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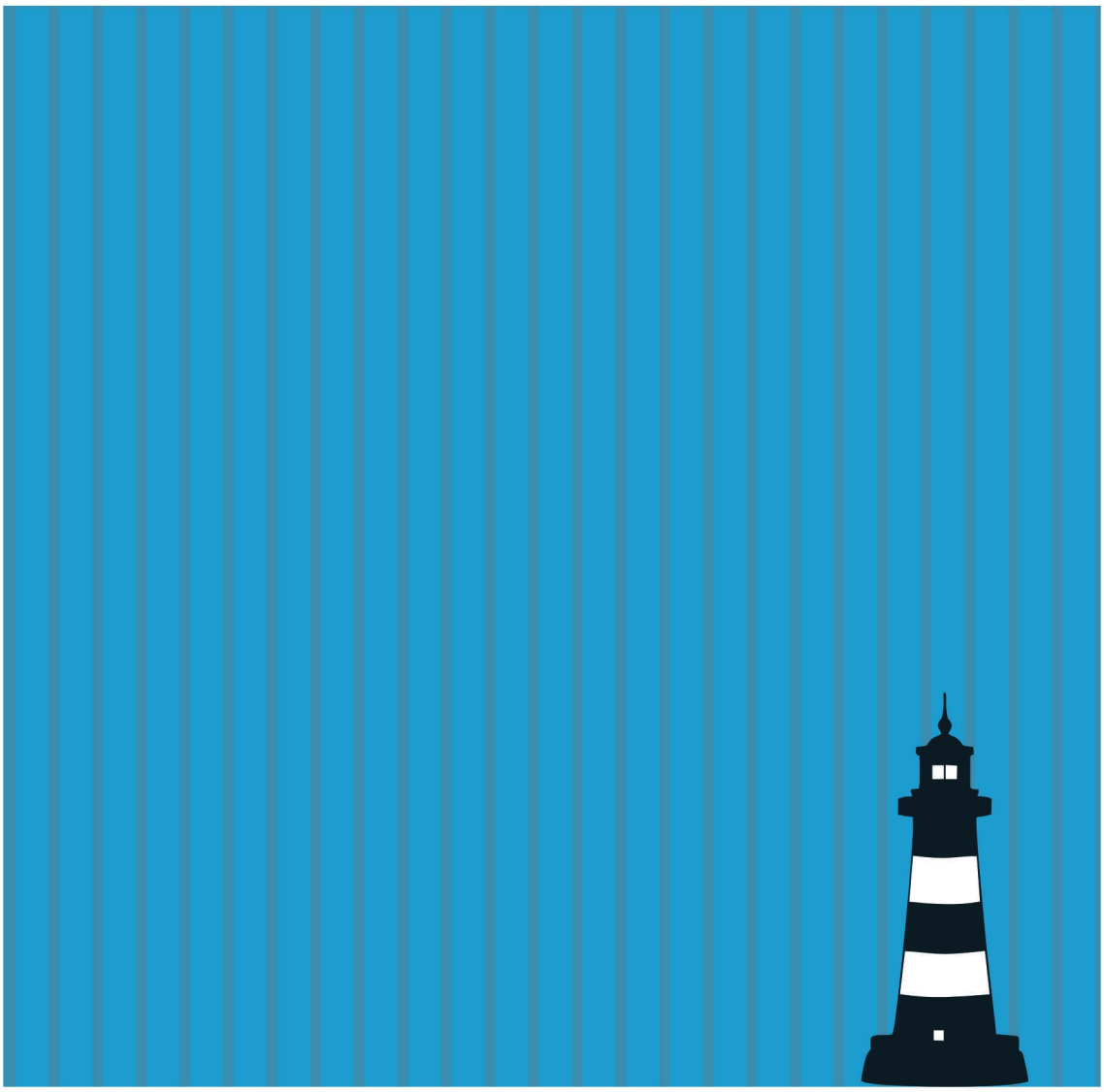

Khyber Pakhtunkhwa Higher Education Academy of Research and Training (HEART)

Message from Patron in Chief

Khyber Pakhtunkhwa Higher Education Academy of Research and Training (HEART) successfully added to the professional capabilities of Associate professors, Assistant professors and Lecturers of the Higher Education Department by imparting quality trainings on updated modules to meet the academic challenges inside the classrooms. Nevertheless, no stone is left unturned to enhance the capacity of DDOs and ministerial staff working in offices at government colleges across Khyber Pakhtunkhwa. The continuous pursuit to excel in the area of training has made HEART a topping professional training institution in KP. Despite the monumental achievements in the area of training however, was deemed unaccomplished as this august academy confronted a demand of initiation of academic research by virtue of the spirit of its act.

An effective Research Wing has been established to look into the intellectual and academic challenges in government colleges and to suggest redressal measures to the government. The Research Wing conceived the idea of *The Lighthouse*, a word suggestive of paving a way forward to the budding creative college faculty and to further glitter the bloomed researchers. The Research Wing tirelessly worked day and night to bring into light the present issues of *The Lighthouse Journal of Literature and Linguistics*, *The Lighthouse Journal of Social Sciences*, *The Lighthouse Journal of Natural Sciences* and *The Lighthouse Journal of Computational and Numerical Sciences*. This is a milestone achievement by the research wing of the academy to provide a first ever forum to the college teachers to get their quality research papers published in journals of their own. This valued contribution by the Research Wing of HEART is also the first ever distinguished endeavor among the five provinces. It is further envisioned to invite researchers from college teachers of other provinces to seek benefit from HEART by publishing their best articles and research papers in the light shedding *The Lighthouse*.

I share the pride of this moment with Ms Seema Rahman, Deputy Directress Academics and Chief Editor of *The Lighthouse* journals, and Mr. Imran Mohsin, the Managing Editor of *The Lighthouse Journal of Natural Sciences*. I am fingers crossed to seeing *The Lighthouse* comes up to gleam and twinkle from afar to the light seekers from all across the country.

Prof. Tasbih Ullah

Director HEART

Patron in Chief

Khyber Pakhtunkhwa Higher Education
Academy of Research and Training (HEART),
Sector F-2, Phase-6, Hayatabad, Peshawar.
+92-91-5863654, hetta.kp@gmail.com

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Contamination Levels of Some Heavy Metals in Selected Vegetables Grown in Urmar Bala of District Peshawar, Khyber Pakhtunkhwa, Pakistan

Arif Ullah Shah, Fazil Wahid, Fazal Dad, Akbar Khan, Hamd Ullah Shah[1]

ABSTRACT

The levels of Cr, Pb, Fe, Ni and Cd have been assesed in edible part of selected vegetables: Bottle gourd (Lagenaria siceraria), Aubergine (Solanum melongena), Lady Finger (Abelmoschus esculentus) and Regde gourd (Luffa acutangula). The range of heavy metals in samples are as: Chromium (Cr) is 0.00 mg/kg in all vegetable samples: Lead (Pb) is from 6.4 mg/kg to 1.35 mg/kg, Iron (Fe) is from 56.45 mg/kg to 31.15 mg/kg, Nickel (Ni) concentration (19.5mg/kg) found only in lady finger (Abelmoschus esculentus), Cadmium (Cd) in the range of 0.75 to 0.6mg/kg. Heavy metals concentration in selected vegetables is in the order of: Aubergine (Solanum melongena) (Pb > Fe > Cd), Bottle gourd (Lagenaria siceraria) (Fe > Cd), Lady Finger (Abelmoschus esculentus) (Fe > Ni > Pb) and Regde gourd (Luffa acutangula) (Fe > Pb). The trend revealed that concentrations of Fe were found maximum in all vegetable except Aubergine (Solanum melongena). When compared with standards, the levels of iron and nickel were found below, but lead and cadmium above their maximum permissible limits according to guidelines set by FAO/WHO.

Keywords: *Heavy metals, Vegetables, Lagenaria siceraria, Solanum melongena, Abelmoschus esculentus, Luffa acutangula, Lead (Pb), Chromium (Cr), Nickel (Ni), Iron (Fe), Cadmium (Cd), Centralized Resource laboratory (CRL).*

INTRODUCTION

Pollution may be organic or inorganic. Inorganic pollution is due to heavy metals in environment [1]. Natural as well as anthropogenic sources are responsible for these elements in aquatic system and soils [2]. Anthropogenic contributions exceed nature due to rapid urbanization and industrial development [3]. Industrial and domestic waste is main cause of heavy metals in surface as well as ground water and soils [4]. Cr, Mn, Ni, Cu, and Fe are required for various functions however when exceeding their maximum permissible level causes negative health effects [5]. Metals are responsible for chronic

[1] Department of Chemistry, Government College Peshawar, Peshawar Khyber Pakhtunkhwa, Pakistan, **Corresponding Author's Email: arifullah751@gmail.com**

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toxicity in humans, based on their concentration, way of exposure and the time of exposure. It is therefore very essential to assess heavy metal contamination and develop plans for their remediation and protect human [6].

Vegetables are very important for human life and animals because they contain proteins, vitamins, iron, calcium and other nutrients [7]. They are also responsible for neutralization of acidic substances form during digestion. [8]. Vegetables are beneficial to maintain health and prevent various diseases [9]. Vegetables receive these metals through their roots from soil and irrigation water, transported to their shoots and finally accumulate in their tissues. Ingestion of heavy metals in water is influenced through deposition, level of heavy metals in soil [10]. The hypothesis about current study is that information about heavy metals analysis in soil, fruits and vegetables of study area is limited. Since its land is most fertile and agriculture is the main source of income. It is irrigated through wastewater from industries and residential area. So it can be contaminated with heavy metals. Therefore it was decided to sought out heavy metal contamination of selected vegetates mostly grown there for the benefits of general public and residence of the area.

RESEARCH METHODOLOGY

Description of study area

This present research study was conducted in summer-2019 by collecting selected vegetables from Urmar Bala of district Peshawar, Khyber Pakhtunkhwa, Pakistan. Geographical coordinates of this area are: 33°57'17.9"N and 71°40'54.8"E. It is located at a distance of 11.2 km / 7.0 mi away from Peshawar. It has very fertile land and different vegetables and fruits are grown here which are consumed locally as well as marketed.

Collection of vegetables samples

Four types of vegetables Bottle gourd (*Lagenaria siceraria*), Aubergine (*Solanum melongena)*, Lady Finger (*Abelmoschus esculentus*) and Regde gourd (*Luffa acutangula*) were collected from four different farms of study area randomly. These are common grown and consumed vegetables in Urmar Bala of district Peshawar, as well as marketed. The samples were kept in polythene bags, labeled and brought to laboratory to department of chemistry at Government College Peshawar, Khyber Pakhtunkhwa, Pakistan. Only edible part was used for analysis.

Samples preparation and treatment

Vegetable samples were cleaned from dust and other undesirable materials first with tap water and then with distilled water. By means of clean knife the vegetables were changed into pieces. These pieces were then dehydrated in the oven at 100 ^{0}C. The dried pieces were powdered through mortar and pestle. The powdered samples were kept in polyethylene bags for further study.

Acid digestion and metal determination of sample

From each vegetable sample, 1 gm was taken in beaker and treated with 3 ml of HNO_3 (Nitric acid) and 1 ml HCl (Hydrochloric acid). To get clear solution for each sample, the di-acid mixture was digested on hot plate oven. After digestion completion, the solution was cooled and then filtered through filter paper. With distilled water, solution was diluted to 50mL. Filtered samples were brought to Centralized Resource laboratory (CRL) University of Peshawar for heavy metals (Cr, Pb, Fe, Ni and Cd) determination through Atomic Absorption Spectrophotometer (Model: AAS 700, Perkin Elmer, USA) . The final concentration of metals in each sample was calculated using formula [12]:

$$\text{Concentration (mg/kg)} = \frac{\text{Concentration (mg/L)} \times V}{W}$$

where V= final volume (50mL) of solution, W= initial weight (1g) of sample used.

ANALYSIS AND DISCUSSIONS

Heavy metals in vegetables

Heavy metals concentrations in different vegetable species of the study area were compared with the standards set by FAO/WHO for vegetables and discussed briefly: **Chromium (Cr):** In current study, chromium was not detected in all vegetables samples of study area.

Lead (Pb): Lead values were observed from 1.35 to 6.4 mg/kg. Its maximum concentration was observed in Aburgine was (6.4mg/kg) while lowest concentration in Regde gourd (1.35mg/kg) but not detected in bottle gourd **(Figure1)** It has been seen that concentration of lead in Aubergine, Lady finger and Redge gourd was above the maximum permissible level (0.1mg/kg) given by FAO/WHO-2011. The decreasing order of lead in vegetable samples was: Aubergine > Lady Finger > Regde gourd > Bottle gourd

Iron (Fe): Iron concentrations in current study were absorbed in the range of 56.45 to 31.15 mg/kg. The highest concentrations of iron was investigated in Regde gourd (56.45 mg/kg) and lowest in lady finger (31.15 mg/kg) **(Figure 2).** Concentration of iron in all vegetables samples were well below the maximum permissible level

(425.5mg/kg) given by FAO/WHO. The decreasing order of iron in current vegetable was: Regde gourd > Bottle gourd > Aubergine > Lady Finger.

Nickel (Ni): It has been found that concentration of Nickel was 19.5 mg/kg in lady finger which is below the maximum permissible level (66.9mg/kg) while in all the remaining vegetables samples its concentration not founded **(Figure 3).**

Cadmium (Cd): Highest concentrations of cadmium was observed in Aubergine was 0.75 mg/kg and lowest concentration in Bottle gourd 0.6mg/kg respectively. It was observed that cadmium concentration in both vegetables samples exceeded the maximum permissible level (0.05mg/kg) according to FAO/WHO-2011. Similarly in Lady Finger and Regde gourd cadmium was not detected **(Figure 4).**

Studies from other countries like Brazil, Vietnam, India and Bangladesh for determination of heavy metals are summarized in **Table 3**. Comparison indicate that presences of Chromium (Cr) in our result are lower than literature values. Similarly presences of Lead (Pb) and Nickel (Ni) in our results are more than in Bangladesh [16], Brazil [13] and Vietnam [14] but lower than India [15]. Levels of Cadmium (Cd) in current study in more than those investigated in Brazil and Vietnam but lower than those in India and Vietnam for lady finger.

Table 1: Common and botanical names of vegetable samples**.**

English name	**Local name**	**Scientific name**	**Edible part**
Bottle gourd	Kadoo	*Lagenaria siceraria*	Fruit
Aubergine	Baingun	*Solanum melongena*	Fruit
Lady finger	Bhindi	*Abelmoschus esculentus*	Fruit
Regde gourd	Turai	*Luffa acutangula*	Fruit

Table No 2: Concentration of heavy matals (mg/kg) in selected vegetables of the study area.

Name of samples	Final concentration (mg/kg)				
	Cr	Pb	Fe	Ni	**Cd**
Bottle gourd	ND	ND	44.45	ND	0.6

Aubergine	ND	6.4	34.75	ND	0.75
Lady finger	ND	3.75	31.15	19.5	ND
Redge gourd	ND	1.35	56.45	ND	ND

