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Message from the Editor-in-Chief

I am pleased to announce second volume and first issue of The Online Science and Technology Journal (TOJSAT) in 2012. In this issue, our journal diffuses its interdisciplinary perspective through various researches in science and technology field. In order to share valuable researches from different fields, this issue sheds a light to open discussion in the academic platform.

This issue represents dynamic development of the journal and underlines how it is strategically explore its academic performance. I would like to thank to editorial board, reviewers and the researchers for their valuable contributions to the journal and this volume and issue.

01 January 2012

Prof. Dr. Aytekin İŞMAN

Editor-in-Chief of TOJSAT

Message from the Editor

Dear Readers,

Welcome to the second volume of The Online Science and Technology Journal which covers the all area of Science and Technology. Globally, this century is called as information era and therefore scientific and technological developments will be most important aspects of the papers.

The Second International Science and Technology Conference (ISTE-C) hold in December 2011 in Istanbul where two continents meet. The previous conference was hold in Famagusta-North Cyprus. The Online Journal of Science and Technology will provide a platform for national and international academics to share their innovative and relevant research efforts.

The journal, which covers all scientific and technological subjects, will be published 4 times a year. Selected papers of the online science and technology conferences will be published in the journal. The main goal of this journal is to be indexed by science citation or social science citation indexes.

01 January 2012

Prof. Dr. M. Şahin DÜNDAR

Editor, TOJSAT

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A CFD MODEL FOR SIMULATING URBAN FLOW IN COMPLEX MORPHOLOGICAL STREET NETWORK

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Abstract: The present study considers an investigations of urban flow in complex morphological street network that is coincidentally similar to ancient Algeria city namely Ghardaïa. This study shows how one can apply a CFD model to simulate the air flow behavior in this urban area.
The computational fluid dynamics software, Fluent 6.3.26 is employed to determine velocity field traversing the streets. Information on the layouts of buildings in the selected area is contained in Google earth.
The AC3D 6.5.28, with its programming facility, has been used to extract the geometry of each building polygon under research. These are then cleaned by Materialise Magics and input into Gambit 2.4.6 the Fluent's mesh generation software to construct the simulations.
Finally, a steady-state simulation results are drawn from the velocity field profile.

Keywords: *CFD, Complex urban fabrics, Buildings, Urban Flow*

1. Introduction

There is a need to properly develop the application of Computational Fluid Dynamics (CFD) methods in support of air quality studies. CFD models are emerging as a promising technology for such assessments, in part due to the advancing power of computational hardware and software (Neophytou, 2005). CFD, simulations have the potential to yield more accurate solutions than other methodologies because it is a solution of the fundamental physics equations and includes the effects of detailed three-dimensional geometry and local environmental conditions. The results of CFD simulations can both be directly used to better understand specific case studies as well as be used to support the development of better-simplified algorithms that may be generally applied (Jal, 2003).

As the 3D solid model of the geometry was not available a captured Google Earth image of the fabric structure was used as a backdrop in the AC3D software, supplied as a utility to Fluent code software. The outlines of the buildings were then traced to create polygons, which were then extruded to produce the individual buildings.

The urban configuration being complex Gambit has been used to develop a specific un-structured meshing grid which takes into account the geometry of the buildings and the surroundings. Special attention was paid to the precise modeling and high grid resolution of the whole built environment

In this work we propose an approach – to model cities as obstacles characterized by an overall city form, building area density and street configuration, morphological characteristics that affect the structure and the intensity of wind entering, leaving, and flowing around and above cities. Studies of the impact of urban morphology on urban airflow are rare, although, ironically, this approach to understanding urban design was promoted by Vitruvius in the first century BC (Vitruvius, 1960). Skote et al. (2005) qualitatively investigated the flow pattern in round compact cities with one or two larger main streets and reinitiated by Hang et al.(2009) for two simple city forms such as a round

city form and a rectangular form each with a single or two intersecting main streets that dominate the otherwise compact city form.
Then the potential of CFD for prediction of wind speeds around a complicated urban environment and a complex fabric structures is investigated in this work.
The city of Ghardaïa, in the M'zab valley, may be taken as the case study with its dense fabric which demonstrates the ideas of building cool, shaded, airy structures in a hot dry climate.
This specific built environment with its complex and organic geometry will help to demonstrate the ability of CFD techniques to tackle the most complicated situations.

2.Mathematical model

2.1 Physical model

The commercial CFD code Fluent 6.3.26 (Fluent Ansys Inc., 2006) was used in this research. The 3D steady RANS equations are solved in combination with the realizable k-ε turbulence model. The realizable k-ε turbulence model is chosen because of its general good performance for wind flow around buildings (Franke et al., 2004). Pressure-velocity coupling is taken care of by the SIMPLE algorithm, pressure interpolation is standard and second order discretisation schemes are used for both the convection terms and the viscous terms of the governing equations.

All the objects that will be included in the simulation must be prepared in a step-by-step procedure starting with the capture of the area of investigation using Google Earth 4.2 Professional (Google Earth, 2007). The image is then imported in the AC3D software where the outlines of the buildings were traced and then extruded to produce the individual buildings (AC3D, 2009). The dimensions and the geometry of the objects are scaled to fit the real size of the configuration.

Fig.1 Ghardaia Complex Urban Morphology

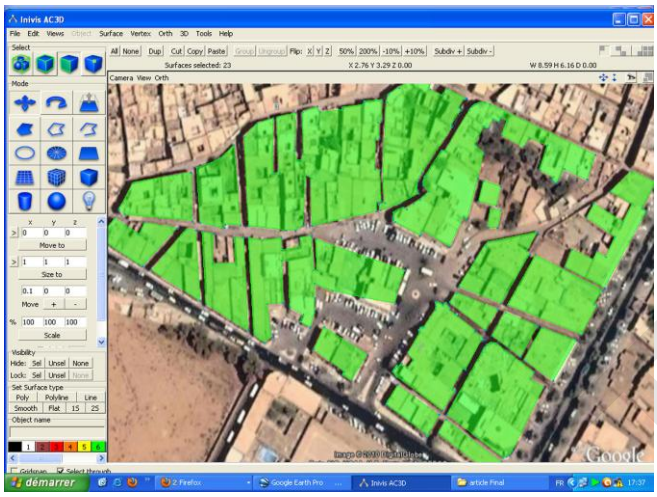

Fig.2 Tracing the Complex Urban Morphology Using AC3D

It is well known that the CAD files created or generated by CAD packages do not necessarily guarantee that the facets are consistent with each other in respect of inward- and outward-looking direction or define closed volumes. Fluent requires that facets should have a direction sense in order that it can know on which side is the fluid and on which the solid; and of course facets which share an edge should be in agreement on the matter. In most cases there a need to fix and repair the overlapping surfaces in order to simulate complicated configurations. In the present case Magics 12.0 software (Materialise Magics, 2007) has been used to correct the file containing the urban geometry of the case study as shown in figures 3 and 4.

Fig.3 Defective 3D Urban Configuration Tracing Result Using AC3D

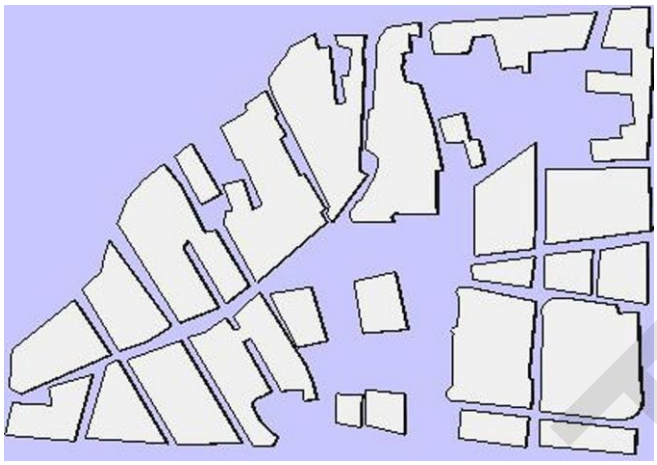

Fig.4 Repaired 3D Urban Configuration Tracing Result Using Magics

2.2 Numerical simulation

The governing equations of incompressible turbulent wind flow around buildings are continuity and the Reynolds-averaged Navier–Stokes equations, expressed as follows:

$$\frac{\partial U_i}{\partial x_i} = 0 \tag{1}$$

$$\frac{\partial U_i U_j}{\partial x_i} = -\frac{\partial P}{\partial x_i} + B_i + \rho \sum_j \left[\nu_L \left(\frac{\partial U_i}{\partial x_j} + \frac{\partial U_j}{\partial x_j} \right) - \overline{u_i u_j} \right] \tag{2}$$

1) Turbulence model

The turbulent stresses appeared in equation (2), use the eddy-viscosity concept to determine the Reynolds stresses from

$$-\rho \overline{u_j u_i} = \mu_\tau \left[\frac{\partial U_i}{\partial x_j} + \frac{\partial U_j}{\partial x_i} \right] - \rho K \frac{2 d_{ij}}{3} \tag{3}$$

Where

$$\mu_\tau = C \rho V_S L_S \tag{4}$$

And where C is an empirical constant, and V_s and L_s are turbulence velocity and length scales which characterize the LARGE-SCALE turbulent motion.

A reasonable value of the kinematic eddy viscosity ν_t can be estimated by taking as $C = 0.01$ and V_S as a typical mean-flow velocity and the length scale *Ls* as ~10% of the flow width

$$\nu_t = \frac{\mu_\tau}{\rho} \quad (5)$$

The standard high-Re form of the *k-ε* model employs the following turbulence transport equations:

$$\rho \frac{\partial}{\partial x_i}\left[U_i K - \frac{\nu_t}{\mathrm{Pr}_K}\frac{\partial K}{\partial x_i}\right] = \rho\left(P_K + \Gamma_b - \varepsilon\right) \quad (6)$$

$$\rho \frac{\partial}{\partial x_i}\left[U_i \dot{o} - \frac{\nu_t}{\mathrm{Pr}_{\dot{o}}}\frac{\partial \dot{o}}{\partial x_i}\right] = \rho \frac{\varepsilon}{K}\left(C_{1e}P_K + C_{3e}\Gamma_b - C_{2e}\varepsilon\right) \quad (7)$$

The kinematic turbulent (or eddy) viscosity is given by:

$$\nu_t = C_\mu C_d \frac{K^2}{\varepsilon} \quad (8)$$

The model constants are: $C_\mu = 0.5478$; $C_d = 0.1643$; Pr_K =1.0; Pr_ε =1.314; C_{1e}=*1.44*, C_{2e}=*1.92* and C_{3e}=*1.0*.

The buoyancy production Γ_b is < 0 for stably-stratified layers, so that K is reduced and turbulence damped. For unstably-stratified layers, Γ_b is positive and turbulence is augmented.

The constant C_{3e} has been found to depend on the flow situation. It should be close to zero for stably-stratified flow, and close to 1.0 for unstably-stratified flows.

The default value of C_{3e} is 1.0.

3. Geometry and Solution Domain

Due to the complex geometry of the urban configuration and the large difference between the smallest and the largest length scales in the domain, generating a computational grid with good quality is not straightforward. In order to predict the flow field around a building with acceptable accuracy, the most important thing is to correctly reproduce the characteristics of separating flows near the roof and the walls. Therefore, a fine grid arrangement is required to resolve the flows near the corners. To handle the complex shaped domain of the case study the pre-processor Geometry and Mesh Building Intelligent Toolkit (GAMBIT) (Ansys Gambit, 2004) was used and results in unstructured grids of tetrahedral cells. The total number of cells is 913487-551593-174698-129211 for very fine, fine, medium and coarse meshings respectively. An overall view of the grids from is shown in figure 5 and a more detailed view is given in figure 6 for only one case.

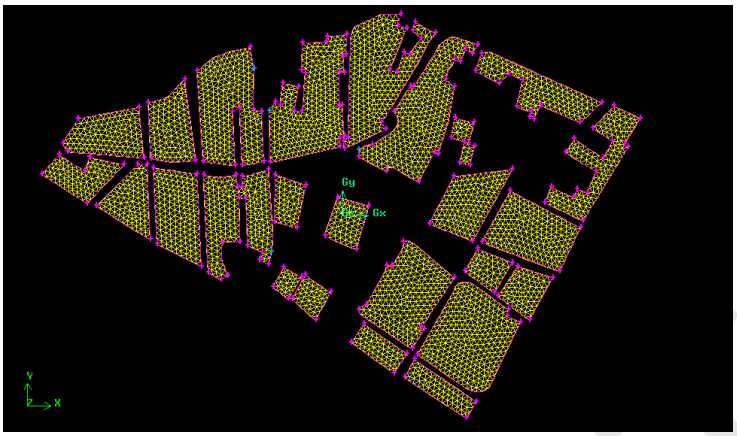

Fig.5 Triangular Paved Mesh on Flow-volume Bottom Face of the Urban Configuration

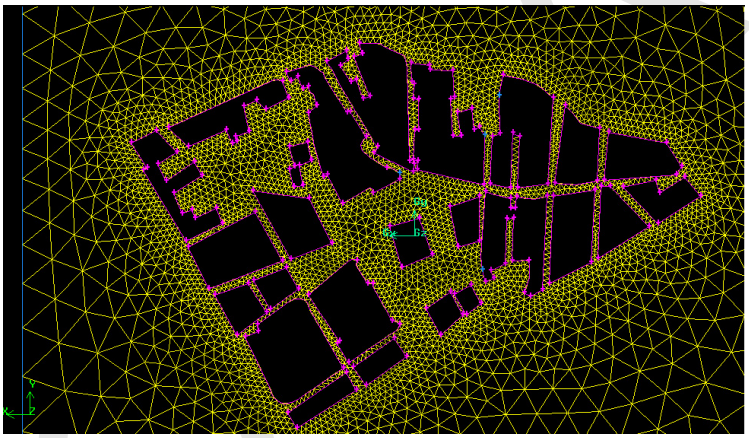

Fig.6 Characteristics of the Tetrahedral Elements Mesh for the Urban Configuration

To allow full control over the grid quality and resolution, the grid is constructed using the control mesh quality size functions to control mesh sizes in the regions adjacent to buildings geometry surfaces.

The size of the domain has been taken as a multiple of the characteristic height of the tallest building which is in this case 18 meters, as recommended by Hall (Hall, 1996) who suggests that the distance between any edge of the domain and the buildings must be at least five times of the characteristic height of the building.
The domain covers the entire area of 350m x 250m, including all the buildings and surrounding areas. The height of 50m from the ground in the vertical direction of the calculation domain provides about 30 m of open space above the tallest building.

4. Boundary conditions

An inflow condition was applied at the leftmost (upwind) side (y-z plane) of the domain as given in Figure 7. An inlet wind speed of 25 m/s has been chosen as representing the average wind conditions in the region. In addition the orientation of the urban configuration has been selected according to the prevailing wind direction. Given that the computational domain is large enough, the boundary conditions for lateral and upper surfaces do not have significant influences on the calculated results around the target building (Mochida et al., 2002; Shirasawa et al., 2003; Yoshie et al., 2005).

A pressure outlet condition was applied at the rightmost (downwind) side (y-z plane) of the domain. The remaining faces of the flow volume are taken as symmetry type expect for the base which has been specified as wall. The values of k-ε were taken as (Robitu et al., 2006).

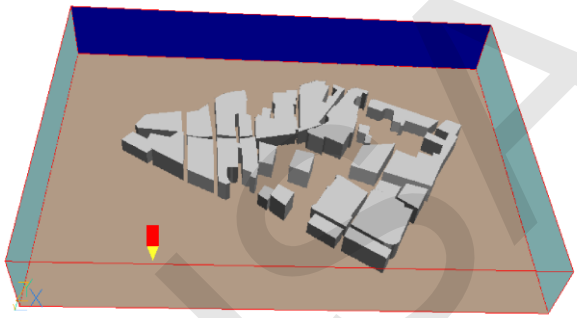

Fig.7 Setup of the 3D urban configuration for simulation

5. Solution of the equations

In Fluent 6.3.26 the pressure-velocity coupling in the incompressible flow is resolved through the SIMPLE algorithm of Patankar and Spalding (Patankar, 1972). The convective and diffusive equations are chosen according to the second order upwind discretization scheme employing a staggered grid. The first-order upwind scheme is not appropriate for all transported quantities, since the spatial gradients of the quantities tend to become diffusive due to a large numerical viscosity. COST also does not recommend the use of first-order methods like the upwind scheme except in initial iterations (Franke et al., 2004).

Calculation needs to be finished after sufficient convergence of the solution. For this purpose, it is important to confirm that the solution does not change by monitoring the variables on specified points or by overlapping the contours among calculation results at different calculation steps. In addition the scaled residuals have been dropped 4 orders of magnitude as suggested by COST (Franke, 2006). A stricter convergence criteria given by relaxation coefficients has been used to resolve the complexity of the case study geometry for the 4 different meshing to achieve the solution of the problem after 78-114-148-189 iterations respectively.

6. Results and discussion

The test of accuracy involved running the same simulation at different resolutions. This involved changing the spacing of the node points in GAMBIT for the creation of the mesh.
The smaller the spacing between node points, the greater the resolution, which means greater accuracy of the model. Ideally one would want the highest resolution possible, but with a higher resolution it takes more processing power to compute the simulation.
Running a simulation at a low resolution may take seconds while at a higher resolution can take hours for the same number of iterations.
To find a good balance between speed and accuracy, this test was run at various resolutions.
More refined grids have been tested and it has been shown that the results remain unchanged.
The comparison between the flux results obtained at outlet area for the last two finer meshes are small and have approximately the same magnitudes 22690 W.

Images were generated to show various views of the velocity fields. The horizontal distribution of airflow at the height of 5 m in the sample area, whose size is 87500 meters square, is shown in Figure 8. At this scale, the shape and arrangement of individual buildings affect the formation of the field of wind.

It is seen from Figures 8 and 9 that the velocity of air flux varies drastically with urban density. In the present case the urban configuration acts as a wind shield reducing the air velocity inside the urban fabric, whereas the maximum velocities are observed at the boundaries of the lot. In large open spaces among buildings, although the wind direction is complicated, the wind speed is relatively low. In addition it is clear that dense and organic forms appear to create a better sheltered outdoor environment.

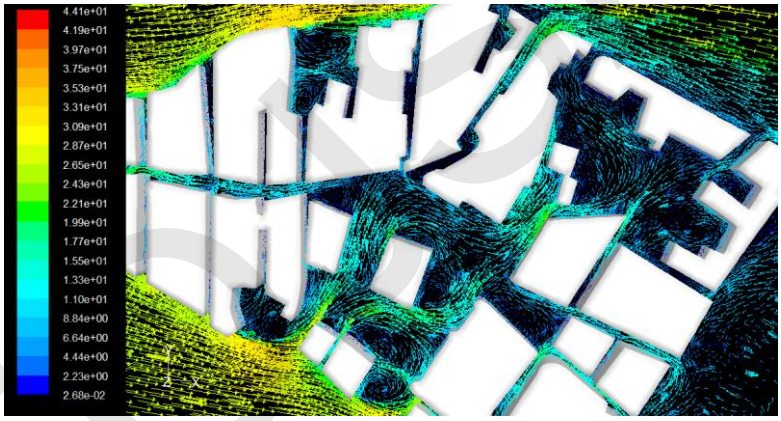

Fig.8 3D view of the horizontal distribution of airflow at the height of 5 m in the sample area

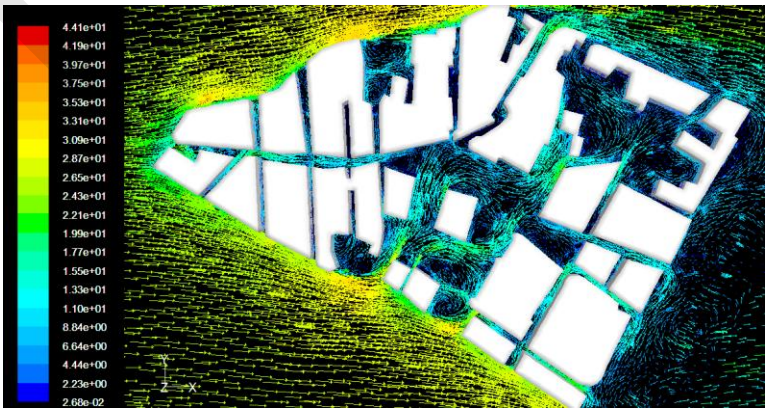

Fig.9 Scaled 3D view of the horizontal distribution of airflow at the height of 5 m in the sample area

Conclusions

Considerable effort has been made in recent years to improve the scientific understanding of air flow phenomena. CFD has been used as a modeling technique requiring long computational times and expensive hardware/software resources. However, recent computer hardware developments have contributed to the spread of CFD modeling, since the speed and memory capacities of PCs are now sufficient for relatively small applications.

However, a large number of research studies have focused on regular new geometric urban configurations overlooking the effects of vernacular old and complex urban fabrics which could be of interest to develop appropriate urban geometries under specific climate conditions.

A numerical study of velocity field in a densely vernacular urban fabric has been undertaken and discussed here. It was found that the velocity distribution can considerably change with the building shape and urban configuration. By using computational fluid dynamics (CFD) useful information can be drawn and used by urban developers, architectural designers and environmental planners to promote natural ventilation, a good measure for reducing energy use in buildings and providing better outdoor air quality. It has been also demonstrated the ability of combining different tools from picture capture, image correction to CFD simulation to assess a natural phenomena.

It should be kept in mind that the main objective of this research is to improve the understanding of the behavior of a system, rather than obtaining results readily comparable with regulatory standards.

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ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF THAI LOCAL FRUIT EXTRACTS: APPLICATION OF A SELECTED FRUIT EXTRACT, *PHYLLANTHUS EMBLICA* LINN. AS A NATURAL PRESERVATIVE IN RAW GROUND PORK DURING REFRIGERATED STORAGE

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Abstract: Crude methanolic extracts of Thai local fruits including *Ardisia polycephala* Wall. (pirangasa), *Elaeocarpus hygrophilus* Kurz. (makoknum), *Limonia acidissima* Linn. (maquid or elephant apple), *Phyllanthus emblica* Linn. (makampom or Indian gooseberry), *Garcinia schomburgkiana* Pierre. (madan) and *Averrhoa carambola* Linn. (mafueng or star fruit) were tested for their antimicrobial and antioxidant activities. Fruit extracts of madan, makampom and makoknum had higher antimicrobial activity, while the extracts of makampom, pirangasa and makoknum had stronger antioxidant activity compared to the others. Then, the extract of makampom (0.25 –2.0%) was used as a natural preservative in raw ground pork during refrigerated storage at 4°C. This extract at 2.0% was the most effective to decrease the number of total viable counts and total *Pseudomonas* in raw ground pork. After 12-day storage at 4°C, total viable counts and total *Pseudomonas* in ground pork samples added with 2.0% makampom extract had low survival rate of 23.27% and 2.06%, respectively. These raw ground pork samples had acceptable appearance with 5.52 pH value. Moreover, addition of 2.0% makampom extract was the most effective to delay lipid oxidation by slowing down the increasing of thiobarbituric reactive substance (TBARS) value of raw ground pork. Then, the chilled raw ground pork added with 2.0% makampom extract was used to produce a seasoned ground pork product. After duo-trio testing, some of 12 taste panels could not detect flavor of this fruit extract in the product.

Keywords: fruit extract, *Phyllanthus emblica*, antimicrobial, antioxidant

INTRODUCTION

Fruit is important as a natural source of antioxidant. Many fruits are rich in some active compounds such as phenolic compounds, vitamins, β-carotene and others which have an important role in antioxidant activity. Some tropical fruits have been reported to have antioxidant activity such as star fruit (*Averrhoa carambola*) (Lim et al., 2007) and Indian gooseberry (*Phyllanthus emblica* or makampom) (Pinsuwan et al., 2007). Epidemiological studies have shown that many phytonutrients in fruits have potentially protective effects against many diseases including cancer, diabetes and cardiovascular diseases caused by oxygen radicals. Phytochemical antioxidants might prevent the oxidative damage (Blasa et al., 2010). In addition, some fruits was reported to have antimicrobial activitiy (Mayachiew and Devahastin , 2008). However, antioxidant and antimicrobial activities of some other Thai local fruits was unknown.

Fruit of *P. emblica* is available in several countries of Southeast Asia. Its extract has been reported to possess antioxidant, antimicrobial, antidiarrheal and spasmolytic activities (Pinsuwan et al., 2007; Mayachiew and Devahastin , 2008; Medmood et al., 2011). Therefore, it is interesting to study the use of *P. emblica* fruit extract as a source of natural preservatives in raw ground pork.

MATERIALS AND METHODS

Microorganisms

Fifteen microbial strains (6 species of bacteria, 4 species of yeasts and 5 species of molds) were used in this study. *Bacillus cereus* DMST 5040, *Escherichia coli* DMST 4212, *Listeria monocytogenes* DMST 11256, *Pseudomonas fluorescens* DMST 20076 and *Salmonella* Typhimurium DMST 0562 were obtained from the culture collection of the Department of Medical Sciences, Ministry of Public Health, Thailand. *Staphylococcus aureus* TISIR 118, *Candida lipolytica* TISTR 5655, *Pichia membranaefaciens* TISTR 5093, *Rhodotorula glutinis* TISTR 5159, *Zygosaccharomyces rouxii* TISTR 5044, *Aspergillus flavus* TISTR 3041, *Aspergillus parasiticus* TISTR 3276, *Aspergillus ochraceus* TISTR 3557, *Fusarium moniliforme* TISTR 3175 and *Rhizopus stolonifer* TISTR 3199 were obtained from the Microbiological Resources Centre for Southeast Asian Region (Bangkok MIRCEN).

Culture preparation

The bacterial cultures (*B. cereus, E. coli, L. monocytogenes, P. fluorescens, S.* Typhimurium and *S. aureus*) were subcultured twice onto Nutrient Agar (NA, Difco) and incubated for 24 h at 37°C, except for *P. fluorescens* incubated at 30°C). Yeast cultures (*C. lipolytica, P. membranaefaciens, R. glutinis* and *Z. rouxii*) were subcultured twice onto Saboraud

Dextrose Agar (SDA, Difco) and incubated at 30°C for 48 h. A loopful of each bacteria and yeast in agar slant was inoculated into NB and SDB, respectively. After incubation, microbial cells were collected by centrifugation at 3000 ×g for 15 min, washed twice and resuspended in 0.1% peptone water. Turbidity was adjusted to match the turbidity of 5 McFarland standard to obtain an inoculum concentration of 10^7 CFU/ml.

To prepare spore suspension of mold, each mold culture was grown in Potato Dextrose Agar (PDA, Difco) at 30°C for 7 days. Conidia were harvested by adding with 5 µl of sterile 0.01% Tween 80 in culture tubes, and the agar surface was scraped. Conidial concentration was adjusted to 10^6 conidia/ml using haemacytometer.

Extraction of Thai local fruits

Six species of Thai local fruits including fruits of *Ardisia polycephala* Wall. (Thai name: pilungasa), *Averrhoa carambola* Linn. (common name: star fruit or Thai name: mafueng), *Elaeocarpus hygrophilus* Kurz. (common name: Spanish plum or Thai name: makoknum), *Garcinia schomburgkiana* Pierre. (Thai name: madan), *Limonia acidissima* Linn. (Thai name: makwit) and *Phyllanthus emblica* Linn. (common name: Indian gooseberry or Thai name: makampom) were purchased at retail in Bangkok, Thailand. These plants were extracted using methanol as a solvent.

To prepare crude methanolic extracts of Thai local fruits, all fruits were washed, cut into small pieces, freeze-dried and powdered. Then, 10 g of each dried fruit were soaked in 100 ml methanol, and shaked at 200 rpm for 24 h at ambient temperature. The mixtures were then filtered. The filtrates were evaporated using vacuum rotary evaporator (BÜCHI Rotavapor R-200/205, Model R205V800, Switzerland), and air dried. Stock solutions of crude methanolic extracts were prepared by diluting 0.2 g dried extracts with 1 ml of 10% dimethyl sulphoxide (DMSO) solution.

Screening of fruit extracts using disk diffusion test

The disk diffusion test was performed using the standard procedure as described by Jorgensen et al. (1999). The inoculum suspension (100 µl) of each microbial strain was added and swabbed onto the surface of Mueller-Hinton Agar (MHA, Difco) for bacteria, SDA for yeasts and PDA for molds. Sterile 6-mm filter paper discs (Whatman) were aseptically placed on MHA, SDA and PDA surfaces. Crude methanolic extracts (15 µl) were immediately added to discs. A 15-µl aliquot of 10% DMSO was also added to a sterile paper disc as a negative control. The plates were incubated at 37°C for 24 h for bacteria, except for *P. fluorescens* incubated at 30°C and at 30°C for 72 h for yeasts and molds. Antimicrobial activity was evaluated by measuring inhibition zone diameters. The experiment was done in triplicate.

Determination of the minimum inhibitory concentrations using agar dilution test

The minimum inhibitory concentrations (MICs) of all fruit extracts against 15 microbial strains were determined using an agar dilution method (Collins et al., 2001). Each fruit extract was diluted to obtain 5 concentrations (200, 140, 102.4, 51.2 and 25.6 mg/ml). Then, 19 ml appropriate agar medium (MHA, SDA or PDA) was poured into each petridish to obtain final concentrations (10, 7, 5.12, 2.56 and 1.28 µg/ml). Negative control was performed using distilled water. Penicillin G (at final concentration of 2000, 1000, 500, 250, 125, 62.5 and 31.25 unit/ml) and fluconazole (at final concentration of 0.1, 0.08, 0.04, 0.02 and 0.01 mg/ml) were tested as positive controls. After surface drying, a loopful of each microbial suspension (spore suspension for molds) was inoculated at the centre of each agar plate. After incubation at appropriate temperature and time, the growth of each microbial strains at different concentrations of fruit extracts was recorded. The lowest concentration of a fruit extract that completely inhibited visible growth of each microbial strain was recorded as the MIC.

Determination of antioxidant activity

Free radical scavenging activity assay (DPPH method)

The free radical scavenging activity of fruit extracts was measured according to the method of Brand-Williams (1995). Each stock solution of extracts and α–tocopherol (10,000 µg/ml, a positive control) were prepared and diluted to the concentrations of 1,000, 500, 100, 10, and 1 µg/ml in methanol. Seventy five microliter of each diluted extract at five concentrations were added to 2.925 ml of a 0.025 g/L DPPH (2, 2-diphenyl-1-picrylhydrazyl) solution in methanol. The reaction mixtures were then incubated in the dark for 30 min. The absorbance at 515 nm was measured at 0 and 30 min of incubation using a UV-Visible spectrophotometer (UNICO, 2800A). To prepare standard curve of DPPH, the absorbance of DPPH at different concentrations was measured at 515 nm. The remaining $DPPH^{\cdot}$ concentration in the reaction mixture was calculated from the DPPH standard curve, and the percentage of the remaining $DPPH^{\cdot}$ was calculated using the following equation:

$$\%DPPH^{\cdot}_{REM} = [DPPH^{\cdot}]_T / [DPPH^{\cdot}]_{T=0}$$

Where $[DPPH^{\cdot}]_T$ and $[DPPH^{\cdot}]_{T=0}$ were the concentration of $DPPH^{\cdot}$ at steady state and zero time, respectively. The percentages of the remaining $DPPH^{\cdot}$ in each reaction mixture of five different concentrations of all extracts were then plotted against µg of extract / mg of $DPPH^{\cdot}$ to obtain the amount of antioxidant or extract necessary to decrease the initial $DPPH^{\cdot}$ by 50% (EC_{50}). The EC_{50} values of all extracts were calculated by the following linear regression of plots, and the antiradical efficiency ($AE=1/EC_{50}$) values were also calculated.

$$[\%DPPH^{\cdot}_{REM}] = b\,[\mu g\ antioxidant/\ mg\ DPPH^{\cdot}] + a.$$

Ferric reducing antioxidant power (FRAP) assay

Antioxidant activity of fruit extracts was determined according to the FRAP method previously described by Lado et al. (2004). To do FRAP assay, 1 mg/ml fruit extract (50 µl) was mixed with 1.5 mL FRAP reagent (25 ml of 300 mM acetate buffer, 2.5 mL of 10 mM TPTZ (2,4,6-tri-2-pyridyl-2-triazine, Fluka, Sigma-Aldrich, Switzerland) in 40 mM HCl and 2.5 mL of 20 mM $FeCl_3.6H_2O$), and incubated at 37°C for 5 min. The absorbance was measured at 594 nm using UV-visible spectrophotometer (UV1601, Shimadzu Scientific Instruments (Oceania) Pty. Ltd., Australia) against blank (FRAP reagent without the sample). The concentration of Fe^{2+}-TPTZ (reducing capacity) was calculated by comparing the absorbance at 594 nm with the standard curve of the Fe (II) standard solutions (ferrous sulfate heptahydrate). Alpha-tocopherol was used as a positive control.

Determination of total phenolic content

Total phenolic content was determined according to the method of Tepe et al. (2005). Each fruit extract (0.1 ml of 10,000 µg/ml crude methanolic extract) was transferred to a flask containing 46 ml distilled water. Folin-Ciocalteu's phenol reagent (Fluka, Sigma-Aldrich, Switzerland) (1 ml) was added, shaken thoroughly, and allowed to stand for 3 min. Then, 3 ml of 2% Na_2CO_3 was added, and allowed to stand for 2 h with intermittent shaking. Then, the absorbance was measured at 760 nm using UV-visible spectrophotometer (UV1601, Shimadzu Scientific Instruments (Oceania) Pty. Ltd., Australia). Standard curve of gallic acid (Fluka, Sigma-Aldrich, Spain) was prepared using the similar procedure. The results were expressed as µg GAE (gallic acid equivalents) /mg extract.

