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Editors Communique

Have we tamed the coronavirus? May be yes,
as pandemics do not die, they can only be faded !

Science and technology has made it possible, in the shortest span of time, it has shown that with firm determination and international cooperation, we can win over the onslaughts of even the worst of the pandemics. COVID-19 is perhaps fading over now, due to our coordinated efforts worldwide. Though we have lost millions, in the two year period, partly due to the mishandling of the viral attacks and somewhat by our own follies and carelessness. Anyway lessons learnt from the past, always make us more stronger and determined. Let us now not relax and work on a better mode, as all is still not well yet. The almost taming of the virus and its cousins have indicated some of the concealed failures, on which we have to focus now. We have to be more vigilant, and even a bit of laxity can spoil the good work done. On societal and governmental parts, utmost care and caution is required on a long term basis.

On behalf of Bioscience Biotechnology Research Communications, we falter at words to express our deep sense of solitude and grief on the catastrophic events of the world wide pandemic, spanning over two years now. We pray for the strength to bear this universal calamity and come up with long lasting fortitude to eradicate it soon.

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Quality publication is one of the ways to keep science alive, and good journals have a leading role to play in shaping science for humanity! As teachers, we have great responsibilities, we have to advocate our students to accomplish and show them the path to test their mettle in hard times to excel, especially in the post COVID 19 era. Science and its advocates will rise more to the occasion and will soon provide succor to the already grief stricken humanity.

Sharique A. Ali, PhD
Editor-in-Chief

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CONTENTS

Predictive Modeling of COVID- 19 Confirmed Cases Using Regressive Objective Regression Methodology

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ABSTRACT

The use of predictive models for the evolution of the pandemic is of great help in decision-making by the authorities. The fundamental objective of this work was to obtain through the Regressive Objective Regression, predictions of confirmed cases of COVID-19 in the Marta Abreu Teaching Polyclinic of the city of Santa Clara. In short-term modeling the model was significant at 19.7% with an error of 0.12. Variables dichotommics, saw tooth and saw tooth inverted and risk returned in 1.3, and 12 cases the trend is negative and not significant. We can conclude that a perfect result was obtained in the long term with the ROR methodology. The short-term ROR model depends on the cases of COVID-19 in the previous case, 3 cases back and 12 cases back without significant trend. The long-term model is perfect and depends on the cases of COVID-19 in 12 cases ago, with a negative trend.

KEY WORDS: CORONAVIRUS; COVID-19; MATHEMATICAL MODELING; ROR.

INTRODUCTION

The world has been involved in an epidemiological crisis caused by the new SARS-CoV-2 coronavirus that gave rise to the contagious and deadly disease COVID-19 (Cortellis, 2020; WHO, 2020).Since the first cases were diagnosed in the city of Wuhan, capital of Hubei province, China, the number of infected and dead people has been increasing, which led the World Health Organization (WHO) to declare a pandemic state for COVID-19 on March 11, 2020 (Sun et al., 2020; Wang et al., 2020; WHO, 2020).

According to the Research Group of Mathematical Models in Science and Technology: Development, Analysis, Numerical Simulation and Control (MOMAT) of the Institute of Interdisciplinary Mathematics of the Complutense University of Madrid, Spain, the application of the Be-

CoDiS (Between-Countries Disease Spread) model in the analysis of the COVID-19 pandemic numerically projects that this viral phenomenon will be present for a prolonged period of time in the world (Ivorra & Ramos, 2020).

Several models and methodologies have been applied in the study, analysis and modeling of COVID-19 in the world, where they stand out: Ordinary Differential Equation of First Order (EDOPO), linear type; Simple linear Regression model; Generalized Logistic Growth Model (GLM); Structured Susceptible-Exposed-Infected-Removed (SEIR)/ SEIR model; the Bayesian Probability Mathematical Model; SIRD model (also employed in Cuba); Conceptual Model and the Simulation Model, among many others (Rue et al., 2017; Simpson et al., 2017; Osés et al., 2021a,b).

The main objective of the present research was to obtain, by means of the Objective Regressive Regression (ORR) methodology, predictions of confirmed cases of COVID-19

in the "Marta Abreu" Teaching Polyclinic in the city of Santa Clara, Villa Clara province, Cuba.

MATERIAL AND METHODS

In the research, the daily data from January to March corresponding to the year 2021 of the number of positive cases to COVID-19 in the Teaching Polyclinic "Marta Abreu" of Villa Clara Cuba were used, from a total of 3 294 cases evaluated in consultation, 58 were positive.

The prognosis was carried out with the use of the methodology of Regressive Objective Regression (ROR) which has been implemented in different variables such as viruses and bacteria circulating in Villa Clara province. Objective Regressive Regression (ORR) modeling is based on a combination of Dummy variables with modeling (ARIMA), where two Dummy variables are created and the trend of the series is obtained; it requires few cases to be used and also allows the use of exogenous variables that make it possible to model and forecast in the long term, depending on the exogenous variable (Osés et al., 2020).

In the ROR methodology, in a first step, dichotomous variables DS, DI and NoC are created, where: NoC: Number of cases of the base, DS = 1, if NoC is odd; DI = 0, if NoC is even, when DI=1, DS=0 and vice versa. Subsequently, the module corresponding to the Regression analysis of the statistical package SPSS version 19.0 (IBM Company) is executed, specifically the ENTER method where the

predicted variable and the ERROR are obtained. Then the autocorrelograms of the ERROR variable will be obtained, paying attention to the maximums of the significant partial autocorrelations PACF. The new variables are then calculated according to the significant Lag of the PACF. Finally, these regressed variables are included in the new regression in a process of successive approximations until a white noise in the regression errors is obtained.

Ethical aspects: The research was subject to ethical standards, where all the information collected and provided was used only for the stated purpose. It did not involve physical or psychological affectations, in order to be able to generate new knowledge without violating the ethical principles established for these cases. On the other hand, all authors involved in the research, publication and dissemination of the results are responsible for the reliability and accuracy of the results shown (DHAMM, 2013).

RESULTS AND DISCUSSION

The great development acquired by different sciences, such as epidemiology, mathematics, statistics and computer science, has led to the generation of new techniques in emerging and recurrent procedures in the field of biostatistics and bioinformatics (spatial statistics, neural networks and functional data analysis, among others) to describe the importance of mathematical models in infectious diseases (Martí & Caylà, 2009).

Table 1. Descriptive statistics of confirmed patients at the "Marta Abreu" Polyclinic. Year 2021

	N	Minimum	Maximum	Half	Typ. desv.
			Descriptive statistic		
Risk COVID-19	3294	.00	1.00	.0176	.13154
N valid (according to list)	3294				
Positives MA	3	7	31	19.33	12.014
N valid (by list)	3				

Figure 1: Distribution of patients according to age. January-March 2021

Age Based Patients Distribution. Marta Abreu Clinic. Santa Clara January-March 2021
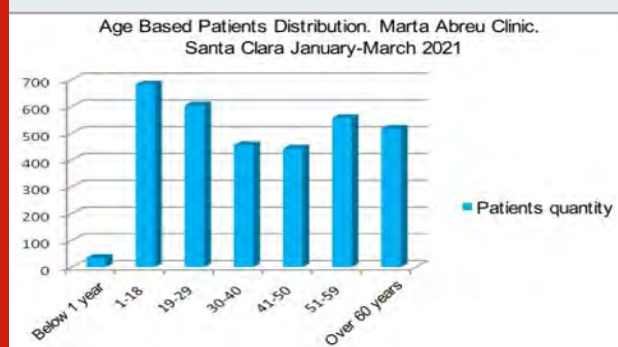
Table 1 calculated the descriptive statistics of the risk of contracting COVID-19, the value of zero was assigned if the PCR was negative, and if the PCR was positive, the value

of Risk 1; the mean value of this variable corresponds to 19.33 cases with a standard deviation of 12.01 cases.

The distribution according to age was irregular, with the highest value corresponding to patients aged 1 to 18 years, followed by those aged 19 to 29 years; children under 1 year of age were also present in the study (Figure 1).

COVID-19 cases were modeled in the short term using the ROR methodology. The model was significant at 19.7% with an error of 0.12 (Table 2). As can be seen in the table the Durbin-Watson statistic is close to 2, so we are in the presence of an adequate explanation of the variance using this model.

1. Predictor variables: Lag12Risk, Lag3Risk, Lag1Risk, DI, SD, NoC.
2. For regression through the origin (the model with no

intersection term), R-squared measures the proportion of the variability of the dependent variable explained by regression through the origin. The above CANNOT be compared with R-squared for models that include an intersection.

3. Dependent variable: RiskCOVID19
4. Linear regression through the origin

The ROR modeling of predictions obtained yielded very significant results for the study of the COVID-19 pandemic in the "Marta Abreu" Teaching Polyclinic, with a Fisher's

F of 22 significant at 100% (Table 3), thus proving that the sample variances are not equal.

Table 3. Analysis of variance of the model for COVID-19

1. Predictor variables: Lag12Risk, Lag3Risk, Lag1Risk, DI, SD, NoC.
2. This total sum of squares has not been corrected for the constant because the constant is zero for regression through the origin.
3. Dependent variable: COVID-19 risk.
4. Linear regression through the origin

Table 2. Summary of the model using Objective Regressive Regression

Model	Summary of model[c,d]				
	R	R squared[b]	R-squared corrected	Standard error of estimation	Durbin-Watson
1	.197a	.039	.037	.12352	2.009

Table 3. Analysis of variance of the model for COVID-19

Model	ANOVA[c,d]				
	Sum of squares	gl	Quadratic mean	F	Sig.
Regression	2.019	6	.336	22.052	.000a
Residual	49.981	3276	.015		
Total	52.000b	3282			

1. Dependent variable: COVID-19 risk.
2. Linear regression through the origin

Next, the model was run only with Lag12 Risk in order to have a long-term valuation by entering the Step POSITIVE variable in the model, obtaining a model that explains 100% of the variance of the model (Table 5).

1. Predictor variables: Step2133, Step1656, Step1655, Step1652, Step1644, Step1336, Step75, Step74, Step14, Step POSITIVE, DS, DI, Lag12Risk, NoC.
2. For regression through the origin (the model with no intersection term), R-squared measures the proportion of the variability of the dependent variable explained by regression through the origin. The above CANNOT be compared to R-squared for models that include an intersection.

The ROR model in question is made up of the following variables, DI and DS which are dichotomous variables, saw tooth DS and inverted saw tooth DI and of the regressed risk in 1,3 and 12 cases (Lag1 Risk; Lag3 Risk and Lag12 Risk). The trend is negative and not significant (Table 4).

Table 4. Results of the application of the ROR model plus trend

Model	Coeficientes[a,b]					Sig.
	Unstandardized coefficients	Typified coefficients				
	B	Error típ.	Beta	t		
	DS	.016	.005	.091	3.316	.001
	DI	.016	.005	.091	3.315	.001
	Tendency	-2.591E-6	.000	-.039	-1.136	.256
	Lag1Risk	.116	.017	.116	6.719	.000
	Lag3Risk	.057	.017	.057	3.326	.001
	Lag12Risk	.065	.016	.069	3.988	.000

1. Dependent variable: COVID-19 risk.
2. Linear regression through the origin

Table 6 shows the analysis of variances using Fisher's test (F). In the results, Fisher's F can't be seen, which infers that

we are dealing with a perfect model, i.e., the result of the variables is equal.

1. Predictor variables: Step2133, Step1656, Step1655, Step1652, Step1644, Step1336, Step75, Step74,

Step14, Step POSITIVE, DS, DI, Lag12Risk, NoC.
2. This total sum of squares has not been corrected for the constant because the constant is zero for regression through the origin.
3. Dependent variable: COVID-19 risk.
4. Linear regression through the origin

Epidemic modeling is historical and has been refined with the advancement of science and technologies, demonstrating the essential role of mathematics in indicating where the disease may move and giving suggestions on how to decide (Guinovart, 2020). COVID-19 disease has been recognized as a global threat, in which predictive models for the epidemiological trend of its prevalence and incidence have

been being used worldwide (Osés et al., 2018; Prades, 2020; Osés et al., 2021c).

In the present research article, predictions were exposed for the cases confirmed to COVID-19 in the "Marta Abreu" Teaching Polyclinic. The increase in the number of children and adolescents infected by the new coronavirus in the mentioned health area showed that they are as susceptible to the disease as the rest of the population. Children and older adults should follow the same biosecurity measures as those prescribed by the Ministry of Public Health for the entire population. Coinciding with the daily reports of MINSAP, the highest number of cases was reported in females (MINSAP, 2021).

Table 5. Model run with Lag12 in the long term

Modelo	Summary of model[c,d]				
	R	R squared[b]	R-squared corrected	Standard error of estimation	Durbin-Watson
1	1.000[a]	1.000	1.000	.0000	1.696

Table 6. Analysis of Variance of the model for COVID-19

Model	ANOVA[c,d]				
	Sum of squares	gl	Quadratic mean	F	Sig.
Regression	52.000	14	3.714	.	.[a]
Residual	.000	3268	.000		
Total	52.000[b]	3282			

The results of the present investigation coincide with those obtained in a similar study conducted in the province of Cienfuegos by Medina (2020); where logistic and exponential models (specifically the Gompertz growth model) were used to obtain estimates. They state that the statistical prediction models obtained gave very significant results for the study of the COVID-19 pandemic in Cuba, which also agrees with the results obtained by other authors in this regard (Medina et al., 2020).

CONCLUSION

The application of ROR modeling provides Public Health authorities and decision makers with information on the short- and medium-term behavior of variables of great interest for understanding the spread of SARS-CoV2. With the use of the ROR methodology, it was possible to predict the future number of positive cases in the "Marta Abreu" health area, making this model a valuable practical tool for correct and timely decision making.

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Women with Postpartum Depression in Kerala: A Survey

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ABSTRACT

The increase in number of Postpartum Depression (PPD) cases in Kerala is increasing day by day. So, a study on the awareness of PPD has become necessary in this scenario. This study tries to quantify the awareness among women in Kerala about PPD and covers how they tackled the issue through various methods used by them to cope with the issue. The study is conducted using an online survey method. A prepared questionnaire was circulated online among 150 young mothers who were born and brought up in Kerala. The questionnaire consisted of eight questions about PPD and baby blues. Each question was provided with options from which the participants had to choose one. The data thus collected were studied and analyzed. The results of the survey were analyzed to arrive at a conclusion. There was active participation from the participants' side. The results showed that the percentage of participants who were well aware of PPD was very low. The study brought to light that, much attention and activities are needed to solve PPD cases in Kerala. Most women who suffer from PPD are reluctant to seek medical help. This situation can change through proper campaigns and other related activities.

KEY WORDS: ANXIETY, AWARENESS, COPING MECHANISM, POSTPARTUM DEPRESSION, TREATMENT.

INTRODUCTION

Postpartum depression is a serious issue faced by women in present-day society. Postpartum depression (PPD) is a medical condition that can be cured with proper care and treatment. In women, depression can occur during and after pregnancy. Depression after delivery can occur as 'baby blues' that last only for one or two weeks after childbirth. It has mild symptoms like mood swings, anxiety, and insomnia. A more severe condition is postpartum depression. It is long-lasting than baby blues and shows intense symptoms including anxiety and panic attacks, sadness, irritability, severe mood swings, appetite problems, difficulty with bonding with your baby, thoughts about harming baby or yourself and severe anger (Jayarajan 2021).

The lack of awareness about Postpartum Depression increases the depth of the problem. Studies show that the lack of early detection of PPD also worsens the condition. The awareness of the real problem, that PPD is a serious issue and can affect the female's later life will bring some changes in the present situation (Zauderer 2009). The purpose of the study is to bring the problem of PPD to the forefront and thus reduce the risk women face due to PPD nowadays. The outcome of this study will help where people are unaware of the seriousness of PPD and where no proper care is given

to women suffering from PPD. The study tries to quantify women awareness of the issue (Jayarajan 2021).

The relevance of the study is high as no such studies have ever been conducted in Kerala in recent times. Moreover, a study like this can contribute to the area of mental health, the overall well-being of a society and the development of the health sector of Kerala.

MATERIAL AND METHODS

An in-depth analysis of Postpartum Depression was conducted using a survey. The study was conducted by circulating the prepared questionnaire among 150 young mothers. The sample of the study was selected after much research on the topic. The ages of the participants were in the range of 25 – 40. Mothers who gave birth in the last 10 years, and who were born and brought up in Kerala were considered for this study.

This was done to examine the recent developments in the area, especially in Kerala. The research subjects were verified to be cognizant of English and all the questions were in English. The participants were well informed about the intention of the survey. They were informed to read the instructions clearly and answer the questions. It was also assured that their details will be kept confidential. The survey mainly aimed at checking the knowledge/awareness women in Kerala had about PPD. Moreover, the study checked how they came to know about PPD and how far they were aware

of the issue. It also helped to collect information about their personal experience of PPD, and how they overcame it. The problem of bonding with the baby was also included as one question.

The questionnaire consisted of 8 questions about PPD and baby blues. These were intended to collect information about the level of awareness women had about PPD, how they came to know about it, have they attended any awareness programs etc. The survey also tried to collect information about the coping mechanism the participants chose to overcome the situation. Responses to this particular question revealed how worse were the condition and what percentage of women seeks medical help.

There were questions about the symptoms they had to suffer and about the time they experienced it. Each of the questions was given options from which the participants can choose one. The number of the options varied from question to question, that is, from 2 – 5. The research subjects were verified to be cognizant of English and all the questions were in English (Jayarajan 2021).The present study has been approved by the Institutional Ethics Committee of Amrita Vishwa Vidyapeetham, Coimbatore. All due permissions have been taken by the concerned authorities including consent etc.

RESULTS AND DISCUSSION

The intensity of the psychic problems faced by women suffering from PPD needs to be discussed in detail, and a long-lasting solution has to be found. An important part of reducing the percentage of patients can be done by giving proper awareness. Not only females but also males and people of other genders have to be educated about the mental condition of women during and after pregnancy. Medical professionals suggest providing proper awareness can reduce the problem in a considerable way. This can contribute a lot to the healthy development of the baby and mother (Zauderer 2009).

Figure 1: Pie chart showing the awareness of participants about Baby Blues
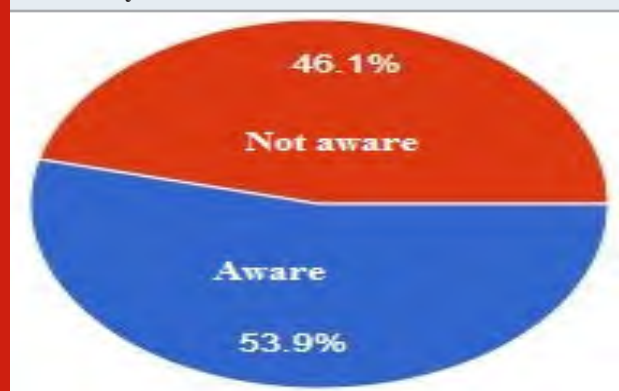
Figure one shows that only about 53.9% of the participants heard about baby blues. This revealed the intensity of the situation. The participants included in the other section, that was, the 46.1%, might be the ones who have gone through

the same. But unawareness of the real problem worsens the condition. As baby blues last only for two or three weeks it is not a dangerous problem as PPD. But even in the case of PPD, a considerable percentage of people who were unaware of PPD was found. Figure 2 shows that 14.8 % of the participants were unaware of postpartum depression. And the results show that people were not much aware of serious problems like PPD as they are of Baby Blues. This condition has to be changed through continuous practices to make the general public aware of PPD and its symptoms (Jayarajan 2021).

Figure 2: Pie chart showing the awareness of participants about PPD
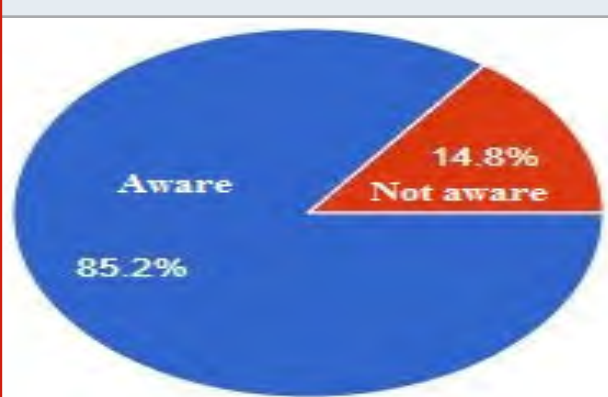
Figure 3: Pie chart showing the response of participants to whether they faced symptoms of PPD
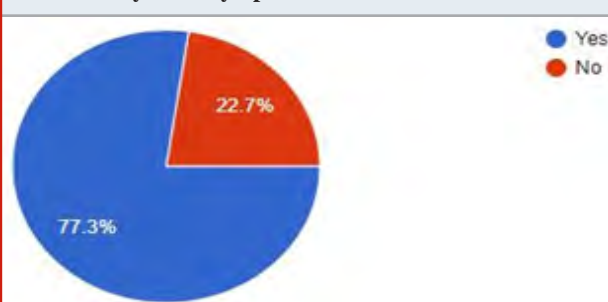
There exists a social stigma in India, especially in Kerala, to consult a psychiatrist or a psychologist (The Hindu 2018). This is another reason which prevents the cure of PPD through proper treatment. People are reluctant to admit the fact that they are facing some mental problems. The same kind of reluctance is there in the case of PPD also. They fear a kind of 'othering' from society and its roots can be traced back to the stigma toward a madness that existed in the middle ages. This tendency limits the possibility to take proper medical care. This can also be controlled by conducting awareness programs and thus normalizing mental problems and depression (Foucault 1988; Kuriakose et al. 2020; Jayarajan 2021).

Figure three and four brings out the seriousness of the situation. In Figure three, one can see that about 77.3% of participants faced the symptoms of PPD. This is proof of the problem faced by women after delivery. Figure four gives

the period for which they faced these difficulties. 19.2% faced symptoms of PPD up to one year after delivery/C-section. For 28.3% it lasted for six months, and for 24.2% it lasted up to one month. Only 28.3% experienced it for two weeks after childbirth (Kuriakose et al. 2020).

Figure 4: Pie chart showing how much time did the participants felt the symptoms

- Two to three weeks after delivery
- One month after delivery
- Lasted up to six months after delivery
- Lasted up to one year after delivery
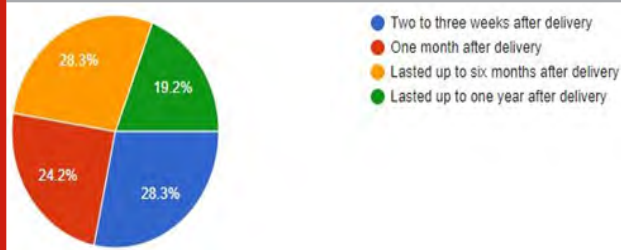
Figure 5: Pie chart showing the level of difficulty the participants faced in bonding with the baby

- Yes
- No
- Only for a short period of time
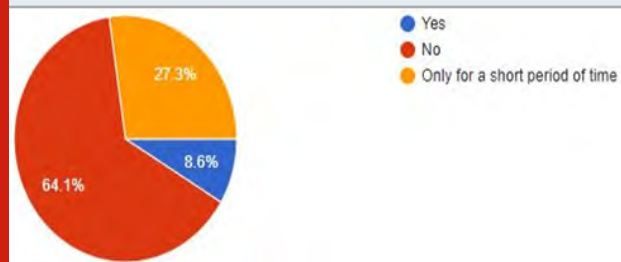
Figure 5 shows that 8.6% of the participants found it difficult to create a bond with the baby and 27.3% of them felt the same for a short period. This is a period that is highly dangerous and may lead up to harming a child and oneself. Recent news reports give evidence of this were mothers in their depressive state harmed babies and some violent acts ended up in their death (Jayarajan 2021). The results of the survey indicate that most of the participants have gone through severe Postpartum Depression and all of them needed medical help. But Figure 6 shows the real scenario of Kerala's treatment of a serious mental issue like PPD (Jayachandran 2021).

