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Removal Capability of Carbon-Soil-Aquifer Filtering System in Water Microbiological Pollutants

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1. Introduction

1.1 Definition and factors

Water can be defined as a clear colourless, nearly odourless and tasteless liquid, H_2O and is very essential for most plant, animal life and the most widely used of all solvents. It can be obtained in many forms such as rain, lake water, column, river, etc. Household used water and other types that are related to our body is very important from the viewpoint of bacterial and microbiology. Microbiology is the study of organisms that are usually too small to be seen by the unaided eye; it employs techniques such as sterilization and the use of culture media that are required to isolate and grow these microorganisms (Prescott et al., 2005).

In rural area of developed, developing and underdeveloped countries, untreated ground and surface waters are used as the sole source of drinking and cooking water. This is due to the general ignorance about water quality and its treatment and also due to relatively poor economy. Ground and surface water are protected from pollution so that raw water can be directly used for drinking and household purposes. The main sources of pollution are municipal and domestic wastewater, industrial as well as irrigation flows, animals wastes; pesticides, fertilizer and human excreta which contaminate ground as well as surface water in rural areas. It affords an opportunity for certain species of flies to lay their eggs, breed, feed on the exposed materials and carry infectious diseases. It is responsible for the incidence of certain diseases, which include *paratyphoid*, *cholera*, *typhoid*, *dysentery*, infant *dicholera* as well as other similar intestinal infection, parasitic infections and chronic disease.

1.2 Principles of microbiology

Biology is the science of life. It has three major divisions: zoology - the study of animals, botany - the study of plants, and microbiology - the study of microbes. These primary

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partitions may be divided further into specialities. For instance, *algology* and *mycology*, the study of algae and fungi respectively, are subdivisions of botany. *Protozoology*, the study of unicellular animals, is a division of zoology whilst bacteriology and virology, the study of bacteria and viruses, are subdivisions of microbiology. Knowledge of the behaviour of micro-organisms is heavily dependent upon biochemistry although knowledge about macroscopic animals and plants may be acquired through studies of anatomy and morphology as well as structure and form.

Life does not have simple definition. It may be characterized by a list of properties which are shared by all living organisms, with the exception of the viruses, and which discriminate them from non-living matter:

Movement: It is characteristic of organisms that they, or some part of them, are capable of moving themselves. Even plants, which at first sight appear to be an exception, display movements within their cells.

Responsiveness: All organisms, including plants, react to stimulation. Such responses range from the growth of a plant towards light to the rapid withdrawal of one's hand from a hot object.

Growth: Organisms grow from within by a process which involves the intake of new materials from outside and their subsequent incorporation into the internal structure of the organisms. This is called assimilation and it necessities some kind of feeding process.

Feeding: Organisms constantly take in and assimilate materials for growth and maintenance. Animals generally feed on ready-made organic matter (*heterotrophic* nutrition) whereas plants feed on simple inorganic materials which they build-up into complex organic molecules (*autotrophic* nutrition).

Reproduction: All organisms are able to reproduce themselves. Reproduction involves the replication of the organism's genetic "blueprint" which is encoded in a nucleic acid. Generally this is *deoxyribonucleic acid* (DNA) but in some viruses it may be *ribonucleic acid* (RNA).

Release of energy: To sustain life an organism must be able to release energy in a controlled and usable form. This is achieved by breaking down *adenosine triphosphate* (ATP). The energy to generate ATP is obtained by the breakdown of food by respiration. The occurrence of ATP in living cells appears to be universal.

Excretion: The chemical reactions that take place in organisms result in the formation of toxic waste products which must be either eliminated or stored in a harmless form.

1.3 Global impact of waterborne disease

Throughout the world, many people do not have access to safe drinking water. As a consequence, there is significant morbidity and mortality due to disease-causing organisms in water. It is estimated that nearly one-fourth of all hospital beds in the world are occupied by patients with complications arising from infection by waterborne organisms (Gerba, 1996). Citing the *WHO/UNICEF Global Water Supply and Sanitation Assessment 2000 Report,* it is estimated that nearly 6000 people, mostly children, die every day because of water related diseases. Even in the United States, an estimated US$20 billion per year in lost productivity has been attributed to diseases caused by waterborne pathogens (Gerba, 1996).

1.4 Microbiological aspects

Drinking water should not contain any microorganisms known as pathogens. It should be free from bacteria indicative of excremental pollution. The primary bacterial indicator recommended for this purpose is the coliform group of organisms as a whole. Although as a group they are not exclusively of faecal origin, they are universally present in large numbers in the faeces of man and other warm-blooded animals, and thus can be detected even after considerable dilution. The detection of faecal (*thermotolerant*) coliform organisms, in particular *Escherichia coli*or *E.coli* provides definite evidence of faecal pollution.

The tiny and invisible microorganisms pose a serious threat to the safety of the world's drinking water. Water when contaminated by microorganisms, particularly by the pathogenic ones, can become a growing peril with the potential to cause significant outbreaks of various types of infectious disease. The list of potentially pathogenic microorganisms transmitted by water is increasing significantly each year. Indeed the distribution of safe drinking water to the home can no longer be taken for granted, not even in the United States and Western Europe. The average person consumes about 2-4 liters of water per day through food and drinks. All these deliberations suggest an urgent need for supply of safe drinking purposes. Pilot and mini scale carbon-soil-sand filtering system has successfully produced potable drinking water in the developed countries for a century. Cost of fabrications, usefulness of local raw materials, ease of operation and maintenance, and low energy requirements make slow carbon-soil-sand filtration system a water treatment technology that is particularly well suited for developing countries.

