Programme à long terme de surveillance et de recherche
sur la pollution dans la Méditerranée
(MED POL Phase II)

LES METHODES DE SURVEILLANCE DE POLLUANTS SELECTIONNES DANS LES EFFLUENTS D'EGOUTS ET LES EAUX COTIERES A USAGE RECREATIF

Rapport sur une réunion mixte OMS/PNUE

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PREFACE

Au cours de la phase pilote du Programme coordonné de surveillance et de recherche sur la pollution dans la Méditerranée (MED/POL-phase I), executée entre 1976 et 1981, le projet sur le contrôle de la qualité des eaux côtières en Méditerranée (MED/POL VII), coordonné en commun par l'OMS et le PNUE, s'est consacré essentiellement aux paramètres bactériologiques et connexes pertinents à la surveillance des eaux côtières utilisées à des fins récréatives, des eaux utilisées pour l'élevage des crustacés et des mollusques et de la chair de ces animaux. Pendant l'exécution de ce projet pilote, à laquelle ont participé 30 laboratoires méditerranéens, les deux principales méthodes d'analyse bactériologique utilisées ont été la méthode de filtration sur membrane (MF) et celle de l'indice (MPN) (nombre le plus probable). Aucune modalité n'a été mise au point pendant l'exécution du projet pilote pour la comparaison réciproque exhaustive de ces deux méthodes.

Au cours de cette même période, des activités ont aussi été entreprises par le Programme des Nations pour l'Environnement (PNUE), en coopération avec l'Organisation mondiale de la Santé, en vue de la préparation de méthodes normalisées pour l'échantillonnage et l'analyse bactériologique et connexe. Ces méthodes sont conçues pour être utilisées par les laboratoires méditerranéens participant au programme à long terme de surveillance et de recherche sur la pollution dans la Méditerranée (MED/POL-phase 2) dans le cadre de la Convention pour la protection de la Méditerranée contre la pollution et des protocoles connexes. Ces méthodes font partie d'une série exhaustive, en préparation avec les institutions spécialisées des Nations Unies intéressées, et sont destinées à couvrir tous les paramètres possibles, énumérés dans les annexes à la convention et dans ses protocoles, mais aussi à pouvoir être utilisées dans des régions autres que la Méditerranée.

Au cours du premier semestre de 1982, quatre méthodes de référence, destinées à couvrir les principaux organismes indicateurs microbiologiques, en vue de la surveillance continue des eaux côtières à usage récréatif, des eaux d'élevage de crustacés et de mollusques et de la chair de ces animaux, ont été définitivement arrêtées, avant mise à l'épreuve, examen et révision. Ces méthodes sont les suivantes :

- numération des coliformes totaux présents dans l'eau de mer, par la méthode de filtration sur membrane;
- identification des coliformes fécaux présents dans l'eau de mer, par la méthode de filtration sur membrane;
- identification des streptocoques fécaux présents dans l'eau de mer, par la méthode de filtration sur membrane;
- recherche des coliformes fécaux présents dans les mollusques bivalves, par la méthode des tubes multiples.

Un certain nombre de laboratoires méditerranéens ont été invités à expérimenter ces méthodes dans leurs propres conditions environnementales. Par la suite, des représentants de ces laboratoires ont été invités à participer à un exercice d'interétalonnage de ces méthodes, qui a été organisé à l'Institut supérieur de la santé à Rome, les 22 et 23 novembre 1982.

Pour faciliter l'application éventuelle des méthodes de référence à d'autres régions, des représentants de trois institutions situées en dehors du bassin méditerranéen ont également été invités à participer à l'exercice d'interétalonnage (deux de la région du Plan d'action du Koweit et un de la région des Caraïbes).

Le premier projet d'une autre méthode de référence "Directives applicables à la surveillance continue de la qualité des eaux côtières utilisées à des fins récréatives et aux eaux d'élevage des crustacés et des mollusques" a également été mis au point au cours du second semestre de 1982.

Au début de 1982, un certain nombre de laboratoires des pays méditerranéens (autres que ceux qui ont participé à la mise à l'épreuve et à l'inter-étalonnage des quatres méthodes de référence microbiologiques) ont été invités à participer à un exercice de comparaison expérimentale des méthodes de filtration sur membrane (MF) et de l'indice (MPN) (nombre le plus probable), et à procéder à des recherches sur les paramètres en cause. Cette étude a été effectuée en coopération avec l'Instituto Superiore di Sanità à Rome, qui a également préparé la synthèse ainsi que l'évaluation nécessaire des résultats obtenus. Cette étude a porté sur les sources de pollution (effluents d'égoûts) ainsi que sur les eaux côtières à usage récréatif et sur des zones-témoins.

La présente réunion de consultation se proposait les objectifs suivantss :

- examiner les résultats de l'étude comparée des méthodes MF et MPN, entreprise par les différents laboratoires;

- revoir les résultats de l'étude sur les méthodes d'échantillonnage et d'analyse utilisées sur les sources de pollution (effluents des d'égoûts), les eaux côtières à usage récréatif et les zones-témoins;

- étudier les résultats de la mise à l'épreuve des méthodes de référence biologique par les laboratoires méditerranéens, ainsi que ceux de l'exercice d'inter-étalonnage, en vue d'identifier les problèmes techniques et autres, y compris ceux afférents au contrôle de la qualité et, à la lumière de ces résultats, revoir les méthodes de référence pertinentes pour les confirmer ou les réviser;

- formuler les recommandations appropriées concernant les méthodes d'échantillonnage et d'analyse relatives aux paramètres en question à l'intention du programme à long terme de surveillance et de recherche sur la pollution dans la Méditerranée (MED/POL - phase II) et d'autres activités régionales du même type.

La réunion a été organisée par l'OMS et le PNUE, en collaboration avec l'Istituto Superiore de Sanità à Rome.

On avait invité à prendre part à la réunion de consultation des représentants des institutions participant : a) à l'étude sur les méthodes d'échantillonnage et d'analyse, y compris la comparaison des méthodes MF et MPN, et b) à la mise à l'essai ainsi qu'à l'inter-étalonnage des méthodes de références bactériologiques, ainsi qu'un certain nombre de spécialistes internationaux dans ce domaine, originaires ou non de la Région européenne. En outre, les organisations et institutions internationales suivantes ont été invitées à se faire représenter : l'Organisation pour l'alimentation et l'agriculture (FAO), la Commission océanographique intergouvernementale (OIC), l'Organisation des Nations Unies pour l'éducation, la science et la culture (Unesco), l'Organisation météorologique mondiale (OMM) et l'Agence internationale de l'énergie atomique (AIEA).

1. Ouverture de la réunion (point 1 de l'ordre du jour)

La réunion avait été organisée à l'Istituto Superiore di Sanità, à Rome, du 24 au 26 novembre 1982. Y ont participé 28 conseillers temporaires originaires notamment de la Règion méditerranéen et d'autres régions. Dix pays méditerranéens et cinq pays non-méditerranéens étaient représentés, de même que le PNUE et le Bureau régional de l'Europe de l'OMS, qui ont envoyé chacun un représentant. On trouvera en annexe 1 une liste des participants.

Le Dr L.J. Saliba, Spécialiste scientifique principal due Plan d'action pour la Méditerranée, Bureau régional de l'Europe de l'Organisation mondiale de la Santé, a ouvert la réunion au nom du Dr Leo A. Kaprio, Directeur régional. Evoquant la réunion en cours, il s'est également référé aux deux exercices dans le cadre duquel celle-ci doit venir s'inscrire, dans le prolongement naturel du projet pilote sur le contrôle de la qualité des eaux côtières (MED/POL VII) exécuté entre 1976 et 1981. L'orateur a exprimé la satisfaction de l'OMS concernant les activités entreprises et les locaux fournis par l'Istituto Superiore de Sanita, tant en liaison avec la réunion de consultation proprement dite et l'exercice conjoint d'inter-étalonnage, que pour l'étude des méthodes d'échantillonage et d'analyse. Il a fait remarquer combien il était juste que la présente réunion soit organisée sur les lieux même de la série de concertations organisées tout au long du projet pilote MED POL VII.

Le Professeur L. Villa, Directeur du Département de microbiologie environnementale à l'Institut supérieur de la Santé a souhaité la bienvenue aux participants au nom du Professeur F. Pocchiari, Directeur de l'Institut. Se disant heureux de ce que l'Institut soit en mesure de poursuivre sa longue collaboration avec l'OMS dans ce domaine important, il a souhaité que la réunion soit fructueuse et couronnée de succès. Quant à l'exercice sur les méthodes d'échantillonnage et d'analyse, l'orateur a signalé que la réaction des institutions participantes est très encourageante. L'éventail des thèmes évoqués est très large, et des données utiles ont été obtenues; cependant, l'ampleur des contributions et la disparité des méthodes employées ont rendu la synthèse et la comparabilité assez malaisée.

2. Portée et objectifs de la réunion (point 2 de l'ordre du jour)

Le Dr L.J. Saliba a exposé la portée et les objectifs de la réunion. Il a décrit la situation d'ensemble à l'achèvement du projet pilote sur le contrôle de la qualité des eaux côtières (MED/POL VII) en 1981. Parmi les questions en suspens, il a signalé la nécessité de procéder à une comparaison exhaustive entre les méthodes MF et MPN, ainsi que de mettre au point des méthodes applicables à l'échantillonnage et à l'analyse des paramètres utilisés. A cet égard, l'étude réalisée par l'Istituto Superiore di Sanità, à laquelle ont collaboré différents laboratoires méditerranéens, répondait au premier de ces impératifs. Ces résultats seront examinés au cours de la réunion. Pour ce qui est des méthodes de référence, les participants à la réunion ne sont pas habilités à adopter une révision définitive. Les méthodes seront soumises à un examen aussi approfondi que possible dans des limites de temps acceptables. Les observations et suggestions des participants seront enregistrées, et il en sera pleinement tenu compte lors de la préparation des versions révisées des méthodes, qui seront alors soumises aux gouvernements des Etats méditerranéens, être adoptées dans les programmes nationaux de surveillance. Ces versions révisées elles-mêmes ne sauraient être en fait décrites commme définitives dans la mesure où elles seront continuellement remises à jour, selon les besoins.

Monsieur F.S. Civili, Spécialiste des sciences de la mer, Unité de coordination du PNUE pour le Plan d'action pour la Méditerranée, a pris la parole au nom de son unité ainsi que du Centre d'activité du Programme des mers régionales. Il a signalé que pour coordonner les composantes scientifiques des différents programmes pour les mers régionales, y compris le Plan d'action pour la Méditerranée, le PNUE a toujours accordé la priorité à l'harmonisation des méthodes d'analyse utilisées par les laboratoires participants. Dans le programme MED/POL et plus particulièrement au cours de la phase pilote, un certain nombre de méthodes communes d'échantillonnnage et d'analyse ont été recommandées et un exercice d'inter-étalonnage a été organisé concernant certains paramètres obligatoires. On souhaitait par là à aider les laboratoires à acquérir des données comparables, nécessaires pour l'évaluation d'ensemble de la situation

MED/POL est maintenant entré dans sa seconde phase, et la surveillance continue est devenue obligatoire pour tous les pays méditerranéens. Il s'ensuit que les données recueillies doivent être désormais tout-à-fait fiables et comparables, pour que le PNUE puisse évaluer l'état de pollution de la Méditeranée. En conséquence, pour assurer la comparabilité des données à l'échelle mondiale, et contribuer ainsi au Système mondial de surveillance continue de l'environnement (GEMS) du PNUE, ou est en train d'élaborer des méthodes de référence et des lignes directrices pour les études sur la pollution marine, qui ont été recommandées aux gouvernements, pour adoption. Ces méthodes et directives sont préparées par le PNUE, en coopération avec les organismes spécialisés intéressés du système des Nations Unies et ont été mises à l'épreuve par un certain nombre de spécialistes des divers domaines. Les méthodes qui font l'objet des discussions ont été élaborées en coopération avec l'OMS.

Enfin, le PNUE a l'intention de renforcer l'élément "inter-étalonnage" du programme MED/POL-phase 2, ainsi que les programmes équivalents, réalisés dans d'autres mers régionales et de soumettre ainsi toutes les données obtenues à un contrôle strict de qualité. A cette fin, des exercices analogues seront ultérieurement organisés tant en Méditerranée que dans d'autres Régions.

3. Election du bureau (point 3 de l'ordre du jour)

Le Professeur L. Villa a été porté à la présidence, le Dr S. Sotiracopoulos a été élu vice-président et M. V. Gauci, rapporteur. Le Dr L.J. Saliba a fait fonction de secrétaire de la réunion.

4. Adoption de l'ordre du jour (point 4 de l'ordre du jour)

L'ordre du jour provisoire a été adopté à l'unanimité.

5. Examen de l'exercice sur les méthodes d'échantillonnage et d'analyse (point 5 de l'ordre du jour)

Des rapports ont été présentés par un certain nombre de participants, et les données reçues avant le 15 octobre 1982 ont fait l'objet d'une synthèse par l'Institut supérieur de la santé, qui l'a présenté en séance (cf. Annexe 1 au présent rapport). Les rapports des institutions participantes sont reproduites aux Annexes 2 à 8. Les points suivants peuvent être retenus, tels que mis en évidence dans les rapports et les débats qui ont fait suite.

5.1 Nombre d'échantillons

Tout laboratoire participant à un programme de surveillance continu ne peut traiter qu'un nombre limité d'échantillons (maximum effectif dépendant des ressources disponibles dans chaque cas), il conviendrait donc de décider laquelle des options suivantes est la plus valable : a) examiner un petit nombre d'échantillons en double et en triple ou b) examiner davantage d'échantillons sans duplication. L'état de pollutin d'un écosystème marin ne saurait faire l'objet d'une évaluation sur la base de quelques échantillons seulement, aussi est-il souvent pratique de sacrifier la répétition à la nécessité d'étudier un plus grand nombre d'échantillons.

5.2 Examen du sable et des sédiments

Les participants ont jugé qu'il est essential que les méthodes recommandées soient simples et explicites, faute de quoi les résultats peuvent n'être pas comparables. Cela a été particulièrement souligné concernant l'examen de sable et des sédiments. Des éléments tels que les méthodes d'échantillonnage, la nature de l'eau de rinçage, l'époque du rinçage, de même que les procédures analytiques adoptées doivent être normalisés. On a mentionné différentes méthodes de transfert des organismes des particules de sable à la phase aqueuse, à savoir notamment l'emploi de mélangeurs, l'utilisation d'ultra-sons et le fait d'ajouter des enzymes à l'eau de rinçage.

5.3 Effets de la méthodologie sur la survie des organismes

On a également soulevé le point de savoir si certaines de ces méthodes sont de nature à porter préjudice aux organismes. La lumière est un facteur très important de mortalité des organismes indicateurs. On a signalé l'existence d'une corrélation étroite entre le nombre de bactéries présent dans l'eau naturelle et servant d'indicateurs et des éléments tels que la durée du jour et le moment de la journée. Il faut donc enregistrer ces facteurs lors des opérations d'échantillonnage. Il importe non pas seulement de normaliser les méthodes d'analyse mais aussi celles de traitement des résultats.

5.4 Organismes en état de stress

La présence dans l'environnement marin d'organismes en état de stress a été évoquée. On a signalé que certaines méthodes permettent de les ranimer, notamment la préincubation des membranes à 36°C ± 1°C pour les coliformes fécaux qui donnerait un nombre plus élevé que l'utilisation dès le départ d'une température d'incubation de 44,5°C ± 0,2°C. On a proposé en outre de recommander un milieu de conservation lorsque le point de prélèvement est éloignés du laboratoire.

La notion de stress s'applique à toute altération des processus métaboliques normaux de la cellule, y compris la dégradation du matériel génétique et des éléments constitutifs du cytoplasme. Des facteurs de stress agissant sur les organismes indicateurs, tels que : températures défavorables ou variables, présence de substances toxiques et concurrence avec un grand nombre d'organismes, sont présents dans l'environnement naturel. Le stress peut entraîner une perte temporaire des caractères biochimiques ou une prolongation de la phase de latence en cours de croissance. Des organismes en état de stress pourraient exercer tant sur les méthodes MPN que MF un effet négatif : certains coliformes fécaux peuvent ne pas produire une colonie identifiable sur la membrane ou du gaz, lorsqu'on adopté la méthode MPN. Les streptocoques fécaux sont particulièrement fragiles à cet égard. Cela pourrait être imputable à leur plus grande longévité que prolonge le contact avec les facteurs de stress. Les résultats des expériences comparées sur des cultures primaires, avec des organismes ranimés ou non i montré que l'adoption d'un procédé de "réanimation" lors de l'isolation de ces organismes indicateurs, issus d'une eau naturelle, est éminemment recommandable. Un certain nombre de méthodes de laboratoire tendent à accentuer le stress, à savoir les cultures primaires dans un milieu inhibant et les températures élevées. Il faut donc les éviter dans toute la mesure possible. Il importe d'améliorer la récupération des organismes indicateurs en état de stress, dans la mesure où les agents pathogènes stressés peuvent être aussi viables et virulents que les pathogènes non stressés. Un certain nombre de méthodes de "réanimation", dont certaines peuvent être utilisées conjointement ont été proposées pour étude ultérieure et adoption subséquente :

1) Incubation primaire à 36°C±1°C pendant deux heures, puis incubation à 44,5°C±0,2°C,

2) culture primaire en milieu non sélectif, applicable tant pour la méthode MPN que pour MF. Dans le premier cas, il est préférable de réaliser l'essai présomptif dans un bouillon de lactose, avec subculture subséquente sur milieu de bile vert-brillant Mac Conkey. Pour ce qui est de la méthode MF, la membrane peut être à l'origine cultivée sur agar-agar, puis transférée sur un milieu sélectif,

3) la méthode des plaques en couches est applicable à MF. La membrane est alors cultivée sur un milieu d'agar-agar constitué par une couche intérieure d'agar-agar sélectif, recouverte d'une couche mince d'agar-agar nutritif. Au cours des deux premières heures de l'incubation, on assiste à un processus de diffusion lente entre les deux couches d'agar-agar.

Au cours de la discussion des organismes atténués et de leur récupération, on a fait remarquer que la "réanimation" se produit aux dépens de la sélectivité et qu'en pratique, il convient de rechercher un compromis.

5.5 Les milieux

L'agar-agar doit être préféré aux tampons absorbants imbibés de milieu liquide, pour la technique MF. On a fait remarquer que certains lots de tampons absorbants contiennent des matières toxiques et qu'il convient de se servir d'un milieu d'agar-agar, susceptible d'être préparé et versé en tant que de besoin, conservé et utilisé si nécessaire, alors que les tampons saturés de milieu liquide doivent être employés le jour même de leur préparation.

5.6 Délai entre le prélèvement et l'analyse

Il a été décidé que dans tous les cas, ce délai doit être maintenu au minimum, si possible inférieur à huit heures. Pendant leur transport au laboratoire, les échantillons devraient être réfrigérés (10°C) et protégés contre la lumière. Il est en effet, avéré que la mortalité en présence de lumière peut être effectivement très élevée. On a suggéré de ne pas prélever plus d'échantillons qu'on ne peut en analyser le même jour. Lorsque cela est impossible, les prélèvements devraient être réfrigérés (4°C) et analysés dans les 24 heures. On a fait état d'une méthode spécifique pour le traitement des échantillons d'eau d'égout après 24 heures. Il s'agit de diluer l'échantillon 1 : 10 avec une solution-tampon phosphatée avant réfrigération. On a signalé que la concentration de coliformes dans l'échantillon original traité le même jour (c'est-à-dire lors du prélèvement) présente une très bonne corrélation avec l'échantillon réfrigéré dilué mis à l'épreuve le jour suivant.

5.7 Comparaison entre méthodes MPN et MF

Les recherches comparées sur le méthodes MPN et MF ont permis d'obtenir une bonne corrélation entre les données obtenues par MF pour le coliformes fécaux au niveau critique, c'est-à-dire entre 100 et 2000 coliformes fécaux/100 ml. On a toutefois fait remarquer qu'il faudrait étudier de façon plus approfondie la corrélation à la concentration significative avant de décider si une étendue d'eau déterminée convient à des usages récréatifs ou pour l'élevage de crustacés et de mollusques. Le choix de méthodes doit être fondé sur d'autres considérations, à savoir des impératifs juridiques, la nature des échantillons et le coût. Pour ce qui est du premier de ces éléments, il apparaît que, pour la plupart des pays participant, la législation en vigueur admet l'une et l'autre méthodes d'analyse. Quant au deuxième, les participants sont convenus que pour les échantillons troubles, la méthode MPN est celle qui s'impose. Il en est de même pour les échantillons fortement contaminés, vu l'anomalie qu'il y a à procéder à une dilution poussée pour ensuite concentrer les organismes sur la membrane. Pour ce qui est du coût, la situation est plus complexe. La MPN demande davantage d'intervention humaine que MF. Les coûts de main-d'oeuvre diffèrent selon les pays, aussi est-il nécessaire de procéder à un calcul des coûts sur l'une et l'autre méthodes dans chaque pays avant de prendre une décision définitive. A cet égard, on a fait état d'une détermination des coûts par le Professeur Geldreich aux Etats-Unis d'Amérique. Ses calculs très complexes pourraient servir de modèle pour les autres pays. Au cours des débats, on a fait remarquer que l'on pourrait envisager, en cas d'urgence, de réutiliser les boîtes de Petri en matière plastique, et même les membranes.

6. Revue des résultats de l'exercice d'inter-étalonnage conjoint pour les méthodes de référence (point 6 de l'ordre du jour)

La réunion a étudié les résultats de l'exercice conjoint d'inter-étalonnage entre méthodes de référence, réalisé dans les laboratoires de l'Institut supérieur de la santé à Rome, les 22 et 23 novembre 1982. On trouvera à l'Annexe 9 une description des opérations, avec des informations générales, sur les laboratoires participant, les méthodes adoptées et les résultats, y comprix ceux des analyses statistiques comprises.

Les participants sont convenus que cet exercice s'est révélé particulièrement utile et ont jugé qu'il convient d'en envisager d'autres dans le cadre du programme de surveillance.

7. Examen des méthodes provisoires de référence (point 7 de l'ordre du jour)

Les participants ont examiné les méthodes de référence provisoires pour la détermination des paramètres bactériologiques (documents : ICP/RCE 211(2)/7, 8, 9, 10 et 11). Avant la discussion générale, les participants ont fait rapport sur les résultats des expériences réalisées avec ces méthodes dans les conditions d'environnement locales. En outre, des rapports écrits ont été envoyés par le Laboratoire d'hygiène du milieu de l'Ecole médicale Hadassah à Jérusalem et le Laboratoire départemental de santé publique à Marseille. A la suite des débats, un certain nombre de modifications des méthodes provisoires de référence on été proposées.

7.1 Méthode de référence N°1 - Directives applicables à la surveillance continue à la qualité des eaux côtières à usage récréatif pour élevage de crustacés et de mollusques

Le projet, pour cette méthode de référence, en est à un stade préliminaire et de nouveaux éléments importants doivent encore y être introduits. On a suggéré que les participants pourraient utilement se borner à formuler des suggestions générales sur la modification du texte actuel et le contenu de la version définitive. Toute fois, les suggestions spécifiques seront également bien accueillies. Il a été admis que les parties pertinentes du texte seront modifiées pour faire une place aux normes applicable aux eaux d'élevage de crustacés et de mollusques et que l'évaluation des résultats (c'est-à-dire l'analyse statistique) sera également prise en compte, soit dans les directives elles-mêmes, soit dans un document distinct. Parmi les points importants de la discussion qui a fait suite, on peut signaler :

Définition des sites de prélèvement

Les prélèvements devraient être réalisés en zone de contact. Ainsi, pour l'eau, on a considéré qu'une profondeur maximum de 1,5 m est le plus appropriée. On a également suggéré d'analyser le sable et les sédiments. On a fait remarquer que les contacts avec le sable (bains de soleil, etc.) sont en général plus prolongés qu'avec l'eau. Les sédiments et plus particulièrement l'interphase sédiments/eau revêtent une importance particulière. On peut en règle générale y isoler davantage de virus et de salmonelles que dans l'eau surjacente. Des méthodes spéciales ont été mises au point aux Etats-Unis d'Amérique pour l'examen de l'interphase air/eau.

Surveillance continue des organismes autres que les espèces-indicateurs

En dehors des coliformes totaux, des coliformes fécaux et des streptocoques fécaux, différents micro-organismes présentent une certaine importance et notamment certains organismes pathogènes. Peut-être conviendrait-il de leur accorder davantage d'attention que dans le passé. On a remarquer que les champignons pourraient constituer de meilleurs paramètres pour la surveillance continue des eaux tropicales et sub-tropicales à usage récréatif.

Paramètres devant faire l'objet d'une surveillance

Il y a été signalé que le programme à long terme de surveillance continue MED/POL ne fait état que d'un paramètre microbiologique obligatoire : les coliformes fécaux. Certains participants ont cependant fait remarquer sans ambiguïté que cela ne signifie en aucune façon que d'autres paramètres soient sans intérêt et ne peuvent pas faire l'objet de recherches. On a remarquer que les coliformes fécaux sont un bon paramètre pour la surveillance des bactéries entéro-pathogènes, tandis que les streptocoques fécaux peuvent être considérés comme un paramètre susceptible de corroborer les conclusions acquises. On sait que les streptocoques fécaux sont plus persistants dans l'environnement marin, de sorte que l'on pourrait les considérer comme des indices d'une pollution plus ancienne. En outre, Streptococcus faecalis var. liquefaciens est notoirement doté d'ubiquité et est déversé en nombre important par les industries alimentaires. On a rapporté pour l'eau de mer une corrélation étroite entre streptocoques fécaux et virus entéro-pathogènes. On dénombre davantage de streptocoques fécaux en milieu KF qu'avec un milieu M-enterococcus, avec incubation dans l'un et l'autre cas, à 36°C. Cela a été attribué à une meilleure récupération des entérocoques issues d'animaux de ferme. On sait qu'il s'agit là d'une source importante de pathogènes humains, et l'on a donc considéré qu'il importe essentiellement de soumettre également à surveillance continue des indicateurs dont on sait qu'ils proviennent de cette source. A cet égard, le milieu KF est donc supérieur au milieu M-enterococcus.

7.2 Méthode de référence N°2 - Numération des coliformes totaux présents dans l'eau de mer par la méthode de filtration sur membrane

Les amendements ci-après ont été proposés :

1) Il convient d'utiliser un incubateur à air à 36°C±1°C;

2) utiliser un milieu M-endo-agar-agar. Après 24 h d'incubation, des colonies rouge à rose, avec un reflet métallique doré sont dénombrés sur les membranes comportant 20 à 80 colonies au total. Inutile de compter les autres colonies;

3) confirmation non systématique; seulement au début et en cas de doute;

4) Bouillon Mac Conkey et Bouillon BGB doivent être utilisés pour la confirmation. Incubation à 36°C±1°C pendant 48 heures.

5) pour ce qui est de l'expression des résultats, celle-ci devrait être simplifiée en arrondissant les dénombrements à deux chiffres significatifs;

6) une culture de bouillon peut être utilisée pour le Test d'estimation de précision.

7.3 Méthode de référence N°3 - Numération des coliformes fécaux présents dans l'eau de mer par la méthode de filtration sur membrane

Les modifications suivantes sont proposées :

1) Utiliser de l'agar-agar M-FC;

2) n'utiliser de l'acide rosolique qu'en cas d'interférence par des organismes non coliformes;

3) utiliser des boîtes de Petri de 5 cm. Les faire incuber dans des récipients rigides et étanches en métal ou en matière plastique, entièrement immergés ou au bain-marie, de sorte que la température d'incubation soit 44,5°C±0,2°C;

4) après 24 h d'incubation, les colonies bleues sont dénombrées sur les membranes présentant 20 à 80 colonies au total. Inutile de dénombrer les colonies qui ne sont pas bleues;

5) il n'y a pas lieu de rechercher systématiquement une confirmation, sauf au début pour s'assurer que l'on dénombre effectivement des coliformes fécaux. Par la suite, la confirmation doit être recherchée en cas de doute sur l'identité des organismes en fonction de la morphologie de la colonie;

6) confirmation par sous-culture sur Bouillon de Mac Conkey ou Bouillon de bile vert brillant, incubation à 44,5°C+0,2°C pendant 24 h;

7) pour ce qui est de l'expression des résultats, celle-ci devrait être simplifiée (arrondir les dénombrements à deux chiffres significatifs);

8) une culture de bouillon peut être utilisée pour le Test d'estimation de précision.

7.4 Méthode de référence N°4 - Détermination des streptocoques fécaux présents dans l'eau de mer par la méthode de filtration sur membrane

Le amendements suivants ont été proposés :

1) utiliser un incubateur à air à 36°C±1°C;

2) employer de l'agar-agar KF. Le milieu doit être d'abord porté à ébullition pour dissoudre l'agar-agar, puis refroidi à environ 50°C, stérilisé par filtration sur membrane de porosité 0,2 um, ajouter TTC et verser; l'indicateur TTC est instable à la chaleur et à la lumière;

3) l'expression des résultats devrait être simplifiée (arrondir les dénombrements à deux chiffres significatifs);

4) une culture de bouillon peut être utilisée pour le Test d'estimation de précision.

5) confirmer s'il y a lieu.

7.5 Méthode de référence N°5 - Détermination des coliformes fécaux présents dans les mollusques bivalves par la méthode MPN

1) Utiliser pour le test de présomption du Bouillon de lactose incubé à 36°C±1°C. Les tubes positifs font l'objet d'une sous-culture sur Bouillon de Mac Conkey ou Bouillon BGB et milieu acqueux à la tryptone, puis incuber au bain-marie à 44,5°C±0,2°C;

2) utiliser comme diluant une solution de phosphate tamponée ou de l'eau peptonée à 0,1%;

3) simplifier les calculs concernant la subdivision de l'échantillon;

4) récrire le tableau MPN de façon à ce qu'il soit exprimé en grammes d'échantillon et non pas en volume.

Il a été décidé que la prochaine version de la méthode de référence N°1 devrait refléter les points généraux et spécifiques évoqués en cours de discussion. On a, de même, décidé que les amendements proposés devraient être incorporés aux versions définitives des méthodes de référence 2, 3, 4 et 5. On trouvera en annexe 12 les grandes lignes de ces quatre méthodes, telles que décidées par les participants.

8. Action future et recommandations (point 8 de l'ordre du jour)

Les participants ont examiné la surveillance continue des aspects sanitaires, dans le cadre de la phase à long terme de MED/POL. En sus des recommandations spécifiquement formulées à l'égard des divers points de l'ordre du jour décrits ci-dessus, les participants ont élaboré les recommandations ci-après :

1) Il conviendrait d'inclure parmi les activités réalisées au titre de la composante "recherche" de MED/POL : a) mise au point de méthodes relatives aux organismes pathogènes et b) étude des facteurs susceptibles d'affecter les résultats (temps de prélèvement, etc.) pour les organismes-indicateurs actuellement surveillés;

2) il est essentiel de procéder à des études destinées à donner une certaine garantie de qualité, et il importe de garantir que les résultats obtenus soient comparables. A cette fin, il est nécessaire de procéder périodiquement à des exercices d'inter-étalonnage. Ces exercices, sur le modèle de celui qui avait été organisé à Rome les 22 et 24 novembre 1982, devraient être réalisés dans des laboratoires centralisés, tant à l'échelle des pays qu'entre pays. Les participants devraient travailler en stricte indépendance, mais chaque numération devrait être effectuée par plusieurs participants;

3) on pourrait aussi envoyer aux laboratoires des milieux et des cultures d'organismes normalisés, et et les participants ont été invités à procéder à trois ou quatre déterminations;

4) il conviendrait de procéder à l'étude des rapports existant entre concentration bactérienne et transmission de la maladie. A cet égard, le participants ont jugé que de telles études sont difficiles compte tenu du nombre d'éléments en cause;

5) une surveillance et un contrôle précis des plages doivent être associés à la surveillance microbiologique;

6) il conviendrait de multiplier les réunions de chercheurs prenant part au programme. A cet égard, les participants ont jugé que l'un des facteurs les plus notables du succès du projet pilote MED/POL VII réside dans la réunion régulière des principaux chercheurs.

ANNEXES

Annex 1

A STATISTICAL ANALYSIS OF THE RESULTS OF THE INTER-LABORATORY EXERCISE ON SAMPLING AND ANALYTICAL METHODS

by
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1. Introduction

This report is a statistical analysis of the data received from Mediterranean institutions participating in the inter-laboratory exercise on the comparison of the membrane filtration (MF) and the most probable number (MPN) methods in determination of bacterial concentrations in coastal water quality monitoring.

Reports were received from the following institutions:

(1) Institut national de la Santé et de la Recherche médicale, Nice, France
(2) The A. Felix Public Health Laboratory, Tel-Aviv, Israel
(3) Institut Pasteur de Tunis, Tunisia
(4) Rudjer Boskovic Institute, Rovinj, Yugoslavia
(5) Istituto Superiore di Sanità, Rome, Italy.

The following data are summarized in Table 1:

- sampling periods
- sampling points
- total number of water, sand and/or sediment samples analysed by each institution
- parameters monitored
- parameters proposed prior to the commencement of the exercise.

Data concerning the methods utilized by each participating institution are summarized in Table 2. In this context, a number of difficulties arose in the comparison of data collected, due to differences in incubation temperature, incubation time and culture media utilized. Detailed analyses of the method and data interpretation techniques used by individual laboratories are given in the relevant reports.

2. Methodology

In the statistical treatment of material, only quantitative data were considered. In particular, sets of data corresponding to specific localities and data including one or more values expressed as "higher than (>)" were omitted for statistical computation purposes.

Log-transformed data were used in correlation analysis, to obtain a Gaussian-type statistical distribution (1). Parametric statistical methods were used. Analysis was initially limited to the parameters monitored by the majority of participating laboratories.