ND: not detected

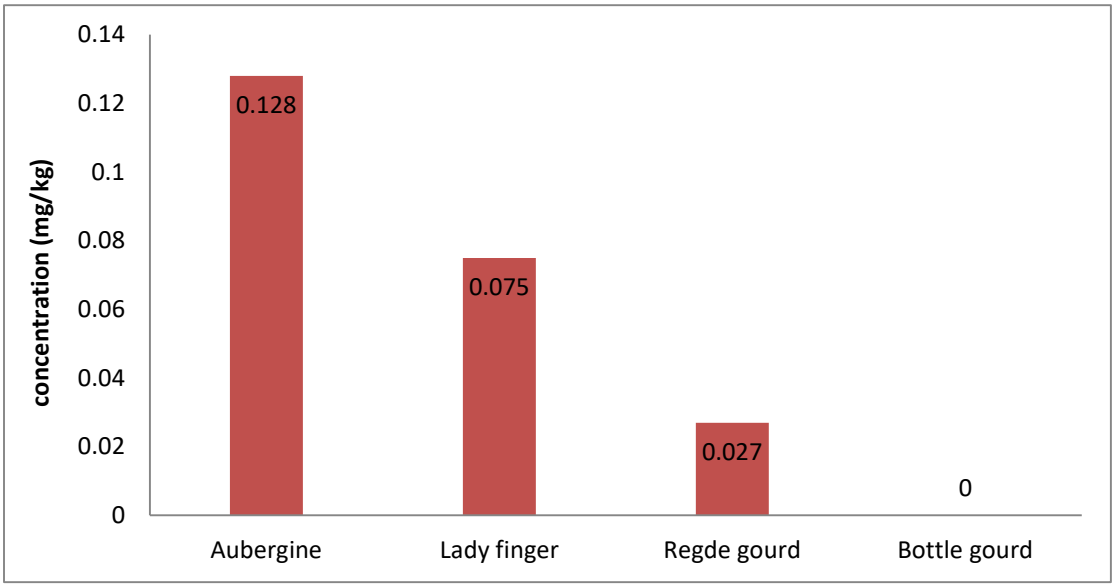

Figure 1: Concentration (mg/kg) of Lead (Pb) in selected vegetables

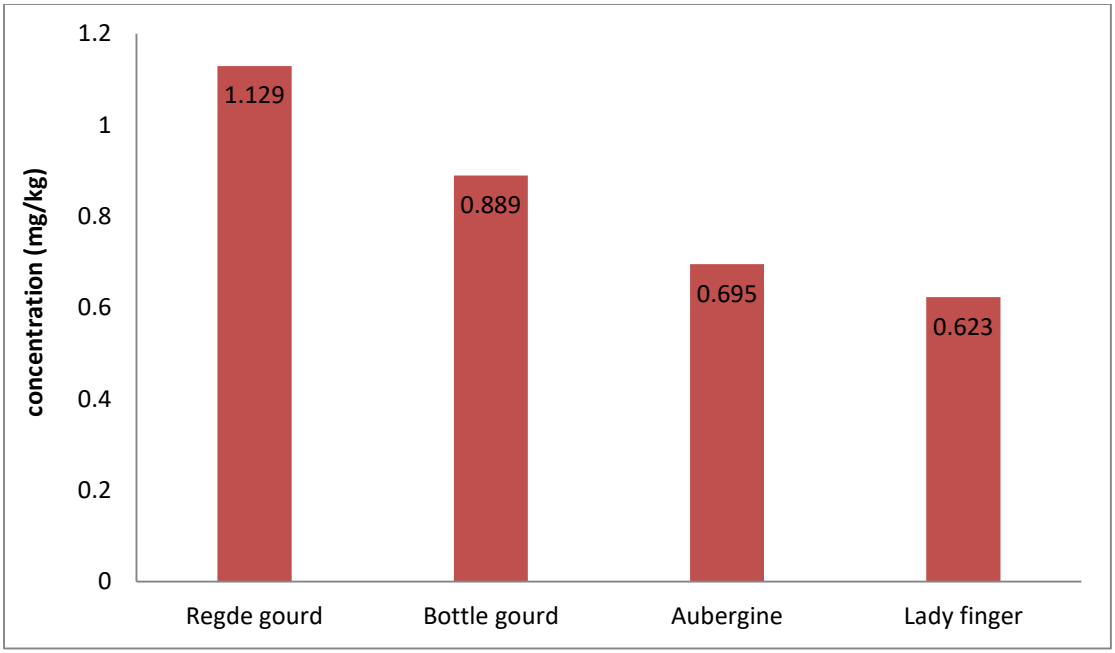

Figure 2: Concentration (mg/kg) of Iron (Fe) in selected vegetables

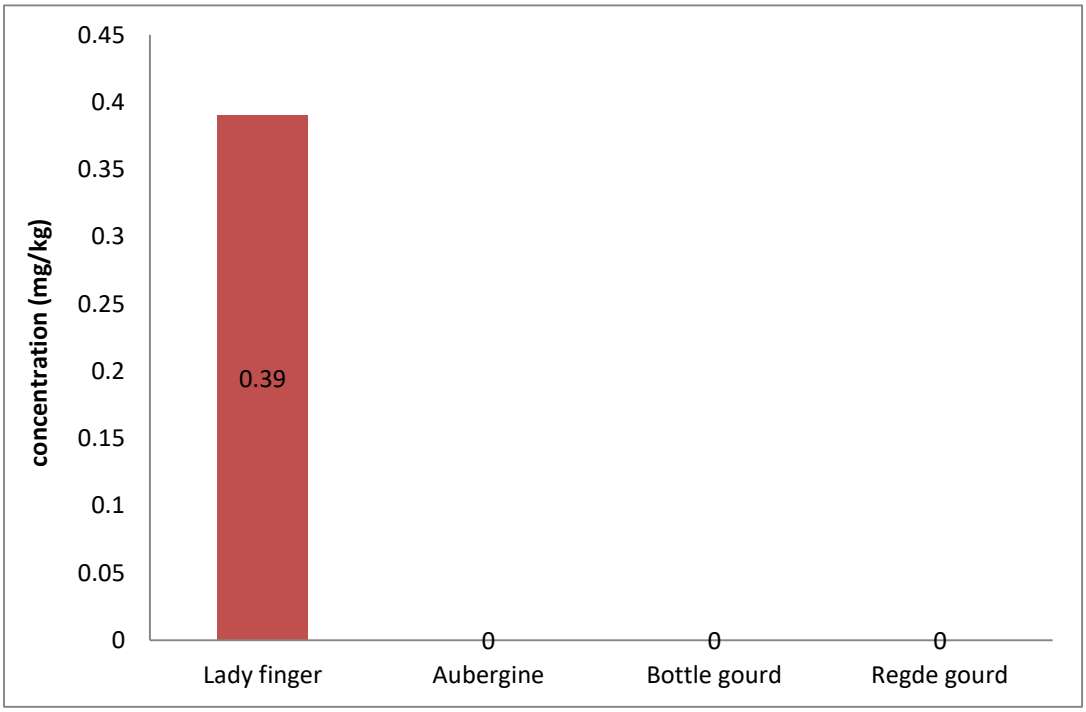

Figure 3: Concentration (mg/kg) of Nickel (Ni) in selected vegetables

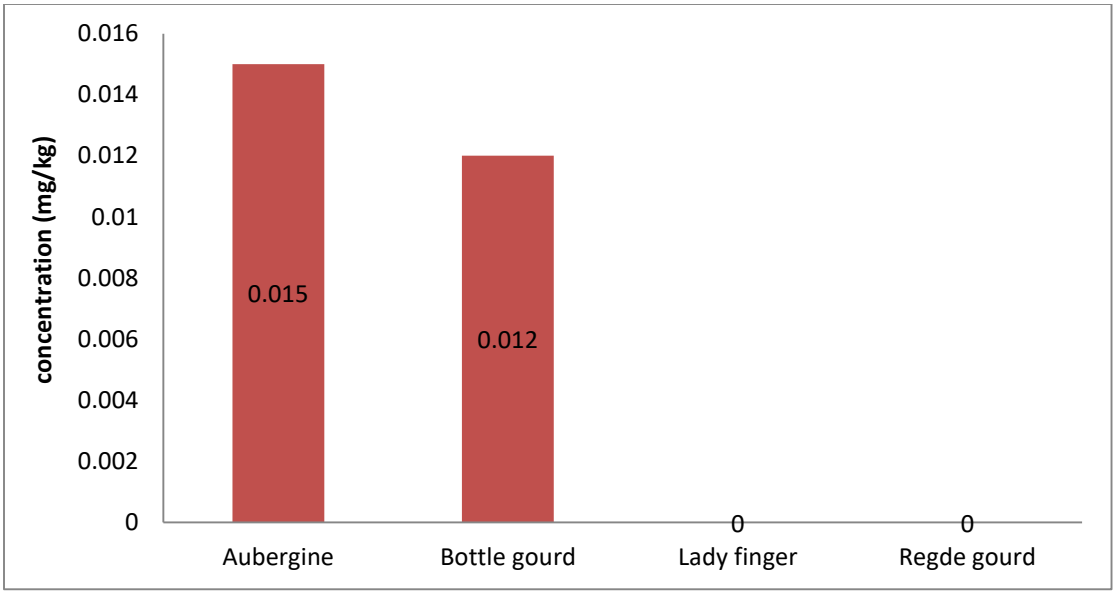

Figure 4: Concentration (mg/kg) of Cadmium (Cd) in selected vegetables

Table 3: Literature comparison of the heavy metals in selected vegetables from different countries.

Country	Vegetables	Cr	Pb	Fe	Ni	Cd	Unit	References
Brazil	Aubergine	0.16	0.44	-	0.13	0.04	(mg/kg)	[13]
	Lady finger	0.21	1.31	-	0.48	0.0		
Vietnam	Aubergine	0.11	0.70	-	-	0.09	(mg/kg)	[14]
	Lady finger	0.58	0.97	-	-	0.96		
India	Lady finger	64.62	26.43	-	58.96	15.04	(mg/kg)	[15]
Bangladesh	Bottle gourd	0.83	1.06	-	-	-	(mg/kg)	[16]

CONCLUSION AND RECOMMENDATIONS

Heavy metals accumulate in vegetables due to their presence in irrigation water and soil. Concentrations of these metals vary among the tested samples of vegetables, which show their different uptake capabilities and accumulation in their edible parts. The vegetables in present study were tested for the levels of heavy metals (Cr, Pb, Fe, Ni and Cd). Among these metals, level of iron was found more in all selected vegetable except Aubergine where lead found maximum. It has been observed that levels of Iron and Nickel in all vegetables samples were below their maximum permissible levels while Lead and cadmium exceeded their permissible limit in most vegetables samples. Therefore, it was concluded that vegetable of this area should not be consumed too much as possible. It is strongly recommended that periodic survey should be conducted on all food committees to protect the health of end user.

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Efficacy of Sovaldi and Rabavirin Combine Therapy in Chronic Hepatitis C Patients in District Peshawar, Khyber Pakhtunkhwa

Muhammad Arshad Khan[1], Arif Lodhi[2], Sohail Ahmad[3], Ejaz Ahmad[4]

ABSTRACT

The efficacy of Sovaldi and Rabivirin Combine therapy in chronic Hepatitis C patients were determined and also find out factors such as gender and age that can influence response to antiviral therapy. A quasi–experimental Study were carried out in Gastroenterology and Pathology department, Hayatabad Medical Complex (HMC), Peshawar, from February 2016 to July 2016. Patients were given 400 mg oral Sovaldi and 800- 1200 mg oral Rabavirin two doses in a day for a period of 180 days. At the end of treatment the response of the combine therapy was depicted from the negative RNA of HCV by PCR after 24 weeks of treatment. Factors like age, gender and serum ALT were examined of the patients and determined the relations of these factors with the efficacy of Sovaldi combine with Ribavirin. Total 113 patients age up to 60 years, were given combine treatment that full filled the inclusion criteria. Sovaldi and Ribavirin in combination therapy showed better response compare to other drugs. Females and young age patients were favorable responders.

Keywords: *Sovaldi, Rabivirin, Serum ALT, PCR, HCV*

INTRODUCTION

Hepatitis C Virus (HCV) which is the main cause of liver diseases including hepatocellular carcinoma and liver failure [1]. It infects about 180 million people all around the word [2]. Hepatitis C virus is one of the main cause of liver diseases all over the world [3]. HCV belong to family flaviviridae which is an RNA virus with a diameter ranges from 40-50nm. HCV genome is a single stranded RNA molecule of 9500 kilo Daltons [4]. HCV, a big problem for the whole world and about 3.3% of world populations are infected with HCV [5].

[1] Department of Chemistry, Govt. Degree College, Chaghar Matti Peshawar, Pakistan
Corresponding Author's Email: makbiochem@gmail.com
[2] Department of Bio Chemistry, Abdul Wali Khan University Mardan, Pakistan
[3] Department of Chemistry, Bacha Khan University Charsadda, Pakistan
[4] PhD Scholar, Bacha Khan University

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In Pakistan HCV has infected round about 10 million people [6]. In USA it is also the main cause of liver disease and was the main reason of liver transplantation in USA till 1996. Currently from 8000 to 10,000 deaths are occurred in USA each year from HCV. And yearly death ratio is estimated to be raised from 30, 000 per anum to 40,000 per anum by the year 2016 due to chronic liver cirrhosis [7]. The ultimate cause of HCV is liver cirrhosis and hepatocellular carcinoma [8]. Egypt is considered at the top as far as prevalence rate in the world is concern. Egypt has more than 15 % of the infected population [9].While Africa continent according to WHO has the most prevalence of regional HCV which is 5.3% [10].

Despite of the modern and latest laboratory technique and apparatus for screening of blood, blood transfusion remains the main cause of transmission of HCV infection, because the unscreened blood are still used in most part of the world. That's why HCV is a blood borne infections. Unhygienic, poor and unsafe injection practices, use of unsterilized surgical apparatus are the most risk factor and cause of HCV transmission in the world. As hepatitis C virus is the main health problem, causing fatal liver cirrhosis and hepatocellular carcinoma in Pakistan [11].An estimated infected people in Pakistan are 4.9 % and the genotype 2 and 3 are predominant in Pakistan [12].Which required 6 months treatment [13].

The treatment plan for the hepatitis C virus disease is, rapidly evolving to get the most satisfactory response and eradication of virus in long term. For this reason, interferon-alpha 2b was the first drug introduced in the market in 1986 for treatment of HCV. The response rate with monotherapy of interferon was 10 – 20% [14]. Later it was noticed that Ribavirin addition to it, its response increases. Result showed that the result of the combine therapy was 26% more compare to monotherapy [15]. But majority of Pakistani population cannot afford Pegylated interferon, thus standard interferon is used by the patients [16]. Apart of 20 different brands of interferon, other drugs like Ribavirin and Sovaldi are also used in Pakistan.

FDA in 2013 approved Sovaldi in combination with others drug for treatment of genotype 2 and 3 of HCV. For genotype 1 and 4 triple treatment, Sovaldi plus interferon and Rivavirin are used [17]. Sofosbuvir (brand name Sovaldi) is a nucleotide analog used in combination with other drugs.

Sovaldi is marketed in 2013 and has higher rate of cure with less side effects. Sovaldi is an RNA polymerase inhibitor drug, hepatitis C virus use polymerase enzyme to replicates its own RNA during the course of disease. Ribavirin is the nucleoside analog 1-3-D ribofuranogy- 1, 2, and 4 – Triazole -3 – Carboxamide also known as virazole that exhibits antiviral activity against RNA viruses in cell culture [18]. Sovaldi which is 400mg daily once orally drug while Ribavirin is given twice from 800 -1200 mg daily orally depend upon the weight of the patient.

The assessment of the efficacy of the drug for primary treatment, a method devised by Japanese Ministry of Health and welfare, described by Yano [19]. A responder

of HCV RNA is defined when a patient has two consecutive undetectable (<100 copies/ml) values. If for 6 months, when HCV RNA result come as negative, then the patient is assigned as sustained response (SR) that does not need second time treatment. In case when the result of HCV RNA come as positive, then the patient is grouped as incomplete response (IR) or partial response (PR), depending upon ALT (Alanine aminotransferase) is normal or Partially responsive (PR). ALT response is defined as 2 consecutive normal ALT values i.e <48IU/L.

RESEARCH METHODOLOGY

Study design and place:

This quasi-experimental study was conducted in the Hayatabad Medical complex (HMC), prevention program of HCV by Gastroenterology and pathology department MTI/HMC, Peshawar Hayatabad. Adult patients of both the gender and age up to 60 years that are infected from HCV virus were studied in this research. All the enrolled patients had detectable levels of RNA of HCV in their blood serum by polymerase-chain- reaction (PCR).

Exclusion criteria

In this study, all the patients would have been excluded if there would have been chances of decomposed cirrhosis, patients that have HIV and HBV infection, that have transplanted organ before, that have received any prior anti HCV therapy, patients that have psychiatric disease, serious heart disease and uncontrolled diabetes.

Inclusion criteria

The normal blood count, RBC, WBC, platelets were also considered. Sovaldi 400mg once a day and Ribavirin 800mg (<75 kg body weight) or 1200mg (>75 kg body weight) pills in two divided doses a day were given to the HCV patients for a 6 months period. For the detection of HCV RNA, PCR has been done before and after the treatment. All proper follow-up of all the patients was ensured after their clinical test. Biochemical and Hematological assessment were finding out of these patients. At the end of 6 months, over all efficacy of Sovaldi and Ribavirin combine therapy was calculated by non-detectable HCV RNA by PCR and the effects of the patients related factors like gender, age and serum ALT were determined and analyzed by applying Chi-Square test, and using SPSS 19.0 Statistical program. In this statistical program, significance were kept below than 0.05 P-value. If value is more than 0.05 then it will be considered as insignificance.

The efficacy of the drug was measured from the end treatment response (ETR). According to NIH, the end treatment response was defined as, at the end of therapy when the patients had undetectable serum HCV RNA levels. Those patients who had HCV RNA detectable after 24 weeks treatment was considered as non-responders patients.

RESULTS AND DISCUSSIONS

A total of 124 HCV patients from district Peshawar were included having chronic hepatitis C. In those 124 patients, 113 patients had completed their treatment and come back with ETR reports. The remaining 11 patients had stop medication due to financial problem or health related issue.

In those patients 74 (66%) were males and 39 (34%) were females. Their ages ranges from 21 to 60 years. Out of all these 113 patients 98 patients showed respond to the combine therapy of Sovaldi and Rabivirin, which was depicted from the negative values of PCR. In which 86.72% patients showed respond among them female showed 89.74% respond better than male which is 85.13%. So, on the basis of gender, female patients showed high response as compare to the male, which was not significant statistically (P>0.05. i-e = .492).

Table 1: Base line characteristics of responders and non-responders patients.

