Examination of bacterial content of sea water essay

Business, Management

Project Title: Examination of bacterial content of sea water samples from selected sites in South East England Introduction Over the past few years coastal beaches across the UK has seen lavished with recognition for their cleanliness, by sections of the media and various organisarions. Majority of these beaches have been awarded the Blue flag award in recognition for meeting set criteria on various standard including cleanliness, water quality, environmental management and environmental education and information programme; an award ran by independent non-profit organisation Foundation for Environmental Education (FEE) and with similar programme ran by The marine conservation society (MCS). The general perception is that this beaches clean due to the reduce numbers of household litters presents, giving the impression that the litter level are the determinant of the cleanliness of the water. This study intends to examine: ? The bacteria population of samples obtained from several site across south east coast of England. ? Identify species of Gram Negative (-) & (+) of each sample.

? identify any faecal coliform bacteria which might suggest water contamination. These indicator organisms are used for indication of water contaminant by human and other warm-blooded animals (APHA 1992). Several sample will be obtained from beach that are participant in some form of recognised sanitary programme and have meet set criteria by a recognised monitoring body. While several more sample will be obtained from location not participating in any such sanitary programmes. This will ensure an even sample distribution of bacterial population and data comparison. Various bacteriological techniques will then be employed to help identify any present bacteria species. Hypothesis Of the four samples; the

bacteria population count would be lower in samples obtained from location awarded the “ blue flag” award. Material, Equipment and Method Material: Petri dish 70% Alcohol Bleach Sterile water/ saline solIodine solutionR2A & R3A AgarMacConkey Agar Masking Tape Acetone Alcohol Safranine Sterile swab Crystal violet dyeMicroscopic oil Micropipette Tips (20- 2000 µl, 200-1000 µl, 1-5mL)Inoculating loops & wire Micro-slideMarking pen Pipette PasteurSterile forceps Mannitol salt agarBlood agar Filter paperSample containers Durham’s (fermentation tube) tubeTDA Ringer solution (Quarter strength)Hydrogen peroxide API Gram (+)(-)selective biochemical test kits/ API 20EIND reagent TMPD or DMPD (Redox indicators) James reagent Equipment : Bunsen burner Stop clockMicropipette (20- 2000 µl, 200-1000 µl, 1-5mL) Glass Rod spreader Google Laboratory coat Rubber glove Autoclave Light Microscope Staining rack Coulter Counter Racks Test tube, bottles, caps & stoppers Tissuepaper Discard jars disinfectant pot Beaker flask Experimental Methodologies The bacteria population first needs to be analysis.

The water sample will be transferred to the laboratory in a cold chain under aseptic conditions to minimise the risk of contamination The sample under investigation would certainly contain mixtures of various organisms. In order to assess the bacterial flora population of the samples, it is essential to use selective and non- selective media because no one medium or temperature will support all present organisms. This will provide suitable favourable growth environment for the various group of organisms. Direct Count Method The bacteria population of an unknown mixture is investigated using the

direct count method. First; the four original samples will be diluted to 10-? to 10-? in peptone / deionised water and 0.

mL of these dilutions will then be streaked in triplicate with the spread plate technique onto the selective (MacConkey, Blood agar, Mannitol salt & eosinomethylen blue agar) and non selective R2A agar, Reasoner & Geldreich (1985), general nutrient agar media. The media will then be incubated par manufacturer instructions at various temperature to allow bacteria growth before been counted. While some colonies might be isolated others might still be clogged together. In these cases isolated and clogged colonies will be counted as one.

A measure of viable bacteria will then be determined in (Colony Forming Unit/millilitre). Some of these colonies might be identifiable visually due to their distinct colonies morphologies, pigmentation, colouration and chemical metabolite. Other will be less distinguishable, requiring further test. To further enhance this method an indicator medium will also be used.

These medium changes colour during fermentation, an indication of pH change due to the presence of acid forming species. Durham tubes will then be employed; when inverted they catch gaseous bubble formed during fermentation. The gas produced at 37C act as a strong indicator to the presence of E. coli Reasoner & Geldreich (1985). Experimental Control The experiment will be replicated and controlled to minimise any inaccuracies and error. Distilled water will be used as negative control, while

contaminated sample will be the positive control to enable any discrepancies in the data to be highlighted.

(Furthermore; coulter counter can be used for the initial experiment; providing the availability of the equipment and its efficiency.) The Gram Stain The Gram stain methods is then employed on obtaining pure culture from subsequent streaking of various bacteria isolates. These isolates are then Gram stained for their Gram group/ identity. There are various modified Gram methods; the appropriate one will be selected in due course. Gram stains method in Brief: First a colony of interest is heat fixed on a microscopic slide and stained with crystal violet solution, then washed off with water after 60sec before been stained with iodine. It’s then left to set for another 60sec then washed with alcohol or acetone.

leave for a few seconds then washed with water before counterstained with safranin or fuchsine for 30s left to set then washed again with deionised water and blot dried with filter paper. Under a microscope Gram positive bacteria will be stained purple while Gram negative bacteria’s are stained pink under the microscope. The bacteria morphologies also gives important clues as to the bacteria class. Biochemical Test The API 20E system is made up of 20 plastic strip of individual miniaturized tests tubes (cupules) each containing a different reagent used to determine the metabolic capabilities and product of bacteria metabolism specific to certain bacteria groups. This process requires the inoculation of the capsule with a saline suspension of a pure bacteria culture with some tube been filled (VP, CIT and GEL) while others (ADH, LDC, ODC, H2S, URE) topped up later with minerals oil to

encourage anaerobic reactions. the capsule will then be incubated humidity chamber for 18-24 hours at 37 degrees after which each capsulated test are assess for colour specific change. Any change will be indicative of a metabolic reaction which would shed light on the bacterial identity.

Other end product might require additional reagent for further identification. The oxidase reaction is however conducted separately. The result are then interpreted numerical via reaction code (7 digits) and associated with the species identification number for the true identity of the bacteria.

Various other API selective bacteria strips will also by used. Limitation of Methodology The Direct count methods has certain limitation; most notable the time it to prepare and incubate the various media. Even though the media for the estimation of the bacteria population is non selective there will be organisms that will find the condition unfavourable, making the result slightly inaccurate. This method also requires a lot of Petri dish and media and the frequent occurrence of false positive result is not uncommon.

Therefore, the use of the coulter counter would be advantageous but it’s not without its own limitation, notably the technical challenge in counting cells as small as 1-2 µm and also the difficulties associated with discriminating cells from detritus and air bubbles and mixture species with overlapping size. Further experimental limitation include human error, risk of contamination during collection, transportation or preparation. These limitations however unavoidable, suggest careful employment of the procedural steps should help yield an improved results accuracy. Statistical Data Handling SPSS

system will be used to compute the data obtained for the various bacteria population count. The mean, standard error of mean, t test , ANOVA and other significant statistical test will be conduct and result's examine for any significant difference. ReferencesAPHA (1992) Standard Methods for the Examination of Water and Wastewater, 15th edn.

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