In order to verify the relationship between the most probable number (MPN) and membrane filtration culture (MF) methods in the determination of faecal coliform (FC) concentrations, different blocks of contamination levels were considered, and correlation analysis performed for each block.

3. Results and discussion

Computed correlation matrices for those parameters monitored by participating laboratories are given in Figures 1 to 5 (W = water, S = sand). In general, a highly significant correlation was found between the MPN and MF values when considering the same parameter and the same seawater sample.

Values for total coliforms (TC) and faecal coliforms (FC) showed a high level of correlation between the two techniques (MF and MPN) in the same water samples. Concentrations of faecal streptococci (FS), where this particular parameter was measured, were generally correlated to TC and/or FC levels.

Data regarding the FC content of sediments appeared to be generally correlated to data on the FC content of the corresponding water samples.

Intra-station contamination variability was observed to range up to a maximum of three orders of magnitude in some cases. A variability of one to two orders of magnitude was very frequent. This variability may be explained as mainly the result of variations in (a) pollutant dispersion phenomena and (b) meteorological patterns. A minor part of it may be due to random errors in the various measurements.

Compared to the overall variability, the differences between the MF and MPN methods were practically negligible (Figure 6).

A comparison of the data obtained from (a) slightly polluted localities and (b) heavily polluted localities indicated that in the presence of a consistent pollution source, all parameters tended to register a corresponding increase. This would account for the correlation levels given above.

The results of the correlation analysis for blocks of contamination levels are given in Appendix 1. Preliminary conclusions which may be drawn from these results are the following.

In the first place, when the contamination level is very low or practically negligible (< 10 FC/100 ml) the two parameters - FC (MPN) and FC (MF) are not correlated. This may be due to the proximity to analytical thresholds and to the consequent low degree of precision, as well as to a real lack of relationship between the parameters in question in this particular range. Results could also be influenced by the limited extension of the range.

Secondly, in considering values higher than 10 FC/100 ml, a highly significant correlation emerges.

Thirdly, values lower than 100 FC/100 ml also correlate, although if the 1-10 FC/100 ml and 10-100 FC/100 ml levels are considered separately, no correlation is evident between the two subsets. Obviously, consistent variations of the contamination level may be appropriately detected in both parameters within the 1-100 FC range. Agreement between them is limited if small variations are considered.

4. Conclusions

In general, despite the remarkable intra-site variability, overall results showed that contaminated and non-contaminated sites can be appropriately distinguished and classified if a reasonable number of analytical determinations are available. The single datum is not usually significant for this purpose. An experimental design, based on an adequate assessment of intra-site variability, would be useful in the programming of seawater and sediment surveys aimed at the detection of prefixed inter-site differences.

It may be reasonably assumed that the MPN and MF methods are both adequate for distinguishing between contaminated and non-contaminated sites. For practical purposes, either method could be adopted, and selection of either method could be based mainly on operational reasons. In any case, if the random statistical fluctuations of values measured in the same sites are considered, the differences between the two methods appear to be negligible from the general point of view. The problem as to which of the two is the more reliable method may occur when particular studies are required, i.e. when the sites under examination are on the borderline from the point of view of bacteriological acceptability.

6. Acknowledgements

The authors are grateful to Mr E. Bianchi and Mr F. Fuglieni for their technical contribution to this study.

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Appendix 1

In order to verify the relationship between FC MPN and FC MF methods, different blocks of contamination levels have been considered, and correlation analysis has been carried out for each block (Figure 7). The following results have been obtained:

	Block of data	R	N	P
1	MPN $\leq$ 10 and MF $\leq$ 10	.29	41	n.s.
2	10 < MPN $\leq$ 100 and 10 < MF $\leq$ 100	.06	40	n.s.
1 + 2	MPN $\leq$ 100 and MF $\leq$ 100	.65	81	.001
3 + 4	MPN > 100 or MF > 100	.844	43	.001
4	MPN > 1000 or MF > 1000	.795	26	.001
2 + 3 + 4	MPN > 10 or MF > 10	.893	83	.001

Some simple preliminary conclusions may be drawn.

When the contamination level is very low or practically negligible (< 10 FC/100 ml), the two parameters, FC (MPN) and FC (MF) are not correlated.

This result may be due to the proximity to analytical thresholds and to the consequent low degree of precision, as well as to a real lack of relationship between the parameters considered, in this particular range. The limited extension of the range may also influence this result.

If values higher than 10 FC/100 ml are considered, a highly significant correlation is found.

Moreover, values lower than 100 FC/100 ml correlated too. If 1-10 FC/100 ml and 10-100 FC/100 ml intervals are considered separately, no correlation is found in the two subsets.

Evidently, consistent variations of the contamination level may be appropriately detected by both parameters in the 1-100 FC range. The agreement between them is limited, if small variations are considered.

In any case, it may be reasonably assumed that both MPN and MF methods are adequate for distinguishing contaminated and non-contaminated sites. As a rule, for practical general purposes, both methods may equally well be adopted. The selection of the MPN or MF method may be based mainly on operative reasons. In any case, if the random statistical fluctuations of values measured in the same sites are considered, the differences between the two methods appear in general negligible. The problem of which method is more reliable may be posed when particular studies are required, for instance on slightly contaminated sites.

Table 1

Results of Inter-Laboratory Exercise on Sampling and Analytical Methods

Participating institute	Testing period	No. of sampling points	Total samples	Water samples	Sand and sed. samples	Bact. parameters (water)	TC water		TC water		FC water		TC sand sed.		FC sand sed.		FS sand sed.		Other parameters
							MPN	MF	MPN	MF	MPN	MF	MPN	MF	MPN	MF	MPN	MF	
INSERM, Nice, France	14.6 - 10.8	3	18	18	-	FC, FS	-	-	+	+	+	+	-	-	-	-	-	-	
A. Felix P.H. Lab., Tel-Aviv, Israel	25.5 - 1.8	3	32	16	16	TC, FC, FS	+	+	+	+	+	+	+	+	+	+	+	+	Wt°, DO, BOD_5
Inst. Pasteur, Tunis, Tunisia	15.5 - 15.8	3	72	36	36	TC, FC	+	-	+	+	-	-	+	-	+	+	-	-	
Rudjer Bosk. Inst., Rovinj, Yugoslavia	20.5 - 29.7	3	36	18	18	TC, FC, FS	+	+	+	+	+	+	-	-	+	-	-	-	BOD_5
ISS, Rome, Italy	18.5 - 31.8	3	40	24	16	TC, FC, FS	+	-	+	+	-	+	+	-	+	-	-	+	At°, Wt°, DO, pH
Methods Arrangement prepared by ISS, April 82	1.5 - 31.7	3	48	24	24	TC, FC, FS	+	+	+	+	+	+	-	-	+	-	-	-	BOD_5, COD, Tgg Tot.Nitr., Amm. Nitr., Org.Nitr. Tot.Phosph.

At° = air temperature (°C); Wt° = water temperature (°C); DO = dissolved oxygen.

TC = total coliforms; FC = faecal coliforms; FS = faecal streptococci.

Table 2

Bacteriological methods

Institute		Total coliforms			Faecal coliforms				Faecal streptococci			
		MPN		MF	MPN		MF		MPN		MF	
		Pres. test	Conf. test		Pres. test	Conf. test	Pres. test	Conf. test	Pres. test	Conf. test	Pres. Test	Conf. test
S.C. 11	Media	---	---	---	lcC. Br.	Eau pept.	m-FC-agar	EMB/ALVBRF	Az.Dx.Br.	EVA Br.	KF-agar	m-enter.agar
INSERM	Time (h)	---	---	---	48	24	24	24	48	48	48	48
Nice, France	Temperature (°C)	---	---	---	44	44	44	44	44	44	44	44
A. Felix	Media	Lact. Br.	BGBB	m-Endo agar LES	Lact. Br.	EC medium	MF Direct m-FC-agar	MF Resusc. LTB	Az.Dx.Br.	EVA Br.	MF-Direct KF-agar	MF-Resusc. TSB
Publ. Health Lab.	Time (h)	48±3	48±3	22	48±3	24	22	2-2 1/2	48±3	48±3	48±3	2-2 1/2
Tel-Aviv, Israel	Temperature (°C)	35±0.5	35±0.5	35±0.5	35±0.5	44.5±0.2	44.5±0.2	35±0.5	35±0.	35±0.5	35±0.5	35±0.555
								m-FC-agar 44.5x22 h				KF-agar 35±0.5x48 h
Institut Pasteur de Tunis	Media	McC. Br. Lact. Br. BGBB	BGBB	Endo agar (Merieux)	McC. Br. Lact. Br. BGBB	BGBB Eau pept.	m-FC-Agar	Not included in Tunis report				
Tunisia	Time (h)	---	---	---	---	---	---					
	Temperature (°C)	37	37	37	37	44	44					
Rudjer-Boskovic Institute	Media	McC. Br.	---	m-Endo agar	McC. Br.	McC. Br.	m-FC-Agar	Az.Dx.Br.	BCP Az.Br.	KF-Agar		
	Time (h)	24	---	24	24	24	24	48	48	48±3		
Rovinj, Yugoslavia	Temperature (°C)	36±1	---	36±1	36±1	44.5±0.2 (gas+indole)	44.5±0.2	36±1	36±1	44.5±0.2		
Institute Superiore di Sanità	Media	Lact. Br.	BGBB	---	Lact. Br.	EC Medium	m-FC-Agar	---	---	KF-Agar		
	Time (h)	48±3	48±3	---	48±3	24	24	---	---	48±3		
Rome, Italy	Temperature (°C)	36±1	36±1	---	36±1	44.5±0.2	44.5	---	---	44±0.2		
Analytical methods (arrangement proposed by Istituto Superiore di Sanità in April 1982)	Media	Lact. Br. or McC. Br.	BGBB	m-Endo agar	Lact. Br. or McC.Br.	EC Medium	m-FC-Agar	Az.Dx.Br.	EVA Br.	KF-Agar		
	Time (h)	48±3	48	24	48	24	24	48±3	48	48±3		
	Temperature (°C)	36±1	36±1	36±1	36±1	44.5±0.2	44±0.2	36±1	36±1	44±0.2		

Lact. Br. = Lactose Broth
BGBB = Brilliant Green Lactose Bile
McC. Br. = MacConkey Broth
Eau pept. = Peptone water
ALVBRF = Agar-lactose-Brilliant Green Phenol-Red
Az.Dx.Br. = Azide Dextrose Broth
EVA Br. = Ethyl Violet Azide Broth
LTB = Lauryl Tryptose Broth
BCPAz. Br. = Bromcresol Purple Azide Broth
TSB = Trypticase Soy Broth

Figure 1

Correlation matrices for determining bacterial concentration

Data from I.N.S.E.R.M. (France)

	$T.C._W$ (MPN)	$T.C._W$ (MF)	$F.C._W$ (MPN)	$F.C._W$ (MF)	$F.S._W$ (MPN)	$F.S._W$ (MF)	$F.C._S$ (MPN)
T.C.(MPN) W	--	--	--	--	--	--	--
T.C. (MF) W	--	--	--	--	--	--	--
F.C.(MPN) W	--	--	1	.841	.825	.832	--
F.C. (MF) W	--	--	.841	1	.796	.790	--
F.S.(MPN) W	--	--	.825	.796	1	.917	--
F.S. (MF) W	--	--	.832	.790	.917	1	--
F.C. (MPN) S	--	--	--	--	--	--	--

N = 18

Figure 2

Data from A. Felix Public Health Laboratory (Israel)

	$T.C._W$ (MPN)	$T.C._W$ (MF)	$F.C._W$ (MPN)	$F.C._W$ (MF)	$F.S._W$ (MPN)	$F.S._W$ (MF)	$F.C._S$ (MPN)
T.C.(MPN) W	1	.949	.949	.947	.604	.700	.905
T.C. (MF) W	.949	1	.975	.989	.659	.719	.868
F.C.(MPN) W	.949	.975	1	.971	.637	.660	.899
F.C. (MF) W	.947	.989	.971	1	.713	.739	.859
F.S.(MPN) W	.604	.659	.637	.713	1	.807	.430
F.S. (MF) W	.700	.719	.660	.739	.807	1	.533
F.C. (MPN) S	.905	.868	.899	.859	.430	.533	1

N = 16

Figure 3

Correlation matrices for determining bacterial concentration

Data from Institut Pasteur de Tunis (Tunisia)

	$T.C._W$ (MPN)	$T.C._W$ (MF)	$F.C._W$ (MPN)	$F.C._W$ (MF)	$F.S._W$ (MPN)	$F.S._W$ (MF)	$F.C._S$ (MPN)
T.C.(MPN) W	1	--	.722	.706	--	--	.241
T.C. (MF) W	--	--	--	--	--	--	--
F.C.(MPN) W	.722	--	1	.493	--	--	.340
F.C. (MF) W	.706	--	.493	1	--	--	.042
F.S.(MPN) W	--	--	--	--	--	--	--
F.S. (MF) W	--	--	--	--	--	--	--
F.C. (MPN) S	.241	--	.340	.042	--	--	1

N = 29

Figure 4

Data from R. Boskovic Institute (Yugoslavia)

	$T.C._W$ (MPN)	$T.C._W$ (MF)	$F.C._W$ (MPN)	$F.C._W$ (MF)	$F.S._W$ (MPN)	$F.S._W$ (MF)	$F.C._S$ (MPN)
T.C.(MPN) W	1	.950	.977	.962	.964	.974	.804
T.C. (MF) W	.950	1	.956	.986	.909	.975	.800
F.C.(MPN) W	.977	.956	1	.967	.955	.978	.811
F.C. (MF) W	.962	.986	.967	1	.912	.988	.808
F.S.(MPN) W	.964	.909	.955	.912	1	.932	.810
F.S. (MF) W	.974	.975	.978	.988	.932	1	.798
F.C. (MPN) S	.804	.800	.811	.808	.810	.798	1

N = 18

Figure 5

Correlation matrix for determining bacterial concentration

Data from Istituto Superiore di Sanità (Italy)

	$T.C._W$ (MPN)	$T.C._W$ (MF)	$F.C._W$ (MPN)	$F.C._W$ (MF)	$F.S._W$ (MPN)	$F.S._W$ (MF)	$F.C._S$ (MPN)
T.C.(MPN) W	1	--	.931	.883	--	.425	.417
T.C. (MF) W	--	--	--	--	--	--	--
F.C.(MPN) W	.931	--	1	.892	--	.422	.488
F.C. (MF) W	.883	--	.892	1	--	.454	.406
F.S.(MPN) W	--	--	--	--	--	--	--
F.S. (MF) W	.425	--	.422	.454	--	1	.303
F.C. (MPN) S	.417	--	.488	.406	--	.303	1

N = 25

Figure 6

Statistical analysis of MPN and MF methods

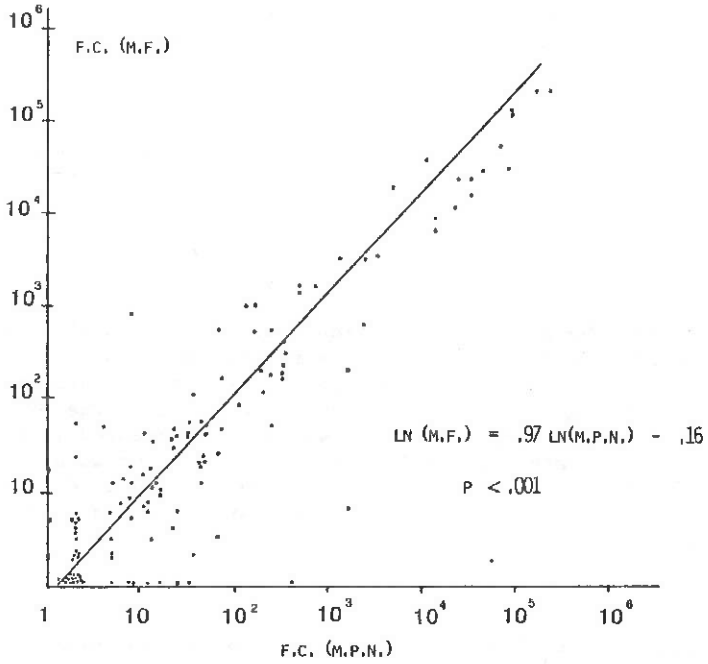
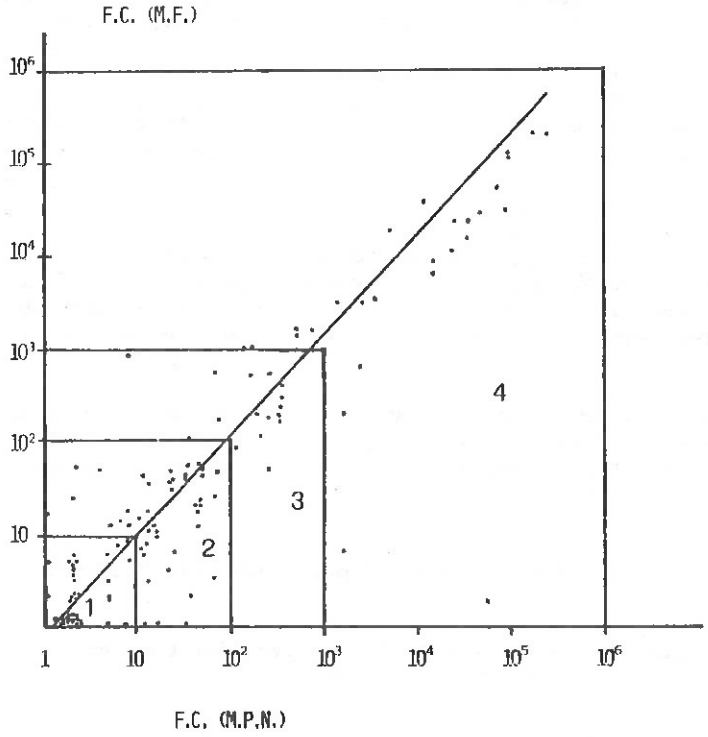


Figure 7

Correlation analysis of blocks of contamination levels



Annex 2

AN EVALUATION OF SOME DIFFERENT METHODS FOR ENUMERATION OF FAECAL COLIFORMS FROM WATER (MOST PROBABLE NUMBER AND MEMBRANE FILTRATION TECHNIQUES)

by
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1. Introduction

The basic methods for the assay of pollution indicators - total coliforms (TC), faecal coliforms (FC) and faecal streptococci (FS) in water are outlined by the American Public Health Association (APHA) (1). These include the multiple tube, or most probable number (MPN) technique and the membrane filter (MF) procedure. Since the acceptance of the MF procedure for the isolation of these indicators, conflicting reports (2, 3, 4, 5) have appeared in the literature regarding the use of membrane filters as a method of evaluating the quality of water. The present study was carried out to determine the efficiency of two modified MF techniques, the LES two-step two-day procedure proposed by Stevens et al. (6) and the two-layer membrane filter procedure proposed by Rose et al. (7) for FC determination.

2. Materials and methods

A total of 110 seawater samples (collected from the Alexandria area) according to the method described by APHA (1) were analysed in the present study.

The MPN procedure was performed by culturing a series of three decimal dilutions per sample, using five tubes for each dilution. Lactose Broth was used for the presumptive test, and FC medium at 44.5°C±0.5°C (water bath) was used for the confirmatory test. The MPN of FC per 100 ml was calculated from the positive EC broth using McCrady's probability tables.

In the MF procedures for FC, two duplications of each sample dilution were filtered through 0.45 µ membrane filters (MILLIPORE) for each bacterial test. One set of membranes was placed on plates of the two-layer medium (bottom layer: 37% M-FC, 1.5% agar in distilled water; top layer: 0.3% beef extract, 0.5% peptone, 0.5% lactose and 1.5% agar in distilled water). These plates were then incubated at 35°C for two hours, then at 44.5°C for 22-24 hours in watertight plastic bags in a water bath.

The other membrane sets were placed on LES minimal holding agar medium (0.05% tryptose, 0.05% dextrose, 0.05% lactose, 0.05% oxgall, 0.04% sodium chloride and 1.5% agar in distilled water). These plates were incubated at 25°C for 18 hours; the membranes were then transferred to absorbent pads in tightly sealed Petri dishes that had been saturated with M-FC Broth and incubated at 44.5°C for 24 hours.

In both MF procedures, the plates which gave 20-60 colonies per membrane were counted, and counts per 100 ml were calculated. Faecal coliform colonies were identified by their blue coloration and the crystallized deposits on their surfaces with the aid of a stereomicroscope.

3. Results

Since FC densities covered many orders of magnitude, all data were expressed and analysed as logarithms (base e).

The results obtained by the different methods used for the detection of the frequency of FC are shown in Tables 1 to 3, comparing the two methods.

Table 1 shows a comparison of the frequency of FC counts/100 ml using the MPN and the LES methods. Out of 110 water samples, 14 (13%) gave FC counts of 1-99, 53 (48%) gave counts of 100-999 and 43 (39%) gave counts of 1000 or more by the MPN method. The corresponding figures for the LES method were 0 (0%), 31 (28%) and 79 (72%) respectively.

The data presented in Table 2 show the frequency of FC counts/100 ml detected by the MPN method as compared to the two-layer method. Out of 110 water samples, 14 (13%) yielded FC counts of 1-99, 53 (48%) yielded counts of 100-999 and 43 (39%) yielded counts of 1000 or more by the MPN method. The corresponding figures for the two-layer method were 2 (1.8%), 37 (34%) and 71 (65%) respectively.

Table 3 presents a comparison of the frequency of FC counts/100 ml detected by the two-layer and the LES methods. Out of the 110 water samples 2 (1.8%) yielded FC counts of 1-99, 37 (34%) yielded counts of 100-999 and 71 (65%) yielded counts of 1000 or more by the two-layer method. The corresponding figures by the LES method were 0 (0%), 31 (28%) and 79 (72%) respectively.

The following results can be computed from these prepared tables considering the combination of the two methods. FC counts of 1-99 were found only in one sample (0.9%) by both the MPN and the two-layer method, counts of 100-999 were found in 25 (23%) by the MPN and the two-layer method, 20 (18%) by the MPN and LES, and 29 (26%) by the two-layer method and LES. The corresponding figures for counts of 1000 or more were 43 (39%), 43 (39%) and 71 (65%) respectively.

A synthesis of the results shown in Tables 1 to 3, to show the agreement between any pair of the methods performed, is given in Table 4.

The percentage of agreement was lowest between the MPN and the LES methods (57%), followed by that between the MPN and the two-layer method (63%). The difference between these figures was not statistically significant ($z = 0.9$, $P < 0.05$). The highest agreement was between LES and the two-layer methods (91%). The difference between this figure and both the above figures was statistically significant ($z = 5.2$ and 6 respectively, $P < 0.05$). Results of the statistical analysis of the data are shown in Figures 1 to 3.

Graphical presentation of the logarithms of the observed values of the LES and the two-layer methods as well as the regression line with zero intercept using the logarithmic transformation for FC counts in both methods are shown in Figure 1. This graph indicates that the fitted line is a good predictor of the relation between these two methods. The regression line with zero intercept was calculated by the method of least squares. This method fits data to an optimized line by the equation $y = bx$, where b is the regression coefficient or slope, y is the dependent variable, and x is the independent variable. Using this technique, the simple regression equation was found to be: $y = 0.91x$ in which y = FC counts/100 ml by the two-layer method and x = FC counts/100 ml by the LES method.

Comparison of the regression line with the equality line, which has a regression coefficient or slope of 1.0 and which could result if the means of the two methods were the same, shows that the regression line lies below the equality line indicating that the LES was superior to the two-layer method.

In both Figures 2 and 3 the regression lines lie below the equality lines indicating the superiority of both the two-layer and the LES methods over the MPN one.

Table 5 shows the geometric mean, arithmetic mean, standard deviation and coefficient of variation of the FC counts/100 ml as detected by the MPN, two-layer and LES methods. The highest geometric mean was found to be that of the LES method (2170) and the lowest value was obtained by the MPN method (545). The geometric mean of the two-layer method was found to be 1135. The calculated ratio of the geometric mean of FC counts using the LES method to that using the MPN method was 3.98:1, that of the two-layer method to the MPN was 2.08:1, and that of the LES method to the two-layer method was 1.9:1.

The coefficient of variation shows the degree of precision in a method of bacterial enumeration, the results with the lowest coefficient of variation being the most precise. In this study the LES method had the lowest coefficient of variation (107) whereas the MPN had the highest one (159). The coefficient of variation for the two-layer method was 140.

4. Discussion and conclusion

The results of this study revealed that the LES method enhanced FC recovery, and had a higher degree of precision than the MPN method. Higher counts were obtained in 47 of the samples examined (43%) with a geometric mean of 2170 and a coefficient of variation of 107. The MPN data reflected a geometric mean of 545 and a coefficient of variation of 159. The ratio of FC counts of the LES method to the MPN method based on the geometric mean was 3.98:1. The highest faecal coliform recovery by the LES method as compared to the MPN method in the present study might be attributed:

(1) to the use of MILLIPORE HC brand of membrane filters, described by Green et al. (8) and Tobin & Dutka (9) as the most efficient one in retaining the bacteria present in the tested water;

(2) to the use of the enriched medium (minimal holding agar) which is easily attacked and metabolized by the faecal coliforms and foster bacterial cell repair (6);

(3) to the resuscitation period at 25°C for 18 hours that affords the injured cells the opportunity to repair themselves, multiply and divide and become insensitive to inhibitory agents in the selective media (2, 10, 11). Results obtained in the present study were in conformity with those obtained previously by several other workers including Goetzee & Pretorius (12), Public Health Laboratory Service (13) and Mara (14).

Comparable results were also obtained by Davenport et al. (15) and Green et al. (16), who attributed these to the resuscitation period (varying from 2 to 6 hours) before exposure to the elevated temperature. Other investigators reported similar results when using first an enrichment medium at low temperature (35°C for 2 to 4 hours) before exposure of the membrane to selective media at an elevated temperature of 44.5°C (4, 10, 17).

Thomas & Woodward (18) reported results disagreeing with the ones described in the present work. These authors used the standard one-step MF technique, i.e. direct incubation of the membrane on M-FC broth at 44.5°C, and observed significantly poorer recoveries of FC organisms by the MF technique than by the MPN method. They also found that the ratio of FC by MPN to MF methods was 1.3:1. This ratio was reported to be 0.92:1, 1.53:1 and 2.2:1 by Adams (19), MacCarthy et al. (20) and Slanetz et al. (17) respectively. The lower counts of the MF technique in these aforementioned studies might be attributed to the use of improper media containing substances having inhibitory effects on non-coliform Gram-negative bacilli that might also have an adverse effect on the growth of coliform organisms. Also the initial shock at 44.5°C adversely affected reproduction of metabolically injured cells.

The data obtained in the present study showed that the two-layer method was superior and more precise than the MPN method. Higher counts were obtained in 41 (37%) of the 110 positive samples with a geometric mean of 1135 and a coefficient of variation of 140; similarly, the MPN data demonstrated a geometric mean of 545 and a coefficient of variation of 159. The ratio of FC counts of the two-layer method to the MPN method based on the geometric mean was found to be 2.08:1.

In addition to the use of the MILLIPORE HC brand of membrane filters, higher FC recoveries by the two-layer method in the present study might be attributed firstly to the fact that the proposed two-layer MF procedure allows for repair and subsequent reproduction of those coliforms which have been debilitated by exposure to the aquatic environment (21); secondly, to the fact that the counts in liquid media are not as accurate as counts on solid media (14); and thirdly, to the easy and accurate counting of the characteristic blue colonies grown on the agar medium after enrichment (20, 22).

However, Stuart et al. (23) found that the two aforementioned methods gave nearly the same counts when they tested chlorinated sewage effluents with some modification in the medium. They attributed their results to the addition of glycerol and acetate, plus reducing agents to the two-layer media. Glycerol and acetate act as intermediates in the glycolytic pathway and repair the enzymatic damages of injured cells, along with reducing agents to inactivate residual chlorine.

As a result of the previous findings it was found that the MPN method gave the least counts as compared to the other two methods. The low recovery of the MPN method as compared to the LES and the two-layer methods respectively might be attributed to the false-negative results obtained in the presumptive tests due to the presence of specific soil organisms or the antagonistic action of Pseudomonas that also suppress coliform growth, so that the minimum concentration of cells required to produce visible gas in the presumptive medium is not obtained within the normal incubation time (24, 25), and the high concentration of heavy metal ions suppresses gas production by coliform bacteria (26). Also the MPN method is a biased estimator of the true density and the amount of bias depends on the number of tubes used in each dilution (27).

In this study the LES method proved to be superior and more precise in FC recovery than the two-layer method. Higher counts were found in 10 (9%) out of the 110 samples with a geometric mean of 2170 and a coefficient of variation 107. The ratio of FC counts of the LES method to the two-layer method based on the geometric mean was found to be 1.9:1. Upon closer examination of the data, it was found that the agreement between these two methods was in 100 (91%) of the 110 samples. The great efficiency of the LES method may be attributed to the longer resuscitation period on the rich nonselective medium which contains simple carbohydrates. Although the LES method gave higher counts, it required incubation on two different media for two days to obtain the

final results. Regarding the two-layer method, it automatically shifts the culture contact from an enrichment growth substrate to the essential differential medium phase, and final results are obtained within 24 hours only. Lin (28) stated that the preenrichment incubation temperature of 35°C (used in the two-layer method) was considered the temperature of choice for the improvement of FC recovery. Ray & Speck (29) and Green et al. (16) reported that for proper enumeration of FC the resuscitation period should be completed before cell multiplication started, the time initiation of multiplication at 35°C being 4 hours. Bissonnette et al.(10) pointed out that the two-layer method avoided the limitation of enrichment techniques due to the fact that considerably more time, equipment and manpower were required for analysis. Finally, Crabow & Preez (30) proved that saturated pads were inconvenient and time-consuming, the pads tended to dry out, and the agar-based medium generally proved to yield higher counts.

In these comparisons the MPN procedure was used as the standard, but it must be noted that there are inherent shortcomings in this technique based on statistical probability, estimates of bacterial density with inherent errors (31). Membrane filtration using the two-layer agar method provides a direct accurate bacterial count, requires only 24 hours to complete, and has the advantage of saving media, chemicals, tubes, racks and space. In contrast, the MPN test requires 2 to 4 days before final results are obtained, and an excessive amount of laboratory work may be involved. In this study, from the 1600 inoculated tubes, 367 positive lactose tubes failed to confirm the presence of faecal coliforms. In other words, about 23% of all the inoculated tubes were processed through the confirmation test without any positive coliform results. One of the disadvantages of the MF technique is the high cost of membranes which have to be imported from abroad. However, Taylor & Burman (17) showed that the membrane could be washed and re-used by sterilizing in 3% (v/v) hydrochloric acid.

Following evaluation of the results obtained during the present study, it is agreed with Geldreich (22, 32, 33) that the MF technique gives the most convenient results, the LES method gives the highest FC counts and two-layer method saves time, media and labour.

5. Conclusion

Three methods were used for the detection of faecal coliforms (FC) in 110 water samples. These methods were the MPN, the LES and the two-layer MF. This work was carried out in order to determine the most efficient method for the isolation and enumeration of FC.

Comparison of the results showed the superiority and the higher degree of precision of both MF procedures over the MPN method.

It was concluded that the MF technique gives the most convenient results; the LES method gives the highest FC counts and the two-layer method saves elaborate time, media and labour.