In the midpoint of international drinking water supply and sanitation decade (1985) only 42% of the world's rural populations and 77% of the world's urban population had access to safe drinking water excluding the Peoples of Republic of China (Rotival, 1987). Extensive research has been performed on the efficiency of carbon-soil-sand filters for treatment of water in normal climates. When source waters are relatively low in temperature and turbidity, this process has been found to be effective in the removal of toxic elements, organics and bacteria (Bellamy et al., 2006, Slezak and Sims , 1984). Tropical rivers and other surface waters, which serve as receiving waters for waste discharges and runoff, also serve as water supplies for downstream communities. Low flow rates of surface waters during dry seasons will result in high levels of toxic and microbial contamination from upstream human waste, agricultural runoff and industrial processing facilities. As concentrations of faceal coliforms in untreated water supplies vary widely in developing countries (Vsscher et al., 1986), polluted source waters were simulated by maintaining a filter influent concentration of approximately 10^6 *E. coli* cells per 100 mL.

Adsorption with carbon-soil-sand filter has been one of the most useful techniques in water treatment. In the past, activated carbon was predominantly used to remove odor and colour producing molecules in water (Suffer and McGuire, 1980). We have previously reported the removal of toxic elements (heavy metals) using the same filtering system (Yusof et al., 2002 and Rahman et al., 2011).

In these papers, the competitive separation and adsorption microbiological pollutants (Coliform, total count etc.) are reported. One of the problems related to groundwater is the reddish colour caused by the presence of iron and manganese. This colour can be seen after it has been exposed to the air, the oxidation of groundwater will promote the precipitation

of iron(III) and manganese ions. A significant removal of iron and manganese was reported by Ahmad et al. (2005). While a fixed bed column or continuously flow study to remove heavy metal specifically cadmium and lead by using granular activated carbon has been successfully removed up to 99 percent (Ahmad et al., 2007).

A culture medium is a solid or liquid preparation used to grow, transport and store micro-organisms also based on Prescott, et. al (2005). The medium must contain all the nutrients the microorganism requires for growth. The isolations and identifications of microorganisms need special media which is essential for testing the sensitivities of water. Sources of energy, *carbon*, *nitrogen*, *phosphorus* and various minerals are required for the growth of microorganisms and the precise composition of a satisfactory medium will depend on the species one is trying to cultivate because nutritional requirements vary so greatly.

Indicator organisms are bacteria that are used as a sign of quality or hygienic status in water. The definition of indicator is the concept of the indicator organisms which is so strictly associated with particular conditions that its presence is indicative of the existence of these conditions. The minimum requirement for an indicator is that it must be a biotype that is prevalent in sewage and excreted by humans or warm-blooded animals. Historically, these conditions have been related to insanitation and public health concerns. Over the years, however, the use of indicator organisms has been extended to provide evaluation of the quality, in addition to the safety, of particular commodities. In addition, the indicator should be present in greater abundance than pathogenic bacteria, incapable of proliferation or at least not more capable than enteric bacteria, more resistant to various disinfectants than the pathogenic bacteria, and qualified by simple and rapid laboratory procedures. To ensure of value in evaluating the risk of disease as well as water quality, the indicator should satisfy the following criteria:

i. The indicator should always be present when the source of the pathogenic microorganisms of concern is present and absent in clean uncontaminated water.
ii. The indicator must present in numbers much greater than the pathogen or pathogens it is intended to indicate.
iii. The indicator should respond to natural environmental conditions and water as well as wastewater treatment processes in a manner similar to the pathogens of interest.
iv. The indicator should be easy to isolate, identify and enumerate.

1.5 Bacteria

The bacteria (blue green bacteria) formerly known as blue-green algae, constitute in the kingdom *Procaryotae*. They are single-celled organisms which use soluble food. From all organisms, this group is the most significant to the public health engineer, since biological wastewater treatment processes rely almost exclusively on the activity of bacteria. Bacteria are relatively endurant in many habitats on earth, growing in soil, acidic hot springs, water, deep in the earth's crust, in organic matter and the live bodies of plants and animals. They constitute the highest population of microorganism in wastewater.

Bacteria can be classified into two major groups: heterotrophic and autotropic depending on the source of nutrients. Heterotrophs utilize organic matter as energy as well as a carbon source for their synthesis. Whereas autotropic bacteria use oxidizing inorganic compounds for energy and carbon dioxide as a carbon source (Hammer, 2008).

Bacteria that cause bacterial infection are called pathogenic bacteria. Pathogenic bacteria are a major cause of human death and disease and cause infections such as cholera, tetanus, typhoid fever, diphtheria, syphilis, foodborne illness, leprosy and tuberculosis. A pathogenic cause for a known medical disease may only be discovered many years after, as was the case with *Helicobacter pylori* and peptic ulcer disease. Bacterial diseases are also important in farm animals such as mastitis, salmonella and anthrax as well as in agriculture, with bacteria causing leaf spot, fire blight and wilts in plants. Some of commonly found bacteria are shown in Figure 1. They are *Bacillus*, *Bordetella*, *Clostridium*, *Escherichia*, *Spirilina*, *Staphylococcus*, *Streptococcus* and *Salmonella*.