Characteristics	Responders	Non-responders	p-value
Total 113	98 (86.72%)	15 (13.27%)	-
Age (years)			
≤ 40 51	45 (88.23%)	6 (11.76%)	0.04
>40 62	53 (85.48%)	9 (14.51%)	
Gender			
Male 74	63 (85.13%)	11 (14.84%)	0.492
Female 39	35 (89.74%)	4 (10.25%)	
Gender age (≤40 & >40)	30 (85.71%)	5 (14.28%)	
Male ≤40 35	33 (84.61%)	6 (15.38%)	-
>40 39	15 (93.75%)	1 (6.25 %)	
Female ≤40 16	20 (86.95%)	3 (13.04%)	
>40 23			
ALT (IU/L)			
≤40 20	16 (80%)	4 (20%)	
41-80 50	45 (90.00%)	5 (10.00%)	0.044
81-120 27	25 (92.59%)	2 (7.40%)	
>120 16	12 (75%)	4 (25%)	
Weight (kg)			
≤70 69	59 (85.50%)	10 (14.49%)	0.633
>70 44	39 (88.63%)	5 (11.36%)	

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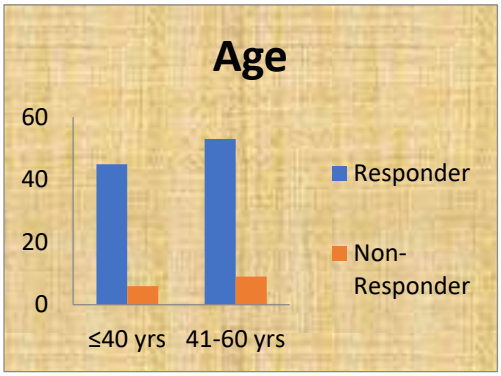

Figure 1 Shows age wise response to drug

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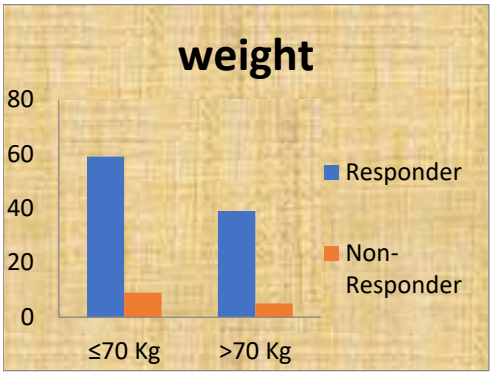

Figure 2 Shows weight wise response to drug

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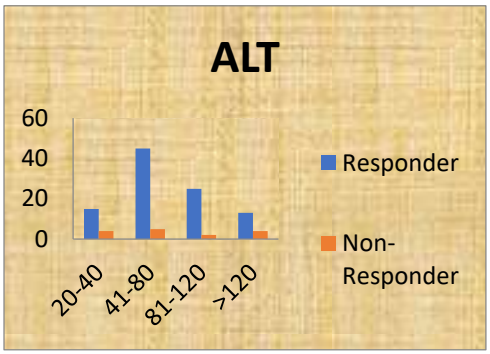

Figure 3 Shows ALT wise response to drug

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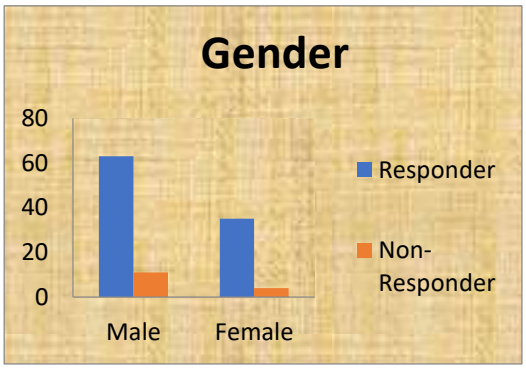


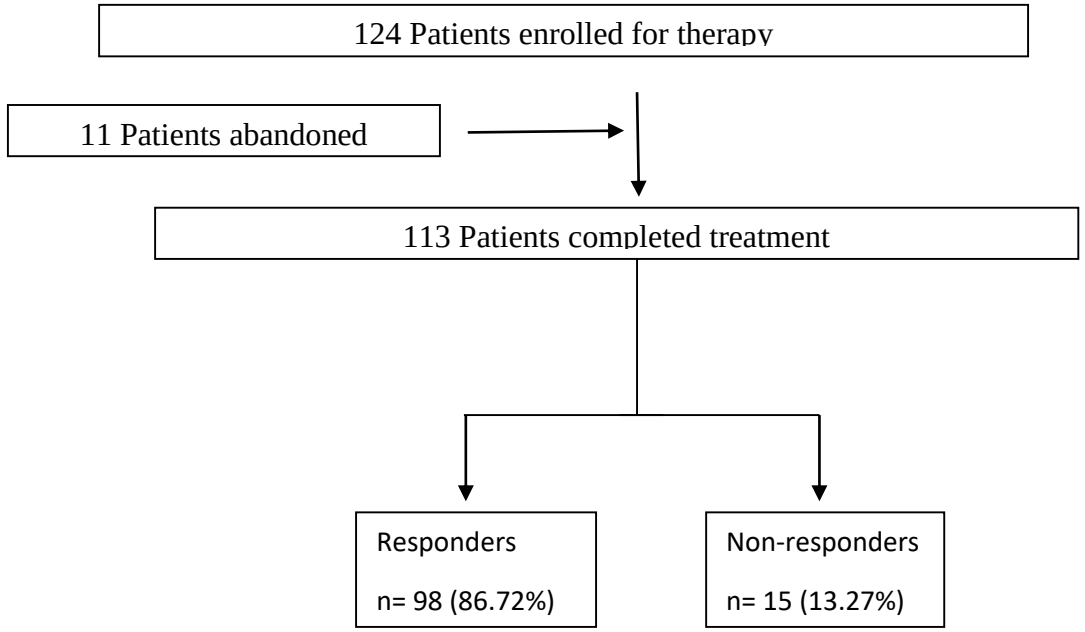
Figure 4 Gender wise response to drug

This Research indicates that younger patients having age less or equal to 40 years, are good responders to the combined therapy of Sovaldi and Ribavirin. Above then 40 years of age, the respond of the patient's decreases considerably, with the age increases as shown in Fig 1.

Patients having weight equal or less than 70kg showed 85.50% respond to the combine Sovaldi and Ribavirin treatment, while the patients having weight greater than 70 kg showed response 88.63% higher than the patients having weight up to 70kg which is statistically not significant (P-Value= 0.633) as shown in Fig 2.

From the ALT levels it is indicated that the patients of HCV showed better respond to Sovaldi combine with Rabivirin drug at markedly high ALT levels. ALT levels increased from the normal i.e. 40 IU/L up to 120 IU/L, than the patients shown

good respond i.e. 90% when the ALT range between 41-80 IU/L and 92.59% when it is raised from 80-120 IU/L levels. These show slightly greater ALT levels, good will be the respond. Further the respond of the patient to the drug decreases, when ALT levels elevated from 120 IU/L i.e. decreased to 75 %. This data is found statistically significant. Thus it is concluded that a slight increase in ALT values induces good result in HCV patients. But at a very high ALT level the respond to the drug is considerably decreases as shown in Fig 3.



DISCUSSION

To select the best treatment, respond in chronic Hepatitis C patients are utmost important. This will help to treat the patients of HCV properly and to find the responders and treat only those patients. In the same way it is highly desirable to find out the biological non-responders in order to abundant their treatment at the start of the treatment because this will not only reduce the cost of the treatment that will help the patient financially but also the adverse effects of the drugs can be avoided.

Keeping all these things in mind, so many pretreatment factors both related to the patient and also related to the virus are evaluated to find out the successful HCV treatment. These factors are co-related and examined clinically related to Sovaldi combine therapy with Rabivirin.

For the patients with genotype 2 and 3 which is predominant in Pakistan, recently FDA- recommended Sovaldi treatment for 24 weeks thus no longer recommended PEG-IFN/RBV treatment [20]. Because PEG-IFN causes some health complications in the patients thus its use should be reduced or abandoned. Sovaldi the safer drug, should be

used for HCV treatment. HCV with genotype 2 and 3 are approximately 30% worldwide [21]. Genotype 3 infected patients show less response, to PEG/IFN [22].

Combination therapy of Sovaldi and Rabivirin increase the response rate for genotype 2 and 3. Response rate in Pakistan for Sovaldi and Rabivirin combine treatment is found in this research which is more than 85%. Genotype 1 and 4 which is mostly present in America and Europe, the response rate found is more than 80% [23].

This study revealed that 98 patients, out of 113 showed response i.e. 86.72% which is depicted from the negative result of PCR. The previous studies indicate that combination therapy in South Asia is the best treatment regime because of genotype 2 and 3 prevalence [24].

Female patients showed better response as compare to the males. Out of 39, 35 showed significant response thus their response rate is nearly 90% as compare to the males which are 85% responders. But this is insignificant statistically. Also the younger patients showed good response as compare to the older one, younger patients age were taken up to 40 years. The good respond shown by younger patients are due to their better immune system and adhesion to the treatment. The older patients showed less response to the therapy and thus have less sustained response regardless of their genotype and other characteristics. Weak immunological response, adverse condition of the disease and other medication use in the old age can reduce the response of the drug. That is why younger patients are good responders to combine therapy of Sovaldi and Rabivirin.

Weight of the patients showed that Patients having weight greater than 70 kg are good responders compare to patients having weight less than 70 kg. But this finding is statistically insignificant having greater P-value than 0.05.

This research also determined that the patients that have slightly or markedly high ALT showed better respond as compare to the normal or elevated ALT levels. Patients having ALT levels between 81-120 IU/L showed greater respond to the drug (92%) as compare to elevated ALT (75%). This difference might be due to the extent of hepatic damage as the level of ALT in the serum reflects the damage of the liver cells. This finding is statistically significant. Research also indicates that viral genotype present in this part of the world is mainly 3a. Out of 113 patients, 64 patients have 3a viral genotype.

So, this study showed that Sovaldi and Ribavirin is more effective to both genotypes 2 and 3 as compare to 1 and 4 in Pakistan. This present data give rise to the need of further research to test the factors like age, gender, weight and serum ALT that effects the response of anti-viral drugs i-e Sovaldi and Ribavirin before and during the treatment on a large scale. I have taken only 113 patients, because Sovaldi is newly marketed in Pakistan, thus the number of patients using this drug are quite limited and many patients still not completed their six months treatment to till date. Secondly the

efficacy of this DAA drug can be depicted after 12 weeks of treatment rather after 24 weeks of treatment which may make the research easier and more result oriented.

CONCLUSION

The oral Sovaldi and Rabivirin combine therapy in chronic Hepatitis C patients has sustained virological response. This combination treatment is interferon free which has so many side effects in patients. Further the patient related factor like gender, age, weight and serum ALT levels to a large extent can predict the response of Sovaldi and Rabivirin combine therapy before and during the therapy. But strictly speaking, gender has no such prominent effect on the response of combination therapy of Sovaldi and Rabivirin. Young age in both genders, high weight in males and high level of ALT favors the response to this antiviral drug.

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Study of Mass Transfer Resistant Effect during Syn Gas and Hydrogen Production through CO2/CH4 Dry Reforming on La/Mg Supported Co-Ni/MSU-S Zeolite

Roohul Amin[1], Bingsi Liu[2], Sana Ullah[3], Ploywarin Sangsomboon[4], Faiq Saeed[2]

ABSTRACT

In this study five different catalysts xLa/MgyCo-7Ni /MSU-S are synthesized through sol-gel procedure and further tested for XRD, HRTEM, FT-IR, H_2-TPR, CO_2-TPD, and O_2-TPO skills. The mass transfer resistant effect, stability and activity of the catalytic samples were observed for syn gas and H_2gas synthesis at different reaction temperatures and various space velocities (GHSV) at atmospheric pressure. Space velocities of 2.4- 3.2 × 10^4 $mL/g^{-1}h^{-1}$ were used for the determination of mass transfer resistant effect, catalytic performance and composition of the catalyst at different reaction temperatures (700-800 ^oC) retaining constant atmospheric pressure during CO_2/CH_4 dry reforming reaction. The reaction outcomes showed that nanoparticles of nickel are highly discreted upon the promotion of Mg, La & Co oxides over MSU-S (mesoporous zeolite) through firmly contact of metal ions with HO-Si-groups of zeolite. Oxides of La (La_2O_3) in 1-3LayCozNi/MSU-S, which showed greater catalytic activity than 1-3Mg supported such catalysts. Among all five catalysts the N-/MSU-S doped with 3%La and 2%Co confirmed the best active catalyst in all respects with stability up to 75 h at of 750 ^oC. The catalyst also showed less mass transfer resistant effect at (GHSV) of 24000 $mL/g^{-1}h^{-1}$ than 32000 $mL/g^{-1}h^{-1}$

Keywords: *mass transfer resistant effect,xLa/mgyCozNi/MSU-S; H2 and syn gas synthesis; methane dry reforming; greater stability and activist*

INTRODUCTION

Hydrogen is clean energy source of energy due to its recent utilization in modern industries and other fuel cells. The synthesis of Hydrogen, ammonia synthesis, from

[1] Department of Chemistry Government College Peshawar, 25120, Pakistan, **Corresponding Author's Email: roohulamin1947@gmail.com**

[2] Department of Chemistry, School of Sciences, Tianjin University, Tianjin 300354, P.R. China

[3] Department of Chemical Engineering and Technology, Tianjin University, Tianjin 300354, P.R. China.

[4] Department of Aviation Services Industry, Faculty of Liberal Arts, Rajamangala University of Technology Thanyaburi, Pathum Thani, Thailand 12110.

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MDR (methane dry reforming) with carbon dioxide is valuable & hot topic toward hydrogen economy research [1].

The preparation of syngas (CO+ H_2) and hydrogen through MDR with CO_2 (CO_2 + $CH_4 \rightleftharpoons 2$ CO + 2 H_2, ΔH^oat 25 ^oC (298K) had gotten significant consideration for usage in environmental optimization and industry [2-4].

The methane reforming reactions are considered as clean and energy efficient technologies either carried out in the presence of CO_2 (dry reforming) [5] or reaction with H_2O (steam reforming) reaction [6].

$$CH_4 + CO_2 \rightleftharpoons 2CO + 2H_2 \qquad \Delta H^o = 247 \text{ kJ/ mol} \qquad (1)$$

$$CH_4 + H_2O \rightleftharpoons CO + 3H_2, \qquad \Delta H^o = 206 \text{ kJ mol} \qquad (2)$$

The production of syn-gas from methane (Eq.1) has been studied extensively as synthetic gas can be used for the preparation of some liquid hydrocarbons of low molecular weights (MW) and other such organic products used in the industry. MDR (methane dry reforming) is superior method than SR (steam reforming) technique (Eq. 2) to accomplish the compulsory syn-gas contents for Fischer-Tropsch reaction to synthesize useful products [7].

It has been pointed out that rare earth metals are best catalysts for MDR reaction [8] but due to the heavy costs of noble metal catalysts researchers prefer to use Ni-based catalysts for this reaction [9-13].

Whereas, the main drawback of MDR reaction is because of decrease in catalytic activity by Coking (carbon deposition) [14] at the surface of catalytic material during decomposition of methane ($CH_4 \rightleftharpoons 2H_2$+ C) and CO bifurcation (2 CO $\rightleftharpoons$ CO_2 + C) Boudouard reaction [15, 16]. The deactivation problem was controlled by different researchers due to incorporation of metals and their oxides like Al_2O_3, CaO, MgO, CeO_2 and La_2O_3, for the betterment of catalytic activity of such catalysts of nickel (Ni) origin [17-21].

The supports such as La_2O_3, ZrO_2 and MgO act along Ni hinder the addition of carbon in nickel based catalysts. Particularly, oxide La_2O_3 reacts with CO_2 and gives $La_2O_2CO_3$, which easily speeds up elimination on the surface carbon containing materials [17].

Additionally, the research on La_2NiO_4/ γ-Al_2O_3 [22], and the comparative studies among $LaNiO_3$ Vrs MCM-41, $LaNiO_3$ Vrs SiO_2 and $LaNiO_3$ Vrs SBA-15 [23], showed insight about increase of surface area activity on basis of thermal stability and the effect of supporting oxide towards the change of CO_2 in syngas.

Further, presence of metallic particles on the surface of MSU-S decrease deposition of carbon. The perovskite [24] and other [25, 26] catalyst utilized for MDR of methane where the mass transfer resistance produce during MDR reaction over La/Mg promoted Co-Ni based on the surface of MSU-S catalysts is not present in scientific reports so far.

Five different catalysts $_x$La/Mg $_y$Co-Ni based MSU-S were assembled by known method (sol-gel), and evaluated by different experimental techniques like H_2 temperature-programmed reduction (H_2-TPR), X-ray diffraction (XRD), The high resolution transmission microscopy (HRTEM), gravimetric/differential scanning calorimetric analysis based on temperature (TG/ DSC), Fourier transform Infra-red (FTIR) and method based on temperature-programming for carbon dioxide and oxygen (CO_2-TPD), (O_2-TPO), respectively. The performance based on these catalysts for MDR reaction of CO_2 were done and evaluated at variation of temperatures and mass transfer resistance effect.

RESEARCH METHODOLOGY

Preparation of supporting material MSU-S and Catalysts

The supporting material MSU-S zeolite was arranged as reported by Pinnavaia *et al.* [27] taking different components are mixed in the following amounts. NaOH (0.507 g), $NaAlO_2$ (0.352 g), deionized water (DW) 10 mL, sodium silicate (25.578 g) at 35 ^oC and stirred for 4h. This gel type of solution was taken in autoclave at 110 ^oC for 12 h. At the end of mixing cetyltrimethylammonium bromide (CTAB) was added at 25 ^oC room temperature (RT) while pH is maintained around 9-10. The gel obtained was heated at 140 ^oC for 48 h in autoclave and dried, followed by calcinations at 550 ^oC in the presence of common air for 6 hrs.

Preparation of catalysts

Five catalytic samples of xLa/Mg-Co zNi supported MSU-S catalysts were made ready by using reported method [28]. Later on these catalysts have given some ordinary names for identification such as, 1%La 2 %Co7 % Ni/MSU-S, 1%Mg2 %Co7 %Ni/MSU-S, 3%La2%Co7Ni/ MSU-S and 3%Mg2%Co7%Ni/MSU-S. All prepared catalysts were sieved via 40-60 meshes before their application for catalytic process.