Figure 6: Pie chart showing how participants with PPD cope up with it

- By seeking medical help
- By seeking the help of a psychologist/psychiatrist
- With the help of family and friends
- By yourself
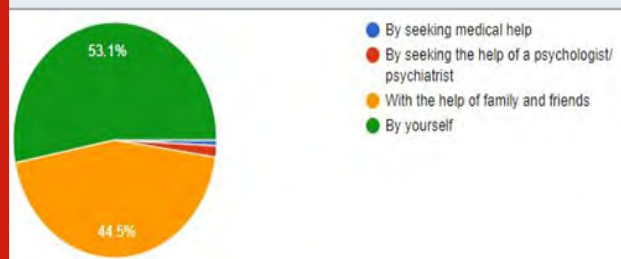
Even though most of the participants faced severe PPD-related problems only 0.8% of them sought medical help and only 1.6% of them were ready to seek the help of a psychologist or psychiatrist. This reveals the pathetic condition of Kerala concerning PPD. This situation can be changed by giving proper awareness about the issue. The collected data shows that only 7% of the participants had attended any awareness programs related to PPD. Most of them learned about this issue through social media. And some of them learned through articles and only 1.7% of them got informed through the newspapers. This shows that the government, health department, as well as the public, have to play a significant role in reducing the difficulties faced by mothers and newborns (Kuriakose et al. 2020).

The study, 'Prevalence of depression among middle-aged women in the rural area of Kerala', conducted by Archana P S, Soumitra Das and Sairu Philip et al., tries to determine the prevalence of depression among women aged 40 – 60 years in the rural area of Kerala using Patient Health Questionnaire 9 (PHQ – 9). The setting of the study is Ambalappuzha, a rural area in the district of Alappuzha. (Archana et al. 2017; Kuriakose et al. 2020). In contrast, this study tried to include young women from all over Kerala who gave birth in the last 10 years. Moreover, this study concentrates only on depression that comes as a result of pregnancy and/or delivery.

CONCLUSION

The findings of the present study quantify the awareness young mothers in Kerala have about PPD. As this study deals with a health-related issue which is related to the well-being of society, it has significance in the present scenario. This study prompts studies in the future that may lead to change in the current situation. Proper awareness about the issue can be given through newspapers, news channels, social media and other media. Constant active participation from the public and initiatives from the government is needed to improve the situation.

ACKNOWLEDGEMENTS

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BMI-For-Age Percentile as Criteria for Assessment of Obesity in Children and Adolescents

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ABSTRACT

The most useful population-level measure of overweight and obesity is BMI. This measure is same for men and women of all ages. But it should not be thought to be as final factor to calculate the same body fat percentage in different individuals. Various proposed reference values for overweight and obesity exist for BMI, but the combined effect of BMI and BMI for age percentile recommendations is scarce. A total 2048 children participated in the epidemiological survey. According to Body Mass Index in the study, 66.26% subjects were found to be malnourished in the total population. The degree of obesity had been observed as Pre-obese 3.81% and Obese-I 3.81% and Obese-II were 0.88%. Pre-obese, obese-I and obese-II boys were more than the girls. According to BMI-for-age percentile, overall, 6.20% of children and adolescents were at risk of overweight and 4.39% were overweight during health survey while 24.80% are underweight.

KEY WORDS: BMI, BMI-FOR-AGE PERCENTILE, OBESITY, OVERWEIGHT.

INTRODUCTION

Overweight and obese children can develop obesity in adulthood and can develop diabetes, hypertension and cardiovascular diseases at an early age (Krushnapriya et al 2015). The highest prevalence rates of childhood obesity have been observed in developed countries; however, its prevalence is increasing in developing countries as well (Popkin and Doak, 1998). Females are more likely to be obese as compared to males, owing to inherent hormonal differences (Gupta, 2009). It is difficult to create one parameter for the measurement of overweight and obesity in children and adolescents because there are number of physiological changes in children and adolescents.

Depending on the age, different methods to measure a body's healthy weight are available. BMI fails to distinguish between fat and fat-free mass (muscle and bone) and may exaggerate obesity in large muscular children. Body mass index (BMI) is a recommended index of obesity by World Health Organization (1996) and is related to various disease risks (Stevens et al, 1998). BMI is the most useful population-level measure of overweight and obesity as it is same for men and women, and for adults of all ages. However, it should not be considered as final parameter to calculate the same body

fat percentage in different individuals. Various proposed reference values for overweight and obesity exist for BMI, but the combined effect of BMI and BMI for age percentile recommendations are scarce. Therefore, the primary aim of this study is to compare the validity of BMI and BMI for age percentile as diagnostic tests for sample of 10-19 years old children and adolescents.

MATERIAL AND METHODS

A total 2048 children participated in the epidemiological survey. Children who had participated in the study also underwent the health examination. There were numerous students who remained absent on the test date and on all subsequent make-up sessions. Many parents requested to excuse their children from the test. For interested children, consent form was signed by the parents before starting the survey work. Children those completed all steps of the survey were included and those who missed even a single step excluded. The World Health Organization, U.S. Centers for Disease Control and Prevention and International Obesity Task Force provide separate definitions of overweight and obesity in children and adolescent. Anthropometric measurements including height, weight, and waist and hip circumferences were taken to explore the incidence of obesity and the various factors associated with it.

Body Mass Index (BMI): This measure was used to assess body weight relative to height. Body mass index is a useful

tool in both clinical and public health practice for assessing nutritional status of the population.

Underweight	BMI less than 18.5
Ideal	BMI 18.5-22.9
Overweight	BMI 23-24.9
Obese – should lose weight	BMI 25.0-29.9
Very obese – lose weight now	BMI greater than 30.0

BMI-for-Age Percentile: The Centers for Disease Control and Prevention (CDC),2000 suggested the levels of concern for children based on the BMI-for-age percentile as follows:-

- Below the 5th percentile : **Underweight**
- 5th percentile < 85th percentile : **Healthy weight**
- 85th percentile to < 95th percentile : **At risk of overweight**
- 95th percentile and above : **Overweight**

A total 2048 children had participated in the epidemiological survey. They had also undergone the health examination. There were numerous students who remained absent on the test date and on all subsequent make-up sessions. Many parents requested to excuse their children from the test. For interested children, a consent form was signed by the parents before starting the survey work. Children those completed all steps of the survey were included and those who missed even a single step were excluded. Anthropometric measurements including height, weight and waist and hip circumferences were taken to explore the incidence of obesity and the various factors associated with obesity.

These measures were used to assess body weight relative to height. Body Mass Index is a useful tool in both clinical

and public health practice for assessing nutritional status of the population. Underweight: BMI less than 18.5; Ideal BMI: 18.5-22.9; Overweight: BMI 23-24.9; Obese: BMI 25.0-29.9; Very obese: BMI greater than 30.0.

The Centers for Disease Control and Prevention (CDC) 2000 suggested the levels of concern for children based on the BMI-For-Age Percentile as Below the 5th percentile: Underweight; 5th percentile < 85th percentile: Healthy weight; 85th percentile to < 95th percentile: At risk of overweight; 95th percentile and above : Overweight. BMI allows comparison between children of the same sex and age (Connor and Arif ; 2021).

RESULTS AND DISCUSSION

A total 2048 children participated in the epidemiological survey. Out of these 2048 children, 1017 were from urban population and 1031 were from rural population. According to body mass index in the study, 66.26% subjects were found to be malnourished in the total population. The degree of obesity had been observed as Pre-obese 3.81% and Obese-I 3.81% and Obese-II were 0.88%. (Table-1Graph-1).

According to BMI-for-age percentile, overall, 6.20% of children and adolescents were at risk of overweight and 4.39% were overweight during health survey while 24.80% are underweight.
Overweight children were highest among urban (6.39%) than rural (2.42%). (Table-2 Graph-2).

Obesity and overweight result from an imbalance in the energy expenditure equation such that either food intake is in excess; energy expenditure is deficit or both. Obesity presently has been properly documented as an ailment in its own right which is preventable through changes in life style especially diet. Obesity is a major determinant of many non-communicable diseases and induces diabetes mellitus and coronary heart disease.

Table 1. Prevalence of overweight and obesity according to BMI Categories										
Group/Sub Group	Below 18.5		18.5-22.9		23.0-24.9		25.0-29.9		Above 30	
	N	%	N	%	N	%	N	%	N	%
All Data	1357	66.26	517	25.24	78	3.81	78	3.81	18	0.88

Figure 1

Prevalence of Obesity according to BMI Categories

<18.5 66%
18.5-22.9 25%
23.0-24.9 4%
25.0-29.9 4%
>30 1%

Physiologically, the body weight is constantly changing. Obesity is the result of excessive expansion of adipose tissue mass. This disorder is often accompanied by abnormalities in systemic carbohydrate and lipid metabolism and the secretion and action of insulin alterations thought to reflect diabetogenic effect of obesity. There remain questions about the best diagnostic criteria for this age group.

According to Dietz and Bellizze, 1999, overweight, during childhood and adolescence, is characterized by a body mass index for age (BMI/age) above the 85th percentile and obesity by a BMI/age above the 95th percentile, based on the National Health and Nutrition Examination Survey

(NHANES II-III) standards. Other authors say it is possible to adopt absolute BMI limits for specific age groups, during the growth period, without considerably compromising diagnostic specificity or sensitivity (Monteiro et al, 1999).

An expert committee in 1997 declared BMI for age to be the principal measure of obesity in childhood. The adoption of regional BMI standards has also been proposed (Anjos et al, 1998).

Table 2. Prevalence of overweight and obesity according to BMI for age percentile Categories

Group/Sub Group	Under Wt.		Healthy Wt		Risk Ov.Wt		Over Wt.	
	N	%	N	%	N	%	N	%
All Data	508	24.80	1323	64.60	127	6.20	90	4.39

Figure 2

	<18.5	18.5-22.9	23.0-24.9	25.0-29.9	>30
Male	64.69	25.37	4.51	4.44	0.99
Female	69.79	24.96	2.23	2.38	0.64

maturation rates, a modified BMI compares heights and weights against growth charts which also consider gender. This index is known as the BMI-for-age percentile (for children 2-19 years of age), and reflects BMI as compared with boys and girls of the same age. Because of that increase, the American Diabetes Association and the American Academy of Pediatrics recommend routine screening of children at higher risk for type 2 diabetes. It is essential to identify the best anthropometric index in any population to predict chronic disease risk.

In the present study, according to BMI-for-age percentile, 24.80% children were underweight in of 10-19 age groups. In the present study 6.20% of children and adolescents were at risk of overweight and 4.39% were overweight during health survey. The results of the present study also shows that it is appropriate to choose BMI / age, based on the international standards, as a diagnostic indicator for overweight and obese children.

Diagnostic criteria should be simple, low cost, reproducible and reliable and offer elevated sensitivity and specificity, thus reducing false positive and negative diagnoses. Based on a pool of studies of BMI profiles by age in a number of different countries, including Brazil, proposed limits for overweight and obesity to be applied to the 2 to 20 age group internationally (Cole et al., 2000).

In this study, the prevalence of obesity was more in boys than in girls. It is possible that the Western feminine self-image and fear of obesity were more marked in girls than in boys. Girls as young as 5 years of age care more about their self-image and associate it to weight status. The same has been reported in other studies where boys had higher prevalence of obesity than girls at all ages (Brown et al, 2002 and Huang et al, 2003).

WHO in 1996 stated that BMI values are not dependent on age or gender. But, they may not narrate equally to levels of obesity in diverse populations (certain racial groups in Africa and Asia) as a result of differing body-proportions. Also, the BMI index may not accurately reflect the fatness levels of people with builds falling far outside the average. As mentioned earlier active persons like sportsmen and sportswomen, will tend to have BMI levels of "overweight" or even 'obese', due to their higher levels of muscle mass. BMI categories also fail to account for varying proportions of fat, bone, cartilage, water weight and more.

Centers for Disease Control and Prevention (CDC), 2000 stated that "overweight" is defined differently for children and teenagers than for adults. Due to growth and differing

Worldwide one in 10 children, aged 5-17 year old, is overweight, a total of 155 million, of which around 30-45 million are obese (Lobstein et al, 2004). The prevalence rate of overweight in children under 5 years of age in developing countries is 3.3% or 17.6 million (Executive Board, 2001). Countries with the highest prevalence of overweight are located mainly in the Middle East, North Africa, and Latin America (de Onis et al, 2000). In the United States, the prevalence of childhood overweight tripled between 1980 and 2000 (Ogden et al, 2002). In Australia, the prevalence of childhood overweight almost doubled between 1985 and 1995 (Magarey et al, 2001). In Europe there are 14 million children who are overweight and 3 million of them are obese. In 2002 nearly a quarter of children within the European Union were overweight, higher than the predicted peak for 2010. (Lobstein et al, 2004). In India prevalence of overweight/obesity was:

- Delhi(North) Overweight, 22%; obesity 6%.(Sharma et al, 2007);
- Uttar Pradesh (North) Obesity, 7.6% (Gupta et al, 1999).

In the present study, as per the BMI for age percentile, 6.20% of children were at risk of overweight and 4.39% were overweight which less than the other studies. But the necessary steps must be taken at this stage to prevent the

agony of obesity and its related complications. Many factors were found to be responsible for the obesity epidemic in children such as increased consumption of energy-dense food, decreasing physical activity and the increasingly easy accessibility of food which can be controlled to prevent it.

Worldwide the urbanization of society is reducing children's physical activity opportunities (Hossain et al, 2007). Increasing calorie consumption is not matched by increased levels of physical activity in children; in fact, children globally are becoming more physically inactive (Anderson and Butcher, 2006). Schools are offering a less healthy environment where children are not protected from bad diets and not encouraged for physically active lifestyles (Story et al, 2006). Because of the link between food advertising and childhood obesity authorities in the UK have banned the advertising of high fat, salt and sugar products in or around programmes made for children (www.ofcom.org.uk/consult/condocs/foodads).

The growing epidemic of childhood overweight and obesity is a major public health concern. Currently 15% of US youth are overweight, a prevalence nearly twice as high in children and three times as high in adolescents compared to 1980 prevalence rates (US Department of Health and Human Services, 2001). Almost two-thirds (60%) of overweight children have at least one cardiovascular risk factor (e.g., hypertension, hyperlipidemia) and the prevalence of type 2 diabetes mellitus is increasing in youth (American Diabetes Association,2000).These trends may seriously compromise the future health and productivity of the US population and add to health care costs.

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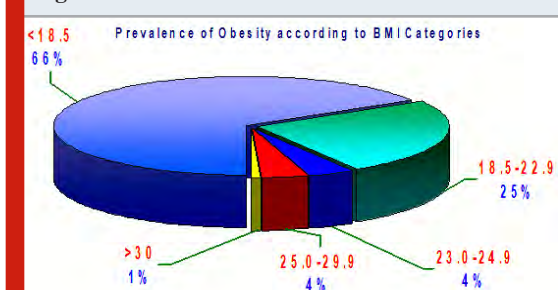
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Obesity and overweight result from an imbalance in the energy expenditure equation such that either food intake is in excess; energy expenditure is deficit or both. Obesity presently has been properly documented as an ailment in its own right which is preventable through changes in life style especially diet. Obesity is a major determinant of many non-communicable diseases and induces diabetes mellitus and coronary heart disease.

Table 1a- Prevalence of overweight and obesity according to BMI Categories

Group/Sub Group	Below 18.5		18.5-22.9		23.0-24.9		25.0-29.9		Above 30	
	N	%	N	%	N	%	N	%	N	%
All Data	1357	66.26	517	25.24	78	3.81	78	3.81	18	0.88

Table 2a. Prevalence of overweight and obesity according to BMI-For-Age Percentile Categories

Group/Sub Group	Underweight		Healthy Weight		Risk Overweight		Overweight	
	N	%	N	%	N	%	N	%
All Data	508	24.80	1323	64.60	127	6.20	90	4.39

Physiologically, the body weight is constantly changing. Obesity is the result of excessive expansion of adipose tissue mass. This disorder is often accompanied by abnormalities in systemic carbohydrate and lipid metabolism and the secretion and action of insulin alterations thought to reflect diabetogenic effect of obesity. There remain questions about the best diagnostic criteria for this age group.

According to Dietz and Bellizze, 1999, overweight, during childhood and adolescence, is characterized by a body mass index for age (BMI/age) above the 85th percentile and obesity by a BMI/age above the 95th percentile, based on the National Hea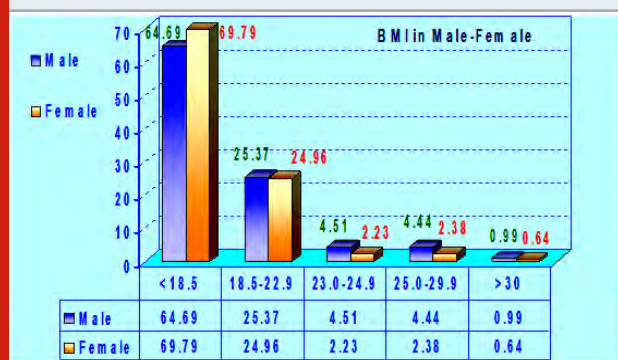lth and Nutrition Examination Survey (NHANES II-III) standards. Other authors say it is possible to adopt absolute BMI limits for specific age groups, during the growth period, without considerably compromising diagnostic specificity or sensitivity (Monteiro et al, 1999). An expert committee in 1997 declared BMI for age to be the principal measure of obesity in childhood. The adoption of regional BMI standards has also been proposed (Anjos et al, 1998).

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Centers for Disease Control and Prevention (CDC), 2000 stated that "overweight" is defined differently for children and teenagers than for adults. Due to growth and differing maturation rates, a modified BMI compares heights and weights against growth charts which also consider gender. This index is known as the BMI-For-Age Percentile (for children 2-19 years of age), and reflects BMI as compared with boys and girls of the same age. Because of that increase, the American Diabetes Association and the American Academy of Pediatrics recommend routine screening of children at higher risk for Type-2 diabetes. It is essential to identify the best anthropometric index in any population to predict chronic disease risk.

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Uttar Pradesh (North) Obesity, 7.6% (Gupta et al, 1999). In the present study, as per the BMI for age percentile, 6.20% of children were at risk of overweight and 4.39% were overweight which less than the other studies. But the necessary steps must be taken at this stage to prevent the agony of obesity and its related complications. Many factors were found to be responsible for the obesity epidemic in children such as increased consumption of energy-dense food, decreasing physical activity and the increasingly easy accessibility of food which can be controlled to prevent it. Overweight and obese children can develop obesity in adulthood and can develop diabetes, hypertension and cardiovascular diseases at an early age (Krushnapriya et al 2015).

Worldwide the urbanization of society is reducing children's physical activity opportunities (Hossain et al, 2007). Obesity in childhood is the most challenging public health issue in the twenty-first century. It has emerged as a pandemic health problem worldwide (Palanikumar and Sunil , 2021)
Data from 2015 demonstrates that approximately 108 million children and 604 million adults globally had a BMI of 30 to classify them as obese; signifies an increase in the prevalence of obesity in almost all countries since 1980 and a doubling in prevalence in 70 countries during that period (NCD Risk Factor Collaboration, 2016).

Increasing calorie consumption is not matched by increased levels of physical activity in children; in fact, children globally are becoming more physically inactive (Anderson and Butcher, 2006). Schools are offering a less healthy environment where children are not protected from bad diets and not encouraged for physically active lifestyles (Story et al, 2006). Because of the link between food advertising and childhood obesity authorities in the UK have banned the advertising of high fat, salt and sugar products in or around programmes made for children (www.ofcom.org.uk/consult/condocs/foodads).

The growing epidemic of childhood overweight and obesity is a major public health concern. Currently 15% of US youth are overweight, a prevalence nearly twice as high in children and three times as high in adolescents compared to 1980 prevalence rates (US Department of Health and Human Services,2001). Almost two-thirds (60%) of overweight children have at least one cardiovascular risk factor (e.g., hypertension, hyperlipidemia) and the prevalence of type 2 diabetes mellitus is increasing in youth (American Diabetes Association,2000).These trends may seriously compromise the future health and productivity of the US population and add to health care costs. A multidisciplinary approach to promote dietary and physical activity changes in the entire family should be used for the treatment and prevention of overweight and obesity in early childhood (Karel et al 2020).

CONCLUSION

BMI categories also fail to account for varying proportions of fat, bone, cartilage, water weight and more. BMI index may not accurately reflect the fatness levels of people with builds falling far outside the average. The boys and girls of the same age have differing growth and maturation rates. Thus a modified BMI compares heights and weights against growth charts which also consider gender specific. For children 2-19 years of age, BMI-for-age percentile is the measure and it reflects BMI as compared with same aged boys and girls. Hence it is essential to identify the best anthropometric index in any population to predict chronic disease risk.

ACKNOWLEDGEMENTS

Conflict of Interests: I declare no conflicts of interest to disclose. This manuscript has not been submitted to, nor is under review at, another journal. The authors have no affiliation with any organization with a direct or indirect financial interest in the subject matter discussed in the manuscript.

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Adsorption of Phenol And Resorcinol on Parthenium Based Activated Carbon (PAC) in Basal Salt Medium: Equilibrium and Kinetics

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ABSTRACT

The adsorption studies were carried out for the removal of Phenol and Resorcinol on *Parthenium* Based Activated Carbon (PAC) at 301 K and neutral pH ~7. To determine the adsorption equilibrium isotherms, the experiments were performed at the initial concentration of adsorbates with adsorbent doses from 1 g/l to 10 g/l. The experimental data were fitted to six isothermal models, and a nonlinear least square method has been used to estimate model parameters. It has been observed that for adsorption on PAC, Freundlich model was best fitted for the study and data was more closely related for the removal of both the pollutants from industrial wastewater. In this study, the adsorption kinetics were also studied in three distinct phases-rapid, medium & amp: slow for the time period till 12 hours. The parameter values found in this study shall be useful in designing and activated carbon adsorber for treatment of industrial wastewater at large scale. The present work shows that Parthenium based activated carbon (PAC) was an effective method for the adsorption of phenol and resorcinol and p-cresol from aqueous solution. The adsorption of phenol and its derivatives are in the following order: Phenol is adsorbed in maximum amount Resorcinol is comparatively adsorbed lesser than phenol.

KEY WORDS: PHENOL, RESORCINOL, ACTIVATED CARBON, ADSORPTION EQUILIBRIUM ISOTHERM, ADSORPTION KINETICS, ADSORPTION STUDIES.

INTRODUCTION

Adsorption is considered as one of the most preferred and cost-effective methods for treatment of waste water contaminant constituents such Phenol and resorcinol the pollution of industrial wastewater, which can reduce the concentration of pollutants discharged into the plant (Agency for Toxic Substances and Disease Registry (ATSDR), 1990; Daifullah and Girgis, 1998; Mourão, Carrott and Ribeiro Carrott, 2006). Naturally, this will inevitably lead to a decline in water quality, especially causing scarcity of drinking water. These organic compounds in water pollution are mainly caused by heavy industrialization (Tanada et al., 1990; Wu and Tseng, 2006) most scientific research is moving towards an innovative process of implementing environmentally friendly treatments. These organic pollutants are non-biodegradable or poorly biodegradable. Like most pollutants, phenol is harmful, and it has been found in the wastewater of many industrial units (D'Ari and Barker, 1985; Rathi and Puranik, 2002, Mourão and Ribeiro Carrott, 2006).

