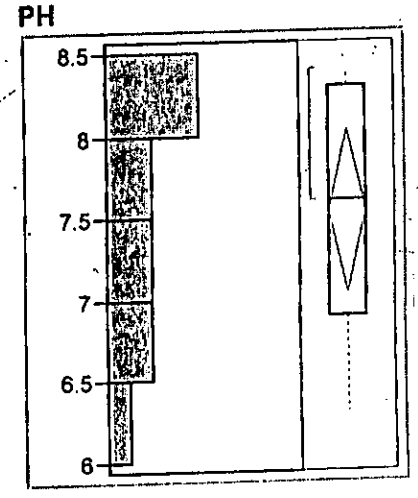
Quantiles

100.0%	maximum	6.6000
99.5%		6.6000
97.5%		6.6000
90.0%		6.5800
75.0%	quartile	6.5000
50.0%	median	5.9000
25.0%	quartile	4.8000
10.0%		3.9600
2.5%		3.9000
0.5%		3.9000
0.0%	minimum	3.9000

Moments

Mean	5.5818182
Std Dev	0.9765431
Std Err Mean	0.2944388
upper 95% Mean	6.2378687
lower 95% Mean	4.9257676
N	11

PH



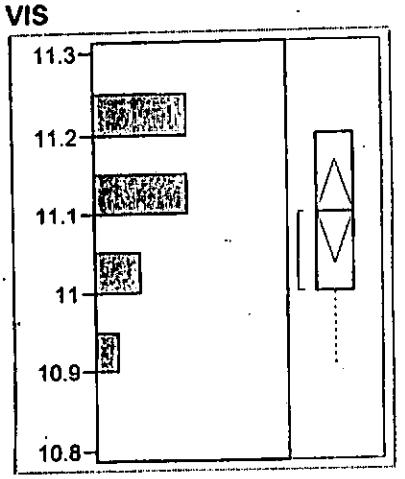
Quantiles

100.0%	maximum	8.4000
99.5%		8.4000
97.5%		8.4000
90.0%		8.4000
75.0%	quartile	8.3000
50.0%	median	7.6000
25.0%	quartile	6.9000
10.0%		6.3600
2.5%		6.3000
0.5%		6.3000
0.0%	minimum	6.3000

Moments

Mean	7.5363636
Std Dev	0.747359
Std Err Mean	0.2253372
upper 95% Mean	8.0384462
lower 95% Mean	7.034281
N	11

VIS



Quantiles

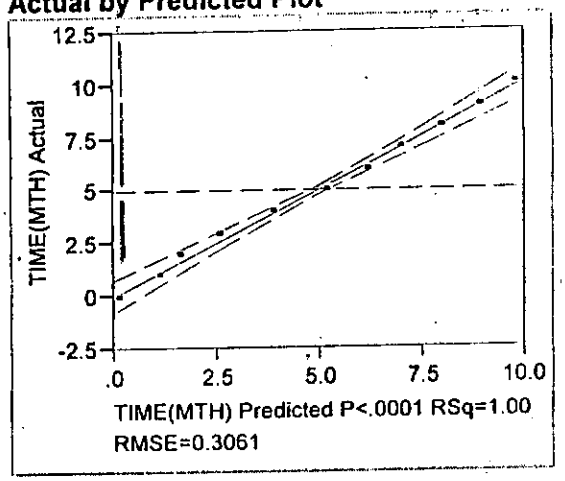
100.0%	maximum	11.200
99.5%		11.200
97.5%		11.200
90.0%		11.200
75.0%	quartile	11.200
50.0%	median	11.100
25.0%	quartile	11.000
10.0%		10.920
2.5%		10.900
0.5%		10.900
0.0%	minimum	10.900

Moments

Mean	11.1
Std Dev	0.1
Std Err Mean	0.0301511
upper 95% Mean	11.167181
lower 95% Mean	11.032819
N	11

DataTable=PS 3

Response TIME(MTH)
Actual by Predicted Plot



Summary of Fit

R-square is the portion of variation attributed to the model, between 0 and 1. Root Mean Squared Error "RMSE" estimates the standard deviation of the residual.

RSquare	0.995741
RSquare Adj	0.991481
Root Mean Square Error	0.306112
Mean of Response	5
Observations (or Sum Wgts)	11

Analysis of Variance

The test that the whole model fits better than a simple mean, i.e. testing that all the parameters are zero except the Intercept

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	5	109.53148	21.9063	233.7802
Error	5	0.46852	0.0937	Prob > F
C. Total	10	110.00000		<.0001

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	24.269018	31.85241	0.76	0.4805
SG	-6.652954	1.888034	-3.52	0.0169
OD	-0.581083	1.2428	-0.47	0.6598
TR	-1.79544	1.599452	-1.12	0.3126
PH	0.2989066	2.381465	0.13	0.9050
VIS	0.0117461	3.238284	0.00	0.9972

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
SG	1	1	1.1635113	12.4168	0.0169
OD	1	1	0.0204850	0.2186	0.6598
TR	1	1	0.1180757	1.2601	0.3126
PH	1	1	0.0014762	0.0158	0.9050
VIS	1	1	0.0000012	0.0000	0.9972

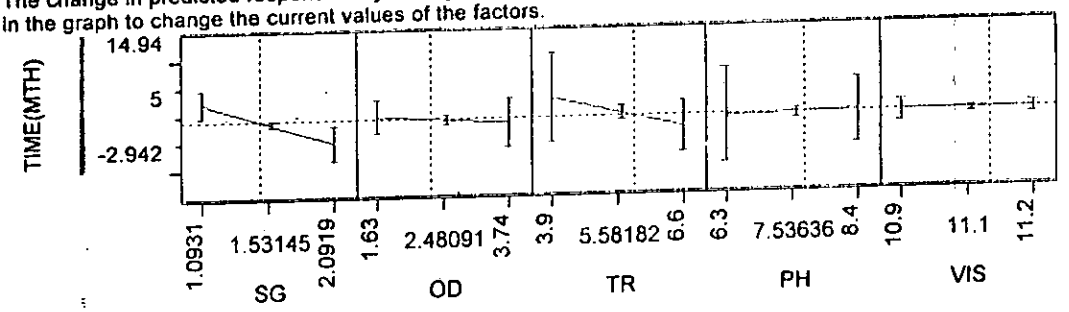
Scaled Estimates

Continuous factors centered by mean, scaled by range/2

Term	Scaled Estimate	Plot Estimate	Std Error	t Ratio	Prob>|t|
Intercept	5		0.092296	54.17	<.0001
SG	-3.322485		0.942884	-3.52	0.0169
OD	-0.613043		1.311154	-0.47	0.6598
TR	-2.423843		2.15926	-1.12	0.3126
PH	0.3138519		2.500539	0.13	0.9050
VIS	0.0017619		0.485743	0.00	0.9972

Prediction Profiler

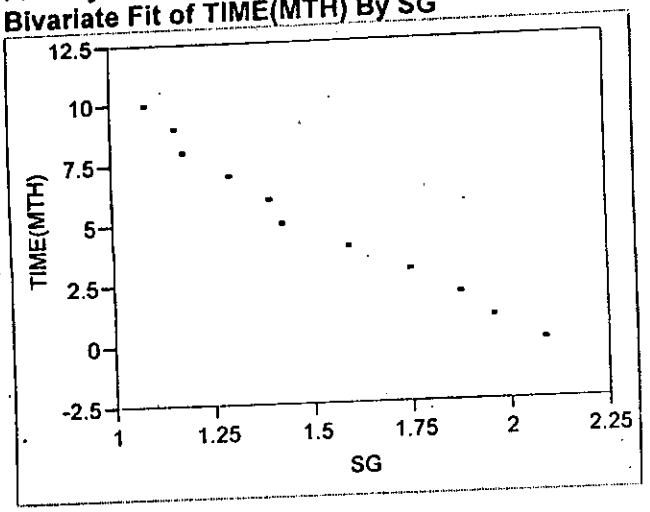
The change in predicted response as you vary one factor at a time, holding the other factors at their current values. Click in the graph to change the current values of the factors.



