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TABLE OF CONTENTS

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EDITORIAL

It is my proud privilege to welcome you all to the Academics World International Conference at Prague, Czech Republic. I am happy to see the papers from all part of the world and some of the best paper published in this proceedings. This proceeding brings out the various Research papers from diverse areas of Science, Engineering, Technology and Management. This platform is intended to provide a platform for researchers, educators and professionals to present their discoveries and innovative practice and to explore future trends and applications in the field Science and Engineering. However, this conference will also provide a forum for dissemination of knowledge on both theoretical and applied research on the above said area with an ultimate aim to bridge the gap between these coherent disciplines of knowledge. Thus the forum accelerates the trend of development of technology for next generation. Our goal is to make the Conference proceedings useful and interesting to audiences involved in research in these areas, as well as to those involved in design, implementation and operation, to achieve the goal.

I once again give thanks to the Academics World, Institute of Research and Journals & TheIIER for organizing this event in Prague, Czech Republic. I am sure the contributions by the authors shall add value to the research community. I also thank all the International Advisory members and Reviewers for making this event a Successful one.

Editor-In-Chief

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EVALUATION OF LACTATE DEHYDROGENASE ACTIVITY AND HISTOPATHOLOGY IN THE LAND SNAIL HELIX VERMICULATA EXPOSED TO HERBICIDE ZOOM.

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Abstract - The present study aimed to investigate the impact of an herbicide Zoom (65,9% of Dicamba and 4,1% of Triasulfuron) against the biomarker of oxidative stress and the histopathology of the hepatopancreas of the band chocolate snails Helix vermiculata (Müller, 1774), known as biomonitors of an ecosystem pollution. According to our results, Oxidative stress studied by the biomarker LDH. Meanwhile, a low effect on the tissue of hepatopancras was observed in the snail's of H. vermiculata after exposure to all the treatment with Zoom.

Keywords - Herbicide, Helix Vermiculata, Zoom, Pollution, Oxidative Stress, Histopathology.

I. INTRODUCTION

After the Second World War, man used chemicals in the fight against crop pests to increase yields and regulate agricultural production and to meet the growing food needs, due to population growth. (Bourbia-Ait Hamlet, 2012). Molluscs are one of the most important groups that contribute to terrestrial biodiversity. There are between 30,000 and 35,000 species of terrestrial molluscs in the world. (Tsai, 2004). These species are often used to control air, soil and water pollution. (Dallinger, 1994). Terrestrial gastropod molluscs have various physiological, biological and ecological characteristics, thus making it possible to compare the influence of these different parameters on their responses to environmental contamination. (Gomot de Vauflery & Pihan, 2000 ; Laskowski & Hopkin, 1996). Our study enters into this theme and aims to evaluate the responses of a bioindicator species of pollution, Helix vermiculata to a type of pesticide (Zoom). Several parameters were studied the activity of LDH and the histopathology of hepatopncreas.

II. MATERIAL & METHODS

1. Test Organisms:

The snails of Helix vermiculata (Müler, 1774) were collected from Bakkaria (3, 5-4, 5 g), Tebessa-Algeria. Animals were acclimated for one month before use. They were maintained in open air glass gages (20 x 20 x 20 cm, with 16 individuals per cage) a bed of sand (5 cm = 2kg) and fed every 3 days on lettuce leaves. Animals were acclimated for one month before use.

2. Experimental Protocol and Treatment:

In the experiment, different concentrations of the herbicide based on their recommended agricultural doses (Dose 1 and Dose 2) were used (Table 1): Dose 1 and Dose 2. The amount of pesticide required was determined by the total area of the experimental glass gages (400 cm^2), with particular concentrations are given in Table 2. For the control treatment, distilled water was used.

	Zoom	Distilled water
Dose 1	30 mg	200 ml
Dose 2	60 mg	200 ml

Table 1. Pesticides concentrations and fertilizer dose.

The snails were weighed with balance of precision (OHAUS, 0,000mg), their food was changed every 3 days and weight were monitored weekly. The snails were considered dead when they did not respond to the gentle mechanical prodding at the anterior of the body.Animal poisoning was carried out for 3 months by food.

3. Biomarker measurement

The LDH assay was based on the conversion of lactate to pyruvate or pyruvate to lactate. The lactate dehydrogenase activity (LDH) was spectrophotometrically measured according to the method of Hill and Lévi (1954) as previously described (Sifi and Soltani, 2019).

4. Histopathology

After 3 months of treatment and after the sacrifice of the snails, 4 individuals randomly selected at the batch level (controls and treated) are intended for histological studies. For the histopathology study of hepatopancreas, the latter undergoes the following steps: fixation, inclusion, cutting, staining and assembly described below for observations in optical microscopy

5. Data Analysis

The means and standard deviation were calculated in duality from independent experiments. Descriptive statics were given as box plots to describe the effects of Zoom on biochemical responses of snails. The data

pertaining were also analyzed with HSD (Tukey) and t Student test (GraphPadPrism version 7).

III. RESULTS

1. Effect of the herbicide Zoom on LDH activity:

The results mentioned in Table 30 show a significant increase in the series treated with the herbicide Zoom by the two doses (Dose 1 and Dose 2), after 1 month of treatment (Control vs Zoom D 1; p=0.0360, Control vs Zoom D 2; p=0.0106), after 2 months of treatment (Control vs Zoom D 1; p=0.0034, Witness vs Zoom D 2; p=0.0013) and after 3 months (Witness vs Zoom D 1; p=0.0021, Witness vs Zoom D2; p=0.0004). No dose effects were recorded during all treatment periods.

	Control	Zoom D 1	Zoom D 2
0	12,05 ± 0,28		
1	11,30 ± 0,02 **a**	13,21 ± 0,80 **b**	13,83 ± 0,35 **b**
2	11,45 ± 0,56 **a**	13,85 ± 0,40 **b**	14,35 ± 0,05 **b**
3	11,25 ± 0,31 **a**	14,01 ± 0,20 **b**	15, 03 ± 0,55**b**

Table 3: Effect of the herbicide Zoom on hepatopancreasLDH activity (µM/min/mg protein).

3. Effect of the herbicide Zoom on the tissue of hepatopancreas

Intertubular connective tissue: Ict; the light of the digestive tubule: L; digestive cells: CD; excretory cells: CE; excretory granules: ge; kernel: N. (A) Control snail, (B) snail treated with Decis insecticide (Dose 1). (C) snail treated with Decis insecticide (Dose 2). Tissue inflammation: , cell degeneration:

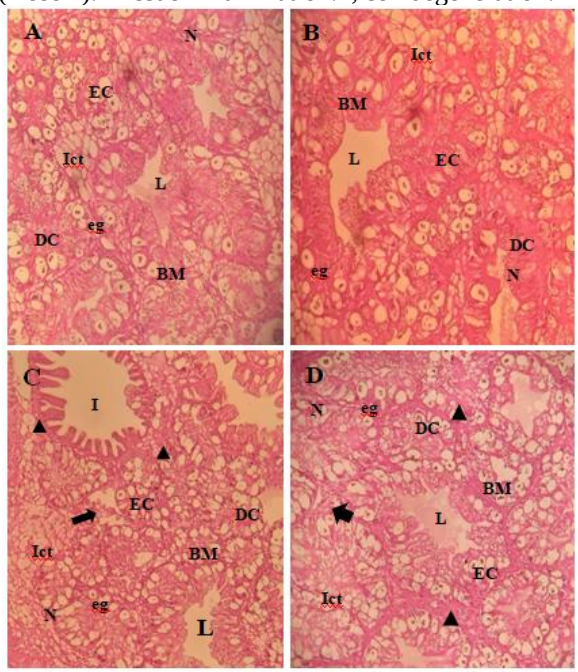

Figure 1: Optical microscope observations of cross-sections of part of the hepatopancreas of Helix vermiculata snails, control (A) and treated (B), (C) with the two doses of Zoom herbicide after 3 months of oral treatment (40×).

A histological section of the hepatopancreas of the control (0 days) and control (3 months) groups are shown in the figure (1A, 2B). Treatment with Zoom herbicide in both doses affects the tissue of the hepatopancreas. An onset of inflammation at the level of the basement membranes of the tubules a narrowing of the lumen in some of them are observed in the histological sections of the snails treated with dose 1 (Fig. 1B) At dose 2, histological examination showed an advanced degradation of the connective intertubular spaces with a partial degradation of the cells (Fig. 1C) compared to the controls.

IV. DISCUSSION

LDH levels are used in toxicology and clinical biology for the diagnosis of cell and tissue damage. LDH levels are used in toxicology and clinical biology for the diagnosis of cell and tissue damage. Similar increases were observed in Lymnaea natalensis exposed to niclosamide (Farid et al, 2009), The same species (E. vermiculata) subjected to sublethal doses of lannate also showed a significant increase in LDH activity compared to control (Khalil, 2016).). There was a significant increase in LDH activity in G. truncatula exposed to cadmium and dimethoate compared to control (Banaee, 2019) in other snail species and pollutants.

Histological analysis of the digestive glands of the snail confirmed inflammation and degeneration of tissues and affected the growth and survival potential (Lee et al. 2002). The possible use of cellular alterations on gastropod hepatopancreas as biomarkers for xenobiotic exposure has been studied (Marigomez et al, 1998; Snyman et al, 2005; Radwan et al, 2008).

Histopathological changes were induced by sublethal concentrations of oxyfluorfen, a herbicide in the digestive gland of Biomphalaria alexandrina snails, and these maternal-aged strains increased with increasing concentrations (Ibrahim & Sayed, 2019).El-Deeb et al. (2015) exposed Biomphalaria alexandrina snails to inorganic fertilizers (high phosphorus and nitrogen content) and recorded histological alterations of the digestive glands.

However, little information is available in the literature regarding the study of biochemical and histological markers of molluscs exposed to insectides (Radwan et al, 2008).

V. CONCLUSION

Our study evaluated the effect of an herbicide in a bioindicator species of pollution, Helix vermiculata on LDH and histopathological parameters. This herbicide induces activation of the detoxification system through an increase in LDH activity.

The histopathological study confirmed the sensitivity of the species compared to the treatment applied through clearly visible tissue damage, at the level of the hepatopancreas.

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IMPACT OF FREQUENCY UP-CONVERSION ON THE PERFORMANCE OF PENDULUM EQUIPPED WITH DYNAMIC VIBRATION ABSORBER

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Abstract - This paper addresses a study on the impact of frequency up-conversion on the vibration behavior of a pendulum designed for an energy harvester mechanism. The equations of motion are developed for 4 degrees of freedom mathematical model using the Lagrange method. The proposed mechanism consists of a pendulum that is located near a barrier and a shock absorber. The presence of a shock absorber reduces the range of displacement of the mass, and at the same time, the frequency up-conversion effect by barrier allows the use of a structure with a frequency higher than the excitation frequency. Both of these effects lead to smaller device sizes. Simulation results reveal that, relative to a simple pendulum structure, the RMS velocity of the mass may be increased, while the displacement of the mass is simultaneously decreased, resulting in an improved energy density.

Keywords: Frequency Up-Conversion, Pendulum Vibration, Low-frequency Excitation.

I. INTRODUCTION

Conventional resonant energy harvesters need to match the excitation frequency with the resonance frequency of the structure for maximum efficiency [1]. Although it is difficult to achieve such a thing in low frequencies, especially in human movement [2]. In addition, in applications such as human movement, on the one hand, the vibration source does not have a fixed frequency and is random in nature, and on the other hand, a harvester usually has a limited bandwidth around the resonance frequency. Also, in small structures, there are problems such as large vibration amplitudes. Large vibration amplitudes occur due to low stiffness to create resonance at low frequencies.

The frequency up-conversion method [3], presented by Kulah and Najafi [4], causes a high frequency oscillation from a low frequency source. Its purpose is also to solve the inherent problems of low frequency vibration energy harvesting. To achieve this phenomenon, a mechanism that uses two resonance structures is needed. In this mechanism, a low frequency resonator is used to excite one or more high frequency resonators. Various methods have been presented in this field [5-8] to match the low frequency resonator with the excitation frequency. These methods do not limit the low frequency resonator vibration range enough and use several structural elements; Therefore, such approaches lead to complex designs that require special manufacturing processes, and as a result, the volume of the device increases.

The alternative approach, to improve the performance of the low frequency harvester, shows that the use of shock absorbers [9] on the one hand reduces the problems related to the large vibration range and on the other hand increases the bandwidth of the device [10]. However, these designs are still dependent on the resonant element with the resonance frequency in the source vibration range and the maximum output power decreases with increasing bandwidth.

In this research, a method for up-conversion of mechanical frequency using a barrier with a high coefficient of restitution is proposed. This barrier is used as a means to achieve the frequency up-conversion effect in the pendulum connected to the supporting structure. In this way, the structural complexity of the device is reduced; because compared to multiple vibrating structures, only one pendulum is needed. At the same time, the vibration amplitude is reduced and as a result, more power density is obtained. Finally, the performance of the structure is compared with a simple pendulum under the same conditions.

II. MATHEMATICAL MODELING

To analyze the dynamic behavior of the mechanism in Fig. 1, the mathematical model of the structure is used using the equations of motion based on the Lagrange method to calculate the velocity and displacement. This structure consists of a pendulum with mass m and length l and a supporting structure of mass M. The mass of the barrier is m_b and its moment of inertia is J_b. The barrier is connected to the absorber with the mass m_a and the moment of inertia J_a by the linear spring k, it is also connected to the supporting structure with a similar spring. Also, the pendulum is connected to the supporting structure by a torsion spring with a coefficient of k_t.

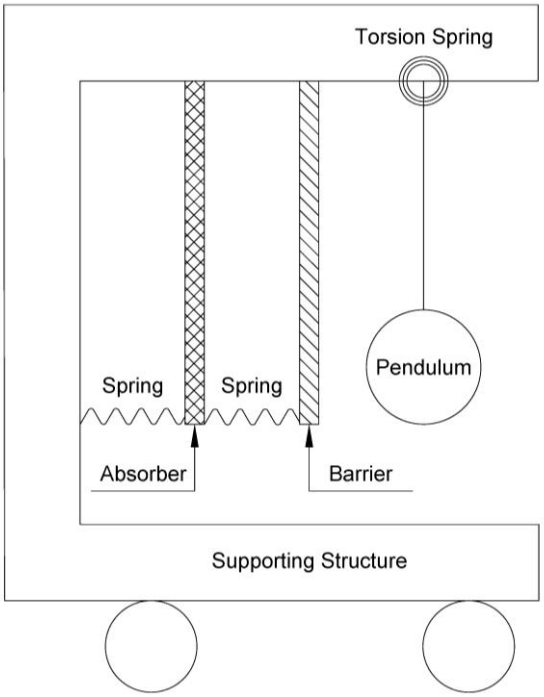

Fig.1. Mechanical frequency up-conversion mechanism

Under external excitation, the supporting structure moves to the sides and creates a relative acceleration opposite to the direction of movement in the pendulum. This causes potential energy to be stored in the pendulum as well. The relative acceleration and potential energy created in the pendulum causes it to collide with the barrier. Following this, several contacts are made between the pendulum and the barrier. By increasing the frequency of contact and reducing the displacement of the mass after each collision, the cycle is repeated until the kinetic energy of the mass is lost through the damping effect.

Hara et al. [11] showed that much higher frequencies can be generated by multiple contacts in a single stimulation cycle. Using this effect, when the excitation frequency is very low and the amplitude is high, it is possible to achieve an oscillation frequency in the pendulum that is significantly higher than its resonance frequency and the source excitation frequency.

2.1. Equivalent mass-spring-damper model

To describe the dynamics of the structure mathematically, e_c is described as the restitution coefficient between the mass and the barrier and the contact time is T_c. These parameters are expressed by Nagurka and Huang [12] in the form of equations (1) and (2) for spring and damper parameters equivalent to k_c and c_c.

$$(1)\quad k_c = m\frac{\pi^2 + (\ln e_c)^2}{T_c^2}$$

$$(2)\quad c_c = -m\frac{2\ln e_c}{T_c}$$

Acceleration of the supporting structure due to external excitation causes acceleration in the mass of the pendulum relative to the support structure. This relative acceleration is applied to the pendulum as a time-varying external force F_e(t). According to equation (3), the excitation force is described by the time-varying acceleration in the support structure (excitation acceleration) a_e(t).

$$(3)\quad F_e(t) = -m.a_e(t)$$

The force exerted on the pendulum by the equivalent spring element of the barrier (k_c) is proportional to the displacement of the pendulum relative to the barrier ($x+d$) and the force applied by the equivalent damper element of the barrier (c_c) is proportional to the relative velocity of the pendulum ($\dot{x}$). d is the distance between the mass and the barrier under the condition that there is no movement in the pendulum. Since the barrier spring and damper elements are not connected to the pendulum, it must be limited by a function of the force exerted on the pendulum by the barrier in the positive direction. Therefore, the force exerted on the pendulum when contacted by the barrier can be expressed as equation (4) using equations (1) and (2).

(4)

$$F_C(x,\dot{x},t) = \max\left[-m\frac{\pi^2 + (\ln e_c)^2}{T_c^2}(x+d) + m\frac{2\ln e_c}{T_c}\dot{x}, 0\right]$$

When the pendulum is in contact with the barrier and to calculate the total force on the pendulum, the sum of all the forces on it can be added together. The condition of contact can also be detected by the displacement of the pendulum relative to the barrier. So $x+d>0$ describes the non-contact state. As stated in section 2, Lagrange's equation is used to obtain the equations of motion, but for a better understanding, the acceleration of the pendulum relative to the support structure can be described by a piecewise nonlinear equation (5) that divides the forces applied to the pendulum by its mass.

$$(5)\quad \ddot{x}(x,\dot{x},t) = \frac{1}{m}\begin{cases} F_e(t), & \mathrm{x+d} > 0 \\ F_e(t) + F_C(x,\dot{x},t), & \mathrm{x+d} \le 0 \end{cases}$$

By solving equation (5), the time response of the velocity and displacement of the pendulum is obtained. By this answer, the RMS velocity of the pendulum can be calculated using equation (6), as the average relative velocity over a period T.

$$(6)\quad v_{RMS} = \sqrt{\frac{1}{T}\int_0^T \dot{x}(t)^2\,dt}$$

Given that the instantaneous kinetic energy of the pendulum relative to the support structure, U_k, is proportional to the square of the relative velocity of the mass. Therefore, an increase in the RMS means an increase in the average kinetic energy of the mass or increasing the transfer of mechanical energy to the mass. Also, the peak-to-peak displacement of the

pendulum can be obtained by equation (7) in a period of displacement. The minimum device volume is also described by the peak-to-peak mass displacement. Therefore, the proportional energy density (u) can be obtained by equation (8) using equations (6) and (7). Energy density is proportional to the average amount of energy that is transferred to the mass for an amount of space occupied per unit of mass.

(7) $x_{pk} = \max[x(t)] - \min[x(t)]$

(8) $u = \frac{v_{RMS}^2}{x_{pk}}$

III. SIMULATION RESULTS AND ANALYSIS

The simulation was performed using MATLAB™ and Simulink® and using the ode5 solver, with a time step of 10 μs. This system is investigated in the time response domain of the pendulum's velocity and displacement, the proportional energy density, and the RMS velocity of the pendulum's mass. Also, to calculate the natural frequency of the proposed mechanism, the linearized model of Lagrange equations was used.

The reference model includes a pendulum and a cart with masses of 20 and 150 g, respectively, a coefficient of restitution of 0.6, contact time of 1 ms, pendulum length of 2.5 cm, and separation distance of 0. A sinusoidal oscillation with acceleration amplitude of 9.81 ms^{-2} at a frequency of 3 Hz is considered as the input excitation to the system. Also, the spring's torsion coefficient is equal to 0.01 $N.m.rad^{-1}$. The barrier mass is 30 g and the moment of inertia is 4×10^{-6} $kg.m^2$ and the absorber mass is 50 g and the moment of inertia is 6.7×10^{-6} $kg.m^2$. Moreover, the spring stiffness equals 1000 N/m.

By solving the linear equations of motion, the natural frequencies were calculated as 0, 35.93, 86.81, and 185.75 Hz, respectively. The time response of the pendulum's velocity and displacement was obtained according to Lagrange's equation and is depicted in Fig. 2.

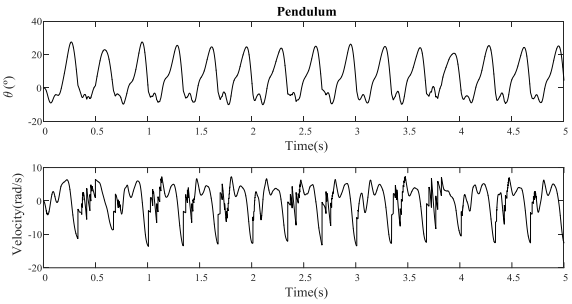

Fig.2. Time response of the pendulum velocity and displacement

The simulation result shows the effect of frequency up-conversion with high fluctuations. Time response of the barrier and absorber relative velocity and displacement, are shown in Fig. 3 and Fig. 4, respectively.

