



**MEDITERRANEAN ACTION PLAN  
MED POL**

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**UNITED NATIONS ENVIRONMENT PROGRAMME**



**WORLD HEALTH ORGANIZATION**

**EPIDEMIOLOGICAL STUDIES RELATED TO ENVIRONMENTAL QUALITY  
CRITERIA FOR BATHING WATERS, SHELLFISH GROWING WATERS  
AND EDIBLE MARINE ORGANISMS (ACTIVITY D)**

**ETUDES EPIDEMIOLOGIQUES RELATIVES AUX CRITERES DE LA QUALITE  
DE L'ENVIRONNEMENT POUR LES EAUX SERVANT A LA BAIGNADE,  
A LA CULTURE DE COQUILLAGES ET A L'ELEVAGE D'AUTRES ORGANISMES  
MARINS COMESTIBLES (ACTIVITE D)**

**Final Report on epidemiological study on bathers from  
selected beaches in Malaga, Spain (1988-1989)**

**Rapport final sur l'étude épidémiologique menée parmi les baigneurs de  
certaines plages à Malaga, Espagne (1988-1989)**

**MAP Technical Reports Series No. 53**

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This volume is the fifty-third issue of the Mediterranean Action Plan Technical Report Series.

This series contains selected reports resulting from the various activities performed within the framework of the components of the Mediterranean Action Plan: Pollution Monitoring and Research Programme (MED POL), Blue Plan, Priority Actions Programme, Specially Protected Areas and Regional Marine Pollution Emergency Response Centre for the Mediterranean Sea.

Ce volume constitue le cinquante-troisième numéro de la série des Rapports techniques du Plan d'action pour la Méditerranée.

Cette série comprend certains rapports élaborés au cours de diverses activités menées dans le cadre des composantes du Plan d'action pour la Méditerranée: Programme de surveillance continue et de recherche en matière de pollution (MED POL), Plan Bleu, Programme d'actions prioritaires, Aires spécialement protégées et Centre régional méditerranéen pour l'intervention d'urgence contre la pollution marine accidentelle.

## GENERAL INTRODUCTION

The United Nations Environment Programme (UNEP) convened an Intergovernmental Meeting on the Protection of the Mediterranean (Barcelona), 28 January - 4 February 1975, which was attended by representatives of 16 States bordering on the Mediterranean Sea. The meeting discussed the various measures necessary for the prevention and control of pollution of the Mediterranean Sea, and concluded by adopting an Action Plan consisting of three substantive components:

- Integrated planning of the development and management of the resources of the Mediterranean Basin (management component);
- Co-ordinated programme for research, monitoring and exchange of information and assessment of the state of pollution and of protection measures (assessment component);
- Framework convention and related protocols with their technical annexes for the protection of the Mediterranean environment (legal component).

All components of the Action Plan are interdependent and provide a framework for comprehensive action to promote both the protection and the continued development of the Mediterranean ecoregion. No component is an end in itself. The Action Plan is intended to assist the Mediterranean Governments in formulating their national policies related to the continuous development and protection of the Mediterranean area and to improve their ability to identify various options for alternative patterns of development and to make choices and appropriate allocations of resources.

### MED POL - Phase I (1976-1980)

The Co-ordinated Mediterranean Research and Monitoring Programme (MED POL) was approved as the assessment (scientific/technical component of the Action Plan).

The general objectives of its pilot phase (MED POL - Phase I), which evolved through a series of expert and intergovernmental meetings, were:

- to formulate and carry out a co-ordinated pollution monitoring and research programme taking into account the goals of the Mediterranean Action Plan and the capabilities of the Mediterranean research centres to participate in it;
- to assist national research centres in developing their capabilities to participate in the programme;
- to analyse the sources, amounts, levels, pathways, trends and effects of pollutants relevant to the Mediterranean Sea;
- to provide the scientific/technical information needed by the Governments of the Mediterranean States and the EEC for the negotiation and implementation of the Convention for the Protection of the Mediterranean Sea against Pollution and its related protocols;
- to build up consistent time-series of data on the sources, pathways, levels and effects of pollutants in the Mediterranean Sea and thus to contribute to the scientific knowledge of the Mediterranean Sea.

MED POL - Phase I was implemented in the period from 1975 to 1980. The large number of national research centres designated by their Governments to participate in MED POL (83 research centres) from 15 Mediterranean States and the EEC), the diversity of the programme and its geographic coverage, the impressive number of Mediterranean scientists and technicians (about

200) and the number of co-operating agencies and supporting organizations involved in it, qualifies MED POL as certainly one of the largest and most complex co-operative scientific programmes with a specific and well-defined aim ever undertaken in the Mediterranean Basin.

#### MED POL - Phase II (1981-1990)

The Intergovernmental Review Meeting of Mediterranean Coastal States and First Meeting of the Contracting Parties to the Convention for the Protection of the Mediterranean Sea against Pollution, and its related protocols (Geneva, 5-10 February 1989), having examined the status of MED POL - Phase I, recommended that during the 1979/80 biennium a Long-term pollution monitoring and research programme should be formulated.

Based on the recommendations made at various expert and intergovernmental meetings, a draft Long-term (1981-1990) Programme for pollution monitoring and Research in the Mediterranean (MED POL-Phase II) was formulated by the Secretariat of the Barcelona Convention (UNEP), in co-operation with the United Nations Agencies which were responsible for the technical implementation of MED POL-Phase I, and it was formally approved by the Second Meeting of the Contracting Parties of the Mediterranean Sea against pollution and its related protocols and Intergovernmental Review Meeting of Mediterranean Coastal States of the Action Plan held in Cannes, 2-7 March 1981.

The general long-term objectives of MED POL-Phase II were to further the goals of the Barcelona Convention by assisting the Parties to prevent, abate and combat pollution of the Mediterranean Sea area and to protect and enhance the marine environment of the area. The specific objectives were designed to provide, on a continuous basis, the Parties to the Barcelona Convention and its related protocols with:

- Information required for the implementation of the Convention and the protocols;
- Indicators and evaluation of the effectiveness of the pollution prevention measures taken under the Convention and the protocols;
- scientific information which may lead to eventual revisions and amendments of the relevant provisions of the Convention and the protocols and for the formulation of additional protocols;
- information which could be used in formulating environmentally sound national, bilateral and multilateral management decisions essential for the continuous socio-economic development of the Mediterranean region on a sustainable basis;
- periodic assessment of the state of pollution of the Mediterranean Sea.

The monitoring of, and research on, pollutants affecting the Mediterranean marine environment reflects primarily the immediate and long-term requirements of the Barcelona Convention and its protocols, but also takes into account factors needed for the understanding of the relationship between the socio-economic development of the region and the pollution of the Mediterranean Sea.

As in MED POL-Phase I, the overall co-ordination and guidance for MED POL-Phase II is provided by UNEP as the secretariat of the Mediterranean Action Plan (MAP). Co-operating specialized United Nations Agencies (FAO, UNESCO, WHO, WMO, IAEA, IOC) are responsible for the technical implementation and day-to-day co-ordination of the work of national centres participating in monitoring and research.

The first eight volumes of the MAP Technical Reports Series present the collection of final reports of the principal Investigators who participated in the relevant pilot projects (MED POL I - MED POL VIII). The ninth volume of the MAP Technical Reports Series is the final report on the implementation of MED POL-Phase I, prepared, primarily, on the basis of individual final reports of the principal investigators with the co-operation of relevant United Nations Agencies (FAO, UNESCO, WHO, WMO, IAEA, IOC).

From the tenth volume onwards, the MAP Technical Report Series contains final reports on research projects, assessment documents, and other reports on activities performed within the framework of MED POL-Phase II, as well as documentation originating from other components of the Mediterranean Action Plan.

This *fifty-third* volume of the MAP Technical Reports Series contains the final reports of the third research project to be completed within the framework of MED POL Phase II in Activity D - "Epidemiological studies related to environmental quality criteria for bathing waters, shellfish-growing waters and edible marine organisms (Activity D)". Final reports on other projects will appear in future issues of the series.

## INTRODUCTION GENERALE

Le Programme des Nations Unies pour l'environnement (PNUE) a convoqué une réunion intergouvernementale sur la protection de la Méditerranée (Barcelone, 28 janvier - 4 février 1975) à laquelle ont pris part des représentants de 16 Etats riverains de la mer Méditerranée. La réunion a examiné les diverses mesures nécessaires à la prévention et à la lutte antipollution en mer Méditerranée, et elle s'est conclue sur l'adoption d'un Plan d'action comportant trois éléments fondamentaux:

- Planification intégrée du développement et de la gestion des ressources du bassin méditerranéen (élément "gestion");
- Programme coordonné de surveillance continue, de recherche, d'échange de renseignements et d'évaluation de l'état de la pollution et des mesures de protection (élément "évaluation");
- Convention cadre et protocoles y relatifs avec leurs annexes techniques pour la protection du milieu méditerranéen (élément juridique).

Tous les éléments du Plan d'action étaient interdépendants et fournissaient le cadre d'une action d'ensemble en vue de promouvoir, tant la protection que le développement continu de l'écorégion méditerranéenne. Aucun élément ne constituait une fin à lui seul. Le Plan d'action était destiné à aider les gouvernements méditerranéens à formuler leurs politiques nationales en matière de développement continu et de protection de zone de la Méditerranée et à accroître leur faculté d'identifier les diverses options s'offrant pour les schémas de développement, d'arrêter leurs choix et d'y affecter les ressources appropriées.

### MED POL - Phase I (1976-1980)

Le programme coordonné de surveillance continue et de recherche en matière de pollution de la Méditerranée (MED POL) a été approuvé au titre de l'élément "évaluation" (scientifique/technique) du Plan d'action.

Sa phase pilote (MED POL-Phase I) avait les objectifs généraux ci-dessous, élaborés au cours d'une série de réunions d'experts et de réunions intergouvernementales:

- formuler et exécuter un programme coordonné de surveillance continue et de recherche en matière de pollution en tenant compte des buts du Plan d'action pour la Méditerranée et de l'aptitude des centres de recherche méditerranéens à y participer;
- aider les centres de recherche nationaux à se rendre plus aptes à cette participation;
- étudier les sources, l'étendue, le degré, les parcours, les tendances et les effets des polluants affectant la mer Méditerranée;
- fournir l'information scientifique et technique nécessaire aux gouvernements des pays méditerranéens et à la Communauté économique européenne pour négocier et mettre en oeuvre la Convention pour la protection de la mer Méditerranée contre la pollution et les protocoles y relatifs;
- constituer des séries chronologiques cohérentes de données sur les sources, les cheminements, les degrés et les effets des polluants de la mer Méditerranée et contribuer par là à la connaissance scientifique de cette mer.

La Phase I du MED POL a été mise en oeuvre au cours de la période 1975-1980. Le grand nombre de centres de recherche nationaux désignés par leurs gouvernements pour participer au MED POL (83 centres de recherche de 15 Etats méditerranéens et de la CEE), la diversité du programme et sa couverture géographique, l'effectif impressionnant de scientifiques et techniciens méditerranéens (environ 200) ainsi que la quantité d'organismes coopérants et d'organisations d'appui qui y étaient engagés permettent sans conteste de caractériser le MED POL comme l'un des programmes de coopération scientifique les plus vastes et les plus complexes, comportant un objectif spécifique et bien défini, qui ait jamais été entrepris dans le bassin méditerranéen.

#### MED POL-Phase II (1981-1990)

La réunion intergouvernementale des Etats riverains de la Méditerranée chargés d'évaluer l'état d'avancement du Plan d'action et première réunion des Parties contractantes à la Convention pour la protection de la mer Méditerranée contre la pollution et aux protocoles y relatifs (Genève, 5-10 février 1979), ayant examiné la situation de la Phase I du MED POL, a recommandé que, durant la période biennale 1979-80, soit formulé un programme à long terme de surveillance continue et de recherche en matière de pollution.

Sur la base des recommandations énoncées lors des diverses réunions d'experts et réunions intergouvernementales, un projet de programme à long terme (1981-1990) de surveillance continue et de recherche en matière de pollution (MED POL - Phase II) a été formulé par le secrétariat de la Convention de Barcelone (PNUE), en coopération avec les organismes des Nations Unies chargés de l'exécution technique de MED POL - Phase I, et il a été officiellement approuvé lors de la deuxième réunion des Parties contractantes à la Convention pour la protection de la mer Méditerranée contre la pollution et aux protocoles y relatifs et réunion intergouvernementale des Etats riverains de la mer Méditerranée chargée d'évaluer l'état d'avancement du Plan d'action, qui s'est tenue à Cannes du 2 au 7 mars 1981.

L'objectif général à long terme de la Phase II du MED POL était de concourir à la réalisation des objectifs de la Convention de Barcelone en aidant les parties contractantes à prévenir, réduire et combattre la pollution dans la zone de la mer Méditerranée ainsi qu'à protéger et améliorer le milieu marin dans cette zone. Les objectifs particuliers étaient de fournir constamment aux Parties contractantes à la Convention de Barcelone et aux Protocoles y relatifs:

- les renseignements dont elles avaient besoin pour appliquer la Convention et les protocoles;
- des indications et une évaluation de l'efficacité des mesures prises pour prévenir la pollution en application de la Convention et des protocoles;
- des renseignements scientifiques qui pourraient servir à réviser et modifier les dispositions pertinentes de la Convention et des protocoles et à rédiger des protocoles additionnels;
- des informations qui pourraient servir à formuler sur les plans national, bilatéral et multilatéral, les décisions de gestion, respectueuses de l'environnement, qui seraient indispensables à la poursuite du développement socio-économique de la région méditerranéenne;
- une évaluation périodique de l'état de pollution de la mer Méditerranée.

La surveillance continue des polluants affectant le milieu marin de la Méditerranée ainsi que la recherche menée à leur sujet répondent en premier lieu aux prescriptions immédiates et à long terme de la Convention de Barcelone et des protocoles y relatifs, mais elles tiennent également compte des facteurs requis pour la compréhension des relations existant entre le développement socio-économique de la région et la pollution de la mer Méditerranée.



Comme lors de la Phase I du MED POL, la coordination et la direction générales de la Phase II étaient assurées par le PNUE, par l'intermédiaire du secrétariat du Plan d'action pour la Méditerranée (PAM). Les organismes spécialisés coopérants des Nations Unies (FAO, UNESCO, OMS, OMM, AIEA, COI) étaient chargés de l'exécution technique et de la coordination quotidienne des travaux des centres de recherche nationaux participant au programme de surveillance continue et de recherche.

Les huit premiers volumes de la Série des rapports techniques du PAM rassemblent les rapports finaux de chercheurs responsables qui ont participé aux projets pilotes correspondants (MED POL I -MED POL VIII). Le neuvième volume de cette même Série se compose du rapport final sur la mise en oeuvre de la Phase I du programme MED POL, établi essentiellement sur la base des rapports finaux individuels des chercheurs responsables avec la coopération des organismes compétents des Nations Unies (FAO, UNESCO, OMS, OMM, AIEA, COI).

A partir du dixième volume, la Série des rapports techniques du PAM, comprend des rapports finaux sur les projets de "recherche", des documents d'évaluation et d'autres rapports d'activités effectués dans le cadre de MED POL-Phase II, ainsi que de la documentation prise dans d'autres domaines du Plan d'action pour la Méditerranée.

Ce cinquante-troisième volume de la Série des rapports techniques du PAM comprend le rapport final sur le troisième projet de recherche à mener à terme dans le cadre de la Phase II du MED POL, dans l'Activité D - "Etudes épidémiologiques relatives aux critères de la qualité de l'environnement pour les eaux servant à la baignade, à la culture de coquillages et à l'élevage d'autres organismes marins comestibles (Activité D)". Les rapports finaux sur d'autres projets figureront dans les prochaines publications de la série.

**EPIDEMIOLOGICAL STUDY ON  
BATHERS FROM SELECTED BEACHES IN MALAGA**

by

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## 1. INTRODUCTION

The discharge of sewage into the marine environment has several potentially adverse consequences, one of which is derived from human population infections. Sewage contains several microorganisms which have been demonstrated to be pathogens and the causative agents of several human diseases. The number of these pathogenic microorganisms is increasing in seawater because of the steady growth of human populations and current methods of sewage disposal. In theory, all the diseases which are spread by the anal-oral route and whose aetiological agents are shed in the faeces of ill individuals or carriers could be contracted by swimming in sewage-polluted seawater. However, in practice, the illnesses directly related to the pollution of seawater is restricted to a few, principally:

- Bacterial diseases, such as salmonellosis (including typhoid and paratyphoid fevers), shigellosis (bacillary dysentery), cholera and bacterial gastroenteritis (Cabelli, 1983).
- Conjunctivitis, otitis and dermatitis produced by microorganisms that can be in sewage (UNEP/WHO, 1988).
- Enteric viral infections caused by enteroviruses (poliovirus, coxsackieviruses A and B, echoviruses), Norwalk, rotaviruses, reoviruses, and adenoviruses.
- Infectious hepatitis, mainly related to the consumption of contaminated shellfish.
- Diseases produced by parasitic microorganisms, such as *Entamoeba*, *Giardia*, *Ascaris* and others.

An epidemiological study may be focused upon different points of view. In the present work, we have reviewed the following questions:

- What are the mechanisms of the human infection?
- Which are the pathogenic microorganisms and what is their relation to the faecal pollution in the sea?
- Is there a consistent and a significant relationship between marine pollution and waterborne infections?
- How can we monitor the problem and what is its extent?

The circumstances under which people may be exposed to the pathogens in the sea at any given time can be conveniently characterized and they depend upon whether people are in contact with non-recreational or recreational seawater, or whether they eat contaminated shellfish. The results from previous prospective epidemiological studies showed that a very limited spectrum of diseases, including typhoid fever, shigellosis, acute gastroenteritis, coxsackie A and B diseases and infectious hepatitis have been clearly associated with swimming in recreational or non-recreational faecally-polluted seawater. The term "swimming" implies that the individual propels himself through the water.

The likelihood of infection in recreational waters depends upon whether a pathogen is in the sewage at any given time, its number, its survival capability, its infective dose, and the degree of dispersal. There is, however little evidence that immersion in recreational water is a common way to acquire the infection. The early literature (Winslow & Moxon, 1928; Moore, 1954) records some outbreaks of typhoid and paratyphoid fevers acquired in highly polluted water. The enteroviruses would seem likely to be transmitted by contaminated water, but their implication as water-borne agents despite the fact that much attention has been paid to the matter, is still inconclusive at present (Hawley *et al.*, 1973; Mosley, 1975).

There is no reason to doubt the infective potential of enteric organisms in seawater, but some aspects related to their dilution into seawater, the small amount of water ingested by the swimmers, and the large inoculum required to initiate an infection process may prevent the population from acquiring these illnesses.

Shellfish-associated infections, especially those of viral aetiology have been more frequently reported by several authors (Mason & McLean, 1962; DiGirolamo *et al.*, 1972; Vickey, 1973; Denis, 1973; Mahoney *et al.*, 1974). The importance of shellfish as a vehicle for enteric infective agents is based on two facts closely related to these animals: their ability to filter waters (which results in a high concentration of the microorganisms in their tissues), and the consumption of raw animals by human.

The use of microbial standards for seawater is basically a surveillance method. They would be helpful for monitoring beaches and even shellfish hygiene if they could predict the occurrence and risk of human infections. Therefore, the level of faecal pollution in marine bathing water measured by the concentration of bacterial indicators may indicate the potential incidence of enteric diseases among swimmers (Cabelli *et al.*, 1979; Cabelli, 1983; Foulon *et al.*, 1983; Favero, 1985). Since, when bathing, the entire body of the swimmer is submerged in the water, illnesses associated with eye, ear, skin and upper respiratory tract must also be considered, and probably other indicator microorganisms and standards are required to trace the transmission of the above mentioned pathogens by the water route.

Ideally, recreational water quality indicators are microorganisms whose densities in the water can be quantitatively related to potential health hazards resulting from recreational use therein; mainly, with infectious enteric diseases, such as cholera or typhoid fever, whose etiological agents are excreted in feces and are spread by the contamination of water and food with faecal wastes.

There are several reasons why the pathogens themselves are not used as microbial indicators. Firstly, there is a wide variety of infectious agents potentially transmitted by the waterborne route, and the density of each will vary both temporally and spatially independent of the others. Secondly, easy and reliable methods for quantifying most of the pathogens are unavailable. Thirdly, because of the temporal variability in pathogen densities in faeces and sewage (and hence their receiving waters), monitoring for the pathogens themselves is more akin to measuring the actual rather than the potential for disease.

Thus, historically the indicator concept was developed in parallel with the establishment of the faecal transmission of the enteric pathogens. Several microorganisms and chemicals have been proposed as indicators being the most important (Colwell, 1978), the coliform group, faecal coliforms, faecal streptococci, sulfite-reducing clostridia, and coliphages. Of these, coliforms and sulfite-reducing clostridia are good indicators mainly to verify the potability of drinking water, but their use as indicators of the degree of faecal pollution degree of natural waters is subject to question.

Many epidemiological studies have been carried out to investigate the relationship between the microbial quality of coastal waters and morbidity among bathers. Stevenson (1953), studying two marine beaches on Long Island Sound found a series of problems related to prospective epidemiological studies. First of all, swimming was not defined rigorously, and for this reason, the illness could not be attributed exclusively to contact of the body orifices with polluted waters. Secondly, the effects of day-to-day fluctuations in the pollution levels at the beaches were not eliminated. Lastly, only one indicator, total coliforms, was used.

Foulon *et al.* (1983) used the beach interview and answer card to obtain epidemiological data about the symptoms occurring in the bathing population. In this case, water samples were also analyzed for classical indicators of faecal pollution (total coliforms, faecal coliforms and faecal streptococci).

Cabelli (1983) established a direct relationship between swimming-associated gastrointestinal illnesses and the microbial quality of bathing water. This author used the interview and subsequent telephone follow-up survey as a method to obtain epidemiological information. In this study, five bacterial indicators (enterococci; total coliforms; faecal coliforms; *Escherichia coli* and *Clostridium perfringens*); two other enterobacteria (*Klebsiella* and *Enterobacter*); and three pathogenic microorganisms (*Aeromonas hydrophila*; *Pseudomonas aeruginosa* and *Vibrio parahaemolyticus*) were tested.

## 2. OBJECTIVES

The main scope of the present study was carry out an epidemiological-microbiological prospective study on a controlled sample, to determine the relationship between the concentration of some pathogenic microorganisms in bathing zones and the appearance of health effects among bathers and non-bathers attributable to the recreational use of water and sand of beaches with different pollution levels.

The objectives of this study, were divided as follows:

### Item I: MICROBIOLOGICAL SCOPE

1. Investigation and performance of different microbiological techniques for the detection and enumeration, in bathing seawater, of the most significant microbial pathogens, such as: *Salmonella* spp; *Staphylococcus aureus*; *Pseudomonas aeruginosa*; *Aeromonas hydrophila*; *Vibrio* spp and *Candida albicans*.
2. Determination of the level of these pathogenic microorganisms on several beaches with different characteristics (faecal pollution levels, number of bathers, etc.).
3. Recovery of dermatophyte fungi and pathogenic yeasts from beach sand.
4. Relationship between the concentrations of different pathogens and faecal indicators (faecal coliforms, *Escherichia coli*, faecal streptococci and coliphages).

### Item II. EPIDEMIOLOGICAL SCOPE

1. To determine the expected background illness rate among non-swimmers.
2. To find the difference of incidence rates among swimmers as compared with non-swimmers.
3. According to the results obtained, to determine the minimum number of persons (sample size) to be included in the final study or phase II (April 1989- April 1990).

Other objectives were to evaluate the design of the questionnaires, the reliability of the information and to find out "confusing factors" to be applied in a subsequent larger-scale study, and to obtain preliminary data regarding swimming-related health risks and swimming-associated morbidity in relation to bacterial pollution of seawater and sand.

### **3. MATERIALS AND METHODS**

#### **3.1 Study zones**

Studies were conducted at two locations: Misericordia beach, near Malaga city, frequented by about 100,000 people from Malaga (Spain), which exhibits high faecal pollution, and Santa Ana beach, a tourist zone near Benalmadena, with low faecal pollution (Figure 1).

Misericordia beach is located in the district of San Andrés. It is 3,100 m long, and receives the discharges of a faecal outfall, the sewage and wastewaters of which are deposited on the coast-line. On the other hand, Santa Ana is on the coast of Benalmadena, a very populated tourist resort. This beach is limited by two artificial docks, which causes marine tides and currents to be very slight. It is 300 m long and 25 m wide. This beach is not affected by outfall sewage discharges.

The selection of these two beaches was based on the results of the study of 10 beaches (Figure 2) according to three items:

- 1) Number of bathers and population definition.
- 2) Pollution degree and well-defined characterization of the faecal sources.
- 3) Proximity to public health centers.

#### **3.2 Study design**

The study was divided into three phases. The first phase, the optimization of the microbiological methods for bathing waters and selection of the beaches, was performed in the winter and spring of 1988; the second phase, a pre-test of microbiological and epidemiological analyses of the selected beaches was carried out during the summer of 1988; and the third phase or Final Study which will consist in a complete epidemiological study will be conducted during 1989 and the first months of 1990.

In the first and second phases all the microbiological studies were completed, but the adaptation of the epidemiological tests to the epidemiological data will require a more detailed revision.

#### **3.3 Microbiological analyses**

Water samples were taken for microbiological analyses at 11-12 a.m. on the same day the beach interviews were conducted at two points along the beach, normally in the swimming zone.

The samples were taken using one litre amber glass bottles and submerged 15-20 cm below the water surface (WHO/UNEP, 1977). The samples were then immediately placed in isothermic containers and carried to the laboratory, where they were tested within 2 h of sampling.

During the summer of 1988, twenty-four samples were taken between June and September.

The following microbiological media and methods were used:

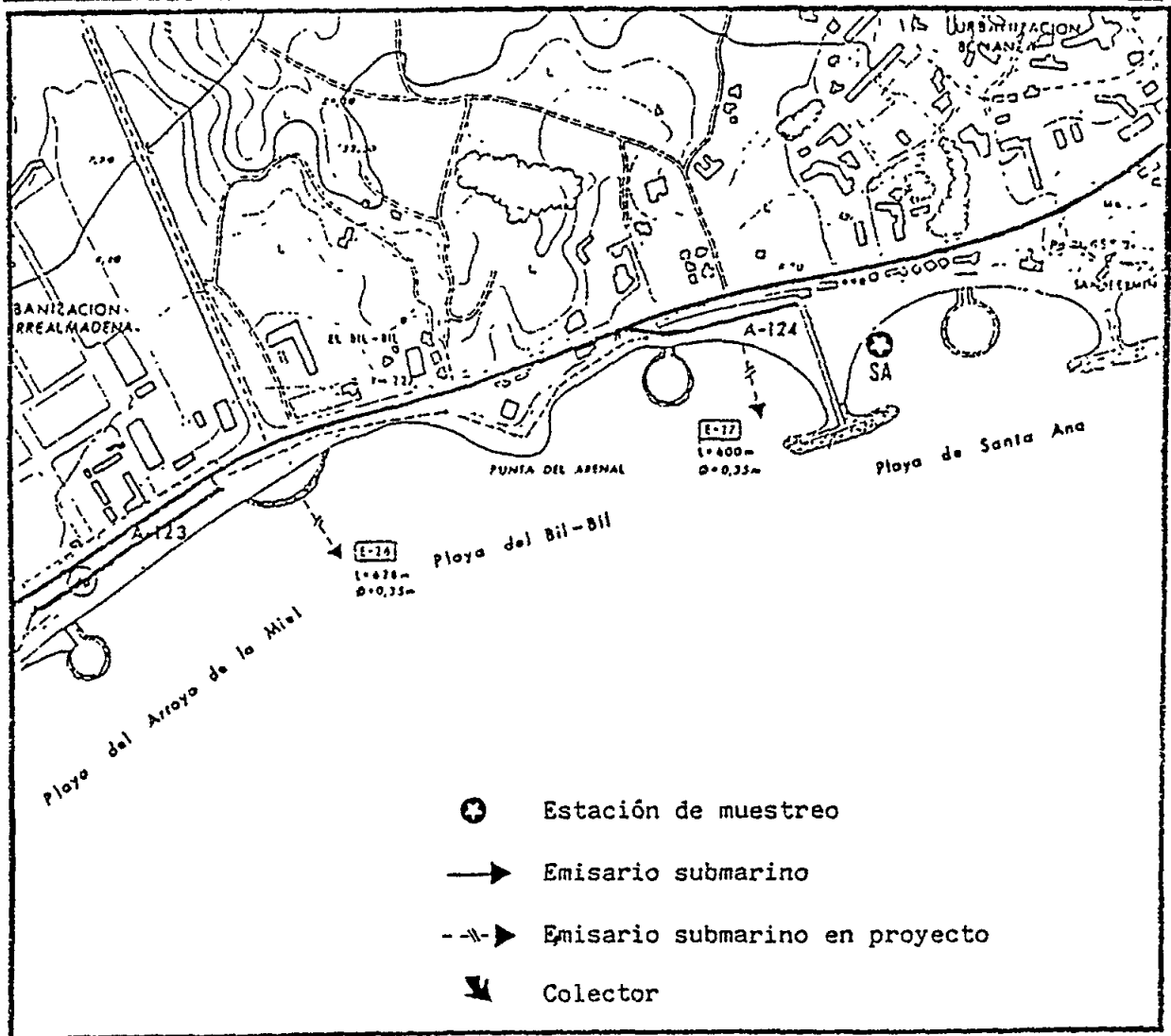
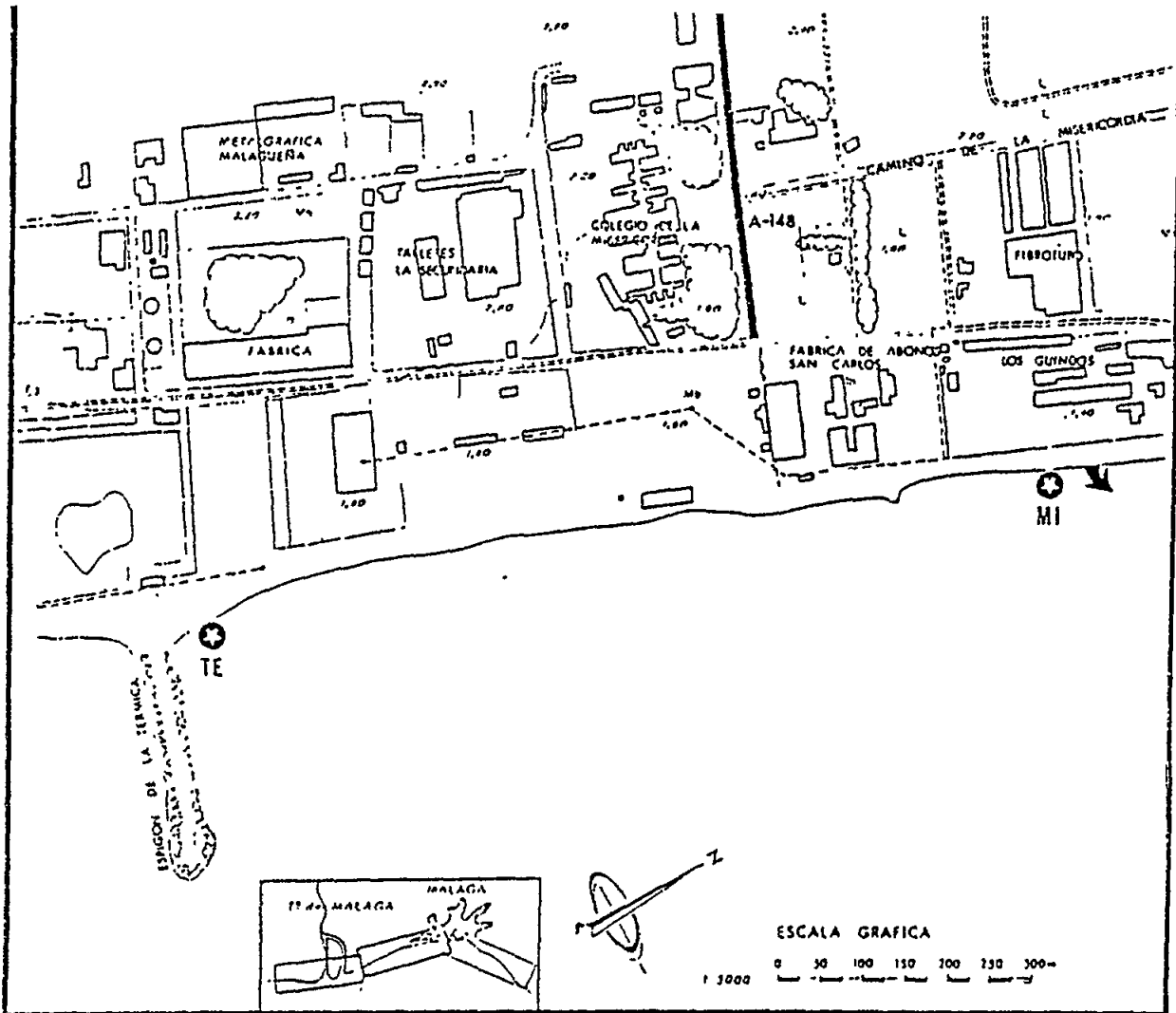


Figure 1 - Map of localization of the beaches studied



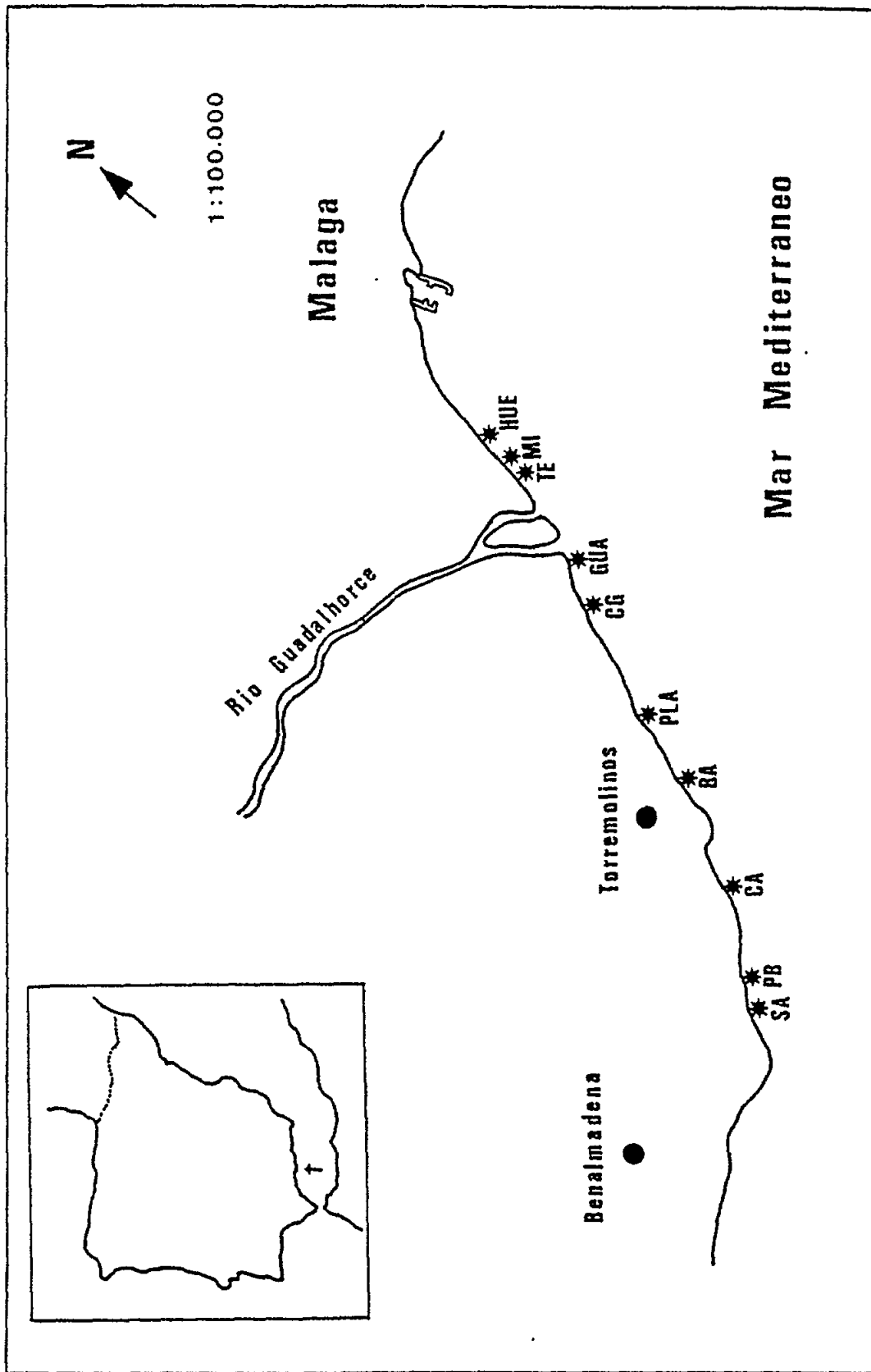


Figure 2 - General localization of the pre-selected beaches along Malaga coast

### 3.4 Media

#### 3.4.1 Enrichment Media

##### a. Peptone Buffered Water (Edel & Kampelmacher, 1973)

Peptone Buffered Water was used for pre-enrichment of *Salmonella* from natural water samples.

Composition (grams per litre of distilled water)

Peptone	10.0
Sodium chloride	5.0
Na <sub>2</sub> HPO <sub>4</sub> . 12 H <sub>2</sub> O	9.0
KH <sub>2</sub> PO <sub>4</sub>	1.5
pH 7.2	

##### b. Modified Rappaport-Vassiliadis broth [NR10(10)/43] (Morinigo et al., 1986)

Selective enrichment broth for the quantitative analysis of *Salmonella* in conjunction with Most Probable Number technique.

Composition

Solution A (grams per litre of distilled water)

Tryptone	5.0
Sodium chloride	8.0
KH <sub>2</sub> PO <sub>4</sub>	1.6

Solution B (grams per litre of distilled water)

Magnesium chloride	400
--------------------	-----

Solution C (grams per litre of distilled water)

Malachite green oxalate	0.4
-------------------------	-----

Solutions A, B and C were mixed in the following proportions: 110, 10 and 1, respectively. The mix was supplemented with 10 mg per ml of sodium novobiocin.

The Xylose-Lysine-Deoxycholate (XLD) agar (Difco) was used to isolate salmonella from the positive tubes with modified Rappaport-Vassiliadis broth.

##### c. Luria broth

This medium was employed both for *Escherichia coli* growth and bacteriophages enrichment.

Composition (grams per litre of distilled water)

Tryptone	10.0
Yeast extract	5.0
Sodium chloride	10.0
pH 7.2	

### 3.4.2 Enumeration media

Media used in combination with the filtration membrane technique.