Application of a selected fruit extract for extending shelf-life of chilled ground pork

A fruit extract with high antimicrobial and antioxidant activities was selected for use as a natural preservative in ground pork during refrigerated storage. Six treatments of ground pork (80% lean meat mixed with 20% pork fat) were prepared. These were treatment 1 (ground pork without fruit extract added, control), treatment 2 (ground pork added with 0.02% BHT, butylated hydroxytoluene) and treatment 3-6 (ground pork added with 0.25, 1.0, 1.5 and 2.0% of a selected fruit extract, respectively). All ground pork samples were added with cell suspension (10^7 cells/ml) of *P. fluorescens* DMST 20076 (10 µl/ 25 g ground pork) to get a final cell concentration of 10^7 cells/ g ground pork. Then, all samples were stored at 4°C for 12 days. At day 0, 1, 3, 7 and 12 days of storage, total viable counts, total psychrotrophic bacteria and total *Pseudomonas* in chilled ground pork samples were analysed by spiral plating technique using the Spiral Plater (Autoplate 4000, Spiral Biotech company, USA) onto Plate Count agar (PCA, incubated at 37°C for 24 h), PCA (incubated at 4°C for 10 days) and *Pseudomonas* isolation agar (PIA, incubated at 30°C for 24 h), respectively. Measurement of pH and color values were also performed using pH meter (Testo 205, Germany) and color value meter (Konica Minolta model CR – 300, Japan), respectively, while TBARS (thiobarbituric reactive substances) values were analysed by using a method of Kirk and Sawyer (1991). Three replicates of experiments were performed.

Statistical analysis

Data were analysed by using analysis of variance to determine if significant differences ($P<0.05$) existed between mean values and using Duncan multiple range test to compare between treatment means.

Use of chilled ground pork for production of a seasoned pork product

This study was aimed at comparing a seasoned pork product made from fresh ground pork without a fruit extract (a control sample) and those made from chilled ground pork added with a selected fruit extract and stored at 4°C for 0, 3 and 7 days. This product contained 87.97% ground pork (with or without the fruit extract), 0.44% sucrose, 4.1% soy sauce, 0.88% tasty sauce, 0.15% salt, 1.47% milk, 2.35% oyster sauce, 1.47% chopped garlic with white pepper, 0.95% olive oil and 0.59% tapioca flour. All ingredients were mixed together. Then, a sensory analysis (a duo-trio test)l was performed in triplicate by 12 untrained taste panels to establish differences between the control sample and the seasoned pork samples made from chilled ground pork stored at different period of time.

To do duo-trio test, the seasoned pork samples including the control sample and the sample made from ground pork added with a fruit extract were divided to small pieces (15 g/piece), and each piece was rounded to a circular shape with 1 inch thick. All samples were fried in palm oil at 160-170°C for 10 min. Then, the cooked samples were served to the 12 untrained panelists. Each panel needed to evaluate 3 samples independently. One sample was coded as "R" and the other two samples were coded with 3 digit numbers. These panels were asked to evaluate "which sample of these two samples was similar to the "R" sample?". The number of the taste panels giving the correct answer was evaluated if the significant differences existed by using a statistical table for duo-trio test (Roessler et al., 1978).

RESULTS AND DISCUSSION

Antimicrobial activity

Extracts of madan, makoknum and makampom exhibited wider inhibition zone against most of bacteria tested by disk diffusion test. However, all fruit extracts could not inhibit growth of all mold species tested, but makoknum and madan extracts could produce inhibition zone only yeast species, *R. glutinis* (Table 1). Madan, makoknum and makampom extracts were selected for MICs determination. Among all fruit extracts tested, fruit extract of madan showed the broadest antimicrobial action to all bacterial species tested (Table 2). Interestingly, it could effectively inhibit foodborne bacterial pathogens (*L. monocytogenes* and *S.* Typhimurium) with the MIC of 2.56 mg/ml. The only yeast species susceptible to madan extract was *R. glutinis*. However, its antimicrobial activity may be due its organic acids. Suntornsuk et al. (2002) reported that the amount of vitamin C in madan fruit juice was 4.6 mg/100 g.

Fruit extract of makampom also exhibited strong inhibitory to some bacteria, especially *P. fluorescens* and *S. aureus* with 2.56 mg/ml MIC (Table 2). Mayachiew and Devahastin (2008) also found that *P. emblica* fruit extract inhibited *S. aureus* with MIC of 13.97 mg/ml. Several components of *P. emblica* fruits may act as antimicrobial agents. They reported that this fruit extract contained gallic acid, hydrolysable tannin and ascorbic acid (11.21%).

Table 1 Antimicrobial activity of Thai local fruit extracts using disk diffusion test

Microbial species	Diameter of inhibition zone (mm)[a] ± SD					
	Ardisia polycephala (pilungasa)	*Averrhoa carambola* (mafueng)	*Elaeocarpus hygrophilus* (makoknum)	*Garcinia schomburgkiana* (madan)	*Phyllanthus emblica* (Imakampom)	*Limonia acidissima* (makwit)
Bacteria						
Bacillus cereus	-[b]	-	11.7± 0.3	11.0 ± 0.5	9.4 ± 0.5	-
Escherichia coli	-	-	-	8.5 ± 0.5	-	-
Listeria monocytogenes	-	-	9.4 ± 0.1	-	-	-
Pseudomonas fluorescens	-	-	11.5± 1.2	10.0 ± 0.3	9.8 ± 0.6	-
Salmonella Typhimurium	-	-	9.0 ± 0.0	11.3 ± 0.2	-	-
Staphylococcus aureus	-	-	8.7 ± 0.3	10.8 ± 0.3	10.3 ± 0.6	-
Yeasts						
Candida lipolytica	-	-	-	-	-	-
Pichia membranaefaciens	-	-	-	-	-	-
Rhodotorula glutinis	-	-	14.7 ± 1.1	21.7 ± 1.1	-	-
Zygosaccharomyces rouxii	-	-	-	-	-	-

[a]Data are mean of three replications.
[b]No inhibition was observed.

Table 2 Minimum inhibitory concentrations of Thai local fruit extracts

Microbial species	Minimum inhibitory concentrations of Thai local fruit extracts (mg/ml)				
	Elaeocarpus hygrophilus (makoknum)	*Garcinia schomburgkiana* (madan)	*Phyllanthus emblica* (makampom)	Pennicillin G*	Fluconazole
Bacteria					
Bacillus cereus	>10	5.12	5.12	250	>0.10
Escherichia coli	>10	5.12	>10	125	>0.10
Listeria monocytogenes	>10	2.56	>10	62.50	>0.10
Pseudomonas fluorescens	7	5.12	2.56	> 2000	>0.10
Salmonella Typhimurium	5.12	2.56	>10	250	>0.10
Staphylococcus aureus	>10	5.12	2.56	31.25	>0.10
Yeasts					
Candida lipolytica	>10	>10	>10	> 2000	0.02
Pichia membranaefaciens	>10	>10	>10	> 2000	0.08
Rhodotorula glutinis	>10	5.12	>10	> 2000	>0.10
Zygosaccharomyces rouxii	>10	>10	>10	> 2000	0.10

* Units/ml for penicillin G

Antioxidant activity

Among all fruit extracts tested, makampom (*P. emblica*) fruit extract had the highest antioxidant activity by DPPH method (EC_{50} of 501.71 μg extract /mg DPPH) and strongest reducing capacity (4.86 mmol/L) by FRAP method, followed by pilangasa, makoknum, madan, mafueng and makwit (Table 3). The lower EC_{50} value of the extract indicates its higher antioxidant activity (Brand-Williams et al., 1995). Their antioxidant activities were related to their phenolic contents. Makampom contained the highest phenolic content (4,220 μg GAE/mg dry extract). Pinsuwan et al. (2007) reported that the alcoholic extract of *P. emblica* possessed high antioxidant capacity with EC_{50} of 1.55 μg/ml and high phenolic content (454.7 mg gallic acid equivalent/g). In addition, Lou et al. (2009) isolated six compounds from *P. emblica* fruit and identified as cinnamic acid, quercetin, 5-hydroxymethylfurfural, gallic acid, β-daucosterol and ellagic acid.

Fruit of pilungasa (*A. polycephala*) was found to possess high antioxidant activity and high phenolic contents. Ahamad et al. (1977) reported that phytochemical constituents in leaves of pirangasa were bauerenol, α-amyrin and β-amyrin. Ruangchakpet and Sajjaanantakul (2007) reported that *E. hygrophilus* (makoknum) fruit at 6 month maturity had high total

phenolics (345.8 mg gallic acid/ 100 g fresh weight) and flavonoid content (49.0 mg catechin/100 g fresh weight). The highest gallic acid content (103.6 mg/100 g fresh weight) was found at 6 month maturity.

Compared to other fruits, mafueng had not much antioxidant activity and total phenolics. Samee et al. (2006) also reported that star fruit (mafueng) had lower antioxidant activity, compared to other fruits tested. Lim et al. (2007) report that star fruit contained antioxidant activity (IC_{50} of 3.8 mg/ml by DPPH method) and total phenolics of 131 mg/100 g fresh fruit.

Table 3 Antioxidant activity and total phenolic content of Thai local fruit extracts

Fruit extracts	DPPH method	FRAP method	Total phenolic content
	EC_{50} (µg extract / mg DPPH)[a] ± SD	Reducing ability (mmol/L)[a] ± SD	(µg Gallic Acid Equivalents (GAE)/mg dry extract) [a] ± SD
Ardisia polycephala (pilungasa)	739.38 ± 15.61	4.72 ± 0.12	1,270 ± 208.71
Averrhoa carambola (mafueng)	17,308.33 ± 339.25	1.27 ± 0.11	116.67 ± 48.21
Elaeocarpus hygrophilus (makoknum)	2,082.49 ± 46.91	2.79 ± 0.08	263.33 ± 108.74
Garcinia schomburgkiana (madan*)*	6,952.48 ± 638.97	2.60 ± 0.05	210 ± 80.32
Phyllanthus emblica (makampom)	501.71 ± 16.61	4.86 ± 0.14	4,220 ± 121.62
Limonia acidissima (makuit)	27,773.4 ± 846.18	0.50 ± 0.01	166.67 ± 39.80
∝-tocopherol	467.55 ± 16.79	4.89 ± 0.08	-[b]

[a] Data are mean of three replications.
[b] Data are not determined.

Application of a selected fruit extract as a natural preservative in chilled ground pork

Makampom extract was selected for use as a natural preservative to extend shelf-life of chilled ground pork. Total microbial counts in ground pork added with 1.5 and 2.0% makampom extract decreased as storage time increased. After 7 days of storage at 4°C, ground pork added with 1.5-2.0% of this fruit extract had lower survival populations of total microorganisms (29-36% survival) than the samples added with lower concentrations of this extract (0.25-1.0% survival) (Figure 1a). Of all, samples added with 2.0% makampom extract had the lowest percentage of *Pseudomonas* survival cells (Figure 1b). However, the number of total psychrotrophs in all treatments of chilled ground pork increased by 0.13-0.51 log unit after 12-day storage. Unlike other treatments, the number of total psychrotrophs in ground pork samples added with 1.5-2.0% makampom extract slightly decreased after 1 day of storage, then gradually increased as the storage time increased (Figure 1c).

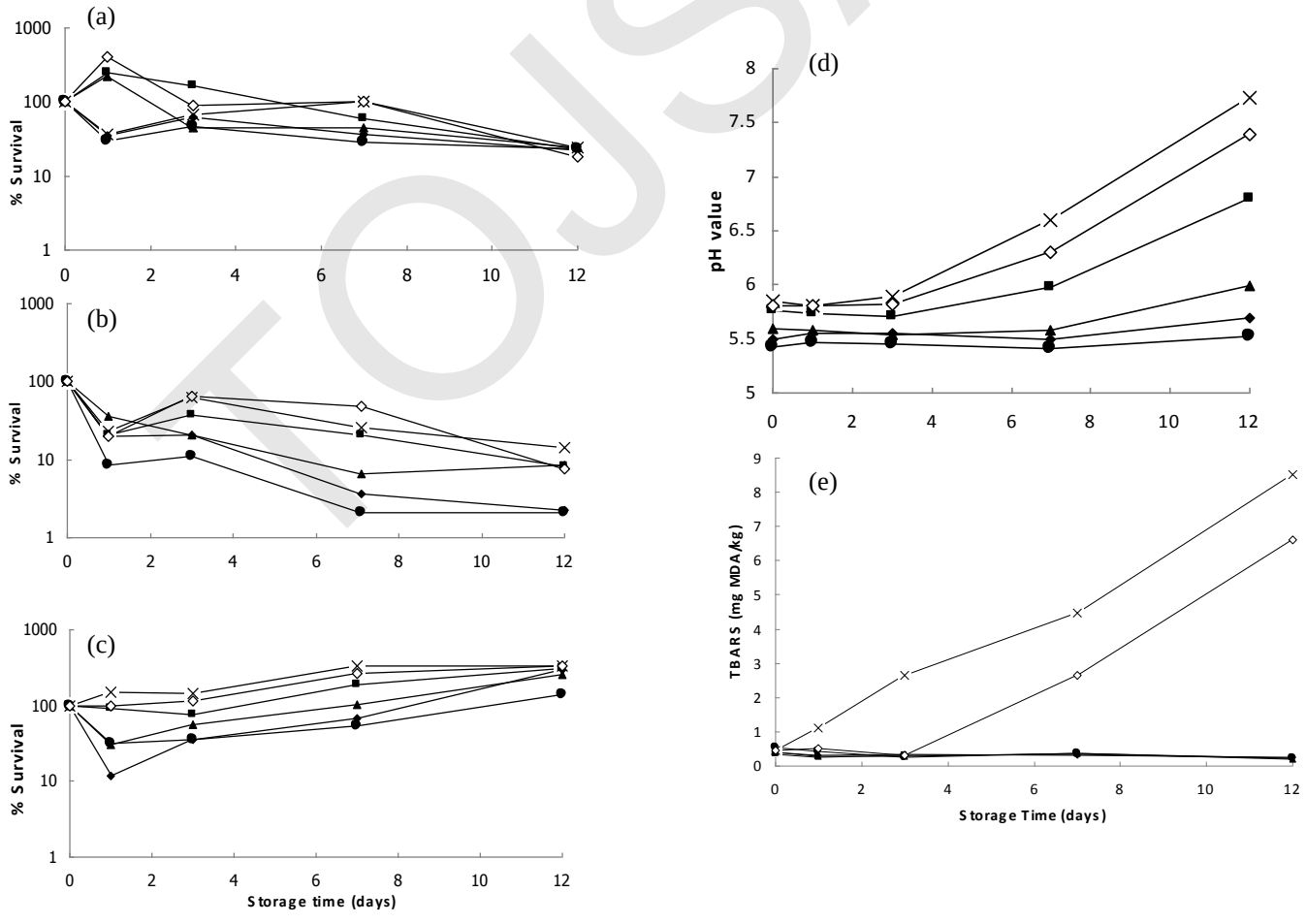

Figure 1 Survival of total microorganisms (a), total *Pseudomonas* (b) and total psychrotroph (c), pH values (d), and thiobarbituric reactive substances (TBARS) values (e) in ground pork added with some preservatives during refrigerated

storage (Symbol: ×, control (no preservative added); ■, 0.25% makampom extract; ▲, 1.0% makampom extract; ♦, 1.5% makampom extract; ●, 2.0% makampom extract, and ◊, 0.02% BHT)

Decreasing of total microbial counts and *Pseudomonas* counts in the samples added with 1.5-2.0% makampom extract was probably due to its active compounds. This indicated that this extract could delay spoilage of chilled ground pork up to 7 days, while the control samples could be kept at 4°C for only 3 days before changing appearance. Medmood et al. (2011) found that crude extract of *P. emblica* fruit contained alkaloids, tannins, terpenes, flavonoids, sterols and saponins.

At the beginning of storage, pH values of ground pork samples without any preservative (control, pH 5.84) and the samples added with BHT (pH 5.81) were higher than the pH values of the samples added with 0.25-2.0% makampom extract (pH 5.76-5.43). The pH values of all pork samples slightly changed or remained constant until 3 days of storage, then increased until the end of storage. The pH values of the control samples and the samples added with 0.02% BHT increased more rapidly (to neutral pH level) than the samples of other treatments. Among all treatments of ground pork, the pH values of ground pork samples added with 1.5 and 2.0% makampom extract were the lowest (5.68 and 5.52, respectively) after 12-day storage (Figure 1d). In the control samples, increasing of pH to neutral level was related with the high number of total viable counts which indicated their spoilage. At 12-day storage, the control samples had green surface with stink odor, while the appearance of the samples added with 1.5-2.0% makampom extract was almost similar to fresh ground pork.

TBARS values of the control samples and the samples added with 0.02% BHT significantly increased more rapidly as the storage time increased ($P<0.05$), compared to other samples. The TBARS values of the samples added with 0.25-2.0% makampom extract slightly changed at each storage time, but no significant different was found between those of the samples with each concentration of the extract ($P>0.05$). Among all treatment samples, the TBARS values of the samples added with 0.25-2.0% makampom extract were the lowest (0.20 – 0.26 mg MDA/kg) at the end of the storage (Figure 1e).

Use of chilled ground pork added with a selected fruit extract to produce a seasoned pork product

Makampom extract at 2.0% was the most suitable to extend shelf-life of chilled ground pork. Therefore, the ground pork added with 2.0% makampom extract and stored for 0, 3 and 7 days at 4°C was used to produce the seasoned pork product (a_w 0.97). After duo-trio test, only 5, 4 and 8 panels (out of 12 panelists) could discriminate between the control samples (pH 5.67) and the samples made from ground pork added with 2.0% makampom extract and stored for 0, 3 and 7 days at 4°C (pH 5.58), respectively. Based on the table of Roessler et al. (1978), it can be concluded that the taste panels could not discriminate between the control samples and the treated samples, when using probability level at 0.05. This indicates that the taste panels could not detect the flavor of 2.0% makampom extract added. Thus, the flavor of this extract should not cause product unacceptability. It is possible to use makampom extract as a natural preservative to extend shelf-life of chilled ground pork.

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BIODEGRADATION OF PESTICIDE: BROMUCONAZOL BY MICROBIAL CONSORTIUM IN BIPHASIC SYSTEM

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Abstract: The bromuconazol is a fungicide which is toxic for the environment. This present study was led with the aim of testing, in vitro, its biodegradation.
The bromuconazol was thus used in the presence of a microbial consortium resulting from a treated soil. A biphasic system MBS / oil of silicone was used during all the period of acclimatization. The fermentation was followed by measure of the optical density, the determination of the dry weight, the emulsifying power as well as the hydrophobicity of the selected consortium.
The results obtained after one year revealed a strong adaptation of this one to the bromuconazol. The use of the biphasic system allowed a better assimilation of the pesticide. The microbiological study allowed identifying a single bacterial strain capable of using the fungicide as unique source of carbon. It is about *Aeromonas hydrophila*. This strain shows a strong hydrophobicity as well as an emulsifying power lived in saw some pesticide.

Keywords: fungicide, Biodegradation, Bromuconazol, biphasic system, Batchs, microbial Consortium. *Aeromonas hydrophila*

INTRODUCTION

The intensification of agriculture has been accompanied by an extensive use of pesticides that generated a contamination of soil and water, major environmental problem of current. This situation is particularly worrying that the use of pesticides should be repeated periodically. This repetition in the long term, necessarily leads to an accumulation of pesticides and their residues in our natural environment, endangering the entire population by their multifaceted toxicity (Bouziani, 2007). The most frequent studies concern the presence of these molecules in the soil (Senesi, 1993), water contamination, biotic and abiotic degradation of these products (Muller et al. 1978; Bollag, 1982), and finally the identification of residues that apparently seem to have a relationship with them (Calderbank, 1989).

The pesticide to which we are interested in this study is bromuconazol, a fungicide widely used around the world. It is a systemic fungicide activity. It belongs to the family of triazoles which are inhibitors of sterol biosynthesis. It is intended to protect cereal crops against fungal diseases. Its target is a cytochrome P450 enzyme encoded by the gene CYP51 (Robbertsee and *al*., 2001). Bromuconazol is an environmental contaminant. Moreover, it is toxic to humans. Repeated exposure can cause health problems, adverse effects on the liver resulting in tissue lesions and impaired functions (anonymous 4, 2008)

The main objective of this study is in this context and aims to study the ability of a microbial consortium obtained from different biotopes, to degrade this fungicide

1. MATERIAL AND METHODS

1.1. Origin of the microbial consortium:

The first sample was taken from a ground agricultural nature located in the region of North- eastern Algeria. This soil is intented for crops of vineyards and has been extensively treated by bromuconazol. The second sample was collected from basins of biological treatment of wastewater also situated in North -eastern Algeria.

1.2. Bromuconazol:

This fungicide comes from Bayer (Rhône-Poulenc Agro- France). It is imported and widely marketed in Algeria as the Vectra. His brute formula is: $C_{13}H_{12}BrCl_2N_3O$ and chemical structure is presented in the following figure.

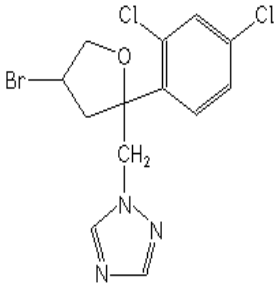

Fig.1. Chemical structure of the herbicide used: bromuconazol

1.3. Selection and acclimatization of microbial consortium:

The acclimatization and selection of the microbial consortium was conducted in biphasic system MBS / silicone oil (batch culture). Thus, at 40 ml of medium basic salin MBS (aqueous phase) are added 10 ml of silicone oil (organic phase). bromuconazol is added in the organic phase at 50µg/ml. The aqueous phase was inoculated with 10 ml of microbial inoculum (biomass). After incubation at 30°C and stirring at 190 rpm, cultures were centrifuged for 20 min at 5000 rpm. The culot obtained were washed with phosphate buffer and again centrifuged. Biomass collected will be used as inoculum for the next fermentation.

1.4. Biodegradation tests of bromuconazol in batch culture:

1.4.1. Biodegradation of bromuconazol in biphasic batch:

After several months of acclimatization of microbial consortium in presence of bromuconazol, biodegradation is followed in biphasic system (medium MBS / silicone oil). At flasks containing a culture medium composed of 40 ml MBS and 10 ml of silicone oil are added 10 ml of the consortium previously acclimated to bromuconazol. The latter is added at a concentration of 50µg/ml. After incubation for one week at 30°C with stirring of 200 rpm, fermentation was monitored by measuring the optical density, the determination of dry weight and changes in the pH of the medium. The emulsifying power and the hydrophobicity of the consortium were also studied.

1.4. 2. Biodegradation of bromuconazol in monophasic batch:

After acclimatization of the microbial consortium in presence of xenobiotic, biodegradation is also followed in the same way in monophasic system, in the absence of the organic phase (MBS without silicone oil).

1.4.3. Biodegradation of bromuconazol in pure culture:

Biodegradation tests of bromuconazol in pure culture were performed in monophasic system. bromuconazol is added at 50µg/ml. The samples are incubated for one week at 30°C, with stirring at 190 rpm. The disappearance of the herbicide in the culture medium was performed using the technique of LC- MS -MS.

2. RESULTS AND DISCUSSION

2.1. Acclimatization of microbial consortium in batch culture:

After 4 weeks of acclimatization of the consortium in the presence of bromuconazol, the results obtained from different batch in biphasic system are shown in the following figure.

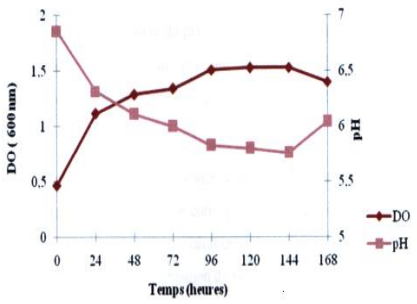

Fig.2. Growth of the microbial consortium in biphasic system (MBS/silicone oil) in the presence of bromuconazol at 50µg/ml

2.2. Study of the performance of biphasic system (MBS / silicone oil):

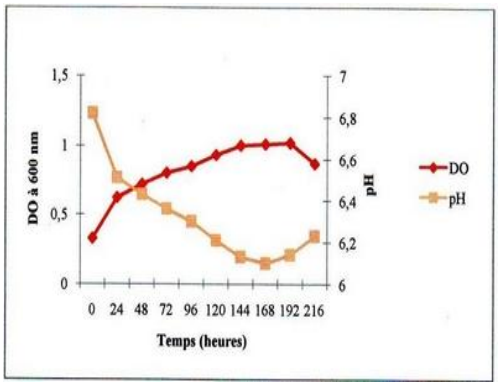

Fig. 3. Growth of the microbial consortium acclimated in batch monophasic

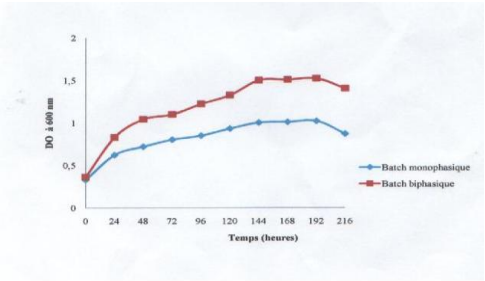

Fig.4. Evolution of the optical density of biomass in batch culture

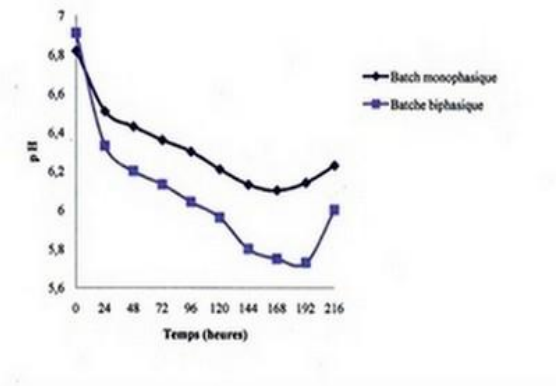

Fig.5. Evolution of pH in batch culture in the presence of bromuconazol

After about five months for the selection and acclimatization of the consortium implicated in the biodegradation of bromuconazol, the study of the biodegradation in batch monophasic (MBS only) was also studied. The aim of this comparative study between the two systems is to determine the usefulness and effectiveness of the organic phase (silicone oil) not only in the degradation process but also with regard to the viability of the consortium involved in the process. The results of test in monophasic systemc show clearly the complete absence of

inhibitory effect of bromuconazol on the growth of microbial consortium previously acclimated. In fact, a typically growth is thus obtained with an increase in the D.O of the culture and acidification of the medium. The maximum growth is obtained after 192 h of incubation at 30°C. The microbial consortium previously acclimated seems able to grow in the presence of bromuconazol as unique source of carbon and energy in monophasic system.

Comparing the curves obtained in monophasic and biphasic system show that the viability of the consortium is maintained in the two types of batch in the presence of bromuconazol. Although the same concentration of substrate (50µg/ml) was added, it does not appear to have the same effect on microbial activity. In the absence of silicone oil, the optical density of the medium reached an average maximum estimated at 1.02, whereas in the presence of the organic phase, the D.O recorded is 1.52. These observations were also found for pH.

2.3. Determination of emulsifying activity of the consortium:

After acclimatization in the presence of the herbicide, the study of the emulsifying activity of the consortium shows that it evolves in a similar way to the cell growth for reaching a maximum production after 144 h of incubation. So, the shape of the curve (Fig.6) shows clearly, a strong emulsifying power exhibited by the microbial culture in the presence of bromuconazol.

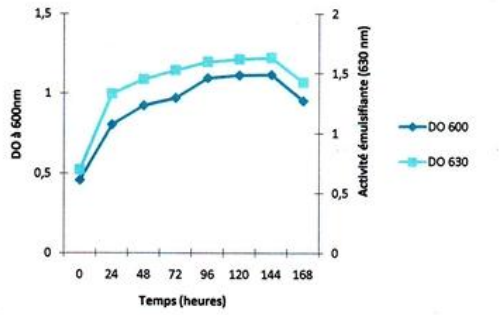

Fig.6. Emulsifying activity of the consortium acclimated to bromuconazol

Fig.7. The emulsifying power of the consortium in the presence of bromuconazol

2.4. Study of the hydrophobicity of the microbial consortium:

The study of the hydrophobicity performed according to the protocol BATH (bacterial adhesion to hydrocarbons) (Rosenberg, 1984) revealed that 55.67 % of the cells are hydrophobic. The hydrophobicity is a key factor in the selection of microorganisms for degradation of xenobiotics, demonstrating the strong adaptation of the culture of the consortium.

2.5. Identification of microorganisms in the consortium degrading bromuconazol:

After a period of acclimatization and adaptation after more than one year in the presence of bromuconazol used as the sole source of carbon and energy, the results obtained after tests of isolation and purification revealed the presence of a single type of colony. After the macroscopic and microscopic examination, the biochemical tests API 20 E and API 20 NE have identified the bacterium implicated in the biodegradation of bromuconazol. It is the species *Aeromonas hydrophila*

2.6. Results of the biodegradation of bromuconazol by *Aeromonas hydrophila*:

Different concentrations of bromuconazol shown in Fig.9 are those obtained after a series of dilutions performed on the initial sample for reasons of handling required by the technique used for the determination of the compound. The form of the curve indicating a gradual disappearance of the herbicide in the culture medium and an increase in the biomass of *Aeromonas hydrophila*, confirming the ability that has this species in the elimination of bromuconazol. The maximum growth, estimated by dry weight is reached after 144 h. The maximum degradation of bromuconazol seems occur during these first 72h.

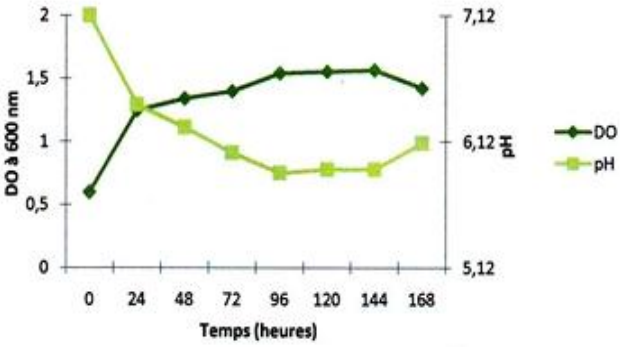

Fig.8. Growth of Aeromonas hydrophila in batch monophasic in the presence of bromuconazol

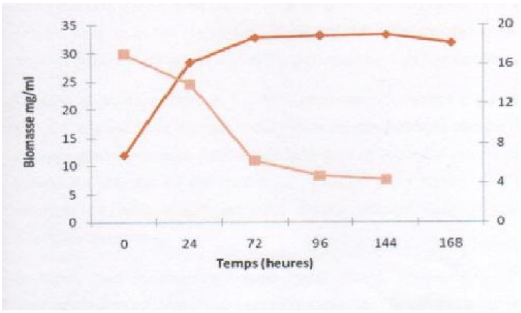

Fig.9. bromuconazol biodegradation by *Aeromonas hydrophila* in monophasic system

CONCLUSION

The use of system biphasic has not only lifted the inhibition due to the substrate, but also allowed better pesticide assimilation compared to that obtained in monophasic system (mineral medium).

After one year of adaptation and acclimatization, the bacterium *Aeromonas hydrophila* has been isolated from a consortium initially inoculated in the MBS culture medium in the presence of bromuconazol.

The disappearance of bromuconazol in the medium culture confirms a real use of herbicide as unique source of carbon and energy by *Aeromonas hydrophila* and the ability possessed by this bacterial species to acclimatize and metabolize the herbicide. This ability is largely due to the presence of the organic phase (silicone oil).

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CHEMICAL MODIFICATION OF WATER HYACINTH FOR THE REMOVAL OF DYESTUFFS

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Abstract: Water Hyacinth (WH), an aquatic weed, is a good heavy metal adsorbent. Their adsorbing capacity and binding selectivity can be increased by converting the WH's hydroxyl functional group into the desired ones. In this report, the chemical modifications of WH by cyanoethylation (WH-CE), amidoximation (WH-AO) were carried out. The chemical testing and the FTIR spectrums of the WH, WH-CE and WH-AO indicated that higher nitrogen content of the modified WH than that of WH was found with the existing of nitrile and amine functional group on WH-CE and WH-AO, respectively. The adsorption of Acid Blue 25 and Basic Blue 9 by WH and WH-AO were studied. Based on Freundlich adsorption isotherm, WH-AO has a much higher dye-adsorption capacity than does WH. The adsorption capacity of WH for BB9 was higher than AB25. For WH-AO, the adsorption capacity of AB25 was higher than WH.

Keywords: dyestufsf; water hyacinth powder; chemical modification of water hyacinth; adsorption

INTRODUCTION

Water hyacinth (Eichhornia crassipes) is a perennial, freshwater, aquatic vascular plant with rounded, upright, shiny green leaves. The petioles of the plant are spongy with many air spaces which contribute to the buoyancy of the hyacinth plant. Water hyacinth (WH) is ranked eighth among the world's top 10 in growth rate and is found abundantly throughout Thailand. It is composed of 43-44% cellulose and 12-13% lignin (Strelpipatkul, 1989). Somboon et al. (1990) has reported that water hyacinth powder (WH) has a very high adsorption capacity for heavy metals (Cr, Cu, Ni, Pb and Zn). Like cotton, WH has the tendency to form a strong bond with direct dyes, reactive dyes, and basic dyes.

Color contamination from textile-dyeing effluents has been the target of great concern in the last few years, mainly due to its unsightliness but also due to its toxicity. The de-colorization of textile wastewater is a worldwide problem to which successful treatment technologies have been applied, including coagulation, adsorption, oxidation, and biological treatment. Coagulation can be used effectively to remove certain types of dyes. Generally, this process is most efficient when dealing with pigment-type materials or dispersed dyes. The process is least efficient when dealing with true water soluble dyes. The oxidation methods are effective only in wastewater having a very low concentration of organic color. The adsorption process by activated carbon and polymer resin is expensive, and it is difficult to regenerate the adsorbent (Bousher, Shen & Edyvean, 1997). These conventional methods are costly and require some skill to operate and to maintain. Consequently, the wastewater has been discharged untreated. There is a need for a more practical technology that is more effective and more selective to organic dyestuffs. In this report, the chemical modification of WH powder into two new products with different functional group was studied.

The main functional group of the natural cellulose (cotton) is the hydroxyl group (OH). The hydroxyl group could bond to the positive charges of the adsorbates such as heavy metal ions or cationic dyes. In case of silk, it is the amino group (negative charge) instead of the hydroxyl group. Thus, silk has the affinity for the anionic dyes. The conversion of the hydroxyl group of the cellulose to the amino group could be performed directly through the carbamoylethylation reaction. The conversion could be achieved by converting the hydroxyl group of WH to the nitrile group (CN) by cyanoethylation reaction with basic catalysts (Parker, 1993). A chemical reaction, to introduce the β-cyanoethyl group (CH_2CH_2CN), involves the addition of acrylonitrile (CH_2=CH-CN) to a compound carrying a reactive hydrogen (reaction 1).