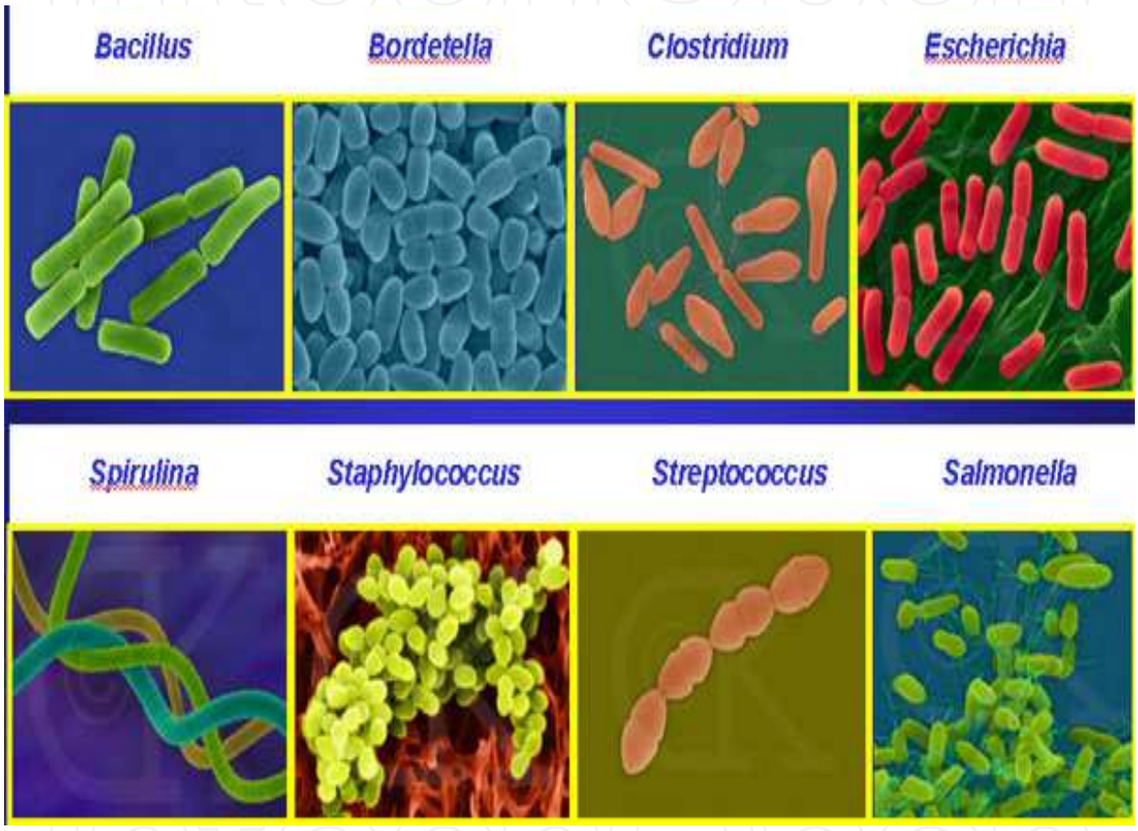

Fig. 1. Commonly found bacteria (Source http://agrobacter wikispaces com/file/list)

It is well known that soil washes into natural bodies of water, particularly after heavy rains. Many different kinds of bacteria also will be carried into water, including soil bacteria and bacteria from the faeces of animals, such as tiny invertebrates, insects, birds, and other "lower animals." In addition, water will often have its own bacterial flora, contributed by its resident species of animals. Bacterial flora from literally hundreds of different species of animals enters natural waters each week. The normal flora of man has been thoroughly studied. The stool flora of man alone may comprise over a hundred different species of bacteria. Although less well studied, other animals have their own characteristic bacterial flora. Many of the bacteria species which form the normal flora of man probably evolved from bacterial species which form the normal flora of lower animals. In the cases that have been carefully studied, it is easy to tell the difference between closely related species with a

sensitive technique, such as DNA-*hybridization*. Often, however, it is not easy to tell the difference between closely related species with the simple tests samples taken from insects, birds, and mammals.

1.6 Coliform

The coliform group was the mainstay of the sanitarian's tools for detecting the presence of faecal contamination in aquatic environments. The broad general characteristics which define this group have allowed it to be one of the most useful of bacterial indicators and at the same time have been responsible for its displacement as an indicator of faecal contamination.

The coliform group is made up of bacteria with defined biochemical and growth characteristics that are used to identify bacteria that are more or less related to faecal contaminants. The total coliforms represent the whole group, and are bacteria that multiply at 37^{o}C. The thermotolerant coliforms are bacteria that can grow at a higher temperature (44.2^{o}C) and *E.coli* is a thermotolerant species that is specifically of faecal origin. A finding of any coliform bacteria, whether thermotolerant or not, in water leaving the treatment works requires immediate investigation and corrective action. There is no difference in the significance of total coliforms, thermotolerant coliforms and *E. coli* in water leaving a treatment works, as they all indicate inadequate treatment, and action should not be delayed pending the determination of which type of coliform has been detected. Upon detection in a distribution system, investigations must be initiated immediately to discover the source of the contamination.

Tests for detection and enumeration of indicator organisms, rather than of pathogens, are used. The cultural reactions and characteristic of this group of bacteria have been studied extensively. Coliform group density is a criterion of a degree of pollution. Membrane filter technique, which involves direct plating for detection and estimation of coliform densities, is as effective as the multiple-tube fermentation test for detecting bacteria of the coliform group. Modification of procedural details, particularly of the culture medium, has made the results comparable with dose given by the multiple-tube fermentation procedure. Although there are limitations in the application of the membrane filter technique, it is equivalent when used with strict adherence to these limitations and to the specified technical details.