- m Endo broth (Difco)

m Endo broth was used for the quantitative recovery of total coliforms, supplemented with 1.2 % of agar-agar (Difco).

- m FC base broth (Difco)

It was employed for the quantitative analysis of faecal coliforms. It was also supplemented with 1.2 % of agar-agar.

- m Enterococcus agar (Merck)

This medium was employed for the selective enumeration of faecal streptococci present in the samples.

- m PA-E agar (de Vicente *et al.*, 1986)

This medium is highly selective in the detection and enumeration of *Pseudomonas aeruginosa* from seawater.

Composition (grams per litre of distilled water)

Yeast extract	1.00
Sodium chloride	5.00
HCl-Lysine	5.00
Xylose	2.50
Ammonium ferric citrate	1.00
Sodium thiosulphate 7H <sub>2</sub> O	3.04
Phenol red	0.08
Agar-agar	15.00
pH 7.2	

The ingredients were dissolved by autoclaving and cooling at 55-60° C. Before spreading the medium onto the Petri dishes, 12 mg/l of kanamycin sulphate (Sigma) and 37 mg/l of nalidixic acid (Sigma) were added.

- BFR-0 agar (Borrego *et al.*, 1987)

This medium was used in the enumeration of staphylococci and particularly *S. aureus*, from water samples.

Composition (grams per litre of distilled water)

Yeast extract	3.0
Tryptone	6.0
Beef extract	1.5
D-mannitol	20.0
Sodium chloride	100.0
Glycine	11.0
Sodium pyruvate	10.0
Potassium thiocyanate	25.0
Phenol red	0.025

Sodium azide	0.049
Agar-agar	15.0
pH 7.2	

- m-CA agar (Buck & Bubucis, 1978)

This medium was used for the selective recovery of pathogenic yeast, especially *Candida albicans*, from sand and water samples.

Composition (grams per litre of distilled water)

Glycine	10.0
Maltose	30.0
Bismut ammonium citrate	5.0
Sodium sulphite	3.0
Chloramphenicol	0.5
Actidione	1.5
Agar-agar	15.0
pH 7.0	

The ingredients were dissolved by boiling at 100° C. After cooling at 45° C, the medium was supplemented with 10 % of yeast nitrogen base (Difco), and the completed medium sterilized by membrane filtration (0.45 µm pore size).

- Thiosulphate Citrate Bile salt Saccharose agar (TCBS) (Difco)

This medium was used in the enumeration of *Vibrio* species, from water samples.

Composition (grams per litre of distilled water)

Yeast extract	5.00
Protease-peptone	10.00
Sodium citrate	10.00
Sodium thiosulphate	10.00
Oxgall	8.00
Saccharose	20.00
Sodium chloride	10.00
Ferric citrate	1.00
Bromothymol blue	0.04
Thymol blue	0.04
Agar-agar	15.00
pH 8.6	

- m-A agar (Rippey & Cabelli, 1979)

This medium was applied for the enumeration of *Aeromonas* species and particularly *A. hydrophila*, from water samples.

Composition (grams per litre of distilled water)

Tryptose	5.00
Thehalose	5.00
Yeast extract	2.00
Sodium chloride	3.00
Potassium chloride	2.00
Magnesium sulphate 7H <sub>2</sub> O	0.20

Ferric chloride. 6 H <sub>2</sub> O	0.10
Bromothymol blue	0.04
Agar-agar	15.00
pH 8	

The ingredients were dissolved at room temperature and afterwards the agar-agar was added, and the completed medium sterilized by autoclaving at 121° C. After sterilization the medium was supplemented with 10 ml/l of ethanol and then, cooled at 50° C in a thermostatic bath. After being homogenized, the medium was supplemented with 20 mg/l of ampicillin (Sigma) and 100 ml/l of sodium deoxycholate (Difco).

### 3.4.3 Other enumeration media

#### a. Tryptose Sulphite Cycloserine (TSC) (Harmon & Kautter, 1978)

This medium, used in combination with the tube poured technique (Bonde, 1977), serves for the quantification of clostridia sulphite reducer microorganisms.

#### Composition (grams per litre of distilled water)

Tryptose	6.30
Yeast extract	2.10
Casitone	2.10
Ferric citrate	0.42
Sodium bisulphite	0.42
Agar-agar	8.40
pH 7.6	

This medium was sterilized at 121° C for 15 minutes, and cooled at 45° C in a thermostatic bath. Before being used, the medium was supplemented with 15.2 ml of a buffered solution of cycloserine (Sigma), previously sterilized by membrane filtration (0.22 µm pore size).

#### Composition (in grams)

KH <sub>2</sub> PO <sub>4</sub>	0.870
K <sub>2</sub> HPO <sub>4</sub>	0.048
D-cycloserine	0.500
distilled water	350ml
pH 8.0	

#### b. Luria agar

Its composition is similar to Luria broth, but it is supplemented with 1.2 % of agar-agar (Difco). Luria agar is employed as bottom layer agar in the double layer technique (Adams, 1959) for detection and enumeration of bacteriophages.

A modification of the medium which consists in the addition of 0.7 % of agar, and the elimination of yeast extract is called soft nutrient agar. It is used as top layer in the double layer technique (Adams, 1959).

### 3.4.4 Solutions

#### a. Phosphate buffered solution (APHA, 1985)

This solution was used as diluent and also for washing the membrane filtration equipment.

Composition (in grams)

Solution I: KH <sub>2</sub> PO <sub>4</sub>	3.4
distilled water	100ml
pH 7.2	

Solution II: Sodium Chloride	8.0
distilled water	100ml
pH 7.2	

To prepare the phosphate buffered solution, 1.25 ml of solution I and 5 ml of the solution II were mixed with 1000 ml of distilled water.

### **3.5 Methods**

#### **3.5.1 Detection and recovery of the microorganisms**

##### **a. Membrane filtration technique**

The principal objective of this method is the concentration of the microbial cells present in a water sample. For this reason, the sample is passed through a membrane filter with a diameter which is smaller than that of the bacteria. Afterwards, the filtered membrane is cultured in an appropriate medium for the detection and selective enumeration of the microorganism to be tested. In the present work, we used the Standard Methods for the Examination of Water and Wastewater guidelines (APHA, 1985).

The media and incubation conditions were the following:

- Total coliforms: m-Endo agar at 36° C for 24 h. The characteristics of the colonies which grow in this medium show a pink or deep red colour with a metallic bright green in the centre, or all over the colony. Counts of 20-80 colonies per petri dishes are considered as optimum.
- Faecal coliforms: m-FC agar at 44.5° C in a thermostatic bath for 24 h. Typical faecal coliform colonies are characterized by their blue colour. In accordance with Presswood & Strong (1978) rosolic acid is suppressed, because the acid can affect the counting negatively. Optimum recount is established between 20 and 60 colonies per filter.
- Faecal streptococci: m-Enterococcus agar at 36° C for 48 h. In order to recover the stressed streptococci, the incubation period is delayed to 72 h (Lin, 1977). The filters that contain between 20 and 100 typical streptococci colonies (pink to red colour and 0.5-2 mm diameter size) are used as optimum count and concentration calculations.
- Pseudomonas aeruginosa: m-PA-E agar at 36° C for 48 h. The typical colonies showed a brownish-gray to black colour, and in most cases, the presence of a diffusible pigment. Optimum recounts of 20-80 colonies per filter are established (de Vicente et al., 1986).
- Staphylococcus aureus: BFR-0 agar at 36° C for 48-72 h. The typical colonies in the medium are differentiated by their yellow colour and also by the yellow halo around the colony. The recount limits range between 20 and 80 colonies per membrane.
- Candida albicans: m-CA agar at 36° C for 48 h. The membrane filter has a 1.2 µm pore size. Typical characteristics of these colonies are their dark-brown colour and mucous aspect. For the concentration calculations, recounts of between 20-80 colonies per membrane dishes are used.

b. Direct count of phages using the double agar layer technique.

This technique is appropriate when the water sample possesses a titre of  $\approx 10$  plaques of lysis forming unit (pfu) per 100 ml and it consists in adding 1 ml of the sample and 0.2 ml of a logarithmic growth culture of the bacterial host to tubes containing soft agar melted at 45° C. After a brief homogenization, the tubes are poured onto Luria agar plates, following the technique described by Adams (1959) and modified by Borrego & Romero (1985). When the top layer of the plates is solidified, they are incubated at 36° C for 12- 18 h in an inverted position.

c. Detection and quantification of *Salmonella* from natural waters.

The method is an adaptation of the multiple tube technique and the protocol used was the following:

Aliquots of 100 ml and 10 ml of the sample were filtered through a membrane filter of 0.45  $\mu$  m pore size, and then put into tubes with 10 ml of buffered peptone water. Aliquots of 1 ml of the sample are inoculated directly into the tubes with the above mentioned medium. In total, three series of three tubes, each containing the pre-enrichment medium, were used. They were incubated at 36° C for 24 h.

A 1:100 portion of each incubated pre-enrichment tube was transferred into another tube with the selective enrichment broth (NR10 (10)/43), which was incubated at 43° C for 48-72 h. A loopful of each incubated tube was streaked onto XLD plates, which were also incubated at 36° C for 24 h.

Typical *Salmonella* colonies grown on XLD plates were identified as *Salmonella* spp by biochemical characteristic profiles (Le Minor, 1984) and serotyping of the isolates (Edwards & Ewing, 1972). All the isolates that coincided with the profile of *Salmonella* spp were confirmed by API 20-E System (BioMerieux).

### 3.6 Statistical analyses

The log-normal distribution of frequencies was chosen for the evaluation of the microbiological quality of the beaches and the selection of two of them for the epidemiological study, according to the results obtained by several authors (UNEP/WHO, 1977; El-Shaarawi & Papes, 1982).

The expression used for the assignment of the frequencies to the microbiological data, ranking from minimum to maximum, was the following (UNEP/WHO, 1983)

$$P(x_i) = (i/n+1) \times 100$$

where,  $P(x_i)$  is the accumulate frequency corresponding to the  $i$  data, expressed in percentages,  $i$  is the order number of the observation considered and  $n$  is the total number of observations.

The results were drawn in log-normal paper.

The relationship between indicator and pathogenic microorganisms concentrations was examined by regression analysis using a STATVIEW 512 PLUS program from Apple Macintosh Plus. The zero concentration of the microorganisms was not computed, and the data were analysed by a mean group, using four groups which represented a month mean. The relationship between indicator and pathogenic microorganisms were represented using mean densities of both by the following log-linear equation:

$$\log y = a + b \log x$$

where,  $x$  was the mean indicator density, and,  $y$  the mean pathogen density.

### **3.7 Epidemiological study**

The design of this phase, conducted on two beaches located in Malaga (Spain), was slightly different from that performed by Cabelli et al. (1982) and by Shuval (1985), and was followed the recommendations for the prospective epidemiological-microbiological studies described in "Health Criteria and Epidemiological Studies Related to Coastal Water Pollution" (WHO/UNEP, 1977) and several WHO/UNEP consultations (1986, 1987).

#### **3.7.1**

The bather definition established by WHO/UNEP (1988) was adopted, with special reference to the groups of people who stay out of the water and on the beach sand almost all the time. Thus, a "bather" is defined as any person present at the bathing beach, and a "swimmer" was defined as a bather whose head had been immersed in water, who had swallowed seawater or whose face had been splashed by waves.

In addition, the following special populations categories were identified:

- Individuals entering the water up to the waist or shoulders, but not immersing the head whatsoever. This group will be of interest for dermatological symptoms.
- Children, particularly infants (0-4 years), not actually entering the sea, but having extended contact with the wet area of beach sand.

#### **3.7.2 Period of study**

The study was carried out on week days and with a high presence of bathers. The period of study was established from June 15th to September 15th, 1988.

#### **3.7.3 Study beaches**

The beaches established and described in the microbiological study, which were surveyed in order to locate relatively "polluted" and "non-polluted" areas, in base to the EEC qualifications, included two beaches of Malaga coast: a) Misericordia beach, highly "polluted" and with a high presence of bathers; and b) Santa Ana beach (Benalmdena) relatively clean and also with a high presence of bathers.

In the same way, the beaches were selected considering the general scope of the present study, and the oceanographic and environmental characteristics. Thus, Misericordia beach is characterized by a high water mass renovation, because this beach is very exposed to the waves and it is an open beach. Santa Ana beach present a low renovation of its water mass, because it is delimited for two artificial break-waters that conform a semiclose space with a narrow seawater mouth.

#### **3.7.4 Study population**

The study population included individuals or groups and, especially, family units with children under ten, that stay on the beach at least one hour and are coincident with the definition of "bathers" and "swimmers" adopted in this study.

The study population involved both local population and tourists, considering that the study populations of both beaches had the same demographic characteristics with the objective of to make possible future comparisons. In particular, the sex, age, source (local or tourist) and bathing habits were considered.



### 3.7.5 Morbidity symptoms

The following morbidity symptoms were studied:

#### a. Enteric diseases

Morbidity analysis for enteric diseases (gastrointestinal) included the following symptomatology: vomiting, diarrhoea, fever, nausea, abdominal pain and combinations of these.

#### b. Non-enteric diseases

Morbidity involved in non-enteric infections included three major areas:

- Affecting ears and eyes (otitis and conjunctivitis) and upper respiratory tract;
- Causing skin rashes (dermatitis);
- Causing urinogenital tract irritation.

Morbidity symptoms were considered "highly credible" if for enteric symptoms there was confinement to bed, vomiting, diarrhoea with fever, nausea, and/or advice of a physician, and for non-enteric symptoms, confirmation in the follow-up interview by phone and/or advice of a physician.

### 3.7.6 Questionnaires

Three questionnaires were prepared for this Phase on the basis of a simplified protocol; two to be conducted on the beach and one for the follow-up by telephone approximately 3 to 10 days after the beach interview.

The first and second questionnaires on the beach (see Appendix 1) were supplied to families of bathers and the same family were not reinterviewed again during the bathing season.

The first questionnaire, namely "week-end interview", included 10 basic items divided into 7 groups, with the following content:

- Questionnaire number, date, time and place;
- Personal identification of the interviewees;
- Permanence time of the interviewees;
- Basic features related to age, sex and the relationship of family members, as well as whether they swim or not in the water and their contact with the beach sand (with towel, deckchair or directly on the sand);
- Symptomatology observed in the family members subsequent to the interview and within 2 to 7 days prior to it. The symptoms covered morbidity defined previously;
- Permanence, previously or not, at another beach or swimming-pool (within 7 days before);
- Contact phone and address of the interviewees to conduct a follow-up 3-10 days after the questionnaire.

The second questionnaire, namely "epidemiological interview" is slightly similar except that it included the position of the interviewees on the beach and number of days they had swim in another beach or swimming pool.

The follow-up questionnaire (see Appendix 1) was also structured in 7 items of questions. The information to be obtained is as follows:

- Symptoms of any family members as a consequence of the use of the beach.
- Influence of several other factors on the symptoms.
- Confirmation of the symptoms by clinician or physician.

Questionnaires on the beach included the following information:

- Information on whether each member of the group was exposed to possible infection from contaminated food and/or beverages.
- Observation of the bathing suit and hair of each member of the group to see if they are wet.
- Observation of the external aspects of the interviewees, specially, skin, eyes and ears.

The interviewer was given precise instructions for collecting answers involving categorizations of swimmers and non-swimmers noting which member entered the water, etc.; comparison of answers and observations for a limited number of families was done to estimate the reliability of the information obtained.

### 3.7.7 Coding of data and computer analyses

All the data from the questionnaires were coded according to a special code sheet for each visit to the beach and for each family group. The data were introduced into a computer, first in a basedate program (dBASE III plus) and then transferred with a test editor to a statistical program (SPSS/+) for data analyses. All the processing was done at an IBM-AT compatible personal computer.

### 3.7.8 Statistical analyses

The classification of subjects according to disease experience and the exposure to an aetiological factor, in a prospective study, was the following:

Exposure (swimmers)	Disease		Total
	+	-	
+	a	c	a + c
-	b	d	b + d
Total	a + b	c + d	a + b + c + d

Data analysis involved calculations of morbidity symptoms incidence rates per 100 persons, swimmers and non- swimmers, for each beach and in all the beaches.

Calculation of incidence rates among swimmers and non- swimmers, standard error of incidence rates, and determination of statistical significance between incidence rates were defined as follows (Mantel & Haenszel, 1959; WHO/UNEP, 1986; Liliendfield, 1986):

$$I_s = \text{Incidence rate among swimmers per 100} = (a/a+c) \times 100$$

$$I_{ns} = \text{Incidence rate among non-swimmers per 100} = (b/b+d) \times 100$$

$$\text{Standard error (SE)} = [r(100-r)/N]^{1/2}$$

r= rate of a symptom  
N= total population

If SE is the standard error and R is the morbidity symptoms, the significance of differences between the incidence rates of groups i and j will be:

Table 1

Selection of the beaches based on XX50, XX84, XX90 and s values

	SA	PB	CA	BA	PLA	CG	GUA	TE	MI	HUE	
TOTAL COLIFORMS	XX50	3.55E3	5.90E3	8.10E2	2.60E3	4.10E3	8.40E4	6.70E4	4.10E4	9.00E4	7.00E5
	XX84	1.10E4	3.00E4	3.70E3	1.48E4	2.10E4	2.40E5	1.42E6	8.20E4	3.00E5	4.10E6
	XX90	1.50E4	4.90E4	5.90E3	2.25E4	3.30E4	3.15E5	2.10E6	1.00E5	4.20E5	6.50E6
	s	1.13	1.63	1.52	1.74	1.63	1.05	0.75	0.69	1.20	1.77
FAECAL COLIFORMS	XX50	3.80E2	6.30E2	1.27E2	5.60E2	1.82E2	9.20E2	2.90E4	1.32E3	1.00E4	2.27E4
	XX84	7.00E2	5.80E3	4.60E2	3.70E3	4.30E2	3.40E3	1.07E5	9.00E3	3.50E4	1.28E5
	XX90	8.50E2	1.12E4	6.80E2	6.30E3	5.80E2	4.70E3	1.48E5	1.38E4	4.90E4	1.90E5
	s	0.61	2.22	1.29	1.89	0.86	1.31	1.130	1.92	1.25	1.73
FAECAL STREPTOCOCCI	XX50	1.10E2	1.15E2	8.10E1	1.10E2	3.45E1	3.45E2	1.56E4	2.10E2	3.00E3	4.60E3
	XX84	4.00E2	9.60E2	3.05E2	9.00E2	6.40E1	1.31E3	8.60E4	1.38E3	9.20E3	2.18E4
	XX90	5.50E2	1.70E3	4.50E2	1.52E3	7.80E1	1.95E3	1.30E5	2.20E3	1.22E4	3.30E4
	s	1.29	2.12	1.32	2.10	0.62	1.33	1.71	1.88	1.12	1.55

Table 2

Counts obtained from bathing waters in Misericordia beach

	FAECAL COLIFORMS			TOTAL COLIFORMS			FAECAL STREPTOCOCCI		
	ufc/100ml	%	Rank	ufc/100ml	%	Rank	ufc/100ml	%	Rank
	3500	62.50	8	5000	37.50	5	6700	79.16	10
	3800	70.83	9	6500	54.16	7	2700	70.83	9
	3800	79.16	10	7600	70.83	9	2100	62.50	8
C	600	20.83	3	3100	12.50	2	600	4.16	1
O	1100	45.83	6	7800	79.16	10	700	12.50	2
U	4800	87.50	11	6100	45.83	6	7100	87.50	11
N	500	12.50	2	400	4.16	1	1200	37.50	5
T	710	37.50	5	4500	24.16	4	1100	20.23	3
S	1800	54.16	7	21000	87.50	11	1260	54.16	7
	675	29.16	4	3900	20.83	3	1150	29.16	4
	140	4.16	1	6500	62.50	8	1240	45.83	6
	480000	95.83	12	710000	95.83	12	460000	95.83	12
N	12			12			12		
x	41789			65200			40487		
s	2.30			2.04			1.98		
XX50	1600			5600			2200		
XX84	16000			43000			16000		
XX90	32000			82000			28000		

Table 3

Counts obtained from bathing waters in Santa Ana beach

	FAECAL COLIFORMS			TOTAL COLIFORMS			FAECAL STREPTOCOCCI		
	ufc/100ml	%	Rank	ufc/100ml	%	Rank	ufc/100ml	%	Rank
	55	54.16	7	750	29.16	4	0	4.16	1
	190	87.50	11	8700	87.50	11	40	79.16	10
	110	79.16	10	6100	79.16	10	0	12.50	2
C	10	12.50	2	510	12.50	2	0	20.83	3
O	0	4.16	1	580	20.83	3	0	29.16	4
U	65	70.83	9	890	37.50	5	60	87.50	11
N	20	29.16	4	2020	54.16	7	0	37.50	5
T	40	37.50	5	1700	45.83	6	0	45.83	6
S	40	45.83	6	2800	70.83	9	30	62.50	8
	60	62.50	8	2060	62.50	8	30	70.83	9
	20	20.83	3	160	4.16	1	20	54.16	7
	2600	95.83	12	6000	95.83	12	2400	95.83	12
N	12			12			12		
X	267.5			2689			215		
s	1.85			1.12			1.79		
XX50	55			1800			17.5		
XX84	350			5500			105		
XX90	550			7400			165		

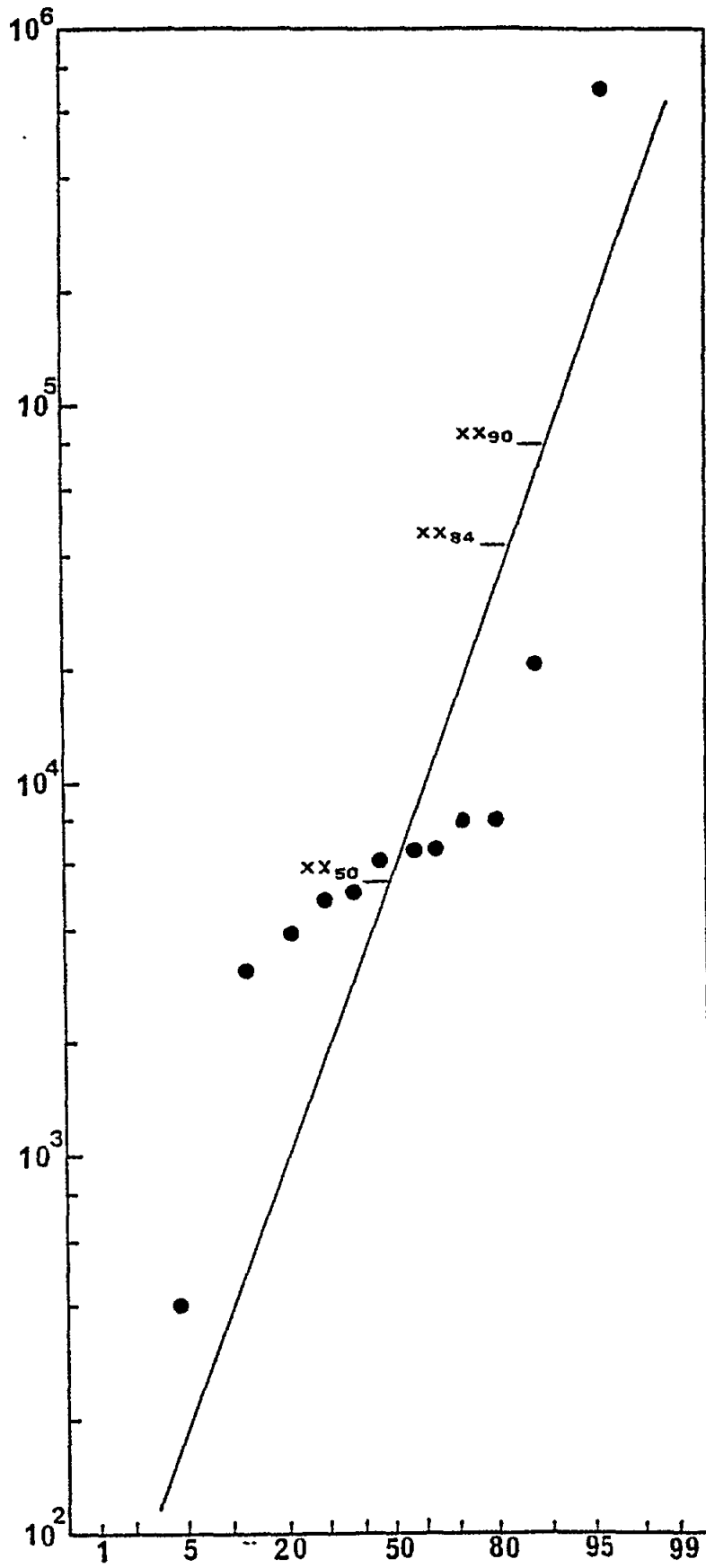


Figure 3 - Log normal analysis of Total Coliforms in Misericordia beach

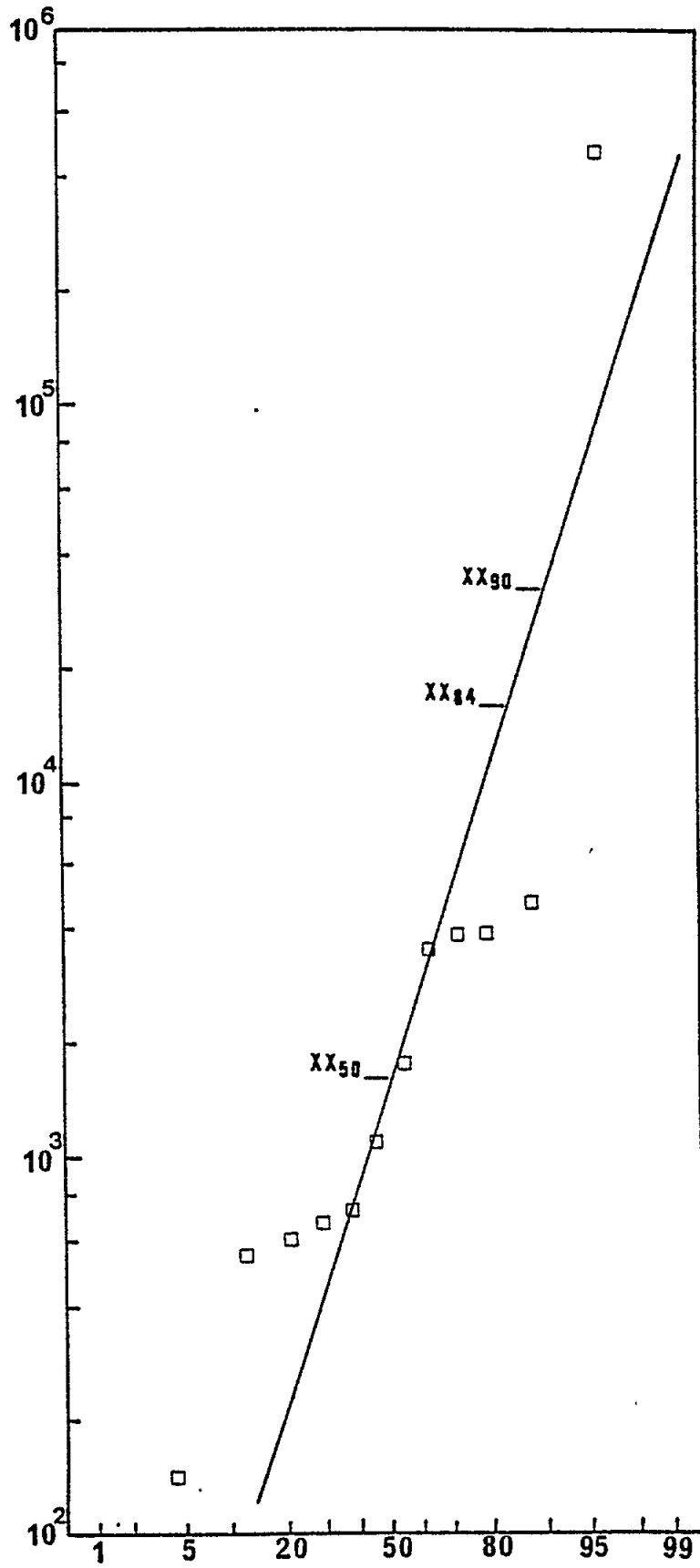


Figure 4 - Log normal analysis of Faecal Coliforms in Misedicordia beach

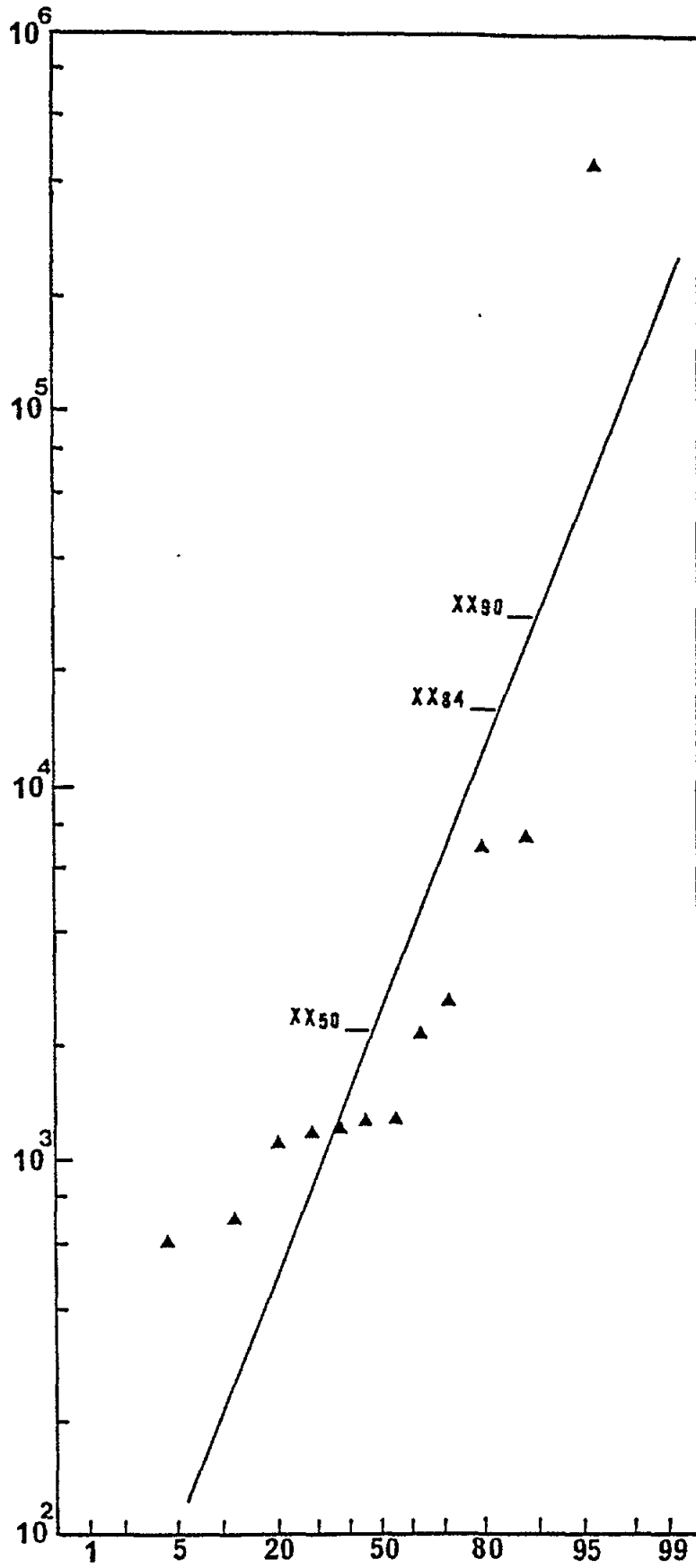


Figure 5 - Log normal analysis of Faecal Streptococci in Misericordia beach



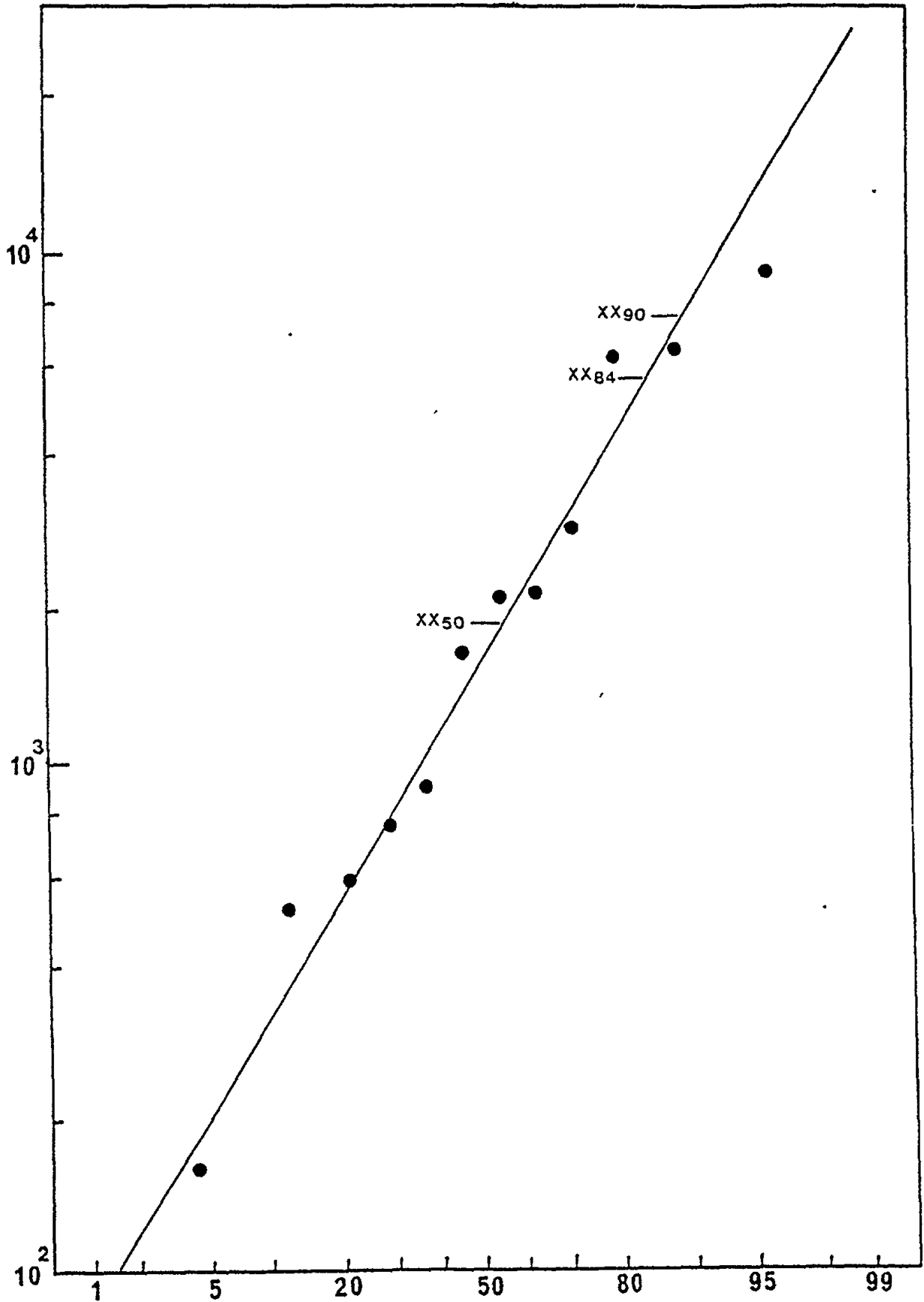


Figure 6 - Log normal analysis of Total Coliforms in Santa Ana beach

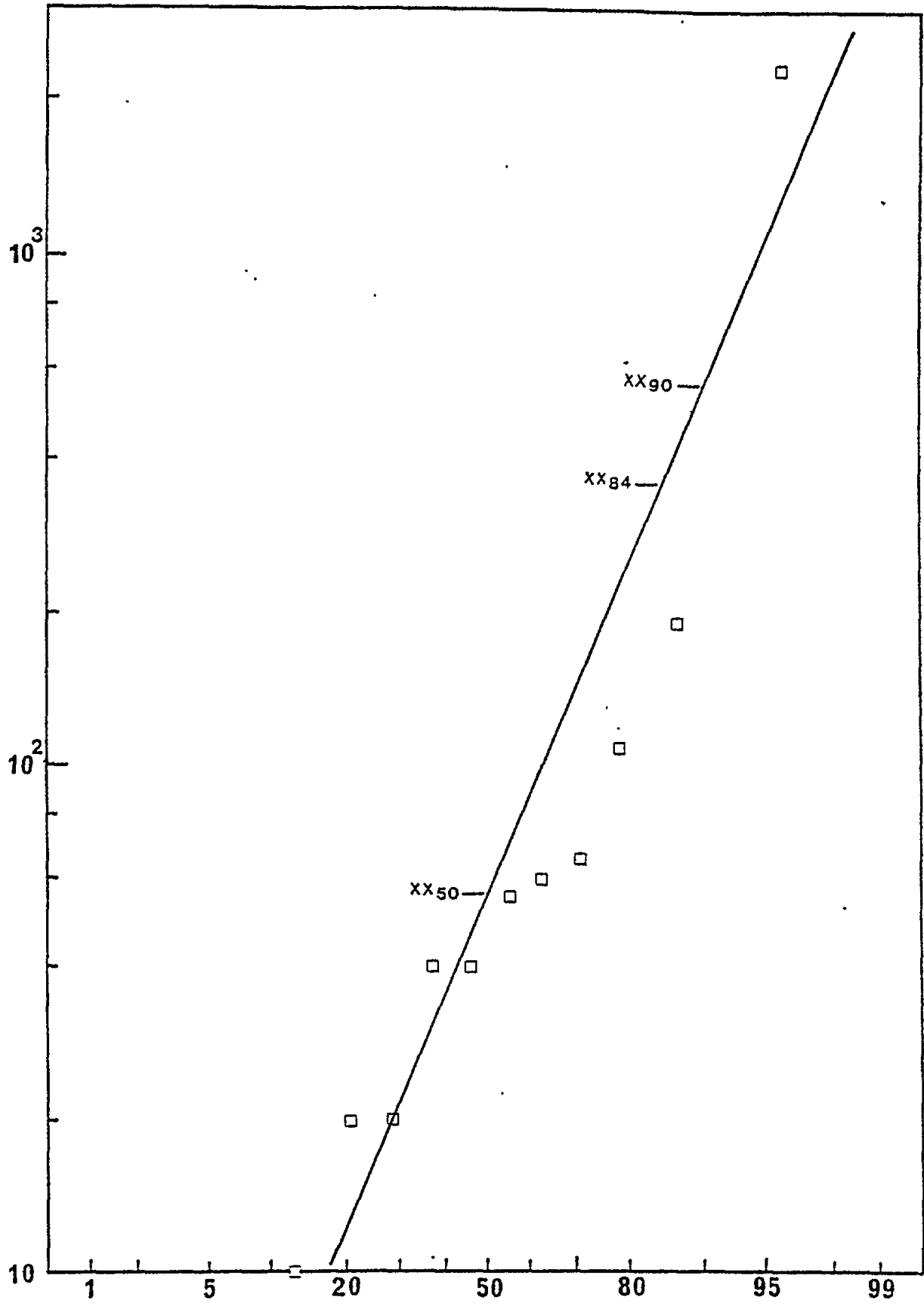


Figure 7 - Log normal analysis of Faecal Coliforms in Santa Ana beach

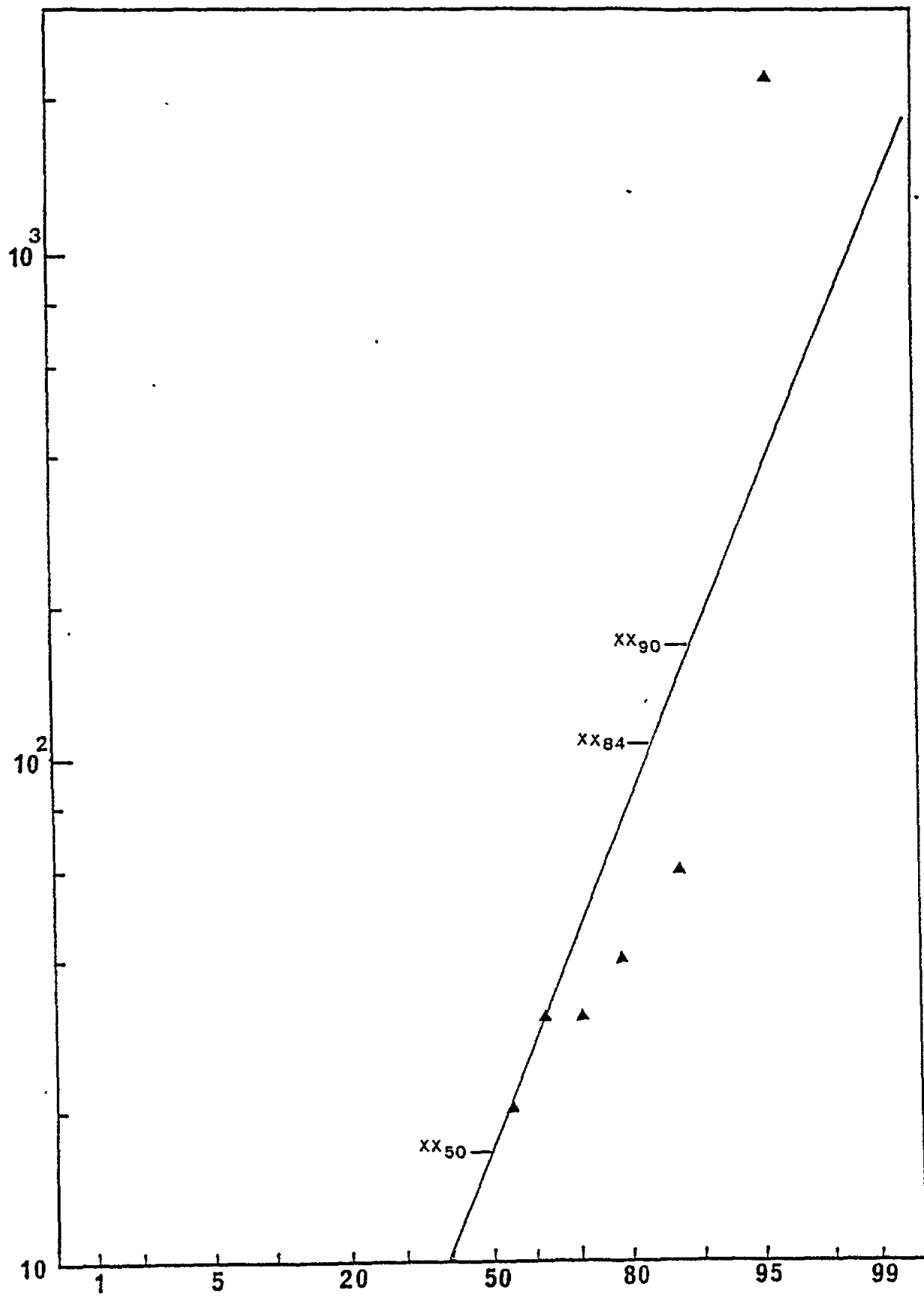


Figure 8 - Log normal analysis of Faecal Streptococci in Santa Ana beach

$$Z = (R_i - R_j) / \sqrt{(SE_i)^2 + (SE_j)^2} \cdot 1/2$$

R = morbidity symptoms incidence rate for i and j groups.

