ORIGINAL RESEARCH

CYCLICAL GROWTHS OF CONTAMINANTS IN DRINKING WATER PACKAGED IN POLYTHENE BAG.

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ABSTRACT

The survival kinetics of contaminants in drinking water packaged in polythene pouches commonly called "pure water" was evaluated using Agar dilution method, with the pH changes being determined concomitantiy.

Contaminants isolated from the different brands studied were many and highly varied. They included *Bacillus* species, 70.0%; *Escherichia coli*, 8.2%; *Klebsiella aerogenes*, 11.8%;

Pseudomoncis species, 7%; Staphylococci, 3.0%. No *Salmonella* or *Shigella* species was found. All the contaminants were found in small numbers of 5-200cfu/ml due possibly to survival in poorly nutritive medium and also suggestive of extrinsic contamination.

When stored at. 8°C in the refrigerator, those packaged water produced significantly ($P<0.05$) lower viable counts of contaminants per sampling period compared to the ones stored at 25°C or 37°C, The pH changes were equally significant ($P<0.05$) and followed similar trend with viable counts.

For each microorganism, survival was highest on the 10-12th day of sampling, thereafter decreasing progressively towards zero, and slowly rising again. This form of cyclical growth pattern has been attributed to marginal survival of these contaminants on the debris of dead relatives, since drinking water is poorly nutritive, and can only favours the survival of non-exacting and saprophytic organisms.

The results of this study has condemnly exposed the inadequacies in production and the poor quality disposition of packaged water stored in plastic pouches. If the issues of leaching of chemical extractives from the polythene bags are well expounded in addition to the gross contamination of the water so obtained in this study, then the total quality picture of this packaged water is that which is totally unfit for consumption.

This situation is outrightly unacceptable as it portends grave danger to the public health of the consumers. When one considers the caliber of people who are at serious risk, the sick/ children/ elderly and even the so called healthy ones/ it demands stringent controls on the production, distribution and even sale of these products.

INTRODUCTION:

The availability of drinking water in polythene pouches popularly called "Pure water" has assumed an explosive dimension in recent years in Nigeria. Apart from the environmental hazards created by the wide spread littering with discarded polythene bags/ the quality of the water leaves much to be desired. The seemingly simplistic mode of production using locally fabricated equipment/ couple with high profitability/ has attracted many individuals into this business. Infact, its production has been dubbed "a poverty alleviation scheme", possibly in recognition of the substandard products, which have been associated with this venture.

Generally, most of the products obtained from this scheme are of very poor quality; Even though WHO and NAFDAC (National Agency for Food/ Drugs and Administration Control) have provided acceptable limit guides; a lot of these products fail to meet them. Chemically most of these water have been found to contain substances such as asbestos, iron, nitrites, lead and other heavy metals all of which are injurious to health (1/2).

The microbiological quality of drinking water is of paramount importance, even when the chemical components have been maintained within acceptable limits (3). Hence a maximum level guide (MLG) of 100cfu/ml has been introduced by NAFDAC. However/ the US Environmental Protection Agency (EPA) has set MLG of 500cfu/ml for drinking water (4). 'This number should exclude all pathogens and opportunistic pathogens/ which can cause infection in humans (5). In this regard/ indicator organisms for faecal contamination e.g. *E. coli*, and other coliforms like *Salmonella* species/ *Shigella* spp should be completely excluded (6,7). Other pathogens such as Staph

aureus, Pseudomonas spp/ Klebsiella spp should equally be absent (8)/ whilst parasites including Entameba histolytica, worms, Giardia lambia should also be excluded (9/10).

Apart from the incidence of microbial and chemical contaminations, which have been widely reported (8/9,10/11,12), there is a possible threat of the release of chemical extractives from the many types of polythene bags used as a primary packaging material. Such leached compound can pose serious chemical hazard to the body, and can also modify the environment of such water to favour enhanced growth of chanced contaminants.

This study is aimed at evaluating the survival kinetics of some microbial contaminants of drinking water in plastic pouches under different storage conditions. This is with a view to predicting their survival profiles/ and possible changes in other water qualities. The results so obtained will be helpful in public health control measures as well as reinforcing the need for quality controls and monitoring of production of these packaged water products.

MATERIALS AND METHODS:

One hundred (100) polythene pouches of drinking water products from 10 producers were studied for microbial contamination and survival at various storage temperatures of 8°C, 25°C and 37°C respectively.

For each product sample, 1 ml aliquot was taken and made up to 10ml with 7ml of sterile water, 1m1 of 3% Tween 80 and 1m1 of 1.8% sodium thiosulphate to produce a 1 in 10 dilution. Sodium thiosulphate was added to inactivate any residual chlorine/ while Tween 80 removed other inhibiting chemical disinfectants. Sampling of the water was carried out daily for 30 days. In order to study the growth of the contaminants in the water samples/ 1 ml of the line 10 dilution .was transferred into 19ml of molten Tryptone soya agar (Oxoid), mixed properly and poured into a sterile petri-dish. The agar was allowed to set, and thenincubated at 37°C for 24h. Formed colonies were counted using New Brunswick colony counter. The isolated colonies were also identified using microbiological methods (9).

