ACKNOWLEDGMENT

I am greatly indebted to thank and deep appreciation to ***Dr. Mohamed Abdel Aziz Toama***, Professor of pharmaceutical Microbiology, Faculty of pharmacy, Cairo University for suggesting the subject, supervising the work, expert advice and the sincere help through out the course of the research.

I am indebted to present my greateful acknowledgement to ***Dr. Zeinab El Demerdash El-Bazza***, Professor of Microbiology (NCRRT) for suggesting the point, supervising the work and sincere help and critical reading of the manuscript.

My deep appreciation is to Shahin A. A., Prof. of Microbiology (NCRRT) for helping in identification of fungi : at the Mycological Center of Assiut University (AUMC).

The deepest thanks are also to the staff of the Drug Radiation Research Department (NCRRT) for their help.

My thanks are extended to the chairman of NCRRT and kind collaboration to the staff members of Gamma irradiation unit (NCCRT) for their cooperation in carrying out the experimental irradiation.

My special cardiac thanks are offered to my ***mother, father and husband*** for their continuous encouragement and support.

ABSTRACT

In the present study, 50 cosmetic cream samples were purchased from the market in Egypt. Cream samples were found to be contaminated with bacteria and fungi in the range of 1.0 x10^5 to 1.0 x 10^8 and 1.3 x 10^3 to 2.1 x 10^6 respectively. The contaminating organisms were gram positive cocci, gram positive rods, gram negative cocci, gram negative rods, gram negative cocci, *Aspergillus* species and *penecillium* species. The effect of consumer handling and time on the microbial levels of the tested cream samples which showed, in the present investigations, no microbial contamination were used. The results reveal that the level of contamination was found to increase with time and during use. The contamination were identified as gram positive rods, gram positive cocci. *Aspergillus* species and *Penicellum* species.

Samples were subjected to gamma radiation, bacterial sublethal dose levels ranged between 1.5 and 20 kGy, 5 and 7kGy, 3 and 5 kGy, while the fungal sublethal dose levels ranged between 3 and 5 kGy, 3 and 5 kGy also 3 and 5 kGy for foundation creams, foundation moisturizing and moisturizing creams, respectively.

Bacteria survived sublethal dose levels were identified as *Staphylococcus haemolyticus, Micrococcus* sp. *Bacillus brevis, Staphylococcus hominis-novo, Bacillus spharicus, Bacillus-pantothenticus* and *Bacillus Alvei* in foundation

cream. While, Acenatobacter baumann / haem, *Bacillus sphaericus* and *Bacillus pumilus* are the bacteria that survived the sublethal dose levels in foundation-moisturizing cream. *Bacillus pantothenticus, Staphylococcus haemolyticus* and *Micrococcus.* sp. were recorded in moisturizing cream samples. The surviving fungi were identified as *Aspergillus niger, Aspergillus fumigatus* in foundation cream, and *Aspergillus niger*, *Aspergillus tamari* and *Penicillium chrysogenum* in foundation-moisturizing cream, while the fungi surviving is *Aspergillus niger* in moisturizing cream.

For foundation cream, the sterilizing doses were calculated to be (6.4 – 23 kGy), (6.1 – 8.6 kGy), (4.7 kGy), and (5.3 – 7.45 kGy) for brands A, B, C and D, respectively. For foundation moisturizing cream, the sterilization doses were calculated to be (6.7 – 10.4 kGy) and (7.75 – 13.4 kGy), for brands F and G respectively. While, for moisturizing cream, the doses were (5.1 – 5.4 kGy) and (8.4 – 8.9 kGy) for brands H and I, respectively. Gamma sterilization doses were applied to the most heavily contaminated samples and results showed no contamination on the irradiated samples.

INTRODUCTION

Cosmetics and to toiletries are in daily use to cleanse, perfume, beautify or decorate the human body. They are mainly applied to the skin or hair, but some such as toothpastes and mouthwashes, are also used internally.

Cosmetics are not intended to permanently alter the physiology of the target organ, although some "health care products may contain an active substance or make medicinal claims. Microbiology of cosmetics is therefore complex due to the wide range of formulations, manufacturing procedures and conditions of consumer use.

There is a wide spread exposure to potential contaminants during manufacture, particularly from raw materials. Water is the most common ingredient and possesses obvious problems, but seemingly innocuous substances such as talc can be contaminated with dangerous pathogens. The principles of Good Manufacturing Practice GMP must always be followed and raw materials, particularly those of natural origin, must be tested for contamination before use and limits of acceptability established. Areas where contamination may be introduced must be identified and controlled. Due to GMP, contamination during actual production is of such a low order that modern cosmetics manufacturing plants can achieve "absence of microorganisms in almost 100% of units produced".

Manufactures also aim, wherever possible, to develop formulations which are incapable of microbial growth. The level of microbial contamination in a non-sterile product such as, cosmetics formulations, is made clear in the microbial limit standards which should be maintained in the products during their use, in spite of the inevitable contamination by the users,

through the addition of a suitable preservative in the products which guarantees the control of microbial growth even before they are marketed.

Cosmetic product are used allover the world and, although aiming at the same high level of consumer protection, their regulations and requirements are quite different from one part of the globe to another. Contaminating microorganisms in cosmetic may cause a spoilage of the product and, when pathogenic, they represent a serious health risk for consumers worldwide (**Behravan *et al.*, 2005).** Therefore, the need to control microbiological contamination of all products for human use and consumption, which support microbial persistence and / or growth, has been of considerable concern to manufacturer. Modern pharmaceutical, cosmetics and toiletries strive for high microbiological standards to protect their products from spoilage on the hand, and their consumers from infection, on the other hand unlike foodstuffs, which are usually kept refrigerated (or thrown away after a few days), a much longer shelf life is expected of personal care products **(Razem *et al.*, 2003).** The microbial contamination of personal care products may occur already in the course of production, through raw materials, ingredients and handling, or the contamination of a final product may ensue through its repeated use by the consumer. A wide range of preservatives has been developed to combat the contamination from the latter source. Maintaining a careful balance between protection against microbial contamination and limiting the health risks of

preservatives has been constituting the art of preservation **(Martin, 1997)**.

The increasing introduction of more vigorous biological standards by regulatory agencies and industry members themselves for pharmaceutical. Medical devices and health care products, has renewed interest in radiation processing as a means of sterilization or reduction in microbial load. Requirements for meeting these strict limits have resulted in revisions to current methods and a search for other technologies to replace or augment some of the processes, which have traditionally been widely employed. There are definite advantages for the introduction of radiation sterilization techniques by local cosmetics manufactures as the preparations will become microbiologically safe preparations. Also, the radio-sterilization as a process that increases the shelf life of the product (a great benefit for the manufacturer). The problem of assuring the complete freedom from pathogenic microorganisms will be solved.

In addition, radiation sterilization, being a cold sterilization method, is quite compatible with most thermally unstable constituents of the cosmetic preparations. It is also compatible with the plastic containers and packaging materials.

Local radiation sterilization facilities are now becoming increasingly available. The application of radiation sterilization in facial creams manufacture could become a method of choice in the near future. Before this could be adopted, however, this

is an urgent requirement to set out the limits of radiation doses, which could be safely applied under local conditions. This should be consistent with meeting the microbial requirements and at the same time insuring the integrity of the preparation and its container.

Aim of the Work:

The present investigation was elucidated to determine the bioburden of some cosmetic facial creams (intact and in use) purchased from different markets in Egypt, isolation and identification of microorganisms from the selected cosmetic cream samples. Study of the gamma radiation effect on the bioburden of the cream samples, dose response study of the radio-resistant microorganisms to gamma radiation and design of the sterilization dose for the selected cosmetic cream products using these radiation resistant microorganisms.

LITERATURE REVIEW

Cosmetic preparations:

Cosmetic preparations are preparations intended for cleansing, beautifying, promoting and altering the appearance of the skin. Cosmetics are classified into: Toilerties, Soaps, shampoos, hair dressings sprays and setting lotions, hair straighteners (relaxers), deodorants, antiperspirants sun-protective agents, skin care products, shaving agents, cosmetic cleansers, astringents, toners, moisturizers, masks, night creams, bath products, make up products: Foundations, eye make up (shadows, liners, mascaras), Lipsticks, rouges, blushers, nail enamel, fragrances products: Perfumes, toilet

water, body silks, bath powders and after shave agents **(FDA, 1997).**

Formulation of foundation creams:

Four basic facial foundation formulations are available, oil-based, water-based, oil-free, and water-free or anhydrous forms. Oil-based products are designed for dry skin, while water-based products can be adapted for all skin types. Oil-free formulations are used in oily skin foundations, while anhydrous forms are extremely long wearing and used for camouflage or theatrical purposes **(Draelos, Z.K., 1993).**

Microbial contamination of cosmetics:

A-Contamination of cosmetics during manufacturing:

a- Raw materials:

The degree of microbial contamination of raw materials depends on their origin. Natural origin mean any plants, animal or mineral material (e.g. gums, sugars, gelatin, hormones, talc or silica). Although method of extraction may reduce the microbial population, there is always the possibility of contamination due to factors such as increased or changed initial population, change in procedures, or plant breakdowns. Active substances of vegetables or animal origin are more often a source of contamination than synthetic ones **(Pederson and Ulrich, 1986; Schiller *et al.*, 1968 and Razem *et al.*, 2003).**

Hefni (1987) stated that materials, which are derived from animals, may be contaminated with animal-borne

pathogens. While, materials of plant origin are more likely to be contaminated with Gram negative bacteria, *Erwinia* spp. and *Pseudomonas* spp. or Gram positive bacteria, *Streptococci*, *Lactobacillus* spp. *Bacillus* spp. and non-myceliated yeasts.

Synthetic compounds are rarely contaminated owing to the use of high temperatures in the manufacturing process, organic solvents, extremes of pH, etc. Although microorganisms may contaminate all types of raw materials, the potential for growth in synthetic materials is very low. In general, contamination of synthetic materials is not detected by currently available microbiological techniques and is considered to be of little practical significance **(Russell, 1988).**

b- Air:

Air is the second most important source of contamination. It can harbor large numbers of microorganisms particularly the spore-forming bacteria; *Bacillus* spp. and *Clostridium* spp. and the non-sporing bacteria; *Staphylococcus* spp., *Streptococcus* spp., *Corynebacterium* spp. and *Enterobacteria* as well as molds, *Penecillium* spp., *Aspergillus* spp., *Mucor* spp. and yeasts **(Evans *et al.*, 1972)**. Air-borne organisms are usually associated with dust and skin scales **(Clegg, 1988).**

c-Water:

Water is the most widely used raw material, which is used as an ingredient, cleansing agent or suspending agent. So, it plays a dual role in cosmetic formulation, being the source of many potentially harmful organisms and providing the vehicle

for the growth of organisms introduced by other sources. The methods of water treatment, its storage and influence on its microbial quality and all these factors must be taken into account when assessing its quality **(Hefni, 1987).**

Microorganisms, particularly Gram-negative rods, can grow rapidly in water to 10^5-10^6 c.f.u/ml under all conditions, clearly showing water to be a high risk raw material. This level of contamination can not be seen with the naked eye. Microorganisms endogenous to fresh water include *Pseudomonas* spp., *Alcaligenes* spp., *Flavobacter* spp., *Serratia* spp. Examination of stored industrial water supplies showed that 98% of the contaminants were Gram-negative bacteria; other organisms isolated were *Micrococcus* spp., yeasts, yeasts like fungi and *actinomycetes* **(Clegg, 1988).**

d-Equipment:

Both compounding and filing equipments act as a contamination source due to direct contact with the product. Between preparations of product batches, growth of microbial contaminants can occur in dead spaces (joints and valves) and such type of contamination is very difficult to eliminate. Cleaning and disinfection measures can be only, carried out effectively if the equipments is capable of being cleaned **(SCF, 2000).**

B-Contamination of cosmetics after manufacturing:

Abdelaziz (1975) found that all non-sterile pharmaceutical preparations are dispensed in plastic and glass containers with variable capacity according to the required volume, these

containers are not sterile, also the cork closures often contain many microorganisms and therefore may act as a source of contamination. Plastic and cork closures may absorb or inactivate preservatives in formulations so, they encourage microbial contamination.

Most foundations are fluids, creams, or pressed powders, all are susceptible to microbial growth. The US Food and Drug Administration which prohibits the distribution of adulterated cosmetics, considers a cosmetic "adulterated" if it contains a substance that may make it harmful to consumers under customary conditions of use. Microbial contamination clearly meets this definition. Cosmetic manufactures can prevent this contamination through proper manufacturing and sanitization procedures. Even if some product is contaminated, normal quality control and microbial plate counts will reveal the problem before the product is released **(Parsons, 1990).**

In Egypt comprehensive surveys were done on the microbial, quality of cosmetics and personal care items **(Hefni, 1987)**; eye shadows, mascaras and face creams, shaving creams and shampoos **(Abdelaziz, *et al.*, 1989)** body lotions and talcum powders **(Ashour *et al.*, 1989).** Face powders, vaseline preparations, lipsticks and other personal care items **(Awwad, 1993).**

The ability of microorganisms to grow on some types of cosmetic products is common in industry knowledge. Many cosmetic formulations if not properly preserved, provide a good medium for microbial growth, accordingly this may

constitute a health hazard to the consumer, keeping in mind that a finished product rejection due to the presence of these microorganisms can be costly and remembering that a final product is a chain of number of links, it makes sense to minimize potential weakness at all links to ensure a good final product raw materials, applicators and primary packaging are all links to the chain whose weakness could be the introduction of harmful microorganisms **(Swinwood and Wilson, 1990).**

Microbial contamination of aqueous consumer products, was found to be by Gram-negative bacteria. Bacteria has been a puzzling problem for some manufacturers because contamination is often sporadic and may occur in products that meet USP and/or Cosmetic, Toiletry and Fragrance Association (CTFA) acceptance criteria. The causes of contamination are believed to be lack of attention to good manufacturing practices resulting in the development of house organisms, inadequate preservative systems and/ or inadequate microbiological test methods and microbial limits for finished products **(Orth *et al.*, 1996).**

Hugbo *et al.* (2003) stated that cosmetic and topical products need not be sterile but may contain low levels of microbial load during use. They determined and compared the levels and types of microbial contaminants in commercial cosmetic products sold in the market and a laboratory prepared aqueous cream. Ten brands of commercially available cosmetic creams and lotions were randomly purchased from the open markets in *Nigeria.* Aqueous cream was also prepared. Their bacterial and fungal loads as well as types were evaluated.

They found that all the products were contaminated to varying degrees. *Staphylococci* and other Gram-positive cocci were the most preponderant; Gram-negative isolates were hardly found. Fungal contaminants consisted largely of *Aspergillus fumigatus, Pencillium* and *Microsporium* species. They concluded that the commercial cosmetic creams and lotions evaluated did not generally meet the standards for microbial limits as specified in official monographs. Such products can adversely affect health status of consumers as well as the stability profiles of the products.

Camapana *et al.* (2006) evaluated the microbial contamination of 91 cosmetics (23 emulsions, 47 tensiolytes, 21 aqueous pastes) in three different states of intact, in use and ending product. Total bacterial count, isolation and identification of pathogenic isolates were performed on the collected cosmetics. About 10.6% of tensiolytes were contaminated with *Staphylococcus warneri, Staphylococcus epidermidis* and *Pseudomonas putida.*

Okeke and Lamikanra (2001) stated that developing countries tend to have lower levels of hygiene and sanitation than industrialized countries, factors that make it possible for such organisms to thrive. The possibility that cosmetic products may function as vehicles of pathogen transfer does not appear to have been investigated to any extent. In addition, to this very important consideration, there is the possibility that bacteria present in unduly high levels at any point during its shelf life could contribute to physical deterioration of the product. The risk of this occurring is higher with many types of

moisturizing creams and lotions which contain special additives (including plant extracts, fatty acids and vitamins) that could serve as substrates for bacteria. They evaluated the bacteriological quality of skin moisturizing products, in Nigeria, and studied factors predisporing their bacterial contamination under tropical conditions. They found that the viable count of bacteria exceeded 10^3 c.f.u ml^{-1} or c.f.u. g^{-1} in 8 (16.3%) commercially available creams and lotions at time of purchase. *Escherichia coli, Pseudomonas* species, *staphylococcus* species and *Bacillus* species were the most commonly recovered bacteria.

The microbial contamination of personal care products may occur already, in the course of production through raw materials ingredients and handling or the contamination of a final product may ensue through its use by the consumer **(Razem *et al.*, 2003).**

Hugbo *et al.* (2003) stated that the tested cosmetic cream products were contaminated to varying degrees. *Staphylococci* and other Gram-positive cocci were the most preponderant; Gram-negative isolates were hardly found. Fungal contaminants consisted largely of *Asp. fumigatus, Pencillium* and *Microsporium* species. Challenge test (re-infection) with *Staphylococcus aureus* revealed that the commercial products have low capacity for suppressing bacterial proliferation such as may be encountered during in-use contamination.

Behravan *et al.* (2005) investigated the bacteriological quality of variety of unused and used cosmetic creams. The pour plate technique was used for aerobic bacterial colony counts, and microorganisms that grew in the culture were Gram-positive *bacilli, Staphylococcus aureus* and non *Escherichia coli* Gram-negative organisms was found to be higher for used cosmetic creams (45%, 38% and 8% respectively) than for unused creams (38%, 25% and 0%, respectively. Viable microorganisms were not recovered from 17% of the unused items whilst only 10% of the used creams did not contain viable microorganisms.

Campana *et al.* (2006) found that intact products showed no bacterial contamination, while species diffused in air, soil and water, such as *Staphylococcus species* and *Pseudomonas* species, were recovered in the in-used and ending tensiolytes, which in one case evidenced c.f.u values more than the standard microbial limit. The likely source of these microorganisms from the hand of users, as moisturizers are often used after or independent of washing. The recovery of these potential pathogenic species in the examined tensiolytes suggested that the preservative system wasn't effective.

Nasser (2007) evaluated the total microbial count of hundred eye make up samples in Egypt. Out of 40 eye shadow samples, 14 samples were found to be contaminated with *Staphylococcus aureus, Bacillus megaterium, Staphylococcus epidermidis* and *Klebsiella pneumonia.* While, 13 samples were contaminated with *Aspergillus Flavus, Aspergillus Niger, Fusarium* and *penicillium* species. Out of 35 mascara samples,

20 samples were found to be contaminated with *Staphyloc-occus Aureus, Staphylococcus warneri, Staphyloc-occus epidermidis* and 6 samples were contaminated with *Aspergillus Niger, Aspergillus Flavus* and *Fusarium* species. On the other hand, 11 samples of eye liner was found to be contaminated with *Bacillus cereus, Staphylococcus Aureus* and *Staphylococcus Epidermidies*. While, 14 samples were contaminated with *Aspergillus Niger*.

Spoilage:

A spoiled products is an important consequence of contamination, the susceptibility to contamination of pharma-ceutical ingredients surfactants, polymers, humectants, fats, oils and sweetening, flavoring and coloring agents, have been described by **(Beveridge, 1983).**

Smart and Spooner, 1972; Wilson *et al.*, 1975; Wilson and Ahearn, 1977; Madden, 1984; Iglewski, 1989 and Swinwood and Wilson, 1990) stated that the ability of microorganisms to grow and reproduce in cosmetic products has been known for many years. Microorganisms may cause spoilage or chemical changes in cosmetic products and injury to the user.

Contaminating microorganisms in cosmetics may cause spoilage of the product and, when pathogenic, they represent a serious health risk for consumers worldwide **(Becks and Lorenzoni 1995; Behravan *et al.*, 2005).**

Other manifestations:

A variety of aroma producing bacteria have long been identified **(Omelianski, 1923)**. Often their unpleasant odors are combined in spoiled products and are particularly disastrous in cosmetics and toiletries which depend on their own specific perfumes. The changes in the product may be due to the metabolism of multiplying organisms **(Smart and Spooner, 1972).**

Spooner (1988) reported that contaminants may be seen as sediment, turbidity or pellicle in liquid products on more solid preparations, colored colonies may be formed. The appearance of large bright yellow *Micrococcus* colonies on white cream. Mold growth is the most common visible manifestations of spoilage creams. It may also be visible on the surface of powders and tablets if they have been stored in damp conditions. Products such as shampoos, which contain a surfactant, are susceptible to contamination by Gram-negative water-borne bacteria.

C- Contamination during the use of cosmetics:

Wilson *et al.* (1975) examined over 150 cosmetic samples of mascaras representing eight popular brands for their susceptibility to microbial contamination during their use by study group members, additional mascaras from patients with symptoms and clinical findings of long-term blepharitis were also investigated. Microbes associated with facial skin and fingers of the study group users were typically isolated from mascaras after use. Establishments of reproducing populations

within cosmetics appeared to be related to the number of usages, personal habits of the user and the formulation of the product. Four patients showed marked clinical improvement when they stopped using the contaminated cosmetics. The application of used eye area makeup prior and following ocular surgery should be avoided.

Eye area cosmetics contaminated with microorganisms during use, particularly mascaras that are applied to the eyelashes with brushes, have been associated with ocular infections **(Ahearn and Wilson, 1976; Wilson and Ahearn, 1977).**

Ahearn *et al.* (1978) reported that microorganisms were isolated from about 60% of the used mascaras of the 159 women in the general study group. The most common bacteria were *Staphylococcus epidermidis*, *Micrococcus* spp. and the most common fungi were *Candida parapsilosis.*

Dawson and Reinhardt, (1981) surveyed 15 different brands of eye shadow on display for customer use in different retail store for microbial contamination. This was the first reported microbial surveillance of in-use eye shadow display testers in retail establishments. Of the 1, 345 individual samples obtained, 67% were contaminated with one or more species of microorganisms representing the genera *Staphylococcus, Micrococcus, Corynebacterium, Acinetobacter, Bacillus,* and *Moraxella.*

Abdelaziz and Alkhofahi, (1989) examined twenty eye cosmetics (AI-Kohl) in-use samples obtained from ladies of different socioeconomic standards, 85% of the samples were contaminated, 5% were heavily contaminated (more than 10^4 c.f.u/g) with bacteria and fungi. Coliofom bacteria in a number of 100 c.f.u/g or more were recovered from 20% of the samples and *Pseudomonas aureuginosa* was detected in one of the samples, some of the detected *Staphylococci* were of the aureus type.

Cosmetic products, in addition to being free from gross microbial contamination and pathogens at manufacture, should be capable of maintaining low contamination levels during use. The use of water and raw materials of suitable quality and good manufacturing practices should generally lead to the production of preparations with low microbial contamination. Adequate preservation and the use of non invasive packages (such as tubes, pumping or narrow orifice containers) increase the chances that contamination levels will remain low during storage and use of the product **(Brannan and Dille, 1990).**

The same authors stated that the dispensing closure used for containers plays an important role in protecting cosmetics from in-use microbial contamination. Their hypothesis was tested by aseptically packing unpreserved shampoo and skin lotion into containers with three different closure types which provided three degrees of protection against consumer and environmental microbial insults. Shampoo was packed in containers with slit-cap, flip cap or

screw cap closures. Skin lotion was packed in, containers with pump-top, flip cap or screw cap. The products were then used by volunteers, under actual in use conditions for 3-(shampoo) or 2-skin lotion (weeks). After use, the products were evaluated for microbial contamination using standard methods for enumeration and identification. The standard screw caped closure provided only minimal protection against microbial contamination of both shampoo (29% contamination incidence) and the skin lotion (71%). The slit cap closure on the shampoo container and the flip cap on the skin lotion container provided slightly enhanced degrees of protection (21 and 39% contamination incidence, respectively). The greatest amount protection (i.e. lowest contamination incidence) was provided by the flip cap closure for the shampoo container (0%) and the pump top closure for the skin lotion container (10%). As a result, closure type plays an important role in protecting poorly preserved cosmetics from in-use microbial contamination.

A survey by **Misilivec *et al.* (1993)** was conducted to assess both the potential health risk from shared use cosmetics caused by microorganisms and the microbial efficacy of preservatives in cosmetics. The study included samples of 3027 shared use cosmetic products were collected from 171 retail establishments nationwide revealed that fungi, were present in 10.4% of the products and 3.9% contained fungal pathogens or opportunistic pathogens. A large share of the fungal isolates were from eye products, fewer were from lip products. Pathogenic or opportunistic infection made up 32.2% of the fungal isolates. A slightly lower percentage of samples that

contained preservatives had fungi, a fact suggesting that preservatives reduce the incidence of fungi in cosmetics. Results of this survey indicate potential microbiological problems concerning the safety of shared used cosmetics.

Orth and Kebara (1998) said that adequately preserved cosmetic and drug products may become contaminated if they are diluted or repeatedly exposed to microorganisms during use. Packaging is a critical components for protection of the product from the time it leaves the manufacturing plant until the product is used up.

Perry, (2001) stated that mascara usage represents the ultimate challenge to a cosmetic. Consumers are not likely to store their shampoo in a car glove box, subjected to extreme temperature changes, but this does happen with mascara. Consumers have also been known to apply mascara whilst driving, poking themselves in the eye with the brush in the process and allowing the introduction of microbes. Even more likely is mascara being stored in a humid bathroom environment where brushes are dropped on the floor and containers are left open to contamination by harmful microorganisms.

Okeke and Lamikanra (2001) studied the following uses of commercially available creams and lotions by volunteers. They found that the recovered proportion of *Escherichia coli* and other Gram-negative organisms increased. They concluded that contaminated cosmetic products are

relatively uncommon but some products present a potential health hazard because they are unable to suppress the growth of organisms of likely faecal origin during use.

Nasser (2007) studies the effect of consumer handling through 28 days on the microbial level and type of eye make up samples. The investigator found that the microbial contamination in case of eye shadow samples were found to be *Staphylococcus epidermidis, Staphylococcus aureus, Staphylococcus albus, Aspergillus* species and *Fusarium* species. The eye mascara samples were found to be contaminated with *Staphylococcus aureus, Bacillus lichiniformis, Bacillus coagulans* and *Fusarium* species. While eye liner samples were found to be contaminated with *Staphylococcus aureus, Bacillus lichiniformis, Aspergillus,* species and *Fusarium* species.

Infections induced due to the use of contaminated cosmetics:

Contaminating microorganisms in cosmetics may cause a spoilage of the products while pathogenic one represent a serious health risk for consumers worldwide **(Becks and Lorenzoni, 1995; Behravan *et al.*, 2005).**

Pathogenic organisms that are encountered cosmetics are variants they include *Pseudomonas aeruginosa, Staphylococcus* species, *Escherichia coli, Kelbsiella* species, *Salmonella* species and Molds.

A-*Pseudomonas aeruginosa:*

Pseudomonas aeruginosa is a potentially harmful organism and has the ability to infect several areas in the body.