Phenol has an unpleasant odor and taste and is toxic to aquatic organisms, plants, and humans at concentrations of 5 μgL-1 Long-term ingestion of phenol at concentrations between 10 and 240 mg L-1 can cause oral irritation, blurred vision, and diarrhea (World Health Organization (WHO), 1971; Fawell and Hunt, 1988). Therefore, this pollutant often appears in rivers, seawater, industrial waste, urban wastewater, and even groundwater. Among all the methods of treatment of wastewater loaded with organic compounds, the adsorption of phenol on solid supports such as activated carbon, clay, and transition metal oxides allows their removal from water with a high removal rate without the addition of chemicals.

Parthenium hysterophorus (Linn), sometimes known as carrot grass, is an invasive species that was introduced to India through a planned import from the United States (PAC). PAC is made by chemical activation using concentrated H2SO4. The produced activated carbon was examined and determined to be a useful adsorbent (Singh et al., 2008). This

paper highlights the use of parthenium activated carbon's adsorption characteristics for the elimination of pollutants like phenol and others. The work being presented aims to investigate the adsorption of phenol and resorcinol on activated carbon made of parthenium.

MATERIAL AND METHODS

Preparation of adsorbent: Activated carbon act as an effective adsorbent material because of its highly porous nature and provides a large surface area for adsorption (Kadirvelu, Sivasankari, et al., 2002; Singh et al., 2008). There are two preferred ways for the preparation of activated carbon first one is called as physical method and a second method called as chemical method. During the preparation of activated carbon there are several parameters which is required and needed to keep in mind such as temperature and heat duration and pressure. Dried activated carbon is pulverized and sieved to get the particles then the next step for the process of preparation of activated carbon is carbonization also called as pyrolysis which is carried out under heat treatment in the absence of air, Dried activated carbon is then pulverized and sieved to get the particles This leads to the initialization of porosity formation in activated carbons (Ajmal et al., 2006; Singh et al., 2008).

Characterisation of Activated carbon: A high porosity and this microporosity enable great adsorption and selectivity, making activated carbon the most effective adsorbate. Both PAC and GAC forms of activated carbon are offered. Basal zone, heterogeneous groups (mostly oxygen-containing surface groups), and inorganic ash are the typical three zones that make up the AC surface (Kumar et al 2003). Adsorption is only permitted in the mesoporous region when the adsorbent possesses liquid

adsorbate, leading to a greater parthenium activated carbon concentration in the ash. The basal zone contains the majority of the adsorption sites for aromatic chemicals (Li et al., 2002). The chemical properties of the carbon surface are defined by heterogeneous groups, who are more active (Kadirvelu, Sivasankari, et al., 2002). Physical, chemical, and electrochemical processes can change the nature of the surface groups (Pandey & Singh, 2014).

Adsorbate solution: For the preparation of synthetic adsorbate solution of phenol and resorcinol. Phenol and Resorcinol were used each with known concentration of 500 mg/l. And for the stock solution preparation and storage of (1%w/v) for both 10g of phenol and resorcinol were dissolved in 90 ml of water separately to make up the volume to 100 ml each. This solution is stored in cool and well- ventilated area and same way phenol is stored. For the experiment, the solution of desired concentrations was prepared by successive dilution of the stock.

Determination of phenol and resorcinol in a solution : The determination of phenol and resorcinol in a solution was done with the help of UV-vis spectrophotometer onto carbonaceous adsorbent at maximum wavelength of 217nm for phenol adsorption and 273nm wavelength for resorcinol adsorption (model Lambada 35; Perkin Elmer, Massachusetts 02451, U.S.A) (Kumar, Kumar and Kumar, 2003; Ajmal et al., 2006).

Experiment procedure: The experimental studies were carried out to observe the adsorption capacity. The process was carried out as follows: stems of parthenium plants were collected from fellow lands near Amity University Noida campus and then the batch experiment was carried out for the study of adsorption potential of *Parthenium* activated carbon.

S. No.	Properties	Parthenium based activated carbon PAC
	Table 1. Major Characteristics of the PAC	
1	BET surface area, m^2/g	350
2	pH (1% solution)	6.8
3	Moisture content (%)	2.6
4	Ash content (%)	7.2
5	Bulk density (g/ml)	0.62
6	Average particle size (mm)	0.512
7	Average pore diameter (Ao)	36.74
8	Water soluble matter (%)	3.2
9	Acid soluble matter (%)	3.8

In this adsorption experiment, 10g of resorcinol aqueous solution sample (whose initial concentration and pH were known) was poured to a known adsorbent dose of 100ml in a 500ml stoppered conical flask and shaken a bit then for a constant time period of 24 hr and at varied temperature adsorption was studied with the help of different isotherm models, where adsorption kinetics were studied by keeping

the temperature constant at 301K, and according to the residual concentration of resorcinol and phenol their percent removal were calculated (Kumar et al 2003; Singh et al., 2008).

The waste effluents concentration varies at high range from 1gm to 1000 gm/l and sometimes more, so the experimental

studies the concentration resorcinol taken was 500 gm/l. The adsorbent dose concentrations in each 500 ml flask used ranges from 1gm then 2gm then 3gm and so on to 10 gm at an initial temperature of 301K with initially maintained at neutral pH 7. The samples were taken at some specific time intervals and filtered this study were carried out on Parthenium activated carbon. The quantity of adsorbed phenol and resorcinol by 1 gram of adsorbent, qt (mg/g) at any given time (t) was calculated by (Pandey and Singh, 2014).

$$qt = [(Co − Ct)\ V]/\ m \qquad (1)$$

where Co is the concentration of phenol and resorcinol at the initial time while Ct is the concentration at time t, respectively, V is the volume of adsorbate solution, and m is the mass of adsorbent dose used in the run. At equilibrium condition, the amount of adsorption per gm of adsorbent, qe (mg/g), was calculated by:

$$qe=[(Co−Ce)V]m \qquad (2)$$

Adsorption equilibrium isotherms: For the study of adsorption different equilibrium isotherms are used. This equilibrium is generally expressed in terms of concentration as of adsorbate (C_e) which is industrial wastewater in liquid state and adsorbent (q_e) on which the process of adsorption occurs at equilibrium conditions which are keeping temperature and pressure constant. These isotherm models help us to study about interaction between adsorbate and adsorbent. In orders to corelate the data six different isotherm models are used which are: Freundlich isotherm model, Langmuir isotherm model, Radke isotherm model, Toth isotherm model, Redlich isotherm models and Fritz isotherm models (Abuzaid & Nakhla, 1996, Li et al., 2001; Nouri, Haghseresht and Lu, 2002).

The Langmuir model equation is expressed as:

$$qe = (Q_0 bC_e)/1 + bC_e \qquad (3)$$

here b is the equilibrium adsorption constant. It is a measurement of adsorption affinity and is associated with the adsorption free energy. The quantity of phenol or resorcinol required to completely create a monolayer on the surface of a gram of activated carbon is known as Q0, which represents the adsorption capacity (Abuzaid and Nakhla, 1996). This model assumes that all of the activated carbon's surface has the same level of activity. Moreover, it is constrained to a particular concentration range.

The Freundlich, an empirical model, can be expressed in the equational form as:

$$qe = K_F Ce^{1/n} \qquad (4)$$

K_F is the measure of the adsorption capacity and n is a measure of the adsorption intensity This model is applicable to the system involving heterogeneous surface and organic compound (e.g. activated carbon—phenolic compound). The Radke–Prausnitz, Toth, and Fritz–Schlunder models

are not common in use to describe adsorption of phenolic compounds on activated carbon.

Kinetic study of adsorption: Following the rate of adsorption and ultimate equilibrium conditions, the fluctuation in the concentration of unadsorbed or remaining phenol and resorcinol in the solution is almost minimal. Pseudo-first order and pseudo-second order kinetic models are the most used ones for phenolic chemical adsorption on activated carbon (Moreno-Castilla et al., 1995; Abuzaid and Nakhla, 1996). The ability of these two categories of kinetic models to predict the adsorption of phenol and resorcinol on PAC is investigated in this work.

As written, the pseudo-first order kinetic model is:

$$Dqt/dt = k1(qe − qt) \qquad (5)$$

where k1 is pseudo-first order adsorption rate constant (min−1). The model with pseudo-second order kinetics can be expressed as:

$$dqt/dt = k2(qe − qt\ )^2 \qquad (6)$$

where k2 is the pseudo-second order adsorption rate constant (g mg−1 min−1). When it is integrated with boundary condition, qt(t = 0) = 0 becomes:

$$ln\ (qe − qt) = ln\ qe − k_1 t \qquad (7)$$

Here, qe and qt represents the amounts of phenolic compounds studied which are phenol and resorcinol adsorbed per unit mass of adsorbent at equilibrium and at time t, respectively, and can be calculated by using the above equations. Based on the above equations order of kinetics is calculated which shows which order of reaction is followed by the studied interactions of adsorption (Moreno-Castilla et al., 1995; Abuzaid and Nakhla, 1996).

RESULTS AND DISCUSSION

Effect of adsorbent's dosage: In order to know the effect of dose of adsorbent on the adsorption of resorcinol and phenol a number of adsorption experiments were carried out with varying amounts of PAC (1–10 g/l) were contacted with *phenol* and *resorcinol* Solutions. The analysis of this experimental series demonstrates that a greater adsorbent dosage increases the amount of available active sites for adsorption, leading to an increase in removal efficiency (Lata, Garg and Gupta, 2007). The % elimination changes very little incrementally as the dosage of PAC is increased further. Adsorbent dosage is not very effective after 99% removal, and the majority of active sites are left vacant. This causes a decrease in the amount of phenol and resorcinol adsorption decreases with increase in adsorbent dose. This research approximates the ideal adsorbent dosage needed to remove phenol and resorcinol under current operating circumstances when employing activated carbons (Kumar, Kumar and Kumar, 2003).

It takes 10 g/l of PAC dosage. The ideal dose of adsorbent for PAC is then required, which is 10 mg/l, to treat 500 mg/l

of phenol and resorcinol solution since the removal rate is so low. The final percentage of removal is represented in the below mentioned data the final removal percentage of phenol is estimated as 93.6% by 10g/l PAC dose, and the final percentage removal of resorcinol is estimated as 87.2% by 10g/l PAC dose (Pandey and Singh, 2014).

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Table 2. Deviation Percentage for Phenol and Resorcinol in different isotherm models

	% Deviation (R^2)	
Isotherm Models	**Phenol**	**Resorcinol**
Freundlich $qe = KFC^{1/n}$	13.5	11.64
Langmuir $qe= (q_o bCe)/1+bCe$	50.75	11.09
Redlich–Peterson $qe= (K1Ce)/1+K2C^b e$	13.45	11.67
Toth $qe= (q^\infty eCe)/[a+Cne]^{1/n}$	14.17	11.92
Radke–Prausnitz $1/qe=1/KC_e+1/KCe^{1/n}$	14.93	9.27
Fritz–Schlunder $qe= \alpha1C^\beta_e 1/1+_{\alpha2}Ce\beta_2$	14.45	11.62

Isotherm studies: By creating a dynamic phase equilibrium between the solid surface of the adsorbent and the liquid adsorbate, equilibrium is studied. This equilibrium is often stated in terms of the adsorbate's concentration and the amount of adsorbate that has been put onto the adsorbent. Adsorbate loading on the adsorbent is shown as (qe) in adsorption equilibrium isotherms, and the liquid phase concentration of adsorbate is shown as (Ce) at equilibrium while maintaining temperature constant. Adsorption isotherm, then, depicts how adsorbate and adsorbent interact. Many models used to characterise the experimental isotherm data are empirical equations based on the form of the isotherm curve to correlate the isotherm data of this sort, six isotherm models are selected (Li et al., 2002).

The data mentioned below shows a comparative result of all the isotherm models studies. The data mentioned below shows that Freundlich isotherm model is best suited for Phenol PAC system, p-cresol PAC (Moreno-Castilla et al., 1995) system and Resorcinol PAC system whereas Langmuir isotherm model is least for Phenol PAC system and Resorcinol PAC system. To represent the whole concentration range with a single isotherm model, six isotherm models were tested. The correlation coefficient and greatest variation from the anticipated value served as the selection criteria for the isotherm model (Kadirvelu, Sivasankari, et al., 2002).

On this basis, Langmuir model could not fit the data for phenol with the maximum deviation percentage of 50.75 %. but it suited for resorcinol with the maximum deviation percentage of 11.09 %. The results of the Toth and Radke-Prausnitz are nearly similar. The only four parameter isotherm model studied is by Fritz Schlunder. This model gave the similar result to three parameter models with maximum deviation percentage for phenol of 14.45% and for resorcinol of 11.62%.

Kinetic study: Kinetics studies were performed in simple glass flask experiments. The calculated amounts of adsorbate were poured into 500 ml stoppered conical glass flask and activated carbon at a range of 1mg/l to 10mg/l variably was added to each flask. These flasks were kept untouched at 301K. The pre-determined amount of stock solution was added to each flask to obtain desired initial phenol and resorcinol concentration with working solution. The flasks were taken out and filtered one at a time at certain time intervals (Kadirvelu, Sivasankari, et al., 2002). The filtrate was used for phenol analysis. These experiments were done for 24 hours. There was very gradual decrease in concentration after 24 hours keeping the adsorbent dosage at 10 g/l, the temperature at 301 K, and the pH of the solution constant at 7. Using Kinetic equation, the values of (qt) corresponding to each expected concentration of phenol and resorcinol at various time intervals were determined.

According to the experiments carried on each set of trials, each activated carbon and phenolic interaction, which is a phenol-PAC system, followed a different pace of reaction. Pseudo first order kinetics was followed by pseudo second order kinetics and the resorcinol-PAC system. The estimation of time for the maximum removal of phenolic compounds were with the help of studying diffusion coefficient as the diffusion coefficient determines the time it takes a solute to diffuse a given distance in a medium (Pandey and Singh, 2014). The maximum time taken for phenol-PAC system was 4hrs with the diffusion coefficient $R^2= 0.975\%$, whereas for resorcinol-PAC system it estimated to be 10hrs, with diffusion coefficient $R^2= 0.9926\%$.

Effect of Temperature: When the temperature rises, phenol and its derivatives' ability to adsorb substances increases. It was well understood that rising temperature would speed up the rate of adsorbate molecule diffusion through the exterior boundary layer and into the pores of the adsorbent particles, resulting in a reduction in the solution's viscosity (Li et al., 2002). The equilibrium capacity of the adsorbate

for a certain adsorbate will also fluctuate with temperature. The following equations were used to determine the thermodynamic parameters such as free energy change (G), entropy (S) and enthalpy change (H):

$$\Delta G^0 = - R \, T \ln Kc$$

$$\Delta G = \Delta H - (T\Delta S)$$

$$Ln \, Kc = \Delta H/RT + \Delta S/R$$

$$\Delta G = + Ve$$

i.e. $\Delta G \propto 1/Temperature$

Kc = equilibrium constant.

Kc = Cas/Ce

Cas = equilibrium const. (mg/L) of adsorbate on the solution.

Ce = equilibrium const. (mg/L) of adsorbate on the solution.

This relation with free energy changes along with temperature pointed towards the direct proportionality of adsorption capacity with temperature. The positive value of (ΔS) shows the affinity of PAC for phenol and it's derivatives (Phenol and Resorcinol). During the adsorption process, some structural changes occur that result in increased randomness at the interface of the solid-solution.

Effect of pH of the solution: The surface charge distribution, which results from the interaction of the surface with adsorbate ions, plays a significant role in the interaction between the adsorbent and the adsorbate. This demonstrates how pH has an impact on the adsorbent's surface charge and the amount of ionisation of the adsorbate, which has an impact on the adsorption phenomena. The initial pH of an aqueous solution, which is defined as the pH at which the surface of activated carbon has zero charge and is thus neutral (Moreno-Castilla et al., 1995; Lata, Garg and Gupta, 2007), is one of the parameters that is frequently examined. As the pH rises over its original pH, the surface of the activated carbon becomes negatively charged and the positive charges are diffused, allowing the adsorption to take place.

Table 3. Effect of Temperature on Phenol, p-Cresol and Resorcinol

S.No.	Adsorbate	Temperature (0K)	ΔG (KJ/mole)	ΔH (KJ/mole)	ΔS (J.K-1 min-1)	R2
1	Phenol	288	2.42	13.24	40.86	0.98
		298	2.04			
		308	1.68			
		318	0.82			
2	p-Cresol	288	3.18	14.02	30.62	0.97
		298	2.67			
		308	1.82			
		318	1.02			
3	Resorcinol	288	4.06	14.46	22.46	0.99
		298	3.46			
		308	2.72			
		318	1.88			

The surface of the activated carbon becomes negatively charged as the pH drops below the starting pH, and the positive ions in the solution are attracted to the surface via electrostatic attraction. Diffusive is now used to help in the adsorption process. The fluctuation in pH has little impact on the effects of pH on the adsorption of phenol and its derivatives. Adsorption isotherm were studies at different pH values. The observed results do not show significant change in the adsorption process at different pH values (AYRANCI and DUMAN, 2005). At low and high pH, their hydrophilic behaviour increases and makes them more surface-active (pH 4 – 9) studies. Preferred pH ~ 7 neutral due to significance difference in adsorption of pollutant.

Effect of Particle Size: The effect of particle size for a const. weight of PAC on the adsorption of Phenol and its derivatives were carried out. The result reveals that the adsorption is higher on smaller particle size of the adsorbent (Kadirvelu, Senthilkumar, et al., 2002; Rajeshwarisivaraj and Subburam, 2002). The reason for the same is that adsorption is a surface phenomenal, so the greater surface area for adsorption lead to the greater accessibility to pores and hence increased the uptake of smaller particles.

CONCLUSION

The present work shows that Parthenium based activated carbon (PAC) was an effective method for the adsorption of phenol and resorcinol and p-cresol from aqueous solution. The adsorption of phenol and its derivatives are in the following order. Shows the best adsorption of phenol then resorcinol. The generalised Freundlich model

was demonstrated to provide the best corelation for the adsorption for the adsorption of Phenol, Resorcinol and P-cresol onto PAC. The Langmuir isotherm model was found the least corelative for the adsorption studies on the PAC-phenol and PAC- Resorcinol system. As a result, it was stated that the phenol-PAC system, P-cresol-PAC system, and resorcinol-PAC system may all be employed with the Freundlich isotherm. Since activated carbon is a costly substance, repension is crucial.

In contrast, PAC is chosen since it is less costly and made from waste or harmful materials (Rajeshwarisivaraj and Subburam, 2002). In India, parthenium is a widespread weed that is inexpensive or free to purchase in the countryside. According to the aforementioned findings, it can be said that the PAC has outstanding adsorptive properties and may be successfully used to remove phenol and its derivative from industrial effluent (Kadirvelu, Sivasankari, et al., 2002; Rajeshwarisivaraj and Subburam, 2002; Singh et al., 2008). The adsorption rate forms from these experiments may be helpful in developing techniques for phenol and its derivatives' elimination from wastewater, that are both affordable and long-lasting (Singh, Vats and Tyagi, 2011; Singh et al., 2013).

ACKNOWLEDGEMENTS

Conflict of Interests: The authors declare no conflict of interests.

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Avifaunal Abundance of Lumding Forest Reserve Area, Assam, India

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ABSTRACT

The solitary aim of our investigation was to explore the avian species richness in Lumding Forest Reserve area, Assam.The survey was cooperated by the team of Zoological Survey of India. After extended survey, 146 species of birds were spotted from Lumding Forest Reserve area, where birds from 17 different orders (Passeriformes, Columbiformes, Accipitriformes , Piciformes, Bucerotiformes ,Gruiformes, Apodiformes, Coraciiformes, Trogoniformes, Cuculiformes, Galliformes, Ciconiiformes, Suliformes , Pelecaniformes , Strigiformes, Anseriformes, Charadriiformes) and 76 families were put down. The bird species thus obtained were compared with the IUCN Red list from where interesting cum astonishing facts were acquired. The IUCN Red list informed us that, 92.51% bird species spotted were under Least Concern category, where as 3.4% were Near Threatened, 2.72% were Vulnerable, 0.68% were Critically Endangered and Endangered. We considered ourselves successful for obtaining such astouned statistics about the avian species plentitude of Lumding Forest Reserve, which will provoke other researchers to come forward and explore more about this place.

KEY WORDS: LUMDING FOREST RESERVE AREA, BIRDS, BIODIVERSITY, IUCN.

INTRODUCTION

Alike other animals, birds too are a remarkable part of ecosystem with amazing ecological values. They cover almost every corner of the planet. Globally there exists around 9000 species of birds of which india contributes 13% with 1300 species (Grimmett et al., 2000). Birds are watcher's delight, with their colorful appreance and melodious chirping, they not only increase the beauty of nature and provide intangible aesthetic enjoyment; they play many roles in ecosysytem (Watanuki et al.,2022).

There exists a strong interdependence between birds and other organisms of this universe. These warm blooded vertebrates help in pollination; birds like bulbul, parakeets help in seed dispersal; act as scavengers; helps in pest control in crop fields; manages wetland grazing species like snails, periwinkles; acts as proficient gardeners(Beal et al., 2021) .

Prior to their sensitiveness to habitat change, birds are considered as one of the most important indicators of environmental change (Pitera et al., 2021). They play significant role in controlling insect outbreak. Birds like

Barn Swallow helps in mosquito control by eating around 850 mosquitoes each day (Vinod et al., 2023).

Lumding is a railway town and a municipal board of Nagaon district. It is the regional divisional headquarter of North –Eastern Frontier Railway, a big important junction and famous for Barak Valley Express, Cachar Express, Hill Queen Express, Agartala-Lumding Express, etc., which passes through scenic routes (Pawar and Salunkhe, 2014). It is also famous for cultural and educational excellence.

Initiated in 1964, The International Union for Conservation of Nature's (IUCN) Red List of Threatened Species has become the most reliable source regarding conservation of floral and faunal species of the earth (Miskelly et al., 2019). It accords information regarding the population, habitat, threats and conservation initiatives that assists us to take necessary steps towards protection. The Red List is an indicator of the exact position of worlds biodiversity (Sauve et al., 2021). It helps to protect natural resources on which we are dependent for survival. Hence, during our investigation, we checked our findings in the Red List to assure their future existence and safety.

MATERIAL AND METHODS

Lumding is a hilly landscape which is covered with dense reserve forest from all sides. Summers are burning here as

well as winters can be chilling. Monsoon covers rainfall around 60-125mm. The only hill station of Assam i.e. Haflong is very closer to Lumding. Climate remaining favourable for agricultural practices (Arya and Rao, 2014).

Species density and diversity can be good indicators for measuring abundance of birds in any locality (Javed and Rahmani, 1993). Our survey was carried out from January,2016 to December,2018, where almost all the seasons were covered like Summer (March-June) and Winter (November-March).

For spotting, following methods were preferred,
(1) point count method, (2) direct count method & (3) area search method.