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Table 1

Comparison of the frequency of faecal coliform counts/100 ml detected by the MPN and LES methods

LES	MPN	FC count 1-99	FC count 100-999	FC count 1000	Total
FC count	1-99	0	0	0	0
	100-999	11	20	0	31
	1000-	3	33	43	79
	Total	14	53	43	110

Table 2

Comparison of the frequency of faecal coliform counts/100 ml detected by the MPN and two-layer methods

Two-layer	MPN	FC count 1-99	FC count 100-999	FC count 1000	Total
FC count	1-99	1	1	0	2
	100-999	12	25	0	37
	1000-	1	27	43	71
	Total	14	53	43	110

Table 3

Comparison of the frequency of FC counts/100 ml detected by the two-layer and the LES methods

LES	Two-layer	FC count 1-99	FC count 100-999	FC count 1000	Total
FC count	1-99	0	0	0	0
	100-999	2	29	0	31
	1000-	0	8	71	79
	Total	2	37	71	110

Table 4

Agreement between the MPN, the two-layer and the LES methods in the estimation of faecal coliforms

First method	Second method	FC counts			
		Equal number by the two methods	Higher counts by 1st method than 2nd method	Lower counts by 1st method than 2nd method	Percentage of agreement
MPN	LES	63	0	47	57
MPN	Two-layer	69	1	40	63
LES	Two-layer	100	10	0	91

Figure 1

Comparison of faecal coliform log counts by two-layer and LES methods

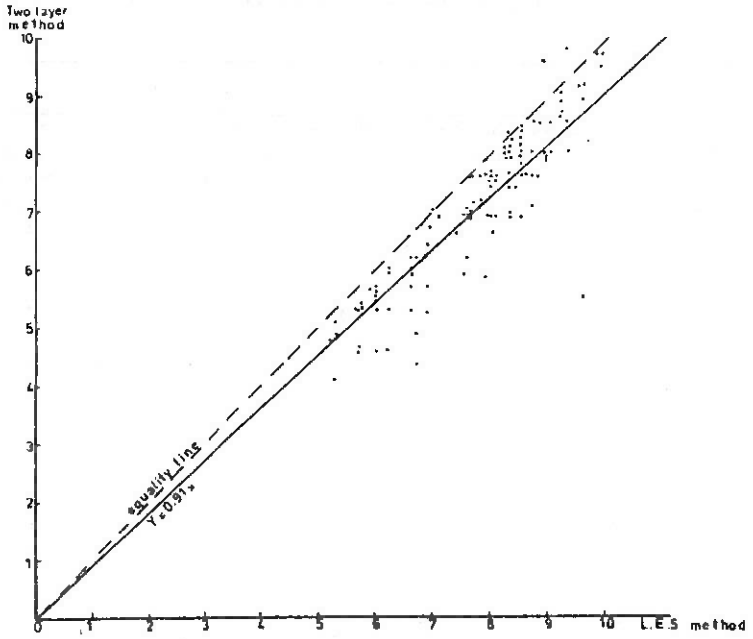


Figure 2

Comparison of faecal coliform log counts by MPN and two-layer methods

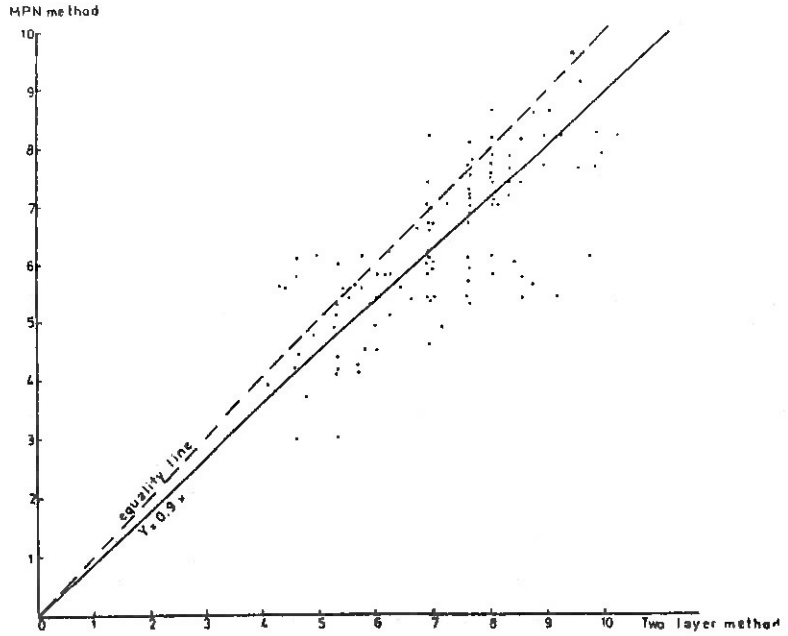
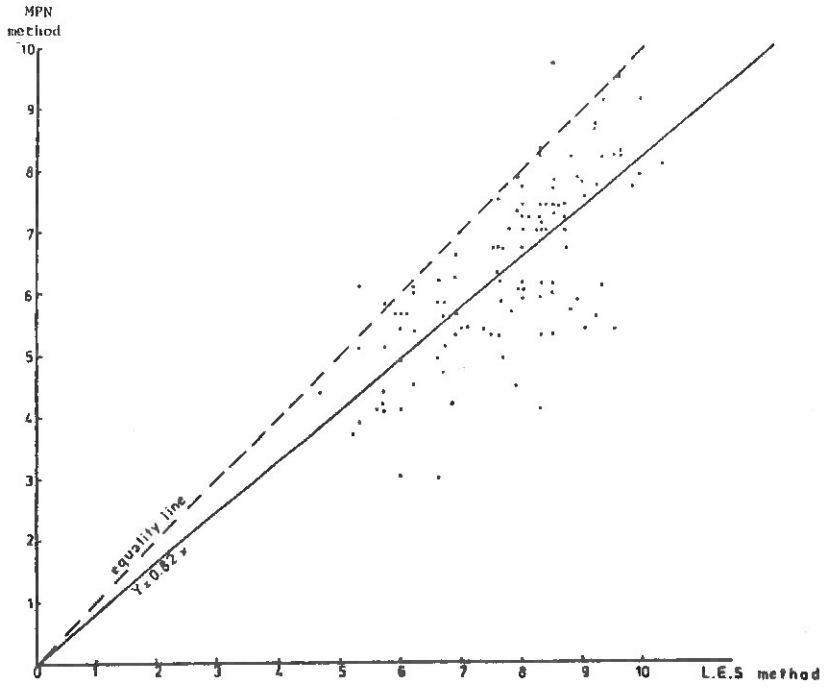


Figure 3

Comparison of faecal coliform log counts by MPN and LES methods



Annexe 3

ETUDE COMPARATIVE DES METHODES UTILISEES EN NUMERATION BACTERIENNE DES EAUX COTIERES DE LA MEDITERRANEE

par
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1. Introduction

La présente étude, entrant dans le cadre des travaux du Service Commun N°11 de l'Institut national de la Santé et de la Recherche médicale (INSERM) pour le Programme MED POL Phase II, a eu pour but de comparer deux méthodologies de numération des germes fécaux (colibacilles et streptocoques) en eau de mer des zones côtières.

A Nice, trois zones ont été choisies :

- la plage de Coco Beach, formée de petites criques rocheuses, située près du Cap de Nice;
- la plage du Forum, au centre de la grande plage de galets de la Baie des Anges, au débouché des principales artères de la Ville de Nice;
- le port de commerce de Nice (Port Lympia), implanté à l'est de la cité; ce site a été sélectionné en considération de sa forte charge bactérienne présumée.

L'exercice d'essai a été conduit de la mi-juin à la mi-août 1982 au cours de six opérations de prélèvements d'eaux de mer côtières effectuées en chacun des trois lieux précités (voir figure 1), soit au total 18 prélèvements.

2. Matériel et méthodes

Prélèvements d'eau de mer

Les prélèvements ont été effectués avec des flacons en verre stérilisés de 1 litre, aux distances suivantes :

Coco Beach :	1 m du bord (rocher)
Forum :	3 m du bord (galets)
Port de Commerce :	0,50 m du bord (béton).

Pour chacun de ces trois sites, 1 à 2 litres ont été échantillonnés à chaque fois au même endroit, à 20 cm sous la surface de l'eau, et les analyses ont commencé une heure après au plus tard.

Volumes d'eau étudiés

Lieux à Nice	Volumes (ml) d'eau filtrés sur membrane (méthode MF)			Volumes (ml) d'eau introduits en tubes (méthode MPN)		
Plage de Coco Beach	100	10	1	10	1	1 à 10^{-1}
Plage du Forum	100	10	1	10	1	1 à 10^{-1}
Port de Commerce	100	1	1 à 10^{-1}	1	1 à 10^{-1}	1 à 10^{-2}

Filtration par membranes (MF)

Les membranes utilisées (SARTORIUS) étaient de 47 mm, 0,45 u de porosité.

Chaque volume d'eau et chaque dilution ont été filtrés 5 fois pour culture sur gélose spécifique à l'aide d'une rampe de filtration (MILLIPORE).

Tubes multiples (MPN)

Chaque volume et chaque dilution ont été répartis dans 5 tubes de verre avec bouchons en coton entouré de gaze.

Prises des échantillons

Elles ont été faites avec des pipettes automatiques PIPETMAN 5000 µl et 1000 µl avec des cônes stérilisés.

Géloses utilisées

1) Coliformes fécaux

a) m FC Broth base (DIFCO) additionné d'acide rosolique
b) EMB (PASTEUR)
Gélose Lactosée Vert Brillant - Rouge de Phénol (PASTEUR).

2) Streptocoques fécaux

a) KF-streptococcus-agar (DIFCO) additionné de TTC
b) Enterococcus-agar (DIFCO).

Bouillons utilisés

1) Coliformes fécaux

a) Mac Conkey Broth (DIFCO) avec cloche
b) eau peptonée exempte d'indole (PASTEUR).

(2) Streptocoques fécaux

a) Azide-Dextrose Broth (DIFCO)
b) EVA Broth (DIFCO).

Incubation

L'incubation a été de 48 h pour les géloses et les bouillons à streptocoques fécaux, ainsi que pour les bouillons Mac Conkey.

Pour les géloses FC, EMB, au Vert Brillant-Rouge de Phénol et pour l'eau peptonée, l'incubation a été arrêtée à 24 h.

Toutes les cultures ont été faites à 44 °C.

Lecture des résultats (effectués par la même personne)

1) Coliformes fécaux

a) Filtrations sur membranes :
- colonies bleu ciel à bleu foncé; pour confirmation certaines colonies ont été repiquées sur gélose EMB ou gélose Lactosée au Vert Brillant-Rouge de Phénol.

b) Tubes multiples :
- croissance, virage du lactose, 5-7 mm de gaz
- en Mac Conkey, confirmation de tous les positifs en eau peptonée.

2) Streptocoques fécaux

a) Filtrations sur membranes :
- colonies roses à rouge foncé sur gélose KF
- repiquage éventuel sur gélose Enterococcus.

b) Tubes multiples :
- croissance et culot foncé en bouillon Azide
- confirmation de tous les tubes positifs en bouillon EVA.

3. Résultats

La présente étude ayant pour but de comparer deux méthodes d'analyse, la moyenne de chaque mesure est présentée avec un intervalle de confiance à 95%. De manière à visualiser les résultats, ces derniers ont été regroupés dans les tableaux 1 et 2 et les figures 2 à 7 (échelle semi-logarithmique, base 10 pour les figures).

3.1 Coliformes fécaux

Les résultats des analyses (figures 2, 4 et 6) par les deux méthodes (filtration sur membranes et tubes multiples) sont compatibles entre eux.

La seule exception concerne le prélèvement effectué à la plage du Forum, le 26 juillet, où un nombre élevé de coliformes fécaux a été mis en évidence (FM = moyenne de 800; tubes multiples = moyenne de 8). Une explication possible de cet écart important serait la présence de nombreux germes alkalinisants (100 colonies rouges par 100 ml sur FM en gélose FC) qui auraient faussé la lecture des bouillons Mac Conkey (tubes multiples).

3.2 Streptocoques fécaux

Les résultats obtenus (figures 3, 5 et 7) ne sont guère différents d'une méthode à l'autre. Pour les deux groupes de germes fécaux, l'intervalle de confiance est plus grand pour la méthode des tubes multiples (MPN).

4. Conclusion

Il apparaît que les deux méthodes de numération - filtrations sur membranes (MF) et tubes multiples (MPN) - donnent des résultats similaires, à l'exception du cas spécial des eaux portuaires.

Ces résultats restent à être interprétés en fonction des données recueillies par l'ensemble des laboratoires ayant participé à l'étude.

Tableau 1

Numérations des coliformes fécaux pour 100 ml d'eau de mer
(moyennes et intervalles de confiance à 95%)

	Date	Filtration sur membranes (5 membranes)			Nombre le plus probable (5 tubes)		
		moyenne	lim. inf.	lim. sup.	moyenne	lim. inf.	lim. sup.
Plage de Coco Beach	14.06.82	4	1	6	2	0,5	7
	21.06.82	9	6	12	17	5	46
	02.07.82	2	1	5	37	23	41
	09.07.82	25	18	31	70	23	170
	26.07.82	14	10	18	7	1	17
	03.08.82	8	6	10	12	3	28
Plage du Forum	14.06.82	165	120	210	79	25	190
	21.06.82	2	1	2	2	1	2
	02.07.82	3	1	5	2	1	7
	09.07.82	13	10	15	17	5	46
	26.07.82	800	660	940	8	1	19
	03.08.82	6	4	8	5	0	13
Port de Commerce	14.06.82	600	480	720	2400	2400	>2400
	21.06.82	1340	830	1850	490	170	1300
	02.07.82	480	220	740	170	50	460
	09.07.82	3020	2110	3930	2400	680	7500
	26.07.82	1600	1340	1860	720	560	880
	03.08.82	3120	2470	3770	1300	350	3000

Tableau 2

Numérations des streptocoques fécaux pour 100 ml d'eau de mer
(moyennes et intervalles de confiance à 95%)

	Date	Filtration sur membranes (5 membranes)			Nombre le plus probable (5 tubes)		
		moyenne	lim. inf.	lim. sup.	moyenne	lim. inf.	lim. sup.
Plage de Coco Beach	14.06.82	51	35	67	23	7	70
	21.06.82	25	11	32	8	1	19
	02.07.82	10	4	16	7	1	17
	09.07.82	37	23	41	79	25	190
	26.07.82	12	9	15	8	1	19
	03.08.82	1	0	2	4	0	11
Plage du Forum	14.06.82	85	74	97	27	9	80
	21.06.82	22	11	32	2	1	7
	02.07.82	21	13	28	46	16	120
	09.07.82	13	10	16	14	4	34
	26.07.82	9	5	13	13	3	31
	03.08.82	2	1	3	2	0	7
Port de Commerce	14.06.82	1800	1520	2080	350	120	1000
	21.06.82	3870	2620	5120	1100	310	2500
	02.07.82	680	350	1010	230	70	700
	09.07.82	2400	1910	2890	1700	430	4900
	26.07.82	720	560	880	110	20	250
	03.08.82	1280	1020	1540	230	70	700

Figure 1

Zones de prelevements à Nice

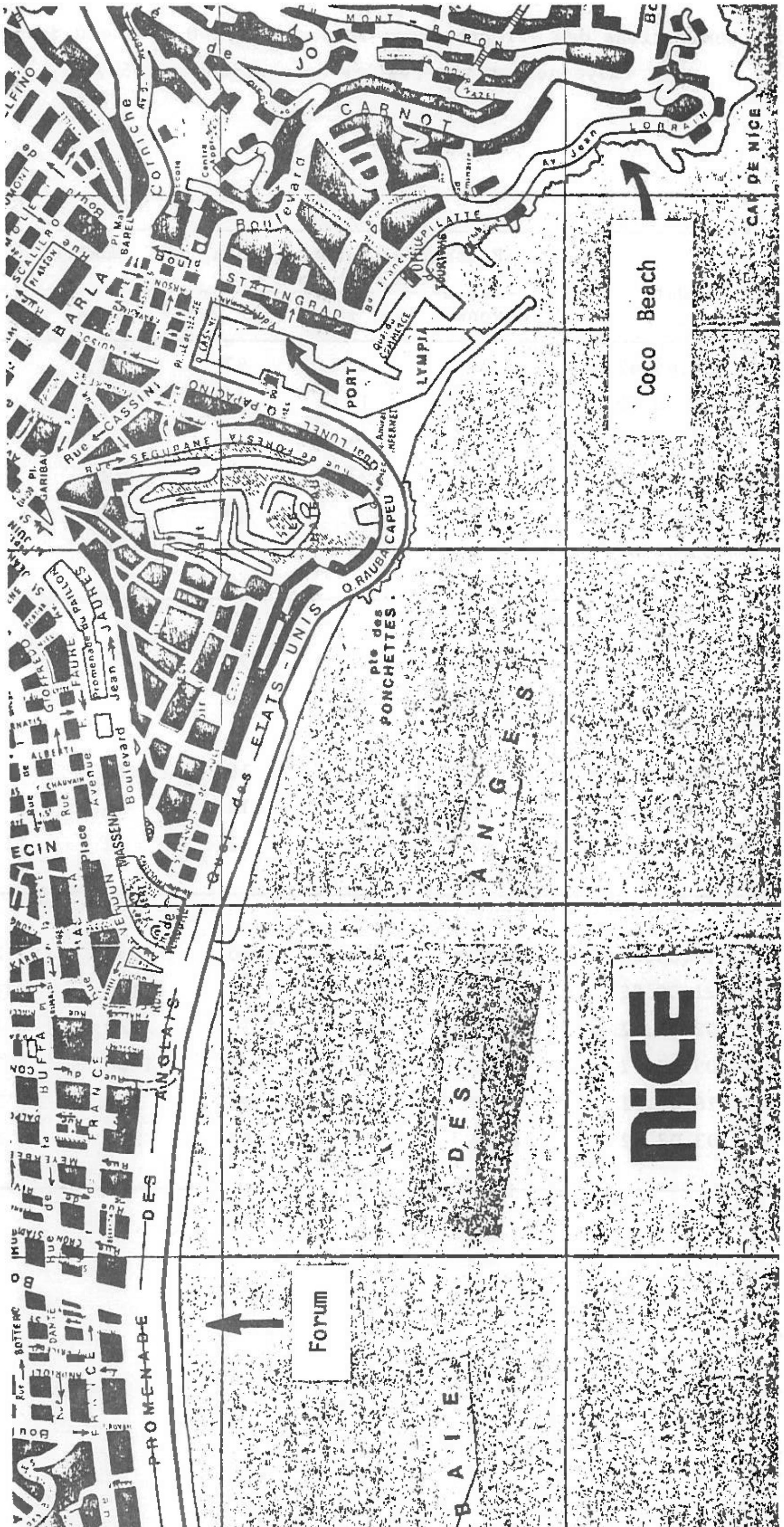


Figure 2

Intervalles de confiance à 95% pour le taux de Coliformes fécaux
(plage de Coco Beach, 2 méthodes de numération)

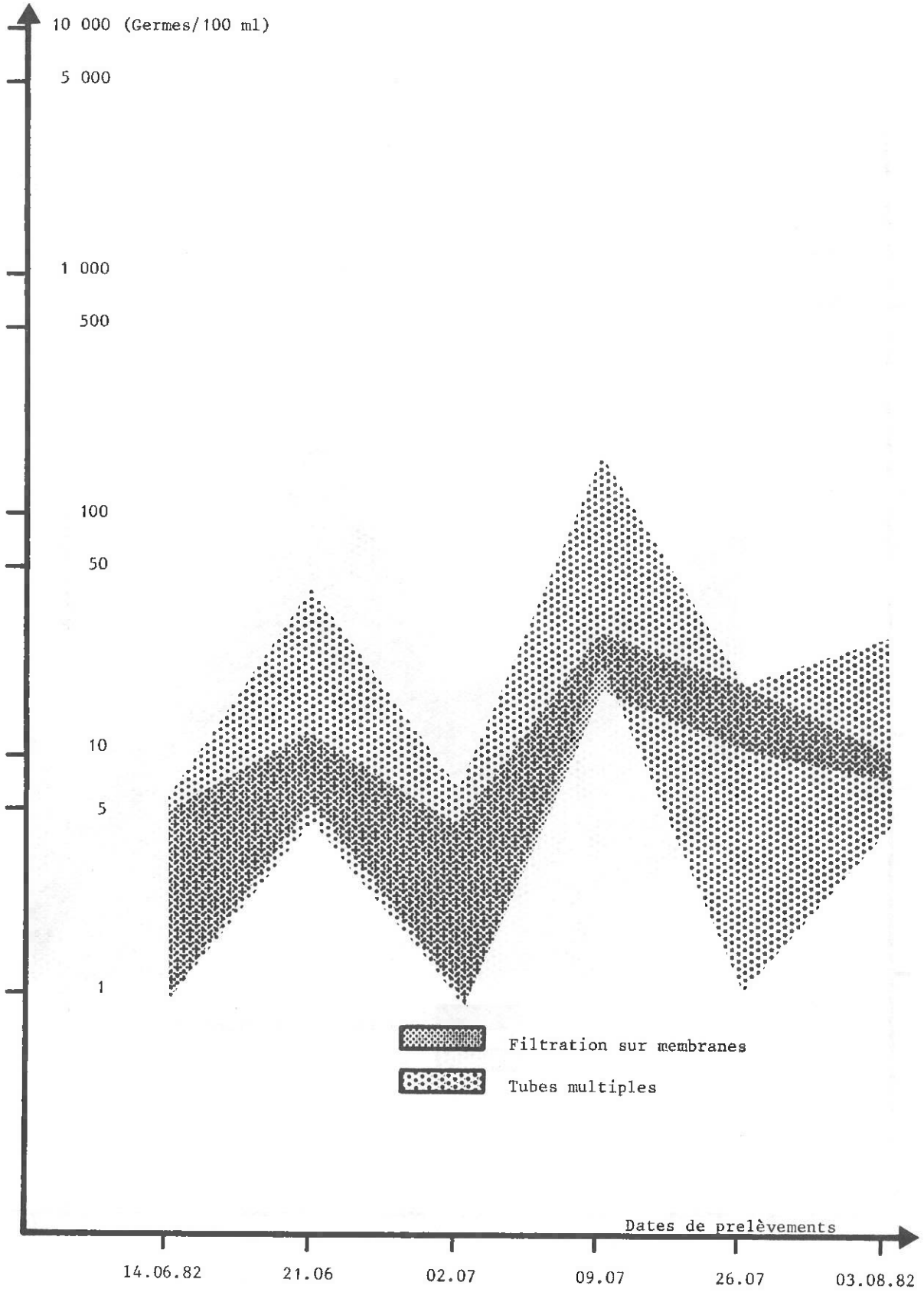


Figure 3

Intervalles de confiance à 95% pour le taux de Streptocoques fécaux
(plage de Coco Beach, 2 méthodes de numération)

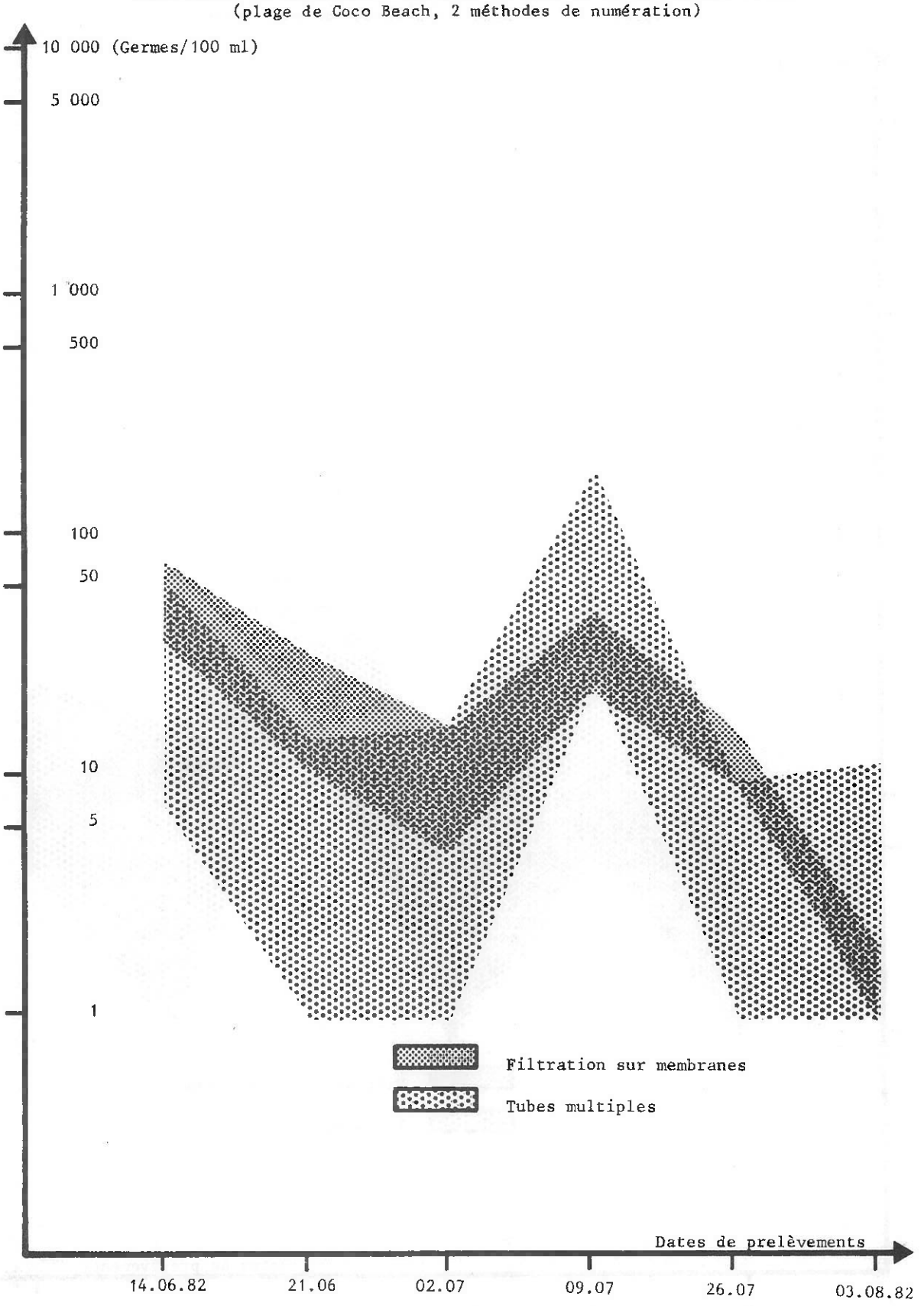


Figure 4

Intervalles de confiance à 95% pour le taux de Coliformes fécaux
(plage du Forum, 2 méthodes de numération)

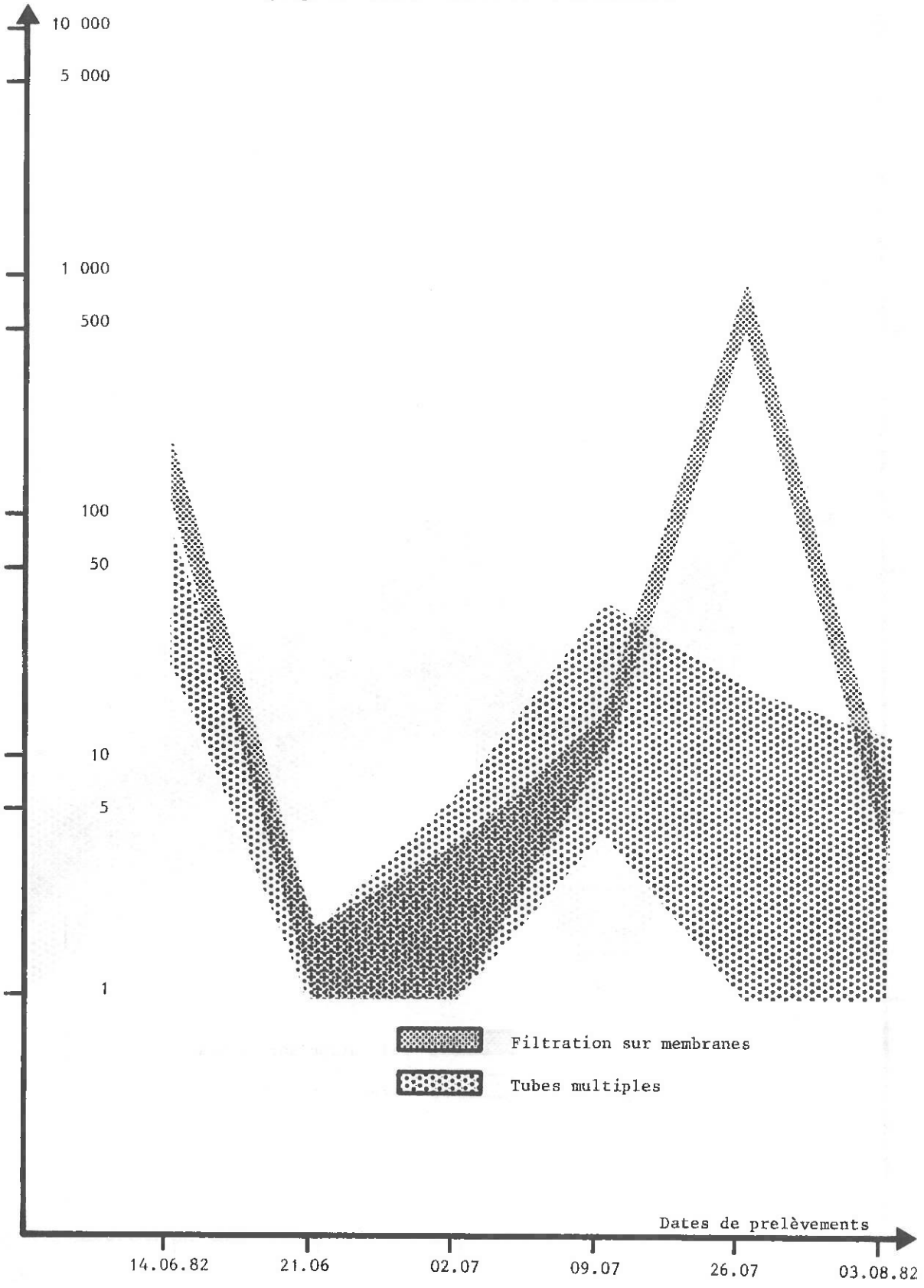


Figure 5

Intervalles de confiance à 95% pour le taux de Streptocoques fécaux
(plage du Forum, 2 méthodes de numération)

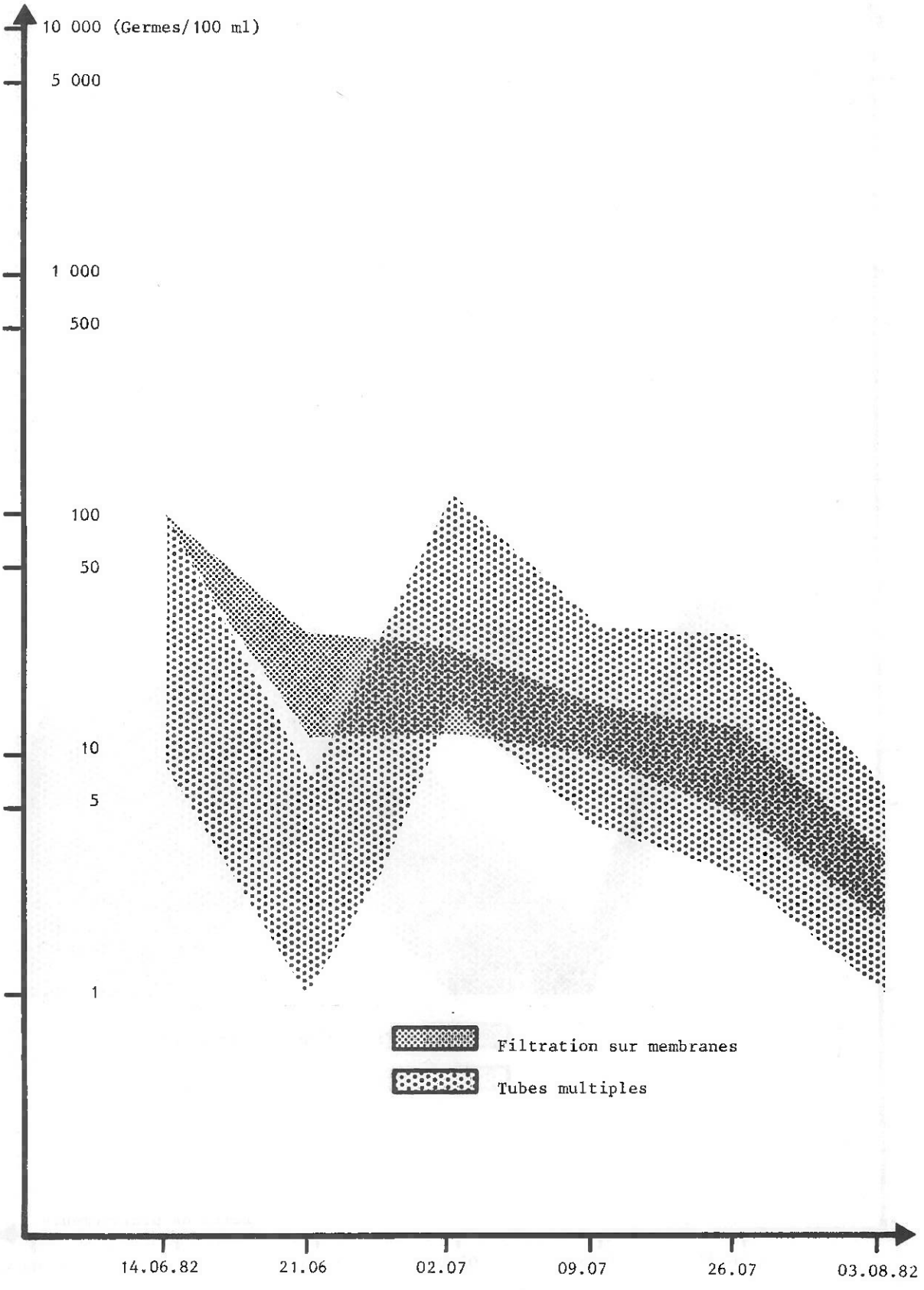


Figure 6

Intervalles de confiance à 95% pour le taux de Coliformes fécaux
(Port de Commerce, 2 méthodes de numération)