Ergun *et al.* (1987) examined the contamination of cosmetics by microorganisms, 14 samples from 64 cosmetics were found to be contaminated with bacteria. Nine of 38 shampoo, 2 of 15 hand cream, 1 of 5 hair cream and 2 of 14 hair tonic. Of the 14 isolates, 3 were *Pseudomonas aeruginosa*, 2 *Escherechia coli*, 2 *Staphylococcus aureus*, 5 *Bacillus subtilis* and 2 *Enterobacter*.

Groves (1998) reported that one of the microorganisms known to cause problems in cosmetics is *Pseudomonas aeruginosa.* Even very low number of this common bacteria can cause eye and skin infections. The minimum infective load is reported to be 2000 to 3000 organisms per ml.

B-*Staphylococcus* species:

Among all species, *Staphylococcus aureus* is a dangerous pathogen. **Kallings *et al.* (1966)** found that *Staphylococcus aureus* contaminated several preparation including hand creams, baby creams and other preparations. *Staphylococcus epidermidis* was isolated from baby powders and wound powders. **Bowman *et al.* (1971)** isolated *Staphylococcus epidermidis* from: lotions and topical ointments.

Staphylococcus species were also detected in 18 samples of both talcum powders and baby lotions, three of

these *Staphylococcus* were of the aureus type **(Ashour *et al.*, 1989)**. Also, *Staphylococcus auerus* was found to contaminate used and unused cosmetic creams **(Behravan *et al.*, 2004).**

Compana *et al.* (2006) reported that about 10.6% of tensiolytes were contaminated by *Staphylococcus warneir, Staphylococcus epidermidis* and *Pseudomonas putida.* On the other hand, **Nasser (2007)** isolated different species of *Staphylococci* from eye make up products.

C-*Escherichia coli:*

Aycliffe *et al.* (1969) found that a considerable proportion (29/70) of soaps and hand creams being used in London, were contaminated with Gram-negative bacteria *Escherichia coli. Escherichia coli* is an opportunistic pathogen produces disease when the resistance of the intestinal or adjacent areas is lowered sufficiently **(Baker and Breach, 1980)**. **Ashour *et al.* (1989)** reported that three samples out of 36 talcum powders contained *Escherichia coli.*

Okeke and Lamikanra (2001) found that *Escherichia coli* contaminated commercially available creams and lotions at time of purchase.

D-*Kelbsiella* species;

The Food and Drug Administration District Laboratory detected *Klebsiella pneumonia,* type 66, in commercial baby powder. The use of baby lotion contaminated with *Klebsiella pneumonia* resulted in five cases of conjunctivitis in the

newborn in Panama Canal Zone **(Bruch, 1971).**

Out of nine items of a specific brand of mascara, one isolates of *Klebsiella pneumonia* was detected **(Abdelaziz *et al.*, 1989)**. While, **Nasser (2007),** detected 10.5% of eye shadow samples to be contaminated with *Klebsiella pneumonia*.

E-*Salmonella* species:

Khatibie (1978) reported that there are nothing mentioned about *salmonella* infection due to use of contaminated cosmetic preparations, but there is a probability that especially several compounds from plant and animal origin are commonly used in preparation of cosmetics which harbor *Salmonella.*

F-Molds:

The members of the genus *Aspergillus* are generally saprophyte in nature which may cause disease in humans. Aspergillosis can cause a localized type of infection involving the nails, feet, external auditory canal or the eye **(Smith, 1957)** or as a pulmonary type infection involving the bronchi and lungs or as disseminated infection usually associated with prolonged therapy or debilitating disease. Filamentous molds were found to contaminate tooth pastes and mouthwashes **(Ashour *et al.*, 1987),** eye shadows, mascaras and face creams **(Abdelaziz *et al.*, 1989)** body lotions and talcum powders **(Ashour *et al.*, 1989),** creams and lotions **(Hugbo *et al.*, 2003)** eye make up products **(Nasser, 2007).** On the other hand, in

Denmark 22% of samples of sun care products which used on beaches contained *Canadida albicans* **(Perry, 2001).**

Danger of microbial toxins:

Consumers may be harmed by the production of toxins or metabolites or by the inactivation of biologically active constituents in a formulation. Many microbiological metabolites possess pharmacological activity and some have been intensively investigated because they also have antibiotic activity. Other molecules with pharmacological activity, are produced by common spoilage organisms such as, species of *Aspergillus* and *Pseudomonas* **(Matthews and Wade, 1977).**

Mycotoxins are highly dangerous metabolites produced by certain fungi **(Wogan, 1975; El-Bazza *et al.*, 1982; 1996).** The presence of mycotoxins, especially the aflatoxins, in pharmaceutical preparation may cause several outbreaks of diseases in man and animals **(Wilson and Miles, 1964 and Katibie, 1978).** Therefore, the use of good pharmaceutical manufacturing practice should avoid the risk of mycotoxicosis by controlling fungal contamination **(Fernandez and Genis, 1979).**

Microbial risk factors for human health from contaminated cosmetics:

Table (1): Microbial risk factors for human health from contaminated cosmetics **(Heinzel, 1999).**

Organisms	**Possible symptoms**
Gram-positive Bacteria:	
Staphylococcus aureus	Pus, sepsis
Streptococcus pyogenes	Ditto
Entrococcus spp.	Infections
Clostridium tetani	Tetanus
Clostridium perifernges	Gas gangrene
Gram-negative bacteria:	
Pseudomonas aeruginosa	Conjunctivitis, pus, infections
Klebsiella spp	Conjunctivitis
Entrerobacteriacea	Enterititis
Fungi:	
Candida albicans	Conjunctivitis
Candida parapsolosis	Conjunctivitis
Maleassezia furfur	dermatomycosis
Trichophyton spp.	dermatomycosis
Trichoderma	Inflammations
Aspergillus spp.	Allergic reactions

Good Manufacturing Practice Guidelines of cosmetics (G.M.P):

Rigorous adherence to good manufacturing practice minimizes the risk of adulteration or misbranding of cosmetics.

In order to comply with G.M.P and Microbial Quality Management, manufactures of cosmetics have to define and allow specific cleaning, sanitation and control measure to keep cosmetics appropriately clean and, free from microorganisms that could be harmful for the consumers or cause adverse effect on the quality of the cosmetics. These proceedings will include procedures to microbiologically control raw materials, bulk and finished products, packaging components, personnel, and equipment **(FDA, 1992a) and (SCCNPF, 1998).**

In addition to sanitary storage and handling of raw materials and sanitary manufacture of finished products, it is recommended that each batch of a cosmetic which isn't self preserving to be tested for microbial contamination before its release for interstate shipment and each cosmetic, particularly each eye area cosmetic, to be tested during product development for adequacy preservation against microbial contamination which may occur under reasonably foreseeable conditions of consumer use **(FDA, 1992b).**

The microbial limits of cosmetics:

Industry has made good progress in producing cosmetics according to guidelines which assure a high safety

standard.

The Cosmetics, Toiletry & perfumery Association (1996) recommends a total viable count of aerobic bacteria, yeast and moulds of less than 100 c.f.u per gram for eye and baby products, and 1,000 c.f.u per gram for other products at completion of manufacture.

The Scientific Committee on Cosmetic products and Non Food products **SCCNPF (1998)** stated that skin and mucous membranes are normally protected from microbial attack by natural mechanical barrier and defense mechanisms. However, protective integuments may be damaged and slight trauma may be caused by the action of some cosmetics that may enhance microbial infection. These situations are of particular concern when cosmetics are used in the eye area or mucous membranes or on damaged skin and when used by children under 3 years, elderly people and people showing compromised immune responses. These are the reasons to define two separate categories of cosmetic products in the microbiological quality control limits. They also stated that in relation with the microbiological quality control of cosmetics, the limits for products specifically intended for children under 3 years, eye area and mucous membranes, is that the total viable count for aerobic mesophyllic microorganisms not more than 10^2 c.f.u/g or ml in 0.5g or ml of the product. While, the limits for the other products is that the total viable count for aerobic mesophyllic microorganisms is not more than 10^3 c.f.u/g or ml, in 0.1 g or ml of the product.

FDA, 2001b stated that eye area products counts should not be more than 500 c.f.u/g or ml and for non eye area counts should not be more than 1000 c.f.u/g or ml. The presence of pathogens such as *Staphylococcus aureus, Pseudomonas auueruginosa* and other species and *Klebsiella pneumonia*, would be of greater particular concern, especially in eye area cosmetic products

Linter and Genet (1998) reported that the previously stated microbial limits by **SCCNPF (1998)** should be maintained in the products during their use, in spite of the inevitable contamination by the users, through the addition of a suitable preservative in the products which guarantees the control of microbial growth even before they are marketed.

There is poor information in the literature regarding the efficacy of preservative systems contained in the cosmetic products to control the microbial contamination of these products during their use by consumers **(Farrington *et al.*, 1994; Okeke and Lamikanra, 2001 and Campana *et al.*, 2006).**

Perry (2001) reported that harmful microorganisms might not be detectable using standard plate count (SPC) techniques and *P.aeruginosa, Staphylococcus aureus* and *C. albicans* are used as indicator organisms. In setting stricter standards, low levels of microorganisms below the limit of detection of the SPC can be determined by enrichment testing. If this approach is pursued then the recovery diluents, selective

media and the incubation conditions should be selected to promote the growth of the chosen indicator microorganisms.

Electromagnetic Radiation:

An entire spectrum of electromagnetic radiation is present in our environment in the from of radio waves, infrared, visible, ultraviolet, x-rays and gamma rays. Gamma rays are electromagnetic radiation with shorter wavelengths in the range of 10^{-11} to 10^{-7} cm, produce both ionizing and excitation in the media through which they travel. The biological effects of this radiation apparently result largely from the ionization which is produced. Those radiations, which originate from atomic nuclei, are termed gamma rays, those, which originate outside the atomic nuclei, are termed x-rays. In all respects other than origin, these two radiations are identical. Gamma rays are produced when an unstable atomic nucleus releases energy to gain stability. These x-rays may have any energy from zero to a maximum which is determined by the kinetic energy of the impinging electrons **(Casarett, 1968).**

Units of radiation:

The units of radiation are the rad or Gray. The rad is a unit for the measurement of the energy absorbed from ionizing radiation by the matter through which the radiation passes. The more modern unit is the Gray (Gy) which measures the energy absorbed, a Gray is defined as: the deposition of energy of one Joule per kilogram of tissue, whereas one rad is the deposition of 10^{-2} joule per kilogram (or 100 erg per gram) energy.

Thus, 1 GY = 100 rad
1 kGy = 100000 rad
Megarad (Mrad) = 1000.000 rad.

The value of absorbed dose depends on both the photon energy of the beam and on the type of the absorbing medium. Dose can be expressed in terms of a total value, measured in rads or Grays. However, it is sometimes more convenient to express it in terms of dose rate, which is the dose absorbed per unit time **George, (1975).**

Total dose = Dose rate x Time

Dose survival Curves:

Dose survival curves illustrate the relationship between numbers of surviving microorganisms and radiation dose. In practice, the necessary data are usually obtained by exposing a number of equalized population to increasing radiation doses and counting the number of survivors **(Ley, 1973).**

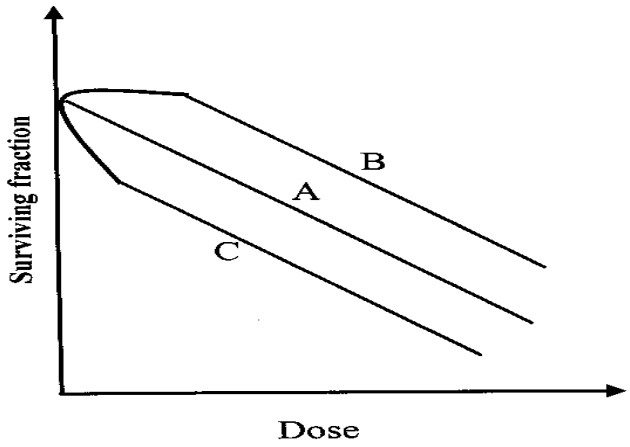

Fig (1): Hypothetical survival curves of irradiated bacteria: curve (A) exponential, curve (B) sigmoidal curve (C) composite, and (n) extrapolation number.

For convenience, it is usually to plot the logarithm of the surviving fraction of cells against the radiation dose. This semi-logarithmic plotting has shown up three types of survival curves first described by **Gunter and Kohn in (1956),** these three types are (A) exponential, curve (B) sigmoidal curve (C) composite (Fig.1).

A-Exponential Inactivation (Type A):

In (Fig.1), curve A represents a straight line or exponential rate of death. Examples of such a response have provided by **Woese (1958, 1959)** who showed that *Bacillus subtilis*, *B. brevis* and *B. mesentericus* were inactivated exponentially when exposed to x-rays.

Silverman *et al.* (1967), who noted a straight-line order of death when *B.globigii* was exposed to gamma radiation, with wet spores irradiated in air, vacuum-dried spores irradiated in air, vacuum-dried spores irradiated in vacuum.

It was reported that *B. licheniformis, B. megaterium, B. sphaericus C, A* and *B.pumilus* E601 exhibit exponential response towards gamma radiation **(Ashour *et al.*, 1993; Salih, 2001 and Nasser, 2007).**

The same response was exhibited by *Staphylococcus aureus* and *Aspergillus flavus* **(El-Fouly *et al.*, 2000)** and by *B. sphaericus* and *Micrococcus luteus* **(Farrag *et al.*, 2000**).

B- Non-Exponential Inactivation (Type B):

A more usual type of response, however is that depicted by curve B in the previous curve is followed by an exponential rate of death **Silverman and Sinsekey, (1977)**, and **Harm (1980).** Curve (B) occurs with the certain non-sporing bacteria such as *Micrococcus radiodurans* **(Bridges, 1976).** Curve B also occurs with various spores, including *Bacillus megaterium, B. mycoides, B. pumilis, B. stearothermophilus, B. subtilis, B. pantothenticus, B. licheniformis, Clostridium sprogenes* and *Cl. Botulinum,* types A, B and E and other *clostridia* **(Ley and Tallentire, 1964; Silverman *et al.*, 1976; Grecz *et al.*, 1967, 1977; El-Bazza *et al.*, 1997 and Nasser, 2007).**

It was demonstrated by **(Ashour *et al.*, 1993)** that *B. stearothermophilus; B. pantothenticus; B. circulans; B. coagulans* and *B. cereus* exhibit non-exponential response towards gamma radiation.

Russell (1982) reported that, the extent of the shoulder can be measured by extrapolating the exponential part of the curve so that it cuts the ordinate, to give the extrapolation number (n) as measured by the distance between the 100% survival axis and the intercept of the exponential part of the curve on the ordinate, the size of the shoulder can be defined also by the intercept of the exponential part of the curve on the 100% survival axis, this is referred to as quasi threshold dose (D_q)**.**

C-Resistant Tail (Type C):

The response depicted by curve C in the figure above, in which an exponential rate of death is followed by a decreasing rate of spore inactivation is encountered less frequently. Spores of *Cl. Botulinum* strains 62 A and 213 B in irradiated chopped ham exhibit a diphasic order of death, an initial rapid death decreasing in slope as dose increases **Greenberg *et al.*(1965).**

Similar tailing off phenomena have been noted by other authors for some sporing and non-sporing bacteria **Erdman *et al.*, (1961b); Wheaton and Pratt, (1962) and Dyer *et al.*, (1966).**

The reason for this tailing-off effect is not known, a slight increase in the resistance of *Cl.botulinum* type A spores by daily exposure to gamma radiation has been found **(Erdman *et al.*, 1961a, b)** and thus the production of radiation resistance mutants remains as possible reason for the tailing phenomenon **(Wheaton and Pratt, 1962).**

It was reported that ionizing radiation affects microorganisms in two ways directly and indirectly. The direct action theory known as the target theory was developed to a large measure by **Lea (1956).**

Woese (1958 and 1959) noted two types of inactivation curves with irradiated spores: a single hit straight line response with *B. subtils, B. brevis, B. mesentericus* and multi hit, initial shoulder response with *B. cerus,* and *B. mycoids.* He also reported that multiple target spores had a higher content of

diplocolinic acids (DPA).

Christensen and Holm (1964) reported that inactivation of bacteria by irradiation does not always cause the immediate death of the organisms. Many biological functions may persist for some hours after the bacteria have subjected to a dose which prevents their multiplication. The ability to multiply thus becomes the decisive criterion for inactivation in relation to sterilization.

Ginoza (1967) stated that the obvious lack of direct quantitative correlation between the initial hit events occurring in bacterial cells and their reproductive death serves as a reminder that the parameters associated with any bacterial curve are too numerous for target theory to be of direct help.

Moseley (1968) also considered that too much attention was being paid to explain variation in resistance in terms of cellular targets rather than to repair of radiation injury. Some bacteria possess the ability to repair the damage to DNA caused by the ionizing radiations at lower doses, and this may account for initial shoulder in the dose-survivor curve.

For the target theory to be applicable, destruction must be influenced by concentration, temperature or dose rate, these factors may affect the radiation sensitivity of spores. With an exponential rate of death, a single hit on the sensitive site (presumably DNA) is responsible for cell death, whereas with survival curves of types (B) in the above figure, several hits (multi hit theory) on DNA are necessary to bring about

inactivation **(Russell, 1982).**

Effect of gamma radiation on bacteria:

The response of bacteria to gamma radiation had been studied by several investigators:

(Christensen, 1973 and Nasser, 2007) isolated a substantial number of bacterial strains with considerably high radiation resistance in comparison with microorganisms in general.

The radiation sensitivity of microorganisms is conveniently expressed in terms of D_{10} **(Sztanyik, 1974).**

D_{10} value:

D_{10} value is defined as: the dose of ionizing radiation required to reduce a given microbial population by a factor of 10 or by one logarithmic cycle (90% kill). The D_{10} value is readily obtained from the linear part of a dose survival curve by reading off the dose required to reduce a surviving fraction through one log cycle **(Christensen, 1970; Ley, 1973 and Russell, 1982).**

D_{10} value may also be obtained from the following equation **(Schmidit and Nank, 1960).**

$$D_{10}\ \text{value} = \frac{\text{Radiation dose(D)}}{\text{LogN}_O - \text{LogN}}$$

Where N_o is the initial number of cells, N is the number of cells surviving the treatment D.

Statement concerning the radiation resistance of different species of microorganisms should always be regarded as relative. The difference in the inherent radiation resistance between strains of bacteria of the same species are significant **(Christensen *et al.*, 1967a, b, c; Ley, 1973; Christensen, 1974).**

Bochkarev *et al.*, (1978) examined the radiation sensitivity of about 8.000 strains of Gram-positive microorganisms of dried culture preparations. From 1500, strains of *Staphylococci* 30% were found to have D_{10} value of 0.1 to 1 kGy and 70% were found to have D_{10}-value of two kGy. 516 strains of Gram-positive spore forming microorganism have D_{10} values ranging from 0.1 to 0.4 kGy.

EL-Shafie, (1982) studies the resistance of *Bacillus cereus* and *Bacillus brevis* and found that D_{10} values were 2.2 and 1.9 kGy, respectively.

Russell, (1982) reported that a considerable variation may exist between different strains of the organisms, for example *Cl. Botulinum* type E spores are considerably less radiation resistant than type A and B, where the response depends on the medium and temperature.

A relatively larger resistant strains of *Bacillus brevis, Bacillus lichiniformis, Staphylococcus aureus, Micrococcus varians, Micrococuus luteus, B. cooagulans, B. circulans* were studied by **El-Tayeb *et al.* (1991)** and the D_{10} values were found to be (1.9, 1.7, 2.18), (2.04, 1.33, 1.63), (0.49, 0.73,

0.95), (1.47); (1.5), (4.42, 1.73, 1.18) and (3.2, 3.64) kGy, respectively.

Ashour *et al.* (1993) studies the response of *Bacillus sterothermophillus, B. pantothenticus, Bacillus licheniformis, B. coagulans, Bacillus laterospores, Bacillus cereus, Bacillus megateriuin, Bacillus pumilis* E601 towards gamma radiation before and after radiation for 2 years. The D_{10} value for these strains before storage ranged from 2.33 to 4.1 kGy.

El-Bazza *et al.* (1997) found that the D_{10} values of *Aspergillus ochraceus* to be 0.33 kGy and two strains of *Aspergillus niger* to be 0.45 and 0.50 kGy. Also, they reported that the D_{10} values for *Bacillus megaterium, Bacillus pantothenticus* and *Bacillus brevis* were found to be 2.58, 3.0 and 1.63 kGy respectively.

Kotiranta *et al.* (1999) reported that four strains of *Bacillus cereus* were sensitive to radiation with gamma rays (D_{10} value = 0.4 kGy).

The D_{10} values of the studied bacterial species of *Micrococcus luteus, M. roseus, M. varians, Staph. Aureus* ATCC, *Staph aureus isolate, B. lichiniformis, B. coagulans, B. pumilis, B. brevis, B. cereus isolate, B. cereus ATCC, B. circulans, B. megaterium isolate, B. megaterium ATCC* and *Pseudomonas capacia* were found to be ranged from 1.8 to 2.83 kGy **(Roushdy *et al.*, 1999).**

The D_{10} value of *B. cereus, Staph aureus* were found to be 1.02, 0.37 kGy respectively, while *Aspergillus flavus* was

0.48 kGy **(El-Fouly *et al.*, 2000).**

The D_{10} values of *B. cereus, M. luteus, B. sphaericus* were found to be 1.0, 1.4, and 1.4 kGy, respectively **(Farrag *et al.*, 2000).**

Razem *et al.* (2003) reported that the D_{10} value for gram negative bacteria was about 0.4 kGy.

Nasser (2007) found that the dose response curves for all the Gram-positive cocci showed and exponential rate of death and the D_{10} values of *Staphylococcus aureus* were found to range from 0.7 kGy to 1.0 kGy, while, the D_{10} value of *Staphylococcus epidermidis* were found to range from 0.7 kGy to 0.8 kGy. The dose response curves for all the Gram-positive spore forming *Bacillus megaterium* showed an exponential rate of death and the D_{10} values were found to be from 1.7 and 1.8 kGy. On the other hand, an initial shoulder following by an exponential rate of death was obtained in case of *Bacillus cereus* (D_{10} = 1.4 kGy) and *Aspergillus niger* showed an exponential rate of death (D_{10} = 0.4 kGy).

Factors influencing radiation resistance of microorganisms:

Species of microorganisms:

Differences in radiation resistance occur within *Bacillus* and *Clostridium*. Among the *Clostridia, Cl. Botulium* types A and B spores are the most resistant. Among *Bacillus species; Bacillus pumilis* strain E601 (ATCC 27142) is probably the most radio resistant organism **(Anellis and Koch,**

1962 and Thornley, 1963).

In general, multi cellular organisms are more sensitive to radiation than are unicellular organisms. Gram-negative bacteria are more sensitive than Gram-positive and bacterial spores are more resistant than vegetative forms. The most radio resistant fungi are about as resistant as those bacterial spores having moderate radiation resistance, and the viruses are generally more radio resistant than bacteria **(Christensen *et al.*, 1982).** The big exception to this generalization *Micrococcus radiodurans* a very radiation resistant coccus that is quite heat sensitive. Thus, the particular species of microorganisms or even serotype or strain is the single important factor in determining the dose needed for a particular microbicidal effects **(Goldblith, 1971).**

Number of microorganisms:

The initial cell density does not appear to affect the radiation resistance of microorganisms **(Farkass *et al.*, 1967)**. Nevertheless, the initial number of organisms or bioburden is of importance in selecting an appropriate radiation dose for sterilization purposes **(Whitby and Gelda, 1979; Shihab, 1992).**

Medium:

Since part of the effect of ionizing radiation on organism is due to indirect action method through free radicals and activated molecules. The nature of the medium in which the organisms are suspended obviously can play an important

role in the dose requirements for a given microbicidal effect. In general the more complex the medium, the greater the competition of the components of the medium for the free radicals and activated molecules produced by the radiation, thus protecting the organism. Conversely, the greatest sensitivity usually occurs when the organism are suspended in the buffers or physiological saline **(Goldblith, 1971).**

Christensen and Sehested (1964) investigated the irradiation sterilization of spores of *B. globigii* and *B. subtilis* in various media (serum broth, water, methanol and phosphate buffered saline) before drying, the spores were washed and unwashed, unwashed spores showed the greatest resistance to ionizing radiation after suspension and drying in serum broth. The non-sporing organism *Streptococcus faceium* was also the most radiation resistance when suspended and dried in serum broth.

Temperature:

The temperature of the medium plays an important role in the dose requirements needed to destroy microorganisms. Organisms are much more sensitive in liquid solutions than when suspended in frozen states. This probably due to interaction with free radicals in liquid solutions and the immobilization of the free radicals and prevention of their diffusion when the medium is frozen. The indirect effects of radiations on microorganisms may be minimized by freezing a suspension to minimize migration of free radicals, or by using

direct preparations in order to restrict moisture content and thus prevent formation of the radicals, or by the addition of other solutes to compete for the free radicals **(Goldblith, 1971).**

Water activity:

The general experience of workers in the radiation field has shown that bacteria are more radiation resistant when dry than in the presence of water. **Powers (1965)** showed that with spores water simultaneously can be both a powerful protective agent and a sensitizing agent, and believed that one may be a physical phenomenon and the other is a chemical one. Nevertheless, it is generally conceded that, in the practical situation, with both foods and medical supplies, bacteria are more resistant in the dry state than when hydrated in suspension, and that this is due to minimization of indirect action in the dry state **(Webb, 1964; Christensen and Sehested, 1964; Christensen and Kristensen, 1981; Kristensen and Christensen, 1981).**

Ionizing radiation can also exerts its effect on microorganisms through the reaction products of the radiolysis of water (indirect action) diffusing into the cell and causing its demise. Since water is the major constituent of a cell, its radiolysis is also of importance **(Yarmenko, 1988).** The following equations represent the radiolysis of water:

$$H_2O \rightarrow H_2O^+ + e^-$$

$$H_2O^+ + H_2O \rightarrow H_3O^- + OH^0$$

$$H_2O^+ + e^- \rightarrow H_2O\text{-}H^o + OH^o$$

$$H^o + HO_2^0 \rightarrow H_2O_2 + 2O$$

The oxygen effect:

Tallentire (1958) showed that dried *B. subtilis* spores were more sensitive when irradiated in air than under reduced pressure. It was also found that the lethal efficiency of a radiation dose depended on the subsequent conditions under which the spores were stored. The highest level of inactivation occurred when post irradiation storage exhibited in oxygen, and the lowest post irradiation storage for 15 min occurred in nitric oxide before exposure to oxygen **(Tallentire and Dickinson, 1962)**. Oxygen present during or after the irradiation process can thus markedly influence the radiation resistance of bacterial spores of these species and a similar oxygen effect takes place with *B. megaterium, B. globigii* and *B. stearothermophilus* **(Silverman *et al.*, 1967).** When dried

spores of *B. megaterium* are irradiated in oxygen, their sensitivity is at highest level, when irradiated in nitrogen the sensitivity is intermediate and when irradiated in the nitric oxide, their radiation sensitivity is least **(Powers, 1965, and Russell, 1982).**

Radiation sensitizing compounds:

A good deal of work has been done in the field of radiation biology on using chemical compounds to protect microorganisms from radiation. Such compounds as N-ethylmalemide, iodoacetic acid, phenyl mercuric acetate and vitamin K_5, its derivatives and analogues have been found to be effective in modifying the viability of bacteria subjected to ionizing radiation **(Bridges, 1961 and 1962).**

Also, among the compounds capable of producing protection are glycerol, dimethylsulphoxide and sulphydryl compounds such as cysteine **(Bridges, 1963).**

Christensen and Sehested, (1964) reported that *Streptococcus faecieum* shows a difference in an inactivation factor or when cultured on blood agar and air-dried from serum broth suspension than when air dried from buffer saline. Inactivation was found to be higher in buffer suspension.