For every sampling time, the pH of the sampled volume was also determined. A plot of number of survivors versus time/ and also pH versus time were obtained at temperatures 8, 25 and 37°C respectively.

RESULTS AND DISCUSSION:

Drinking water product is not expected to be sterile, nonetheless, water products devoid of microbial contaminants should be the desired choice, and target. All the water samples studied were contaminated; ranging from Staph. aureus, Micrococcus species. Bacillus subtilis, B. cereus, B. licheniforms, Escherichia coli, Pseudomonas aeruginosa, Klebsiella aerogenes, to Streptococcus species, and Chromobacter species

Microbial distribution showed that 60% of the water contained Bacillus species, 20% of samples contain E. coli and Klebslella aerogenes respectively and 7% Pseudomonas species. Staphylococci were present in 3% of the total samples. No Salmonella species was isolated. Other workers have isolated pathogenic fungi, animal parasites and viruses (5,6). Instances of typhoid infections traceable to the consumption of these water-based products have equally been reported (7,8).

All the isolates occurred in very low numbers of 5 - 200cfu/ml, an indication of possible extrinsic contamination, and due to growth in poorly nutritive medium. The high incidence of contamination of the drinking water pouches found in this study may be due to a combination of several factors; the most significant of which is non-adherence to Good Manufacturing Practice (4). Others include poor state of the manufacturing environment, dirty filling equipment, contaminated packaging materials, unhygienic handling of the products, and lack of microbiological in-house controls (4,10,11).

The high incidence of Bacillus subtilis including the gram-positive isolates which are airborne contaminants is most probably due to the use of untreated air (1 2). Most of the production outfits for these water products have not been built to specification. The production and filling areas equally lack facilities to enhance hygiene and guarantee a controlled environment. Products made in such environments are bound to be contaminated. The polythene bags are generally not sanitized prior to filling with treated water,

and the operatives are in various degrees of filthiness. It is therefore not surprising that most of these contaminants may also have been introduced during manufacture, handling and filling of the bags with purified water. In most factories there is overcrowding of the production floor with operatives involved in making the bags and filling with water. The consequence of such is the generation of microorganisms and even other particulates which eventually then contaminate the water.

The process of filling the bags entails the formation of the bags from polythene film made into sheets, which had hitherto been printed with the packaged information. The bags are then filled with purified water and sealed using a hot plate. A survey of most producers showed that they carry out these processes under very filthy environmental conditions. Most of the filling areas and equipment are handicapped by a number of designing faults and products made in these conditions should be expected to be contaminated with aerial microorganisms such as the aerobic spore bearers and Gram positive cocci, whereas products made in clean rooms have lower incidence of contamination (13). Hence the isolation of Bacillus subtilis. Streptococcus spp which are airborne contaminants is a serious indictment of the unhygienic state of the factory environment.

In most factories, it was also found that the operatives are highly deficient in basic hygienic protocols, in addition to current techniques of handling and filling the products. Besides, the absence of protective clothing and face mask, these operatives generate much of the contaminants for the water product. Therefore, contaminants including E. coli, Strept.viridans, Sfaph. aureus may have been associated with touch contaminations (14). Pseudomonas spp and Klebsiella spp may have originated from environmental sources such as sinks, dirty cleaning equipments, rilling equipment and other wet sites (15). Though these organisms could also have originated from the processed water through improper treatment, the odoriferous nature of the products could be traced to the activities of microbial contaminants particularly the saprobes (16).

It was observed that there were changes in the pH of the water during the period of storage (Figs, 1.0, 2.0). The pH changes of the waters stored at 37^{0C} over those at 25^{0C} were significant ($P<O.05$). So also were the pH changes of waters stored at 8^{0C}. These changes may be attributed to the growth and activities of the contaminants in the water products as well possible leaching of extractives from the bag. However, the increases observed in the pH with time of sterile water stored in the plastic bags is an indication of leaching of substances from the polyethylene bag into the water (Fig. 3.0). This is because since sterile water contains no microorganisms, the only contributor to such pH changes will reside in the polyethylene bag.

Polyethylene plastics have been known to leach materials into the products stored in them (17). It is therefore, possible that additives such as fillers, colorants, and plasticizers may be released into the drinking water by the polyethylene bag. The effect of such extractives on health may depend on the magnitude and toxicity of such materials (18).