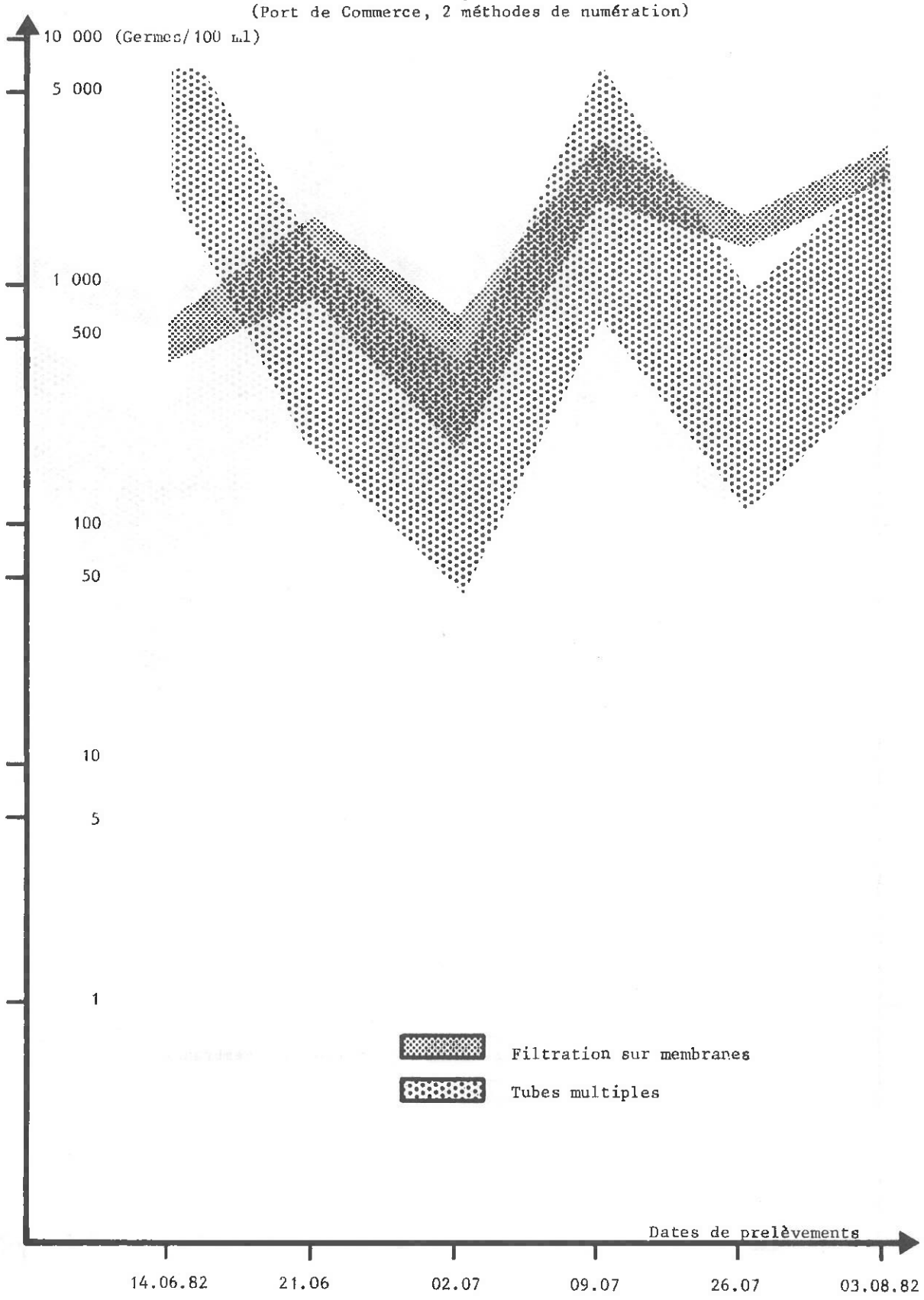
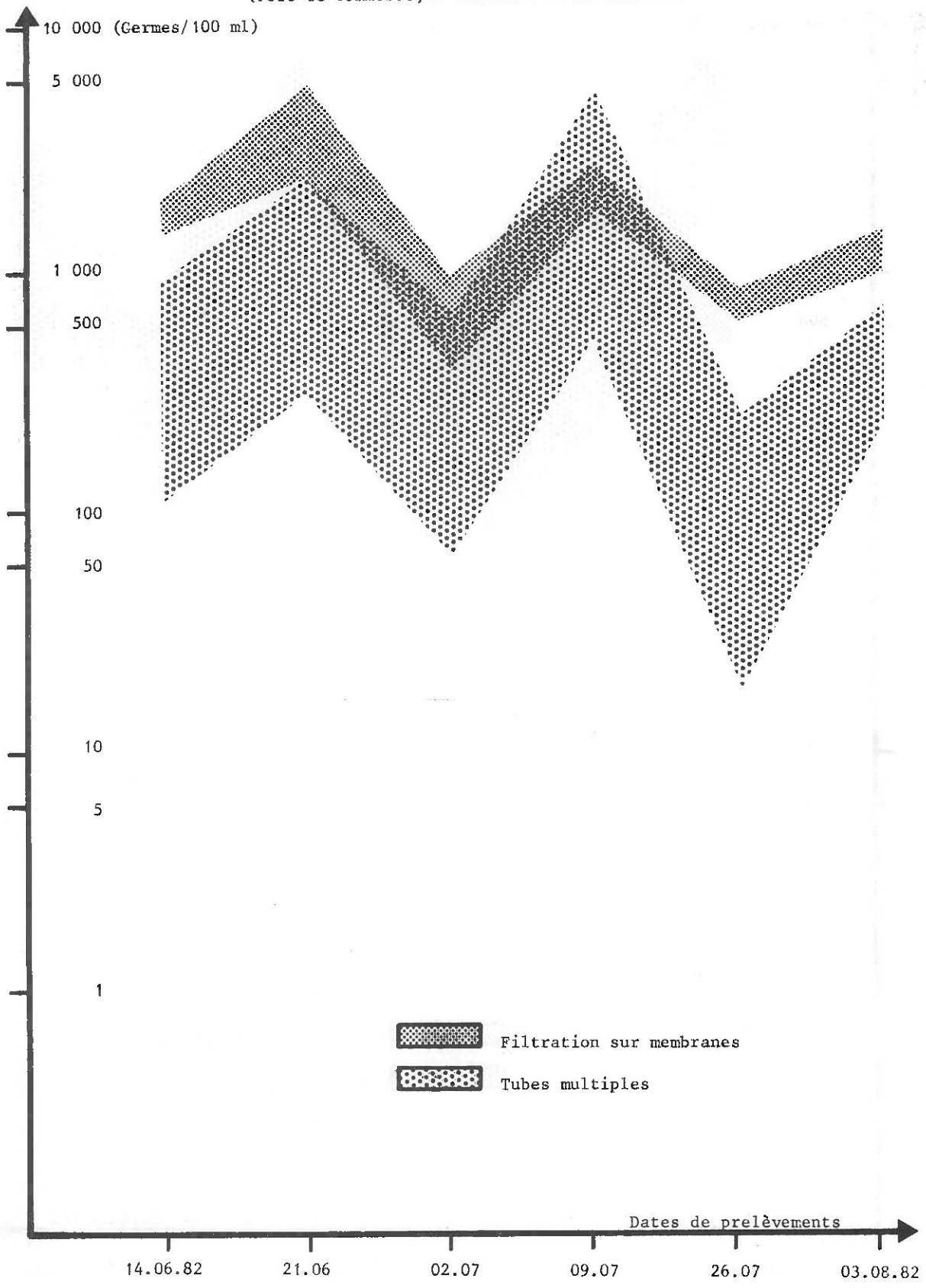


Figure 7

Intervalles de confiance à 95% pour le taux de Streptocoques fécaux
(Port de Commerce, 2 méthodes de numération)



Annex 4

COMPARISON OF METHODS FOR MONITORING INDICATOR ORGANISMS IN MARINE WATER

by
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1. Introduction

The purpose of the present study was to compare the most probable number (MPN) and membrane filtration (MF) methods in monitoring the major bacteriological parameters for assessing the quality of coastal water in the Mediterranean. This would be useful in the eventual recommendation of the most suitable one as a standard procedure.

In previous comparisons of these two methods in coastal water (1, 2), the number of coliforms obtained by the MPN procedure was usually higher than that obtained by the MF method, which raised the question of the rate of recovery of stressed organisms by the accepted one-step MF method. An attempt was made in the present study to elucidate this point by examining parallel samples for faecal coliforms and faecal streptococci with and without resuscitation.

2. Materials and methods

2.1 Testing period

Sampling started on 25 May 1982 and continued until the first week in August. Two of the sampling points selected on the first sampling day gave very low results and were replaced by others on the following sampling day. The number of samples was therefore six in one sampling point and only five in the other two.

2.2 Sampling points

No means of sampling at the pollution source itself, which was approximately 300 m offshore, was available. The closest accessible location was approximately 500 m north of the sewage outfall at Tel-Baruch, where bathing is forbidden. The other sampling points were: Sheraton Beach, circa 900 m south of the outfall, and Bugrashov Beach, circa 1800 m south of the outfall.

Two litres of water and about 200 g of sediment were taken at each point and transported to the laboratory within 1 - 1 1/2 hours. The water temperature was measured at the time of sampling.

Total coliforms

MPN: Presumptive test in Lactose Broth confirmed in Brilliant Green Bile Broth, both incubated at 35°C±0.5°C for 48±3 hours. Four to five dilutions of each sample were inoculated in a 5-tube series and the appropriate three dilutions were selected for reading results.

MF: Two to three dilutions were filtered from each sample. The membranes were placed on M-endo-agar LES and incubated at 35°C±0.5°C for 22 hours.

Faecal coliforms

MPN: From each positive tube of the presumptive test for total coliforms two drops were inoculated into a tube of EC medium using a Pasteur pipette. The tube was incubated at 44.5°C±0.2°C (water bath) for 24 hours. Tubes showing any amount of gas were considered positive.

MF: A double series was filtered from each dilution. One was placed directly on M-FC-agar and incubated at 44.5°C±0.2°C, the other was placed on pads saturated with Lauryl Tryptose Broth (3) (in the covers of the Petri dishes) and incubated at 35°C±0.5°C for 2 - 2 1/2 hours, then transferred to the M-FC-agar and incubated at 44.5°C±0.2°C for circa 22 hours.

Faecal streptococci

MPN: Presumptive test in Azide-Dextrose Broth, as outlined for total coliforms, confirmed in EVA Broth, both incubated at 35°C±0.5°C for 48±3 hours.

MF: In view of the existence of two sets of directions for faecal streptococci, one calling for incubation at 35°C±0.5°C (3) and the other at 44°C±0.2°C (4), both temperatures were tested on one sampling day. Counts obtained at 35°C±0.5°C were considerably higher, especially in the least polluted points, therefore all further examinations were incubated at 35°C±0.5°C for 48±3 hours.

A double series was filtered for each dilution, one set of filters was placed directly on KF-agar and the other was resuscitated on pads saturated with Trypticase Soy Broth for 2 - 2 1/2 hours at 35°C±0.5°C then transferred to KF-agar and incubated for 2 days.

From each filter, several colonies representative of the various groups were selected and transferred to blood agar plates, then to brain heart infusion broth. They were examined microscopically (Gram stained) and for production of oxidase.

2.3 Examination of sediment

From the sediment samples, 50 or 100 g portions (wet weight) were weighed and 10 volumes of phosphate buffer with 0.1% peptone added to each. The diluted samples were shaken vigorously by hand and as soon as most of the sand had settled the liquid was decanted and used for inoculation. The results obtained were multiplied by 10 and calculated for 100 g of sample.

2.4 Other parameters

Dissolved oxygen and BOD_5 were determined in all water samples. Salinity was measured only on one sampling day.

2.5 Estimation of precision

The precision of the membrane filtration technique was checked by filtering triplicate samples from a triplicate dilution series of faecal coliforms and faecal streptococci in marine water (using strains isolated during the present work), and subjecting the results to analysis of variance to determine the standard error. The number of organisms in the suspensions was also determined on plate count agar.

3. Results

The data obtained for each sampling point are presented in Tables 1 to 3. Results show that when total coliforms were chosen as the parameters for assessing the bacterioloigcal quality of water or sediment, and the limit set at 1000 organisms per 100 ml of water or 100 g of sediment, one sample of water and two samples of sediment from the Sheraton Beach would exceed that limit when monitored by the MPN method, while complying with it by the MF procedure. In the case of faecal coliforms, using the MPN method, this limit was exceeded only in one sample of sediment. All other samples fell into the same classification by either method. The number of faecal streptococci recovered was much higher by the MF method with KF-medium and the resuscitation step increased their number considerably. The incubation temperature of 35°C±0.5°C was found to be more efficient in recovering faecal streptococci (as illustrated in Table 4).

Salinity was measured on one day only (18 July). Levels were 35.2°/oo at Tel-Baruch and 35.49°/oo at the Sheraton and Bugrashov Beaches.

The estimation of precision is summarized in Table 5.

4. Discussion

The results for total coliforms were higher by the MPN procedure in 75% of the samples, by the MF method in 15% of the samples and equal for both methods in 10% of the samples. When all data were subjected to the Student's t-test, the difference was not statistically significant. However, when the two unusually high readings at Tel-Baruch on 18 July were excluded the difference was statistically significant.

Faecal coliform readings were higher by the MPN technique in 44% of the samples, by the MF in 31% of the samples and equal by both methods in 25% of the samples. Differences were not statistically significant.

Several authors have reported the MPN procedure as being more efficient in recovering total and faecal coliforms from stream water and sewage (5) from wastewater effluent (6) and from seawater (7). On the other hand, Dutka & Tobin (8) achieved maximum estimates of coliforms in the Lower Great Lakes region of Canada by the MF procedure with M-Endo-agar LES. They compared four procedures and found that each was selective for different genera of Enterobacteriacea.

According to Rose et al. (9), a two-layered medium proposed by them, which included two hours' pre-incubation at 35°C, recovered 3.8 to 7 times more faecal coliform colonies from marine water than the direct M-FC procedure. In the present study, the additional resuscitation step recovered a larger number of faecal coliforms only in a few samples and its use would not therefore appear to be justified, particularly in clean beaches, in view of the considerable amount of time and work involved.

In polluted water however (Tel-Baruch), the difference between the number of faecal coliforms recovered after resuscitation and without it was statistically significant (P 0.1).

Since the data presented here were collected during a short period of time in a limited area, it appears that for final selection of the most suitable method for monitoring faecal coliforms in the Mediterranean more data from a wider area should be compared.

Counts of faecal streptococci were considerably higher at an incubation temperature of 35°C±0.5°C than at 44.5°C±0.2°C (Table 4) and increased significantly with a 2 - 2 1/2 hour resuscitation period on Trypticase Soy Broth.

In 90% of the samples, counts were much higher by the direct MF method at 35°C±0.5°C than by the MPN procedure (Tables 1 to 3) and analysis of all data showed that the difference was statistically significant (P 0.1). After resuscitation the difference was statistically significant at a higher level (P 0.01).

No explanation can be offered for the enormous discrepancy between the recovery rate of faecal streptococci by MF on KF-medium and MPN methods. Among the colonies that were confirmed, 10-30% were not streptococci (there were micrococci and even a few strains of Pseudomonas) and this rate did not account for the difference. The ratio between the MF and the MPN densities was from 0.6 to 92, with 220 in one sample and 355 in another. Kenner et al. (10), who introduced the KF-medium, obtained ratios of 0.61 to 20 with one of 200. Different media and procedures for recovering faecal streptococci from water have been compared by various authors (10-14) and all of them reported that KF and PSE-agar gave the highest recovery rates. Several authors prefer the PSE-medium, as it requires only 24 hours incubation versus 48 hours on KF-medium. Daust & Litzky (12) obtained the highest counts from marine water on PSE agar and from sewage on KF-agar; Brodsky & Schiemann (13) found that KF medium was not as selective as PSE agar - only 65% of all colonies they confirmed were faecal streptococci compared to 90% from PSE.

In conclusion it can be said that the data presented in this study, as well as that available in the literature, indicate that for faecal streptococci the MF procedure with KF-medium is superior to the MPN method, as it gives better results in a shorter time (two days instead of four) with less work involved. Since the PSE-medium has the advantage of shorter incubation time, and has been reported by some authors to be more selective in some environments, it may be desirable to compare the two media for selecting the most suitable one for Mediterranean waters.

5. Acknowledgement

The author is grateful to Dr Israel Cohen for his assistance in the statistical analysis of the data.

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Table 1

Comparison of indicator organism densities per 100 ml of water or 100 g of sediment at the Tel-Baruch coast by MPN and MF methods

Date	Temp. °C	Total coliforms		Faecal coliforms			Faecal streptococci			DO	BOD_5
		MPN	MF-D	MPN	MF-D	MF-R	MPN	MF-D	MF-R		
Water											
6.6.82	25	920	340	350	170	110	27	2500	8700	6.3	1.8
22.6.82	27	35000	5600	4900	8000	22000	2400	3600	3500	5.0	2.0
4.7.82	27.8	28000	24000	22000	11000	22000	5400	13000	21000	5.5	5.15
18.7.82	28.5	35000	100000	11000	38000	41000	9200	14000	20000	5.0	6.2
1.8.82	29.5	160000	95000	92000	30000	48000	11000	10000	21000	5.2	2.0
MEAN*		22000	13000	8200	7000	10000	2000	7000	11000		
SD		7	11	8	9	13	12	2	2		
Sediment											
6.6.82		17000	7500	3500	4600	4500	24000	71000	98000		
22.6.82		4600	2800	4900	4400	20000	16000	21000	27000		
4.7.82		5400	15000	3500	6000	3000	24000	15000	22000		
18.7.82		16000	500000	9200	14000	14000	2200	2000	38000		
1.8.82		17000	2800	17000	900	800	3300	8100	7800		
MEAN*		10000	28000	6000	4400	5000	9200	13000	28000		
SD		2	14	2	3	4	3	4	3		

* Geometric mean rounded up to 2 significant figures
MF-D: membrane filtration, direct
MF-R: membrane filtration with resuscitation

Table 2

Comparison of indicator organism densities per 100 ml of water or 100 g of sediment at the Sheraton Beach by MPN and MF methods

Date	Temp. °C	Total coliforms		Faecal coliforms			Faecal streptococci			DO	BOD_5
		MPN	MF-D	MPN	MF-D	MF-R	MPN	MF-D	MF-R		
Water											
25.5.82	25	240	160	240	50	270	<2	34	-	7.3	3.1
6.6.82	25	1600	320	220	110	110	2	710	1800	6.5	1.5
22.6.82	27	130	14	8	8	42	2	440	1200	5.5	1.2
4.7.82	27.8	110	110	26	40	42	70	3500	7000	6.0	1.6
18.7.82	28.5	23	26	8	18	10	170	1100	2700	6.0	0.9
1.8.82	29.5	49	19	13	6	6	170	940	2500	5.5	0.6
MEAN*		190	60	32	24	38	14	580	2500		
SD		4	4	5	3	4	11	5	2		
Sediment											
25.5.82		24000	960	1300	1200	1200	310	820			
6.6.82		9400	390	1100	900	1200	4600	7500	18000		
22.6.82		490	20	50	20	40	490	3200	17000		
4.7.82		50	40	20	50	<20	330	7000	9000		
18.7.82		49	50	49	70	20	1100	5000	17000		
1.8.82		20	10	<20	20	<20	13	42	420		
MEAN*		390	73	94	110	70	390	1800	7100		
SD		18	6	8	6	10	7	7	5		

* Geometric mean rounded up to 2 significant figures
MF-D: membrane filtration, direct
MF-R: membrane filtration with resuscitation

Table 3

Comparison of indicator organism densities per 100 ml of water or 100 g of sediment at the Bugrashov Beach by MPN and MF methods

Date	Temp. °C	Total coliforms		Faecal coliforms			Faecal streptococci			DO	BOD_5
		MPN	MF-D	MPN	MF-D	MF-R	MPN	MF-D	MF-R		
Water											
6.6.82	25	49	2	2	2	350	970	710	1700	6.5	0.6
22.6.82	27	49	8	2	2	6	2	150	150	5.7	1.5
4.7.82	27.8	170	100	110	38	60	49	1300	3500	6.3	1.2
18.7.82	58.5	46	28	46	20	19	130	410	1100	6.0	1.0
1.8.82	29.5	33	40	23	39	34	220	400	1000	5.7	0.6
MEAN*		57	18	14	8	12	44	500	1400		
SD		2	5	6	7	5	14	2	2		
Sediment											
6.6.82		230	200	50	50	200	80	3600	6100		
22.6.82		130	20	20	20	20	490	2000	12000		
4.7.82		700	160	700	100	20	1400	8000	24000		
18.7.82		20	20	20	10	10	220	1600	1400		
1.8.82		50	10	20	10	20	330	650	1300		
MEAN*		100	42	37	18	24	330	2300	5000		
SD		5	4	6	3	3	3	3	4		

* Geometric mean rounded up to 2 significant figures
MF-D: membrane filtration, direct
MF-R: membrane filtration with resuscitation

Table 4

Effect of incubation temperature on recovery of faecal streptococci on KF-medium (number of organisms per 100 ml water or 100 g sediment)

Location		Incubation temperature			
		35°C±0.5°C		44.5°C±0.2°C	
		MF-D	MF-R	MF-D	MF-R
Tel-Baruch	water	3600	3500	2300	2400
	sediment	21000	27000	22000	20000
Sheraton	water	440	1200	400	20000
	sediment	3200	17000	900	4800
Bugrashov	water	150	930	40	170
	sediment	2000	12000	200	8800
MEAN*		1800	5300	470	2300
SD		6	4	13	6

* Geometric mean rounded up to 2 significant figures
MF-D: membrane filtration, direct
MF-R: membrane filtration with resuscitation

Table 5

Estimation of Precision of Membrane Filtration

Bacterial suspension	Total coliforms		Faecal coliforms		Faecal streptococci	
	Mean	SE	Mean	SE	Mean	SE
A	132	2.3	119	3.2	15	0.33
B	143	7.3	123	11.7	22	3.3
C	131	4.0	110	13.1	22	5.5
MSE		8.6		17.8		6.5
F-test		NS		NS		NS

Annex 5

A COMPARISON OF MICROBIOLOGICAL METHODS FOR MONITORING INDICATOR ORGANISMS IN SEAWATER AND SAND

by
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1. Introduction

The purpose of this study was to compare two different analytical methods, most probable number (MPN) and membrane filtration (MF) for determination of the faecal coliform density in coastal seawater samples. In addition, total coliforms and faecal streptococci were determined in water samples and total and faecal coliforms and faecal streptococci in sand samples.

The following data were also determined: sea and sky conditions, surface current, wind, last relevant precipitation, air and water temperature, dissolved oxygen (DO) and pH.

2. Materials and methods

2.1 Testing periods

Sampling was performed between 18 May and 31 August 1982, the following being the sampling dates: 18.5.82; 1.6.82; 15.6.82; 26.6.82; 13.7.82; 27.7.82; 10.8.82; 31.8.82.

Hydrographic and meteorological conditions on each sampling day are listed in Table 1.

2.2 Sampling points

The following sampling stations (on the coast of the Province of Latina, between Torre Astura and Torre Paola) were selected (Figures 1 and 2):

No. 1: "Canale Caterattino" - a small canal receiving sewage discharges only in the summer. Water samples were collected near the outfall of the canal into the sea. Sand samples were collected on the shoreline, near the outfall of this canal.

No. 2: beach, at km 25.6 on the coast road - clean area. Samples of seawater and sand were collected.

No. 3: eliminated.

No. 4: "Rio Martino" - canal receiving most of the municipal industrial wastewater of Latina (80 000 inhabitants approx.). The samples were collected from the bridge, at km 17.7 on the coast road.

No. 5: at km 17.1 on the coast road (approx. 500 m north-north west of the outfall of Rio Martino into the sea) under the possible influence of this pollution source.

No. 6: beach, located 500 m south east of station No. 7.

No. 7: "Fosso Mascarello" - canal receiving the municipal and industrial wastewater of the area (40 000 inhabitants approx.).

No. 8: beach, located 500 m north west of station No. 7.

2.3 Sampling methods

Water samples were collected in sterile 1 litre bottles. In sampling points 2, 5, 6 and 8 (seawater) the samples were collected by hand at about 10 m from the shoreline. In sampling points 1, 4 and 7 collection was performed by sampling device. In sampling points 1, 2, 5, 6 and 8 sand samples were collected using a sterile wide-mouthed container.

All samples were collected between 9 a.m. and 3 p.m. and transported in cooling containers (4-10°C) protected against ultraviolet irradiation and examined in the laboratory. The maximum transportation and storage time was 24 hours).

In all, 56 water and 40 sand samples were collected and analysed.

2.4 Analytical methods

2.4.1 Water samples

Total coliforms (MPN): presumptive test tubes of Lactose Broth were incubated at 36°C±1°C (total incubation time = 48 hours). Positive tubes were confirmed in Brilliant Green Lactose Bile Broth tubes and incubated at 36°C±1°C for 48 hours.

Faecal coliforms (MPN): positive test tubes of Lactose Broth were confirmed in EC Broth tubes and incubated at 44.5°C±0.2°C for 24 hours.

Faecal coliforms (MF): each sample (or its dilution) was filtered by MF. The membrane was then incubated on M-FC-agar at 44.5°C±0.2°C for 24 hours.

Faecal streptococci (MF): each sample (or its dilution) was filtered by MF. The membrane was then incubated on KF-agar at 44°C±0.2°C for 48±3 hours.

Other parameters (dissolved oxygen, pH, air and water temperature) were determined by standard technical methods.

2.4.2 Sand samples

Total coliforms, faecal coliforms and faecal streptococci were determined also in sand samples collected from the shoreline. Sand was examined by adding approximately 50 g of sand to a beaker containing 50 ml of physiological saline, followed by analysis for total coliforms, faecal coliforms and faecal streptococci, as follows:

Total coliforms (MPN): presumptive test tubes of Lactose Broth were incubated at 36°C±1°C. Positive tubes were confirmed in Brilliant Green Lactose Bile Broth tubes and incubated at 36°C±1°C for 48 hours.

Faecal coliforms (MPN): positive test tubes of Lactose Broth (see total coliforms) were confirmed in EC broth tubes and incubated at 44.5°C±0.2°C for 24 hours.

Faecal streptococci (MF): the sample was filtered by MF and the membrane incubated on KF-agar at 44°C±0.2°C for 48±3 hours.

3. Results

Results obtained are summarized in Tables 2 to 8.

Sampling station No. 1 (Canale Caterattino) showed a high density of bacterial indicators (total and faecal coliforms and faecal streptococci) only in water samples collected in August. The other water samples showed acceptable levels of bacterial indicators. This conforms with the seasonal presence of small discharges (see 2.2). The sand samples showed a constant level of bacterial indicators, which were subject to no fluctuation during the period of study. The DO concentration was at acceptable levels with the exception of the sample collected in August, this correlating with the density of bacterial indicators in the water samples.

Sampling station No. 2 (beach, km 25.6) showed very low levels of bacterial indicators in both water and sand samples. The only exception was the relatively high density of faecal streptococci in the water sample collected on 15.6.82. The DO concentration and pH values were at excellent levels in all the samples examined.

Sampling station No. 4 (Rio Martino) showed very high levels of bacterial indicators in all the samples and correspondingly low values of DO. The pH values were acceptable.

Sampling station No. 5 (beach, km 17.1) showed relatively high levels of bacterial indicators only in the sample collected on 15.6.82. The levels however did not exceed the limits prescribed by the Italian regulations for bathing (100 FC/100 ml). The values of DO and pH were satisfactory in all the samples.

The levels of bacterial density in sand samples were acceptable, though levels found in sampling station No. 2 indicated the influence of the pollution source at station No. 4.

Sampling station No. 6 showed high levels of bacterial indicators in all the water and sand samples (with the exception of the water sample collected on 13.7.82), exceeding the limits of the Italian regulations. The high levels found were due to the influence of the pollution source at station No. 7. The levels of bacterial density in the sand were very high in all the samples. The values of DO and pH were satisfactory in all the samples.

Sampling station No. 7 (Fosso Mascarello) showed very high levels of bacterial indicators in all the samples, which did not correlate to the DO values. The pH values were acceptable.

Sampling station No. 8 showed very high levels of bacterial indicators in the water samples collected on 1.6.82, 13.7.82 and 10.8.82; medium levels in the water samples collected on 18.5.82; and low levels in samples collected on 15.6.82, 26.6.82, 27.7.82 and 31.8.82. Levels of DO and pH were satisfactory. All the sand samples showed a relatively high density of bacterial indicators.

4. Discussion

The levels of the various parameters are shown in Figure 3, as measured in the first sampling site in eight successive weeks. The total coliform (TC), faecal coliform (FC) and faecal streptococci (FS) levels in water samples (the first three graphs in Figure 3) appear to show comparable time trends. The TC, FC and FS levels for sand appear more stable than the corresponding levels in seawater (variances of log-transformed TC and FC values in the sand were significantly less than the corresponding ones in the seawater samples). Analogous results have been recorded in other consistently contaminated sites. It is considered that further data would be necessary to confirm this.

Figure 4 shows contamination levels as measured in five sampling sites three successive weeks. The various indicators generally assume maximum and minimum values in the same sites, thus showing that they basically record the same contamination phenomenon. Significant correlations were found among the various parameters.

Figure 5 shows a highly significant correlation between the FC (MPN) and FC (MF) values. The two parameters appear to be basically equivalent (on the average, MF values were somewhat lower than MPN values, in particular when contamination levels were low). Log-transformed values have been considered, because such transformation enabled a Gaussian-like statistical distribution to be obtained, thus simplifying the statistical treatment of data (Geldreich, 1975).

Figure 6 shows a highly significant correlation between log-transformed FC (MPN) data and log-transformed TC (MPN) data, and Figure 7 the correlation between log-transformed FC (MF) and TC (MPN) data. Figure 8 shows the correlation between log-transformed FS (MF) and FC (MF) log data. Figure 9 shows the correlation between the TC and FC (MPN) log-values in the seawater and in the corresponding sand samples respectively.

All the data obtained indicate that the TC, FC and FS levels in the seawater and in the sand samples are generally correlated, thus indicating that these parameters basically obtain a measure of the same pollution level. The high variability of levels measured in successive samplings is also evident. In any case, the data shown allow an easy classification of site contamination level, if several measurements are considered, and the mean value is assumed as the best indicator (as a rule, the geometric mean or the log-mean or the median value). Single values are scarcely significant for this purpose. The measurement repeatability, as tested by the means of replicated analyses on the same samples, appeared to be characterized by a log-data standard deviation (napierian logarithms) of about 0.7 - 0.8, corresponding to about a factor of 2 for original data in the case of TC and FC, and slightly better for FS. This variability may account only for a minor part of overall variability of measured values.