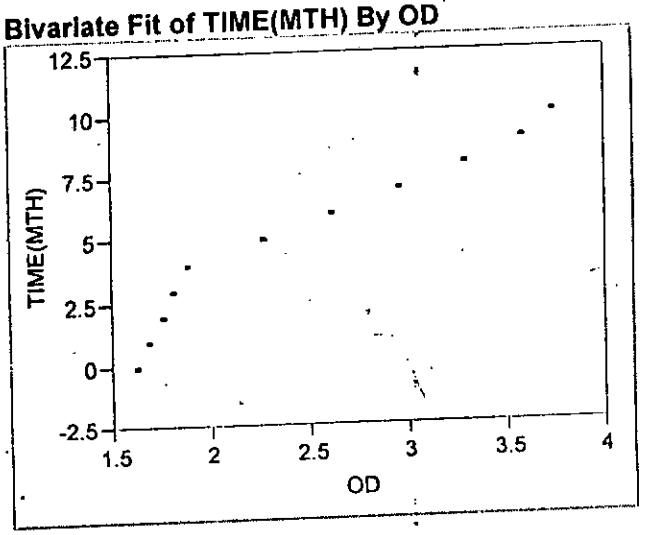
DataTable=PS 3

Fit Y by X Group

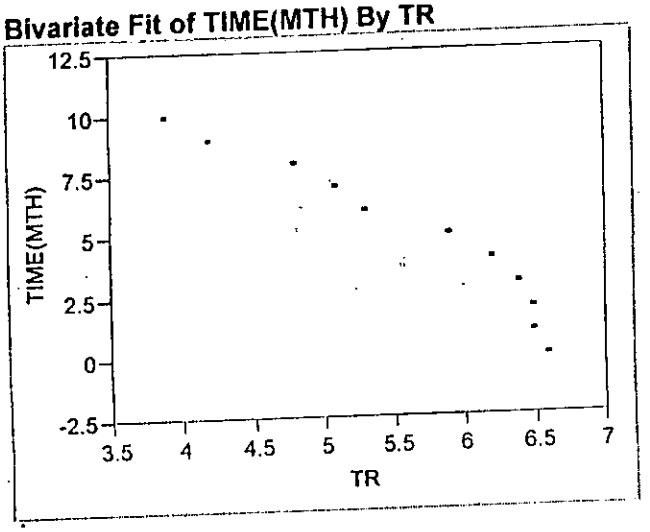
Bivariate Fit of TIME(MTH) By SG



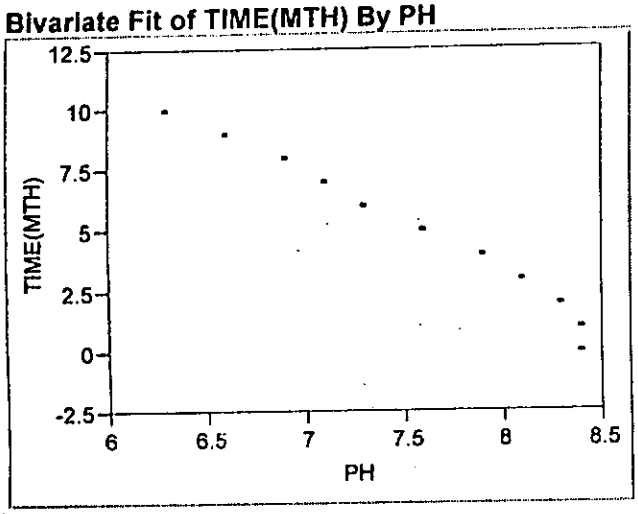
Bivariate Fit of TIME(MTH) By OD



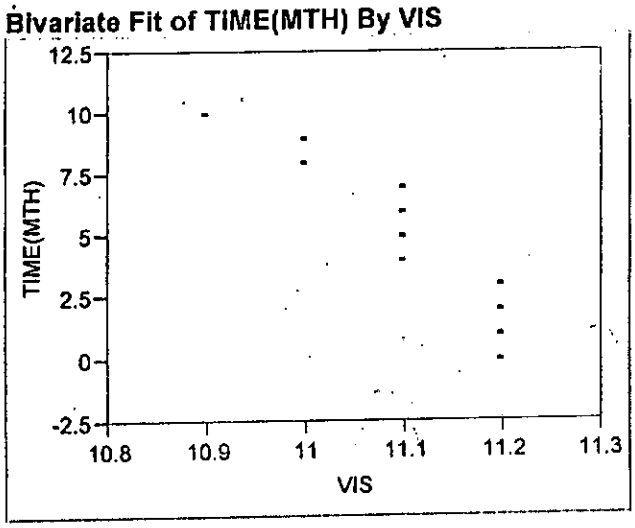
Bivariate Fit of TIME(MTH) By TR



Bivariate Fit of TIME(MTH) By PH



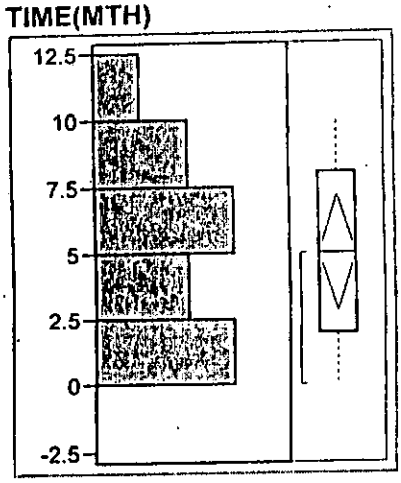
Bivariate Fit of TIME(MTH) By VIS



DataTable=PS 4

Distributions

TIME(MTH)



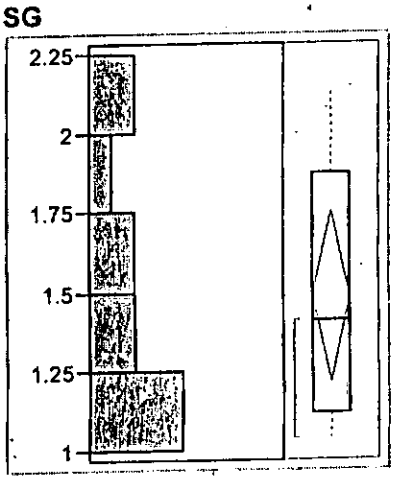
Quantiles

100.0%	maximum	10.000
99.5%		10.000
97.5%		10.000
90.0%		9.800
75.0%	quartile	8.000
50.0%	median	5.000
25.0%	quartile	2.000
10.0%		0.200
2.5%		0.000
0.5%		0.000
0.0%	minimum	0.000

Moments

Mean	5
Std Dev	3.3166248
Std Err Mean	1
upper 95% Mean	7.2281389
lower 95% Mean	2.7718611
N	11

SG



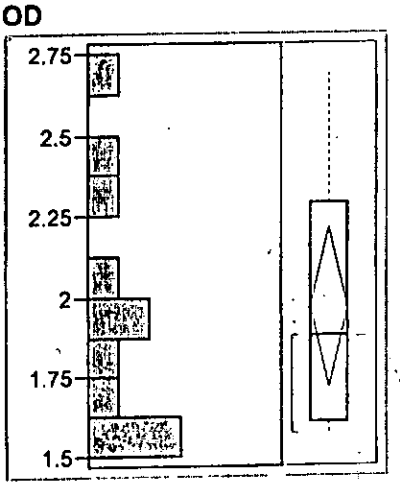
Quantiles

100.0%	maximum	2.1331
99.5%		2.1331
97.5%		2.1331
90.0%		2.1237
75.0%	quartile	1.8726
50.0%	median	1.4116
25.0%	quartile	1.1193
10.0%		1.0486
2.5%		1.0378
0.5%		1.0378
0.0%	minimum	1.0378

Moments

Mean	1.4840455
Std Dev	0.4005004
Std Err Mean	0.1207554
upper 95% Mean	1.7531053
lower 95% Mean	1.2149856
N	11

OD



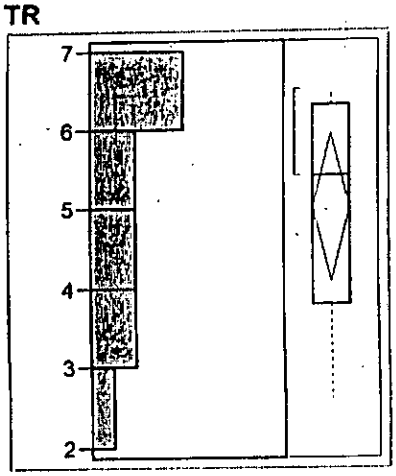
Quantiles

100.0%	maximum	2.6900
99.5%		2.6900
97.5%		2.6900
90.0%		2.6440
75.0%	quartile	2.2900
50.0%	median	1.8800
25.0%	quartile	1.6100
10.0%		1.5740
2.5%		1.5700
0.5%		1.5700
0.0%	minimum	1.5700

Moments

Mean	1.9654545
Std Dev	0.3733996
Std Err Mean	0.1125842
upper 95% Mean	2.2163078
lower 95% Mean	1.7146013
N	11