Figure 1: Study area map

Figure 2: Graphical representation showing order wise abundance of avian fauna in Lumding Forest Reserve area
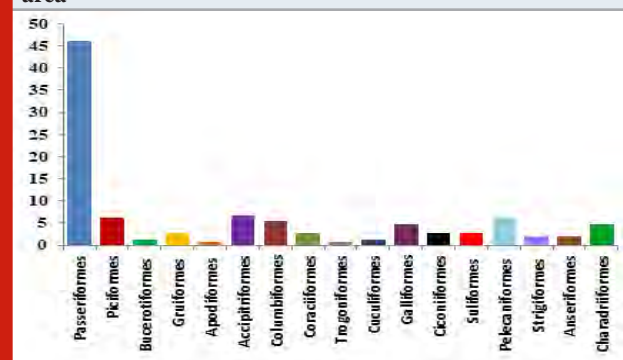
Bird counting was performed from 5.00-8.00 am (morning) and 4.00-6.00 pm (evening). Various species of birds were observed during survey peroid. Observation was performed with the help of field binoculars (10x40). Pictures were captured with Digital Canon EOS 1300D, 18MP DSLR. Renouned Ornithologist Dr. Ghosh and his team from Zoological Survey of India, remained physically present and helped in species identification. Species identification was confirmed with the help of " Books of Birds of the Indian subcontinent" (Grimmett et al., 2000) and book of Salim Ali "The Book of Indian Birds", Wikipedia and other resources.

RESULT AND DISCUSSION

North-East India refers to the easternmost region of India consisting of contiguous seven sister states, Sikkim and parts of North Bengal (district of Darjeeling , Jalpaiguri and Kochbihar) (Javed and Rahmani, 1993). North East India is ethnically distinct from the other states of India, linguistically the region is distinguished by preponderance of Tibeto- Burman languages. Strong ethnic cultures that had escaped sanskritization effects permeate the region. The eight states from a special category is officially recognized (Peralta et al., 2020).

Figure 3: Graphical representation showing IUCN status of avian fauna spotted in Lumding Forest Reserve area
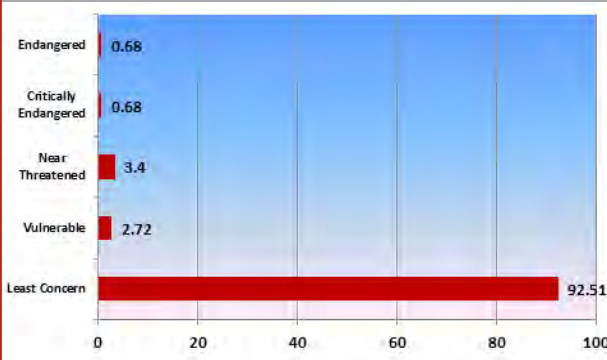
Figure 4: Pictures of some birds spotted from Lumding Forest Reserve area

Figure 5: Pictures of some birds spotted from Lumding Forest Reserve area

Paradise Flycatcher;   Brown Fish Owl;   Green Bee Eater;   Coot;

Crow Pheasant;   Yellow Wagtail;   Pied Harrier;   Black Kite;

Black Baza;   Common Pochard;   Grey Hornbill;   Pintail duck;

Ruby Throated Hummingbird;   Black Winged Kite;   Bittern;   Alexandrine Parakeet;

Night Heron;   Spur Winged Lapwing;   Tiger Bittern;   Red Whiskered Bulbul;

Lumding Reserve Forest is a compact large block of forest, measuring 252.9 sq. Kms and comparatively has less human disturbance. This forest gives shelter to a large variety of floral as well as faunal species (Arya and Rao, 2014). As the forest area of neighbouring Karbi Anglong district and N.C.Hills district are affected by shifting cultivation, the herbs of Asiatic Elephant takes shelter in Lumding Reserve Forest (Joshi and Shrivastava, 2012). The principal type of forest found in Lumding is degraded moist mixed deciduous forest type (Khan, Rao and Wani, 2012).

Lumding Reserve Forest is also habitat for a huge variety of medicinal plants like Sarpagandha, Basaka, Ahoi, Hilikha, etc. Among all the floral species Teak and Gamari are found to dominate in Lumding Reserve Forest (Javed and Rahmani, 1993). Among the climbers *Mikennia sp.* predominates where canopy is open and other climbers are *Bridelia sp.*, B*utea parviflora, disoscorea sp., Clematis sp*. Himalayan black bear, Clouded Leopard, Crab eating mongoose, Slow loris, Assamese Macaque, Hoolock gibbon, Asiatic jackal,etc. comes under faunal diversity of Lumding. 146 species of birds were spotted from Lumding Forest Reserve area during our survey; Of which, 17 different avian orders including Passeriformes, Columbiformes, Accipitriformes, Piciformes, Bucerotiformes ,Gruiformes, Apodiformes, Coraciiformes, Trogoniformes, Cuculiformes, Galliformes, Ciconiiformes, Suliformes, Pelecaniformes, Strigiformes, Anseriformes, Charadriiformes were detected.

Figure 1. Checklist of avian fauna of Lumding Forest Reserve area

SL NO.	ORDER	FAMILY	COMMON NAME	SCIENTIFIC NAME	Feeding habits*	IUCN Red List (2019-22) status**
1	Passeriformes	Pycnonotidae	Red vented bulbul	*Pycnonotus cafer*	Nectivorous, Frugivorous, Insectivorous, Palynivorous	Least Concern
2		Campephagidae	Scarlet minivet *speciosus*	*Pericrocotus* Carnivorous	Insectivorous, Concern Ophiophagous	Least Concern
3		Oriolidae	Black headed oriole	*Oriolus larvatus*	Frugivorous, Insectivorous	Least Concern
4			Indian Golden oriole	*Oriolus kundoo*	Frugivorous, Insectivorous, Nectivorous	Least Concern
5		Pittidae	Black crown pitta	*Erythropitta ussheri*	Carnivorous	Least Concern
6			Indian pitta	*Pitta brachyura*	Carnivorous, Insectivorous,	Least Concern
7		Orididae	Maroon oriole	*Oriolus traillii*	Insectivorous, Nectivorous	Least Concern
8		Alaudidae	Eastern skylark (Oriental skylark)	*Alauda gulgula*	Granivorous	Least Concern

Table 1a

9		Dicruridae	Racket tailed drongo	*Dicrorus paradiseus*	Insectivorous, Nectivorous	Least Concern
10			Black drongo	*Dicrorus macrocercus*	Insectivorous,	Least Concern
11			Ashy drongo	*Dicrorus leucophaeus*	Insectivorous	Least Concern
12			Bronzed drongo	*Dicrurus aeneus*	Insectivorous	Least Concern
13		Nectariniidae	Nepal sunbird	*Aethopyga nipalensis*	Nectivorous	Least Concern
14		Sturnidae	Hill myna	*Gracula religiosa*	Omnivorous, Insectivorous, Frugivorous	Least Concern
15		Laniidae	White crowned shrike	*Eurocephalus anguitimens*	Insectivorous, Carnivorous	Least Concern
16			Bull headed shrike	*Lanius bucephalus*	Insectivorous, Carnivorous	Least Concern
17			Long tailed fiscal shrike	*Larius collaris*	Insectivorous , Carnivorous	Least Concern
18		Leiothrichidae	Jungle babbler	*Argya striata*	Insectivorous	Least Concern
19		Pycnonotidae	Black headed bulbul	*Pycnonotus atriceps*	Insectivorous, Frugivorous	Least Concern
20		Hirundinidae	Red rumped swallow	*Cecropis daurica*	Insectivorous	Least Concern
21		Passeri	Fairy blue bird	*Irena puella*	Insectivorous, Nectivorous	Least Concern
22		Chloropseidae	Gold fronted leafbird	*Chroropsis aurifrons*	Omnivorous	Least Concern
23			Orange bellied leafbird	*Chloropsis hard wickii*	Insectivorous, Nectivorous	Least Concern
24		Pycnonotidae	Brown eared bulbul	*Hypsipetes amaurotis*	Insectivorous, Frugivorous, Nectivorous	Least Concern
25		Mectarinidae	Large browed wagtail	*Motacilla moderaspatensis*	Insectivorous	Least Concern
26		Estrildidae	Spotted munia	*Lonchura punctulata*	Granivorous	Least Concern
27			Black headed munia	*Lonchura malacca*	Granivorous	Least Concern
28		Ploceidae	Baya weaver bird	*Ploceus philippinus*	Granivorous, Insectivorous	Least Concern
29		Dicruridae	Lesser racket tailed drongo	*Dicrurus remifer*	Insectivorous	Least Concern
30		Muscicapidae	White rumped magpie	*Copsychus malabaricus*	Insectivorous , Carnivorous	Least Concern
31		Sturnidae	Common myna	*Acridotheres tristis*	Insectivorous, Granivorous, Carnivorous, Omnivorous	Least Concern
32			White headed starling	*Sturnia erythropygia*	Insectivorous	Least Concern
33			Brahminy starling	*Sturnus pagodarum*	Omnivorous	Least Concern
34			Jungle myna	*Acridotheres fuscus*	Omnivorous	Least Concern
35			Indian Pied myna	*Gracupica contra*	Omnivorous	Least Concern
36		Motacilladae	Yellow wagtail	*Motacilla flava*	Insectivorous	Least Concern
37			Citrine wagtail	*Motacilla citriola*	Insectivorous	Least Concern
38		Turdidae	Common black bird	*Turdus merula*	Omnivorous	Least Concern
39		Vangidae	Indian wood shrike	*Tephrodornis pondicerianus*	Insectivorous	Least Concern

Table 1b

40		Motacilladae	Indian paddy field pipit	*Anthus rufulus*	Insectivorous	Least Concern
41		Paridae	Great tit	*Parus major*	Insectivorous	Least Concern
42		Alaudidae	Black lark	*Melanocorypha yeltoniensis*	Insectivorous	Least Concern
43			Sand lark	*Alaudala raytal*	Insectivorous	Least Concern
44		Corvidae	Jungle crow	*Corvus culminatus*	Frugivorous	Least Concern
45			House crow	Corvus splendens	*Omnivorous*	Least Concern
46		Monarchidae	Paradise flycatcher	*Terepsiphone paradisi*	Insectivorous	Least Concern
47		Muscicapidae	Blue flycatcher	*Cyornis tickelliae*	Carnivorous	Least Concern
48		Chloropseidae	Leaf bird	*Chloropsis flavipennis*	Insectivorous, Frugivorous, Nectivorous	Vulnerable
49		Laniidae	Brown shrike	*Lanius cristatus*	Insectivorous	Least Concern
50		Muscicapidae	Shama bird	*Copsychus malabaricus*	Insectivorous, Frugivorous	Least Concern
51			Slaty backed forktail bird	*Enicurus schistaceus*	Carnivorous	Least Concern
52			Plumbeous redstart	*Rhyacornis fuliginosa*	Insectivorous, Carnivorous	Least Concern
53		Leiothrichidae	Grey sibia	*Heterophasia gracilis*	Insectivorous, Frugivorous	Least Concern
54			Jungle babbler	*Turdoides striata*	Insectivorous, Granivorous, Nectivorous	Least Concern
55			Common babbler	*Argya caudata*	Omnivorous	Least Concern
56			White crowned shrike	*Eurocephalus ruppelli*	Carnivorous	Least Concern
57		Psittaculidae	Alexandrine parakeet	*Psittacula eupatria*	Omnivorous	Near Threatened
58		Laniidae	Red breasted parakeet	*Psittacula alexandri*	Frugivorous, Granivorous	Near Threatened
59		Pnoepygidae	Pygmy cupwing	*Pnoepyga pusilla*	Insectivorous	Least Concern
60		Pellorneidae	Brown capped babbler	*Pellomeum fuscocapillus*	Insectivorous	Least Concern
61			Marsh spotted babbler	*Pellomeum palustre*	Insectivorous	Vulnerable
62		Paradoxornithidae	Grey headed	*Psittiparus gularis* parrot bill	Insectivorous	Least Concern
63		Corvidae	Red whiskered bulbul	*Pycnonotus jocosus*	Insectivorous, Frugivorous	Least Concern
64			Black browed treepie	*Dendrocitta frontalis*	Carnivorous	Least Concern
65		Passeridae	House sparrow	*Passer domesticus*	Insectivorous	Least Concern
66			Tree sparrow	*Passer montanus*	Insectivorous	Least Concern
67		Paridae	Oriental tit	*Parus minor*	Omnivorous, Molluscivorous	Least Concern
68		Anatidae	Pin tail duck	*Anas acuta*	Granivorous	Least Concern
69	Piciformes	Megalaimidae	Large green barbet	*Psilopogon zeylanicus*	Frugivorous, Insectivorous	Least Concern
70			Coppersmith barbet	*Psilopogon haemacephalus*	Frugivorous	Least Concern

Table 1c

71		Picidae	Golden backed woodpecker	*Dinopium benghalense*	Insectivorous	Least Concern
72			Large golden backed woodpecker	*Chrysocolaptes guttacristatus*	Frugivorous, Insectivorous, Granivorous	Least Concern
73			Rufous piculet	*Sasia abnormis*	Insectivorous, Carnivorous	Least Concern
74			Darjeeling pied woodpecker	*Dendrocopos darjellensis*	Insectivorous	Least Concern
75			Heart spotted woodpecker	*Hemicircus canente*	Insectivorous	Least Concern
76			Large yellow fronted woodpecker	*Melanerpes flavifrons*	Insectivorous, Frugivorous	Least Concern
77			Rufous woodpecker	*Micropternus brachyurus*	Insectivorous, Frugivorous, Nectivorous	Least Concern
78	Bucerotiformes	Bucerotidae	Grey hornbill	*Ocyceros birostris*	Granivorous	Least Concern
79		Upupidae	Hoopoe	*Upupa* sp	Insectivorous, Carnivorous	Least Concern
80	Gruiformes	Rallidae	Coot	*Fulica atra*	Omnivorous	Least Concern
81		Rallidae	Common moorhen	*Gallinula chloropus*	Omnivorous	Least Concern
82			Western swamphen	*Porphyrio porphyrio*	Omnivorous	Least Concern
83		Ardeidae	Little egret	*Egretta garzetta*	Carnivorous	Least Concern
84	Apodiformes	Trochilidae	Ruby throated humming bird	*Archilochus colubris*	Insectivorous,	Least Concern
85	Accipitriformes	Accipitridae	Indian white rumped vulture	*Gyps bengalensis*	Carnivorous	Critically Endangered
86			Shikra (Indian shikra)	*Accipiter badius*	Insectivorous	Least Concern
87			Pariah kite	*Milvus migrans*	Carnivorous, Avivorous	Least Concern
88			Pied harrier	*Circus melanoleucos*	Insectivorous, Avivorous	Least Concern
89			Crested serpent eagle	*Spilornis cheela*	Insectivorous, Ophiophagous	Least Concern
90			Black winged kite	*Elanus caeruleus*	Insectivorous	Least Concern
91			Black eared baza	*Aviceda leuphotes*	Insectivorous	Least Concern
92			Changeable hawk eagle	*Nisaetus cirrhatus*	Avivorous	Least Concern
93			Crested serpent eagle	*Spilornis cheela*	Carnivorous, Avivorous, Carnivorous	Least Concern
94			White tailed eagle	*Haliaeetus albicilla*	Carnivorous	Least Concern
95	Columbiformes	Columbidae	Spotted dove	*Stiloopelia chinensis*	Frugivorous, Granivorous, Insectivorous	Least Concern
96			Ring necked dove	*Streptopelia capicola*	Frugivorous, Granivorous, Insectivorous	Least Concern
97			Emerald dove	*Chalcophaps indica*	Frugivorous	Least Concern

Table 1d

98			Red turtle dove	*Streptopelia tranque baricha*	Granivorous,	Least Concern	
99			Green imperial pigeon	*Ducula aenea*	Granivorous	Near Threatened	
100			Rock pigeon	*Columba livia*	Granivorous, Frugivorous, Insectivorous	Least Concern	
101			Imperial pigeon	*Dacula* sp	Frugivorous	Least Concern	
102			Spotted dove	*Spoilopelia chinenois*	Insectivorous	Least Concern	
103	Coraciiformes	Meropidae	Chestnut headed bee eater	*Merops leschenaulti*	Insectivorous	Least Concern	
104			Green bee eater	*Merops orientalis*	Insectivorous , Carnivorous	Least Concern	
105		Alcedinidae	White breasted kingfisher	*Halcyon smyrnensis*	Insectivorous, Carnivorous	Least Concern	
106			Common kingfisher	*Alcedo anthis bengalensis*	Carnivorous, Insectivorous, Piscivorous	Least Concern	
107	Trogoniformes	Trogonidae	Red headed trogon	*Harpactes erythrocephalus*	Insectivorous	Least Concern	
108	Cuculiformes	Cuculidae	Crow pheasant	*Centropus sinensis*	Carnivorous	Least Concern	
109			Asian koel	*Eudynamys scolopaceus*	Omnivorous	Least Concern	
110	Galliformes	Phasianidae	Black francolin	*Francolinus francolinus*	Insectivorous	Least Concern	
111			Grey partridge	*Perdix perdix*	Insectivorous, Granivorous	Least Concern	
112			Jungle bush quail	*Perdicula asiatica*	Frugivorous, Granivorous	Least Concern	
113			Red jungle fowl	*Gallus gallus*	Frugivorous	Least Concern	
114			Kalij pheasant	*Lophura leucomelanos*	Omnivorous	Least Concern	
115		Dicacidae	Plain coloured flower pecker	*Diacacum minullum*	Frugivorous, Nectivorous	Least Concern	
116		Pycnorotidae	Black bulbul	*Hypsipetes leucocephalus*	Insectivorous, Granivorous	Least Concern	
117	Ciconiiformes	Ciconiidae	Greater adjutant	*Leptoptilos dubius*	Omnivorous, Carnivorous	Endangered	
118			Lesser adjutant	*Leptoptilos javanicus*	Omnivorous, Carnivorous, Piscivorous	Vulnerable	
119			Black necked stork	*Ephippiorhynctus asiaticus*	Carnivorous	Near Threatened	
120			Scaly breasted stork	*Anastomus oscitans*	Carnivorous, Molluscivorous	Least Concern	
121	Suliformes	Phalacrocoracidae	Little cormorant	*Microcarbo niger*	Carnivorous	Least Concern	
122			Large cormorant	*Phalacrocorax carbo*	Carnivorous	Least Concern	
123			Indian cormorant	*Phalacrocorax fusicollis*	Carnivorous	Least Concern	
124		Anhingidae	Darter	*Anhinga* sp	Piscivorous	Near threatened	
125	Pelecaniformes	Ardeidae	Great egret	*Ardea alba*	Carnivorous	Least Concern	
126			Medium egret	*Ardea intermedia*	Carnivorous	Least Concern	
127			Cattle erget	*Bubulcus ibis*	Carnivorous	Least Concern	

Table 1e

128			Yellow bittern	*Ixobrychus sinensis*	Carnivorous	Least Concern
129			Chinese pond heron	*Ardeola bacchus*	Carnivorous	Least Concern
130			Indian pond heron	*Ardeola grayii*	Carnivorous	Least Concern
131			Night heron	*Nycticorax nyctanassa*	Insectivorous, Avivorous, Piscivorous	Least Concern
132			Bittern	*Botaurus stephens*	Carnivorous	Least Concern
133			Tiger heron	*Tigrisoma lineatum*	Carnivorous	Least Concern
134	Strigiformes	Strigidae	Jungle owlet	*Glaucidium radiatum*	Insectivorous	Least Concern
135			Brown fish owl	*Ketupa zeylonensis*	Piscivorous	Least Concern
136		Tytonidae	Barn owl	*Tyto alba*	Carnivorous	Least Concern
137	Anseriformes	Anatidae	Common pochard	*Aythya ferina*	Molluscivorous, Insectivorous	Vulnerable
138			Lesser whistling duck	*Dendrocygna javanica*	Gregarious	Least Concern
139			Brahminy duck	*Tadorma ferruginea*	Omnivorous	Least Concern
140	Charadriiformes	jacanidae	Pheasant tailed jacana	*Hydrophasianus chirurgus*	Insectivorous, Molluscivorous	Least Concern
141			Bronze winged jacana	*Metopidius indicus*	Insectivorous	Least Concern
142		Charadriidae	Red wattled lapwing	*Vanellus indicus*	Insectivorous, Granivorous, Molluscivorous	Least Concern
143			Spur winged lapwing	*Vanellus spinosus*	Insectivorous	Least Concern
144		Laridae	River tern	*Sterna aurantia*	Insectivorous, Piscivorous	Vulnerable
145			Indian robin	*Saxicoloides fulicatus*	Insectivorous	Least Concern
146		Scolopacidae	Long toed stint	*Calidris subminuta*	Insectivorous, Molluscivorous, Granivorous	Least Concern

***Piscivorous:** fish eater birds; Palynivorousbirds: pollen eater birds; Nectivorous: necter eater birds; Ophiophagous: snake eater birds; Mucivorous: mucus eater birds; Molluscivorous: mollusce eater birds; Granivorous: grain eater birds; Frugivorous: fruit eater birds; Carnivorous: meat eater birds; Avivorous: bird eater birds.

****Extinct in the wild (EW) - Known only to survive in captivity; Extinct (EX) - No known individuals remaining; Critically Endangered (CR) - Extremely high risk of extinction in the wild; Endangered (EN) - High risk of extinction in the wild; Vulnerable (VU) - High risk of endangeredment in the wild; Near Threatened (NT) - Likely to become endangered soon; Least Concern (LC) - Lowest risk. Does not qualify for a more at risk category; Data Deficient (DD) - Not enough data to assess its risk of extinction; Not Evaluated (NE) - Has not yet ben evaluated against the criteria.

Noticably, Passeriformes showed highest species frequency with 46.25% abundance which was followed by Accipitriformes (6.8%), Piciformes and Pelecaniformes(6.12%) ;Columbiformes(5.44%); Galliformes and Charadriiformes(4.76%); Gruiformes, Coraciiformes, Suliformes, Ciconiiformes(2.72%); Strigiformes, Anseriformes(2.04%); Cuculiformes, Bucerotiformes (1.36%); Trogoniformes, Apodiformes (0.68%). Under 17 spotted orders, total 76 families were recorded. Passeriformes came out with maximum number of families (44).

The IUCN status of avian species richness of Lumding revealed that 92.51% birds of Limding are Least Concern indicating their sufficient existance, where as 3.4% are Near Threatened, 2.72% are Vulnerable, 0.68% are Critically Endangered and Endangered. Birds like River tern, Common pochard, Lesser adjutant, Greater adjutant, Indian white rumped vulture, Marsh spotted babbler, Black necked stork, Alexandrine parakeet, Darter which were found under Critically Endangered, Near Threatened, Vulnerable and Endangered category of IUCN demands our attention to work for their conservation and protection from becoming extinct.

CONCLUSION

Its extremely worrying that the number of Critically Endangered birds on the IUCN Red List continues to increase, despite successful conservation initiatives around the world", says Simin Stuart, Chair of IUCN's Species Survival Commission. Hence, it becomes immense important to work for the safety and security of birds at global level. Although our work was very small but we expect that it will motivate other researchers to work in this field so that rare species of birds can servive for long.