Yields in cyanoethylation are generally high. The reaction is strongly exothermic and is usually carried out at moderate temperature in solvent such as dioxane or t-butanol. The cyanoethylated product can be subjected further to the usual nitrile

reactions such as hydration, hydrolysis and reduction. Morita, Higuchi & Sakata (1987) converted the cyanoethylated wood to the amidoximated wood by reacting with methanol saturated with hydroxylamine hydrochloride at 60ºC. The reaction is shown in reaction (2).

$$R\text{-}OH + CH_2{=}CH\text{-}CN \xrightarrow{OH^-} R\text{-}CH_2\text{-}CH_2\text{-}CN \qquad (1)$$

$$R{-}CN \xrightarrow[67°C]{NH_2OH{-}HCl} R{-}\overset{NH_2}{\underset{|}{C}}{=}NOH \qquad (2)$$

MATERIALS

Water Hyacinth Powder

WH stalk was washed with tap water and cut into pieces of 0.20 cm or less in length. The cut WH pieces were dried overnight at room temperature before being ground and sieved to a size between 0.15 and 0.85 mm (20 - 100 mesh). The ground powder was oven-dried overnight at 103°C prior to use.

Dyestuffs

Dyestuffs selected for study and associated information are indicated in Table 1. The chemical structure of each dye is presented in Fig. 1. Dyestuffs are used as received without any purification. They are dried at 60ºC for 3 hours prior to use.

Table 1. Dyestuffs selected for the studies.

Name of dyestuffs	Abbreviation	Type of Dye	CI No.
Acid blue 25	AB25	Acid	62055
Basic blue 9	BB9	Basic	52015

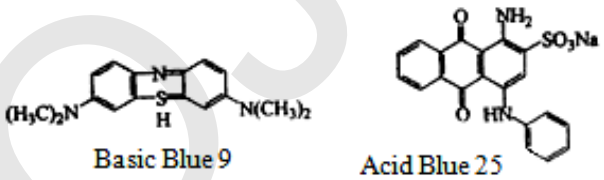

Figure 1. Chemical structure of dyes.

METHODS

Chemical Modification of WH

The hydroxyl functional group (OH) of WH is chemically converted into the amine group (NH_2) by indirect conversion to the nitrile group (CN) via the cyanoethylation reaction and then converting into the amino group via the amedoximation.

Cyanoethylation of WH: Place WH into 4.0% NaOH saturated with NaI for 30 min. The mixture is pressed to a wet pickup of 150% with a piece of cloth. The damped WH is quickly placed in a three-necked round-bottomed flask and equips the flask with a reflux condenser. Heat the mixture to a constant temperature of 60ºC after adding acrylonitrile. After 12 hours, allow the flask to cool and neutralizes the mixture with 0.1 M acetic acid. Wash the product in the Buchner funnel, using DI water, until a constant pH of the rinsed water is observed. The product (WH-CE) is dried in a vacuum oven at 70ºC.

Amidoximation of WH: Place WH-CE into methanol saturated with hydroxylamine hydrochloride in a round bottom flask and refluxed the mixture 67ºC for 15 hours. The product was washed in the Buchner funnel with deionized water until a constant pH of the rinsed water is observed, then washed the product with methanol and dried in a vacuum oven at 70ºC. This product is called WH-AO.

Determination of Wavelength at Maximum Adsorption

The dye adsorption spectrum of each dye is determined by dissolving dye in deionized water. The dye solutions were scanned with a UV-VIS Spectrophotometer. The wavelength at the maximum adsorption (λ_{max}) was determined from the spectrum.

Adsorption Isotherm Study

The adsorption isotherms were determined by shaking fixed weights of WH or WH-AO with known volume of dye solutions having concentrations ranging from 50 to 550 mg-dye/l, at room temperature.

RESULTS AND DISCUSSION

Some Physical Properties of WH Powder

The surface area of WH powder (0.15 - 0.85 mm) is 1.01 m^2/g, indicating a non-porous solid. The pH_{zpc} was determined by the method described by Huang & Ostovic (1987). The pH_{zpc} of WH is about 6.3, indicating that WH has a weakly acidic surface which is of the cationic type.

WH is a weak cationic ion exchanger, the total cation exchange capacity was found to be 1.0 meq/g. The total exchange capacities for a number of commercial resins ranged from 2.5 to 4.9 meq/g of resin on a dry basis. The total exchange capacity of WH is low because of its low surface area.

Chemical Properties of WH and the Modified WH

Solubility tests are performed on WH, WH-CE and WH-AO. The solubility test in acid or a basic solvents can reveal whether the compound in a base (amine), an acid, or a neutral substance. WH, WH-CE and WH-AO are all soluble in sulfuric acid (Table 2). The solubility of WH-CE, but not WH, in pyridine indicates that some of the OH groups of WH are converted into the nitrile groups, as revealed by the IR spectrum. Aromatic amine (amide) is soluble in pyridine. WH-CE and WH-AO are soluble in pyridine, while only WH-AO is soluble in benzaldehyde. The solubility tests and along with the IR-spectra of WH-AO show that it is quite possible that WH-AO contains the amine functional groups.

Table 2. Some chemical testing of WH and modified WH

Type of WH	Solubility Test			Amide Test		Amine Test		Nitrogen Content, %
	H_2SO_4	Pyridine	Benzaldehyde	Ammonia smell	Color of Litmus paper	Nitrous acid test	Hinsberg's test	
WH	Y	N	N	N	Red	No bubble	N	0.28
WH-CE	Y	Y	N	Y	Blue	No bubble	N	7.81
WH-AO	Y	Y	Y	Y	Blue	Bubble at 4°C	N	7.73

Note: Y = positive test and N = negative test

The smell of ammonia resulting from the WH-CE and WH-AO tests confirms the presence of the CN group on WH-CE, and the presence of either the CN group or the amide group on WH-AO. If most of the CN functional groups on WH-CE are converted, then the smell of ammonia will indicate the presence of the amide groups on WH-AO. The positive nitrous acid test on WH-AO indicates the presence of the primary amine. The differing results from the nitrous acid test indicate that WH-AO contains the amino group. The ammonia smell from the nitrous acid test comes from the presence of the amide group, not the CN group. The Hinsberg's reagents do not dissolve the WH and all of the modified WH and thus no reaction occurred. The Hinsberg's test cannot differentiate the modified WH, and thus the presence of the amine group cannot be determined.

The nitrogen (N) content of WH and the modified WH was determined by the Total Kjedahl Method (TKN). The fact that the N content of WH-CN (about 7.81%) is higher than that of WH (about 0.28%) confirms the conversion of the OH group of WH into the CN group of WH-CE. The N content of WH-AO is not twice as much as that of WH-CE could be because of the N content of C=N cannot be determined by the Kjedahl method.

Thin layer chromatography can be performed on WH-CE and WH-AO only because of their ability to be dissolved in pyridine. The retardation factor (R_f) value of WH-CE and WH-AO are 0.88 and 0.63, respectively. It is clearly shown by the R_f values that the CN functional groups of WH-CN are consumed, and the new products (WH-AO) have different functional groups.

The Spectrum of WH and the Modified WH

WH is a natural fiber, which is primarily composed of cellulose, lignin, and wax. The IR-spectrum of WH would therefore contain many bands at the different absorption regions. The WH IR-spectrum cannot be accurately interpreted to identify its functional groups. It can, however, be used as one of the tools to differentiate the modified WH. In the infrared spectrum of WH (Fig. 2), the broad band between 2,800 and 3,000 cm^{-1} results from C-H stretching vibrations. The band at 665.59 cm^{-1} is from the rocking of the CH_3 and CH_2 groups.

The spectrum of WH-CN is shown in Fig. 3. The OH functional group of WH (or WH-OH) is converted into the nitrile group by cyanoethylation (reaction 2).The strong band at 2,252.51cm^{-1} result from C≡N stretching. The broad band of O-H stretching centered at 3,476.39 cm^{-1} is still apparent. The adsorption bands in the range of 2,850 to 2,950 cm^{-1}, and at 1,677.05 cm^{-1} and 1,104.38 cm^{-1}, indicate the stretching frequencies of C-H, C=C, and C-O-C, respectively.

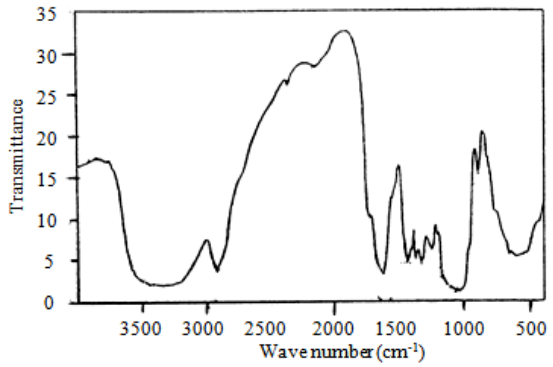

Figure 2. FTIR-Spectrum of WH

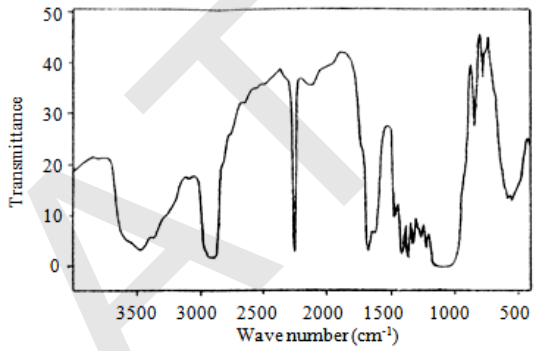

Figure 3. FTIR-Spectrum of WH-CE

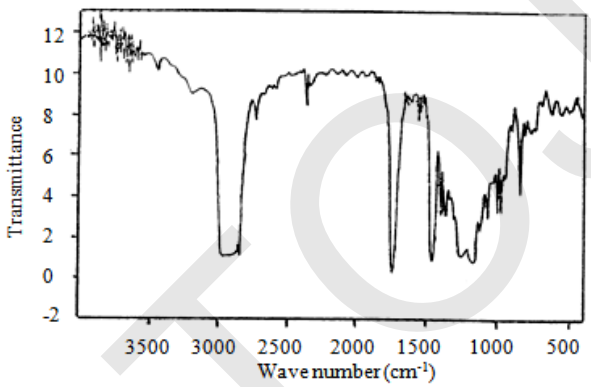

Figure 4. FTIR-Spectrum of WH-AO

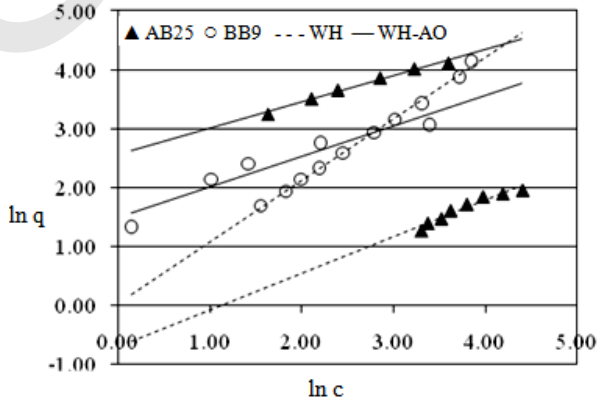

Figure 5. Freundlich adsorption isotherm.

The spectrum of WH-AO is shown in Fig. 4. The fact that there is no broad absorption band around 3,500 cm^{-1} indicates that most of the hydroxyl groups are largely consumed. The intensity of the C=N vibration (1,630 - 1,680 cm^{-1}) lies between that of the C=O vibration (1,600 - 1,950 cm^{-1}) and the C=C vibration (1,560 - 1,680 cm^{-1}). In the spectrum of WH-CN there is, however, no band in the range of 1,680 to 1,950 cm^{-1} as indicated in Fig. 3. It is possible that the strong band at 1,742.68 cm^{-1} (Fig. 4) is the vibration of C=N and not that of C=O or C=C. There is a weak absorption at around 3,440 cm^{-1} which is most likely the stretching vibration of either the primary amines or the secondary amines. Most of the primary amines show two spikes in the N-H stretching. The secondary amines generally show one N-H spike in the absorption range of 3,400 to 3,530 cm^{-1}. If the band at 3,440 cm^{-1} is one of the stretching vibrations of the OH group, then it should be a broad band. There are two bands at 1,180 and 1,280 cm^{-1} that could be the stretching vibrations of the C-N (aromatic) group. The band at 1,280 cm^{-1}also could be, however, the C-O-C stretching vibration.

Adsorption Isotherm

Adsorption isotherm data were fitted to the linear form of the Freundlich model. The plots of ln q against ln C, for the adsorption of dyes by WH and WH-AO gave a straight line indicates that the adsorption process conforms to Freundlich adsorption isotherms. The values of K, n and the corresponding correlation coefficients (r), are presented in Table 3.

Table 3. Freundlich adsorption constants.

Adsorbent	Dyestuffs	K	n	r
WH	AB25	0.5034	1.62	0.938
WH	BB9	1.0500	0.96	0.998
WH-AO	AB25	12.8726	2.22	0.988
WH-AO	BB9	4.4817	1.96	0.916

The adsorptions of AB25 and BB9 by WH and WH-AO are shown in Fig. 5. The OH functional group of WH is first converted to the CN group and then to the NH_2 group. The WH-AO should, however, contain some OH, CN, and NH_2 groups. The OH group is a negatively charged group, while both CN and NH_2 are positively charged groups. The adsorption of AB25, an anionic dye, by WH- AO is much higher than that by WH, indicating the net positive charge of WH-AO. The positive charges of both CN and NH_2 on WH-AO can form hydrogen bonding with =O groups of AB25 molecules. At a high equilibrium dye concentration, the adsorption of BB9 by WH-AO is lower than that by WH, due to the repellency between the positive charge of dye and the positive charge of WH-AO.

CONCLUSION

The hydroxyl functional groups of WH are chemically converted to the nitrile group by cyanoethylation reaction. Some part of these nitrile groups are converted to the amine group by amidoximation reaction. AB25, an anionic dye, has a net negatively charge while that of BB9, a cationic dye, has a net positively charge. WH-AO has a higher positive charge comparing to WH and therefore can adsorption AB25 much more than BB9. The lower adsorption of BB9 by WH-AO is due to the higher repellence force between the positive charge of BB9 and the positive charge of WH-AO.

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COMMON SPORTS-RELATED INJURIES AND THE EFFECTIVENESS OF REHABILITATION IN THE PREVENTION OF REOCCURRENCE

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Abstract: Injuries among student athletes are a major concern, especially when the prevalence of injury is high among load-bearing sports (e.g. basketball, volleyball, football, soccer). The purpose of this study was to determine the most common injuries among college-aged individuals that participated in load-bearing sports, to determine the most common method of treatment/rehab for these injuries, and the prevalence of reoccurrence. We hypothesized that ankle and knee injuries would be the most prevalent type of injury with electric stimulation and bracing as the most common form of treatment. Also, we precluded that higher reoccurrence in ankle injuries would predominate over other reoccurrence injuries.

Data collected through a survey showed that the most common injuries were to the lower extremities, which accounted for 21% of our findings with ankle injuries (ankle sprains) being the most common. However, 41% of athletes still had reoccurrence injury to the ankle following treatment. We conclude that while ankle injuries are among the most prevalent injuries among college-aged athletes, further studies are warranted to determine an effective treatment for these injuries in the prevention of injury reoccurrence.

Keywords: Rehabilitation, Sports related injuries.

INTRODUCTION

In recent years, more focus has been given to sport-related injuries, particularly in lower extremities, which can be attributed to the types of movement, frequency of participation, and intensity of the sport (Agel et al., 2007; Sharpe et al., 1997).

According to the National Collegiate Athletic Association (NCAA), injury surveillance data for women's basketball indicated the most common sports-related injuries were to the lower extremities. Specifically, more than 60% of all game and practice injuries were to the lower extremity including ankle, knee, and upper leg muscle injuries (Agel et al., 2007). In addition, studies pertaining to female soccer players reported that ankle injuries (i.e. ankle sprains) were among the most common impairments at the collegiate level (Sharpe et al., 1997; Elkstrand and Tropp, 1990; Garrick and Requa, 1973; Knapik et al., 1991). Based on previous studies, individuals that experience ankle sprains are more likely to endure reoccurrence (Elkstrand and Tropp, 1990; Garrick and Requa, 1973; Jones et al., 1993; Milgrom et al., 1991).

PURPOSE AND HYPOTHESIS

The purpose of this study is to: i) determine the most prevalent sports-related injuries and the most common form of treatment for these injuries and ii) determine the most common modality of treatment and its effectiveness in preventing re-injury.

Based one previous studies, we hypothesized that ankle and knee injuries would be the most frequent type of injury with electric stimulation and bracing as the most common form of treatment. In addition, we hypothesized that ankle injuries lead to a higher rate of reoccurrence compared to other injuries.

METHODS

Experimental Protocol

84 student athletes of the Ohio Dominican University male and female basketball and soccer teams were asked to participate in a survey questionnaire regarding past injuries.

Experimental Measurement and Data Collection

Each team was given a survey questionnaire proctored by a co-investigator involved in the study.

The purpose of the study, the details of the survey, and information of the right to refuse participation was explained to each subject.

Those who chose to participate signed a form of consent and all data was kept in a locked cabinet accessible only to the co-investigators of the study. The study was approved by the Ohio Dominican University Institutional Review Board and complied with the regulations and rules set forth by the Declaration of Helsinki.

Each survey included information regarding: type of sport, position of the athlete, years of participation at the collegiate level, past injuries (upper and lower extremity), treatment for those injuries, and any record of reoccurrence based on modality of treatment and/or rehab.

RESULTS

Table 1 – Survey response of all reported sports injuries and applied methods of treatment**.**

	Injuries						
Treatment	Ankle Sprain	Hamstring	Groin	Shin Splits	Knee	Concussion	Total # of Athletes
Surgery	0	0	0	0	4	0	4
Cast	2	0	0	0	0	0	2
Brace	7	0	0	0	4	0	11
Sling	0	0	0	0	0	0	0
Rehab	1	2	0	0	5	0	8
Chiropractor	0	0	0	0	1	0	1
Ultrasound	3	4	1	1	4	0	13
Electric Stimulation	9	4	1	1	4	0	19
Rest	6	2	1	1	4	0	14
Functional Bracing	3	0	0	0	3	0	6
Cortisone Shots	0	0	0	0	1	0	1
Anti-inflammatory	2	1	0	1	3	0	7
Pain medication	1	1	0	1	3	0	6
Ice	2	1	1	0	1	0	5
Muscle Relaxants	0	2	3	1	0	0	6
None	1	0	0	0	0	0	1

Table 2 – Most common reported injuries and prevalence for each sport.

	Men's Soccer	Men's Basketball	Women's Soccer	Women's Basketball
Ankle Sprain	7	9	8	3
Hamstring	1	3	3	0
Groin	3	1	1	0
Shin Splits	1	0	5	1
Knee	4	3	4	3
Concussion	4	0	2	2

Table 3 – Number of post-rehabilitation reoccurrence injuries for each sport.

	Men's Soccer	Men's Basketball	Women's Soccer	Women's Basketball
Ankle Sprain	4	3	4	0
Hamstring	1	0	0	0
Groin	0	0	0	0
Shin Splits	0	0	1	0
Knee	1	0	0	0
Concussion	2	0	0	1

Figure 1 – Most prevalent load-bearing sport-related injuries among college-aged women's and men's basketball and soccer teams.

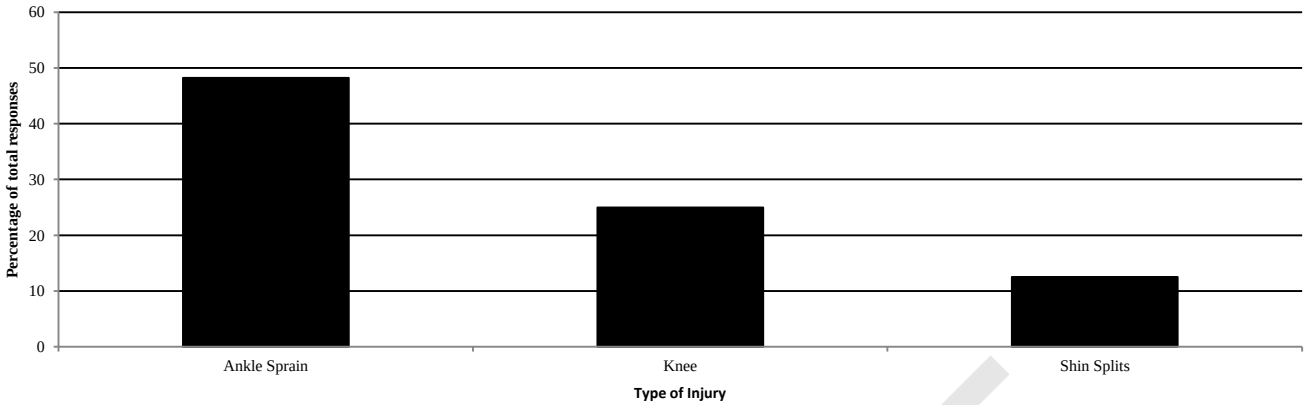


Figure 2 – Most common methods of treatment for all injuries among college-aged women's and men's basketball and soccer teams.

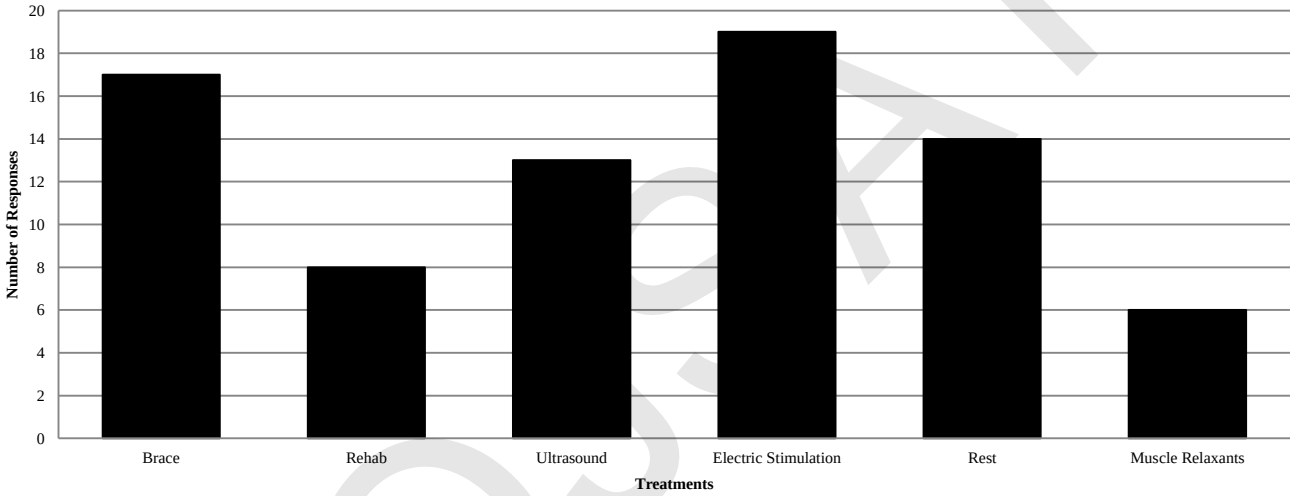


Figure 3 – Most common methods of treatment for ankle sprains among college-aged women's and men's basketball and soccer teams.

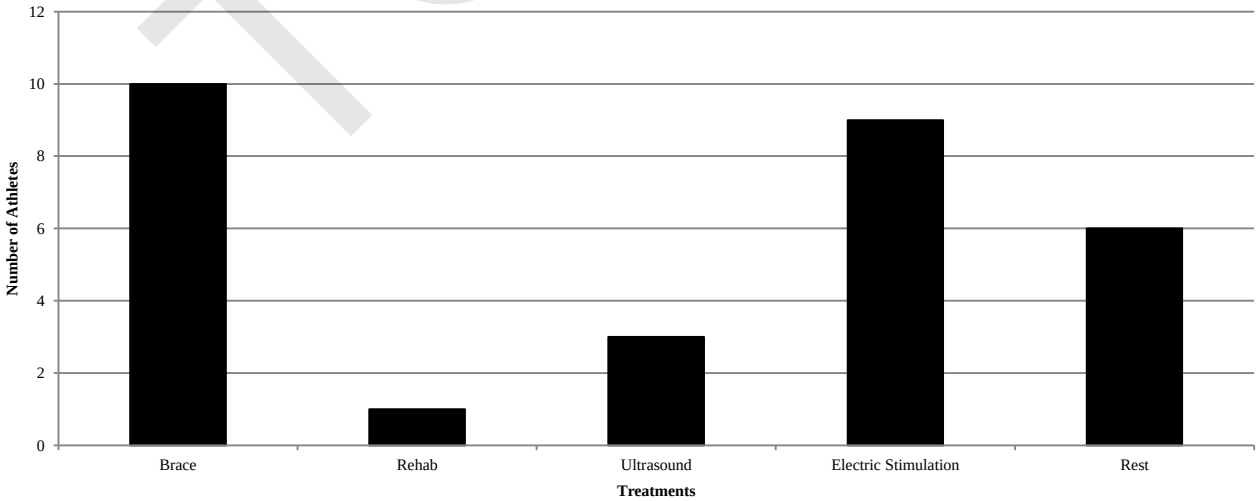
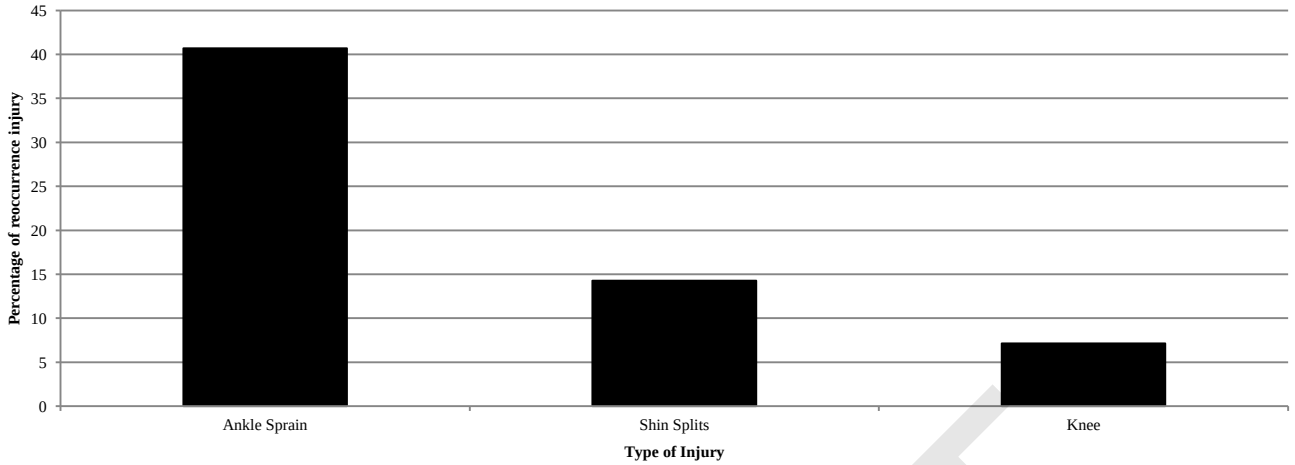


Figure 4 – Most prevalent post-rehabilitation reoccurrence injuries among college-aged women's and men's basketball and soccer teams.



SUMMARY OF RESULTS

The most common sports-related injuries, by percentage, were ankle sprains, knee injuries, and shin splits. The most common injuries were to the lower extremities, which accounted for 21% of our findings. In particular, ankle injuries (ankle sprains) were the most common type of injury. Bracing, electric stimulation, rest, and ultrasound were among the most common forms of treatment. 41% of the athletes still had reoccurrence injury to the ankle following treatment.

CONCLUSIONS

Based on the present study, ankle injuries are among the most prevalent injuries among college-aged athletes participating in load-bearing sports. Electric stimulation is the least effective treatment in rehabilitation of ankle sprains in preventing reoccurrence.

LIMITATIONS

Data obtained from subjects that did not fill out the survey in accordance to the directions were excluded from the study. While we understand this is a survey, all subjects were informed that all responses had to be completed to the best of their knowledge. While the data was collected from ODU women's and men's basketball and soccer teams, a larger pool of athletes would ensure more definitive findings as to injuries and treatments.

FUTURE STUDIES

Future studies would include surveying a larger pool of athletes over a wide range of sports. One possible study would be to survey NCAA athletes preceding their season. Additional studies could focus on discrepancies between gender-related injuries. Other research can focus on the effects of various playing surfaces on sports-related injuries.

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COSMETIC FORMULATIONS CONTAINING BLUEBERRY EXTRACTS (*VACCINIUM MYRTILLUS* L.)

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Abstract: The blueberry is a fruit originally native to North America. Consumption has increased globally, mainly due to its reputation for boosting health and longevity. Currently, the cosmetic products market is focused on formulations containing substances with antioxidant activity. The biological properties of the blueberry have already been linked to their polyphenolic content. The aim of this study was to develop and evaluate cosmetic formulations containing Brazilian freeze-dried blueberry extract. Extracts of ripe blueberry fruits were optimized varying the parameters time of extraction and heat, and temperature. The extract with higher polyphenolic content was lyophilised and added to a non-ionic cream in concentrations of 4% and 8%. Rheological behaviour, pH, spreadability, sensorial characteristics and free antiradical activity were tested. Preliminary results suggest that the formulation developed has potential as an antioxidant cosmetic product, though more analysis will be required before the product can go to market.

Key words: blueberry, cosmetic, antioxidant, formulations.

INTRODUCTION

The blueberry was introduced into Brazil in the 1980's by the Brazilian Agricultural Research Corporation (Embrapa/ CPACT, Pelotas/RS), with the main target cultivation area being in the south of the country due to the favorable climate conditions for its growth. The plant is described as a small bush bearing bittersweet berry fruits of a dark blue-purple coloration when ripe. Among those fruits studied having antioxidant potential, the blueberry shows a greater polyphenol concentration, both in the pulp and peel. High levels of anthocyanidins are found mostly in the water-soluble purple pigment of the peel. These molecules promote collagen synthesis, this being one of the main structural components of dermal connective tissue, providing benefits to the skin and also supporting the vascular system. Additionally, these antioxidant molecules are able to prevent the deleterious effects of oxidation, inhibiting the onset of lipid peroxidation, sequestering free radicals and protecting aerobic organisms from oxidative stress, which is defined as an increase in the formation of reactive oxygen species (ROS)(Colleti, 2009 ; Lüdke, 2007 ; H. Rodrigues & al., 2003 ; S. Rodrigues, Gularte, Pereira, Borges, & Vendruscolo, 2007; Silveira, Vargas, & Rosa, 2007).

Polyphenols are a secondary product of plant metabolism, constituting a complex phytochemical group of more than 8000 known structures. These substances are divided into: anthocyanins, flavans, flavanones, flavones, flavonols and isoflavonoids. The flavonoids are among the most important phenolic compounds found in the blueberry and present the greater therapeutic activity. From the age of 20 onwards, almost imperceptibly, the human skin begins to lose some properties of strength and self-regeneration. It is a slow and irreversible process, which varies according to skin type. This progression is dependent on several endogenous (chronological aging) and exogenous (photoaging) factors, with signs appearing from an age range as early as the thirties or more subtly being perceived by the sixties. The causes of skin aging are related to age, genetic tendencies, environmental factors and lifestyle (Buchli, 2002; Dalcin, Schaffazick, & Guterres, 2003 ; Krambeck, 2009). It is suggested by some researchers that imbalances in the body's antioxidant defense mechanism can be one of the main reasons for the aging process, with increases in ROS production leading to oxidative stress (Lima & ABDALLA, 2001). Thus, there is a constant preoccupation in cosmetology to prevent and mitigate skin aging by means of research and study of effective antioxidants agents. This study aimed to produce and evaluate potential antioxidant cosmetic formulations containing lyophilized (freeze-dried) Brazilian blueberry extract.

MATERIALS AND METHODS

Materials

Blueberry fruits were obtained from the city of Arvorezinha in Rio Grande do Sul, Brazil. Harvesting of the berries took place when they had reached the stage of maturity at which they would be sent to market, and only fruits showing no signs of damage, disease or pest attack were chosen. Subsequently, the fruits were refrigerated at -5±2ºC, before being crushed to obtain the extract. Analysis of the collected extract was performed three times.

Extraction optimization

Different parameters were investigated in order to achieve a blueberry extract rich in polyphenols for use in a cosmetic formulation. A hydroalcoholic solvent (10% v/v) was used, acidified to pH 3 with tartaric acid at a ratio of 1:2 (drug/solvent). The extraction parameters evaluated were: temperature, heating and maceration times (Table 1) (Magri & Heberlé, 2009) .

Table 1. Parameters for the blueberry extraction optimization.

Extracts	Maceration time (days)	Temperature (°C)	Heating time (min)
E 1	3	40°C	240
E 2	3	70°C	240
E 3	7	70°C	180
E 4	7	70°C	240
E5	7	70°C	300
E6	14	70°C	180
E7	14	70°C	240
E8	14	70°C	300
E9	21	70°C	180
E 10	21	70°C	240
E 11	21	70°C	300

Determination of phenolic compounds using the Folin-Ciocalteu method.

The total phenolic compound of extracts was determined using the Folin-Ciocalteu reagent, following the method described by Singleton and Rossi (Singleton & Rossi, 1965). Sample readings were made using a spectrophotometer (Cary 100 Bio, Varian) at 765 nm. The total phenolic compound content was obtained through building a calibration curve, using gallic acid in concentrations of 50 to 500 $\mu g.mL^{-1}$, as the standard substance with results being expressed in mg of EGA (equivalent to gallic acid) per L of extract.

Extract Lyophilization

The tested extract with the biggest total polyphenol content was freeze-dried (Lyophilized Liotop Model: L202) and frozen at -30°C.