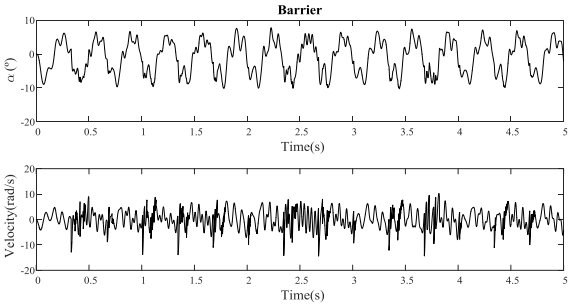

Fig.3. Time response of the barrier velocity and displacement

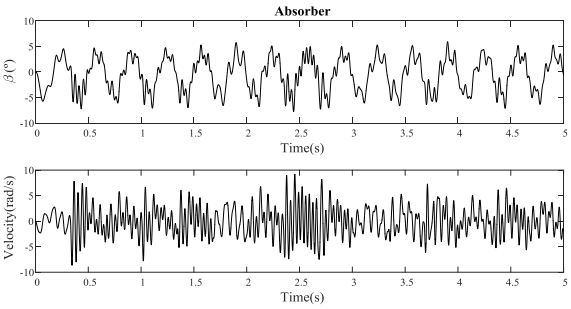

Fig.4. Time response of the absorber velocity and displacement

3.1. Comparison of the proportional energy density

To investigate the proportional energy density of the frequency up-conversion mechanism compared to the model without an absorber and its effect on the transfer of total energy to the mass, a simulation was performed between two reference models. The stimulation frequency in the range of 3 to 6.5 Hz with an increase of 0.5 Hz was considered as input. Then the proportional energy density of each component was obtained as a function of the excitation frequency according to Fig.5.

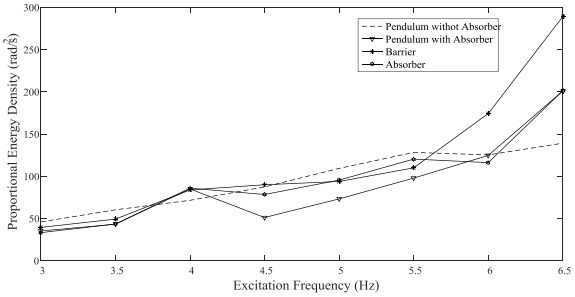

Fig.5. Comparison of the proportional energy density of the proposed mechanism with the simple pendulum

From Fig. 5, the maximum and minimum average proportional energy density in the excitation frequency range is related to the barrier with the value of 116.28 and the pendulum with the value of 88.97 $rad.s^{-2}$ in the model equipped with the absorber.

Also, the average proportional energy density for the simple pendulum equals 95.95 rad.s^{-2}.

3.2. Comparison of the RSM velocity

The maximum RMS velocity that can be achieved in the frequency up-conversion mechanism in the range of different separation distances to the barrier was obtained as a function of the excitation frequency and is shown in Fig. 6.

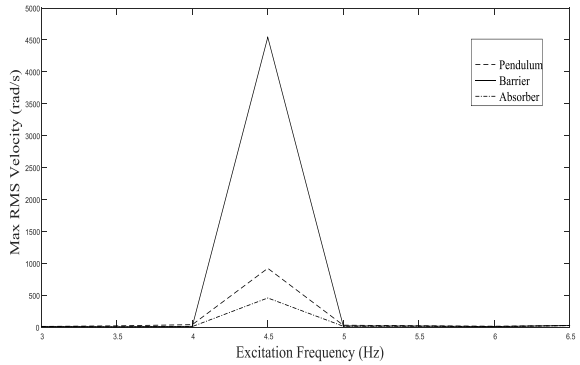

Fig.6. Comparison of the maximum RMS velocity in the proposed mechanism

It can be seen from Fig. 6 that the maximum RMS velocity of the barrier at the frequency of 4 Hz is significantly higher compared to other components. Therefore, considering this factor as well as the average energy density, the barrier at this excitation may be suitable for implementation in a low frequency energy harvester.

IV. CONCLUSION

This paper dealt with the performance of the proposed frequency up-conversion mechanism. For this purpose, a mathematical model was developed and simulations were performed. The proposed mechanism created the up-conversion effect in the pendulum and barrier. Then, the behavior of the structure compared to the simple pendulum was analyzed. Results show the advantage of this structure in increasing the transfer of energy to the mass and at the same time reducing the displacement of the pendulum. Since the resonance frequency of the structure is more than the excitation frequency, therefore, there is no need to match the excitation frequency, and it is possible to implement such a mechanism in low frequency energy harvesting devices.

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THE IMPACT OF TRANSFORMATIONAL LEADERSHIP ON THE ROLE OF SCHOOL ADMINISTRATION IN SPREADING THE CULTURE OF ENTREPRENEURSHIP

(A Field Study on Preparatory Schools in the State of Libya**)**

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Abstract - The current study aimed to assess the impact of the transformational leadership style on the role of school administration in teaching entrepreneurship for middle school students from the point of view of teachers and school administrators, through testing the modified role of the organizational climate. By analyzing the questionnaire data that was obtained from a convenance sample of (172) individuals from the study sample and to review the results of the study that were obtained. The researcher analyzed the data obtained from 172 preparatory school teachers and principals in Libya. The program 28SPSS and AMOS26 were used to analyze the data obtained. The study results concluded:

- The presence of a significant and statistically significant effect of transformational leadership on the role of school administration in spreading the culture of pioneering education in middle school schools in the State of Libya.

There is a significant effect of the transformational leadership on the role of school administration in spreading the culture of pioneering education, the axis of spreading the culture of entrepreneurship inside and outside the school in middle school schools in the State of Libya. And I found the most influential dimensions of transformational leadership: intellectual influence and idealized influence

- There is a significant effect of the transformational leadership on the role of school administration in spreading the culture of pioneering education, the axis of professional development for teachers in the field of entrepreneurship in middle school schools in the State of Libya. The most influential dimensions are: intellectual influence ideal influence, and mental stimulation.
- There is a significant effect with a statistical significance for transformational leadership on the role of school administration in supporting and financing pilot projects in middle school schools in the State of Libya. The most influential axes were: ideal influence, intellectual influence and mental stimulation.

The organizational climate modifies the relationship between transformational leadership and the role of school administration in spreading the culture of entrepreneurial education.

Keywords - Transformational Leadership, Organizational Climate, Entrepreneurship

I. INTRODUCTION

The problem of unemployment is one of the problems that trouble societies, as it affects them security, socially, and economically. Governments always seek to find a solution to this problem by eliminating its causes, which may be due to the weak wages available for holders of higher degrees and those with professional qualifications, or the incompatibility between education outputs. and the labor market.

According to the statement of the Ministry of Labor in Libya for the year 2019, unemployment in Libya is of forms and types, and between full unemployment, which means complete cessation of work, lack of opportunities to engage in labor activities, and partial unemployment, Libya suffers from force majeure, which is caused by the exit of international companies from the country from what This led to the suspension of workers from work due to the security situation, and the collapse of the wheel of the economy and development in the country.

This type of unemployment in Libya, and as a result of the instability, has greatly increased with the passage of days and the succession of crises, and the exit of companies and embassies that provided many jobs with good income.

A report of the International Labor Organization revealed that Libya occupied the second place in the Arab world with the highest unemployment rates. According to the report, which monitored unemployment rates in 2017, the unemployment rate in Libya reached 17.7%, behind Palestine, which ranked first with 26%.

It is noteworthy that the unemployment rate in the country recorded a significant decrease in the year 2011 by 15.2%, before it increased due to the security and political events in the country, and their direct impact on the economic situation in various sectors.

Unemployment in Libya is a strange and undesirable being and does not live in Libya, which is not its home.

Wars and conflicts in the country, which create a psychological condition for the worker that makes him ascetic in production and his unwillingness to develop the wheel of development, due to the lack of the minimum requirements of life, as well as his displacement from his place of work and residence.

Experts point out that unemployment in Libya was an important motive for thousands of Libyan youth to join armed organizations, especially if we know the huge salaries they receive monthly, ranging between 2000-3,000 dollars.

Among the strategic solutions adopted by a number of developed and developing countries alike is to resort to entrepreneurship as a great source for establishing emerging businesses and consolidating the culture of self-employment in societies, creating urgent and permanent job opportunities for citizens, opening up broad and wide horizons for innovation and encouraging initiatives (Ahmed, 2013).

Entrepreneurial education contributes to preparing and rehabilitating human capital. It also helps to develop the learner's capabilities in a way that makes him a good and effective citizen that contributes to building the nation and positively interacts with the surrounding business environment (Mubarak, 2014).

At the present time, the role of the school should not be limited to indoctrination and memorization only, but rather go beyond that to educate students and educate them in building the best directions for the future profession.

Culture, with all its components, plays an important and decisive role in the growth of entrepreneurship. Entrepreneurial culture requires encouraging business practice and motivating the community through learning the principles of entrepreneurship and developing skills and general features of it. The requirement for this education to be based on creativity, innovation and idea generation (Al-Shamimary and Al-Mubarik, 2014).

Orienting education towards the needs of the labor market is one of the necessities of the educational process at the present time. Education is seen in one of its aspects as a means of preparing highly qualified and highly efficient manpower for productive life and work. If the educational process expands horizontally or vertically without being accompanied by employment, uses and projects. The productivity required by society and demanded by the labor market, and from which other sectors benefit, the economic return of this process becomes decreasing (Badran and Sheikh, 2013).

The study (Al-Rubaie, 2017) indicated that the process of harmonizing education outcomes with the labor market in Libya has a number of challenges, the most important of which are:

The percentage of representation of higher education outputs in unemployment rates is much greater than the percentage of their participation in the labor market.

The dominance of the public sector over the labor market and the job guarantees granted by this sector led to the division of this market and the emergence of excessive demand for public sector jobs. Disguised and explicit unemployment, low labor productivity, and the emergence of quantitative imbalances in the composition of the labor force.

Therefore, the researcher found the necessity of activating the role of the school administration in teaching its students the basics of entrepreneurship, and providing them with some entrepreneurial skills so that they can contribute economically and solve the problem of unemployment. The proposed study seeks to answer the following questions:

• What is the impact of the transformational leadership style on the role of school administration in teaching entrepreneurship for middle school students from the teachers' point of view?

Transformational leadership theory begins with the concept of Burns (1978) & Bass (1985). Bass (1985) argues that transformational leaders stimulate their subordinates to formnew perceptions of leadership through new perspectives caused by intellectual stimulation. A leader is able to make perceptions as individuals who can support and pay attention to the people they lead through a balance of individuality, motivation and inspiring charisma.

Transformational leadership focuses on bringing about innovative change in institutions through followers who are willing to commit to the organization's vision and long-term goals (Sullivan & Decker, 2009). Transformational leadership style is able to have a significant influence on followers because transformational leaders can pay attention to their followers in self-development, change awareness about problems by looking at problems in new ways, and inspire followers to work hard in achieving organizational goals (Robbins & Judge, 2015).

II. LITERATURE REVIEW & HYPOTHESIS DEVELOPMENT

Most of the Arab and foreign studies in which companies and other institutions participated, in a form that focused on the importance of entrepreneurial education, a course in the development of societies, varied in terms of the fields and forms they dealt with. Al-Osaimi study study (2010) Al-Lakhawi study study (2008), the study of the True Man study (Troe men, 2003), the study of the study of the impact of entrepreneurship, the study of the study of Al-Salibi and Assi (2010), while the study of the study of Jawad and Thalith (2010) is a model proposal, a study study The impact of programs in developing entrepreneurial skills such as the study of Al-Maliki (2015) study (cindy, harry & wang) -chan, 2010) studies that dealt with the study of the instructions contained in the study and vision of Ramadan (2012) study (segumpan) &abuzahari, 2012) an easy-to-use educational interface The occasion for creativity as a study by Abu Zariqa (2006). Al-Khasawneh Study Study (2014) Obstacles to the development of creativity among students, and Al-Omari study (2011) sought to propose the Al-Khasawneh study strategy for leadership education. And the study (saeed, Moreno &yousafzai, 2014) aimed to measure the impact of university support on students' guidance, the study (Chang &rieple, 2013) and (anumu, 2014) to develop students' entrepreneurial skills, while the goal of Al-Ani and Al-Haritha (2015) and (pihie, bagheri&sani 2012),

Al-Ani and Ismail (2011) revealing the availability or possession of entrepreneurship and its relationship to some variables. It also tried to identify the reality of entrepreneurship, as the study of Muhammad and Abdul-Karim (2011)
The study of Al-Ani and Al-Harithiya (2015) the study (anumu, 2014) the study of Al-Kaf (2014) the study of Al-Ani and Ismail (2011), the study of Al-Omari (2011), the study of Muhammad and Abdul Karim (2011) the study of the Al-Osaimi study (2010) the study of the Balwani study (2008) and a simple study in the introduction to the methodology used in studying the methodology used in studying the methodology and the research study and Assi (2010) the experimental, the Khasawneh study (2014) the awareness method, the study (Govin, 2009) the comparative approach. The study of Jawad and Shifa (2010) field surveys.

As was the case in the studies conducted by the university on the transformational exchange on the pioneering role of school administration.
Most of the studies collected in your country are in Libya.

Based on the previous presentation of previousstudies, the study hypotheses can be formulated as follows:

H1: " There is a statistically significant effect of transformational leadership on the role of school administration in spreading the culture of pioneeringeducation in middle school schools in the State of Libya".

H1.a There is a significant effect of the idealinfluence dimensionon the role of school administration inspreading the culture of pioneering education in middle school schools in theState of Libya

H1.b :There is a significant effect of the Intellectual Stimulation on the role of school administration in spreading theculture of pioneering education in middle school schools in the State of Libya

H1.cThere is a significant effect ofInspirational Motivation on the role of school administration in spreading theculture of pioneering education in middle school schools in the State of Libya

H1.d :Thereis asignificant effect of the individual consideration dimension on the role ofschool administration in spreading the culture of pioneering education in middleschool schools in the State of Libya

H2: Organizational Climate moderates the relationship between transformational leadership and spreading the culture of pioneering education in middleschool schools in the State of Libya

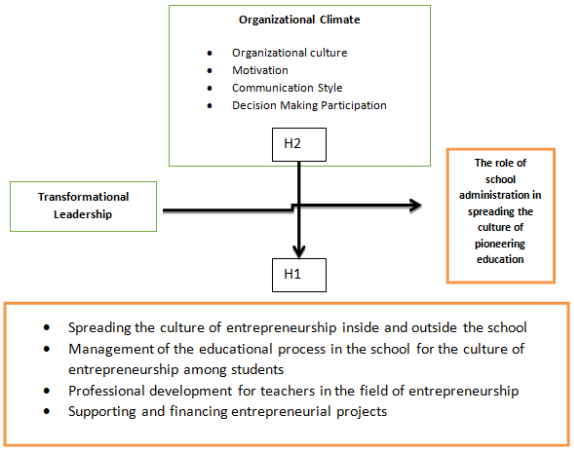

Figure (1-1) Research Model
Source: Prepared by the researcher based on the literature of previous studies

III. METHODOLOGY

This study uses quantitative approach where a questionnaire survey method is used to collect data
To achieve the purpose of the study and to test the hypotheses on which it was built, the researcher created a questionnaire, which is one of the primary data that is used in social and administrative sciences, such as questionnaire, observation, and personal interview.
The study population consisted of teachers and administration workers in middle schools in Libya. The researcher took a convenience sample of teachers in preparatory schools in the State of Libya.
Based on the foregoing, (350) questionnaires were distributed, and (322) questionnaires were retrieved, or (59.2%), and (29) questionnaires, or (11.2%) were excluded from the original distributor.
The study sample consisted of 61.5% of the sample males and 38.5% females, for the qualification variable, a high qualification holders of 63.6%, while 30.6% qualified above average. For the age variable, category (from 35 to less than 40) (32%, category) of 40 to less than 45) at a rate of 29.4%, 45 years and older by 64.7%. With regard to the job level at the manager level (61.8%), the managerial level (29.4%), the teacher (8.8%), and with regard to the experience level, 70.6% Of the sample have experience of more than 15 years, followed by 26.5% have experience from 10 to less than 15 years. A previously (350) were distributed and (322) questionnaires were retrieved, or (59.2%), and (29) questionnaires were excluded. That is (11.2%) out of the distributor.
The study adopted the five-gradual Likert Scale in the questionnaire to give more flexibility to the sample members in choosing

After the researcher completed the process of collecting the necessary data and information about the variables of this study, the stage of data analysis comes to answer the questions of the study and test its hypotheses.

IV. RESULTS

H1: " Thereis astatisticallysignificant effect of transformationalleadershipon the role of school administration in spreading the culture of pioneeringeducation in middle school schools in the State of Libya".

H1.aThere is a significant effect oftransformational leadership on the role of school administration in spreading the culture of entrepreneurial education. The axis of spreading the culture of entrepreneurship inside and outside the school in middle school schools in the State of Libya

variable	B	std. Error standard error	β . values	value (t)	P-value
(Constant)	0.165	0.163		1.008	0.314
TransformationalLeadership	0.968	0.041	0.818	23.36	0
R^2	0.818				
*At the significance level of α≤0.05					

Table (1) Regression analysis of the impact of transformational leadership on the axis of spreading the preparatory schools in Libya

As it is clear from the statistical results contained in Table) 1 ,(and from the follow-up to the values of the (T) test, that the transformational leadership variable has a positive impact on spreading the culture of entrepreneurship inside and outside the school in middle school schools in the State of Libya , where the calculated (T) values reached23.360 ,(which are significant values at the level of significance) α ≤ , (0.05and it was found that the transformationalleadership variable predicts about81.8 %as an impact factor of the changes occurring in spreading theculture of entrepreneurship inside and outside the school , which proves thevalidity and acceptance of the first sub-hypothesis.

H1.b :There is a significant effect of transformational leadership on the role of school administration in spreading the culture of entrepreneurial education. The axis of managing the educational process in the school for the culture of business administration among students in middle schools in the State of Libya

variable	B	std. Error standard error	β . values	value (t)	P-value
(Constant)	0.434	0.126		3.437	0.001
Transformational Leadership	1.063	0.032	0.896	33.225	0
Total Impact Factor R^2	96 0.8				
*At the significance level of α≤0.05					

Table (2) Regression analysis of the impact of transformational leadership on the axis of of managing the educational process in the school for the culture of business administration

It is also evident from thestatistical results contained in Table) 2 ,(and fromthe follow-up to the values of the (T) test that the transformationalleadership variable has a positive impact on the management of the educationalprocess in the school for the culture of business management among students inmiddle school schools in the State of Libya , where the values of (T) reachedCalculated) 33.225 ,(which are significant values at the level of significance) α ≤ ,(0.05and it was found that the transformationalleadership variable predicts about89.6 %as an impact factor of changes occurring in the management ofthe educational process in the school for the business culture of students , which proves the validity and acceptance of the second sub-hypothesis.

H1.cThere is a significant effect of transformational leadership on the role of school administration in spreading the culture of entrepreneurial education. The axis of Professional development for teachers in the field of entrepreneurship in preparatory schools in Libya

variable	B	std. Error standard error	β	(t)	P-value
(Constant)	0.612	0.15		4.07	0
TransformationalLeadership	1.11	0.038	0.871	29.106	0
TotalImpact Factor R^2	0.871				
*At the significance level of α≤0.05					

Table (3) Regression analysis of the impact of transformational leadership on The axis of Professional development for teachers in the field of entrepreneurship in preparatory schools in Libya

It is also evident from thestatistical results presented in Table) 3 ,(and fromthe follow-up to the values of the (T) test that the transformationalleadership variable has a positive impact on theprofessionaldevelopment of teachers in the field of entrepreneurship inmiddle schoolschools in the State of Libya , where the calculated (T) values reached29.106 ,(which are significant values at the level of significance) α ,(0.05 ≤and it was found that the transformationalleadership variable predicts about87.1 %as an impact factor of changes in the professional developmentof teachers in the field of entrepreneurship, which proves the validity andacceptance of the third sub-hypothesis.

H1.dThere is a significant effect of transformational leadership on the role of school administration in spreading the culture of entrepreneurial education.