Thus, two standard methods are presented for the detection of bacteria of the coliform group. It is customary to report results of the coliform test by the multiple-tube fermentation procedure as a Most Probable Number (MPN) index. This is an index of the number of coliform bacteria that, more probably than any other number, would give the results shown by the laboratory examination; it is not an actual enumeration. By contrast, direct plating methods such as membrane filter procedure permit a direct count of coliform colonies. In both procedures coliform density is reported conventionally as the (most probable number) MPN or membrane filter count per 100 mL. Use of either procedure permits appraising the sanitary quality of water and the effectiveness of treatment process. *E. coli* infection often causes severe bloody diarrhoea and abdominal cramps; sometimes the infection causes none bloody diarrhoea or no symptoms. Usually little or no fever is present, and the illness resolves in 5 to 10 days. To some people, particularly children under 5 years of age and the elderly, the infection can also cause a complication called hemolytic uremic syndrome, in

which the red blood cells are destroyed and the kidneys fail. About 2%-7% of infections lead to this complication. In the United States, hemolytic uremic syndrome is the principal cause of acute kidney failure in children, and most cases of hemolytic uremic syndrome are caused by *E. coli.*

1.7 Fungi

Fungi are multi-cellular, non-photosynthetic, heterotrophic organisms. Fungi are obligate aerobes that are reproduced by a variety of methods including fission, budding, and spore formation. Their cells require only half as much nitrogen as bacteria so that in a nitrogen-deficient wastewater, they predominate over the bacteria. Fungi are plants that are unable to do photosynthesis such as yeast and moulds. Yeast is normally used for fermentation in making bread, cake and alcohol. Moulds are filament in shape that lives in acidic condition. They reduce the efficiency of secondary sedimentation tank and cause unpleasant smell and taste.

Few members of the group are readily visible when they are present. It is their fruiting body which is seen rather than the vegetative body of the organisms. Perhaps the most familiar is the mushrooms, whose Greek name *mykes* gives rise to the term applied to the scientific study of fungi and mycology.

The fungi have highly distinctive biological organization. Although some aquatic fungi and the yeasts are unicellular they are readily distinguishable from bacteria by their large cells and membrane-bound nuclei. Some aquatic fungi do show resemblances to flagellate protozoa. Fungi occupy a wide variety of habitats including the sea and fresh waters. However, the majority occupy moist habitats on land and are abundant in soil. At least 100,000 species are known. Although some produce macroscopic fruiting bodies (e.g. mushrooms) the overwhelming majority are microscopic. The fungi are heterotrophic organisms acquiring organic materials for their nutrition. Those which feed on dead organic materials are described as *saprophytic*. Saprophytes bring about the decomposition of plant and animal remains and in doing so release simpler chemical substances into the environment. In soil this is vital significance in maintaining fertility by recycling essential plants nutrients.

Fungi are present in, and have been recovered from, diverse, remote, and extreme aquatic habitats including lakes, ponds, streams, estuaries, marine environments, wastewaters, sludge, rural and urban storm-water runoff, well waters, acid mine drainage, asphalt refineries, jet fuel systems, and aquatic sediments. Fungi are widely distributed and are found wherever moisture is present. Glycogen is the primary storage polysaccharide in fungi. Most fungi use carbohydrates (preferably glucose or maltose) and nitrogenous compounds to synthesise their own amino acids and proteins. Identification of fungi which are larger than bacteria is dependent on colonial morphology on a solid medium, growth as well as reproduction morphology and for yeasts, physiological activity in laboratory cultures.

Increasing numbers of fungi usually indicate the increasing organics loadings in water or soil. Large numbers of similar fungi suggest excessive organic load while a highly diversified mycobiota indicates populations adjusted to the environmental organics. Despite

their wide occurrence, little attention has been given to the presence and ecological significance of fungi in aquatic habitats. The relevance of fungi and their activities in water is emphasized by increasing knowledge of their pathogenicity for humans, animals, and plants; their role as food or energy sources; their activity in natural purification processes; and their function in sediment formation. Quantitative enumeration of fungi is not equivalent like the unicellular bacteria because a fungal colony may develop from a single cell (spore), an aggregate of cells (a cluster of spores or a single multi-celled spore), or from a mycelial or pseudo-mycelial fragment (containing more than one viable cell). It is assumed that each fungal colony developing in laboratory culture originates from a single colony-forming unit (CFU) which may or may not be a single cell.

The advantages of fungi are for food and food preparation such as edible fungi and fermentation of bread, wines and beers; for medicine e.g. Penicillin is the best known antibiotic and it is actually made from a mould; and decomposers of organic material. The disadvantages of fungi are poisonous (i.e. wild fungi can be both delicious and deadly poisonous. The high concentration of metals such as arsenic, cadmium, copper and lead in wild fungi) causing fungal diseases to plants (i.e. mildew, smuts, ruts, etc).

1.8 Algae

Algae are plant-like organisms that usually photosynthetic and aquatic but do not have true root, stem, leaf, vascular tissues and have simple reproductive structures. They range from tiny single cells to branched forms of visible length that appear as attached green slime. They may be either unicellular or multi-cellular. There are a wide variety of algal species in various shades of commonly green, brown and red. They are distributed worldwide in sea, in freshwater, waste water, marine water, and non-marine water such as mud and sand. Typical green algae are *Oocystis* and *Pediastrum*. Whereas the blue green algae that associated with polluted water are *Anacystis*, *Anabaena*, and *Aphanizomenon*.