Gamma radiation sterilization:

Industry uses gamma radiation sterilization because of its superior reliability, safety, and cost savings over the Ethylene oxide fumigation method. Ethylene oxide has many

processing variables and is toxic and expensive. The Environmental Protection Agency has recently declared Ethylene oxide as both mutagenic and carcinogenic. The residual Ethylene oxide in hospital products has been reported to adversely affect hospital workers. Unlike Ethylene oxide fumigation, radiation sterilization imparts no toxic residuals. The discoloration and embitterment problems experienced earlier with some products sterilized by gamma radiation are being overcomed with the introduction of new manufacturing materials and lower radiation dosages. Other benefits, of radiation sterilization include the option of sterilizing some materials that could not otherwise be sterilized and using new types of packaging to better protect the products and increase shelf-life. The emphasis now being placed on cost containment for health care products will be another significant part of the answer to why industry uses gamma radiation sterilization **(Sparks, 1984).**

Ionizing radiation, which include high-energy electrons and electromagnetic gamma radiations, are lethal to microorganisms. Their use for sterilization has some clear advantages over other methods in that they have high penetrability and cause a minimal temperature rise in the irradiated products. Sterilization can therefore, be carried out, if desired, on the finally packaged product and is applicable to heat sensitive and ethylene oxide incompatible materials. A vast amount of knowledge has been accumulated on the radiation inactivation of microbial populations, where lethality is generally measured by the loss of colony forming ability of

the cells, when placed in suitable growth media. Generally, components irradiated in the dry state are much more irradiation stable than those irradiated in the aqueous solution, a factor which is particularly relevant when considering decontamination of specific highly contaminated components. In considering complete products, it must be emphasized that the stability of an individual component may be quite different when irradiated alone or as apart of a product. No doubt that the higher microbiological standards demanded for cosmetics will make gamma radiation a very useful tool for reducing microbial loads of contaminated cosmetic raw materials **(Jacobs, 1981).**

Swinwood and Wilson, (1990) reported that the increasing introduction of more rigorous microbiological standards by regulatory agencies and industry members themselves for pharmaceutical, medical devices, and health care products, has renewed interest in radiation processing as a mean of sterilization or reduction in microbial load. Requirements for meeting these stricter limits have resulted in revisions to current methods and a search for other technologies to replace or augment some of the processes which have traditionally been widely employed. They also reported that the irradiation of Talc, Gelatin, kaolin, and bentonite revealed that bioburden studies showed no microorganisms contamination following irradiation.

Control of microbial contamination by gamma radiation:

There has been an increasing awareness of the problem

of microbiological contamination of pharmaceutical and cosmetic preparations. While, such products with the exception of parental and ophthalmic preparations, do not generally have to be sterile, there is a recognized need for reducing their initial microbial contamination, particularly in the light of more vigorous microbial safety standards that are being introduced in both the pharmaceutical and cosmetic industries **(Jacobs, 1981).**

Ley (1976) reported that ionizing radiation, mainly in the form of gamma rays from the radioisotope Co-60, is being increasingly used for the inactivation of contaminants in cosmetics and toiletry preparation. The treatment is applied to the product in its final pack, and therefore, it is important to recognize that the properties of the packaging materials could be affected particularly if high radiation doses are used. The low radiation doses used for the control of contaminants in cosmetic products are quite unlikely to cause changes in packing materials which will limit the use of the process, with the possible exception of the discolouration of glass.

Progress in the technology of radiation sterilization including the development of large radiation sources, makes the method of decontamination must be feasible. In the case of pharmaceutical and cosmetic creams and ointments and other cosmetic preparations, reducing the microbial load of individual highly contaminated components may be practicable by use of gamma radiation **(Jacobs, 1981).**

The need to control microbiological contamination of all products for human use and consumption, which support

microbial persistence and / or growth, has been of considerable concern to manufactures. Modern food, pharmaceuticals, cosmetics and toiletries industries strive for high microbiological standards to protect their products from spoilage and their consumers from infection. Unlike foodstuffs, which are usually kept refrigerated or thrown away after a few days, a much longer shelf life is expected of personal care products **(Razem *et al.*, 2003).**

A standard hygiene control system is vital in order to lower the level of contamination to safe level. Ionizing radiation **(Tilquin, 1991 and Sainz Vidal *et al.*, 1999).** U.V light **(Harris, *et al.*, 1993),** heat **(Link and Buttner, 1992)** and chemicals **(Berth and Wolffbrandt, 1992)** are widely used for decontamination purposes. However, the use of any of these methods is limited by many factors, mainly the nature of the material to be decontaminated **(Tilquin, 1991 and White *et al.*, 1996)** and availability of the sterilizing means **(Brinston, 1995).**

Although, the radiation for decontamination is not very old, it has found its way into many applications. However, there is still much to be investigated to improve the efficiency and to find an ideal procedure, which can decontaminate items with minimal alteration of their components **(Tilquin, 1991).**

As a part of the effort dedicated to improve the efficiency of radiation sterilization, a number of theoretical models relating radiation dose and microbial contamination

load have been proposed **(Dwyer *et al.*, 1985 and Fitch *et al.*, 1985).** These models have mainly been used to predict the efficiency of radiation sterilization through calculating the probability of occurrence of a contaminated item after the delivery of radiation dose **(Salih, 2001).**

When a population of microorganisms is exposed to lethal treatment (antibiotics, ionizing radiations, high heat or preservatives). Microbial death occurs if the killing is fast enough. If the rate of killing is too slow, some members of the population may undergo genetic and metabolic changes that enable them to survive and grow **(Orth *et al.*, 1996).** Therefore, it would be desirable to know the rate of death required to kill bacteria fast enough to prevent adaptation / survival in aqueous cosmetic products **(Orth *et al.*, 1998).**

One of the microorganisms known to cause problems in cosmetics is *Pseudomonas aeruginosa*. Even a very low number of this common bacteria can cause eye and skin infections. The minimum infective count is reported to be 2.000 to 3.000 organisms **(Groves, 1998)**. Fortunately, *Pseudomonas aeruginosa* is very sensitive to radiation processing, and relatively low doses can eliminate *Pseudomonas* as a contamination risk **(Reid and Feirand, 1998).**

Choice of radiation sterilization dose:

In considering the choice of organisms for various sterilization processes, proposed that the number of test

organisms should represent the maximum contamination likely to be encountered in practice, together with a safety margin, and suggested that was convenient to aim at the total sterilization of a test object containing 10^5 to 10^6 organisms **(Kelsy, 1961).**

Christensen and Holm (1964) reported that radiation sterilization dose necessary to achieve sterility depends on several factors including the species and the number or organisms present before irradiation. The environment of the organisms during and after irradiation and the standard required permissible level of surviving organisms. The expression of "permissible level of surviving organisms" doesn't involve a change in definition of sterility, as it is previously understood, the absence of lining microorganisms, implying in practice no demonstrable microorganisms capable of multiplication. The individual object is either sterile or not, but the exponential course of most of the inactivation curves in all methods of sterilization means that it is impossible to indicate any dose whereby all microorganisms will be inactivated. All what can be done is to determine the probability of demonstrating microorganisms in a given sample.

The most important considerations in selecting a particular sterilizing dose are destruction of microorganisms, and no alterations in the product being sterilized. Thus a knowledge of the type and number of organisms per unit prior to sterilization is of considerable value **(Osterberg, 1973)** and

various studies have been devoted to this problem. **Armbrust, 1975** proposed radiation pasteurization of pharmaceuticals and raw materials, while, **Hangay (1978)** extended the concept to include cosmetics and raw materials.

Several theoretical studies have been made of the influence of the initial numbers of contaminants and their radiation resistance on the quality assurance of sterilized products **(Ley, 1971; Tallentire *et al.*, 1971; Ley *et al.*, 1972; Tallentire, 1973; Tallentire and Khan, 1975, 1978; Khan *et al.*, 1977).** The principle of this development of mathematical model, using sub process data, which examines the dependence on radiation dose of the proportion of items contaminated in a population undergoing irradiation. This model is based upon the concept that sterility testing of samples after sub-sterilizing radiation doses would give a number of positive samples from which it would be possible to deduce likelihood of positive at much higher radiation doses **(Kirshahbaum, 1971; Outschoorn 1977**; **Whitby and Gelda, 1979).**

USP XXI (1985) stated that the radiation techniques are available for sterilization of appropriate articles. The choice of the sterilization dose should be determined by the knowledge of the microbial bio-burden (types and numbers) and the nature of the article to be sterilized. A dose of 25kGy had been selected as starting point, but many articles including radiation sensitive articles have low or susceptible bio-burdens, can be sterilized effectively at lower absorbed dose levels.

Also, 25 kGy was an unrealistic dose as much as it is wasteful, in some instances damage to products and generally prohibitive **(Boegl, 1985).**

De Riso (1986) reported that the microbial death generally follows the first order kinetics of a mono molecular reaction, therefore the death rate can be represented by a straight line when the logarithm of the numbers of survivors is expressed as a function of exposure time (Fig. 2).

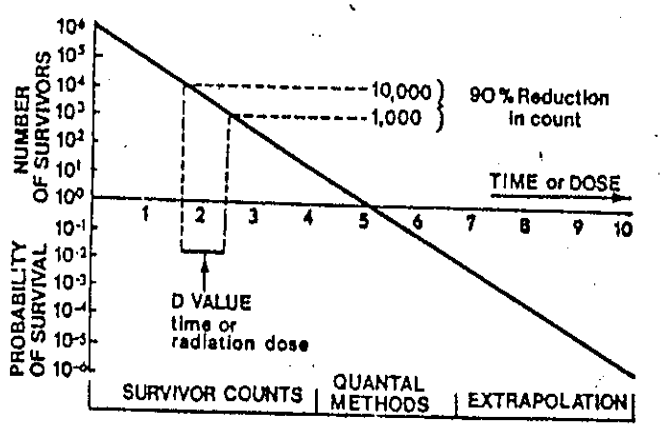

Fig (2): Microbial survival versus process dose

The same author also reported that at low process doses, the number of survivors could be counted to establish points on the survival curve. At somewhat larger doses, one would expect a fraction of the samples to contain surviving microorganisms. This area is called the quantal zone. In this region, one uses mathematical approaches such as the most probable number (MPN) method **(Stumbo, 1973)** for

calculating D-values based on fraction of replicate units that test sterile among samples exposed to several time increments within this region **(Pflug, 1979).** At higher doses beyond the quantal region, recovery of survivors exceeds the sensitivity and practicality of a sterility test. One must extrapolate to the probability of an organism surviving. The probability of survival that corresponds to the process of exposure time or radiation dose is refered to as the sterility assurance level (SAL). In practice, the SAL for most terminally sterilized products is 10^{-6} or one in one million **(Shihab El-Din, 1992).**

The pharmaceutical industry has lead the way in the change of attitudes from the quality control (QC) to the quality assurance (QA) approach. Treatment by irradiation is a particularly suitable measure to be integrated into a comprehensive QA approach in the processing of biological materials for microbial safety. Ionizing radiation is a universal, nonselective biocidal agent; it acts on all forms of life, in all parts of an irradiated volume; it can be delivered in precise portions of dose, and there is a well-defined relationship between the microbial kill and irradiation dose. An inverse proportionality between the logarithm of the viable cell count and absorbed dose holds for most pure microbial cultures. As consequence, the dose required to achieve the reduction of the number of microorganisms initially present No, to a selected endpoint level of microbial purity, N_D, can be determined.

$$D = D_{10} (\text{Log No} - \text{Log } N_D).$$

D_{10} is the decimal reduction dose, which is characteristic of microbial species and irradiation conditions.

In natural materials one would expect mixed populations of microorganisms rather than a single culture. Consequently the mentioned relationship would not be expected to hold throughout the entire dose range, while D_{10} value in the initial linear part would assume the meaning of the dose needed for the first 90% reduction of the viable cell count. Nevertheless, Total Aerobic Plate Count (TAPC) is a useful indicator of the microbiological status of the materials, both before and after irradiation **(Clegg, 1988; Razem *et al.*, 2001).**

The sterilization doses were calculated by using the survival curves of the radiation resistant isolates. The radiation sterilization doses of eye make up cosmetic preparations were calculated by the knowledge of the average bioburden on the eye make up samples, the radiation resistance of the contaminant (with the highest D_{10} value) and the sterility assurance level (SAL, $1:10^{-6}$) required for eye make up products after sterilization.

The calculated sterilization doses for eye shadow samples were found to be ranged between 6.1 kGy and 16 kGy. The dose for eye mascara were between 7.5 kGy and 8.3 kGy. While the sterilization doses for eye liner samples were found to be from 5.3 kGy to 12.3 kGy **(Nasser, 2007).**

MATERIALS AND METHODS

A-Materials:

1- Cosmetic cream samples:

A total of fifty (50) cosmetic cream samples were purchased from the market of different localities in Egypt. The investigated samples were 25 foundation cream samples of five (5) different brands, ten (10) foundation moisturizing cream samples of two (2) different brands and fifteen (15) moisturizing cream samples of three (3) different brands (Table 2).

Table (2): Cosmetic cream samples of different brands used in the present investigation.

Cosmetic face cream	Brand code	Brand name.	Number of Samples
Foundation cream	A	Engy	5
	B	Pop	5
	C	Joly	5
	D	Nancy	5
	E	Dhior	5
Total (F.)	5	5	25
Foundation-moisturizing	F	Bai-Meng	5
	G	Louys	5
Total (F. M.)	2	2	10
Moisturizing cream	H	Johonsons	5
	I	Lux	5
	J	Dhior	5
Total (M.)	3	3	15
Total.	10	10	50

F: foundation cream
F. M. : Foundation moisturizing

M.: Moisturizing

2- Chemicals:

Peptone, Lab-Lemco were product of oxoid. Yeast extract and beef extract were products of BBL. Agar-Agar and tryptone were the products of Difco. Other chemicals used in the present study were of the reagent grade.

3- Media:

a- Medium used for total aerobic count and isolation of the bacterial contaminants:

- Nutrient Agar:

Lab-Lemco	1.0 gm
Yeast extract	2.0 g
Peptone	5.0 gm
Sodium chloride	5.0 gm
Agar	15.0 gm
Dist. Water	1000.0 ml
pH was adjusted to	7.4 ± 0.2

b- Medium used for total count and isolation of the fungal contaminants

- Sabouraud's Agar:

Peptone	10.0 gm
Glucose	4.0 gm
Agar	15.0 gm
Dist. Water	1000.0 ml
Ph was adjusted to	5.6 ± 0.2

4- Microorganisms:

A total of seventy eight (78) microbial contaminants were isolated from cosmetic face cream products obtained from the market. The isolated microbial contaminants were 43

bacterial isolates and 35 fungal isolates. The contaminants were purified and maintained on nutrient agar for bacteria and on Sabouraud's agar for fungi. All cultures were stored at 4ºC and subcultured monthly on the same medium.

B-Methods:

Microbial evaluation of the tested cosmetic cream samples BP, 1988 and FDA, 2001 b):

Samples were analysed as soon as possible after purchasing, the samples were kept at room temperature. Samples were not incubated, refrigerated, or freezed before or after analysis.

Surfaces of samples containers were disinfected with aqueous mixture of 70% ethanol (v/v) before opening of the containers in a Laminar flow cabienet.

Diluent used for the Cosmetic cream samples (Nasser, 2007):

For the bacterial and fungal counts on detection, the diluent used was 0.1% tween-peptone (0.1% w/v peptone water containing 0.1% v/v tween 80, pH 7).

a- Preliminary contamination test: (Detection lest):

For detection of the contaminated cream samples. One ml of each of the tested samples were suspended in test tubes containing 9 ml sterile tween-peptone. The test tubes were shaken well on vortex (type paramix II No. 65, West

Germany). For bacterial detection, 0.1 ml was taken from each test tube and streaked on nutrient agar plates. The plates were incubated at 35ºC ± 2 for 72 hours. While, for fungal detection, 1 ml was taken from each test tube and mixed in plates containing sabourad's agar. The plates were incubated at 28ºC ± 2 for 3-7 days.

b- Evaluation test (Behravan *et al.*, 2004):

1- Total aerobic bacterial counts:

The spread plate technique was used, one ml of each cosmetic cream sample was mixed with 9 ml sterile tween-peptone, and ten fold serial dilutions were made in the same diluent. Then, 0.1 ml was taken from each suitable dilution and spread in duplicate sterile plates containing nutrient agar using a presterilized bent glass rod for each dilution. The medium was let to absorb the inoculum before invertion. Inverted plates were incubated at 35ºC ± 2ºC and examined daily up to 72 hours. Then, suitable dilutions were counted and the results were recorded.

2- Total fungal counts:

One ml of each cosmetic cream sample was mixed with 9 ml sterile tween-peptone, then ten fold serial dilutions were made in the same diluent. One ml was taken from each dilution and mixed with sabouraud's agar in sterile duplicate plates. The contents were allowed to solidify. Then plates were incubated at 28ºC ±2 and examined daily up to 7 days. Suitable dilutions were counted and the results were recorded.

Isolation of the microbial isolates:

According to the morphological characters of the microbial isolates. Calonies were selected, and spread nutrient agar for bacteria, and on Sabourud's agar for fungi, purified and then kept on slants of the same medium at 4ºC for further investigations.

Identification of the bacterial isolates:

All morphologically dissimilar colonial types were cultured onto Mac-Conkey agar and Baird parker media. Gram stains of all morphological dissimilar colonial types obtained in pure cultures were carried out. The identification procedures were carried out as follows:

Identification of the Gram positive rods:

Identification of the Gram positive rods were performed according to Cowan and Steel's, (1985).

Acetyl Methyl Carbinol production (Voges Proskauer test) (VP test):

The glucose - peptone medium tubes were inoculated with 24 hours culture of the tested microorganism and incubated up to 5 days at 32°C. The tubes were tested for positive VP reaction by adding 0.6 ml of 5% α-naphthol solution followed by 0.2 ml of 40% aqueous KOH. The tubes were shaken, sloped and examined after 15 min. A positive reaction was indicated by the appearance of a strong red color after 15 minutes.

Citrate utilization:

The surface of Simmon's citrate agar plates were inoculated with a loopfull of 24 hour culture of the tested microorganism after suspension in sterile distilled water, plates are then incubated at 32°C for up to 7 days. The plates were examined daily for growth and colour change. Positive citrate utilization is indicated by the appearance of growth and blue coloration in the medium.

Casein Hydrolysis:

Casein agar plates were streaked with one loopfull of a 24-hour culture of the tested organisms. The plates were incubated at 32°C for up to 14 days. Clearing zones around the bacterial growth indicated hydrolysis of casein.

Gelatin hydrolysis:

Tubes of nutrient gelatin were stab inoculated with a 24 hours culture of the organisms to be tested. The tubes were incubated at 32°C for up to 14 days. Every 2-3 days, the tubes were cooled in the refrigerator for 2 hours and then examined for liquefaction.

Growth on7% Sodium chloride Broth:

Tubes of 7% Sodium chloride Broth were inoculated with 24 hours culture of the tested organisms. The tubes were incubated at 32°C, and examined visually for bacterial growth up to 14 days.

Nitrate reduction test:

Nitrate broth tubes (Pot, nitrate, 1.0 gm and nutrient broth, 1000 ml), are to be inoculated with 24 hours culture of the organisms. The tubes are incubated at 32°C for 5 days, then 0.1 ml of nitrate reagent is added (1 ml of reagent A = sulphanilic acid, 0.8gm and acetic acid, 100.0 ml and 1 ml of reagent B = α-napthylamine, 0.5gm and acetic acid, 100.0 ml). The appearance of red colour in in the nitrate broth tubes indicate the positive reaction due to reduction of nitrate to nitrite.

Starch Hydrolysis:

Starch agar plates were inoculated by surface streaking with one loopfull of a 24 hrs culture of the tested organisms .The plates were incubated at 32°C for 5 days .The plates were then flooded with Lugol's iodine solution. The presence of clear zones around the colonies indicates positive reaction.

Growth at pH 5.7:

Nutrient broth tubes having a pH of 5.7 were inoculated with one loopfull of 24 hours culture of the tested organisms. The tubes were incubated at 32°C for 24 hours. The tubes were examined for growth as indicated by the appearance of turbidity.

Growth at 45°C:

Tubes of nutrient broth were inoculated with one loopfull for 24 hrs of the tested organisms. The tubes were

incubated at 45°C up to 72 hours. The tubes were examined daily for turbidity of the medium.

Identification of Gram positive cocci:

The Gram positive cocci showing growth on Baird Parker medium were identified using the automated "Microscan" which contains a variety of biochemical tests to identify the Staphylococcus species through an automated procedure that contains biochemical interpretations as follows:

Well	Reagent	Positive	Negative
CV MS NOV OPT NACL BAc		Growth	No Growth
NIT	Add drop 0.8% sulfanilic Acid and I drop of 0.5% N, NDimethyl-alpha-naphtyl amine. Wait 5 mins for the reaction to develop.	Pink to red	Clear (colorless)
PGR PHO PGT	Any shade of yellow should be interpreted as positive use The NOVas a negative control	Yellow	Clear (colorless)
IDX		Any shade of Blue may be a precipitate.	Clear (colorless or white precipitate
VP	Add I drop of 40% KOH and 1 drop of 5%Alpha Naphthol ,Wait at least 20 min for reaction to develop	Pink to red	Clear (colorless) to Brown, May be cloudy or very pale pink
BE		Dark Brown to Black	Clear (colorless) to Light brown
PYR	Add 2 drops Peptidase reagent .Wait for 2 mins for the reaction to develop.	Red / orange to Red	Yellow to orange /Red

ARG		Pink / orange to pink Pink	Yellow to orange
URE		Pink	Yellow to orange

Well	Reagent	Positive	Negative
RAF LAC TRE MNS SOR ARA RBS INU MAN PRV	Any hint of orange should be considered negative	Yellow	Orange to Red
BL	Add 1 drop Penicillin solution. Incubate 30 min. Add 1 drop Iodine reagent .Record within 5 min.	colorless	Black
HEM		Beta	Alpha or gamma

Identification of Gram negative rods:

Culture (24 hours) of the Gram negative rods was inoculated on the surface of the MacConkey Agar. The plates were incubated at 35+ 2°C for 48 hrs and examined for growth and the test resultant growth was identified using the automated "MicroScan" which contains a variety of biochemical tests to identify the gram negative bacteria through an automated procedure that contains biochemical interpretations as follows:

Well	Reagent	Positive	Negative
GLU		Strong yellow only Some non-fermentors may produce a golden color which should be considered negative.	Orange to red
SUC SOR RAF		Yellow 10 yellow orange	Orange to red

RHA ARA INO ADO MEL			
URE		Magenta to pink	Yellow, Orange or light pink
H_2S		Black precipitate or Black Button	No Blacking
IND	Add 3 drops (or 1 drop if panel is read visually) of Microscan kovac's Reagent color develops immediately.	Pink to Red	Pale yellow to orange

Compare LYS, ARG and ORN with DCB. The DCB well must be over layer with oil for the following results to be valid. For non-fermenters, a positive test must be significantly more purple than the base control, if the base control is purple, the basal medium has been, alkanized and the LYS, ARG and ORN should all be recorded as negative, *Achromobacter* species and *Ochrobacterum anthropi* tend to do this.

LYS ARG ORN		Purple Gray Purple	Fermenters: Yellow Non-Fermenters; Colorless to gray
TDA	Add 1 drop of 10% ferric chloride Color develops immediately	Any shade of Brown	Yellow to Orange
ESC		Light Brown to black	Beige to colorless
VP	Add 1 drop of 40% KOH and 1 drop of 5% Alpha Naphthol, Wait at least 20 min for reaction to develop.	Red to colorless	Colorless, pale pinl non-produce a color after 18 hours incubation

	Yellow	Colorless
ONPG	Compare any ONPG well which looks clear to the cetrimide well. If the ONPG shows any yellow as compared to the cetrimide well, record is positive.	
CIT MAL	Blue to Blue-Green	Green to Yellow

TAR ACE		Any blue shade is positive	
CET		Growth	No Growth
OF/G		Yellow	If of base is Green: Green to blue If of base is blue to Blue
		Green	Blue
		Yellow to Green	
		Compare OF/G well with OF base control	
NIT	Add drop 0.8% sulfanilic Acid and 1 drop of 0.5% N,N-Dimethyl-alpha-naphtyl amine. Wait 5 mins for the reaction to develop	Red	Colorless to Pale pink
P_4 K_4 Cl_4 Fd_{64} To_4 Cf_4		Growth (resistant)	No Growth (Susceptible)

Principles of identification of reactions:

Carbohydrates fermentation:

(GLU, SUC, SOR, RAF, RHA, ARA, IND, ADO, MEL):

The fermentation of a specific carbohydrate results in acid formation and a pH drop is detected by the phenol red indicator.

Urea (URE):

The enzyme urease splits urea forming ammonia, the resulting increase in pH drop is indicated by the phenol red indicator.

Hydrogen Sulphide (H_2S):

Hydrogen sulphide gas is produced from sodium thiosulphate and reacts with ferric ions in the medium to produce a black precipitate.

Indole (lND):

The metabolism of tryptophan results in the formation of basic amines which are detected by addition of Kovac's reagent if indole is present a red color is observed.

Lysine. Arginine, Ornithine (LYS, ARG, ORN):

Decarboxylation of these amino acids results in the formation of basic amines which are detected by the bromo-cresol purple indicator.