ACKNOWLEDGEMENTS

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Standardization and Evaluation of Buffers: A One Step DNA Extraction Protocol from Microbial Cultures

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ABSTRACT

Bacteria are one of the most diverse organisms that exist. Every day labs are flooded with cultures to detect the type of microorganisms and their antibiotic resistance patterns to treat patients. However, this reporting takes 3 days. Molecular biology is a field that is associated with quicker reporting. This work is an attempt to make molecular testing easier and quicker to help with one step DNA extraction method. 3 different buffers were tested for DNA extraction from cultures. The result of the best buffer is represented in agarose gel electrophoresis. Efficient extraction of DNA from bacteria was seen. The first step to standardization of buffers for the one-step extraction of DNA from bacteria. The buffers need to be tested and standardized with a wider variety of samples of both gram-positive and gram-negative organisms and an efficient method of extraction from the primary samples instead of the culture plates has to be standardized. This is a pilot study to screen the buffers for their effectiveness.

KEY WORDS: BACTERIAL DNA, GRAM-POSITIVE, GRAM NEGATIVE, BUFFERS, AGAROSE GEL ELECTROPHORESIS.

INTRODUCTION

Infection by microorganisms is an overwhelming scenario that causes serious implications in the patients and sometimes even causes death. Bacteria are a major cause of these infections. Bacteria are defined as a group of small, single-celled organisms. The extent of damage is measured by the route, number, mode of transmission, and stability of the host. While the nose, throat, and cuts in the skin are foregrounds of microbial infection, the lungs, bowel, urinary tract, vagina, etc are also sites of serious infections, (Cars & Nordberg 2005, Weber & Stilianakis 2008 Reynolds & Kollef 2021).

The major mode of infection is entry through the skin and replication in the tissue. The incidence of infectious diseases was estimated at 71.8 per 10,000 population in 2022. These infectious diseases kill about nine million individuals per year among which children under the age of 5 are a major part. Bacterial diseases causing infection have reached various advanced levels of testing. Techniques of Lateral flow assay, ELISA, and molecular testing techniques have been employed (Lamballerie et al., 1992, Boehringer and O'Farrell, 2021).

However, for infections in wounds, the techniques of microbial identification and consequently identification of the antibiotics to treat the infection is a 3-day process. The samples for anti-microbial testing are in the order of sputum, urine, high vaginal swab, wound swab, and finally blood. Anti-microbial resistance due to prolonged use of antibiotics is one of the biggest challenges of the microbial industry today. Some of the major bacteria that cause infections are *Klebsiella spp, Moraxella spp.,Escherichia spp., Pseudomonas spp., Staphylococcus spp.* etc., (Bharathi et al., 2010, Reynolds & Kollef 2021).

The constant evolution of bacteria poses a major challenge in identification. Testing like miRNA and SNP may be an apt solution (Ali et al., 2017). The bacterial cell wall is made up of rigid structures of uniform thickness around the cell. This rigidness ensures the various types of shapes like spiral, rod, and coccus and patterns of colonization. Based on cell wall components bacteria are divided into Gram-positive and Gram-negative bacteria (Schleifer et al., 1972). The presence of a peptidoglycan layer in the outer layer of the Gram-negative bacteria makes the need for the buffer to lyse the cell wall different from that of the gram-positive bacteria.

This study deals with the standardization of a buffer that extracts bacterial DNA from overnight culture plates in one step. The purity of the DNA obtained was screened with A

260/ 280 and confirmed with Agarose gel electrophoresis. The results displayed below are of the best methods that yielded a good quantity of DNA.

MATERIALS AND METHODS

The bacteria for testing was taken from ATCC strains. The confirmation was done by staining and biochemical studies. One gram- Positive and one gram-negative bacteria were taken for study.Gram Positive – *Staphylococcus aureus*-ATCC @ 25923 Gram Negative - *Escherichia coli* ATCC 25922. The same was inoculated for overnight growth in nutrient broth. One ml of broth was taken centrifuged and used for the rest of the experiment.

Buffers: The below table contains the constituents of the best 3 buffers which were taken for further studies.

Absorbance at 260 nm, 280nm, and 230nm.:NanoDrop® ND-1000 was used to take the readings (5). Quantification of DNA : dsDNA concentration = 50 μg/mL × OD_{260} × dilution factor.

Gel Electrophoresis: A 1% gel was used for the analysis of the result. Only pure DNA was used for this analysis. The gel pictures of the best results are represented in the results section. The running buffer of TAE and TBE was used. To visualize DNA EtBr (Ethidium Bromide) was added and visualized through a UV transilluminator and documented through a gel documentation system (Lee et al., 2012).

RESULTS AND DISCUSSION

All experiments have been carried out in triplicate. The below results are the representatives of the same.The purity of the DNA obtained is seen from the A260/280 ratio. A value between 1.7 -2.0 is considered pure for ds DNA. A lower value indicated the presence of phenol, proteins, and other contaminants as they are easily absorbed at 280nm. A higher value of the ratio shows the presence of RNase. In this experiment as proteinase K is not used to avoid the barrier of storage the need to rule out contamination becomes important. DNA Purity is calculated using the below formula and represented in the table (Ning et al., 2009).

Table 1. Buffers and their ingredients		
Buffer 1:	**Buffer 2:**	**Buffer3:**
Tris HCL	$MgCl_2$	Tris HCL
KCL (Pottasium chloride)	EDTA	TE buffer
Mgcl₂(Magnesium chloride)	SDS	$MgCl_2$
SDS 2% (Sodium Dodecyl Sulfate)	Triton X100	EDTA
Triton X	PVP (Polyvinylpyrrolidone)	SDS
Ethanol	CH3COOK	PVP
	TE buffer (Tris –EDTA)	CH_3COOK
	Ethanol	Ethanol
		NaCl(Sodium Chloride)

DNA Purity (A_{260}/A_{280}) = (A_{260} reading – A_{320} reading) ÷ (A_{280} reading – A_{320} reading) (Tiwari et al., 2017).. A260/230 is needed to rule out contaminants such as guanidine thiocyanate, guanidine HCL, triazole, phenol, etc. A ratio between 2.0 to 2.2 is considered pure for this ratio.The

below table contains the results of the nanodrop for the evaluation of the buffers: The results of the effective buffers are only represented in the table. buffers and methods which showed values of ratios below 1.5 or above 2.2 for ratios A260/A280 are not represented.

Table 2. Nanodrop reading of the DNA extact and quantification					
S.no.	Buffer name	Method	A260/280	A2260/230	DNA Quantification ng/μl
1.	Buffer 1	A	1.68	2.2	4.1
		B	1.75	2.19	8.3
2.	Buffer 2	A	1.85	2.18	27.2
		B	1.71	2.1	10.7
3.	Buffer 3	A	1.72	2.19	11.2
		B	1.91	2.02	33.4

This result shows that buffer 2 is more efficient for S. aureus – gram-positive, while buffer 3 is more efficient for E.coli – gram-negative. This shows PVP and CH_3COOK increase the efficiency of extraction. CH_3COOK helps with the breaking down of proteins (Angela 2017). PVP increases the DNA yield (Khan et al., 2007). Buffer 2 with the second detergent, Triton X is more efficient in the case of Gram –Positive bacteria. While Tris HCL and Nacl proved greater efficiency in the breakdown of the peptidoglycan layer presenting Gram Negative bacteria. Tris HCL helps in the maintenance of the buffer layer whereas NaCl proves efficient with the lysis of the peptidoglycans by creating pores on the surface of the cell wall.

Figure 1: Lane 1 – ladder Lane 2 and 3, Buffer 3 – *Escherichia coli*. Lane 4 and 5 , Buffer2 – *Staphylococcus aureus*
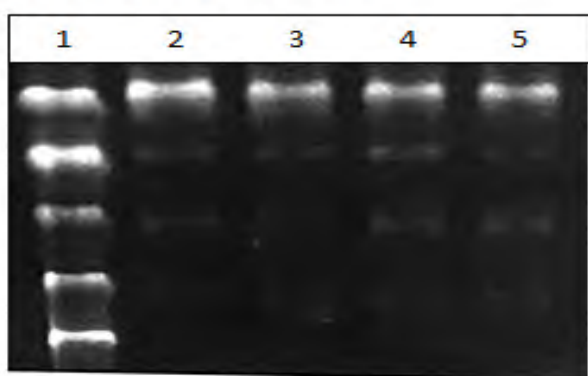
Figure 2: DNA after ethanol wash. Lane 1: DNA Ladder Lane 2 : gram positive bacteria from kit method. Lane 3 : gram negative bacteria from kit method . Lane 4 and 5- Gram negative bacteria from buffer 3. Lane 6 and 7 Gram positive bacteria from buffer 2
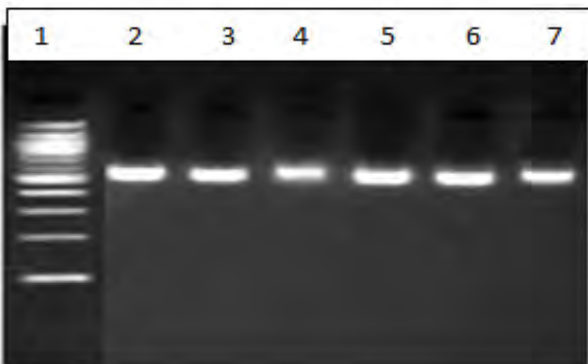
Separation of DNA is seen. Addition of Ethanol to collect the DNA yields pure DNA that can be used for further study.

Efficient extraction comparable to a CE-approved extraction kit was seen.

CONCLUSION

Efficient extraction of DNA from bacteria was seen. The first step to standardization of buffers for the one-step extraction of DNA from bacteria. The buffers need to be tested and standardized with a wider variety of samples of both gram-positive and gram-negative organisms and an efficient method of extraction from the primary samples instead of the culture plates has to be standardized. This is a pilot study to screen the buffers for their effectiveness.

Conflict of Interest: Authors have no conflict of interest

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Didactic Strategy for the Use of Virtual Itineraries in the Training of Graduates in Education Geography

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ABSTRACT

The training of teachers has always been a necessity of the planet aligned to the space and time where this process takes place. Future training of graduates in education, Geography who study geographic space need novel ways to appropriate these contexts and knowledge. The objective of this research was to propose a didactic strategy with the use of virtual didactic itineraries, in the teaching-learning process for the training of education, Geography in the first year of the career: Bachelor of Education. Geography, through the subject Introduction to Labor Practice, which contributed to deepen the knowledge about different landscapes and at the same time developed professional skills related to the study of the landscape. For the development of the research, information collection methods were used (participatory observation, document review, interview, expert criteria) and information processing methods (analysis - synthesis, inductive-demonstrative, logical history, descriptive statistics, modeling and analysis). The methods allowed to determine the needs of the students from the cognitive, educational and procedural point of view, regarding the teaching-learning process. The didactic strategy was implemented in practice, achieving very satisfactory results from the 2019-2022 academic years. It allows contributing to the development of professional pedagogical skills while deepening the knowledge of different landscapes in the students of the Degree in Education. Geography of the Faculty of Secondary Education of the Central University "Marta Abreu" of Las Villas, Villa Clara, Cuba.

KEY WORDS: DIDACTIC STRATEGY; GEOGRAPHY; LABOR PRACTICE; TEACHING-LEARNING PROCESS; VIRTUAL ITINERARIES.

INTRODUCTION

The International Geographical Union and the Commission on Geographical Education (1992, 2000 and 2007) propose International Declarations for the teaching of Geography where proposals are made to promote the idea of fostering knowledge of the environment close to the society where we live. Threats and risks often arise from the lack of knowledge of the inhabitants themselves, from forgetting the history accumulated in each of them (Sánchez, 2020).

The 1992 Declaration outlines that, when selecting geographic content, one can opt for or combine regional studies (e.g., studies of the region, country) or thematic studies (e.g., through the study of physical or human geography, or through centers of interest, such as environmental quality or population growth). In any case, it is pointed out that there should be a balance in the scale of analysis: local, regional,

national or global (International Geographical Union and the Commission on Geographical Education, 1992). The authors of the research consider that it is oriented to study not only the near or local space but also the distant or international space, for which it is necessary to use information and communication technologies for the purpose of teaching Geography, so it is essential for the training of teachers to develop skills related to these media.

This statement also addresses the methods for its teaching, the statement points out that they should stimulate in students the desire to engage in questioning and inquiry. It is essential that students exercise and develop geographical skills with a tendency to search for solutions to the problems posed in the organization of space, present and future (International Geographical Union and the Commission on Geographical Education, 1992). Another Declaration of great interest is the Seoul Declaration of 2000, which proclaims how geographic education is fundamental for people to develop different capacities in relation to cultural diversity such as:

The ability to defend and be sensitive to human rights;

The ability to understand, accept and appreciate cultural diversity;

The ability to understand, empathize and criticize alternative points of view about people and their social conditions;

To be aware of the impact of their own lifestyles on their social contexts;

Appreciate the need to protect the environment and provide environmental justice to regions and local communities that have suffered environmental devastation;

Ability to act as an informed and active member of both their own society and the global society (International Geographical Union and the Commission on Geographical Education, 2000).

It is a Declaration that insists on intercultural studies from the perspective that geographic education also helps to understand the need to protect the environment at different scales, both local and international, and contributes to educate people active in the global society. (International Geographical Union and the Commission on Geographical Education, 2000).

Subsequently, the 2007 Lucerne Declaration on Geographical Education for Sustainable Development prioritizes issues affecting ecological-holistic thinking, and focuses on three a

A. The Contribution of Geography to Education for Sustainable Development.

B. Criteria for Developing a Geographic Curriculum for Education for Sustainable Development.

C. The Importance of Information and Communication Technologies (ICT) in Education for Sustainable Development in Geography (International Geographical Union and the Commission on Geographical Education, 2007).

This same Declaration emphasizes that the teaching of Geography and Education for Sustainable Development are closely linked, because the acquisition of geographic competencies can improve the understanding of sustainable development. For this reason, it insists on the three competence areas: knowledge, skills and attitudes.

Through geographic knowledge and understanding of natural systems, interaction between ecosystems, and socio-economic systems helps students understand the world through key geographic concepts such as: location, distribution, distance, movement, region, scale, spatial association, spatial interaction, and change over time; Work on communication skills, reasoning, and practical and social skills help explore geographic issues at a range of levels from local to international. With the development of attitudes and values to seek solutions to local, regional,

national and international questions and problems on the basis of the Universal Declaration of Human Rights (International Geographical Union and the Commission on Geographical Education, 2007).

Education, in its dynamics, does not escape the significant changes in its teaching-learning process (TLP), by taking into account the growing use of information and communication technologies, as essential elements for the fulfillment of the programs offered by educational institutions (Lima and Fernandez, 2016). Current education does not take advantage of the technological resources at its disposal, much less, all the possibilities offered by connectivity (Garrison, 2016). Another problem is related to the consensus about the common denomination to express e-learning (Lin et al., 2017).

The authors of this research consider that for the success of this purpose it is necessary to train teachers capable of fulfilling this social task, therefore their training must meet the demands of contemporary Geography teaching. With this purpose of studying the geographic space in an integral way at all scales (global, regional and local), from the ASP, multiple researchers at the international and national level have addressed, in a precise and contextualized manner, the subject matter of the research, the following stand out in recent years (Vilarrasa, 2003; Berger, 2006; Ruiz and González, 2007; Maderuelo, 2007; Sánchez, 2010; Garrido, 2014; Liceras, 2018; Alcántara and Medina, 2019).

Many strategies, projects, research, study programs and other elements such as work practice and field work have been proposed, but there are insufficient proposals from science for the use of connectivity as a teaching tool for the study of landscapes at different scales (Liceras, 2018; Ruiz and González, 2007; Berger, 2006).

From the use of the observation method, it has been possible to observe: weak preparation of the graduates in Education. Geography for the use of virtual didactic itineraries, insufficiencies related to this strategy such as: scarce use of didactic itineraries to promote the study of geographical space and scarce use of local values and technological means available in educational institutions, in addition, geographical knowledge is prioritized while professional skills are little promoted from the (TLP), scarce theoretical and methodological foundations for the use of didactic itineraries to contribute to the training of graduates in education, Geography and the contributions related to the study of geographic space at different scales in a virtual way are insufficient.

In order to carry out this didactic strategy, it is important how this knowledge is transmitted, therefore, active methodologies based on collaborative and/or individual learning are needed. The combination of other teaching strategies and the variety of didactic resources is also a necessity to favor motivation, such as: case studies, field work, didactic itineraries, games and simulations, involving the use of new technologies, handling of Geographic Information Systems, digital cartography, statistical data, use of the web, creation of a notebook, portfolio or dossier,

virtual trips, project work, worksheets, analysis of images, stories and poems, creation and solution of problems, among others, that help them to understand and deepen their knowledge, to acquire geographic skills and abilities, and to become aware of the world in which they live, in order to be sensitive to the problems that affect them, both near and far. Elements that should characterize the Geography TLP for the training of graduates in Education, Geography and thus influence the future of the new generations of this society to ensure the Education for Sustainable Development to which we aspire (De la Calle, 2012).

Although different agencies have emphasized the implementation of a sustainable model with an increasing amount of didactic resources, there is still progress to be made in linking science in society. It also states that this is possible through the use of geographic information technologies that allow the creation of virtual databases using virtual globes such as Google Earth and other geometric applications (Oyarce, 2018).

A didactic itinerary is a route or path to follow with different stopping points or points of interest in certain elements of heritage or cultural value. A field trip, previously organized in the classroom by the teacher, and which aims to make a series of stops in those places that you want to make known to the students, but that do not have to be heritage or cultural, because, if we want to know, for example, the commercial activities of a city, such as the market and the different types of existing trade, this didactic tool can also be used (Liceras, 2018; Campo et al., 2016).

According to the same author, it is a tool that provides the keys to the understanding and teaching of geographic space, constituting rich and deep learning moments that awaken interest, while teaching the student to observe, value and reflect on the surrounding environment (Liceras, 2018).

Virtual didactic itineraries allow the study of various localities through the unobstructed observation of the surrounding landscape and the understanding of the history of the land recorded in rocks, structures, and geographical features (Migon, 2017). In addition, the place is identified from points or sites of interest that allow to recognize and understand a geographical space as a whole according to Palacio et al. (2016).

Taking into consideration what has been expressed in previous paragraphs, the objective of this research is to propose a didactic strategy to use virtual didactic itineraries in the TLP for the training of graduates in Education. Geography.

MATERIAL AND METHODS

The research was conducted at the Faculty of Pedagogical Sciences "Félix Varela Morales" (Figure 1) of the Central University "Marta Abreu" of Las Villas, in the city of Santa Clara, province of Villa Clara, Cuba (Figure 2)

For the development of the research, the authors assume the classification of methods given by Bermúdez & Rodríguez

(2016) and Expósito et al. (2021). Information collection methods and methods for processing the information collected were used. The information collection methods used were the following:

Figure 1: Satellite photo of the location of the Faculty of Pedagogical Sciences "Félix Varela Morales" of the Central University "Marta Abreu" of Las Villas, Santa Clara, Villa

Figure 2: Map of the province of Villa Clara, with its 13 municipalities. Taken from Google Scholar.

Participatory observation: to detect the inadequacies of the Teaching-Learning Process (TLP) in the sample and evaluate the transformations experienced by the students during the application of the didactic strategy.

Document review: to verify the shortcomings and potentialities of the sample and the TLP being developed.

Interview: to verify the geographic knowledge, professional skills and professional motives and interests of the students before and after applying the didactic strategy.

Expert criteria: to evaluate the didactic strategy developed and improve it according to the suggestions made by the experts in order to achieve the proposed objective.

An expert is assumed to be a person or persons with experience in the subject, with the capacity to make judgments, criteria and evaluations that allow for the improvement of the proposal developed. The competence of the experts was determined by the K coefficient, calculated

according to the opinion of each of the candidates, regarding their level of knowledge on the subject to be addressed and the relations with the sources to argue their criteria (Armiñana, 2020).

Table 1. Level of competence of the experts consulted for the assessment of the strategy.

EXPERT	K	PROFICIENCY LEVEL
1	1	High
2	1	High
3	1	High
4	1	High
5	0,97	High
6	0,89	High
7	1	High

The evaluation criteria used to determine the level of competence of the experts were: high competence (0.8 to 1); medium competence (0.5 to 0.7) and low competence less than 0.5. The application of the competence coefficient made it possible to select 7 highly competent experts, on the basis of their knowledge in the training of graduates in Education, Geography and Higher Education.

The aspects on which they had to make their judgment were: objective of the strategy, logical structure, contextualization, contribution to the training of B.Ed., Geography, uniformity for the treatment of geographic knowledge and professional skills in the career of B.Ed. in Geography. Geography.

For the evaluation of these aspects, a scale from 1 to 5 was proposed, where (1) is inadequate, (2) not very adequate, (3) moderately adequate (4) quite adequate and (5) very adequate. The methods used to process the information collected (intellectual) were as follows:

Analysis-synthesis: to analyze the subject of study, approached by various researchers at national and international level; in the choice of methods; the analysis and synthesis of the results of the initial and final survey, and in the design of the didactic strategy.

Inductive-demonstrative: to verify from the bibliographic exploration and applied methods, the knowledge, skills and professional pedagogical attitudes of the sample of selected students of the Bachelor's degree in Education. Geography and to demonstrate the certainty of the research proposal.

Historical-logical: to implement the theoretical and methodological foundations related to the evolution of the use of virtual didactic itineraries in Cuba and the world.

Modeling: to model the didactic strategy in the Bachelor's Degree in Education. Geography; starting from the general objective, the stages, actions and the interrelations among them to fulfill the proposed objective.

The methods of processing the information collected (Statistics) were descriptive statistics for the analysis of frequency distribution tables and elaboration of graphs; which allowed quantifying the results for their subsequent description in the results.

The population coincides with the sample according to the interests of the researchers, and consisted of 14 students, which is the totality of the enrollment of the first year of the Bachelor's Degree in Education. Geography of the Regular Day Course 2021.

RESULTS AND DISCUSSION

The application of the scientific methods allowed obtaining the following results:

The review of guiding documents such as the Study Plan and Model of the Professional of the Bachelor's degree programs in Education. Geography, evidenced that the professional skills of landscape interpretation and work with technological media are oriented from the objectives and curricular contents of the career. The use of virtual didactic itineraries as a didactic strategy is not required.

An initial interview was carried out individually with the 14 students of the first year of the Regular Day Course of the Geography career. The objective was to ascertain the geographic knowledge, professional skills and motives and interests of the students about the TLP they would like to receive, as well as the elements that give them pleasure during the Geography TLP.

In the first question related to the geographical phenomena found in a given landscape, only 14.0%, recognized them, but did not establish the relationships between the phenomena that make it up. In the second question, 10.0% of the students identified the problems of a given landscape, but failed to explain them in context. In the third question, the same number of students recognize the potential of this discipline for the study of geographic space, through virtual itineraries, but none of them managed to exemplify it.

In the fourth question, 3.0% of the sample, in the fourth question, show the professional skills achieved and no student is capable of proposing other examples to develop it in the school. Question 5 addresses the level of satisfaction of the students with the TLP they receive, which is 50.0%. With this instrument it was evidenced the characteristics that students demand in the TLP for the formation of the future B.A. in Education., Geography, being these:

• Expanding their knowledge.
• Develop cartographic skills.
• Knowledge exchange between students and teachers.
• Collaborative learning.
• Variety of learning environments, both real and virtual.
• Scientific research.
• Search for information from different sources.
• Students as protagonists of this process.
• Use of different forms of organization.

- Varied means of teaching.
- Use of the most effective methods for learning.
- Development of positive human attitudes and values.