The axis of supporting and financing entrepreneurial projects in middle school schools in Libya Table No.4 (regression analysis of the impact of transformational leadership on the axis of project support

variable	B	std. Error standard error	β . values	value (t)	p-value
(Constant)	0.38	0.182		2.081	0.038
TransformationalLeadership	1,049	0.046	0.81	22.688	0
Total Impact Factor R²	0.81				
*At the significance level of α≤0.05					

Table 4: the regression analysis of Entrepreneurship and its financing inmiddle school schools in Libya

It is also clear from thestatistical results contained in Table) 4 ,(and fromthe follow-up of the T-test values that the transformational leadershipvariable has a positive impact on the axis of supporting and financing pilotprojects in middle school schools in the State of Libya , where the calculatedT-values amounted to) 22.688 , (which are significant values at the level of significance) α ,(0.05 ≤and it was found that the transformationalleadership variable predicts about81 %as an impact factor of the changes occurring in the axis ofsupport and financing of entrepreneurial projects , which proves the validityand acceptance of the fourth sub-hypothesis.

The second main hypothesis: The organizational climatemodifies the relationship between the transformational leadership style and therole of school administration in spreading the culture of entrepreneurialeducation.

TableNo. (5-291Multiple regression analysis of theimpact of the organizational climate variable as a modified variable

variable	std. Error standard error	β . values	value (t)	R 2 Impact factor	p-value
Transformational Leadership	398	0.121	3.298	0.813	0.001
transformational leadership organizational climate	0.053	0.015	0.279	822	0
*At the significance level of α≤0.05					

Table 5: The impact of transformational leadership on the role of school administration inspreading the culture of entrepreneurial education

It is also evident from Table (5) that the value of the impact factor of transformational leadership is)R2 = .813 ,(which expresses transformational leadership on the role of school administration in spreading the culture of entrepreneurial education. When noting the significance limit, we find that theP-Value is less than 0.01%, and when testing theroleof the organizationalclimateas a modified variable, we findthat the value of the impact factor increased to) R2 = .822 ,(which means that the organizational climate modifies the impact of transformational leadership on The role ofschool administration in spreading the culture of entrepreneurial education , and the two variables together predict the change in the level of improving thequality of service provided by) 82.2 .(%Which confirms the validity of the second main hypothesis

V. CONCLUSION

- There is a statistically significant impact of transformational leadership on the role of school administration in spreading the culture of pioneering education in middle school schools in the State of Libya.
- There is a significant effect of the transformational leadership on the role of school administration in spreading the culture of entrepreneurial education, the axis of spreading the culture of entrepreneurship inside and outside the school in middle school schools in the State of Libya. And I found the most influential dimensions of transformational leadership: intellectual arousal and idealized influence

The presence of a significant effect with a statistical significance for transformational leadership on the

role of school administration in spreading the culture of pioneering education, the axis of professional development for teachers in the field of entrepreneurship in middle school schools in the State of Libya. The most influential dimensions are: intellectual arousal, ideal influence, and mental stimulation.

- There is a significant effect with a statistical significance for transformational leadership on the role of school administration in supporting and financing pilot projects in middle school schools in the State of Libya. The most influential axes were: ideal influence, intellectual arousal and mental stimulation.

The organizational climate modifies the relationship between transformational leadership and the role of school administration in spreading the culture of entrepreneurial education

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ECOLOGICAL ASSESSMENT OF THE STATE OF WATER BODIES IN NORTHERN KAZAKHSTAN ON THE EXAMPLE OF LAKE MAI-BALYK

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Abstract - This article presents the results of a comprehensive study of the ecological state of Lake Maybalyk in Akmola region. The main hydrochemical (pH, O_2 content, chromaticity, transparency, turbidity, NO_3^-, NO_2^-) and hydrobiological (zooplankton state, ichthyofauna) indicators were studied. It is established that all the studied indicators are within the normal range, which indicates the absence of external factors causing pollution of this reservoir during the studied time periods. Ecological assessment of the ichthyofauna, the forage base of Lake Maybalyk shows its prospects for fishery use.

Keywords - Hydrobionts, Ecomonitoring, Hydrochemistry, Hydrobiology

I. INTRODUCTION

Ecosystems of water bodies are one of the complex ecosystems in which a huge number of processes occur between biotic and abiotic components. [1]. Currently, Kazakhstan is faced with the problem of serious deterioration of the state of natural resources and the environment in all the most important environmental indicators. Kazakhstan ranks second in terms of the total volume of environmental pollution by organic substances among the countries of Central and Eastern Europe, Central Asia [2].

Every year, thousands of chemicals with unpredictable effects enter water basins, many of which are new chemical compounds. [3-7]. The most reliable indicator of changes in ecosystems and habitat under the influence of anthropogenic pollution of water or soil are the parameters of the state of individual biological objects or their groups, therefore, biological systems of different levels of organization are the central link of environmental monitoring: individual organisms, populations, communities [8-11].

The importance of hydrobionts in ecosystems is great and varied. Hydrobionts oxidize organic substances dissolved in water, thereby participating in the self-purification of water bodies, and are used as indicators of their saprobity [12-14]. The main components of the majority of freshwater basin ecosystems are algae, protozoa, worms, crustaceans and mollusks [15-16]. Based on the above, it is necessary to conduct a comprehensive environmental assessment in water bodies in order to increase the efficiency of resource use (water, land, biological, etc.) and their management, as one of the main priorities for the transition to a «green economy». This study is of interest in a conversation with the studied one of the main ecological components of water resources – the stability of the ecosystem and anthropogenic impact on the structure of biodiversity, which determines the main system of natural use and the permissible volume of withdrawal of bioresources in accordance with the requirements of the environmental legislation of the Republic of Kazakhstan.

II. DETAILS EXPERIMENTAL

Field expeditionary work to collect materials for scientific research was carried out in the period from 05/27/2021 to 10/27/2021. Hydrochemical observations were carried out simultaneously with the main hydrobiological studies. Sampling was carried out from the surface and bottom layers of water according to generally accepted methods [17-19].

The study of the hydrochemical regime of water was carried out with the help of a field laboratory for water quality analysis. The determination of dissolved oxygen was carried out by a thermo-oximeter. The study of the oxygen regime was carried out both from the surface of the reservoir and from the depth of the lake, to calculate the value of the oxygen balance.The pH of the water was measured by the pH meter testo 206 - TestoAGGermany. Nitrates were determined by visual colorimetric method. Chlorides were determined by argentometric titration. The chromaticity was determined by the visual colorimetric method. In the course of the work, the turbidity index was calculated and the normalized

index of suspended sediments was determined using Quantum GIS (QGIS) software using satellite images from Landsat 5 and Sentinel-2.

Turbidity index:

$$TI = \frac{Blue}{(Blue + Green + Red)}$$

NDSSI (normalized difference suspended sediment index) - was used by many authors [20] to develop models of suspended sediments in rivers, lakes, estuaries and many other water bodies.

$$NDSSI = \frac{(Blue - NIR)}{(Blue + NIR)}$$

The index is applied by subtracting the near infrared range from the blue range and calculating the result by the sum of both ranges. NDSSI also range from -1 to +1, where higher values indicate the presence of cleaner water, and lower values indicate the presence of more turbid water or land [20].

Zooplankton samples were taken by filtering 100 liters of water through the Upstein plankton network. The samples were fixed with 40% formalin to a final concentration of 4%. Identification of planktonic invertebrates to the species was carried out by the determinants [21-28].

The study for the presence of toxic elements in fish (lead, cadmium) was carried out on a TA-Lab voltammetric analyzer. Determination of radionuclides of caesium-137 and strontium-90 on the beta-gamma spectrometric complex "Progress BG"). A totalof 15 fishspecimenswereexamined.

III. RESULTS AND DISCUSSION

Lake Maybalyk, Tselinograd district of Akmola region is located in the south-east of the city of Nur-Sultan (coordinates 50°59'25" N71°30'11"E) (Fig. 1).

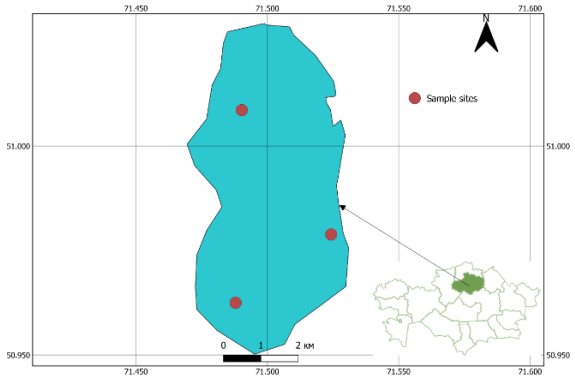

Fig. 1. Location of the study areas (Source: Authors)

The area of the lake is 2000 hectares. The salinity of Lake Maybalyk varies depending on the degree of filling from a salty reservoir (water salinity up to 20–27 g/l) in the years of shallowing to a slightly brackish lake (water mineralization up to 1.0–1.5 g/l in summer and 2.4 g/l) in the years of the highest water content. The soils in the adjacent territory are mainly meadow-chestnut, which are represented by carbonate, saline and alluvial rocks. Coastal vegetation is represented by reeds (Fig. 2).

Fig. 2. Lake Maybalyk, 2021 (Source: Authors)

About 100 species of plants grow east of Lake Maybalyk, more than 200 species of birds live, including breeding and migrating species, more than 30 species of mammals are found. The ichthyofauna is represented by the following species: perch (Percafluviatilis), roach (Rutilusrutilus), crucian carp (Carassius), carpbream (Abramisbrama), a small number of tench (Tincatinca), carp (Cyprinus), ruffe (Gymnocephaluscernuus). The temperature regime of the reservoir during the study period was 15.6-20.1 °C.

The smell of water was equal to 1 point, which indicates that the smell was detected experimentally in the laboratory. The characteristic of waters by color was in the range from 20 to 45 degrees, and refers to water bodies with low color (table 1).

Reservoir	Chromaticity, ^{0}C	Transparency, cm
LakeMaybalyk	35	42

Table 1: Physical indicators of water in Lake Maybalyk

The oxygen concentration in water determines the direction and speed of the processes of chemical and biochemical oxidation of organic and inorganic compounds. A decrease in oxygen content adversely affects the state of water bodies. During the period of expedition trips, a low content of the oxygen regime in Lake Maibalyk was noted with an oxygen balance of 0.2 to 1.8 mg/l. According to the oxygen content, the water of Lake Maybalyk can be classified as “moderately polluted water” (Table 2).

Reservoir	O_2 content in water, mg/l		
	nearthe surface	nearthebottom	O_2balance
LakeMaybalyk	3,9	2,1	1,8

Table 2:Oxygen regime of Maybalyk lake, mg/l

The content of biogenic elements (NO_3^-, NO_2^-) did not exceed the norms of maximum permissible concentrations. Depending on the pH level, Lake Maybalyk belongs to the reservoirs of the slightly alkaline group of waters (Table 3).

Reservoir	NO_3^- mg/l	NO_2^- mg/l	pH, mg/l
LakeMaybalyk	2,3	0,01	7,6

Table 3:The content of biogenic elements and the hydrogen index of Lake Maybalyk, mg/l

According to the data obtained on the turbidity index and the normalized index of suspended sediments, in 2016 there was a peak in the smallest amount of suspended substances in the lake in August, however, already in 2021 the index went into the negative side. However, thedecreaseinminusisnotserious (Figure 3-4).

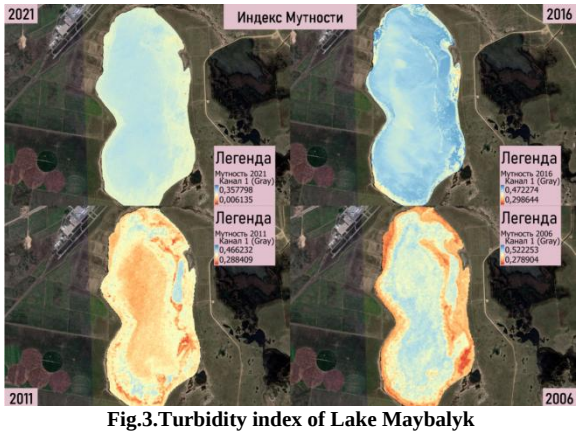

Fig.3.Turbidity index of Lake Maybalyk

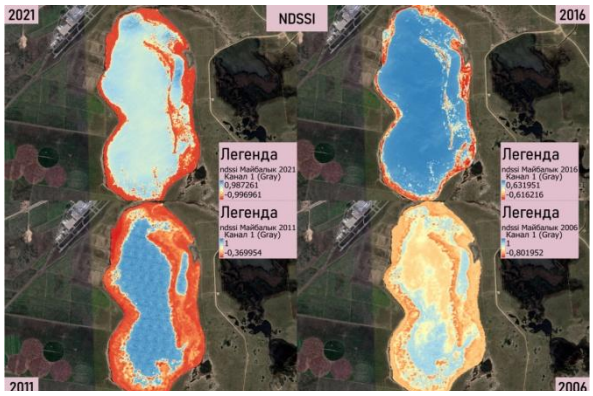

Fig.4.Normalized index of suspended sediments of Lake Maybalyk

In Lake Maybalyk, the diversity of planktonic invertebrates numbered 14 taxa, of which 6 rotifers, 4 branchiform, 3 paddleheads, 1 facultative plankters. Background species were rotifers Asplanchnagirodi, Brachionusangularis, Filinialongiseta, branchiform-Bosmina (Bosmina) longirostris, Ceriodaphniareticulata and cyclops Thermocyclopstaihokuensis. The number of planktonic animals was at a high level (Table 4). Its basis is 64.1%, formed by paddleheads. Thermocyclopstaihokuensis accounted for 40.4%, E. affinis – 23.8% of the total population.

Subdominated rotifers – 31.4%. Filinialongiseta formed the vast majority of the indicator – 22.6%, three times less, but almost equally complemented by Asplanchnagirodi (3.7%) and Brachionusangularis (4.5%). Zooplankton biomass averaged 2025.36 mg/m3 (Table 4). Copepods dominated in biomass (85.6%), with the absolute leader Th. taihokuensis.

Station	Rotatoria	Cladocera	Copepoda	Other	Total
Number, copies/m³					
1	73652	2322	127870	0	207161
2	57244	3731	138143	20	202869
3	41499	5825	85583	0	138711
average	57465	3959	117199	7	182914
Biomass, mg/m³					
1	142,81	27,29	1832,46	0	2228,28
2	117,43	37,10	2484,65	0,0931	2639,28
3	199,13	111,55	886,55	0	1208,51
average	153,13	58,65	1734,55	0,03	2025,36

Table 4: Quantitative indicators of zooplankton communities in Maybalyk Lakes, June 2021.

The biomass of zooplankton communities in Lake Maybalyk was 2025.4 mg/m3, which were formed by branchous and paddlefoot. They accounted for up to 66.6-96.3% of the total indicators of zooplanktocenoses.

In the veterinary laboratory in Nur-Sultan of the Akmola regional branch of the RSE at the Republican Veterinary Laboratory of the Committee for Veterinary Control and Supervision of the Ministry of Agriculture of the Republic of Kazakhstan, samples of freshly frozen fish caught in the reservoirs of the Akmola region on June 30 (9 samples) and August 16 (6 samples) 2021 were analyzed for the content of toxic elements (lead, cadmium) and radio-nuclides (cesium – 137, strontium 90).

According to the results of the study of this laboratory, no violations of veterinary and sanitary rules and safety requirements were found in all samples. The studied substances were found in significantly smaller quantities than the established regulatory indicators.

Table 5 shows data on the content of toxic and radioactive elements in fish samples (crucian carp) caught at the beginning of the summer period in Lake Maybalyk.

№	Name of the substance	Normalized values of indicators	Research results
1	Cesium-137, Bk/kg	not more than 130 Bq/kg	22,90 ±13,10
	Strontium 90, Bq/kg	not more than 100 Bq/kg	23,40±11,00
	Cadmium, mg/kg	not more than 0.2 mg/kg	0,072±0,0024
	Lead, mg/kg	not more than 0,1 mg/kg	0,060±0,017
2	Cesium-137, Bk/kg	not more than 130 Bq/kg	22,89 ±11,00
	Strontium 90, Bq/kg	not more than 100 Bq/kg	23,39±13,00
	Cadmium, mg/kg	not more than 0.2 mg/kg	0,054±0,0021
	Lead, mg/kg	not more than 0,1 mg/kg	0,063±0,019
3	Cesium-137, Bk/kg	not more than 130 Bq/kg	22,50 ±12,10
	Strontium 90, Bq/kg	not more than 100 Bq/kg	22,40±10,00
	Cadmium, mg/kg	not more than 0.2 mg/kg	0,073±0,0024
	Lead, mg/kg	not more than 0,1 mg/kg	0,063±0,019

4	Cesium-137, Bk/kg	not more than 130 Bq/kg	22,50 ±12,10
	Strontium 90, Bq/kg	not more than 100 Bq/kg	22,40±10,00
	Cadmium, mg/kg	not more than 0.2 mg/kg	0,073±0,0024
	Lead, mg/kg	not more than 0,1 mg/kg	0,063±0,019
5	Cesium-137, Bk/kg	not more than 130 Bq/kg	21,90 ±14,10
	Strontium 90, Bq/kg	not more than 100 Bq/kg	23,40±10,00
	Cadmium, mg/kg	not more than 0.2 mg/kg	0,064±0,0028
	Lead, mg/kg	not more than 0,1 mg/kg	0,053±0,017
6	Cesium-137, Bk/kg	not more than 130 Bq/kg	24,90 ±13,10
	Strontium 90, Bq/kg	not more than 100 Bq/kg	23,40±10,03
	Cadmium, mg/kg	not more than 0.2 mg/kg	0,074±0,0024
	Lead, mg/kg	not more than 0,1 mg/kg	0,063±0,019
7	Cesium-137, Bk/kg	not more than 130 Bq/kg	21,90 ±14,10
	Strontium 90, Bq/kg	not more than 100 Bq/kg	20,40±15,00
	Cadmium, mg/kg	not more than 0.2 mg/kg	0,064±0,0021
	Lead, mg/kg	not more than 0,1 mg/kg	0,063±0,018
8	Cesium-137, Bk/kg	not more than 130 Bq/kg	22,80 ±15,00
	Strontium 90, Bq/kg	not more than 100 Bq/kg	23,39±10,01
	Cadmium, mg/kg	not more than 0.2 mg/kg	0,060±0,0024
	Lead, mg/kg	not more than 0,1 mg/kg	0,063±0,019
9	Cesium-137, Bk/kg	not more than 130 Bq/kg	22,90 ±15,10
	Strontium 90, Bq/kg	not more than 100 Bq/kg	23,40±10,00
	Cadmium, mg/kg	not more than 0.2 mg/kg	0,074±0,0024
	Lead, mg/kg	not more than 0,1 mg/kg	0,063±0,019

Table5: The results of the examination of the content of toxic and radioactive elements in the samples of the ichthyofauna of the reservoirs of the Akmola region at the beginning of the summer period (30.06.21)

The indicators of the content of heavy metals and radionuclides in the tested fish samples are significantly less than the normative ones, which indicates the absence of contamination of the studied reservoirs with these toxic and radioactive elements at the beginning of the summer period. Also, apparently, during the entire summer season in the studied reservoirs, the ichthyofauna is not exposed to harmful effects of heavy metals and radioactive particles. This is evidenced by the data obtained after analyzing samples of fish caught in the late summer period in the same reservoirs.

IV. CONCLUSIONS

Thus, for the first time in Kazakhstan, a comprehensive study of the ecological state of Lake Maybalyk was conducted. Water monitoring was carried out according to the main hydrochemical and hydrobiological indicators in the spring, summer and autumn of 2021. It is established that all the studied indicators are within the normal range, which indicates the absence of external factors causing pollution of this reservoir during the studied time periods. Ecological assessment of the ichthyofauna, the forage base of Lake Maybalyk shows its prospects for fishery use.

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INTERSEMIOTIC TRANSLATIONAS A DIALOGUE OF CULTURES

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Abstract - The search for solutions to translation problems has a high level of importance in terms of modern globalization, as the translation itself may determine the success or failure of intercultural dialogue. To put it another way, successful translation is one of the keys for ensuring successful cultural dialogue. In the process of cultural dialogue, it acquires special importance insofar as translation is not only a means, the result of the transformation of the original text, but also a manifestation of national and universal values. It becomes more conspicuous and appealing when it is the result of inter-semiotic translation of the source text. Following R. Jacobson, we consider intersemiotic transformation, intersemiotic translation to be the delivery, transmission of verbal information by non-verbal means. Among those, we are considering a screen adaptation. Screening – target text, as the product of inter-semiotic transformation (so-called inter-semiotic translation). This occurs when the means of transfer of information is changed and transformed.
Transformation takes place when:
- Nonverbal codes are added to source verbal information – sound, mimics, gestures, costumes, decorations, background display, motion, music etc.
- There is a compensation of verbal information taking place. The verbal text is not sounded in entirety. For instance, the description of towns, the physical description of the people, mills - we see all of these visually and not verbally. That is where the compensation for the verbal text takes place. Actually, there are two parts to the verbal text. The first is that represented by the images (mimics, gestures, decorations for instance); the second one is that which is chosen and selected by the screenplay writer and film director and which is sounded and "spoken"; here, we need to take the opportunity to discuss what is "most important" and "less important" information. With "most important", it is impossible to omit or represent by non-verbal forms. Whereas "less important" information is not necessary to sound and it is often sufficient to reflect it in non-verbal forms. We consider intercultural transformation not only the transformation of the verbal characteristics of the text. In this case, we are dealing with a transformation cultural information encoded in the text (both national and universal) into a language understandable for the bearer of culture.