Algae are photoautotrophic that use carbon dioxide or bicarbonate as carbon source and inorganic nutrients of nitrogen as ammonia or nitrate and phosphate. Algae have chlorophyll that can manufacture their own food through the process of photosynthesis. They conduct photosynthesis within membrane bound structure called chloroplast. In the presence of sunlight, the photosynthetic production of oxygen is greater that the amount used in respiration. At night they use up oxygen in respiration. If the daylight hours exceed the night hours by a reasonable amount, there is a net production of oxygen. They produce more oxygen than all the plants in the world.

The good of algae are: form important food source for many animals. e.g: little shrimps and huge whales; most important at the bottom of food chain with many living things depend upon them; and have an economic importance because they are a source of carotene, glycerol, and alginates and can be converted into a food source for aquaculture. While the bad of algae are: too much algae does suffocate the lake, so it will kill many fish; blue green-algae are very toxic and algal toxin can seriously affect human and animal; and toxic blue-green algal blooms cause a rash known as “swimmer's itch”, while powerful neuromuscular toxins released by other cyanobacteria (blue green algae) can kill fish or animals that drink the water.

1.9 Filtration

Filtration is most often a polishing step to remove non-settleable flocs remaining after chemical coagulation and sedimentation or precipitant particles of softened water. Under certain conditions, filtration may serve as the primary turbidity removal process especially in direct filtration of raw water.

The normally used filtration process involves passing the water through a stationary bed of granular medium. Solids particles in the water are retained in the interstices of the filter media. Several modes of operation are possible in granular medium filtration. These include upflow, downflow, pressure and vacuum filtration. The most common practice is downward gravity flow filtration, with the weight of the water column above the filter providing the driving force.

The solids removal operation with granular medium filter involves several complicated processes. The most obvious processes include straining, flocculation and sedimentation. The straining process occurs at the interface between filter media and water. Initially, materials larger than the pore openings at the interface are strained. In the filtration process, conditions within pores of a filter bed promote flocculation. Flocs grow in size and become trapped in the interstices. Other processes are also important since most of the solids existence in settled water is too small to be completely removed by straining. Removal of particles and flocs in the filter media depends on transport mechanisms that carry the solids through the water to the surface of the filter grains, and on retention of the solids by the medium once contact has occurred. Transport mechanisms include gravity settling, inertial impaction, diffusion of colloid, Brownian movement and van der Waals forces (Kim and Whittle, 2006). Retention of solids once contact has occurred can be attributed primarily to electrochemical forces, van der Waals force, and physical adsorption (Yaroshevskaya, 2007).

With chemical preconditioning of the water, a well-designed and operated filter should remove virtually all solids down to the submicron size. Removal begin at the top portion of the filter. As pore opening are filled by the filtered material, increased hydraulic shear sweeps particles farther into the bed. The ideal filter media should be coarse enough for large pore openings to retain huge quantities of particles or floc however sufficiently fine to avoid passage of small floc. It should has an optimum filter depth to produce relatively long operation filter run and graded to permit effective cleaning during backwash. Dual-media filter of coarse burnt oil palm shell granules or anthracite overlaying the sand media provide higher porosity at the upper layer as well as higher filter run of more than three times than the conventional sand filter as recorded by Ahmad et al. (2009).

2. Methodology

2.1 Sampling protocols

Sampling site was whole area of Terengganu DarulIman, one of the east coast states, which was divided by 7 districts and these are Kemaman, Dungun, Marang, Kuala Terengganu, Hulu Terengganu, Setiu and Besut. Two samples were taken randomly from every single district, except Kuala Terengganu, and from there 4 samples were taken. All together 16 samples were taken in the whole Terengganu area.

Chosen places are in Kemaman, Kampong (Kpg.) Baharu and Esso petrol pump. Two samples were taken from there. Kpg. Kemenyer and Kpg. Pasir were chosen in Dungun districts and two samples were taken from there to be analyzed. Kuala Terengganu districts which consists of Tanjung, Pantai Tok Jembal, MengabangTelipot 1 and MengabangTelipot 2 were places of sampling where 4 samples were taken. Hulu Terengganu, there were two samples taken that was in Kpg. Bt. Gemuroh and Kpg. Nibong. Marang district, two samples was taken in Kpg. Lubok Perah and Wakaf Tapai. In Setiu, Kpg. Guntung Luar and Kpg. Tembila were chosen and two samples were taken there. In Besut, Kpg. Bt. Bunga and Kpg. Pasir Aka were chosen and two samples were taken there.

For the field method, refer to Figure 2 and the explanation in sampling procedure for the well.