The p values of Z are found in the table of cumulative normal distribution.

To summarize the information contained in the microbiological and epidemiological data, a principal component analysis (PCA) was performed. A logarithmic transformation was used to approximate the distributions to normality and to reduce the proportion of total variance of the data. PCA was performed using the SPSS/+ statistical package.

## 4. RESULTS

### 4.1 Selection of the study zone

The selection of two beaches to carry out the epidemiological study was based, mainly on two aspects:

- Degree of pollution of the water, selecting one beach with a low faecal pollution level, and another with a high level of pollution.
- Type of bathing population. Our aim was to study a beach attended mainly by tourists and visitors, and another by local residents.
- Proximity to Public Health Centers.

To select both beaches, a previous study of the degree of pollution by measuring of indicator microorganisms (total coliforms, faecal coliforms and faecal streptococci) was carried out. For this purpose, the log-normal analysis of ten beaches along the Malaga coast was used and the results are shown in Table 1.

According to the results obtained, three beaches belong the category "low degree of pollution", were selected: Santa Ana in Benalmadena and Carihuela and Playamar in Torremolinos. On the basis of two other conditions above mentioned, Santa Ana beach was finally chosen. On the other hand, belonging to "high degree of pollution" category, three beaches fulfil the first requirement, Guadalhorce, Misericordia and Huelin, all of them located in Malaga city. Due to its large number of bathers, Misericordia beach was selected for the subsequent epidemiological study.

### 4.2 Microbiological quality of the bathing waters

The microbiological quality of the bathing waters allowed us to establish the influence that the discharges of sewage or wastewaters exert on the marine environment. This parameter was evaluated by the analyses of faecal pollution indicators.

To obtain the frequency distribution of the classical indicators used, the graphical log-normal statistical model was applied from the data specified in Tables 2 and 3, obtaining the regression lines and the XX50, XX84 and XX90 values.

Figure 3 depicts the log-normal distribution of total coliforms in Misericordia beach; it can be observed that the values of XX50, XX84 and XX94 were as high as 5.6E3, 4.3E4 and 8.2E4, respectively. The standard deviation (S) obtained from the expression ( $S = \frac{LXX84 - LXX50}{2.303}$ ), was 2.04.

The log-normal distribution of faecal coliforms values on this beach is represented in Figure 4. The XX50, XX84 and XX90 values obtained from this microbial parameter were: 1.6E3, 1.6E4 and 3.2E4, and the "S" value calculated was 2.30.

Faecal streptococci log-normal distribution for Misericordia beach is drawn in Figure 5. For this microbial parameter the following values were obtained: 2.283, 1.6 E4 and 2.8 E4 for XX50, XX84 and XX94, respectively, the "S" value being 1.98.

Figures 6, 7 and 8 represent the log-normal distributions of total coliforms, faecal coliforms and faecal streptococci, respectively on Santa Ana beach (Benalmadena). For total coliforms (Figure 6) the concentrations obtained were 1.8 E3, 5.5 E3 and 7.4 E3 for XX50, XX84 and XX90 values, respectively. Figure 7 shows the regression line and XX50, XX84 and XX90 values, which were 5.5 E1, 3.5 E2 and 5.5 E2, respectively. Finally, faecal streptococci concentrations obtained for XX50, XX84 and XX90 were 1.75 E1, 1.05 E2 and 1.65 E2, respectively (Figure 8).

### 4.3 Quality criteria

The criteria and standards derived by several international and national organizations and institutions for the interpretation of the microbial quality of recreational bathing waters are defined by different quality parameters which are specified in Table 4.

The quality levels obtained from the log-normal analyses of the two beaches studied (Tables 2 and 3) applying the different quality criteria, depend on the standard used. In the present study we applied three standard criteria: California Department of Public Health (CDPH,1943); EEC(1976); WHO/UNEP (1977, 1981) and Spanish Ministry of Public Works and Urbanism (MOPU, 1977). Santa Ana was the only beach that fulfils the MOPU and UNEP/WHO criteria.

### 4.4 Microbial analyses in the beaches selected

These microbiological analyses were carried out in the summer of 1988, including the evaluation of the following microbial parameters: 1) Indicator microorganisms (faecal coliforms, *Escherichia coli*; faecal streptococci and coliphages); 2) Pathogenic microorganisms (*Salmonella* spp, *Pseudomonas aeruginosa*, *Aeromonas hydrophila*, *Staphylococcus aureus*, *Vibrio* spp. and *Candida albicans*); 3) Pathogenic microorganisms in the beach sand (dermatophyte fungi and the pathogenic yeast *Candida albicans*).

Table 5 summarizes the microbial densities (mean values), the range and the number of samples obtained in both beaches studied, and defined previously as beaches with "high" and "low" degree of pollution. As it can be seen, there is a significant difference ( $p < 0.01$ ) between the pollution degrees analyzed by indicator mean concentrations in both bathing waters, so the highest mean concentration in Santa Ana beach was 4.07 E2 for faecal streptococci and compared to Misericordia beach, the highest score was 3.24 E3 for coliphages. There is also a significant difference between the concentration ranges, the upper limit in Santa Ana beach being 1,800 versus 15,000 in Misericordia beach. However, the lower limits on the two beaches were very similar, varying between 7 and 180 for Misericordia and 0 and 40 for Santa Ana beach.

For the pathogenic microorganisms we have appreciated a similar behaviour between the two selected beaches. We have never isolated *Salmonella* spp and *Staphylococcus aureus* from the two beaches during the time of the study, although previously we were able to recover both pathogens from these bathing waters and other. The most reliable explanation is that, in the time studied, the environmental conditions were extreme: high water temperature; high insolation grade; high salinity, all of which exert a very negative effect on these sensitive pathogens and cause their recovery and enumeration to fail.

The numbers of *P. aeruginosa* and *C. albicans* were similar in the two bathing waters (39 vs 10 for *P. aeruginosa* and 37 vs 9 for *C. albicans* on Misericordia and Santa Ana beaches, respectively). These results are accounted for the fact that these pathogenic microorganisms are

Table 4

Criteria and standard of microbiological quality for bathing waters

INDICATOR MICROORGANISM			
STANDARD	TOTAL COLIFORMS	FAECAL COLIFORMS <u>Escherichia coli</u>	FAECAL STREPTOCOCCI
a C.D.P.H	XX80 = 1000 TC/100 ml		
b UNEP/WHO		XX50 = 100 Ec/100 ml XX90 = 1000 Ec/100 ml	
c EEC	XX80 = 500 TC/100 ml XX95 = 10000 TC/100 ml	XX80 = 100 FC/100 ml XX95 = 2000 FC/100 ml	XX90 = 100FS/100 ml
d MOPU		XX50 = 299 Ec/100 ml XX90 = 1000 Ec/100 ml	

- a: California Department of Public Health (1943)
- b: United Nations Environment Programme / World Health Organization (1979, 1981)
- c: European Economic Community Council (1975)
- d: Ministerio de Obras Públicas y Urbanismo. Gobierno de España. (1977).

Table 5

Microbial densities in bathing waters and sand during summer 88  
in the beaches studied

	MISERICORDIA BEACH			SANTA ANA BEACH		
	N	Mean <sup>a</sup>	Range	N	Mean <sup>a</sup>	Range
FAECAL COLIFORMS	12	2608	7-12000	12	20.5	0-80
<u>Escherichia coli</u>	12	2608	7-12000	12	17.5	0-61
FAECAL STREPTOCOCCI	12	2835	130-11500	12	407	40-1800
Coliphages	12	3240	180-15000	12	30	5-120
<u>Salmonella</u> spp	12	0	0	12	0	0
<u>Pseudomonas aeruginosa</u>	12	39	0-120	12	10	0-80
<u>Vibrio</u> spp	12	3375	250-12000	12	735	100-2800
<u>Aeromonas hydrophila</u>	12	3298	80-11800	12	11	0-50
<u>Staphylococcus aureus</u>	12	0	0	12	0	0
<u>Candida albicans</u>	12	37	3-150	12	9	0-40
<sup>b</sup> FUNGI (sand)	12	22	10-50	12	13	5-20
<sup>b</sup> <u>C. albicans</u> (sand)	12	1.5	0-5	12	9	0-30

N: Number of samples  
a: cfu/100 ml of water  
b: cfu/1 g of sand

**Table 6**  
 Correlation coefficients matrix between the logs of indicator  
 microorganism concentrations in the two beaches studied

	FAECAL COLIFORMS		Escherichia coli		FAECAL STREPTOCOCCI		COLIPHAGES	
	MIS	SA	MIS	SA	MIS	SA	MIS	SA
FAECAL COLIFORMS	1.00	1.00						
Escherichia coli	* 0.930	0.998 *	1.00	1.00				
FAECAL STREPTOCOCCI	* 0.973	* 0.732 #	* 0.959	0.695 #	1.00	1.00		
COLIPHAGES	* 0.872	0.259 NS	* 0.850	0.283 NS	* 0.949	0.176 NS	1.00	1.00

\*: Significant at p 0.001  
 #: Significant at p 0.01  
 NS: No significant



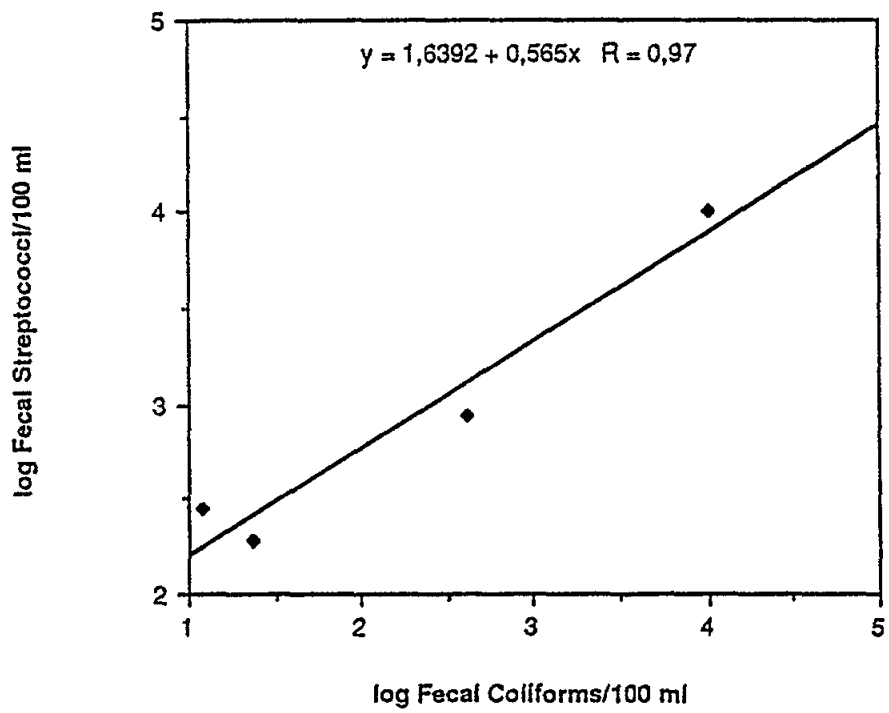
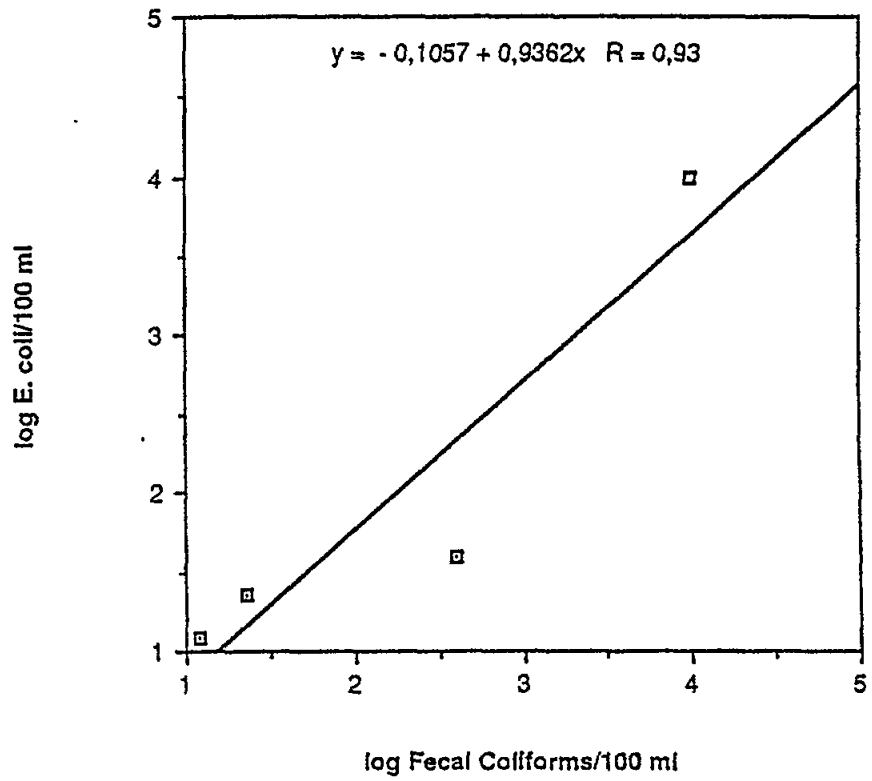


Figure 9 - Relationships between indicators in bathing of Misericordia beach

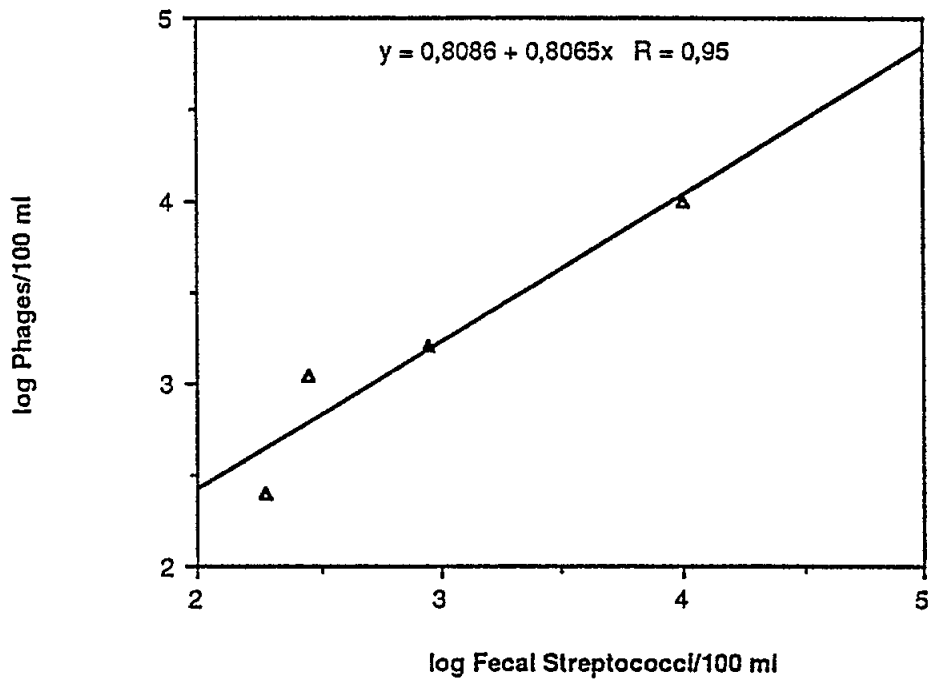
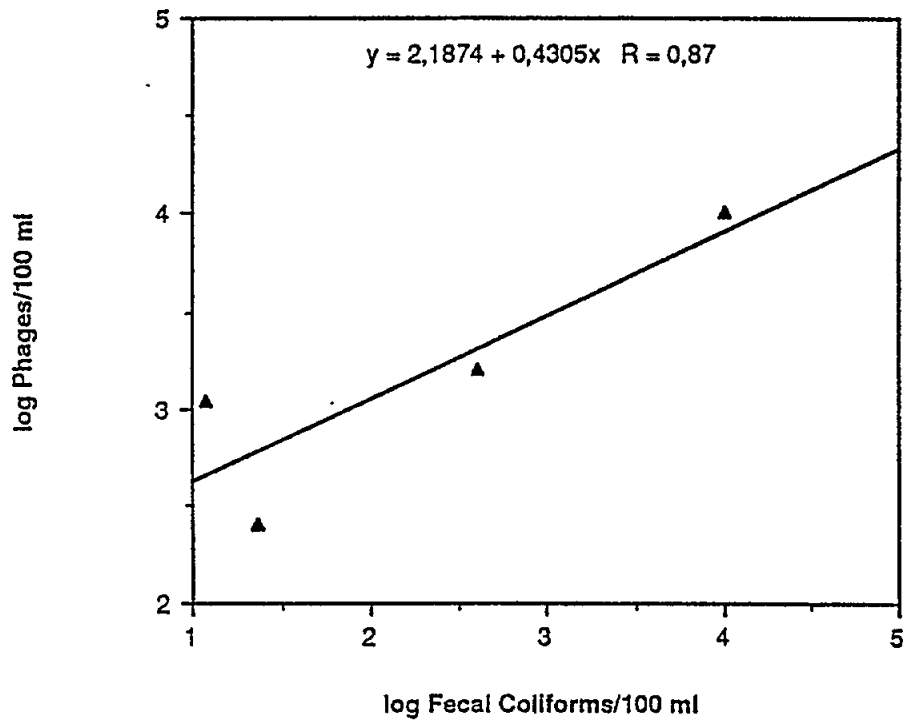


Figure 10 - Relationship between indicators in bathing waters of Misericordia beach

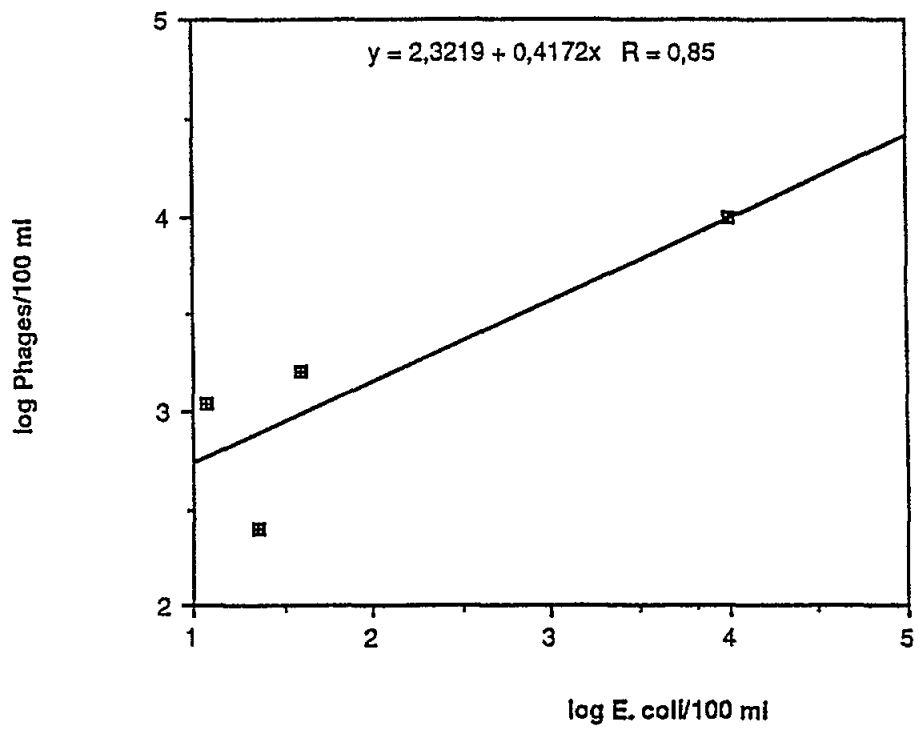
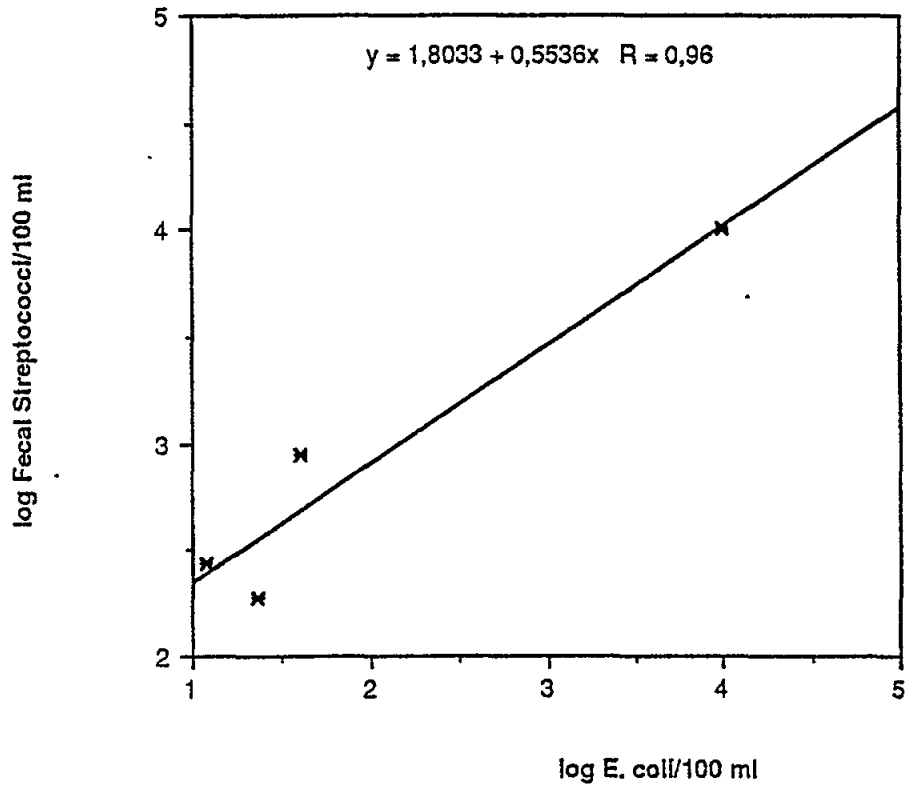


Figure 11 - Relationship between indicators in bathing waters of Misericordia beach

Table 7

Correlation between indicators and pathogens in Misericordia beach waters

INDICATOR MICROORGANISMS	PATHOGENIC MICROORGANISMS			
	<i>P.aeruginosa</i>	<i>A.hydrophila</i>	<i>Vibrio spp</i>	<i>C.albicans</i>
FAECAL COLIFORMS	r 0.878 p< 0.001	r 0.989 0.001	r 0.378 NS	r 0.933 0.001
<i>Escherichia coli</i>	r 0.736 p< 0.01	r 0.815 0.001	r 0.071 NS	r 0.909 0.001
FAECAL STREPTOCOCCI	r 0.897 p< 0.001	r 0.922 0.001	r 0.161 NS	r 0.982 0.001
COLIPHAGES	r 0.945 p< 0.001	r 0.857 0.001	r 0.001 NS	r 0.923 0.001

NS: No significant (p> 0.1)

\*: The best correlation.

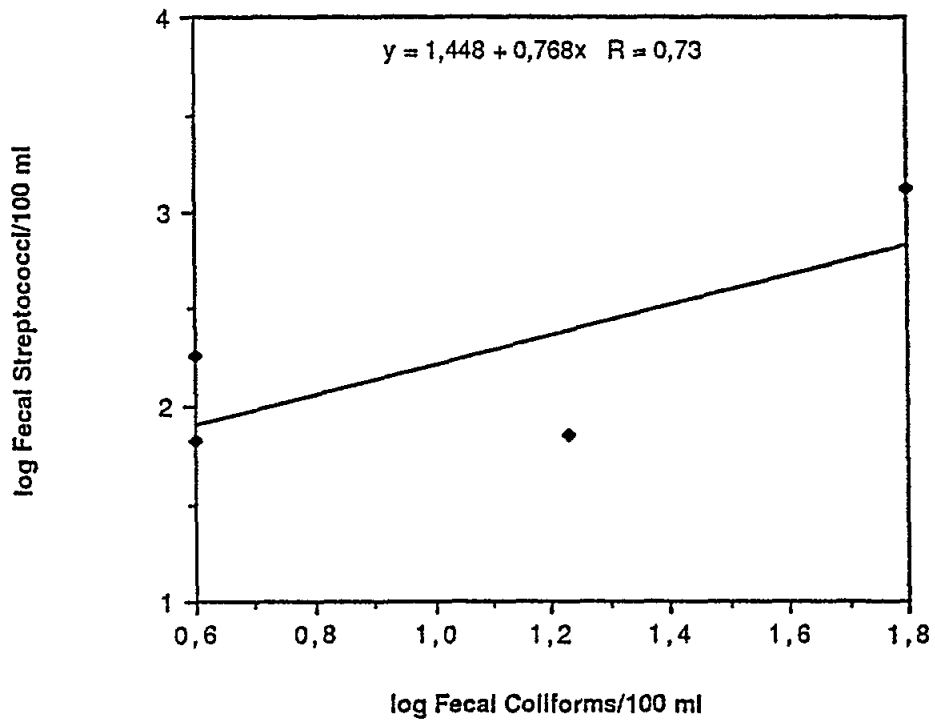
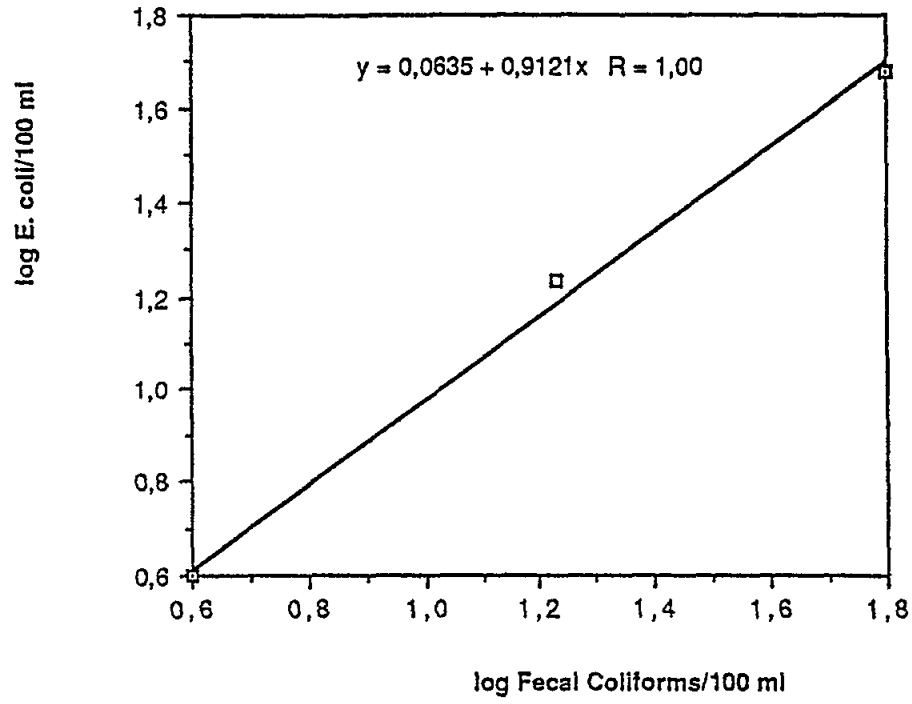


Figure 12 - Relationship between indicators in bathing waters of Santa Ana beach

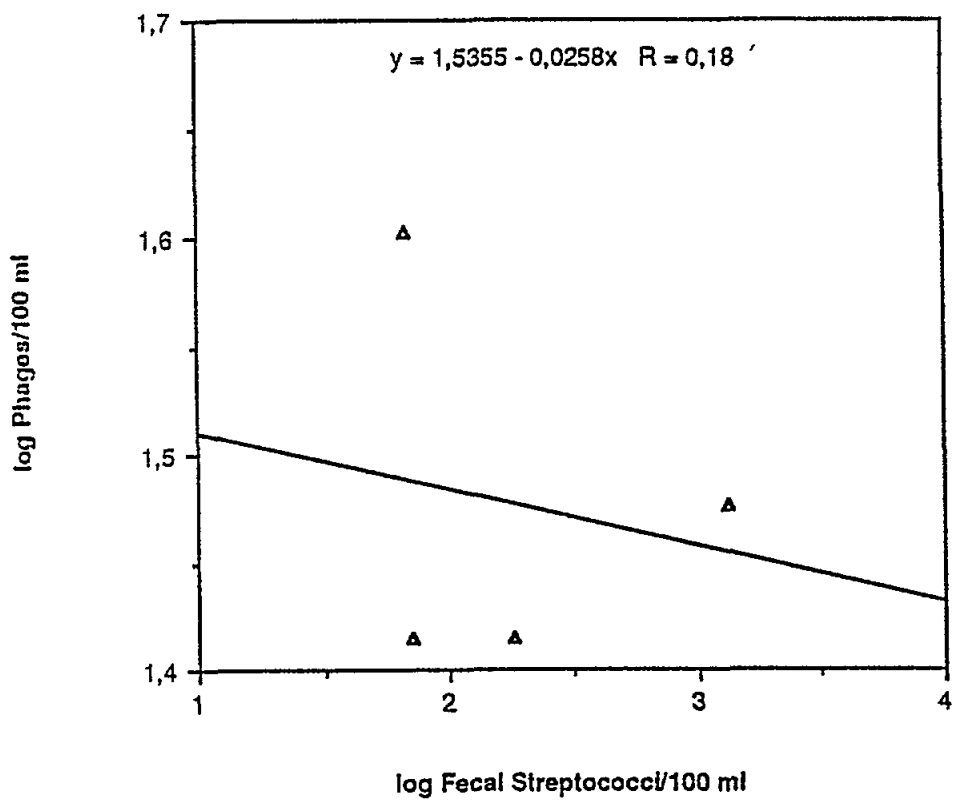
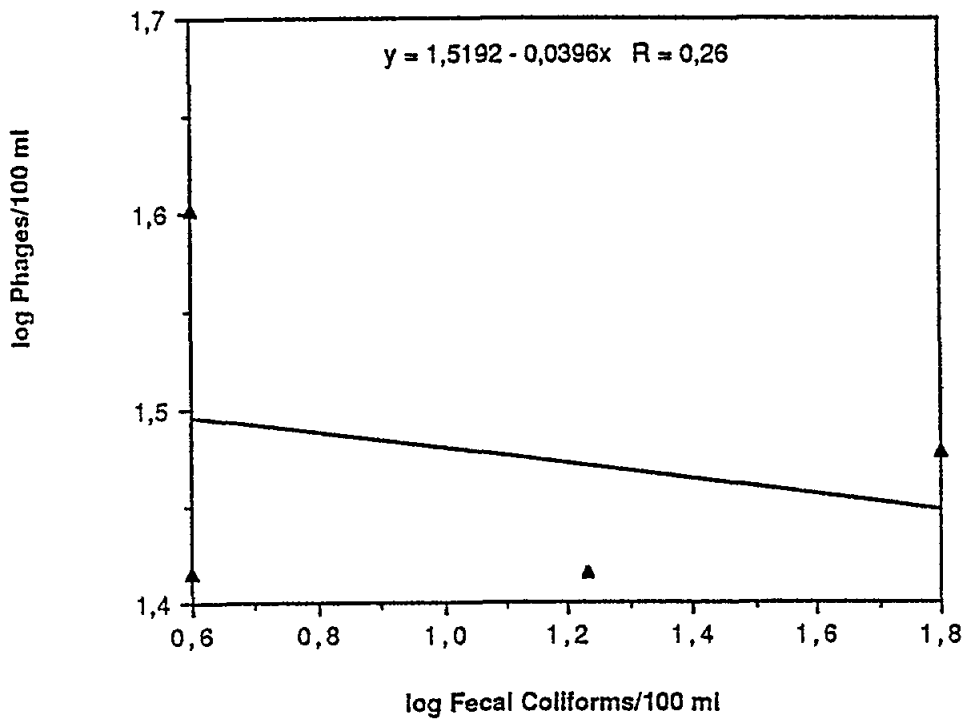