Key words - Intersemiotic Translation, Dialogue of Cultures, Nonverbal Codes, Transformation of Cultural Information, Compensation of Verbal Information

NEOMYCIN REMOVAL USING FUNGAL BIOTECHNOLOGY

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Abstract –

Problem statement: Recalcitrant compounds including antibiotics often remain in the effluents from wastewater treatment plants (WWTPs). The increased concentration levels of antibiotics like the aminoglycoside neomycin in wastewater streams leading to WWTPs thus constitute a breeding ground for antibiotic resistant bacteria. In this study, it was investigated whether it is possible to use fungal species to biodegrade this compound.

Methods: The biodegradation of neomycin was investigated using the white rot fungus species *Trametes versicolor* (phylum Basidiomycota) and the ericoid mycorrhizal species *Rhizoscyphusericae* (phylum Ascomycota). The study was conducted in the presence (co-metabolism) and absence of external nutrients. The experimental design included the nutrients glucose and ammonium tartrate which were added at different concentration levels to determine their influence on the biodegradation degree. All experiments were conducted at pH 4.0 with the aim to suppress bacterial growth. Liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS) was used for neomycin quantification purposes. The initial concentration of the aminoglycoside was 34 mg L^{-1} and was motivated by a large-scale application in which these concentration levels are relevant.

Results: At selected co-metabolic conditions, approximately 70% of neomycin was removed by each species. *Rhizoscyphusericae* caused a removal of 60% in the absence of external nutrients. It was shown that this mycorrhizal species could use the antibiotic as growth substrate while the biodegradation using *Trametes versicolor* was directly correlated to the amounts of the external nutrients.

Conclusion: This study indicates that there are species, belonging to different phyla that can be used in fungal biotechnology to biodegrade aminoglycosides like neomycin. Biodegradation at non-cometabolic conditions as was demonstrated by the use of *Rhizoscyphusericae* is of interest since the production costs in large scale applications will be reduced.

Keywords – Neomycin, Biodegradation, LC-HRMS, *Trametesversicolor*, *Rhizoscyphusericae*

THE EFFECT OF QUALITY DIMENSIONS ON INFORMAL FAST FOOD RESTAURANTS IN SOUTH AFRICA

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Abstract – The informal fast-food restaurant sector has been growing rapidly in South Africa over the past decades. The sector is important for the development of township economies, as it creates opportunities for self-employment and jobs in township communities. The quality dimensions such as food, service and physical environment of a food restaurant are crucial determinants of customer satisfaction and continual business growth. The objective of this paper is to examine the influence of quality dimensions on customer satisfaction in an informal micro-fast-food restaurant; quality dimensions are important to the survival and growth of informal micro-fast-food restaurants. A survey was conducted on customers of informal micro-fast-food restaurants using a convenience sampling method. The collected data was transferred to the Statistical Package of Social Science (SPSS) for data analysis. The results showed how to enhance customer satisfaction and increase loyalty by means of improving quality dimension of restaurant, in particular the setting and environment of business. The results further suggest that the continuous improvement of the ambiance of the restaurant as it is essential to creating an atmosphere in the restaurant that will attract more customers.

Keywords - Quality Dimension, Customer Satisfaction, Informal Sector, Micro Fast Food Restaurants.

I. INTRODUCTION

The informal micro-fast-food restaurant (IMFFR) sector has been growing rapidly in South Africa over the past decades, more specifically in townships. The sector is important for the development of township economies, as it creates opportunities for self-employment and jobs in township communities (Mhlanga, 2018; Petersen & Charman, 2018a; Petersen & Charman, 2018b; Knox et al., 2019). The rapid growth of the IMFFR in townships brings along a progressively multifaceted and highly competitive sector, according to Mhlanga (2018). In such environment, restaurant owners should have an understanding of the market environment and its influence on the restaurant's performance, and more importantly it growth prospects. In this study, the micro-fast-food restaurant sector in South Africa is typically located in the informal trading sector along the township streets and side-walks near busy transport nodes and marketplaces. They operate in areas of heavy foot traffic, as indicated by Battersby et al., (2016) and Knox et al. (2019), where people conveniently buy a meal or snack near or on the way to and from work or school during the day and into the evening. The micro fast food restaurants prepare and sell a range of affordable food products such as traditional meals, sandwiches, roasted meat, deep fried foods, boiled eggs, cold drinks and many others. Customers can take these snacks away with them or eat them at the seating areas provided, which often are inadequate. According to Musara and Nieuwenhuizen (2020), given the high proportion of this informal enterprises that operate in the food restaurant sector, it is difficult to measure the full size of the sector or the number of people who depend on the incomes that they generate. However, it is estimated that globally more than 60 percent of employed people earn their livelihoods in the informal economy, with figures going up to 86 percent in Africa, as analysed by Statistics South Africa (StatsSA) (2016), Medina et al. (2017) and International Labour Organisation (2018). In South Africa, 20 percent of the people are employed and earn their livelihoods in the informal sector (Rogan & Skinner, 2020; StatsSA, 2020), which suggests that the South Africa's economy, while significant, is comparatively small, combined with high levels of unemployment, with the official and expanded rates of unemployment in the first quarter of 2020 at 30 percent and 40 percent respectively (StatsSA, 2020).

Generally, the informal sector provides employment with below-average wages for survival and informal workers and informal micro-entrepreneurs operating at the survivalist level, as established by Mahadea and Zogli (2018) and Etim and Daramda (2020), underlining the importance for the micro-enterprises to gradually step from survivalist to developmental levels in the business life-cycle. In order for the enterprises to survive, grow and generate greater profits, the micro-fast-food restaurants must pay more attention to price, service quality, food quality and physical environment (Kotler &Amstrong, 2010; Zhang & Moon, 2020). Maintaining customer satisfaction is very important because it can lead to repeat purchase by customers and increased sales. The objective of this paper is to examine the influence of quality dimensions on customer satisfaction in an IMFFR, the dimensions are important to the survival and growth of businesses. Presented here are the objectives of the paper based on the importance of quality dimensions on IMFFR in the North West province, South Africa.

The importance of the informal economic sector in South Africa

The informal economy plays an integral role in addressing the socio-economic issues confronting many developing nations (Gurtoo& Williams, 2009; Webb et al., 2013; Autio& Fu, 2015; Williams & Youssef, 2015). South Africa's informal sector accounts for a significant contribution towards the total employment in the country. StatsSA (2015), in its third quarter, revealed that over 2 million South Africans are employed in the informal sector. This accounts for about 16.7% of the total employment in South Africa. Similarly, Rogan and Skinner (2017) observed that about 2.5 million people in South Africa are employed in the informal sector, of whom about 1.5 million people are self-employed. Rogan and Skinner (2017) further note that, for the period between 2008 and 2014, informal sector entrepreneurship continued to contribute between 16% and 18% of South Africa's total employment figures. The contribution of informal sector entrepreneurship towards the gross domestic product (GDP) is also remarkable, as analysed by Musara and Nieuwenhuizen (2020). Saunders and Loots (2005), in their study of informal sector contribution to the GDP, established that the informal sector entrepreneurship averaged 9.5% of the country's GDP during the period from 1967 to 2002. The South Reserve Bank (SARB) (1999) also estimated that the contribution of the informal sector towards the country's GDP was at approximately 7% in 1999. In relatively recent estimates, StatsSA (2018) and SARB (2019) have estimated that the informal sector contributes up to 5.5% of the country's GDP.

Generally, the informal sector, consisting – of unpaid workers in family enterprises, casual wage employment, seasonal employment, home-based worker, employment for immigrants, street vending, are the means for survival for poor communities in townships and rural areas (Mahadea&Zogli, 2018), which is also prevalent when there are slower growth and cycles of downturn in the economy, resulting in a decline in the national GDP as a result of external factors such as the Coronavirus pandemic that has adversely impacted the economy and people's livelihoods. Consequently, these have widened the output gap and have led to an expanding inequality in income distribution, as supported by Etim and Daramda (2020). The inadequacies of government and the formal sector to provide employment for communities, in particular in the township and rural areas, have meant that the communities have been involved in the informal sector out of necessity and survival (Petersen &Charman, 2018a; Etim&Daramda, 2020; Musara&Niewenhuizen, 2020). The informal micro-fast-food restaurants should be considered the grass roots of economic self-reliance and entrepreneurship with the township economy, as explained by Petersen (2020). Many of these enterprises, as explained by Botha (2012), represent an important financial income stream and subsidised food source for township communities who may otherwise be unable to access formal employment.

The South African government has not yet understood the influence of the micro-environment on informal micro-fast-food restaurants, resulting in high restaurant failure (Maumbe, 2012; Mhlanga, 2018). According to StatsSA (2016), approximately 62% of micro-fast-food restaurants fail during their first year of operation, and 87% fail within five years. The numbers are startling considering the importance of micro-businesses for the South African, as the economy struggles with a high unemployment rate (StatsSA, 2021).

The importance of quality dimensions on customer satisfaction

Beyond the formal retail economy in the streets of Central Business Districts (CBDs) and shopping malls, as indicated by Ligthelm (2008), Charman et al. (2017) and Petersen and Charman (2018a), lies a real of business activity that is typified by micro-enterprises in rural-township settlements. These businesses are considered to be informal in that they generally lie outside the legal and institutional regulatory framework, and are generally unregistered (Statistics SA, 2010). The enterprises form part of the informal economy, and among these enterprises operating in the informal economy are micro-fast-food restaurants. The micro-fast-food restaurants are facing intense competition from established fast food restaurants in South Africa (Nair, 2016). Among the established brands is Kentucky Fried Chicken (KFC) which is the biggest player with over 771 restaurants across the country (Sullivan, 2018; Masocha&Mandipaka, 2020). The IMFFRs are also facing stiff competition from major supermarkets, namely: Checkers, Pick'n Pay, Spar, Shoprite, and other retail chains (Insight Survey, 2016). These major supermarkets now offer increasingly popular ready-to-eat meals in their deli sections (Veitch, 2017).

These competitive nature of the fast-food-chain industry present micro fast food restaurant managers with a special challenge regarding how to maintain profitability in a shrinking market while providing the customers with high quality products and services (Mhlanga et al. 2014; Goko, 2017). In order to compete successfully, the micro-fast-food restaurants must pay more attention to quality dimensions namely: price, service quality, product quality, and physical environment. If customers are satisfied with the quality dimension, they will repeat purchases, show loyalty, and act as representatives for the micro fast food restaurants by telling other people of their experience (Nindani et al., 2018). On the contrary,

should customers be dissatisfied with the quality dimension of the restaurant, as assessed by Nair (2013), it will have an adverse effect on the restaurant's image and probably their long-term survival. The results of Bowen and Chen's (2001) study verified the nonlinear and asymmetric relationship between customer satisfaction and customer loyalty, and more importantly the importance of the correlation with business growth. However, the relationship between satisfaction and loyalty is expected to be dependent on the quality dimensions produced. In the IMFFR industry, every restaurant must strive to attain customer satisfaction as this leads to a stronger competitive position under intense competitive environment, as explained by Samudro et al. (2020), as these micro-businesses are not only competing with homogeneous businesses that sell foods having great similarities often using the same components, but are competing with established restaurants and supermarkets that have superior resources in general.

II. RESEARCH METHOD

Secondary data research was undertaken in order to lay the theoretical foundation upon which this paper is based, and a survey instrument was used to collect primary data from customers of an IMFFR in Mahikeng, South Africa (Leedy&Ormrod, 2013), to gather their views concerning the quality dimensions offered by the restaurant.Positivism philosophy was applied in this paper because positivism philosophy assists in addressing the objective of this paper, namely to examine the influence of quality dimensions on customer satisfaction in a micro fast food restaurant, and to determine the relationship between variables.

A core characteristic of the non-probability sampling technique, as proposed by Ivankova (2015), is that samples are selected based on the subjective judgement of the researcher, rather than random selection. Furthermore, a non-probability convenience sampling was used in this paper, and the target group was customers of the IMFFR situated in the township of Mahikeng that represented the research population with a predetermined sample size of 180 customers. A questionnaire was used to gather customers' viewpoints and perceptions of the quality dimensions offered by the restaurant, that is the product, service, physical environment and price.The questionnaire consisted of the following sections, starting with section (A) obtaining a demographic description of the research population and their level of association with the restaurant. This was followed by section (B), a series of questions concerning customers' insight concerning the restaurant's product and service provided. The following section (C) dealt with measuring the respondents' level of satisfaction with the restaurant in general. Lastly, section (D) dealt with general comments. In designing the questionnaire as research instrument to collect the quantitative data needed, validity and reliability were considered. For measuring the variables of this study in the quantitative method approach relevant items were selected from various authors (Sun, 2011; Trentin et al. 2012; Cruz, 2015; Khoironi et al., 2018) that were guided and aligned to the study's objectives and literature review section to form the measurement scales for the study. The measurement scales were used to measure the customers' perception concerning the quality dimensions offered by the restaurant.

In light of the research objectives and nature of the study, fieldwork was conducted by distributing questionnaires to the business site by the researcher. The questionnaires were placed at the restaurant for respondents to complete whilst waiting for their meal or after consumption. Participation in the study was voluntary and data was collected for a period of four months. From the 180 questionnaires that were distributed to respondents for data collection, 132 were completed and then data cleaning process was undertaken to remove incomplete and incorrect data for analytical purposes and as a result only 104 questionnaires were analysable. The questionnaire was assigned numeric codes for analytical purposes.Having collected the quantitative data, the next step was to analyse the data. All responses from the survey questionnaires were assigned numeric codes, as explained in the data collection process section, and subsequently inserted into the Statistical Package for the Social Science (SPSS) that included bi-variant, multivariate and regression analysis, as mentioned by Field (2013).

III. RESULTS AND DISCUSSION

The findings comprise of the demographic description of the customers of an IMFFR and the customers' views on the quality of product offered by the restaurant and their level of satisfaction with the restaurant. The results indicate that of the 104 customers who participated in the survey, 68.3% were customers were students, with 82.7% of participants being below the age of 30 years. From the 104 customers that participated in the survey, only 48 (46.2%) answered the question relating to their income level, and 26 out of 48 customers revealed that they have an income of R1 000 or less in a given month.Customers' preference for a particular product may be as a result of how the customer rated the quality and the degree to which it satisfied their stated needs, as suggested by Agwu and Afieroho (2016). In addition, Table 1 reveals that most customers were satisfied with the quality of food as it had a mean score of 1.45. Overall, customers indicated that they were satisfied with the quality dimensions of the restaurant, however they were neutral and slightly dissatisfied with the

restaurant's comfort and sitting arrangement as it has the highest mean score of 2.68.

Subsequently, it has the highest standard deviation score of 1.176, which indicates that the data points are spread out over a large range of values, and the results proposes that customers had dissimilar views with regards to comfort and sitting availability of the restaurant; and this point is substantiated by a high median score of 3. As shown in Table 2, customer loyalty has no relationship with value for money; the result obtained is -0.008, which is close to 0. If the correlation coefficient is 0, no relationship exists between the variables, whereas, the correlation between food quality and value for money is positively significant as the value is close to 1. There is a positive relationship between the variables, and this means that if one variable moves a given amount, the second moves proportionally in the same direction. A positive correlation coefficient less than 1 indicates a less than perfect positive correlation, with the strength of the correlation growing as the number approaches 1. It is important to note that customers of the restaurants are well satisfied with the price of the food as they get value for their money. The results will suggest that the food offered at the restaurant is reasonably priced, and can be afforded by its customers, specifically as the greatest number of its customers are students who have a low monthly income allowance.

Statement	N	Mean statistics	Median statistics	Std. dev statistics
The food is served hot and fresh	104	1.41	1.00	.719
The food is tasty and rich in flavour	104	1.39	1.00	.703
The quality of food is excellent	104	**1.45**	1.00	.695
The food meets the price value	104	1.68	1.00	.839
Menu has a good variety of items	104	1.89	2.00	1.023
Availability of sauces and beverages	104	2.26	2.00	1.005
Comfort and sitting availability	104	2.68	3.00	1.176
Friendly service	104	1.36	1.00	.723
Staff knowledgeable of items sold	104	1.46	1.00	.835
Cleanliness and hygiene	104	1.91	2.00	.883

Table 1: Quality Dimensions

Pearson correlations					
Variables		Occupation status	Value for money	Customer loyalty	Food quality
Occupation status	Pearson correlation	1	.088	.097	.153
	Sig. (2-tailed)		.375	.328	.120
	N	104	104	103	104
Value for money	Pearson correlation	.088	1	-.008	.631**
	Sig. (2-tailed)	.375		.934	.000
	N	104	104	103	104
Customer loyalty	Pearson correlation	.097	-.008	1	.104
	Sig. (2-tailed)	.328	.934		.295
	N	103	103	103	103
Food quality	Pearson correlation	.153	.631**	.104	1
	Sig. (2-tailed)	.120	.000	.295	
	N	104	104	103	104
**. Correlation is significant at the 0.01 level (2-tailed).					

Table 2: Correlation of variables – Pearson correlation coefficient

Statement	N	Min	Max	Mean statistics	Std. dev statistics	Std. error mean	Skewness
Quality of food	104	1	5	1.67	1.065	.104	1.869
Quality of service	104	1	5	1.67	1.028	.101	1.953
Setting and environment	104	1	5	2.47	1.292	.127	.464
Product price	104	1	5	1.80	.969	.095	1.397
Overall satisfaction level	**104**	**1**	**5**	**1.90**	**1.09**	**.110**	**1.420**

Table 3: Measurement of overall satisfaction

Customer satisfaction is the result of the correlation between a customer's expectation and experience. As observed from Table 3, the customers' overall satisfaction levels were high as it had an aggregate mean score of 1.90, which indicates that an overwhelmingly majority of customers from the study were satisfied with the performance of the restaurant. In addition, the satisfaction level with the quality of food and service both had a mean score of 1.67, and this indicates that the majority of the customers were satisfied. However, it is important to note that the results further revealed that customers were relatively neutral concerning the setting and environment of the restaurant. The more satisfied the customers are with the quality of food and overall performance of a restaurant, the more likely they will repeat they purchase, become loyal customers, share their positive experiences, recommend restaurants to their friends and family, and share their good experiences online with strangers. Specifically, IMFFRs are reliant on positive word of mouth, existing customers disseminating information to other people or prospect customers on their encounter with the restaurant, more importantly because micro-businesses do not have the advertising budget of established fast-food brands.

IV. CONCLUSION

These restaurants are essential for township economies in South Africa, as they provide community members an opportunity to start their own enterprises and be economically self-reliant, and typically will create employment for those members of the community who might not acquire jobs in the registered business market segment. In general, the informal sector is important to the country's economy as it significantly contributes towards the total employment in the country. The purpose of this research was to examine the importance of the quality dimensions on customer satisfaction in an informal micro-fast-food restaurant. The findings revealed an aggregated mean score of 1.90 for the level of satisfaction with quality dimensions offered by the restaurant, which clearly indicates that customers were satisfied with the restaurant. In addition, customers who participated in the study indicated that they were very satisfied with the taste and rich flavour of the food. However, customers had differing views concerning the seating and comfort of the restaurant as the statement had the highest standard deviation score as compared to others as previously shown. Therefore, in order to enjoy customer loyalty and an increased market share, the quality dimensions of the restaurant must be constantly improved upon. In addition, the restaurant should adopt a learning approach to gain knowledge and be committed to customer satisfaction through continuous improvement such as the improvement of the ambiance of the restaurant, as it is crucial to create an atmosphere in the restaurant conducive to the type of food the restaurant serves, as well as the type of customers the restaurant attracts. A need exists for further research into the examining the importance of total quality management in the growth of informal micro-fast-food restaurants.

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STUDY ON USE OF MODIFIED HIGH EFFICIENCY ATTACHED GROWTH FIXED BED BIOREACTOR PROCESSES FOR EDUCATIONAL INSTITUTE

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Abstract –

The goal of decentralized treatment systems is to reduce large sewage flows to the centralized facilities and can eliminate or reduce disposal cost to receiving water bodies. This study describes the modified high efficiency attached growth fixed bed bioreactor process and presents application of waste water generated from educational institute. The optimum hydraulic retention time of the system was 3 hour and average reduction of 79.99% COD, 89.5 BOD were achieved. The modified attached growth fixed bed bioreactor is capable of removing organic matter from educational institute waste water and provides robust, efficient and cost-effective method.