Table 1

Sea and meteorological conditions on the sampling day

Sampling date	Sea conditions	Surface current m/min	Wind	Sky	Last relevant precipitation
18.5.82	calm	10 from E	weak from SW	clear	+ 4 days
1.6.82	calm	absent	weak from E	clear	+ 4 days
15.6.82	rough	absent	strong from E	clear	2 days
26.6.82	slightly rough	absent	weak from E	clear	+ 4 days
13.7.82	calm	3 from E	absent	clear	+ 4 days
27.7.82	slightly rough	absent	weak from E	clear	+ 4 days
10.8.82	calm	1 from E	weak from E	overcast	2 days
31.8.82	calm	20 from W	weak from W	clear	+ 4 days

Table 2

Monitoring results: sampling station No. 1

Date	Hour	Surface water/100 ml				Sand/100 g			Optional parameters		Other parameters	
		Total coli. MPN	Faecal coli. MPN	Faecal coli. MF	Faecal strep. MF	Total coli. MPN	Faecal coli. MPN	Faecal strep. MF	DO mg/l	pH	water temp. °C	air temp. °C
18.5.82	11.40	1600	350	300	70	230	90	70	10.5	8.3	23	20
1.6.82	11.30	1600	33	40	93	1100	95	10	7.0	7.9	26	29
15.6.82	9.30	70	49	51	39	210	70	20	10.7	8.3	21	22
26.6.82	9.30	23	23	11	58	210	70	0	7.2	8.3	26	22
13.7.82	9.30	14	14	19	16	230	230	40	6.8	8.2	27	28
27.7.82	9.00	46	46	58	10	430	90	40	6.9	8.2	27	29
10.8.82	12.30	>2400	1600	>300	>300	90	40	10	4.3	7.8	26	30
31.8.82	14.20	>2400	>2400	>300	>300	150	90	10	1.0	7.2	28	29

Table 3

Monitoring results: sampling station No. 2

Date	Hour	Surface water/100 ml				Sand/100 g			Optional parameters		Other parameters	
		Total coli. MPN	Faecal coli. MPN	Faecal coli. MF	Faecal strep. MF	Total coli. MPN	Faecal coli. MPN	Faecal strep. MF	DO mg/l	pH	water temp. °C	air temp. °C
18.5.82	12.00	5	<2	0	0	0	0	10	11.5	8.1	23	20
1.6.82	11.40	<2	<2	0	86	0	0	0	11.0	8.3	23	29
15.6.82	9.50	11	11	15	>300	40	30	30	13.7	8.4	17	22
26.6.82	9.55	8	8	5	76	0	0	0	11.4	8.2	22	22
13.7.82	9.45	<2	<2	0	7	150	90	30	9.8	8.2	27	28
27.7.82	9.15	2	2	0	7	90	40	30	11.3	8.1	22	29
10.8.82	12.50	2	2	0	5	0	0	0	10.0	8.3	27	29
31.8.82	14.40	<2	<2	0	4	0	0	0	9.7	8.3	27	29

Table 4

Monitoring results: sampling station No. 4

Date	Hour	Surface water/100 ml				Sand/100 g			Optional parameters		Other parameters	
		Total coli. MPN	Faecal coli. MPN	Faecal coli. MF	Faecal strep. MF	Total coli. MPN	Faecal coli. MPN	Faecal strep. MF	DO mg/l	pH	water temp. °C	air temp. °C
18.5.82	12.30	23000	13000	8000	13000	====	====	====	1.0	7.2	21	20
1.6.82	12.15	70000	70000	51000	1000	====	====	====	1.2	7.0	26	24
15.6.82	10.15	1600000	1600000	>300000	6000	====	====	====	1.0	7.3	22	22
26.6.82	10.20	240000	93000	110000	1000	====	====	====	0.8	7.6	25	25
13.7.82	10.15	1600000	1600000	>300000	13000	====	====	====	0.6	7.2	26	29
27.7.82	9.45	1600000	43000	28000	13000	====	====	====	0.8	7.4	26	28
10.8.82	11.50	>2400000	430000	>300000	45000	====	====	====	0.9	7.2	27	28
31.8.82	13.55	>2400000	>2400000	>300000	21000	====	====	====	0.6	7.5	25	28

Table 5

Monitoring results: sampling station No. 5

Date	Hour	Surface water/100 ml				Sand/100 g			Optional parameters		Other parameters	
		Total coli. MPN	Faecal coli. MPN	Faecal coli. MF	Faecal strep. MF	Total coli. MPN	Faecal coli. MPN	Faecal strep. MF	DO mg/l	pH	water temp. °C	air temp. °C
18.5.82	12.35	240	79	46	3	90	3	80	8.0	8.0	23	22
1.6.82	12.25	43	23	27	40	>11000	4600	40	9.7	8.2	26	26
15.6.82	10.35	150	110	86	>300	150	70	30	11.3	8.1	18	22
26.6.82	10.35	<2	<2	0	20	90	42	40	9.1	8.3	22	25
13.7.82	10.30	<2	<2	0	38	90	40	10	7.4	8.2	27	27
27.7.82	10.00	34	34	39	24	230	230	40	9.5	8.2	22	26
10.8.82	11.30	33	26	6	13	90	40	10	8.3	8.2	26	27
31.8.82	13.40	<2	<2	5	15	10	10	10	11.0	8.4	27	28

Table 6

Monitoring results: sampling station No. 6

Date	Hour	Surface water/100 ml				Sand/100 g			Optional parameters		Other parameters	
		Total coli. MPN	Faecal coli. MPN	Faecal coli. MF	Faecal strep. MF	Total coli. MPN	Faecal coli. MPN	Faecal strep. MF	DO mg/l	pH	water temp. °C	air temp. °C
18.5.82	13.00	350	70	3	6	>11000	>11000	900	9.0	8.1	24	22
1.6.82	12.40	>2400	>2400	>300	5	640	390	500	10.8	8.2	26	24
15.6.82	10.45	>2400	1600	>300	100	1500	280	80	11.1	8.3	25	22
26.6.82	11.00	1600	1600	210	50	>24000	280	80	8.2	8.1	27	25
13.7.82	11.00	46	46	12	45	>24000	280	250	8.1	8.2	29	27
27.7.82	10.10	350	350	>300	>300	>24000	4600	520	8.3	8.3	28	28
10.8.82	11.00	350	350	180	>300	4600	200	80	7.9	8.2	28	27
31.8.82	13.15	>2400	>2400	>300	82	1500	390	28	10.6	8.3	28	27

Table 7

Monitoring results: sampling station No. 7

Date	Hour	Surface water/100 ml				Sand/100 g			Optional parameters		Other parameters	
		Total coli. MPN	Faecal coli. MPN	Faecal coli. MF	Faecal strep. MF	Total coli. MPN	Faecal coli. MPN	Faecal strep. MF	DO mg/1	pH	water temp. °C	air temp. °C
18.5.82	13.05	>2400000	1600000	>300000	13000	====	====	====	10.5	7.8	20	22
1.6.82	12.50	160000	160000	205000	1000	====	====	====	10.1	7.8	21	25
15.6.82	11.00	33000	33000	22000	2000	====	====	====	11.3	7.9	19	22
26.6.82	11.20	350000	240000	210000	1000	====	====	====	10.4	7.8	21	25
13.7.82	11.30	33000	33000	15000	900	====	====	====	9.3	7.9	21	28
27.7.82	10.30	2400000	2400000	>300000	1000	====	====	====	9.0	7.6	22	29
10.8.82	10.40	>2400000	350000	>300000	>300000	====	====	====	10.3	7.7	19	26
31.8.82	13.00	23000	5000	18000	850	====	====	====	11.2	8.0	21	27

Table 8

Monitoring results: sampling station No. 8

Date	Hour	Surface water/100 ml				Sand/100 g			Optional parameters		Other parameters	
		Total coli. MPN	Faecal coli. MPN	Faecal coli. MF	Faecal strep. MF	Total coli. MPN	Faecal coli. MPN	Faecal strep. MF	DO mg/1	pH	water temp. °C	air temp. °C
18.5.82	13.20	1600	350	215	14	>11000	>11000	900	9.4	8.1	27	22
1.6.82	13.00	1600	1600	>300	>300	11000	95	500	8.8	8.2	30	25
15.6.82	11.15	8	8	0	>300	280	30	80	12.1	8.1	19	23
26.6.82	11.30	17	17	0	32	430	230	80	10.7	8.4	22	25
13.7.82	12.00	350	350	>300	>300	150	90	250	8.5	8.3	29	28
27.7.82	11.00	13	13	0	3	430	430	520	11.4	8.3	23	28
10.8.82	10.20	240	240	180	>300	930	110	80	10.5	8.3	24	23
31.8.82	12.40	<2	<2	0	0	60	30	9	11.7	8.4	22	27

Figures 1 and 2

Selected sampling stations, Province of Latina

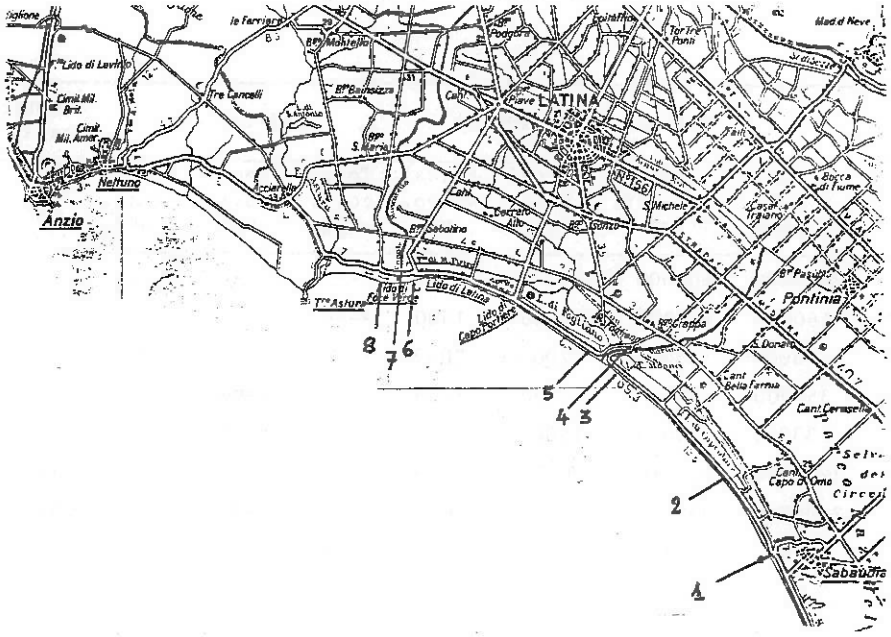


Figure 3

Levels of selected parameters measured at Sampling Site 1 in 8 successive weeks

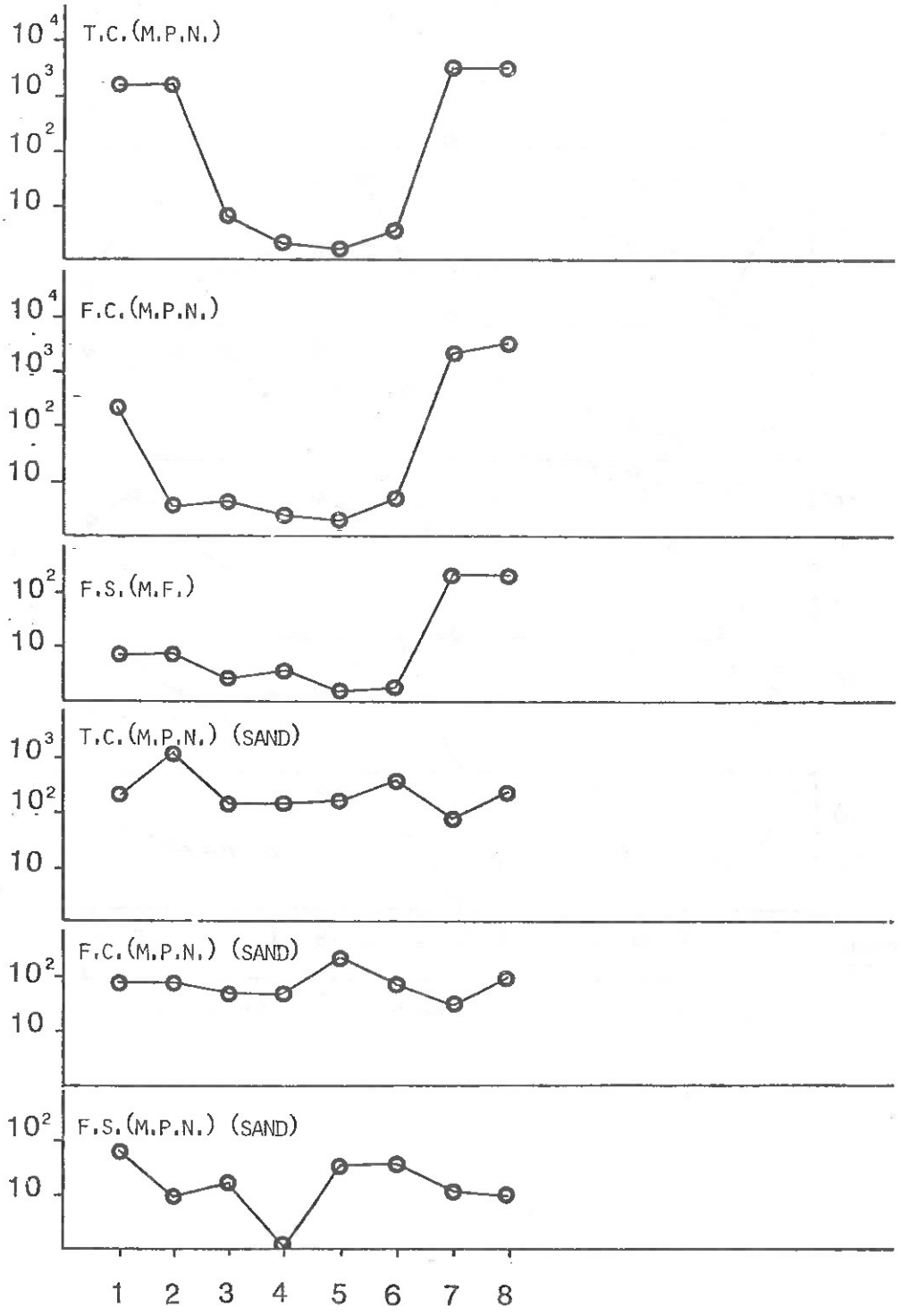


Figure 4

Levels of contamination as measured in 5 sampling sites 3 successive weeks

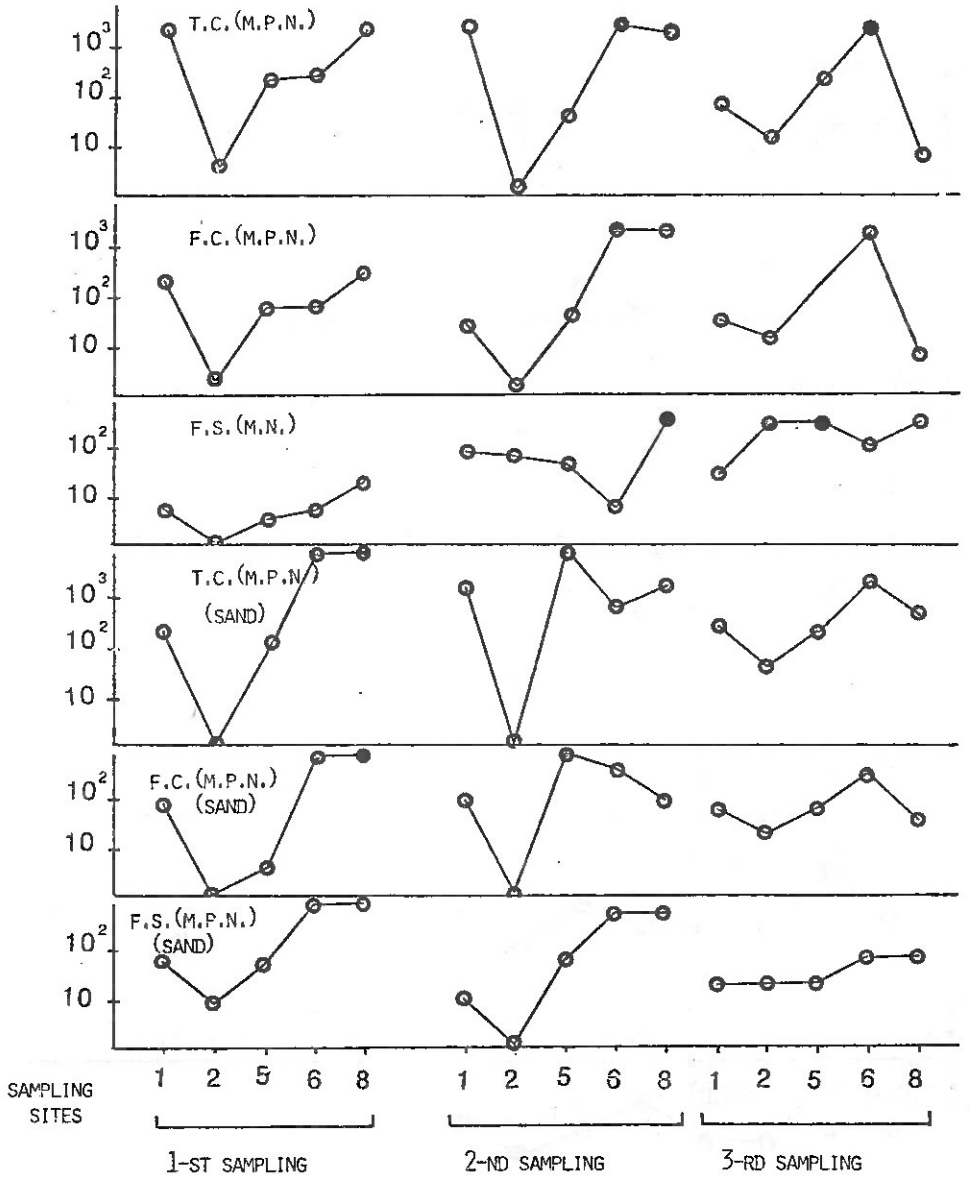


Figure 5

Statistical analysis of FC (MPN) and FC (MF) values

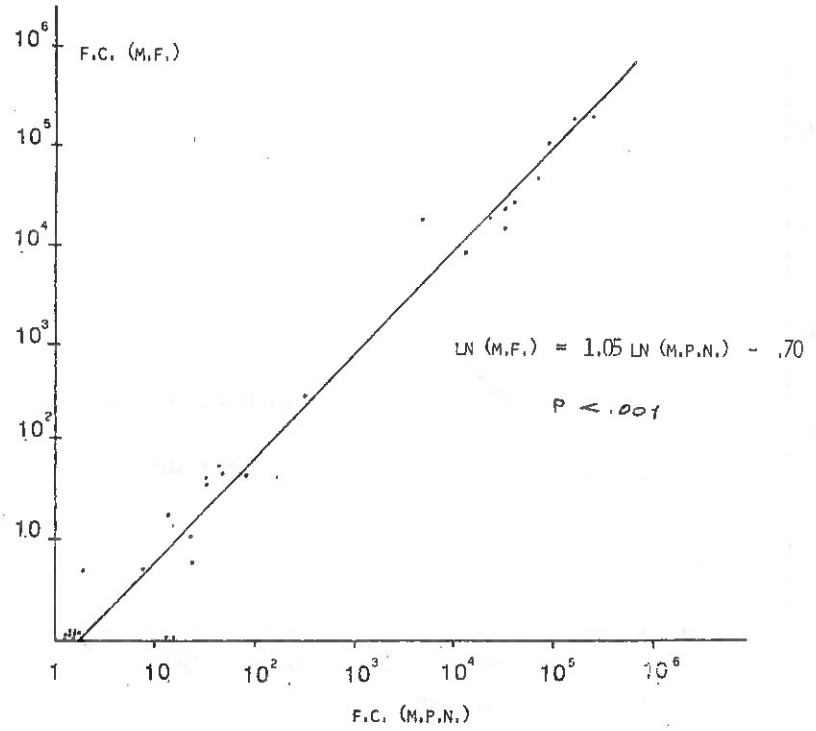


Figure 6

Statistical analysis of log-transformed FC (MPN) data and log-transformed TC (MPN) data

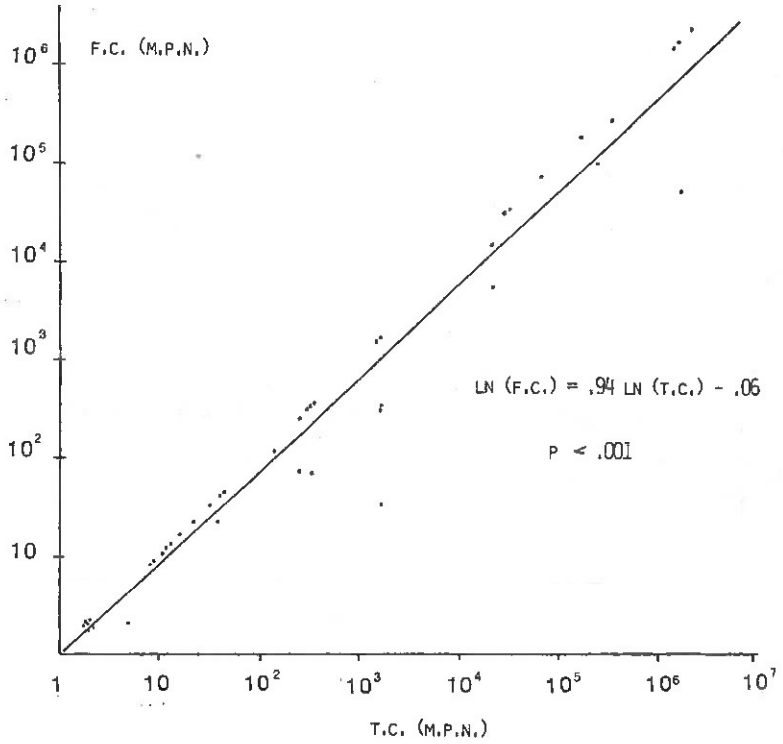


Figure 7

Statistical analysis of log-transformed FC (MF) data and log-transformed TC (MPN) data

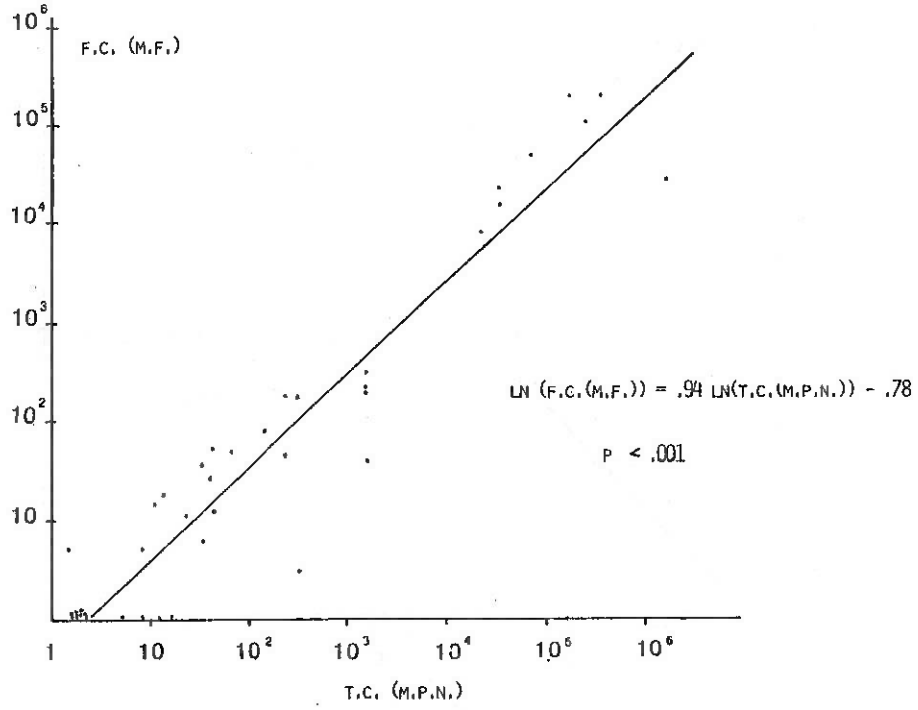


Figure 8

Statistical analysis of log-transformed FS (MF) and log-transformed FC (MF) data

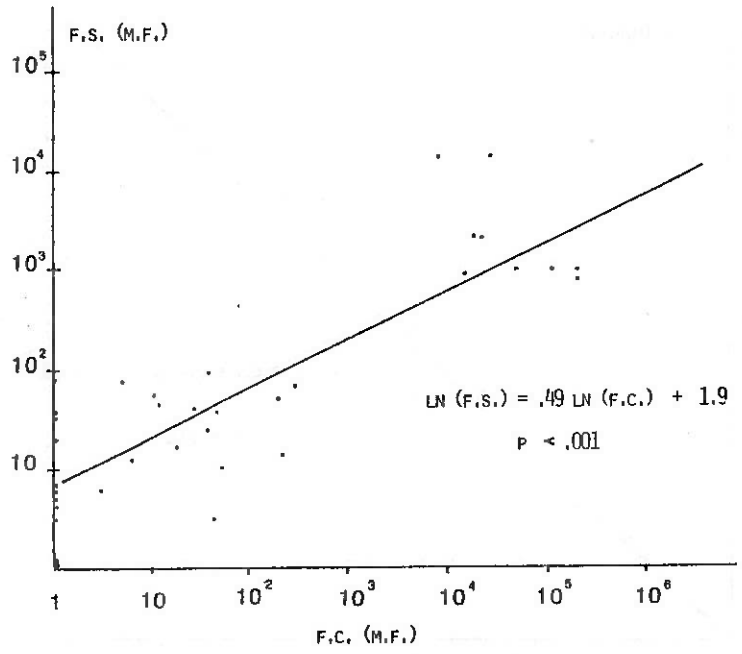
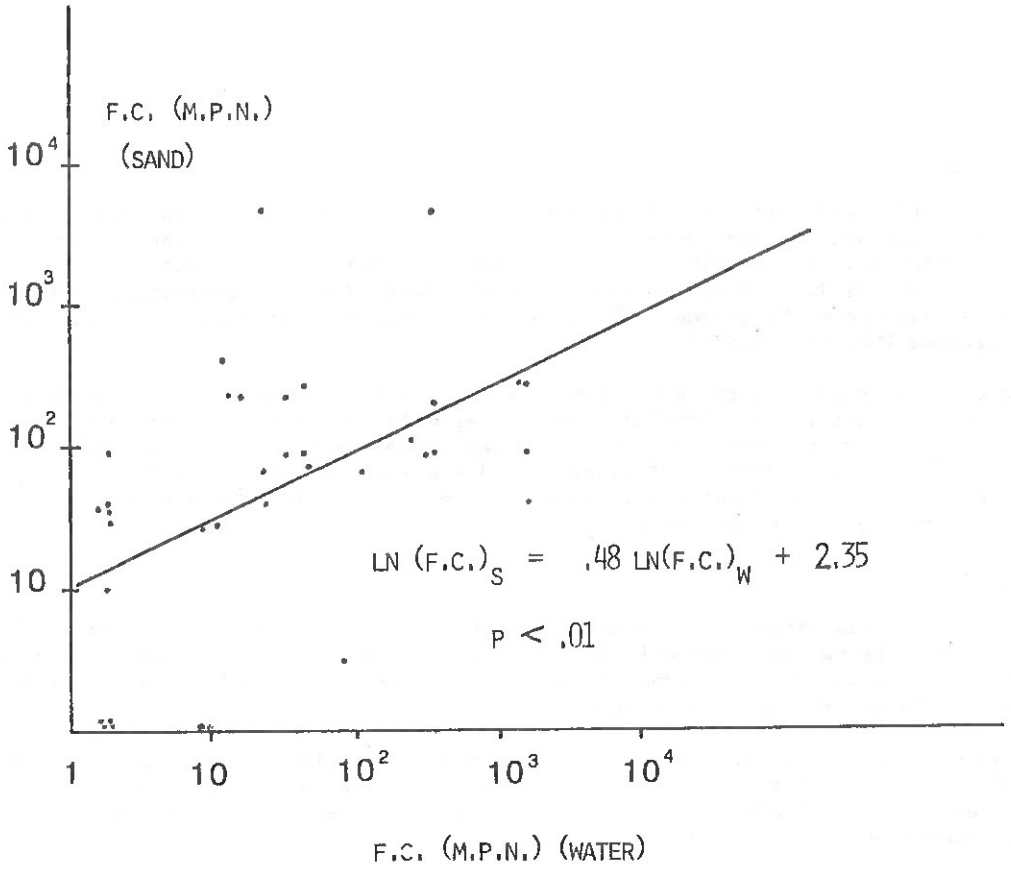


Figure 9

Statistical analysis of log-transformed FC (MPN) values in seawater and sand samples



Annex 6

A COMPARATIVE STUDY OF THE MEMBRANE FILTRATION AND MOST PROBABLE NUMBER METHODS IN THE MICROBIOLOGICAL ANALYSIS OF SEAWATER

by
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1. Introduction

Since 1976, the Territorial Health Promotion Service of the city of Tarragona, Regional Government of Catalonia, has been developing a system of microbiological control of the waters of the province's beaches. In 1978-1979, it participated in the pilot project on Coastal Water Quality Control (MED VII), jointly coordinated by the World Health Organization (WHO) and the United Nations Environment Programme (UNEP), as part of the Mediterranean Pollution Monitoring and Research Programme (MED POL Phase I).

During the course of both these projects, repeated reference was made to the need for unifying sampling procedures, analytical techniques and criteria for interpreting findings. For this reason and on the basis of work performed to date, the Institution expressed its willingness to participate in the study on methods of sampling and analysis of bacteriological parameters in coastal water quality, carried out under the joint sponsorship of WHO and UNEP and coordinated by the Istituto Superiore di Sanità, Rome.

2. Scope and purpose

The purpose of this study was to compare the membrane filtration and most probable number methods, which are the two most commonly used in analysis of the microbiological quality of coastal waters with regard to those microorganisms indicating faecal contamination, i.e. total coliforms (TC), faecal coliforms (FC) and faecal streptococci (FS).

Three sampling stations were selected. These represented three different levels of faecal contamination, ranging from a coastal area with little or no contamination to an urban wastewater outfall. In each case, repeated samples were taken and analysed microbiologically, using the two methods mentioned above.

3. Sampling

The sampling points selected were:

(a) the submarine outfall of urban wastewater from the city of Tarragona (length 1000 m, outfall depth 20-25 m);

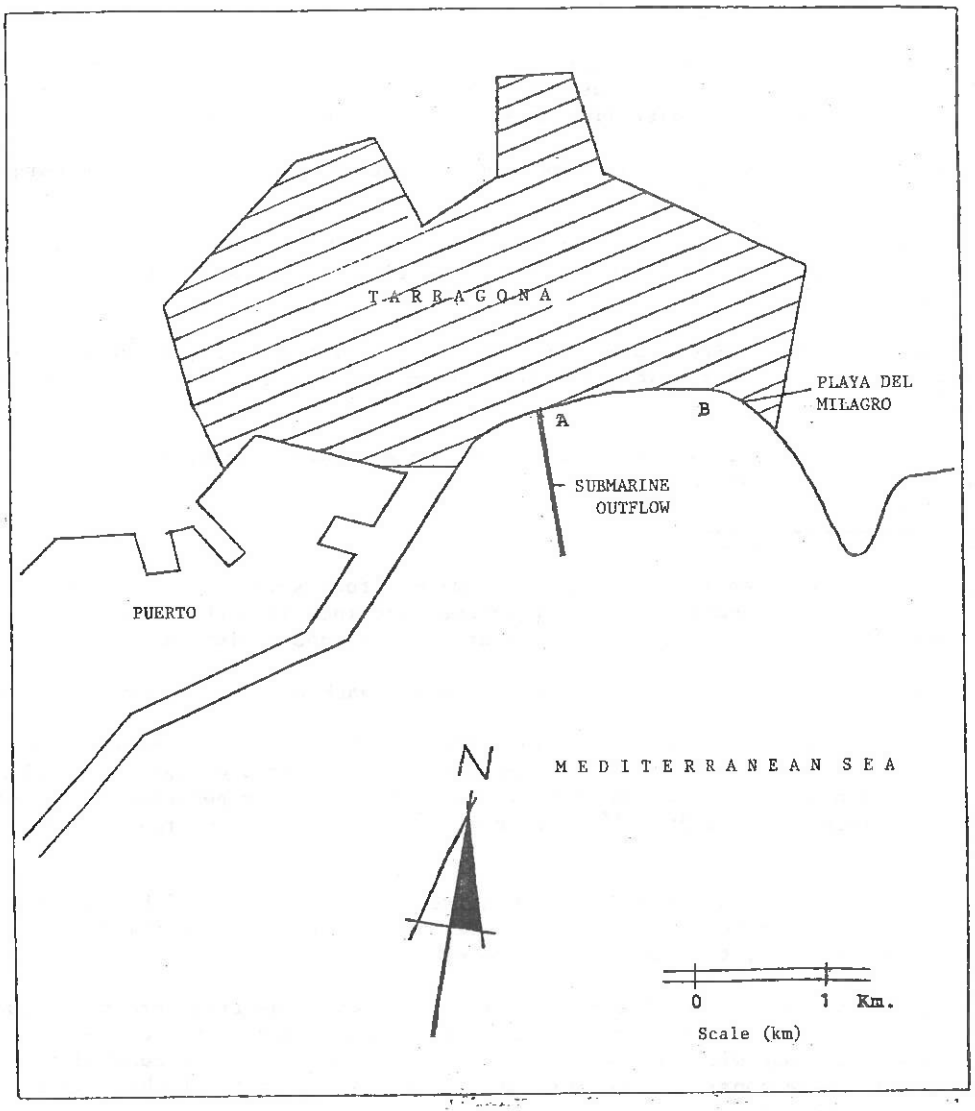
(b) the Playa del Milagro, Tarragona, which is affected by submarine outfall A (diagram 1);

(c) the Playa de la Mora, Tarragona, which is exceptionally clean and hygienic.

Sampling was performed between 10 August and 6 September 1982, most of the samples being taken between 8 a.m. and 10 a.m., using amber glass bottles with ground-glass stoppers, sterilized in a Pasteur oven. At points B and C, sampling was performed by direct access up to 10-15 m from the coastline, at a depth of 1.00-1.50 m, the bottle being dipped 15-20 cm below the surface of the water. Sampling at point A was performed directly at the pumping station for the submarine wastewater outfall.

As soon as samples were collected, they were placed in a thermally insulated container and protected from the light until they reached the laboratory. The time between the collection of the first sample and its arrival at the laboratory was in all instances less than two hours, and the samples were analysed immediately.

Diagram 1. Situation of sampling points A and B (Playa del Milagro, Tarragona)



4. Analytical techniques

The analytical techniques used were those described by APHA (1975), WHO/UNEP (1977) and UNEP (1981).

In addition to the comparison made between the membrane filtration and most probable number methods, analysis of TC and FC using the membrane filtration method was carried out in duplicate, using absorbent pads and agar respectively, thus giving a third set of results for these microorganisms.

The use of Escherichia coli (EC) as an indicator microorganism was regarded for the purposes of evaluating the water quality of a coastal area as practically the equivalent of FC.

4.1 Membrane filtration method

For analysis of the three categories of microorganisms of the indicator type, membranes used were Millipore Standard HAWG, 47 mm in diameter and 0.45 µm in pore size. Filtration funnels and supports were made of plastic and sterilized with ultraviolet rays. Culture media were prepared daily, and the volume of water filtered was decided in the light of the concentration of microorganisms anticipated, so as to make the resulting number of colonies 20-80 per plate.

The average number of filtrations per sample of water analysed was eight: three for TC, three for FC, and two for FS. In the case of highly polluted water samples, a series of prior dilutions was carried out, using distilled water, sterile and stoppered.

Culture media and incubation conditions used for each of the indicator microorganisms were as follows.

(1) Total coliforms: M-Endo broth MF on absorbent pads or M-Endo MF on agar, depending on whether pads or agar were used, on a hermetic petri dish, 47 mm in diameter, incubated for 24 hours at 36 ± 1°C.

(2) Faecal coliforms: M-FC broth on absorbent pads or M-FC agar, depending on whether pads or agar were used, on a hermetic petri dish, 47 mm in diameter, incubated for 24 hours at 44 ± 0.2°C.

(3) Faecal streptococci: Agar-M-Enterococci, on a hermetic petri dish, 47 mm in diameter, incubated for 48 hours at 36 ± 1°C.

4.2 Most probable number method

For analysis of the three indicator microorganisms, four series of five tubes were used to determine the most probable number of microorganisms contained in each sample of water. For the analysis of samples of highly polluted water, a sterile phosphate plug was used as a dilutant.

The culture media and the incubation conditions for each of the microorganisms were as follows.

(1) Total coliforms: MacConkey broth incubated at 36 ± 1°C for 48 hours. Tubes were considered positive when they turned the medium over and produced gas in a Durham tube. Thus, the presumptive number of coliform bacteria was obtained. The positive tubes were redispensed in brilliant green broth at 36 ± 1°C for 48 hours, giving the confirmatory number of coliform bacteria.

(2) Faecal coliforms: MacConkey broth incubated at 36 ± 1°C for 48 hours. The reading was taken as in determining TC. The positive tubes were redispensed in brilliant green broth at 44 ± 0.2°C for 24 hours, thus giving the number of FC.

(3) Faecal streptococci: for the presumptive test, azide dextrose broth was used, incubated at 36 ± 1°C for 48 hours. The reading was taken by turbidity. The redispensing of the positive tubes was done with ethyl violet azide broth (EVA broth) incubated at 36 ± 1°C for 48 hours. Tubes were considered positive if there was turbidity of the medium and a purple bead appeared at the base.

5. Results and statistical analysis

The results obtained from microbiological analyses of TC, FC and FS found at the three sampling points, carried out in accordance with the two methods, can be seen in Tables 1, 2 and 3. The number of samples taken at the submarine outfall was greater because of the variability of the microbiological characteristics of the wastewater. In the case of TC and FC, the results of the analyses made using the membrane filter method, with absorbent pads instead of agar, were also added.

For a statistical comparison of the series of microbiological results obtained from each water sample using the two methods, a parametric statistical method of "comparison of pairs" known as the "t-test" was applied. In order to apply this "t-test", the two series of microbiological results to be compared have to be regarded as adjusting to a normal distribution, which is actually the case if the logarithm of the microbiological concentrations obtained is considered.