Figure 13 - Relationship between indicators in bathing waters of Santa Ana beach

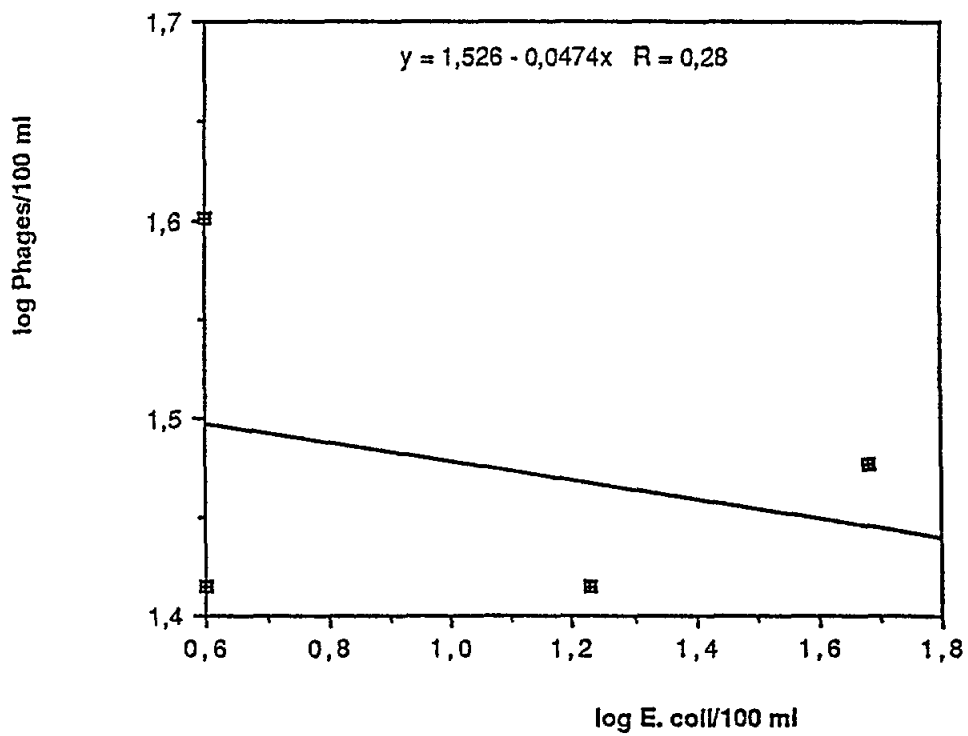
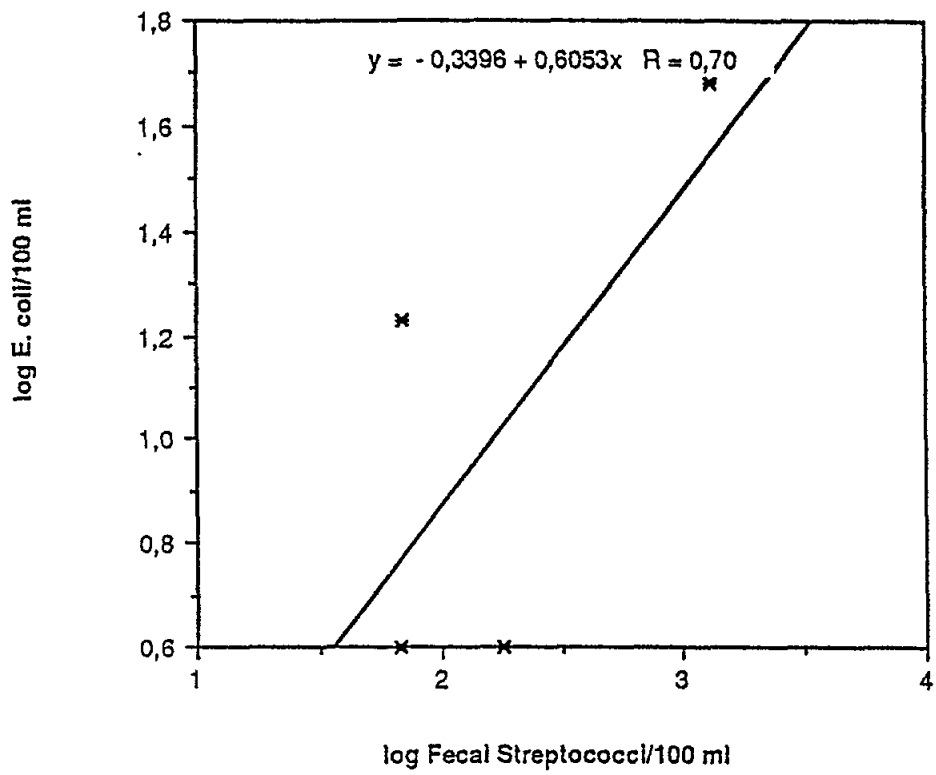


Figure 14 - Relationship between indicators in bathing waters of Santa Ana beach

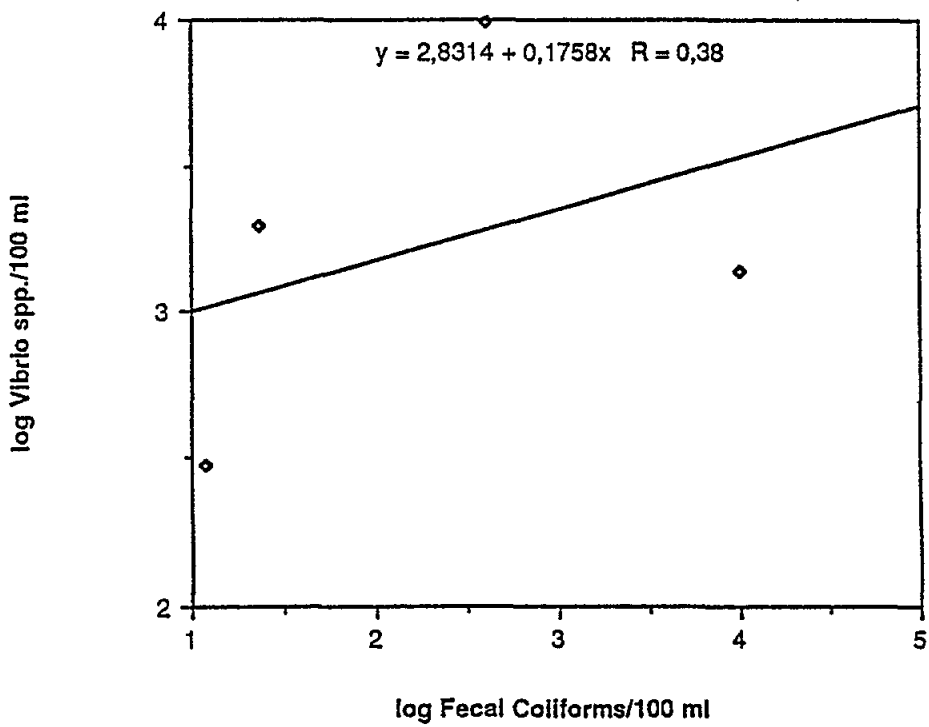
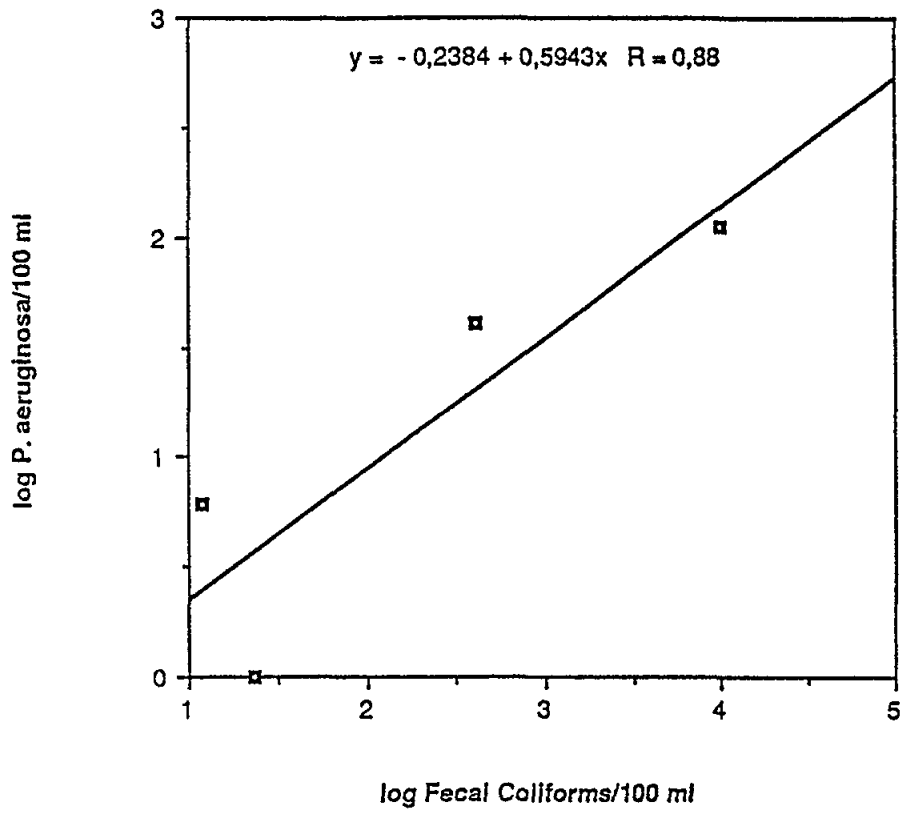


Figure 15 - Relationship between Faecal Coliforms and pathogenic microorganisms in Misericordia beach



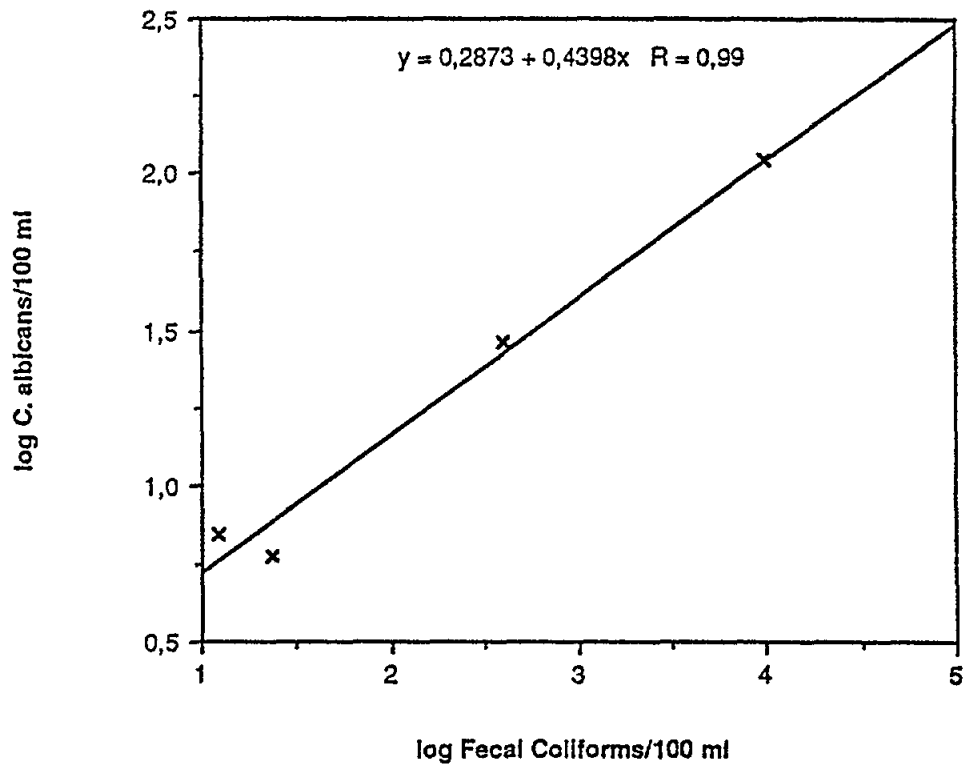
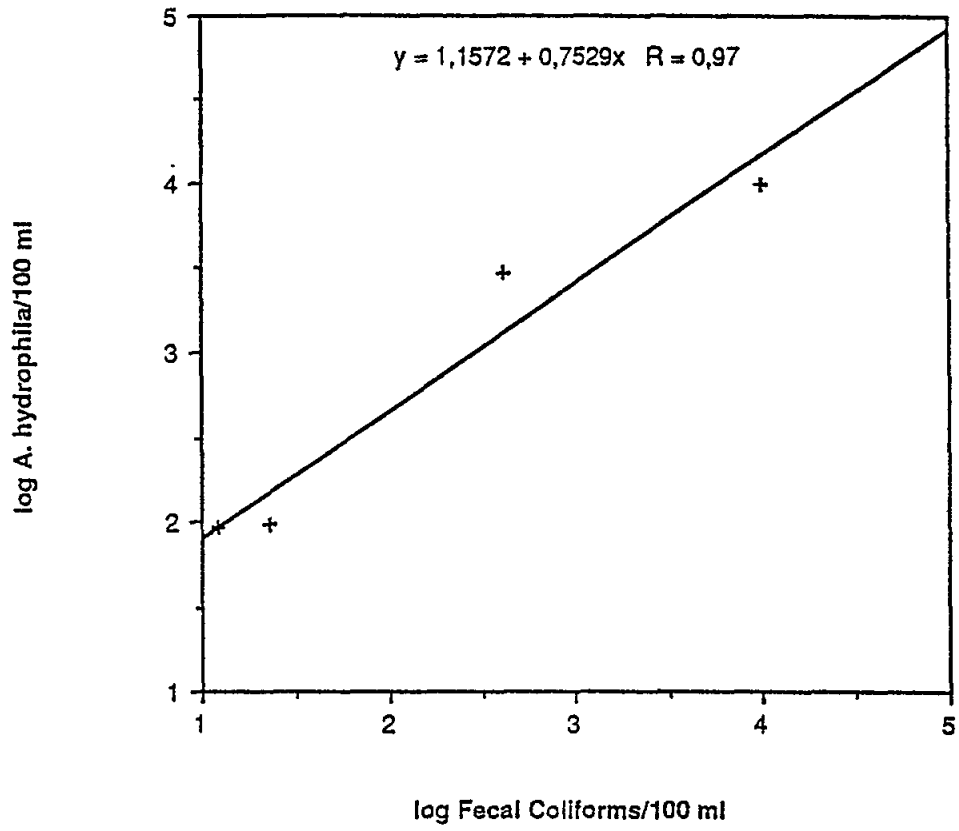


Figure 16 - Relationship between Faecal Coliforms and pathogenic microorganisms in Misericordia beach

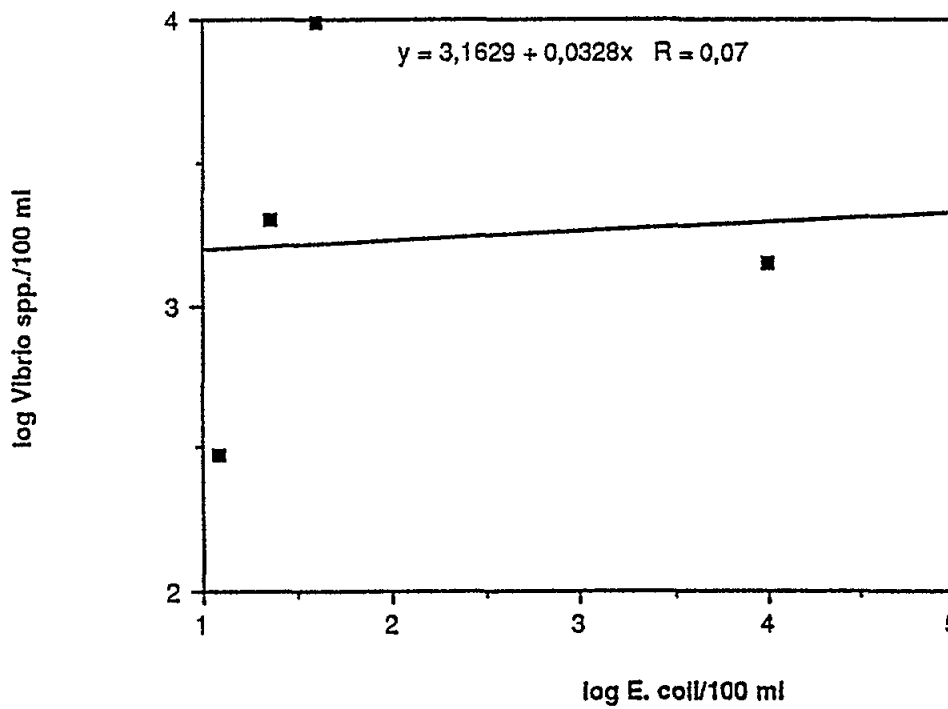
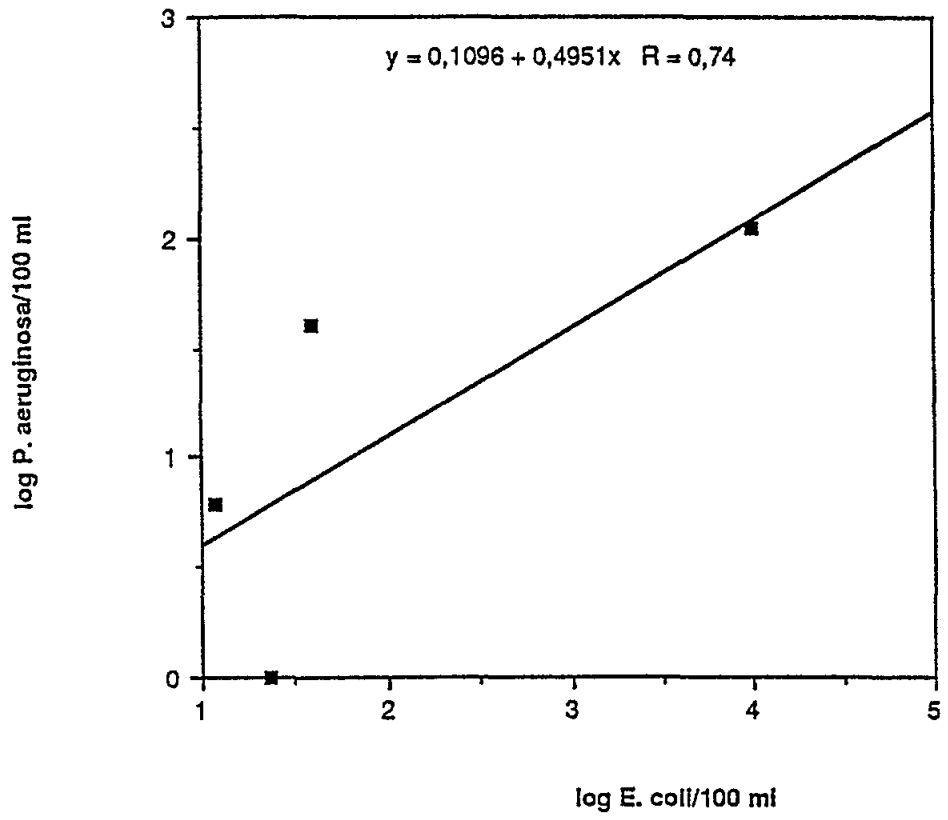


Figure 17 - Relationship between *Escherichia coli* and pathogenic microorganisms in Misericordia beach

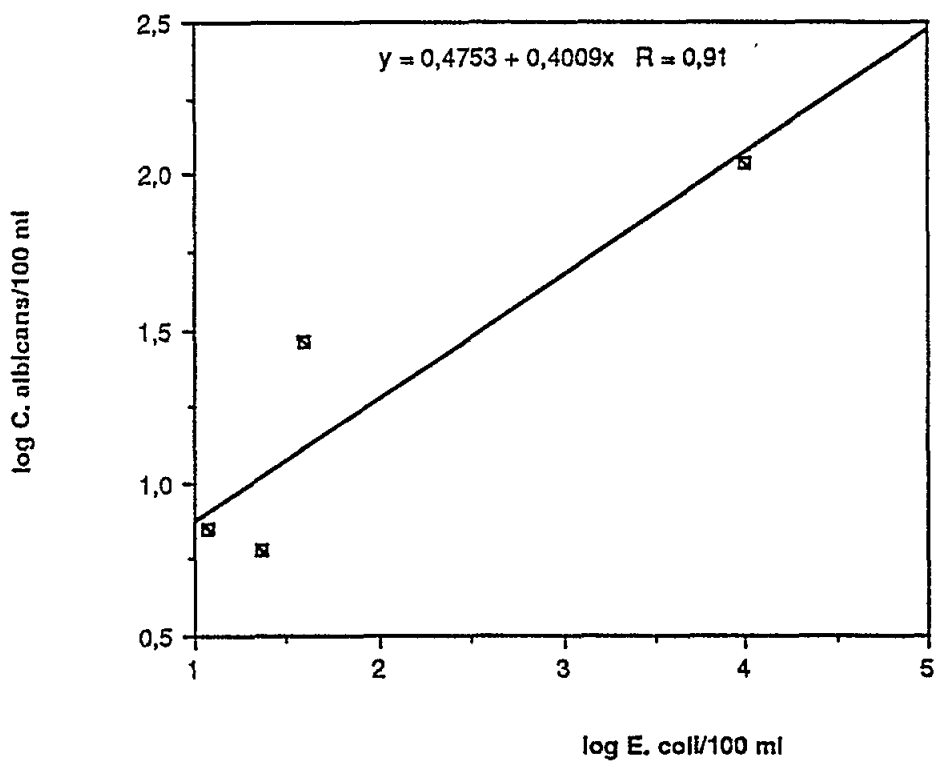
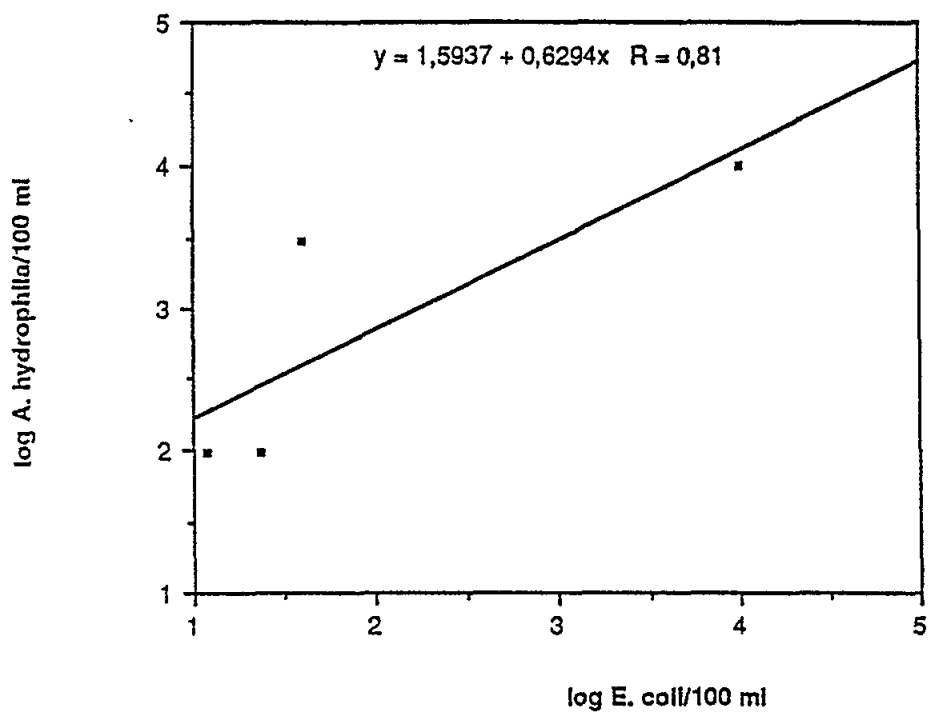


Figure 18 - Relationship between *Escherichia coli* and pathogenic microorganisms in Misericordia beach

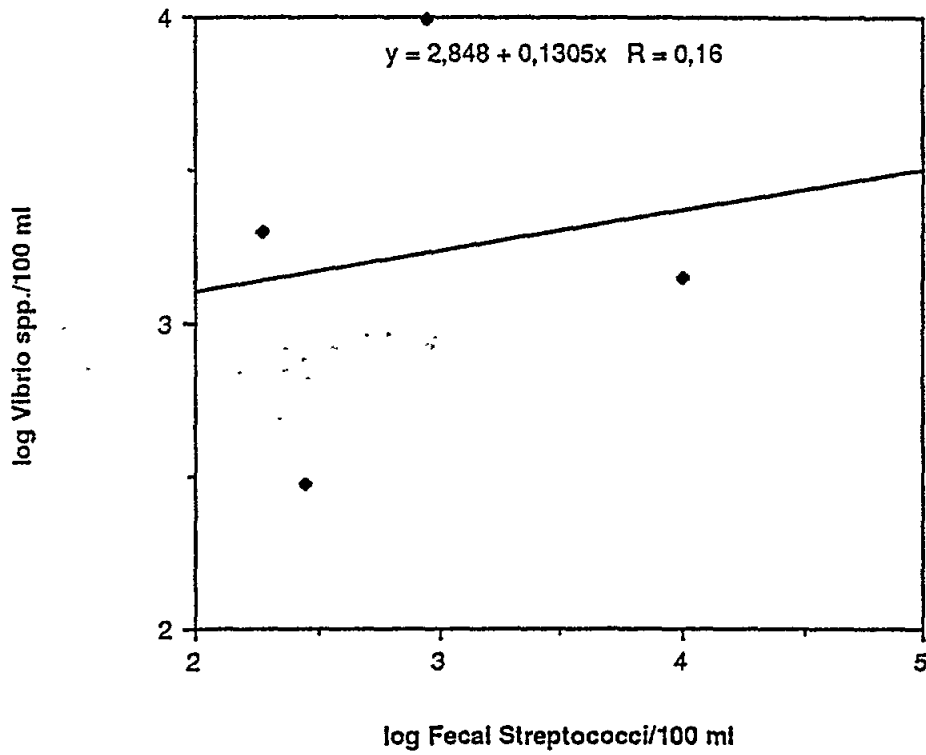
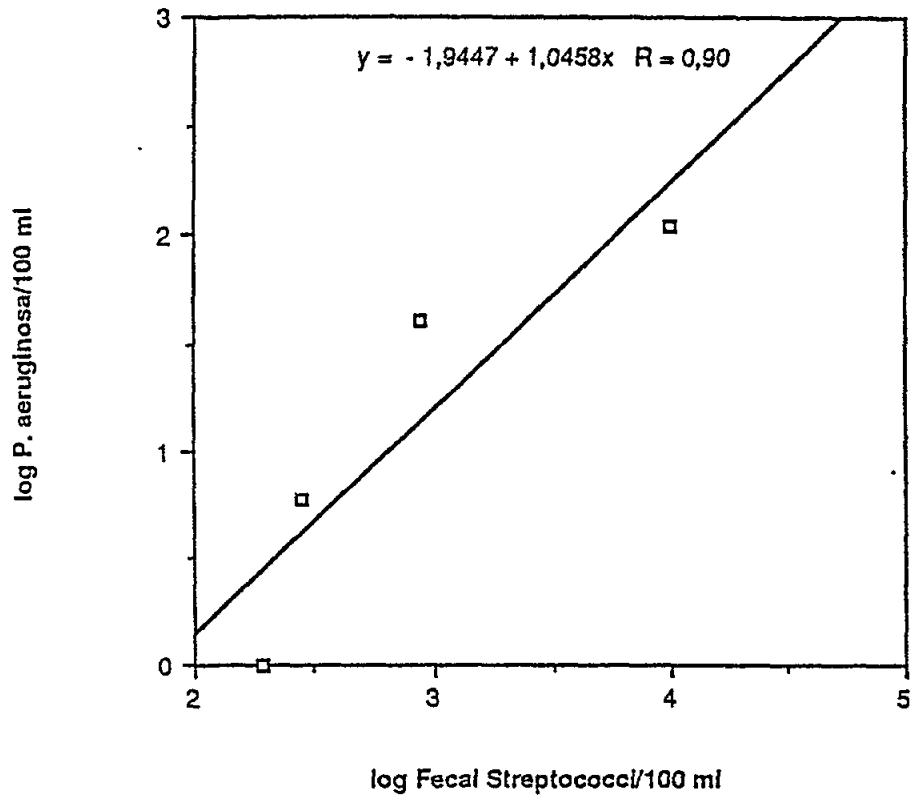


Figure 19 - Relationship between Faecal Streptococci and pathogenic microorganisms in Misericordia beach

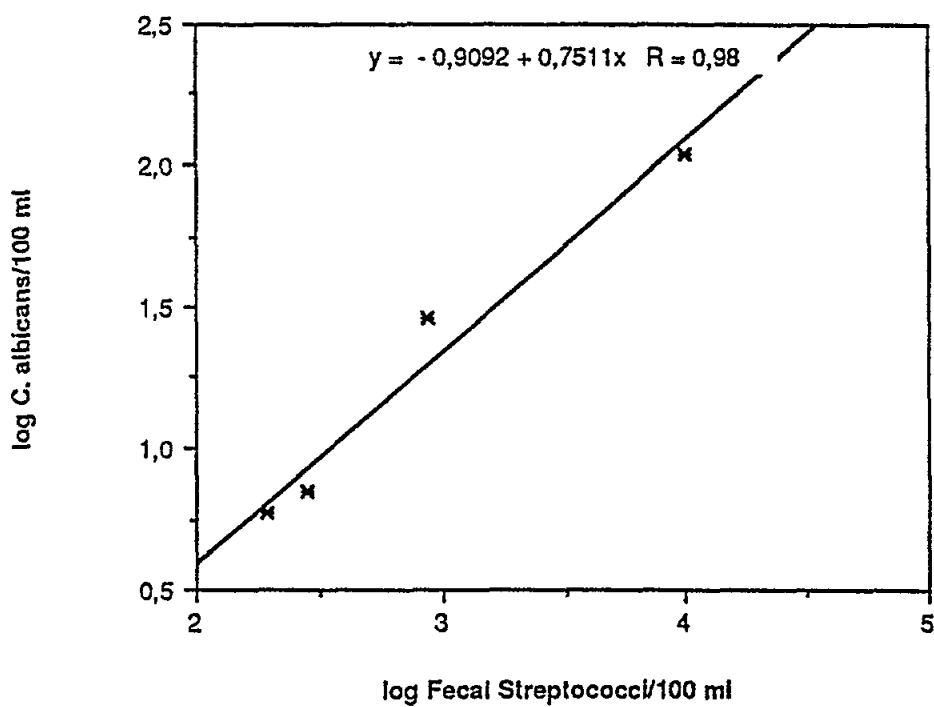
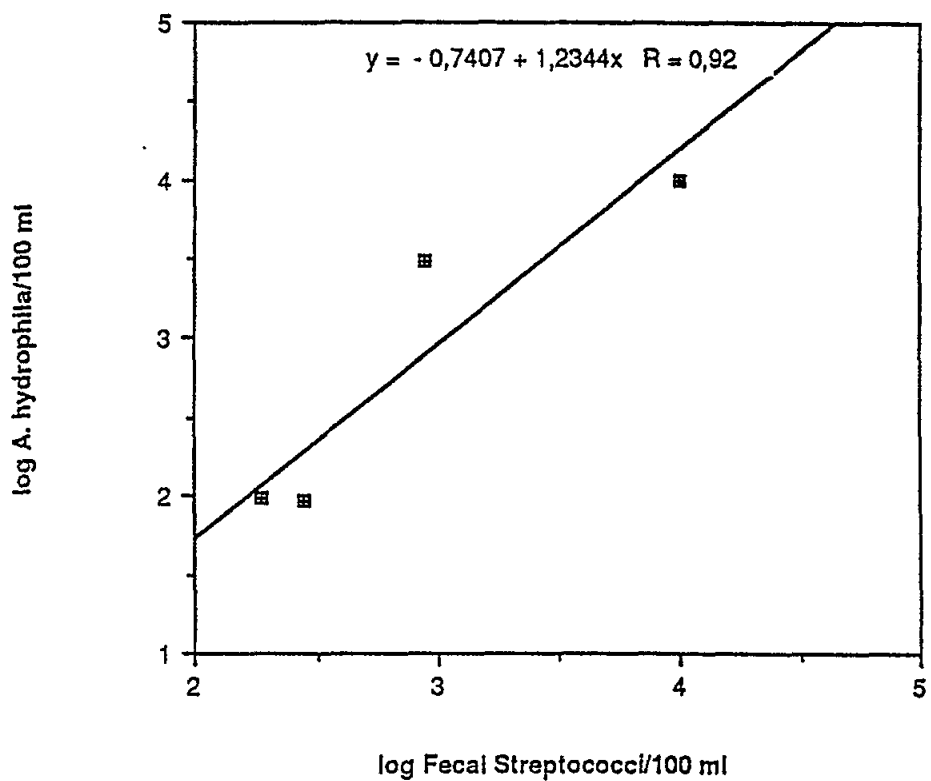


Figure 20 - Relationship between Faecal Streptococci and pathogenic microorganisms in Misericordia beach

discharged into the bathing waters by the own bathers, and they do not depend on the faecal degree of pollution. However, to confirm this assumption we must carry out a regression analysis between those microorganisms and the faecal pollution indicators.

*Vibrio* spp and *A. hydrophila* concentrations are higher than the indicator microorganism concentrations on Misericordia beach. This fact shows that these pathogens are not exclusively of faecal origin. However, on Santa Ana beach the *A. hydrophila* concentration was similar to those obtained for *C. albicans*, *P. aeruginosa*, and some indicator microorganisms, whereas *Vibrio* spp presented a higher number.

Finally, the analysis of the sand in both beaches revealed a similar number of pathogenic fungi and yeast in both zones, which implies that these microorganisms can be discharged by the bathers or beach towels.

#### **4.5 Regression analyses between the microbial indicators**

The regression analysis between the indicators studied in both beaches is shown in Table 6, and Figures 9-14. In all the cases, except for coliphages on Santa Ana beach, the correlations between microbial indicators were high and significant at levels of  $p < 0.001$  and  $p < 0.01$ .

The best correlations were found between faecal coliforms and faecal streptococci for Misericordia beach ( $r=0.973$ ) and faecal coliforms and *Escherichia coli* for Santa Ana beach ( $r=0.998$ ). On the contrary, the lowest significant correlation was obtained between *E. coli* and coliphages for Misericordia beach ( $r=0.850$ ,  $p < 0.001$ ), and *E. coli* and faecal streptococci for Santa Ana beach ( $r=0.695$ ,  $p < 0.01$ ).

To establish the source and type of pollution that affect those beaches, the ratios between faecal coliforms and faecal streptococci or coliphages and *E. coli* vs faecal streptococci or coliphages were estimated. The results obtained (data not shown) indicate that the FC/FS ratio range between 0.039 and 1 for Misericordia beach, and between 0.075 and 2.1 for Santa Ana beach. From these results, we can conclude that the origin of faecal pollution was remote and at a long distance from the pollution source (Borrego et al., 1982a, b).

#### **4.6 Correlation between indicator and pathogenic microorganisms in the bathing waters**

Tables 7 and 8 summarize the correlation established between the indicators and the pathogens studied, except for *Salmonella* spp and *S. aureus* (see item 4 for explanation).

As it can be seen in Table 7 on Misericordia beach the indicator microorganisms yield a good correlation with the pathogenic microorganisms studied, except for *Vibrio* spp. The best correlation for *P. aeruginosa* was established with coliphages ( $r=0.945$ ,  $p < 0.001$ ) and the lowest with *E. coli* ( $r=0.736$ ,  $p < 0.01$ ). For *A. hydrophila*, faecal coliforms are the best correlated indicator ( $r=0.968$ ,  $p < 0.001$ ) and *E. coli* the worst ( $r=0.815$ ,  $p < 0.001$ ). Finally, *E. coli* shows the best correlation level ( $r=0.991$ ,  $p < 0.001$ ) with *C. albicans*, and *E. coli* the lowest ( $r=0.909$ ,  $p < 0.001$ ).

Figures 15-22 show the regression lines obtained by adjustment by the minimum square method between indicator and pathogenic microorganisms. As it can be seen, the slopes of the regression lines are near 1 in the following relationships: Faecal coliforms/*A. hydrophila*; Faecal streptococci/*P. aeruginosa*; Faecal streptococci/*A. hydrophila*; Faecal streptococci/*C. albicans*; Coliphages/*P. aeruginosa*; Coliphages/*A. hydrophila* and coliphages/*C. albicans*, which together with the high and significant correlation coefficient values demonstrate that these are the best indicators for those pathogens.

Table 8 and Figures 23-30 give correlation coefficient figures and curves obtained by the regression analyses between indicator and pathogenic microorganisms on Santa Ana beach. The correlation levels and the number of indicators correlated with pathogens are lower than those obtained for Misericordia beach. Thus, FC and *E. coli* are only correlated with *A. hydrophila* and *C. albicans*, whereas FS is correlated with *Vibrio* spp and *C. albicans*, and coliphages with *A. hydrophila*. The best correlation for *A. hydrophila* is obtained with FC ( $r=0.657$ ,  $p<0.02$ ), for *Vibrio* spp with FS ( $r=0.495$ ,  $p<0.1$ ), and for *C. albicans* again with FC ( $r=0.719$ ,  $p<0.01$ ). *P. aeruginosa* is not significantly correlated with any of the indicator microorganisms.

#### **4.7 Correlation between indicator and pathogenic microorganisms in the beach sand**

Due to the high incidence of skin infections among bathers, in the present study we carried out analyses of the main pathogenic microorganisms involved in these kinds of infections from the sand of the beaches selected.

Table 9 and Figures 31 to 38 show the regression analyses between the indicator levels in the bathing waters and the pathogens in the sand. As it can be seen in Table 9 all the indicators have a high significant regression coefficient with the dermatophyte fungi on Misericordia beach, especially coliphages ( $r=0.896$ ,  $p<0.001$ ). Although, *C. albicans* only *E. coli* showed a significant relationship ( $r=0.597$ ,  $p<0.05$ ). In the case of Santa Ana beach, FS showed the best correlation with dermatophyte fungi ( $r=0.948$ ,  $p<0.001$ ) and coliphages were the indicator best correlated with *C. albicans* ( $r=0.777$ ,  $p<0.01$ ).

From these results, we can conclude that the level of pollution and the concentrations of the indicators in the bathing waters reflect in a reliable way the presence and levels of pathogenic microorganisms in the sand.

#### **4.8. Epidemiological results**

##### **4.8.1 Demography**

The participants observed during the period studied (sample from bathing season Jul-Sep. 1988) included a total of 435 family interviews; of these families, a total of 803 individuals participated in the sample of 20th July and 3rd August ("week-end questionnaire") and a total of 644 individuals participated in the sample of 17th August and 5th September ("epidemiological questionnaire"). Table 10 shows the distribution and the percentage of the participants in the four samples, with a total of 1447 individuals ( $n=1447$ ).

The distribution of samples by beaches (Benalmadena and Misericordia), sex and age studied (Tables 11 to 14) was very similar between both bathing beaches and it may be considered as a normal distribution for the age groups with two well differentiated curves for the 0-10 year-age group and the over 10 year-age group, as a logical consequence of the sampling design selected (families with young children).

Tables 15 to 18 show the distribution of individuals by origin and beaches, respectively. On Benalmadena beach most bathers were tourists (71%), mainly from other regions of Spain, and on Misericordia beach local bathers predominated (63%).

##### **4.8.2 Swimming and sand conditions**

Table 19 shows the distribution of the second questionnaire study sample, by permanence ("hours on the beach") and time ("days on this beach") on the beaches of Benalmadena and Misericordia. Eighty-six percent of the individuals who stayed on the beach between 1 and 5 hours and 48.6% stayed more than 3 days on the same beach.