Keywords – Decentralized waste water, Educational waste water, Removal of COD, Attached growth, optimum Hydraulic retention time

DETERMINATION OF THE ACTIVITY OF ENZYMES PRODUCED BY INTESTINAL BACTERIA IN THE PRESENCE OF RESISTANT DEXTRIN

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Abstract — The aim of the study was to determine the activity of enzymes produced by intestinal bacteria in the presence of resistant dextrin. For this purpose, the concentration of β-glucosidase and β-glucuronidase was determined by spectrophotometric method in the post-culture fluid obtained as a result of joint cultivation of intestinal microorganisms of the genera: Lactobacillus, Enterococcus, Bifidobacterium, Clostridium, Escherichia, Prevotella and Bacteroides, which were isolated from the faeces of children. On the basis of the obtained results, it was found that the resistant dextrin caused a decrease in the activity of all tested enzymes. The concentration of enzymes was lower compared to the control samples, where the carbon source was glucose. The activity of the enzymes varied depending on the cultivation time and reached the highest values at 96 or 168 hours of cultivation.

Keywords — Faecal Enzymes, Intestinal Bacteria, Resistant Dextrin

I. INTRODUCTION

The group of microorganisms that inhabit the human body is 10^{14} (100 trillion) cells, belonging to approximately 500-1500 species. Their number is almost 10 times greater than that of the human body. The composition of the microbiota includes indigenous species, which constitute a protective barrier against the penetration of pathogens and have an impact on the formation of the immune system and allochthonous ones that enter from the external environment - they can be pathogenic. The system of microorganisms and their numbers are individual for each representative of the human species and are specific for particular places in the body [2].

The intestinal microbiota plays an important role in metabolic processes carried out in the human body. It produces enzymes that are not encoded by the human genome and are responsible, for example, for the breakdown of polysaccharides, polyphenols and for the synthesis of vitamins [1, 3].

The metabolic activity of the intestinal microflora, in addition to its positive effect on the human body, may also cause harmful effects, such as, for example, colorectal cancer. Intestinal bacteria have the ability to activate procarcinogenic compounds that enter the intestines with food and those that enter the colon with bile. In addition, they are able to enzymatically remove the detoxification group that is attached to the carcinogen in the liver. Another way of creating harmful metabolites by the gut bacteria is by converting endogenous substances via metabolic pathways into carcinogens or substances that induce tumor formation [3].

Fecal enzymes that have a significant impact on the formation of carcinogenic compounds are: β-glucosidase, α-glucosidase, β-galactosidase, α-galactosidase, β-glucuronidase, nitroreductase, azoreductase and 7-α-dehydroxylase. Compounds that arise as a result of their excessive activity include phenolic and indole compounds, flavonoid aglycones, azo dyes, estrogens, as well as secondary bile acids. All of the above-mentioned products may adversely affect human health [5].

Resistant dextrins are starch dextrinization products resistant to the action of hydrolytic enzymes in the human gastrointestinal tract. They contain short chains of linked glucose oligomers / polymers along with numerous types of digestible glycosidic linkages different from the typical glycosidic linkages present in starch. It is the formation of these bonds that makes the end products unavailable for human digestive enzymes, demonstrating the properties of resistant starch, which includes resistant dextrins. Resistant dextrins have been found to have many beneficial properties, resulting in their use in functional foods [4, 6].

Hence, the study investigated the activity of two faecal enzymes (β-glucosidase and β-glucuronidase) produced by deer bacteria and whether resistant dextrin will have a positive effect on the production of these enzymes.

II. MATERIAL AND METHODS

The scope of work included the determination of the concentration of fecal enzymes (β-glucosidase and β-glucuronidase) in post-culture fluid obtained as a result of co-culturing intestinal microorganisms of the genus: *Lactobacillus*, *Enterococcus*, *Bifidobacterium*, *Clostridium*, Escherichia, *Prevotella* and *Bacteroides* which were isolated from the faeces of children. Bacterial cultures were incubated in a medium containing resistant dextin (appropriate samples) or

glucose (control samples) for 168 h. After 24 h, 48 h, 72 h, 96 h and 168 h of culture, post-culture fluid was collected in which the activity of fecal enzymes was determined.

The enzymes activity was measured by spectrophotometric method. Activity units were calculated as the amounts (µmol/L) of phenolphthalein (for β-glucuronidase) and nitrophenyl (for β-glucosidase) released per 1 hour, per 1 ml (U) per 1 mg of protein (U/mg).

III. RESULTS

In culture with resistant dextrin, the activity of β-glucosidase was variable and dependent on the time of the culture. At 24 hours of culture, the concentration of this enzyme ranged from 0.04 to 0.67 µM*U/mg (0.40 µM*U/mg on average). In the following hours of cultivation, an increase in the activity of this enzyme was found. The highest concentration of β-glucosidase was found at 96 hours of culture (from 0.34 to 0.77 µM*U/mg, average 0.58 µM*U/mg). At 168 hours of cultivation, the concentration of β-glucosidase fluctuated around similar values (Figure 1A).

In culture with glucose, the β-glucosidase activity was also variable and time-dependent, and also higher than when resistant dextrin was used as the carbon source. For all samples, the highest concentration was recorded at the 96 hour of culture (from 0.72 to 1.04 µM*U/mg, average 0.94 µM*U/mg) and it was on average

0.36 µM*U/mg higher than in a resistant dextrin culture (Figure 1B).

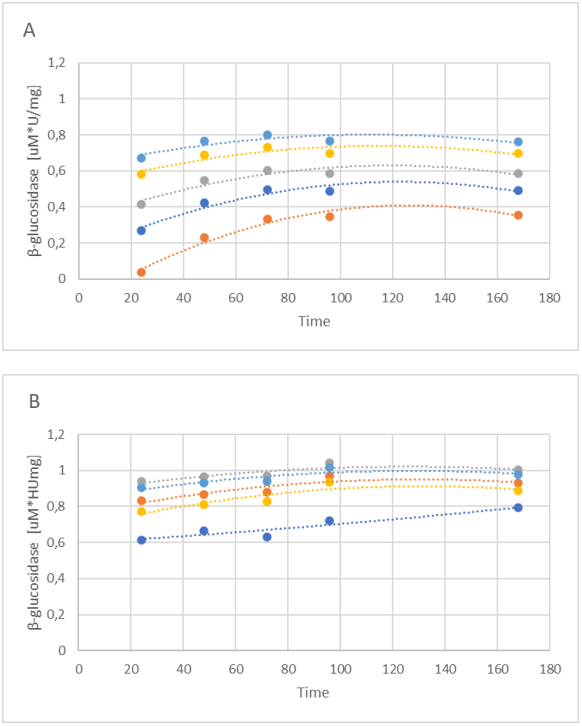

Figure 1. β-glucosidase activity in medium with resistant dextrin (A) and glucose (B).

The β-glucuronidase activity was also varied and depended on the cultivation time. The lowest activity of this enzyme in the samples with resistant dextrin was recorded at 24 hours of cultivation (from 0.43 to 2.53 µM*U/mg, average 1.49 µM*U/mg). With the passage of time of cultivation, the activity of this enzyme increased and its highest value was recorded at 168 hours of cultivation (from 1.23 to 2.91 µM*U/mg, average 2.15 µM*U/mg) (Figure 2A).

β-glucuronidase was more active in glucose culture than in the dextrin resistant trials. At 24 hours of culture, the concentration of this enzyme ranged from 2.93 to 4.08 µM*U/mg (mean 3.63 µM*U/mg) and was, on average, higher by 2.14 µM*U/mg than in the resistant dextrin culture. Observing the dynamics of the activity of this enzyme, it was noted that it was the highest at 168 hours of cultivation (from 4.59 to 5.08 µM*U/mg, on average 4.89 µM*U/mg) and it was on average higher by 2.74 µM * H / mg than trials with resistant dextrin (Figure 2B).

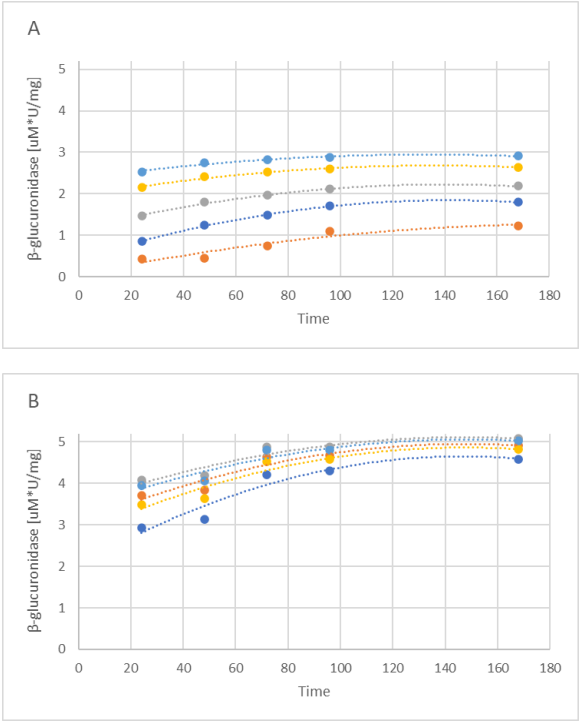

Figure 2. β-glucuronidase activity in medium with resistant dexrite (A) and glucose (B)

IV. CONCLUSION

The activity of faecal enzymes (β-glucosidase and β-glucuronidase) varied and depended on the time of culture. The tested fecal enzymes obtained the highest activity at 96 or 168 hours of cultivation. The resistant dextrin decreased the concentration of all tested faecal enzymes. The concentration of enzymes was lower compared to the control samples (with glucose).

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THE USE OF TECHNOLOGY IN THE TEACHING AND LEARNING OF AFRICAN LANGUAGE – A SOCIOLOGICAL APPROACH

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Abstract –

Technology has brought about new knowledge forms in all aspects of human life. The new knowledgeforms have transformed not only human lives but also human ways of doing things. Education has alsobenefited from technological transformation which is witnessed through the delivery of teaching andreception of learning with technology. The use of technology in teaching has been a fundamental themein the 21st century as the world tends towards the fourth industrial revolution. Using Bourdieu's socialtheory, this paper seeks to gain an understanding of how the use of technology is impacted by the socialcontext of language lecturers. The aim of this study, therefore, is to investigate how language lecturers'social context influences their use of technology in teaching African languages. To pursue the above aim,an in-depth survey is conducted with 34 languages lecturers from 3 universities in KwaZulu-Natalusing a stratified sampling method. This study provides a detailed account and analysis of lecturers'social context regarding the use of technology for their teaching. In addition, the value lecturers place ontechnology in teaching African languages shall be discussed. Finally, obtainable, and sustainable ways ofmaking the use oftechnologies in teaching African languages more effective considering the social contextof language lecturers are recommended for lecturers and other educational stakeholders.

Keywords – Bourdieu; Social Context; Language Lecturers; Language Teaching And Learning; African Languages

PILOT RESEARCH ON INTEGRATED TRANSPORT SYSTEM AND SHARING ACCOMMODATION IN THE NETHERLANDS

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Abstract - This paper shows preliminary results from international research on the impact of an integrated transport system on sharing accommodation (i.e., Airbnb). The article focuses mainly on the findings of the author's own field research, which mainly focused on P+R parking lots. The next part focuses on the evaluation of the results of the questionnaire survey, which is based on the expert's opinion level. In this contribution the attention is mainly paid to the evaluation of the potential of tourism in the researched region. The article also shows what kind of tourists visit the given region the most, i.e., who visit the Netherlands the most.

Keywords - Airbnb, Integrated Public Transport, P+R Parking Lots, Sharing Accommodation, Sharing Economy.

I. INTRODUCTION

The sharing economy is a concept that has been around for a very long time. The prerequisites for sharing are the existence of a good to be shared, the willingness to share under certain conditions, and the willingness of people to deal with such an offer. The development of Internet communication technologies and the massive development of electrical engineering, combined with the minimization of devices and the change in the lifestyle of people all over the world, are the main causes of the huge expansion of this industry [1]. For example, according to Eurostat an annual number of guest nights at short-stay accommodation offered via sharing economy platforms in the European Union is 511 939 000, domestic 169 736 000 and international 342 202 000 [2]. The most popular sharing accommodation platform is Airbnb. Airbnb was founded in August 2008 and is based in San Francisco. Since then, it has grown to more than 4 million hosts who have accommodated more than 1 billion guests in approximately 100,000 cities worldwide [3].

Sharing accommodation, i.e., Airbnb is dependent on transport availability. No accommodation provider can do without the accommodation being accessible by public transport or individual transport. According to [4], the concentration of Airbnb accommodation becomes greater the closer it is to major public transport connections. They also report that a greater number of sharing accommodation is located in areas with better transport accessibility.

There are certain economic benefits associated with the flourishing of software platforms and peer-to-peer services. Reference [5] rank among the biggest advantages of these platforms primarily the reduction of translation costs, improvement of resource redistribution, information efficiency, pricing efficiency etc. In addition to the positive aspects of the sharing economy, there are also a number of negatives. Airbnb is criticized for increasing the cost of living for local residents. Many cities, not only European ones, have started to regulate short-term rentals that are connected to this service. Another significant problem is noise, clutter and constant changing tenants [6].

The need for relocation has been present since the beginning of humanity, and nowadays more and more demands are being placed on transport options. Road passenger transport is constantly growing, because it offers fast and affordable transport. The Integrated Transport System (ITS) is used for efficient transport, which connects individual types of urban and regional transport into a coordinated unit. Transport within the ITS is provided by various means of transport (e.g., bus, trolleybus, train, metro, tram, ship, cable car, etc.). In order for everything to work properly, ITS coordinator is established to coordinate timetables, tariffs, and information, because the various means of transport are operated by different carriers. ITS offers a number of advantages, such as connectivity, easy purchase of a single ticket, uniform tariffs, easily accessible and quality information, or the potential to relieve the city of individual car traffic, but also significant barriers [7], [8]. Many authors, e.g., [9]–[12], conducted studies on various aspects and the entire context of ITS implementation. ITS simplifies passenger transport and increases the overall volume of passenger transport.

As stated by [13], integration is needed in any system that consists of several parts that must complement each other. With proper integration, the system works efficiently. The transport system is made up of individual transport networks, their users, providers, means of transport and management institutions with operators. Thanks to the integration, the system effectively serves the transportation needs of the entire company, is operated with minimal costs and has the lowest possible impact on the environment. The demand for transport is derived from the total available journey, with the most complex being securing a part of the journey outside the main mode used (e.g.,

journey from or to the railway station). Integrated systems should bring system synergy or lower costs, according to [14], ITS performance can be measured and analysed using overall quality (i.e., link quality and capacity, transmission and endpoints, and factors such as intermodality and interoperability).

The integration focuses mostly on the integration of public transport and its vehicles. But it is also important to focus on the integration of individual and public transport systems. This way of integration can be ensured by building connecting transport infrastructure, such as park-and-ride car parks or multimodal terminals. The principle is to park cars in a parking lot on the outskirts of the city, and then users continue by public transport to the city centre. The implementation is closely linked to information devices and traction control systems using traffic telematics[15].

II. METHODOLOGY

The aim of the paper is to evaluate the approach to tourism, especially to transport services and sharing economy in the Netherlands. The following hypotheses were established:

- H1: Recreational tourism has the greatest potential in the Netherlands.
- H2: Selected areas of the Netherlands are mainly visited by foreigners.
- H3: Airbnb is the second most used accommodation after hotels.
- H4: Public transport is used by tourists more than individual transport.

The data collection was directly in the Netherlands in the capital Amsterdam and the city Rotterdam (see in the Figure 1). The constructed questionnaire, which was used previously in the state of Mexico, was used for the research in the Netherlands, so that it is possible to subsequently compare individual states. The questionnaire for experts in the field of tourism (academic staff and experts from practice) contained 15 questions, of which questions 1-3 were opened and aimed at determining the respondent´s scope. Questions 4-14 were closed and their aim was to find out opinions about tourism, especially accommodation and transport services in the area. The 15th question was used to gain additional contacts to experts, so it was possible to use the "snowball" method to pack and expand potential respondents. The questionnaire was submitted in English and additional interviews were conducted with some respondents to better understand their views. Out of the total number of 59 (35 + 24) contacts, 21 questionnaires were obtained from tourism experts. The return of the questionnaires was therefore 35.59%.

Data collection took place in the Netherlands in the period 06.04.-13.04.2022. During this research, tourism experts were personally approached and at the same time the current state of ITS (especially public transport and P+R car parks) and shared services was observed and researched. The experts were addressed by email before, during the survey and after our return to the Czech Republic. The obtained data were sorted, cleaned and coded to Excel. After obtaining the necessary data, the data were statistically processed, basic statistical characteristics (average, mode, median, etc.) were created and selected questions were graphically processed, which serve to refute or confirm the established hypotheses.

Fig. 1: Location of cities in the Netherlands
Source: Own (with using Google maps)

III. RESEARCH

A. Park and Ride

Parking is often perceived as a "passive" element of the transport system, but it can also be classified as an "active" element of the development of public transport, as the provision of parking spaces can also influence e.g., journey time, destination safety or greater use of modes of transport that compete with the car [16]. The P+R system includes car parks on the outskirts of cities and offers benefits for travellers who leave their cars there and use public transport to the city centre. Reference [15] defines park and ride as a parking lot that allows a transfer between an individual public transport (taxi) or public transport (bus, metro, railway) and an individual private car (or bicycle). He also mentions that it is necessary to make this system more attractive for users, for example with a discounted price, so that they decide to use it. The P+R scheme can be seen in Figure 2.

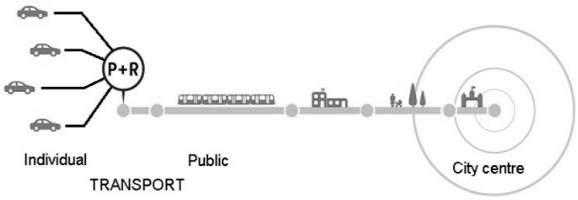

Fig. 2 P+R scheme
Source: Own by: [17]

During research in the Netherlands, P+R and the transport system in the capital city of Amsterdam were tested. Currently, the number of P+R parking lots in European cities is increasing. The whole of the Netherlands has many bicycle parking spaces (which is related to B+R). The capital city of Amsterdam has 10 P+R parking spaces, the location can be seen in Figure 3.

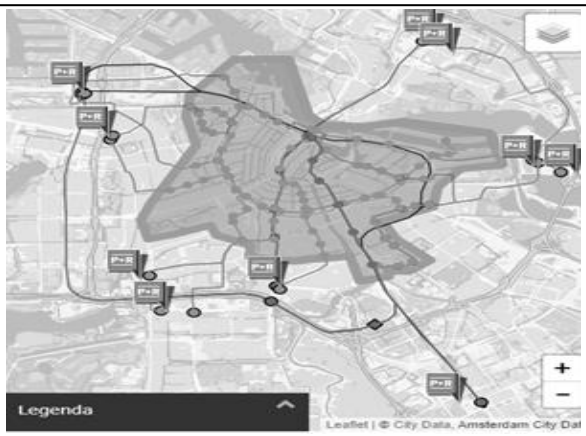

Fig. 3 P+R in Amsterdam
Source: [18]

The P+R RAI parking lot, which was closest to our accommodation, was selected for investigation. To use discounted all-day parking, after parking the vehicle, it was necessary to buy a special ticket in a blue machine, which was very well marked at the parking lot. This ticket must be used to ride to the area designated as "centre" within one hour, and the passenger must not forget to mark the ticket when boarding and exiting the means of transport. When returning to the car park, this process is repeated with the same ticket. When paying the parking fee, the vehicle's license plate number is entered, interest in the P+R discount is selected and the used ticket is loaded. At that point, the price is reduced, the parking fee is paid and the visitor can leave.

P+R prices in Amsterdam - parking in the P+R parking lot costs €1 or €8 for 24 hours (can be used for a maximum of 96 hours). The weekday rate for arrivals before 10:00 a.m. is €8 for the first 24 hours and then €1 for each day. When arriving after 10:00 a.m., the rate is €1 per 24 hours, likewise on the weekend the time of arrival does not matter and the price is €1/day. Ticket prices are as follows:

- 1 or 2 persons: €5.50
- 3 persons: €6.50
- 4 persons: €7.50
- 5 persons: €8.50

Discovered advantages and disadvantages - the sophisticated website, which describes in detail how to use P+R in Amsterdam, can be evaluated very positively. It is also possible to find the current availability of parking spaces in the given locations. Directly at the RAI parking lot, the P+R parking option is conveniently marked, the ticket machines are well marked and easy to use. The system of marking the ticket when boarding and after exiting can be inconvenient for passengers because they have to think about it (if they don't mark the ticket at the given time, the parking discount will not be recognized). However, this system enables, for example, the subsequent acquisition of statistical data on the use of ITS during the P+R service. The disadvantage of P+R RAI was that it is possible to park here only for 24 hours. Furthermore, we discovered a shortcoming that the camera that captures vehicle license plates does not appear to be related to foreign license plates. In the Czech Republic, the license plate starts with e.g., 7C8 and the camera took this as 7CB. After paying for our license plate, the barrier did not want to let us drive away because the loaded license plate had not been paid for. However, this problem can be quickly solved with the called service, who verified the fact and opened the gate for us.