Characterization of catalysts

XRD was taken at rate of 1 ^oC/min scanning at PAN analytical automatic diffractometer taking Ni-filtered Cu K_α radiation (λ = 0.154 06 nm) at setting of 40 kV and 50 mA.

The FTIR spectra were taken on BIO-RAD FTS 3000 spectrophotometer, whereas TG/DSC was taken in air on a STA 409 PC/PG at 30-850 ^{o}C at a heating rate of 10^{o}C/min. The Tecnai G2 F20 microscope present in Hong Kong used for High Resolution TEM images of unused and spent 3%La 2%Co 7%Ni/MSU-S catalyst.

The techniques like CO_2 & O_2TPD, Hydrogen TPR and oxygen TPO along TCD detector were taken by gas chromatograph (GC) system present in university laboratory at building 7 [28].

The catalytic activity and stability

The MDR was done by a quartz reactor at 1 atm with monitoring temperature of by K-type thermocouple during reaction. Quantities of methane and Carbon dioxide were monitored by controller for mass flow (D07-7B/ZM, made in Beijing Chinese Company. The ratio of carbon dioxide and methane at 1:1 with speed of 60 mL/min having speed of gas hour space velocity (GHSV) of 24000 - 32000 mL $g^{-1}h^{-1}$ at 700–750 ^{o}C during reaction. The changing of methane, carbon dioxide and selectivities for syngas and hydrogen and further yield of carbon (Coking) are calculated by using formulas as reported in literature (28.

RESULTS AND DISCUSSIONS

XRD Pattern study

The XRD (small-angle) for MSU-S supporting material (Fig. 1A) and wide angle XRD were done. They showed an ordered hexagonal structural shape [29, 30] and curves at $2\theta = 1.98°$, 3.4° and 3.9° having similarity with cards (100), (110) and (200) pattern of P6mm [31] The XRD patterns of fresh and spent catalysts are recorded as shown in the figure. These peaks at $2\theta = 43.3^{o}$ and 37.3^{o} and 62.7^{o} were accredited to NiO (24). XRD study showed that after reactivity, there were distinctive diffraction curves of Ni^{o} at $2\theta = 51.8^{o}$ and 43.9^{o} (26) due to changing of NiO species into Ni^{o} at spent 3La2Co7Ni/MSU-S catalyst Fig 1B(c).

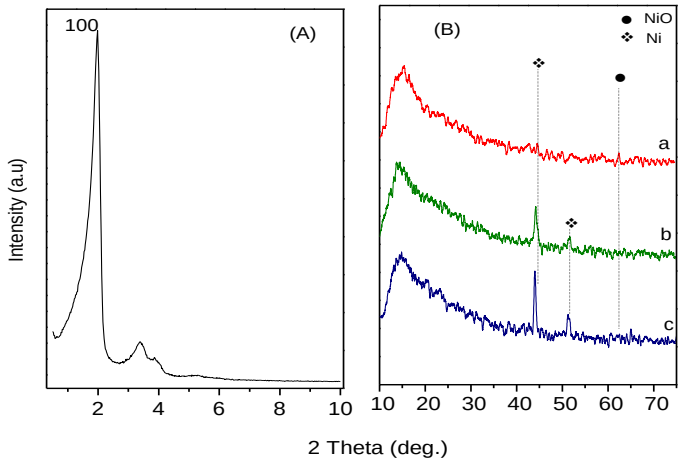

Fig. 1 (A) XRD (small-angle) of MSU-S fresh (B) Large XRD for unused and spent (a, b) 3%La 2%Co 9%Ni based MSU-S, (c) spent 3%La 2%Co 9%Ni based MSU-S during 75 h reaction.

H_2-TPR

Fig. 2 exhibited the H_2-TPR profiles, which can be seen that 3La2Co 7Ni/MSUS-S (Fig. 2a) having peak at 359 ºC which due to Co^{3+} to Co^{2+} [31]. The H_2-TPR peak at 449 was showed NiO reduction to metallic Niº [20, 32]. The graphs at 618 & 597°C showed La_2CoO_4 reduction [33] while graph over 679 °C & 719 °C confirmed reduction of La_2NiO_4 [33]. While the reduction curve at 343°C is noted in the case of 3%Mg 2%Co and 7 % Ni based MSUS-S (Fig. 2b), showing reduction of Co^{+2} [31] as well as the curve of 453°C exhibited NiO reduction. However there was no spinel structure, such as La_2CoO_4 or La_2NiO_4 was found in the H_2-TPR pattern of 3%Mg 2%Co and 7 % Ni based MSUS-S, probable due to metallic Mg. Meanwhile the study of Xu *et al* [34] and Djaidja *et al* [35], confirmed the reduction at 859 °C was accredited Al_2O_3 and NiO reduction as shown in Fig. 2b.

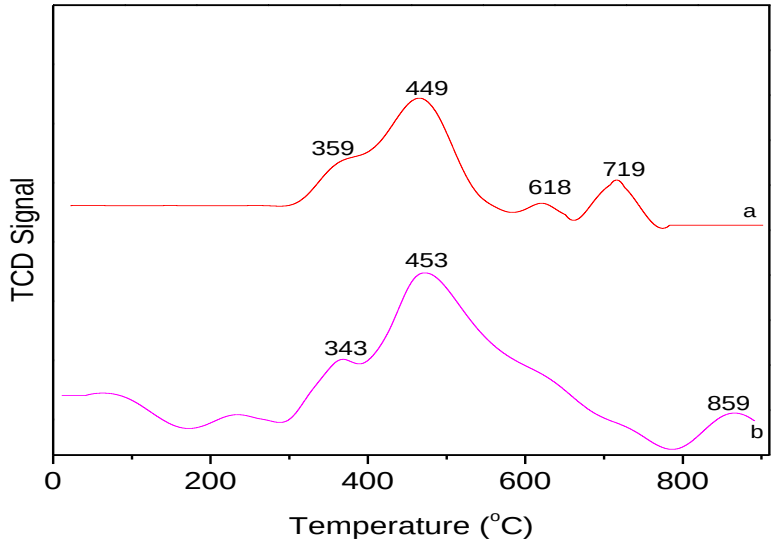

Fig. 2 H_2-TPR pattern of fresh (a) 3%La (b) 3 %Mg 2%Co and 7 % Ni based MSUS-S catalyst.

CO_2 –TPD

The CO_2-TPD (Fig. 3b) which realized CO_2 desorption peaks for different catalysts showing 3 types of positions on catalysts. The weak curves at 164 and 173 °C for CO_2 desorption may accredited for carbon dioxide adsorption.

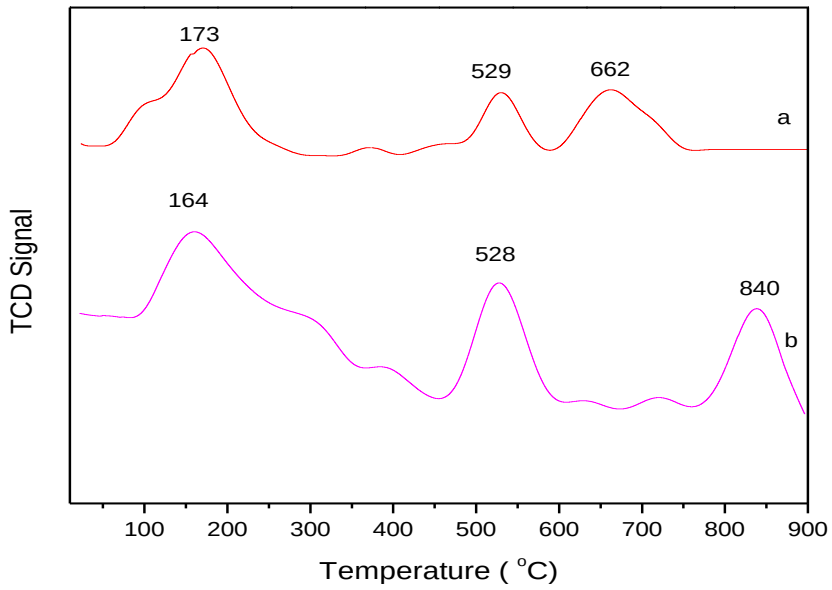

Fig. 3 The CO_2-TPD unused catalysts (a) 3%La (b) 3%Mg 2%Co and 7 % Ni based MSUS-S

FT-IR spectra

Figure 4 showed the FT-IR spectrum with peaks for absorption at range of 3438 & 1630 cm^{-1} [38] were stretching and bending vibrations respectively for OH groups [39]. Curves for symmetric and asymmetric stretching for Si-O-Si showed at 1390, 1076, 810 and 460 cm^{-1} for mesophorous material [40].

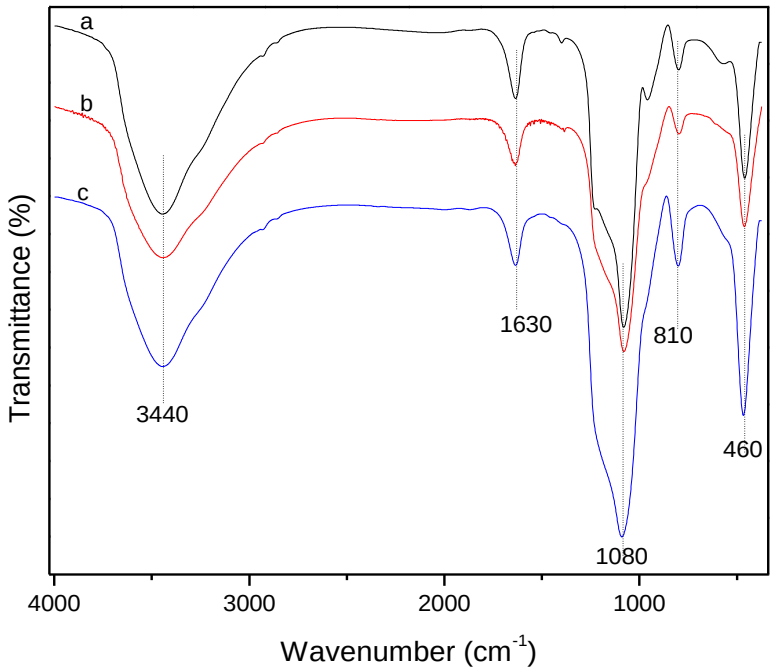

Fig. 4 FT-IR spectrum of zeolite (a) MSU-S; fresh (b) spent at 10 °C (c) spent at 75 °C 3% La 2%Co and 7 % Ni based MSUS-S catalyst

According to the report of Camblor *et al* [41] the peaks at 956 cm^{-1} weak curves at and pointed for the defect sites of (Si-OH).

HRTEM

The high resolution images of TEM for unused and spent 3%La 2%Co and 7 % Ni based MSUS-S is shown in Fig. 5. It is confirmed that support and all other catalysts are stable as fresh and after reaction. Fig 5B showed ordered hexagonal mesoporous arrays pattern [30], the both black strips with 2.6 nm distance (Fig. 5B) confirmed with results of reported literature [43]. The nanoparticles of Co, Ni and La are highly distributed on MSU-S zeolite and showed amorphous shape of zeolite. The TEM studies exhibited that supporting zeolite remained intact after reforming reaction (Fig. 5C and 5D) and particles retained with constant size in Fig 5 C & D. It is confirmed from HRTEM images that the said catalyst was stable at high temperature.

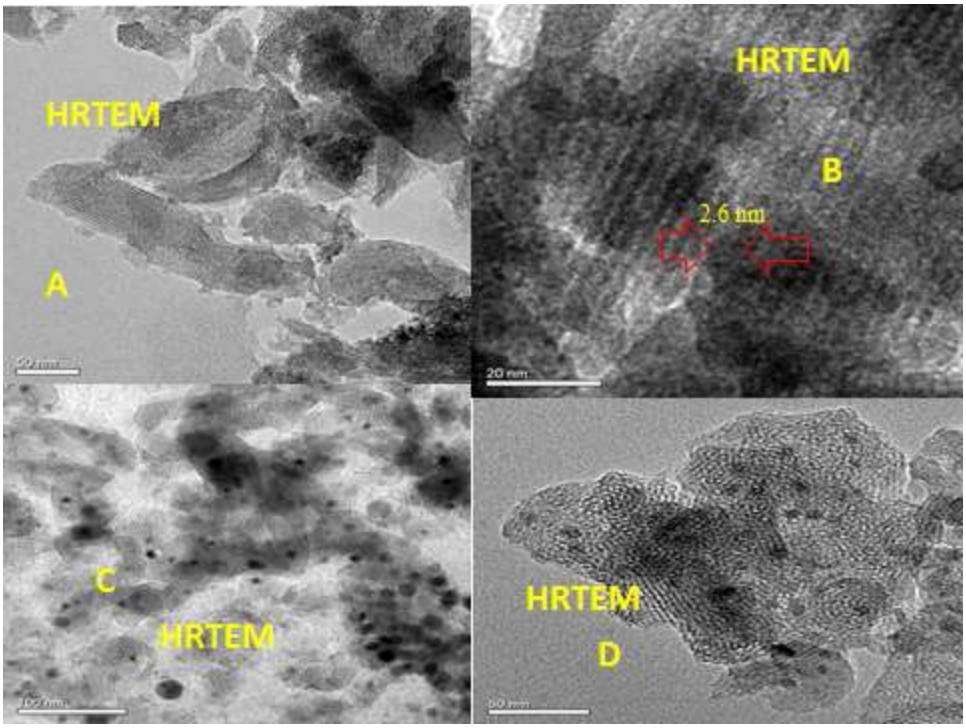

Fig. 5 HRTEM unused (A, B) & spent (C, D) 3%La 2%Co & 7 % Ni based MSUS-S at 750 ºC.

Studies of catalytic performance

Catalytic performance of xMg/La-yCo-Ni based MSU-S

The catalytic activity was done for selectivities for Carbon monoxide and hydrogen, conversion of methane and carbon dioxide, as well as coking was given in (Fig. 6A-C). It is confirmed that the change of methane on 3%La 2%Co and 7 % Ni based MSUS-S is higher than all other catalytic materials with speed rate of 24-32 L g^{-1} h^{-1} at time on stream of 10h.

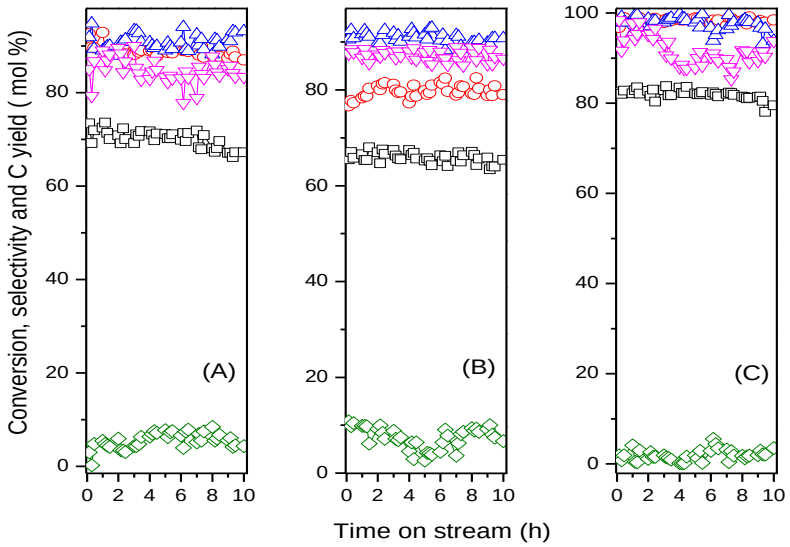

Fig. 6 Selectivity CO (△) H_2 (▽) Conversions CH_4 (□) CO_2(○), carbon (◇) at (a) 1 %La (b) 3 % Mg (c) 3% La 2%Co and 7 % Ni based MSUS-S at 700 ºC, with 24 L $g^{-1}h^{-1}$ GHSV, CH_4/CO_2 =1:1. Study showed that best catalyst (3La 2Co7Ni/MSUS-S) performed an efficient work than all other four catalysts. The reason for best performance is the presence of basic oxide La_2O_3 which react with CO_2 and form $La_2O_2CO_3$ species [37]. The H_2-TPR result showed the reduction of La_2NiO_4 [33] to form basic oxide La_2O_3. This basic oxide (La_2O_3) interacted with CO_2 to reduce the carbon deposition. The reason for low performance is the carbon deposition (coking), for other catalysts.

$H_2 + CO_2 \rightleftharpoons H_2O + CO$; $\Delta H_{298} = 41.2$ kJ/mol (1)

$2CO \rightleftharpoons CO_2 + C$; $\Delta H_{298} = -171$ kJ/mol (2)

$CH_4 \rightleftharpoons 2H_2 + C$; $\Delta H_{298} = 75$ kJ/mol (3)

This deposition of carbon (coking) is from (Eq. (2 & 3) side reactions.

Studies of mass transfer resistant effects

Experiments proved that high temperature is encouraging for the occurrences of MDR reaction which is actually endothermic in nature. So, therefore, the reaction at high temperature such as 750 ºC showed better performance than lower temperature 700 ºC. The conversions of methane and carbon dioxide on best catalyst (3%La 2%Co and 7 % Ni based MSUS-S) remained more than 90% & more than 97%, respectively (Fig. 7A-C) at 750ºC. Further results showed change of methane and carbon dioxide remain constant

with rise in (GHSV), confirmed increase in mass velocities of the reactants as Zhang reported [44], that rise of mass flow at rate of 10×10^{-5} g h/mL, the mass transfer resistance becomes ignorable. This is suggested that catalytic activity remained high and stable at 24 L g^{-1} h^{-1} as compared with 32 L g^{-1} h^{-1}.