With the participant observation of teaching activities, a total of seven were observed, with the purpose of verifying the development of the Geography TLP in the main integrating discipline and the subjects of the academic year, distributed as follows: two of Physical Geography, one of Seminar and another of Lecture, two of Cartography, one Lecture and another Practical Class, three Workshops, two of Work Practice and another of Research Methodology. The following difficulties were identified:

- Scarce treatment of the knowledge of cause-effect and geographical regularities.
- Poor adjustment of the process to the students' motivations and interests.
- Insufficient linkage of the subjects of the academic year with professional pedagogical skills.
- Scarce technological resources are used.
- Insufficient representations are used to illustrate knowledge.
- A large amount of information is transmitted, with little explanation of how to transmit it.
- Little use of varied learning environments (virtual or real) and at different scales (global, regional and local).
- Insufficient use of knowledge to strengthen emotions, feelings, values and positive attitudes towards the profession.

The analysis of the documents consulted, the results of the interview with students and teachers, as well as the participant observation, made it possible to determine the following Potentialities: The use of technological means, the study of various spaces and at different scales is included in the normative guiding documents such as Study Plan and Model of the professional of the Bachelor's Degree in Education. Geography, the use of technological means, the study of various spaces and at different scales.

- The motivation and interest of the students for the profession and the career.
- The experience of the teaching staff.

The main inadequacies in the teaching-learning process of Geography for the formation of the Bachelors in Education, Geography were:

- Little use of the Geography ADP for educational work and the development of professional skills.
- Little use of other forms of organization such as: seminar, practical class, workshop and geographic excursion.
- Insufficient use of the technological resources available to them.
- Insufficient motivation, meaning and involvement of students in learning.
- Insufficient use of the potential of the study plan for the benefit of the Geography teacher's training.
- Lack of interest by the teacher of how his student learns

and how much he contributed to his formation as a teacher and as a man for life in society.
- Insufficient treatment of cause-effect knowledge and geographical regularities.

Based on the results achieved with the applied methods, the didactic strategy is proposed with the use of virtual didactic itineraries in the TLP for the training of graduates in Education, Geography, which was submitted to the experts' criteria. From the analysis carried out by the experts, the following results are specified:

- 93, 3%, 6 of the experts consulted, estimated that the objective of the strategy was adequate.
- For 5 of the experts, 86.6%, the structure and contextualization of the strategy was very adequate, while the rest considered it to be quite adequate.
- In relation to the actions to achieve unity in the treatment of professional knowledge and skills, only 20.0% considered it to be quite adequate, while 80.0% considered it to be very adequate.
- 93.3% considered the way to establish integration between professional knowledge and skills to be very adequate; only one expert considered it to be not very adequate.
- 100% of the experts consulted agree that the proposal contributes to the training of graduates in Education, Geography of the first year of these careers, they evaluate this aspect as very adequate.

The following are some of the assessments made by the experts. What they expressed has been taken literally.

Expert 1 "I consider that the didactic strategy is pertinent, applicable, shows a logical structure; the professional knowledge and skills have a specific character for the Geography career".

Expert 2 "The theoretical and methodological conception of the proposal responds to the proposed objective".

Expert 4 "The actions conceived in stages respond to a logical, coherent procedure, which integrates professional knowledge and skills in correspondence with the professional's Model".

Expert 5 "I consider that the strategy is novel and opportune to incorporate students who are starting out in a more dynamic and motivating process, so that they acquire knowledge that they can apply in the profession and in daily life and that a change of attitude is evidenced in them, the transformation into a professional consistent with the world where he/she lives. It is necessary to involve the faculty so that, together with the students, the objective proposed in the strategy can be achieved".

Expert 7 "The proposal evidences the principles of education and instruction and preparation for life, which are not circumscribed to a career, discipline or year, but should be approached in an integral manner in all university processes, with the participation of students and professors in achieving a better teacher training".

The criteria, judgments and suggestions issued by the experts were given by: to continue perfecting the actions to achieve an adequate use of the Internet, to establish adjustments for its use in the strategy, taking into account the diagnosis of the group. After reviewing the proposal by the experts, the researchers made adjustments, reworked the didactic strategy and sent it back to the experts, who evaluated it and expressed that it was pertinent to be applied. The following is the model of the Didactic Strategy designed (Fig. 3).

Figure 3: Didactic Strategy Modeling.
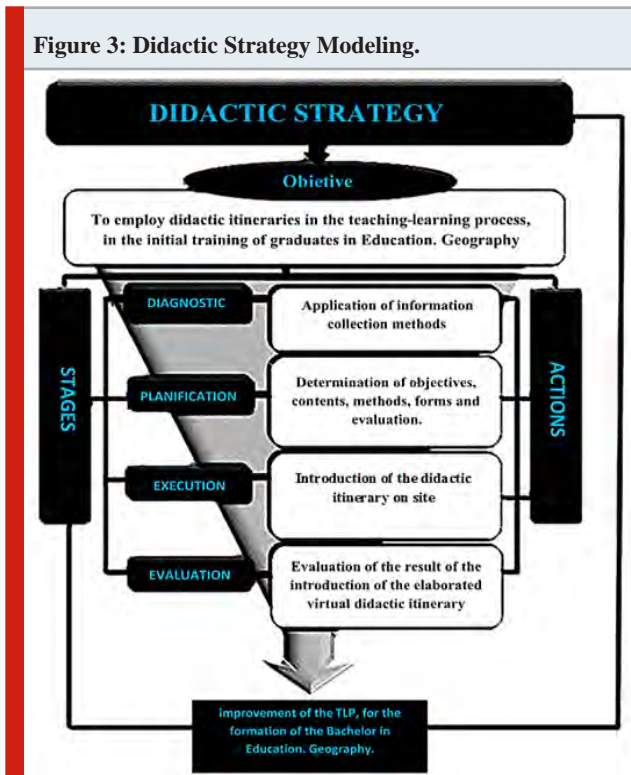
The execution of this didactic strategy was carried out through the use of virtual didactic itineraries in four steps: preparation, research, realization and conclusions, each with specific characteristics, where the student is the protagonist of the activity and the teacher serves as a guide, controller and animator.

Preparation stage

* It began with the elaboration of a program of Introduction to Labor Practice for the use of virtual didactic itineraries.
* The proposal was presented to the discipline and year group for its approval, planning and integration of different contents and subjects of the year.
* Administrative procedures were carried out.
* The necessary material resources for the activity were organized.
* Virtual didactic itineraries, points of interest or stops were analyzed and selected with the students, using cartographic means, taking into account the combination of different scales: global, regional and local.
* The motivation of the activity was carried out.

* Each of these virtual didactic itineraries was visited and studied in order to know the potentialities and possibilities offered by the networks for their study, as well as to define which one is the most suitable for the proposed objectives.
* A preliminary study of the itinerary to be carried out was carried out.
* The students were organized into study teams and those in charge were selected.
* The information search and scientific research activities carried out by the students in the following stage were planned and oriented, as well as the bibliography they used or the Internet sites they consulted.

In the research stage:

* The teacher examined various means to interest and motivate his students for the topic of the activity.
* The students elaborated and applied research techniques such as: interview, survey or any other that the teacher determined, to specialists or experts on the topic.
* Maps of different scales and themes were consulted to contrast and compare the regularities of the phenomenon in different contexts (digital maps).
* The didactic potentialities of each studied itinerary were determined.
* Students located on maps the areas of study and traced the itinerary to be followed together with their teacher, according to their cognitive interests.
* The students searched for information in different internet sites about the proposed itineraries and elaborated the observation guide for each studied landscape, then compared it with other teams and reached a consensus about the worksheet to be solved during the realization.
* The students, with the help of their teacher, analyzed the information obtained and determined the didactic resources to be addressed during the activity and determined the points of interest during the tour.
* They expressed their opinions about the results obtained in the research.
* A portfolio or dossier was prepared with the information collected, to be consulted in the next stage.

In the realization stage:

* The teacher guided, oriented, directed, proposed, facilitated, asked questions where to direct the observation and what elements to look for, at the end settles the knowledge.
* The students, organized in work teams, investigated what was guided, taking into account the didactic points of interest.
* Procedures such as observing, identifying, describing, inventorying, analyzing, analyzing, understanding, relating, explaining, evaluating, concluding, comparing, extrapolating, generalizing and enjoying while learning were developed.
*
* The perception of the landscape was enhanced through the following sequence: perceive-observe-read-analyze-conclude, from different angles of vision or

perspectives.
- The observations, descriptions and activities carried out made up the reading of the study landscape.
- Virtual spatial boundaries were established.
- The most relevant elements of the landscape were distinguished and described, going through the entire study area.
- The observation and perception of the landscape was compared with the research carried out.
- A common objective perspective was established for all.
- The reading of the landscape began taking into account the following levels: perception, analysis, structure and application.
- The points of interest were located and located with the use of the superimposition of images and digital maps.
- A dense description of the landscape was made, taking into account the relationship between components, causes and reasons for processes and phenomena.
- The didactic principles of Geography were addressed, such as: location, distribution, permanence, change and continuity.
- The aesthetic, patrimonial and identity values of the study landscape, the history and traces of history in it were specified.
- Nature-society relationships were established, environmental damage was identified and actions to minimize it were proposed.
- Interdisciplinary relationships were established.
- The causes of the processes and phenomena studied were understood, explained and valued, taking into account the contents studied, observed and investigated as a result of thinking.
- The feelings experienced during the activity, the elements that provoked those emotions, as well as the most significant lived experiences for the students were described.
- The study of global spaces and local landscapes, as well as the virtual local, were compared with the real local.
- Human values such as solidarity, responsibility, love of nature, humility, honesty, patriotism, sense of belonging and identity were strengthened.
- Professional skills and didactic procedures were developed and the didactic potentialities of the activity were addressed, as well as its contribution to their training.
- An integral evaluation of the studied space and its potentialities for the development of the Geography teaching process was carried out.

In the conclusion stage.

- Conclusions were reached and the fulfillment of the objectives of each didactic itinerary was verified.
- The experience was reflected and recapitulated, deepening in the contributions of the activity to the integral formation of the students.
- The results were presented in the form of seminars or round tables.
- The presentation of the work was organized in exhibitions or publications where there were exhibitions of samples of natural elements, photographs, creative works made by the students, posters, murals, banners, collages, models, maps or computer products, such as web pages or interactive blogs or publications in social networks and creation of groups.
- The teacher evaluated his students, comprehensively and individually, taking into account their evolution during the whole process of the activity.
- The activities carried out were specified, the best and most complete works were selected, as well as the most outstanding students and teams in the activity. In addition, the main difficulties and achievements were specified and the students proposed goals for the next virtual didactic itinerary.

At the end of these steps and actions, a new one is started, which allowed its improvement throughout the research. A total of 6 virtual didactic itineraries were carried out, which make up this elaborated didactic strategy. At the end of its application, a final interview was applied to the students of the first year of the Bachelor's Degree in Education. Geography, with the objective of verifying the geographic knowledge and professional skills acquired and the degree of satisfaction they have in relation to the actions developed. The results obtained are presented below.

The first question related to the total determined, 99.0% of the students answered satisfactorily. Regarding the recognition of environmental problems and explanation of the same in the context (second question), 97.0% already do it correctly.
100.0% of the students recognize the potential of this discipline for the study of geographic space and exemplified it (question 3).

Question 4, which refers to the professional skills achieved, 99.0% manage to do it correctly and 90.0% are able to propose other examples to develop it at school.

Question 5 addresses the students' level of satisfaction with the process achieved, reaching 100%.

The graph (Fig. 4) shows the satisfactory results achieved by the students after applying the didactic strategy.

The results showed that the students in the sample acquired geographic knowledge while developing professional skills, according to the requirements of the TLP of Higher Education. From the actions carried out, it was possible to contribute to the formation of graduates in Education, Geography with greater deepening and integration of knowledge to a geographic space and how to carry out similar activities in the students of the Geography career in the Faculty of Secondary Education of the Central University "Marta Abreu" of Las Villas, Villa Clara, Cuba.

In the research process, it was possible to confirm that the use of virtual didactic itineraries is an effective, efficient and effective didactic strategy for the training of graduates in Education. Geography, as part of the initial training of this professional, where the teacher is the most important

element in the intellectual, affective and moral formation, focused on achieving changes in attitudes in the subjects involved, which does not differ from what is expressed by (Marrero and Méndez, 2018).

As results of the use of virtual didactic itineraries in the teaching-learning process for the training of Bachelors in Education. Geography the authors consider that:

It contributed to enhance the training of future Bachelors in Education. Geography in the following elements: greater participation of the students, as active agents within the process; they became researchers at the same time they acquired new knowledge; they were motivated and interested not only in Geography but also in their profession; this form of professional performance became a reference for them; it promoted the search for these particularities in their localities and the study of them; it promoted the systematic application of this form of professional performance;

strengthened values and positive attitudes for a culture of TLPce; promoted Education for Sustainable Development; developed professional skills and the ability to solve problems related to their profession; promoted the use of technologies as a means of teaching, the use of social networks; favored the development of skills such as observing, describing, explaining, characterizing, arguing and valuing; developed the perception of the landscape.

The influence of these technological tools in the TLP is evident in self-learning, the acquisition of knowledge, the development of personal and interpersonal skills, the promotion of creativity, and the stimulation of collaborative and committed attitudes, in addition to the use of technologies focused on the landscape as an educational resource (Botella and Hurtado, 2016). The combination of strategies and skills contributes to the achievement of different objectives, competencies and contents that characterize the training process of future teachers.

In order to teach school geography, didactic itineraries are an important resource that should be known and practiced as active teaching. In addition, it promotes motivation in students to disconnect the rhythm and routines of daily classes or to discover new emotions by performing different experiences (Campo et al., 2016).

There is correlation between students' effort, active participation in the virtual environment and reading the document prepared by the student with the evaluation of their academic performance (Dominguez et al., 2016).

The teaching-learning process contributed to the study of different geographical spaces at different scales, as Marrón (2007) points out, when the student is able to explain the diversity of existing spaces, the territorial and social inequalities that occur on the planet and within the countries or nations themselves, the effects that human action is having on Nature and the environment and the causes that motivate them, and try to provide solutions, since it is necessary to put limits to an unlimited development in a space - the planetary - that is limited.

Batllori (2011) suggests the need to combine the large scale with the small scale because it provides a more complex view of reality. The use of virtual tools has advantages such as: lower costs. They are more motivating for learning. It has no defined limits, neither of time nor of space. It can be accessed from anywhere in the world. It can be documented with a great variety of materials and in very diverse formats.

It allows unlimited interactivity. It is an experiential experience in which concepts, skills and procedures are developed and emotions are produced. It promotes a deep knowledge of space. It demands the active participation of students. It integrates the stages of traditional fieldwork. It reinforces real observation with digital literacy while obtaining deep knowledge of the place (González and Marrón, 2012).

The authors agree with the criteria expressed by Évora (2011) when he asserts that the strategy, in the educational field, constitutes the pedagogical direction to transform the real state to the desired state of the object to be modified, from the conditioning of the system of actions between the directing subsystem and the directed subsystem to achieve the proposed objectives at its highest level.

We also agree with Charbonet (2009), when he asserts that strategies arise from dissatisfactions or contradictions between the actual and the desired state, pursue defined objectives, are organized by stages, require transforming actions that cause changes in the actual state and in their conception are present control and feedback mechanisms. In this sense, the didactic strategy assumes the postulates issued by these authors.

For Chávez et al. (2019) and coinciding with Orfa et al. (2019), the strategy becomes the teacher's actions with the purpose of facilitating training and learning, using didactic techniques which allow building knowledge in a creative and dynamic way.

The actions implemented in the didactic strategy influence the transformation of the personality of the future teacher of Geography; manifested in the acquisition of knowledge, its implementation in new situations, teamwork for the solution of problems related to the pedagogical task, willingness for active and conscious participation in environmental activities, and the planning, preparation and implementation of actions that can be put into practice from the Cuban school.

The criteria issued by the teachers and experts consulted ratify the importance of the studies conducted by Villamandos et al. (2019), related to the search for alternatives as the didactic strategy for the development of the TLP in Geography students.

The strategy perfected how to express the scientific results achieved by the students in order to socialize them, which

can be applied in performance contexts similar to the one in which the research is developed; in the opinion of the researchers, actions should be conceived in the strategy that involve Educational Research Methodology, which is the subject that has within its task, to prepare the student for this purpose.

Figure 4: Results of the interviews before and after applying the strategy.
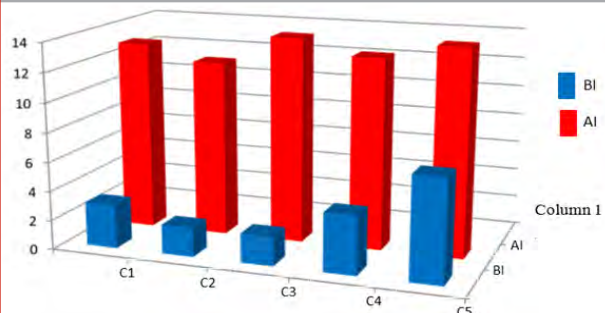
The authors agree with García & Reques (2012) about the use of these tools in the teaching of Geography where it should be kept in mind that the Internet is a means not an end, that data should not be confused with information, nor information with knowledge.

In addition, García and Pascual (2012) point out the dual role of the teacher as a model of discipline, authority and for teaching to think, and as a social animator who creates learning situations and promotes their development in an adequate manner. It is also necessary to count on the disposition of students towards learning with the use of Information and Communication Technologies.

In this regard, Esteban et al. (2018) expressed about adapting to the changes that today's society demands, where the master class has already been left behind to give way to the class with technological, audiovisual and interactive media, which has greater prominence.

On the other hand, the training of students must be in accordance with today's society, in an integral way where it enables them to apply professional knowledge, in a responsible and regulated way under the respect, not only to the procedures and adequate decision-making processes, but also within the respect for values and attitudes proper to the profession such as professional ethics, as expressed by González and Méndez (2017), elements with which the researchers agree.

In conclusion, the actions applied as a result of the didactic strategy contributed to improve the TLP in the students of the first year of the Regular Day Course of the Geography career of the Faculty of Secondary Education of the Central University "Marta Abreu" of Las Villas.

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Localization of neuropeptide Y(NPY) Immunoreactivity in the Proximal and Distal Intestinal Region of Teleost Fish, *Notopterus Notopterus*

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ABSTRACT

The purpose of the present study was to investigate the immunocytochemical aspects of the intestine of the teleost fish *N.notopterus*. The distribution and relative frequency of the endocrine cells in the intestine were studied immunocytochemically using Streptavidin biotin peroxidase complex method. The aim was analyse the distribution and localization of immunoreactivity in the intestine of *Notopterus notopterus* against the antisera Neuropeptide Y (NPY). The samples were taken from the proximal and distal intestine. The NPY immunoreactive endocrine cells were found in the distal intestine at low frequencies than proximal intestine. These immunoreactive cells were distributed among more in mucosa epithelium and very low in the lamina propria and submucosa of intestine of *N.notopterus*.

KEY WORDS: IMMUNOCYTOCHEMISTRY , INTESTINE, TELEOST, *NOTOPTERUS NOTOPTERUS*

INTRODUCTION

The intestine in the carnivorous fish is shorter than that of omnivores and herbivores. The intestine of the fish plays an important role in digestion absorption of dietary nutrients and also involved in immunological functions (Cao et al.2011; Salianas et al .2011).Morphometric features are important in identifying the fish species and their interaction in various habitats like freshwater, rivers and seas (Akombo et al.2011). Gastrointestinal tract (GI) of fishes can be divided into four topographical regions headgut, foregut, midgut and hindgut. Although in some cases there is no clear morphological distinction between the midgut and hindgut, morphometric characteristic of intestine of *N. notopterus* was performed .

The endocrine cells secreted the gastrointestinal hormones which are distributed throughout the mucosa of gastrointestinal tract(GI). A great variety of endocrine type secretions are generated in the mucosa of the gastrointestinal tract of fish. Peptides are the name given to these secretions. For the last 15 years more than 45 gastrointestinal

peptides have been determined in studies on fish GI tract. Neuropeptide Y(NPY) was isolated and sequenced for the first time from swine brain (Tatemoto, 1982, Bjorgen and Koppang 2021).

It has been characterized as one of the most highly conserved neuroendocrine peptide through out the evolution. NPY has been involved in the regulation of a wide range of physiological central effects like the control of appetite, body weight homeostasis, the modulation of reproductive processes. This paper examines the distribution and localization of endocrine cells in the proximal and distal intestine of teleost fish *N. notopterus* using immunocytochemical study.

The Asian Knifefish *(N. notopterus)* is a carnivorous and predatory fish. Intestine of the carnivorous fishes is short or more or less straight, because meaty foods can be digested more readily than vegetable ones. This fish accepts most kind of live frozen food. Some specimen accepts pellets and dry food. It is available in India, Pakistan, Bangladesh, Nepal, Thailand, Malaysia, and Indonesia.. Body is highly compressed, dorsal and ventral profile almost equally convex. Hence in the present investigation, immunocytochemical were carried out.

MATERIAL AND METHODS

Sample collection: Twelve live adult *N. notopterus* of either sex with body length ranging from 19-22 cm , weighing 80-100 gm were collected from fresh water pond called as Chatri Talab, Amravati, Maharashtra, India (200 56' N, 770 47' E). Fishes were anaesthetized with 2-phenoxy ethanol (1:2000) for 10-15 mins in the anaesthetic tank. After being anaesthetized, the fish was scarified by transcardial perfusion by ice cold Phosphate Buffer Saline (PBS, pH 7.45) solution and processed for immunocytochemical studies.

ImmunocytochemicalStudy: For immunocytochemical study, the intestine was excised, separated into proximal and distal region and fixed in Bouins fluid for 24 hours. After fixation, fixative was removed by using cold sucrose solution prepared in PBS. The tissue were cryoprotected in 10% sucrose for 2 hours, 20% sucrose for 2 hours and 30% sucrose for overnight at 4^0 C .Cryoprotected tissues were embedded in 15% Polyvinyl Pyrrolidone Phosphate (PVP) on cryoprotected peg.12-20 micron thick sections were obtained by cryostat -29^0 C and spread on the slides subbed with poly L-lysine (sigma).The slides were preserved in deep freezer and proceed for immunocytochemical staining. Immunocytochemical staining was carried out by using Streptavidin–biotin peroxidase complex method-The slides were transferred to humidity chamber and sections were washed in PBS (3×5 min) and then washing with Triton X 0.5 % (20 min).

Table 1. Morphometric characteristic of *Notopterus notopterus*

Notopterus notopterus	Fish total body length(cm)	Fish standard length(cm)	Digestive tract length(cm)	Intestine length (cm)	Weight (gm)
Mean ± SD	20 ± 1	19.5 ± 1	6.333 ± 0.3	5 ± 0.5	90 ± 10

Table 2. Morphological different cells in proximal and distal intestine of *Notopterus notopterus*

Types of cells	Proximal per villi/per sec	Distal per villi/per sec
Round	++	++
Tadpole	+++	++
Ovale	++	+
Triangular	++	+
Sac like	+	+
Rod like	+	+
Elongated	+	+
Cucurbit like	+	+

+ -- average , ++ --moderate, +++ -- maximum

Table 3. Meannumber of endocrine cells per intestinal folds (Mean ± SD) in *N.notopterus* (120 intestinal fold were examine for NPY antisera)

	NPY like immunoreactivitiy from 120 folds divided into 3 groups	
Intestinal region (N.notopterus)	Proximal	Distal
Mean ± SD	68.66 ± 1.52752	35.6 ± 6.4291005

The sections were treated with blocking solution 1% BSA (30 min).3)The sections were incubated with primary antibody (NPY Sigma ,Cat # N-9528) at dilution of 1:2000 with PBS containing 0.3 % Triton X-100 1% BSA and then washed with PBS(3×5min).4) The sections were incubated with secondary antibody (Biotin IgG complex, Sigma, Cat #BA2) for 2 hours and then washed in PBS (5 min).4) The sections were incubated with Streptavidin peroxidase conjugate at dilution (1:100) for 2 hours and then washed in PBS(3×5 min) and then double distilled water (2×5 min).5) For visualization of reaction 3-Amino -9-ethylcarbazole (AEC) solution were applied on sections for 5-10 min until reddish brown colour appear .6)Then slides were washed in double distilled water (2×5 min) and mounted with glycerol gelatin.7) The sections were examined with light microscope and photograph were taken.