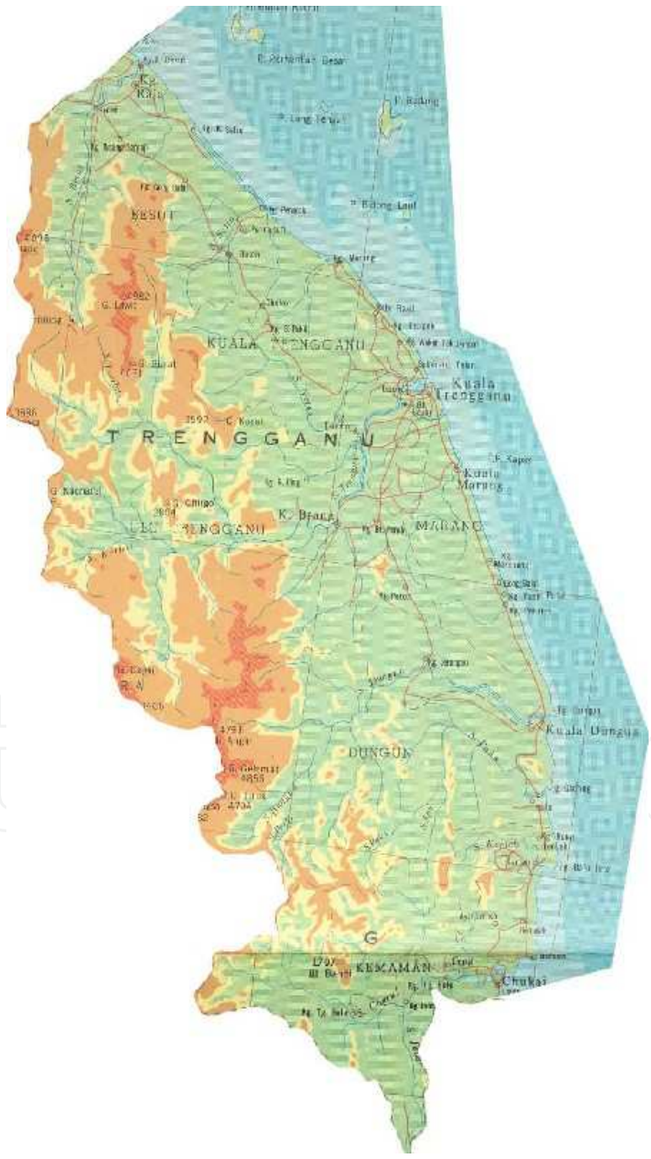

Fig. 2. Sampling Map

2.2 Filtration system

The setup of the filtration system consisting of the carbon-soil-aquifer filtering system is shown in Figure 3. The experimental set-up consists of two identical columns, though only final setup was diagramed. Filter column height is 18 cm, external diameter is 8.5 cm, internal diameter is 7.3 cm and its constructed material is polyethylenetetrathelate (PET). Granulated activated carbon, modified activated carbon and red soil particles were prepared from locally available raw materials. Sand and silica particles were collected from locally available sources.

2.3 Sampling procedure and preserved for microbiological test

In Figure 3, firstly, the metal bucket was cleaned until it is free from earth and rubbish to avoid contamination. Next, a little methylated spirit was poured into the bucket, light it and allowed the burning alcohol to run over the walls of the bucket so as to sterilise the inner surface. After that, the bucket was lowered into the well and natural water sources, and making sure that the rope does not enter the water or the inside of the bucket, as well as the bucket should not touch the side of the well. When the bucket is full of water, it was carefully raised. Next, the string fixing the protective cover was untied and the stopper removed. Then, he sample bottle was filled with the water from the bucket. Finally, the bottle was capped and the protective sheet fixed in place with string.

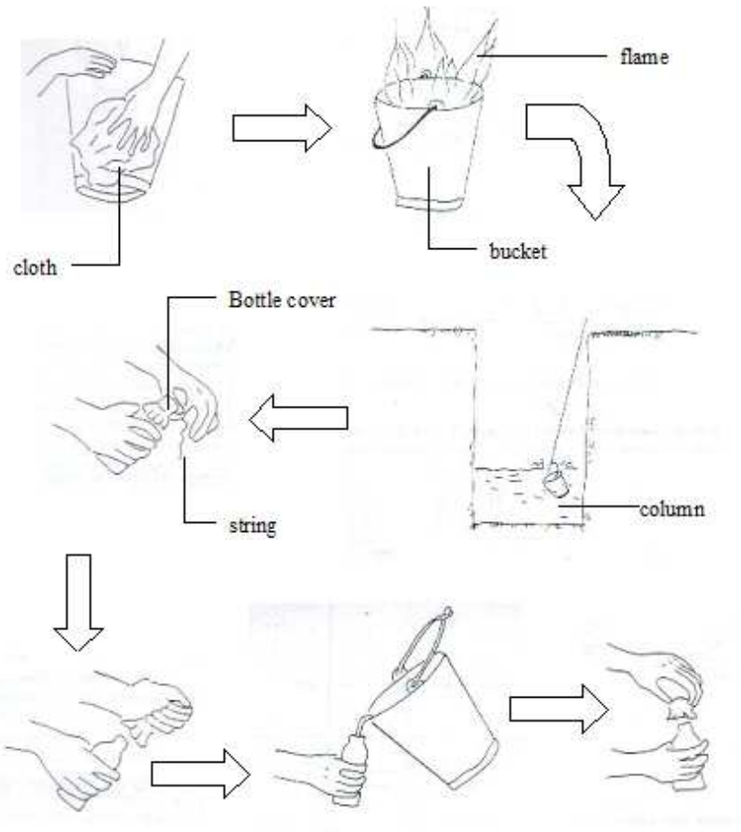

Fig. 3. Sampling method for dug well

The sequence of the components, its thickness and quantity inside the filtration system is shown in Figure 4. The Teflon plastic bottles were cleaned until free from earth and rubbish to avoid contamination. Next, a little methylated spirit was poured into the bottle, light it and allowed the burning alcohol to run over the inside wall of the bottle so as to sterilise the inner surface. When the bottle is full of water, it is carefully transferred, and then bottles are capped and protective sheet fixed in place with a piece string.