Overall, the use of P+R in Amsterdam can be evaluated positively, as parking in the centre and at hotels are very expensive. In this way, a person leaves the vehicle on the side lines, easily buys a ticket, travels to the city by integrated transport, which is numerous and well connected, and does not have to worry about his vehicle any further. Thus, tourists can more easily choose between available accommodations, for example, it is not a problem to find an Airbnb without the possibility of parking. Many surveys show that P+R reduces the number of cars in city centres. However, as mentioned by [19], it is necessary to investigate whether the population of the peripheries attracts P+R to reach it by car, while public transport connections could travel to the centre and from the near peripheries. In this case, although there would still be a reduction in traffic density in the centre, there would also be a decrease in interest in public transport around the cities or in cycling.

B. Questionnaire survey

In the questionnaire we include a question about tourism potential in the region (What is the potential of tourism in the region?), see Table I. We used Likert Scale where the respondents evaluate potential by using points, 5 points mean the most, 1 point the least (1 = the least, 5 = most).

QUESTION NO. 4	TOTAL	1 (%)	2 (%)	3 (%)	4 (%)	5 (%)	TOTAL (%)
Recreational	21	5	5	10	19	62	100
Cultural and Social	21	0	5	14	24	57	100
Spa	21	10	43	24	19	5	100
Congress service	21	0	5	48	29	19	100
Rural tourism	21	10	14	29	33	14	100
Nature, nature parks and natural heritage	21	10	10	43	33	5	100

Table I: What is the potential of tourism in the region?
Source: Own

According to the results we can see, hypothesis number one can be considered as confirmed. The experts evaluate recreational tourism as the greatest potential in the Netherlands, followed by cultural and social. The least is evaluated spa tourism and nature tourism.

In the questionnaire we include a question about the type of tourist who are visiting the Netherlands (What kind of tourists visits the region?), see Table II. We used Likert Scale where the respondents evaluate the certain tourist type by using points, 5 points mean the most, 1 point the least (1 = the least, 5 = most).

QUESTION NO. 10	TOTAL	1 (%)	2 (%)	3 (%)	4 (%)	5 (%)	TOTAL (%)
Families with children	21	0	0	48	48	5	100
Young people, students	21	0	0	5	19	**76**	100
Exclusive clientele	21	0	0	24	43	33	100
Seniors	21	0	24	43	29	5	100
Migrants	21	0	10	52	33	5	100
Foreign	21	5	0	5	33	57	100

Table II: What kind of tourists visits the region?
Source: Own

According to the results we can see, hypothesis number two cannot be considered as confirmed. Experts consider young people/students to be the segment that most visits the Netherlands, followed by foreign and exclusive clientele.

In the questionnaire we include a question related to the most used type of accommodation and about the most used mode of transport in the Netherlands (How often do tourists use mention services during their stay in a given destination?), see Table III. We used Likert Scale where the respondents evaluate the certain tourist type by using points, 5 points mean the most, 1 point the least (1 = the least, 5 = most).

QUESTION NO. 11	TOTAL	1 (%)	2 (%)	3 (%)	4 (%)	5(%)	TOTAL (%)
Hotels	21	0	5	0	14	**81**	100
Pensions	21	0	14	33	29	24	100
Airbnb	21	0	24	33	38	5	100
Public Transport	21	0	5	0	29	**67**	100
Taxi	21	0	19	48	5	29	100
Uber	21	0	19	33	33	14	100
Individual Transport	21	5	24	10	48	14	100

Table III: How often do tourists use mention services during their stay in a given destination?
Source: Own

According to the results we can see, hypothesis number three can be considered as confirmed. Experts consider Hotels as the mainly used type of accommodation during the tourist stay and Airbnb is the second most used type. Hypothesis number four can be considered as confirmed, as well. The public transport is the most used mode of transport, according to expert opinion level. The second most used transport mode is surprisingly the Taxi, which can also be due to P+R parking lots, when it could be more convenient for tourists to use a taxi service than to move around anunknown city with their own car.

IV. CONCLUSION

The whole of the Netherlands has many bicycle parking spaces (which is related to B+R). The capital city of Amsterdam has 10 P+R parking spaces. Overall, the use of P+R in Amsterdam can be evaluated positively, as parking in the centre and at hotels are very expensive. In this way, a person leaves the vehicle on the side lines, easily buys a ticket, travels to the city by integrated transport, which is numerous and well connected, and does not have to worry about his vehicle any further. Thus, tourists can more easily

choose between available accommodations, for example, it is not a problem to find an Airbnb without the possibility of parking. Many surveys show that P+R reduces the number of cars in city centres.

According to the results we can see, hypothesis number one can be considered as confirmed. The experts evaluate recreational tourism as the greatest potential in the Netherlands, followed by cultural and social. The least is evaluated spa tourism and nature tourism.

According to the results we can see, hypothesis number two cannot be considered as confirmed. Experts consider young people/students to be the segment that most visits the Netherlands, followed by foreign and exclusive clientele

According to the results we can see, hypothesis number threecan be considered as confirmed. Experts consider Hotels as the mainly used type of accommodation during the tourist stay and Airbnb is the second most used type. Hypothesis number fourcan be considered as confirmed, as well. The public transport is the most used mode of transport, according to expert opinion level. The second most used transport mode is surprisingly the Taxi, which can also be due to P+R parking lots, when it could be more convenient for tourists to use a taxi service than to move around an unknown city with their own car.

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DIALYSIS-DRIVEN CATALYTIC PROCESS FOR THE SIMULTANEOUS REDUCTION OF NITROAROMATICS AND SEPARATION OF AROMATIC AMINES

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Abstract –

Problem statement
Due to the extensive use, increased discharge into water, as well as toxic, mutagenic and cancerogenic character, aromatic nitro compounds (ANCs) are recognized as one of the most hazardous pollutants of anthropogenic origin[2]. Fortunately, ANCs can be recognized as important substrates for the synthesis of aromatic amines (AAMs), that are the key building-blocks for the manufacturing of large-scale pharmaceuticals. In this context, the present study provides a new catalysis-dialysis process, that enables the simultaneous catalytic hydrogenation of ANCs and separation of resultant AAMs.

Methods
The catalysis-dialysis process was performed with the use of nanocomposite membranes (NCMs) loaded with Au nanoparticles (AuNPs). They were obtained by forming poly(vinyl chloride) films with the aid of cyclohexanone (CH) or tetrahydrofuran (THF), then the so-prepared films were functionalized with the selection of amines. Finally, the reduction-coupled adsorption of Au(III) allowed to fabricate and stabilize AuNPs within the membranes. The nanomaterials were analyzed using scanning transmission electron microscopy (STEM), and Fourier-transformation infrared spectroscopy (FT-IR). The catalytic reaction was carried out using UV-Vis spectrophotometryfor the model process where 4-nitrophenol (4-NP) is being reduced to 4-aminophenol (4-AP). A two-chamber dialysis unit was used for this purpose.

Results and Conclusion
NCMs with AuNPs allowed to effectively reduce 4-NP and to separate the resultant 4-AP. Each of the synthesized materials led to the 100% conversions of 4-NP. The 4-AP separation efficiency was linked with the greater in the case of membranes obtained in the presence of THF. This suggests the NCMs were efficient catalysts, that separation properties were linked with the diffusion channels present in the materials. The outcome of the research suggests, that the novel catalysis-dialysis process could be a tempting alternative towards traditional hydrogenation reaction, as the applied NCMs offer an additional functionality, enabling the production of AAMs from waste sources.

Keywords – Nanoparticles, Nanocomposite, Membranes, Catalyst, Nitroaromatics.

PRODUCTION OF HYDROGEN AND CARBON MONOXIDE FUEL GAS FROM PINE NEEDLES

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Abstract - Forestry wastes are readily available in large quantities around the world. Based on European Green Deal for the deployment of renewable and decarbonized energy by 2050, as well as global energy crisis, energy recovery from such wastes reducing greenhouse gas emissions is very attractive. Gasification has superior environmental performance to combustion, producing a clean fuel gas utilized in internal combustion engines, gas turbines, solid oxide fuel cells, or for synthesis of liquid bio-fuels and value-added chemicals.In this work, pine needles, which are abundantly found in Mediterranean countries, were gasified by either steam or carbon dioxide via a two-step process to improve reactivity and eliminate tar, employing a fixed bed unit and a thermal analysis system. Solid, liquid and gaseous products from the whole process were characterized and their energy potential was determined. Thermal behavior, reactivity, conversion and energy recovery were examined. The gasification process took place above 650°C. At 950°C conversion and energy recovery were 77% dry and 2 under a flow of steam and 85% dry and 2.9 under a flow of carbon dioxide, respectively. Organic matter was almost completely converted to syngas, the yield of which varied between 89% and 99%. The higher heating values of biochar, bio-oil and pyrolysis gas were 27.8 MJ/kg, 33.5 MJ/kg and 13.6 MJ/m^3. Upon steam or carbon dioxide gasification, the higher heating value of syngas produced was 11.5 MJ/m^3 and 12.7 MJ/m^3, respectively.

THE EFFECT OF DIMETHYL SULFOXIDE ON GROWTH OF PSEUDOMONAS AERUGINOSA

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Abstract - Dimethyl sulfoxide (DMSO) is a commonly used solvent for synthetic and natural antibacterial compounds. Experiments that use DMSO to determine Minimal inhibitory concentration (MIC) must take into account that the solvent can have an effect upon bacterial growth. The aim of this study was to explore how the growth of *Pseudomonas aeruginosa* (PA) was affected by different concentrations of DMSO. Six PA clinical isolates and ATCC PA strain 27853 were used. MIC assays were conducted using the microtiter broth dilution method. DMSO at concentration of 1%–8% v/v were used to evaluate the impact of DMSO upon the growth and bio film formation using microtiter-plate test. The results were analyzed using an ANOVA. The statistical significance was set as $P < 0.05$. The MIC and minimal bacteriostatic concentration (MBC)for PA were 25% and 50% respectively. There was a significant decline in the growth of PA and development of biofilms when the concentration of DMSO at was 4%, 6% and 8%. Growth and biofilm formation inhibition was also detected at lower concentrations of DMSO (1% and 2%. Studies into antimicrobial susceptibility should consider the effect ofthis common solvent have upon compounds that are being tested, such as traditional antibiotics and novel antimicrobials.

Keywords - Cystic Fibrosis CF, Pseudomonas aeruginosa, DMSO, Solvents, Growth, Biofilm

I. INTRODUCTION

Cystic fibrosis (CF) is a genetic, autosomal recessive disease that predominately affects Caucasian populations of European ancestry, with an approximate rate of 1 in 2500 births (1). The symptoms of CF are related to a defective gene product named CF transmembrane conductance regulator, which is widely distributed throughout the tissue in affected people(2). The failure or altered expression of this gene product leads to abnormal mucous secretions that occlude the airway and ductal lumens(3).

In people with CF, these secretions result in recurrent pulmonary infections, as well as pancreatic insufficiency and intestinal obstruction syndromes. Pseudomonas aeruginosa (PA) leads to chronic infections, and together with related pulmonary inflammation, it is the major cause of death in patients with CF (4). The biofilm-forming capability and extensive virulence factors of PA mean that it causes diverse infections. The intercellular quorum-sensing (QS) communication system in PA is pivotal to its virulence(5, 6). Treating PA with antibiotics is impeded by high levels of pan-resistant and multi-resistant isolates in CF patients(6). The high level of antibiotic resistance in PA organisms has motivated researchers to try to discover new sources of antimicrobials that may be more effective against these organisms.

Dimethyl sulfoxide (DMSO) is used as a solvent to dissolve water-insoluble drugs or other test samplesin both *in vivo* and *in vitro* experiments(7). Researchers including (7-9) have published review papers on DMSO activity, which has also been the subject of several recent studies by pharmaceutical researchers and manufacturers. These publications report that DMSO is effective as an anti-inflammatory adjunct and bacteriostatic agent, as well as a local analgesic, tranquilizer, and diuretic. (10, 11) report that DMSO can increase the action of drug substances by promoting penetration across biologic membranes, but these findings are controversial. The experiments conducted for the present study found that sub-inhibitory concentrations of DMSO could significantly inhibit biofilm production and growth of PA. Since there have been no previous reports on the inhibitory effects of solvents such as DMSO on PA growth, we decided to study the extent to which DMSO is effective against certain reference and clinically isolated strains of PA. The present study could provide valuable basic information about how DMSO can act as both an antimicrobial agent against PA and as a solvent.

II. METHODOLOGY

Bacterial isolates and their identification

This study used American Type Culture Collection(ATCC) PA 27853 standard strain and six clinical isolates, PA1, PA2, PA3, PA4,PA5, and PA6. PA ATCC is from the international PA panel and its whole genome sequence is available(12). The clinical isolates taken from the sputum of CF patients were obtained from the microbiology department at Prince Hamza Hospital in Jordan and the microbiology laboratory at Jordan University. The PA bacteria were identifiedby Gramstain, the production of green pigments on nutrient agar, growth on MacConkey agar, oxidase test, motility, growth on cetrimide agar,

and the ability to grow at 42°C. This was confirmed using the VITEK2 computer automatic bacteria identification system (Bio Merière, Lyon, France).

Culture conditions

The selective medium used for the isolation of PA was Pseudomonas cetrimideagar (OxoidTM). The sputum samples collected for CF patient were enriched using Brain-Heart Infusion (BHI) broth medium (OxoidTM). Following this, the enriched samples were cultured on cetrimideagar using the streak plate and pour plate method. Subcultures were initiatedfrom one single colony for every clinical isolate, which were grown on Pseudomonas cetrimideagar plates at 37°C. Fresh cultures from each PA strain were prepared in a concentration of 0.5 McFarland Scale (1.5×10^8 CFU/mL), and were used in the assessment experiments.

Minimal inhibitory concentration (MIC) assays

The microtiter broth dilution method was used to determine the MIC(13).This involved mixing varying volumes of DMSO and MH broth in different wells of 96-well assay plates (tissue culture-treated polystyrene; Costar 3595, Corning Inc., Corning, NY). To achieve a concentration of 50%, the stock solution was prepared by mixing 190-µ of broth with 190-µLDMSO then concentrations of 25%, 12.5%, 6.25%, and 3.75% were produced through serial two-fold dilutions. The turbidity of the 0.5 McFarland standard was matched by adding 20-µL of bacteria at a density of 5×10^5 CFU/mL of inoculum, which was prepared from the overnight growth of the test organism. The total volume in each of the wells was 400 µL. The growth control was produced by inoculating a tube containing the growth medium with 20-µL of inoculum. The lowest concentration of DMSO, where no visible growth was seen in the tubes, was the MIC endpoint.

MBC determination

Following the MIC determination of the DMSO, inoculated aliquots of 50-µLfrom all tubes with no visible bacterial growth on BHI agar plates, which were then incubated for 24 hours at 37°C. Observing the pre- and post-incubated agar plates for the presence or absence of bacteria allowed the MBC endpoint to be determined.This was reached when 99.9% of the bacterial population was killed by the lowest concentration of the antimicrobial agent.

Microtiter-plate test for biofilm assay

The sub-inhibitory concentration of DMSO impact on PA was assessed by mixing varying volumes of MH broth and DMSO in different wells of 96-well assay plates so that the concentration of the solvent ranged from 8% to 1 % v/v.as seen in Table 1. Tryptone Soy Broth was used to prepare 20 µL ofstandardized bacteria suspensions from each strain of PA that had been cultured overnight; the tests were performed in triplicate. The positive control contained PA with broth but no DMSO, while the negative control wells contained broth only.

Concentration of DMSO	Volume added	
	broth	DMSO
1%	376	4
2%	372	8
4%	364	16
6%	356	24
8%	348	32

Table 1: varying volumes of MH broth and DMSO used in Microtiter-plate test for biofilm assay so that the concentration of the solvent ranged from 8% to 1 % v/v.

The plates were covered and incubated aerobically for 24 hours at 37°C. The content of each well was aspirated then the wells were washed three times with 250 µL of sterile physiological saline. To remove all non-adherent bacteria, the plates were vigorously shaken then the remaining attached bacteria were fixed using 200 µL of 99% methanol per well. After 15 minutes, all of the plates were emptied and left to dry; once dry, the plates were Gram stained for five minutes with 0.2-mL per well of 2% Hucker's Crystal Violet. The plates were rinsed under running tap water to remove excess stain and after air drying, the dye bound to the adherent cells was re-solubilized using 160 µL of 33% (v/v) glacial acetic acid per well. An automated ICN Flow TitertekMultiscan Plus reader was used to measure the optical density (OD) of each well. To assess growth, the reading was performed three times, (i) before and (ii) after incubation, and(iii) after the biofilm assay. To normalize the measurement of biofilm formation to bacterial growth, the ratio of A570/A600 was used.

Statistical analysis

An ANOVA test was used to identify statistical differences between the results obtained by the microtiter-plate tests with and without the addition of DMSO. Meanwhile, Tukey's test was used to compare the OD values obtained in the microtiter-plate tests performed with and without the addition of DMSO at different dilutions. P values of <0.05 were considered statistically significant.

III. RESULTS

MIC and MBC of DMSO

After 24 hours of incubation under aerobic conditions at 37°C, turbidity was not observed in the test tubes of 50% and 25% dilution (Figure 1), exhibiting inhibition of PA bacteria with MIC of 25; however, turbidity was seen in dilutions of 12.5% and below. For the tubes that showed no visible sign of growth/turbidity in MIC determination (50% and 25%), the streak plate method was used to inoculate test microorganisms onto sterile nutrient agar plates. After the plates were incubated at 37°Cfor 24 hours,

no bacterial growth was observed at 50% concentration, but microbial growth was noted in the plate with 25% concentration, thus confirming that MBC is 50%.