RESULTS AND DISCUSSION

The sections obtained from the proximal and distal intestinal tissue of *N. notopterus* were formed by mucosa, submucosa , muscularis and serosa layer which is similar to the basic organization of intestinal wall of other teleost fishes according to the histological studies (Fig.1).

In the present investigation, essentially NPY immunoreactive (IR) endocrine cells were observed in the epithelium of intestinal mucosa and few existed in the lamina propria and submucosa of the *Notopterus notopterus*. Density of NPY IR endocrine cells were more pronounced in the proximal intestine than the distal intestine. All NPY IR cells were stained dark brown colour. Two types of NPY IR endocrine cells were observed in both proximal and distal intestine –open type and closed type. Numerous open type tadpole like NPY IR endocrine cells having long cytoplasmic processes extending to the lumen and basal process extending to the lamina propria were observed in the mucosal epithelium (Fig.2,4).

Open type rod like,(Fig2),Spindle shaped(Fig.6),Pyramid like with short cytoplasmic process extending to the lumen and basal broad process extending to the

lamina propria,(Fig.,6),elongated (Fig.5), Piriform like(Fig.6),Triangular like (Fig.6).NPY IR endocrine cells were observed in the intestinal mucosal epithelium. Open type Sac like NPY IR endocrine cells with apical cytoplasmic processes extending to the lumen, the basal part narrow extending to the lamina propria & middle part of the cell body to be broader are observed in the apical, middle and basal part of the intestinal mucosal epithelium. (Fig.3)Closed type oval like and round like(Fig.7) NPY IR endocrine cells were observed in the intestinal mucosal epithelium.

Figure 1: Transverse section through proximal intestine showing different layers Mucosa (MU), Sub mucosa (SM), Muscularis (MU), Longitudinal Muscle Layer (LML),Circular Muscle Layer (CML), Lamina propria (Lp) and Lumen(Lu) (X 100).

Figure 2: Magnified view of open type tadpole like and rod like NPY IR endocrine cells.

Figure 3: Magnified view of open type sac like and pyramid like NPY IR endocrine cells.

Figure 4: Open type triangular and tadpole like NPY IR endocrine cells.

Figure 5: Magnified view of open type triangular and elongated NPY IR endocrine cells (X400).

Figure 6: Magnified view of open type pyramid like and spindle shaped open type NPY IR endocrine cell (X1000).

Fig.1   Fig.2   Fig.3   Fig.4   Fig.5   Fig.6
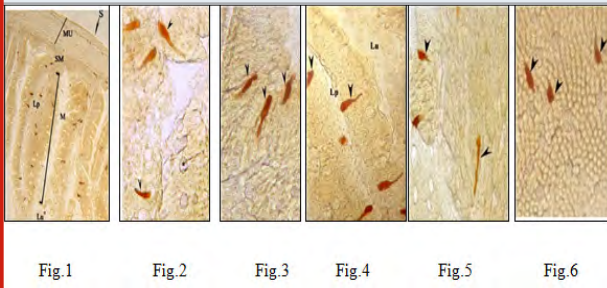
Tadpole like open type NPY IR endocrine cells with one long cytoplasmic processes (fig.8), two long cytoplasmic processes(fig.11) extended to the lumen and basal process extending to the lamina propria are observed in the intestinal mucosal epithelium.Open type elongated, Spindle shaped and Piriform like (fig.8), Cucurbit like(fig.9) NPY IR endocrine cells were observed in the intestinal mucosal epithelium Open type triangle like NPY IR endocrine cells with long cytoplasmic process extending to the lumen and basal process to the lamina propria in the intestinal mucosal epithelium.(fig.8).

Sac like open type IR endocrine cells were observed in the intestinal mucosal epithelium (fig.10) and in sub-mucosa. Closed type oval like NPY IR endocrine cells were observed

in the lamina propria. Closed type round like NPY IR endocrine cells were observed in the intestinal mucosal epithelium (fig.12).

Figure 7: Magnified view of closed type round NPY IR endocrine cells.

Figure 8-12: Transverse section through distal intestine of Notopterus notopterus showing NPY immunoreactive (IR) endocrine cells.

Figure 8: Open type tadpole like, piriform, trianglular, spindle shaped NPY IR endocrine cell (X400).

Figure 9: Open type cucurbit like NPY IR endocrine cell (X400).

Figure 10: Magnified view of sac like NPY IR endocrine cell (X1000).

Figure 11: Magnified view of elongated NPY IR endocrine cell with two long cytoplasmic processes (X1000).

Figure 12: Closed type round NPY IR endocrine cell (X400).

Fig.7   Fig.8   Fig.9   Fig.10   Fig.11   Fig.12
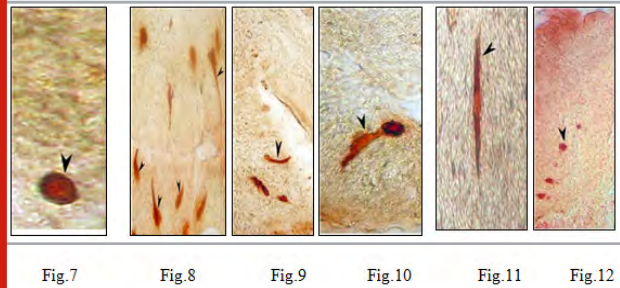
Studies concerning regulatory peptides in fish intestine have been carried out since last several years. But intestinal NPY like endocrine cells have not been previously documented in species of *N.notopterus*. In the present, we have studied the distribution and localization of NPY regulatory peptide from intestine of the fish *N.notopterus*. In the present study immunoreactive endocrine cells were detected more in the mucosal epithelium of proximal intestine than distal intestine. Similar results of NPY were reported in *Anguilla anguilla* (Domeneghini et al.2000 ; Youson et al.2001) and with variation to above results less amount of NPY immunoreactive cells were observed in the intestine of Salmotrutta (Dezfuli et al. 2000) and *Stizostedianlucio perca* (Ozen et al.2010).

Open and closed types of endocrine cells were observed in the intestine of *N.notopterus* in the present study. In open type, hormones were carried to the gut lumen (lumen endocrine)by long apical cytoplasmic processes (Liu et al. 2003; Pan and Fang 1995) sand in closed type cells it is covered by lumen epithelia (Liu et al.2003 ;Talatar and Simsek 1993).The endocrine cells of the gastrointestinal tract were different in the relative frequency of regional distribution and cell types between species (Leet al.2004;

Pan et al. 2000;Reverter et al.2001; Salianas et al.2011 Bjorgen and Koppang 2021).

CONCLUSION

The distribution and relative frequency of immunoreactive endocrine cells in *N. notopterus* are similar to that of other teleost fishes. However few difference in morphology of immunoreactive endocrine cells were observed. From morphometric analysis of the intestine of *N. notopterus* it was confirmed that intestine is relatively shorter in length as compared to other teleost fishes. NPY may involved in many physiological functions like regulation of food intake and modulation of reproductive processes. These studies could be extended to study for the immunology of this fish.

Authors' Contribution: YG, TK and SC, were responsible for conceptualization and design of the research. TUK, YG were responsible for specimen collection. SC and TK were responsible for result analysis and interpretation. TUK and YG were responsible for laboratory techniques. All authors contributed equally in literature research, manuscript preparation, editing and review.

Statement of Ethics: The study was approved by the Institutional Animal Ethics Committee ((IAEC) Of Government Vidharbha Institute of Science and Humanities, Amravati.

Declaration of Conflicting Interests: The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ecology of Biofouling Phytoplankton in Chinnamuttom Harbour Waters Southeast Coast of India

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ABSTRACT

Phytoplankton are the primary source of food, attributed most zooplankton communities and larval stages, and many meroplankton such as bivalves, crustaceans, and fishes. In the present study, phytoplankton samples were collected from surface water and substratum of the ship hull during the period of June 2015 to May 2016. Population density recorded from water and substratum of the ship hull ranged from 1.5e+4 to 3.4e+4 cells/L. Shannon-Wiener diversity index (H′) of station I and station II ranged from 5.448 to 6.134 bits/ind. Simpson's richness range was 0.973 to 0.984 at station I and station II. Pielou's Evenness index (J') was found to be from 0.950 to 0.979 and Chlorophyll 'a' range was 0.277 to 1.326 mg/m3 in the station I and station II. During this entire study period, 74 species of phytoplankton and 54 species of phytoplankton in station II have been recorded. Data of the present study show that significant differences occurred in over all percentage composition of phyhtoplankton species, both in in water and substratum. In water samples, recorded data showed that 85% were of *Bacillariophyceae*, 8 % of *Chlorophyceae* and, 7% of *Dinophyceae*. On the other hand, 75 % *Bacillariophyceae*, 7 % *Chlorophyceae*, 16% *Dinophyceae* and 2% *Cyanophyceae* were recorded in the substratum. The marine biofouling phytoplankton density, diversity, richness, evenness and chlorophyll'a' were recorded in their maximum levels during summer season, and minimum were obtained in the monsoon. The diatoms were the predominant groups among the phytoplankton. Among the diatoms, species like *Nitzschia longissima, Coscinodiscus centralis* and *Fragilaria* sp. were record almost in all the seasons in the present study.

KEY WORDS: CHLOROPHYLL, PHYTOPLANKTON, POPULATION DENSITY, SUBSTRATUM, WATER

INTRODUCTION

Phytoplankton by virtue of drifting habits in illuminated waters of our planet and short turnover periods link the oceanic food web and its trophic marine structures. These are extremely diverse energy sources, distributed across major eukaryotic lineage, in food web of the aquatic ecosystems, playing a key role in planktonic communities, ecological structure, function, and the dynamic fate of the biota, (Mattei and Scardi 2021). In marine ecosystems, the phytoplankton (<1μm to ≈1mm length in diameter) represent at least five eukaryotic phyla, which are one of the world's simplest, basic and dominant key producers of the primary marine food chain, (Sun et al. 2000).

Anthropogenic discharge of nutrient load to sea waters, increase of global carbon emission and their mix with wide oceanic surface water, multitude seasonal hydrogeochemical changes impacts on the primary producers. Hence about 45% of global net primary productivity plays a critical role in biogeochemical functions and production of climatically active gases. The earlier estimate of Geider et al. (2014) indicates that, the extensive range in size and desperate genetic diversity challenges the phytoplankton quantification and characterization, (Kim and Kim 2021). Species composition, density, abundance, richness and primary productivity of phytoplankton vary in coastal geography, which is liable to physiological and hydro-

biological fluctuation (Al-amri et al. 2020). Physicochemical values, phytoplankton and zooplankton species, and their composition, abundance, seasonal variations have been studied in various regions of Indian coastal waters (Vajravelu et al. 2018).

Significantly, the phytoplankton are the major contributors in absorbing atmospheric carbon than the terrestrial plants, also, these plankton takes a major role in global carbon dioxide sequestration, oxygen production, nutrient cycle, and carbon fixation and contributes to global warming and the existence of aquatic lifeforms (Paffenhofer, 1993). Consequently, assessing the species composition and their density diversity is necessary to assess a healthy ecosystem.

Changes in phytoplankton ecology and diversity are driven by several major unresolved conceptual challenges, perhaps the foremost of which is the marine pollution of fouling. Analysis of diversity and ecology of phytoplankton has largely benefited many research fields in biology as it is entering a new era with the advent of fostering the improvement of the predictive tool of phytoplankton distribution at global scales. Hence, the present study-was focused on diversity, density, species composition, richness, and evenness of phytoplankton community in the fishing harbor environment concerning the prevailing hydrographical conditions of Chinnamuttam area which harbours waters of southeast coast of India.

MATERIAL AND METHODS

Sampling program: A total of four sampling sessions were conducted during the study period i.e. June 2015 to May 2016, between the hours of 06.00 hrs and 12.00 hrs.

Collection of samples: The phytoplankton sampling was done using standard Indian Ocean hand net (48μm) by towing horizontally in water surface for 30 minutes, as well the sampling from substratum of ship hull was collected by scraping using sterile spatula (Manickam et al. 2017).

Preparation of the sample: 10ml/L Lugol's iodine was added into samples and kept standing for 24hrs to ensure complete sedimentation followed by centrifugation if necessary. The supernatant liquid was removed using pipette and the sample was further concentrated up to 10-100ml depending on the number of plankton.

Fixation and Preservation of samples: The formalin was added in the ratio of 5-10 to 90 parts(v/v) and the bottle was inverted for fine dispense. The pH of the solutions was maintained at 7.6 – 8.3.

Observation of phytoplankton: The observation of phytoplankton was done by exposing the concentrated sample to the Light microscope (COSLAB). The phytoplankton observed under microscope were taken with the support of a digital camera (MDCE-5C).

Identification of phytoplankton: The identification of phytoplankton was carried out with the help of standard books, identification manuals and classical works of Subramanyan (1946); Subramanyan (1968); Venkataraman (1939); Tomas (1997and Mitra et al. (2004).

Plankton enumeration using Sedgwick-Rafter Cell method: One ml of well mixed plankton samples was poured into the Sedgewick-Rafter cell using graduated pipette and uniformly spread as thin layers. The plankton numbers were counted by selecting squares randomly. The plankton density was estimated using the following formula. Calculation N = n x v/V Where, N = Total number of plankton cells / L, n = Average number of plankton cells in 1 ml of sample v = Volume of plankton concentrate, V = Total volume of water filtered (L).

Diversity Analysis of plankton samples: The diversity indices (DI) of phytoplankton in Chinnamuttom harbour was calculated using Shannon Weiner's Index (H'), Simpson richness index (D') and Evenness index (E'), which was calculated by using PAST– Palaeontological Statistics Ver. 2.00 software packages.

Chlorophyll- 'a' : 250 ml of collected water sample was filtered through (0.45 diameter GF/ C filter paper) Millipore filtering unit. Then filtered paper was soaked in acetone and kept in dark for 20–24 hours under refrigeration to extract the chlorophyll. The centrifuged concentration of chlorophyll extracts measured spectrometric at 665nm (APHA, 2010).

RESULTS AND DISCUSSION

Species composition: The phytoplankton samples collected from Chinnamuttom fishing harbour was analysed for the one-year period from 2015 to 2016. In the one year study period, totally 74 phytoplankton species were recorded, viz.. 57 species of Bacillariophycea, 9 species of Dinophycea and 8 species of Chlorophycea in water sample, whereas in the substratum of the ship hull 59 phytoplankton species were recorded. Among the total of 59 species, 45 Bacillariophycea species, 8 Dinophycea species, 4 Chlorophycea species, and 2 species of Cynophycea were noted. Similar, study was conducted recently in southeast coast of India and recorded five different classes (Vajravelu et al. 2017)

Among the recorded species of sampling stations (Bacillariophyceae, Dinophyceae, Cyanophyceae and Chlorophyceae) diatoms were found to be dominant contributor (75–85%) of the total phytoplankton. Especially, species such as Nitzchia spp., constituted the maximum numbers followed by Navicula spp., and Coscinodiscus spp. The domination in phytoplankton group was in the order: Diatom > Dinoflagellates > Green algae > Blue-green algae.

Percentage composition: Phytoplankton comprised of Bacillariophyceae (diatoms), Dinophyceae (Dinoflagelates), Chlorophyceae (green algae) and Cyanophycean (blue green algae). The recorded phytoplankton number from the collected samples were composed of 85% by Bacillariophyceae, 8% Dinophyceae and 7% by Chlorophyceae, were recorded in water sample (Figure 1

and 2). All these groups were present in all four seasons. In substratum of the ship hull, the recorded compendial count of 75% Bacillariophyceae, 16% Dinophyceae, 7% Chlorophyceae and 2% Cyanophycean (Figure 1). Therefore, totally 74 and 59 phytoplankton species were recorded in water and substratum of the ship hull in Chinnamuttom harbour respectively during the study period.

Figure 1: Percentage composition of phytoplankton in Chinnamuttom Harbour water during June 2015-May 2016
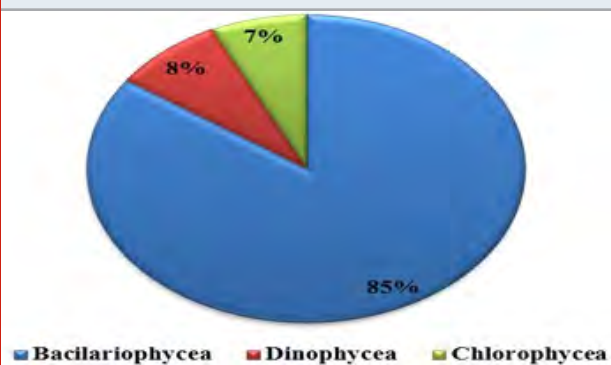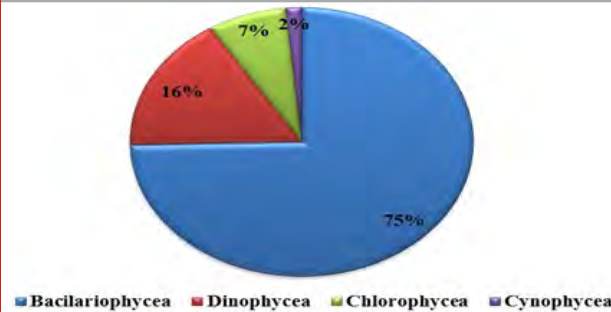

Figure 2: Percentage composition of phytoplankton in Chinnamuttom Harbour substratum during June 2015 - May 2016

Population density: Population density of phytoplankton was recorded from water and substratum of the ship hull, in the range between 15000 - 34000 cells/L. The minimum density was recorded during monsoon (1500 cells/L) in the substratum of the ship hull, while the maximum value (34000 cells/L) was noticed during summer in the water sample (Figure 3). In the present study, the phytoplankton population density was found to be high (15000 cells/L) during summer could be 77 attributed to stability in hydrographical conditions, and minimum density (34000 cells/ L) was obtained during monsoon due to low salinity and high turbidity due to rainfall (Senthilkumar et al. 2002). A similar increased number of phytoplankton species significant with high salinity increase in Bay of Bengal were reported (Rajasekar et al. 2005).

Species richness: An analysis of the phytoplankton species richness at Chinnamuttom harbour was noticed to be the range between 0.973 and 0.984. The minimum (0.973) richness during monsoon in substratum, and maximum

(0.984) was during summer in the water sample (Figure 5).

Figure 3: Seasonal variations in phytoplankton density during June 2015 - May 2016
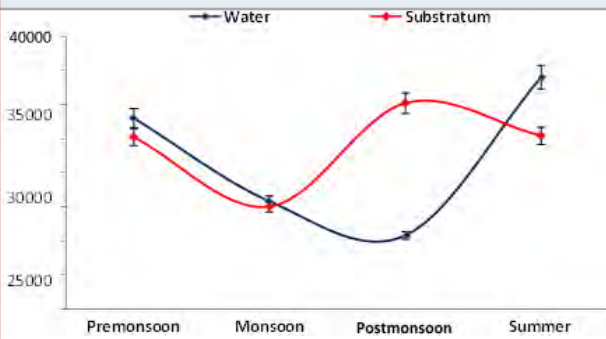
Figure 4: Seasonal variations in phytoplankton species diversity during June 2015-May 2016
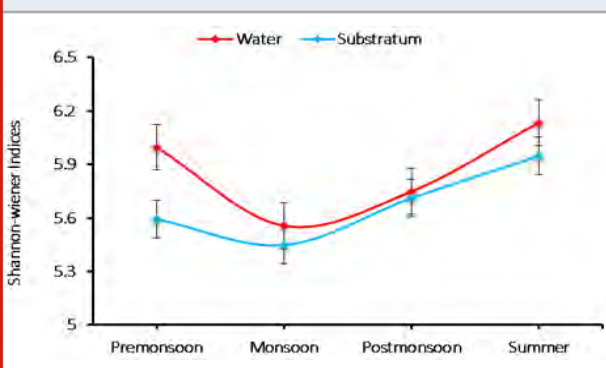
Figure 5: Seasonal variations in phytoplankton species richness during June 2015-May 2016
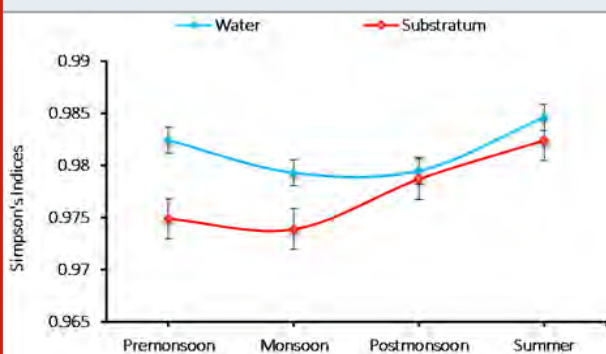
The richness index of species (0.973-0.984 cells/L) are between 0.973 and 0.984. In substratum, minimum species richness was 0.973 during monsoon, and the maximum was 0.984 during summer in the water. The recorded species richness variation was correlated with the recorded salinity values (Mani, 1992).

The species evenness: The evenness index was varied 0.950 to 0.979 cells/L in phytoplankton species. The minimum species evenness was recorded 0.950 cells/L during

monsoon in the substratum and the maximum 0.979 cells/L was observed during summer in

Figure 7: Seasonal variations in chlorophyll 'a' concentration during June 2015-May 2016
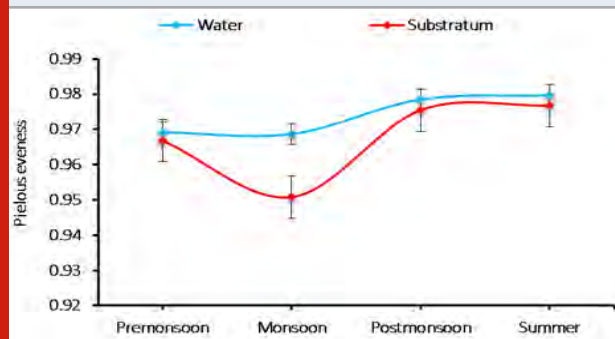
Chlorophyll 'a: chlorophyll 'a' ranged between (0.277 to 1.326 mg/m3), the higher concentration of 1.067mg/m3 was found in summer season. Significant high population of phytoplankton density during summer season was reported in Palk Bay coral reef region (Sridhar et al.,
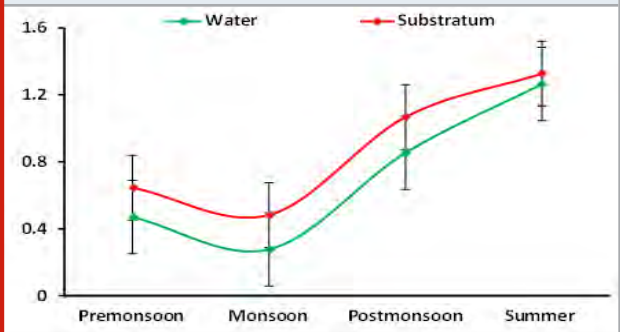
2010). The 0.277 mg/m3 concentration of low chlorophyll 'a' was obtained on monsoon season may be due to low phytoplankton production owing to high turbidity and low light availability due to high land drainage and cloudy sky (Kawabata et al. 1993, Godantaraman 2002, Rajasekar et al. 2005). The phytoplankton density, richness, composition, diversity, and evenness indices are significant to the variations in seasonal physicogeological condition variation (Figure 7).