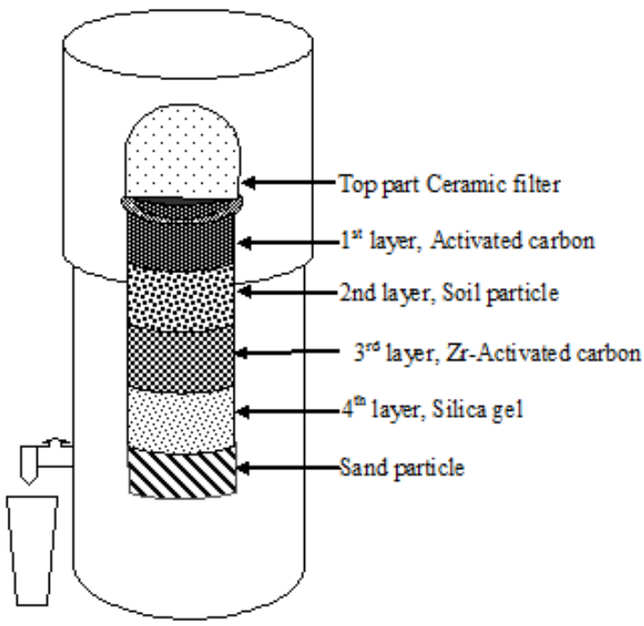

Fig. 4. Constructed filter unit (Yusof et al., 2002 and M. M. Rahman et al., 2011)

2.4 Preparation of media for coliform and total count

Endo broth: About 2.4 g of (7.5 g agar 1.25 g Yeast Extract + 2.5g Peptone) MF Endo Broth mixture was dissolved in 50 mL of distilled water containing 1.0 mL ethanol. Then this was sterilized in an autoclave at 121^0C, 15 psi for 20 minutes. The broth was cooled to room temperature before incubation. This medium was freshly prepared to ensure accurate results used for coliform count.

Exactly 1.25 g of tryptone was added to 50 mL of deionized water and to it was added 3.75 g agar. The mixture was then gently heated. The pH was adjusted to 8.6 to 9.0 and then 1.25 g of glucose was added and then autoclaved. Yeast and mould count was then done.

Appropriate volume of mixture was filtered through a sterile 47-mm, 0.45µm, grid membrane filter (cellulose nitrate membrane filter), under partial vacuum. The funnel was rinsed with three 20 to 30ml portions of sterile dilution water. An exact amount of 100 ml of water sample was poured through the funnel. If the water was heavily polluted, the water sample will be diluted using a dilution bottle 3 to 5 times dilution with 90 ml sterile dilution water. The filter was placed on the agar in the petri dish. Dishes were placed in close fitting box containing moistened paper towels. Incubation at 44.5± 0.5°C for 24 hours was done if using the EMB Agar medium. Duplicate plates may be incubated for other time and temperature conditions as desired. Membrane filter technique is shown in Figure 5.

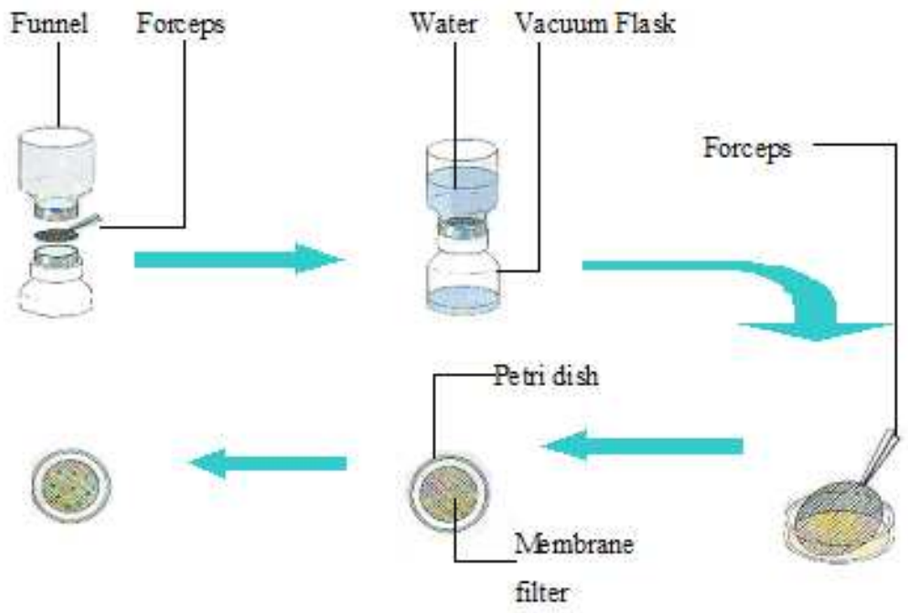

Fig. 5. Membrane filter technique

2.5 *E. coli* (faecal)

Colonies on membrane filters were counted by viable count or using a stereoscopic microscope at 10 to 15 x magnification. Preferably, the petri dish was placed on the microscope stage slanted at 45^0 and light source was adjusted vertically to the colonies. Optimal colony density per filter is 20 to 200. If colonies are small and there is no crowding, a higher limit is acceptable.

All colonies were counted on the membrane when there were 1 to 2 or fewer, colonies per square. For 3 to 10 colonies per square count 10 squares and obtain average count per square. For 10 to 20 colonies per square count 5 square and obtain average count per square. Multiply average count per square by 100 times reciprocal of the dilution to give colonies per millilitre. If there are more than 20 colonies per square, record count as >2000 times the reciprocal of the dilution. Report averaged counts as estimated colony-forming units (CFU). Make estimated counts only when there are discrete, separated colonies without spreaders

2.6 Fungi – Membrane filter method

Sample preparation: Each plate was marked with a sample number, dilution, date, and any other necessary information before examination. Duplicate plates were prepared for each volume of sample or dilution examined. All samples were thoroughly mixed or dilutions done by rapidly making about 25 complete up-and-down movements. Optionally, the use of a mechanical shaker to shake samples or dilution blanks for 15 second would be useful.