Tryptophan Deaminase (TDA):

Bacteria capable of deaminating tryptophan produce indole pyruvic acid, which reacts with ferric ammonium citrate in the medium after the addition of ferric chloride to produce a brown color.

Esculin Hydrolvsis:

Hydrolysis of esculin is detected by ferric ammonium citrate in the medium, which reacts with hydrolytic products to from a black precipitate.

Voges-proslauer(VP):

Acetylmethylcarbinol produced from glucose, reacts with 40% KOH and 5% Alpha Naphthol to form a red color.

Galactosidase (ONPG):

β-galactosidase hydrolyses ortho nitrophenyl β-D-galactopyranoside, which liberates the yellow -colored ortho-nitro phenol.

Citrate, Malonate, Acetamide, Tartarate (CIT, MAL, ACE, TAR):

The utilization of these substrates as the sole source of carbon for metabolism results in a rise in pH that is indicated by bromo-thymol blue indicator.

Oxidation -Fermentation (OF/G):

The oxidation of glucose results in acid fom1ation and a ph drop is detected by bromo-thymol blue indicator. The (OF/G) is compared to (OF/B) base to determine if any acid has been produced.

Nitrate (NIT):

Reduction of nitrate to nitrite is detected by the fom1ation of a red color following the addition of 0.8% Sulfanilic Acid and 0.05% N, N-dimethyl-alpha-naphthyl amine, which produce a red color in the presence of nitrite.

Cetrimide (CET):

Tolerance to cetrimide is demonstrated by growth in Muller-Hinton broth supplemented with cetrimide.

Penicillin, Kanamycin, Collistin, Cephalothin, Nitrofurantoin, Tobramycin (P_4, K_4, Cl_4, Fd_{64}, To_4, Cf_4):

Resistance to specific concentrations of these antimicrobial agents is demonstrated by growth.

Identification of the fungal isolates:

The fungal isolates grown on Sabouraud's agar medium were examined morphologically and then identified microscopically according to **Moubasher (1993)** and Manual of clinical microbiology **(2001),** at Assiut University, Mycological Center, Faculty of Science, Assiut – Egypt. (AUMC).

Gamma Irradiation Studies

Effect of gamma radiation on the total microbial counts in the cosmetic cream samples:

Preliminary test:

One ml of each cosmetic cream sample, in sterile test tubes were exposed to gamma radiation doses (5, 10, 15, 20, 25 and 30 kGy). Each irradiated sample was suspended in 9 ml tween-peptone. The test tubes were shaken well on the vortex. From each sample, 0.1 ml was taken and streaked on nutrient agar plate for detection of bacterial growth. While, 1 ml was taken and put in sterile plate and mixed with Sabouraud's agar for detection of fungal growth. The plates were incubated at 35ºC ± 2 up to 72 hours and at 28ºC ± 2 up to 7 days for

observation of bacterial and fungal growth, respectively.

Determination tests:

According to the results of the preliminary tests, each cosmetic cream sample was irradiated at the suitable irradiation doses from 1-30 kGy. After irradiation, the number of surviving colonies were counted on nutrient agar and Sabouraud's agar for bacteria and fungi, respectively. The mean of the duplicate plates was reported as CFU/ml and log number of survivors was determined at each dose level.

Isolation of the most radioresistant bacterial and fungal microorganisms:

From each cosmetic cream sample exposed to gamma radiation the microorganisms which could survive the highest radiation dose, under treatment, were isolated on nutrient agar for bacteria and on Sabouraud's agar for fungi. The isolated microorganisms were taken as the radioresistant isolates, identified and then subjected for studying the response to gamma radiation.

Study of the response of the radioresistant bacterial strains to gamma radiation

This study was carried out according to Nasser (2007) with some modifications as follows:

The radioresistant bacterial strains of the different cosmetic cream brands were used. A 24 hours of each radioresistant bacterial culture was surface inoculated on

nutrient agar in sterile duplicate plates. The plates were incubated at 35ºC ± 2ºC for 48 hours. After incubation, the heavy bacterial growth was scrapped off by sterile glass slide and transferred into small porcelain mortar in a laminar flow cabinet. Sterile saline-tween (Saline solution containing 0.1% tween 80) was added dropwise to the mortar and the mixture was homogenized using sterile pestle so as to have a thick suspension.

Aliquots of 20 µl of the resulting dense suspension of each radioresistant bacterial strain was withdrawn using a sterile micropipette, transferred into test tubes. The test tubes were exposed at room temperature to gamma radiation doses in the range of 0.0 kGy to 30 kGy. After irradiation, 10 ml sterile tween-saline solution was added to each irradiated tube and shaken on the vortex and one tenth serial dilutions of each irradiated bacterial strain were done in the same solution. From each appropriate dilution, 0.1 ml was taken and spread on duplicate nutrient agar plates. The plates were incubated at 35ºC ± 2ºC up to 72 hours. The mean of the bacterial counts of the duplicate plates were recorded as CFU/ml at each irradiation dose level and then, log No. of survivors were determined.

Study of the response of the radioresistant fungal strains to gamma radiation (EL-Fouly *et al*, 2000 and Bazza *et al.*, 2001):

The most radioresistant fungal strains of different

brands were used. Each radioresistant fungus was cultivated by spreading on Sabouraud's agar plate and incubated at 28ºC ± 2 for 14 days. After incubation, 2 discs (~ 10^6-10^8 spores) of each mold (1 cm diameter) were taken from each plate, using a sterile cork porer, and then transferred to sterile test tube. The test tubes containing the agar discs were exposed at room temperature to gamma radiation doses ranging from 0.5 to 7.0 kGy and non-irradiated agar discs served as normal control. After irradiation, the discs in each tube were suspended in 9 ml sterile saline-tween solution, shaked on the vortex and then, one tenth serial dilutions of the spore suspension were done in the same solution. One ml of each appropriate dilution was mixed with Sabouraud's agar in duplicate plates and then incubated at 28ºC ± 2 for 3-5 days. After incubation, the numbers of surviving colonies were counted and the mean counts of the duplicate plates were recorded as CFU/ml and log numbers of survivors were determined.

Construction of dose response curves of the microbial isolates:

The survival curves were obtained by plotting the logarithm of the number of microbial survivors versus the radiation doses in kGy. The D_{10} values which is the measure for the radiation resistance of the microorganisms to gamma radiation, can be read directly from the curves by finding the gamma radiation dose which reduces the microbial population by one logarithmic cycle.

Calculation of the sterilization dose:

The radiation sterilization dose for any product can be calculated by the knowledge of the average bioburden on this product, the radiation resistance of the most radiation resistant contaminant and the ultimate purpose for the use of the product (sterility assurance level, SAL) required for this product **(Nasser, 2007).**

Therefore, for determination of the sterilization dose of certain brand of the tested cosmetic creams, the survival curves of the most radiation resistant microorganisms were used. A parallel curve to that of the radiation resistant microorganism is drawn starting at initial count equals the average bioburden on the product wanted to be sterilized. In case of straight line survival curves, the curve is extrapolated to the negative side of the Y-axis. For the topical cosmetic creams, one per thousand (10^{-3}) is a good level for sterility. To get the sterilization dose for the product, a parallel line to the X-axis is drawn starting at log cycle (-3) on the negative side of the Y-axis until it meets the extrapolated part of the curve, then a parallel line to the Y-axis is drawn until it meets the X-axis to give the required sterilization dose which realize the sterility assurance level of one per thousand (10^{-3}). In case of shouldered survival curves, the size of the shoulder was defined by the intercept on the 100% survival axis (quasi- threshold dose, Dq). The Dq value was added to the determined dose value.

Applicability of radiation sterilization dose:

The calculated radiation sterilization doses for cosmetic cream samples were applied on the most contaminated samples of each individual brand. The probability of sample contamination was studied by determination of the viable count on the samples after application of the calculated sterilization doses, as mentioned before.

RESULTS

In the present study 50 cosmetic cream samples were purchased from the market in Egypt. The samples were twenty five (25) foundation cream, ten (10) foundation moisturizing cream and fifteen (15) moisturizing cream (Table. 2).

Evaluation of the microbial contamination of cosmetic cream samples:

1- Detection of the bacterial and fungal contamination:

Detection of the bacterial and fungal contamination were performed on plates containing nutrient agar and sabouraud's agar, respectively.

The results in Table (3) show that the microbial contamination differ between the different cream types and brands. The results also showed that the samples were contaminated with bacteria and / or fungi. Out of 25 foundation cream samples representing 5 brands, 12 samples were found to be contaminated, out of 10 foundation moisturizing cream samples representing 2 brands, 6 were contaminated, while, out

of the 15 moisturizing cream samples, representing 3 brands, 4 were found to be contaminated. The percent of contaminated samples is illustrated in Table (4).

2- Determination of the microbial contamination

The microbial counts as cfu/ml are illustrated in Tables (5-6). Results reveal that the level of microbial contamination of the tested cosmetic cream samples differs between the different types and between samples of the same brand.

The results in Table (5) reveal that out of the 25 foundation cream samples, 10 samples were found to be contaminated with bacteria and 9 samples were found to be contaminated with fungi.

Out of (10) foundation-moisturizing cream samples, (6) samples were found to be contaminated with bacteria and (6) samples with fungi, while, out of the 10 moisturizing cream samples, 4 samples were found to be contaminated with bacteria, while, they showed fungal contamination on (4) samples.

Results show that there are 43 bacterial contaminants and 35 fungal contaminants isolated from the tested cosmetic cream samples of the different brands.

Results also show that a total of 25, 8 and 10 bacterial isolates, while 20, 10 and 5 fungal isolates were isolated from samples of different brands of foundation cream, foundation-moisturizing cream and moisturizing cream, respectively.

The presented data is summarized in Table (7). The data show that 10 foundation cream samples were contaminated with bacteria in the range of $1.0x10^5$ to $2.5x10^7$ cfu/ml and 9 samples were found to be contaminated with fungi in the range of $1.3x10^3$ to $1.5x10^6$ cfu/ml.

Six foundation-moisturizing cream samples were contaminated with bacteria in the range of $4.0x10^5$ to $6.6x10^7$ cfu/ml and 6 samples with fungi in the range of $1.3x10^4$ to $7.0 x10^5$ cfu/ml.

Four moisturizing cream samples were contaminated with bacteria in the range of $7.5x10^6$ and $1.5x10^8$ cfu/ml while, they showed fungal contamination ranged between $9.5x10^4$ and $2.1x10^6$ cfu/ml.

The percent of contamination with bacteria were found to be 40%, 60%, 26.67%, with fungi were 36%, 60%, 26.67% and with bacteria and fungi were 48%, 60%, 26.67% for foundation cream, foundation-moisturizing and moisturizing cream, respectively.

Identification of the microbial isolates:

Identification of the isolated bacteria was done by the method of cowan and steel's (1985) for gram positive rods while gram positive cocci and gram negative rods were identified using automated "micro scan".

Identification of fungi was done according to **Moubasher (1993)** and Manual of clinical microbiology **(2001).**

Tables (8-9) illustrate the microscopical examination of the gram stained bacteria. The order of contamination on foundation cream samples is gram positive cocci > gram positive rods > gram negative cocci and gram negative rods. While, on foundation moisturizing cream, the order is gram positive rods > gram positive cocci > gram negative rods. On the other hand, the order of contaminants on moisturizing cream samples is gram positive cocci > gram negative rods > gram positive rods.

The results of evaluation of the fungal contamination on the tested cream samples is illustrated in Tables (10-11). The results show that *Aspergillus* sp. is predominate over *Penecillum* sp. as the only fungal contaminants.

Microbial evaluation of the tested cosmetic cream samples during in-use:

The effect of consumer handling and time on the microbial levels of the tested cream samples was done on samples which showed, in the present investigations, no detectable microbial contamin-ation was used.

Samples were subjected to share use by consumers (different consumers apply daily the selected test cream samples of a certain brand) and the level of contamination through 36 days was determined.

Results in Tables (12-17) reveal that the level of contamination was found to increase with time and during use.

After 36 days of use, the bacterial contamination of foundation cream, foundation moisturizing cream and moisturizing cream reach to $1.5x10^8$, $2.0x10^7$ and $1.0x10^7$ and for fungi it reachs to $7.5x10^6$, $4.2x10^6$ and $4.3x10^7$, respectively.

The predominant bacterial contaminations on the tested cream samples were identified as gram positive rods, and sometimes the contamination occur with gram positive cocci. While, the fungal contaminations were identified as *Aspergillus* sp. or / and *Penicellium* sp.

The results of the present investigations are summarized in Table (18)

Effect of gamma radiation on the total microbial counts in the cosmetic cream samples:

The contaminated cosmetic cream samples, were exposed to gamma radiation in the range of 5 to 30 kGy, the results of this preliminary test were illustrated in Table (19). Therefore, the suitable radiation doses for each cream sample was choosen in the determination test.

According to the results of the preliminary test, each contaminated cosmetic cream sample was irradiated at the suitable irradiation doses.

Gamma radiation effect on bacterial and fungal counts of foundation cream samples were illustrated in Figs (2-5) and (10-13), respectively. The results reveal that the bacterial

sublethal dose levels ranged between 1.5 and 20 kGy, while the fungal sublethal dose levels ranged between 3 and 5 kGy. Gamma radiation effect on bacterial and fungal counts of foundation-moisturizing cream samples were illustrated in Fig (6-7) and (14-15), respectively. The results reveal that the bacterial sublethal dose levels ranged between 5 and 7 kGy, while the fungal sublethal dose levels ranged between 3 and 5 kGy.

The results of the effect of gamma radiation on the bacterial and fungal counts of moisturizing cream samples were illustrated in Fig (8-9) and (16-17), respectively. The results reveal that the bacterial sublethal dose levels ranged between 3 and 5 kGy, while, the fungal sublethal dose levels ranged between 3 and 5 kGy.

Identification of microorganisms isolated from irradiated cream samples:

The results of identification of the bacteria and fungi surviving the sublethal dose levels on the irradiated cream samples were illustrated in Tables (20 and 21).

The bacteria surviving the sublethal dose levels from (1.5 – 20 kGy) were identified as *Staphylococcus haemolyticus, Micrococcus* sp. *Bacillus brevis, Staphylococcus hominis-novo, Bacillus sphaericus, Bacillus pantothenticus* and *Bacillus Alvei* from foundation cream. The bacteria surviving the sublethal doses from (5-7 kGy) were identified as *Acenaobacter*

baumann/haem, *Bacillus sphaericus* and *Bacillus pumilus* from foundation moisturizing cream.

While, those surviving the sublethal dose from (3-5 kGy) were identified as *Bacillus pantothenticus*, *Staphylococcus haemolyticus* and *Micrococcus* sp. from moisturizing cream (Table. 20).

The fungi surviving the sublethal dose levels from (3-5 kGy) were identified as *Aspergillus niger* and *Aspergillus fumigatus* from foundation cream. The fungi surviving the sublethal doses from (3-5 kGy) were identified as *Aspergillus niger*, *Aspergillus tamarii* and *Penicillium chrysogenum* from foundation-moisturizing cream, while those surviving the sublethal dose from (3-5 kGy) were identified as *Aspergillus niger* from moisturizing cream, Table (21). The results are summarized in Table (22).

Response study of the radiation resistant microbial strains to gamma radiation:

The results of the response of the radioresistant microbes to gamma radiation (the survival curves) are graphically represented in figures (18-35).

The D_{10} values or the decimal reduction doses were calculated from the graphs and were found to range from (0.4 to 2 kGy), (0.9 to 1.25 kGy), (0.47 to 1.0 kGy) in case of microbial strains isolated from foundation cream, foundation-moisturizing cream, and moisturizing cream, respectively. Results of this part are summarized in Tables (23 and 24).

Calculation of the sterilization dose:

The sterilization dose was calculated by using the survival curves of the radiation resistant isolates. The radiation sterilization doses of cosmetic face creams preparations were calculated by the knowledge of the average bioburden on the cosmetic cream samples, the radiation resistance of the contaminant (with the highest D_{10} value) and the sterility assurance level (SAL $1:10^{-3}$) required for cosmetic creams products after sterilization.

The results of determination of the gamma sterilization doses of cosmetic cream samples of the studied brands were illustrated in Fig (36-53) and Table (25).

For foundation cream, the calculated sterilization doses were calculated for the samples of the different brands to be (6.4 and 23.0 kGy), (6.1 to 8.6 kGy), (4.7 kGy), and (5.3-7.45 kGy) for brands A, B, C and D, respectively.

For foundation moisturizing cream, the sterilization doses were calculated to be (6.7 and 10.4 kGy) and (7.75 – 13.4 kGy) for brands F and G.

For moisturizing cream, the doses were (5.1 and 5.4 kGy) and (8.4 and 8.9 kGy) for brands H and I. It is clear from the results that the maximum doses concerning the present investigations are 23., 13.4 and 8.9 kGy for sterilization of studied foundation cream foundation-moisturizing cream and moisturizing cream, respectively.

The gamma sterilization doses were applied on the most heavily contaminated samples and results showed no detected contamination on the irradiated samples Table (26).

Table (2): Cosmetic cream samples used in the present investigations.

Cosmetic cream		**Brand code**	**Number of samples**
	Foundation cream	A B C D E	5 5 5 5 5
Total F.C.	1	5	25
	Foundation moisturizing cream	F G	5 5
Total F.M.C.	1	2	10
	moisturizing cream	H I J	5 5 5
Total M.C.	1	3	15
Total	3 Types	10	50 Samples

F.C. : Foundation cream
F. M. C. foundation moisturizing cream
M.C. : Moisturizing cream

Table (3): Screening for the microbial contamination on cosmetic cream samples of different brands.

Cream Type	Brand	Sample No.	Locality	Microbial contaminant	
				Bacteria	Fungai
Foundation cream	A (Engy)	1	Maadi (M)	+	+
		2	El Rehab	+	+
		3	Alexandria	+	+
		4	Marsa Matrouh	+	+
		5	(MM) Hurgada (H)	+	+
	B (Pop)	6	Maadi (M)	+	+
		7	Heliopolis	-	-
		8	Nasr city	-	-
		9	El-Rehab	-	-
		10	Alexandria (E)	+	+
	C (Jolly)	11	Maadi (M)	+	-
		12	El-Rehab	+	-
		13	Alexandria	-	-
		14	Marsa Matrouh	-	-
		15	(MM) Hurgada (H)	-	-
	D (Nancy)	16	Maadi (M)	+	-
		17	Helwan	-	+
		18	Zamalik	-	-
		19	Heliopolis	-	-
		20	El Rehab	-	+
	E (Dhior)	21	Maadi (M)	-	-
		22	El Rehab	-	-
		23	Alexandria	-	-
		24	Marsa Matrouh	-	-
		25	Hurgada (H)	-	-
Foundation moisturizing cream	F (Bai Meng)	26	Maadi (M)	+	+
		27	Helwan	+	+
		28	Heliopolis	-	-
		29	Nasr city	-	-
		30	El-Rehab	+	+
	G (Luoys)	31	Maadi (M)	-	-
		32	Heliopolis	+	+
		33	Nasr city	+	+
		34	El-Rehab	-	-
		35	Alexandria	+	+

To be Continued

Cream Type	Brand	Sample No.	Locality	Microbial contaminant	
				Bacteria	Fungai
moisturizing cream	H (Johnson)	36	Maadi (M)	-	-
		37	Helwan	+	+
		38	Zamalik	+	+
		39	Heliopolis	-	-
		40	Nasr city	-	-
	I (Lux)	41	Maadi (M)	+	+
		42	Helwan	-	-
		43	Zamalik	+	+
		44	Heliopolis	-	-
		45	Nasr city	-	-
	J (Dhoir)	46	Maadi (M)	-	-
		47	Helwan	-	-
		48	Zamalik	-	-
		49	Heliopolis	-	-
		50	Nasr city	-	-

+ detected growth **- Not detected growth**

Table (4): Percent of the microbial contamination in the tested cosmetic creams of different brands.

Cream Type	brand	No. of *S.	No. of **C.S.	% of C. S
Foundation cream	A	5	5	100.0
	B	5	2	40.0
	C	5	2	40.0
	D	5	3	60.0
	E	5	-	0.0
Total	5	25	12	
Foundation moisturizing cream	F	5	3	60.0
	G	5	3	60.0
Total	2	10	6	
moisturizing cream	H	5	2.0	40.0
	I	5	2.0	40.0

	J	5	0.0	0.0
Total	3	15	4	

***S: Samples.**

****C.S.: Contaminated samples.**

Table (5): Determination of the bacterial counts and number of isolates in cosmetic cream samples of different brands.

Cream Type	Brand	Locality	Sample No.	Bacterial Count (cfu/ml)	Number of bacterial isolates
Foundation cream	A	Maadi	1	1.9×10^7	2
		El Rehab	2	9×10^6	3
		Alexandria	3	4.7×10^6	2
		Marsa	4	1.0×10^7	3
		Matrouh	5	2.4×10^6	2
		Hurgada			
	B	Maadi	6	1.0×10^5	4
		Alexandria	10	3.6×10^6	2
	C	Maadi	11	3.0×10^5	1
		El-Rehab	12	2.5×10^7	3
	D	Maadi	16	1.5×10^5	3
Foundation moisturizing cream	F	Maadi	26	8.5×10^6	1
		Helwan	27	4.0×10^5	2
		El-Rehab	30	2.1×10^6	2
	G	Heliopolis	32	6.6×10^7	1
		Nasr city	33	5.8×10^7	1
		Alexandria	35	2.5×10^6	1
Moisturizing cream	H	Helwan	37	1.0×10^7	4
		Zamalik	38	3.0×10^7	3
	I	Maadi (M)	41	7.5×10^6	2
		Zamalik	43	1.5×10^8	1
Total	8	20	20	---	43

Table (6): Determination of the fungal counts and number of isolates in cosmetic cream samples of different brands.

Cream Type	Brand	Locality	Sample No.	fungal Count (cfu/ml)	Number of fungal isolates
Foundation cream (F. C.)	A	Maadi	1	7.5×10^4	1
		El Rehab	2	1.5×10^5	2
		Alexandria	3	1.5×10^6	2
		Marsa	4	1.2×10^6	3
		Matrouh Hurgada	5	1.3×10^3	1
	B	Maadi	6	5.6×10^3	5
		Alexandria	10	8.5×10^4	2
	D	Helwan	17	2.3×10^5	2
		El-Rehab	20	4.0×10^5	2
Foundation moisturizing cream (F M C)	F	Maadi	26	2.5×10^4	2
		Helwan	27	7.0×10^5	1
		El-Rehab	30	7.5×10^4	2
	G	Heliopolis	32	1.3×10^4	2
		Nasr city	33	7.8×10^4	2
		Alexandria	35	5.0×10^5	1
moisturizing cream (M.C)	H	Helwan	37	2.1×10^6	2
		Zamalik	38	9.5×10^4	1
	I	Maadi (M)	41	1.2×10^5	1
		Zamalik	43	1.8×10^6	1
Total	7	19	19	--	35

Table (7): Survey on the microbial contamination of the tested cosmetic cream samples of different brands.

Type	**Foundation cream**	**F. M cream**	**M. cream**
Brands	5	2	3
Samples	25	10	15
C.S.B	10	6	4
%	40.0	60.0	26.67
L.C.B. (cfu/ml)	1.0×10^5 - 2.5×10^7	4.0×10^5- 6.6×10^7	7.5×10^6- 1.5×10^8
C.S. F	9	6	4
%	36.0	60.0	26.67
L.C.F (cfu/ml)	1.3×10^3-1.5×10^6	1.3×10^4 – 7×10^5	9.5×10^4-2.1×10^6
S with B & F %	12 48.0	6 60.0	4 26.67
NCS	13	4	6
%	52.0	40.0	40.0

CSB : contaminated samples with bacteria
LCB : Level of contamination with bacteria
CSF : Contaminated samples with fungi
LCF : Level of contamination with fungi
NCS : Non contaminated samples.

Table (8): Microscopical identification of the bacterial contaminants of the tested cosmetic creams samples.

Cream Type	Brand	Sample No.	Number of Bact. isolates	Bacterial identification
Foundation cream	A	1	1	G + ve rods
			1	G + ve cocci
		2	2	G + ve cocci
			1	G + ve rods
		3	1	G - ve rods
			1	G + ve rods
		4	1	G + ve rods
			2	G + ve cocci
		5	1	G + ve rods
			1	G + ve cocci
	B	6	3	G + ve cocci
			1	G - ve cocci
		10	2	G + ve rods
	C	11	1	G + ve rods
		12	2	G + ve rods
			1	G + ve cocci
	D	16	3	G + ve cocci
Total	**4**	10	25	-
Foundation moisturizing cream	F	26	1	G - ve rods
		27	1	G + ve rods
			1	G + ve cocci
		30	1	G + ve rods
			1	G + ve cocci
	G	32	1	G + ve rods
		33	1	G + ve rods
		35	1	-
Total	**2**	6	8	8
Moisturizing cream	H	37	1	G + ve rods
			1	G - ve rods
			2	G + ve cocci
		38	3	G + ve cocci
	I	41	1	G + ve cocci
			1	G - ve rods
		43	1	G + ve cocci
Total	**2**	4	10	-

Table (9): Evaluation of the bacteria contaminating each type of the tested cosmetic creams.

Cream type	No. of C.S.	Total No. of bacteria	Identification	No. of B.C.	Percent (%)
Foundation cream	**10**	25	+ ve Cocci	13	52.0
			+ ve rods	10	40.0
			- ve cocci	1	4
			- ve rods	1	4
Foundation moisturizing cream	**6**	8	+ ve Cocci	2	25
			+ ve rods	5	62.5
			- ve rods	1	12.5
moisturizing cream	**4**	10	+ ve Cocci	7	70
			+ ve rods	1	10
			- ve rods	2	20

C.S : contaminated Samples

B.C. : Bacterial contaminants

Table (10): Identification of the fungal contaminants of the tested cosmetic creams samples.

Cream Type	Brand	Sample No.	Number of fungal isolate	Fungal identification
Foundation cream	A	1	1	*Aspergillus. sp*
		2	2	*Penecillium sp.* *Aspergillus Niger*
		3	2	*Aspergillus. sp* *Aspergillus Niger*
		4	3	*Aspergillus sp.* *Aspergillus Niger* *Penecillium sp.*
		5	1	*Aspergillus Niger*
	B	6	4	*Aspergillus. sp* *Aspergillus. sp* *Aspergillus Niger* *Aspergillus. sp*
		10	2	*Aspergillus Niger* *Aspergillus. sp*
	D	17	2	*Aspergillus. sp* *Aspergillus Niger*
		20	2	*Aspergillus sp.* *Aspergillus Niger*
Total	3	9	19	19
Foundation moisturizing cream	F	26	2	*Aspergillus Niger* *Aspergillus. sp*
		27	1	*Aspergillus Niger*
		30	2	*Aspergillus Niger* *Aspergillus sp.*
	G	32	2	*Aspergillus Niger* *Penecillium sp.*
		33	2	*Aspergillus Niger* *Penecillium.sp*
		35	1	*Aspergillus Niger*
Total	2	6	10	10
Moisturizing cream	H	37	2	*Aspergillus Niger* *Aspergillus. sp*
		38	2	*Aspergillus Niger* *Aspergillus. sp*
	I	41	1	*Aspergillus Niger*

		43	1	*Aspergillus Niger*
Total	2	4	6	6

Table (11): Evaluation of the fungi contaminating each type of the tested cosmetic creams.