Table 1. Results of microbiological analyses carried out at Playa del Milagro, submarine outfall, point A

Date	Temperature	Total coliforms per 100 ml			Faecal coliforms per 100 ml			Faecal streptococci per 100 ml	
		MF (pads)	MF (agar)	MPN	MF (pads)	MF (agar)	MPN	MF (agar)	MPN
10.8.82	24.8°	$3.0.10^8$	$4.0.10^8$	$5.0.10^7$	$5.5.10^7$	$8.0.10^7$	$2.0.10^7$	$\geqslant 10^5$	$3.3.10^6$
11.8.82	25.0°	$9.6.10^7$	$2.4.10^7$	$2.0.10^5$	$6.0.10^6$	$1.0.10^5$	$\geqslant 10^5$	$1.1.10^6$	$2.0.10^3$
12.8.82	25.0°	$1.2.10^8$	$6.0.10^7$	$2.4.10^7$	$\geqslant 10^5$	$\geqslant 10^5$	$2.0.10^5$	$1.9.10^6$	$7.0.10^4$
16.8.82	26.5°	$1.4.10^7$	$1.7.10^7$	$2.4.10^7$	$1.1.10^7$	$1.1.10^7$	$2.4.10^7$	$5.6.10^5$	$1.6.10^6$
23.8.82	25.5°	$4.0.10^7$	$6.3.10^7$	$9.2.10^7$	$3.4.10^7$	$2.2.10^7$	$2.0.10^5$	$2.6.10^6$	$5.4.10^6$
24.8.82	24.5°	$2.3.10^7$	$2.4.10^7$	$3.4.10^6$	$\geqslant 10^5$	$\geqslant 10^5$	$2.0.10^5$	$1.8.10^6$	$9.4.10^5$
30.8.82	24.8°	$5.2.10^7$	$7.2.10^7$	$1.6.10^8$	$3.4.10^7$	$1.2.10^7$	$1.7.10^6$	$6.4.10^5$	$5.4.10^6$
31.8.82	24.0°	$1.6.10^7$	$3.5.10^7$	$7.9.10^6$	$8.4.10^6$	$1.0.10^6$	$7.9.10^6$	$3.7.10^6$	$1.3.10^6$
6.9.82	25.5°	$3.4.10^7$	$3.6.10^7$	$3.5.10^7$	$1.7.10^7$	$1.1.10^7$	$1.4.10^6$	$9.6.10^5$	$2.4.10^6$
6.9.82	25.4°	$2.6.10^7$	$2.0.10^7$	$2.4.10^7$	$4.5.10^6$	$8.0.10^6$	$9.0.10^5$	$1.7.10^5$	$2.3.10^5$

Table 2. Results of microbiological analyses carried out at Playa del Milagro, submarine outfall, point B

Date	Temperature	Total coliforms per 100 ml			Faecal coliforms per 100 ml			Faecal streptococci per 100 ml	
		MF (pads)	MF (agar)	MPN	MF (pads)	MF (agar)	MPN	MF (agar)	MPN
10.8.82	24.3°	190	130	70	40	5	2	5	2
11.8.82	24.0°	100	15	2	5	5	2	31	6
12.8.82	24.6°	700	260	49	12	4	5	7	2
16.8.82	26.0°	60	44	26	29	24	21	3	2
24.8.82	24.4°	550	46	5	20	7	2	4	4
30.8.82	24.7°	1100	470	170	110	49	70	8	2
31.8.82	25.0°	50	80	9	0	7	4	0	2

Table 3. Results of microbiological analyses carried out at Playa del Milagro, submarine outfall, point C

Date	Temperature	Total coliforms per 100 ml			Faecal coliforms per 100 ml			Faecal streptococci per 100 ml	
		MF (pads)	MF (agar)	MPN	MF (pads)	MF (agar)	MPN	MF (agar)	MPN
10.8.82	24.3°	70	70	2	2	2	2	10	7
11.8.82	24.5°	64	44	< 2	0	0	< 2	0	< 2
12.8.82	24.4°	94	54	11	4	3	4	3	5
16.8.82	26.0°	53	26	7	0	2	< 2	9	6
23.8.82	24.5°	61	23	2	5	16	< 2	2	< 2
24.8.82	24.4°	280	119	2	2	70	< 2	4	5
30.8.82	24.3°	13	10	< 2	0	6	< 2	0	< 2
31.8.82	25.0°	44	4	6	2	0	2	0	< 2

If the logarithms of the microbiological concentrations obtained using the membrane filter method are represented as:

$$X_1, X_2, \ldots\ldots X_i \ldots\ldots X_n$$

with a mean of μ_x and a standard deviation of δ^2_x,

and if the logarithms of the microbiological concentrations obtained using the most probable number method are represented as:

$$Y_1, Y_2, \ldots\ldots Y_i \ldots\ldots Y_n$$

with a mean of μ_y and a standard deviation of δ^2_y,

then the "t-test" consists in obtaining the difference for each pair of results relating to the same water sample and to the same microorganism (X_i, Y_i), i.e.:

$$\begin{aligned} d_1 &= X_1 - Y_1 \\ d_2 &= X_2 - Y_2 \\ &\vdots \\ d_i &= X_i - Y_i \\ &\vdots \\ d_n &= X_n - Y_n \end{aligned}$$

This series of differences d_i will also follow a normal distribution whose mean is μ_d, and whose standard deviation is δ_d.

Before the two series of results X_i, Y_i can be regarded as not presenting significant differences at level of confidence α, the following hypothesis must hold good:

$$H_o : \mu_d = \mu_X - \mu_Y = 0$$

where

$$\left| \frac{\mu_d \sqrt{n}}{\delta_d} \right| < t_{1-\frac{\alpha}{2},n-1}$$

and μ_d is the mean of the differences
δd is the standard deviation
α is the level of confidence
n is the number of differences involved
t is the probability as shown in Table 4.

If H_o does not hold good, there are two alternative hypotheses:

(a)

$$H_1 : \mu_d = \mu_X - \mu_Y > 0$$

where

$$\frac{\mu_d \sqrt{n}}{\delta_d} > t_{1-\alpha,n-1}$$

i.e. the resulting figures for series X_i (membrane filter method) are higher than those for series Y_i (most probable number method);

(b)

$$H_1 : \mu_d = \mu_X - \mu_Y < 0$$

where

$$\frac{\mu_d \sqrt{n}}{\delta_d} < t_{1-\alpha,n-1}$$

i.e. the figures for series Y_i (most probable number method) are higher than those for series X_i (membrane filter method).

Table 4. Probabilities "t" for the application of the "t-test"

The probability of a difference numerically greater than t is twice that shown at the head of the table.

Simplified tables of t distributions

Degree of freedom	Probability of a difference greater than t											
n	.003	.01	.025	.05	.1	.15	.2	.25	.3	.35	.4	.45
1	63.657	31.821	12.706	6.314	3.078	1.963	1.376	1.000	.727	.510	.325	.158
2	9.925	6.965	4.303	2.920	1.858	1.386	1.061	.816	.617	.445	.289	.142
3	5.841	4.541	3.182	2.353	1.638	1.250	.978	.765	.584	.424	.277	.137
4	4.604	3.747	2.776	2.132	1.533	1.190	.941	.741	.569	.414	.271	.134
5	4.032	3.365	2.571	2.015	1.476	1.156	.920	.727	.559	.408	.267	.132
6	3.707	3.143	2.447	1.943	1.440	1.134	.906	.718	.553	.404	.265	.131
7	3.499	2.998	2.365	1.895	1.415	1.119	.896	.711	.549	.402	.263	.130
8	3.355	2.896	2.306	1.860	1.397	1.108	.889	.706	.546	.399	.262	.130
9	3.250	2.821	2.262	1.833	1.383	1.100	.883	.703	.543	.398	.261	.129
10	3.169	2.764	2.228	1.812	1.372	1.093	.879	.700	.542	.397	.260	.129
11	3.106	2.718	2.201	1.796	1.363	1.088	.876	.697	.540	.396	.260	.129
12	3.055	2.681	2.179	1.782	1.356	1.083	.873	.695	.539	.395	.259	.128
13	3.012	2.650	2.160	1.771	1.350	1.079	.870	.694	.538	.394	.259	.128
14	2.977	2.624	2.145	1.761	1.345	1.076	.868	.692	.537	.393	.258	.128
15	2.947	2.602	2.131	1.753	1.341	1.074	.866	.691	.536	.393	.258	.128
16	2.921	2.583	2.120	1.746	1.337	1.071	.865	.690	.535	.392	.258	.128
17	2.898	2.567	2.110	1.740	1.333	1.069	.863	.689	.534	.392	.257	.128
18	2.878	2.552	2.101	1.734	1.330	1.067	.862	.688	.534	.392	.257	.127
19	2.861	2.539	2.093	1.729	1.328	1.066	.861	.688	.533	.391	.257	.127
20	2.845	2.528	2.086	1.725	1.325	1.064	.860	.687	.533	.391	.257	.127
21	2.831	2.518	2.080	1.721	1.323	1.063	.859	.686	.532	.391	.257	.127
22	2.819	2.508	2.074	1.717	1.321	1.061	.858	.686	.532	.390	.256	.127
23	2.807	2.500	2.069	1.714	1.319	1.060	.858	.685	.532	.390	.256	.127
24	2.797	2.492	2.064	1.711	1.318	1.059	.857	.685	.531	.390	.256	.127
25	2.787	2.485	2.060	1.708	1.316	1.058	.856	.684	.531	.390	.256	.127
26	2.779	2.479	2.056	1.706	1.315	1.058	.856	.684	.531	.390	.256	.127
27	2.771	2.473	2.052	1.703	1.314	1.057	.855	.684	.531	.389	.256	.127
28	2.763	2.467	2.048	1.701	1.313	1.056	.855	.683	.530	.389	.256	.127
29	2.756	2.462	2.045	1.699	1.311	1.055	.854	.683	.530	.389	.256	.127
30	2.750	2.457	2.042	1.697	1.310	1.055	.854	.683	.530	.389	.256	.127
oo	2.576	2.326	1.960	1.645	1.282	1.036	.842	.674	.524	.385	.253	.126

Source: Hoel, P.G. A first course in the theory of modern statistical methods. New York, Wiley, 1963 (28).

Table 4 taken from Statistical methods for research workers, with the kind permission of the author, Professor R.A. Fisher, and the publishers, Oliver and Boyd.

On applying the method to the results obtained from the microbiological analyses with a confidence interval $\alpha = 95\%$, the following results are obtained.

Sampling point A - submarine outfall

(1) Total coliforms: MF (agar)/MPN

$n = 10$
$\mu_d = 0.415$
$\delta_d = 0.737$
$t = 2.262$

$$\left|\frac{0.415.\sqrt{10}}{0.737}\right| = 1.779 < 2.262$$

i.e. the two series of results do not present significant differences with a confidence interval of 95%.

(2) Total coliforms: MF (pads)/MPN

$n = 10$
$\mu_d = 0.423$
$\delta_d = 0.925$
$t = 2.262$

$$\left|\frac{0.423.\sqrt{10}}{0.925}\right| = 1.446 < 2.262$$

i.e. the two series of results do not present significant differences with a confidence interval of 95%.

(3) Total coliforms: MF (pads)/MF (agar)

$n = 10$
$\mu_d = 0.0087$
$\delta_d = 0.271$
$t = 2.262$

$$\left|\frac{0.0087.\sqrt{10}}{0.271}\right| = 0.102 < 2.262$$

i.e. the two series of results do not present significant differences with a confidence interval of 95%.

(4) Faecal coliforms: MF (agar)/MPN

$n = 7$
$\mu_d = 0.586$
$\delta_d = 0.955$
$t = 2.447$

$$\left|\frac{0.586.\sqrt{7}}{0.955}\right| = 1.624 < 2.447$$

i.e. the two series of results do not present significant differences with a confidence interval of 95%.

(5) Faecal coliforms: MF (pads)/MPN

$n = 7$
$\mu_d = 0.777$
$\delta_d = 0.857$
$t = 2.447$

$$\left|\frac{0.777.\sqrt{7}}{0.857}\right| = 2.399 < 2.447$$

i.e. the two series of results do not present significant differences with a confidence interval of 95%.

(6) Faecal coliforms: MF (pads)/MF (agar)

$n = 8$
$\mu_d = 0.390$
$\alpha_d = 0.672$
$t = 2.365$

$$\left|\frac{0.390.\sqrt{8}}{0.672}\right| = 1.641 < 2.365$$

i.e. the two series of results do not present significant differences with a confidence interval of 95%.

(7) Faecal streptococci: MF (agar)/MPN

$n = 9$
$\mu_d = 0.298$
$\alpha_d = 1.140$
$t = 2.306$

$$\left|\frac{0.298.\sqrt{9}}{1.140}\right| = 0.784 < 2.306$$

i.e. the two series of results do not present significant differences with a confidence interval of 95%.

Sampling point B - Playa del Milagro

(1) Total coliforms: MF (agar)/MPN

$n = 7$
$\mu_d = 0.636$
$\alpha_d = 0.319$
$t = 2.447$

$$\left|\frac{0.636.\sqrt{7}}{0.319}\right| = 5.273 > 2.447$$

Hypothesis H_o does not hold good, and we apply the terms of hypothesis H_1:

$t = 1.943$

$$\frac{0.636.\sqrt{7}}{0.319} = 5.273 > 1.943$$

i.e. the figures for the series representing the membrane filter method are higher than those for the series using the most probable number method.

(2) Total coliforms: MF (pads)/MPN

$n = 7$
$\mu_d = 1.035$
$\delta_d = 0.634$
$t = 2.447$

$$\left|\frac{1.035.\sqrt{7}}{0.634}\right| = 4.318 > 2.447$$

Hypothesis H_o does not hold good, and we apply the terms of hypothesis H_1:

$t = 1.943$

$$\frac{1.035.\sqrt{7}}{0.634} = 4.318 > 1.943$$

i.e. the figures for the series representing the membrane filter method are higher than those for the series using the most probable number method.

(3) Total coliforms: MF (pads)/MF (agar)

$n = 7$
$\mu_d = 0.399$
$\delta_d = 0.434$
$t = 2.447$

$$\left|\frac{0.399.\sqrt{7}}{0.434}\right| = 2.432 < 2.447$$

i.e. the two series of results do not present significant differences with a confidence interval of 95%.

(4) Faecal coliforms: MF (agar)/MPN

$n = 7$
$\mu_d = 0.198$
$\delta_d = 0.269$
$t = 2.447$

$$\left|\frac{0.198.\sqrt{7}}{0.269}\right| = 1.947 < 2.447$$

i.e. the two series of results do not present significant differences with a confidence interval of 95%.

(5) Faecal coliforms: MF (pads)/MPN

$n = 7$
$\mu_d = 0.402$
$\delta_d = 0.617$
$t = 2.447$

$$\left|\frac{0.402.\sqrt{7}}{0.617}\right| = 1.723 < 2.447$$

i.e. the two series of results do not present significant differences with a confidence interval of 95%.

(6) Faecal coliforms: MF (pads)/MF (agar)

$n = 7$
$\mu_d = 0.203$
$\delta_d = 0.548$
$t = 2.447$

$$\left|\frac{0.203.\sqrt{7}}{0.548}\right| = 0.979 < 2.447$$

i.e. the two series of results do not present significant differences with a confidence interval of 95%.

(7) Faecal streptococci: MF (agar)/MPN

$n = 7$
$\mu_d = 0.305$
$\delta_d = 0.364$
$t = 2.447$

$$\left|\frac{0.305.\sqrt{7}}{0.364}\right| = 2.217 < 2.447$$

i.e. the two series of results do not present significant differences with a confidence interval of 95%.

Sampling point C - Playa de la Mora

(1) Total coliforms: MF (agar)/MPN

$n = 8$
$\mu_d = 0.938$
$\delta_d = 0.626$
$t = 2.365$

$$\left|\frac{0.938.\sqrt{8}}{0.626}\right| = 4.237 > 2.365$$

Hypothesis H_o does not hold good, and we apply the terms of hypothesis H_1:

$t = 1.895$

$$\frac{0.938.\sqrt{8}}{0.626} = 4.237 > 1.895$$

i.e. the figures for the series representing the membrane filter method are higher than those for the series using the most probable number method.

(2) Total coliforms: MF (pads)/MPN

$n = 8$
$\mu_d = 1.271$
$\delta_d = 0.476$
$t = 2.365$

$$\left|\frac{1.271.\sqrt{8}}{0.476}\right| = 7.551 > 2.365$$

Hypothesis H_o does not hold good, and we apply the terms of hypothesis H_1:

$t = 1.895$

$$\frac{1.271.\sqrt{8}}{0.476} = 7.551 > 1.895$$

i.e. the figures for the series representing the membrane filter method are higher than those for the series using the most probable number method.

(3) Total coliforms: MF (pads)/MF (agar)

$n = 8$
$\mu_d = 0.333$
$\delta_d = 0.318$
$t = 2.365$

$$\left| \frac{0.333 . \sqrt{8}}{0.318} \right| = 2.961 > 2.365$$

Hypothesis H_o does not hold good, and we apply the terms of hypothesis H_1:

$t = 1.895$

$$\left| \frac{0.333 . \sqrt{8}}{0.318} \right| = 2.961 > 1.895$$

i.e. the figures for the series representing the membrane filter method using pads are higher than those for the same method using agar.

(4) Faecal coliforms: MF (agar)/MPN

$n = 8$
$\mu_d = 0.275$
$\delta_d = 0.657$
$t = 2.365$

$$\left| \frac{0.275 . \sqrt{8}}{0.657} \right| = 1.184 < 2.365$$

i.e. the two series of results do not present significant differences with a confidence interval of 95%.

(5) Faecal coliforms: MF (pads)/MPN

$n = 8$
$\mu_d = 0.063$
$\delta_d = 0.239$
$t = 2.365$

$$\left| \frac{0.063 . \sqrt{8}}{0.239} \right| = 0.734 < 2.365$$

i.e. the two series of results do not present significant differences with a confidence interval of 95%.

(6) Faecal coliforms: MF (pads)/MF (agar)

$n = 8$
$\mu_d = -0.338$
$\delta_d = 0.601$
$t = 2.365$

$$\left| \frac{-0.338 . \sqrt{8}}{0.601} \right| = 1.590 < 2.365$$

i.e. the two series of results do not present significant differences with a confidence interval of 95%.

(7) Faecal streptococci: MF (agar)/MPN

$n = 8$
$\mu_d = 0.111$
$\delta_d = 0.202$
$t = 2.365$

$$\left| \frac{0.111 . \sqrt{8}}{0.202} \right| = 1.557 < 2.365$$

i.e. the two series of results do not present significant differences with a confidence interval of 95%.

The entire statistical analysis of the results is summarized in Tables 5 and 6.

Table 5. Summary comparison of the results of microbiological analysis obtained using the MF and MPN methods

Sampling point	Faecal contamination	Total coliforms	Faecal coliforms	Faecal streptococci
A	High	$10^8 - 10^7$ MF = MPN	$10^7 - 10^6$ MF = MPN	$10^6 - 10^5$ MF = MPN
B	Lower	$10^3 - 10^2$ MF > MPN	$10^2 - 10$ MF = MPN	$10 - 0$ MF = MPN
C	Minimal	$10^2 - 10$ MF > MPN	$10 - 0$ MF = MPN	$10 - 0$ MF = MPN

N.B. The concentrations of microorganisms are expressed in 100 ml of the sample.

Thus, the series of results of the microbiological analyses obtained by the MF and MPN methods do not present significant differences with a confidence interval of 95%, except in the case of lower concentrations of TC, where the figures obtained by the MF method are slightly higher than those using the MPN method.

Table 6. Summary comparison of results obtained from analyses of total coliforms and faecal coliforms by the MF method, using absorbent pads or agar

Sampling point	Faecal contamination	Faecal coliforms	Faecal streptococci
A	High	$10^8 - 10^7$ Pads = agar	$10^7 - 10^6$ Pads = agar
B	Lower	$10^3 - 10^2$ Pads = agar	$10^2 - 10$ Pads = agar
C	Minimal	$10^2 - 10$ Pads > agar	$10 - 0$ Pads = agar

N.B. The concentrations of microorganisms are expressed in 100 ml of the sample.

Thus, the series of results of analyses of TC and FC obtained by the MF method using pads or agar do not present significant differences with a confidence interval of 95%, except in the case of lower concentrations of TC, where the use of pads gives slightly better results than those obtained when agar is used.

6. Comparative economic assessment

With a view to making a comparative economic assessment of the microbiological analyses of the three indicator microorganisms, according as one or other method is used, the following factors were taken into account:

(1) cost of culture media, reagents and dilutants;

(2) cost of disposable material;

(3) cost of depreciation on the inventory;

(4) time spent in preparing and carrying out the analyses.

This last factor was not included in the cost estimates. A breakdown of costs is given in Tables 7-12.

A summary of the economic assessment is given in Table 13.

Table 7. Average cost of culture media per tube or plate

Culture medium	Price per flask (pesetas)	Number of tubes/ plates per flask		Total cost per tube/plate (pesetas)	
		Double	Single	Double	Single
EVA broth	7 539	648	1 297	11.6	5.8
Azide dextroze broth	7 000	648	1 297	10.8	5.4
MacConkey broth	7 300	648	1 297	11.26	5.6
Brilliant green	5 131	648	1 297	7.92	3.96
M-FC broth	7 670	6 191		1.2	
Agar (FC)	-	-		2.5	
M-Endo broth	8 031	1 179		6.8	
Agar (TC)	-	-		8.1	
M-Enterococcus agar	10 175	1 513		6.7	
Bacto agar	4 884	3 623		1.3	

Table 8. Average cost of culture per sample analysed

		Total coliforms	Faecal coliforms	Faecal streptococci	Total cost (pesetas)
MF	Number of plates	3	3	2	-
	Average cost	24.3	7.5	13.4	45.2
MPN	Number of tubes (presumptive)	20	-	20	-
	Number of tubes (confirmed)	20	20	20	-
	Average cost	252	79.2	251	582.2

Table 9. Average cost of disposable material per sample of water analysed

MPN method		
Tubes	Average lifetime 1.5 years	
	51 pesetas per tube 100 tubes per sample	13 pesetas per sample
Durham tubes	Average lifetime 1.5 years	
	25 pesetas per D. tube 60 D. tubes per sample	3.8 pesetas per sample
Total		16.8 pesetas
MF method		
Plates	Use and discard	
	29.6 pesetas per plate 8 plates per sample	236.8 pesetas per sample
Membranes	Use and discard	
	36.0 pesetas per membrane 8 membranes per sample	288 pesetas per sample
Total		524.8 pesetas

Table 10. Cost of depreciation of apparatus (MPN method)

MPN method		
Incubator (stove) 37°C	Cost:	43 227 pesetas
	Average lifetime (depreciation):	10 years
	Average number of analyses per week:	7
	Average depreciation cost:	33.2 pesetas
Incubator (stove) 44°C	Cost:	43 227 pesetas
	Average lifetime (depreciation):	10 years
	Average number of analyses per week:	7
	Average depreciation cost:	16.6 pesetas
Autoclave	Cost:	400 000 pesetas
	Average lifetime (depreciation):	10 years
	Number of times used per analysis:	2
	Average number of analyses each week:	7
	Average depreciation cost:	153.0 pesetas
Total depreciation cost (average)		202.8 pesetas

Table 11. Cost of depreciation of apparatus (MF method)

MF method		
Filtering equipment	Cost:	153 000 pesetas
	Average lifetime (depreciation):	10 years
	Average number of analyses per week:	56
	Average depreciation cost:	58.8 pesetas
Incubator (stove) 37°C	Cost:	43 227 pesetas
	Average lifetime (depreciation):	10 years
	Average number of analyses per week:	56
	Average depreciation cost:	2.07 pesetas
Incubator (stove) 44°C	Cost:	43 227 pesetas
	Average lifetime (depreciation):	10 years
	Average number of analyses per week:	56
	Average depreciation cost:	2.07 pesetas
Autoclave	Cost:	400 000 pesetas
	Average lifetime (depreciation):	10 years
	Average number of analyses per week:	56
	Number of times used per analysis:	1
	Average depreciation cost:	76.5 pesetas
Total depreciation cost (average)		88.0 pesetas

Table 12. Time spent in preparing and carrying out analyses

		MF	MPN
Auxiliary staff	Preparation of culture media and cleaning of tubes	2 hours	4 hours
Technical staff	Dispensing	1/2 hour	1/2 hour
	Redispensing		1/2 hour
Total time	Auxiliary staff	2 hours	2 hours
	Technical staff	1/2 hour	1 hour

Table 13. Total cost and time spent for carrying out a microbiological analysis

	FM	MPN
Average cost of cultures	45.2 pesetas	582.2 pesetas
Average cost of disposables	524.8 pesetas	16.8 pesetas
Average cost of depreciation on apparatus	88.0 pesetas	202.8 pesetas
Total cost (average)	658.0 pesetas	801.8 pesetas
Time spent (auxiliary staff)	2 hours	4 hours
Time spent (technical staff)	1/2 hour	1 hour
Total time (average)	2 1/2 hours	5 hours

Thus, the average cost of a microbiological analysis of TC, FC and FS is higher using the MPN method than using MF.

The time spent in preparing and carrying out microbiological analyses of TC, FC and FS using the MPN method is approximately double that using the MF method.

7. Conclusions

The following can be concluded from the results obtained.

(1) The mean concentrations of microorganisms indicating faecal contamination of urban wastewater in the case of a Spanish city with a population of approximately 100 000 are TC (10^8-10^7), FC (10^7-10^6) and FS (10^6-10^5). These concentrations are expressed in 100 ml of the sample.

(2) The mean concentrations of microorganisms indicating faecal contamination of the waters in the case of a beach affected by a submarine outfall of specific dimensions and characteristics are TC (10^3-10^2), FC (10^2-10) and FS (10-0). These concentrations are expressed in 100 ml of the sample.

(3) The mean concentrations of microorganisms indicating faecal contamination of the waters in the case of a virgin beach, in the sense of one not affected by wastewater effluents, are TC (10^2-10), FC (10-0) and FS (10-0). These concentrations are expressed in 100 ml of the sample.

(4) The analysis of TC gives values which present no significant differences whether the method is MF or MPN until a certain minimum bacterial concentration has been reached which can be set at 10^3. For lower concentrations, the MF method gives slightly better results than MPN.

(5) For the analysis of FC, it is immaterial, as far as obtaining results is concerned, whether the MF or MPN method is used, since the results of the series obtained by either method present no significant difference.

(6) For the analysis of FS, it is immaterial, as far as obtaining results is concerned, whether the MF or MPN method is used, since the results of the series obtained by either method present no significant difference.

(7) For the analysis of TC using the MF method, it is immaterial whether a solid culture medium (agar) or a liquid medium (absorbent pads) is used, except after a certain minimum bacterial concentration which may be set at 10^2. For lower concentrations, the use of a liquid medium (absorbent pads) gives results which are slightly better than those using a solid medium (agar).

(8) For the analysis of FC using the MF method, it is immaterial whether a solid culture medium (agar) or a liquid (pad) is used, since the results obtained from the series produced by either method present no significant difference.

(9) The total incubation time for the various microorganisms indicating faecal contamination is less with the MF method than with MPN, which means that the results are obtained more quickly.

(10) The MPN method requires far more laborious and lengthy preparation of the culture media, in terms of time and materials, than the MF method.

(11) The time required for carrying out an analysis using the MPN method is twice that needed for the MF method.

(12) The MF method has a greater degree of precision, since it provides a direct colony count instead of the statistical approximation required for obtaining results by the MPN method.

(13) The MF method isolates the bacteria from the liquid in which they are suspended, thus making it possible to analyse water samples containing enzymes and other substances which inhibit growth.

(14) The MF method is definitely indicated for the analysis of highly mineralized waters, which can produce false reactions in MPN liquid media.

(15) The MF method is not to be recommended for waters with a high suspended matter content, since the matter in suspension retained in the membrane filter inhibits the perfect diffusion of the nutritive substance through its pores to the bacteria deposited on its upper surface and thus has an unfavourable effect on the growth of the colonies.

(16) The MF method is not to be recommended for waters with a high suspended matter content, since the filters rapidly become choked up and filtering of representative volumes is difficult.

(17) The MF method enables a larger volume of samples to be analysed than MPN.

(18) The MF method allows for the filtration of samples in situ and the transfer of the membranes to the laboratory in a preserving medium.

(19) The average total cost of microbiological analysis of TC, FC and FS is higher with the MPN method than with the MF method.

8. Acknowledgements

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Annexe 7

MISE AU POINT DES TECHNIQUES D'ECHANTILLONNAGE ET D'ANALYSE POUR LA SURVEILLANCE DE LA POLLUTION

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1. Introduction

Conformément au programme de travail concernant la mise au point des techniques d'échantillonnage et d'analyse pour la surveillance de la pollution proposé par l'Institut Supérieur de la Santé, Rome, l'Institut Pasteur de Tunis a procédé, entre le 15 mai et le 15 août 1982, à des prélèvements hebdomadaires d'eau de mer et de sédiments en trois points sur le littoral de la région de Tunis. Les trois points sont les suivants :

- plage d'Hammam-Lif : milieu marin pollué;
- plage de la Marsa : milieu marin contaminé (sous l'influence du milieu pollué à Hammam-Lif);
- plage Raoued : milieu marin propre.

A partir de ces points nous avons prélevé au total 72 échantillons (répartis en trois groupes de 24 échantillons (12 échantillons "eau de mer" et 12 échantillons "sédiments"). Afin d'attribuer statistiquement plus de crédibilité à nos investigations, nous avons doublé le nombre des échantillons, effectuant des prélèvements chaque semaine, au lieu de tous les 15 jours. L'horaire des prélèvements est donné au tableau 1.

Les analyses de ces échantillons ont porté sur la détermination du nombre le plus probable/100 ml (NPP) des coliformes totaux, des coliformes fécaux et d'Escherichia coli. Concernant les dénominations NPP des coliformes fécaux et NPP des E. coli, nous admettons comme E. coli les coliformes qui manifestent à la fois la faculté de fermenter, avec dégagement de gaz, le lactose à 44 °C et de produire l'indole à 44 °C, à part leur conformité aux tests JMVIC.

Nous considérons comme "coliformes fécaux" l'ensemble des E. coli et des coliformes positifs au lactose à 44 °C et négatifs à l'indole.

La technique des tubes multiples dont la phase confirmative exige l'épreuve des deux caractères thermophiles (fermentation du lactose avec dégagement de gaz et production d'indole à 44 °C) nous donne la possibilité de différencier le nombre le plus probable des E. coli.

La technique de la membrane filtrante, qui est conçue à la base exclusivement d'un troisième caractère thermophile (notamment la faculté de certaines souches de coliformes qui circulent dans le milieu marin à croître et à proliférer en colonies sur les membranes filtrantes incubées à 44 °C) nous donne la possibilité d'établir seulement le nombre le plus probable des coliformes fécaux et ce dans un sens beaucoup plus large, étant donné que la faculté de croissance à 44 °C est plus commune aux coliformes que celle de fermenter le lactose ou de produire l'indole à cette même température.

Si l'objectif est d'établir laquelle des deux techniques est prioritaire en colimétrie du milieu marin, dès maintenant il faut préciser le paramètre (NPP des coliformes totaux, fécaux ou E. coli) qui servira à faire le choix. Il est important aussi de préconiser le procédé standard à appliquer pour la détermination de ce paramètre.

Dans cet esprit, il est utile de mentionner qu'en ce qui concerne la technique des tubes multiples, et notamment le bouillon lactosé utilisé pour effectuer la phase présomptive de cette technique, l'Institut Supérieur de Santé de Rome a recommandé d'appliquer l'un des deux milieux, soit Mac Conkey Broth, soit Lactose Broth, laissant le choix aux participants de l'étude.

Lors du Programme MED POL VII, nous avons préféré utiliser la technique des tubes multiples comme étant, selon notre expérience, plus valable que celle de la membrane filtrante en appliquant simultanément deux bouillons lactosés - le Brilliant Green Lactose Bile Broth (agréé en France) et le Lactose Broth (agréé aux USA).

Nous avons constaté qu'en utilisant le Lactose Broth, les nombres les plus probables des coliformes totaux et des coliformes fécaux étaient beaucoup plus élevés que ceux obtenus par le Brilliant Green Lactose Bile Broth, ce qui exprime que la sensibilité de la technique des tubes multiples est dépendante du type de bouillon utilisé. Afin de confirmer cette constatation et d'arriver à une meilleure compréhension sur la valeur réelle des deux techniques (tubes multiples et membrane filtrante), nous avons effectué dans le cadre de ce projet la colimétrie de chaque échantillon en appliquant la technique des tubes multiples simultanément en trois variantes, en ce qui concerne le type de bouillon lactosé utilisé.

Pour la première variante, nous avons utilisé Mac Conkey Broth (production déshydratée DIFCO), pour la deuxième variante Lactose Broth (production déshydratée DIFCO), pour la troisième variante Brilliant Green Lactose Bile Broth (production déshydratée PASTEUR). Les volumes d'échantillons ensemencés dans ces différents bouillons lactosés sont : 5 tubes de 10 ml, 5 tubes de 1 ml et 5 tubes de 0,1 ml.

Dans le cas de ces trois variantes, la phase "confirmative" a été effectuée par réensemencements dans le bouillon lactosé bilié au vert brillant - épreuve de la fermentation du lactose à 44 °C - et dans l'eau peptonée - épreuve de la production d'indole à 44 °C - à partir de toutes les cultures propres à la phase présomptive qui ont manifesté la fermentation du lactose avec dégagement de gaz à 37 °C.

En ce qui concerne la technique de la membrane filtrante, nous avons utilisé les membranes et l'équipement MILLIPORE, et la gélose Endo (production déshydratée MERIEUX dans le cas des coliformes totaux et la gélose CF DIFCO pour les NPP des coliformes fécaux). Les résultats obtenus sont présentés au tableau 2.