Filtration: Appropriate volumes of well shaken samples or dilution were filtered in triplicates, through membrane filter with a pore diameter of 0.45 or 0.80µm. The filters were then transferred and incubated at 15°C for 5 days in a humid atmosphere. Alternatively, incubate-ion can also be performed at 20°C for 3 days, or longer depending on the fungi

present. Using a binocular dissecting microscope at a magnification of 10×, all colonies on each selected plate were then counted. If counting must be delayed temporarily, plates are held at 4°C for not longer than 24 hours. Ideal plates should have 20 to 80 colonies per filter.

2.7 Total count of bacteria – Membrane filter method

Appropriate volume of water samples were filtered through a sterile 47-mm, 0.45µm, grid membrane filter, under partial vacuum. The funnel was rinsed with three 20 to 30ml portions of sterile dilution water. The 100 ml of water sample was poured into the funnel. Dilution is required if the water was heavily polluted, and done with a dilution bottle 3 to 5 times dilution with 90 ml sterile dilution water. The filter was placed on the agar in the petri dish. The dishes were later placed in close fitting box containing moistened paper towels and incubated at 35± 0.5°C for 24 hours if the nutrient agar medium was used. Duplicate plates may be incubated for other time and temperature conditions as desired.

Counting the colonies was done on membrane filters by viable count or using a stereoscopic microscope at 10 to 15x magnification. Preferably, the petri dish was placed on a microscope stage slanted at 45^{o} and the light source adjusted vertical to the colonies. Optimal colony density per filter is 20 to 200. If the colonies are small and there is no crowding, a higher limit is acceptable.

Counting was done on all colonies on the membrane when there are 1 to 2 or fewer, colonies per square. For 3 to 10 colonies per square count 10 squares an average count per square was obtained. For 10 to 20 colonies per square, counting was done on 5 squares and an average count per square obtained. Multiplying the average count per square by 100 times reciprocal of the dilution will give colonies per millilitre. If there are more than 20 colonies per square, the count was recorded as >2000 times the reciprocal of the dilution. The average count was reported as estimated colony-forming units. Estimated counts are made only when there are discrete, separated colonies without spreaders.

3. Discussion

Environmentally polluted water samples were taken from various sources such as rivers, columns, tube well, sea and water falls. This naturally polluted water was filtered continuously through the filtering column for 10 days. The results of coliform and TC in raw and treated shown in Table 1 and Table 2. Coliform count is the most important microbiological count for drinking water. Coliforms are quantified to assess water treatment effectiveness and the integrity of the distribution system. They are also used as a screening test for recent feacal contamination. Treatment that provides coliform-free water should also reduce the pathogens to a minimal level. A major shortcoming is that coliforms under certain circumstances may proliferate in the bio-films of water distribution systems, clouding their use as an indicator of external contamination.

From the results in Table 1 it was observed that all raw water samples contain coliform except raw water from locations 10, 13 and 16. The sample taken from location 16 was observed to contain uncountable coliforms but treated water using filter column contained no coliforms. Therefore, it could be confirmed that the filtering column has produced coliform free water and safe for drinking. From Table 2, it was found that the total count includes three types of microorganisms; bacteria, yeasts and moulds which presence in the

raw water samples. Filtrate water supplies were selected at random from the stock. After 24 and 72 hours of observation the results showed that the yeasts and moulds could be distinguished from each other. The 24 hour observation could not distinguish between the yeast and mould but with the 72 hours observation yeast and mould were detected.

Sample ID	WHO Std.	Raw Water	Treated Water
1	0/100 ml	02	0
3	0/100 ml	01	0
6	0/100 ml	01	0
7	0/100 ml	20	0
8	0/100 ml	01	0
9	0/100 ml	23	0
10	0/100 ml	0	0
11	0/100 ml	11	0
12	0/100 ml	01	0
13	0/100 ml	0	0
14	0/100 ml	04	0
15	0/100 ml	25	0
16	0/100 ml	Uncountable	0

Table 1. Coliform count in the raw and treated water samples

Sample ID	WHO Std	Raw water/10 ml		Treated water/10 ml	
		24 hours	72 hours	24 hours	72 hours
1	<25/10mL	28	36/14	09	11/1
3	<25/10mL	38	40/38		
6	<25/10mL	02	4/1		
7	<25/10mL	06	20/2		
8	<25/10mL	25	30/18	13	18/12
9	<25/10mL	21	32/6		
10	<25/10mL	15	15/3		
11	<25/10mL	70	78/12	11	12
12	<25/10mL	Uncountable	/6	09	10/9
13	<25/10mL	0	13/3		
14	<25/10mL	18	22/88		
15	<25/10mL	Uncountable	/25	02	12/3
16	<25/10mL	08	8/8		
utm tap	<25/10mL			00	00

Table 2. Total count in raw and treated water samples

4. Conclusion

A newly fabricated filter unit as shown in Figure 4 has been found to be significantly improved for its removal capability of microbiological hazardous materials from the filtered water. It provides a safe drinking water in the point of view of microbiological, toxicity, softening hardness and pH value. All kinds of parameters for drinking water have fulfilled the requirements as provided by this water filter unit.

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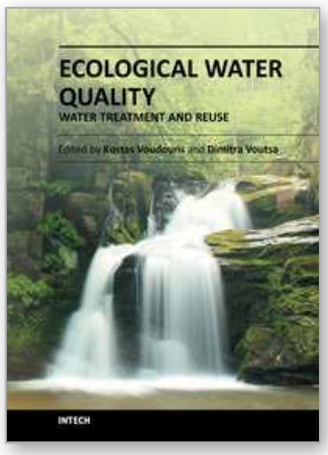

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