TR



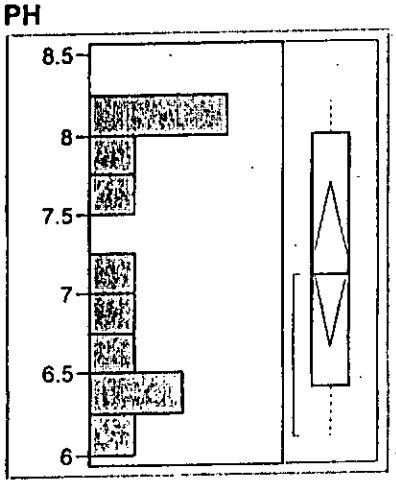
Quantiles

100.0%	maximum	6.5000
99.5%		6.5000
97.5%		6.5000
90.0%		6.4800
75.0%	quartile	6.3000
50.0%	median	5.4000
25.0%	quartile	3.8000
10.0%		2.7000
2.5%		2.6000
0.5%		2.6000
0.0%	minimum	2.6000

Moments

Mean	5
Std Dev	1.3928388
Std Err Mean	0.4199567
upper 95% Mean	5.9357219
lower 95% Mean	4.0642781
N	11

PH



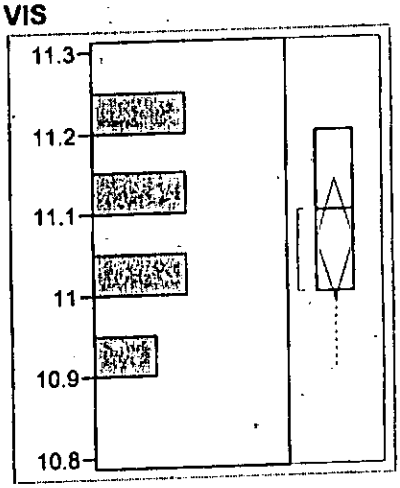
Quantiles

100.0%	maximum	8.2000
99.5%		8.2000
97.5%		8.2000
90.0%		8.1800
75.0%	quartile	8.0000
50.0%	median	7.1000
25.0%	quartile	6.4000
10.0%		6.1400
2.5%		6.1000
0.5%		6.1000
0.0%	minimum	6.1000

Moments

Mean	7.1727273
Std Dev	0.7798601
Std Err Mean	0.2351367
upper 95% Mean	7.6966444
lower 95% Mean	6.6488101
N	11

VIS



Quantiles

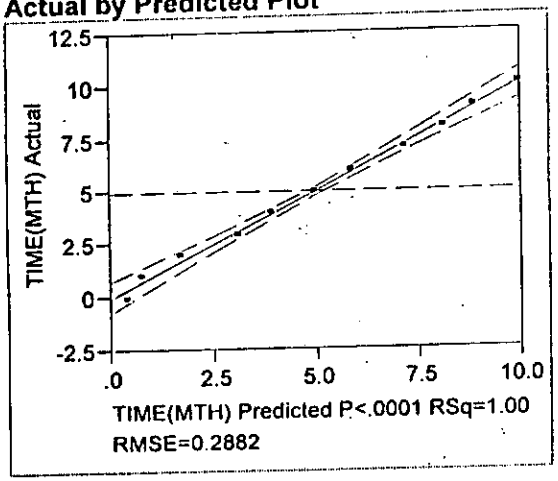
100.0%	maximum	11.200
99.5%		11.200
97.5%		11.200
90.0%		11.200
75.0%	quartile	11.200
50.0%	median	11.100
25.0%	quartile	11.000
10.0%		10.900
2.5%		10.900
0.5%		10.900
0.0%	minimum	10.900

Moments

Mean	11.063636
Std Dev	0.1120065
Std Err Mean	0.0337712
upper 95% Mean	11.138883
lower 95% Mean	10.988389
N	11

DataTable=PS 4

Response TIME(MTH)
Actual by Predicted Plot



Summary of Fit

R-square is the portion of variation attributed to the model, between 0 and 1. Root Mean Squared Error "RMSE" estimates the standard deviation of the residual.

RSquare	0.996225
RSquare Adj	0.99245
Root Mean Square Error	0.288191
Mean of Response	5
Observations (or Sum Wgts)	11

Analysis of Variance

The test that the whole model fits better than a simple mean, i.e. testing that all the parameters are zero except the Intercept

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	5	109.58473	21.9169	263.8870
Error	5	0.41527	0.0831	Prob > F
C. Total	10	110.00000		<.0001

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	-2.465596	42.46613	-0.06	0.9559
SG	-3.839115	1.36221	-2.82	0.0372
OD	2.534578	1.768174	1.43	0.2112
TR	-0.40556	0.80467	-0.50	0.6357
PH	-0.642703	1.150123	-0.56	0.6004
VIS	1.3394471	3.888119	0.34	0.7445

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
SG	1	1	0.65968399	7.9428	0.0372
OD	1	1	0.17065662	2.0548	0.2112
TR	1	1	0.02109775	0.2540	0.6357
PH	1	1	0.02593549	0.3123	0.6004
VIS	1	1	0.00985676	0.1187	0.7445

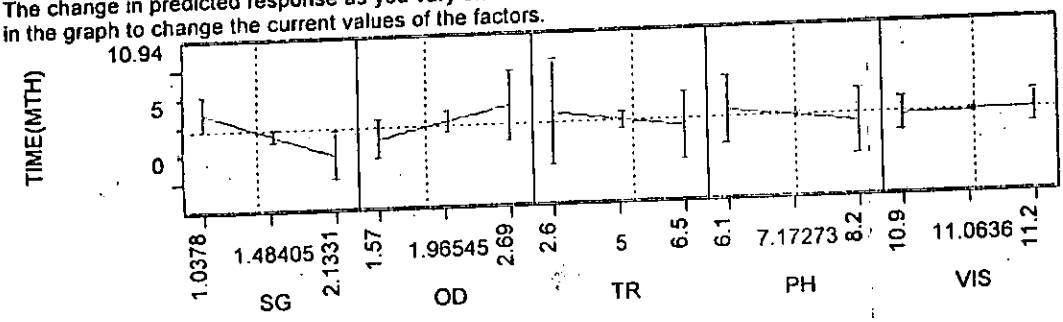
Scaled Estimates

Continuous factors centered by mean, scaled by range/2

Term	Scaled Estimate	Plot Estimate	Std Error	t Ratio	Prob>|t|
Intercept	5		0.086893	57.54	<.0001
SG	-2.102491		0.746015	-2.82	0.0372
OD	1.4193637		0.990178	1.43	0.2112
TR	-0.790842		1.569107	-0.50	0.6357
PH	-0.674839		1.207629	-0.56	0.6004
VIS	0.2009171		0.583218	0.34	0.7445

Prediction Profiler

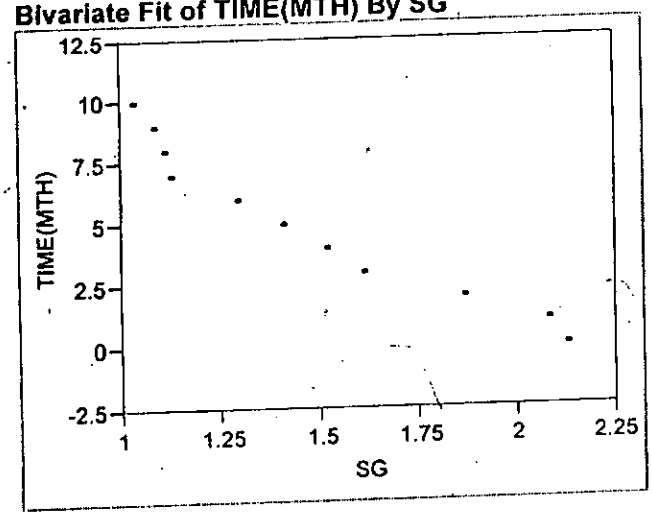
The change in predicted response as you vary one factor at a time, holding the other factors at their current values. Click in the graph to change the current values of the factors.