Cream Type	No of C. S.	Total No. of fungi	Identification	No. of F.C.	Percent
Foundation cream	9	19	*Aspergillus. sp* *Penecillium.sp*	17 2	89.47 10.53
Foundation moisturizing cream	6	10	*Aspergillus. sp* *Penecillium.sp*	8 2	80.0 20.0
Moisturizing cream	4	6	*Aspergillus. sp* *Penecillium.sp*	6 --	100.0 ---

C.S : contaminated Sample

F.C. : Fungal contaminants

Table (12): Effect of sharing consumers handling and time on the bioburden of cosmetic foundation cream sample No (9) of brand (B).

Time (days)	Bacteria		Fungi	
	Count (cfu/ml)	microorganisms	Count (cfu/ml)	microorganisms
Control **6**	- 5.5 x 10^2	- G + ve rods G + ve rods	-- 1.0 x 10^1	- *Penicillium* sp *Penicillium* sp

16	7.5 x 10^4	G +ve rods	1.5 x 10^3	*Penicillium* sp
26		G + ve rods		*Penicillium* sp
36	1.0 x 10^5		3.0 x 10^5	
	1.6 x 10^6		7.5 x 10^6	

Table (13): Effect of sharing consumers handling and time on the bioburden of cosmetic foundation cream sample No (18) of brand (D).

Time (days)	Bacteria		Fungi	
	Count (cfu/ml)	**microorganisms**	**Count (cfu/ml)**	**microorganisms**
Control	-	-	--	-
6	5.0 x 10^2	G + ve rods Spore former	5.0 x 10^1	*Aspergillus* sp.
16	5.0 x 10^5	G + ve rods Spore former	1.0 x 10^3	*Aspergillus* sp.
26	9.5 x 10^6	G +ve rods Spore former	2.5 x 10^4	*Aspergillus* sp.
36	1.1 x 10^7	G + ve rods Spore former	7.5 x 10^5	

Table (14): Effect of sharing consumers handling and time on the bioburden of cosmetic foundation cream sample No (22) of brand (E).

Time (days)	Bacteria		Fungi	
	Count (cfu/ml)	microorganisms	Count (cfu/ml)	microorganisms
Control	-	-	-	-
6	1.0 x 10^3	G + ve rods	1.5 x 10^1	*Aspergillus* sp.
16	2.5 x 10^5	G +ve rods	2.5 x 10^4	*Aspergillus* sp.
36	1.35 x 10^7	G + ve rods	3.5 x 10^5	*Aspergillus* sp. *Penicillium* sp
	1.5 x 10^8		7.5 x 10^6	*Aspergillus* sp. *Penicillium* sp

Table (15): Effect of sharing consumers handling and time on the bioburden of cosmetic (foundation –moisturizing) cream sample No (31) of brand (G).

Time (days)	Bacteria		Fungi	
	Count (cfu/ml)	microorganisms	Count (cfu/ml)	microorganisms
Control	- 2.0 x	- G + ve rods	-- 0.4 x	- *Aspergillus* sp.

6	10^2	G + ve rods	10^2	*Aspergillus* sp.
16	4.5 x	G +ve rods	(<10)	*Aspergillus* sp.
26	10^4	G + ve rods	1.0 x	*Aspergillus* sp.
36	6.0 x	G + ve cocci	10^3	
	10^6		3.3 x	
	2.0 x		10^5	
	10^7		4.2 x	
			10^6	

Table (16): Effect of sharing consumers handling and time on the bioburden of cosmetic moisturizing cream sample No (40) of brand (H).

Time (days)	**Bacteria**		**Fungi**	
	Count (cfu/ml)	**microorganisms**	**Count (cfu/ml)**	**microorganisms**
Control	-	-	-	-
6	2.5 x 10^2	G + ve rods	1.0 x 10^1	*Aspergillus* sp.
16	8.0 x 10^3	G + ve rods	1.5 x 10^3	*Aspergillus* sp.
26	6.0 x 10^6	G +ve rods	5.0 x 10^6	*Aspergillus* sp.
36	1.0 x 10^7	G + ve rods	4.3 x 10^7	*Aspergillus* sp.

Table (17): Effect of sharing consumers handling and time on the bioburden of cosmetic moisturizing cream sample No (45) of brand (I).

Time (days)	Bacteria		Fungi	
	Count (cfu/ml)	**microorganisms**	**Count (cfu/ml)**	**microorganisms**
Control	--	-	-	-
6	1.0 x 10^2	G + ve rods	0.5 x 10^2 (< 10)	*Aspergillus* sp.
16	7.0 x 10^4	G + ve rods		*Aspergillus* sp.
26	17.5 x 10^6	G +ve rods	1.0 x 10^4	*Aspergillus* sp.
36	2.0 x 10^7	G + ve cocci	2.5 x 10^5	
			3.0 x 10^6	

Table (19): Preliminary test for detection of the lethal dose for microorganism from irradiated cream samples of different brands.

Cream type	brand	Sample No.	Lethal dose kGy	
			Bacteria	Fungi
Foundation cream	A	1	2	5
		2	3	7
		3	3	7
		4	7	5
		5	25	7
	B	6	7	7
		10	7	7
	C	11	5	--
		12	3	--
	D	16	5	--
		17	7	7
		20	7	7
Foundation moisturizing	F	26	9	7
		27	7	7
		30	7	5
	G	32	9	7
		33	9	7
		35	7	5
Moisturizing cream	H	37	5	5
		38	5	5
	I	41	5	5
		43	7	7

Table (20): Identification of bacteria isolated at the sublethal doses from irradiated cream samples of different brands.

Cosmetic cream	Brand	Sample No.	Number of RBI	Identification	SRD
Foundation cream	A	1 2 3 4 5	1 1 1 1 1	Staphylococcus haemolyticus Micrococcus sp. Bacillus brevis Bacillus brevis Micrococcus sp.	1.5 2 2 5 20
	B	6 10	1 1	Staphylococcus hominis-novo Bacillus sphaericus	5 5
	C	11 12	1 1	Bacillus pantothenticus Bacillus alvei	3 2
	D	16	1	Micrococcus sp.	3
Total	4	10	10	10	--
Foundation moisturizing cream	F	26 27 30	1 1 1	Acenatobacter baumann/haem Bacillus sphaericus Bacillus punilus	7 5 5
	G	32 33 35	1 1 1	Bacillus sphaericus Bacillus sphaericus Bacillus pumilus	7 7 5
Total	2	6	6	6	--
Moisturizing cream	H	37 38	1 1	Bacillus pantothenticus Staphylococcus haemolyticus	3 3

	I	41 43	1 1	Micrococcus sp. Micrococcus sp.	3 5
Total	2	4	4	4	--
Total 3	8	20	20	20	--

RBI : Resistant bacterial isolates

SRD: Sublethal radiation doses at which the bacteria were isolated.

Table (21): Identification of the fungi isolated at the sublethal doses from irradiated cream samples of different brands.

Cosmetic cream	**Brand**	**Sample No.**	**RFI**	**Identification**	**SRD**
Foundation cream	A	1 2 3 4 5	1 1 1 1 1	Aspergillus Niger Aspergillus Niger Aspergillus Niger Aspergillus fumigatus Aspergillus Niger	3 5 5 3 5
	B	6 10	1 1	Aspergillus Niger Aspergillus Niger	5 5
	D	17 20	1 1	Aspergillus Niger Aspergillus fumigatus	5 5
Total	3	9	9	9	--
ion moisturizing	F	26 27 30	1 1 1	Aspergillus Niger Aspergillus Niger Aspergillus tamarii	5 5 3

	G	32 33 35	1 1 1	Aspergillus Niger Pencillium chrysogenum Aspergillus Niger	5 5 3
Total	2	6	6	6	--
Moisturizing cream	H	37 38	1 1	Aspergillus Niger Aspergillus Niger	3 3
	I	41 43	1 1	Aspergillus Niger Aspergillus Niger	3 5
Total	2	4	4	4	--

RFI : Resistant fungal isolates

SRD: Sublethal radiation doses at which the fungi were isolated.

Table (22): The total number of resistant microbial isolates from irradiated cream samples.

Cream type	**For bacteria**		**For fungi**	
	No. of I.C.S.	**No. of RBI**	**No. of I.C.S.**	**No. of RFI**
Foundation	10	10	9	9
Foundation moisturizing	6	6	6	6
Moisturizing	4	4	4	4
Total	20	20	19	19

I.C.S: Irradiated contaminated samples

RBI : Resistant bacterial isolate

RFI: Resistant fungal isolates

Table (23): The D_{10} value of the radioresistant bacterial isolates from cosmetic cream samples representing each brand.

Cosmetic cream	Brand	Samples No.	RBS	D_{10} (kGy)
Foundation cream	A	5	*Micrococcus sp.*	2.0
	B	6	*Staphylococcus. Hominis-Novo*	0.85
	B	10	*Bacillus spharicus*	0.85
	C	11	*Bacillus pantothenticus*	0.4
	D	16	*Micrococcus sp.*	0.4
Foundation moisturizing cream	F	26	*Acenatobacter baumann/ haem*	1.1
	G	32	*Bacillus sphaericus*	1.25
Moisturizing cream	H	37	Bacillus panto tenticus	0.5
	H	38	*Staphylococcus haemolyticus*	0.47
	I	43	*Micrococcus sp.*	0.75

Samples No: Sample number
RBS: Radioresistant bacterial strain

Table (24): The D_{10} value of the radioresistant fungal isolates from cosmetic cream samples representing each brand.

Cosmetic cream	Brand	Samples No.	RFS	D_{10} (kGy)
Foundation cream	A	3	*Aspergillus niger*	0.7
	B	10	*Aspergillus niger*	0.75
	D	20	*Aspergillus fumigatus*	0.8
	D	17	*Aspergillus niger*	0.95
Foundation moisturizing cream	F	27	*Aspergillus niger*	0.9
	G	32	*Aspergillus niger*	1.0
	G	33	*Penicilium chrysogenum*	0.95
Moisturizing cream	I	43	*Aspergillus niger*	1.0

RFS: Radioresistant fungal strain

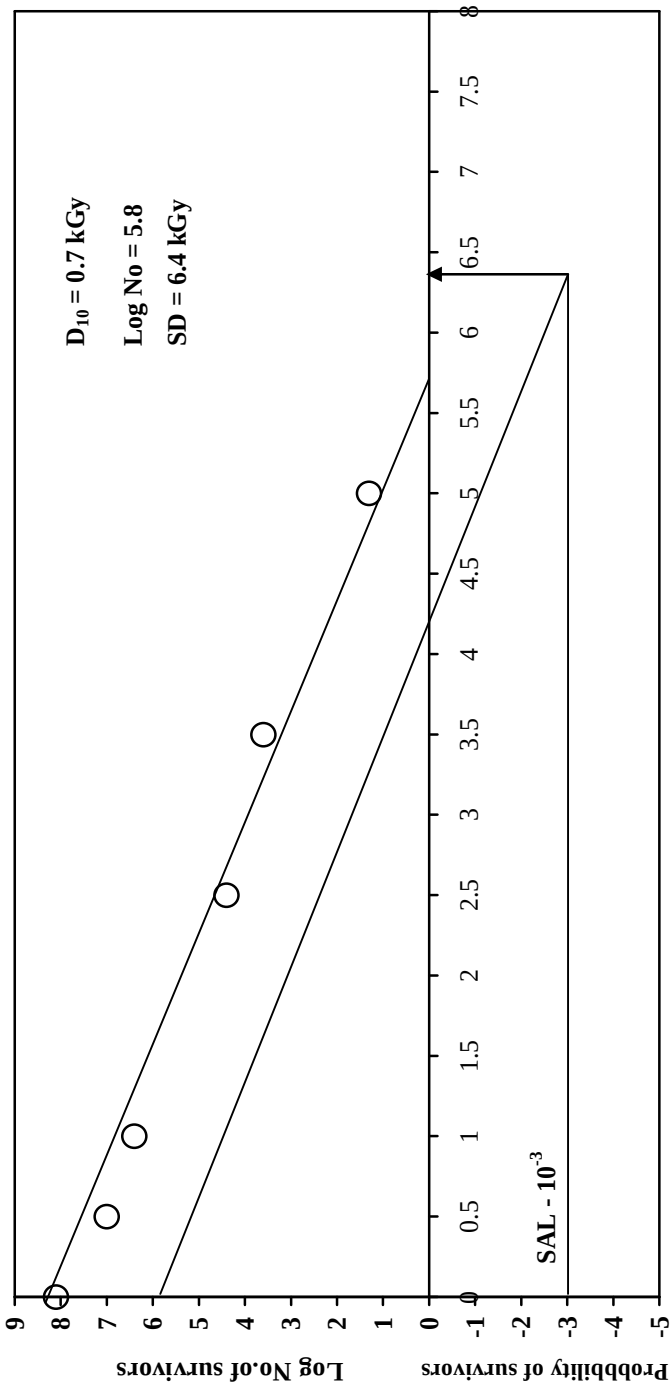

Fig (36): Calculation of the sterilization dose of foundation cream sample No (3) of brand (A) using *Aspergillus niger* as the most radioresistant isolate

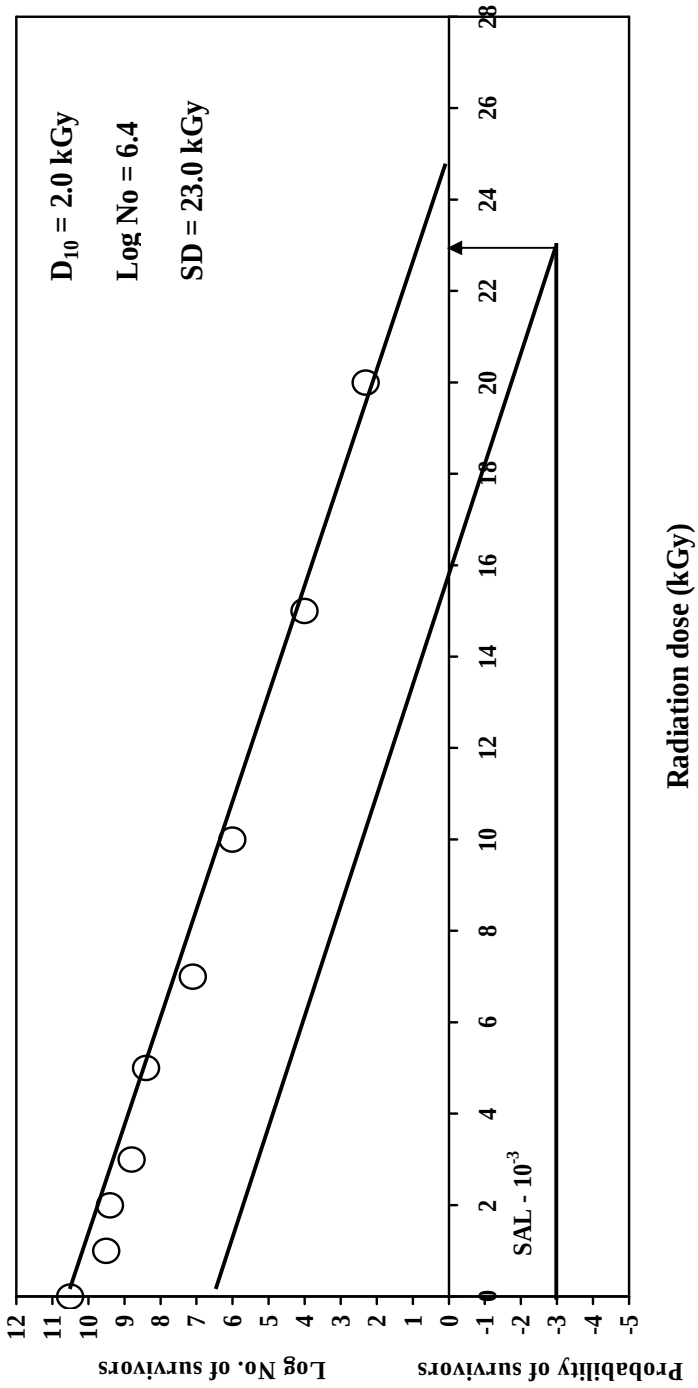

Fig (37): Calculation of the sterilization dose of foundation cream sample No (5) of brand (A) using *Micrococcus* sp. as the most radioresistant isolate

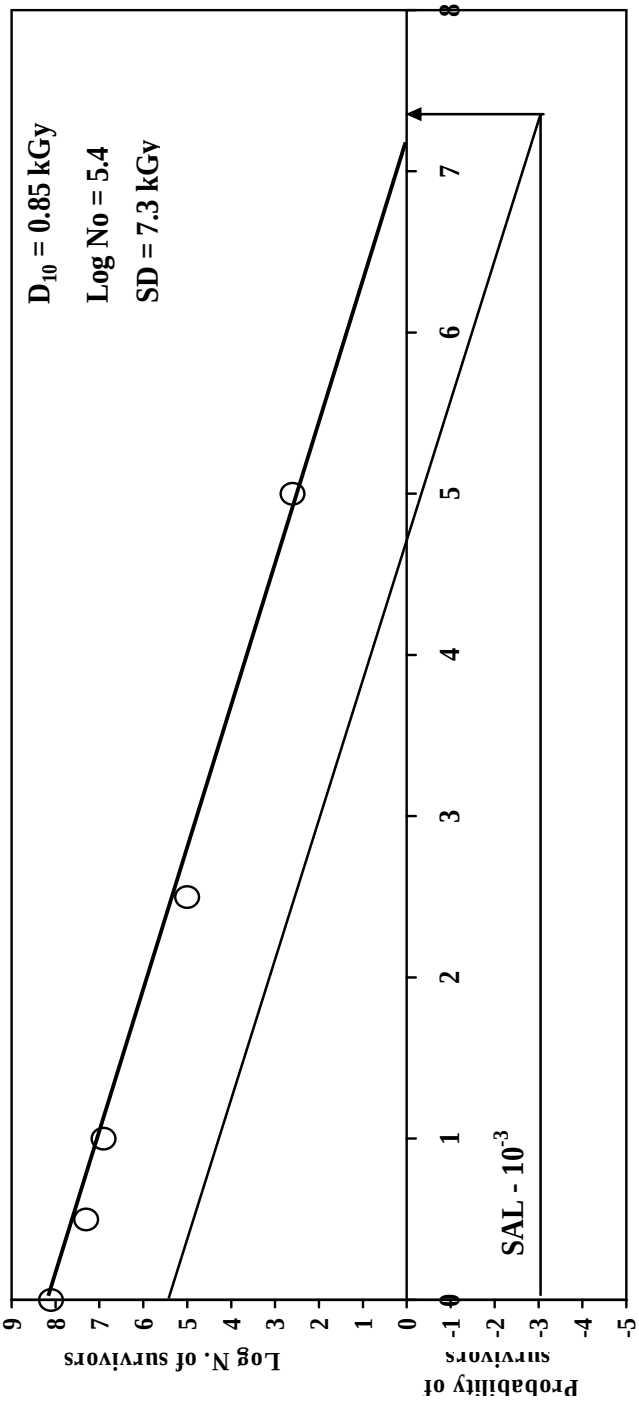

Fig (38): Calculation of the sterilization dose of foundation cream sample No (6) of brand (B) using *Staphylococcus hominis-novo* as the most radioresistant isolate

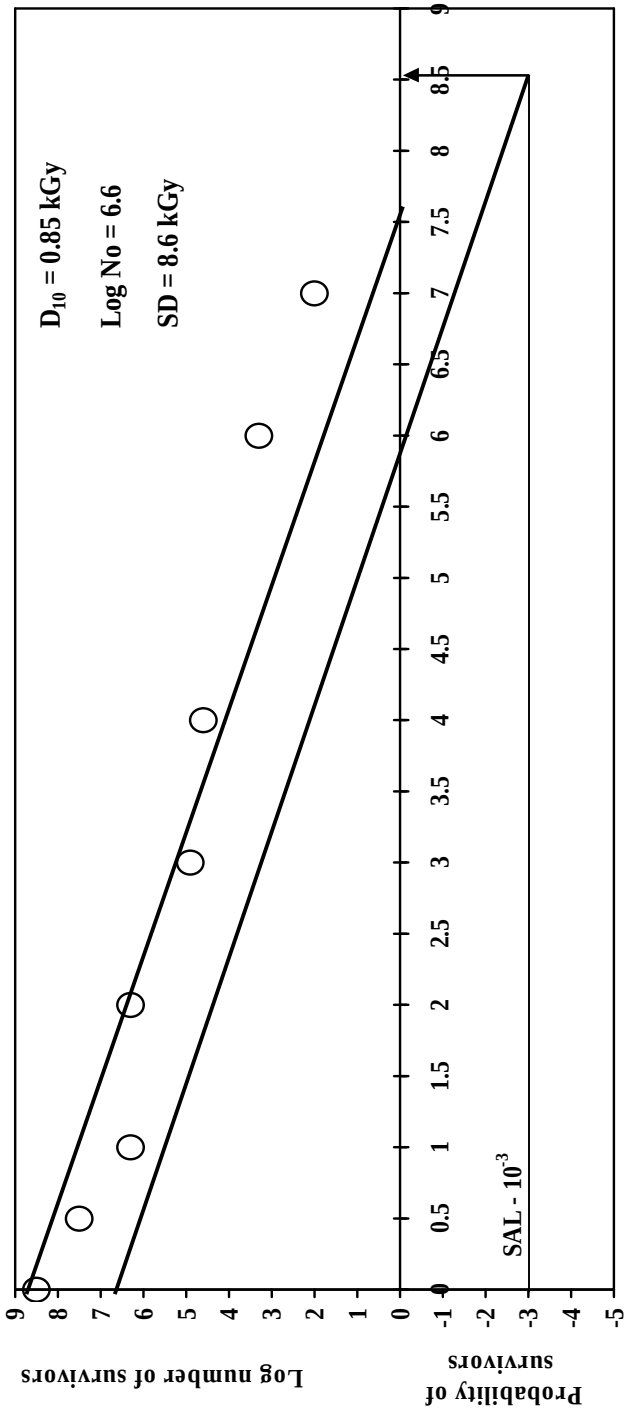

Fig (39): Calculation of the sterilization dose of foundation cream sample No (10) of brand (B) using *Bacillus spharicus* as the most radioresistant isolate

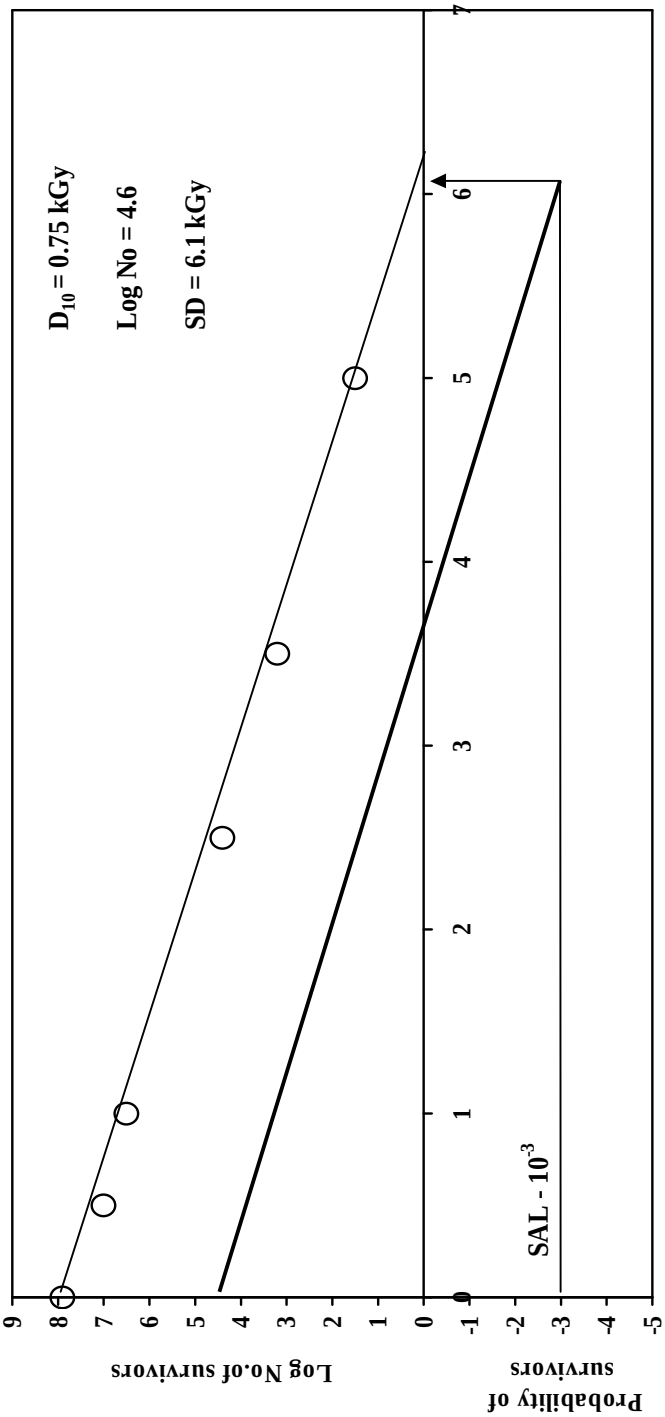

Fig (40): Calculation of the sterilization dose of foundation cream sample No (10) of brand (B) using *Aspergillus niger* as the most radioresistant isolate