Emulsion Preparation

A non-ionic base cream as described in the National Formulary was chosen as the base for the formulation. The aqueous phase was heated to 80ºC in a glass beaker using a magnetic stirrer with heater (Model MA-085, Marconi), and the oil phase melted in a porcelain mortar using a hot water bath (Model MA-156, Marconi) at 75ºC. The aqueous phase was then poured over the oil phase and manually stirred until cool (Brasil, 2005) , with the lyophilized blueberry extract being subsequently incorporated. Two preparations were produced with each containing concentrations of 4% and 8% blueberry extract, respectively.

Organoleptic characteristics

Each preparation was evaluated for appearance and color. A visual assessment was made by adding a sample portion to a glass plate, placed over a white background, and comparing it to the original non-ionic base cream (Brasil, 2004).

pH Determination

The pH of each preparation was determined using a sample diluted by purified water (1:10 p/v) obtained by reverse osmosis (Marconi), and using a previously calibrated pH meter (DM-20, Digimed) (Amaral & Vilela, 2003; Brasil, 2010 ; Gil, Matias, & Serrano, 2005).

Assessment of Spreadability

The sample was applied to a glass support plate (20cm x 20cm) positioned over a sheet of graph paper and centralized using a circular plate with a central hole. This plate was subsequently removed and replaced at one minute intervals with plates of predetermined weights, with the diameter of the spread of the sample being measured. The spreadability (Ei), at 25° C is calculated by the equation: (Isaac *et al.*, 2008 ; Spellmeier & Heberlé, 2007; Zanin, Miguel, Chimelli, & Dalmaz, 2001).

$$Ei = d2 \times \pi / 4$$

Where: Ei: spreadability of sample to weight i (mm^2); d: mean diameter (mm).

Viscosity Determination

A study of the rheological behavior of the formulations was made with a rotational viscometer (DV-I+, RV series, Brookfield) using the spindle SC4-29, inserted on a sample without air bubbles and with a stable temperature (Brasil, 2004). This research evaluated viscosities from $0.1s^{-1}$ to $500s^{-1}$, and from $500s^{-1}$ to $0.1s^{-1}$, with a 1 minute delay between measurements.

Activity of free antiradicals

This analysis was made through use of the stable free radical DPPH (2.2-diphenyl-1-picryl-hydrazyl-hydrate) (Elmastas *et al.*, 2006). A DPPH methanolic solution of $50.0\mu g.mL^{-1}$ was prepared, with 1.0mL then being added to a 3.0mL methanolic solution of the formulation samples, at concentrations of $1.0\mu g.mL^{-1}$, $5.0\mu g.mL^{-1}$, $20.0\mu g.mL^{-1}$, $40.0\mu g.mL^{-1}$, $60.0\mu g.mL^{-1}$ and $100.0\mu g.mL^{-1}$. The mixtures were vigorously stirred and kept in the dark at room temperature for 30 minutes. After this time, the absorbance of the samples (n=3) and of a control (1.0mL solution of DPPH $50.0\mu g.mL^{-1}$ with 3.0mL of methanol) were read by a spectrophotometer (Cary 100–Bio, Varian) at a wavelength of 517 nm, which corresponds to the

maximum absorption for the free radical being used. Methanol was used as blank solution (Lange, Heberlé, & Milão, 2009). The ability of the extracts to reduce the free radicals is calculated according to the equation:

% inhibition of DPPH = [(A0 – A1) / A0 x 100]
Where:
A0 = absorbance of the control reaction
A1 = absorbance of the samples.

Statistical Analysis

Anova and Tukey's test was used to analyze the obtained results with a degree of confidence of 95% (p=0.05), through the Statistical Support System, SAE program (Ahlert, 2005).

RESULTS AND DISCUSSION

Extraction optimization

Overall, the extraction parameters affect the quality of the extraction solutions. According to Figure 1 the extract E2 showed the greatest polyphenol content (1420.45mg/L ± 9.39).

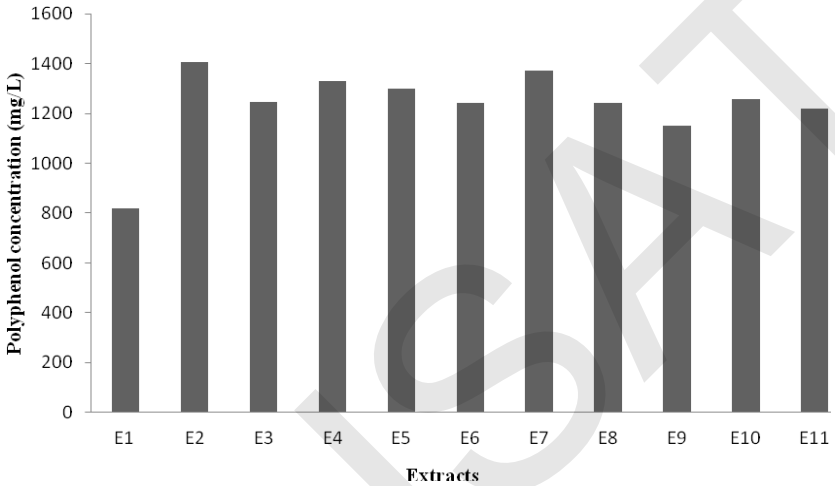

Figure 1: Total polyphenol content of extracts tested.

Extract lyophilization

The lyophilized extract maintained the purple color of the liquid extract, presented a pH 3.72 ± 0.02 and had a yield of 3%.

Organoleptic characteristics

The product containing 4% extract presented a pink color and glossy appearance, with this color becoming more intense in the product containing an 8% concentration of extract.

pH determination

The formulation containing 4% extract of lyophilized blueberry had a pH of 3.32 (±0.006), whilst that containing 8% extract had a pH of 3.20 (±0.01). There was no statistically significant difference between the pH values of the formulations, however, these values are not suitable for dermatologic use as products remaining on the skin for prolonged periods must have a pH of between 5.5 and 6.5, compatible with the pH of the human skin (Isaac, *et al.*, 2008). Therefore, an adjustment was made to the pH of the formulations with the addition of 10µL and 20µL of AMP-95 to the products containing 4% and 8% extract, giving pH values of 5.52 (±0.08) and 5.60 (±0.07), respectively.

Determination of viscosity and spreadability

Rheology is the study of the flow or deformation of a material when subjected to a tension. It is important for quality control of the intermediate or final product as well as determining the shelf life and product acceptability to the consumer, and includes the analysis of viscosity and spreadability parameters. The viscosity of a fluid is given as its resistance to flow or movement. The higher the viscosity, the slower the speed at which the fluid moves, having a lower spreadability. The formulations showed non-Newtonian behavior (Figure 2) as the curve does not pass through the origin but intersects the shear stress axis, called the transfer value, and characteristic of plastic material where the flow does not begin until its transfer value is reached. The viscosity of a plastic decreases with increasing shear rate and this behavior makes the formulations suitable for topical use, making it easier to use and requiring the application of pressure to start the flow, thus preventing container leakage (Mariott, 2005, Sinko, 2008).

The thixotropy can be evaluated by the area of hysteresis on the rheogram (Figure 2), where the descending curve appears shifted to the left of the ascending curve. This parameter indicates the ability and time it takes for the formulation to return to its structure after the removal of applied tension. Achieving topical formulations with a thixotropic character is very desirable as they become more fluid during application, making it easier to spread but recovering the initial viscosity as soon as application has ended, thus avoiding product leakage. However, it is important that the thixotropic value is not too high as the product will run off the skin after application due to very slow recovery of its structure (Gaspar & Maia Campos, 2003; Mariott, 2005; Sinko, 2008). The formulations analyzed presented similar rheological characteristics with no significant differences between the base values of maximum viscosity.

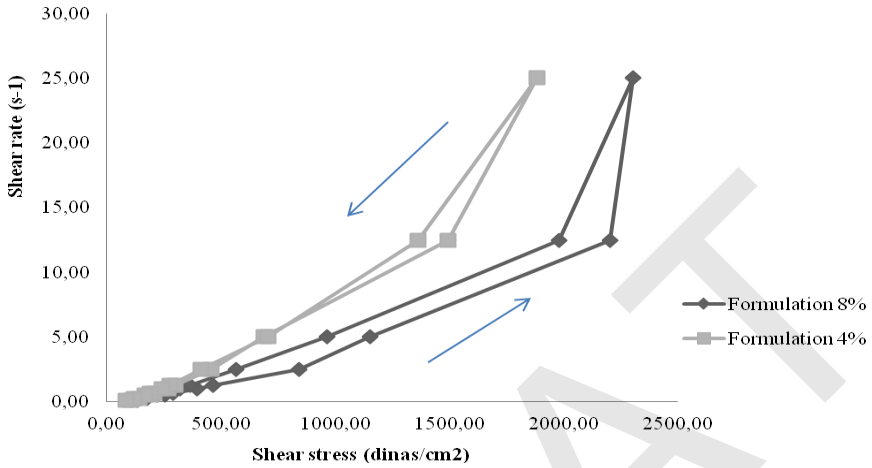

Figure 2: Rheogram of the produced formulations with 4% and 8% lyophilized blueberry extract.

Figure 3 shows the spreadability of formulations with both showing similar behavior and no significant difference between the base values of maximum spreadability.

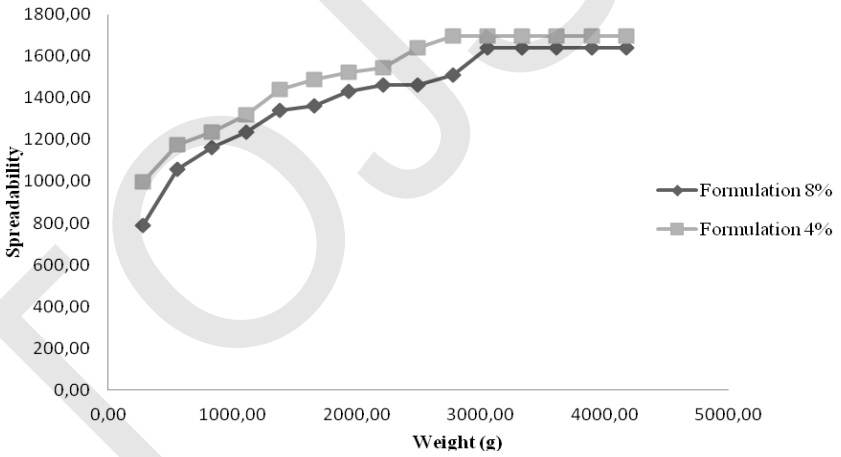

Figure 3. Spreadability of produced formulations with 4% and 8% lyophilized blueberry extract.

Determination of antioxidant activity

The evaluation model for antioxidant activity uses DPPH based on the ability of the stable free radical 2.2-diphenyl-1-picryl-hydrazyl-hydrate to react with substances that donate hydrogen, including compounds. (Mensor *et al.*, 2001). The antioxidant activity of lyophilized blueberry extract was compared with the standard natural and synthetic quercetin and BHT antioxidants (Figure 4). At the highest concentration, the extract demonstrated DPPH sequestering ability (radical scavenging activity) similar to the standard, however statistically significant differences were observed for all tested concentrations (Figure 4).

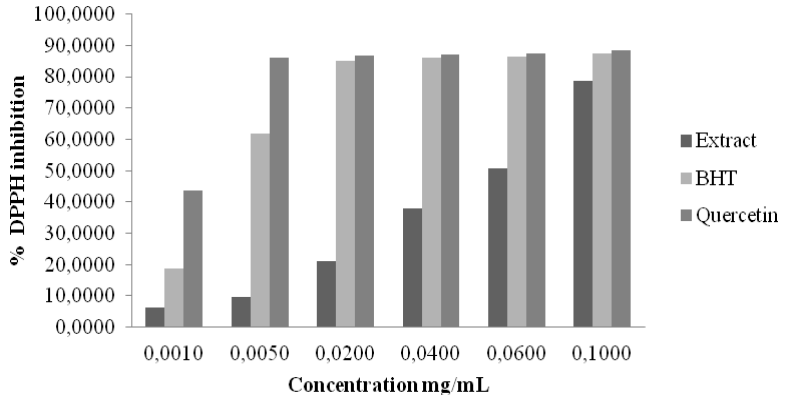

Figure 4. Antioxidant activity of blueberry extract, BHT and quercetina.

The results for the formulations with 4% extract, including the form alkalinized with AMP-95, showed a significant difference only at a concentration of 0.0200 mg/mL (Figure 5). No significant difference for all concentrations was observed in the formulations containing 8% extract. These results demonstrate that adjusting the pH did not alter the antioxidant activity.

When comparing the antioxidant activity of the prepared formulations with two anti-aging skin products, one national and one imported, they produced the same inhibition of free radicals in comparison to the imported cosmetic product for the two lower concentrations, as shown in Figure 5. As regards the national cosmetic product, the formulations also showed no significant difference for the highest concentration, and for the other tested concentrations showed a higher antioxidant activity than both the national and imported cosmetic products.

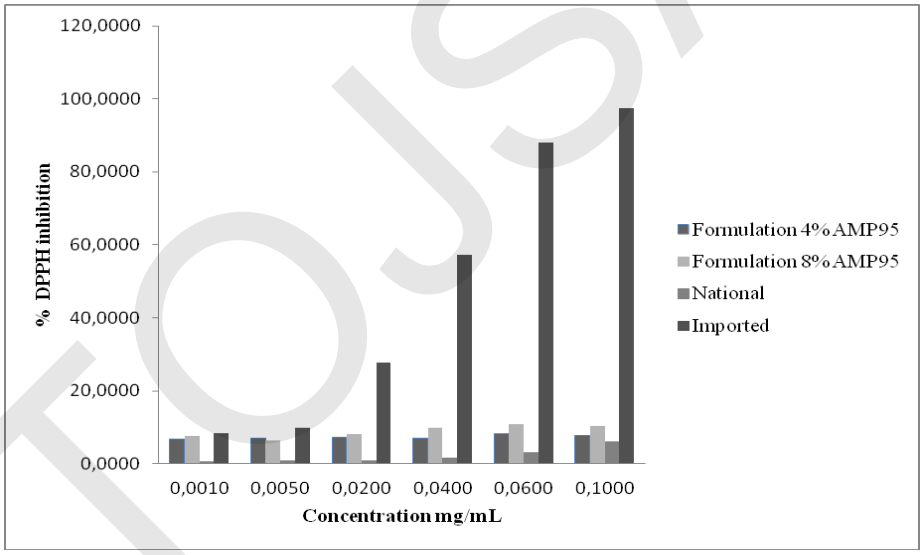

Figure 5. Antioxidant activity for the alkalinized formulations and the national and imported cosmetic product.

CONCLUSIONS

It can be concluded from the tests that the formulations containing 4% and 8% freeze-dried blueberry extract showed no significant difference in relation to spreadability and viscosity. In terms of antioxidant activity there was a difference only at the concentration of 0.06mg/mL. Adjusting the pH of the formulations did not alter their antioxidant activity, and they presented a superior antioxidant activity than the national cosmetic product analyzed. Based on these results it would appear that the formulations produced for this study show potential for development as antioxidant cosmetic products, though additional research is needed to continue the process of their development as an anti-aging product, such as those found on the market.

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EVALUATION OF THE NUMBER OF TRANSFER UNITS(NTU) AND THE COLUMN HEIGHT BY USING ON-LINE TEMPERATURE MEASUREMENTS FOR A PILOT SCALE PACKED BATCH DISTILLATION COLUMN

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Abstract: There have been relatively few experimental attempts at evaluating the column height and comparing experimental results obtained from a pilot scale packed batch distillation column with theoretical ones.

In the present study, the pilot plant packed batch distillation column was used to distillate the binary methanol-water mixture, and to obtain on-line temperature values. The top temperature changes with time were observed at steady-state and dynamic conditions. The column was operated initially for approximately one hour at the total reflux. In this case, There were no feed and product flows. Temperature samples were taken on-line from the top and bottom of the column. Temperature profiles observed on the computer were recorded. Refractive index of the samples were determined. When the temperatures were constant, the system was at a steady-state condition for total reflux. After the system reached the steady-state condition the reflux ratio was adjusted to a certain level. From experimental top and bottom temperature values, two models were obtained between mol fraction and temperature. The mass transfer coefficient was calculated, and the final mol fraction of reboiler was determined. The column height was calculated and compared with the real packed height. It is noted that the equation used for mass transfer coefficient is considerable well.

Keywords: Batch distillation, packed column, packed height, mass transfer coefficient

INTRODUCTION

The investigation of distillation columns is mainly based on two different approaches: fundamental studies, for example concerning mass transfer behaviour of packed sections, etc. and alternatively, integral studies on columns provided with trays or packing (Elgue, Prat, Cabassud, Lann and Cezerac, 2004; Zuiderweg, 1999). There has been many articles to produce a certain amount of product at desired composition either in minimal production time or with maximal economic profit (Betlem, Krijnsen and Huijnen, 1998; Muddu, Narang and Patwardhan, 2010). The investigation of column performance can be identified by studying the liquid composition profile.

The batch distillation have been widely used in the separation processes because of its easy operation and maintenance. Noda, Kato, Chida, Hasebe and Hashimoto (2001) discussed the optimal structure and operation of a batch distillation column separating ternary mixture from the viewpoint of energy conservation, and derived the optimal reflux operation for three types of batch distillation columns; rectifying, stripping and total reflux columns. They noted that the energy consumption of the total reflux column is reduced when the optimal operation is used. Kim and Han (1999) showed that the dynamic model gives an accurate design for the operation of a batch distillation column. The variations of reflux ratio and top product composition are obtained.

Sadeghifar and Kadri (2011) developed a general and accurate method for efficiency calculation of the distillation columns packed with structured packings. They noted that for distillation columns with a large number of components (except for total reflux conditions), it is too difficult to obtain the realistic value of the experimental efficiency. An approximate estimation of the experimental efficiency is also sometimes impossible. Senol (2001) suggested that the increased amount of effectives area in randomly packed distillation column, as compared with the wetted one, is due to tendency toward rippling, wave and droplet formation in the falling liquid film. It is shown that the observed and predicted behaviors of the relative proportion of effective interfacial area are overly sensitive to the vapor and liquid loads, as well as to the packing properties.

Rejl et. al. (2006) measured volumetric mass transfer coefficients in liquid an vapour phases in distillation column by the method consisting of a fitting of the concentration profile of liquid phase along the column obtained by the integration of a differential model to the experimental one. It is noted that the concentration profiles obtained by the integration of the differential model of the distillation column using the coefficients from absorption correlation are differed from the experimental profiles. Liu, Yu, Yuan and Liu (2009) proposed a numerical method for modeling the distillation process in a randomly packed column. They showed that the predicted height equivalent of theoritical plate of the distillation column concerned is in satisfactory agreement with the reported experimental data.

In the present study, it is shown that mass transfer coefficient and the column height can be predicted much more simply using experimental top and bottom temperature data while being able to achieve the required separation in a pilot scale packed batch distillation column.

METHODS AND PROCEDURES

Distillation system consists of a glass flask (100 L), a heating mantle (2000 W), a packed 80 mm internal diameter column filled with 1 in Rasching rings, a valve adjusting reflux ratio at the top of the column and a condenser. System has two thermocouples to measure the temperature of top product and the boiler (See Figure 1).

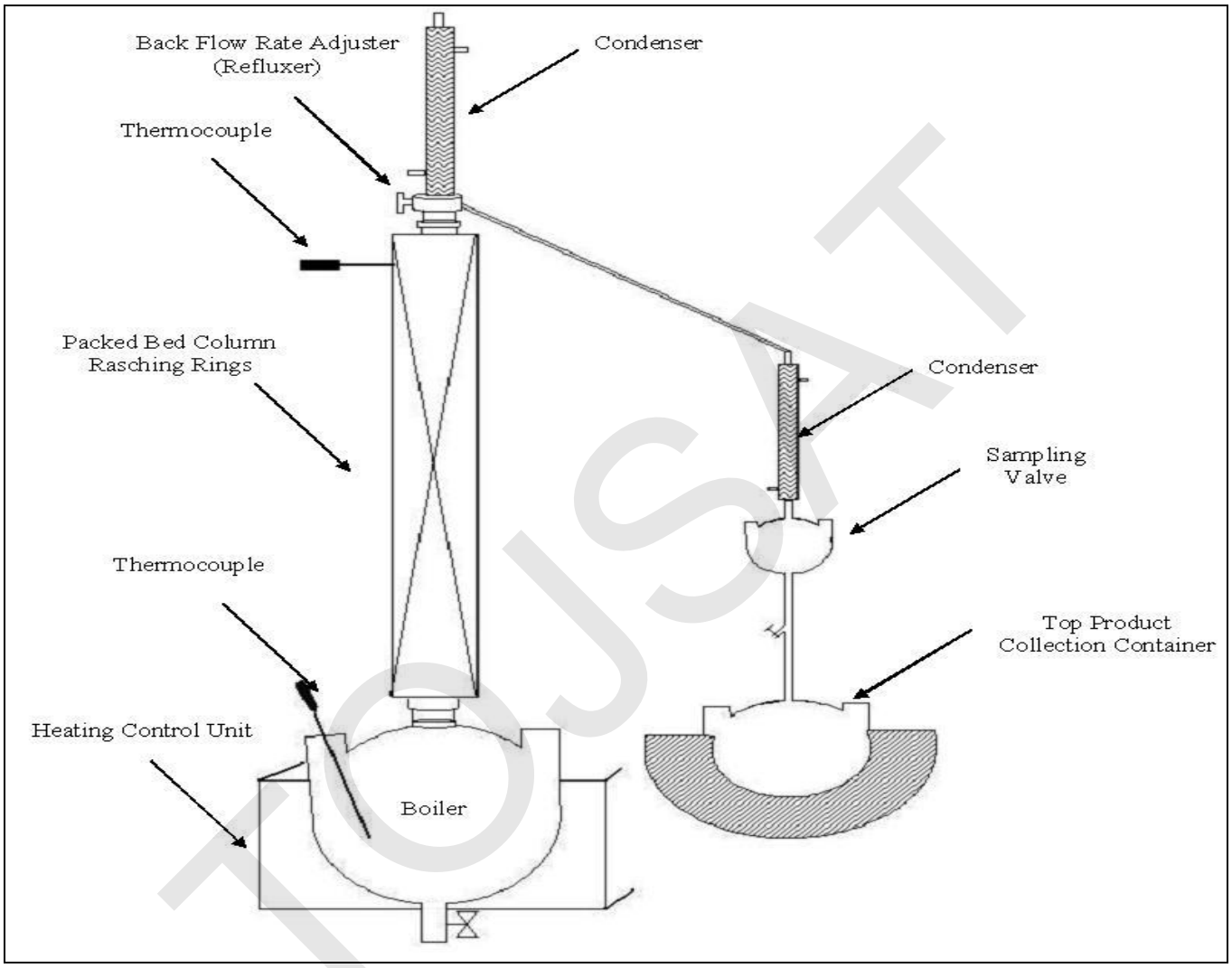


Figure 1. Experimental system

To operate the experimental system, certain volume and concentration of methanol-water mixture is filled into boiler. Cooling water inlet valve opening is provided for condenser. The button on the distillation column control panel that gives energy to the system is turned on. Thus, heating mantle begins to heat the mixture in the vessel. Batch distillation is operated at the total reflux until the steady state condition occurs. When the steady-state condition is reached in the column, reflux valve is adjusted to a desired value and the top product is obtained by batch distillation. Experimental data for the distillation column is given in Table 1.

Table 1 Experimental data for distillation column

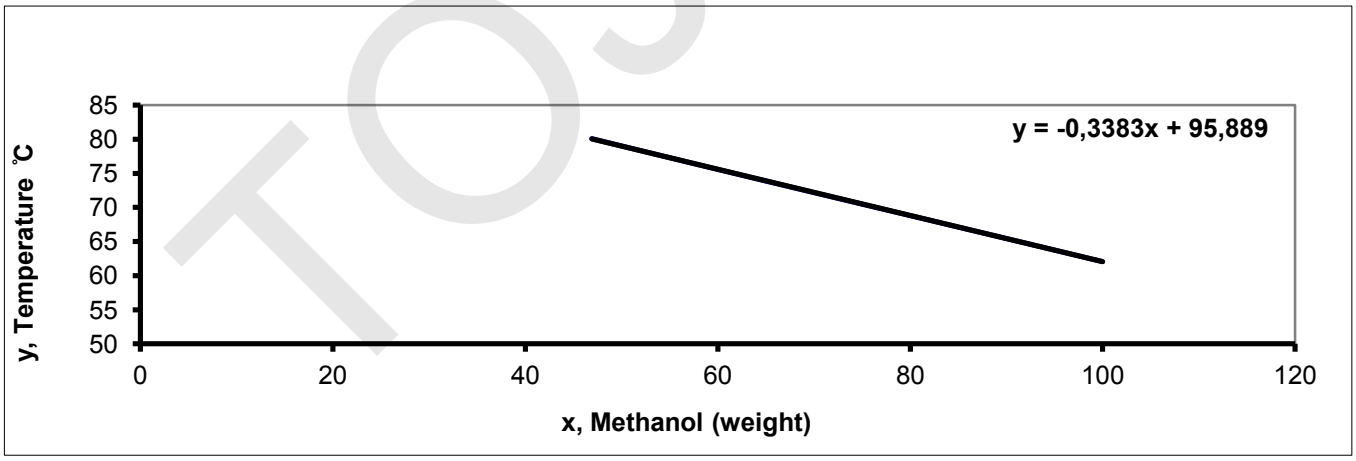
Initial reboiler amount (F)	55 L
Initial reboiler methanol mol fraction (Xs_1)	0.222
Reboiler temperature	81.7 °C
Top product flow rate (D)	0.14 mL/s
Total top product amount (d)	2.726 L
Vapour flow rate (V)	0.24 mL/s
Liquid flow rate (L)	0.10 mL/s
Average top product temperature	67.8 °C
Average top product density (ρ_{ort})	910.407 kg / m^3
Average top product molecular weight (M_w)	21.559 kg / kmol
Reflux ratio	0.682

RESULTS

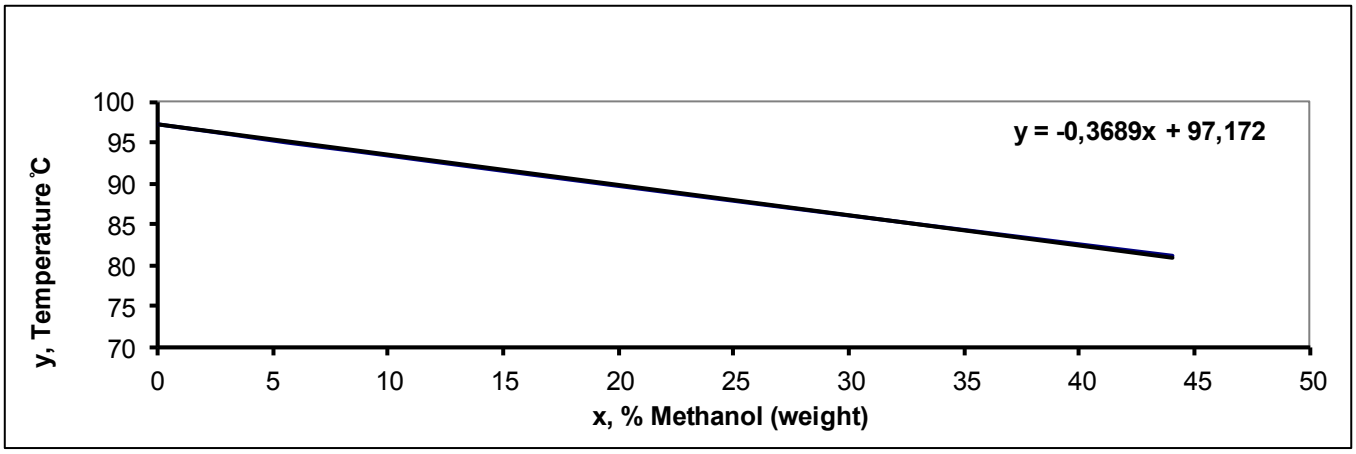
55 L mixture with 22% methanol concentration is fed to boiler. The temperature change is monitored during the distillation (Table 2). Samples are taken from the product collection container at a certain time intervals (10 min). The sample concentrations are determined by reading the indices of refraction from refractometer (see Figure 2 and Table 2). At the same time the top product is read on the temperature indicator.

Table 2 Experimental temperature data and calculated methanol mole fractions

Time (min)	Top Product Temperature (°C)	Reboiler Temperature (°C)	Calculated Top Product Methanol Mole Fractions X_D (See Figure2-b)	Intercept: $\frac{X_D}{R+1}$	Calculated Reboiler Methanol Mole Fractions X_S (See Figure2-a)	$\frac{1}{X_D - X_S}$
0	60.4	81.7	0,994	0,591	0,530	2,155
10	60.9	82.0	0,971	0,577	0,400	1,751
20	63.2	82.4	0,868	0,516	0,220	1,543
30	66.3	82.5	0,743	0,442	0,150	1,686
40	71.9	82.6	0,550	0,327	0,080	2,128
50	72.6	82.7	0,529	0,315	0,070	2,179
60	73.0	82.7	0,517	0,307	0,065	2,212



(a)



(b)

Figure 2 . Calibration plots a) for reboiler b) for top product

At the end of a certain operating time, to measure the flow rate of steam, refluxer is fully opened and the top of the product volume collected per unit time is measured in container. The concentration of the bottom product is determined by reading reflactive index. Total top product volume is measured at the end of the distillation time.