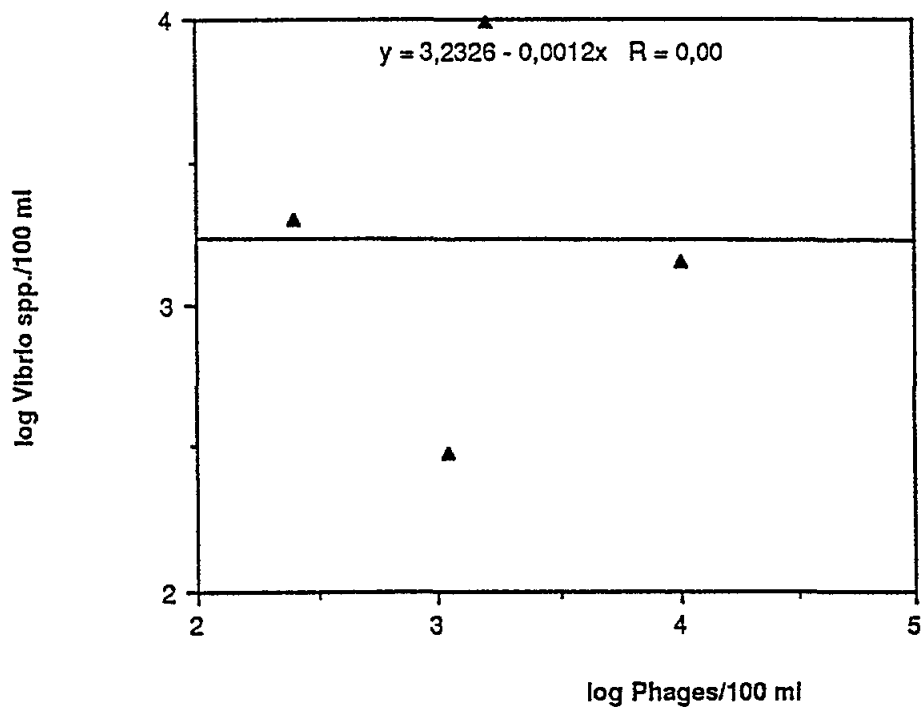
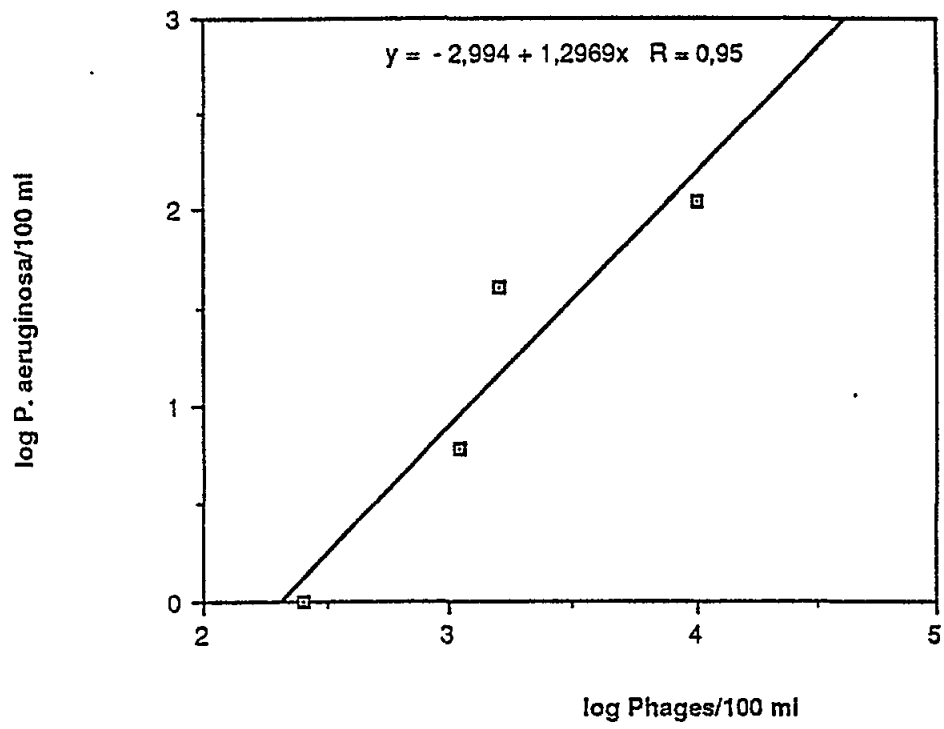


Figure 21 - Relationship between coliphages and pathogenic microorganisms in Misericordia beach



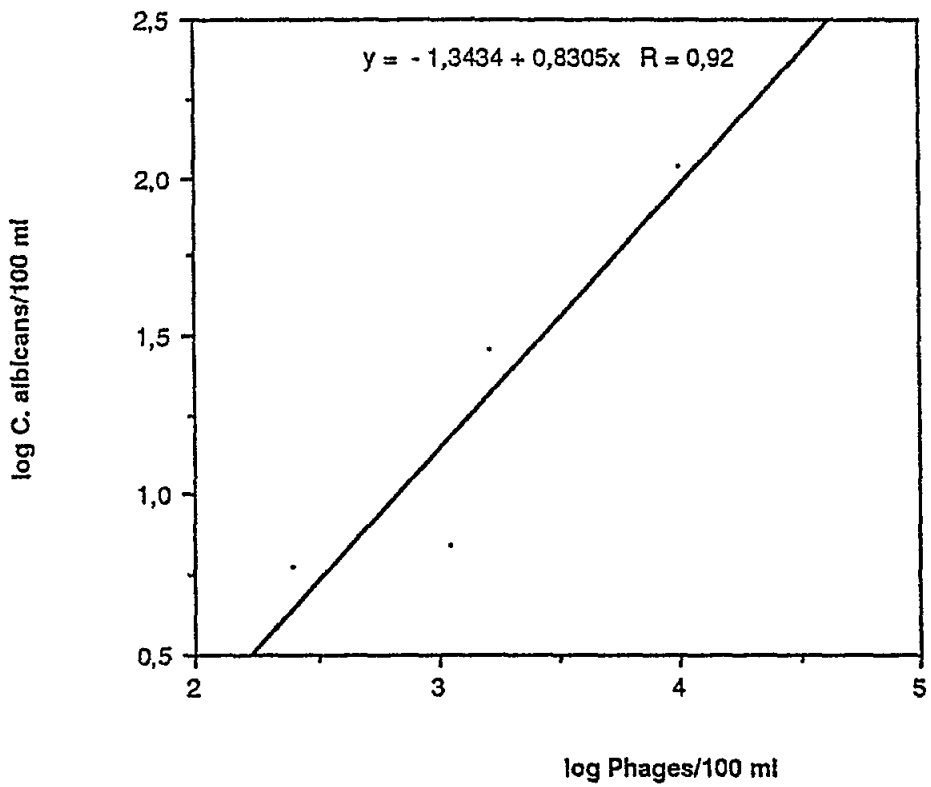
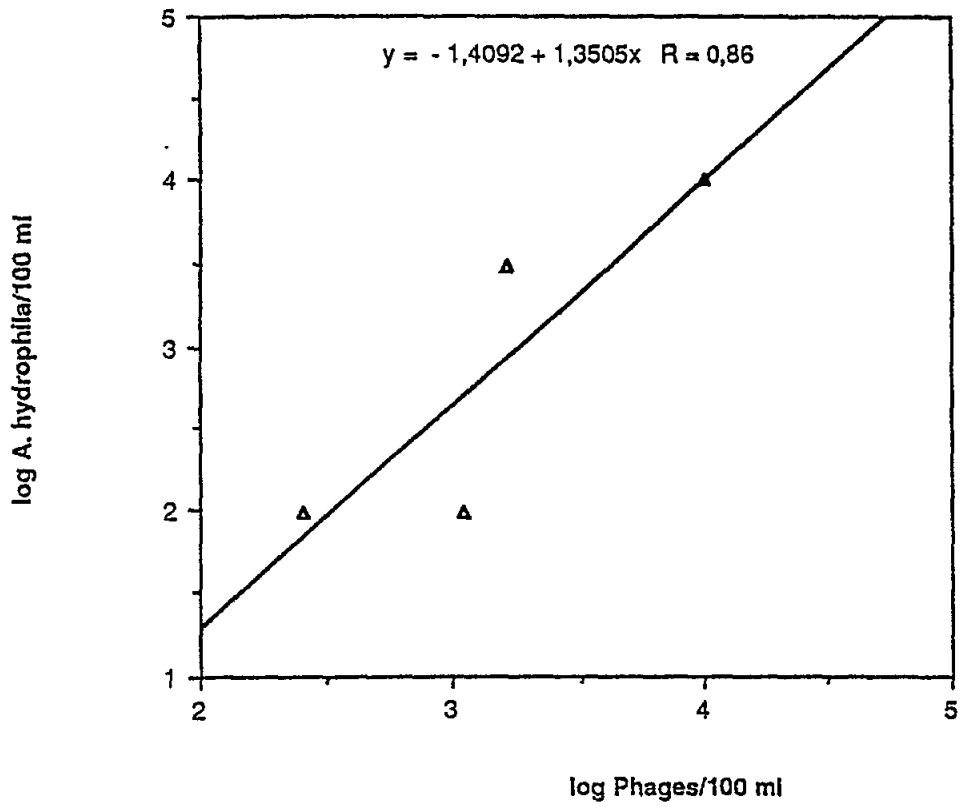


Figure 22 - Relationship between Coliphages and pathogenic microorganisms in Misericordia beach

Table 8

Correlation between indicators and pathogens in Santa Ana beach waters

INDICATOR MICROORGANISMS	PATHOGENIC MICROORGANISMS				
	<i>P.aeruginosa</i>	<i>A.hydrophila</i>	<i>Vibrio spp</i>	<i>C.albicans</i>	
FAECAL COLIFORMS	r	*		*	
	0.291	0.657	0.145	0.719	0.01
	NS	0.02	NS	0.01	0.01
<i>Escherichia coli</i>	r				
	0.346	0.699	0.179	0.692	0.01
	NS	0.01	NS	0.01	0.01
FAECAL STREPTOCOCCI	r			*	
	0.416	0.016	0.495	0.655	0.02
	NS	NS	0.1	0.02	0.02
COLIPHAGES	r				
	0.329	0.493	0.382	0.463	NS
	NS	0.1	NS	NS	NS

NS: No significant (p > 0.1)

\*: The best correlation.

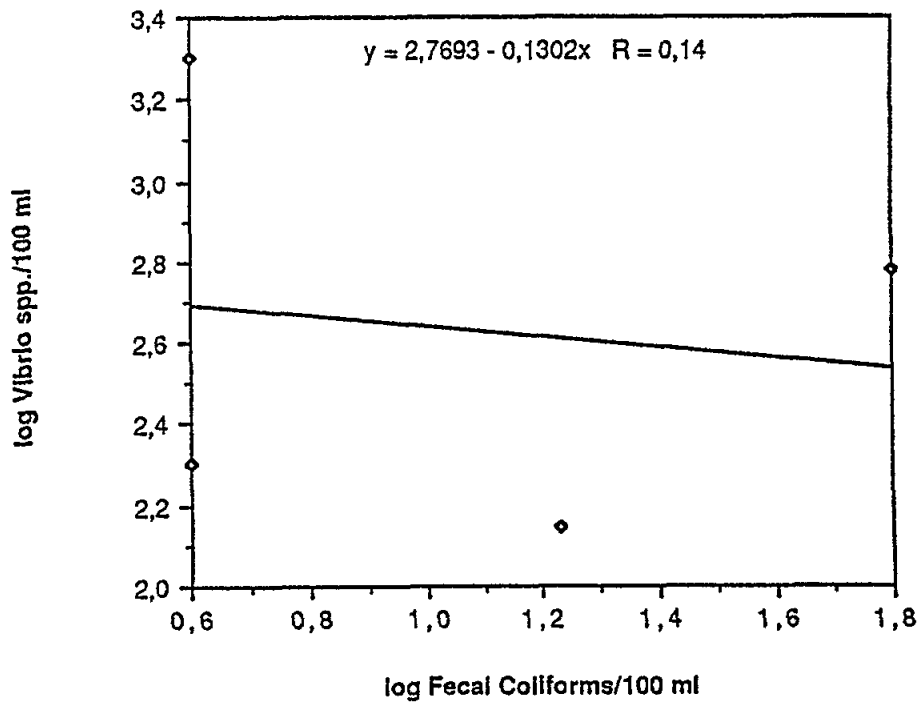
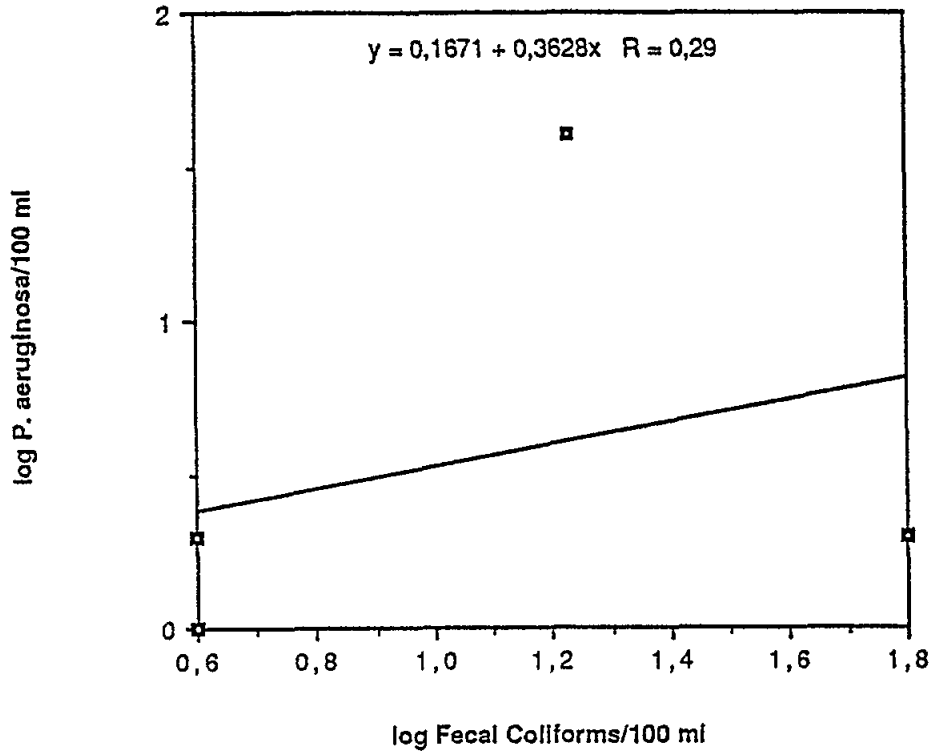


Figure 23 - Relationship between Faecal Coliforms and pathogenic microorganisms in Santa Ana beach

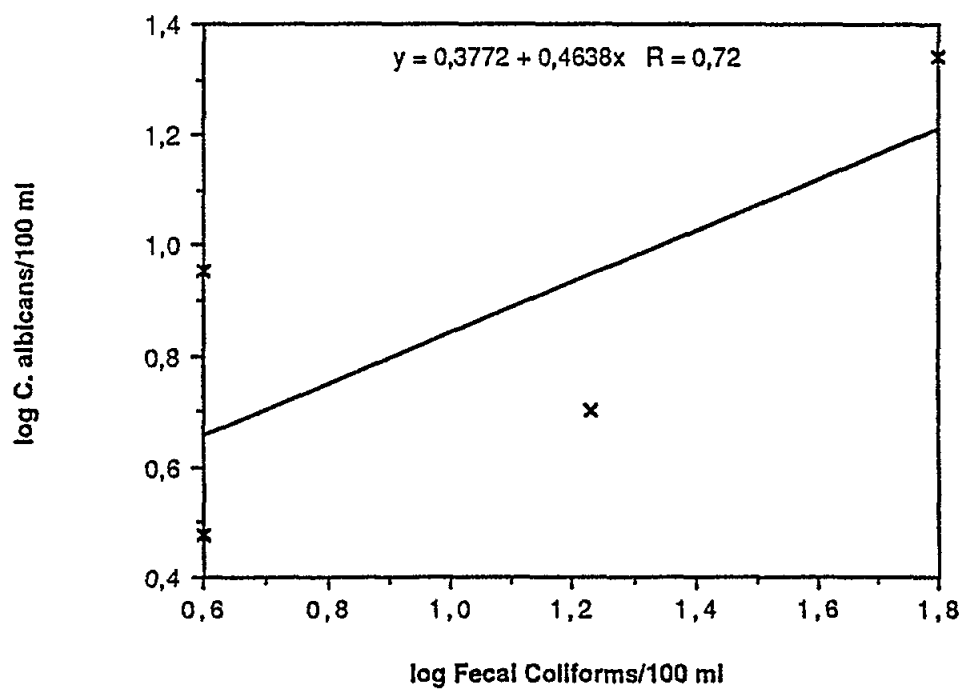
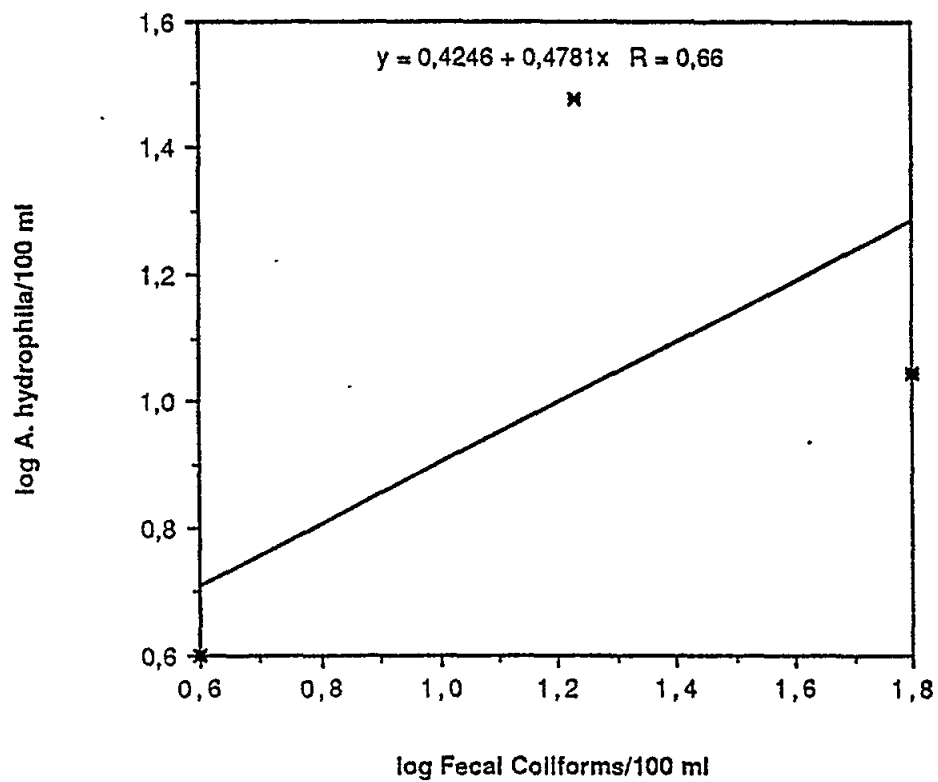


Figure 24 - Relationship between Faecal Coliforms and pathogenic microorganisms in Santa Ana beach

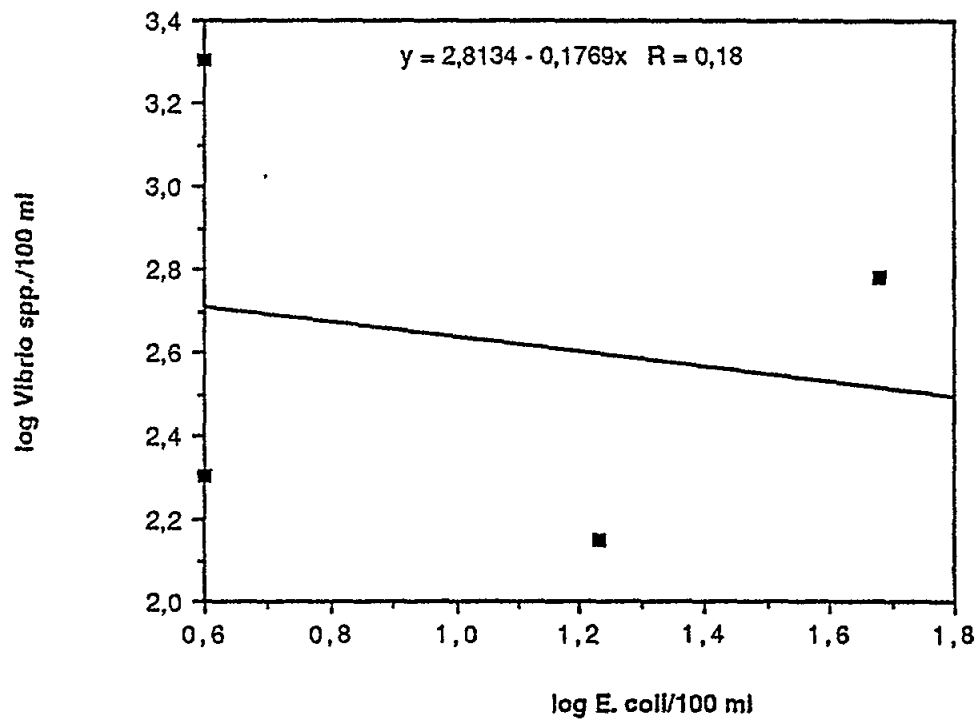
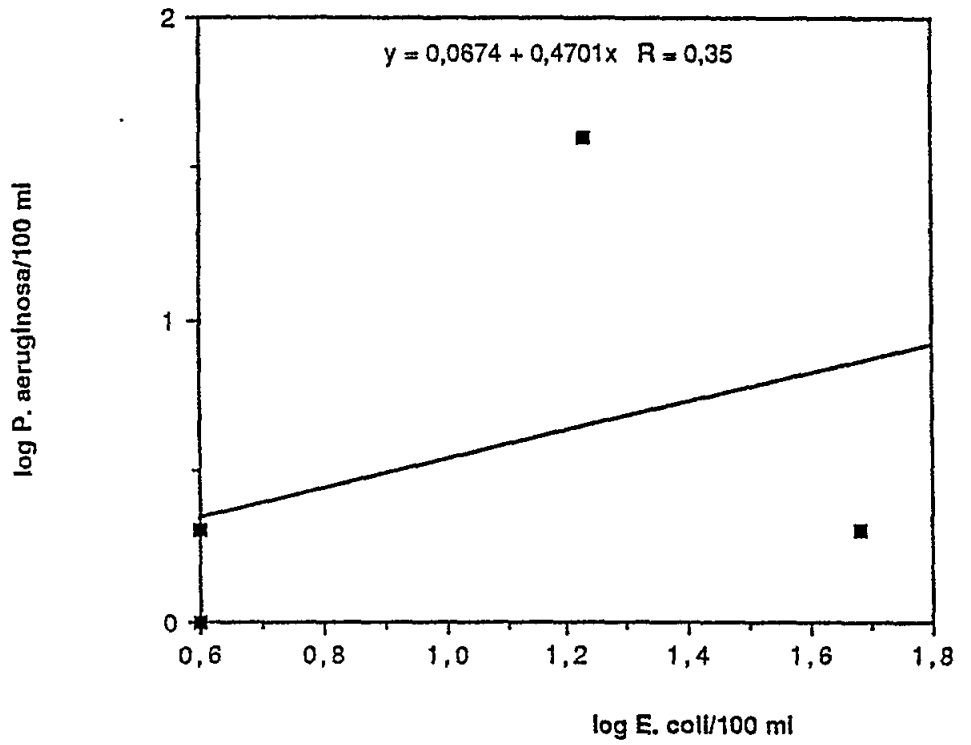


Figure 25 - Relationship between *Escherichia coli* and pathogenic microorganisms in Santa Ana beach

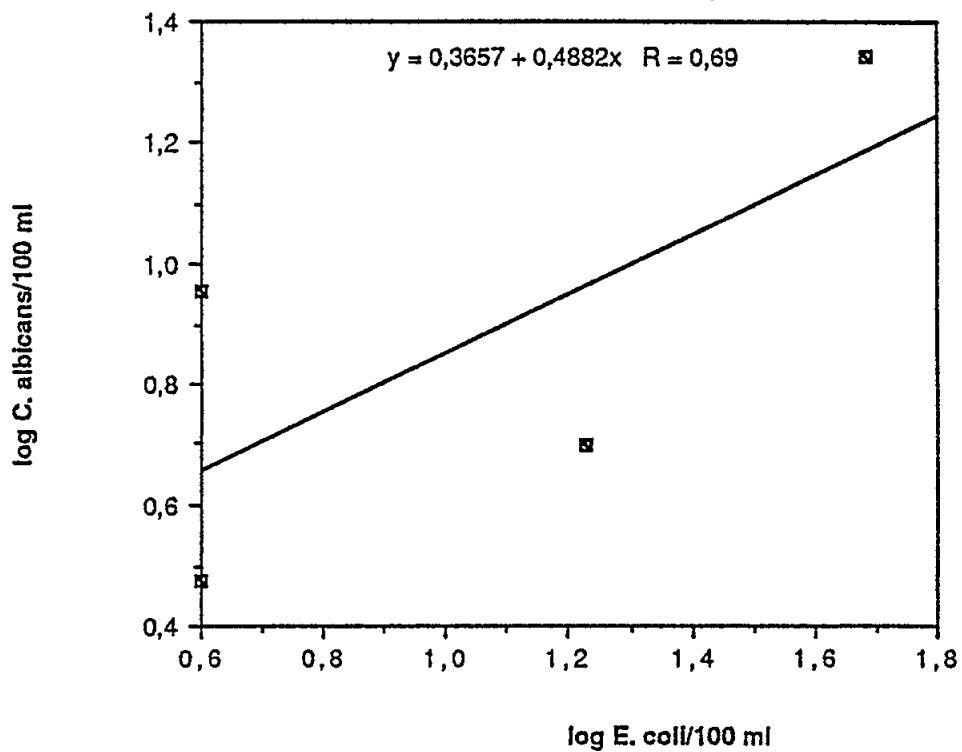
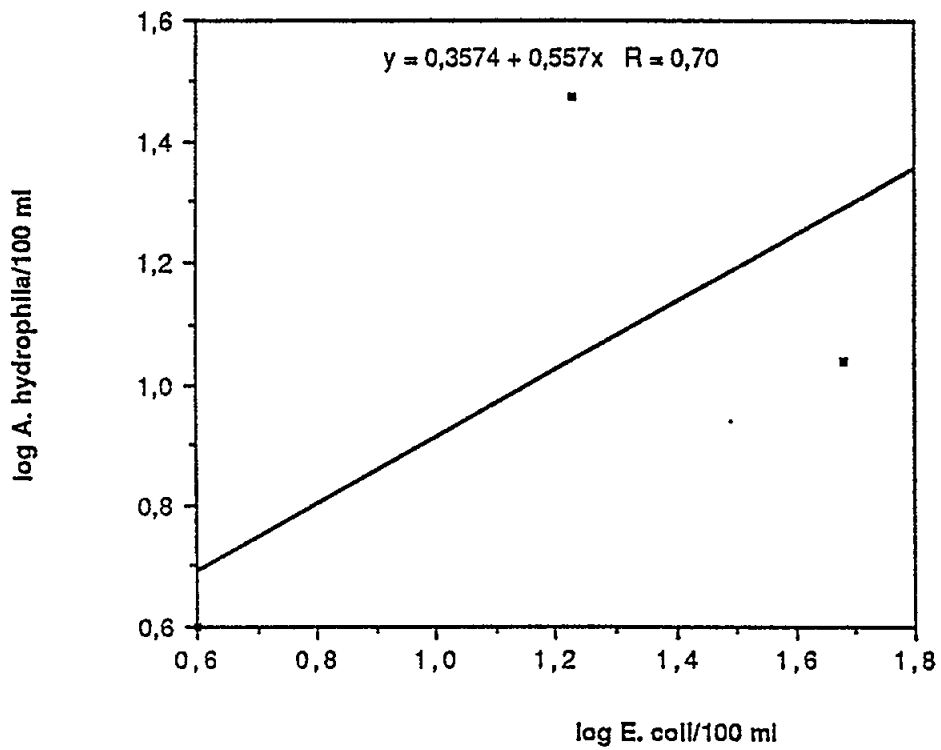


Figure 26 - Relationship between *Escherichia coli* and pathogenic microorganisms in Santa Ana beach

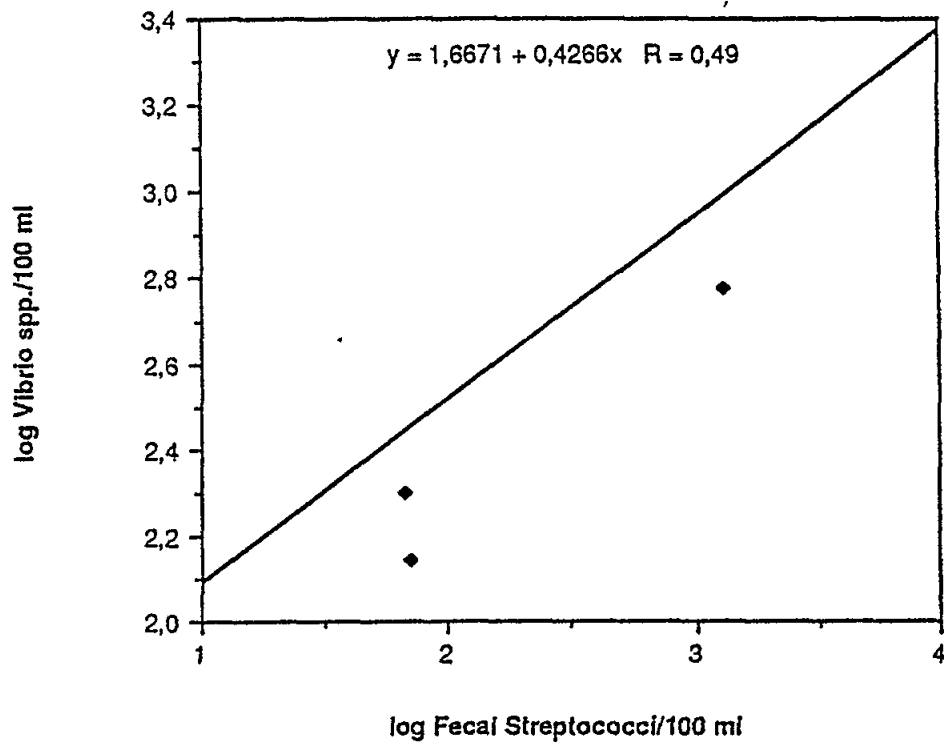
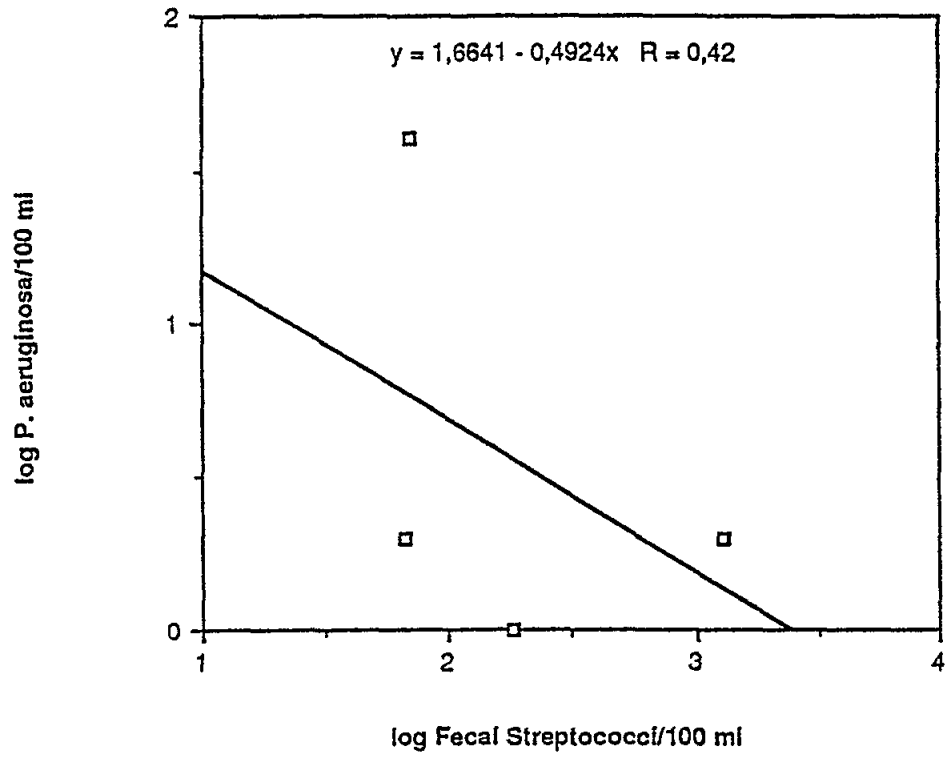


Figure 27 - Relationship between Faecal Streptococci and pathogenic microorganisms in Santa Ana beach

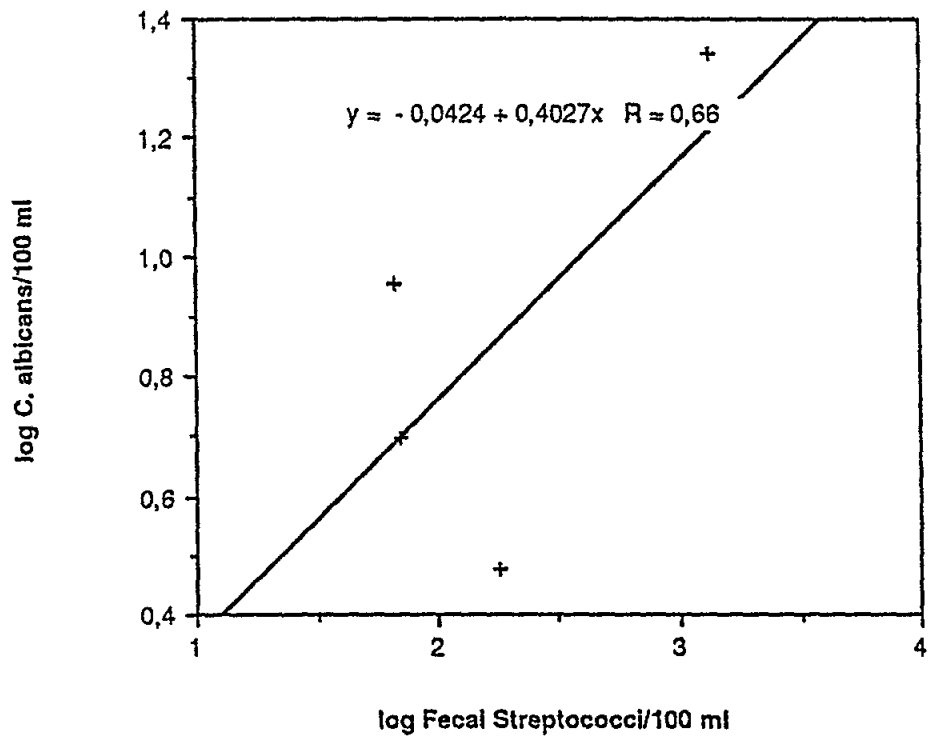
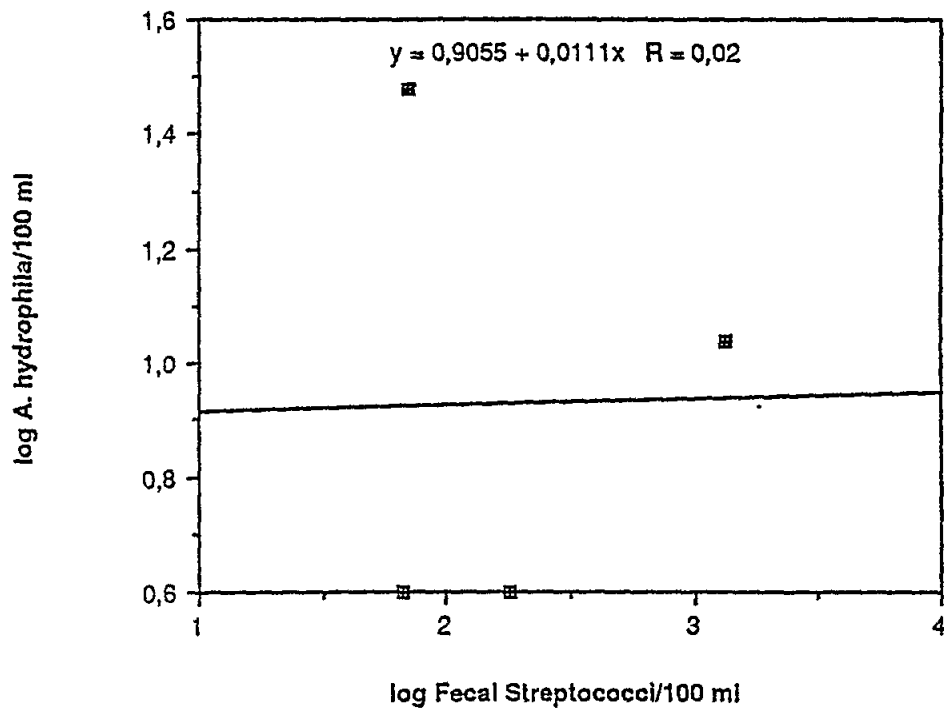


Figure 28 - Relationship between Faecal Streptococci and pathogenic microorganisms in Santa Ana beach



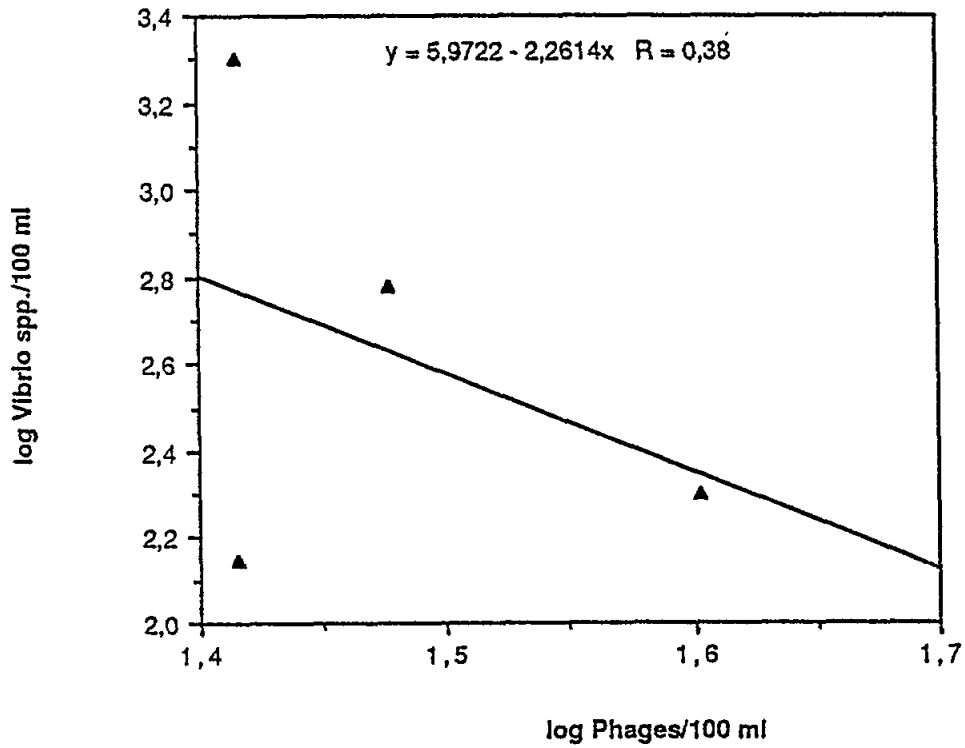
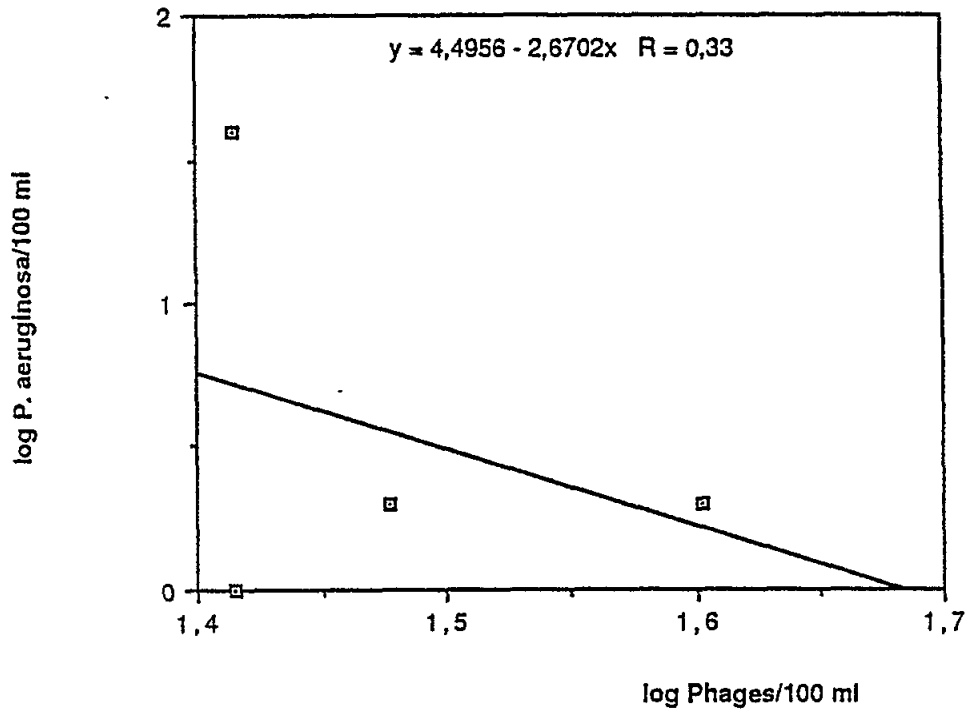