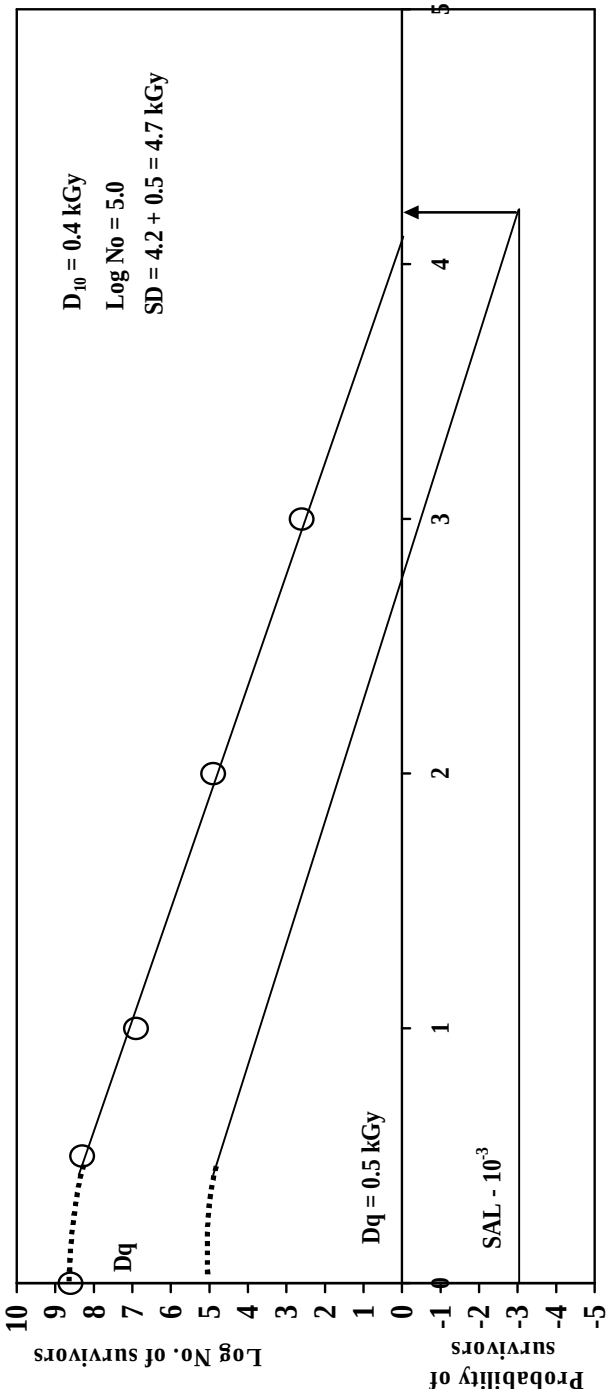

Fig (41): Calculation of the sterilization dose of foundation cream sample No (11) of brand (C) using *Bacillus* pantothenticus as the most radioresistant isolate

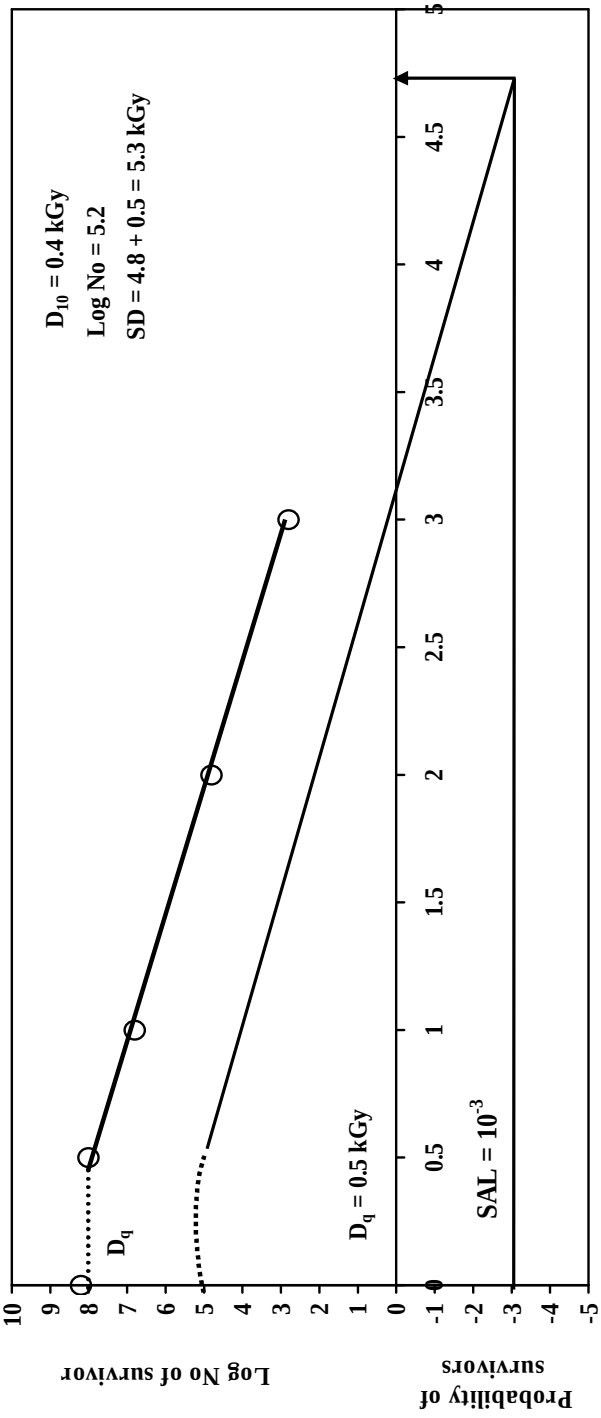

Fig (42): Calculation of the sterilization dose of foundation cream samples No (16) of brand (D) using *Micrococcus* sp. as the most radioresistant isolate

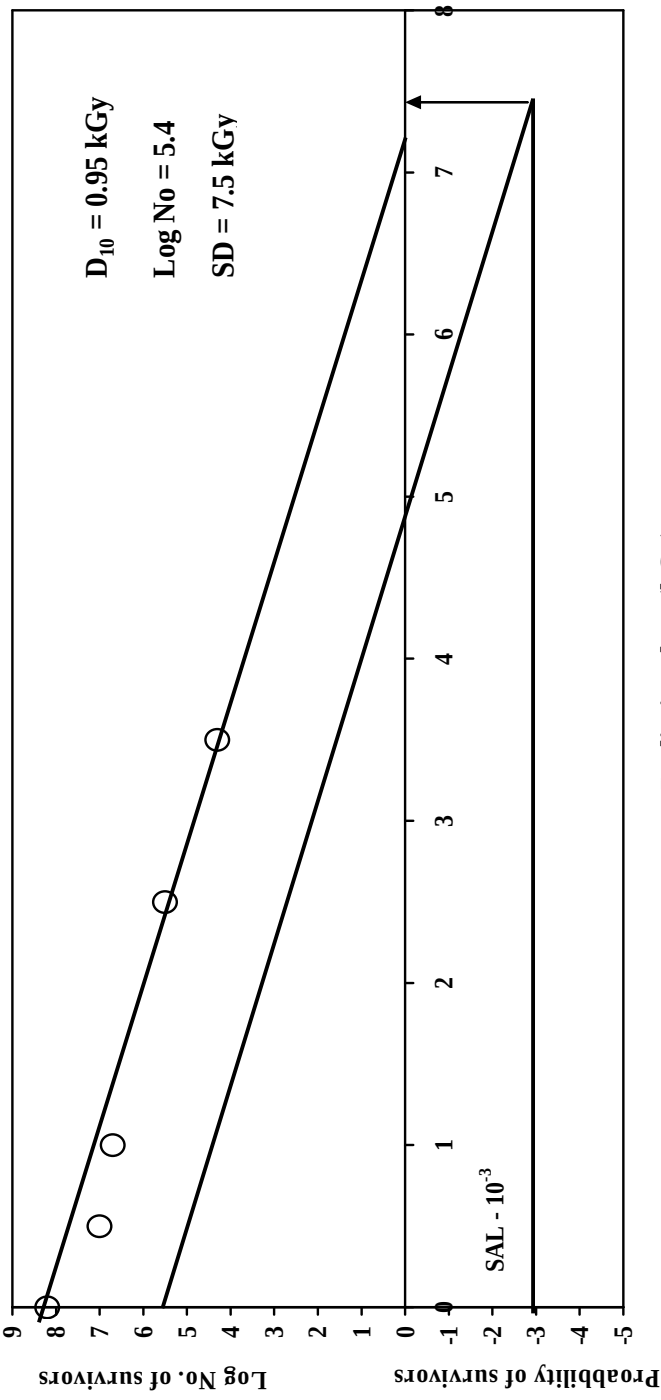

Fig (43): Calculation of the sterilization dose of foundation cream sample No (17) of brand (D) using *Aspergillus niger* as the most radioresistant isolate

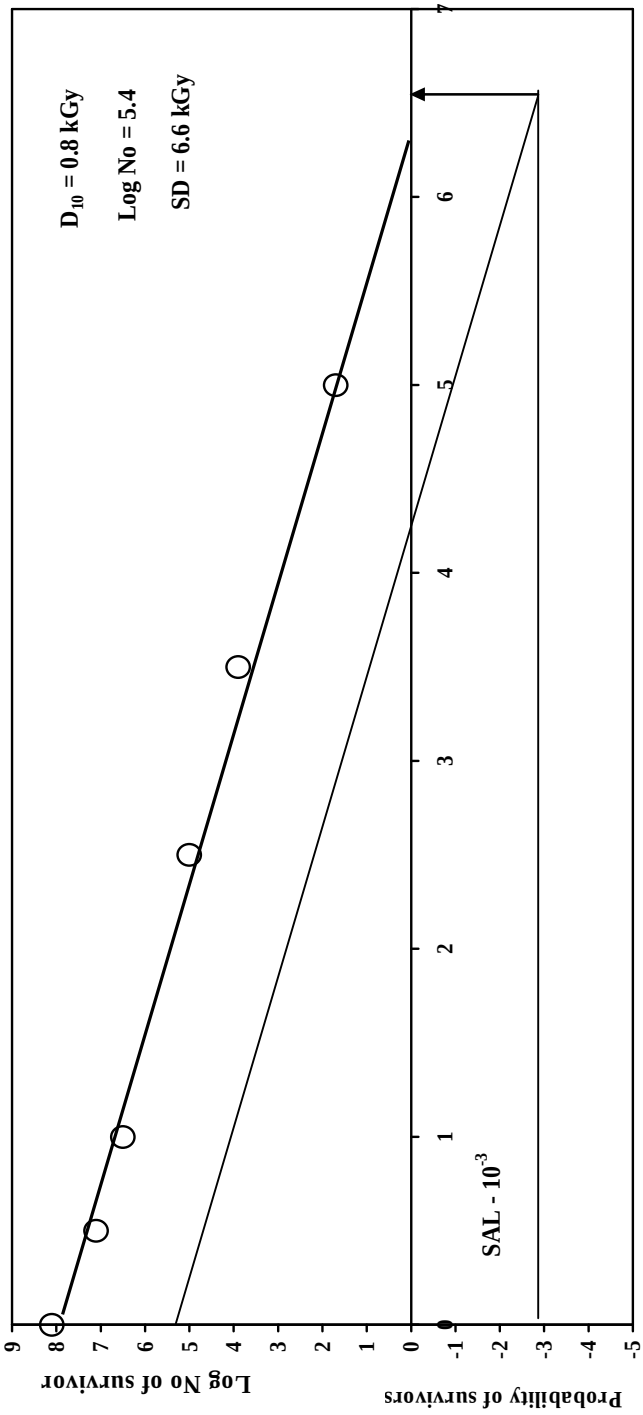

Fig (44): Calculation of the sterilization dose of foundation cream sample No (20) of brand (D) using *Aspergillus* fumigatus as the most radio-resistant isolate

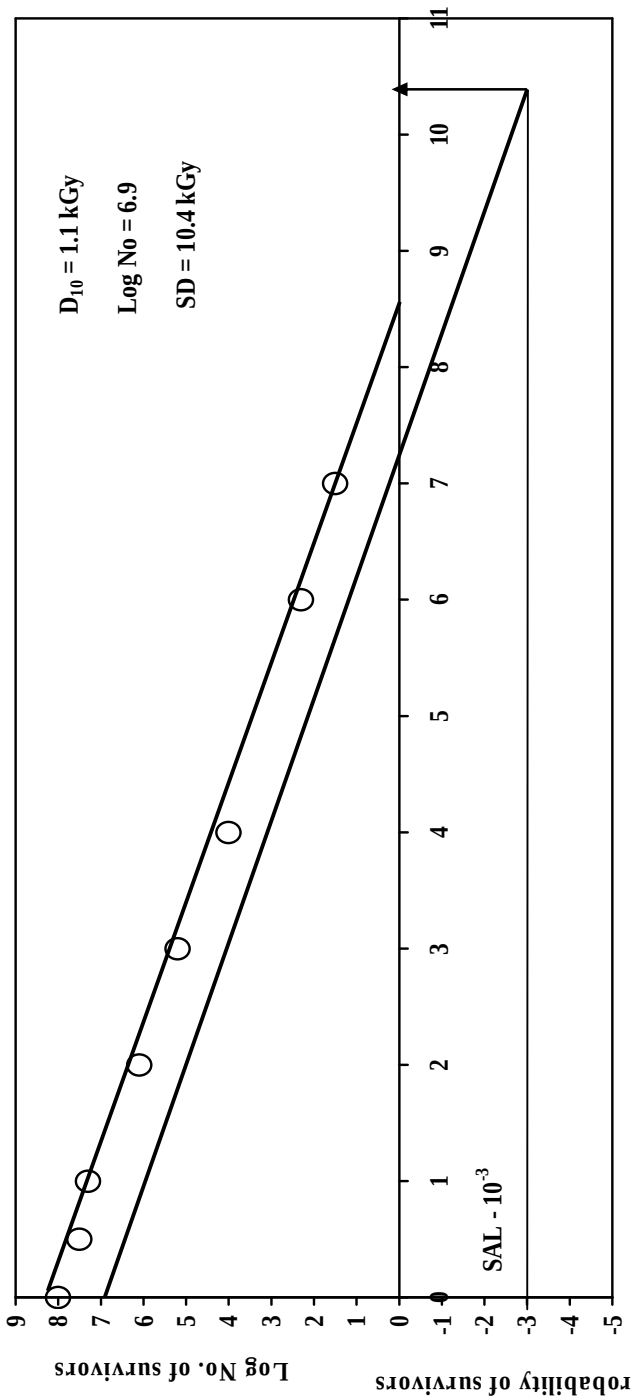

Fig (45): Calculation of the sterilization dose of foundation-mosturizing cream sample No (26) of brand (F) using *Acenatobacter baumann / haem* as the most radioresistant isolate

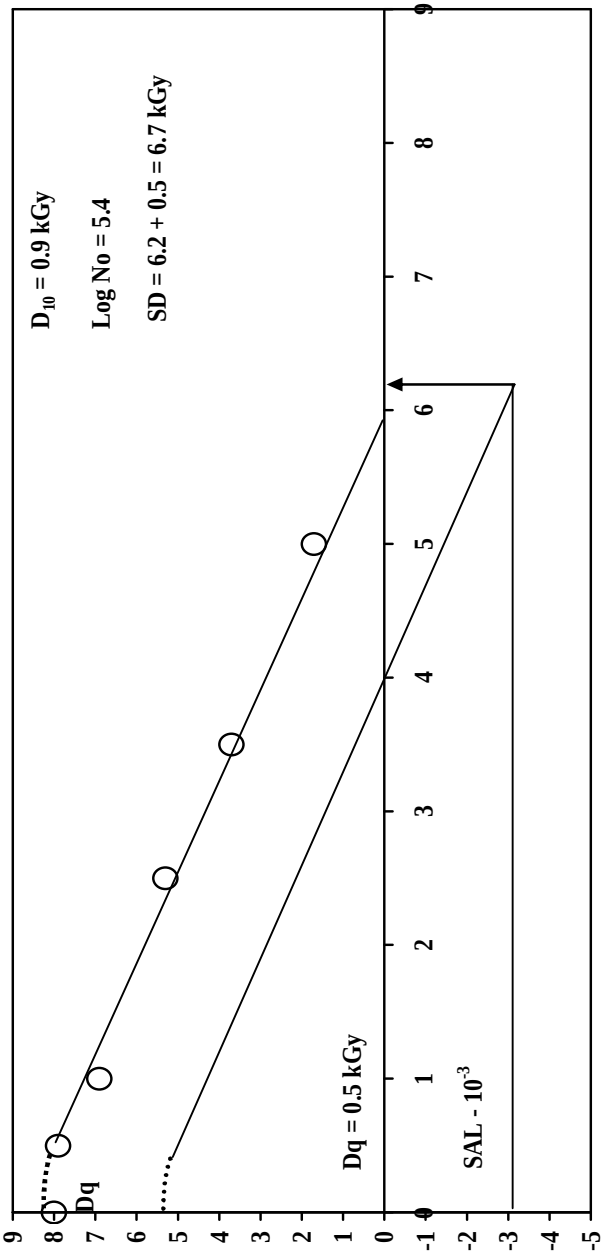

Fig (46): Calculation of the sterilization dose of foundation-moisturizing cream sample No (27) of brand (F) using *Aspergillus niger* as the most radioresistant isolate

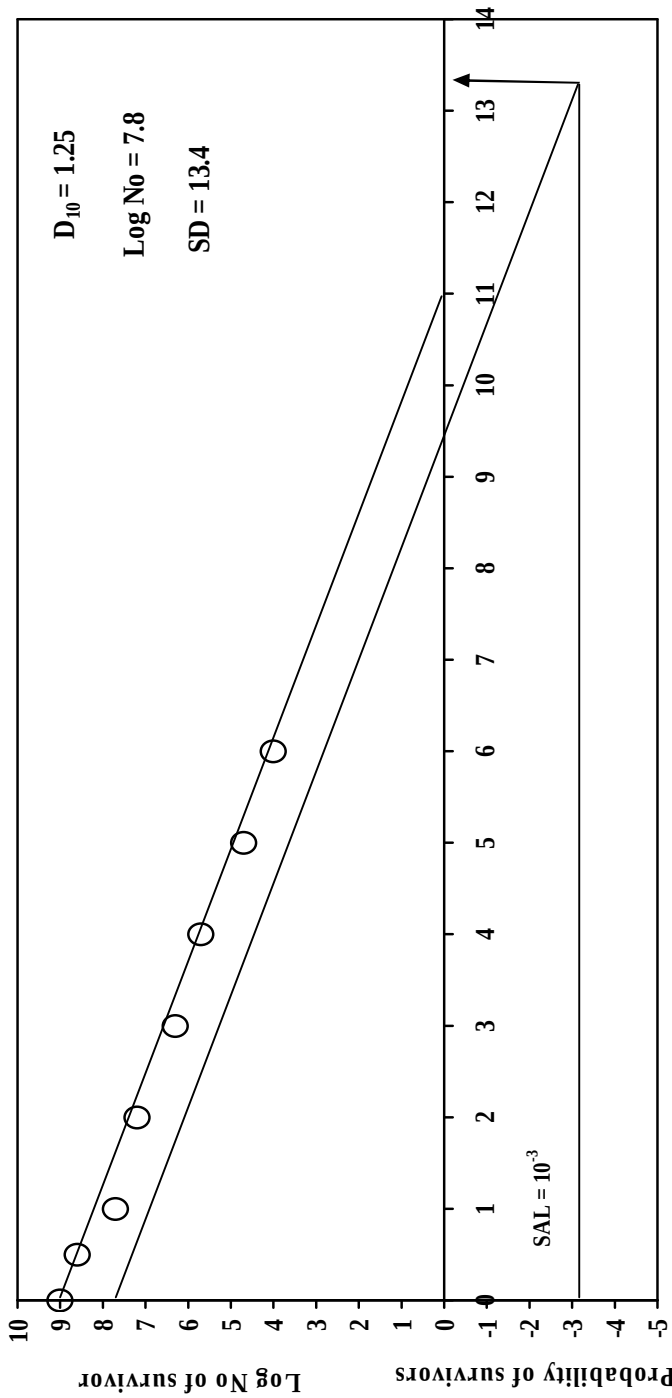

Fig (47): Calculation of the sterilization dose of foundation-moisturizing cream sample No (32) of brand (G) using *Bacillus spharicus* as the most radio-resistant

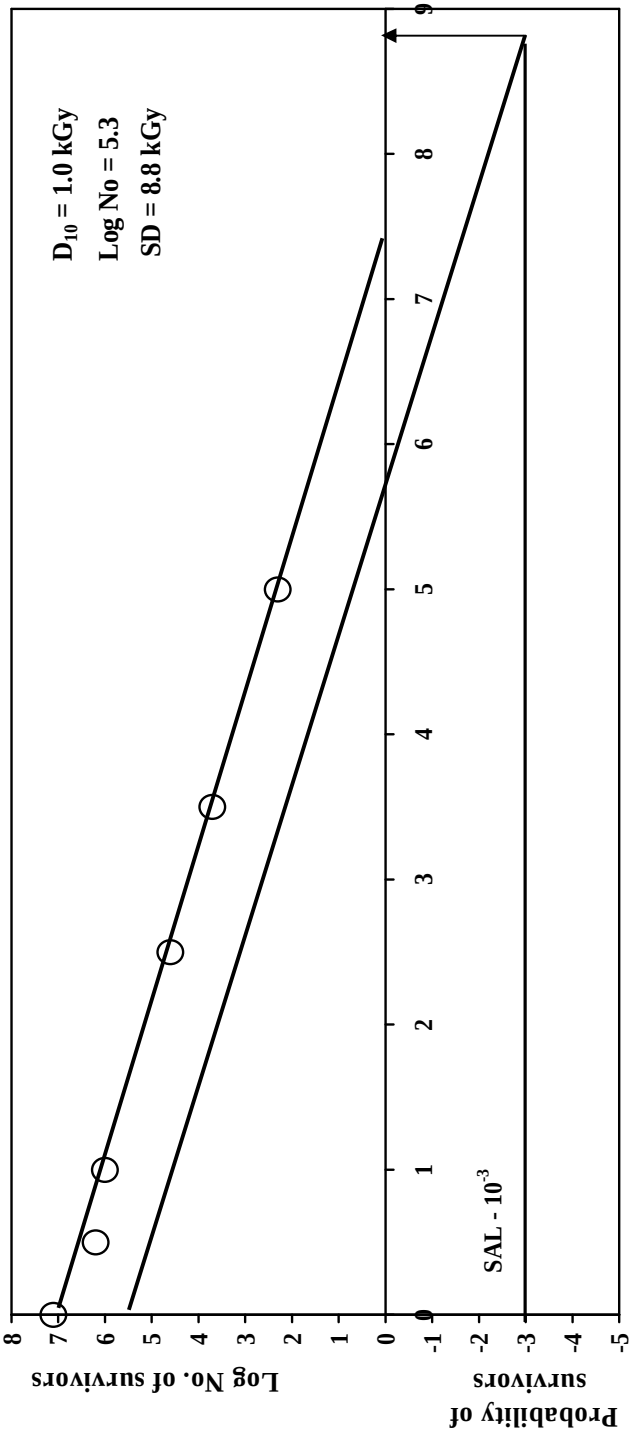

Fig (48): Calculation of the sterilization dose of foundation-moisturizing cream sample No (32) of brand (G) using *Aspergillus niger* as the most radioresistant isolate

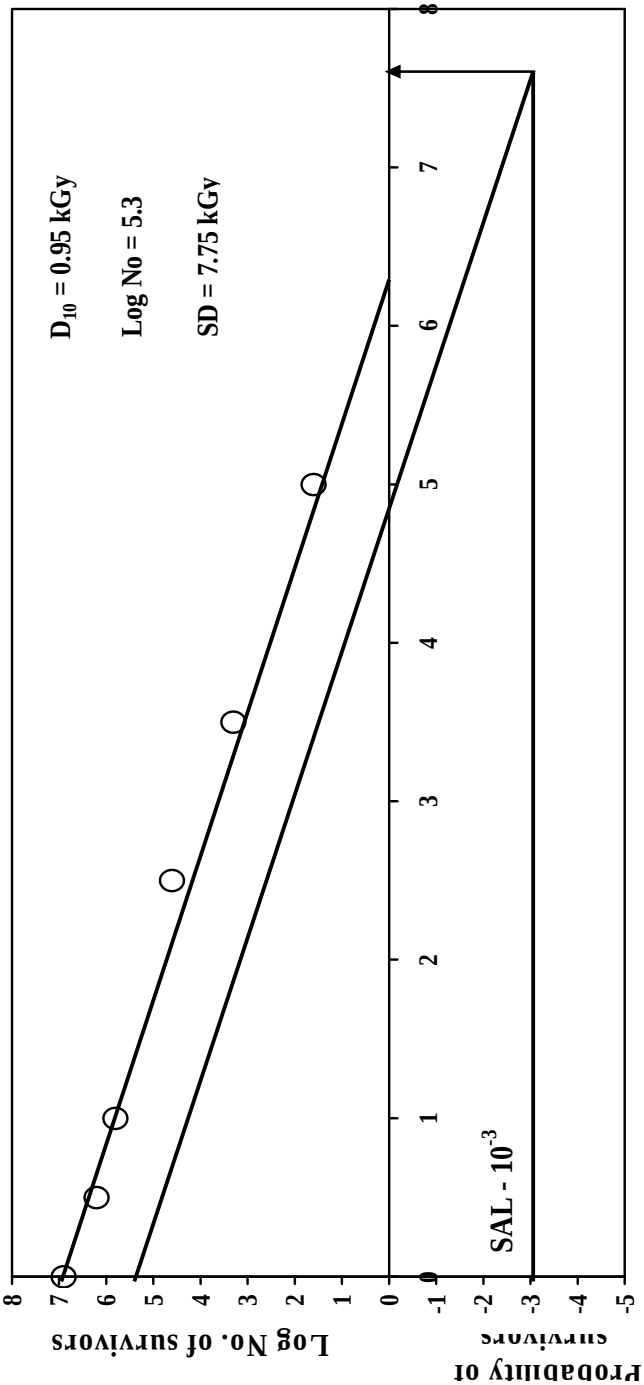

Fig (49): Calculation of the sterilization dose of foundation-moisturizing cream sample No (33) of brand (G) using *Penicillium chrysogenum* as the most radioresistant isolate