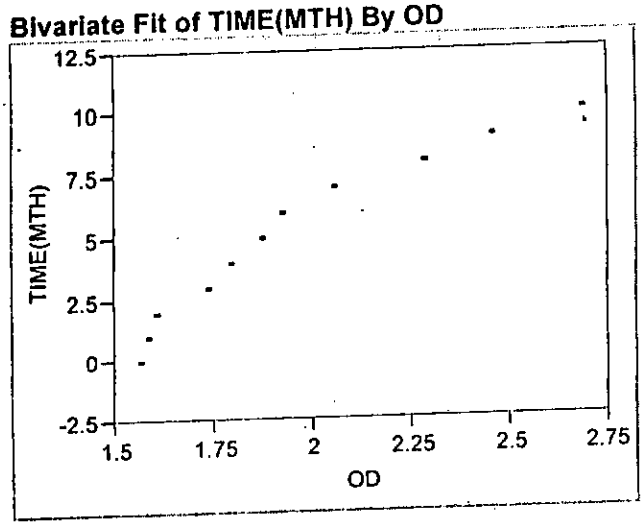
DataTable=PS 4

Fit Y by X Group

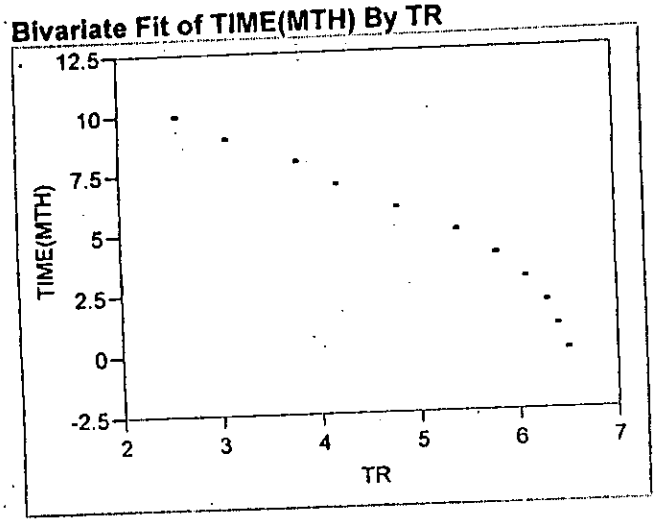
Bivariate Fit of TIME(MTH) By SG



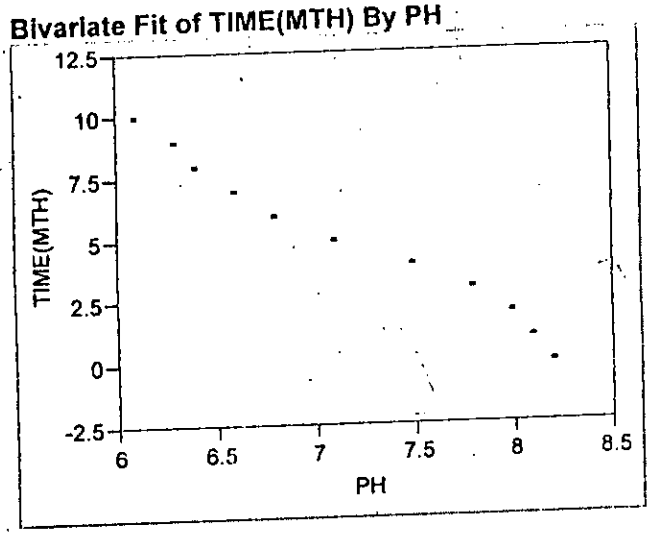
Bivariate Fit of TIME(MTH) By OD



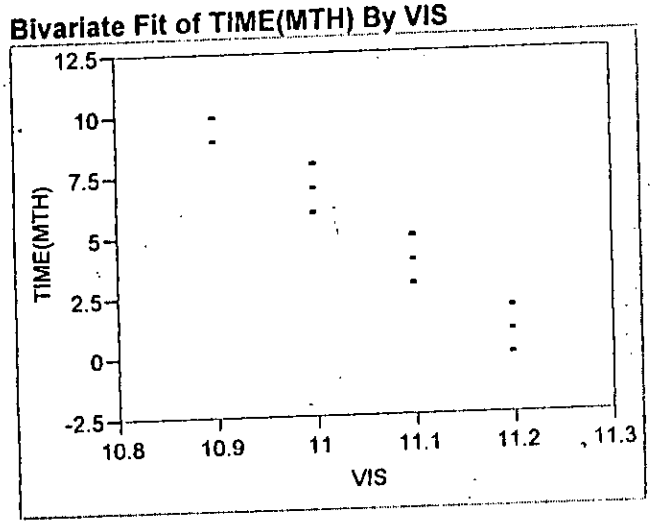
Bivariate Fit of TIME(MTH) By TR



Bivariate Fit of TIME(MTH) By PH



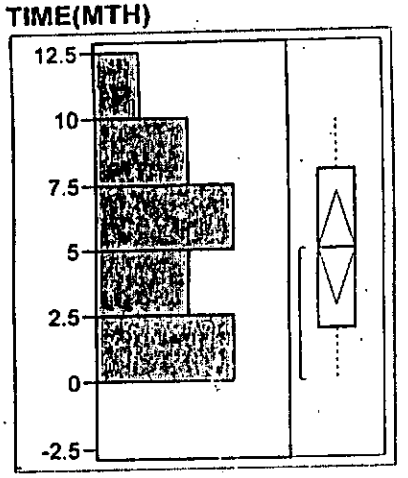
Bivariate Fit of TIME(MTH) By VIS



DataTable=PS 5

Distributions

TIME(MTH)



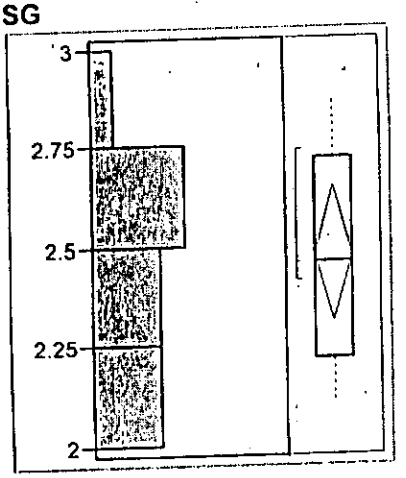
Quantiles

100.0%	maximum	10.000
99.5%		10.000
97.5%		10.000
90.0%		9.800
75.0%	quartile	8.000
50.0%	median	5.000
25.0%	quartile	2.000
10.0%		0.200
2.5%		0.000
0.5%		0.000
0.0%	minimum	0.000

Moments

Mean	5
Std Dev	3.3166248
Std Err Mean	1
upper 95% Mean	7.2281389
lower 95% Mean	2.7718611
N	11

SG



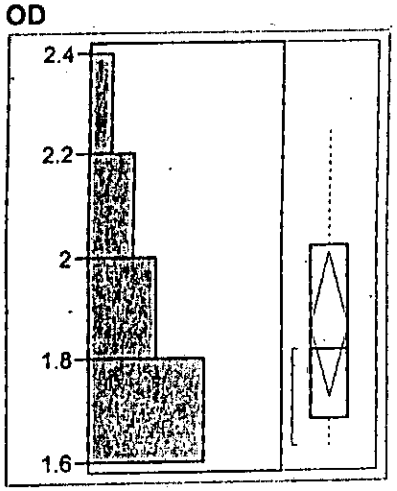
Quantiles

100.0%	maximum	2.8658
99.5%		2.8658
97.5%		2.8658
90.0%		2.8405
75.0%	quartile	2.7163
50.0%	median	2.4583
25.0%	quartile	2.2178
10.0%		2.1204
2.5%		2.1056
0.5%		2.1056
0.0%	minimum	2.1056

Moments

Mean	2.4757091
Std Dev	0.2524405
Std Err Mean	0.0761137
upper 95% Mean	2.6453009
lower 95% Mean	2.3061173
N	11

OD



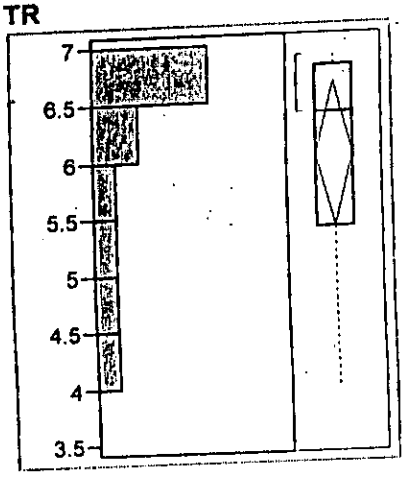
Quantiles

100.0%	maximum	2.2500
99.5%		2.2500
97.5%		2.2500
90.0%		2.2360
75.0%	quartile	2.0200
50.0%	median	1.8200
25.0%	quartile	1.6800
10.0%		1.6340
2.5%		1.6300
0.5%		1.6300
0.0%	minimum	1.6300

Moments

Mean	1.8645455
Std Dev	0.2124318
Std Err Mean	0.0640506
upper 95% Mean	2.0072591
lower 95% Mean	1.7218318
N	11

TR



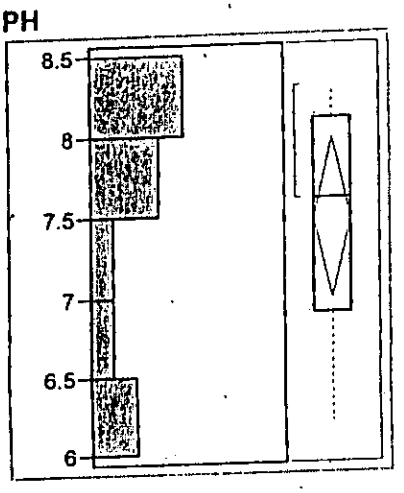
Quantiles

100.0%	maximum	6.9000
99.5%		6.9000
97.5%		6.9000
90.0%		6.8800
75.0%	quartile	6.8000
50.0%	median	6.4000
25.0%	quartile	5.4000
10.0%		4.1600
2.5%		4.0000
0.5%		4.0000
0.0%	minimum	4.0000