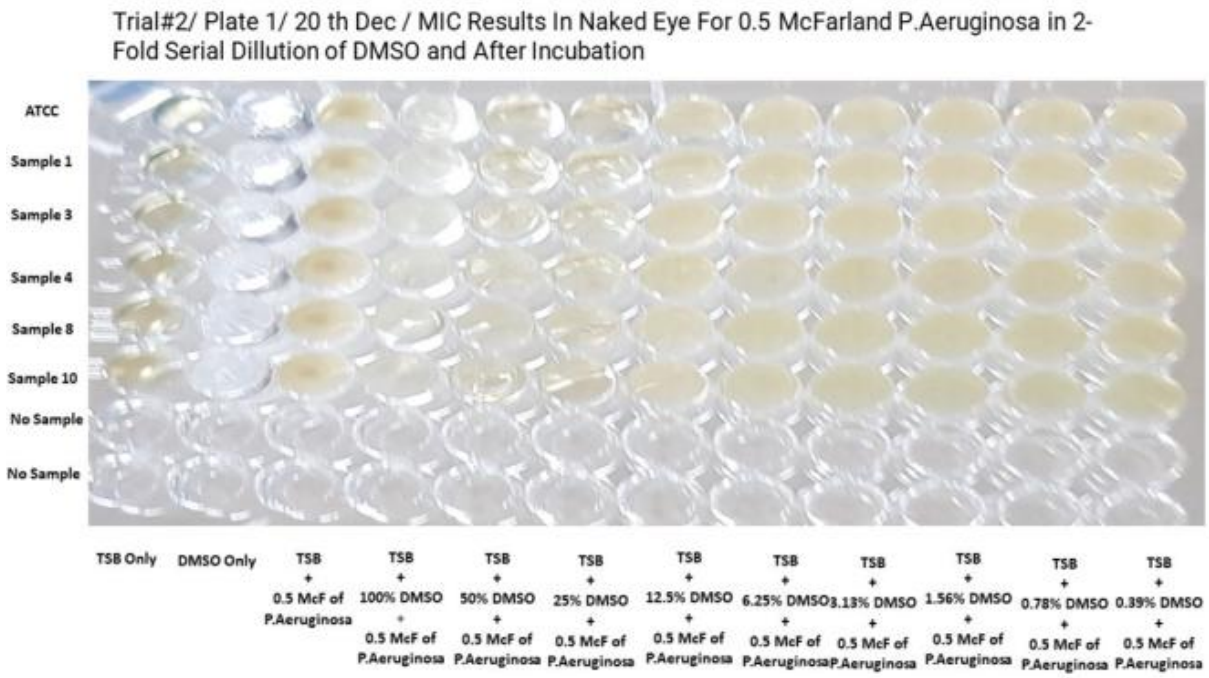

Figure 1: Inhibition of growth by DMSO in P. aeruginosa. The MIC of DMSO was 25% (turbidity was not observed in the test tubes of 50% and 25% dilution)

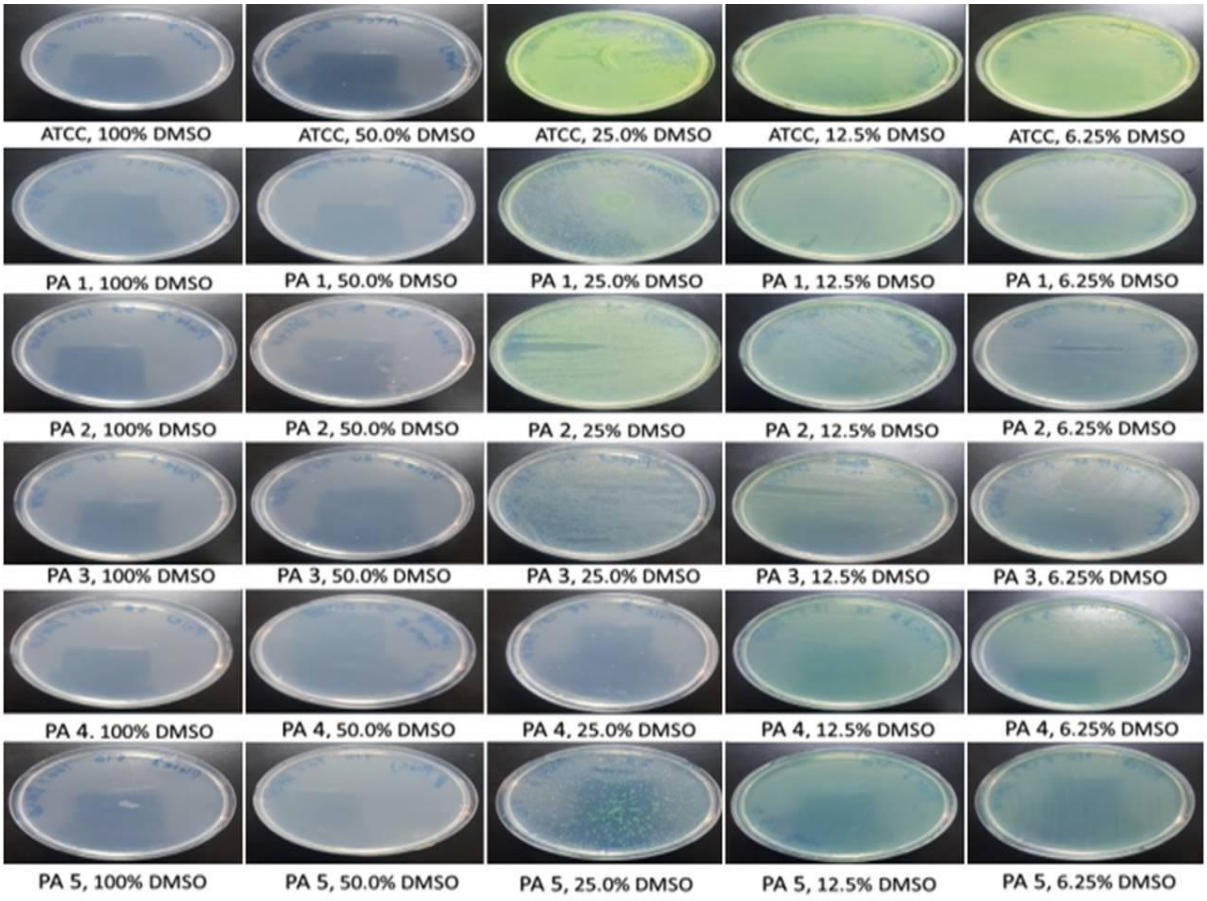

Figure 2: MBC essay show that the MIBC of DMSO upon PA. No bacterial growth was observed at 50% concentration, but microbial growth was noted in the plate with 25% concentration, thus confirming that MBC is 50%

The effect of DMSO on growth and biofilm formation was determined after 24-hour growth in the wells of a microtiter plate. Both biofilm formation and planktonic growth were statistically significant ((F (4, 16.64) = [6.659], (p=0.002), (F (9, 22.64) = [11.659], (p=0.002), respectively), inhibited by the presence of DMSO at concentrations of 8%, 6%, and 4% (Figure 3 and 4). Reduced growth and biofilm formation was also observed at DMSO concentrations of 2% and 1%, but this was not statistically significant compared to control.

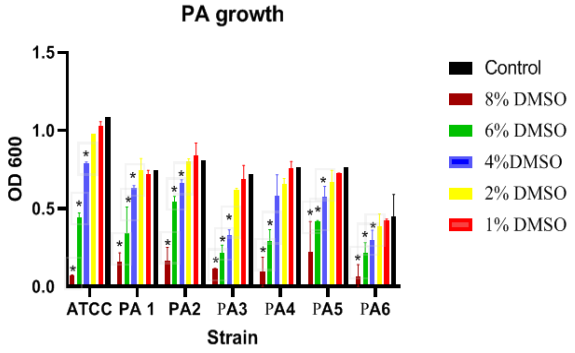

Figure 3: the impact of different DMSO dilution upon PA growth, *statistical significant with p value <0.05

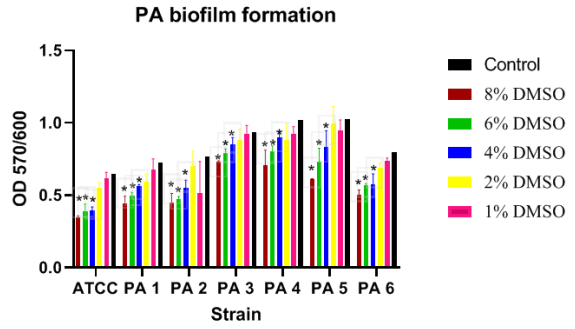

Figure 4: the impact of different DMSO dilution upon PA Biofilm formation. *statistical significant with p value <0.05

IV. DISCUSSION

As antimicrobial resistance has begun to emerge as a global issue of concern, this has motivated research into new drugs based on novel antimicrobial agents, as well as how existing drugs may be repurposed. According to (14), these novel antimicrobial molecules may be derived from repurposing existing drugs, sources such as synthetic biology and medicinal chemistry, or phytochemicals as natural sources. However, these diverse sources share one issue, which is the relative inability to find appropriate solvents that can be used to dissolve these novel molecules and test antimicrobial susceptibility. (15) argue that it is pivotal to explore the use of suitable organic solvents because such molecules are insoluble in water or aqueous solutions. DMSO is an established solvent for the solubilization of conventional antibiotics and other difficult- to-dissolve anti-infective molecules(16).

The Clinical & Laboratory Standards Institute (17) protocols for antimicrobial susceptibility testing also recommend the use of this solvent to dissolve insoluble antibiotics. Therefore, it is critical to investigate whether DMSO itself has potential antimicrobial effects. Deutch (18) highlights that although DMSO is a good solvent for the solubilization of conventional antibiotics and novel anti-infective molecules under antimicrobial investigation, its use also runs the risk of inhibiting the bacterial species under examination, as well as the potential reporting of false-positive effects.

The effect of DMSO upon PA growth and biofilm formation is shown in Figures 1 and 2. Both growth and the formation of biofilms were significantly impacted when the concentration of DMSO was ≥ 4%. When the concentration of solvent was lower, there was no significant effect upon growth and biofilm formation. The results presented here are consistent with those of Basch and Gadebusch (19), who also found DMSO to be perceptibly capable of inhibiting various microorganisms, including Pseudomonas aeruginosa, Mycobacterium tuberculosis; Diplococcus, Neisseria, Haemophilus, and Corynebacteriumat DMSO dilution of 5-10% . Furthermore, there is agreement between this study and the findings of the study performed by Randhawa (20), who used the agar diffusion method to establish the impact upon dermatophyte exposed to different concentrations of DMSO (ranging from 0.125% to 10%). The researchers noted that in 10% DMSO, fungi did not grow at all; however, when the DMSO was between 1.25% and 5%, the growth showed a linear dose-inhibition relationship, which differed significantly to the control. The effect of DMSO was variable < 1% varied across species (20).Comparable results were obtained using *Escherichia coli, Pseudomonas aeruginosa, and Bacillus megaterium* by Ansel, Norred (21)who found a slight reduction in growth rate over a six-hour period at 5% DMSO, but this was considerably decreased at 10% DMSO, where the number of viable bacteria decreased slightly over a six-hour period(21).

In a study into the hemolytic activity of DMSO, de Abreu Costa, Henrique Fernandes Ottoni (22) found that unless the cells were protected by an extracellular material, such as sodium chloride, DMSO could freely penetrate erythrocyte membranes, causing osmotic hemolysis, as well as causing the red blood cells to lyse when the concentration of DMSO was approximately 25%. Comparing the responses of red blood cells and the bacteria to DMSO, both types of cells could be sensitive and could be destroyed by DMSO. Although this paper does not seek to explain the mechanism of the bacteriostatic activity of DMSO, the findings above and the unique solvent characteristics of DMSO suggest that it is not only capable of penetrating cells, but it also alters bacterial

cells in a way that considerably reduces the division of cells (22).

Reduce the biofilm formation of PA reported in this paper is supported by a study performed recently by Azam and Khan (23)who found that as an important polar aprotic solvent, DMSO could affect the expression of an abundance of QS-related genes at a non-inhibitory concentration (2% v/v) at different strain of PA. Azam and Khan (23)concluded that DMSO could significantly attenuate QS-controlled virulence factor production in PA, including biofilm formation and the production of elastase, LasA protease, rhamnolipids, and pyocyanin, which are positively controlled by the rhl QS system.

The finding of this study suggested that when researchers use solvents such as DMSO, they should include an appropriate control in their study so as to identify the potential inhibition of bacterial growth. Similarly, Pérez Aranda, Alcudia Cruz (24)suggested that DMSO should be diluted at least ten-fold (1:10), and should not be used undiluted when working with some type of bacteria. Moreover, it may be that DMSO could beused in combination with antibiotics, although possible interactions with the antibiotics must also be considered. Mi, Wang (25) reported that DMSO inhibits the action of some reactive oxygen species-dependent antibiotics against *E. coli*.Guo, Wu (26) investigated the effectiveness of DMSO on the efficacy of six different antibiotics against PA. Unlike what was reported for *E. coli*, Guo, Wu (26) found that DMSO at 1% or 2% made PA slightly more sensitive to ciprofloxacin (the MIC decreased from 0.4 g/mL to 0.2 g/mL), and slightly less sensitive to ceftazidime and chloramphenicol (a two-fold increase); no change was observed in the MICs of tetracycline, carbenicillin, or meropenem. It therefore appears that the interaction of DMSO with antibiotics could be both antibiotic and bacterium species-specific, so this effect should be considered when using DMSO and antibiotics concurrently.

It is important that solvents used in biological tests provide maximum solvation, but must also be well-matched to the system being tested. Those solvents that are not water-based can be toxic to the test organisms. Prior to conducting the research experiments, toxicity tests should be conducted to establish the maximum concentration of solvent that can be used without initiating toxicity; these tests should include controls with potential solvent toxicity. It is essential that the ultimate concentration of organic solvent does not disrupt the bioassay. Also, it is critical to be mindful of that solvents act selectively, affecting some organisms more than others even when the concentration of solvent is consistent. Best practice is to use the lowest possible concentration of organic solvent in the assays; however, regulating the concentration can be a challenge with some natural products, in which the efficiency of extracting the solvent can be inconsistent. Despite the apparent absence of effect on the growth of bacteria, the lower concentrations of solvents could have an impact on the antibacterial compound being evaluated.

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DOES HERDING LEAD SIZE GROWTH OF CRYPTO CURRENCY MARKET?

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Abstract –
Herding behavior of investors are of interest for many parties in financial markets. Motivation of these investors are addressed and discussed in this paper with the aim of shedding light to the transactions in crypto currency markets. Information is hard to achieve as there are limited amount of analyst that fully understand the block chain mechanism and they hardly produce reports about the new projects. Investors either may not access to the reports or have hardship to absorb the information. Therefore, they may risk to herd deliberately. When investors move together they may lead to bubbles or crashes in the market and objective of this study is to analyze the possible future effects of herding in crypto exchanges.

Keywords – Herding, Block Chain, Decentralized Finance

I. INTRODUCTION

Decentralized finance led to attract risk takers to take positions in the crypto currency markets. Valuation of these crypto currencies are simply harder than other assets as it is not easy to find the intrinsic values. The prospectuses of these currencies have to be read carefully but they may contain pitfalls for financially illiterate investors. By intuition, majority of investors may not differentiate the differences among tokens via prospectuses but trade based on other investors' transactions.

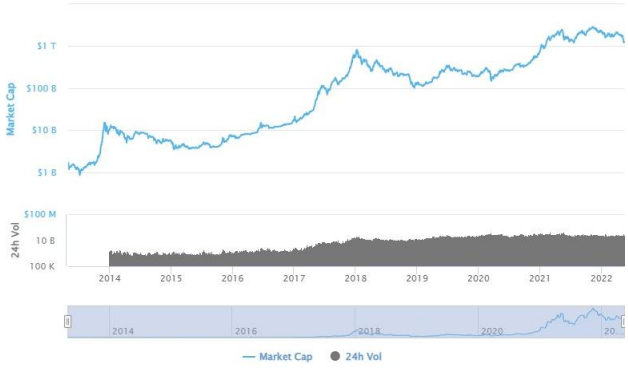
This study aims to analyze the relationship between herding and the market capitalization of the crypto currency market. Herding generally leads to mispricing and investors incurring losses. Investors buy overpriced assets or sell underpriced assets. Regarding with crypto currencies this may not be the case as it is almost impossible to reach intrinsic value. Expectations regarding with these projects determine the price and demand has to be created for these projects to survive in the short run. With the availability of financing created, the projects may evolve to a more robust and sustainable state. In order for these projects to endure, herding may be detrimental due to additional price fluctuations or may be beneficial as they help in terms of additional demand. The objective of this paper to discuss the possible effects of herding to crypto world.

II. MARKET CAPITALIZATION OF THE CRYPTO WORLD

The growth of the crypto world is a well-known fact despite increasing concerns. Particularly, regulators and some scholars keep frowning to this emerging market but it is apparent that funds are channeled to these markets.

2.1 Size of Crypto World

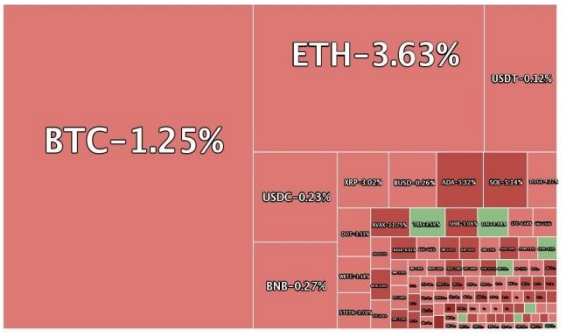
As of 19 May 2022, there are 527 exchanges and 19499 Cryptos. 24-hr trading volume of the market is around $86.95B. the daily volume was 10.07% lower the day before; indicating the demand is alsovolatile. $ 7.84 of the total daily trading volume belongs to DeFi and $77.19Bof the total daily trading volume belongs to stable coins; 9.01% and 88.77% respectively. The total market capitalization is approximately1.25 Trillion USD that recently plummeted from 2 Trillion levels since the tapering process has started in United States.



Source: Coin market cap
Figure 1: Market Capitalization of Crypto Currencies

2.2 Domination of Leading Coins in the Crypto Market.

Top two crypto coins rule the market. Bitcoin has a domination in the market 42.73% and Ethereum has a market share of 18.03%. For instance, Litecoin has the third highest percentage of 0.36%. Stellar has 0.24% and Monero has 0.23%. The rest of the market has 38.1%.



Source: CoinGecko

Figure 2: Cryptocurrencies Tree Map (Top 100 by Market Capitalization)

2.3 Motivation for Herding in Crypto World and Discussion for the Possible Outcomes of Herding

Investors are inclined to herd when they trade depending on their social mood [8] and [9]. Mood congruency effect assumes investors tend to remember more about what their mood conforms with. Some scholars argue that regulatory policies that are mood congruent may increase herding as well [1]. Some investors may even join the herd intentionally to enjoy the power of the group they are in. The motivation of such investors are mainly; imperfect information, concern for the reputation and the compensatory structures [2].

Evidently, herding destabilizes markets and may pose a threat to some financial markets where additional oscillations of prices may lead to crashes.

2.4 Literature Review

Herding has been studied widely in literature but regarding with cryptocurrencies only recently there were studies [10], [6]. Size is one of the determinants of herding. Herding seems to be present with smaller stocks in which obtaining information may be more difficult [4] and [5].

Kallinterakis and Wang have studied factors of herding in the virtual world for the period December 2013 and July 2018. They provide evidence that herding is stronger when market is up, less volatile and when the trading volume is high [7].

III. METHODOLOGY

To state herding in a market; Cross sectional absolute deviation is calculated as follows [3]:

$$CSAD_t=\beta_0+\beta_1|R_{m,t}|+\beta_2R^2_{m,t}+u_t \quad (1)$$

Where, $R_{m,t}$ is the average return of all cryptocurrencies on day *t* and $CSAD_t$is the daily cross-sectional absolute deviation:

$$CSAD_t=\sum_{i=1}^{n}|R_{i,t}-R_{m,t}|/n \quad (2)$$

n is the number of traded cryptocurrencies on day *t* and $R_{i,t}$ is cryptocurrency *i*'s return on day *t*, calculated as the first logarithmic difference of its closing prices. A significantly negative β_2 may indicate the presence of herding.

The following models are constructed to assess the presence of herding in markets:

$$CSAD_t=\beta_0+\beta_1D^{up}|R_{m,t}|+\beta_2(1-D^{up})|R_{m,t}|+\beta_3D^{up}R^2_{m,t}+\beta_4(1-D^{up})R^2_{m,t}+u_t \quad (3)$$

$$CSAD_t=\beta_0+\beta_1D^{high\text{-}VT}|R_{m,t}|+\beta_2(1-D^{high\text{-}VT})|R_{m,t}|+\beta_3D^{high\text{-}VT}R^2_{m,t}+\beta_4(1-D^{high\text{-}VT})R^2_{m,t}+u_t \quad (4)$$

$$CSAD_t=\beta_0+\beta_1D^{high\text{-}VL}|R_{m,t}|+\beta_2(1-D^{high\text{-}VL})|R_{m,t}|+\beta_3D^{high\text{-}VL}R^2_{m,t}+\beta_4(1-D^{high\text{-}VL})R^2_{m,t}+u_t \quad (5)$$

Where:

$D^{up}=1$ on up market days ($R_{m,t}>0$), zero otherwise;

$D^{high\text{-}VT}=1$ on high volatility days, zero otherwise. Volatility is assumed to be the squared value of $R_{m,t}$and is considered to be high (low), if it is above (below) its 30-day moving average;

$D^{high\text{-}VL}=1$ on high volume days, zero otherwise. Volume is calculated to be high (low), if it is above (below) its 30-day moving average [3].

The authors believe that crypto currency trades are shorter term and 30-day moving average is long for crypto currency markets thus, our model will incorporate 15-day moving average for both volume and volatility.

IV. CONCLUSION

Herding in crypto markets is a known phenomenon as investors cannot be informed simultaneously about the project or it requires longer time period to analyze a crypto project. The crypto market is very volatile and investors need to time the market so they do not want to late to act. The authors of this paper believe that investors are willingly taking part in the herding process and try to reap profits by timing the waves of trades.

For further research, a model is proposed to provide evidence for the presence of herding in crypto currency market. Shorter term moving averages for calculating volume and volatility may enhance the model that points herding in a market.

Herding also may be part of the reason for the increase of crypto prices in 2021 and the substantial fall in 2022. Presence of herding in smaller market

capitalization crypto coins is planned to be analyzed in a further article.

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COMBINATION TECHNIQUES FOR FAST AND LOW-WASTE IDENTIFICATION AND SEPARATION OF FRAGRANCES FROM PERSICARIA ODORATA

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Abstract - Fragrance of Persicaria odorata has been using for decades in ASEAN states, from food to perfume industries. Identification and separating of its effective volatile substances for its true scent require high resources and time. Therefore, this study used combination techniques of extraction, distillation and chromatography to develop faster and lower-waste method. It was found that the most abundance fragrances were aldehydes. Real fragrances content in essential oil was 54.61% while extracts varied from more than twenty to over eighty percentages with lesser chemical diversity. This procedure may be applied to experimental planning and increasing in fragrance separation efficiency.

Keywords - Fragrance, Persicaria odorata, Separation, Solid-phase microextraction

I. INTRODUCTION

Persicaria odorata is in family Polygonaceae, generally known as Phak Phai in northern Thailand. It is a wet biennial plant with herbal fragrances [1]. People in Association of Southeast Asian Nations (ASEAN) countries, especially in Thailand and Malaysia, consumes it as a food ingredient or side-dish and even use it as perfumes at the semi-industrial level [2]–[3]. Due to high quantity of feed with low yield, the separation of the important substances is therefore at high risk.