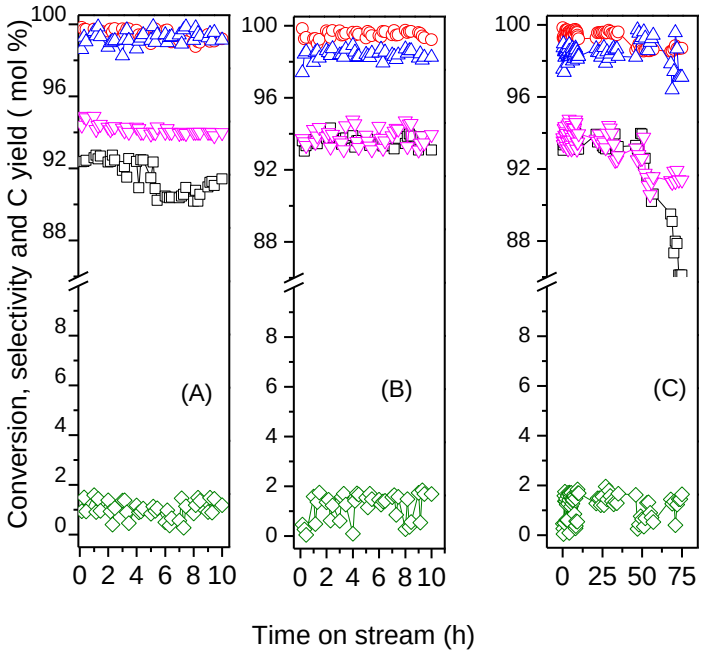

Fig. 7 Selectivity CO (△) and H_2 (▽) Conversions CH_4 (□) and CO_2(○) as well as carbon (◇) over 3% La 2%Co and 7 % Ni based MSUS-S (A) 10 h at 750 °C with GHSV=32 L $g^{-1}h^{-1}$ (B) 10 h and (C) 75 h at 750 °C with GHSV=24 L $g^{-1}h^{-1}$, CH_4/CO_2 =1:1.

The thermal stability and catalytic activity of La_2O_3 supported with (1: 3) for the said catalyst interrelated with variation of La/Ni in weight ratios. These catalysts played very vital role in DRM reaction, where the changing rate of CH_4 into product in descending order of 82% to 71% with change mass ration of La (1:3) at reaction temperature of 700 °C (Fig. 6C, A). Furthermore the amount of water collected in ice trap during catalyst evaluation studies at 1% La based catalyst was more than that collected over 3% based catalyst. The reason for such type of reaction is named reverse water gas shift (RWGS) for H_2 and carbon dioxide. The greater amount of La and La_2O_3 is promising material for the removal of deposited carbon during reaction.

The coking determined over spent 3%La doped catalyst was observed in least amount than over other catalysts.

CONCLUSION

These five xLa/Mg yCo zNi supported MSUS-S were synthesized by known method (sol–gel). In MDR reaction, catalyst 3%La 2%Co and 7 % Ni based MSUS-S remained the best at 700-750 ^{o}C. At this temperature the change (conversion ratio) of CH_4 and CO_2 remained highest. The unused and spent catalysts characterized via FT-IR, XRD, Carbon dioxide-TPD, OxygenTPO, Hydrogen TPR, and HRTEM. The results of evaluation and characterization for all catalysts indicated MSU-S as a good hydrothermal support. MSU-S has noted stable during MDR at 750 ^{o}C for long time duration of 75 h time on stream. The best catalyst (3La2Co7Ni/MSUS-S) with mesoporous structure was found intact at 750 ^{o}C. It was confirmed that 3% La 2%Co and 7 % Ni based MSUS-S catalyst showed outstanding performance and remained thermally stable for synthesis gas and H_2 yield at 750 ^{o}C for 75 h time on stream. Studies of mass transfer resistant showed that catalyst 3La2Co7Ni/MSUS-S exhibited better performance with flow of lower GHSV.

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Water Analysis Collected from Different Areas of Charsadda District Khyber Pakhtunkhwa (Pakistan) for Microbial Risk Assessment and Different Constituents in Water Bodies

Roohul Amin[1], Tauheed Ullah[2], Muhammad Haris[2], Qazi Muhammad Sharif[3], Muhammad Sajjad[1], Hussain Gulab[4], Sana Ullah[4]

ABSTRACT

In this research work five water samples are collected from different places of district Charsadda (KP), Naguman River, Sardaryab, Arat area Prang, charasadda bazar, and one sample is collected form district malakand. The purpose of research is the study of different organic and inorganic impurities present in these water samples. Initially pH, conductance, alkalinity, sulphate and chloride contents are determined in all samples and it is found that sample S4 collected from Charsadda bazaar has the worst results than other. The same sample S4 along with other samples were analyzed for the presence of biological impurities and sample S4 was declared as the worst once again. In the result of our finding, we conclude that sample S4 is the worst one which may be due to the anthropogenic activity such as entry of municipal waste in water bodies below earth surface.

Keywords: *Quantitative analysis, microbial risk assessment, censored data, acidity of water, waterborne disease*

INTRODUCTION

The provision of health effectiveness water supply system, along with administrative regulations and laws regarding security and check on drinking-water supply is considered first and foremost duty of Government. The basic and important aim of good system of water supply is the proper intake, treatment and delivery of pure and healthy water to inhabitants at appropriate and suitable quality and quantity [1, 2]. The network of water-supply is functional, it is possible that components of the system will fail, leave the obligation and function to provide supply of water to public. These failures result in a lack of water supply in good way and sometime also cause health problems.

[1] Department of Chemistry GC Peshawar , University of Peshawar, **Corresponding Author's Email: roohulamin1947@gmail.com**
[2] Department of Chemistry GPGC Charsadda, Bacha Khan University Charsadda
[3] PCSIR Laboratories Complex, Peshawar , 25120
[4] Department of Chemistry Bacha Khan University Charsadda

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Divya Bhardwaj and Neetu Verma stated that water in limited natural resource, therefore, its preservation is vital for conservation and safety of our natural environment [3]. Many water quality methods for monitoring are used to measure its concentration. Water quality can be determined through quality index which is analyzed through [4] different methods such as pH level, Turbidity, Conductivity, dissolved oxygen (DO) methods. The impact of water quality parameters are given along description that how water can be used on basis of various parameters. Murthal Sonepat described the water Quality Index (WQI) for quality determination of drinking water for different aims [5]. The WQI predicts the suitability of water for drinking and industrial purposes as well as for aquatic organisms. It can be measured from 0 to 100. The higher value of WQI shows better quality of water. WQI values may be affected by various water quality parameters like dissolved oxygen (DO), turbidity, pH level and electrical conductivity [6].

Jean-Claude described the treatment processes for different types of waste waters, such as industrial, municipal and agricultural including residuals managements. This controlling and assessment of quality is based on various methods such as chemical, physical and biological [7]. The waste management of solid and hazardous materials include the source characterization which effects and control the gaseous emissions [8].

According to Jerry L. Wilhm and Troy C. Dorris (1968) the foundation of water quality criteria by examination of the benthic macro invertebrate community as reflected in the diversity index, $d=-\Sigma (ni/n) \log 2 (ni/n)$, is proposed [9]. The index expressed the relative influence of each component collected is independent of sample size and dimensions.

According to A. Vogelpohl, S.-U.Geissen (1997) the hydroxyl radicals are the major oxidants which govern the advanced oxidation processes (AOPs) used in water technology and also applicable for cloud water. The effectiveness of various hydroxyl groups are quantified for calibration. The generalization of results depends on governing the lifetime of hydroxyl groups. The connection between climatic hydrological and QWI parameters of concerned River Mekong was observed by L.Prathumratana and S.Sthiannopka in their study. This river is passing via four countries/areas (Cambodia, Thailand, Vietnam and Lao PDR) [10].

The secondary data of this research done on river Mekong for hydrological and climatic study is based on parameters such as precipitation, evaporation, average air temperature, mean water level and discharge ratio of flow. The parameters for water quality are included TSS, conductivity, pH, alkalinity, presence of radicals such as PO_4^{3-}, NO^{3-}, SO_4^{2-},TP , DO, COD and metals like Ca, Cl, Fe, Mg, Na, K, and Si. This relationship of different parameters was determined by using Pearson's correlation method. The precipitation range (0.375- 0.661), air temperature range (0.515–0.621), discharge flow range (0.526–0.659) with weak negative correlation of evaporation in the range of (0.169–0.468), the values for these parameters are noted [11]. The relationship of QWI showed that five parameters have precipitation values. These are PO_4^{3-}, TP, TSS,

NO_3^-, and COD also having weak to fair positive correlations with mean water level and discharge flow.

In the research of Elise Barbot and Natasa S. Vidic (2001) the exponential increase in energy was discussed and critically evaluated. In this study for the comparison Chloride was used as a reference because its concentration changes with the passage of time. Some cations in large concentration (Ca, Mg, Sr) were well-correlated with concentration of chloride whereas barium exhibited strong effect on geographic location. The analysis of water quality in this work is helpful toward water management strategies for the development of unconventional gas resources [13-15]. In this work water samples collected from different areas of district Charsadda, KP was analyzed for organic and inorganic impurities and for microbial risk assessment.

METHODOLOGY

Water sample collection

In this work various sample were collected from different places for analyzing of organic and inorganic components in water bodies. Water samples were collected in clean plastic bottles from following areas, such as Malakand, Naguman, Arat Parang (Persian well rural area) Charsadda Bazar (Charsadda main city) and Sardaryab. These research samples are labeled as S1, S2, S3, S4 and S5 respectively.

Analysis for pH and conductance

The samples were collected in clean bottles and carried for further analysis. These samples were checked for different experimental tests which are given below. The common pH meter and conductometer available in college laboratory was used for pH and conductance measurement. The pH is one of the most important parameter which showed variation in water varieties and nature of water body. If a sample has dissolved inorganic constituents, these were checked for conductivity and determined quality of water.

Determination of Total suspended solids (TSS) and total dissolved solids (TDS)

The (TDS) and (TS) are solid particles present in water samples which are filtered. These are present in water samples in different concentration depending on the nature and quality of water body. Dissolved minerals, gases and organic constituents may produce aesthetically displeasing color, taste and odor. Some dissolved organic chemicals may deplete the dissolved oxygen in water bodies and some constituents interrupted these substances via biological oxidation, whereas other toxic components have been considered carcinogenic.

Procedure for Determination of TDS and TSS

Both TDS and TSS are determined by procedures of Jhon Moore and Elizebth A Moore [13], additionally distilled water is used for washing all apparatus used in this experiments. The given water sample is filtered and the filtrate is heated to dryness in a weighted china dish to some constant weight at 179-181^{o}C. Similarly research samples is filtered and residue were dried to a constant temperature at 103-105^{o}C. The increase in weight of the filter paper showed the total suspended solids (TSS). The difference between the total solids (TS) and total dissolve solids (TDS) may provide an estimate of the total suspended solids.

Determination of Alkalinity, Sulphates and Chloride Contents.

The alkalinity, sulphate and Chlorides contents were determines by known procedures of Jhon Moore and Elizebth A Moore [13]. The alkalinity of the given samples is investigated to calculate its effect on the nature of water using formula given below.

$$\text{Alkalinity} = \frac{V(H2SO4)\ \text{x}\ \text{N(H2SO4)}\ \text{x}\ \ \text{Eq.wt of CaCO3 x 1000}}{Volume of sample}$$

$$\mathbf{S}= \frac{N(iodine)\ \text{x Eq.wt of SO3 X (Q}-\text{B) x 1000}}{Volume of sample}$$

Where B is blank titration, While Q is volume of iodine used in sample

Similarly sulphate content in water samples were checked in all water samples under study in this research work for softness and hardness of water.

Sulphate contents:

The samples are analyzed to find sulphate content. Which are in the given table 2 and also shown in fig no 4.The sulphate content were calculated by the given formula.

$$\mathbf{S}= \frac{N(iodine)\ \text{x Eq.wt of SO3 X (Q}-\text{B) x 1000}}{Volume of sample}$$

Where B is blank titration, while Q is volume of iodine used in sample

Sulphate content shows the nature of water i.e softness and hardness. As $MgSO_4$ and $CaSO_4$ make water hard which are not good for health and washing purposes.

Chloride determination of water samples:

All the three samples were analyzed for the determination of chloride contents through different experiments that gives results differ from each other with a bit margin. The chlorides contents of different water sample are given in descending order below.

Total dissolve solids (TDS) and Total suspended solids (TSS) determination.

Dissolved solids and suspended solids also play a key role on the nature of water samples. Water with greater quantity of TSS and TDS are not good for health.

Biological impurities (Microbial study)

The presence of microorganism is determined in the PCSIR laboratories complex Peshawar by standard analytical method [14]. This technique of determination of microorganisms in water bodies it the most important method used for the quality estimation and suitability of drinking water. This technique is used to determine the presence of different microorganism in the water samples.

RESULT AND DISCUSSION

The experimental work was carried out in several parts and hence the data was collected from the following experiments for TDS and TSS, alkalinity and sulphate determination. The samples which were collected from different/ various areas are further investigated for pH values (Fig 1).

Table 1: pH and conductance values at different temperatures

Samples	0-20° C		20-30° C		30-40° C	
	pH	Conductance (mV)	pH	Conductance (mV)	pH	Conductance (mV)
S1	7.69	233	7.35	117	7.11	011
S2	7.53	219	7.22	129	7.09	023
S3	7.41	223	7.19	113	7.05	009
S4	7.55	231	7.31	115	7.13	003
S5	6.97	211	6.99	122	7.01	007

The pH values given at different temperatures showed change in pH. Sample S1 and S4 have basic pH at 0-20° C while sample S5 has acidic nature in the temperature range of 0- 20°C.While all samples showed natural behavior in the temperature range of 30-40° C as shown in table 1

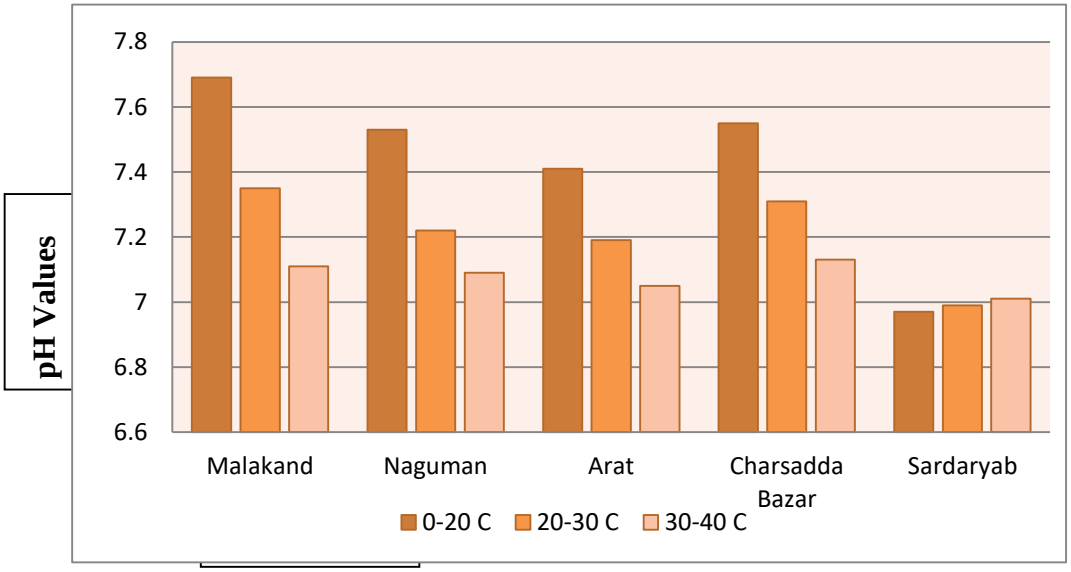

Fig. 1 pH values at different temperature

Conductance Determination

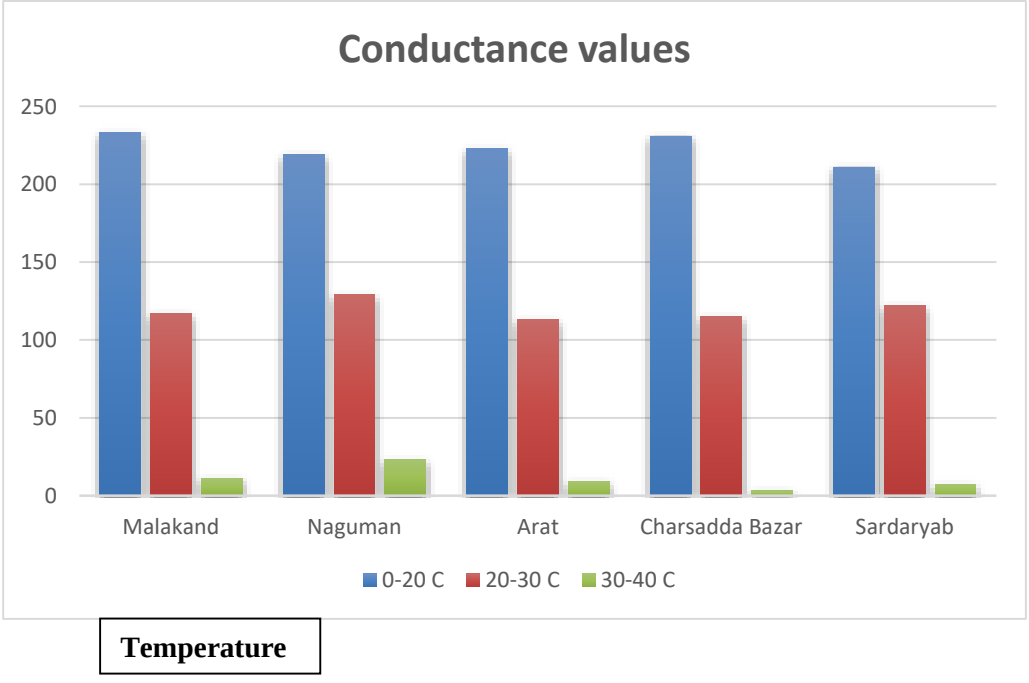

Figure 2. Conductance values at different temperatures.