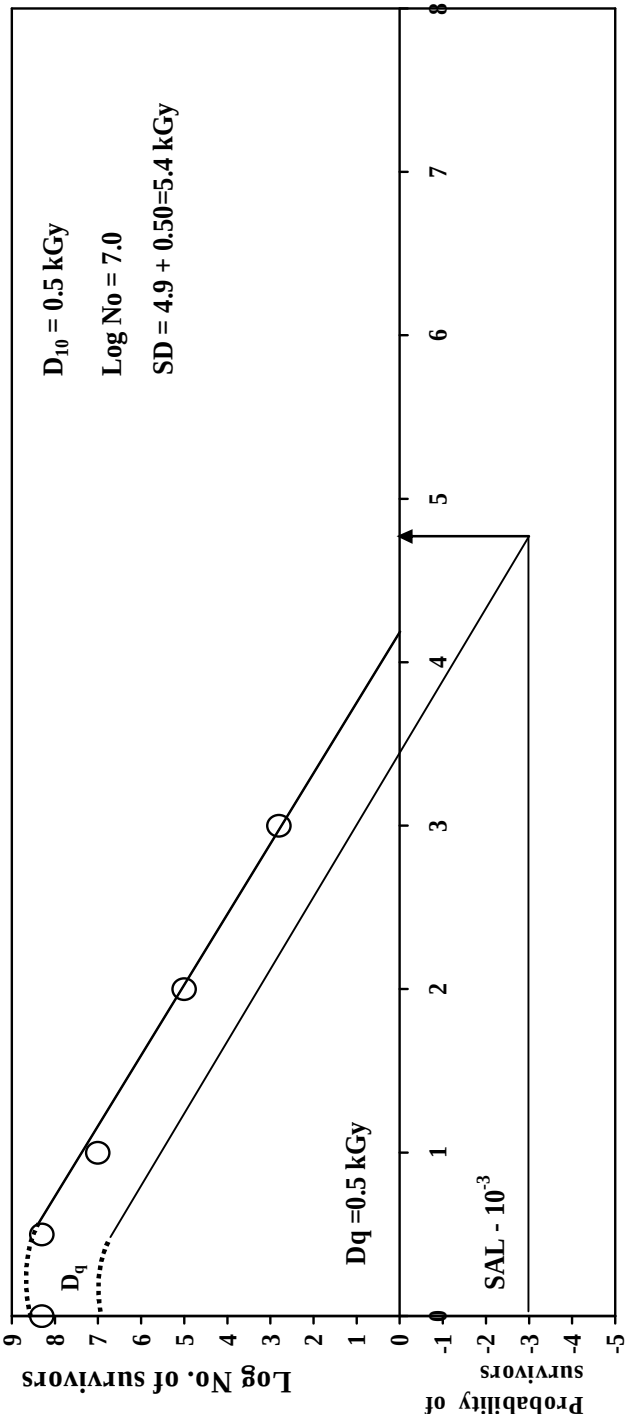

Fig (50): Calculation of the sterilization dose of moisturizing cream sample No (37) of brand (H) using *Bacillus pantothenticus* as the most radioresistant isolate

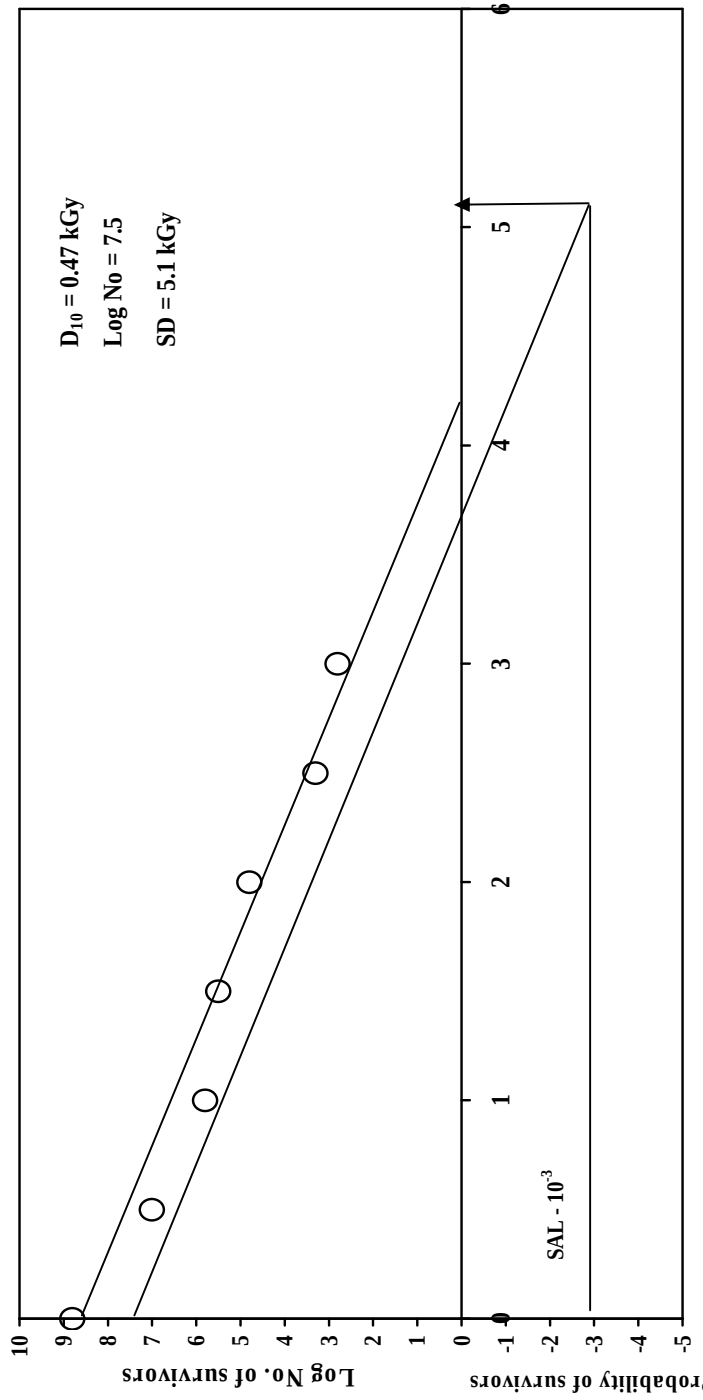

Fig (51): Calculation of the sterilization dose of moisturizing cream sample No (38) of brand (H) using *Staphylococcus haemolyticus* as the most radioresistant isolate

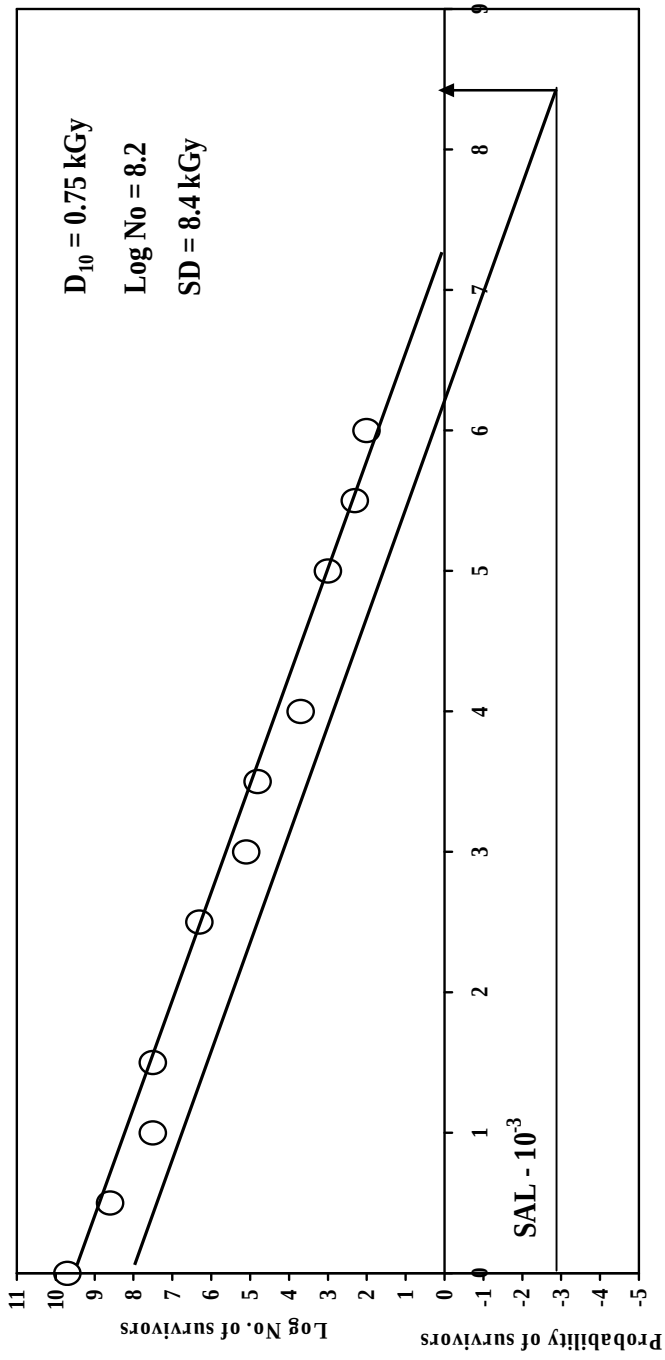

Fig (52): Calculation of the sterilization dose of moisturizing cream sample No (43) of brand (I) using *Micrococcus* sp. as the most radioresistant isolate

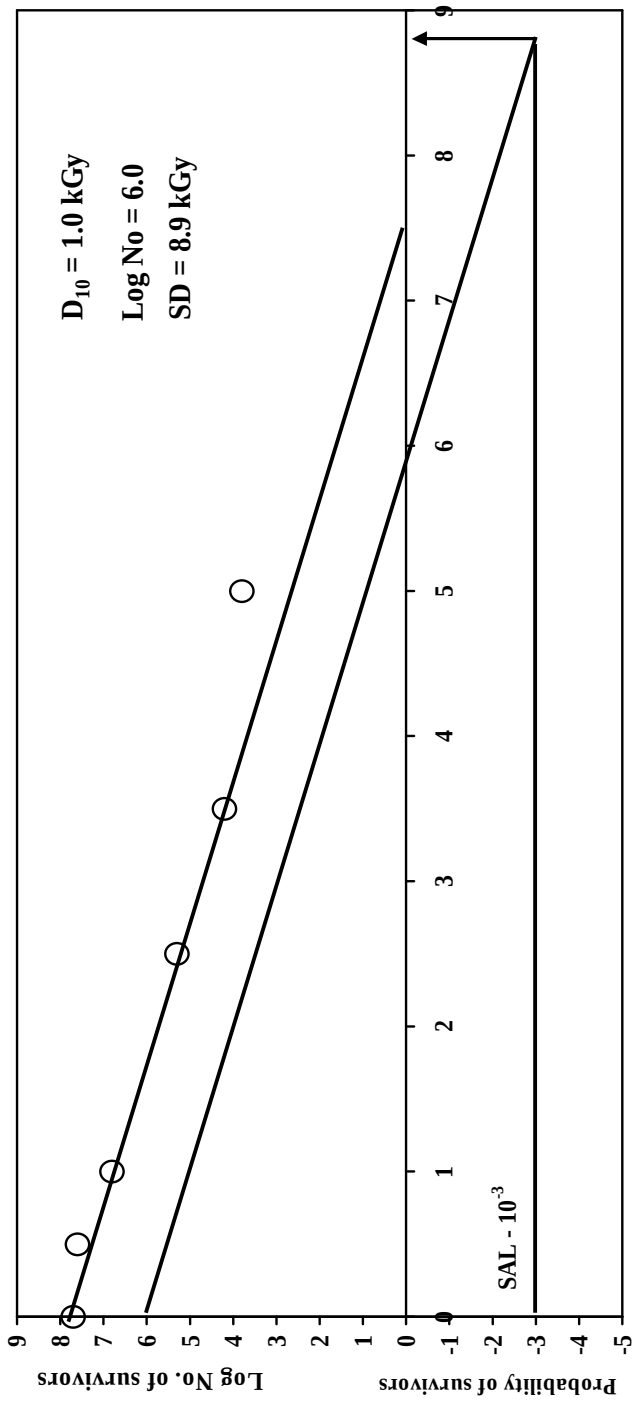

Fig (53): Calculation of the sterilization dose of moisturizing cream sample No (43) of brand (I) using *Aspergillus niger* as the most radioresistant isolate

Table (25): Determination of the gamma sterilization doses of cosmetic cream samples of different brands.

Cosmetic cream	brand	Sample No.	No.	Log No.	R. S.	D_{10} kGy	SD kGy
Foundation cream	A	3	$7.0x10^5$	5.8	*Aspergillus niger*	0.7	6.4
		5	$2.4x10^6$	6.4	*Micrococcus sp.*	2.0	23.0
	B	6	$2.8x10^5$	5.4	*Staph. Hominis-Novo*	0.85	7.3
		10	$3.6x10^6$	6.6		0.85	8.6
		10	$5.0x10^4$	4.6	*Bacillus spharicus*	0.75	6.1
					Aspergillus niger		
	C	11	$1.0x10^5$	5.0	*Bacillus pantothenticus*	0.4	4.7
	D	16	$1.5x10^5$	5.2	*Micrococcus sp.*	0.4	5.3
		17	$2.3x10^5$	5.4		0.95	7.45
		20	$4.0x10^5$	5.6	*Aspergillus niger*	0.8	6.6
					Aspergillus fumigatus		
Foundation moisturizing cream	F	26	$8.5x10^6$	6.9	*Acenatobacter bauman/name.*	1.1	10.4
		27	$2.3x10^5$	5.4	*Aspergillus niger*	0.9	6.7
	G	32	$6.6x10^7$	7.8	*Bacillus sphaericus*	1.25	13.4
		32	$2.5x10^5$	5.3		1.0	8.8
		33	$2.5x10^5$	5.3	*Aspergillus niger*	0.95	7.75
					Penecillium chrysogenum		
Moisturizing cream	H	37	$1.0x10^7$	7.0	*Bacillus pantothenticus*	0.5	5.4
		38	$3.0x10^7$	7.5	*Staphylococcus haemolyticus*	0.47	5.1
	I	43	$1.5x10^8$	8.2	*Micrococcus sp.*	0.75	8.4
		43	$1.8x10^6$	6.0	*Aspergillus niger*	1.0	8.9

No : bioburden of the samples
R.S. : radioresistant microbial strain
SD : The calculated sterilization doses.

Table (26): Irradiation of the most heavily contaminated samples with the determined sterilization doses.

Cosmetic cream	Brand	Sample No*	Dose kGy	Microbial contamination			
				Bacteria (cfu/ml)		Fungi (cfu/ml)	
				B. ir	A. ir	B. ir	A. ir
Foundation cream	A	1	23	$1.9x10^7$	--	$7.5x10^4$	--
	B	10	8.6	$3.6x10^6$	--	$8.5x10^4$	--
	C	12	4.7	$2.5x10^7$	--	--	--
	D	16	7.45	$1.5x10^5$	--	--	--
Foundation moisturizing cream	F	26	10.4	$8.5x10^6$	--	$2.5x10^4$	--
	G	33	13.4	$5.8x10^7$	--	$7.8x10^4$	--
Moisturizing cream	H	38	5.1	$3.0x10^7$	--	$9.5x10^4$	--
	I	43	8.9	$1.5x10^8$	--	$1.8x10^6$	--

***: The heavily contaminated samples.**
-: Non detected growth
B. ir : Before irradiation
A. ir : After irradiation.

DISCUSSION

The Federal Food Drug and cosmetic (FD & C, Act) defines cosmetics as articles applied to human body, for cleansing, beautifying, promoting attractiveness, or altering the appearance without affecting the body's structure or functions. Included in this definition are products such as facial make up preparations.

There is a wide spread exposure to potential contaminants during manufacture, particularly from the raw materials, the principles of Good Manufacturing Practice GMP must always be followed.

The ability of microorganisms to grow in some types of cosmetic products is common in industry knowledge. Many cosmetic formulations, if not properly preserved, provide a good medium for the growth of bacteria, yeasts and moulds and as such may constitute a health hazard to the consumer keeping in mind that a finished product rejection due to the presence of these microorganisms can be costly, remembering that a final product is a chain made of number of links, it makes sense to minimize potential weakness at all links in the chain whose weakness could be the introduction of harmful microorganisms **(Swinwood and Wilson, 1990).**

The warm humid conditions that are characteristic of a tropical environment are conductive to the growth of microorganisms which are responsible for a number of

infectious diseases and the spoilage of cosmetic and pharmaceutical products **(Ballereau, *et al.*, 1997 and Rosas, *et al.*, 1997).** Developing conutries also tend to have lower levels of hygiene and sanitation than industrialized countries, factors that make it possible for such organisms to thrive.

Microbiological quality assurance is an important aspect of the manufacturing process of personal care products. The majority of laboratories continue to use conventional culture based techniques which on the contaminating organisms forming visible colonies on agar plates **(Morris, 1998).**

In the present study 50 cosmetic cream samples were purchased from the market in Egypt.

The samples were twenty five (25) foundation cream, (10) foundation moisturizing cream and (15) moisturizing cream.

Detection of the bacterial and fungal contaminations were performed on plates containing nutrient agar and sabouraud's agar, respectively.

The results shows that the microbial contaminations with bacteria and fungi differ between the different cream types and brands.

Out of 25 foundation cream samples representing 5 brands, 12 samples were found to be contaminated. Out of 10

foundation moisturizing cream samples representing 2 brands, 6 were contaminated, while, out of the 15 moisturizing cream samples, representing 3 brands, 4 were found to be contaminated.

The level of microbial contamination of the tested cosmetic cream samples differs between the different types and between samples of the same brand. The results also show that, out of the 25 foundation cream samples, 10 samples were found to be contaminated with bacteria and 9 samples were found to be contaminated with fungi. Out of 10 foundation-moisturizing cream samples 6 samples were found to be contaminated with bacteria and 6 samples with fungi, while, out of the 10 moisturizing cream samples, 4 samples were found to be contaminated with bacteria and they showed fungal contamination.

The present investigation show that 43 bacterial contaminants and 35 fungal contaminants were isolated from the tested cosmetic cream samples of the different brands.

The results show that a total of 25, 8 and 10 bacterial isolates, while 20, 10 and 5 fungal isolates were isolated from samples of different brands of foundation cream, foundation-moisturizing, and moisturizing cream, respectively.

The data show that 10 foundation cream samples were contaminated with bacteria in the range of $1.0x10^5$ to $2.5x10^7$ cfu/ml and 9 samples were found to be contaminated with

fungi in the range of $1.3x10^3$ to $1.5x10^6$ cfu/ml.

Six (6) foundation-moisturizing cream samples were contaminated with bacteria in the range of $4.0x10^5$ to $6.6x10^7$ cfu/ml and six samples with fungi in the range of $1.3x10^4$ to $7x10^5$ cfu/ml.

Four (4) moisturizing cream samples were contaminated with bacteria in the range of $7.5x10^6$ and $1.5x10^8$ cfu/ml while, they showed fungal contamination ranged between $9.5x10^4$ and $2.1x10^6$ cfu/ml.

The % of contamination with bacteria were found to be 40%, 60%, 26.67%. For fungi the % were 36%, 60%, 26.67%. For bacteria and fungi the % were found to be 48%, 60%, 26.67%, for foundation cream foundation-moisturizing cream and moisturizing cream, respectively.

The level of microbiological contamination in a non sterile product, such as cosmetic formulations is made clear in the microbial limit standards (Cosmetics, Toiletries, and Fragrance Association, Inc., 1996). These values should be maintained in the products during their use, in spite of the inevitable contamination by the users, through the addition of suitable preservative in the products which guarantees the control of microbial growth even before they are marketed and during their use by consumers. **(Farrington *et al.*, 1994; Linter and Genet, 1998.; Okeke and Lamikanra, 2001).**

Contaminating microorganisms in cosmetics may cause spoilage of the product and when pathogenic, they represent a serious health risk for consumers.

Hugbo *et al.* (2003) in Nigeria, Stated that tested cosmetic cream products were contaminated to varying degrees.

Campana *et al.* (2006) in Italy evaluated (47 tensiolytes, 21 aqueous pastes) in three different states of intact, in use and ending product.

Total bacterial count, isolation and identification of pathogenic isolates were performed on the collected cosmetics, about 10.6% of tensiolytes were contaminated with *Staphylococcus warneri, Staphylococcus epidermidis* and *Pseudomonas putida.*

In the present study the results of identification of the bacterial contaminants on the cosmetic cream samples reveal that the order of contamination on foundation cream samples is gram positive cocci > gram positive rods > gram negative cocci and gram negative rods.

On foundation moisturizing cream, the order is gram positive rods > gram positive cocci > gram negative rods. On the other hand, the order of contamination on moisturizing cream samples is gram positive cocci > gram negative rods > gram positive rods.

The results of evaluation of the fungal contamination show that *Aspergillus* sp. predominate over *Penecillum* sp. as the only fungal contaminants world wide **(Becks and Lorenzoni, 1995.; Behravan *et al.*,2005).**

Most contaminations are probably introduced into the cosmetic creams by the frequent and common use of fingers and multiple- use applicators volunteers.

Since microorganisms are ever-present in the home, especially in woman and moist areas, cosmetics and toiletries are exposed to contamination with both spoilage and potentially hazardous microorganisms during their use. From the moment the product is opened until the consumer discard it, it is subjected to constant and variable microbial contamination from the domestic environment and the consumer's hands and body fluids **(Perry, 2001).**

In the present study the effect of consumer handling and time on the microbial levels of the tested cream samples which showed no microbial contamination were subjected to share use by consumers, the level of contamination through 36 days was determined. Results reveal that level of contamination was found to increase with time and during use. After 36 days of use, the bacterial contamination of foundation cream, foundation moisturizing cream and moisturizing cream reach to $1.5x10^8$, $2.0x10^7$ and $1.0x10^7$ and for fungi, it reaches to $7.5x10^6$, $4.2x10^6$ and $4.3x10^7$, respectively.

The predominant bacterial contamination on the tested cream samples were identified as gram positive rods, and some times the contamination occur with gram positive cocci. While, the fungal contaminations were identified as *Aspergillus* sp or / and *Penicellium* sp. Other investigators **(Abdel-Aziz and Alkofahi, 1989)** found that *Bacillus* species, *Staphylococcus* species, *Pseudomonas* species, *Pseudomonas vulgaris* and *serratia marcescens* were recovered from the in-use cosmetic samples in different percentages and some of the detected *Staphylococcus* were aureus type and one isolate of *Pseudomonas aeruginosa.*

The presented data are consistent with those obtained by **Misilivec *et al.* (1993)** who studied the potential health risk from shared use cosmetic caused by microorganisms. The study included samples of 3027 shared use cosmetic products were collected from 171 retail establishments nation wide revealed that fungi, were present in 10.4% of products and 3.9 contained fungal pathogens or opportunistic pathogens, they reach to 32.2% of fungal isolates. Also, **Orth and Kebara (1998)** reported that adequately preserved cosmetic and drug products may become contaminated if they are diluted or repeatedly exposed to microorganisms during use.

The need to control microbiological contamination of all products for human use and consumption, which support microbial persistence and / or growth has been of considerable concern to manufactures. Pharmaceutical, cosmetics and

toiletries, industries strive for higher microbiological standards to protect their products from spoilage. Requirements for meeting these stricter limits has resulted in revisions to current methods and reach for other technologies to replace or augment some of the processes which have traditionally been widely employed this renewed interest in radiation processing as a means of sterilization or reduction in microbial load. **(Swinwood and Wilson, 1990.; Razem *et al.*, 2003).**

The mixed contaminated cosmetic cream samples were exposed to screening doses of gamma radiation in the range of 0.0 to 30 kGy. According to the results of the preliminary test each cosmetic cream sample was irradiated at room temperature at the suitable irradiation doses, Non irradiated samples served as controls, total aerobic plate count (TAPC) is a useful indicator of the microbiological status of the materials, both before and after irradiation **(Razem *et al.*, 2001).** So, in the present study, the results of the effect of gamma radiation on the microbial contaminants on the cosmetic cream samples, using the total aerobic plate count technique, reveal that the bacterial and fungal counts decrease with increasing the radiation doses.

The bacterial sublethal dose levels ranged between 1.5 and 20 kGy, 5 and 7 kGy, 3 and 5 kGy. While, the fungal sublethal dose levels ranged between 3 and 5 kGy; for irradiated foundation cream, foundation-moisturizing cream and moisturizing cream, respectively.

The bacterial and fungal organisms survived the higher radiation doses under test (the sublethal dose levels) were isolated as the radioresistant microorganisms.

The present study showed that 10 radioresistant bacteria and 9 radioresistant fungi were isolated from 10 irradiated samples of foundation cream. The bacterial isolates were identified as *Staphylococcus haemolyticus, Micrococcus* sp., *Bacillus brevies, Staphylococcus hominis-novo, Bacillus sphaericus, Bacillus pantothenticus* and *Bacillus alvei.* While, the fungal isolates were identified as *Aspergillus niger* and *Aspergillus fumigatus.*

Six radioresistant bacteria and 6 redioresistant fungi were isolated from 6 irradiated samples of foundation moisturizing cream. The bacterial isolates were identified as *Acenatobacter baumann / haem, Bacillus sphaericus* and *Bacillus pumilus.* While, the fungal isolates were identified as *Aspergillus niger, Aspergillus tamari,* and *Penicillium chrysogenum.*

Four radioresistant bacteria and 4 radioresistant fungi were isolated from 4 irradiated samples of moisturizing cream. The bacterial isolates were identified as *Bacillus pantothenticus, Staphylococcus haemolyticus* and *Micrococcus* sp. While the fungal isolates were identified as *Aspergillus niger.*

Our results are consistent with those obtained by

Nasser (2007) who reported that the number of surviving bacterial organisms in mixed population, decrease by increasing the gamma radiation doses.

The isolation of the most radiation resistant *Bacillus* sp. from mixed bacterial population were performed by **Ashour *et al.* (1990). Nasser (2007)** isolated from irradiated mixed population of bacteria, *Staphylococcus aureus* at 4-7 kGy, *Bacillus megatericum* at 13-14 kGy, *Staphylococcus epidermidis* at 5 kGy, *Bacillus cereus* at 11 kGy and *Aspergillus niger* at the dose level of 3 kGy.

In the present investigations, further studies were carried out on the different bacterial and fungal strains survived the most higher radiation doses. Therefore, *Aspergillus niger*, *Micrococcus* sp, *Staphylococcus. Hominis-novo, B. sphaercius, Bacillus pentothenticus, Aspergillus fumigatus* were choosen from foundation cream. *Acenatobacter baumann/haem, Aspergillus niger, Bacillus sphaericus* and *Penecillium chrysogenum* were choosen from foundation moisturizing cream. *Bacillus pantothenticus, Staphylococcus haemolyticus, Micrococcus* sp. and *Aspergillus niger* were chosen from moisturizing cream.

Dose response studies were carried out on the most radioresistant bacterial and fungal strains under test, by studying their response towards gamma radiation through plotting their dose response curves.

It is clear that 7 bacterial and 7 fungal strains exhibited exponential response towards gamma radiation (straight line curve). These strains are *Micrococcus* sp. *Staphylococcus hominis-novo, Micrococcus* sp, *Bacillus sphaericus, Aspergillus niger* and *Aspergillus fumigatus* from foundation creams. *Acenatobacter baumann/ haem, Bacillus sphaericus, Aspergillus niger* and *Penicillium chrysogeum* from foundation moisturizing creams, and *Staphylococcus haemolyticus, Micrococcus* sp. and *Aspergillus niger* from moisturizing creams.