Moments

Mean	6.0272727
Std Dev	0.9456119
Std Err Mean	0.2851127
upper 95% Mean	6.6625434
lower 95% Mean	5.392002
N	11

PH



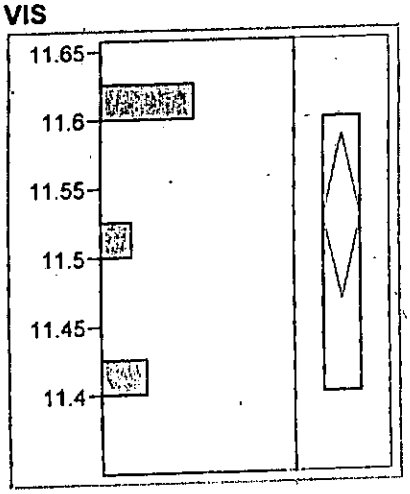
Quantiles

100.0%	maximum	8.3000
99.5%		8.3000
97.5%		8.3000
90.0%		8.3000
75.0%	quartile	8.1000
50.0%	median	7.6000
25.0%	quartile	6.9000
10.0%		6.2400
2.5%		6.2000
0.5%		6.2000
0.0%	minimum	6.2000

Moments

Mean	7.4818182
Std Dev	0.7305042
Std Err Mean	0.2202553
upper 95% Mean	7.9725776
lower 95% Mean	6.9910588
N	11

VIS



Quantiles

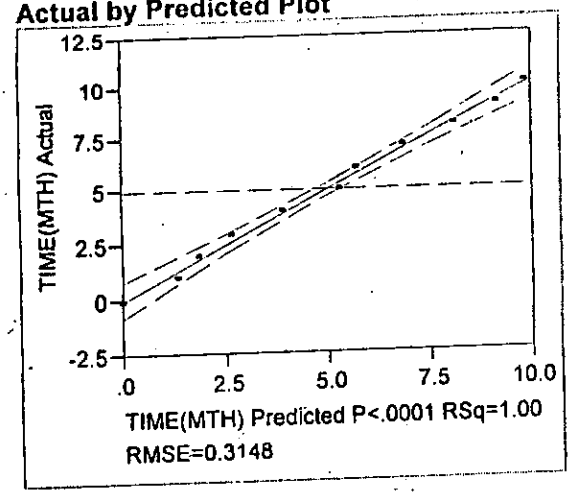
100.0%	maximum	11.600
99.5%		11.600
97.5%		11.600
90.0%		11.600
75.0%	quartile	11.600
50.0%	median	11.600
25.0%	quartile	11.400
10.0%		11.400
2.5%		11.400
0.5%		11.400
0.0%	minimum	11.400

Moments

Mean	11.527273
Std Dev	0.0904534
Std Err Mean	0.0272727
upper 95% Mean	11.58804
lower 95% Mean	11.466505
N	11

DataTable=PS 5

Response TIME(MTH)
Actual by Predicted Plot



Summary of Fit

R-square is the portion of variation attributed to the model, between 0 and 1. Root Mean Squared Error "RMSE" estimates the standard deviation of the residual.

RSquare	0.995496
RSquare Adj	0.990991
Root Mean Square Error	0.314792
Mean of Response	5
Observations (or Sum Wgts)	11

Analysis of Variance

The test that the whole model fits better than a simple mean, i.e. testing that all the parameters are zero except the intercept

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	5	109.50453	21.9009	221.0112
Error	5	0.49547	0.0991	Prob > F
C. Total	10	110.00000		<.0001

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	0.4004296	53.03388	0.01	0.9943
SG	-10.5699	1.967235	-5.37	0.0030
OD	3.9713807	8.134019	0.49	0.6460
TR	0.6006672	0.766823	0.78	0.4689
PH	-0.744179	1.958925	-0.38	0.7196
VIS	2.1956779	3.241915	0.68	0.5283

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
SG	1	1	2.8607324	28.8689	0.0030
OD	1	1	0.0236222	0.2384	0.6460
TR	1	1	0.0608031	0.6136	0.4689
PH	1	1	0.0143010	0.1443	0.7196
VIS	1	1	0.0454550	0.4587	0.5283

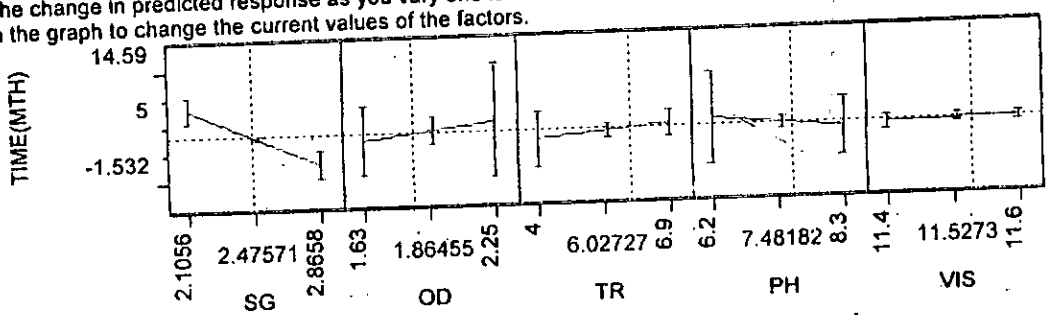
Scaled Estimates

Continuous factors centered by mean, scaled by range/2

Term	Scaled Estimate	Plot Estimate	Std Error	t Ratio	Prob>|t|
Intercept		5	0.094913	52.68	<.0001
SG		-4.017619	0.747746	-5.37	0.0030
OD		1.231128	2.521546	0.49	0.6460
TR		0.8709675	1.111893	0.78	0.4689
PH		-0.781388	2.056871	-0.38	0.7196
VIS		0.2195678	0.324191	0.68	0.5283

Prediction Profiler

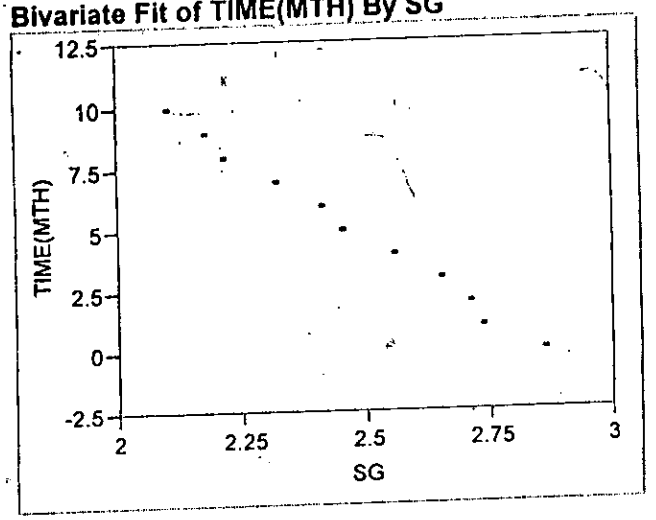
The change in predicted response as you vary one factor at a time, holding the other factors at their current values. Click in the graph to change the current values of the factors.