Equations used to calculate top product and reboiler methanol mole fractions (X_D and X_S) are given as follows:

Calibration Equation for Top Product **:** Y = -0.3689*X + 97.172

(3.1)

$$X_D = \frac{(X_A/M_A)}{\left(\frac{X_A}{M_A}\right) + \left(\frac{(100 - X_A)}{M_B}\right)}$$

(3.2)

Calibration Equation for Reboiler : Y = -0.3383*X + 95.889

(3.3)

$$X_S = \frac{(X_A/M_A)}{\left(\frac{X_A}{M_A}\right) + \left(\frac{(100 - X_A)}{M_B}\right)}$$

(3.4)

Initial methanol amount (S_1) in the reboiler before the distillation is determined as:

t = 0 $\rho_{methanol}$ = 721.6 kg/m^3 , ρ_{water} = 970.2 kg/m^3

Average Molecular Weight (Mw) = 0.283 * 32.04 + 0.717 * 18.02 = 21.986 kg / kmol

(3.5)

Average Density (ρ_{av}) = $0.283 * 721.6 + 0.717 * 970.2 = 899.871\ kg/m^3$

(3.6)

$$S_1 = \frac{\rho_{av} * V}{M_w} \Rightarrow S_1 = \frac{\left(899.871\ \frac{kg}{m^3}\right) * (55 * 10^{-3} m^3)}{(21.986\ kg/kmol)} = 2.251\ kmol$$

(3.7)

Methanol amount(S_2) in the reboiler after the distillation is evaluated as below:

Amount of mixture in the boiler ; V = F - D V = 55 – 2.726 = 52.274 L

(3.8)

t = ∞ $\rho_{methanol}$ = 741.3 kg/m^3 , ρ_{water} = 967.5 kg/m^3

Average Molecular Weight (Mw) = 0.2524 * 32.04 + 0.7476 * 18.02 = 21.559 kg / kmol

(3.9)

Average Density (ρ_{av}) = $0.252 * 741.3 + 0.748 * 967.5 = 910.407\ kg/m^3$

(3.10)

$$S_2 = \frac{\rho_{av} * V}{M_w} \Rightarrow S_2 = \frac{\left(910.407 \frac{kg}{m^3}\right) * (52.274 * 10^{-3} m^3)}{(21.559\ kg/kmol)} = 2.207\ kmol$$

(3.11)

$$V = 0.24 \frac{ml}{s} * 3600 \frac{s}{hr} * 910.407 \frac{kg}{m^3} * \frac{1 m^3}{10^6 ml} * \frac{1\ kmol}{21.559\ kg} = 0.037 \frac{kmol}{hr}$$

(3.12)

$$L = 0.10 \frac{ml}{s} * 3600 \frac{s}{hr} * 910.407 \frac{kg}{m^3} * \frac{1 m^3}{10^6 ml} * \frac{1\ kmol}{21.559\ kg} = 0.015 \frac{kmol}{hr}$$

(3.13)

D = V – L = 0.037 – 0.015 = 0.022 kmol / hr

(3.14)

Column Cross-sectional Area= $S = \pi * (0.08)^2 / 4 = 0{,}005\ m^2$

(3.15)

$$G_y = \frac{V}{S} = \frac{0.037}{0.005} = 7.4 \frac{kmol}{m^2.hr} \quad and \quad G_x = \frac{L}{S} = \frac{0.015}{0.005} = 3.0 \frac{kmol}{m^2.hr}$$

(3.16)

Mass transfer coefficient is written as follows (Sahay and Sharma,1973; Karacan, Hapoğlu, Cabbar, and Alpbaz, 1997)

$$K_y a = 1.28 * 10^{-5} * (V)^{0.64} * (L)^{0.48}$$

(3.17)

$$K_y a = 1.28 * 10^{-5} * (7.4 * 10^3)^{0.64} * (3.0 * 10^3)^{0.48} = 0.179 \frac{kmol}{m^2.hr}$$

(3.18)

To determine Height of Transfer Units (H_{Theo}) and Number of Transfer Units (N_{Theo}) for multistage batch distillation, the equations given below and Table 3 and 4 are utilized.

$$In \frac{S_1}{S_2} = \int_{X_{S2}}^{X_{S1}} \frac{dX_S}{X_D - X_S} = (1 | X_D - X_S)_{average} * (X_{S1} - X_{S2}) \Rightarrow 0.01974 = 1.54 * (0.222 - X_{S2})$$

(3.19)

$S_1 / S_2 = 0.014$, $X_{S1} = 0.222$, $X_{S2} = 0.209$

$$y_{average} = \frac{S_1 * X_{S1} - S_2 X_{S2}}{S_1 - S_2} \Rightarrow y_{average} = 0.874$$

(3.20)

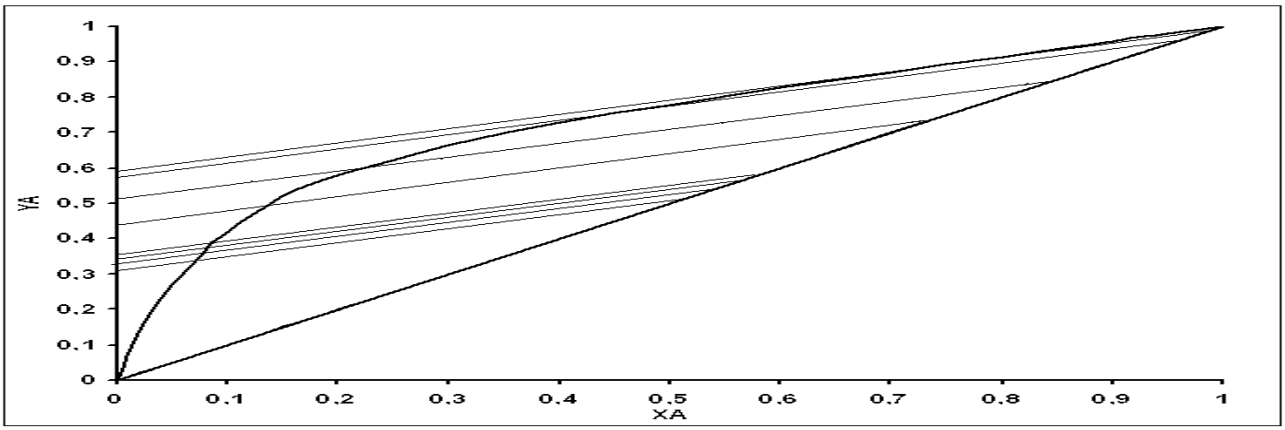


Figure 3. Equilibrium curve and Operation lines for the case studied

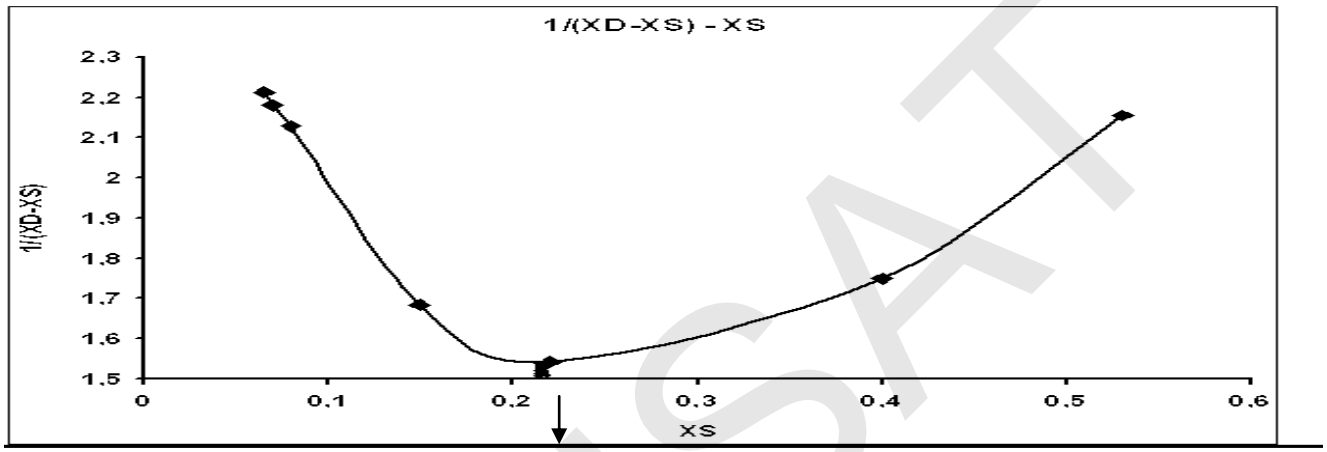


Figure 4. Relationship between $1/(X_D-X_S)$ and X_S values.

$$H_{Theo} = \frac{G_Y}{K_y a} = \frac{7.4}{0.179} = 41.37 \quad (3.21)$$

$$N_{Theo} = \int_{y_1}^{y_2} \frac{dy}{y - y^*} = \frac{y_2 - y_1}{\Delta y_L} = N_{Theo} = \frac{(y_w - y_F)}{\Delta y_L} = \frac{(0.209 - 0.222)}{-0.379} = 0.0343 \quad (3.22)$$

$$\Delta y_L = \frac{(y_2 - y_2^*) - (y_1 - y_1^*)}{In\frac{(y_2 - y_2^*)}{(y_1 - y_1^*)}} = \Delta y_L = \frac{(0.209 - 0.59) - (0.222 - 0.60)}{In\frac{(0.209 - 0.59)}{(0.222 - 0.60)}} = -0.38 \quad (3.23)$$

Column Height (Z) is determined as follows:

$$Z_{Theo} = H_{Theo} * N_{Theo} => \ Z_{Theo} = 41.37 * 0.0343 = 1.42\ m \quad (3.24)$$

The column height is evaluated as 1.42 m and by comparing with the real packed height of 1.25 m, correction factor (e) is calculated for the column studied as follows:

$$Z_{Real} = Z_{Theo} * e \ => \ e = \frac{Z_{Real}}{Z_{Theo}} = 0.88 \quad (3.25)$$

it is shown that the equation used for mass transfer coefficient can be written as follows:

$$K_y a = e^{-1} * 1.28 * 10^{-5} * (V)^{0.64}(L)^{0.48}$$
(3.26)

CONCLUSION

The evaluated packed distillation column height is in satisfactory agreement with the real one. The separation efficiency of packed column is generally expressed in terms of height equivalent of theoritical plate (HETP). For distillation at total reflux, HETP may be calculated by the following equation:

$$HETP = H_{Theo} * e = (Z_{Theo} * e)/N_{Theo} = Z_{Real}/N_{Theo}$$
(3.27)

This work provides a new tool for those concerned with the design or batch performance of packed distillation columns. It gives a simple method to assess the sensitivity of a packed bed to batch distillation.

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ISOLATION, CHARACTERIZATION AND MICROENCAPSULATION OF PROBIOTIC *Lactobacillus curvatus* G7 FROM CHICKEN CROP

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Abstract: The controlled release of bioactive substances to their site of action in the GIT is essential in modern drug and food industries. The major obstacles that probiotic bacteria should overcome are stomach acidity and bile salts. In this research a *Lactobacillus curvatus* strain was isolated from chicken crop; it was identified based on morphological and biochemical characteristics and tested for its probiotic properties. Furthermore; the survival of free and microencapsulated *Lb curvatus* in 1 % sodium alginate was evaluated in GIT-like conditions. The results showed that 27.87 % of the free cells were found to be resistant to acidic conditions (pH 2) after 1 hour of incubation, while only 2.09 % survived after 2 hours of incubation therefore the bacteria could not be capable of resisting in the stomach. Microencapsulation improved the viability particularly after 2 hours for the reason that 11.36 % of the cells survived after 2 hours. On the other hand, in bile salts, the percentage of survival of the free cells of *Lb curvatus* was 47 after 4 hours of incubation and decreased to 40 after 8 hours. However, the microencapsulated form resists more since 66 % of the cells survived after 4 hours and more than 52 % survived in bile salts after 8 hours. It appears evidently that cell entrapment in sodium alginate protects the bacteria from gastric and intestinal hostile conditions.

Keywords: *Lactobacillus curvatus*, probiotic, microencapsulation, chicken crop

INTRODUCTION

Probiotics are defined by the FAO as "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host" **(FAO report).**

Currently, probiotics are being used extensively in veterinary to replace the use of antibiotics. In poultry farming, probiotics are essentially used to provide beneficial microorganisms that were basically absent in chicken's digestive tract, thus, the least can profit by favorable effects offered by the introduced microorganisms **(Lutful Kabirm, 2009; Gournier-Château et *al*., 1994)**. The two main commercial preparations are targeting the crop and the anterior small intestine as well as the caecum **(Fuller et Turvey, 1997).** The effects of some probiotic bacteria were reported; they include modification of the microbial composition and metabolic activity of the intestinal flora, inhibition of infective pathogens like *Escherichia coli, Salmonella typhimurium* and *Staphylococcus aureus* by competitive exclusion, and enhancing the growth and development indexes in chicken **(Higgins et al., 2010, Awad et al. 2009, Reque et al. 2000).** However, during GI passage, cultures are required to tolerate the low pH of the stomach, and the antimicrobial activity of bile salts, for that reason, it is important to find methods for enhancing the viability of microbial cells in the digestive tract, one of them is microencapsulation which consists of the technology for packaging active materials in miniature, sealed capsules that can release their contents at controlled rates under specific conditions according to **Shahidi and Han (1993)**. Several studies have shown that microencapsulation of bacteria with alginate at different concentrations or other gels protects them against acid stress, allowing the cells to survive in the stomach and to be delivered in the intestine **(Lee et al. 2004, Crittenden et al. 2006).** Generally, most of the researchers are in agreement that alginate is the most suitable material for encapsulating food ingredients even though the recent studies are providing new improvements in capsule texture and rheology characteristics.

In the present study, a lactic acid bacterium from the crop content of chickens was isolated, identified and assessed for its ability to inhibit the growth of some pathogenic bacteria and to attach to intestinal epithelium. In addition, the tolerance of the bacterium to GIT-like conditions was evaluated before and after microencapsulation in 2 % sodium alginate.

MATERIALS AND METHODS

Isolation of lactic acid bacteria

A 10 g sample of the content of local chicken crop was serially diluted in normal saline, then; the appropriate dilutions were plated on MRS agar and incubated for 24 hours at 37°C. The obtained colonies were cultured in MRS broth and further purified.

Test organisms

The following strains were used as test organisms for antimicrobial activity; *Escherichia coli, Klebsiella* spp. from a local rabbit GIT, *E. coli* ATCC 25929 and *Staphylococcus aureus.*

Identification of LAB

The isolated strains were identified based on their morphological and biochemical properties according to **Bergey (1994).** The tests included gram stain, catalase, arginine dihydrolase, acetoine production, citrate utilization, growth in hypersaline solution, fermentation type and sugars fermentation. The obtained results were analyzed by API-LAB program at the "Laboratoire de Biologie des Microorganismes et Biotechnologie" at Es-Senia University, Oran.

Antibacterial activity

The antibacterial activity of Lb. curvatus culture, the cell-free supernatant and the NaOH neutralized supernatant (pH 6) against the cited bacteria was evaluated according to **Tagg et al. (1976)** based on disc diffusion method.

Assay of the *in vitro* adherence of LAB to epithelial cells

The method described by Lin et al. 2007 was used for the assay of the in vitro adherence of LAB to epithelial cells. Segment of chicken crop were opened and washed with sterilized phosphate-buffer saline (PBS, pH 7.2). It was held in PBS at 4 °C for 30 min to remove the surface mucus and then washed three times with PBS. Epithelial cells were scrapped into sterilized PBS. The cell suspension was examined by microscopy to ensure that contaminated bacteria had been removed and the epithelial cell concentration was adjusted to approximately $5x10^4$ cells/ml. The adherence of LAB strain to the epithelial cells was assayed as follows: the overnight culture of LAB in MRS broth was centrifuged and the cell pellet was resuspended to approximately 1.108 CFU/ml in PBS (pH 7.2). One milliliter of the bacterial suspension was mixed with 1 ml of the suspension of epithelial cells from chicken. The mixture in a tube was rotated at 20 rev/min at 37 °C for 30 min. The adhesion was observed using light microscopy (magnification fold, 100x) after stained with 0.5% crystal violet for 5 min **(Lin et al. 2007)**.

Microencapsulation of LAB in 2% sodium alginate

Alginate (2 % w/v) capsules containing the *Lb. curvatus* cells were prepared by dissolving 2 g of sodium alginate in 80 mL distilled water under constant mechanical stirring, and heating at 80ºC. The solution was autoclaved and cooled to 40ºC to which 20 mL of a freshly prepared cell suspension was added and homogenized. The final solution contained approximately 88.10^{11} UFC/mL. The mixture was injected through a needle into 100 mL of autoclaved and pre-cooled 0.05M CaCl2 crosslinking bath. The resultant capsules were allowed to harden in the cross-linking solution for 30 min, and then washed three times with distilled water **(Boyaval *et al.*, 1985)**.

Survival of LAB in acidic conditions

The viability of free and microencapsulated cells of *Lb. curvatus* in acidic conditions was tested by incubating MRS broth (pH 2) inoculated with approximately 10^{10} UFC/ml (free or encapsulated cells) for 2 hours at 37° C. A viable count on MRS agar was carried out at 1h intervals over the assay period after appropriate serial dilution in normal saline. The plates were incubated at 37ºC for 48 h. For microencapsulated cells, the count was determined after lysis of the capsules in 2M M phosphate buffer (pH7)

Tolerance to bile

The viability of free and microencapsulated cells of *Lb. curvatus* in bile conditions was studied by incubating MRS broth supplemented with 0.3% bile salts with approximately 10^{10} UFC/ml (free or encapsulated cells) for 8 hours at 37° C. A viable count on MRS agar was carried out at 1h intervals over the assay period after appropriate serial dilution in normal saline. The plates were incubated at 37ºC for 48 h. For microencapsulated cells, the count was determined as described before.

RESULTS AND DISCUSSION

Eighteen strains were isolated on MRS medium from chicken crop, after biochemical identification it appeared that most of them belonged to *Lactobacillus curvatus. Lactobacillus curvatus* J7 was chosen for further investigations.

Antimicrobial activity test

The antimicrobial activity of the selected bacterium against some bacteria was evaluated in three ways in order to determine the nature of the inhibitory element. The crude culture, the crude cell-free supernatant as well as the neutralized supernatant were used to analyze the antagonistic effect; the results are shown in **table 1**.

Table 1 Effect of different culture fractions on some test microorganisms.

Test microorganisms / Tested fractions	**Inhibition zone diameter (mm)**			
	Klebsiella	***E. coli***	***E. coli*** ATCC 25929	***Staphylococcus aureus***
Crude culture	**21**	**18**	**19**	**17**
Cell-free supernatant	**19**	**20**	**23**	**22**
Neutralized supernatant	**12**	**14**	**14**	**15**

The culture of *Lb. curvatus* showed a good inhibitory effect against the four tested strains, the inhibition zone diameters are ranging from 17 mm for *S. aureus* to 21 mm for *Klebsiella*, in addition, the cell-free supernatant displayed also an inhibitory effect whereas the neutralized supernatant did not lost the whole inhibitory activity although the diameters of the zones are less important (from 12 for *Klebsiella* to 15 for *S. aureus*). Several mechanisms have been reported to describe antagonistic action of probiotic bacteria such as competitive exclusion, production of antimicrobial compounds, modulation of immune response, alternation of intestinal bacterial metabolic activity, alteration of microecology of the animal intestine, and inhibition of bacterial translocation. The production of antimicrobial agents could be easily demonstrated *in vitro* by the disc diffusion assay; they include fatty acids, organic acids, hydrogen peroxide, and diacetyl, acetoin and the small, heat-stable inhibitory peptides called 'bacteriocins'**(Soomro et al., 2002; Simova et al., 2009)**.

In our experiment, the probiotic *Lb. curvatus* decreased the growth of the tested microorganisms not only by the production of lactic acid but other substances could be involved like bacteriocins or hydrogen peroxide this was confirmed by the residual activity found in the neutralized supernantants.

***In vitro* adhesion test**

The adhesion test of *Lb. curvatus* to epithelial cells was conducted as described before as it is one of the most important criteria to select probiotic bacteria **(Roy et *al.*, 2006)**; the results shown in **figure** 1 indicated that the cells of the lactic acid bacterium are adherent to the selected epithelial tissue.

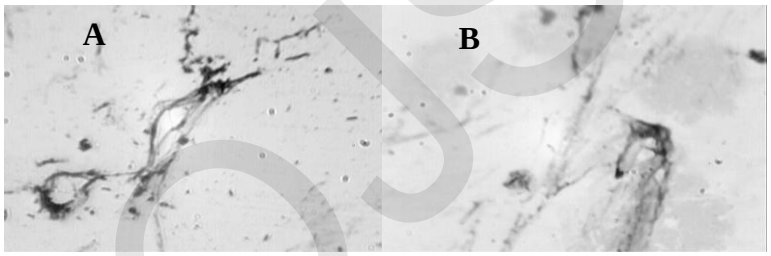

Fig. 1 Adhesion of *Lb. curvatus* to epithelial cells (A: positive result, B: negative control).

As described **by Lin *et al.* 2007**, *Lb. fermentum* cells; isolated from chicken crop highly attach the epithelial cells, which make them; in addition to the other properties; a good candidate to be selected as a probiotic.

The mechanism of adhesion of these cells is not completely understood, although it was suggested that some lactic acid bacteria like *Lb. plantarum* and *Lb. rhamnosus* are capable of colonizing the lower digestive tract for a long period resulting in the inhibition of pathogenic bacteria by competing to specific receptors required for adherence **(Robin and Rouchy, 2001; Roy et *al.*, 2006).**

Survival test

Microencapsulation of *Lb. curvatus* was conducted by using 1% sodium alginate; the microcapsules prepared by extrusion technique were spherical and uniform in size (3 mm), each bead contains approximately 9.10^{12}UFC. The survival of free and microencapsulated cells in acidic pH of the stomach was evaluated by a 2 h *in vitro* SGJ survival assay (Figure 2).

The viability of the free cells decreased intensively after the first hour of incubation in acidic conditions, it reached approximately 28 %; moreover, only 2 % of the cells remained viable after 2 hours, however, the cells in a microencapsulated state are slightly more resistant since after one hour, 40% of cells survived and after 2 hours, the viability attained 11%.

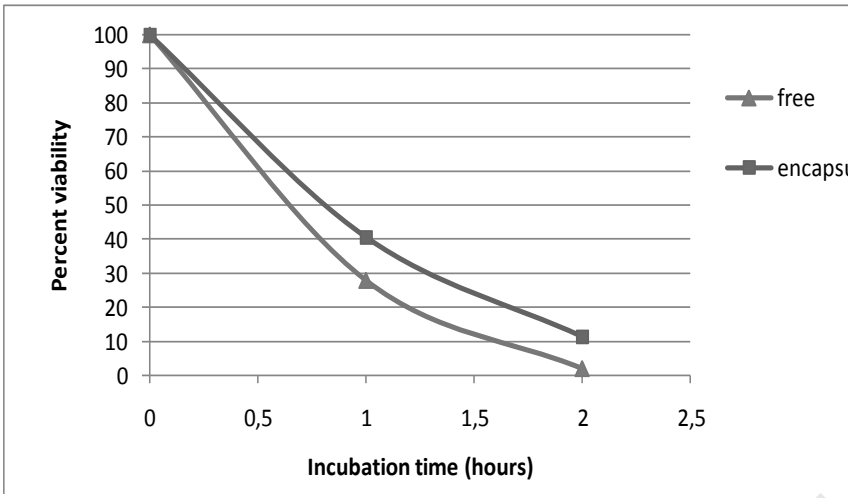

Fig. 2 Effect of acidic pH (2) on the survival of free and microencapsulated *Lb. curvatus* cells.

The chicken GIT contained a complex microbial community distributed unequally in its different compartments; the normal flora consists mainly of lactic acid bacteria particularly lactobacilli, enterobacteria and other groups are also found **(Gabriel et *al*. 2005; Lin et *al*. 2007).** Gizzard's microbial community is less abundant due to the high acidity; the hostile conditions of the duodenum reduced as well the incidence of microbes, although some lactobacilli, enterococci and coliforms were isolated (**Fuller, 1984).** Probiotics must then survive the transit through the gizzard to exert beneficial effects; therefore, resistance to a low pH (2) for at least 2 hours is required for a probiotic cell to be delivered effectively to the intestine. Several microencapsulation materials were used to protect probiotic cells including sodium alginate, carraghenane, pectin, whey proteins… (**Voo *et al.* 2011**; **Kailasapathy, 2002**). However; alginate matrix system is the most widely used and investigated biopolymer for cell bioencapsulation. It is biocompatible, and it can gel at mild condition with the presence of calcium cations. In a related study; *Lb. acidophilus* and *Lb. rhamnosus* were significantly protected from stomach conditions (**Ding and Shah 2009**), similarly; microencapsulated *Lb. acidophilus* and *Bifidobacterium sp*. showed 16 % and 16.7 % increase in viability after incubation at pH 2 for 2 hours when compared to free cells **(Vidhyalakshmien, 2009)**. **Lee and Heo (2000**) showed that *Bifidobacterium longum* encapsulated in calcium alginate containing 2.0, 3.0, and 4.0% sodium alginate tolerated significantly incubation in a simulated gastric juice (pH 1.5) better than free cells. The death rate of the cells in the beads decreased proportionally with an increase in both the alginate gel concentration and bead size.

Figure 2 shows that *Lactobacillus curvatus* was most likely to survive the passage through the stomach, furthermore microencapsulation within alginate capsules resulted in an approximate 5.4-fold increase in the survival of cells in pH 2 after 2 hours of incubation.

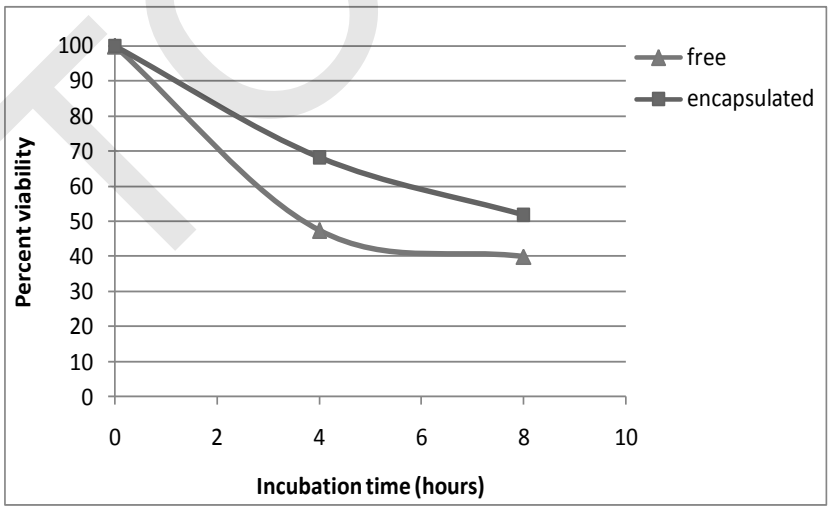

Fig. 3 Effect of bile salts (0.3%) on the survival of free and microencapsulated *Lb. curvatus* cells.

Viability of probiotic cells in the presence of bile salts was conducted as described by incubating the cells in MRS medium supplemented with 0.3 % bile salts. Results showed in **figure 3** indicated that the viable count of free cells decreased by approximately 53 % after 4 hours of incubation, it decreased to reach 40 % after 8 hours with an average cell concentration of about (152.10^{12} UFC/mL), moreover; the gel-enclosed cells resisted more, more than

68 % of the cells were found to survive bile treatment for 4 hours; and more than 50 % tolerated the treatment after 8 hours.

Bile salts are the second barrier that probiotic cells should bypass to attain their site of action. In general, the required concentration of bile salts considered necessary to screen for resistant strains for human and animal use is 0.3% (**Pacheko et al. 2010, Lin et al. 2007**). Several studies reported the improvement of cell viability when exposed to bile salts by microencapsulation, **Ding and Shah** (2009) found that *Lb. plantarum* and *Bifidobacterium lactis type Bi-07* were slightly sensitive to bile toxicity (39 % of the cells survived the treatment); however, microencapsulation in 3% alginate enhanced the viability by 2-fold. In a different study; *L. bulgaricus* KFRI 673, an acid-sensitive strain was found to survive SGI exposure when protected in alginate microparticles coated with a high molecular weight chitosan (**Lee et al. 2004).** Conversely, *Bifidobacterium infantis, Lactobacillus casei* and *L. acidophilus* encapsulated in symbiotic beads composed of Hi-Maize starch (a prebiotic) and sodium alginate did not demonstrate a significant increase in survival when subjected to *in vitro* high acid and bile salt conditions **(Sultana et al .2000**).

In this study; *Lb. curvatus* cells were found to be relatively resistant to bile toxicity, in addition; the use of alginate gel improved their tolerance, as approximately 1.3 –fold increase in viability was observed after 8 hours of treatment.

In conclusion, the isolated *Lb. curvatus* was found to present good probiotic properties, it displayed antimicrobial activity against some selected pathogenic bacteria, and a substantial adhesion capacity. Furthermore, the viability in GIT like conditions was increased by the alginate microencapsulation, which provides additional evidence that alginate-based microparticles are suitable for food ingredient delivery.

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PRODUCTION AND CARACTERIZATION OF BACTERIOCIN OF *Lactobacillus plantarum* F12 WITH INHIBITORY ACTIVITY AGAINST *Listeria monocytogenes*

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Abstract: Thirty five lactic acid bacterial isolates from different origins (fermented Milk, chicken, olive oil, butter and newborn feces) were tested for their ability to produce bacteriocins against 12 indicator strains. These isolates presented a broad inhibitory spectrum against many indicator strains such as Methycilin resistant *Staphyococcus aureus*, *Escherichia coli*, *Salmonella* sp., *Listeria monocytogenes*… Only six isolates produced antimicrobial activity in the neutralized cell-free supernatant treated with catalase against indicator strains. The bacteriocin produced by *Lactobacillus plantarum* F12 was characterized and showed sensitivity to proteolytic hydrolysis (trypsine, chymotrypsine and pronase) and resistance to α-amylase and lipase. The activity of bacteriocin remained constant after heating at 100°C for 30 min and no change in activity was recorded after 4 h at pH 6.0. Bacteriocin production is dependent on biomass concentration; it's started at the beginning of the log phase of the bacterial growth till reached its maximum level at the stationary phase at 37°C as the optimum temperature of production. Due to the inhibitory effect of this bacteriocin on *L. monocytogenes*, it can be used to prevent food spoilage by this pathogen.

Keywords: antimicrobial activity, bacteriocin, Lactic acid bacteria, *Lactobacillus plantarum*, *Listeria monocytogenes*

INTRODUCTION

Lactic acid bacteria (LAB) are microorganisms widely used in food industry in a variety of fermentations such as the development of meat products, vegetables and many dairy products including fermented milk, cheese, yogurt and butter (Dortu & Thonart, 2009; Makhlouf, 2006). LAB produce organic acids such as lactic, acetic acid and hydrogen peroxide which possess antimicrobial activity against several pathogenic and spoilage microorganisms (Benabbou, 2009). LAB represent a major class which produces bacteriocins that become a current subject for several researches. These bacteriocins are now being explored for their potential utility in human and animal health applications, food biopreservation and agricultural uses (Parada *et al*., 2007; Todorov *et al*., 2011b).

Bacteriocins differ from most therapeutic antibiotics in being proteinaceous agents that are rapidly digested by proteases in the human digestive tract. They are ribosomally synthesized peptides, and this fact creates the possibility of improving their characteristics to enhance their activity and spectra of action (Parada *et al*., 2007). In additions, it has been shown that some strains of LAB possess interesting health-promoting properties; one of the characteristics of these properties is the potential to combat gastrointestinal pathogenic bacteria such as *Helicobacter pylori*, *Escherichia coli* and *Salmonella*. The antimicrobial spectrum frequently includes spoilage organisms and food-borne pathogens such as *Listeria monocytogenes* and *Staphylococcus.* The activity against Gram-negative bacteria such as *E. coli* and *Salmonella* has been shown, but usually only when the integrity of the outer membrane has been compromised, for example after osmotic shock or low pH treatment, in the presence of a detergent or a chelating agent, or after pulsed an electric field or high-pressure treatment. An experimental focus on bacteriocin production by probiotics LAB strains has indicated that this potential might play a considerable role during in vivo interactions occurring in the human gastrointestinal tract, for instance towards *H. pylori* (De Vuyst & Leroy, 2007; Osmanagaoclu & Beyatli, 2002;).

Several authors have recommended the use of bacteriocins combined with other preservation methods to create a series of hurdles during the manufacturing process to reduce food spoilage by microorganisms. In fact, it has been proven that application of chemical preservatives, physical treatments (heat), or new mild non-thermal physical methods (pulsed electric field, HHP, vacuum, or modified atmosphere packaging), which increase the permeability of cell membranes, positively affects the activity of many bacteriocins. Notably, combined treatments of bacteriocins with selected hurdles affecting outer-membrane permeability increase the effectiveness of some LAB bacteriocins against Gram-negative cells, which are generally resistant (Ananou *et al.*, 2007; Galvez *et al.*, 2007; Dortu & Thonart, 2009).

The main purpose of our work was to select bacteriocinogenic strains from a group of LAB with antimicrobial activity isolated from different origins, to characterize the produced bacteriocin and to determine the antimicrobial spectrum of this bacteriocin produced by the selected isolate.

MATERIALS AND METHODS

Bacterial strains and culture conditions

Lactic acid bacterial isolates used in this study were previously isolated and identified from different origins (Raibe " traditional fermented milk", chicken, olive oil, butter and newborn feces) **(Table 1)**. Isolates were grown at 37°C in Man Rogosa Sharpe (MRS) broth (Biokar Diagnostics, France) (g/l: 10g glucose, 10g beef extract, 5g yeast extract, 0.5g sodium acetate, 2g Bipotassic phosphate, 2g ammonium citrate, 0.2g magnesium sulfate, 0.05g manganese sulfate, 1ml Tween 80, pH 6.5). To confirm the purity of the isolates each strain was individually streaked on MRS agar plates (MRS broth + 15g agar) and single colonies were isolated and tested for antimicrobial activity. Indicator strains used for determining antimicrobial activity were grown on nutrient agar. The antimicrobial activity and bacteriocin assay were realized on Muller-Hinton agar.

Table 1: The total number of LAB used in this study and their origin

Test strains	**origin**
Lb. brevis H27 ThT	Traditionally extracted olive oil
P. acidilacticii	Commercial strain
Lb. plantarum	Olive oil
Lb. bifermentans, *Lb. plantarum* R17, *Lc .lactis sp lactis* R4, *Lb. delbrueckii sp lactis* R4, *Lb. curvatus* R12	Raibe "traditional fermented Milk"
Lc. lactis sp pac B7, *Lb. Curvatus* BJ, *Lb. delbrueckii sp bulgaricus* B8,	Traditional butter
Lb. delbrueckii sp delbrueckii, *Lb. bifermentans*, *St. thermophilus*, *Lc. Lactis sp cremoris* B13,*Lb. delbrueckii sp delbrueckii*, *Lb .plantarum*, *Lb. delbrueckii sp delbrueckii*, *Lc. Reffinolactis*, *Lc helveticus*, *Lb. curvatus*, *Lc .lactis sp cremoris*, *St. salivaricus sp thermophilus*, *Lc. Cremoris*, *Lc. lactis sp diacetylactis*	Butter
Lb. cremoris NNN105	Goat butter
Lb .fermentum G8, *Lb. fermentum* G12, *Lb. plantarum* G13	Chicken gizzard
Lb. plantarum F12, *Lb. curvatus* G6, *Lb. casei ssp tolerans* G4, *Lactobacillus sp.* B5, *Lb. gasseri*, *Lb. plantarum*	Newborn feces

Antimicrobial activity assay

The isolated strains were grown in MRS broth (pH 6.5) inoculated with 1% of an overnight culture and incubated at 37°C for 18-24 h. After incubation, cells were removed from the growth medium by centrifugation (6000×g for 20 min, 4°C).The cell-free supernatant was sterilized by filtering through a 0.22 µm Millipore filter. The antimicrobial spectrum of the bacteriocin from LAB was determined using the well diffusion method (WDM) (Tagg and Mc-Given, 1971). The indicator bacteria were cultured on nutrient agar for 24 h at 37°C, and used to prepare cell suspensions in 9 ml normal saline. Twenty ml of Muller Hinton agar cooled to 45°C was mixed with 110µl of the indicator strain suspension, pooled in a Petri dish and incubated aerobically for 2 to 4h at 37°C. Six mm wells were made and filled with 100 µl of the supernatants. Plates were incubated at 37°C for 24h. Inhibition zones were determined by measuring the diameter of the clear zones around the well.

Screening for bacteriocin producing strains

The cultures of LAB that showed antimicrobial activity against indicator bacteria based on the well diffusion assay were tested for their potential to produce bacteriocins. The assay of bacteriocin was carried out as follow; the cell-free supernatants of LAB were adjusted to pH 6.0-6.5 using NaOH 5N to exclude the antimicrobial effect of organic acids. Inhibitory activity of hydrogen peroxide was eliminated by the addition of catalase at a final concentration of 1mg/ml. The catalase-treated samples were incubated for 2h at 37°C, after incubation the treated and neutralized cell-free supernatants were then tested for antagonistic activity against indicator bacteria by the WDM as described above (Ghalfi et al., 2006). Bacteriocin activity was expressed in arbitrary units (AU/ml). One AU was defined as the reciprocal of the highest level of dilution resulting in a clear zone of growth inhibition (Rajaram *et al*., 2010). Zone of 1 mm and above was considered as inhibition.

Characterization of bacteriocin

The bacteriocin samples were characterized with respect to thermal and pH stability, and susceptibility to denaturation by enzymes. The effect of temperature on the bacteriocin was tested by heating the cell-free supernatants to 40, 60, 80 and 100°C during 60 min. Aliquots of each treatment were taken after: 0, 15, 30 and 60 min. 100µl of each heat-treated sample were used for the well diffusion method, the residual activity was determined using methycilin-resistant *Staphylococcus aureus* (MRSA) as indicator organism. The effect of pH on the bacteriocin was determined by adjusting the cell-free supernatant between pH 2.0 and 12.0 with sterile 1N

HCl or 5N NaOH. The adjusted supernatants were incubated for 4 h at room temperature, 100µl of each sample were tested by the WDM using MRSA as indicator organism and the residual activity was determined.

The supernatants were treated with the following enzymes at a final concentration of 1mg/ml: lipase (Sigma), trypsine (Sigma), α-chymotrypsin (Merck), pronase E (Merck), α- amylase (Fluka). 5µl of the enzyme solution were added to 100µl of the cell-free supernatant. Controls consisted of only cell-free supernatant and tris-HCl buffer. Both the samples and the controls were incubated at 37ºC for 2 hours and heated in boiling water for 5 min to inactivate the enzymes. The remaining bacteriocin activity was determined by the WDM described above using the MRSA as indicator organism. All enzymes were used at a final concentration of 1mg/ml and maintained in tris-hydrochloric buffer (pH 8.0).