Figure 29 - Relationship between Coliphages and pathogenic microorganisms in Santa Ana beach

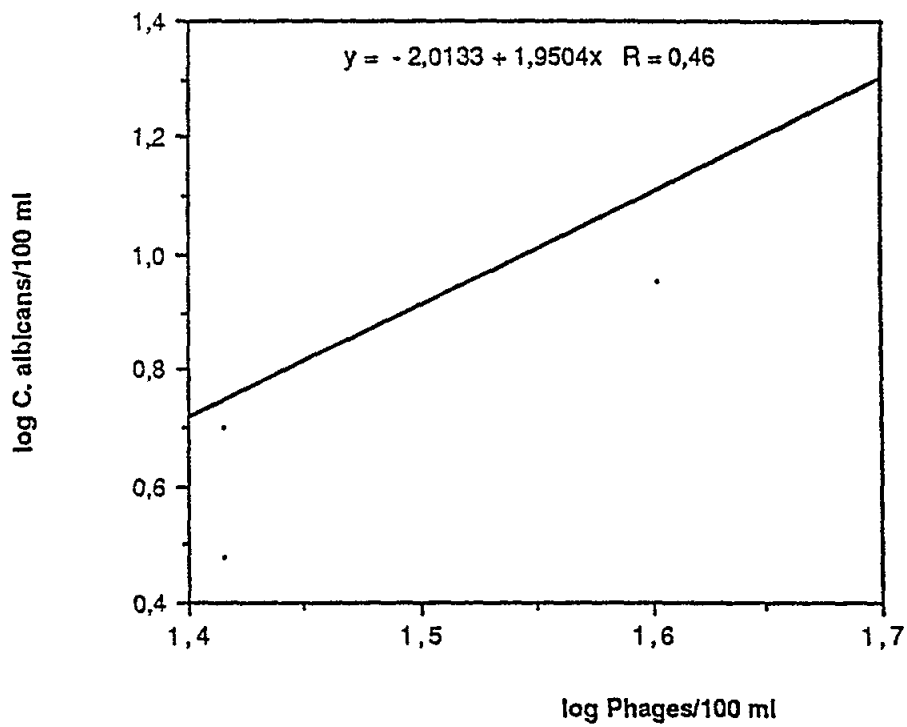
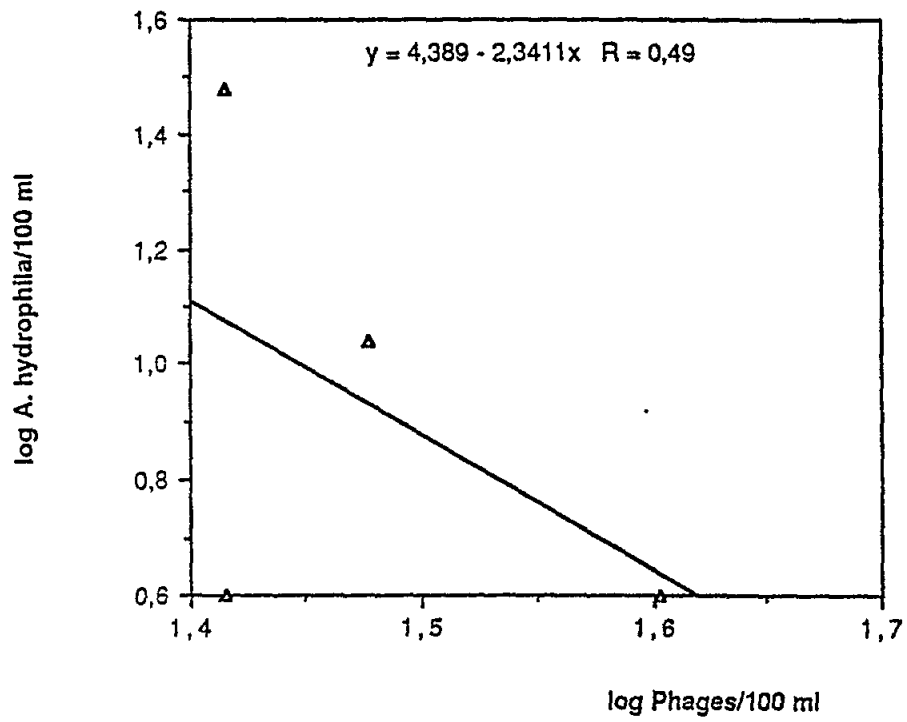


Figure 30 - Relationship between Coliphages and pathogenic microorganisms in Santa Ana beach

Table 2  
Correlations between pathogenic microorganisms and indicators in sand of  
the two studied beaches

INDICATOR MICROORGANISMS	MISERICORDIA BEACH		SANTA ANA BEACH	
	Dermatophyte Fungi	<i>C. albicans</i>	Dermatophyte Fungi	<i>C. albicans</i>
FAECAL COLIFORMS	r p<	0.574 0.466 NS	0.479 0.1	0.082 NS
<i>Escherichia coli</i>	r p<	0.478 0.597 *	0.431 NS	0.031 NS
FAECAL STREPTOCOCCI	r p<	0.678 0.02	0.948 0.001	0.479 0.1
COLIPHAGES	r p<	0.869 0.001	0.025 NS	0.777 0.01

NS: No significant (p> 0.1)

\*: The best correlation.

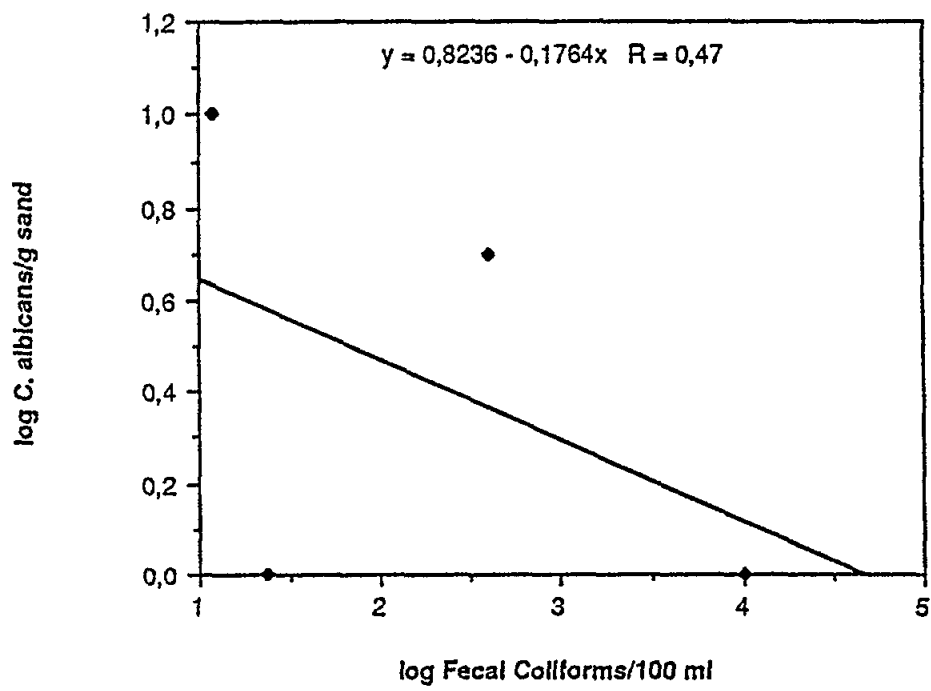
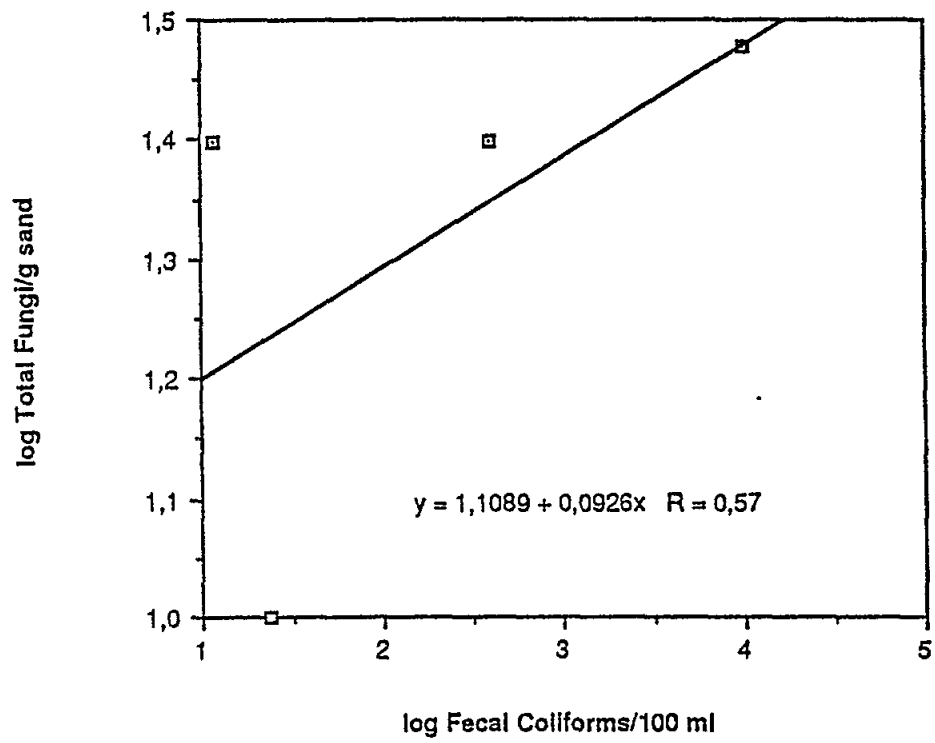


Figure 31 - Relationship between indicators and pathogenic microorganisms in sand of Misericordia beach

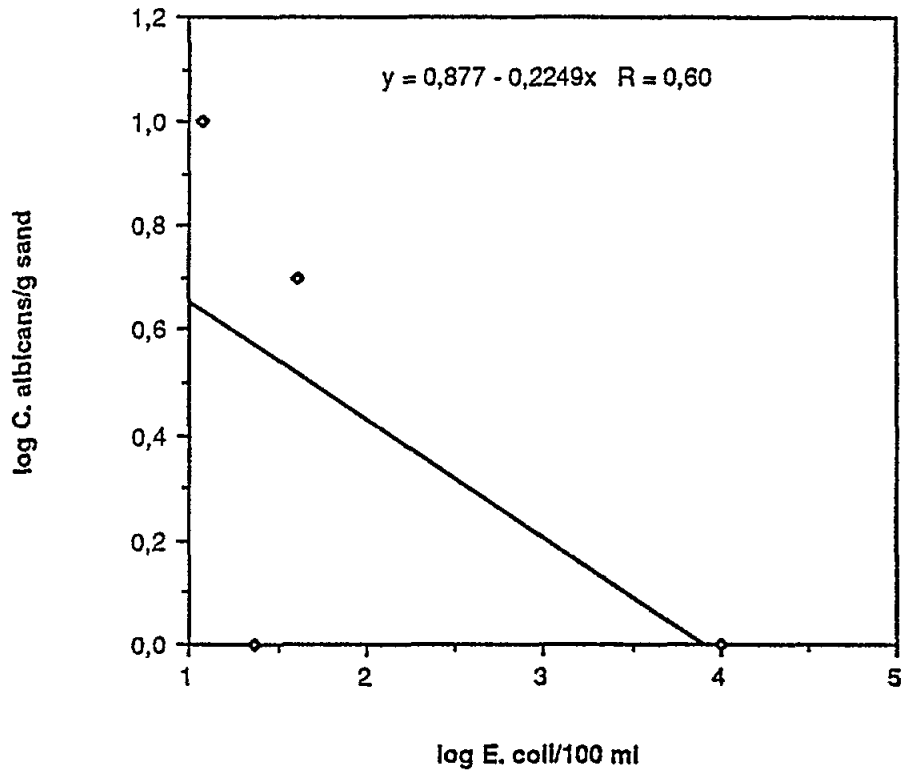
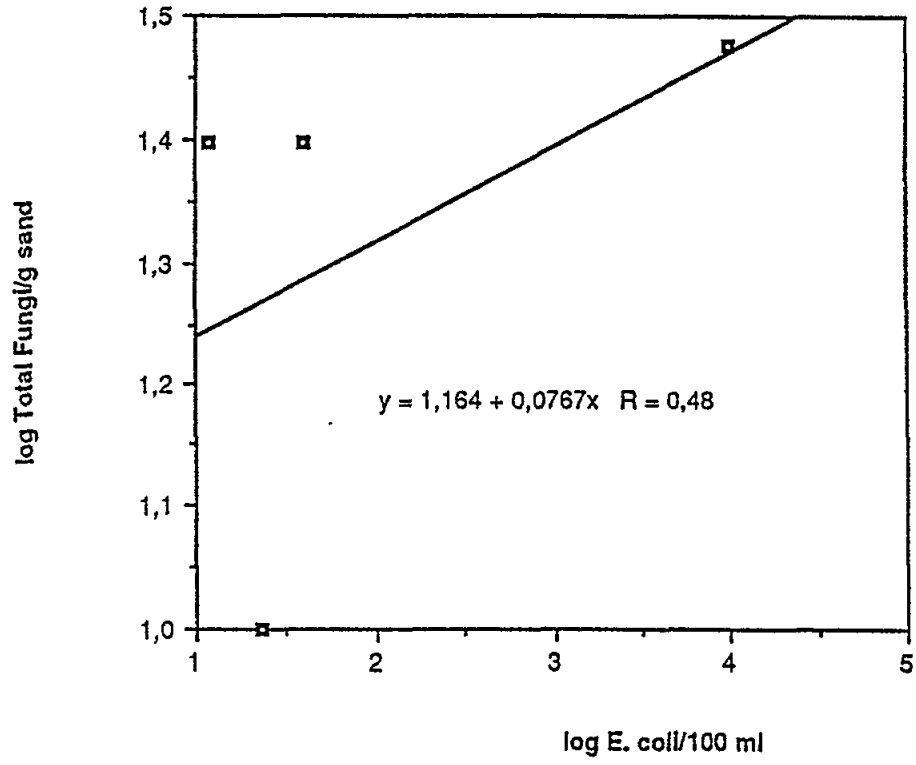


Figure 32 - Relationship between Indicators and pathogenic microorganisms in sand of Misericordia beach

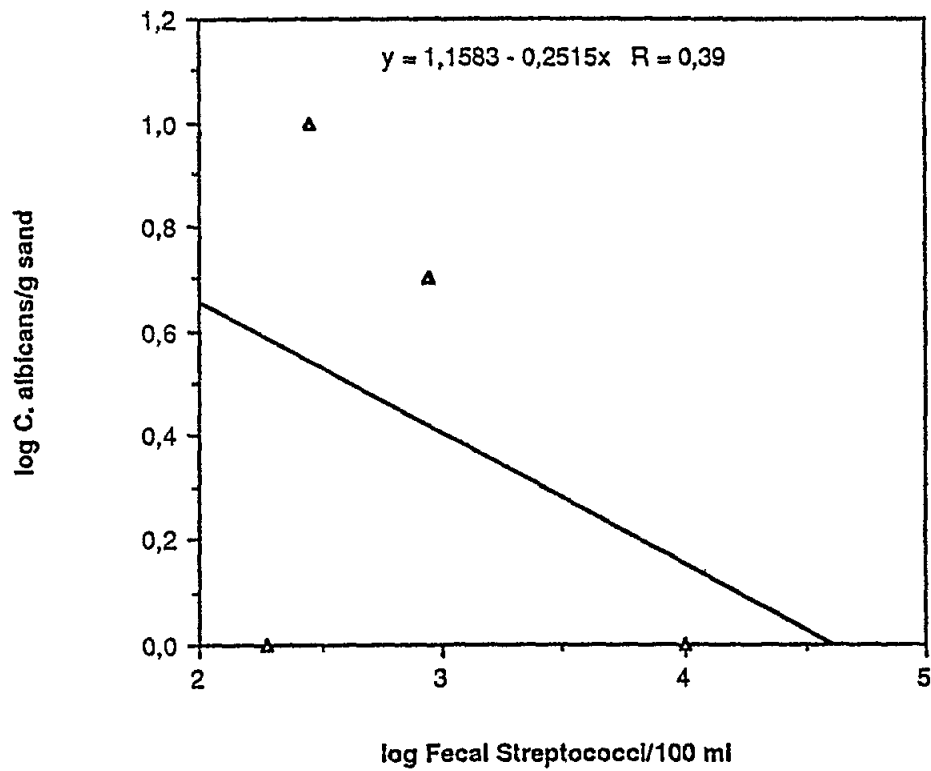
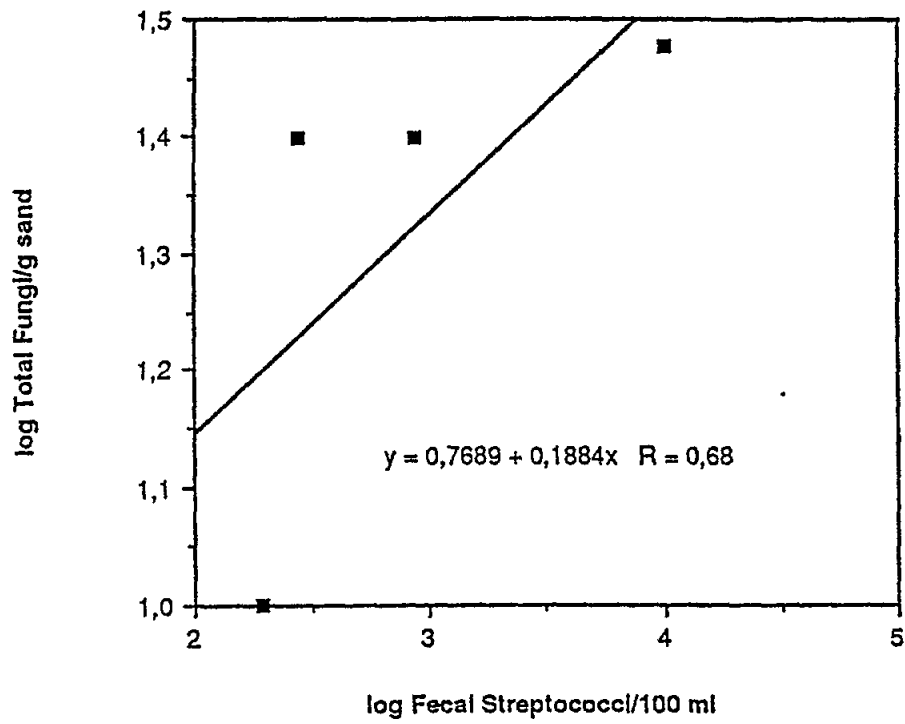


Figure 33 - Relationship between Indicators and pathogenic microorganisms in sand of Misericordia beach

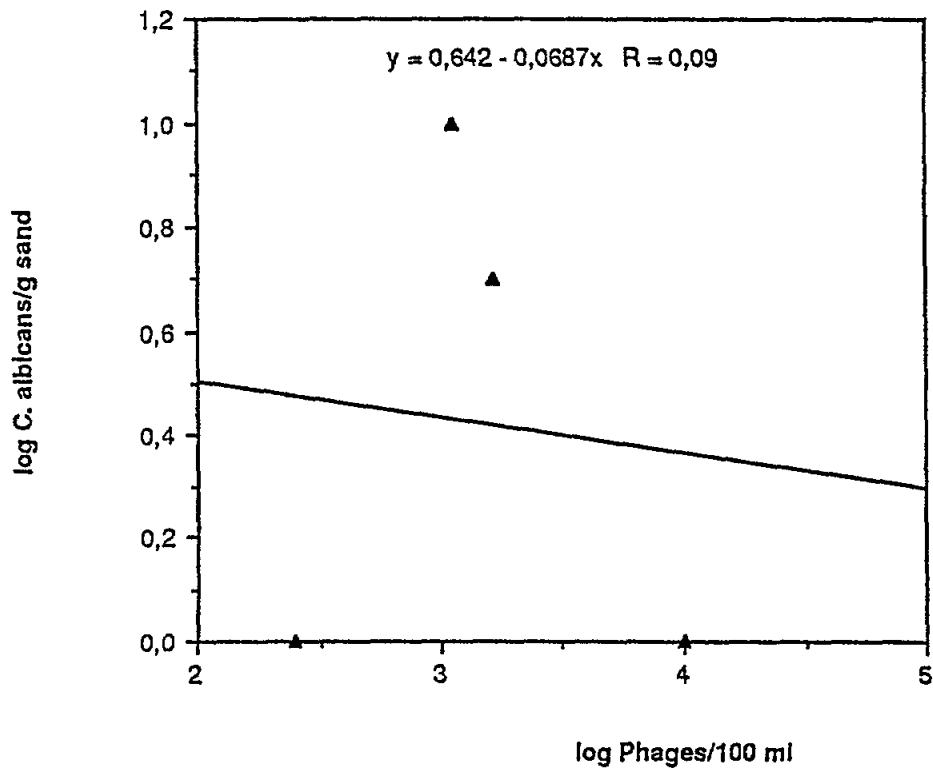
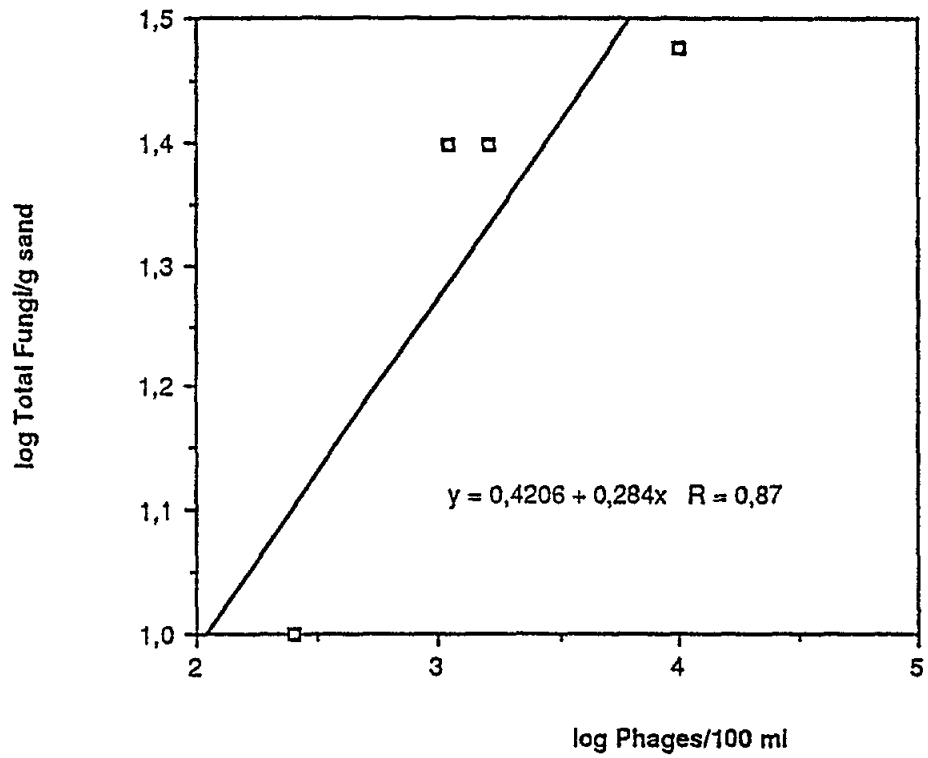


Figure 34 - Relationship between Indicators and pathogenic microorganisms in sand of Misericordia beach

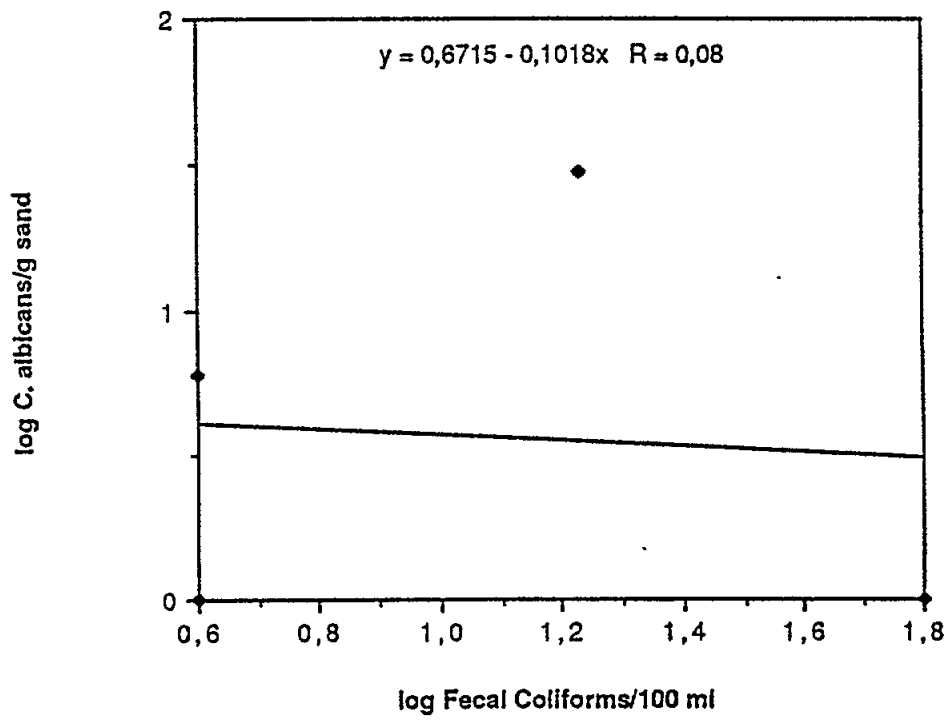
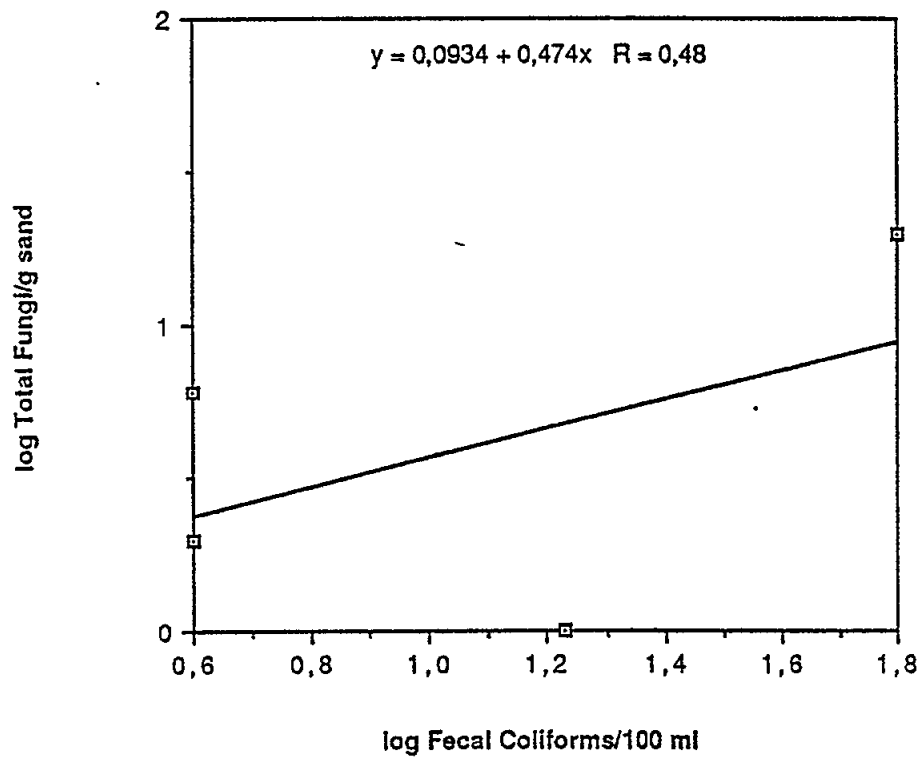


Figure 35 - Relationship between Faecal Indicators and pathogenic microorganisms in sand of Santa Ana beach



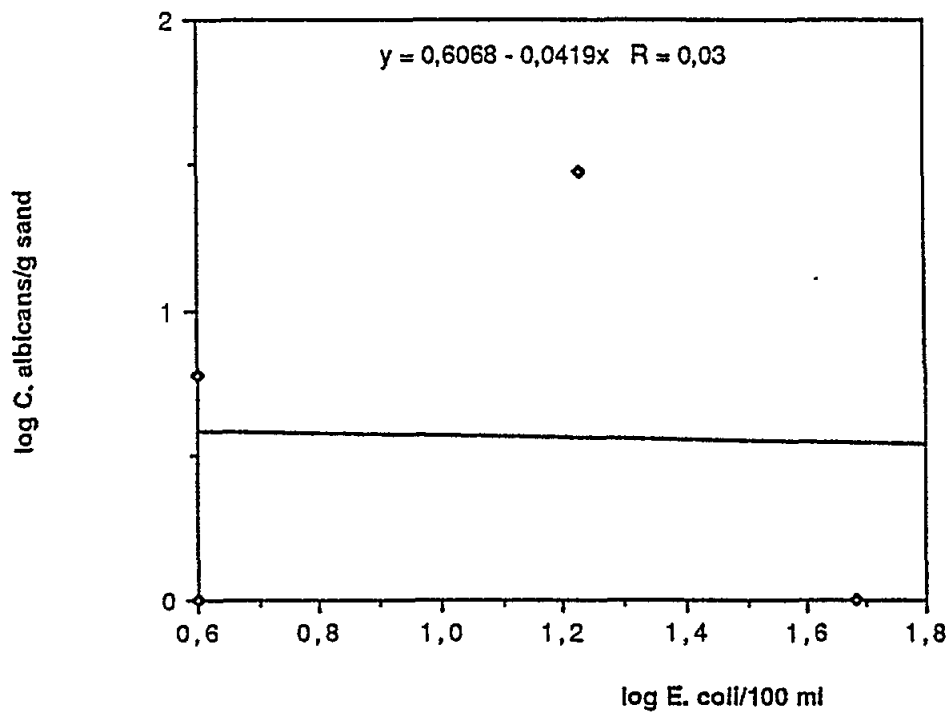
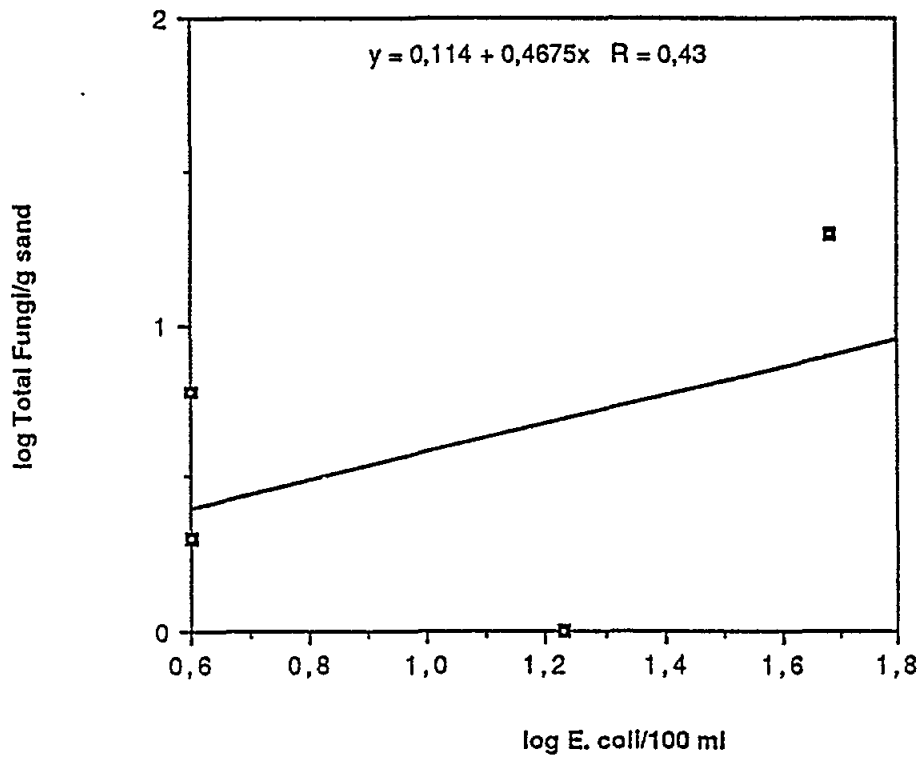


Figure 36 - Relationship between *Escherichia coli* and pathogenic microorganisms in sand of Santa Ana beach

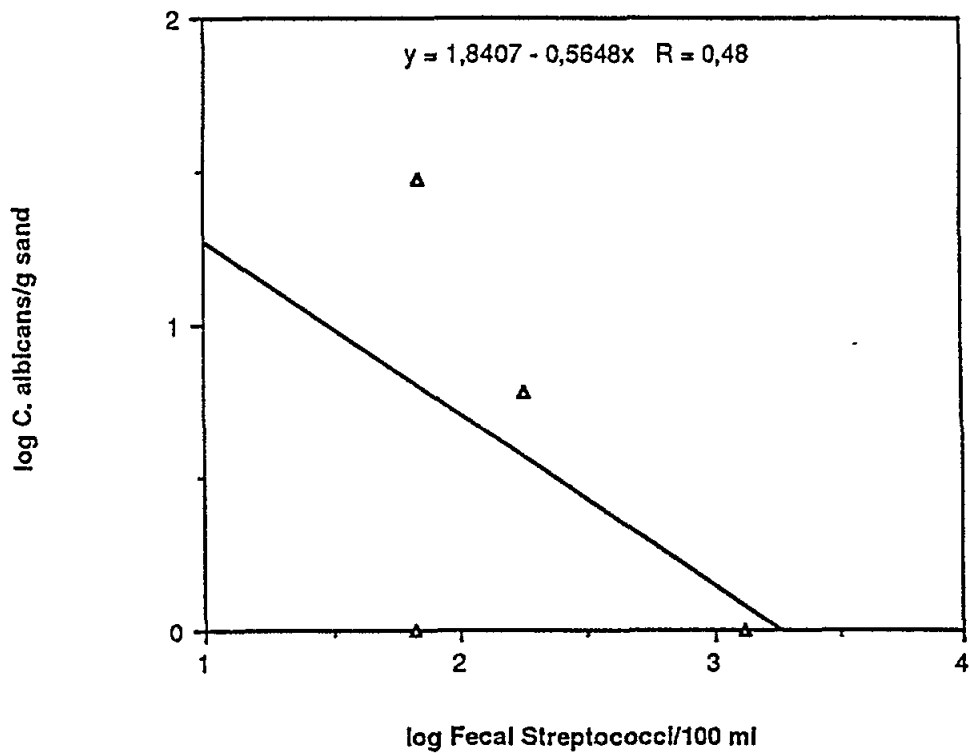
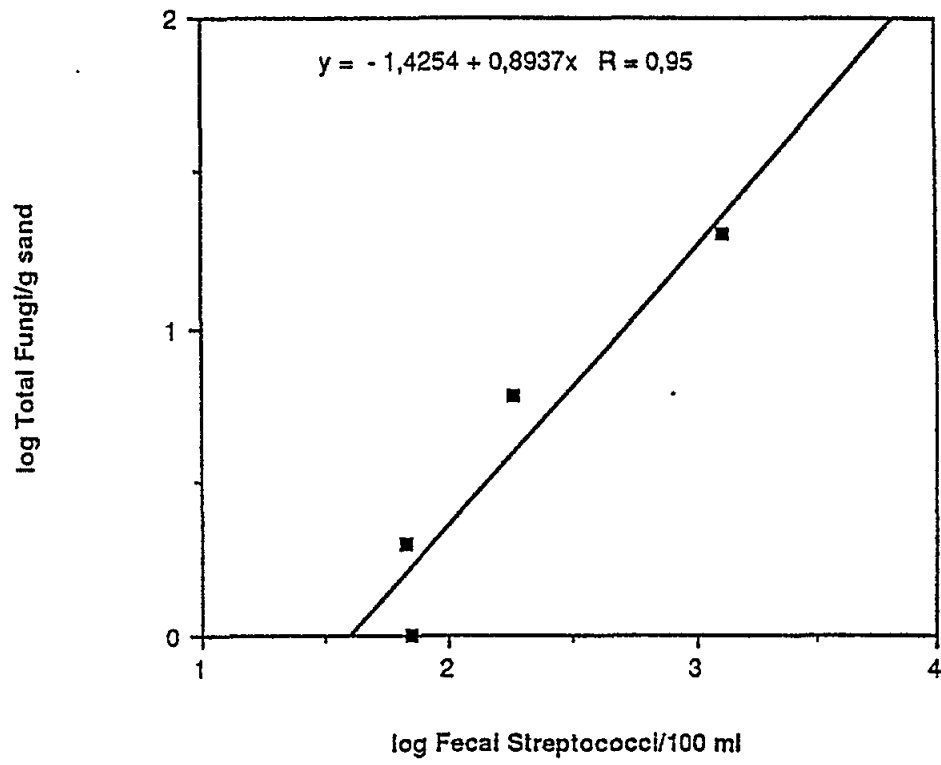


Figure 37 - Relationship between Faecal Streptococci and pathogenic microorganisms in sand of Santa Ana beach

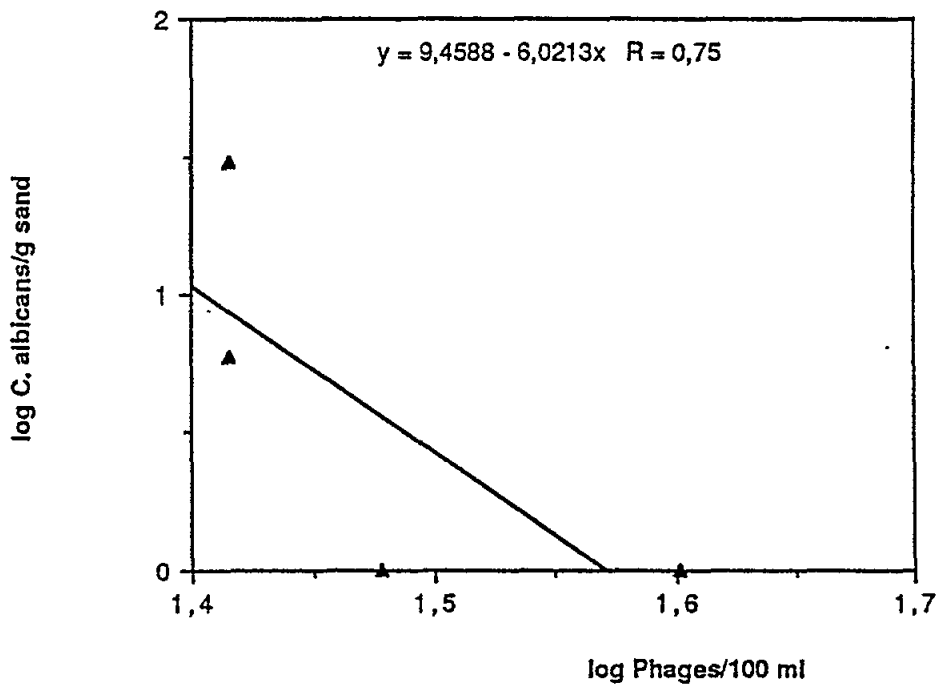
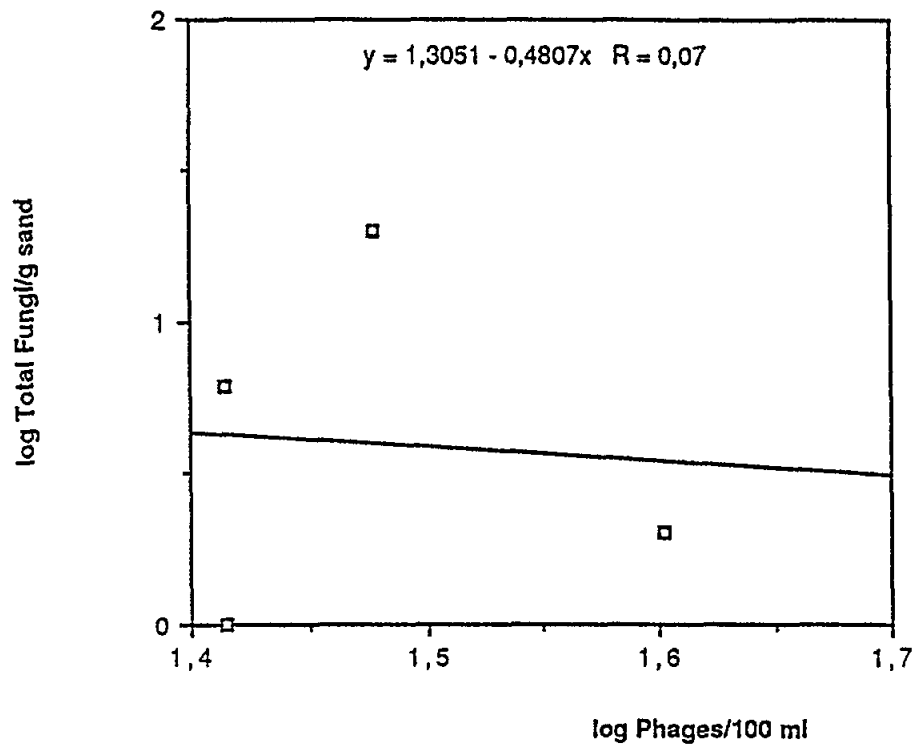


Figure 38 - Relationship between Coliphages and pathogenic microorganisms in sand of Santa Ana beach

Table 10

Distribution of the participants in the samples tested.