The samples were analyzed to find its conductance at different temperature range. Which are plotted in the given table 1 and also shown in Fig. 2. The values of conductance are more at low temperature 20-30 ºC while its value is greater at high temperature as shown in table 1.

TDS (total dissolve solid), TSS (total suspended solid)

The research samples were further analyzed to obtain the total dissolved solid in the given samples. The result of TDS is given below in table 2. Similarly the collected water samples were further analyzed for total suspended solid (TSS).

Table 2: TDS, TSS, Sulphate, alkanity and Chloride contents of different water samples

SAMPLES	TDS	TSS	Sulphate contents (g/L)	Alkalinity contents (mg/L)	Chlorides mg/L
S1	63.5	52.2	604.45	603.86	201.33
S2	55.2	52.4	589.56	598.08	215.33
S3	41.5	52.0	572.95	621.89	233.33
S4	58.5	52.3	616.35	615.76	243.67
S5	32.1	52.1	607.16	628.14	217.56

The amount of total dissolved solid and total suspended solid are shown in fig 3. Sample S5 has least amount of TDS while water sample 1 (S1) has maximum amount of TDS. Similarly sample S3 has least value of TSS and sample S2 has maximum value.

Sulphate contents:

The samples were analyzed to find sulphate content. Which are in the given table 2 and also shown in fig no 4.The sulphate content were calculated by the given formula.

$$S = \frac{N(iodine) \text{ x Eq.wt of SO3 X (Q}-\text{B) x } 1000}{Volume of sample}$$

Where B is blank titration, while Q is volume of iodine used in sample

Sulphate content shows the nature of water i.e. softness and hardness. As $MgSO_4$ and $CaSO_4$ make water hard which is not good for health and washing purposes. Sample S1, S4 and S5 have greater sulphate content, while S3 has the least sulphate content.

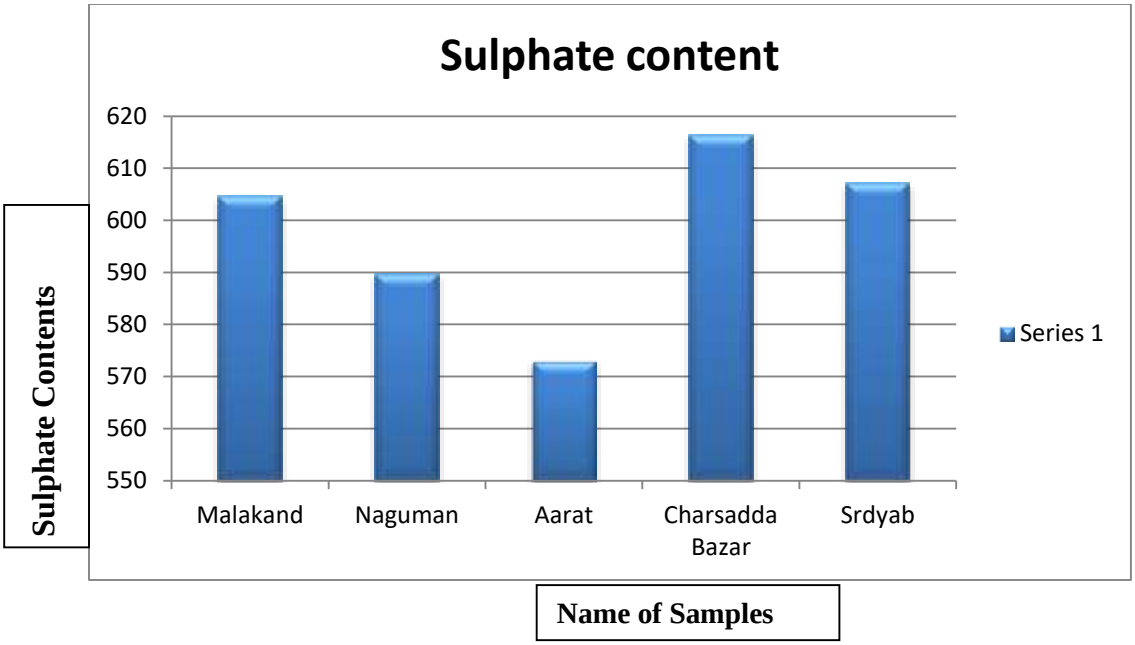

Fig no 3.sulphate contents of different water samples

Alkalinity content:

The samples were analyzed to find alkalinity content and results are plotted in the given table 2 and also shown in fig no 5.The formula through which the alkalinity is calculated is given below.

$$\text{Alkalinity} = \frac{V(H2SO4) \text{ x } \mathrm{N(H2SO4)} \text{ x Eq.wt of } \mathrm{CaCO3} \text{ x } 1000}{Volume of sample}$$

Sample 5 has greater alkalinity content, and S2 has lower alkalinity content. The alkalinity of all sample is given below in descending order S5>S3>S4>S1>S2.

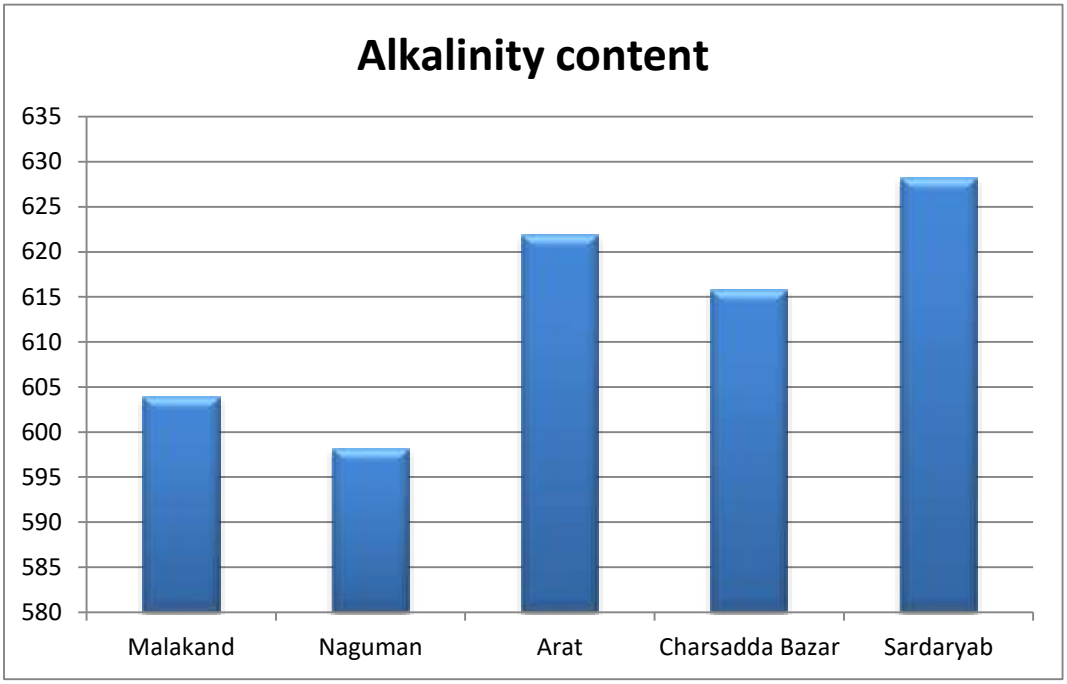

Fig.5 Alkalinity determination

Chloride determination of water samples

All the three samples were tested for the determination of chloride contents through different experiments that gives results differ from each other with a bit margin which are showing in table 2. The chlorides contents of different water sample are given in descending order below.

S4>S3>S5>S2>S1

The above results showed the total amount of chloride present in all research samples of water, which is given the figure below.

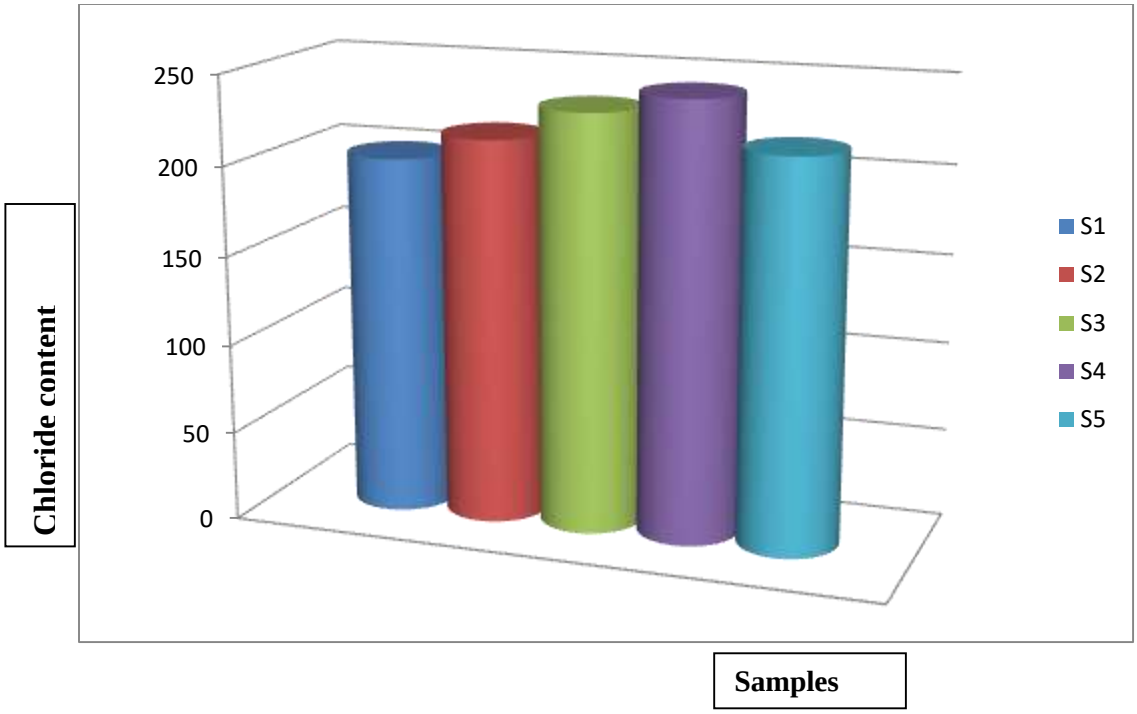

Fig 6: Chloride contents of different water samples.

Presence of Microorganisms in the research samples

The following three research samples were analyzed for the presence of microorganisms in the PCSIR laboratories Peshawar. The result of experiments are given below in fig 7.

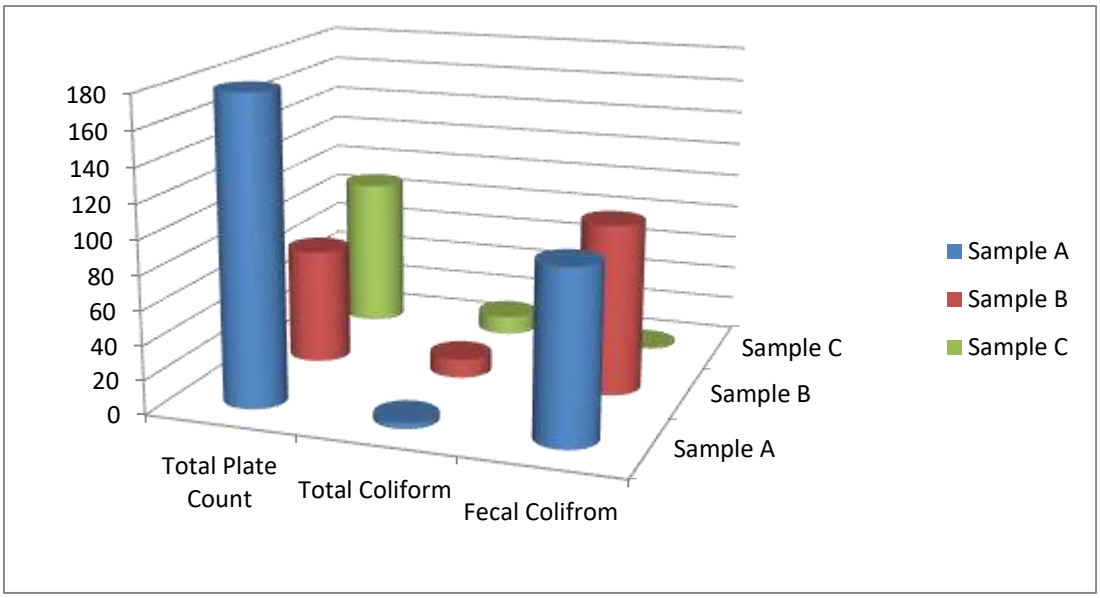

Fig 7: showing different values of organic contents in water samples

Three water samples were characterized for the presence of Microorganism (bacteria) in PCSIR laboratories Peshawar are mentioned below along name and the area from where these are collected.

Sample A =Nauguman

Sample B = Charsadda bazaar

Sample C = Sardaryb

The sample B = Charsadda bazar

In these samples, the water collected from Charsadda bazaar has the worst result for the presence of microorganisms.

CONCLUSION

In this research work water samples were collected from five different places and these samples were marked named like S1, S2, S3, S4, S5. All the samples were characterized for various parameters. Such as pH. Conductance, Alkalinity, Sulphates and chloride contents.

In the result pH level for S3 is comparatively better than other. Similarly, conductance falls in the normal range, alkalinity levels of S3 is little more than other which showed that more base present in water sample. The Sulphate content of S5 is comparatively more and showing poor quality of water for drinking and cloth washing.

So, the given result and studies showed that the sample S3 is the better one as it contains low level of contamination as compared to others and sample S4 is the worst. The bacteriological analysis of different water samples showed that sample B has the large amount of microorganisms which is not drinkable as it contains contamination while the sample A & C contain less amount of contamination.

In the result of this study, it is concluded that water collected from Charsadda bazaar have poor qualities. Therefore, this water is not good for drinking purposes.

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In Silico Elucidating the Common Gene-Regulatory-Network(S) Of Accelerated Cell Death (Acd11) from *Arabidopsis Thaliana* and Its Orthologs

Shahid Ali[1], Humayun[2], Mushtaq Ali[3], Muhammad Ali[4], Fazal Raziq[5], Saadat Khan[6]

ABSTRACT

The Arabidopsis accelerated cell death 11 (acd11) is a genetic model for studying immune response activation and localized cellular suicide that prevent pathogen spread during plant infection. In this research article we predicted the common gene-regulatory-network of accelerated cell death (acd11) from Arabidopsis thaliana and its orthologs with the help of well-known bioinformatics tools. The most common orthologs were extracted from Phytzome and PLAZA on the basis of protein sequence similarity using BLASTp. Developmental expression analysis from Genevestigator concluded the acd11 is highly expressed at last developmental stage and co-expressed with Autophagy related and Disease resistant genes. PPI from STRING network confirm that its interacting partners are EDS1 and LAZ1 both of which play an important role in disease resistant and programmed cell death respectively. Protein disorder from DisEMBL confirm its diverse functioning properties and non-stable structure. Ligand binding study from RaptorX predicted that residues in the Active site pocket Asparagine and Histidine.

Keywords: *Arabidopsis accelerated cell death 11, acd11, BLASTp*

INTRODUCTION

Due to the hypersensitive (HR) response in plants, which causes PCD, the plant host resistance (R) protein identifies pathogen avirulence component. In the model plant Arabidopsis, recessive acd11 is a lesion mimic mutant that activates HR-like PCD in the absence of pathogen infection. Before the plant flowers, Acd11 starts to degrade the chlorophyll in the two-leaf stage, consuming the entire plant (Brodersen *et al*., 2002). Sphingolipid transfer proteins that help sphingosine transfer across membranes in vitro are homologous to ACD11 (Brodersen *et al*., 2002). ACD11 has similarity to GLTP, a protein that facilitates the transfer of glycoshingolipids. Although mammalian GLTP is

[1] Assistant Professor, Govt. Postgraduate College Swabi, **Corresponding Author's Email: profshahid.ali@gmail.com**
[2] Assistant Professor, Govt. Degree College Yar Hussain, Swabi
[3] Assistant Professor, Govt. Postgraduate College, Swabi
[4] Assistant Professor, GDC Toru, Mardan
[5] Assistant Professor, GPGC Mardan
[6] Assistant Professor, Govt. Commerce College, Peshawar

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structurally and biophysically well described, its biological function is unknown (Brown and Mattjus, 2007). The positively charged triad's clustered Lys/Arg residue in ACD11, which is best positioned to bind phosphate, explains why the protein is unable to bind sugar head groups or transfer glycolipids (Peterson *et al*., 2008).

ACD11 in yeast forms interactions with PRA7 and PRA8, while VAP27-1 has a stronger interaction with an ACD11 homolog rather than the ACD11 protein itself. While its interactors have varying degrees of membrane association, ACD11 is mostly a cytosolic protein. ACD11, in conjunction with the proteins PRA and VAP, may control membrane trafficking (Peterson *et al*., 2009).