On the other hand, the results show that 3 bacterial and one fungal strains exhibited non exponential response towards gamma radiation and this is manifested by the microorganisms straight line curves proceeded by an initial shoulder.

These strains are *Bacillus pantothenticus, Micrococcus* sp. from foundation cream, *Aspirgillus niger* from foundation moisturizing cream and *Bacillus pantotheticus* from moisturizing cream.

Radiation resistance can be associated with the D_{10} value (the dose of gamma radiation required to reduce a microbial population by 90%, **Miller and Berube, 1978**).

In the present study, the D_{10} values were calculated from the dose response curves of the most radioresistant microbial strains.

The Data show that the D_{10} values of microorganisms

isolated from foundation cream were found to be: *Micrococcus* sp (2.5 kGy), *Staph. hominis-novo* (0.85 kGy), *Bacillus sphaericus*,(0.85 kGy), *Bacillus pantothenticus* (0.4 kGy), *Micrococcus* sp (0.4 kGy), three strains of *Aspergillus niger* (0.7, 0.75, 0.95 kGy) and *Aspergillus fumigatus* (0.8 kGy). On other hand the D_{10} values of *Acenatobacter baumann/haem*, was found to be (1.1 kGy), for *Bacillus sphaericus*, (1.25 kGy), two strains of *Aspergillus niger* were found to be (0.9, 1.0 kGy) and *Penecillium chrysogenum* was (0.95 kGy) from foundation-moisturizinf cream.

While for moisturizing cream the D_{10} values were found to be: *Bacillus pantothenticus*, (0.5 kGy), *Staphylococcus haemolyticus*, (0.47 kGy), *Micrococcus* sp (0.75 kGy) and *Aspergillus Niger* was (1.0 kGy).

In other study **Bochkarev *et al.* (1978)** examined the radiation sensitivity of about 8.000 strains of Gram-positive microorganisms of dried culture preparations. From 1500 strains of *Staphylococci* 30% were found to have D_{10} value of 0.1 to 1 kGy and 70% were found to have D_{10} value of 2 kGy.

It was reported that Gram-positive cocci showed an exponential rate of death **(El-Shafei, 1982 ; El-Tayeb, 1991 and Roushdy *et al.*, 1999)** and the D_{10} values of *Staphylococcus aurues* were found to range from 0.49 to 0.95 kGy **(El-Tayeb, 1991).**

The exponential rate of death of *Bacillus megaterium* was also, obtained by **Ashour *et al.* (1990)** and **Salih (2001).**

Ashour *et al.* (1993) studied the response of *Bacillus sterothermophillus, B. pantothenticus, B. licheniformis, B. coagulans, B. laterospores, B. cereus, B. megaterium* and *B. pumilis E601* towards gamma radiation before and after radiation for 2 years. The D_{10} values for these strains were found to be ranged from 2.33 to 4.1 kGy.

Kotiranta *et al.* (1999) reported that four strains of *Bacillus cereus* were sensitive to gamma radiation and the D_{10} values was found to be 0.4 kGy.

The D_{10} values of bacterial species of *Micrococcus luteus, M. roseus, M. varians, Staphylococcus aureus ATCC, Stap. Aureus, Bacillus lichiniformis, B. coagulans, B. pumilis, B. brevis, B. cereus* isolates, *B. circulans, B. megaterium, B. cereus ATCC, B. megaterium ATCC,* and *Pseudomonas cepacia* were found to range from 1.8 to 2.83 kGy **(Roushdy *et al.*, 1999).**

The D_{10} values of *B. cereus, Staph. aureus* were found to be 1.02 and 0.37 kGy, respectively. While *Aspergillus flavus* was 0.48 kGy **(El-Fouly *et al.*, 2000).** The D_{10} values of *B.cereus, M.luteus, B. Sphaericus* were found to be 1.0, 1.4 and 1.4 kGy, respectively **(Farrag *et al.*, 2000).** The D_{10} values of 4 different *Bacillus* strains were found to range from 2.3 – 2.9 kGy **(Bashandy and Hassan, 2005**).

Abostate *et al.* (2006) found that *Bacillus cereus* strains exhibited exponential rate of death and the D_{10} values

were calculated to be 1.9 and 2.2 kGy.

Nasser (2007) reported that the dose response curves, for all the gram positive cocci showed an exponential rate of death and the D_{10} values of *Staphylococcus aureus* were found to range from 0.7 kGy to 1 kGy, while, the D_{10} values of *Staphylococcus epidermidis* were found to range from 0.7 kGy to 0.8 kGy, the dose response curves two gram positive spore forming *Bacillus megaterium* showed an exponential rate of death and the D_{10} values were found to be 1.7 and 1.8 kGy. On the other hand, an initial shoulder followed by an exponential rate of death was obtained in case of *Bacillus cereus* (D_{10} = 1.4 kGy) and *Aspergillus niger* showed an exponential rate of death (D_{10} = 0.4 kGy).

The D_{10} value of *Bacillus cereus* which showed an exponential rate of death was calculated to be 0.9 kGy by **El Tablawy and Elhifnawy (2009).**

Our results and that obtained by other investigators show that the resistance and response of microorganisms towards gamma radiation differ between the different microbial strains and the different chains of the same microorganisms. This may be attributed to the species number of organism and the different factors during the irradiation process **(Goldblith, 1971; Whitby and Gelda, 1979; Christensen, *et al.*, 1982 and Russel, 1982)**.

The cosmetics industry's commitment to produce high quality products coupled with an increasing awareness of microbial hazards by consumers has led to a search for more and varied alternate sources for sterilization. One of these options is the use of gamma processing to ensure the absence of undesirable microorganisms in the cosmetic products **(Swinwood and Wilson, 1990)**. Progress in the technology of radiation sterilization including the development of large radiation sources makes this method of decontamination most feasible.

Although, the use of radiation decontamination is not very old, it has found its way into many applications. However, there is still much to be investigated to improve the efficiency and to find an ideal procedure, which can decontaminate items with minimal alteration of their components **(Tilquin, 1991 ; Sainz vidal *et al.*, 1999; and Salih, 2001).** Ionizing radiation is very practical in inducing sterility without noticeable changes in the appearance or structure of the irradiated materials.

Nevertheless, the technology is still limited in the use because of unavailability of suitable radiation sources mainly in some of undeveloped countries, the dose of ionizing radiation and the microbial contamination load are among the factors that determine the efficiency of radiation sterilization **(Salih, 2001).**

Christensen and Kristensen (1981) reported that

bacteria demonstrated and the assumption that a medical device with an average total count of 20 contaminating organisms per product unit prior to irradiation should comply with an established norm ($1:10^6$) or suggested norm ($1:10^3$) respectively, for radiation sterilized devices. The minimum dose necessary for routine sterilization was estimated as about 38 kGy and about 20 kGy, respectively.

The data concerning the presented investigation show that the maximum irradiation doses are 23.0, 13.4 and 8.9 kGy for sterilization of the tested foundation cream, foundation moisturizing cream and moisturizing cream, respectively.

The highest sterilization doses for cosmetic cream samples was high in comparison to that reported by **Razem *et al.* (2003)** who was found to be 1.9, 2.2 and 5.3 kGy for eye make up cosmetic product. It should be taken into consideration that the types of contaminants were different, in our study, the contaminants include radiation resistant gram positive bacteria, gram negative bacteria and molds. While, in case of the mentioned authors, the contaminants were mainly radiation sensitive gram negative bacteria and molds.

In the present study, the calculated sterilization doses were applied on the heavily contaminated samples representing each individual brand. The study revealed that, no microbial contaminants were detected after the application of the irradiation process.

Boegl (1985) reported that the early work on pharmaceuticals and cosmetics relied on the application of 25 kGy dose. However, such a high dose was unrealistic as much as it is wasteful and damaging to products. The 25 kGy assumes that contamination levels of the pre-sterilized products are high.

The USPXXI (1985) stated that a dose of 25 kGy frequently has been selected as starting point, but many articles, including radiation sensitive articles having low or susceptible bioburdens, can be sterilized effectively.

CONCLUSIONS

The real problem was not only the heavy contamination on the cosmetic cream samples but also, the contamination with pathogens, which played a great role.

The consumers may play an important role in contaminating their cosmetic cream samples during in-use.

Cosmetic cream samples were generally found to be contaminated (intact and in-use) with (and, or) gram positive cocci, gram positive rode, gram negative cocci, gram negative rods, *Aspergillus* species, and *Penicillium* species.

The gamma radiation technology can offer the process of decontamination of cosmetics as a mean of approaching a higher standard of microbiological safety limits, decreasing the

bioburden on cosmetics and elimination of pathogenic microorganisms.

Sterility assurance of cosmetic creams can be satisfactorily maintained through adequate radiosterilization processing. Sources of bacterial contamination should be eliminated, the degree of sensitivity of the contaminating microorganisms to gamma radiation must be known and minimal level of gamma irradiation should be applied to ensure acceptable level of sterility.

RECOMMENDATIONS

The shelf life for cosmetic creams is more limited than for other products. Because of repeated exposure during use by the consumer and the risk of skin infections.

Consumers should be aware that a product's safety may expire long before the expiration date if the product has not been properly stored. Cosmetics have not been improperly stored, for examples, exposed to high temperatures or sunlight, or opened and examined by consumers prior to final sale, may deteriorate substantially before the expiration date.

On the other hand, products stored under ideal conditions may be acceptable long after the expiration date has been reached.

The radiation sterilization could be applied to the cosmetic cream in doses less than those applied in case of

pharmaceutical preparations, also this technology can eliminate many microbial problems that couldn't be eliminated by the preservatives which don't guarantee the sterility. However, the chemical and physical changes that may occur after the radiation doses must be investigated. The radiation sterilization is a feasible technology that provides a unique sterilizing procedure for cosmetics that is applied to the products in their final package ensuring the product's safety until reaching the consumer.

SUMMARY

In the present study 50 cosmetic cream samples were purchased from the market in Egypt. The samples were twenty five (25) foundation cream, representing 5 brands, 12 samples were found to be contaminated. Out of 10 foundation moisturizing cream samples representing 2 brands, 6 were contaminated. While, out of 15 moisturizing cream samples, representing 3 brands, 4 were found to be contaminated.

A total of seventy-eight (78) microbial contaminants were isolated from cosmetic face cream products obtained from the market. The isolated microbial contaminants were 43 bacterial isolates and 35 fungal isolates.

Evaluation of the total microbial count reveals that 10 foundation cream samples were contaminated with bacteria in the range of 1.0×10^5 to 2.5×10^7 cfu/ml and 9 samples were found to be contaminated with fungi in the range of 1.3×10^3 to 1.5×10^6 cfu/ml. Six (6) foundation-moisturizing cream samples were contaminated with bacteria in the range of 4.0×10^5 to 6.6×10^7 cfu/ml and 6 samples with fungi in the range of 1.3×10^4 to 7.0×10^5 fu/ml.

Four (4) moisturizing cream samples were contaminated with bacteria in the range of 7.5×10^6 and 1.5×10^8 cfu/ml while, they showed fungal contamination ranged between 9.5×10^4 and 2.1×10^6 cfu/ml.

The percent of contamination with bacteria were found to be 40%, 60%, 26.67%, with fungi were 36%, 60%, 26.67% and with bacteria and fungi were 48%, 60%, 26.67% for foundation cream, foundation-moisturizing and moisturizing cream, respectively.

The order of contamination on foundation cream samples is gram positive cocci > gram positive rods > gram negative cocci and gram negative rods. While, on foundation moisturizing cream, the order is gram positive rods > gram positive cocci > gram negative rods. On the other hand, the order of contaminants on moisturizing cream samples is gram positive cocci > gram negative rods > gram positive rods.

The results of evaluation of the fungal contamination on the tested cream samples show that *Aspergillus* sp. predominante over *Penecillum* sp. as the only fungal contaminants.

The effect of consumer handling and time on the microbial levels of the tested cream samples which showed, in the present investigations, no microbial contamination reveal that the level of contamination was found to increase with time and during use.

After 36 days of use, the bacterial contamination of foundation cream, foundation-moisturizing cream and moisturizing cream reach to 1.5×10^8, 2.0×10^7 and 1.0×10^7

cfu/ml and for fungi it reaches to 7.5 x 10^6, 4.2 x 10^6 and 4.3 x 10^7 cfu/ml, respectively.

The predominant bacterial contaminations on the tested cream samples were identified as gram positive rods and gram positive cocci. While, the fungal contamination were identified as *Aspergillus* sp. and *Pencillium* sp.

The contaminated cosmetic cream samples were exposed to gamma radiation in the range of 5 to 30 kGy, then suitable radiation doses for each cream samples was chosen in the determination test. The results of the effect of gamma radiation on the bacterial and fungal counts of foundation cream samples reveal that the bacterial sublethal dose levels ranged between 1.5 and 20 kGy, while the fungal sublethal dose levels ranged between 3 and 5 kGy.

The effect of gamma radiation on the bacterial and fungal counts of foundation moisturizing cream samples, reveal that the bacterial sublethal dose levels ranged between 5 and 7 kGy, while the fungal sublethal dose levels ranged between 3 and 5 kGy.

The effect of gamma radiation on the bacterial and fungal counts of moisturizing cream samples, reveal that the bacterial and fungal sublethal dose levels ranged between 3 and 5 kGy.

The bacteria surviving the sublethal dose levels from (1.5 – 20 kGy) were identified as *Staphylococcus haemolyticus, Micrococcus* sp. *Bacillus brevis, Staphylococcus hominis-novo, Bacillus sphaericus, Bacillus pantothenticus* and *Bacillus Alvei* from foundation cream. The bacteria surviving the sublethal doses from (5-7 kGy) were identified as *Acenatobacter baumann / haem, Bacillus sphaericus* and *Bacillus pumilus* from foundation moisturizing cream, while those surviving the sublethal dose from (3-5 kGy) were identified as *Bacillus pantothenticus, Staphylococcus haemolyticus* and *Micrococcus* sp. from moisturizing cream. The fungi surviving the sublethal dose levels from (3-5 kGy) were identified as *Aspergillus niger* and *Aspergillus fumigatus* from foundation cream.

The fungi surviving the sublethal doses from (3-5 kGy) were identified as *Aspergillus niger, Aspergillus tamari* and *Pencillium chrysogenum* from foundation-moisturizing cream, while those surviving the sublethal dose from (3-5 kGy) were identified as *Aspergillus niger* from moisturizing cream.

The D_{10} values or the decimal reduction doses were calculated from the graphs and were found to range from (0.4 to 2.0 kGy), (0.9 to 1.25 kGy), (0.47 to 1.0 kGy) in case of microbial strains isolated from foundation cream, foundation-moisturizing cream, and moisturizing cream, respectively.

The sterilization dose was calculated by using the survival curves of the radiation resistant isolates. The radiation sterilization doses of cosmetic face cream preparations were

calculated by the knowledge of the average bioburden on the cosmetic cream samples, the radiation resistance of the contaminant and sterility assurance level SAL (1: 10^{-3}) required for cosmetic creams products after sterilization.

For foundation cream, the calculated sterilization doses were calculated for the samples of the different brands to be (6.4 and 23 kGy), (6.1 to 8.6 kGy), (4.7 kGy) & (5.3 – 7.45 kGy) for brands A, B, C and D, respectively.

For foundation moisturizing cream, the sterilization doses were calculated to be (6.7 & 10.4 kGy) and (7.75 & 13.4 kGy) for brands F and G.

For moisturizing cream, the doses were (5.1 & 5.4 kGy) and (8.4 & 8.9 kGy) for brands H and I.

The calculated sterilization doses were applied on the heavily contaminated samples representing each brand. No microbial contaminants were detected. This suggests the success of the process of microbial decontamination of cosmetic face creams by gamma radiation. The maximum doses concerning the present investigations are 23.0 kGy, 13.4 kGt and 8.9 kGy for foundation cream, foundation moisturizing cream and moisturizing cream respectively.

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الملخص العربى

فى هذه الدراسة تم شراء خمسين من مستحضرات كريم الوجه من أسواق القاهرة ، كانت هذه العينات عبارة ع
.ن خمس وعشرون عينة كريم أساس تمثل خمس ماركات ، وقد وجد أن إثنا عشر عينة منهم ملوثة بالميكروبات ، وأيضا عشر عينات من كريم الأساس المرطب يمثلوا ماركتين ، ست عينات منهم وجدوا ملوثين. بينما خمس عشر عينة من الكريم المرطب يمثلون ثلاث ماركات أربع منهم وجد أنهم ملوثين.

فى الدراسة الحالية تم تقدير التلوث الميكروبى الكلى للخمسين عينة من مستحضرات كريم الوجه. وأسفرت النتائج أن عشر عينات من كريم الأساس ملوثة بالبكتريا فى حدود (١ x ١٠ ^{٥} إلى ٢.٥ x ١٠ ^{٧} مستعمرة / ملى) و تسع عينات ملوثة بالفطريات فى حدود (١.٣ x ١٠ ^{٣} إلى ١.٥ x ١٠ ^{٦} مستعمرة ملى).

وأيضا ستة عينات من كريم الأساس المرطب وجدوا ملوثين بالبكتريا فى حدود (٤ x ١٠ ^{٥} إلى ٦.٦ x ١٠ ^{٧} مستعمرة / ملى). وستة عينات ملوثة بالفطريات فى حدود (١.٣ x ١٠ ^{٤} إلى ٧ x ١٠ ^{٥} مستعمرة / ملى).

على الصعيد الآخر أربعة عينات من الكريم المرطب وجدوا ملوثين بالبكتريا فى حدود (٧.٥ x ١٠ ^{٦} و ١.٥ x ١٠ ^{٨} مستعمرة / ملى) بينما أظهروا تلوث فطرى فى حدود (٩.٥ x ١٠ ^{٤} و ٢.١ x ١٠ ^{٦} مستعمرة / ملى).

وقد وجد أن نسبة العينات الملوثة بالبكتريا هى ٤٠% ، ٦٠% ، ٢٦.٦٧% ونسبة الملوثة بالفطريات كانت ٣٦% ، ٦٠% ، ٢٦.٦٧% ونسبة

الملوثة بالبكتريا والفطريات معا كانت ٤٨% و ٦٠% و ٢٦.٦% لعينات كريم الأساس ، وكريم الأساس المرطب والكريم المرطب على التوالى.

بالنسبة لترتيب التلوث البكتيرى فى عينات كريم الأساس هو المكورات الموجبة الجرام > البكتريا العضوية الموجبة الجرام > المكورات السالبة الجرام والبكتريا العضوية السالبة الجرام ، بينما الترتيب فى كريم الأساس المرطب هو البكتريا العضوية الموجبة الجرام > المكورات الموجبة الجرام > البكتريا العضوية السالبة الجرام.

وقد وجد أن ترتيب التلوث الميكروبى فى الكريم المرطب هو المكورات الموجبة الجرام > البكتريا العضوية السالبة الجرام > البكرتيا العضوية موجبة الجرام .

أما بالنسبة للفطريات فقد تم تقدير التلوث للعينات تحت البحث التى أظهرت أن أجناس الأسبرجيليس تفوق أجناس البنيسيليوم وهما يمثلان التلوث الفطرى الموجود بالعينات.

وتم دراسة التلوث الميكروبى لعينات مستحضرات التجميل المختلفة أثناء الإستخدام التى لم تظهر تلوث ميكروبى فى هذه الدراسة ، وقد وجد أن مستوى التلوث يزيد بزيادة الوقت وأثناء الإستخدام.

بعد ستة وثلاثون يوما من الإستخدام وجد أن التلوث البكتيرى لكريمات الأساس وكريمات الأساس المرطبة والكريمات المرطبة تصل إلى ١.٥ x ١٠ ^{٨} ، ٢.٠ x ١٠ ^{٧} و ١.٠ x ١٠ ^{٧} مستعمرة/ ملى. أما بالنسبة للتلوث الفطرى فقط وصل إلى ٧.٥ x ١٠ ^{٦} و ٤.٢ x ١٠ ^{٦} و ٤.٣ x ١٠ ^{٧} مستعمرة / ملى على التوالى.

وقد تم تعريف التلوث البكتيرى ، إتضح أن التلوث البكتيرى بالبكتريا العضوية السالبة الجرام قد فاق التلوث البكتيرى بالمكورات موجبة الجرام فى

العينات تحت الإختبار وأيضا إتضح من الدراسة أن هذه العينات ملوثة بفطريات مثل أجناس أسبرجيليس وأجناس بنيسيليوم.

تم دراسة تأثير أشعة جاما على عينات كريمات التجميل الملوثة وذلك بتعريضها للجرعات الإشعاعية التى تتراوح ما بين ٥ إلى ٣٠ كيلو جراى وإختيار الجرعات المناسبة لكل عينة كريم فى هذا الإختبار.

أظهرت نتائج تأثير أشعة جاما على مستوى التلوث البكتيرى والفطرى لعينات كريم الأساس أن مستوى الجرعة قبل المميتة للبكتريا تتراوح ما بين ١.٥ إلى ٢٠ كيلو جراى بينما مستوى الجرعة قبل المميتة للفطريات تتراوح ما بين ٣ على ٥ كيلو جراى. أما تأثير أشعة جاما على مستوى التلوث البكتيرى والفطرى على عينات كريم الأساس المرطب أظهرت أن مستوى الجرعة قبل المميتة للبكتريا تتراوح ما بين ٥ على ٧ كيلو جراى بينما مستوى الجرعة قبل المميتة للفطريات تتراوح ما بين ٣ إلى ٥ كيلو جراى.

وبالنسبة لتأثير أشعة جاما على مستوى التلوث البكتيرى والفطرى لعينات الكريم المرطبة أظهرت أن مستوى الجرعة قبل المميتة للبكتريا وكذا للفطريات تتراوح ما بين ٣ إلى ٥ كيلو جراى.

البكتريا الناجية من مستوى الجرعة قبل المميتة التى تتراوح ما بين ١.٥ إلى ٢٠ كيلو جراى وجدت أنها ستافيلوكوكس ، هيموليتيكس ، أجناس ميكروكوكس ، باسيليس بريفيس ، ستافيلوكوكس هومينيس نوفو وباسيليس سفيريكس وباسيليس بانتوثينتيكس وباسيلس الفى فى كريمات الأساس ، أما البكتريا الناجية من مستوى جرعة الإشعاع قبل المميتة التى تتراوح ما بين ٥ إلى ٧ كيلو جراى هى اسينياتوباكتر بومان هيم ، باسيليس سفيريكس ، باسيلس بيوميلس من عينات كريم الأساس المرطب ، بينما البكتريا الناجية من جرعة الإشعاع قبل المميتة التى تتراوح ما بين ٣ إلى ٥ كيلو جراى هى باسيلس بانتوثينتيكس وستافيلوكوكس هيموليتيكس وأجناس ميكروكوكس من عينات الكريم المرطبة.

أما الفطريات الناجية من جرعة الإشعاع قبل المميتة التى تتراوح ما بين ٣ إلى ٥ كيلو جراى عرفت بأسبرجيليس نيجر ، أسبرجيليس فيوميجاتس من عينات كريم الأساس. والفطريات التى نجت من جرعة الإشعاع قبل المميتة التى تتراوح ما بين ٣ إلى ٥ كيلو جراى عرفت بأسبرجيليس نيجر وأسبرجيليس تاماراى وأيضا بنيسيليوم كريسوجينم فى عينات كريم الأساس المرطب ، بينما الذى نجا من جرعة الإشعاع قبل المميتة التى تتراوح ما بين ٣ إلى ٥ كيلو جراى عرف باسبراجيليس نيجر فى عينات الكريم المرطبة.

وقد تم تقدير الجرعة (د ١٠) وهى تعتبر مقياس للمقاومة الإشعاعية من منحنيات الإستجابة لكل سلالة ميكروبية ، وقد وجد أنها تتراوح من ٠.٤ إلى ٢ كيلو جراى ، ٠.٩ إلى ١.٢٥ كيلو جراى و ٠.٤٧ إلى ١.٠ كيلو جراى وذلك فى أنواع الميكروبات المعزولة من عينات كريم الأساس ، كريم الأساس المرطب والكريم المرطب على التوالى.

وقد تم إستخدام منحنيات الإستجابة للإشعاع للسلالات المختبرة فى تقدير الجرعة الإشعاعية اللازمة لتعقيم مستحضرات كريم الوجه بمعلومية متوسط الحمل الميكروبى لعينات الكريم المختلفة وأكثر الميكروبات مقاومة للإشعاع ، وكذلك درجة العقامة المطلوبة لهذا المنتج بعد التعقيم وهى (واحد فى الألف) ، وجد أن الجرعات الإشعاعية المحسوبة لتعقيم الماركات المختلفة لكريم الأساس هى ٦.٤ و ٢٣ كيلو جراى ، ٦.١ إلى ٨.٦ كيلو جراى ، ٤.٧ كيلو جراى و ٥.٣ إلى ٧.٤٥ كيلو جراى للماركات أ ، ب ، ت ، و ث على التوالى.

أما عينات كريم الأساس المرطب فجرعات التعقيم المحسوبة كانت ٦.٧ و ١٠.٤ كيلو جراى ، ٧.٧٥ إلى ١٣.٤ كيلو جراى للماركات ح ، خ وأخيرا فى عينات الكريم المرطب جرعات التعقيم المحسوبة كانت ٥.١ و ٥.٤ كيلو جراى و ٨.٤ و ٨.٩ كيلو جراى للماركات د ، و ، ذ.

تم إستخدام الجرعة المحسوبة فى تعقيم العينات الأكثر تلوثا لكل ماركة ولم يتضح أى تلوث ميكروبى بعد إختبار العينات المعقمة ، وهذا يـــرجح نجـــاح الجرعات المحسوبة فى تعقيم هذه المستحضرات.

وقد وجد أن أعلى جرعات إشعاعية للتعقيم تم تســـجيلها كانـــت ٢٣.٠ كيلو جراى ، ١٣.٤ كيلو جراى ، ٨.٩ كيلو جراى لعينات كريم الأساس وكريم الأساس المرطب والكريم المرطب على التوالى.

STUDIES ON DECONTAMINATION OF COSMETIC CREAMS BY GAMMA RADIATION

BY

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THESIS

Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Pharmaceutical Sciences

IN

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2011

دراسات على إزالة التلوث الميكروبى لكريمات التجميل بواسطة أشعة جاما

رسالة مقدمة من

هدير عبد الفتاح طاهر

للحصول على درجة الماجستير فى العلوم الصيدلية

فى الميكروبيولوجى

تحت إشراف

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