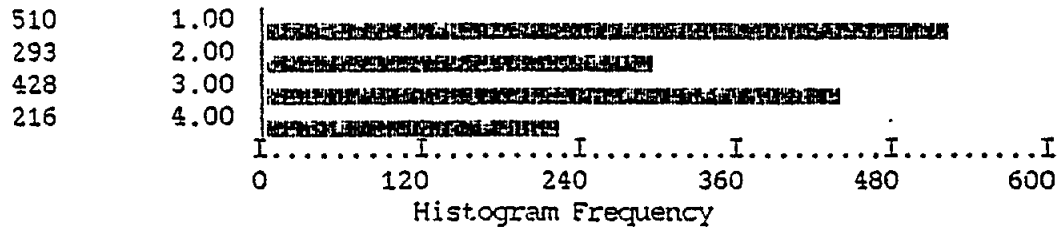
SAMPLE

Value Label	Value	Frequency	Percent	Valid Percent	Cum Percent
20JUL	1	510	35.2	35.2	35.2
3ALG	2	293	20.2	20.2	55.5
17ALG	3	428	29.6	29.6	85.1
5SEP	4	216	14.9	14.9	100.0
	TOTAL	1447	100.0	100.0	

SAMPLE

COUNT

VALUE



Valid Cases

1447

Missing Cases

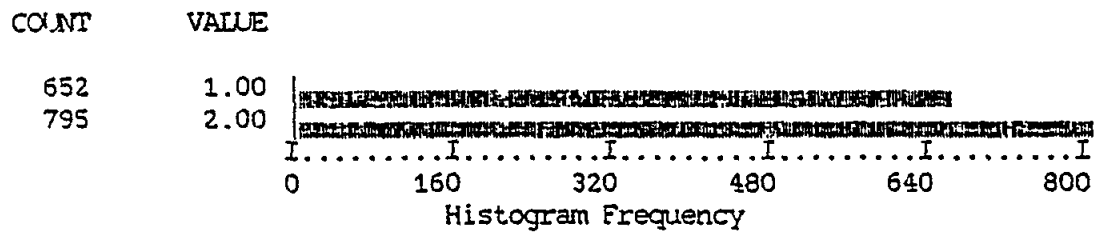
0

Table 11

Distribution of the participants in the samples by beaches

BEACH

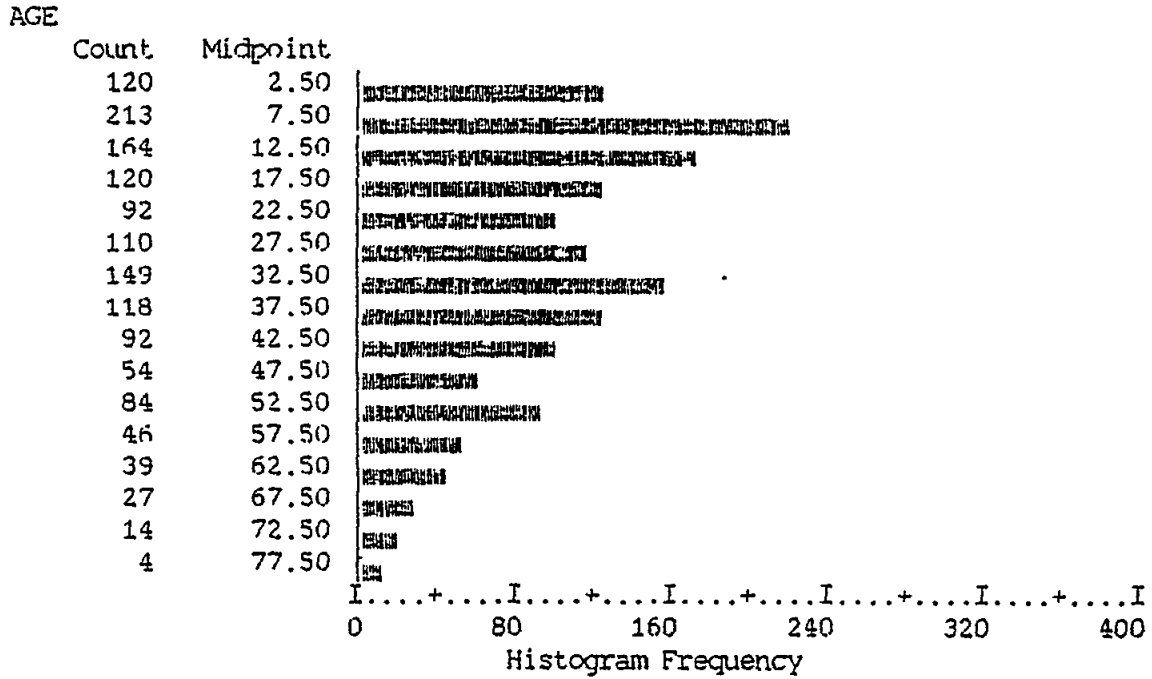
Value Label	Value	Frequency	Percent	Valid Percent	Cum Percent
Benalmadena	1	652	45.1	45.1	45.1
Misericordia	2	795	54.9	54.9	100.0
	TOTAL	1447	100.0	100.0	



Valid Cases 1447 Missing Cases 0

Table 12

Distribution of the participants in the samples by sex and age

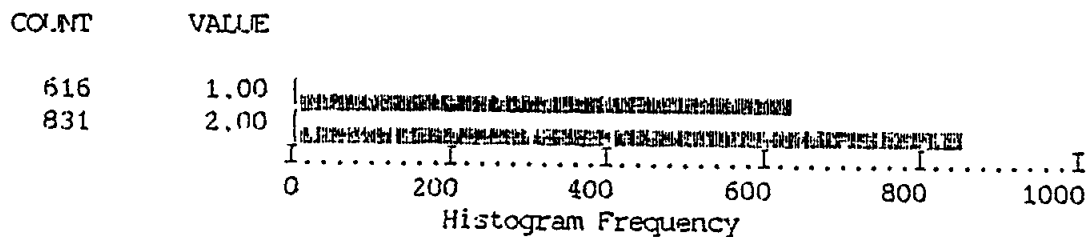


AGE	Mean	Std Err	Median
	26.794	.482	25.000
	Mode	Std Dev	Variance
	30.000	18.352	336.808
	Kurtosis	S E Kurt	Skewness
	-.629	1.999	.522
	S E Skew	Range	Minimum
	.064	90.000	.100
	Maximum	Sum	
	90.100	38770.900	

Valid Cases 1447 Missing Cases 0

SEX

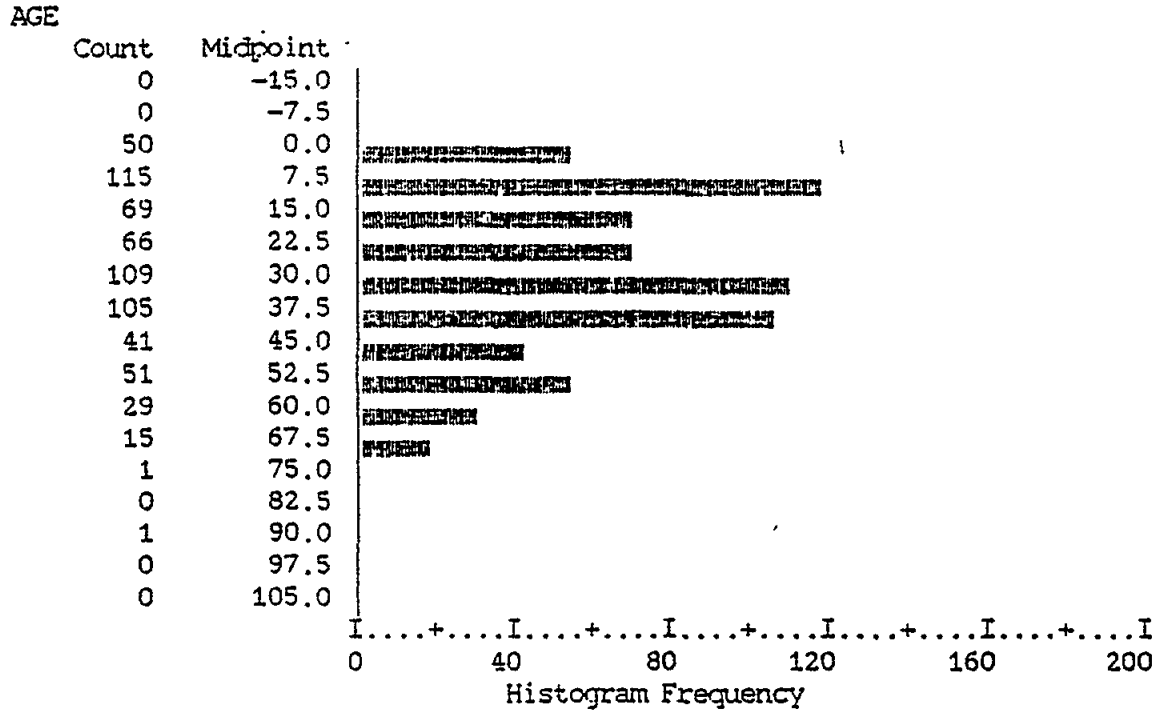
Value Label	Value	Frequency	Percent	Valid Percent	Cum Percent
Male	1	616	42.6	42.6	42.6
Female	2	831	57.4	57.4	100.0
	TOTAL	1447	100.0	100.0	



Valid Cases 1447 Missing Cases 0

Table 13

Distribution of the participants in the samples by age  
in Santa Ana beach

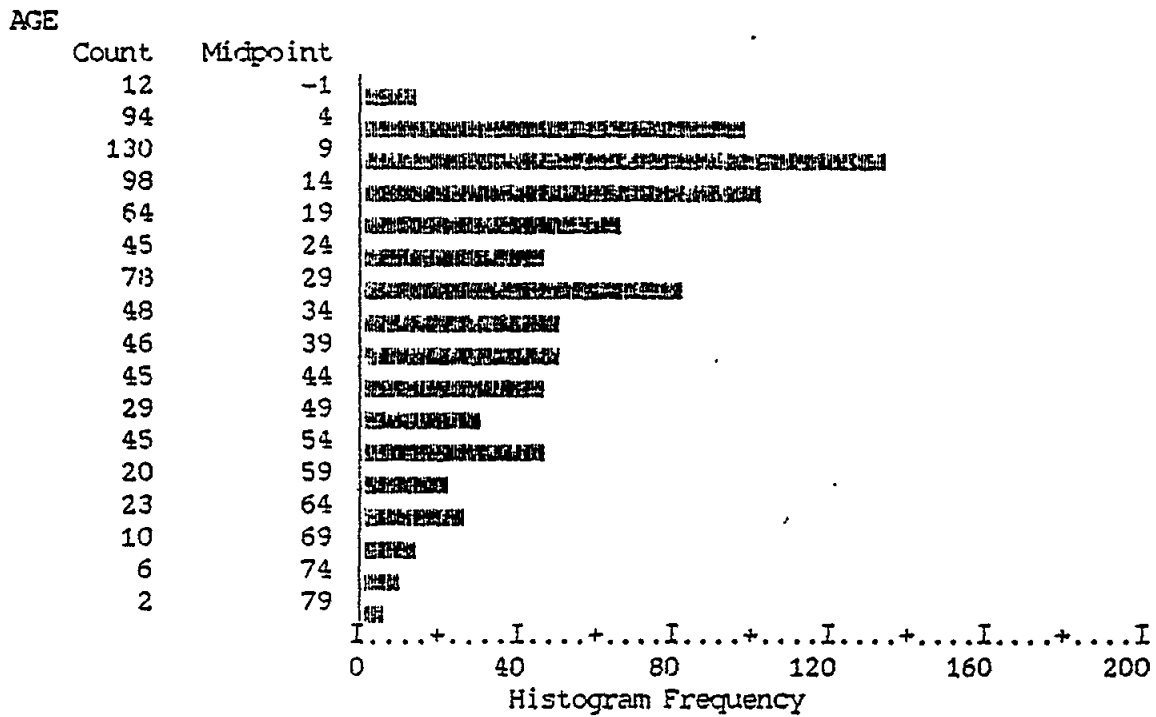


AGE					
Mean	27.725	Std Err	.701	Median	28.000
Mode	3.000	Std Dev	17.894	Variance	320.193
Kurtosis	-.614	S E Kurt	1.997	Skewness	.361
S E Skew	.096	Range	90.000	Minimum	.100
Maximum	90.100	Sum	18077.000		

Valid Cases	652	Missing Cases	0
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Table 14

Distribution of the participants in the samples by age  
in Misericordia beach



AGE					
Mean	26.030	Std Err	.663	Median	21.000
Mode	8.000	Std Dev	18.696	Variance	349.557
Kurtosis	-.593	S E Kurt	1.998	Skewness	.652
S E Skew	.087	Range	77.100	Minimum	.900
Maximum	78.000	Sum	20693.900		

Valid Cases 795 Missing Cases 0



Table 15

Distribution of individuals by their origin

Origin					
Value Label	Value	Frequency	Percent	Valid Percent	Cum Percent
No answer		305	21.1	21.1	21.1
ALGECIRA		2	.1	.1	21.2
ALICANTE		3	.2	.2	21.4
ANTEQUER		10	.7	.7	22.1
ASTURIES		2	.1	.1	22.3
BADAJOS		6	.4	.4	22.7
BARCELON		3	.2	.2	22.9
BENALMAD		2	.1	.1	23.0
BILBAO		7	.5	.5	23.5
C.REAL		2	.1	.1	23.6
CACERES		2	.1	.1	23.8
CORDOBA		54	3.7	3.7	27.5
CUENCA		2	.1	.1	27.6
ESTEPONA		2	.1	.1	27.8
JAEN		45	3.1	3.1	30.9
LEON		7	.5	.5	31.4
LUCENA		3	.2	.2	31.6
MADRID		111	7.7	7.7	39.3
MALAGA		576	39.8	39.8	79.1
MARSELLA		7	.5	.5	79.5
NK/NA		204	14.1	14.1	93.6
OSUNA		4	.3	.3	93.9
PARIS		6	.4	.4	94.3
PTO.LLAN		3	.2	.2	94.5
S.SEBAST		3	.2	.2	94.7
SEVILLA		44	3.0	3.0	97.8
SUIZA		3	.2	.2	98.0
TOLEDO		10	.7	.7	98.7
TORREMOL		6	.4	.4	99.1
VALENCIA		6	.4	.4	99.5
ZARAGOZA		7	.5	.5	100.0
TOTAL		1447	100.0	100.0	

Table 16

Cross-tabulation of the distribution of individuals  
by their origin and beach sampled

Crosstabulation:      ORIG      Origin  
                            By BEACH

BEACH →	Count	Benalmad ena	Miserico rdia	Row Total
ORIG		1	2	
No answer		227	78	305
ALGECIRA			2	2
ALICANTE		3		3
ANTEQUER		4	6	10
ASTURIES		2		2
BADAJOS		6		6
Column Total		652	795	1447
		45.1	54.9	100.0

Crosstabulation:      ORIG      Origin  
                            By BEACH

BEACH →	Count	Benalmad ena	Miserico rdia	Row Total
ORIG		1	2	
BARCELON			3	3
BENALMAD		2		2
BILBAO		4	3	7
C.REAL		2		2
CACERES		2		2
CORDOBA		44	10	54
Column (Continued) Total		652	795	1447
		45.1	54.9	100.0

Table 17

Cross-tabulation of the distribution of individuals  
by their origin and beach sampled

Crosstabulation:      ORIG      Origin  
                            By BEACH

BEACH →		Count	Benalmad ena	Miserico rdia	Row Total
ORIG			1	2	
	CUENCA		2		2
	ESTEPONA			2	2
	JAEN		14	31	45
	LEON		5	2	7
	LUCENA		3		3
	MADRID		81	30	111
	Column Total		652 45.1	795 54.9	1447 100.0

Crosstabulation:      ORIG      Origin  
                            By BEACH

BEACH →		Count	Benalmad ena	Miserico rdia	Row Total
ORIG			1	2	
	MALAGA		123	453	576
	MARSELLA			7	7
	NK/NA		53	151	204
	OSUNA		4		4
	PARIS			6	6
	PTO. LLAN		3		3
	Column (Continued) Total		652 45.1	795 54.9	1447 100.0

Table 18

Cross-tabulation of the distribution of individuals  
by their origin and beach sampled

Crosstabulation:      ORIG      Origin  
By BEACH

BEACH\>	Count	Benalmad ena 1	Miserico rdia 2	Row Total
ORIG				
S.SEBAST		3		3
SEVILLA		40	4	44
SUIZA		3		3
TOLEDO		10		10
TORREMDL		6		6
VALENCIA		6		6
Column Total		652 45.1	795 54.9	1447 100.0

Crosstabulation:      ORIG      Origin  
By BEACH

BEACH\>	Count	Benalmad ena 1	Miserico rdia 2	Row Total
ORIG				
ZARAGOZA			7	7
Column Total		652 45.1	795 54.9	1447 100.0

Number of Missing Observations = 0

Table 19

Distribution of the individuals depending on their permanence  
(in hours) and days on the beach

PERM Hours on the beach

Value Label	Value	Frequency	Percent	Valid Percent	Cum Percent
	0	13	2.0	2.0	2.0
	1	8	1.2	1.2	3.3
	2	101	15.7	15.7	18.9
	3	186	28.9	28.9	47.8
	4	163	25.3	25.3	73.1
	5	104	16.1	16.1	89.3
	6	40	6.2	6.2	95.5
	7	12	1.9	1.9	97.4
	8	10	1.6	1.6	98.9
	9	4	.6	.6	99.5
	67	3	.5	.5	100.0
	TOTAL	644	100.0	100.0	

TBEACH Days in this beach

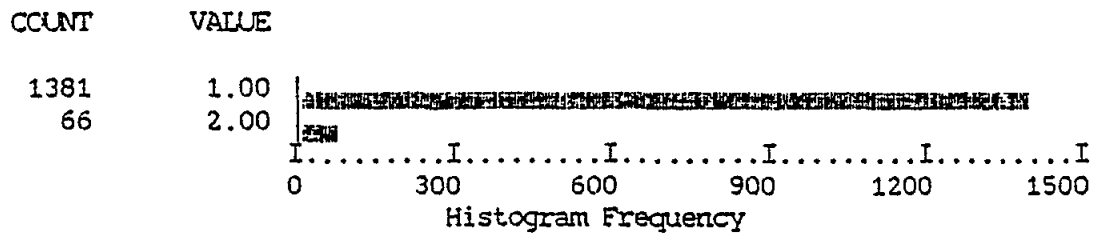
Value Label	Value	Frequency	Percent	Valid Percent	Cum Percent
	0	133	20.7	20.7	20.7
	1	74	11.5	11.5	32.1
	2	47	7.3	7.3	39.4
	3	34	5.3	5.3	44.7
	4	39	6.1	6.1	50.8
	5	43	6.7	6.7	57.5
	6	6	.9	.9	58.4
	7	36	5.6	5.6	64.0
	8	2	.3	.3	64.3
	10	2	.3	.3	64.6
	14	7	1.1	1.1	65.7
	15	37	5.7	5.7	71.4
	16	9	1.4	1.4	72.8
	17	8	1.2	1.2	74.1
	18	7	1.1	1.1	75.2
	20	7	1.1	1.1	76.2
	21	2	.3	.3	76.6
	30	25	3.9	3.9	80.4
	40	4	.6	.6	81.1
	45	8	1.2	1.2	82.3
	60	71	11.0	11.0	93.3
	70	2	.3	.3	93.6
	75	3	.5	.5	94.1
	90	25	3.9	3.9	98.0
	120	11	1.7	1.7	99.7
	210	2	.3	.3	100.0
	TOTAL	644	100.0	100.0	

Table 20

Percentage of swimmers and non-swimmers of the sample

SWIM      Swimming status

Value Label	Value	Frequency	Percent	Valid Percent	Cum Percent
Swimmer	1	1381	95.4	95.4	95.4
Non-swimmer	2	66	4.6	4.6	100.0
	TOTAL	1447	100.0	100.0	



Valid Cases      1447      Missing Cases      0

Table 21

Cross-tabulation of the population depending on the swimming status considering the age and beach factors

Crosstabulation: SWIM Swimming status  
 By AGE  
 Controlling for BEACH  
 = 1 Benalmadena

AGE->	Count	0-4 year	5-9 year	10-18 year	more y.	Row Total
SWIM		1.0	2.0	3.0	4.0	
1 Swimmer	53	81	87	400	621	95.2
2 Non-swimmer	6	1	6	18	31	4.8
Column Total		59	82	93	418	652
		9.0	12.6	14.3	64.1	100.0

Crosstabulation: SWIM Swimming status  
 By AGE  
 Controlling for BEACH  
 = 2 Misericordia

AGE->	Count	0-4 year	5-9 year	10-18 year	more y.	Row Total
SWIM		1.0	2.0	3.0	4.0	
1 Swimmer	56	129	164	411	760	95.6
2 Non-swimmer	5	2	5	23	35	4.4
Column Total		61	131	169	434	795
		7.7	16.5	21.3	54.6	100.0

Number of Missing Observations = 0

Table 22

Sample distribution by sand status

SAND	Sand status				Valid	Cum
Value Label	Value	Frequency	Percent	Percent	Percent	Percent
NK/NA	0	15	1.0	1.0	1.0	1.0
Towel	1	714	49.3	49.3	50.4	
Deckchair	2	398	27.5	27.5	77.9	
Nothing	3	313	21.6	21.6	99.5	
Towel & deckchair	4	6	.4	.4	99.9	
Deckchair & nothing	6	1	.1	.1	100.0	
	TOTAL	1447	100.0	100.0		

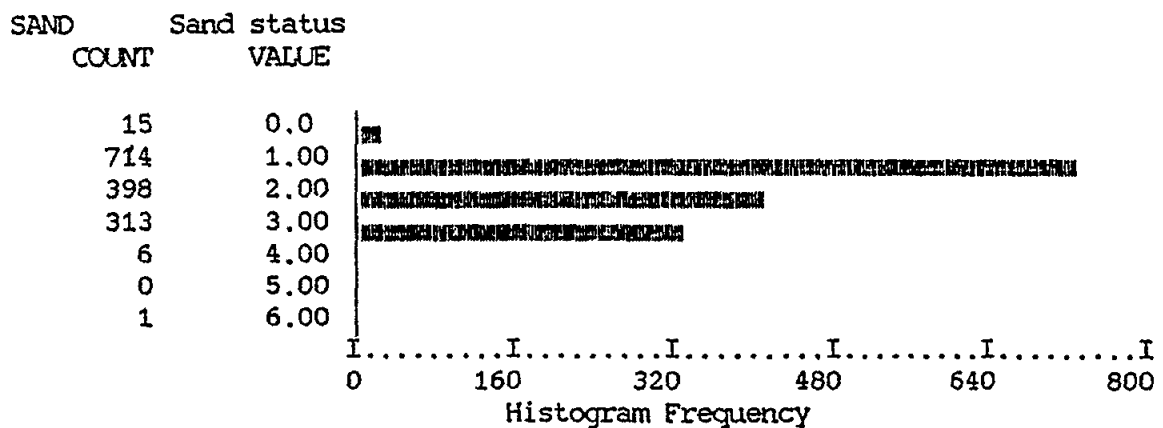




Table 23

Cross-tabulation by sand status and age in Santa Aba beach

Crosstabulation: SAND Sand status  
 By AGE  
 Controlling for BEACH  
 = 1 Benalmadena

AGE-->	Count	0-4 year	5-9 year	10-18 ye ar	more y.	Row Total
SAND		1.0	2.0	3.0	4.0	
	0	2	1	2	3	8
NK/NA						1.2
	1	14	19	36	194	263 40.3
Towel						
	2	16	12	27	207	262 40.2
Deckchair						
	Column Total	59 9.0	82 12.6	93 14.3	418 64.1	652 100.0

Crosstabulation: SAND Sand status  
 By AGE  
 Controlling for BEACH  
 = 1 Benalmadena

AGE-->	Count	0-4 year	5-9 year	10-18 ye ar	more y.	Row Total
SAND		1.0	2.0	3.0	4.0	
	3	26	50	28	10	114 17.5
Nothing						
	4				4	4 .6
Towel & deckchai						
	6	1				1 .2
Deckchair & noth						
	Column Total	59 9.0	82 12.6	93 14.3	418 64.1	652 100.0

Table 24

Cross-tabulation by sand status and age in Misericordia beach

Crosstabulation: SAND Sand status  
 By AGE  
 Controlling for BEACH  
 = 2 Misericordia

AGE→	Count	0-4 year	5-9 year	10-18 year	more y.	Row Total
SAND		1.0	2.0	3.0	4.0	
	0		1	3	3	7
NK/NA						.9
	1	19	29	97	306	451
Towel						56.7
	2	3	7	8	118	136
Deckchair						17.1
	Column Total	61	131	169	434	795
		7.7	16.5	21.3	54.6	100.0

Crosstabulation: SAND Sand status  
 By AGE  
 Controlling for BEACH  
 = 2 Misericordia

AGE→	Count	0-4 year	5-9 year	10-18 year	more y.	Row Total
SAND		1.0	2.0	3.0	4.0	
	3	39	94	61	5	199
Nothing						25.0
	4				2	2
Towel & deckchai						.3
	Column Total	61	131	169	434	795
		7.7	16.5	21.3	54.6	100.0

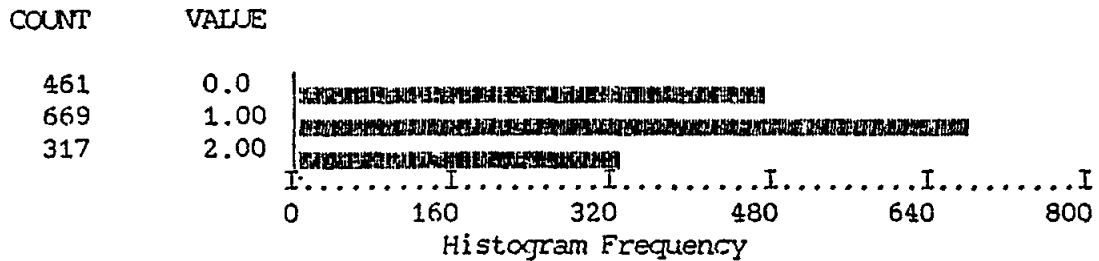
Number of Missing Observations = 0

Table 25

Distribution and cross-tabulation of swimmers who use other beaches

OBEACH Other beaches

Value Label	Value	Frequency	Percent	Valid Percent	Cum Percent
NK/NA	0	461	31.9	31.9	31.9
Yes	1	669	46.2	46.2	78.1
No	2	317	21.9	21.9	100.0
TOTAL		1447	100.0	100.0	



Valid Cases 1447 Missing Cases 0

Crosstabulation: BEACH  
By OBEACH Other beaches

OBEACH→	Count	NK/NA 0	Yes 1	No 2	Row Total
BEACH					
1 Benalmadena	652	228	307	117	652 45.1
2 Misericordia	795	233	362	200	795 54.9
Column Total	1447	461	669	317	1447 100.0

Number of Missing Observations = 0

Table 26

Frequency of symptoms presented by the individuals

SYMP      Symptoms and diseases					Valid	Cum
Value Label	Value	Frequency	Percent	Percent	Percent	Percent
NK/NA	0	6	.4	.4	.4	
Cough	1	27	1.9	1.9	2.3	
Vomiting	2	4	.3	.3	2.6	
Diarrhoea	4	10	.7	.7	3.2	
Fever	5	1	.1	.1	3.3	
Skin	6	82	5.7	5.7	9.0	
Ears	7	17	1.2	1.2	10.2	
Normal	8	1267	87.6	87.6	97.7	
Vaginal inf.	9	3	.2	.2	97.9	
Cough & diarr.	14	3	.2	.2	98.1	
Cough & Fev.	15	2	.1	.1	98.3	
Cough & skin	16	4	.3	.3	98.5	
Cough & ears	17	1	.1	.1	98.6	
Vomiting & naus.	23	5	.3	.3	99.0	
Vomiting & diarr.	24	1	.1	.1	99.0	
Nausea & diarr.	34	1	.1	.1	99.1	
Diarr. & fever	45	1	.1	.1	99.2	
Diarr. & skin	46	2	.1	.1	99.3	
Skin & ears	67	3	.2	.2	99.5	
Vaginal & skin	96	1	.1	.1	99.6	
Cough & vom. & diarr	124	2	.1	.1	99.7	
Vom. & Diarr. & Fev.	245	3	.2	.2	99.9	
Naus. & Diarr. & Fev	345	1	.1	.1	100.0	
	TOTAL	1447	100.0	100.0		

Table 27

Cross-tabulation of the symptoms presented by swimmers and non-swimmers in Santa Ana beach

Crosstabulation: SWIM Swimming status  
 By SYMP Symptoms and diseases  
 Controlling for BEACH  
 = 1 Benalmadena

SYMP→	Count Col Pct	NK/NA 0	Cough 1	Diarrhoe a 4	Skin 6	Ears 7	Row Total
SWIM							
1 Swimmer	621 95.2	4 80.0	19 100.0	2 100.0	26 100.0	4 100.0	
2 Non-swimmer	31 4.8	1 20.0					
Column Total	652 100.0	5 .8	19 2.9	2 .3	26 4.0	4 .6	

Crosstabulation: SWIM Swimming status  
 By SYMP Symptoms and diseases  
 Controlling for BEACH  
 = 1 Benalmadena

SYMP→	Count Col Pct	Normal 8	Vaginal inf. 9	Cough & diarr. 14	Cough & Fev. 15	Vomiting & naus. 23	Row Total
SWIM							
1 Swimmer	621 95.2	555 95.0	1 100.0	3 100.0	1 100.0	1 100.0	
2 Non-swimmer	31 4.8	29 5.0					
Column Total	652 100.0	584 89.6	1 .2	3 .5	1 .2	1 .2	

(Continued)

Table 28

Cross-tabulation of the symptoms presented by swimmers and non-swimmers in Santa Ana beach

Crosstabulation: SWIM Swimming status  
 By SYMP Symptoms and diseases  
 Controlling for BEACH = 1 Benalmadena

SYMP→	Count Col Pct	Nausea & diarr. 34	Diarr. & skin 46	Skin & e ars 67	Cough & vom. & d vom. & d 124	Vom. & D iarr. & 245	Row Total
SWIM							
Swimmer	1	1 100.0	1 100.0	1 100.0	1 100.0	1 100.0	621 95.2
Non-swimmer	2						31 4.8
Column Total		1 .2	1 .2	1 .2	1 .2	1 .2	652 100.0

Crosstabulation: SWIM Swimming status  
 By SYMP Symptoms and diseases  
 Controlling for BEACH = 1 Benalmadena

SYMP→	Count Col Pct	Naus. & Diarr. & 345	Row Total
SWIM			
Swimmer	1		621 95.2
Non-swimmer	2	1 100.0	31 4.8
Column Total		1 .2	652 100.0

Table 29

Cross-tabulation of the symptoms presented by swimmers and non-swimmers in Misericordia beach

Crosstabulation: SWIM Swimming status  
 By SYMP Symptoms and diseases  
 Controlling for BEACH  
 = 2 Misericordia

SYMP->	Count Col Pct	NK/NA	Cough	Vomiting	Diarrhoe	Fever	Row Total
		0	1	2	a 4	5	
SWIM							
Swimmer	1	1 100.0	7 87.5	3 75.0	8 100.0	1 100.0	760 95.6
Non-swimmer	2		1 12.5	1 25.0			35 4.4
Column Total		1 .1	8 1.0	4 .5	8 1.0	1 .1	795 100.0

Crosstabulation: SWIM Swimming status  
 By SYMP Symptoms and diseases  
 Controlling for BEACH  
 = 2 Misericordia

SYMP->	Count Col Pct	Skin	Ears	Normal	Vaginal inf.	Cough & Fev.	Row Total
		6	7	8	9	15	
SWIM							
Swimmer	1	56 100.0	12 92.3	651 95.3	2 100.0	1 100.0	760 95.6
Non-swimmer	2		1 7.7	32 4.7			35 4.4
(Continued) Column Total		56 7.0	13 1.6	683 85.9	2 .3	1 .1	795 100.0

Table 30

Cross-tabulation of the symptoms presented by swimmers and non-swimmers in Misericordia beach

Crosstabulation: SWIM Swimming status  
 By SYMP Symptoms and diseases  
 Controlling for BEACH = 2 Misericordia

SYMP→	Count Col Pct	Cough & skin 16	Cough & ears 17	Vomiting & naus. 23	Vomiting & diarr 24	Diarr. & fever 45	Row Total
SWIM							
1 Swimmer	4 100.0	1 100.0	4 100.0	1 100.0	1 100.0	760 95.6	
2 Non-swimmer						35 4.4	
Column Total		4 .5	1 .1	4 .5	1 .1	1 .1	795 100.0

Crosstabulation: SWIM Swimming status  
 By SYMP Symptoms and diseases  
 Controlling for BEACH = 2 Misericordia

SYMP→	Count Col Pct	Diarr. & skin 46	Skin & e ars 67	Vaginal & skin 96	Cough & vom. & d 124	Vom. & D iarr. & 245	Row Total
SWIM							
1 Swimmer	1 100.0	2 100.0	1 100.0	1 100.0	2 100.0	760 95.6	
2 Non-swimmer						35 4.4	
Column Total		1 .1	2 .3	1 .1	1 .1	2 .3	795 100.0

Number of Missing Observations = 0



Table 31

Percentages of total symptoms for the  
0-4 year-old-age group

Crosstabulation: SWIM Swimming status  
By SYMP Symptoms and diseases  
Controlling for AGE = 1.0 0-4 year

SYMP→	Count	NK/NA	Cough	Vomiting	Diarrhoe a	Fever	Row Total
SWIM		0	1	2	4	5	
Swimmer	1	1	3	1	2	1	109 90.8
Non-swimmer	2	1		1			11 9.2
Column Total		2 1.7	3 2.5	2 1.7	2 1.7	1 .8	120 100.0

SYMP→	Count	Skin	Ears	Normal	Vomiting & naus.	Cough & vom. & d	Row Total
SWIM		6	7	8	23	124	
Swimmer	1	14		82	1	1	109 90.8
Non-swimmer	2		1	8			11 9.2
Column Total		14 11.7	1 .8	90 75.0	1 .8	1 .8	120 100.0

SYMP→	Count	Vom. & D iarr. &	Row Total
SWIM		245	
Swimmer	1	3	109 90.8
Non-swimmer	2		11 9.2
Column Total		3 2.5	120 100.0

Table 32

Percentages of total symptoms for the  
5-9 year-old-age group

Crosstabulation: SWIM Swimming status  
By SYMP Symptoms and diseases  
Controlling for AGE = 2.0 5-9 year

SYMP->	Count	NK/NA	Cough	Vomiting	Diarrhoe	Skin	Row Total
SWIM		0	1	2	4	6	
Swimmer	1	2	5	1	1	12	210 98.6
Non-swimmer	2						3 1.4
Column Total		2 .9	5 2.3	1 .5	1 .5	12 5.6	213 100.0

SYMP->	Count	Ears	Normal	Cough & diarr.	Cough & Fev.	Cough & skin	Row Total
SWIM		7	8	14	15	16	
Swimmer	1	4	178	1	1	3	210 98.6
Non-swimmer	2		3				3 1.4
Column Total		4 1.9	181 85.0	1 .5	1 .5	3 1.4	213 100.0

SYMP->	Count	Vomiting & naus.	Row Total
SWIM		23	
Swimmer	1	2	210 98.6
Non-swimmer	2		3 1.4
Column Total		2 .9	213 100.0

Table 33

Percentages of total symptoms for the  
10-18 year-old-age group

Crosstabulation: SWIM Swimming status  
By SYMP Symptoms and diseases  
Controlling for AGE  
= 3.0 10-18 year

SYMP→	Count	NK/NA	Cough	Vomiting	Skin	Ears	Row Total
SWIM		0	1	2	6	7	
1 Swimmer	1	1	7	1	17	5	251 95.8
2 Non-swimmer	2		1				11 4.2
Column Total		1 .4	8 3.1	1 .4	17 6.5	5 1.9	262 100.0

SYMP→	Count	Normal	Cough & diarr.	Cough & skin	Cough & ears	Skin & ears	Row Total
SWIM		8	14	16	17	67	
1 Swimmer	1	215	1	1	1	1	251 95.8
2 Non-swimmer	2	9					11 4.2
Column Total		224 85.5	1 .4	1 .4	1 .4	1 .4	262 100.0

SYMP→	Count	Cough & vom. & d	Naus. & Diarr. &	Row Total
SWIM		124	345	
1 Swimmer	1	1		251 95.8
2 Non-swimmer	2		1	11 4.2
Column Total		1 .4	1 .4	262 100.0

Table 34

Percentages of total symptoms for more than  
18 year-old-age group

Crosstabulation: SWIM Swimming status  
By SYMP Symptoms and diseases  
Controlling for AGE = 4.0 more y.