Monitoring of bacteriocin production

One ml of an 18h-old culture was used to inoculate 100 ml of MRS broth and incubated at 37°C for 48 hours. Samples were taken after time interval and examined for bacterial growth (OD 660nm), changes in culture pH, and antimicrobial activity against MRSA. The WDM was used and the activity expressed as AU/ml as described previously.

Effect of temperature on bacteriocin production

To determine the optimum temperature for bacteriocin production, we used 100ml Erlenmeyer flask. In each flask, 50ml of MRS broth was inoculated with 0.5ml of an overnight culture. The Erlenmeyer flasks were incubated at different temperatures: 30, 37 and 40°C. Samples were collected after 24h and examined for bacteriocin production as described earlier.

RESULTS AND DISCUSSION

Screening for bacteriocin producing isolates

Thirty five LAB isolated from different origins were examined for displaying bacteriocin activity against a set of 12 indicator strains. These strains presented a broad inhibitory spectrum since they were able to inhibit many of the indicator strains tested such as *E. coli* ATCC29522, *K. oxytoca*, *K. pneumoniae*, *Proteus mirabilis*, *Salmonella* sp. *S. aureus* ATCC29523, *P. aeruginosa*, *E. coli* ATCC25922, MRSA, *B. subtilis*, *E. coli* ATCC28484, *L. monocytogenes* and the pathogenic *Klebsiella* 111. The inhibitory effect, which was observed by the formation of clear and distinct zones around the wells, may be due to the production of several antimicrobial compounds like organic acids, hydrogen peroxide or bacteriocins (Labioui *et al*., 2005).

The activity of the inhibitory agent was tested under conditions which eliminate the possible effect of organic acids by adjusting the pH of the cells-free supernatant to 6.0 and of hydrogen peroxide by catalase treatment. Six of 35 strains (*Lb.plantarum* F12, *Lb.curvatus* G6, *Lb. gasseri*, *Lb. plantarum*, *Lb. casei* ssp *tolerans* G4, and *Lactobacillus* sp. B5) produced antimicrobial activity in the neutralized cell-free supernatant against four indicator strains (MRSA, *Bacillus subtilis*, *L. monocytogenes* and pathogenic *Klebsiella* 111). When the cell-free supernatant was treated with catalase (1mg/ml) the six strains confirmed their activity only against three indicator strains (MRSA, *L. monocytogenes* and *B. subtilis*). The diameters of inhibition zones of the indicator strains by the cell-free supernatant neutralized and treated with catalase are ranging from 14 to 20 mm**.** The highest diameter (20mm) was obtained with the cell-free supernatant of *Lb. plantarum* F12 and *Lb. curvatus* on *B. subtilis*, whereas the lowest diameter was obtained with the cell free supernatant of *Lactobacillus* sp. B5 against MRSA.

The fact that, the cell-free supernatants (neutralized and treated with catalase) inhibited the growth of the indicator strains gives evidence that the antimicrobial activity is due to the production of bacteriocins (Tatsadjieu *et al.,* 2009). Gram-positive indicator bacteria are much more sensitive to bacteriocin of our LAB strain than Gram-negative indicator bacteria. These results indicated that our LAB had an inhibitory spectrum towards closely related Gram-positive bacteria. The resistance of Gram-negative bacteria is attributed to the particular nature of their cell membrane; the mechanism of action described for bacteriocin involved a phenomenon of adsorption. Ivanova *et al*. (2000) found that, the bacteriocin produced by *Lactococcus lactis* subsp. *lactis* B14 inhibited only wide range of strains from the group of closely related LAB. The known bacteriocins does not still act on the sorts taxonomic close, for example, nisin has an inhibitory effect against a wide variety of Gram-positive food-borne pathogens and spoilage microorganisms and can also act on several Gram-negative bacteria when the integrity of their outer membranes is disrupted (Savadogo *et al.,*2004). The isolate *Lb. plantarum* F12 was selected for further studies. *L. monocytogenes* has become one of the most significant food borne pathogens. In food industry, the control of this pathogen remains a challenge because of its widespread occurrence and its ability to survive and persist even in hostile environment (Hartmann *et al*., 2011). For this reason we tested the ability of bacteriocins produced by *Lb. plantarum* F12 to inhibit this bacterium. Hartman *et al*. (2011) observed that the cell-free supernatant produced by eight antagonistic bacteria strains were able to inhibit *L. monocytogenes* in different food matrices. In another study, Singh and Prakash, (2009) found that, several LAB strains isolated from cottage cheese are capable of inhibiting pathogenic microorganisms in the food environment and display crucial antimicrobial properties with respect to food preservation and safety. They can also be used more specifically to inhibit certain high–risk bacteria like *L. monocytogenes* in food. Application of bacteriocins may help reduce the use of chemical preservatives and /or

the intensity of heat and other physical treatments, satisfying the demands of consumers for foods that are fresh tasting, ready to eat, and lightly preserved.

Characterization of bacteriocin

The effect of heating, pH and enzymes were studied in this work by using MRSA as indicator strain. Based on the results showed in **Fig. 1**, the inhibitory compounds produced by the tested isolate were considered to be heat stable. The activity of bacteriocin produced by *Lb. plantarum* F12 remained constant after heating at 100°C for 30 min followed by subsequent decline after 60 min. Similar results were recorded for a number of bacteriocins produced by *Lactobacillus* spp. and *Lactococcus* spp.. In addition, lacticin NK24 produced by *Lc. lactis* NK24, lost only 87.5% of its activity after 30 min at 100°C and was completely inactivated after 15min at 121°C (Todorov *et al*., 2011b).On the other hand, Sarika *et al*. (2010) observed that, the bacteriocin GP1 produced by *Lb. rhamnosus* had a remarkable stability over heat treatment even at the autoclaving temperature for 20 min. Heat stability of *Lb. plantarum* F12 at 100°C is important if the bacteriocin is used as a food preservative, because many procedures of food preparation involve a heating step.

As shown in **Fig. 2**, the antimicrobial activity of *Lb. plantarum* F12 is significantly influenced by pH. In this respect, it was observed that the residual activities were significantly higher in the range of pH 6.0 to pH 10.0 then those at pH 2.0, 4.0 and 12.0; with a maximum activity at pH 6.0, suggesting that compounds other than acids inhibited growth of MRSA. These observations are in agreement with those reported by Ogunbanwo *et al*. (2003) who showed that *Lc. brevis* excreted other compounds such as bacteriocins that inhibited the growth of pathogens. According to these results, we can say that the antimicrobial activity of *Lb. plantarum* F12 presents stability in the range of pH from 2.0 to 12.0. This property has been considered highly useful for their application as food preservative.

The effect of various enzymes on the inhibitory agent was studied. Complete inactivation or significant reduction in activity was observed after treatment of the cell-free supernatant with chymotrypsine, trypsine and pronase which confirmed the proteinaceous nature of the active agent. The other enzymes tested in our study (amylase and lipase) did not cause inactivation. This confirmed that carbohydrate and lipid moieties if existing were not required for the inhibitory activity. Similar results were recorded by Todorov *et al*. (2004) for bacteriocins produced by *Lc. plantarum* ST13BR whereas Ivanova *et al*. (2000) observed that trypsine, chymotrypsine and rennin had no effect on bacteriocin produced by *Lc. lactis* subsp. *lactis* b14 isolated from boza Bulgarian Traditional cereal beverage.

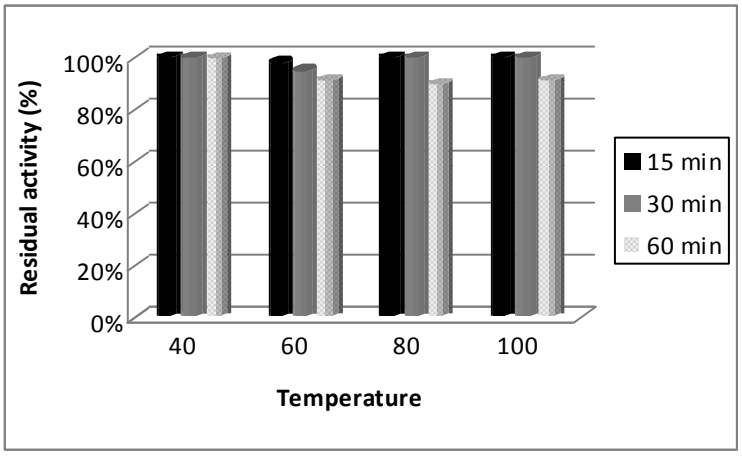

Fig. 1: Effect of temperature on bacteriocin activity produced *Lb. plantarum* F12.

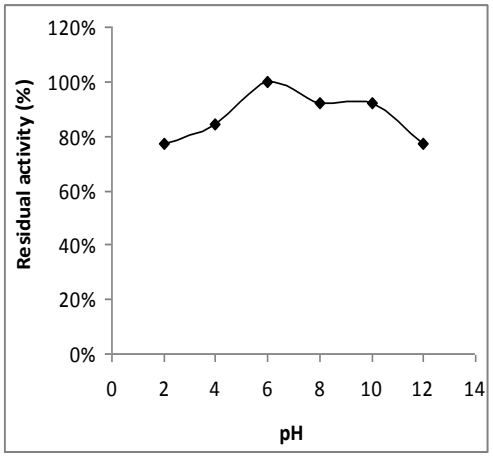

Fig. 2: Effect of pH on bacteriocin activity produced by *Lb. plantarum* F12.

Monitoring of bacteriocin production

Bacteriocin production was monitored during 48 hours of growth in MRS broth. **Fig. 3** illustrates the growth, pH and the level of *Lb. plantarum* F12 bacteriocin production through 48 hours of incubation. Data showed that bacteriocin production started at the beginning of the log phase of the bacterial growth (after 4 h), and increased gradually with bacterial growth till it reached its maximal level (2416 AU/ml) after 30 hours of incubation (in stationary phase). After 32 h of incubation a decrease in bacteriocin production was observed (2166 UA/ml). During the same period of growth the pH of the medium decreased from 6.5 to 4.5. The growth of *Lb. plantarum* F12 increased gradually and reached its optimum after 32 h and remained more or less constant during the following 16 hours. Several studies have shown that bacteriocin production is dependent on biomass concentration. Todorov and Dicks, (2005) reported that optimal levels of plantaricin ST194BZ, produced by *Lb. plantarum* ST194BZ, were obtained in growth media that supported high biomass production, such as MRS. A similar bacteriocin production profile was reported for bacteriocin ST311LD produced by *E. faecium* ST311LD isolated from fermented olives, in which maximal bacteriocin production was reported after 20 hours growth in MRS broth, followed by a decrease in activity in the following 5 hours. The decrease in activity of bacteriocins produced by *Lb. plantarum* F12 at the end of the monitored period could be explained by the degradation of the bacteriocin by extracellular proteolysis enzymes, similar decreases have also been observed for bacteriocins produced by *Enterococcus faeciu*m ST311LD (Todorov &Dicks, 2005), *Enterococcus mundtii* ST4SA and *Pediococcus acidilacticii* NRRL B5627 (Todorov *et al*., 2011a).

Effect of temperature on bacteriocin production

The effect of temperature on bacteriocin production by *Lb. plantarum* F12 was tested in Erlenmeyer flasks cultures containing sterile MRS and maintained at different temperatures (30, 37 and 40°C). **Fig. 4** shows the effect of temperature on bacteriocin production. The optimum temperature for the production of bacteriocin was 37°C, thus the bacteriocin activity at this temperature was higher than that observed at 30 and 40°C. According to these results we can say that, the optimum temperature for production and the one for growth are correlated, as observed elsewhere for lactocin A, enterocin 1146, lactocin S and nisin Z (Todorov *et al*., 2004). So, growth temperature seems to play an important role on bacteriocin production. Different results were recorded by Mataragas *et al*. (2003), as they found that the optimum temperature for the production of bacteriocins produced by *Leuconostoc mesenteroides* L124 and *Lb. curvatus* L442 was 25°C and was lower than that of growth (30°C).

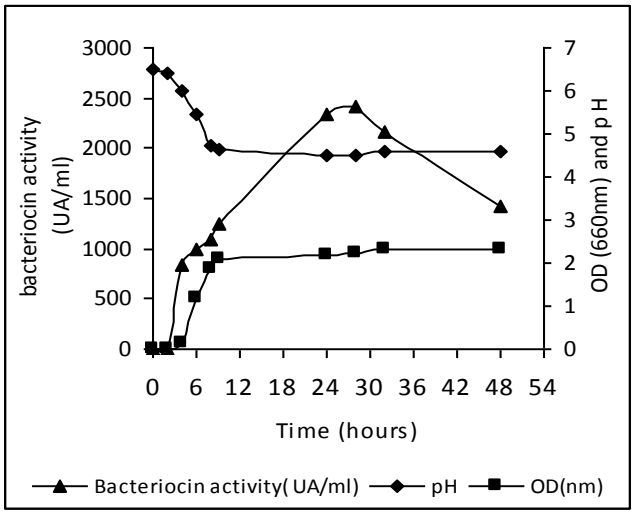

Fig. 3: Monitoring of bacteriocin production from *Lb. plantarum* (F12) in MRS medium at 37°C during 48 h.

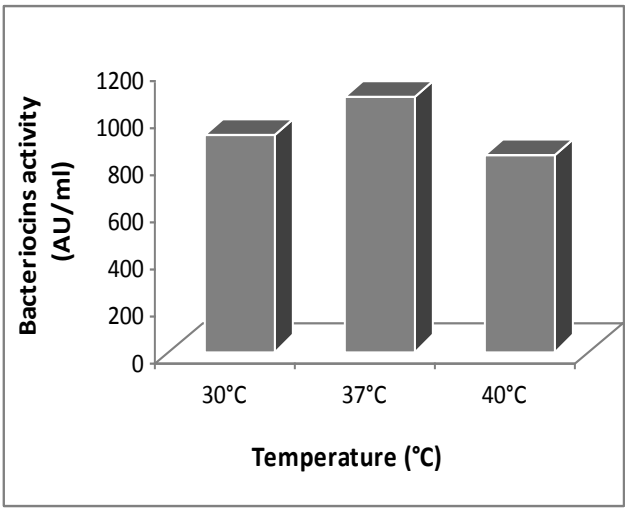

Fig. 4: Effect of temperature on bacteriocin production from *Lb. plantarum* F12 in MRS medium at 37°C.

Acknowledgement: Authors are very grateful to Dr. Tayeb Idoui and Mrs. Fathia Bahri for providing bacterial isolates.

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SMALL ROV TO DETECTION AND IDENTIFICATION OF DANGEROUS UNDERWATER OBJECTS

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Abstract: A small unmanned underwater vehicle (UUV) to inspection of an undersea space is presented in the paper. Its behavior is controlled by a trained pilot. Correct detection and identification of targets depends on vehicle's precise displacement along a predefined route. Nowadays, the UUVs are equipped with an automatic control system to execute some basic maneuvers without constant human interventions. Hence, in the paper, an autopilot assuring an appropriate vehicle's movement is described. Selected results of computer simulations illustrating a quality of the underwater mission are inserted.

Keywords: - underwater vehile, autopilot, modelling, simulation.

1. INTRODUCTION

A described system to detection and identification of dangerous objects located in the underwater space is a floating platform designed and built basis on a special kind of the UUV, called remotely operated vehicle (ROV). It is equipped with a comprehensive set of devices and sensors to achieve a high quality of operational work. The set mounted on the vehicle's body consists of: lamps and TV cameras, a scanning sonar, an inertial navigation unit, a doppler velocity log, a transponder/responder for hydroacoustic navigation and a manipulator (see Fig. 1).

The ROV operates in crab-wise manner in four degrees of freedom (DOF) with small roll and pitch angles that can be neglected during normal operations. Its behaviour is controlled by a trained pilot located on a board of the mother-ship or an offshore structure. A typical mission of detection and identification of dangerous objects consists of two phase. The first one, called a transition phase, is a displacement to a target area from a launch point. During the second phase, called detection phase, a searched object is found by the pilot. A pilot's work is supported by a computer which provides required information, integrating sonar and cameras images with data from a navigation system and other sensors (see Fig. 2 and Fig. 3).

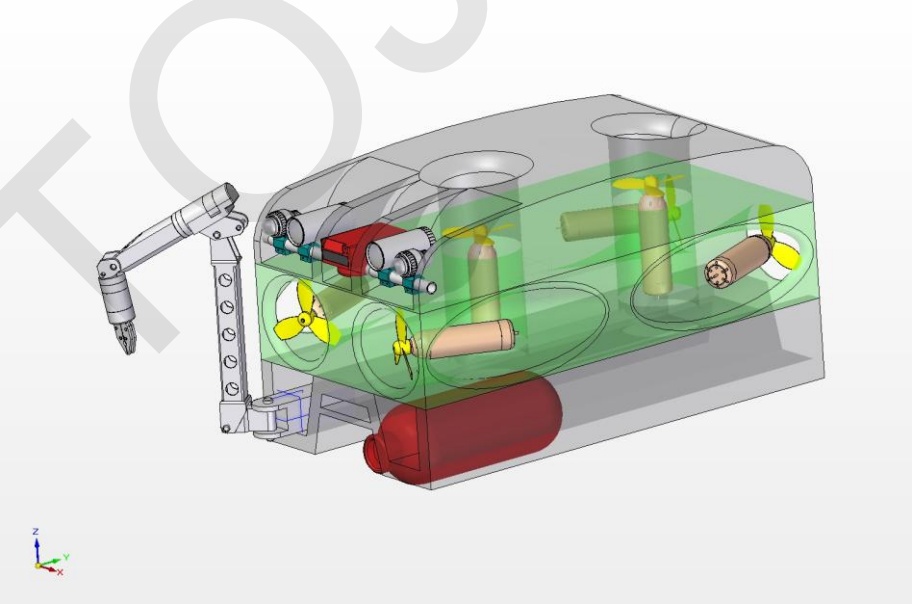


Figure 1. Virtual vision of ROV.

To execute some basic manoeuvres without a constant pilot's supervision, contemporary ROVs are often and often equipped with an automatic control system. An interesting review of classical and modern techniques useful to steering of the UUVs vehicles has been provided in Craven *et al.*, 1998. Automatic control of such underwater apparatus is a difficult problem due to their nonlinear dynamics. Moreover, the dynamics can change according to the alteration of configuration to be suited to the mission. Hence, an autopilot, responsible for keeping desired positions and orientations of the ROV during the transition phase, should be flexible and self-adapting to varying motion conditions.

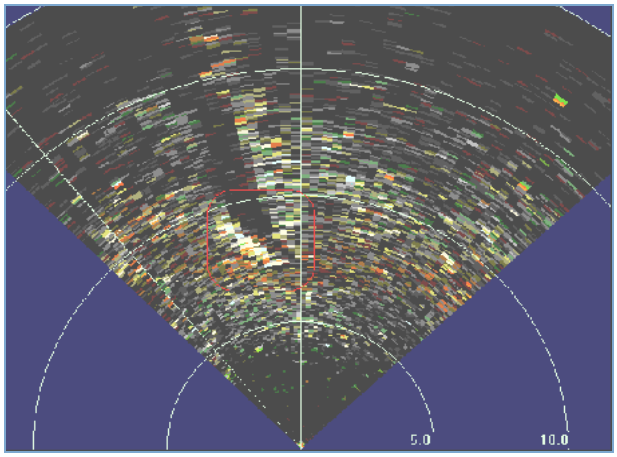


Figure 2. Screen display with sonar image.

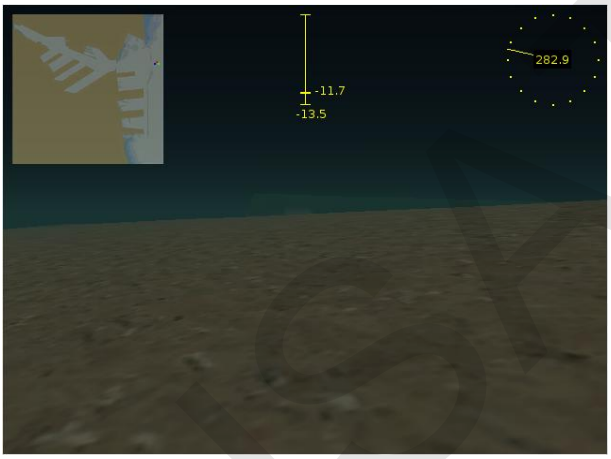


Figure 3. Screen display with TV image and navigation data.

2. EQUATIONS OF MOTION

A general motion of the underwater vehicle of six DOFs describes the following vectors (Fossen, 1994; Fossen, 2011) :

$$\begin{aligned} \boldsymbol{\eta} &= [x, y, z, \phi, \theta, \psi]^T \\ \mathbf{v} &= [u, v, w, p, q, r]^T \\ \boldsymbol{\tau} &= [X, Y, Z, K, M, N]^T \end{aligned} \tag{1}$$

where:

$\boldsymbol{\eta}$	–	position and orientation vector in the inertial frame;
x, y, z	–	coordinates of position;
ϕ, θ, ψ	–	coordinates of orientation (Euler angles);
$\mathbf{v}$	–	linear and angular velocity vector with coordinates in the body-fixed frame;
u, v, w	–	linear velocities along longitudinal, transversal and vertical axes;
p, q, r	–	angular velocities about longitudinal, transversal and vertical axes;
$\boldsymbol{\tau}$	–	vector of forces and moments acting on the vehicle in the body-fixed frame;
X, Y, Z	–	forces along longitudinal, transversal and vertical axes;
K, M, N	–	moments about longitudinal, transversal and vertical axes.

Nonlinear dynamical and kinematical equations of motion in the body-fixed frame can be expressed as:

$$\begin{aligned} \mathbf{M}\dot{\mathbf{v}} + \mathbf{C}(\mathbf{v})\mathbf{v} + \mathbf{D}(\mathbf{v})\mathbf{v} + \mathbf{g}(\boldsymbol{\eta}) &= \boldsymbol{\tau} \\ \dot{\boldsymbol{\eta}} &= \mathbf{J}(\boldsymbol{\eta})\mathbf{v} \end{aligned} \tag{2}$$

where:

- **M** – inertia matrix (including added mass);
- **C(v)** – matrix of Coriolis and centripetal terms (including added mass);
- **D(v)** – hydrodynamic damping and lift matrix;
- **g(η)** – vector of gravitational forces and moments;
- **J(η)** – velocity transformation matrix between the body fixed and the inertial frames.

3. ADAPTIVE ALGORITHM OF CONTROL

The algorithm of control worked out basis on a simplified ROV model proposed in Fossen, 1994:

$$\mathbf{M}_d \dot{\mathbf{v}} + \mathbf{D}_d(\mathbf{v})\mathbf{v} = \boldsymbol{\tau} \tag{3}$$

where all kinematics and dynamics cross-coupling terms are neglected, so $\mathbf{M}_d$ and $\mathbf{D}_d(\mathbf{v})$ are diagonal matrices. Uncertainties in the above model are compensated by a control system.

The expression (3) for motion in four DOFs, (surge, sway, heave and yaw), takes a form (Garus, 2007):

$$\begin{aligned} m_X \dot{u} + d_X |u|u &= \tau_X \\ m_Y \dot{v} + d_Y |v|v &= \tau_Y \\ m_Z \dot{w} + d_Z |w|w &= \tau_Z \\ m_N \dot{r} + d_N |r|r &= \tau_N \end{aligned} \tag{4}$$

Define the following vectors $\boldsymbol{\tau} = [\tau_X, \tau_Y, \tau_Z, \tau_N]^T$ and $\mathbf{p} = [m_X, d_X, m_Y, d_Y, m_Z, d_Z, m_N, d_N]^T$ the expression (4) can be written as:

$$\boldsymbol{\tau} = \mathbf{Y}(\mathbf{v}, \dot{\mathbf{v}})\mathbf{p} \tag{5}$$

where $\mathbf{Y}(\mathbf{v}, \dot{\mathbf{v}})$ is a known matrix of measured signals, usually referred as the regressor matrix, (for more details see Spong *et al.*, 1998), and has the form:

$$\mathbf{Y}(\mathbf{v}, \dot{\mathbf{v}}) = \begin{bmatrix} \dot{u} & |u|u & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & \dot{v} & |v|v & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & \dot{w} & |w|w & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & \dot{r} & |r|r \end{bmatrix} \tag{6}$$

A structure of the proposed control system is depicted in Fig. 4.

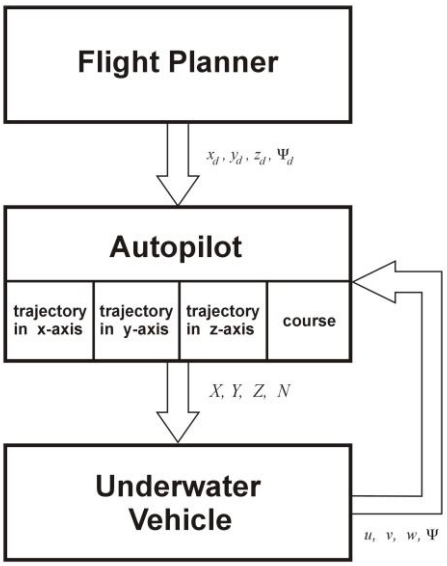


Figure 4. Block diagram showing structure of control system.

4. SIMULATION STUDY

Numerical simulations have been made to confirm validity of the proposed control algorithm under the following assumptions:

1. the ROV can move with varying linear velocities *u*, *v*, *w* and angular velocity *r*;
2. its velocities *u*, *v*, *w*, *r* and coordinates of position *x*, *y*, *z* and heading ψ are measurable;
3. the desired route is given by means of set of way-points $\{(x_{di}, y_{di}, z_{di})\}$;
4. segments of the predefined route between two successive way-points are defined as smooth and bounded curves;
5. the command signal $\boldsymbol{\tau}$ consists of four components: $\tau_1 = \tau_X = X$, $\tau_2 = \tau_Y = Y$, $\tau_3 = \tau_Z = Z$ and $\tau_4 = \tau_N = N$ calculated from the control law (5).

A regulation problem has been examined under interaction of environmental disturbances, i.e. a sea current. To simulate such influence on vehicle's motion its velocity V_c was assumed to be slowly varying and having a fixed direction. For computer simulations the disturbance was calculated by using the 1st order Gauss-Markov process (Song *et al.*, 2003):

$$\dot{V}_c + \mu V_c = \omega \tag{11}$$

where ω is a Gaussian white noise, $\mu \geq 0$ is a constant and $0 \leq V_c(t) \leq V_{c\max}$.

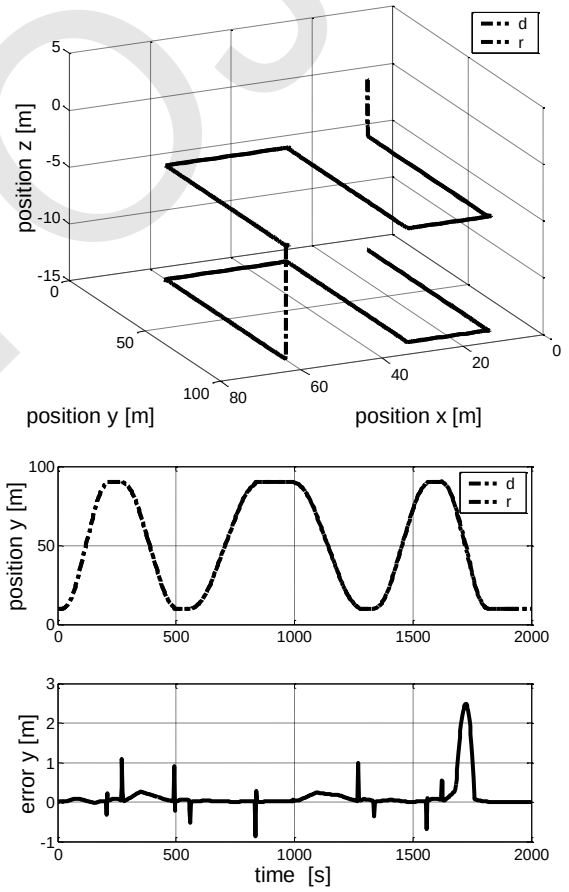
Some results of simulations are depicted in Fig. 5. The case study showed that the proposed adaptive algorithm enhanced good quality of movement along the desired route.

5. CONCLUSIONS

This paper has described the using of the adaptive algorithm for control of positions and orientations of the remotely operated vehicle designed to detection and recognition of dangerous targets in the underwater space.

It can be concluded from the obtained results that the proposed approach provides the automatic control system being robust and having good performance.

Another advantage of the discussed control system is its flexibility with regard to the change of dynamic properties of the ROV according to the alteration of configuration to be suited to the mission.



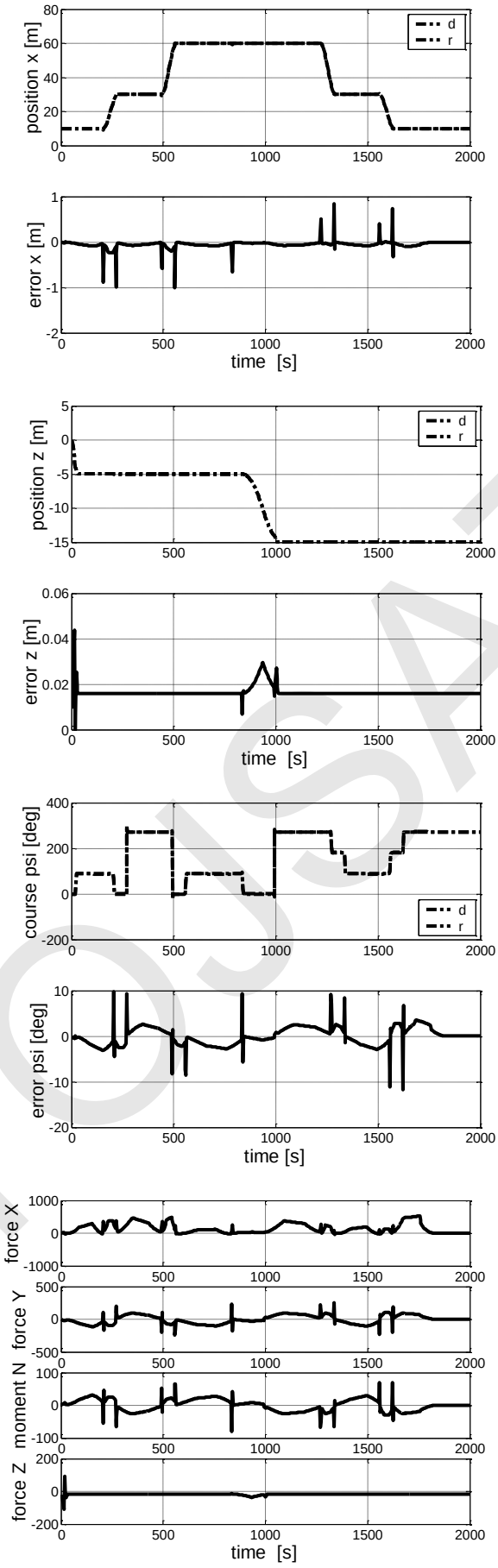


Figure 5. Results of track-keeping control: desired (d) and real (r) path in 3D space (upper plot), *x*-, *y*-, *z*-position and error of position ($2^{nd} \div 4^{th}$ plots), course and error of course (5^{th} plot), commands (low plot).

6. ACKNOWLEDGMENT

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THE CHEMICAL INVESTIGATION ON WATER POLLUTION OF KURNOOL DISTRICT BY WATER QUALITY ANALYSIS

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Abstract: This study consisted of the determination of the trace metal ions and some physiochemical properties in drinking water samples from the neighboring villages of Nandyal region, Kurnool district, where drinking water samples are not treated before it is consumed. The purpose was to ascertain the quality of water from these sources. Samples were taken from ten sampling points and analyzed for the following parameters Fe, Cu, Mn, Zn, Al, pH,EC,NO3-, SO_4 , and F- using the procedure outline in the plain test photometer method. The data showed the variation of the investigated parameters in samples as follows: pH 5.47-7.39, conductivity (EC) 49-1168 μs/cm, turbidity 4.68-73.34JTU, F - 0.54 to 1.29mg/L.NO_3-11.19 to 39.76 mg/L , $SO_4$2- 41.2 to 73.0 mg/L Cu 1.25 to 2.96 mg/L. Fe 0.08-0.94mg/L, Zn 5-19 mg/L, Mn 0.004-0.016 mg/L and Al 0.07-0.18 mg/L , The concentrations of most of the investigated parameters in the drinking water samples from Nandyal region were within the permissible limits of the World Health Organization drinking water quality guidelines.

Key words : drinking water, Nandyal Rural region areas, World Health Organization, Trace metals, physiochemical Properties.

1.INTRODUCTION

Good drinking water quality is essential for the well being of all people. Unfortunately in many countries around the world, including India, some drinking water supplies have become contaminated, which has impacted on the health and economic status of the populations Contaminants such as bacteria, viruses, heavy metals, nitrates and salt have found their way into water supplies as a result of in adequate treatment and disposal of waste industrial discharges, and over-use of limited water resources Even other Chemicals to be harmful to human health. Unfortunately, this problem arose because the groundwater was extracted for drinking without a detailed chemical investigation. The natural water analyses for physical and chemical properties including trace element contents are very important for public health studies. These studies are also a main part of pollution studies in the environment (Kot., *et al*,. 2000; Soylak. *et al*., 2002 a). According to our literature review Some physical and chemical properties of the samples were determined by using standard analytical methods.

2.MATERIALS AND METHODS

2.1 Sample collection

The drinking water samples were collected in prewashed (with detergent, diluted HNO3 and doubly de- ionized distilled water, respectively) polyethylene bottles. pH and conductivity of the samples were measured while collecting the samples. Each water sample was taken four times at four different sampling periods approximately three month apart. Samples were collected in January, April,July and October ;2009.The determinations of the physical and other chemical properties of the water samples were performed on the same day of sampling. For surface water sampling, the bottles and caps were rinsed three times with water to be sampled during sampling and

for ground water, the samples were obtained directly from the water pump after allowing the water to run for at least five minutes and each sample bottle and its cap rinsed three times. These samples were subsequently stored at 4 °C for as short a time as possible before analysis to minimize physicochemical changes (Anonymous, 1996).Because very little particulate matter was present in the sample, filtration was not considered necessary.