2. Résultats et discussion

Les NPP/100 ml obtenus par la technique des tubes multiples en utilisant simultanément les trois bouillons lactosés, répartis selon la provenance des échantillons, sont présentés dans les tableaux 3, 4 et 5. Selon ces tableaux, 27,77% des échantillons se sont avérés exempts de coliformes en appliquant ces trois variantes.

Il faut noter que dans la majorité des échantillons "positifs", la concentration des coliformes est minime comparée aux normes propres à l'eau de mer polluée. Cela est dû au fait que le déversement de l'eau d'égout, non épurée dans le point de prélèvement choisi comme "pollué", était éliminé immédiatement après le début de cette étude, en conséquence de la mise en exploitation de la station d'épuration des eaux d'égout.

Cette contamination quoique limitée s'est avérée suffisante à l'étude de la sensibilité de la technique des tubes multiples en fonction du type de bouillon lactosé utilisé. Pour démontrer cette dépendance nous avons gradué en trois niveaux (sensibilité élevée, sensibilié moyenne et sensibilité limitée) les trois bouillons lactosés ci-dessus mentionnés. Dans ce but, nous avons comparé les valeurs des NPP des E. coli, des coliformes fécaux et des coliformes totaux propres à chaque échantillon qui a révélé la présence de coliformes.

Le niveau "sensibilité élevée" est attribué au bouillon lactosé qui a manifesté le NPP (exprimé en chiffres) le plus élevé. Sur ce principe sont évalués les niveaux "sensibilité moyenne" et "sensibilité limitée". Les résultats de cette classification sont présentés dans le tableau 6. Compte tenu de ces résultats comparatifs on peut justifier la conclusion que l'utilisation des bouillons lactosés Lactose Broth s'avère la plus favorable à la sensibilité de la technique des tubes multiples en ce qui concerne la mise en évidence de la présence de coliformes et l'évaluation de leur concentration dans le milieu marin (soit l'eau de mer, soit les sédiments).

La priorité du Lactose Broth par rapport au Mac Conkey et Brilliant Green Lactose Bile Broth est bien confirmée surtout par les chiffres propres aux NPP/100 ml des E. coli présentés dans le tableau 7. Cette priorité du bouillon Lactose Broth provient du fait que les E. coli, après avoir quitté leur habitat naturel (l'organisme humain et animal) pour passer dans les effluents, et enfin dans l'eau de mer, ne trouvent pas toujours dans l'environnement des conditions propices à leur survie. Souvent au moment des prélèvements d'eau de mer et de leur ensemencement au laboratoire, ils sont déjà d'une vitalité fort affaiblie. Aussi est-il important d'éviter l'utilisation, à l'étape présomptive de la technique des tubes multiples, des bouillons lactosés sélectifs (Mac Conkey Broth et Brilliant Green Lactose Bile Broth), qui risquent de gêner plus encore la croissance et la prolifération de ces germes et leur mise en évidence au laboratoire. Il faut rappeler que le Lactose Broth offre aux coliformes des conditions de réanimation pendant la phase de latence, à l'image de celles qu'offre l'eau peptonée aux denrées alimentaires pour leur préenrichissement.

Afin de faire le choix de laquelle des deux techniques (tubes multiples ou membrane filtrante) doit être préparée dans la pratique de la colimétrie du milieu marin, nous avons comparé les données de notre étude portant sur les NPP/100 ml des E. coli à celles concernant la technique de la membrane filtrante. Les données utilisées à cette comparaison sont présentées aux tableaux 8 et 9.

En comparant les valeurs des NPP des coliformes fécaux obtenues par la technique de la membrane filtrante aux valeurs concernant les NPP des E. coli déterminées par la technique des tubes multiples (il est difficile de les différencier par la technique de la membrane filtrante pratiquée à 44 °C), on constate que par cette technique dans toutes ses variantes à niveaux différents on arrive à déceler la présence des coliformes dans un nombre plus élevé d'échantillons (en pourcentage) que par la technique de la membrane filtrante (voir tableaux 7 et 8).

3. Conclusion

En conclusion des résultats de cette étude, on peut dire que la technique des tubes multiples s'avère plus efficace que celle utilisant la membrane filtrante quant à la détermination du degré de pollution de l'eau, et ce, utilisant l'un quelconque des trois bouillons lactosés. Par ailleurs, il est à remarquer que le Lactose Broth s'est manifesté bien plus sensible que les autres bouillons lactosés lors de la détermination du degré de pollution de l'eau pour les différents coliformes.

Cependant faut-il rappeler que le nombre d'échantillons utilisés à cette étude est trop réduit pour pouvoir s'assurer une conclusion statistique crédible sinon valable sur la supériorité de la sensibilité de la technique des tubes multiples sur celle des membranes filtrantes.

Tableau 1

Horaire des prélèvements

Date de prélèvement	Points de prélèvements					
	Pollué		Contaminé		Propre	
	Eau	Sédiment	Eau	Sédiment	Eau	Sédiment
	N° d'échantillon		N° d'échantillon		N° d'échantillon	
24.05.82	1	2	3	4	5	6
31.05.82	7	8	9	10	11	12
10.06.82	13	14	15	16	17	18
17.06.82	19	20	21	22	23	24
21.06.82	25	26	27	28	29	30
26.06.82	31	32	33	34	35	36
07.07.82	37	38	39	40	41	42
15.07.82	43	44	45	46	47	48
19.07.82	49	50	51	52	53	54
27.07.82	55	56	57	58	59	60
03.08.82	61	62	63	64	65	66
10.08.82	67	68	69	70	71	72

Tableau 2

Résultats obtenus par la méthode utilisant les membranes filtrantes
(exprimés en nombre de colonies métalliques/100 ml à 44 °C

Propre		Contaminé		Pollué	
Numéro des échantillons	Nombre des colonies à 44 °C	Numéro des échantillons	Nombre des colonies à 44 °C	Numéro des échantillons	Nombre des colonies à 44 °C
5	1	3	1	1	4
11	–	9	3	7	3
17	0	15	0	13	1
23	2	21	–	19	7
29	0	27	7	25	35
35	0	33	2	31	10
41	6	39	22	37	–
47	0	45	26	43	52
53	0	51	0	49	40
59	2	57	12	55	40
65	0	63	12	61	–
71	5	69	–	67	20
6	0	4	0	2	0
12	2	10	–	8	20
18	1	16	18	14	150
24	1	22	56	20	5
30	0	28	40	26	–
36	0	34	8	32	–
42	20	40	–	38	–
48	4	46	0	44	–
54	2	52	20	50	–
60	4	58	–	56	–
66	–	64	–	62	260
72	–	70	–	68	0

Tableau 3

Comparaison du nombre le plus probable/100 ml des coliformes totaux, des coliformes fécaux et des E. coli évalué par la technique des tubes multiples appliquée en trois variantes selon le type de bouillon lactosé utilisé (Mac Conkey Broth, Lactose Broth, Brilliant Green Lactose Bile Broth)

Echantillons prélevés au point pollué
Nature de l'échantillon : eau de surface

Type de bouillon utilisé	Formule des tubes positifs			Nombre le plus probable, 100		
	Coliformes totaux	Coliformes fécaux	E. coli	Coliformes totaux	Coliformes fécaux	E. coli
Mac Conkey Broth	5-5-2	5-5-2	5-3-0	542	542	79
Lactose Broth	5-5-3	5-5-2	5-3-1	920	542	109
Brilliant G.L.B. Broth	5-1-0	0-0-0	0-0-0	33	<2	<2
Mac Conkey Broth	5-5-5	5-3-0	5-3-0	>2400	79	79
Lactose Broth	5-5-5	5-3-1	5-3-0	>2400	109	79
Brilliant G.L.B. Broth	5-2-0	4-2-0	3-2-0	49	22	14
Mac Conkey Broth	5-5-1	5-2-0	5-1-0	348	49	33
Lactose Broth	5-5-2	5-2-2	5-1-1	548	94	46
Brilliant G.L.B. Broth	5-1-0	5-1-0	5-1-0	33	33	33
Mac Conkey Broth	5-5-5	5-5-5	5-5-5	>2400	>2400	>2400
Lactose Broth	5-5-5	5-5-5	5-5-5	>2400	>2400	>2400
Brilliant G.L.B. Broth	5-5-5	5-5-5	5-5-4	>2400	>2400	1600
Mac Conkey Broth	5-5-2	5-4-2	2-2-0	542	221	9
Lactose Broth	5-5-2	5-5-2	5-1-1	542	542	46
Brilliant G.L.B. Broth	5-4-2	5-4-1	3-2-0	221	172	14
Mac Conkey Broth	5-3-2	5-2-0	5-1-0	109	49	33
Lactose Broth	5-5-1	4-2-1	4-1-0	348	26	17
Brilliant G.L.B. Broth	5-3-1	5-1-1	4-1-0	109	46	17
Mac Conkey Broth	5-5-5	5-5-5	5-5-5	>2400	>2400	>2400
Lactose Broth	5-5-5	5-5-2	5-3-2	>2400	542	141
Brilliant G.L.B. Broth	5-5-5	5-5-5	5-4-5	>2400	>2400	426
Mac Conkey Broth	5-3-3	0-0-0	0-0-0	175	<2	2
Lactose Broth	5-4-3	5-3-2	5-1-1	278	141	63
Brilliant G.L.B. Broth	5-1-1	4-1-0	1-1-0	46	17	4
Mac Conkey Broth	5-5-5	5-5-5	5-5-5	>2400	>2400	>2400
Lactose Broth	5-5-5	5-5-5	5-5-5	>2400	>2400	>2400
Brilliant G.L.B. Broth	5-5-5	5-5-5	4-5-5	>2400	>2400	1600
Mac Conkey Broth	5-4-4	5-4-3	0-0-0	345	278	<2
Lactose Broth	5-5-3	5-5-3	2-1-0	920	920	7
Brilliant G.L.B. Broth	5-4-4	5-5-0	1-5-0	345	240	24
Mac Conkey Broth	5-4-5	5-4-2	5-3-1	426	221	109
Lactose Broth	5-5-5	5-5-5	5-4-1	>2400	>2400	172
Brilliant G.L.B. Broth	5-4-2	5-4-2	4-2-1	221	221	26
Mac Conkey Broth	5-4-5	5-3-4	4-3-1	426	212	33
Lactose Broth	5-3-3	5-2-1	3-2-1	175	70	14
Brilliant G.L.B. Broth	5-4-1	5-3-1	5-2-0	172	109	49

Tableau 3 (suite)

Comparaison du nombre le plus probable/100 ml des coliformes totaux, des coliformes fécaux et des *E. coli* évalué par la technique des tubes multiples appliquée en trois variantes selon le type de bouillon lactosé utilisé (Mac Conkey Broth, Lactose Broth, Brilliant Green Lactose Bile Broth)

Echantillons prélevés au point pollué
Nature de l'échantillon : sédiment

Type de bouillon utilisé	Formule des tubes positifs			Nombre le plus probable, 100		
	Coliformes totaux	Coliformes fécaux	E. coli	Coliformes totaux	Coliformes fécaux	E. coli
Mac Conkey Broth	-	-	-	-	-	-
Lactose Broth	5-5-2	5-5-2	5-3-2	542	542	141
Brilliant G.L.B. Broth	5-5-0	2-1-0	0-0-0	240	7	<2
Mac Conkey broth	5-5-4	4-5-4	3-3-3	1600	1600	21
Lactose Broth	5-5-5	5-5-3	5-5-0	>2400	920	240
Brilliant G.L.B. Broth	5-5-4	5-5-3	5-3-2	1600	920	141
Mac Conkey broth	5-5-4	5-5-4	4-2-0	1600	1600	22
Lactose Broth	5-5-4	5-5-0	5-4-0	1600	240	130
Brilliant G.L.B. Broth	5-5-2	0-1-1	0-0-0	542	4	<2
Mac Conkey broth	5-1-1	2-0-0	1-0-0	46	5	2
Lactose Broth	5-1-0	5-0-0	3-0-0	33	23	8
Brilliant G.L.B. Broth	5-0-2	3-0-2	3-0-2	43	13	13
Mac Conkey broth	0-0-0	0-0-0	0-0-0	<2	<2	<2
Lactose Broth	0-0-0	0-0-0	0-0-0	<2	<2	<2
Brilliant G.L.B. Broth	0-0-0	0-0-0	0-0-0	<2	<2	<2
Mac Conkey broth	0-0-0	0-0-0	0-0-0	<2	<2	<2
Lactose Broth	0-0-0	0-0-0	0-0-0	<2	<2	<2
Brilliant G.L.B. Broth	0-0-0	0-0-0	0-0-0	<2	<2	<2
Mac Conkey broth	0-0-0	0-0-0	0-0-0	<2	<2	<2
Lactose Broth	0-0-0	0-0-0	0-0-0	<2	<2	<2
Brilliant G.L.B. Broth	0-0-0	0-0-0	0-0-0	<2	<2	<2
Mac Conkey broth	0-0-0	0-0-0	0-0-0	<2	<2	<2
Lactose Broth	1-2-1	1-1-1	1-1-0	8	6	14
Brilliant G.L.B. Broth	0-0-0	0-0-0	0-0-0	<2	<2	<2
Mac Conkey broth	0-0-0	0-0-0	0-0-0	<2	<2	<2
Lactose Broth	3-0-0	3-0-0	2-0-0	8	8	5
Brilliant G.L.B. Broth	0-0-0	0-0-0	0-0-0	<2	<2	<2
Mac Conkey broth	0-0-0	0-0-0	0-0-0	<2	<2	<2
Lactose Broth	0-0-0	0-0-0	0-0-0	<2	<2	<2
Brilliant G.L.B. Broth	0-0-0	0-0-0	0-0-0	<2	<2	<2
Mac Conkey broth	5-5-5	5-5-5	5-5-5	>2400	>2400	>2400
Lactose Broth	5-5-5	4-5-5	3-4-4	>2400	1600	24
Brilliant G.L.B. Broth	5-3-5	5-3-4	0-3-0	253	212	6
Mac Conkey broth	5-3-2	5-2-2	2-2-1	141	94	12
Lactose Broth	5-2-2	5-1-1	4-1-1	94	46	21
Brilliant G.L.B. Broth	4-1-0	4-1-0	1-0-0	17	17	2

Tableau 4

Comparaison du nombre le plus probable/100 ml des coliformes totaux, des coliformes fécaux et des E. coli évalué par la technique des tubes multiples appliquée en trois variantes selon le type de bouillon lactosé utilisé (Mac Conkey Broth, Lactose Broth, Brilliant Green Lactose Bile Broth)

Echantillons prélevés au point contaminé
Nature de l'échantillon : eau de surface

Type de bouillon utilisé	Formule des tubes positifs			Nombre le plus probable, 100		
	Coliformes totaux	Coliformes fécaux	E. coli	Coliformes totaux	Coliformes fécaux	E. coli
Mac Conkey broth	5-5-3	1-0-0	0-0-0	920	2	<2
Lactose Broth	5-5-3	3-1-1	2-0-0	920	14	5
Brilliant G.L.B. Broth	3-0-0	3-0-0	2-0-0	8	8	5
Mac Conkey broth	5-1-0	4-1-0	1-1-0	33	17	4
Lactose Broth	5-5-2	4-2-1	2-1-0	542	26	7
Brilliant G.L.B. Broth	5-3-1	4-0-0	2-0-0	109	19	5
Mac Conkey broth	4-0-0	2-0-0	2-0-0	13	5	5
Lactose Broth	4-0-0	1-0-0	1-0-0	13	2	2
Brilliant G.L.B. Broth	2-0-0	2-0-0	1-0-0	5	5	2
Mac Conkey broth	3-1-0	3-0-0	0-0-0	11	8	<2
Lactose Broth	5-1-0	4-1-0	4-1-0	33	17	17
Brilliant G.L.B. Broth	3-1-0	3-1-0	3-0-0	11	11	8
Mac Conkey broth	4-3-0	4-2-0	4-2-0	27	22	22
Lactose Broth	5-0-0	5-0-0	4-0-0	23	23	13
Brilliant G.L.B. Broth	5-0-0	5-0-0	3-1-0	23	23	11
Mac Conkey broth	1-0-0	1-0-0	1-0-0	<2	<2	<2
Lactose Broth	5-0-0	5-0-0	2-0-0	23	23	5
Brilliant G.L.B. Broth	3-0-0	2-0-0	2-0-0	8	5	5
Mac Conkey broth	0-0-0	0-0-0	0-0-0	2	2	2
Lactose Broth	5-0-0	4-0-0	3-0-0	23	13	8
Brilliant G.L.B. Broth	5-2-0	5-2-0	5-2-0	49	49	49
Mac Conkey broth	5-0-0	5-0-0	2-0-0	23	23	5
Lactose Broth	5-0-0	1-0-0	1-0-0	23	2	2
Brilliant G.L.B. Broth	3-0-0	1-0-0	1-0-0	23	2	2
Mac Conkey broth	2-0-0	1-0-0	0-0-0	5	2	<2
Lactose Broth	5-0-0	2-0-0	2-0-0	5	5	5
Brilliant G.L.B. Broth	0-0-0	0-0-0	0-0-0	<2	<2	<2
Mac Conkey broth	4-0-0	3-0-0	0-0-0	13	2	<2
Lactose Broth	5-4-0	3-2-0	3-2-0	130	14	14
Brilliant G.L.B. Broth	3-0-0	2-0-0	2-0-0	8	5	5
Mac Conkey broth	5-4-1	3-3-1	3-0-0	172	21	8
Lactose Broth	5-1-1	5-1-0	0-0-0	46	33	<2
Brilliant G.L.B. Broth	5-3-1	3-2-1	3-0-0	109	17	8
Mac Conkey broth	5-3-2	5-2-2	2-2-1	141	94	12
Lactose Broth	5-2-2	5-1-1	4-1-1	94	46	21
Brilliant G.L.B. Broth	4-1-0	4-1-0	1-0-0	17	17	2

Tableau 4 (suite)

Comparaison du nombre le plus probable/100 ml des coliformes totaux, des coliformes fécaux et des E. coli évalué par la technique des tubes multiples appliquée en trois variantes selon le type de bouillon lactosé utilisé (Mac Conkey Broth, Lactose Broth, Brilliant Green Lactose Bile Broth)

Echantillons prélevés au point contaminé
Nature de l'échantillon : sédiment

Type de bouillon utilisé	Formule des tubes positifs			Nombre le plus probable, 100		
	Coliformes totaux	Coliformes fécaux	E. coli	Coliformes totaux	Coliformes fécaux	E. coli
Mac Conkey Broth	4-1-0	2-0-0	1-0-0	17	5	2
Lactose Broth	5-1-0	3-1-0	3-0-0	33	11	11
Brilliant G.L.B. Broth	5-0-0	5-0-0	3-0-0	23	23	8
Mac Conkey Broth	3-2-0	3-2-0	2-2-0	14	14	9
Lactose Broth	5-1-0	5-1-1	5-1-1	33	46	46
Brilliant G.L.B. Broth	4-2-0	4-2-0	4-2-0	22	22	22
Mac Conkey Broth	5-5-1	5-1-0	3-0-0	348	33	8
Lactose Broth	4-4-2	4-3-0	3-2-0	40	27	14
Brilliant G.L.B. Broth	-	-	-	-	-	-
Mac Conkey Broth	5-3-2	3-1-0	2-1-0	141	11	7
Lactose Broth	5-3-2	4-1-0	4-1-0	141	17	17
Brilliant G.L.B. Broth	5-2-1	4-0-0	3-0-0	70	13	8
Mac Conkey Broth	5-2-2	3-2-1	2-1-1	94	17	9
Lactose Broth	5-4-2	5-3-1	5-3-1	221	109	109
Brilliant G.L.B. Broth	5-2-2	4-2-2	1-0-0	94	32	2
Mac Conkey Broth	5-3-1	5-3-1	3-2-0	109	109	14
Lactose Broth	5-0-1	5-0-1	5-0-0	31	31	23
Brilliant G.L.B. Broth	4-2-0	2-0-0	0-0-0	22	5	<2
Mac Conkey Broth	5-3-1	2-0-0	1-0-0	109	5	2
Lactose Broth	5-0-1	5-0-0	3-0-0	31	23	8
Brilliant G.L.B. Broth	4-2-0	3-0-2	3-0-2	22	13	13
Mac Conkey Broth	4-2-2	2-1-1	1-0-0	32	9	2
Lactose Broth	5-0-0	5-0-0	4-0-0	23	23	13
Brilliant G.L.B. Broth						
Mac Conkey Broth	4-3-0	4-3-0	3-3-0	27	27	17
Lactose Broth	5-3-2	5-3-2	0-0-0	141	141	<2
Brilliant G.L.B. Broth	5-1-0	4-1-0	0-0-0	33	17	<2
Mac Conkey Broth	5-0-0	1-0-0	1-0-0	23	2	2
Lactose Broth	5-0-1	4-0-1	4-0-1	31	17	17
Brilliant G.L.B. Broth	5-1-0	3-0-0	2-0-0	33	8	5
Mac Conkey Broth	0-0-0	0-0-0	0-0-0	<2	<2	<2
Lactose Broth	3-0-0	3-0-0	2-0-0	8	8	5
Brilliant G.L.B. Broth	0-0-0	0-0-0	0-0-0	<2	<2	<2
Mac Conkey Broth	3-3-2	3-3-2	1-0-1	21	33	4
Lactose Broth	4-2-2	3-2-0	2-0-0	32	14	5
Brilliant G.L.B. Broth	3-1-1	3-0-0	1-0-0	14	8	2

Tableau 5

Comparaison du nombre le plus probable/100 ml des coliformes totaux, des coliformes fécaux et des E. coli évalué par la technique des tubes multiples appliquée en trois variantes selon le type de bouillon lactosé utilisé (Mac Conkey Broth, Lactose Broth, Brilliant Green Lactose Bile Broth)

Echantillons prélevés au point propre
Nature de l'échantillon : eau de surface

Type de bouillon utilisé	Formule des tubes positifs			Nombre le plus probable, 100		
	Coliformes totaux	Coliformes fécaux	E. coli	Coliformes totaux	Coliformes fécaux	E. coli
Mac Conkey Broth	3-3-0	1-1-0	1-0-0	17	4	2
Lactose Broth	4-2-0	0-1-0	0-0-0	22	2	<2
Brilliant G.L.B. Broth	1-0-0	1-0-0	1-0-0	2	2	2
Mac Conkey Broth	4-1-0	1-0-0	1-0-0	17	2	2
Lactose Broth	5-1-1	1-0-0	0-0-0	36	2	<2
Brilliant G.L.B. Broth	0-0-0	0-0-0	0-0-0	<2	<2	<2
Mac Conkey Broth	0-0-0	0-0-0	0-0-0	<2	<2	<2
Lactose Broth	2-0-0	0-0-0	0-0-0	5	<2	<2
Brilliant G.L.B. Broth	0-0-0	0-0-0	0-0-0	<2	<2	<2
Mac Conkey Broth	4-1-0	2-1-0	0-0-0	17	7	<2
Lactose Broth	5-0-0	1-0-0	0-0-0	23	2	<2
Brilliant G.L.B. Broth	0-0-0	0-0-0	0-0-0	<2	<2	<2
Mac Conkey Broth	3-0-0	0-0-0	0-0-0	8	<2	<2
Lactose Broth	4-0-0	1-0-0	0-0-0	13	2	<2
Brilliant G.L.B. Broth	0-0-0	0-0-0	0-0-0	<2	<2	<2
Mac Conkey Broth	1-2-0	1-2-0	1-0-0	6	6	2
Lactose Broth	5-2-1	3-1-0	1-0-0	70	11	2
Brilliant G.L.B. Broth	0-0-0	0-0-0	0-0-0	<2	<2	<2
Mac Conkey Broth	4-1-0	1-1-0	0-0-0	17	4	<2
Lactose Broth	5-0-0	5-0-0	0-0-0	23	23	<2
Brilliant G.L.B. Broth	4-0-0	2-0-0	0-0-0	13	5	<2
Mac Conkey Broth	2-0-0	0-0-0	0-0-0	5	<2	<2
Lactose Broth	1-0-0	0-0-0	0-0-0	2	<2	<2
Brilliant G.L.B. Broth						
Mac Conkey Broth	1-0-0	0-0-0	0-0-0	2	<2	<2
Lactose Broth	3-0-0	0-0-0	0-0-0	8	<2	<2
Brilliant G.L.B. Broth	0-0-0	0-0-0	0-0-0	<2	<2	<2
Mac Conkey Broth	5-0-1	2-0-0	0-0-0	31	5	<2
Lactose Broth	5-1-0	2-0-0	0-0-0	33	5	<2
Brilliant G.L.B. Broth	1-2-1	1-2-1	1-1-0	8	8	4
Mac Conkey Broth	4-0-0	2-0-0	0-0-0	13	5	<2
Lactose Broth	5-0-0	2-0-0	1-0-0	23	5	2
Brilliant G.L.B. Broth	1-0-0	0-0-0	0-0-0	2	<2	<2
Mac Conkey Broth	4-0-0	0-0-0	0-0-0	13	<2	<2
Lactose Broth	4-1-0	1-0-0	1-0-0	17	2	2
Brilliant G.L.B. Broth	1-0-0	0-0-0	0-0-0	2	<2	<2

Tableau 5 (suite)

Comparaison du nombre le plus probable/100 ml des coliformes totaux, des coliformes fécaux et des E. coli évalué par la technique des tubes multiples appliquée en trois variantes selon le type de bouillon lactosé utilisé (Mac Conkey Broth, Lactose Broth, Brilliant Green Lactose Bile Broth)

Echantillons prélevés au point propre
Nature de l'échantillon : sédiment

Type de bouillon utilisé	Formule des tubes positifs			Nombre le plus probable, 100		
	Coliformes totaux	Coliformes fécaux	E. coli	Coliformes totaux	Coliformes fécaux	E. coli
Mac Conkey Broth	4-0-0	1-0-0	0-0-0	13	2	<2
Lactose Broth	5-1-0	1-0-0	0-0-0	33	2	<2
Brilliant G.L.B. Broth	3-1-0	2-0-0	0-0-0	11	5	<2
Mac Conkey Broth	0-0-0	0-0-0	0-0-0	<2	<2	<2
Lactose Broth	2-1-0	0-0-0	0-0-0	9	<2	<2
Brilliant G.L.B. Broth	0-0-0	0-0-0	0-0-0	<2	<2	<2
Mac Conkey Broth	1-0-0	1-0-0	1-0-0	2	2	2
Lactose Broth	2-0-0	2-0-0	1-0-0	5	5	2
Brilliant G.L.B. Broth	1-0-0	1-0-0	0-0-0	2	2	<2
Mac Conkey Broth	0-0-0	0-0-0	0-0-0	<2	<2	<2
Lactose Broth	3-2-0	0-0-0	0-0-0	14	<2	<2
Brilliant G.L.B. Broth	0-0-0	0-0-0	0-0-0	<2	<2	<2
Mac Conkey Broth	-	-	-	-	-	-
Lactose Broth	2-0-0	2-0-0	1-0-0	5	5	2
Brilliant G.L.B. Broth	1-0-0	0-0-0	0-0-0	2	<2	<2
Mac Conkey Broth	0-0-0	0-0-0	0-0-0	<2	<2	<2
Lactose Broth	4-0-0	1-0-0	1-0-0	13	2	2
Brilliant G.L.B. Broth	0-0-0	0-0-0	0-0-0	<2	<2	<2
Mac Conkey Broth	5-0-0	2-0-0	0-0-0	23	5	<2
Lactose Broth	5-0-0	2-0-0	0-0-0	23	5	<2
Brilliant G.L.B. Broth	3-1-0	2-0-0	1-0-0	11	5	2
Mac Conkey Broth	2-0-0	0-0-0	0-0-0	5	<2	<2
Lactose Broth	3-0-0	0-0-0	0-0-0	8	<2	<2
Brilliant G.L.B. Broth						
Mac Conkey Broth	3-0-0	0-0-0	0-0-0	8	<2	<2
Lactose Broth	3-0-0	0-0-0	0-0-0	8	<2	<2
Brilliant G.L.B. Broth	0-0-0	0-0-0	0-0-0	<2	<2	<2
Mac Conkey Broth	5-3-1	2-2-0	0-0-0	109	9	<2
Lactose Broth	4-0-0	4-0-0	0-0-0	13	13	<2
Brilliant G.L.B. Broth	3-2-0	0-1-0	0-0-0	14	2	<2
Mac Conkey Broth	-	1-0-0	0-0-0	-	2	<2
Lactose Broth	-	2-0-0	0-0-0	-	<2	<2
Brilliant G.L.B. Broth	-	0-0-0	0-0-0	-	<2	<2
Mac Conkey Broth	0-0-0	0-0-0	0-0-0	<2	<2	<2
Lactose Broth	0-0-0	0-0-0	0-0-0	<2	<2	<2
Brilliant G.L.B. Broth	0-0-0	0-0-0	0-0-0	<2	<2	<2

Tableau 6

Comparaison de la sensibilité (exprimée en pourcentage) de la technique des tubes multiples selon le type de bouillon lactosé utilisé en ce que concerne la détermination des nombres les plus probables de coliformes

Type de bouillon lactosé	Nombre le plus probable des coliformes totaux/100 ml			Nombre le plus probable des coliformes fécaux/100 ml			Nombre le plus probable des E. coli/100 ml		
	Degré de sensibilité								
	élevé	moyen	limité	élevé	moyen	limité	élevé	moyen	limité
Lactose Broth	79,41	14,71	5,88	71,01	24,63	2,89	76,81	20,27	2,89
Mac Conkey Broth	41,17	47,11	11,76	49,27	28,98	21,73	46,37	33,33	20,28
Brilliant G.L. Bile Broth	19,11	45,58	35,29	30,43	47,82	21,73	40,57	43,47	15,98

Tableau 7

Comparaison du degré de sensibilité (exprimé en pourcentage) des trois bouillons lactosés dans la détermination de la concentration des E. coli dans les échantillons analysés

Type de bouillon utilisé	NPP - coliformes totaux/100 ml	NPP - coliformes fécaux/100 ml	NPP - E. coli/100 ml
Lactose Broth	79,41	71,01	76,81
Mac Conkey Broth	41,17	49,27	46,37
Brilliant G.L. Bile Broth	19,11	30,43	40,57

Tableau 8

Comparaison des résultats obtenus par la méthode des tubes multiples et par la membrane filtrante concernant le NPP/100 ml des E. coli

PROPRE					CONTAMINEE					POLLUEE				
Numéro des échantillons	Nombre des colonies à 44 °C 100 ml	NPP/100 ml sur			Numéro des échantillons	Nombre des colonies à 44 °C 100 ml	NPP/100 ml sur			Numéro des échantillons	Nombre des colonies à 44 °C 100 ml	NPP/100 ml sur		
		L*	B*	M*			L*	B*	M*			L*	B*	M*
EAUX DE SURFACE														
5	1	<2	2	2	3	1	5	5	<2	1	4	109	<2	79
11		<2	<2	2	9	3	7	5	4	7	3	79	14	79
17	0	<2	<2	<2	15	0	2	2	5	13	1	46	33	33
23	2	<2	<2	<2	21	-	17	8	<2	19	7	240	1600	>2400
29	0	<2	<2	<2	27	7	13	11	22	25	35	46	14	9
35	0	2	<2	2	33	2	5	5	2	31	10	17	17	33
41	6	<2	<2	<2	39	22	8	49	<2	37	-	141	426	2400
47	0	<2	<2	<2	45	26	2	2	5	43	52	63	4	<2
53	0	<2	<2	<2	51	0	5	<2	<2	49	40	>2400	48	2400
59	2	<2	4	<2	57	12	14	5	<2	55	40	7	11	2
65	0	2	<2	<2	63	12	<2	8	8	61	-	172	26	109
71	5	2	<2	<2	69	-	21	2	12	67	20	14	49	33
SEDIMENTS														
6	0	<2	<2	<2	4	0	11	8	2	2	0	141	<2	-
12	2	<2	<2	<2	10	-	46	22	9	8	20	240	141	21
18	1	2	<2	2	16	18	14		8	14	150	130	<2	22
24	1	<2	<2	<2	22	56	17	8	7	20	5	8	13	2
30	0	2	<2		28	40	109	2	9	26	-	<2	<2	<2
36	0	2	<2	<2	34	8	23	<2	14	32	-	<2	<2	<2
42	20	<2	2	<2	40	-	8	13	2	38	-	<2	<2	<2
48	4	<2	<2	<2	46	0	13	<2	2	44	-	4	<2	<2
54	2	<2	<2	<2	52	20	<2	<2	17	50	-	8	<2	<2
60	4	<2	<2	<2	58	-	17	5	2	56	-	<2	<2	<2
66	-	<2	<2	<2	64	-	5	<2	<2	62	260	24	6	2400
72	-	<2	<2	<2	70	-	5	2	4	68	0	21	2	12

* L = Lactose Broth
B = Brilliant Green Lactose Bile Broth
M = Mac Conkey Broth

Tableau 9

Comparaison de la sensibilité des deux techniques (tubes multiples et membranes filtrantes exprimée en pourcentage par rapport aux nombres des échantillons analysés)

Point de prélèvement	Technique tubes multiples supérieure à celle de la membrane filtrante	Technique membrane filtrante supérieure à celle des tubes multiples	Egalité
Propre	42,8	33,33	23,8
Contaminé	70,58	29,41	0
Pollué	87,5	12,5	0

Annex 8

A CONTRIBUTION TO THE COMPARATIVE STUDY OF ANALYTICAL METHODS USED FOR DETECTION OF MAJOR BACTERIAL INDICATORS OF POLLUTION CAUSED BY FAECAL SEWAGE

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This study is designed to contribute to the comparative study of analytical methods used for detection of the major bacterial indicators of pollution caused by faecal sewage.