Generally, it was found that water stored at 8^{0C} produced significantly ($P<O.05$) lower counts of the contaminants compared to the ones stored at 37^{0C} or 25^{0C} (Figs. 4.0 - 8.0), a similar trend which was also observed with pH changes at these temperatures. This is in conformity with established fact that storage of products at lower temperature less than 10^{0C} hinders growths and proliferation of microorganisms and enhances stability of any product not sensitive to cold. One peculiar observation of the growth of the contaminants in the water samples was-the peak and trough contours manifested by each bacterial contaminant studied.

Microbial contaminants including Bacillus subtilis, Escherichia coil, Klebsiella aerogenous, and Pseudomonas aeruginosa which could have been introduced extrinsically all showed similar pattern of growth profiles (Figs 4.0 - 7.0). For these organisms, growth progressed from few colonies, rose to a peak, then declined to zero. With the exception of Staphylococcus aureus (Fig. 8.0) which may have been introduced intrinsically due to poor water treatment and purification techniques, the original count of 100 cfu/ml increased slightly to about 140 cfu/ml, and then decreasing finally towards zero.

For each microbial contaminants studied at a

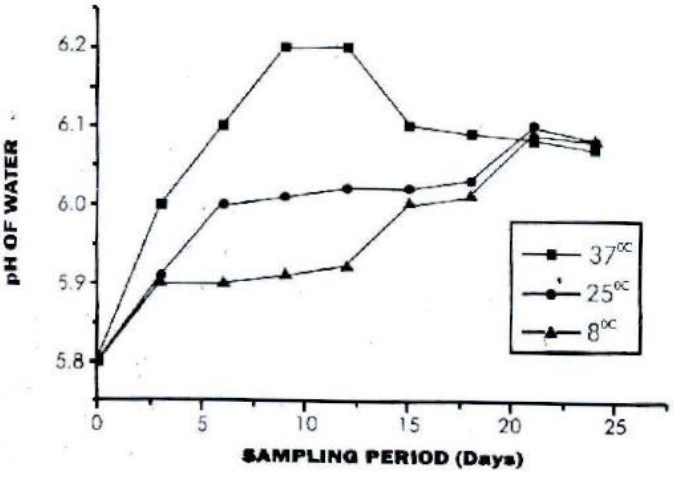
particular storage temperature, it was generqlly observed that the highest number of survivors were recorded on the 10th - 12th day of sampling, then decreased progressively towards zero, thereafter, rising again towards the 4th week (Figs 4.0 - 9.0). All contaminants actually grew irrespective of the fact that their original numbers were low. Since drinking water contains no preservatives and also having poor nutritive quality, it is only non-exacting organisms that can survive in such products if poorly prepared. The cyclical pattern of growth seen here may be due to marginal survival on the debris of dead organisms hitherto present in the packaged water. This is a characteristic feature of post kinetic phase of decline, the so-called phase of survival. It is important to note that potable water should be produced with the quality target of containing nil microorganisms, since it has now been established that they can actually grow over stipulated limit when stored at ambients temperatures. In this regard therefore, all standards which are geared towards sustaining and enhancing Good Manufacturing Practice should be vigorously pursued. Such areas as adequate water treatment and purification, suitable production and filling environment, and measures to limit contamination of packaging materials. The contributions of the operatives in terms of being dedicated and conscientious in achieving the desired quality of the table water needs to adequately motivated.

CONCLUSION:

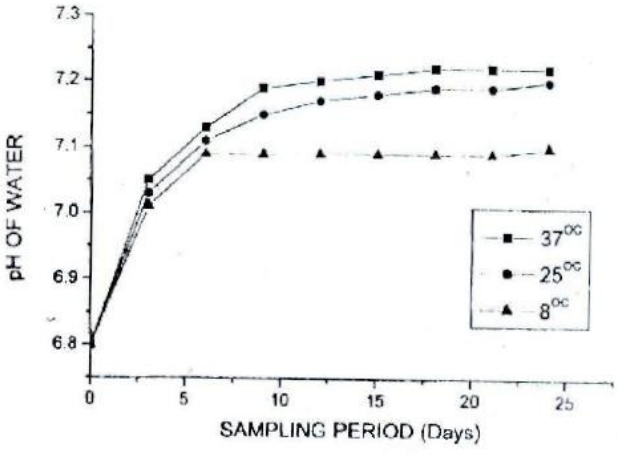
It is thus obvious that drinking water when stored for a period of time in a particular container system can generate greater tendency for physical, chemical and microbiological interactions, leading to higher chance of being unfit for consumption. The product of such interactions will lead to an altered environment in favour of the growth of contaminations or generate obnoxious characteristics unsuitable for consumption. For plastic bags which are prone to permeability to a lot of materials, and leaching of components into the water, these drawbacks will predictably lead to instability of the packaged water. These effects if they are highly exaggerated can precipitate undesirable consequences on the public health of its consumers. There is therefore, an urgent need to seriously monitor production and quality of the water products, which have become a common-place product in the country.