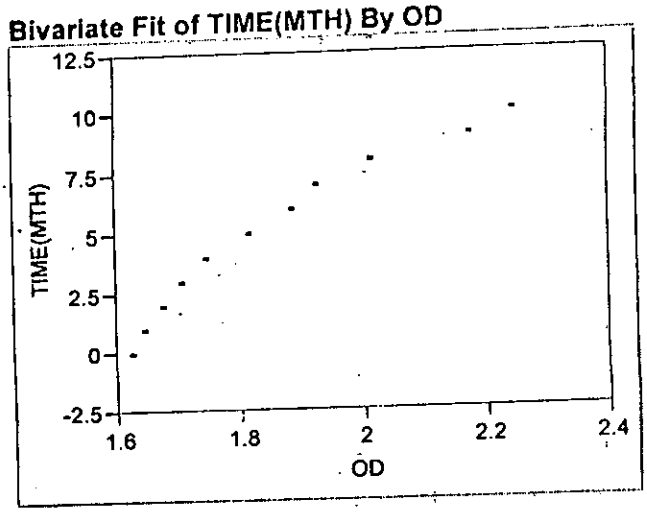
DataTable=PS 5

Fit Y by X Group

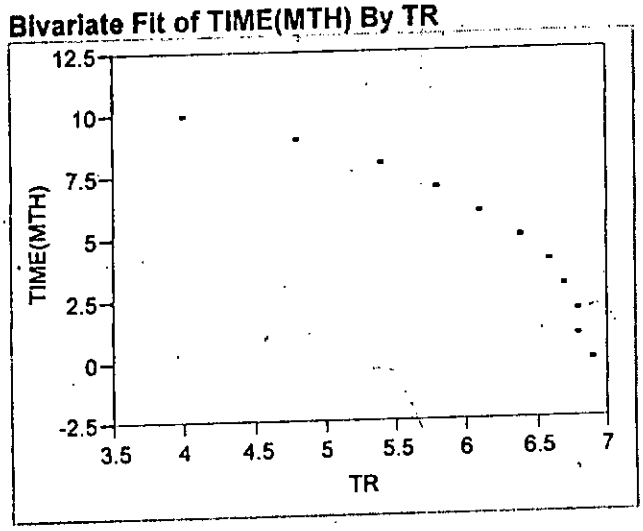
Bivariate Fit of TIME(MTH) By SG



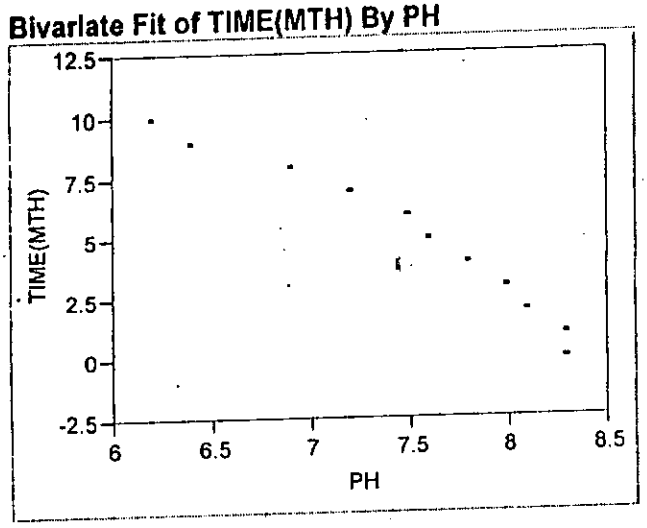
Bivariate Fit of TIME(MTH) By OD

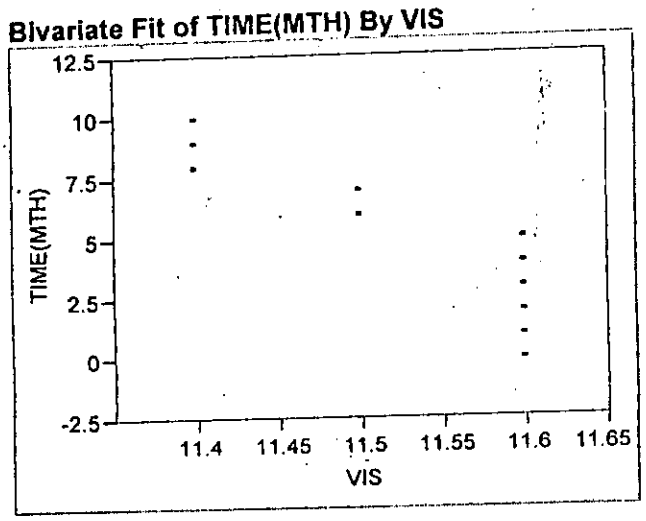


Bivariate Fit of TIME(MTH) By TR



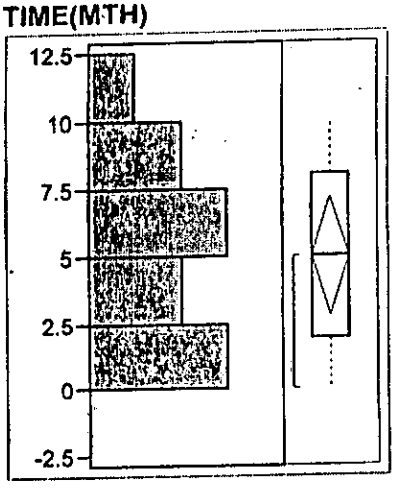
Bivariate Fit of TIME(MTH) By PH




Bivariate Fit of TIME(MTH) By VIS
12.5
10
7.5
5
2.5
0
-2.5
TIME(MTH)
11.4
11.45
11.5
11.55
11.6
11.65
VIS

DataTable=PS 6

Distributions
TIME(MTH)



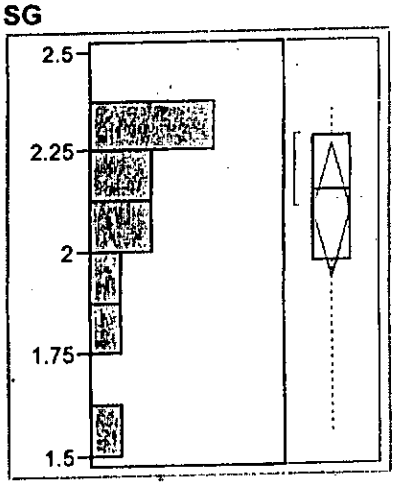
Quantiles

100.0%	maximum	10.000
99.5%		10.000
97.5%		10.000
90.0%		9.800
75.0%	quartile	8.000
50.0%	median	5.000
25.0%	quartile	2.000
10.0%		0.200
2.5%		0.000
0.5%		0.000
0.0%	minimum	0.000

Moments

Mean	5
Std Dev	3.3166248
Std Err Mean	1
upper 95% Mean	7.2281389
lower 95% Mean	2.7718611
N	11

SG



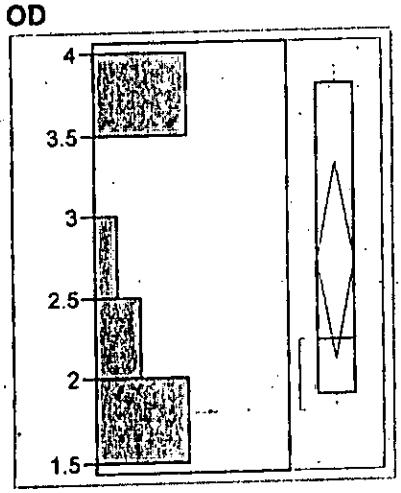
Quantiles

100.0%	maximum	2.3614
99.5%		2.3614
97.5%		2.3614
90.0%		2.3471
75.0%	quartile	2.2851
50.0%	median	2.1485
25.0%	quartile	1.9765
10.0%		1.5896
2.5%		1.5487
0.5%		1.5487
0.0%	minimum	1.5487

Moments

Mean	2.0953182
Std Dev	0.2493362
Std Err Mean	0.0751777
upper 95% Mean	2.2628245
lower 95% Mean	1.9278119
N	11

OD



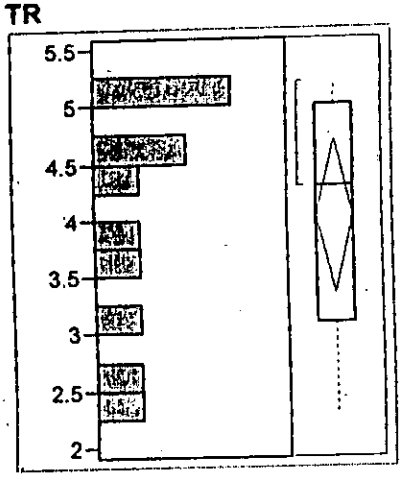
Quantiles

100.0%	maximum	3.9100
99.5%		3.9100
97.5%		3.9100
90.0%		3.9000
75.0%	quartile	3.8000
50.0%	median	2.2200
25.0%	quartile	1.8900
10.0%		1.7960
2.5%		1.7900
0.5%		1.7900
0.0%	minimum	1.7900

Moments

Mean	2.7090909
Std Dev	0.9076944
Std Err Mean	0.2736802
upper 95% Mean	3.3186883
lower 95% Mean	2.0992935
N	11

TR



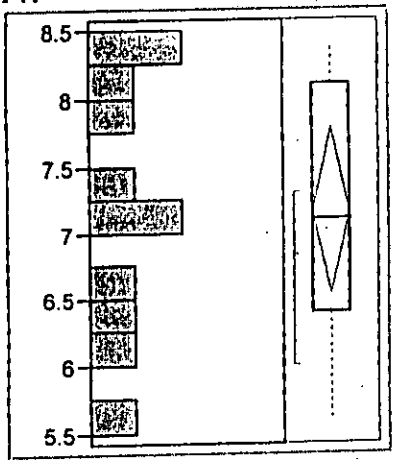
Quantiles

100.0%	maximum	5.2000
99.5%		5.2000
97.5%		5.2000
90.0%		5.1800
75.0%	quartile	5.0000
50.0%	median	4.3000
25.0%	quartile	3.1000
10.0%		2.3600
2.5%		2.3000
0.5%		2.3000
0.0%	minimum	2.3000

Moments

Mean	4.0272727
Std Dev	1.0149787
Std Err Mean	0.3060276
upper 95% Mean	4.7091447
lower 95% Mean	3.3454007
N	11