2.2. Methodology

Analytical water test tablets (photometer grade) reagents for specific test were used for the preparation of all solutions. Each sample was analysis for , Fe, Cu,Mn, Zn, Al, NO3- , SO4 2-, and F- using procedures outline in the Palintest Photometer Method (Palintest Photometer 5000) for the examination of water and waste water.

3.RESULTS AND DISCUSSION

The average physical and chemical properties of the drinking water samples including pH, electrical conductivity, turbidity, fluoride, nitrate ,sulphate from these sample points(1,2,3,4,5,6,7,8,9,10) were given in Table 1. The pH values were in the range of 5.47 to 7.39. Minimum pH (5.47) was observed from an well in Panyam rural area(1) and a maximum of (7.39) was observed from the Panyam stream(2) at Nandyal Rural area. The pH levels were lower than permissible limit (6.5-8.5) in 10% villages, the rest were within optimum limit. The recommended permissible limit for electrical conductivity (EC) is 300 µs/cm. By analyzing the results 80% villages showed EC lower than permissible limit The value for EC ranged from 49 to 275 µs/cm, except that of the groundwater samples from Konidedu(4) and Alamur(7) which recorded 963 µs/cm and 1168 µs/ cm respectively. Turbidity is a measure of the cloudiness of water. It has no health effects. However,turbidity can interfere with disinfection and provide a medium for microbial growth. Turbidity may indicate the presence of disease causing organisms. These organisms include bacteria, viruses, and parasites that can cause symptoms such as nausea, cramps, diarrhea, and associated headaches . All the samples have turbidity values greater than the WHO permissible value of 10 JTU except that of the groundwater sample from Neravada(5) and Kowluru(6) villages which recorded values of 5.38 JTU and 4.68 JTU respectively. Fluoride (F-) varied from 0.54 to 1.29 mg/L.Minimum (0.54 mg/L) and maximum (1.29 mg/L) concentration of F- was observed from Odugandla(9) and Balapanuru(10) villages respectively (Table 1). Permissible limit for F concentration is 1-1.5 mg/L according to WHO (2003).The data revealed that 50 % villages are with in limit.. Nitrate in the investigated samples were found to be in a range of 11.19 to 39.76 mg/L .The range of sulphate (SO42-) in the samples was 41.2 to 73.0 mg/L but was negligible at Maddur (8)throughout the area.

Table 1: The physical and chemical parameters of the drinking water samples

Sampling site	Sample site code	Water Type	pH	EC µs/cm
Panyam Rural area	1	Surface	6.78	67
Panyam Stream	2	Surface	7.37	129
Panyam Lake	3	Surface	7.17	184
Konidedu	4	Ground	5.47	963
Neravada	5	Ground	6.23	213
Kowlur	6	Ground	6.68	198
Alamur	7	Ground	7.12	1168
Maddur	8	Tap Water	7.43	95
Odiguntla	9	Tap Water	7.29	102
Balapanur	10	Tap Water	7.09	116

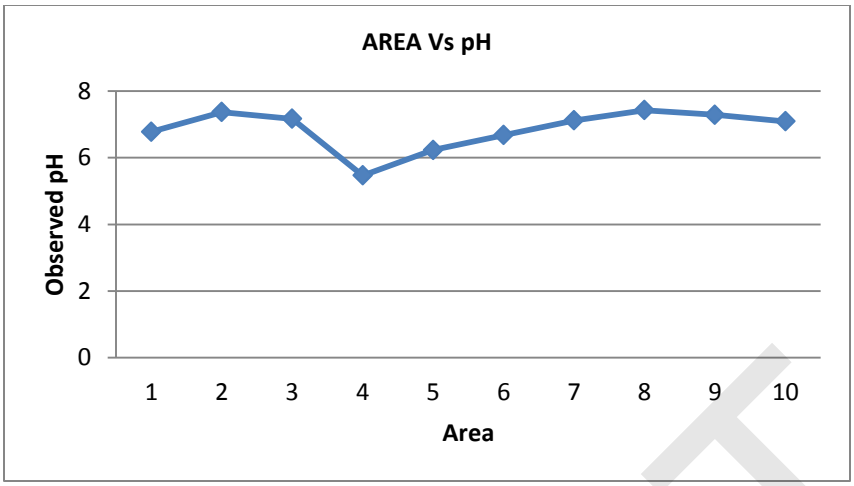

Figure: 1: Graphical representation between Area Vs Observed pH

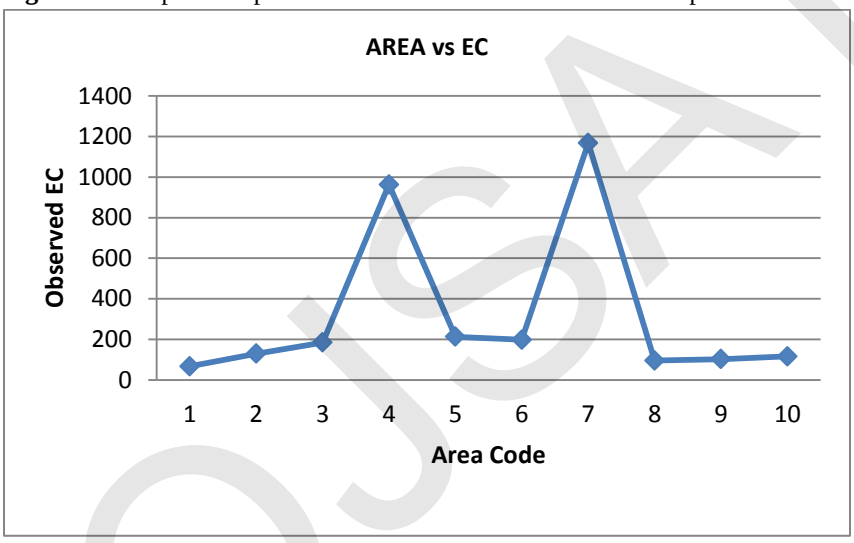

Figure: 2: Graphical representation between Area Vs Observed EC

Table 2: The physical and chemical parameters of the drinking water samples

Sampling site	Sample site code	Water Type	Turb. NTU	F (mg/l)
Panyam Rural area	1	Surface	36.0	1.03
Panyam Stream	2	Surface	43.67	1.18
Panyam Lake	3	Surface	51.53	0.99
Konidedu	4	Ground	62.98	0.68
Neravada	5	Ground	5.35	0.92
Kowlur	6	Ground	4.68	0.74
Alamur	7	Ground	73.34	1.05
Maddur	8	Tap Water	32.76	1.23
Odiguntla	9	Tap Water	26.88	0.54
Balapanur	10	Tap Water	29.0	1.29

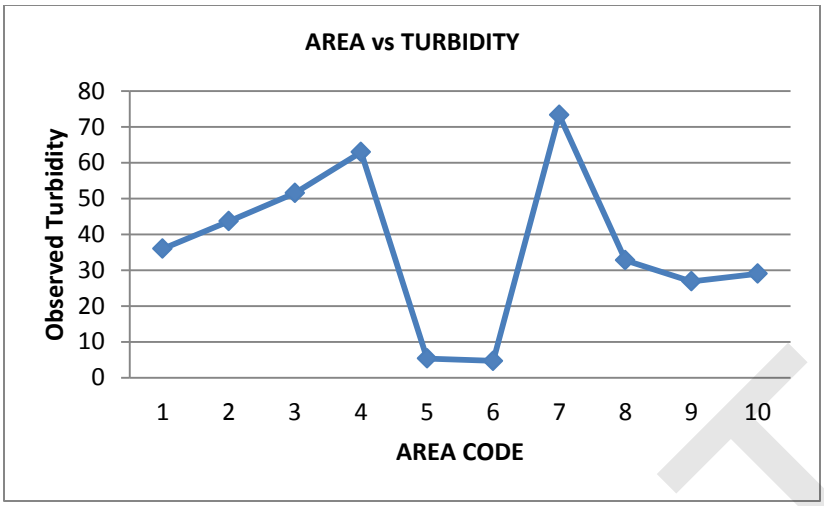

Figure: 3: Graphical representation between Area Vs Observed Turbidity

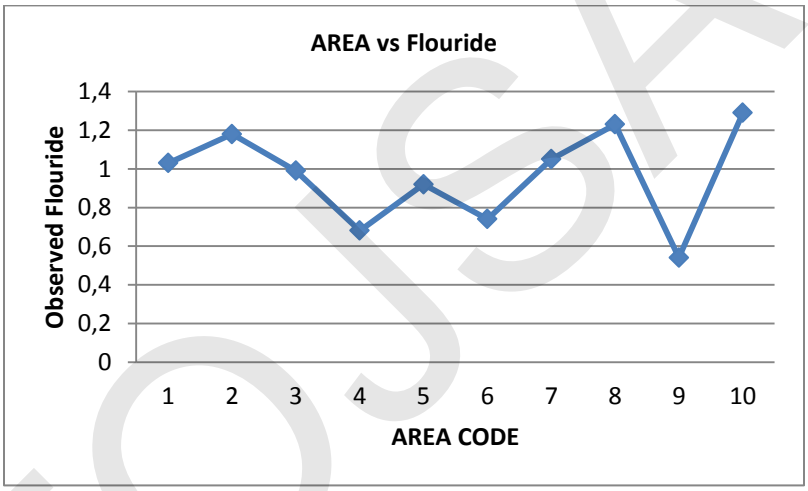

Figure: 4: Graphical representation between Area Vs Observed Flouride

Table 3: The physical and chemical parameters of the drinking water samples

Sampling site	Sample site code	Water Type	NO_3^- (mg/l)	SO_4^{2-} (mg/l)
Panyam Rural area	1	Surface	11.19	41.9
Panyam Stream	2	Surface	18.12	54.5
Panyam Lake	3	Surface	27.07	41.2
Konidedu	4	Ground	24.37	59.2
Neravada	5	Ground	32.49	68.7
Kowlur	6	Ground	19.45	53.4
Alamur	7	Ground	22.56	52.9
Maddur	8	Tap Water	39.76	--
Odiguntla	9	Tap Water	32.88	73.0
Balapanur	10	Tap Water	29.69	48.4

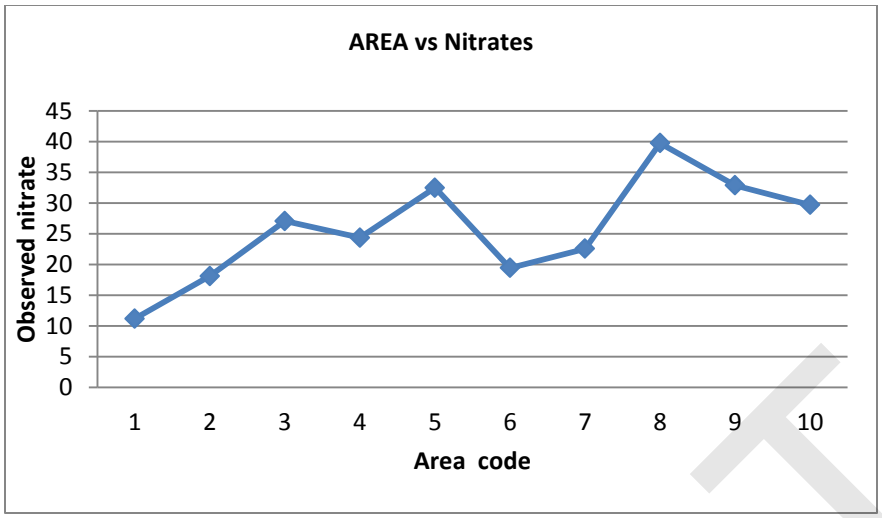

Figure: 5: Graphical representation between Area Vs Observed Nitrates

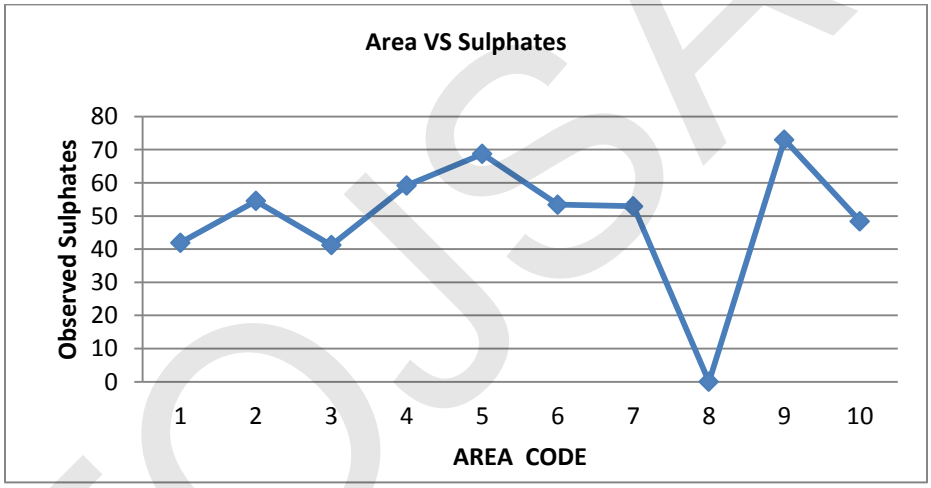

Figure: 6: Graphical representation between Area Vs Observed Sulphates

The concentrations of the major ions were below the permissible limits given by the WHO. The concentrations of trace metals (Cu, Fe, Zn, Al and Mn) ions in the drinking water samples are presented in Table 2. The lowest and the highest levels of trace metals detected ranged between 0.004 mg/L - 0.016mg/l for manganese in the sample from Konidedu and 2.96 mg/L for copper from the K.c.canal at Maddur villages. The highest level of total trace metals ions were found in the water sample from K.C Canal at Maddur Average copper concentrations in the drinking water samples were in the range of 1.25 to 2.96 mg/L. The levels in all the stations were above the limit of 1.0 mg/L permitted by WHO in drinking water. This indicates that the local mineral deposit in the catchment area studied may have high levels of copper. Copper is an essential nutrient, but at high doses it has been shown to cause stomach and intestinal distress, liver and kidney damage, and anemia (US EPA, 2003). The highest iron level was found in the sample from Kowlur as 0.94 mg/L and the lowest in Panyam as 0.08 mg/L, almost all the samples contain higher amount of iron except in Panyam where it was below the acceptance limit of iron which is 0.1 mg/ L permitted by the WHO. The levels of zinc in the samples were in the range of 5 mg/L to 19 mg/L . 80% village are between limit according to WHO . Average manganese levels were found to be in the range of 0.042 mg/L to 0.63 mg/L. 70% villages water samples were with in the WHO permitted limit Aluminum concentration in the drinking water samples were in the range from a limit which is 0.05 mg/L. Aluminum concentration in the drinking water samples

were in the range from a minimum of 0.07 mg/L from Panyam stream canal stream at Balapanur to a maximum of 0.18 mg/L from the river at Nandyal rural area. Aluminum was considerable below the limit of 0.5 mg/L permitted by WHO in drinking water. A linear regression correlation test was performed to investigate correlations between metal concentrations. The whole data were subjected to statistical analysis and correlation matrices were produced to examine the interrelationships between the investigated metal concentrations. Correlations between metal concentrations in water samples have been widely studied by a number of authors (Mohmood,*et al*, 1998 and Asubiojo, *et al*, 1997).

Table 4: The concentration of trace metals ion in the drinking water samples

Sampling site	Sample site code	Water Type	Cu	Zn
Panyam Rural area	1	Surface	1.25	8
Panyam Stream	2	Surface	1.76	5
Panyam Lake	3	Surface	1.98	7
Konidedu	4	Ground	1.47	12
Neravada	5	Ground	2.38	16
Kowlur	6	Ground	2.67	11
Alamur	7	Ground	2.12	19
Maddur	8	Tap Water	2.96	9
Odiguntla	9	Tap Water	1.98	8
Balapanur	10	Tap Water	2.25	13

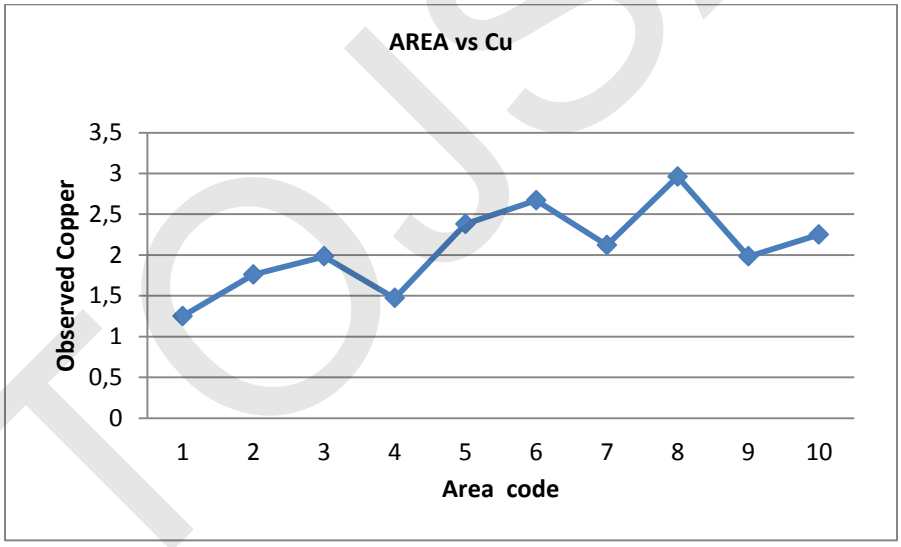

Figure: 7: Graphical representation between Area Vs Observed Copper

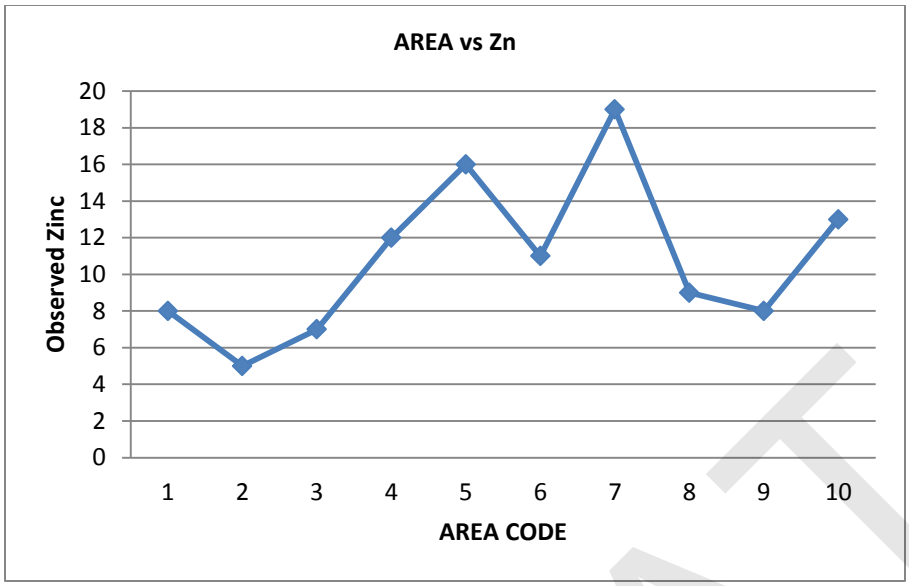

Figure: 8: Graphical representation between Area Vs Observed Zinc

Table 5: The concentration of trace metals ion in the drinking water samples

Sampling site	Sample site code	Water Type	Mn	Fe
Panyam Rural area	1	Surface	0.042	0.08
Panyam Stream	2	Surface	0.054	0.12
Panyam Lake	3	Surface	0.23	0.25
Konidedu	4	Ground	0.058	0.76
Neravada	5	Ground	0.63	0.68
Kowlur	6	Ground	0.08	0.94
Alamur	7	Ground	0.39	0.89
Maddur	8	Tap Water	0.066	0.27
Odiguntla	9	Tap Water	0.414	0.34
Balapanur	10	Tap Water	0.313	0.48

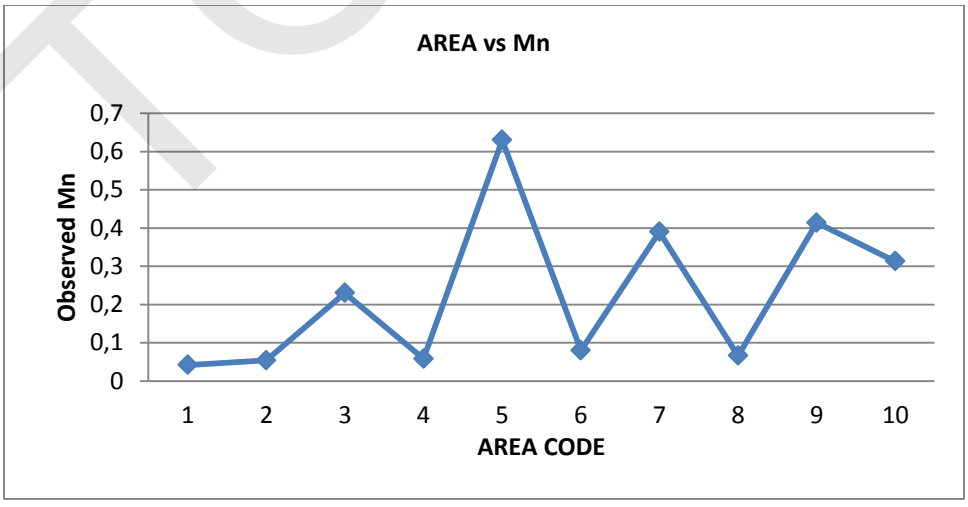

Figure: 9: Graphical representation between Area Vs Observed Manganese

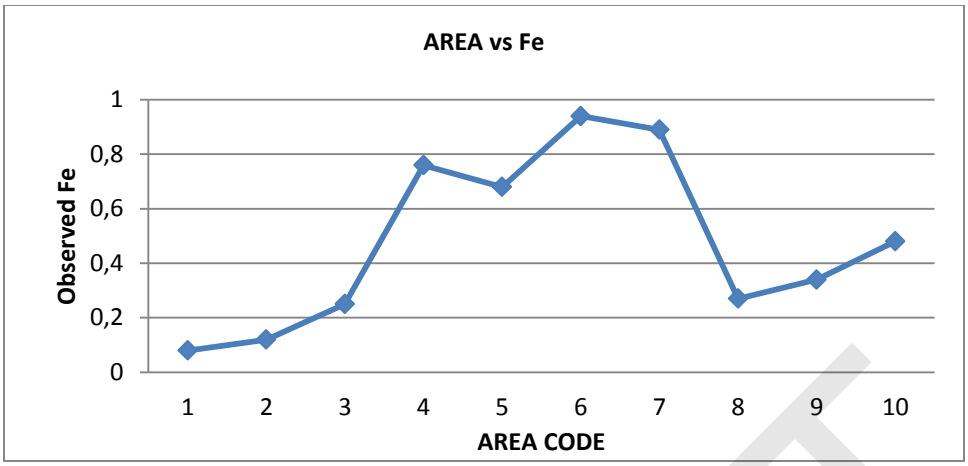

Figure: 10: Graphical representation between Area Vs Observed Fe

Table 6: The concentration of trace metals ion in the drinking water samples

Sampling site	Sample site code	Water Type	Al
Panyam Rural area	1	Surface	0.10
Panyam Stream	2	Surface	0.07
Panyam Lake	3	Surface	0.09
Konidedu	4	Ground	0.08
Neravada	5	Ground	0.11
Kowlur	6	Ground	0.15
Alamur	7	Ground	0.14
Maddur	8	Tap Water	0.12
Odiguntla	9	Tap Water	0.16
Balapanur	10	Tap Water	0.18

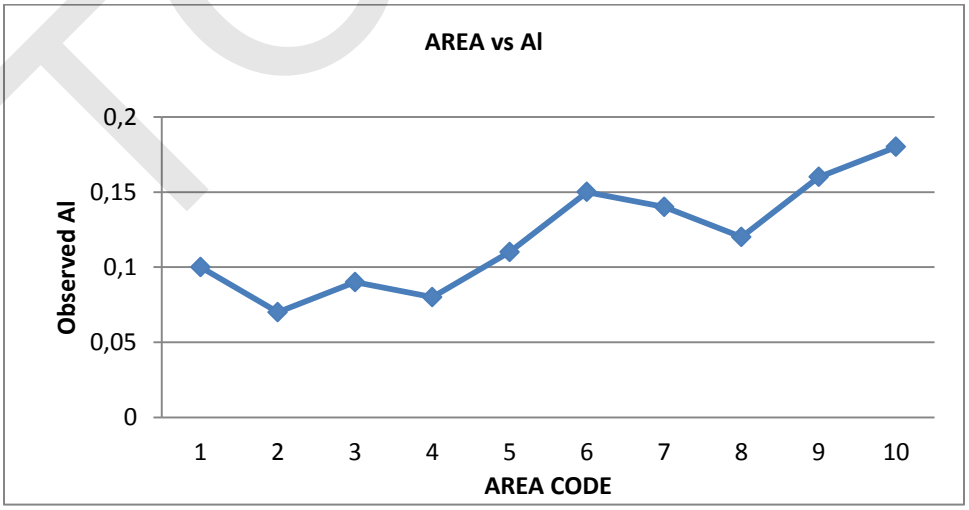

Figure: 11: Graphical representation between Area Vs Observed Aluminium

4. CONCLUSION

In conclusion, the concentrations of the investigated major ions and trace metal ions in the drinking water samples from these communities in the Nandyal region, Iddia were found below the guidelines for drinking waters given by the World Health Organization (WHO). Further research on other communities in this region for drinking water analyses is required as levels of contaminants may vary due to different soil types, water chemistry and different human activities. No correlations were found between metal concentrations in the drinking water samples.

5. ACKNOWLEDGEMENT

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TRAFFIC POLLUTANTS LEVELS AT DIFFERENT DESIGNS OF KING FAHD ROAD, SAUDI ARABIA: COMPARATIVE STUDY

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Abstract: Dammam and Khobar governorates constructed tunnels and bridges on King Fahd road. This study aimed at comparing traffic pollutants' levels on normally designed parts and at about 50m from the portals of tunnels and bridges. This study was conducted during March to August 2009. Particulate matter; traffic vapors and gases; and noise were measured at roadside level at the three designs of King Fahd Road in the two governorates. Levels of particulate matter, total volatile organic compounds, sulfur dioxide, carbon monoxide, and noise at the normally designed parts of King Fahd road [687(25.4)µg/m^3, 1.0(1.0)] 0.1(0.2), 4.0(5.0) ppm and 76.5(6.3)dB] were higher than that at tunneled [359.1(231.0), 1.0(1.0), ND(0.1), 2.0(2.0) and 72.8(5.3)] and bridged parts [420.4(259.8), ND(1.0), ND(0.0), 1.0(2.0) and 72.2(5.3)] respectively. Therefore, it can be concluded that traffic pollutants at tunneled and bridged parts of King Fahd road were lower than that at normally designed parts.

Key Words: Carbon monoxide, particulate matter, road re-design, sulfur dioxide, total volatile organic compounds, traffic gases, traffic pollution, traffic vapors.

1. INTRODUCTION

In the past, the major sources of poor air pollution were industrial activities and domestic heating. Nowadays; traffic pollution is predominant and significantly contributes in the urban air quality problems especially in roads of condensed traffic. Traffic congestion contributes to traffic pollution, and hence affects public health, and may cause annoyance particularly for those live, or work in heavy traffic roads. Traffic emissions and noise levels are higher in congested, stop-and-go and idling traffic than they are when traffic is moving at a steady speed (Balbus et al., 2010; Ingle, Wagh, Pachpande, Patel, & Attarde, 2005; Kassomenos, Karakitsios, & Papaloukas, 2006; Potoglou & Kanaroglou, 2005).

Traffic pollution is a drastic public health problem in both developed and developing nations (Issever et al., 2005). It is usually associated with human health hazards including asthma exacerbations and other cardiovascular illness (de Kok, Driece, Hogervorst, & Briede, 2006; Linn & Gong, 1999). Moreover, traffic pollutants, of which particulate matter (PM), total volatile organic compounds (VOCs) sulfur dioxide (SO_2), and carbon monoxide (CO), have significant effects on emergency department visits for asthma among children less than 2 years and elderly of more than 75 years (Villeneuve, Chen, Rowe, & Coates, 2007). According to World Health Organization, 2004 atmospheric pollution is the cause of 2.4 million deaths per year worldwide (Chimonasa & Gessner, 2007). In addition to their adverse effects on public health, traffic pollutants have also great impact on the environment that causes public health problems. These environmental impacts include depletion of ozone layer, generation of tropospheric ozone, greenhouse effects and acid deposition phenomenon (EPA, 2004; Fenger, 2009).

Dammam is the capital of the Eastern Province of Saudi Arabia. It is about 400 km away from Riyadh. It is the major seaport of the region. Khobar is another large city in the Eastern Province. It is one of the main commercial centers. In addition, there is increase in the migration of people to Dammam and Khobar governorates for getting job and studying. King Fahd Road is one of the heavy traffic roads that connects the two governorates Dammam and Khobar and penetrates them deeply. The two fractions of the road penetrate Dammam and Khobar are characterized by length, multiple activities, and heavy traffic. There are industries, universities and different governmental and commercial centers in the King Fahd Road. Hence, air pollution abatement will remain a challenge because of increasing demands for transportation (Potoglou & Kanaroglou, 2005). Recently, increasing traffic flow on Dammam and Khobar roads leads to traffic congestion that necessitates developing and implementing transportation control strategies, of which road redesign is one of the important strategies (NG, 2000; Orubu, 2004).

So, the two governorates implemented projects of re-designing King Fahd Road. Their objectives were to ease and speed up the transportation and reduce travelling time and time wasted at intersections (UNITED NATIONS, 2002). This took place by considering the two fractions in the road penetrate Dammam and Khobar governorates. Then, each road fraction was divided into three parts. The first part was left with normal design (normally designed parts). The second and third parts were re-designed by construction of tunnels and bridges (tunneled and bridged parts). On each of the tunneled and bridged parts the road was divided into two alternative pathways, one of which was the tunnel or bridge (below or above the roadside level) and the other was the normal road (at the roadside level). Hence, vehicles at the modified parts of King Fahd road were distributed between the two alternative pathways. Consequently, traffic congestion and idling traffic was greatly reduced and traffic flow was increased.

The research questions were whether the traffic pollutants at the roadside level on normally designed parts and at re-designed parts at about 50 m from the portals of tunnels or bridges are similar or not? And which road design is the best from air pollution and public health points of view? So this study aims at comparing traffic pollutants' levels on normally designed parts of King Fahd road and at about50 m from the portals tunnels and bridges at the roadside level and thus recommending the best design from air pollution and public health points of view.

2. MATERIAL AND METHODS

The study was conducted during March to August 2009 (after nearly one year of completing construction of tunnels and bridges under study) at the fractions of King Fahd road penetrate deeply in Dammam and Khobar governorates.

The sampling stations were located on the Curbside at the roadside level on the normally parts and at 50 m from the portals of both tunnels and bridges to be away from intersections and the portals of tunnels and bridges. The first parts in each fraction represented normally designed parts of road in both Dammam and Khobar (two sampling stations). The second and third parts represented parts of road modified by constructing tunnels and bridges in the two governorates (four sampling stations). Therefore, six sampling stations were included in the two governorates. Sampling occurred during the morning rush hours (6 am – 8 am) at the working days (Saturday – Wednesday).

Four traffic pollutants were measured during the present study including: particulate matter (PM) which was measured in micrograms per cubic meter ($\mu g/m^3$); total volatile organic compounds (VOCs), sulfur dioxide (SO_2), and carbon monoxide (CO) which were measured in parts per million (ppm); in addition to noise levels in decibel (dB). The EntryRAE (PGM-3000) Multi-Gas Monitor was used for measurement of VOCs and the VRAE Hand Held 5 Gas Surveyor (Model 7800 Monitor) was used for measurement of SO_2 and CO. For Quality Assurance purposes, data of the two gas monitors were calibrated against known concentrations of these gases. Noise levels were measured using Sound Level Meter Model CA832 calibrated at 114 dB. At each sampling station, 25 readings (over two-hour period) were directly recorded on the basis of 5 minutes averages for each gaseous pollutant and noise levels. Therefore, in the present study, there were 6000 records for each of the VOCs, SO_2, CO and noise levels. Half of the 6000 records were taken from Dammam and the other half from Khobar.

Particulate Matters were sampled on 60 mm diameter glass fiber filters by the pre-calibrated Hand Held Battery Portable air sampler on the basis of two- hour samples. After sampling, the filters were transferred to the Occupational Health and Air Pollution Research Unit Laboratories in High Institute of Public Health, Alexandria University, for further gravimetric determination and calculation of PM concentrations in $\mu g/m^3$. Therefore, there were 600 PM records, 300 of which from Dammam and the other 300 from Khobar.

Statistical Analysis

Data entry, statistical analysis and graphical presentation of data were done at High Institute of Public Health, Alexandria University using SPSS-16 package (Chicago, Illinois, 2007). The used statistical analysis were: Kolmogorov-Smirov and Shapiro- Wilk tests of normality, descriptive statistics (median and interquartile range), Kruskal Wallis test as a significance test of more than two independent samples and Mann Whitney Test as a significance test of two independent samples.

3. RESULTS AND DISCUSSION

Traffic pollutants levels at roadside on normally designed parts of King Fahd Road were higher than that at 50 m from the portals of tunnels and bridges. The total volatile organic compounds (VOCs), sulfur dioxide (SO_2) and carbon monoxide (CO) showed highly significant Kolmogrov-Smirnov and Shapiro-Wilk tests of normality ($p<0.05$ at 95% C.I). Therefore, VOCs, SO_2 and CO were non-parametric variables (did not follow normal distribution). Particulate matter (PM) and noise levels proved non-significant Kolmogrov-Smirnov and Shapiro-Wilk tests ($p>0.05$ at 95%C.I). Hence, they were parametric variables (follow normal distribution). For simplification, all numeric variables were assumed to be non-parametric(Glasser, 2005). Therefore, the data were expressed as [median (Inter-quartile range IQR)] and the tests of significance used were the non-parametric tests (Kruskal-Wallis and Mann Whitney tests).