The prevalence of gastroenteritis and other water-borne infections are clear indications of this suspicions. Such infections such as diarrhea, cholera and other gastroenteritis which have high mortalities particularly in the young and elderly; and is a constant reminder of the need to produce good quality water.

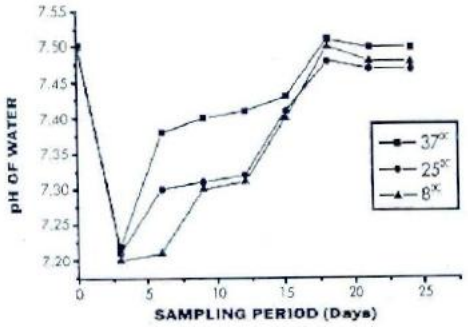
VARIATIONS IN pH OF PACKAGED WATER STORED IN POLYTHENE BAGS



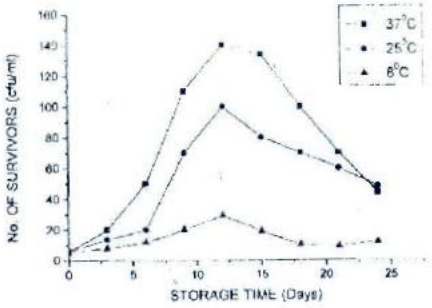
VARIATIONS IN pH OF PACKAGED WATER STORED IN POLYTHENE BOTTLES



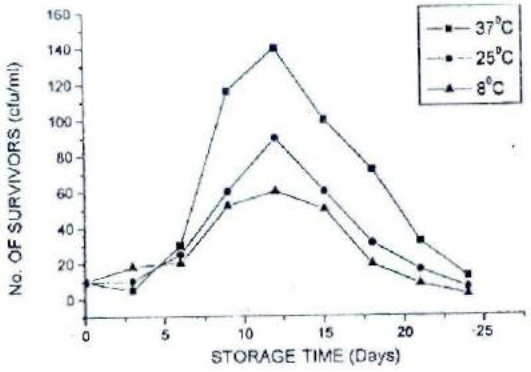
CHANGES IN pH OF STERILE WATER STORED IN POLYTHENE BAGS



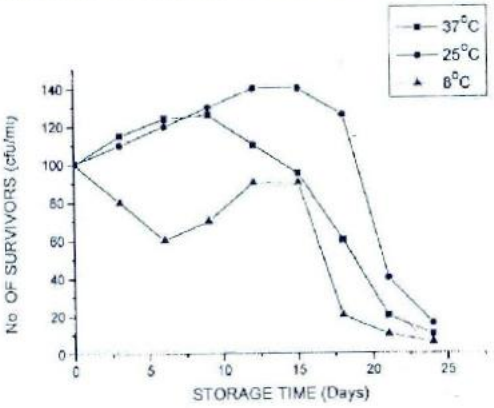
VARIATIONS IN GROWTH OF *PSEUDOMONAS AERUGINOSA* IN PACKAGED WATER STORED IN POLYTHENE BAGS.



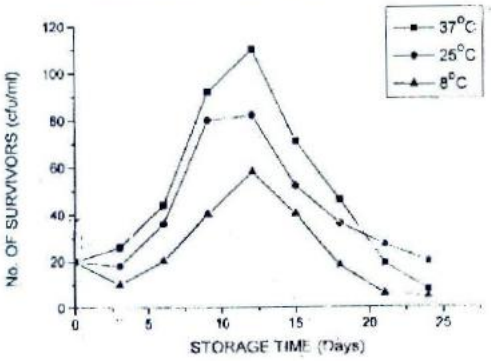
VARIATIONS IN GROWTH OF *BACILLUS SUBTILIS* IN PACKAGED WATER STORED IN POLYTHENE BAGS



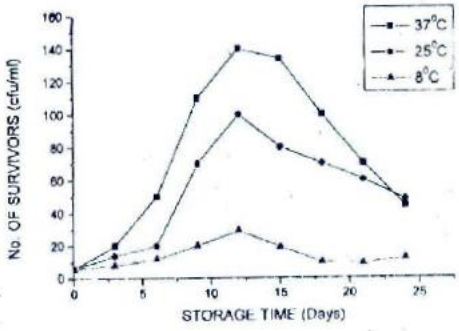
VARIATIONS IN GROWTH OF *ESCHERICHIA COLI* IN PACKAGED WATER STORED IN POLYTHENE BAGS



VARIATIONS IN GROWTH OF *KLEBSIELLA AEROGENES* IN PACKAGED WATERS STORED IN POLYTHENE BAGS



VARIATIONS IN GROWTH OF *STAPHYLOCOCCUS AUREUS* IN PACKAGED WATER STORED IN POLYTHENE BAGS



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