1. Areas studied

Three sampling points were selected in the area of Rovinj, a small town of 10 000 inhabitants, situated on the West Istrian coast of the North Adriatic, with facilities for accommodating up to 40 000 tourists:

- station no. 1: near an island not under the direct influence of domestic wastewater;
- station no. 2: a beach close to the town harbour, where the influence of sewage discharge in the vicinity is noticeable;
- station no. 3: in the harbour itself, where most of the sewage outfalls discharge into the sea.

2. Methodology

2.1 Sampling methods

Seawater samples were taken with a sterile glass sampler (using an extension arm) 10 m from the coastline, and 20-50 cm below the surface. Sediment samples were taken using a Pfleger corer. The surface layer of the sample was transferred by a spatula to a sterile Petri dish. Samples were transported to the laboratory in a cooling container. The interval between sample collection and processing was never more than three hours.

The following parameters were analysed: total coliforms, faecal coliforms, faecal streptococci and BOD_5 in water, and faecal coliforms in sediments.

2.2 Analytical methods

Total coliforms: the membrane filtration (MF) technique was used as described in the relevant WHO/UNEP reference method (1). The most probable number (MPN) technique was used, as described in the WHO/UNEP Guidelines for health-related monitoring of coastal water quality (2). In the former method, samples were incubated for 24 hours at 36°C±1°C using Mac Conkey Broth. Gas and acid production were identified and used for the MPN calculation.

Faecal coliforms: the same methods as described above for total coliforms were used. In the MF technique, samples were incubated for 24 hours on solid M-FC-agar at 44.5°C±0.2°C, blue colonies being counted. In the MPN technique, samples were incubated for 24 hours at 36°C±1°C using Mac Conkey Broth. Positive tubes were tested by transfer to individual Mac Conkey tubes and incubated at 44.5°C±0.2°C for 24 hours. Confirmation was performed using the indole production test, acid, gas and indole production in the tubes being used for the MPN calculation.

Faecal streptococci: the membrane filtration technique as described in the WHO/UNEP Guidelines (2) was used, while the MPN method was used as described in the APHA Standard Methods (3). In the former, samples were incubated for 48±3 hours at 44.5°C±0.2°C on KF-streptococcus-agar, dark red colonies being counted. In the latter, samples were incubated for 48 hours at 36°C±1°C on Azide-Dextrose Broth. Due to lack of Ethyl-Violet-Azide Broth, the confirmatory test was performed using a Bromocresol-purple Azide Broth. Incubation was performed at 36°C±1°C for 48 hours. Increasing turbidity and colour change to yellow were used for MPN calculation. Bromcresol-purple Azide Broth (g/l) was prepared as follows: peptone from casein - 10.00; yeast extract - 10.00; D-glucose - 5.0; NaCl - 5.0; K_2HPO_4 - 2.7; KH_2PO_4 - 2.7; NaN_3 - 0.5; Bromcresol- purple - 0.032.

BOD_5: the technique described in the APHA Standard Methods (3) was used for determination of the biochemical oxygen demand. Samples were incubated in 250 ml bottles.

Faecal coliforms in sediment: the MPN technique described above for determination of faecal coliforms in seawater was used for this parameter.

3. Results and discussion

The results obtained during the experimental period are presented numerically in Table 1 and graphically in Figures 1 to 3. Analysis of the data shows significant differences in the level of pollution between the three stations (Table 1). The concentration of bacterial indicators as well as the biochemical oxygen demand value confirm that station no. 1 is located in an unpolluted area, station no. 2 is subject to wastewater pollution, and no. 3 is polluted by discharges from sewage outfalls. Although the values of BOD_5 obtained showed differences in pollution levels between stations, correlation between the BOD_5 values and the concentration of bacterial indicators was obtained only at station no. 3 ($r = 0.87$-89). The number of faecal coliforms recorded in sediments confirmed the influence of location of sewage outfalls on the quality of the seawater in the various stations.

Figures 1-3 show some discrepancy between the results obtained using the MF and MPN techniques respectively. Higher values of bacteria were obtained in 67% of samples determined by the MF technique, as compared to those obtained using the MPN method. Extreme differences were noticed for faecal streptococci at station no. 2 where in 5 out of 6 samples, MF values were out of the MPN 95% confidence limits. Statistical evaluation of the results showed significant differences between MF values and MPN values only in the case of faecal streptococci at station no. 2 ($P = 0.001$).

The phenomenon whereby the membrane filter technique produces lower counts than the multiple tube test or most probably number (MPN) techniques in the bacteriological examination of chlorinated wastewaters has not previously been recorded in the coastal waters of Rovinj.

In comparing the relative merits of the MF and MPN techniques, the precision obtained with a single membrane filter was five times greater than that of a five-tube MPN (4). Also, the MF method gave much better replication (5). Results obtained in the present experiment confirmed previous results recorded in MF/MPN comparisons in the analysis of coastal waters from the Rovinj area, although the media used were different (6).

Although the MF and MPN tests for the enumeration of total and faecal coliforms as well as for that of faecal streptococci do not always yield exactly the same results, it is clear that both methods do supply substantially the same information.

In summary, membrane filtration using M-FC-agar provides a direct bacterial count and requires only 24 hours to complete. A commercial product which automatically switches incubation temperature is available to make this procedure more practical for routine application.

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Table 1

The variation of parameters covered by methodology testing exercises

Station No.	date of sampling	hour of sampling	Total coliforms MF	Total coliforms MPN	Faecal coliforms MF	Faecal coliforms MPN	Faecal streptococci MF	Faecal streptococci MPN	Faecal coliforms in sediments n/1 g	BOD_5 mgO_2/l
			n/100 ml							
1	20.05.82	10.00	9	0	2	0	0	2	0	0.83
	4.06.82	08.45	0	0	0	0	0	0	0	0.38
	17.06.82	09.00	50	2	16	0	12	0	0	0.81
	22.06.82	09.10	3	2	1	2	1	0	0	0.36
	14.07.82	12.45	3	8	1	0	0	8	2	0.08
	29.07.82	09.00	22	2	5	0	1	0	4	0.40
2	20.05.82	09.30	280	350	205	204	200	110	2	0.86
	4.06.82	09.25	800	920	575	70	420	79	0	0.97
	17.06.82	09.20	610	240	550	240	320	13	7	0.60
	22.06.82	10.50	1 050	140	1 015	140	290	27	14	1.36
	14.07.82	13.15	70	79	55	33	90	7	5	2.11
	29.07.82	09.40	2 600	540	400	350	615	110	2	1.15
3	20.05.82	10.30	3 400	5 400	3 300	3 500	6 650	7 200	33	2.10
	4.06.82	09.40	20 000	14 000	16 000	13 000	13 600	3 500	49	2.55
	17.06.82	10.30	87 500	24 000	21 500	24 000	47 200	7 900	220	6.92
	22.06.82	10.10	135 000	92 000	119 000	92 000	98 000	54 000	110	7.58
	14.07.82	13.20	1 050	330	1 000	170	1 130	60	22	2.70
	29.07.82	09.45	2 700	3 500	1 700	490	1 250	700	240	1.44

Figure 1

The variation of total coliforms, faecal coliforms and faecal streptococci in six water samples taken at Station No. 1*

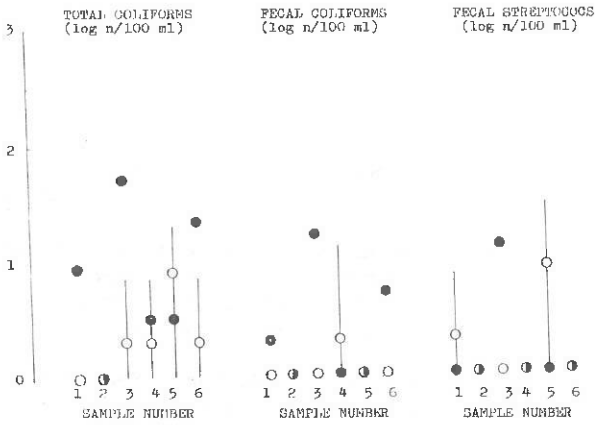


Figure 2

The variation of total coliforms, faecal coliforms and faecal streptococci in six water samples taken at Station No. 2*

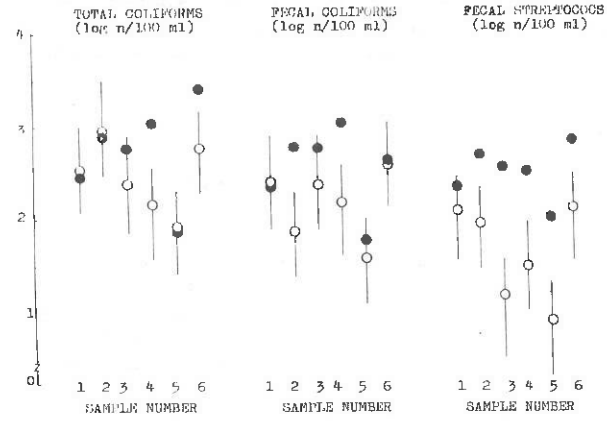
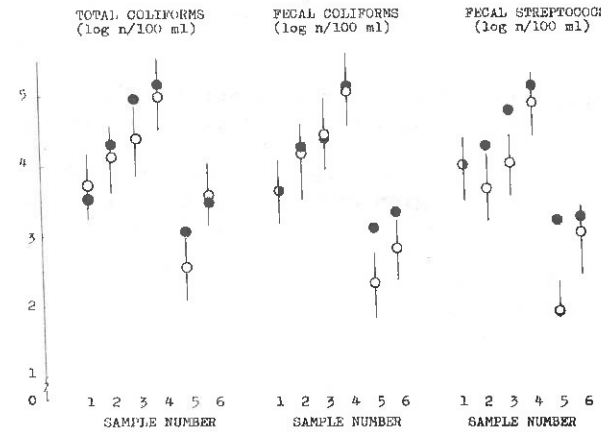


Figure 3

The variation of total coliforms, faecal coliforms and faecal streptococci in six water samples taken at Station No. 3*



*Results of the MF method indicated by (●), results of the MPN method indicated by (o) with 95% confidence limit.

Annex 9

REPORT ON THE JOINT INTERCALIBRATION EXERCISE ON MICROBIOLOGICAL METHODS

1. Introduction

During the course of the pilot phase of the Joint Coordinated Mediterranean Pollution Monitoring and Research Programme (MED POL Phase II), draft reference methods were produced for use in the long-term monitoring programme. These methods included the following:

- determination of total coliforms in seawater by the membrane filtration culture method;
- determination of faecal coliforms in seawater by the membrane filtration culture method;
- determination of faecal streptococci in seawater by the membrane filtration culture method;
- determination of faecal coliforms in bivalves by the multiple test-tube method.

The above four methods were developed by UNEP's Regional Seas Programme Activity Centre (RS/PAC) in cooperation with the WHO Regional Office for Europe. By mid-1982, all four had reached a sufficient stage of development to be tested by individual laboratories, following which an intercalibration exercise could be organized prior to final review.

2. Organization

A selected number of Mediterranean institutions were invited to test the reference methods under their local environmental conditions during the late summer/early autumn of 1982. They were also invited to send representatives to participate in a joint intercalibration exercise in Rome on 22-23 November 1982, and in a consultation meeting to review the methods, also in Rome, from 24 to 26 November 1982.

The following laboratories were invited:

- Centre de Recherche océanographique et des Pêches, Alger, Algeria;
- Cellule de Lutte contre les Pollutions marines, Service maritime des Bouches-du-Rhône, Marseille, France;
- Environmental Pollution Control Project, Athens, Greece;
- Environmental Health Laboratory, Hadassah Medical School, Jerusalem, Israel;
- Institute of Water Supply and Wastes Disposal, University of Naples, Italy;
- Public Health Laboratory, Department of Health and Environment, Valletta, Malta;
- Institut national d'Hygiène, Ministère de la Santé publique, Rabat, Morocco;
- Jefatura Provincial de Sanidad, Malaga, Spain;
- Environmental Engineering Department, Middle East Technical University, Ankara, Turkey;
- Institute of Biology, University of Ljubljana, Portoroz, Yugoslavia.

Selection of laboratories was made on the basis of (a) participation in MED POL Phase I (MED VII - Coastal Water Quality Control), (b) intended participation in the long-term phase of MED POL, (c) geographical distribution. Because of financial limitations, account was also taken of invitations already issued for participation in related activities during 1982, and plans for similar exercises during 1983.

Nine Mediterranean laboratories accepted the invitation to test the methods and sent representatives to participate in the joint intercalibration exercise and consultation meeting.

By arrangement with UNEP's Regional Seas Programme Activity Centre, the three following laboratories outside the Mediterranean region were also invited and accepted to participate in the testing and send representatives to the joint intercalibnfation exercise and consultation meeting:

- Environment Pollution Division, Ministry of Health, Sha'ab, Kuwait;
- Bacteriological Laboratory, Ministry of Health, Oman;
- The Caribbean Environmental Health Institute, St Lucia, West Indies.

Also by arrangement with UNEP's Regional Seas Programme Activity Centre, the following experts were invited to attend the joint intercalibration exercise and the consultation meeting in an advisory capacity: Dr G.I. Barrow (United Kingdom), Dr M. Bernhard (Italy), Professor E. Geldreich (USA) and Mr K. Krongaard Kristensen (Denmark).

Copies of the reference methods were transmitted to the laboratories for testing during late August/early September 1982.

3. Proceedings and results

The joint intercalibration exercise was held in the laboratories of the Istituto Superiore di Sanità, Rome, from 22 to 25 November 1982. A list of participants is appended. On the last two days (24 and 25 November), activities were limited to analysis of results, and the time-table arranged for cohesion with the consultation meeting.

The exercise was limited to the first three reference methods, i.e.:

- determination of total coliforms in seawater by the membrane filtration culture method;
- determination of faecal coliforms in seawater by the membrane filtration culture method;
- determination of faecal streptococci in seawater by the membrane filtration culture method.

It was originally intended that, apart from determination of the three parameters (total coliforms, faecal coliforms and faecal streptococccci) by the membrane filtration (MF) method, participants could also carry out parallel determinations on the same samples, utilizing the most probable number (MPN) method. Detailed instructions were therefore prepared in this regard for the following samples:

No. 1 - effluent (fresh water) - MF and MPN
No. 2 - "clean" area (seawater) - MF and MPN
No. 3 - intermediate area (seawater under the possible influence of a pollution source from which sample No.1 was taken) - MF and MPN
No. 4 - sand from sampling point No. 2 - MPN
No. 5 - sand from sampling point No. 3 - MPN.

During the exercise, considering the available time, it was decided to limit work to determination of the three parameters by the MF method only (i.e. as detailed in the draft reference methods). Only samples Nos 1, 2 and 3 were therefore utilized.

Participants were divided into six teams of two persons each. Results obtained by individual teams were more or less in agreement, although differences were statistically significant, even if slight, in some cases. A preliminary analysis of part of the data was also carried out.

The original instructions as given to participants, the results obtained and the preliminary statistical analysis, are reproduced herewith.

4. Conclusions

Considering that this was the first laboratory exercise of this nature organized within the framework of MED POL, the results can be described as satisfactory, and have provided the basis on which further intercalibration exercises both at national and regional levels can be organized in future years. In addition, the occasion provided the opportunity for participants to compare techniques during actual work-performance, rather than through discussion only.

Instructions given to participants in intercalibration exercise on microbiological reference methods

1. SAMPLES

No. 1 = Effluent (fresh water).
No. 2 = Clean area (sea water).
No. 3 = Intermediate area (sea water under the possible influence of the pollution source No. 1).
No. 4 = Sand of sampling point No. 2.
No. 5 = Sand of sampling point No. 3.

2. SAMPLING METHOD

The samples are collected directly by hand, in sterilized containers, and are carried out by cooling containers (4-10°C). Storage and transportation time = maximum 24 hours.

3. ANALYTICAL METHODS

3.1 WATER SAMPLES (No. 1, 2 and 3)

3.1.1 TOTAL COLIFORMS

3.1.1.a Membrane filter method

3.1.1.a.1 M-Endo-Agar

Filter 100 ml of sample (or its dilution). Incubate 24 hours at 36±1°C on solid M-Endo-Agar. After incubation, read for typical colonies (pink or red). Calculate for bacterial density (see Form A).

3.1.1.a.2 M-FC-Broth

Filter and incubate as in 3.1.1.a.1 on M-FC-Broth. After incubation, read for typical blue colonies. Calculate for bacterial density (see Form A).

3.1.1.b Most probable number method (MPN)

Inoculate Lactose Broth tubes with the sample or its dilutions. Incubate 18-24 hours at 36±1°C.

Read for gas production. For negative tubes, incubation is continued for another 24 hours (total incubation time = 48±3 hours).

Positive tubes are tested by transferring a small inoculum from each positive tube to individual Brilliant Green Lactose Bile Broth tubes and incubating at 36±1°C for 48 hours.

Read for gas production. Calculate for MPN (see Form A).

3.1.2 FAECAL COLIFORMS

3.1.2.a Membrane filter method

3.1.2.a.1 M-FC-Agar

Filter as in 3.1.1.a.1.

Incubate 24 hours on solid M-FC-Agar at 44±0.2°C (water-bath). After incubation, read for blue colonies. Calculate for bacterial density (see Form A).

3.1.2.a.2 M-FC-Broth

Filter as in 3.1.1.a.1.

Incubate 24 hours on M-FC-Broth at 44.5±0.2°C (water-bath). After incubation, read for blue colonies. Calculate for bacterial density (see Form A).

3.1.2.b Most Probable Number Method (MPN)

Inoculate tubes, incubate and read for gas production, as in 3.1.1.b. Positive tubes are tested by transferring a small inoculum of each positive tube to individual EC-Broth tubes and incubating at 44.5±0.2°C for 24 hours in a water-bath. Calculate for MPN (see Form A).

3.1.3 FAECAL STREPTOCOCCI

3.1.3.a Membrane filter method

3.1.3.a.1 KF-Agar (at 44°C)

Filter as in 3.1.1.a.1. Incubate 48±3 hours at 44±0.2°C on KF-Agar (water-bath). After incubation read for red colonies or colonies with a red center. Calculate for bacterial density (see Form B).

3.1.3.a.2 KF-Agar (at 36°C)

Filter as in 3.1.1.a.1. Incubate 48±3 hours at 36±1°C on KF Agar. After incubation read for red colonies or colonies with a red center. Calculate for bacterial density (see Form B).

3.1.3.a.3 M-Enterococcus Agar

Filter as in 3.1.1.a.1. Incubate 48 hours at 36±1°C. After incubation read for pink or red colonies. Calculate for bacterial density (see Form B).

3.1.3.b.1 Most Probable Number Method (MPN) at 36°C

Inoculate tubes of Azide Dextrose Broth with the sample or its dilutions. Incubate at 36±1°C for 24+24 hours. Read for turbidity. Positive tubes have to be confirmed on EVA Broth (24+24 hours at 36±1°C). Read for turbidity and formation of a "purple" bottom. Calculate for MPN (see Form B).

3.1.3.b.2 Most Probable Number Method (MPN) at 44°C

Inoculate tubes of Azide Dextrose Broth with the sample or its dilutions. Incubate at 44±0.2°C for 24+24 hours (water-bath). Read for turbidity. Positive tubes have to be confirmed on EVA Broth (24+24 hours at 44±0.2°C in water-bath.) Read for turbidity and formation of "purple" bottom. Calculate for MPN (see Form B).

Participating Laboratory _____

Country _____

Sample n° ____

FECAL STREPTOCOCCI - MF METHOD

dilution	inoculum	KF-AGAR 44°C	KF-AGAR 36°C	M-Enteroc. Agar 36°C
10^0	10 ml			
10^0	1 u			
10^{-1}	1 u			
10^{-2}	1 u			
10^{-3}	1 u			
10^{-4}	1 u			
10^{-5}	1 u			
10^{-6}	1 u			

FECAL STREPTOCOCCI - MPN METHOD

dilution	inoculum	incubation hours	Pres. Test. 36°C: Az. Dext. Br.	Pres. Test. 36°C: EVA broth I	Pres. Test. 36°C: EVA Broth II	Pres. Test. 36°C: FS MPN/100 ml	Pres. Test 44°C: Az. Dext. Br.	Pres. Test 44°C: EVA broth I	Pres. Test 44°C: EVA Broth II	Pres. Test 44°C: FS MPN/100 ml
10^0	10 ml	24								
		48								
10^0	1 u	24								
		48								
10^{-1}	1 u	24								
		48								
10^{-2}	1 u	24								
		48								
10^{-3}	1 u	24								
		48								
10^{-4}	1 u	24								
		48								
10^{-5}	1 u	24								
		48								
10^{-6}	1 u	24								
		48								

Participating Laboratory

Country ---

Sample n° ---

Fecal coliforms - Multiple Tube Test method

dilution	inoculum ml		L.B.	E.C. I	E.C. II	FC MPN /100 ml
10^{0}	10	24				
		48				
10^{0}	1	24				
		48				
10^{-1}	1	24				
		48				
10^{-2}	1	24				
		48				
10^{-3}	1	24				
		48				
10^{-4}	1	24				
		48				
10^{-5}	1	24				
		48				
10^{-6}	1	24				
		48				

Form C (Sand-Sediment)

SUMMARY RESULTS OF INTERCALIBRATION EXERCISE

Sample No.	Sample Test Amount	Results obtained (median and range) Team 1	Team 2	Team 3	Team 4	Team 5	Team 6
Faecal Coliforms							
1	0.1 ml	190(156-210)	190(151-224)	110(102-118)	152(148-186)	157(138-183)	∞
1	0.01 ml	15 (10-22)	18 (17-26)	22 (17-54)	20 (0-24)	28 (27-29)	30 (24-32)
2	100 ml	1 (1-2)	5 (4-8)	2 (1-3)	4 (2-4)	5 (5-5)	5 (2-6)
2	10 ml	1 (0-1)	0 (0-1)	0 (0-0)	0 (0-0)	1 (1-2)	0 (0-0)
3	100 ml	11 (9-12)	11 (9-12)	18 (12-19)	6 (6-6)	10 (8-15)	10 (8-14)
3	10 ml	3 (2-3)	3 (2-3)	1 (0-1)	3 (0-5)	1 (0-1)	3 (0-6)
Faecal Streptococci							
1	1 ml	67 (65-72)	47 (41-52)	60 (55-83)	67 (62-74)	52 (51-53)	51 (50-56)
1	0.1 ml	6 (3-8)	3 (2-5)	9 (4-10)	6 (5-7)	5 (2-6)	7 (7-8)
1	0.01 ml	1 (0-1)	-	0 (0-1)	1 (0-2)	1 (0-3)	0 (0-1)
2	100 ml	7 (1-8)	5 (2-6)	7 (2-8)	5 (4-5)	5 (4-7)	5 (3-7)
2	10 ml	0 (0-1)	-	0 (0-0)	1 (0-1)	1 (0-1)	0 (-1)
3	100 ml	10 (7-13)	12 (4-16)	11 (7-17)	11 (10-11)	13 (6-14)	13 (12-24)
3	10 ml	3 (2-3)	-	1 (1-1)	1 (0-1)	1 (0-3)	1 (0-2)

INTERCALIBRATION EXERCISE: PRELIMINARY ANALYSIS

The analysis has been limited to the first collaborative experiment, concerning water samples at 0.1 and 0.01 dilution factor.

The following hypotheses have been assumed:

- A Poissonian statistical distribution of values,
- An approximate Gaussian behaviour of such distribution in the range of data examined.

Under these conditions, confidence limits of detected values have been computed, as shown in Fig. 1.

The expected values of the measured parameter, as estimated on the basis of the two determinations available for each participant (0.1 and 0.01 dilution factor samples), are reported in Fig. 2, together with the corresponding confidence limits. The width of last confidence interval is due to the lack of the 0.1 dilution datum.

The agreement among the estimates appears to be good. However, the hypothesis of some slight difference "among participants", statistically significant, may be posed. Moreover, at least in one case, a slight significant difference may be hypothesized between the two estimates carried out at the two different dilution factors.

In any case, these nearly negligible differences may be considered as largely acceptable, if considered in the light of practical needs in environmental monitoring. If this reasonable point of view is assumed, the experiment outcomes appear to be fairly satisfying. Analogous conclusions may be expected for the two other series of measurements. Due to the little numbers of F.C. counted, the Gaussian approximation of Poissonian statistical distribution appears not acceptable in this case. Therefore, a reliable data evaluation requires much more computer work, and a simple estimate is not possible.

In any case, all the hypotheses here presented need to be verified with further study and have to be considered as a rough preliminary estimate. The comments of participants are necessary to design a complete analysis of data.

Figure 1

Confidence limits of detected values

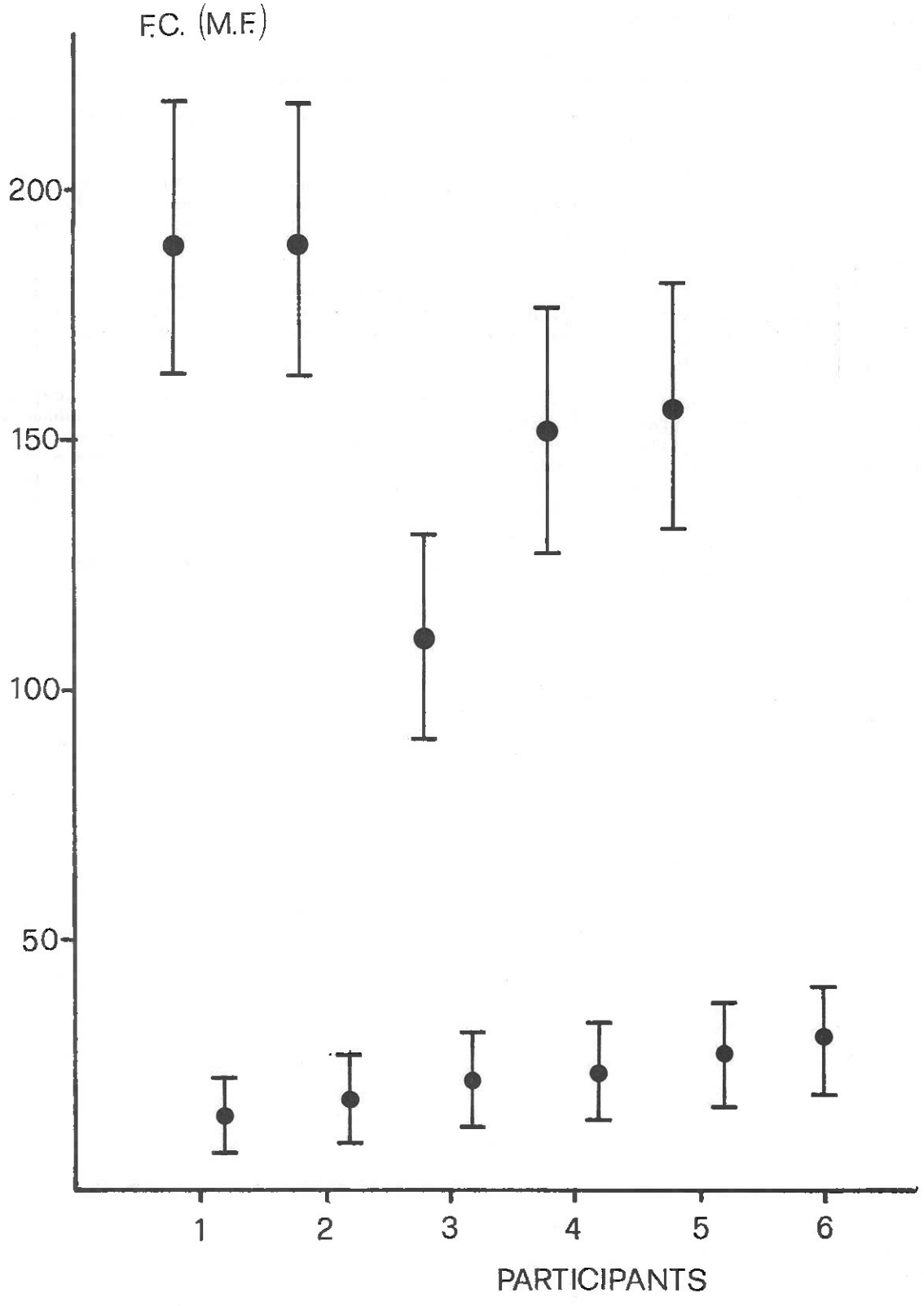
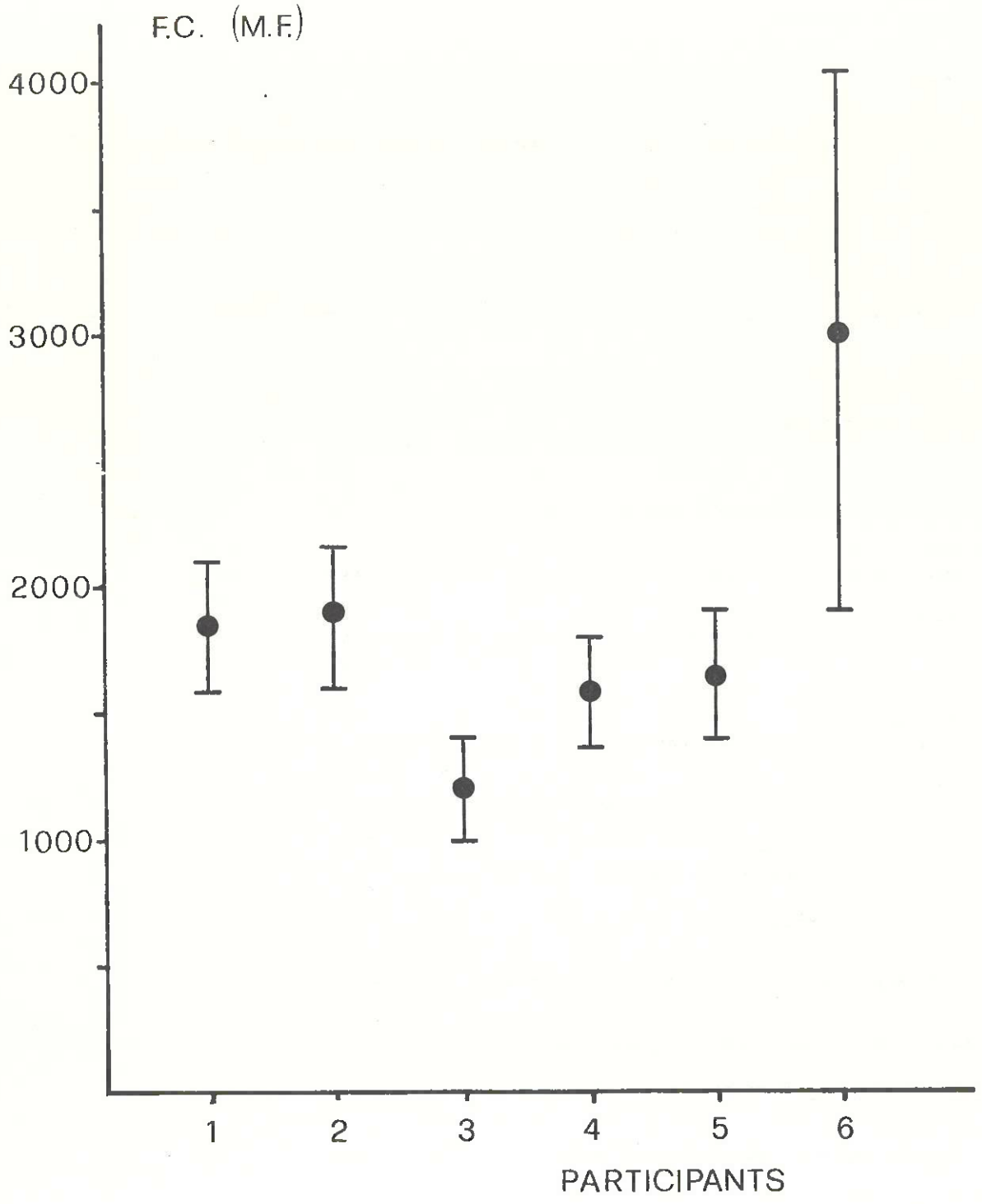


Figure 2

Expected values and confidence limits of measured parameters



PARTICIPANTS

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Annex 10

OUTLINE SUMMARIES OF REFERENCE METHODS, AS MODIFIED

Summary A: total coliforms in sea water (Reference Method No. 2)

Method:	membrane filtration
Medium:	M-endo agar (not to be autoclaved)
Incubation temperature:	36±1°C (air incubator)
Time:	24 hours
Colour of colonies:	Pink to red with golden sheen
Confirmation:	AG on Mac Conkey broth 48 hours at 36±1°C, or G on brilliant green broth 48 hours at 36±1°C (not systematic, carried out only initially and thereafter when in doubt)

Summary B: faecal coliforms in sea water (Reference Method No. 3

Method:	membrane filtration
Medium:	m-FC agar (not to be autoclaved) add rosolic acid only if necessary
Incubation temperature:	44.5±0.2°C (water-bath)
Time:	24 hours
Count:	blue colonies
Confirmation:	AG on Mac Conkey broth 24 hours at 44.5°C, or G on brilliant green broth 24 hours at 44.5°C, and Indole + in tryptone water 24 hours at 44.5°C (not systematic, carried out only initially and thereafter when in doubt)

Summary C: faecal streptococci in sea water (Reference Method No. 4)

Method:	membrane filtration
Medium:	KF agar (not to be autoclaved)
Incubation temperature:	36±1°C (air incubator)
Time:	48 hours
Colonies:	pink to maroon
Confirmation:	not necessary

Summary D: faecal coliforms in bivalves (Reference Method No. 5)

Method:	MPN
Medium:	lactose broth (Presumptive Test) at 36±1°C (48 hours) Mac Conkey broth at 44.5±0.2C (24 hours or brilliant green broth at 44.5±0.2°C (24 hours and Indole test at 44.5±0.2°C (24 hours)

Annex 11

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