PH



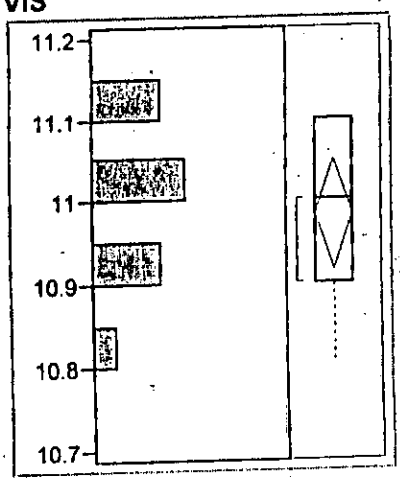
Quantiles

100.0%	maximum	8.4000
99.5%		8.4000
97.5%		8.4000
90.0%		8.3800
75.0%	quartile	8.1000
50.0%	median	7.1000
25.0%	quartile	6.4000
10.0%		5.6800
2.5%		5.6000
0.5%		5.6000
0.0%	minimum	5.6000

Moments

Mean	7.1545455
Std Dev	0.9352686
Std Err Mean	0.2819941
upper 95% Mean	7.7828674
lower 95% Mean	6.5262235
N	11

VIS



Quantiles

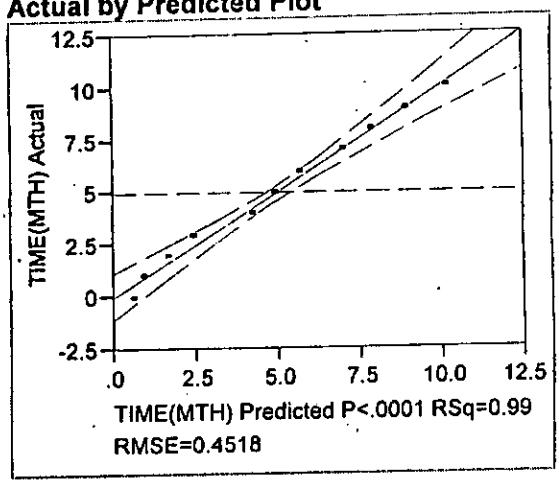
100.0%	maximum	11.100
99.5%		11.100
97.5%		11.100
90.0%		11.100
75.0%	quartile	11.100
50.0%	median	11.000
25.0%	quartile	10.900
10.0%		10.820
2.5%		10.800
0.5%		10.800
0.0%	minimum	10.800

Moments

Mean	10.981818
Std Dev	0.098165
Std Err Mean	0.0295979
upper 95% Mean	11.047766
lower 95% Mean	10.91587
N	11

DataTable=PS 6

Response TIME(MTH)
Actual by Predicted Plot



Summary of Fit

R-square is the portion of variation attributed to the model, between 0 and 1. Root Mean Squared Error "RMSE" estimates the standard deviation of the residual.

RSquare	0.990724
RSquare Adj	0.981447
Root Mean Square Error	0.451753
Mean of Response	5
Observations (or Sum Wgts)	11

Analysis of Variance

The test that the whole model fits better than a simple mean, i.e. testing that all the parameters are zero except the Intercept

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	5	108.97960	21.7959	106.8005
Error	5	1.02040	0.2041	Prob > F
C. Total	10	110.00000		<.0001

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	17.346952	54.56624	0.32	0.7634
SG	-0.306647	2.834668	-0.11	0.9181
OD	1.0694062	0.928199	1.15	0.3014
TR	1.1678518	2.222819	0.53	0.6218
PH	-3.794724	1.217567	-3.12	0.0264
VIS	0.7143369	5.239226	0.14	0.8969

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
SG	1	1	0.0023882	0.0117	0.9181
OD	1	1	0.2708976	1.3274	0.3014
TR	1	1	0.0563338	0.2760	0.6218
PH	1	1	1.9823321	9.7135	0.0264
VIS	1	1	0.0037938	0.0186	0.8969

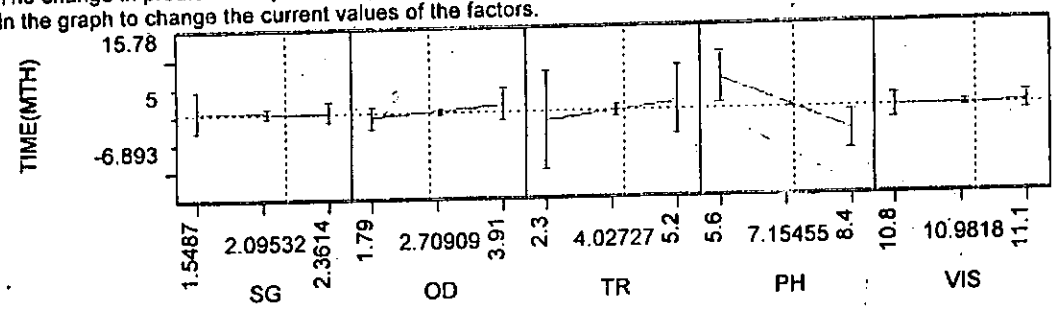
Scaled Estimates

Continuous factors centered by mean, scaled by range/2

Term	Scaled Estimate	Plot Estimate	Std Error	t Ratio	Prob>|t|
Intercept	5		0.136209	36.71	<.0001
SG	-0.124606		1.151867	-0.11	0.9181
OD	1.1335705		0.983891	1.15	0.3014
TR	1.6933851		3.223088	0.53	0.6218
PH	-5.312613		1.704594	-3.12	0.0264
VIS	0.1071505		0.785884	0.14	0.8969

Prediction Profiler

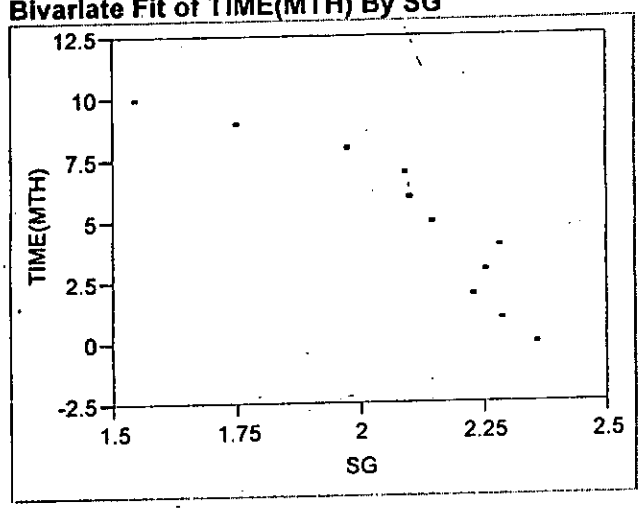
The change in predicted response as you vary one factor at a time, holding the other factors at their current values. Click in the graph to change the current values of the factors.