To regulate programmed cell death (PCD), it is essential to maintain a dynamic balance between Cer and C1P as per the studies by Chen *et al.* (2009), Pata *et al.* (2010), and Berkey *et al.* (2012). The deficiency of *acd11* results in altered C1P levels and increased phyto-cer levels, indicating a functional connection between *acd11* expression and sphingolipid metabolic regulation in plants. Despite the low sequence similarity among ACD11 and other GLTP homologs, such as human CPTP, Simanshu *et al*. (2014) have demonstrated the existence of conserved structural similarity between Arabidopsis ACD11, human GLTP, and human CPTP.

OBJECTIVES

1. Identification and extraction of orthologs of ACD11 from *Arabidopsis thaliana* among plant species.

2. Screen for common interacting protein with ACD11 and its orhtologs to analyze and deduce the gene regulatory network of ACD11.

3. Prediction of 3D structure and interacting residue of proteins by determining its interface residue, intrinsic protein disorder.

LITERATURE REVIEW

Simanshu *et al.* (2014) have demonstrated that ACD11, encoded by the *acd11* gene, is a lipid transfer protein that promotes intermembrane transfer of sphingosine and sphingomyelin but not Cer or glycolceremides. *Acd11* is a lesion mimic mutant in the model plant *Arabidopsis*, and in its recessive form, it triggers HR-like PCD even without pathogen infection. At the two-leaf stage, *Acd11* induces chlorophyll degradation, which eventually engulfs the entire plant before flowering. Despite being similar to three known sphingolipid transfer proteins, ACD11 has been demonstrated to aid the transfer of sphingosine between membranes in laboratory experiments.

The discovery of lesion mimic mutants (LMM), such as the hypersensitive response (HR), which is associated with pathogen resistance, has proven to be a useful tool in studying programmed cell death (PCD) pathways in plants (Lorrain *et al.*, 2003).

The incompatibility reaction, a cell death response that takes place when cells of different genotypes fuse in filamentous fungi, is governed by a group of loci called *het* loci (heterokaryon incompatibility loci). In *Podospora anserina*, the genes upregulated during this cell death response are involved in a process similar to autophagy, and ACD11 homolog, called HET-C protein, is implicated in vegetative incompatibility reaction in the fungus (Pinan-Lucarre *et al.*, 2003).

Plants rely on programmed cell death (PCD) as an essential response mechanism to various internal and environmental signals to achieve proper development and survival. Unlike animal apoptosis, PCD in plants is characterized by the absence of engulfment by neighboring cells. PCD occurs in plants during development, virulent infections, and the hypersensitive response (HR) brought about by avirulent stress effectors, as noted by Lam and colleagues in 2004.

The plant surveillance system has evolved to recognise pathogen-secreted chemicals in order to initiate a defense response when plant pcd coexists with disease resistance, an occurrence known as the hypersensitive response. These released compounds function as virulence factors in plants lacking genetic disease resistance, acting through mostly unidentified methods. If the function of ACD11 is lost, the result is a lethal condition. The recessive homozygous mutant of *acd11* grow normally until they develop 2-4 leaves. After that, they exhibit accelerated cell death similar to that observed in lesion mimic mutant, which is a plant with spontaneous lesions on the leaves (Greenberg *et al*., 2004).

Broderson *et al.* (2005) have reported that salicylic acid (SA) levels increase in response to infections that induce programmed cell death (PCD), and salicylate hydroxylase, which is encoded by the bacterial nahG gene, can prevent PCD development. SA is believed to play a role in the initiation of PCD linked to pathogen defense responses. PCD in acd11 is reliant on the plant hormone SA and can be hindered by expressing a bacterial SA hydroxylase (nahG) or through mutation in PAD4 and EDS1.

Airene et al. (2006), Brown and Mattjus (2007), and Peterson et al. (2008) have suggested that ACD11 has a GLTP fold and acts as a glycolipid transfer protein, based on its predicted structural homology model. Meanwhile, Hofius et al. (2007) reported that programmed cell death (PCD) during the hypersensitive response (HR) in plants is triggered by recognition of pathogen avirulence factors by the plant host resistance (R) protein.

According to research by various authors, including Brown and Mattjus (2007), GLTP and FAPP2 are homologs of ACD11 that are involved in the transfer of glycosphingolipids between membranes, although the exact role of GLTP remains unknown. ACD11 and ACD5 are involved in regulating the balance of Cer and C1P levels, which control PCD related to HR. Additionally, ACD11 may play a role in regulating membrane trafficking through interactions with PRA and VAP proteins, despite its inability to transfer glycolipids due to the Lys/Arg residue cluster. Studies also

suggest that ACD11 expression is involved in regulating sphingolipid metabolism in plants, and its deficiency can lead to inappropriate HR activation by LAZ5 (Chen *et al*., 2009; Pata *et al*., 2010; Berkey *et al*., 2012; Peterson *et al*., 2008, 2009; Wang *et al*., 2008; Palma *et al*., 2010).

LAZ1, a DFU300 transmembrane protein, serves as a regulator of PCD associated with HR and suppresses cell death in acd11 and certain types of HR cell death (Malinovsky et al., 2010). Although ACD11 and other GLTP homologs, including human CPTP, have low sequence homology, crystallographic data has established their conserved structural homology (Simanshu et al., 2014).

RESEARCH METHODOLOGY

The research methodology of this research article is described in the following subsections.

Orthologous genes of ACD11 that is identifie and selected on the basis of protein sequence similarity from PLAZA, PHYTOZOME (Goodstein *et al*., 2012) and NCBI Databases.

Protein sequences of the orthologous genes will be retrieved from Phytozome for further analysis.

Gene regulatory network will be predicted on the basis of tissue or conditional specific co-expressional behavior of genes *acd11,* using expression browser Databases such as eFP and tools like Planex (Yim*et al*., 2013), PlaNet (Mutwil *et al*., 2011) and Genevestigator (Zimmerman *et al*., 2004),

PPI network of ACD11 and orthologous genes will be retrieved from STRING (Szklarczyk *et al*., 2011) to predict common interacting protein. 3D structure of all interacting protein will be predicted from PHYRE2 and HHPred.

DisEMBL is a useful method for predicting protein structure disorder, and RaptorX is a web-based tool used for determining the functional role of proteins by generating reliable three-dimensional atomic models of proteins. These tools are important for target selection and constructing biochemical studies, particularly in genomics and structural biology projects. In addition, protein sequences and structures can be utilized to forecast residues that participate in ligand binding sites (LBS) (Linding and Jenssen *et al.*, 2003; Wang and Li *et al*., 2016).

RESULT AND DISCUSSIONS

Identification of orthologous for the reference Protein ACD11

For the reference gene ACD 11 of Arabdosis Thaliana were retrieved and identified through MSA BLAST tool form NCBI and Phytozome on the basis of protein

structure similarity. From the following retrieved data of orthologues from databases, further selection was made on the basis of their availability for protein interaction and co-expression analysis.

Table 1. Representing ACD 11 (AT2G34690) orthologous genes with GeneInfo Identifiers from different plant species Data obtained from PLAZ and Phytozome BLASTp search.

GENE ID PLAZA	**GENE ID PHYTOZOME**	**ORGANISM**	**PROTEIN LEGTH**	**E-VALUE**	**BIT SCORE**	**% IDENTITY**
CRU_004G15840	Carubv10023844m.g	*C.rubella*	207	4e-140	400	92.75
TC0002G01160	Thecc1EG006094	*T. cacao*	207	5e-108	316	72.46
GR08G04690	Gorai.008G046900	*G. raimondii*	207	2e-106	312	71.98
MD10G020560	MDP0000162581	*M.domestica*	207	2e-103	304	71.01
RC28617G00100	**28617.t000010**	***R. communis***	**214**	**2e-103**	**305**	**69.16**
PPE_004G08510	Prupe.4G089300	*Prunus persica*	201	5e-102	302	70.65
FV3G15140	gene28951-v1.0-hybrid	*Fragaria vesca*	201	1e-99	295	71.64
SL02G070250	**Solyc02g070250.2**	***S.lycopersicum***	**205**	**1e-96**	**287**	**68.78**

ZM01G55250	**GRMZM2G058872**	***Zea mays***	**209**	**1e-96**	**289**	**67.46**
OS03G57140	**LOC_Os03g57140**	***Oryza Sativa j***	**209**	**9e-93**	**277**	**61.72**
CS00010G00480			171	9e-87	262	74.27
MT2G029520	1g027852m.g	*Citrus cinensis*	**209**	**5e-86**	**260**	**61.24**
PT06G05180	**Medtr2g030530**	***M.truncata***	**171**	**5e-83**	**253**	**71.35**
	Potri.006G051800	***P.trichocarpa***				
GLYMA15G13470	**GM.15G13470**	***Glycine max***	**209**	**7e-99**	**293**	**70.05**

The plant species selected for further study have almost the same number of Amino acids in polypepetide chain with variations between 214 aa to 171 aa. The selection of species cover both dicots (*S.lycopersicum, M.truncata, P.trichocarpa, R.cummunis*) and monocots (*Zea mays and Oryza sativa*), for predication their protein structure, function and interactions to validate the reference gene and protein regulatory network analysis.

Extraction of protein sequences of ACD11 orthologs

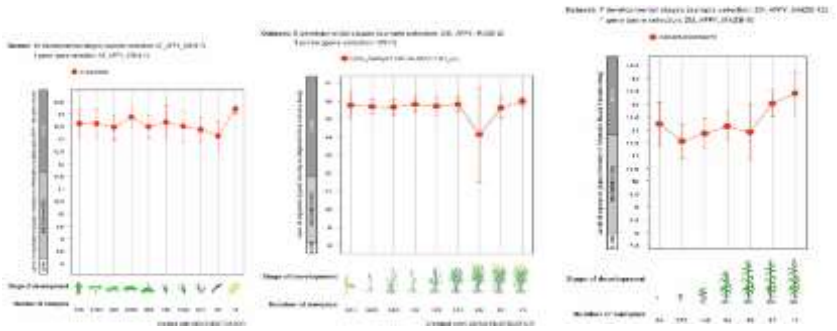
Protein sequences in FASTA format of the orthologues gene were retrieved from the Phytozome database for further analysis of protein structure prediction, ligand binding, protein interaction, protein disorder all based on the sequence of proteins.

Predicting developmental stages co-expression of ACD11 and its orthologues

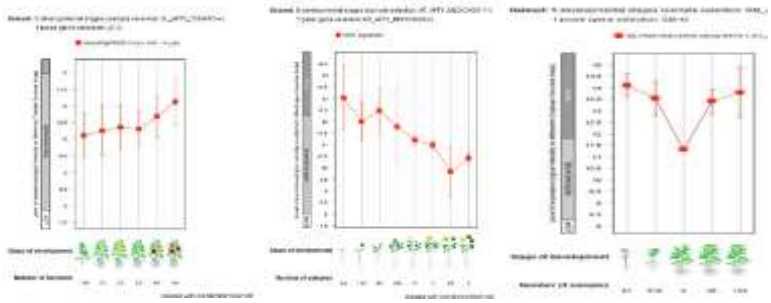
The expression of genes was examined during plant development and at the anatomical level by accessing the Affymetrix array database using the Genevestigator response viewer. Microarray data from the Arabidopsis Gene Chip platform was obtained

and analyzed by querying gene member IDs. The database contains microarray data from only the wild-type background, and the expression level was assessed in the late stages of plant development, including senescence. ACD11 loss-of-function mutants exhibit accelerated cell death similar to that observed in lesion mimic mutants, and the protein may play a functional role during plant maturation and senescence (Greenberg et al., 2004).

The co-expressed gene were retrieved form the data for the refence gene as well as of their orthologues.



(a) (b) (c)



(d) (e) (f)

Figure 1 Developmental stage-dependent expression analysis of the reference gene and its orthologues, in most cases the genes expression is at highest level in the last developmental stages of plant. (a) Arabidopsis thaliana (b) Oryza staiva (c) Zea mayz (d) Lycopersicum esculentum (e) Medicago truncatula (f) Glycine max

Co-expression were observed under the stress conditions (Perturbations) by default with a 50 limit of expressed genes. Positive correlation of the expressed genes under the same conditions were analyzed Pearson correlation through Genevistiagtor affymytrix biochips. All genes of the orthologues were put to the same conditions to confirm validation of the co-expressed genes with ACD11 of Arabidopsis thaliana. As it

is evident from the result that stress and autophagy related proteins are highly co-expressed in the reference gene as well as in its orthologues, which reflects a strong association of these proteins in programmed cell death regulation of the plant. The programmed cell death observed in *acd11* is controlled by the hormone salicylic acid (SA) and can be inhibited by the expression of a bacterial SA hydroxylase (nahG) or by mutations in PAD4 and EDS1, as reported by Broderson *et al. (*2005) and Feys *et al.* (2001).

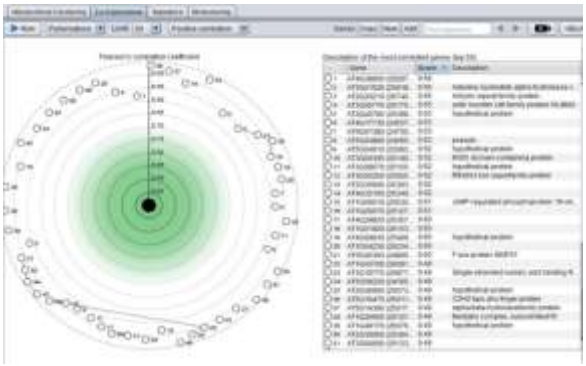


Figure 2*: Conditional stress co-expression analysis of reference gene ACD11 using Genevestigator co-expression tool.*

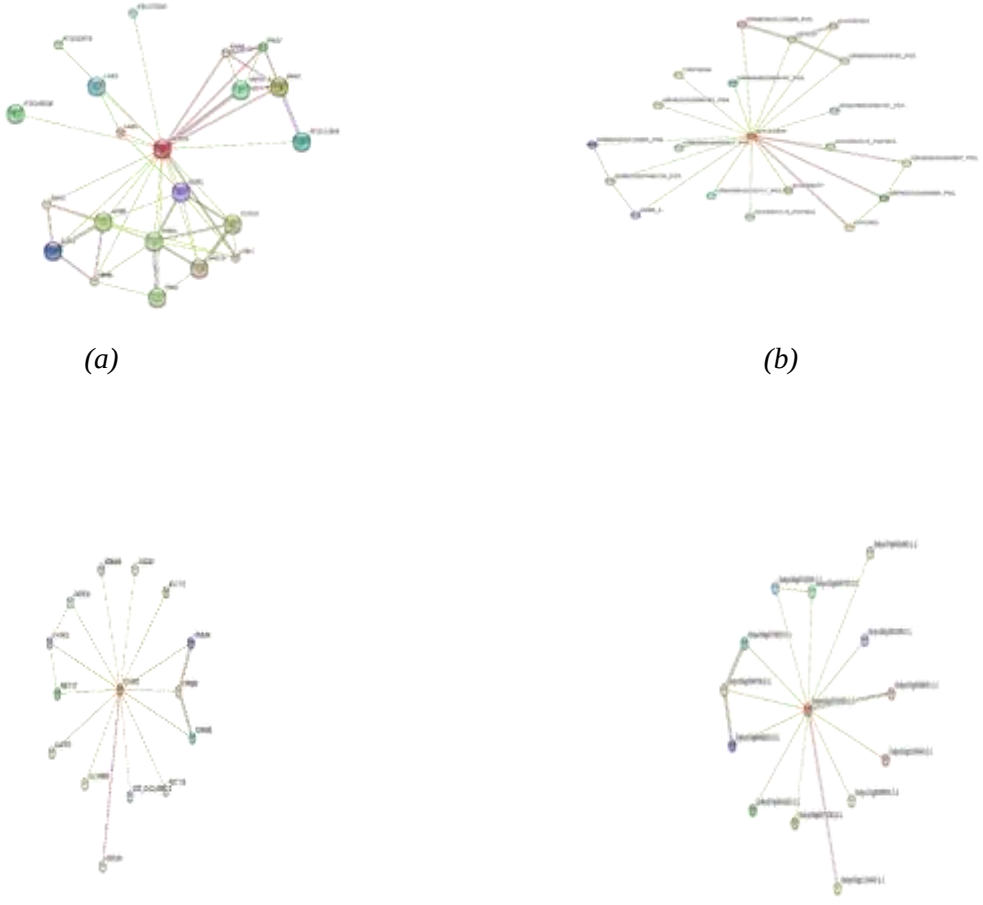
Table 2: *Co-expressed genes of the reference gene and its orthologues under stress conditions using Genevestigator tool.*

Protein	*Arabidopsis Thaliana*	*Glycine max*	*S.lycopersicum*	*Oryza sativa*	*Zea mays*
Syntaxin	AT4G17730 AT4G32150 AT1G26670 AT3G60600 AT1G16240 AT2G36900	GLYMA01G00760 GLYMA14G02120 GLYMA09G13840 GLYMA16G08200 GLYMA02G42280	SOLYC05G013050 SOLYC01G100820 SOLYC03G122090 SOLYC02G032930	OS03G0137400	GRMZM5G806784
UQ Ligase	AT4G09830 AT2G36060 AT5G32440	GLYMA10G43120 GLYMA06G02390	SOLYC04G080810	OS08G0170900 OS11G0483600 OS08G0540300	GRMZM2G171430

Zinc finger	AT5G16470	GLYMA14G16717 GLYMA12G13270 GLYMA10G43090 GLYMA07G12170	SOLYC03G063320 SOLYC09G008470	OS07G0167200 OS06G0697200	GRMZM2G049296 GRMZM2G000014
Stress related protein	AT1G3700	GLYMA10G28300 GLYMA03G41030	SOLYC09G090370 SOLYC01G080410 SOLYC09G072670 SOLYC01G104600 SOLYC07G044850	OS07G0589400 OS05G0122700	GRMZM2G106960
Autophagy related protein	AT2G45980	----------	SOLYC06G034160 SOLYC03G096790 SOLYC03G113000	OS03G0137400	GRMZM2G056572 GRMZM2G171435
Ras rekated protein	----------	GLYMA09G01950 GLYMA05G32520	SOLYC06G065230 SOLYC01G104700	----------	----------

Protein-protein interaction network(s) and 3D structure prediction

The PPI network of ACD11 and its orthologous genes was obtained from the STRING database to predict its interactions with other proteins. The analysis revealed ACD11's interaction with EDS1, LSD1, LAZ1, LCB, and FMO. LAZ1, which belongs to the DFU300 transmembrane protein family, regulates plant PCD associated with HR and is involved in acd11 cell death. Loss of LAZ1 function suppresses cell death in acd11 and certain types of HR cell death. The STRING database is a valuable tool for predicting protein interactions and understanding their functional impact (Malinovsky *et al*., 2010).