SYMP→	Count	NK/NA	Cough	Diarrhoe a	Skin	Ears	Row Total
SWIM		0	1	4	6	7	
1 Swimmer	1	1	11	7	39	7	811 95.2
2 Non-swimmer	2						41 4.8
Column Total		1 .1	11 1.3	7 .8	39 4.6	7 .8	852 100.0

SYMP→	Count	Normal	Vaginal inf.	Cough & diarr.	Cough & Fev.	Vomiting & naus.	Row Total
SWIM		8	9	14	15	23	
1 Swimmer	1	731	3	1	1	2	811 95.2
2 Non-swimmer	2	41					41 4.8
(Continued) Column Total		772 90.6	3 .4	1 .1	1 .1	2 .2	852 100.0

Table 35

Percentages of total symptoms for more than  
18 year-old-age group

Crosstabulation: SWIM Swimming status  
By SYMP Symptoms and diseases  
Controlling for AGE = 4.0 more y.

SYMP→	Count	Vomiting & diarr · 24	Nausea & diarr. 34	Diarr. & fever 45	Diarr. & skin 46	Skin & e ars 67	Row Total
SWIM							
1 Swimmer	1	1	1	1	2	2	811 95.2
2 Non-swimmer	2						41 4.8
Column Total		.1	.1	.1	.2	.2	852 100.0

SYMP→	Count	Vaginal & skin 96	Row Total
SWIM			
1 Swimmer	1	1	811 95.2
2 Non-swimmer	2		41 4.8
Column Total		1	852 100.0

Number of Missing Observations = 0

Table 36

Percentages of total symptoms for the 0-4 year-old-age group in Santa Ana beach

Crosstabulation: SWIM Swimming status  
 By SYMP Symptoms and diseases  
 Controlling for AGE = 1.0 0-4 year  
 BEACH = 1 Benalmadena

SYMP->	Count	NK/NA	Cough	Diarrhoe  a	Skin	Normal	Row Total
SWIM		0	1	4	6	8	
Swimmer	1		3	1	6	42	53 89.8
Non-swimmer	2	1				5	6 10.2
Column Total		1 1.7	3 5.1	1 1.7	6 10.2	47 79.7	59 100.0

SYMP->	Count	Vom. & D  iarr. &   245	Row Total
SWIM			
Swimmer	1	1	53 89.8
Non-swimmer	2		6 10.2
Column Total		1 1.7	59 100.0

Table 37

Percentages of total symptoms for the 5-9 year-old-age group in Santa Ana beach

Crosstabulation: SWIM Swimming status  
 By SYMP Symptoms and diseases  
 Controlling for AGE = 2.0 5-9 year  
 BEACH = 1 Benalmadena

SYMP->	Count	NK/NA	Cough	Skin	Normal	Cough & diarr.	Row Total
SWIM							
Swimmer	1	2	3	3	70	1	81 98.8
Non-swimmer	2				1		1 1.2
Column Total		2 2.4	3 3.7	3 3.7	71 86.6	1 1.2	82 100.0

SYMP->	Count	Cough & Fev.	Vomiting & naus.	Row Total
SWIM				
Swimmer	1	1	1	81 98.8
Non-swimmer	2			1 1.2
Column Total		1 1.2	1 1.2	82 100.0

Table 38

Percentages of total symptoms for the 10-18 year-old-age group in Santa Ana beach

Crosstabulation: SWIM Swimming status  
 By SYMP Symptoms and diseases  
 Controlling for AGE = 3.0 10-18 year  
 BEACH = 1 Benalmadena

SYMP→	Count	NK/NA	Cough	Skin	Ears	Normal	Row Total
SWIM		0	1	6	7	8	
Swimmer	1	1	3	2	2	77	87 93.5
Non-swimmer	2					5	6 6.5
Column Total		1	3	2	2	82	93 100.0

SYMP→	Count	Cough & diarr.	Cough & vom. & d	Naus. & Diarr. &	Row Total
SWIM		14	124	345	
Swimmer	1	1	1		87 93.5
Non-swimmer	2			1	6 6.5
Column Total		1	1	1	93 100.0



Table 39

Percentages of total symptoms for the more than 18 year-old-age group in Misericordia beach

Crosstabulation: SWIM Swimming status  
 By SYMP Symptoms and diseases  
 Controlling for AGE = 4.0 more y.  
 BEACH = 1 Benalmadena

SYMP→	Count	NK/NA	Cough	Diarrhoe a	Skin	Ears	Row Total
SWIM		0	1	4	6	7	
Swimmer	1	.1	10	1	15	2	400 95.7
Non-swimmer	2						18 4.3
Column Total		1 .2	10 2.4	1 .2	15 3.6	2 .5	418 100.0

SYMP→	Count	Normal	Vaginal inf.	Cough & diarr.	Nausea & diarr.	Diarr. & skin	Row Total
SWIM		8	9	14	34	46	
Swimmer	1	366	1	1	1	1	400 95.7
Non-swimmer	2	18					18 4.3
Column Total		384 91.9	1 .2	1 .2	1 .2	1 .2	418 100.0

SYMP→	Count	Skin & e ars	Row Total
SWIM		67	
Swimmer	1	1	400 95.7
Non-swimmer	2		18 4.3
Column Total		1 .2	418 100.0

Table 40

Percentages of total symptoms for the 0-4 year-old-age group in Misericordia beach

Crosstabulation: SWIM Swimming status  
 By SYMP Symptoms and diseases  
 Controlling for AGE = 1.0 0-4 year  
 BEACH = 2

SYMP->	Count	NK/NA	Vomiting	Diarrhoe	Fever	Skin	Row Total
SWIM		0	2	4	5	6	
Swimmer	1	1	1	1	1	8	56 91.8
Non-swimmer	2		1				5 8.2
Column Total		1	2	1	1	8	61 100.0

SYMP->	Count	Ears	Normal	Vomiting & naus.	Cough & vom. & diarr.	Vom. & D	Row Total
SWIM		7	8	23	124	245	
Swimmer	1		40	1	1	2	56 91.8
Non-swimmer	2	1	3				5 8.2
Column Total		1	43	1	1	2	61 100.0

Table 41

Percentage of total symptoms for the 5-9 year-old-age group in Misericordia beach

Crosstabulation: SWIM Swimming status  
 By SYMP Symptoms and diseases  
 Controlling for AGE = 2.0 5-9 year  
 BEACH = 2

SYMP->	Count	Cough	Vomiting	Diarrhoe	Skin	Ears	Row Total
SWIM							
1 Swimmer	1	2	1	1	9	4	129 98.5
2 Non-swimmer	2						2 1.5
Column Total		2	1	1	9	4	131
		1.5	.8	.8	6.9	3.1	100.0

SYMP->	Count	Normal	Cough & Vomiting	Row Total
SWIM				
1 Swimmer	1	108	3	129 98.5
2 Non-swimmer	2	2		2 1.5
Column Total		110	3	131
		84.0	2.3	100.0

Table 42

Percentage of total symptoms for the 10-18 year-old-age group in Misericordia beach

Crosstabulation: SWIM Swimming status  
 By SYMP Symptoms and diseases  
 Controlling for AGE = 3.0 10-18 year  
 BEACH = 2

SYMP->	Count	Cough	Vomiting	Skin	Ears	Normal	Row Total
SWIM		1	2	6	7	8	
1 Swimmer	1	4	1	15	3	138	164 97.0
2 Non-swimmer	2	1				4	5 3.0
Column Total		5	1	15	3	142	169
		3.0	.6	8.9	1.8	84.0	100.0

SYMP->	Count	Cough & skin	Cough & ears	Skin & ears	Row Total
SWIM		16	17	67	
1 Swimmer	1	1	1	1	164 97.0
2 Non-swimmer	2				5 3.0
Column Total		1	1	1	169
		.6	.6	.6	100.0

Table 43

Percentage of total symptoms for the more than 18 year-old-age group in Misericordia beach

Crosstabulation: SWIM Swimming status  
 By SYMP Symptoms and diseases  
 Controlling for AGE = 4.0 more y.  
 BEACH = 2

SYMP->	Count	Cough	Diarrhoe	Skin	Ears	Normal	Row Total
SWIM		1	4	6	7	8	
Swimmer	1	1	6	24	5	365	411 94.7
Non-swimmer	2					23	23 5.3
Column Total		1	6	24	5	388	434
		.2	1.4	5.5	1.2	89.4	100.0

SYMP->	Count	Vaginal inf.	Cough & Fev.	Vomiting & naus.	Vomiting & diarr.	Diarr. & fever	Row Total
SWIM		9	15	23	24	45	
Swimmer	1	2	1	2	1	1	411 94.7
Non-swimmer	2						23 5.3
Column Total		2	1	2	1	1	434
		.5	.2	.5	.2	.2	100.0

SYMP->	Count	Diarr. & skin	Skin & ears	Vaginal & skin	Row Total
SWIM		46	67	96	
Swimmer	1	1	1	1	411 94.7
Non-swimmer	2				23 5.3
Column Total		1	1	1	434
		.2	.2	.2	100.0

Number of Missing Observations = 0

Table 44

Percentage of symptoms by beaches grouped into population classes (see text)

Crosstabulation: SWIM Swimming status  
By SYMP Symptoms and diseases

SYMP→	Count Col Pct						Row Total
		0	8	10	11	12	
SWIM							
1 Swimmer	5 83.3	1206 95.2	32 94.1	43 95.6	91 100.0	1381 95.4	
2 Non-swimmer	1 16.7	61 4.8	2 5.9	2 4.4		66 4.6	
Column Total	6 .4	1267 87.6	34 2.3	45 3.1	91 6.3	1447 100.0	

Crosstabulation: SWIM Swimming status  
By SYMP Symptoms and diseases

SYMP→	Count Col Pct		Row Total
		13	
SWIM			
1 Swimmer	4 100.0		1381 95.4
2 Non-swimmer			66 4.6
Column Total	4 .3		1447 100.0

Table 45

Percentage of symptoms by beaches grouped into population classes in Santa Ana beach

Crosstabulation: SWIM Swimming status  
 By SYMP Symptoms and diseases  
 Controlling for BEACH = 1 Benalmadena

SYMP→	Count Col Pct	0	8	10	11	12	Row Total
SWIM							
Swimmer	1	4 80.0	555 95.0	10 90.9	23 100.0	28 100.0	621 95.2
Non-swimmer	2	1 20.0	29 5.0	1 9.1			31 4.8
	Column Total	5 .8	584 89.6	11 1.7	23 3.5	28 4.3	652 100.0

Crosstabulation: SWIM Swimming status  
 By SYMP Symptoms and diseases  
 Controlling for BEACH = 1 Benalmadena

SYMP→	Count Col Pct	13	Row Total
SWIM			
Swimmer	1	1 100.0	621 95.2
Non-swimmer	2		31 4.8
	Column Total	1 .2	652 100.0

Table 46

Percentage of symptoms by beaches grouped into population classes in Misericordia beach

Crosstabulation: SWIM Swimming status  
 By SYMP Symptoms and diseases  
 Controlling for BEACH = 2 Misericordia

SYMP->	Count Col Pct						Row Total
		0	8	10	11	12	
SWIM							
Swimmer	1	1 100.0	651 95.3	22 95.7	20 90.9	63 100.0	760 95.6
Non-swimmer	2		32 4.7	1 4.3	2 9.1		35 4.4
	Column Total	1 .1	683 85.9	23 2.9	22 2.8	63 7.9	795 100.0

Crosstabulation: SWIM Swimming status  
 By SYMP Symptoms and diseases  
 Controlling for BEACH = 2 Misericordia

SYMP->	Count Col Pct		
		13	Row Total
SWIM			
Swimmer	1	3 100.0	760 95.6
Non-swimmer	2		35 4.4
	Column Total	3 .4	795 100.0



Table 47

Percentage of symptoms in Santa Ana beach for the 0-4 and 5-9 year-old-age groups

Crosstabulation: SWIM Swimming status  
 By SYMP Symptoms and diseases  
 Controlling for AGE = 1.0  
 BEACH = 1 Benalmadena

SYMP→		Count					Row Total	
SWIM			0	8	10	11	12	
	1	Swimmer		42	2	3	6	53 89.8
	2	Non-swimmer	1	5				6 10.2
	Column Total		1	47	2	3	6	59
			1.7	79.7	3.4	5.1	10.2	100.0

Crosstabulation: SWIM Swimming status  
 By SYMP Symptoms and diseases  
 Controlling for AGE = 2.0  
 BEACH = 1 Benalmadena

SYMP→		Count					Row Total	
SWIM			0	8	10	11	12	
	1	Swimmer	2	70	3	3	3	81 98.8
	2	Non-swimmer		1				1 1.2
	Column Total		2	71	3	3	3	82
			2.4	86.6	3.7	3.7	3.7	100.0

Table 48

Percentage of symptoms in Santa Ana beach for the 10-18 and more than 18 year-old-age groups

Crosstabulation: SWIM Swimming status  
 By SYMP Symptoms and diseases  
 Controlling for AGE = 3.0  
 BEACH = 1 Benalmadena

SYMP→	Count					Row Total	
		0	8	10	11	12	
SWIM							
Swimmer	1	1	77	2	5	2	87 93.5
Non-swimmer	2		5	1			6 6.5
	Column Total	1	82	3	5	2	93
		1.1	88.2	3.2	5.4	2.2	100.0

Crosstabulation: SWIM Swimming status  
 By SYMP Symptoms and diseases  
 Controlling for AGE = 4.0  
 BEACH = 1 Benalmadena

SYMP→	Count					Row Total	
		0	8	10	11	12	
SWIM							
Swimmer	1	1	366	3	12	17	400 95.7
Non-swimmer	2		18				18 4.3
	Column Total	1	384	3	12	17	418
		.2	91.9	.7	2.9	4.1	100.0

SYMP→	Count	Row Total
	13	
SWIM		
Swimmer	1	400 95.7
Non-swimmer	2	18 4.3
	Column Total	418
		.2 100.0

Table 49

Percentage of symptoms in Misericordia beach for the 0-4  
and 5-9 year-old-age groups

Crosstabulation: SWIM Swimming status  
By SYMP Symptoms and diseases  
Controlling for AGE = 1.0  
BEACH = 2 Misericordia

SYMP→		Count					Row Total	
SWIM			0	8	10	11	12	
	1	1	40	7			8	56
	2		3	1	1			5
	Column Total	1	43	8	1	8		61
		1.6	70.5	13.1	1.6	13.1		100.0

Crosstabulation: SWIM Swimming status  
By SYMP Symptoms and diseases  
Controlling for AGE = 2.0  
BEACH = 2 Misericordia

SYMP→		Count					Row Total	
SWIM			8	10	11	12		
	1	108	3	6		12		129
	2	2						2
	Column Total	110	3	6	12			131
		84.0	2.3	4.6	9.2			100.0

Table 50

Percentage of symptoms in Misericordia beach for the 10-18  
and more than 18 year-old-age groups

Crosstabulation: SWIM Swimming status  
By SYMP Symptoms and diseases  
Controlling for AGE = 3.0  
BEACH = 2 Misericordia

SYMP→		Count	8	10	11	12	Row Total
SWIM	1	138	1	8	17	164	97.0
	2	4	1			5	3.0
	Column Total	142	1	9	17	169	
	Total	84.0	.6	5.3	10.1	100.0	

Crosstabulation: SWIM Swimming status  
By SYMP Symptoms and diseases  
Controlling for AGE = 4.0  
BEACH = 2 Misericordia

SYMP→		Count	8	10	11	12	13	Row Total
SWIM	1	365	11	6	26	3	411	94.7
	2	23					23	5.3
	Column Total	388	11	6	26	3	434	
	Total	89.4	2.5	1.4	6.0	.7	100.0	

The percentage of swimmers and non-swimmers for the whole sample and by age and beach is shown in Tables 20 and 21. In the whole sample 95.4% of the individuals were swimmers, with a low percentage of non-swimmers (4.6%) and a similar distribution by age and beach for both beaches. The percentages of swimmers (about 89%) and non-swimmers (10%) in the 0 to 4 year old-age group was similar for Benalmadena and Misericordia and slightly different for the rest of the age groups.

Sand condition is reflected in Table 22 for the whole sample, and in Tables 23 and 24 by age and beaches. A percentage of 53% (Benalmadena) and 69% (Misericordia) of children belonging to the 0-10 age group did not use anything to stay on the sand, using 49.3% of the interviewees towels and 27.5% deckchairs to stay on the sand.

Table 25 shows the distribution of swimmers who use other beaches. For Benalmadena beach, 47% of bathers used another one and for Misericordia beach only 45%. As it will be noted later, this variable allows us to establish one of the main "confounding factors" to study the swimming-associated morbidity between swimmers and non-swimmers, for both enteric and non-enteric diseases.

Finally, considering the above mentioned results for both beaches studied, the "typical bather" interview presents the following characteristics: stays on the beach about 3 hours; goes to the same beach for more than 3 days; is a swimmer; stays on the beach with no contact with the sand (adults use towels and children nothing); and usually changes to another beach.

#### **4.8.3 Swimming-related health risk**

According to the objectives established in this first part of the study, the first question to be answered was which disease are swimming-related, dividing these diseases into two principal groups: enteric and non-enteric diseases, with special reference to skin diseases (dermatitis).

Tables 26 to 30 show the percentages of total symptoms studied for all bathers and beaches, indicating a preliminary view of the results. It can be seen from these Tables that skin, total enteric and otitis syndromes are the most probable symptoms with a great variety of symptoms, including cough, vomits, nausea, fever, etc., and with a clear difference for enteric diseases between Benalmadena and Misericordia beaches.

Tables 31 to 43 show the percentages of total symptoms for the 0-4-year old-age group and for all ages and by beaches. Tables 44 to 50 show the percentages of symptoms by beaches, grouped in to six classes (0, No answer/Don't know; 8, normal; 10, enteric symptoms; 11, otitis, conjunctivitis and respiratory symptoms; 12, dermatitis symptoms; and 13, urinogenital symptoms). The rates for enteric symptoms, for otitis, conjunctivitis and respiratory symptoms, for dermatitis and for urinogenital symptoms are 2.2; 2.9; 6.2 and 0.2%, respectively among swimmers.

From these results, the incidence rates per 100 persons among swimmers and non-swimmers both on Benalmadena and Misericordia beaches and for age groups of the whole sample (n=1447 ind.) were calculated (Table 51).

This preliminary analysis showed that the morbidity for enteric symptoms was higher among swimmers than among non-swimmers especially for the 0-4-age-group. Morbidity for non-enteric symptoms was similarly higher among swimmers except for otitis, conjunctivitis and respiratory symptoms on Misericordia beach, where the incidence rate was 0.2% among non-swimmers and 0% among swimmers and the 0-4 age group showing a similar behavior to the enteric symptoms.

Table 51

Incidence rates per 100 persons among swimmers and non-swimmers of 0-4 year old-age group and all ages, for selected symptoms and diseases and for Misericordia and Santa Ana beaches.

	INCIDENCE RATES PER 100 PERSONS			
	Santa Ana		Misericordia	
	Swimmers	Non-swimmers	Swimmers	Non-swimmers
<u>Age group: 0-4</u>				
No. of persons	53	6	56	5
Total enteric (%)	3.7	0	7.1	1.7
Otitis, conjunctivitis and respiratory (%)	5.6	0	0	0.2
<u>Age group: Rest ages</u>				
No. of persons	568	25	704	30
Total enteric (%)	1.4	0.2	2.1	0
Otitis, conjunctivitis and respiratory (%)	3.5	0	2.8	0
Dermatitis	3.8	0	7.8	0
Urinogenital	0.2	0	0.4	0

Table 52

Cross-tabulation of sand related health risk  
(swimmers minus non-swimmers) by sand status

SAND STATUS	SKIN DISEASES	NON-SKIN DISEASES
NK/NA	1	14
Towel	52	662
Deckchair	12	386
Nothing	25	288
Towel and deckchair	0	6
Towel and nothing	1	0

#### 4.8.4 Sand related health risk

Cross-tabulation for sand condition and skin diseases for beaches and age groups are shown in Table 52. Only bathers showed the symptoms studied, and for dermatitis there was a higher incidence among the bathers than used towels or stayed on the sand directly.

#### 4.8.5 Confounding factors related to swimming-health effects

In order to estimate the percentage of factors that can lead into error in the study, a small representative subsample was obtained from the total sample (n=1447 individuals) selecting these swimmers who showed some of the symptoms described, keeping under strict control the following variables or confounding factors:

- Confusion due to contaminated food or beverages.
- Did not visit any beach or swimming-pool a week prior to the beach interview.
- Did not have any of the morbidity symptoms under study for a week prior to the health interview.
- Allergic symptoms for dermatitis diseases.

The incidence rates corrected in this manner for this subsample range between 45% (enteric) and 40% (dermatitis) depending upon the morbidity studied, thus obtaining for the total sample (n=1447) and estimate ( $p < 0.01$ ) of the incidence rate loss due to other factors which are not directly related to the water quality.

#### 4.8.6 Sample size

Taking into account the aims of this study as well as the information obtained in the above section 8.5. for estimating the definitive size of the sample the following parameters obtained from the present pilot study or pre-test have been employed:

Table 53

Swimming-associated incidence rate (swimmers minus non-swimmers) per 100 persons for Misericordia and Santa Ana beaches, for the 0-4 year-old-age group

SYMPTOMS	SWIMMING-ASSOCIATED INCIDENCE RATE		
	Misericordia	Santa Ana	p*
Total enteric	7.1	3.7	0.05
Otitis, conjunctivitis and respiratory	0	5.6	0.001
Dermatitis	14.2	11.3	N.S.

\* Level of significance of differences of incidence rates between Misericordia and Santa Ana beaches  
N.S. No statistical difference

Table 54

Swimming-associated incidence rate (swimmers minus non-swimmers) per 100 persons for Misericordia and Santa Ana beaches, for all ages for whole samples

SYMPTOMS	SWIMMING-ASSOCIATED INCIDENCE RATE		
	Misericordia	Santa Ana	p*
Total enteric	2.8	1.6	N.S.
Otitis, conjunctivitis and respiratory	2.6	3.7	N.S.
Dermatitis	8.2	4.5	0.05
Urinogenital	0.3	0.02	N.S.

\* Level of significance of differences of incidence rates between Misericordia and Santa Ana beaches  
N.S. No statistical difference



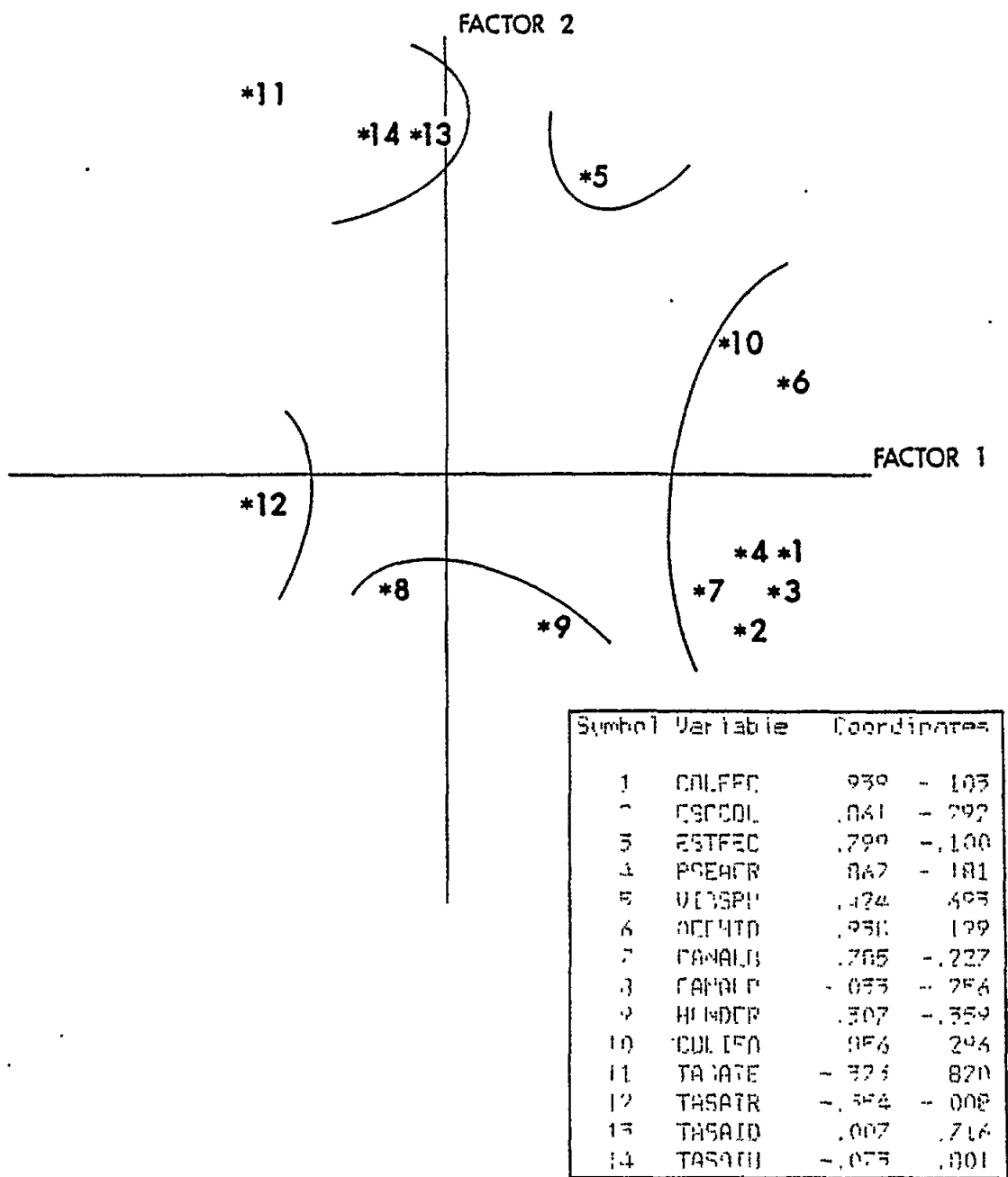


Figure 39 - Graphic representation of PCA

- 1) The incidence morbidity for the dermatitis symptoms among non-swimmers for each beach.
- 2) The significance of the incidence rate difference among swimmers as compared with non-swimmers for each beach.
- 3) Based on 5% probability of type-one error.

For dermatitis symptoms and Santa Ana beach, the incidence morbidity among non-swimmers and swimmers was about 1% and 4.5%, respectively. Considering the results of the last item, where a 40% percentage was calculated due to the different confusing factors, it can be estimated that the real incidence rate among swimmers in Santa Ana beach is ranged between 2.2 and 2.6%.

Considering these results and the tables proposed by WHO to estimate the final number of individuals in each group (swimmers and non-swimmers) so that a relative statistically significant risk can be detected ( $p < 0.05$ ), an estimated value of the minimal sample size of 3521 individuals (167 non-swimmers and 3354 swimmers) must be calculated.

For dermatitis symptoms and Misericordia beach was obtained an incidence rate among swimmers of 8.2%, and a real incidence morbidity compressing between 4.5 and 4.9%. For these results and to obtain a relative statistically significant risk ( $p < 0.05$ ) between swimmers and non-swimmers. The minimal sample size calculated is 1740 individuals (80 non-swimmers and 1660 swimmers).

#### **4.8.7 Preliminary analysis of swimming-associated morbidity by bathing beaches**

In order to study whether the swimming-associated morbidity is related to bathing water pollution swimming-associated health risk has been compared for all the study population, for bathing beaches with significance differences of bacterial indicators -Benalmadena and Misericordia beaches- Tables 53 and 54 show the incidence rates and level of significance of difference between both beaches for all age groups and for the 0 to 4 year age group.

Table 53 shows that total enteric and dermatitis symptoms were significantly swimming-associated for the "polluted" beach (Misericordia) whereas only otitis, conjunctivitis and respiratory symptoms were significantly swimming-associated for "clean" beach (Benalmadena). The incidence rates were the highest for dermatitis (8.2) on Misericordia beach.

Table 54 shows that swimming-associated morbidity total enteric and dermatitis symptoms indicated a significant difference for the 0 to 4 year age group for the Misericordia beach ("polluted"), whereas, only otitis, conjunctivitis and respiratory symptoms were significant for Benalmadena. Incidence rates were also the highest for dermatitis (14.2) on Misericordia beach.

#### **4.8.8 Relationship between the microbial concentrations (indicators and pathogens) and the presence of disease**

To test the possible relationships between the indicator and pathogenic microorganism concentrations and the presence of enteric and non-enteric diseases, a statistically multivariant technique was used, which allow us to study globally the microbiological and epidemiological data.

Initially, emphasis was placed on the use of PCA because: (i) PCA is a mathematically well defined procedure; (ii) computer programs are readily available in existing software packages; and (iii) PCA is the multivariate technique that almost directly accesses in the data and its sources (Cuadras, 1981).

The graphical representation of PCA from the results obtained in this Phase (Phase-I or Pre-Test) is exposed in Figure 39. The first and second components explained 68% of the total variation of the data (40% for the first and 28% for the second).

An examination of the variables plotted in these components revealed three clusters of variable. The first cluster grouped the variables that presented a high positive coefficient with the first axis, including the faecal indicators such as faecal coliforms, *E. coli*, faecal streptococci, and coliphages, and some pathogenic microorganisms, *P. aeruginosa*, *A. hydrophila*, *C. albicans*, and pathogenic fungi in the sand. This relationship between indicators and some pathogenic microorganisms was previously established in the correlation analysis carried out in the item 6 of this chapter, being higher the relationship in Misericordia beach than in Santa Ana.

The incidence morbidity rates for the enteric, skin and urinogenital infections presented a high positive coefficient with the second axis, related also with *Vibrio* spp. and coliphages. No relationship of this second cluster of variables is shown with the first axis, and therefore, with the microorganisms including in it. However, in general it can be seen that *Vibrio* spp. and coliphages are the microorganisms better correlated with the enteric, skin and urinogenital infections.

Other variables (incidence rates of respiratory, otitis and conjunctivitis, and concentration of *C. albicans* in sand) do not showed relationship with the first and second axis, with negative or low coefficients.

## 5. DISCUSSION

In the Consultation on Microbial Pollution of Mediterranean Coastal Areas and Associated Health Effects, convened by WHO/UNEP within the framework of MED POL Phase II in Athens (September, 1987), it was considered necessary to review the existing informations about the diseases in the Mediterranean region caused by pathogenic microorganisms and contractible through exposure to contaminated seawater and/or sand in marine coastal recreational areas. In this regard, it was also pointed out that, so far, insufficient attention appeared to have been accorded to pathogenic fungi and in the identification of indicator microorganisms for non-gastrointestinal pathogens.

In particular, attention was drawn to the need for further adequately conducted microbiological-epidemiological studies under specific conditions to the Mediterranean region and also have to take into account the conditions prevailing in the different countries. Finally, it was considered that immediate action should take the form of relatively simpler pilot studies.

The results presented in this Final Report included the data corresponding to the Phase-I or Pre-Test microbiological-epidemiological study carried out over 1988 in two beaches on the Malaga coast (Spain). For both beaches differences in the incidence rates of enteric and non-enteric infections between swimmers and non-swimmers were detected, being these differences statistically significant only for the skin infections in the age group of 0-4 years.

Misericordia beach present a high concentration of faecal coliforms, *E. coli*, faecal streptococci, *Vibrio* spp., *A. hydrophila*, and coliphages in seawater, and *C. albicans* in sand, in comparison Santa Ana beach. In same way, the concentration limits established in the guidelnes and standards for bathing seawater by WHO/UNEP, EEC and MOPU, are exceeded for Misericordia beach. However, there is not a significant difference between both beaches for the their content in *P. aeruginosa* in seawater and dermatophytic fungi in sand.

### Preliminary conclusions (pre-test)

1. The densities of faecal indicator microorganisms (faecal coliforms and faecal streptococci), *Vibrio* spp., *Aeromonas hydrophila*, and coliphages were higher at Misericordia beach than at the Santa Ana beach ( $p < 0.005$ ) due to it is located near to the sewage outfall.
2. No significant differences were found for *Pseudomonas aeruginosa*, *Candida albicans* and total fungi densities, in seawater and sand, between the beaches studied.
3. A total of 1447 persons were interviewed in this Phase I. Of the total sample, 95.4% were defined as "swimmers". The final size sample to study were defined according to: (i) incidence morbidity for dermatitis symptoms among non-swimmer; (ii) magnitude of the excess incidence among swimmer as compared with non-swimmer; and (iii) 5% probability of type-one error; resulting a estimated sample size of 3520 persons for Santa Ana beach (3355 swimmers and 165 non-swimmers) and of 1740 individuals for Misericordia beach (1660 swimmers and 80 non-swimmers).
4. The preliminary data showed that the risk of enteric symptomatology was found to be higher among swimmers than among non-swimmers for the most polluted beach for the 0-4 year old age group and for all ages.
5. There was a significant excess in the rates of dermatitis and respiratory symptoms for the 0-4 year old age group among swimmers relative to non-swimmers and all ages, but this excess was not associated with the bacterial densities in seawater.
6. No statistically significant relationship were found between the incidence of swimming-associated enteric symptom rates and faecal indicators.
7. The densities of total fungi in the sand were similar for both beaches, and no ration were found between the incidence of sand status-associated dermatitis symptom rates and dermatitis pathogens.

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Appendix 1

Questionnaires utilized in the beach  
and follow-up surveys

NATIONAL HEALTH SCHOOL. MADRID  
DEPART MENT OF MICROBIOLOGY. UNIVERSITY OF MALAGA.  
Epidemiological survey

0. Survey code .....  
1. Date: .../.../.... Time: .....  
2. Beach: ..... COD.??

3. Who is interviewed?: mother ; father ; grandparents ;  
others .....COD.  
4. Where do they come from?:.....COD.  
5. How long do they stay on the beach (in hours)?: ..?

	6. Family dates:			7. Bathing:		8. How does he/she stay?:			
	N	Relation.	Age	Sex	YES	NO	Towell	Deckchair	Nothing
1	.....								
2	.....								
3	.....								
4	.....								
5	.....								
6	.....								
7	.....								

9. Which area of the beach are they staying at?:.....

	10. Present and former symptoms:									
	N	Relation	Cold	Vomit	Nausea	Diar.	Fever	Derm.	Ears	Normal
1	.....									
2	.....									
3	.....									
4	.....									
5	.....									
6	.....									
7	.....									

(write down the number of days the symptoms last)

11. How many days has he/she been bathing on this or others beaches  
1. 2. 3. 4. 5. 6. 7.

On this beach								12. Which beach?:
On other beaches								
On swimming-pools								

13. Contact telephone  
Tf: .... Town: .....  
14. Remarks:



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 DEPART MENT OF MICROBIOLOGY. UNIVERSITY OF MALAGA.  
 Epidemiological survey. Follow-up study

To be filled by the interviewer:

0. Survey code --- Telephone : ... ..  
 1. Date of the survey on the beach .../.../...

2. Date when this survey is filled in .../.../...

3. Who answers ?: Father  Mother  Grandparents   
 Others: ..... COD.

4. On which beach have they spent more time ? : .....

5. How many days ? :

6. Has any member of the family been to any other beach or swimming-pool since the survey on the beach ?:

	1	2	3	4	5	6	7
Member of the family	...	...	...	...	...	...	...
Which beach ? :	.....						
Days	...	...	...	...	...	...	...
Has he/she swim ? :	...	...	...	...	...	...	...

7. Symptoms shown by any member of the family since the survey on the beach.

N	Relationship	Cold	Vomit	Nausea	Dia-rrhoea	Der-mat.	Ears	Normal
1	.....	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
2	.....	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
3	.....	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
4	.....	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
5	.....	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
6	.....	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
7	.....	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

8. Has he/she been to the doctors?: YES  NO  9. Where ? : .....

10. Has he/she undergone any medical?: YES  NO  11. Results: .....

12. Remarks :

## PUBLICATIONS OF THE MAP TECHNICAL REPORTS SERIES

1. UNEP/IOC/WMO: Baseline studies and monitoring of oil and petroleum hydrocarbons in marine waters (MED POL I). MAP Technical Reports Series No. 1. UNEP, Athens, 1986 (96 pages) (parts in English, French or Spanish only).
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