3.1. Traffic Pollutants at the Two Governorates

The levels of PM [545.2(210.6) $\mu g/m^3$], VOCs [1.0(2.0) ppm], SO_2 [0.1(0.1) ppm], CO [3.0(3.0) ppm], and noise [74.5(5.9)dB] on King Fahd road in Dammam were higher than that in Khobar [287.9(428.6)$\mu g/m^3$], [Non-detected ND (1.0) ppm], [ND(0.1) ppm], [2.0(2.8) ppm], and [73.1(6.4) dB] respectively (table 1). This may be attributed to higher traffic volume as a result of higher commercial activities and presence of educational institutions on King Fahd road in Dammam. Mann-Whitney test revealed the highly significant differences of the five traffic pollutants' levels between the two governorates. This means that differences of traffic pollutants' levels between the two governorates were not due to chance.

Table 1. Comparison of medians of PM, vocs, SO_2, CO and noise at fractions of King Fahd Road in Dammam and Khobar Governorates during the period from during March to August 2009

	Dammam					Khobar					Mann-Whitney Test
	No	Median	Q1	Q3	IQR	No	Median	Q1	Q3	IQR	
PM	30	545.2	476.86	687.4775	210.6	30	287.9	245.2	673.9	428.6	<0.05
VOCs	300	1.0	ND	2.0	2.0	300	ND	ND	1.0	1.0	<0.05
SO_2	300	0.10	ND	0.1	0.1	300	ND	ND	0.1	0.1	<0.05
CO	300	3.0	1.0	4.0	3.0	300	2.0	1.0	3.8	2.8	<0.05
Noise	300	74.5	71.8	77.7	5.9	300	73.1	69.6	76.0	6.4	<0.05

PM	Concentration of particulate matter ($\mu g/m^3$)	CO	Concentration of carbon monoxide (ppm)
VOCs	Concentration of total volatile organic compounds (ppm)	Noise	Noise level (dB)
SO_2	Concentration of sulfur dioxide (ppm)	IQR	Inter-quartile range = Q3 – Q1
ND	Non-detected		

3.2. Traffic Pollutants at Normally Designed and Modified Parts of the Road

The levels of PM, VOCs, SO_2, CO, , and noise levels at the normally designed parts of road [687(25.4)$\mu g/m^3$, 1.0(1.0) 0.1(0.2), 4.0(5.0), ppm and 76.5(6.3)dB respectively] were higher than that at tunneled [359.1(231.0) $\mu g/m^3$, 1.0(1.0), ND(0.1), 2.0(2.0) ppm, and 72.8(5.3) dB] and bridged parts [420.4(259.8) $\mu g/m^3$, ND(1.0), ND(0.0), 1.0(2.0) ppm and 72.2(5.3) dB respectively] (figures.1, 2, 3, 4, 5). This may be due to different designs of King Fahd road in which the vehicles on tunneled or bridged parts were distributed between the two alternative pathways. This may reduce both traffic congestion, and idling and increase traffic flow. Hence, traffic pollutants at tunneled and bridged parts were consequently reduced [(4)].These findings were supported by two studies in Trento (Heimann et al., 2007), and Sydney(NSW, 2010) [(18)]. which concluded that emissions released from bridges and tunnels respectively reduce surface traffic congestion and improve roadside air quality(LightHouse, 2007).

It is also clear from figure 1 that at the roadside level, PM at tunneled parts of road [359.1(231.0)$\mu g/m^3$] were lower than that at bridged parts [420.4(259.8) $\mu g/m^3$) (NSW, 2010)[(18)]. In addition, PM levels at tunneled and bridged parts were 52% and 61% of that at normally designed parts respectively. This may be attributed to the aerodynamic diameter and collision of PM with walls and ceilings of the tunnels that may enhance settling at tunneled parts and consequently, reduce PM concentrations at the roadside level. In addition, PM excitation and dispersion at bridged parts of road reduce the settling velocity and enhance suspension in the atmosphere consequently increase PM concentrations at the roadside level(EPA, 2010). Kruskal Wallis test indicated the highly significant variation of PM among the three designs ($p<0.05$, at 95% C.I). Mann Whitney test showed significant differences between PM concentrations at the roadside levels of normal and tunneled, normal and bridged and tunneled and bridged parts of King Fahd Road ($p<0.05$, at 95% C.I). This indicated that the observed differences of PM concentrations at different road designs were realistic and were not due to chance.

Considering traffic vapors (VOCs), figure2 indicates the same VOCs levels at the roadside of tunneled and bridged parts. At bridged parts, the better vertical and horizontal diffusion and dilution of VOCs as a result of higher sources' heights reduce VOCs concentrations at roadside level while at tunneled parts the ceilings and walls of tunnels may absorb VOCs and hence, reduce their concentrations at roadside level (Heimann, et al., 2007 ; TAN, Vergel, & Camagay, 2006). Kruskal Wallis test indicated the highly significant variation in VOCs levels among the three road designs ($p<0.05$, at 95% C.I). Further analysis using Mann Whitney test revealed highly significant differences of VOCs at the roadside level between normal and tunneled and normal and bridged parts ($p<0.05$, at 95% C.I.) and non-significant difference between tunneled and bridged parts ($p>0.05$). This means that the observed variations were not due to chance.

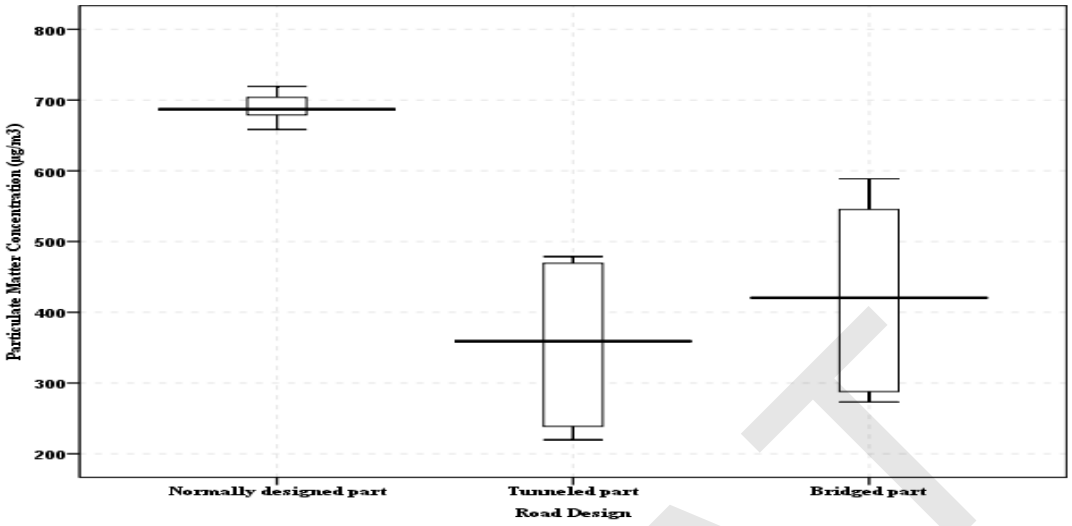

Figure (1). Particulate matter levels at normally designed parts and at 50m from the portals of tunnels and bridges on King Fahd Road in Dammam and Khobar Governorates during the period from March to August 2009

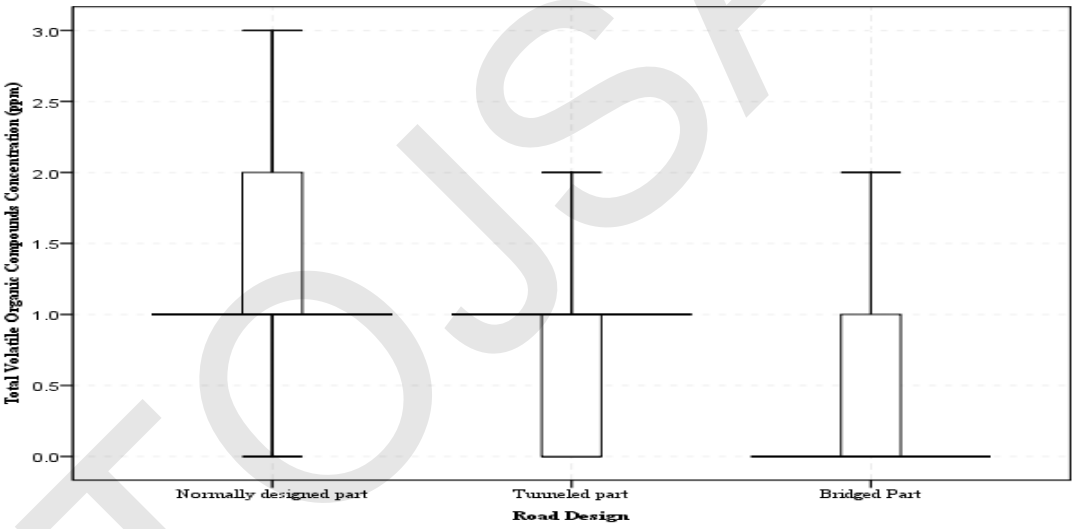

Figure (2). Total volatile organic compounds levels at normally designed parts and at 50m from the portals of tunnels and bridges on King Fahd Road in Dammam and Khobar Governorates during the period from March to August 2009

Regarding traffic gases, SO_2 at the roadside level confirmed higher levels at tunneled [ND (0.1) ppm] than at bridged parts [ND (0.0) ppm] (figure 3). This may be due to the lower vertical and horizontal diffusion and hence lower dilution at tunneled than that at bridged parts. Kruskal Wallis test, indicated the highly significant variation of SO_2 levels among the three road designs ($p<0.05$, at 95% C.I). Further analysis using Mann Whitney test revealed highly significant differences of SO_2 levels between normal and tunneled, normal and bridged and tunneled and bridged parts ($p<0.05$, at 95% C.I). Therefore, the observed differences were realistic and were not due to chance.

The lower levels of CO at bridged [2.0(2.0ppm)] than at tunneled parts [1.0(2.0)] of road were owing to higher diffusion that increased by increasing heights of emission sources (figure-4). Kruskal Wallis test revealed the highly significant variation of CO at the roadside among the three road designs ($p<0.05$, at 95% C.I). Further analysis using Mann Whitney test revealed highly significant differences of CO between normal and tunneled, normal and bridged and tunneled and bridged parts ($p<0.05$, at 95% C.I). Hence, the observed differences were not due to chance.

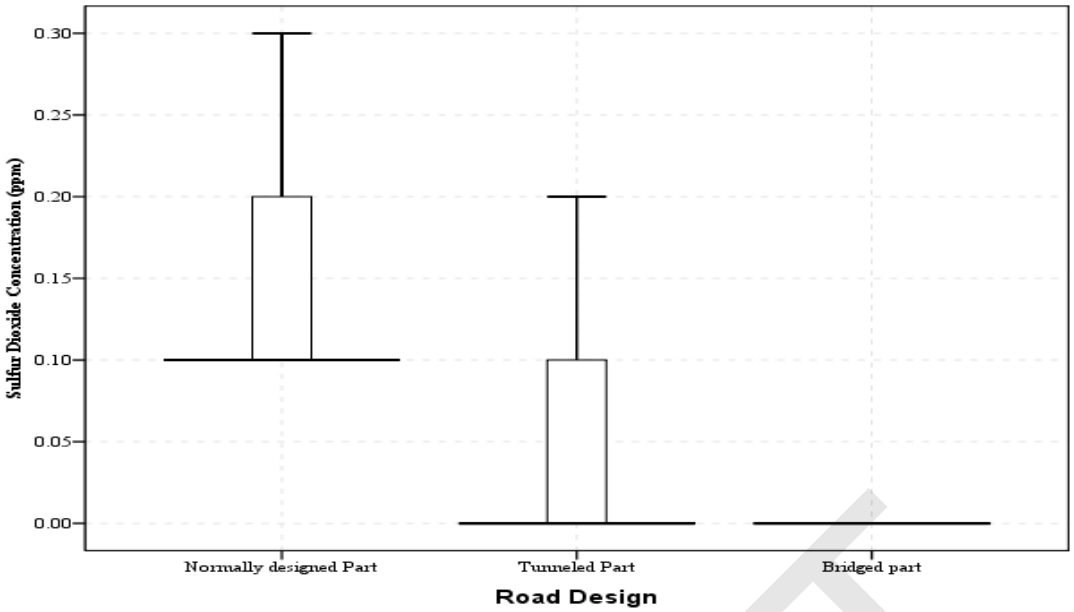

Figure (3). Sulfur dioxide levels at normally designed parts and at 50m from the portals of tunnels and bridges on King Fahd Road in Dammam and Khobar Governorates during the period from March to August 2009

Considering noise levels, figure 5 indicates that noise levels at the roadside of the bridged parts [72.8(5.3) dB] were slightly lower than that at tunneled parts [72.2(5.3) dB].This ensures that traffic is the main source of noise in King Fahd road. Kruskal Wallis test revealed the highly significant variation of noise among the three road designs ($p<0.05$, at 95% C.I). Further analysis by Mann Whitney test revealed highly significant differences of noise levels between normal and tunneled, normal and bridged and tunneled and bridged parts ($p<0.05$, at 95% C.I). Therefore, the observed differences were realistic and were not due to chance.

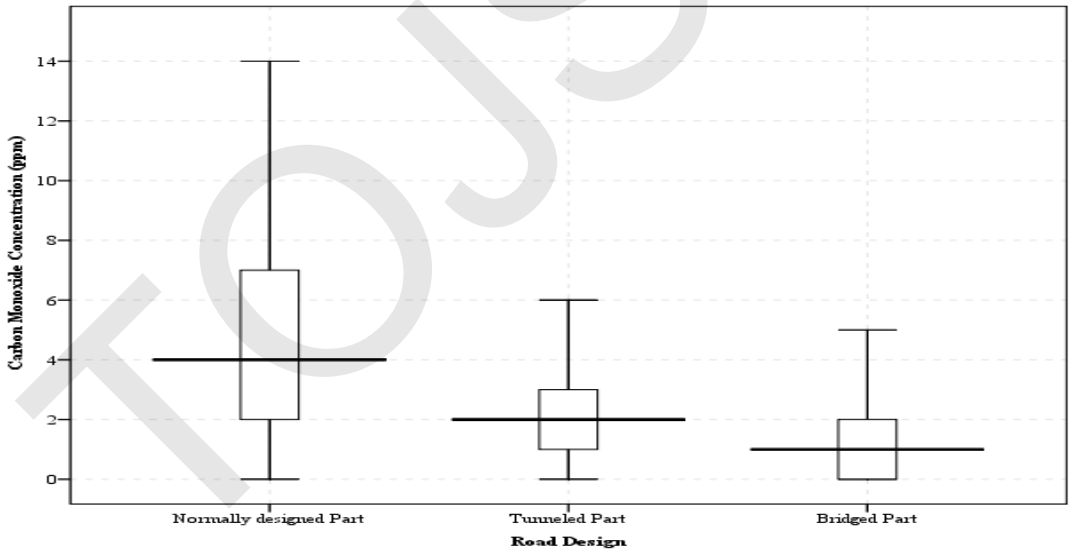

Figure (4). Carbon monoxide levels at normally designed parts and at 50m from the portals of tunnels and bridges on King Fahd Road in Dammam and Khobar Governorates during the period from March to August 2009

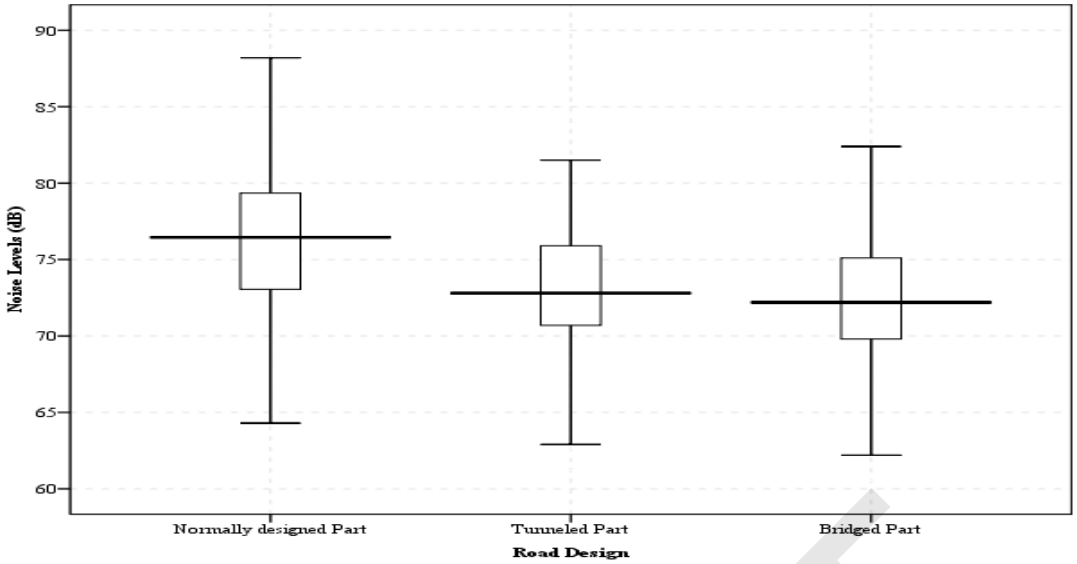

Figure (5). Noise levels at normally designed parts and at 50m from the portals of tunnels and bridges on King Fahd Road in Dammam and Khobar Governorates during the period from March to August 2009

All of the above results indicate the positive impact of the alternative roads on reducing traffic congestion and traffic pollutants levels. Both tunnels and bridges improve air quality at roadside level. The choice between tunnels and bridges depends mainly on the nature of traffic pollutants That is to say, in case of higher PM concentrations, tunnels are more recommended while in case of higher traffic gases and noise levels bridges are more recommended.

3.3. CONCLUSIONS

Traffic pollutants at the roadside level of tunneled and bridged parts of King Fahd road were lower than that at normally designed parts. Particulate matters (PM) at tunneled parts were lower than that at bridged parts. Traffic gases (SO_2, CO), and noise levels were lower at bridged than at tunneled parts. Traffic vapors (VOCs) levels were similar at both tunneled and bridged parts.

It is recommended to design and implement tunnels and/or bridges as one of the transportation control strategies in heavy traffic roads to reduce traffic congestion and mitigate traffic pollution. This mainly has positive impacts on the public health.

Further studies are recommended to assess the importance and effect of using other emission control technologies (catalytic converter, and electric transport; continuous maintenance of vehicles and transformation into cleaner fuel) on reduction of traffic pollutants on roads. In addition, improvement of public transportation and encouragement of their use may also lead to reduction of traffic density and traffic pollutants.

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WATER EFFECT ON DETERIORATIONS OF ASPHALT PAVEMENTS

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Abstract: Water has lots of adverse effects on pavement performance. In fact, moisture damage in asphalt pavements is global concern. Moisture damage can be defined as the loss of strength and durability in asphalt mixtures caused by the presence of water. Hence, it's the need to correctly identify the problem and isolate issues of contributing factors like material variability and construction practices for a better understanding of water effect on pavement deterioration. This study has discussed some of major failure mechanisms associated with the presence of water. In addition this study has also summarized some of the widely used methodology for the evaluation of water susceptibility. It was found that the empirical nature of test methods and the inherent variability of the results are the two primary challenges that impede the reliable characterization and assessment of water effect on pavement deterioration.

Keywords : Asphalt pavements, water effect, pavement deteriorations, stripping

INTRODUCTION

Moisture damage can be defined as the loss of strength and durability in asphalt mixtures caused by the presence of water. Moisture damage is induced by the loss of bond between the asphalt cement or the mastic (asphalt cement, the mineral filler and small aggregates) and the fine and coarse aggregate. Moisture damage accelerates as moisture permeates and weakens the mastic, making it more susceptible to moisture during cyclic loading. Finally, moisture damage mechanisms results in the following distresses.

- Stripping: Debonding of aggregates and binder at the bottom of HMA layer.
- Bleeding: Formation of asphalt binder film on the pavement.
- Rutting: Surface depression along wheel path.
- Corrugation and Shoving: Plastic movement typified by ripples or an abrupt wave across the pavement surface.
- Cracking, Water Bleeding and Pumping.
- Raveling: Progressive disintegration of HMA layer.
- Localized failures: Progressive loss of adhesion between binder and aggregates or progressive loss of cohesion in aggregates and in binder.

Historically, six contributing mechanisms have been identified associated with moisture damage: detachment, displacement, spontaneous emulsification, pore pressure induced damage, hydraulic scour, and the effects of the environment on the aggregate–asphalt system. However, it is to be mentioned that moisture damage is not limited to a single mechanism but is the outcome of a combination of these mechanisms (Little and Jones, 2003). Santucci and Aschenbrener (2003) have identified the following factors that contribute to adverse effects of water in asphalt pavement.

Table 1: Factors Contributing Water Induced Distresses (after Santucci and Aschenbrener, 2003)

Mix Design	• Binder and aggregate chemistry • Binder content • Air voids • Additives
Production	• Percent aggregate coating and quality of passing the No. 200 sieve • Temperature at plant • Excess aggregate moisture content • Presence of clay
Construction	• Compaction—high in-place air voids • Permeability—high values • Mix segregation • Changes from mix design to field production (field variability)
Climate	• High-rainfall areas • Freeze–thaw cycles

	• Desert issues (steam stripping)
Other Factors	• Surface drainage • Subsurface drainage • Rehab strategies—chip seals over marginal HMA materials • High truck ADTs.

Identification of the Problem

For a better understanding of water effect on pavement deterioration, it's the need to correctly identify the problem and isolate issues of contributing factors like material variability and construction practices. To this end, current study is intended to discuss the mechanisms associated with water induced damages in pavement. In order to fulfill this objective, this paper addresses following issues:

- Identification of the problem.
- Fundamental concepts- binder and aggregate interaction and representative failure mechanisms.
- Test methods to characterize moisture sensitivity.

FUNDAMENTAL CONCEPTS

Before delving deeper in the mechanisms of water induced distresses, sources of water ingress and egress should be identified. Current engineering practice is predicated on the fact that water enters the pavement despite the efforts to prevent it. The presence of water in the pavement is mainly due to infiltration through the pavement surfaces and shoulders, melting of ice during freezing/thawing cycles, capillary action, and seasonal changes in the water table. The significance of the respective routes depends on the materials, climate, and topography. Elsayed and Lindly (1996) noted that prior to the study by Ridgeway (1982), high water table and capillary water were thought to be the primary causes of excess water in pavements. However, crack and shoulder infiltration, and to some extent subgrade capillary action, are also considered to be the major routes of water entry to the pavement (Dawson and Hill, 1998). A simplified schematic for routes of ingress and egress of water is provided in Figure 1.

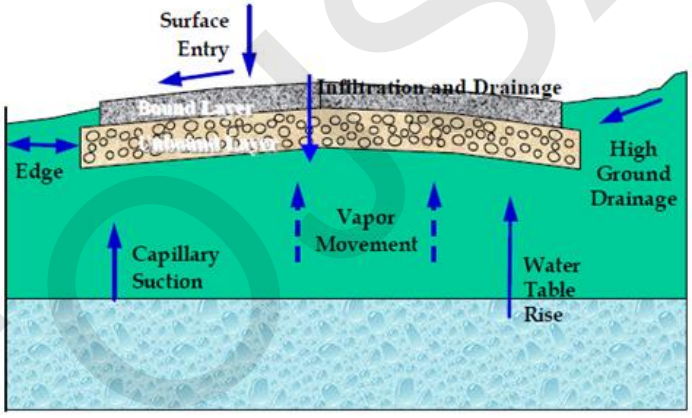

Figure 1: Possible Sources of Water in Pavement (after Elsayed and Lindly, 1996)

The majority of studies on moisture or water damage in asphalt mixtures deals with an observed phenomenon called stripping. Stripping is the dis-placement of asphalt films from aggregate surfaces that occurs when the aggregate has greater affinity for water than the asphalt. It has been speculated that asphalt may be able to strip from an aggregate under dry conditions, especially after it has aged many years, but most losses of adhesion are attributed to the action of water.

The aggregates and asphalt for mixtures susceptible to stripping can be treated with a variety of anti-stripping additives; these additives commonly include the following:

- Liquid anti-stripping additives
- Portland cement
- Hydrated lime

Studies done by Terrel and Al-Swailmi (1994), Kiggundu and Roberts, (1988), Taylor and Khosla (1983) revealed at least five different mechanics of stripping: detachment, displacement, spontaneous emulsification, pore pressure, and hydraulic scour. Kiggundu and Roberts (1988) mentioned additional mechanisms that may play a significant role in moisture damage. These incorporate pH instability and the effects of the environment or climate on asphalt–aggregate material systems.

Moisture Damage Theories

No single theory properly explains moisture damage. Considering this, Kiggundu and Roberts (1988) attempted to combine some of the theories discussed earlier. They tabulated the primary and secondary contribution relationships shown in Table 2. This table attempts to relate theories that explain loss of adhesion to stripping mechanisms. For example, the mechanism of pH instability is, according to Kiggundu and Roberts, explained by both chemical reaction theory and physical and chemical components of interfacial energy theory. Detachment, as a second example, is assumed to be explained by physical and chemical aspects of interfacial energy theory as well as physical aspects of mechanical interlock theory. The physical aspects are manifested, according to Kiggundu and Roberts, by surface energy, while the chemical aspects are attributed to the effects of polarity of the molecules present at the common boundary. Even with this attempt to simplify the interaction of different theories and mechanisms, the interactive complexity of the processes becomes clearly evident. For example, surface bond is not solely a physical process because surface bond is dictated by the chemical nature of bonding at the asphalt and aggregate surface as well as by the presence of broken bonds or incomplete coordination of atoms due to broken bonds resulting in an increase in free energy.

Table 2: Proposed Relationships between Theories of Adhesive Bond Loss and Stripping Mechanisms (After Kiggundu and Roberts, 1988)

		THEORY								
		Mechanical Interlock			Chemical Reaction			Interfacial Energy		
	Proposed Operating Mode	P	C	P-C	P	C	P-C	P	C	P-C
Stripping Mechanism	Detachment	S						S	W	
	Displacement					S		S		
	Spontaneous Emulsification				S	W				
	Film Rupture	S								
	Pore Pressure	S								
	Hydraulic Scouring	S								
	pH Instability					S				S

P= Physical C= Chemical P-C= Physical- Chemical S = Primary Contributor W= Secondary Contributor

TEST METHODS TO CHARACTERIZE MOISTURE SENSITIVITY

Numerous tests have been used to evaluate moisture susceptibility of HMA; however, no test to date has attained any wide acceptance (Roberts et al., 1996). In fact, just about any performance test that can be conducted on a wet or submerged sample can be used to evaluate the effect of moisture on HMA by comparing wet and dry sample test results

The tests that have been developed can be classified into two main categories based on the type of outcome: qualitative and quantitative. Qualitative tests provide a subjective evaluation of the stripping potential and include

- Boiling water test.
- Freeze–thaw pedestal test.
- Quick bottle test.
- Rolling bottle method.

The quantitative tests provide a value for a specific parameter such as strength before and after conditioning. These tests include

- Immersion–compression test.
- Indirect tensile test.
- Marshall immersion test.
- Double punch method.
- Resilient modulus tests.

On the other hand, the tests for identifying the moisture damage potential of an asphalt-aggregate mixture can be divided into two major categories based on mixture type: those on loose mixtures and those on compacted mixtures (Mansour et al., 2003). Tables 3 and 4 summarize the tests for moisture sensitivity on loose and compacted mixtures, respectively.

Table 3: Moisture Sensitivity Tests on Loose Samples

Test	ASTM	AASHTO	Other
Methylene blue			Technical Bulletin 145, International Slurry Seal Association
Film stripping			(California Test 302)
Static immersion	D 1664*	T182	
Dynamic immersion			
Chemical immersion			Standard Method TMH1 (Road Research Laboratory 1986, England)
Surface reaction			Ford et al. (1974)
Quick bottle			Virginia Highway and Transportation Research Council (Maupin 1980)
Boiling	D3625		Tex 530-C Kennedy et al. 1984
Rolling bottle Net adsorption			Isacsson and Jorgensen, Sweden, 1987 SHRP A- 341 (Curtis et al. 1993)
Surface energy			Thelen 1958, HRB Bulletin 192 Cheng et al., AAPT 2002
Pneumatic pull-off			Youtcheff and Aurilio (l997)

Table 4: Moisture Sensitivity Tests on Compacted Specimens

Test	ASTM	AASHTO	Other
Moisture vapor susceptibility			California Test 307 Developed in late 1940s
Immersion-compression	D1075	T165	ASTM STP 252 (Goode 1959)
Marshal immersion			Stuart 1986
Freeze-thaw pedestal test			Kennedy et al. 1982
Original Lottman indirect tension			NCHRP Report 246 (Lottman 1982); Transportation Research Record 515 (1974)
Modified Lottman indirect tension		T283	NCHRP Report 274 (Tunnicliff and Root 1984), Tex 531-C
Tunnicliff-Root	D 4867		NCHRP Report 274 (Tunnicliff and Root 1984)
ECS with resilient modulus			SHRP-A-403 (Al-Swailmi and Terrel 1994)
Hamburg wheel tracking			1993 Tex-242-F
Asphalt pavement analyzer			
ECS/SPT			NCHRP 9-34 2002-03
Multiple freeze-thaw			

Tests on Loose Mixtures

These are the tests conducted on asphalt-coated aggregates in the presence of water. Examples incorporate boil, film strip, and static/dynamic immersion tests. Major advantage of these tests is that they are simple to conduct and less costly to run than tests conducted on compacted specimens. The major disadvantage is that the tests are not capable of taking the pore pressure, traffic action, and mix mechanical properties into account. The results are mostly qualitative, and interpretation of the results becomes a subjective matter depending on the evaluator's experience and judgment. Loose mixture tests are best used for comparison between different aggregate- asphalt mixtures in terms of compatibility, strength of adhesion, and stripping. Mixtures failing in these tests, on the basis of some pre-established criteria, have the potential to strip and should be avoided. However, good results should not mean that a mix can be used, since the effects of the other contributing factors are overlooked in these tests.

In recent years, significant amount of research has been carried out to establish relationship between surface free energy and moisture damage potential. The principle behind using the concept of surface free energy is that the cohesive bonding within asphalt and the adhesive bonding between asphalt and aggregate are related to the surface free energy of the asphalt and aggregate. Researchers at Texas A&M University demonstrated the effectiveness of this concept by using three different aggregates (one granite and two limestone aggregates) and two of the SHRP asphalts (AAM and AAD).

The permanent deformation on compacted specimens using compressive testing correlated well with measured values of surface free energy of the asphalts and aggregates used in the research when tested in dry and wet conditions.

Tests on Compacted Mixtures

These tests are conducted on laboratory compacted specimens or field cores or slabs. Typical compacted mixture tests include indirect tensile freeze-thaw cyclic with modulus and strength measurement, immersion-compression, abrasion weight loss, and sonic vibration tests. The major advantage of these tests is that the mix physical and mechanical properties, water/traffic action, and pore pressure effects can be taken into account. Major disadvantages of these tests are the requirement of more elaborate testing equipment, longer testing times, and more laborious test procedures.

The AASHTO Standard Method of Test T283, "Resistance of Compacted Bituminous Mixture to Moisture Induced Damage," is one of the most commonly used procedures for determining HMA moisture susceptibility. This test is a modified version of Lottman Indirect Tension Test. The test involves curing of loose mixtures for 16 hours at 60° C, followed by an aging period of 2 hours at 135° C. At least six specimens are prepared and compacted. The compacted specimens are expected to have air void contents between 6.5% and 7.5%. Half of the compacted specimens are conditioned through a freeze (optional) cycle followed by a water bath. First, vacuum is applied to partially saturate specimens to a level between 55% and 80%. Vacuum-saturated samples are kept in a -18° C freezer for 16 hours and then placed in a 60° C water bath for 24 hours. After this period the specimens are considered conditioned. The other three samples remain unconditioned. All of the samples are brought to a constant temperature, and the indirect tensile strength is measured on both dry (unconditioned) and conditioned specimens. Test results are reported as a tensile strength ratio:

$$TSR = \frac{S_2}{S_1}$$

where, TSR = Tensile strength ratio,
S_1 = average dry sample tensile strength and
S_2 = average conditioned sample tensile strength.

The Hamburg Wheel Tracking Device (HWTD) is used to measure combined effects of rutting and moisture damage by rolling a steel wheel across the surface of an asphalt concrete specimen that is immersed in hot water. Originally, both beam and cylindrical samples were tested with device. However, with the increase in use of superpave gyratory compactor (SGC), researchers have adopted a testing protocol using cylindrical specimens compacted in the SGC as shown in Figure 2.

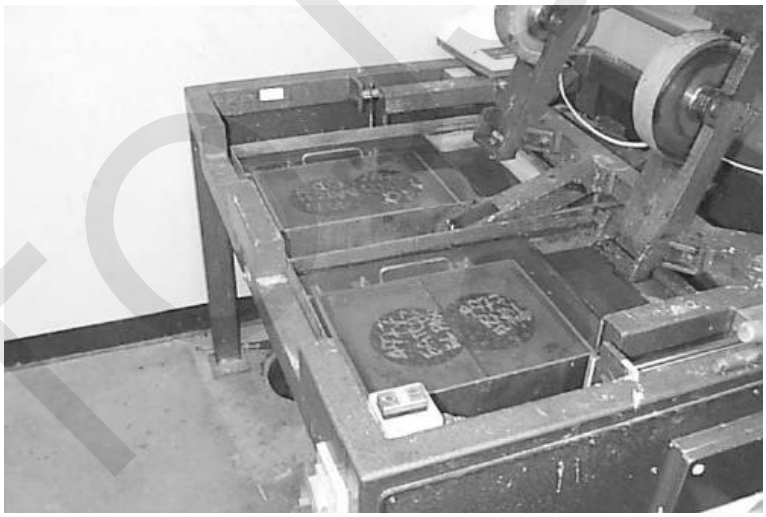

Figure 2: HWTD with Cylindrical Specimens

CONCLUSIONS

Water effect on pavement deterioration is a complex phenomenon involving thermodynamic, chemical, physical, and mechanical processes that contribute to pavement deterioration. This study has discussed some of major failure mechanisms associated with the presence of water. In addition this study has also summarized some of the widely used methodology for the evaluation of water susceptibility. It was found that the empirical nature of test methods and the inherent variability of the results are the two primary challenges that impede the reliable characterization and assessment of water effect on pavement deterioration. This study successfully conveys the fact that water effect on pavement deterioration is an open ended problem which is to be solved by the broader understanding of representative failure mechanism and site-specific treatments applicable to the problem.

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