(a) *(b)*

(c) *(d)*

Figure 3: PPI of the reference gene and orthologues retrieved from STRING database network. (a) Arabidopsis Thaliana (b) Zea mayz (c) Lycopersicum esculentum (d) Oryza sativa.

node1	node2	node1 accession	node2 accession	node1 annotation	node2 annotation	score
ACD11	ACD5	AT2G34690.1	AT5G51290.1	ACCELERATED CELL DEATH 11	ACCELERATED CELL DEATH 5	0.902
ACD11	AT1G14340	AT2G34690.1	AT1G14340.1	ACCELERATED CELL DEATH 11	RNA recognition motif-containing ...	0.800
ACD11	AT1G23070	AT2G34690.1	AT1G23070.1	ACCELERATED CELL DEATH 11	uncharacterized protein	0.588
ACD11	AT1G77220	AT2G34690.1	AT1G77220.1	ACCELERATED CELL DEATH 11	uncharacterized protein	0.579
ACD11	AT5G45230	AT2G34690.1	AT5G45230.1	ACCELERATED CELL DEATH 11	TIR-NBS-LRR class disease resist...	0.580
ACD11	BPA1	AT2G34690.1	AT5G16840.2	ACCELERATED CELL DEATH 11	binding partner of acd11 1	0.938
ACD11	EDS1	AT2G34690.1	AT3G48090.1	ACCELERATED CELL DEATH 11	enhanced disease susceptibility 1	0.753
ACD11	EDS16	AT2G34690.1	AT1G74710.2	ACCELERATED CELL DEATH 11	Isochorismate synthase 1; Involve...	0.641
ACD11	FMO	AT2G34690.1	AT1G12200.1	ACCELERATED CELL DEATH 11	flavin monooxygenase; Catalyzes ...	0.597
ACD11	FMO1	AT2G34690.1	AT1G19250.1	ACCELERATED CELL DEATH 11	flavin-dependent monooxygenase...	0.597
ACD11	LAZ1	AT2G34690.1	AT4G38360.2	ACCELERATED CELL DEATH 11	LAZARUS 1	0.721
ACD11	LAZ5	AT2G34690.1	AT5G44870.1	ACCELERATED CELL DEATH 11	LAZARUS 5	0.759
ACD11	LCB2	AT2G34690.1	AT5G23670.1	ACCELERATED CELL DEATH 11	long chain base2; Serine palmitoyl...	0.758
ACD11	LSD1	AT2G34690.1	AT4G20380.8	ACCELERATED CELL DEATH 11	zinc finger protein LSD1	0.711
ACD11	PRA7	AT2G34690.1	AT1G55190.1	ACCELERATED CELL DEATH 11	PRA1 family protein F2; May be in...	0.894
ACD11	PRA8	AT2G34690.1	AT3G13720.1	ACCELERATED CELL DEATH 11	PRA1 family protein F3; May be in...	0.939
ACD11	SAG13	AT2G34690.1	AT2G29350.1	ACCELERATED CELL DEATH 11	senescence-associated gene 13	0.645
ACD11	SBH1	AT2G34690.1	AT1G69640.1	ACCELERATED CELL DEATH 11	sphingoid base hydroxylase 1; Inv...	0.645
ACD11	SBH2	AT2G34690.1	AT1G14290.1	ACCELERATED CELL DEATH 11	sphingoid base hydroxylase 2; Inv...	0.621
ACD11	VAP27-1	AT2G34690.1	AT3G60600.1	ACCELERATED CELL DEATH 11	vesicle associated protein; May pl...	0.861

Table 3*. Protein interacting partners of ACD11 and its orthologues*

	A.thaliana	*S.lycopersicum*	*G.max*	*P.trichocarpa*	*O.sativa*	*Zea mays*
EDS1	At3g48090	solyc06g071280	glyma04g34800	Poptr_0015s08100	Loc_os09g22450	grmzm2g045716
LSD1	at4g20380	solyc02g069720	glyma05g30490	Poptr_0011s15810	Loc_os12g41700	grmzm2g114613
LAZ1	at4g38360	solyc07g054320	glyma06g00300	10Poptr_0009s167	Loc_os06g51100	grmzm2g000427
LCB2	at5g23670	solyc06g072320	glyma02g07250	Poptr_0012s10630	Loc_os01g70380	grmzm2g010202
FMO	at1g12200	solyc07g042430	glyma17g05160	Poptr_0001s36280	Loc_os03g08410	grmzm2g000427

Phyre2 was used to predict the 3D structure of the reference protein and its orthologues, and HHPred was used to validate the structure by comparing the secondary structure of the reference protein to that of its orthologues. Although ACD11 has low sequence homology with other GLTP homologs, crystallographic data has shown conserved structural homology between Arabidopsis ACD11, human GLTP, and human CPTP. The 3D model for ACD11 was retrieved from the PDB database with ID 4NT1.

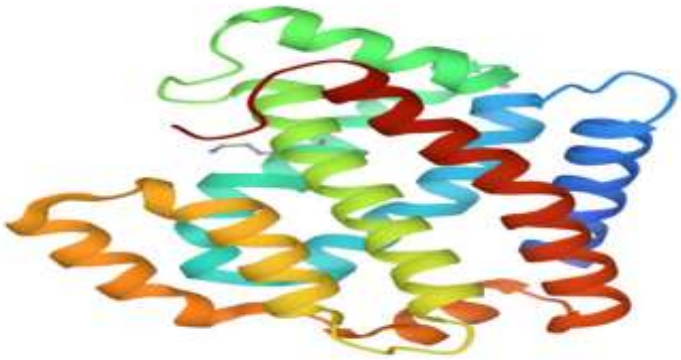


Figure 4: *3D Structure of the ACD11 retrieved from Phyre2*

Prediction of ACD11 protein disorder and ligand binding residue and its characterization

It is noted that DisEMBL is a web-based tool used to study and predict Intrinsic Disorder Proteins (IDPs), and that understanding protein function and folding pathways is important for protein disorder. IDPs are thought to become ordered only when bound to another molecule or when biochemical environment changes. Loops/coils are not necessarily disordered, but protein disorder is only found within loops. Disordered regions in a protein's flexibility facilitate different conformational possibilities and are therefore important for functionality as these regions may contain functional sites or linear motifs. The functional diversity of the first and middle segments of the protein structure is revealed by Disorder by Hot-loops and Loop-coils.

Table 4*. Protein disorder by Hot-loop*

S.NO	PROTEIN	RESIDUE POSITION	RESIDUES
1	*A.thaliana*	1-9, 92-107, 121-131, 199-206	MADSEADKP, RKAGSHTRNLLRVKRG, SEGDNSLKDPA, SKQLGIDW
2	*B.rapa*	**1-19**, 32-47, 62-73	**MDYVAKVEDLAKASSSVST**, CVRKAGSHTRNLLRVK, ASEGDNSLKDPA
3	*S.lycopersicum*	1-11, 99-112, 126-136, 201-210	MANHVAEEKPL, AGSHTRNLLRVKRG, SEGNSLKDPAS, FTSRDLGTDW
4	*G.max*	1-8, 92-111, 124-132	MTEGNGDK, NTVRKGGSHTRNLLRVKRGL, TEGNSLRDP

5	*V.vinifera*	86-104, 120-127	IEGNCVRKAGSNSRNLLRV, ASDDNSLR
6	*O.sativa*	1-10, 77-108, 183-192	MGSSDGDKPL, ISKLPEMVELDIQKGTVRQAGSHT RNMLRVKR, FIRASGPVIL
7	*Z.mays*	1-14, 22-59, 126-161, 233-258	MNALKHRKARQHQ, QKNKKGEGKNSRLLPRACQ HPRSASSPKAAMGSSQAD, KSISTLPSMVERDIQ TDTVRKPGSHTRNLLRVKRGI, NFVRSSAP VICYVDDLFTSRNLGIDW
8	*P.trichocarpa*	1-10, 73-107	MGDLETEKPL, KSIGTLQSVLDKDVERNS VRKGGSHSRNLLRVKRG

Table 5. *Protein disorder by Loop/coil*

S.NO	PROTEIN	RESIDUE POSITION	RESIDUES
1	*A.thaliana*	1-8, 24-36, 121-141, 194-206	MADSEADK, VNSPNPEVPVTQF, SEGDNSLKDPATKSYAQVFA, DNLFLSKQLGIDW
2	*B.rapa*	63-85	SEGDNSLK DPASKSYDQV FRPHH
3	*S.lycopersicum*	1-9, 30-49, 126-145, 201-210	MANHVAEEK, LDEAAKMEVAPFSHACTLVS, SEGNS LKDPASKAYTQVFAP, FTSRDLGTDW
4	*G.max*	1-8, 22-53, 92-104, 125-136	MTEGNGDK, NVFTDSQSAEAEVKVAPFSHACSLVSPLFGC L, NTVRKGGSHTRNL, EGNSLRDPASKA
5	*V.vinifera*	26-50, 83-103, 120-133	SQTVDIEVAPFSHACSLVSPLFGCL, DHDIEGNC VRKAGSNSRNLLR, ASDDNSLRNPASTA
6	*O.sativa*	1-9, 26-36, 77-103, 199-207	MGSSDGDKP, QQAPGPAMEVG, ISKLPEMVELDIQK GTVRQAGSHTRNM, TSRNLGMDW
7	*Z.mays*	1-10, 24-61, 77-89, 127-155, 205-212, 235-258	MNALKHRKAS, NKKGEGKNSRLLPRACQ HPRSASSPKAAAMGSSQADKP, KQQPAVPMDAGAF, SISTLPSMVERDIQTDTVRKPGSHTRNLL, VRSSAP VICYVDDLFTSRNLGIDW
8	*P.trichocarpa*	1-8, 30-50, 83-102,	MGDLETEKPL, KSIGTLQSVLDKDVERNS VRKGGSHSRNLLRVKRG

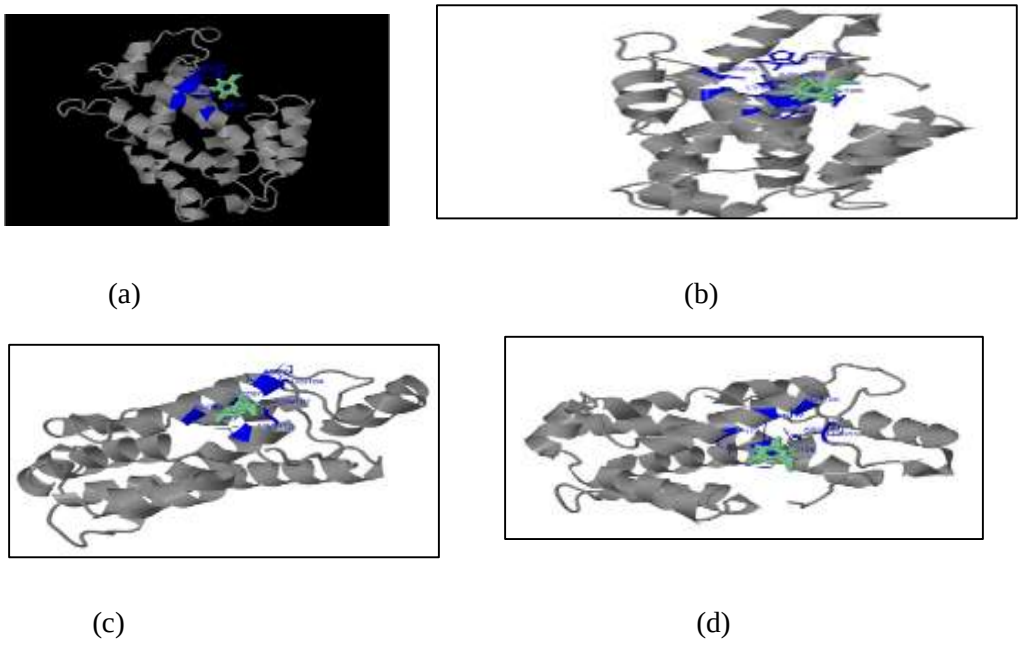
121-139,
197-209

Table 6. *Protein disorder by the method of beta-aggregation*

S.NO	PROTEIN	RESIDUE POSITION	RESIDUES
1	*A.thaliana*	20-24, 75-82, 108-115	LAIIV, ISTLVVMM, LDMVKVLF
2	*B.rapa*	17-24, 131-135	VSTLVVMM, VIAYL,
3	*S.lycopersicum*	113-120, 192-202	LDMVKVLF, TVILYIDKLFT
4	*G.max*	113-118, 191-200	MVRVLF, LIQYIDKLFV
5	*V.vinifera*	188-192	VILYI
6	*O.sativa*	45-56, 110-121, 190-194	VSVLFGCLGIAF, IDMVKILFEQIL, VILYV
7	*Z.mays*	65-69, 96-107, 161-172	IAVSF, VSVLFGCLGIAF, IDMVKVLFEQIL
8	*P.trichocarpa*	206-217	QSTFYLTWCTII

Ligand Binding residues of the reference protein ACD11 and its orthologues were predicted through RaptorX. The residues involved in forming the binding pockets for the ligands are given in Table 3.

EIS(N-{(2S,3R,4E)-3-hydroxy-1-[(3-O-sulfo-beta-D-galactopyranosyl) oxy]octadec-4-en-2-yl}dodecanamide) binding residues of acd11 and its orthologous protein model obtained from RaptorX server are shown in figure 4. The residues in the binding site pockets are the same for ACD11 and its orthologues. In active site pocket of the reference protein and its orthologues the most prominent and interactive amino acids are Asparagine and Histidine ranging at the same position as shown in the table. 7



(a) (b)

(c) (d)

Figure 5: *Ligand binding site and residue prediction of the reference protein and its orthologues retrieved from RaptorX. (a) Arabidopsis thaliana (b) Glycine max (c) Oryza sativa (d) Zea mayz*

Table 7. *Binding residues and their score*

	A.thaliana	*G.max*	*O.sativa*	*Z.mays*
Binding residues And score	60ASP 0.66 97HIS 0.67	74ASP 0.68 104HIS 0.66	69ASP 0.70 99HIS 0.66	120ASP 0.66 150HIS 0.64

CONCLUSION

In the present study we identified, predicted and comprehensively analyzed the accelerated cell death protein ACD11 of Arabidopsis thaliana and its orthologues like *Glycine max, Poplus trichocarpa, Solanum lycopersicum* form dicot plants and *Oryza sativa* and *Zea mays* from monocots across the diverse group of plant species. By taking advantage of the available computational tools, we perform complete analysis of developmental expression, protein-protein interaction, Co-expression, protein structure prediction and determination of intrinsic protein disorder, and finally ligand residues of the selected proteins. It is obvious form the result analysis that ACD11 and its orthologues mostly express in the last developmental stages of the plant i.e. senescence, which is confirming the role of ACD11 in cell death mechanism of the plant and other associated pathways.

From the study of the Co-expression the result shows that some the membrane bounding proteins SNARE, disease resistance and Autophagy related genes are co-expressed with acd11 and their orthologues, which validate again that during the cell death response either due to biotic or abiotic stress the disease resistance and autophagy related genes are also regulated. Form protein-protein interaction it is clear that the interacting partners of the ACD11 and their orthologues from diverse plants species is Enhanced disease resistance protein EDS1and LAZ1, so it is evident from both the results that there is a strong relationship between the co-expressed and the interacting proteins leading to a regulatory pathway of ACD11. Intrinsic disorder protein study predicts the flexibility in protein conformation from which it can be assumed that this protein has the capability of diverse functioning properties. From ligand binding study it is deduced that the common residues in the active site pocket of the protein are ASPRGINE and HISTDINE with high score of average distance and contact of the residue. Future work will elaborate more about the interacting protein partners and co-expressed proteins to determine a common gene-regulatory network of ACD11 and its role in programmed cell death.

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Khyber Pakhtunkhwa Higher Education Academy of Research and Training (HEART)

Sector F-2, Phase-6, Hayatabad, Peshawar
Tel. +92-91-5863654
Email: hetta.kp@gmail.com
Website: www.kpheart.edu.pk