Table 1. List of phytoplankton species recorded in Chinnamuttom Harbour during June 2015 – May 2016

| S. No.	Species Name	Water	Substratum
	BACILLARIOPHYCEAE (Diatoms)		
1	*Asterionellopsis glacialis*	+	+
2	*Bacteriastrum comosum*	+	+
3	*B. paradaxa*	+	+
4	*B. delicatula*	+	+
5	*Biddulphia aurita*	+	-
6	*B. sinensis*	+	+
7	*B. mobiliensis*	+	+
8	*Chaetoceros affinis*	+	+
9	*C. compressus*	+	+
10	*C. dydymus*	+	-
11	*C. longissimi*	+	+
12	*C. lorenzianus*	+	+
13	*C. messanensis*	+	+
14	*C. paruvianus*	+	+
15	*Coscinodiscus centralis*	+	+
16	*C. concinnes*	+	+
17	*C. gigas*	+	+
18	*C. jonesianus*	+	+
19	*C. marginatus*	+	+
20	*C. radiates*	+	+
21	*Diploneis robusta*	+	+
22	*Ditylum brightwelli*	+	+
23	*Fragilaria* sp.	+	+
24	*F. intermedia*	+	-
25	*F. oceanica*	+	+
26	*Gyrosigma balticum*	+	+
27	*Hemidiscus cuneformis*	+	+
28	*H.hardmannianus*	+	-
29	*Hyalodiscus stelliger*	+	-
30	*Lauderia annulate*	+	-
31	*Leptocylindrus danicus*	+	-
32	*Navicula forcipate*	+	+
33	*N. granulate*	+	-
34	*N. longa*	+	+
35	*Nitzschia Closterium*	+	+
36	*N. longissimi*	+	+
37	*N. paradoxa*	+	+
38	*N. sigma*	+	+
39	*N. sigmoidea*	+	+
40	*Odontella mobiliensis*	+	+
41	*O. sinensis*	+	+
42	*Planktoniella sol*	+	+
43	*Pleurosigma angulatum*	+	+
44	*P. elongatum*	+	+
45	*Rhizosolenia setigera*	+	-
46	*R .robusta*	+	-
47	*R. setigera*	+	+
48	*R. styliformis*	+	+
49	*Skeletonema costatum*	+	+
50	*Thalassionema nitzschiodes*	+	+
51	*T. mobilienis*	+	+
52	*T. subtilis*	+	+
53	*Thalassiothix frauenfeldii*	+	+
54	*T. longissimi*	+	+

55	*Triceratium favus*	+	+
56	*Triceratium robertsianum*	+	-
57	*T. reticulatum*	+	-
	DINOPHYCEAE (Dinoflagellates)		
58	*Ceratium extensum*	+	+
59	*C. declinatum*	+	+
60	*C. furca*	+	+
61	*C. harridum*	+	+
62	*C. macroceros*	+	+
63	*C. minutam*	+	+
64	*C. trichoceros*	+	+
65	*Dinophysis caudate*	+	+
66	*Dinophysis* sp.	+	-
	CYANOPHYCEAE(Blue-Greens)		
67	*Trichodesmium erythraeum*	-	+
68	*Spirulina meneghiniana*	-	+
	CHOLOPHYCEAE(Greens)		
69	*Chlorella vulgaris*	+	-
70	*C. marina*	+	+
71	*Clostriopsis longissimi*	+	+
72	*Tetraselmis gracilis*	+	+
73	*Oedogoniumde sikaeharyii*	+	+
74	*Pediastrum simplex*	+	-
75	*Spirogyra indica*	+	-
76	*Spirogyra* sp	+	-

+ Presence; - Absence

Figure 8: Dominant phytoplankton species recorded at Chinnamuttom harbour during May -June months

Nitzschia longissima　　　*Navicula* sp.

Coscinodiscus sp.　　　*Fragilaria* sp.

Asterionella sp.

CONCLUSION

Data of the present study show that significant differences occurred in over all percentage composition of phyhtoplankton species, both in in water and substratum. In water samples, recorded data showed that 85% were of *Bacillariophyceae*, 8 % of *Chlorophyceae* and, 7% of *Dinophyceae*. On the other hand, 75 % *Bacillariophyceae*, 7 % *Chlorophyceae*, 16% *Dinophyceae* and 2% *Cyanophyceae* were recorded in the substratum. The marine biofouling phytoplankton density, diversity, richness, evenness and chlorophyll'a' were recorded in their maximum levels during summer season, and minimum were obtained in the monsoon. The diatoms were the predominant groups among the phytoplankton. Among the diatoms, species like *Nitzschia longissima, Coscinodiscus centralis* and *Fragilaria* sp. were record almost in all the seasons in the present study.

ACKNOWLEDGEMENTS

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Biomedical Communication

Knowledge and Management Assessment of Temporomandibular Joint (TMJ) Disorders Among Dental interns at King Saud University

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ABSTRACT

The temporomandibular joint (TMJ) complex is mainly composed of bone, cartilage, muscles, ligaments, and neurovascular channels that supply the tissues. The temporomandibular disorders (TMD) are collective term embracing a number of clinical problems that involve the masticatory muscles, the temporomandibular joints (TMJs) and associated structures, or both. The most common symptoms of temporomandibular joint disorders (TMDs) are pain, noises in the joint, limitation of mouth opening, or a combination of these. The objective of this study was to assess the knowledge and management of temporomandibular joint (TMJ) disorders among dental interns at King Saud University, Saudi Arabia. The sample size of this study consisted of 100 dental interns at King Saud University. Informed consent from the participants was obtained. The participants were informed that their responses and personal information would be kept confidential. A cross-sectional survey was prepared in English language using Google Forms. The survey contains 12 questions was distributed among the selected sample of dental interns at King Saud University. The average knowledge score of the dental interns on the knowledge regarding management of (TMJ) disorders was 4.04 (SD = 1.42). The maximum score obtained was 8 out of 8, the minimum was zero. that the majority of the dental interns (67%) had fair knowledge. The finding of the present study showed, there was a fair level of knowledge about (TMJ) disorders among dental interns. Many interns are unable to diagnose and treat patients suffering from temporomandibular joint disorders.

KEY WORDS: TEMPOROMANDIBULAR JOINT, KNOWLEDGE AND MANAGEMENT, DISORDERS AMONG.

INTRODUCTION

The temporomandibular joint (TMJ) complex is mainly composed of bone, cartilage, muscles, ligaments, and neurovascular channels that supply the tissues. The temporomandibular disorders (TMD) are collective term embracing a number of clinical problems that involve the masticatory muscles, the temporomandibular joints (TMJs) and associated structures, or both. The most common symptoms of temporomandibular joint disorders (TMDs) are pain, noises in the joint, limitation of mouth opening, or a combination of these (Glick et al.,, 2015). The most common musculoskeletal diseases affecting the temporomandibular joint and associated structures in the orofacial region

are temporomandibular joint disorders. Many dentists are unable to diagnose and treat patients suffering from temporomandibular joint disorders. the misdiagnosis of the orofacial pain of the temporomandibular joint associated with the dentoalveolar region, which leads to incorrect management (Al-Huraishi et al., 2020).

TMDs are diagnosed using a variety of methods including clinical examination, radiographic image such as: panoramic X-rays, conventional tomography (CT), Digital Volume Tomography, Arthrography, Magnetic Resonance Imaging (MRI), and Cone Beam-Computed Tomography (CBCT) (Petersson 2010). Pain relievers, nonsteroidal anti-inflammatory medications (NSAIDs), muscle relaxants, anticonvulsants (gabapentin), and tricyclic antidepressants (TCAs) are among the medications used to treat TMDs (López-Frías 2019).

In another study, Rabaa Aboubakr and Elkwatehy (2021) found that the knowledge level about TMDs was insufficient among undergraduates and dental intern students. The aim of this study was to assess the knowledge and management of temporomandibular joint (TMJ) disorders among dental interns at King Saud University, Saudi Arabia.

MATERIAL AND METHODS

The sample size of this study consisted of 100 dental interns at King Saud University. Informed consent from the participants was obtained. The participants were informed that their responses and personal information would be kept confidential. A cross-sectional survey was prepared in English language using Google Forms. The survey contains 12 questions was distributed among the selected sample of dental interns at King Saud University. The first three questions were designed based on an established Likert scale, the questions were related to the ability of undertake a clinical examination regarding TMJ, assess the treatment needs and management of TMJ and the third question was related to satisfaction of lectures and clinical training giving at undergraduate curriculum. The followed eight questions were to measure and assess the knowledge and management regarding temporomandibular joint (TMJ) disorders.

Data Analysis: All questionnaire data were included for analysis purposes in the SPSS 22 software (SPSS, Chicago, USA). Each element has been assigned to the value 1 in case of a correct answer and 0 if it is incorrect. Descriptive statistics were used to show the results in terms of frequencies, percentages, means and the standard deviation.

Figure 1
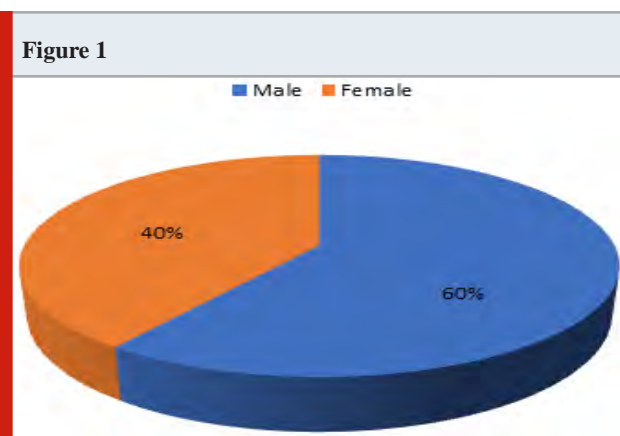
Table 1. The total number of participants in this study (N=100):

		Frequency	Percent
GENDER	Male	60	60.0%
	Female	40	40.0%
	Total	100	100.0%

RESULTS AND DISCUSSION

The total number of participants in this study was 100. Participants were recruited in this survey, 60% males and 40% females.

Competencies to undertake a clinical examination, assess the treatment needs and management regarding temporomandibular joint (TMJ) disorders: The participants were asked question about their competencies to undertake a clinical examination, assess the treatment needs and management regarding temporomandibular joint (TMJ) disorders. The results were presented in the following tables:

Table 2. Competency to undertake a clinical examination (N=100):

Responses	No	Percent	Mean	SD
Strongly agree	5	5.0	3.03	0.96
Agree	28	28.0		
Neutral	36	36.0		
Disagree	27	27.0		
Strongly disagree	4	4.0		
Total	100	100.0		

Figure 2
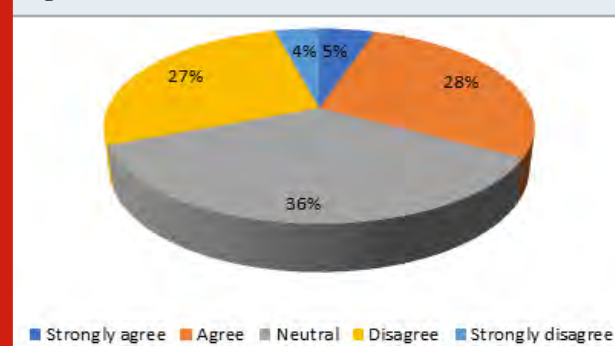
Table 3. Competency to assess the treatment needs and management (N=100):

Responses	No	Percent	Mean	SD
Strongly agree	2	2.0	2.84	0.84
Agree	19	19.0		
Neutral	43	43.0		
Disagree	33	33.0		
Strongly disagree	3	3.0		
Total	100	100.0		

As shown in Table 2, that only 33% of dental interns were able to undertake a clinical examination regarding (TMJ) disorders and reach to the correct diagnosis, while 31% of

them reported they do not have the ability to undertake a clinical examination regarding (TMJ) disorders and reach to the correct diagnosis. However, the high percentage of participants were not sure about that i.e. (did not give an exact response). Moreover, the average response toward this question was (3.03± 0.96) which tend to be neutral.

As shown in Table 3, the high percentage of participants (43%) (mean ±SD 2.84, 0.84) were not sure about their ability to assess the treatment needs and management regarding (TMJ) disorders, while 36% were not able to do that. However, only 21% of dental interns were able to assess the treatment needs and management regarding (TMJ).

Figure 3

■ Strongly agree ■ Agree ■ Neutral ■ Disagree ■ Strongly disagree
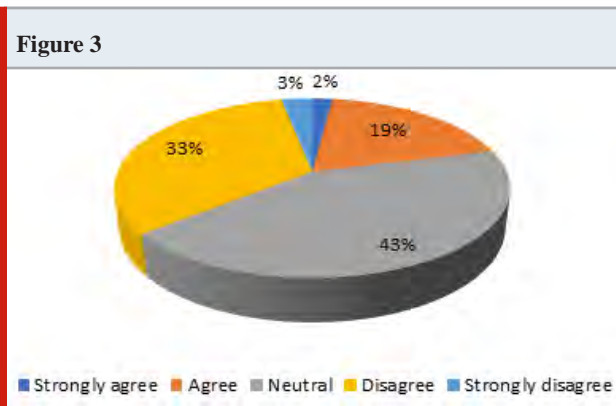
Table 4. Satisfaction of lectures and clinical training (N=100):

Responses	No	Percent	Mean	SD
Strongly agree	3	3.0	2.40	1.07
Agree	12	12.0		
Neutral	31	31.0		
Disagree	30	30.0		
Strongly disagree	24	24.0		
Total	100	100.0		
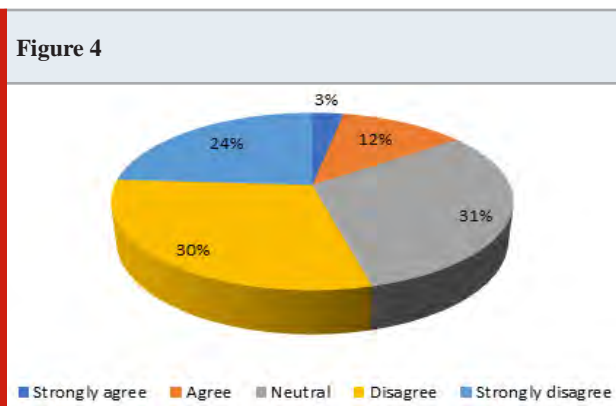
Figure 4

■ Strongly agree ■ Agree ■ Neutral ■ Disagree ■ Strongly disagree

Satisfaction of lectures and clinical training during undergraduate curriculum to diagnosis and manage (TMJ) disorders: The participants were asked question

about if they are satisfied about the coverage of lectures and clinical training during undergraduate curriculum to diagnose and manage (TMJ) disorders. The result was presented in the following table:

As shown in Table 4, more than half of dental interns (54%) reported that lectures and clinical training covered during undergraduate curriculum were not enough to diagnose and manage (TMJ) disorders. However, 31% were neutral about that i.e. (did not give an exact responses). On the other hand, only 15% of participants reported that lectures and clinical training covered during undergraduate curriculum were enough to diagnose and manage (TMJ) disorders.

Knowledge and management of (TMJ) disorders among dental interns at King Saud University: In this section of the study the knowledge and management of (TMJ) disorders were assessed among dental interns at King Saud University in Saudi Arabia. As shown in Tables 5, regarding dental interns' knowledge about (TMJ) disorders at King Saud University, that the overall percentage of correct answers to this subscale was 46.5%.

However, the results revealed that 68% of dental interns were knowledgeable that Ankylosis of the temporomandibular joint (TMJ) most often results from Trauma or infection. Moreover, 56% of dental interns had the knowledge that the most probable diagnosis for the case (A well-nourished 41-year-old female patient present with a chief complaint of painful limited mouth opening, her mouth deflects to the right side while opening, she reported receiving a trauma from her teenage son one week ago) was acute disc displacement without reduction in the right TMJ.

Moreover, the majority of dental interns involved in this study failed to answer the item (A 23-year-old former ballet dancer came to your clinic complaining of a loud clicking sound in both sides of her TMJs without any pain, her mouth opening is 55mm, with slight deviation to the right then to the left, started suddenly 16 months ago, during examination; clicking sound existed even after mandibular advancement, the most probably diagnosis for this case is) correctly by 76%. Also, 62% of dental interns failed to answer item (Which of the following statements is true regarding the temporomandibular joint?) correctly. As shown in Tables 6, regarding dental interns' knowledge about (TMJ) disorders at King Saud University, that the overall percentage of correct answers to this subscale was 36%.

However, the results revealed that the most item pertained to management of (TMJ) disorders that dental interns aware about the treatment of dislocation, that 70% of dental interns had the knowledge that dislocation is treated by forcing the mandible downward and backward. As well, 51% of dental interns had the knowledge that best way to control painful anterior disc displacement cases was by administering non-steroidal anti-inflammatory drugs.

Moreover, that the most item that dental interns failed to answer correctly was "A 35-year-old female teacher came to your clinic complaining of severe pain in the left side

of her jaw started three months ago, her medical history was significant for systemic osteoarthritis, that she's been diagnosed last year. Her pain is severe with dull aching quality. She is using paracetamol, but it doesn't help anymore, her mouth opening is limited because of the pain. The best way to control the pain in this case is achieved by:", 90% of them failed to answer this item correctly.

Table 5. Frequency distribution of true and false answers on [knowledge about TMJ disorder (n=100)

Items	Answers	
	Correct	Incorrect
Which of the following statements is true regarding the temporomandibular joint?	38%	62%
Ankylosis of the temporomandibular joint (TMJ) is defined as immobility or fusion of the joint. This condition most often results from which of the following?	68%	32%
A 23-year-old former ballet dancer came to your clinic complaining of a loud clicking sound in both sides of her TMJs without any pain, her mouth opening is 55mm, with slight deviation to the right then to the left, started suddenly 16 months ago, during examination; clicking sound existed even after mandibular advancement, the most probably diagnosis for this case is	24%	76%
A well-nourished 41-year-old female patient present with a chief complaint of painful limited mouth opening, her mouth deflects to the right side while opening, she reported receiving a trauma from her teenage son one week ago. What is the most probable diagnosis for this case?	56%	44%

Table 6. Frequency distribution of true and false answers on [management regarding TMJ disorder (n=100)

Items	Answers	
	Correct	Incorrect
A 35-year-old female teacher came to your clinic complaining of severe pain in the left side of her jaw started three months ago, her medical history was significant for systemic osteoarthritis, that she's been diagnosed last year. Her pain is severe with dull aching quality. She is using paracetamol but it doesn't help anymore, her mouth opening is limited because of the pain.	10%	90%
The best way to control the pain in this case is achieved by: In painful anterior disc displacement cases, which one of the following modalities is the best way to control pain?	51%	49%
A 23-year-old former ballet dancer came to your clinic complaining of a loud clicking sound in both sides of her TMJs without any pain, her mouth opening is 55mm, with slight deviation to the right then to the left, started suddenly 16 months ago, during examination; clicking sound existed even after mandibular advancement, the most probably diagnosis for this case is:	13%	87%
Dislocation is treated by forcing the mandible	70%	30%

Also, 87% of dental interns involved in this study failed to answer the item "A 23-year-old former ballet dancer came to your clinic complaining of a loud clicking sound in both sides of her TMJs without any pain, her mouth opening is 55mm, with slight deviation to the right then to the left, started suddenly 16 months ago, during examination; clicking sound existed even after mandibular advancement, the most probably diagnosis for this case is".

Knowledge scores regarding Knowledge and management of (TMJ) disorders among dental interns at King Saud University: The knowledge score was classified as follow: Poor knowledge (<4) , Fair knowledge (4-6) and good knowledge (7-8) (Table 7).

Table 7. Knowledge scores regarding TMJ (n=100)

Knowledge Level	No.	Percentage			
Poor (<4)	31	31%			
Fair (4-6)	67	67%			
Good (7-8)	2	2%			
Total	100	100.00%			
Correct answers		Min.	Max.	Mean	SD
		0	8	4.04	1.42

As shown in the above Table, that the average knowledge score of the dental interns on the knowledge regarding management of (TMJ) disorders was 4.04 (SD = 1.42). The maximum score obtained was 8 out of 8, the minimum was zero. As presented in Table 6, that the majority of the dental interns (67%) had fair knowledge. Many studies have been done to assess the knowledge and management regarding TMDs among general practitioners and dental students but none of them has been done on dental interns. This the study has been conducted on dental Interns only.

According to the current survey, the average score of the dental interns on the knowledge and management regarding (TMJ) disorders was fair. The participants were asked question about if they are satisfied about the coverage of lectures and clinical training during undergraduate curriculum to diagnose and manage (TMJ) disorders, the result was more than half of dental interns (54%) reported that lectures and clinical training covered during undergraduate curriculum were not enough to diagnose and manage (TMJ) disorders.

Many studies have been conducted to assess the level of knowledge and management regarding TMDs among general dental practitioners (GDPs) and dental students. Aboubakr and Elkwatehy (2021) study which was conducted among undergraduate dental students and interns revealed that the knowledge level among the participants regarding TMDs was not sufficient as the percentage of participants who answered correctly to the questions was less than 50% for all questions except in two questions they were more than 50% which were asking about the common medications prescribed for TMDs, another survey by Elyas

et al. (2021) revealed that general dental practitioners had fair level of knowledge regarding TMDs.

This finding is also in accordance with a survey by Patil and Iyengar (2016) showed evident that GDPs had fair level of knowledge regarding TMDs, whereas the majority of the experts had a good level of knowledge. Thus, a significant difference in the level of knowledge was observed between the two groups. Also, Rahmeier et al. (2021) revealed that the students had little knowledge regarding TMD and bruxism due to the insufficient credit hours offered by the curriculum. Le Resche et al. (1993) reported that general dental practitioners (GDPs) had low knowledge level regarding pathophysiology, diagnosis and treatment in comparison to TMDs specialists. However, Ashwin and Siri (2018) revealed good knowledge level among their postgraduate participants. In addition, López Frías et al. (2019) showed sufficient knowledge among postgraduate students regarding etiology and diagnosis of TMDs in comparison to lower knowledge in management of the same condition among their GDPs participants.

In dentistry and educational programs regarding TMDs, importance is given to applying and converting learned knowledge to diagnose and manage TMDs in clinical practices. Thus, the current study emphasizes the importance of developing and improving undergraduate curricula to provide dental practitioners with both theoretical and practical knowledge regarding the diagnosis and management of TMDs. Continuing dental education programs, fellowships, and TMJ workshops may help dental practitioners to improve their skills and knowledge.

CONCLUSION

The anatomy and pathophysiology of the TMJ are important topics in the curriculum of dental students that help with the proper diagnosis and management of TMJ disorders. The finding of the present study showed, there was a fair level of knowledge about (TMJ) disorders among dental interns. Many interns are unable to diagnose and treat patients suffering from temporomandibular joint disorders. Further studies on a larger scale with a larger sample size are required to get a proper overview regarding this topic among dental interns.

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