Separation of fragrance analyte fractions from extract and isolation of mixture from essential oil require large amount of plant sample, chemicals and time [4]. General technique and procedure for separation like chromatography, both common and high-performance mostly need high amount of precursor to get enough amount in fraction to use in further levels [5]–[7]. It is not suitable to identify which substances are the true fragrances that play as the key role in real scent, every fraction and step need to be checked. This leads to so much loss of real effective substances.

Gas chromatography coupled with mass spectroscopy (GC–MS) is one of the fastest and reliable technique for identifying volatile components. It requires the lowest amount of sample, among chromatographic techniques [8]. However, it still needs liquid-form volatile inlet. Solid-phase microextraction (SPME) which is a technique capable of capturing substances like fragrances above head space (HS) of sample [9], can therefore be of great support. However, both techniques still not quite capable for quantitative collection. This study aims is to develop a procedure using many extractions and identification techniques together to point out for separation of the interesting fragrances from *P. odorata* with minimization of time, chemicals and solvents.

II. METHODS

A. Plant Material

Fresh *P. odorata* leaves were purchased from a local market of Chiang Mai, Thailand. The leaves were washed and dried in an oven at 60°C for 12 hours.

B. Solvent Extraction

Dried *P. odorata* leaves were grounded into fine powder. Each 25 g of the dried powder was immersed and extracted with 500 mL of four different solvents; hexane (QRëC, New Zealand), ethyl acetate (KemAus, Australia), dichloromethane (J.T.Baker, USA) and methanol (Macron Fine Chemicals, USA) at 25°C; for 24 hours. The extracts were filtered and concentrated using a rotary evaporator to obtaining hexane extract (Hex Ext), ethyl acetate extract (EA Ext), dichloromethane extract (DCM Ext) and methanol extract (MeOH Ext), separately.

C. Oil Isolation

Essential oil of 500 g fresh *P. odorata* leaves were isolated by hydro-distillation with modified Clevenger apparatus for 3 hours. The oil was collected and stored in the amber vial at 4°C.

D. Solid-phase Microextraction

Divinylbenzene/Carboxen/polydimethylsiloxane (DVB/CAR/PDMS) SPME fiber was purchased from Supelco (USA). The fiber was heated in GC–MS inlet for 10 minutes before each sampling in head space (HS). Fresh *P. odorata* leaves 5 g in 100 mL sealed beaker and 50 mg of its essential oil in 10 mL sealed tube were set for 15 minutes at 40°C. The extracted fiber was ported to GC–MS with spitless mode.

E. Chromatographic Conditions

Each 1 μL of P. odorata extracts and oil were carried into GC–MS (Agilent 7820A – HP 5977E) with

HP-5MS capillary column (30 m x 0.25 mm I.D. x 0.25 μm film thickness). The carrier gas was helium at flow rate of 1.0 ml/min. Injection port and detector temperatures were 250°C C and 280°C, respectively. Oven temperature program was started at 40°C for 3 minutes and rated at 30°C/min to 140°C before rated at 15°C/min to 150°C, then rated at 6°C/min to 180°C, and final was rated at 15°C/min to 220°C. Mass spectra were obtained by electron ionization at 70 eV and scan mass was ranged from 33 to 600 amu. Retention index (RI) was calculated following Kovats procedure [10].

III. RESULTS

Maceration extractions of *P. odorata* by hexane, ethyl acetate, dichloromethane and methanol yielded 0.42%, 1.43%, 0.90% and 1.02%, respectively. Pale greenish-yellow essential oil was obtained from hydro-distillation with modified Clevenger apparatus in 0.21% yield. Identification of the chemical components was based on mass spectra library of NIST14. The overall composition of *P. odorata* showed 106 compounds, 42 compounds of which were volatile organic compounds by SPME. As shown in table 1, comparisons of direct injection to SPME; essential oil, Hex Ext, DCM Ext, EA Ext and MeOH consisted of 16 (54.61%), 5 (22.15%), 9 (80.92%), 7 (75.64%) and 7 (46.90%) fragrances, respectively. The major compounds of essential oil were dodecanal (26.72%, fragrance), 1-decene (10.68%, non-fragrance) and decanal (7.01%, fragrance). Hex Ext majorities were 1,1,2,2-tetramethyl-cyclopropane (21.53%, non-fragrance), dodecanal (15.92%, fragrance) and 2,3,3,4-tetramethyl-pentane (10.01%, non-fragrance). DCM Ext major compounds were dodecanal (58.75%, fragrance), decanal (10.79%, fragrance) and caryophyllene (5.16%, fragrance). EA Ext majorities were dodecanal (55.97%, fragrance), decanal (10.27%, fragrance) and caryophyllene (5.01%, fragrance). Major compounds of MeOH Ext were dodecanal (27.57%, fragrance), caryophyllene (8.32%, fragrance) and humulene (7.02%, non-fragrance).

For direct injection, fragrances that could be found only in essential oil were (*E*)-3-hexen-1-ol, 6-methyl-5-hepten-2-ol, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-benzene, [4a*R*-(4aα,7α,8aβ)]-decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-naphthalene, [1*S*-(1α,7α,8aα)]-1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-naphthalene, β-bisabolene, (*R*)-1-methyl-4-(6-methylhept-5-en-2-yl)cyclohexa-1,4-diene, [*S*-(*R**,*S**)]-3-(1,5-dimethyl-4-hexenyl)-6-methylene-cyclohexene and (2*S*,4a*R*,8a*R*)-4a,8-dimethyl-2-(prop-1-en-2-yl)-1,2,3,4,4a,5,6,8a-octahydronaphthalene.

Undecanal was found in only direct injection of Hex Ext.

No.	RT	Compounds	RI	SPME (%)	Direct injection (%)				
				Fresh	Oil	Hex Ext	DCM Ext	EA Ext	MeOH Ext
1	4.64	3-Methyl-cyclopentanol	847	ND	ND	5.59	ND	ND	ND
2	4.69	(*R*)-(+)-3-Methylcyclopentanone	852	ND	ND	3.61	ND	ND	ND
3	4.73	(*E*)-2-Hexenal	855	0.23	ND	ND	ND	ND	ND
4	4.77	(*E*)-3-Hexen-1-ol	859	1.56	0.06	ND	ND	ND	ND
5	4.93	4-Methyl-2-oxepanone	874	ND	ND	0.28	ND	ND	ND
6	5.05	Hexan-2,4-dione,enol	884	ND	ND	0.29	ND	ND	ND
7	5.11	1-Nonene	890	0.08	ND	ND	ND	ND	ND
8	5.22	Methoxy-phenyl-oxime	900	0.14	ND	ND	ND	ND	ND
9	5.45	2,3,3,4-Tetramethyl-pentane	928	ND	ND	10.79	ND	ND	ND
10	5.49	2,5-Hexanedione	932	ND	ND	1.67	ND	ND	ND
11	5.53	(1*R*)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	938	0.32	ND	ND	0.44	0.27	1.50
12	5.55	α-Pinene	939	ND	ND	0.76	ND	ND	ND
13	5.63	1-(3-Ethyloxiranyl)-ethanone	950	ND	ND	4.35	ND	ND	ND
14	5.70	2,2-Dimethyl-pentanoic acid,ethenyl ester	958	ND	ND	4.95	ND	ND	ND
15	5.81	1,1,2,2-Tetramethyl-cyclopropane	972	ND	ND	21.53	ND	ND	ND
16	5.84	2-Pentanone	976	ND	ND	2.56	ND	ND	ND
17	5.89	(1*S*)-6,6-Dimethyl-2-methylene-bicyclo[3.1.1] heptane	982	0.36	ND	ND	ND	ND	ND
18	5.95	6-Methyl-5-hepten-2-one	988	0.05	ND	ND	ND	ND	ND
19	5.98	6-Methyl-5-hepten-2-ol	993	0.34	0.06	ND	ND	ND	ND
20	5.99	2,3,3-Trimethyl-1-butene	994	ND	ND	2.46	ND	ND	ND
21	6.02	Carbonic acid,hexadecyl prop-1-en-2-yl ester	998	ND	ND	ND	ND	ND	4.85
22	6.03	2-Methyl-6-hepten-1-ol	999	ND	0.01	ND	ND	ND	ND

23	6.04	Decane	1000	ND	ND	ND	ND	0.73	ND
24	6.17	2-Methyl-2-(1-methylethyl)-oxirane	1019	ND	ND	0.85	ND	ND	ND
25	6.29	Eucalyptol	1038	0.19	ND	ND	ND	ND	ND
26	6.37	β-Ocimene	1050	0.04	ND	ND	ND	ND	ND
27	6.65	1-Undecene	1091	0.12	ND	ND	ND	ND	ND
28	6.70	Undecane	1099	1.77	0.03	ND	ND	ND	1.60
29	6.74	Nonanal	1105	ND	0.04	ND	ND	ND	ND
30	6.79	3-Butyl-2,4-pentanedione	1114	ND	ND	0.10	ND	ND	ND
31	6.81	(*E*)-4,8-Dimethylnona-1,3,7-triene	1117	0.09	ND	ND	ND	ND	ND
32	7.03	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	1151	ND	ND	ND	ND	ND	2.49
33	7.08	Camphor	1158	0.06	ND	ND	ND	ND	ND
34	7.21	1-Nonanol	1179	ND	0.08	ND	ND	ND	ND
35	7.29	1-Dodecene	1191	ND	ND	ND	ND	1.10	ND
36	7.34	Dodecane	1199	ND	ND	ND	ND	0.32	ND
37	7.48	Decanal	1218	9.24	7.01	3.10	10.79	10.27	4.70
38	7.59	*cis*-3-Hexenyl isovalerate	1235	0.20	ND	ND	ND	ND	ND
39	7.63	2,6,6-Trimethyl-1-cyclohexene-1-carboxaldehyde	1240	ND	0.03	ND	ND	ND	ND
40	7.84	Cyclodecane	1270	ND	ND	ND	ND	ND	1.66
41	7.86	1-Decanol	1273	3.25	4.88	ND	0.71	ND	ND
42	8.04	Tridecane	1298	0.07	ND	ND	ND	ND	ND
43	8.14	(*E*)-2-Decen-1-ol	1311	ND	1.85	ND	ND	ND	ND
44	8.15	Undecanal	1311	0.83	ND	0.29	ND	ND	ND
45	8.67	1-Nonene	1373	0.21	ND	ND	ND	ND	ND
46	8.82	1-Tetradecene	1390	ND	ND	ND	ND	1.69	ND
47	8.90	(1*S*,5*S*)-2-Methyl-5-((*R*)-6-methylhept-5-en-2-yl)bicyclo[3.1.0]hex-2-ene	1400	ND	0.12	ND	ND	ND	ND
48	8.97	[1*S*-(1α,2β,4β)]-1-Ethenyl-1-methyl-2,4-bis(1-methylethenyl)-cyclohexane	1407	0.30	ND	ND	ND	ND	ND
49	9.07	Dodecanal	1417	37.82	26.72	15.92	58.75	55.97	27.57
50	9.17	4,11,11-Trimethyl-8-methylene-, [1*R*-(1*R**,4*Z*,9*S**)]-bicyclo[7.2.0]undec-4-ene	1427	0.21	ND	ND	ND	ND	ND
51	9.33	Caryophyllene	1443	14.69	6.97	1.96	5.16	5.01	8.32
52	9.38	*trans*-α-Bergamotene	1447	2.90	ND	0.20	0.41	0.40	0.71
53	9.49	(*E*)-β-Famesene	1459	1.28	ND	ND	ND	ND	ND
54	9.54	(1*S*,5*S*,6*R*)-6-Methyl-2-methylene-6-(4-methylpent-3-en-1-yl)bicyclo[3.1.1]heptane	1464	ND	0.35	ND	ND	ND	ND
55	9.59	(1*R*,9*R*,*E*)-4,11,11-Trimethyl-8-methylenebicyclo[7.2.0]undec-4-ene	1468	0.31	ND	ND	ND	ND	ND
56	9.63	Cyclododecane	1473	3.29	ND	ND	ND	ND	ND
57	9.65	1-Decene	1475	ND	10.68	ND	ND	0.35	ND
58	9.67	*Z*,*Z*,*Z*-1,5,9,9-Tetramethyl-1,4,7,-cycloundecatriene	1476	5.78	ND	0.97	3.05	3.03	ND
59	9.70	Humulene	1479	ND	ND	ND	ND	ND	7.02
60	9.79	(1*R*,4*R*,5*S*)-1,8-Dimethyl-4-(prop-1-en-2-yl)spiro[4.5]dec-7-ene	1489	ND	0.92	ND	ND	ND	ND
61	9.83	1-(1,5-Dimethyl-4-hexenyl)-4-methyl-benzene	1492	1.21	0.75	ND	ND	ND	ND
62	9.87	(-)-α-Panasinsen	1496	1.51	ND	ND	ND	ND	ND
63	9.93	Selina-3,7(11)-diene	1502	ND	0.61	ND	ND	ND	ND
64	9.96	4a,8-Dimethyl-2-(prop-1-en-2-yl)-1,2,3,4,4a,5,6,7-octahydronaphthalene	1504	1.33	ND	ND	ND	ND	ND
65	10.00	[4a*R*-(4aα,7α,8aβ)]-Decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-naphthalene	1508	1.15	0.43	ND	ND	ND	ND
66	10.01	[1*S*-(1α,4α,7α)]-1,2,3,4,5,6,7,8-Octahydro-1,4,9,9-tetramethyl-4,7-methanoazulene	1509	ND	0.87	ND	ND	ND	ND
67	10.04	[1*S*-(1α,7α,8aα)]-1,2,3,5,6,7,8,8a-Octahydro-1,8a-dimethyl-7-(1-methylethenyl)-naphthalene	1511	1.39	1.29	ND	ND	ND	ND

68	10.06	2,4-Di-tert-butylphenol	1513	ND	ND	ND	ND	0.97	ND
69	10.11	β-Bisabolene	1525	0.95	0.34	ND	ND	ND	ND
70	10.15	(*R*)-1-Methyl-4-(6-methylhept-5-en-2-yl)cyclohexa-1,4-diene	1528	0.24	0.49	ND	ND	ND	ND
71	10.27	(1α,4aβ,8aα)-1,2,3,4,4a,5,6,8a-Octahydro-7-methyl-4-methylene-1-(1-methylethyl)-naphthalene	1537	0.12	ND	ND	ND	ND	ND
72	10.30	[*S*-(*R**,*S**)]-3-(1,5-Dimethyl-4-hexenyl)-6-methylene-cyclohexene	1539	0.20	0.19	ND	ND	ND	ND
73	10.34	7-epi-α-selinene	1537	ND	ND	ND	0.44	ND	ND
74	10.35	(2*S*,4a*R*,8a*R*)-4a,8-Dimethyl-2-(prop-1-en-2-yl)-1,2,3,4,4a,5,6,8a-octahydronaphthalene	1544	2.12	1.05	ND	ND	ND	ND
75	10.50	1-Ethenyl-1-methyl-2-(1-methylethenyl)-4-(1-methylethylidene)-cyclohexane	1550	ND	0.03	ND	ND	ND	ND
76	10.53	(1*S*,2*R*,5*R*)-2-Methyl-5-((*R*)-6-methylhept-5-en-2-yl)bicyclo[3.1.0]hexan-2-ol	1552	ND	ND	0.23	ND	ND	ND
77	10.56	Dodecanoic acid	1555	ND	5.42	ND	ND	ND	6.56
78	10.70	(*E*)-3,7,11-Trimethyl-1,6,10-dodecatrien-3-ol	1570	0.11	ND	ND	ND	ND	ND
79	10.76	1,1-Dimethoxy-heptane	1572	ND	ND	ND	ND	ND	1.98
80	10.98	Cetene	1591	ND	ND	ND	ND	1.59	ND
81	11.17	Caryophyllene oxide	1606	ND	1.73	ND	ND	ND	ND
82	11.26	Tetradecanal	1613	1.01	1.41	ND	0.75	ND	ND
83	11.48	(1*R*,3*E*,7*E*,11*R*)-1,5,5,8-Tetramethyl-12-oxabicyclo[9.1.0]dodeca-3,7-diene	1630	ND	0.76	ND	ND	ND	ND
84	11.79	10,10-Dimethyl-2,6-dimethylenebicyclo[7.2.0]undecan-5β-ol	1654	ND	0.69	ND	ND	ND	ND
85	11.85	9,11-Dimethyltetracyclo[7.3.1.0(2.7).1(7.11)]tetradecane	1659	ND	ND	ND	ND	ND	2.68
86	11.91	1-(2-Methoxyphenyl)-2,5-dihydro-1H-pyrrole-2,5-dione	1663	ND	1.81	ND	ND	ND	ND
87	12.03	[2*R*-(2α,4aα,8aβ)]-1,2,3,4,4a,5,6,8a-Octahydro-4a,8-dimethyl-2-(1-methylethenyl)-naphthalene	1672	ND	0.19	ND	ND	ND	ND
88	12.10	(*E*)-2-Tetradecene	1677	ND	0.33	ND	ND	ND	ND
89	12.11	Alloaromadendrene	1678	ND	0.03	ND	ND	ND	ND
90	12.17	1-(1,5-Dimethyl-4-hexenyl)-4-methyl-3-cyclohexen-1-ol	1683	ND	0.85	ND	ND	ND	ND
91	12.60	1,2,4-Trimethyl-cyclopentane	1718	ND	ND	ND	ND	ND	1.52
92	12.72	3,7,11-Trimethyl-2,6,10-dodecatrien-1-ol	1728	ND	0.22	ND	ND	ND	ND
93	12.97	(*Z*,*E*)-3,7,11-Trimethyl-2,6,10-dodecatrienal	1751	ND	0.10	ND	ND	ND	ND
94	13.10	Tetradecanoic acid	1762	ND	0.82	ND	ND	ND	ND
95	13.20	7-Isopropenyl-1,4a-dimethyl-4,4a,5,6,7,8-hexahydro-3H-naphthalen2one	1770	ND	0.12	ND	ND	ND	ND
96	13.34	Drim-7-en-11-ol	1783	0.12	2.94	ND	0.86	0.69	2.49
97	13.44	1-Octadecene	1792	ND	ND	ND	ND	1.19	ND
98	13.45	Aromandendrene	1792	ND	0.06	ND	ND	ND	ND
99	13.87	Dodecyl 2-methylbutanoate	1834	ND	0.19	ND	ND	ND	ND
100	13.91	Isovaleric acid,dodecyl ester	1837	ND	0.26	ND	ND	ND	ND
101	13.94	Neophytadiene	1841	ND	ND	0.65	2.33	2.21	4.69
102	14.00	6,10,14-Trimethyl-2-pentadecanone	1847	ND	0.09	ND	ND	ND	ND
103	14.12	Pentadecanoic acid	1859	ND	0.03	ND	ND	ND	ND
104	14.74	Hexadecanoic acid,methyl ester	1923	ND	ND	ND	ND	ND	2.34
105	14.87	Palmitoleic acid	1938	ND	0.07	ND	ND	ND	ND
106	14.95	Isophytol	1946	ND	0.06	ND	ND	ND	ND
		Total (%)		97.20	84.05	83.10	83.68	85.79	82.68
		Unknowns (%)		2.80	15.95	16.90	16.32	14.21	17.32

Table 1: Fragrances and composition of fresh plant, oil and extracts from P. odorata identified by HS-SPME/GC–MS and GC–MS.
ND: not determined; RT: retention time; RI: retention index; Hex Ext: hexane extract; DCM Ext: dichloromethane extract; EA Ext: ethyl acetate extract; MeOH Ext: methanol extract.

IV. CONCLUSION

This study concluded that the majority substances that cause fragrance in *P. odorata* was aldehyde such as dodecanal, filled with over forty minors. The proper solvent for extraction could be dichloromethane which was able to extract the greatest amount of fragrances, following by ethyl acetate; however, they gradually

lacked a variety of chemicals. It could be observed that essential oil had the highest number of fragrance substances, even though its real volatile content only accounted as half of the oil.

According to the database, reliable identifications of composition can be obtained by GC–MS. Since HS-SPME can provide information of which compound is a volatile, comparison of extract chemical compounds can directly point out the analytical fragrances for separation and saving time, chemicals and solvents before further steps. The combination of techniques met the optimum of interested benefit point, with this system from all these techniques, 20 out of 42 fragrances from 106 compositions could be separated

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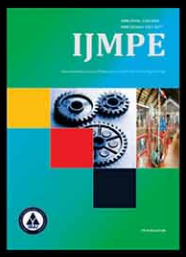

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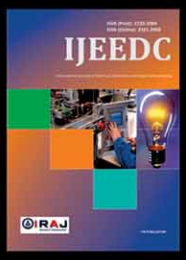

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