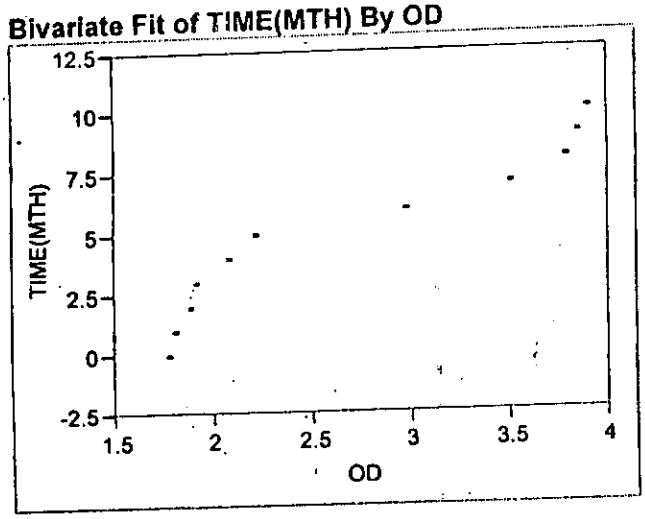
DataTable=PS 6

Fit Y by X Group

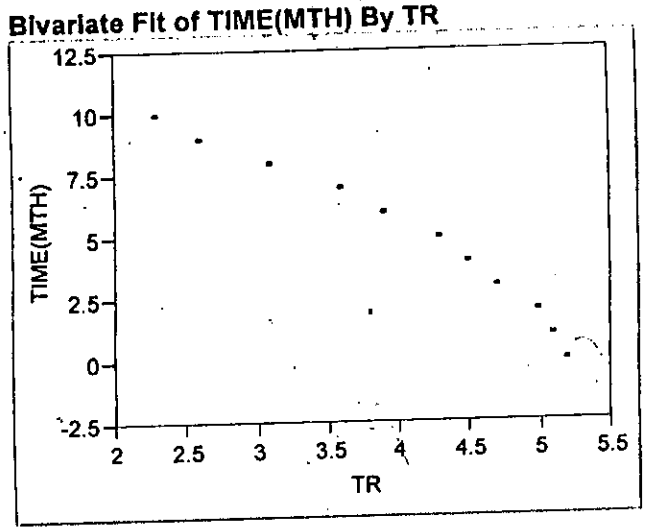
Bivariate Fit of TIME(MTH) By SG



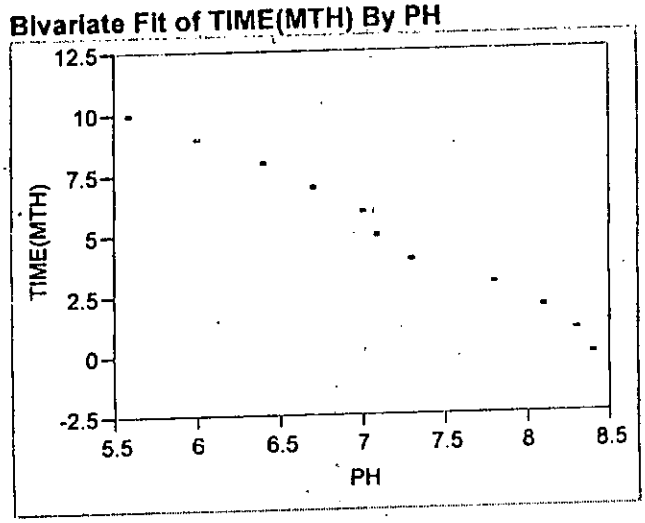
Bivariate Fit of TIME(MTH) By OD

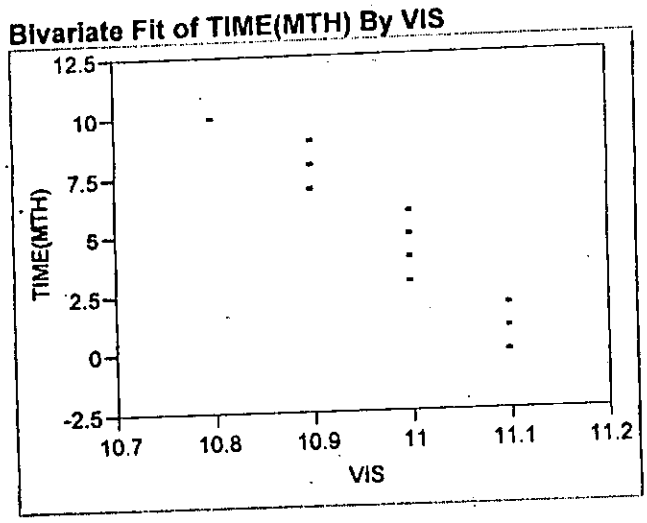


Bivariate Fit of TIME(MTH) By TR



Bivariate Fit of TIME(MTH) By PH




Bivariate Fit of TIME(MTH) By VIS
12.5
10
7.5
5
2.5
0
-2.5
TIME(MTH)
10.7
10.8
10.9
11
11.1
11.2
VIS

APPENDIX 111

Culture Media and Reagents

1. **Liquid Media**

(a) **Nutrient broth**	g/L
Beef extract	10
NaCl	5
peptone	10
Distilled water	1000ml

(b) **Czapek's broth medium of Saad (1992)**	g/L
Carboxymethyl cellulose (CMC)	30.0
Sodium nitrate	3.0
Magnessium sulphate	0.5
Potassium chloride	0.5
Iron (111) sulphate	0.01
Di- potassium hydrogen phosphate	1.0

(c) **Peptone water**	g/L
Peptone	10
NaCl	5
Distilled water	1000ml

(d) **Yeast Extract Trypticase Soy Broth**	g/L
Pancreatic digest of casein	17.0
Papaic digest of soybean meal	3.0
NaCl	5.0
Di basic potassium phosphate	2.5
Glucose	2.5
Yeast extract	3
pH	7.3 at 25^{0}C

(e) **Koser's Citrate medium**	g/L
Sodium ammonium phosphate	1.5
Potassium dihydrogen phosphate	1.0
Magnessium sulphate	0.2
Sodium citrate	2.5
Bromothymol blue	0.016

2. Solid Media

(a) Nutrient agar	g/L
Lab-Lemco beef extract	1.0
Yeast extract	2.0
Peptone	5.0
NaCl	5.0
Agar	15.0
pH	7.4

(b) MacConkey Agar	g/L
Peptone from casein	17.0
Peptone from meat	3.0
Sodium chloride	5.0
Lactose	10.0
Bile salt mixture	1.5
Neutral red	0.03
Crystal violet	0.001
Agar-agar	13.5

(c) Potato Dextrose Agar	g/L
Potato extract	4.0
Dextrose	20.0
Agar	15.0

(d) Christensen's Urea Medium	g/L
Urea	1
NaCl	5
KH_2PO_4	2
Agar	20
pH	6.8

(e) Aesculin Medium	g/L
Peptone water	100ml
Aesculin	0.1
Ferric citrate	0.05
New Zealand powdered agar	1.0
pH	7.4

(f) Yeast Extract Agar	g/L
Yeast extract	3
Peptone	5
New Zealand Powdered Agar	15
pH	7.2

3. Chemical Reagents

(a)	**Kovac's Reagent**	g/L
	P-Dimethylaminobenzaldehyde	5.0
	Amyl alcohol	75.0ml
	Concentrated HCl	25ml
(b)	**Solution 1 (Suspending buffer)**	
	Glucose	50mM
	Tris HCl, pH 8.0	25mM
	EDTA, pH,8.0	10mM
	Keep ice cold	
(c)	**Solution 11 (Lysing buffer)**	
	NaOH	0.2N
	SDS	1%
(d)	**Solution 111 (pH lowering buffer/acetate buffer)**	
	Potassium acetate	60ml
	Glacial acetic acid	11.5ml
	Distilled water	28.5ml
	Keep ice cold	
(e)	**TBE buffer**	
	Tris base	54
	Boric acid	27.5
	EDTA (0.5M)	20ml
(f)	**TENS solution**	
	Tris EDTA	10mM
	Sodium hydroxide	0.1N
	SDS	0.5
(g)	**Running gel preparation**	
	TBE	5ml
	Distilled water	45ml
	Agarose	1
	Ethidium bromide	7µl
(h)	**Methylene blue**	
	Methylene blue	0.5
	Distilled water	100ml

BIOMÉRIEUX

[redacted]

ID 32 E V2.0 Printout Export New test Modify

REFERENCE
NO 11 SPREAD OBIDI

DATE
2/16/07

COMMENT

API

ID32

ID 32 C
ID 32 E
ID 32 STAPH
rapid ID 32 A
rapid ID 32 E
rapid ID 32 STREP

GOOD IDENTIFICATION

Strip	ID 32 E V2.0
Profile	3 1 0 0 0 0 0 0 0 3
Note	

Significant taxa	**% ID**	**T**	**Tests against**			
Pseudomonas aeruginosa	95.6	0.6	ODC	1%	LIP	86%

Next taxon	**% ID**	**T**	**Tests against**			
Burkholderia cepacia	3.3	0.51	ADH	2%	URE	2%

[redacted]

API 20 E V4.0 Printout Export New test Modify

REFERENCE
MRS OBIDI E.COLI
COMMENT

DATE
2/1/07

API
API 10S
API 20 A
API 20 C AUX
API 20 E
API 20 NE
API 20 STREP
API 50 CHB
API 50 CHE
API 50 CHL
API CAMPY
API CANDIDA
API CORYNE
API LISTERIA
API NH
API STAPH
RAPID 20 E

ID32

GOOD IDENTIFICATION

Strip	API 20 E V4.0							
Profile	7 1 4 4 5 1 2 5 7							
Note								
Significant taxa	**% ID**	**T**	**Tests against**					
Escherichia coli 1	**97.9**	**0.6**	**ADH**	**1%**	**MEL**	**75%**		
Next taxon	**% ID**	**T**	**Tests against**					
Salmonella arizonae	**1.7**	**0.22**	**CIT**	**75%**	**H2S**	**99%**	**IND**	

BIOMÉRIEUX

API 20 E V4.0 Printout Export New test Modify

REFERENCE
MRS OBIDI PROTEUS

DATE
2/1/07

COMMENT

API
API 10S
API 20 A
API 20 C AUX
API 20 E
API 20 NE
API 20 STREP
API 50 CHB
API 50 CHE
API 50 CHL
API CAMPY
API CANDIDA
API CORYNE
API LISTERIA
API NH
API STAPH
RAPID 20 E
ID32

EXCELLENT IDENTIFICATION

Strip	API 20 E V4.0
Profile	0 5 3 6 0 0 0 5 7
Note	

Significant taxa	**% ID**	**T**	**Tests against**
Proteus mirabilis	**99.9**	**1.0**	

Next taxon	**% ID**	**T**	**Tests against**				
Proteus vulgaris	**0.1**	**0.14**	**ODC**	**0%**	**IND**	**92%**	**SAC**