ACKNOWLEDGMENTS

Government College University, Faisalabad, hosted the 28th Pakistan Congress of Zoology (International).

The Zoological Society of Pakistan expresses its deep gratitude to the Vice Chancellor, Government College University, Faisalabad and faculty members and students of the Department of Zoology and Faculty of Sciences for extending warm hospitality.

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TWENTY EIGHTH PAKISTAN CONGRESS OF ZOOLOGY (INTERNATIONAL)

GOVERNMENT COLLEGE UNIVERSITY, FAISALABAD

March 18 – 20, 2008

PROGRAMME

TUESDAY, MARCH 18, 2008

08:30 AM Registration
10:00 AM Inauguration: Recitation from the Holy Quran
10:05 AM Welcome Address by the Vice Chancellor, Government College University, Faisalabad
10:15 AM Address by the President, Zoological Society of Pakistan
10:25 AM Distribution of Medals and Awards
10:45 AM Address by the Chief Guest
11:15 AM Vote of Thanks by the Dean, Faculty of Sciences
11:25 AM Refreshment

JOINT SESSION I: (Plenary Lectures)

Chairperson: Dr. Syed Arif Ali Zaidi
Co-chairperson: Prof. Dr. Syed Shahid Ali

Speakers: 1. **Prof. Dr. Nasiruddin**
Distinguished National Professor & Director, Institute Molecular Sciences & Bionfirmatics, Lahore
Functional implications of post-tranlational modification in proteins.

2. **Prof. Dr. Dr. A.R. Shakoori**
Distinguished National Professor & Director, School of Biological Sciences, University of the Punjab, Lahore.
Heavy metal resistant protozoans, as component of consortia for bioremediation of industrial waste water.

01:00 PM Lunch and Prayer

HALL – 1

SECTION I: CELL BIOLOGY, BIOCHEMISTRY GENETICS, MOLECULAR BIOLOGY, PHYSIOLOGY, GENETICS

SESSION I

	Chairperson:	Dr. Syed. Arif A. Zaidi
	Co-chairperson:	Dr. Arif Siddiqui
02:00 AM	Paper reading	
04:30 PM	Tea Time	

SESSION II

	Chairperson:	Prof. Dr. Syed Shahid Ali
	Co-chairperson:	Dr. Farhat Jabeen
05:00 PM	Paper reading	
06:30 PM	Prayer	

SESSION III

	Chairperson:	Prof. Dr. M. Naeem Khan
	Co-chairperson:	Dr. Muhammad Hassan
06:45 AM	Paper reading	
08:00 PM	Dinner	

HALL – 2

SECTION II: PEST AND PEST CONTROL

SESSION I

	Chairperson:	Prof. Dr. Mushtaq A. Saleem
	Co-chairperson:	Dr. Abdul Ghafoor
02:00 PM	Paper reading	
04:30 PM	Tea Time	

SESSION II

	Chairperson:	Prof. Dr. Imtiaz Ahmad
	Co-chairperson:	Dr. Tayyaba Sultana
05:00 PM	Paper reading	
06:30 PM	Prayer	

SECTION III: ENTOMOLOGY

SESSION I

	Chairperson:	Prof. Dr. Syed Kamaluddin
	Co-chairperson:	Dr. Abida Butt
06:45 PM	Paper reading	
08:00 PM	Dinner	

HALL – 3

SECTION V: FISHERIES, ECOLOGY, WILDLIFE, FRESHWATER BIOLOGY, MARINE BIOLOGY

SESSION I

	Chairperson:	Prof. Dr. Ahmad Nadim Sheri
	Co-chairperson:	Prof. Dr. Javed Ahmad
02:00 AM	Paper reading	
04:30 PM	Tea Time	

SESSION II

	Chairperson:	Prof. Dr. Shahid M. Rana
	Co-chairperson:	Dr. Ali Mohammad Yousuf Zai
05:00 PM	Paper reading	
06:30 PM	Prayer	

SESSION III

	Chairperson:	Dr. Abdul Aziz Khan
	Co-chairperson:	Dr. Shahnaz A. Rana
06:45 AM	Paper reading	
08:00 PM	Dinner	

WEDNESDAY, MARCH 19, 2008

JOINT SESSION II: (Plenary Lectures)

Chairman: Prof. Dr. Shahzad A. Mufti
Co-chairman: Prof. Dr. M. Akhtar

09:00 AM 1. **Dr. Naeem Rashid**
School of Biological Sciences, University of the Punjab, Lahore.
Subterranean environment: A promising source for the isolation of psychrotrophs.

2. **Prof. Dr. M. Nasim Siddiqi**
Ex-chairman, Department of Zoology, University of Peshawar.
Spedeogy – an emergence science.

3. **Dr. Khalid Khan**
Provincial Chief of Sciences & Technology, NWFP, Peshawar
Revival of the dying art of the taxonomy.

HALL – 1

SECTION I: CELL BIOLOGY, BIOCHEMISTRY, GENETICS, MOLECULAR BIOLOGY, PHYSIOLOGY, GENETICS

SESSION IV

Chairperson: Prof. Dr. Muhammad Ali
Co-chairperson: Dr. Akram Shah
10:00 AM Paper reading
11:00 PM Tea Break

SESSION V

Chairperson: Prof. Dr. Naeem Rashid
Co-chairperson: Dr. Farah R. Shakoori

11:30 AM Paper reading
01:00 PM Lunch and Prayer

SESSION VI

Chairperson: Prof. Dr. Shamsuddin Shaikh
Co-chairperson: Dr. Abdul Rehman
02:00 PM Paper reading
04:30 PM Tea Break
05:00 PM Executive Council Meeting

SESSION VII

Chairperson: Prof. Dr. Nuzhat Ahmad
Co-chairperson: Dr. Shahid Nadeem
05:00 PM Paper reading
06:30 PM Prayer
08:00 PM Dinner

HALL – 2

SECTION III: ENTOMOLOGY

SESSION II

Chairperson: Prof. Dr. M.S. Wagan
Co-chairperson: Dr. Khalid Mehmood
10:00 AM Paper reading
11:00 PM Tea Break

SESSION III

Chairperson: Prof. Dr. M.K. Lohar
Co-chairperson: Dr. Alazeb
11:30 AM Paper reading
01:00 PM Lunch and Prayer

SESSION III (Continued)

Chairperson: Prof. Dr. M. Suleman
Co-chairperson: Dr. Inayat Ali Shahjehan

SECTION IV: PARASITOLOGY

SESSION I

	Chairperson:	Prof. Dr. Juma Khan Kakar
	Co-chairperson:	Dr. Zafar Iqbal
02:00 PM	Paper reading	
04:30 PM	Tea Break	
05:00 PM	Executive Council Meeting	

SESSION II

	Chairperson:	Prof. Dr. F.M. Bilqees
	Co-chairperson:	Prof. Dr. A.G. Arijo
05:00 PM	Paper reading	
06:30 PM	Prayer	
08:00 PM	Dinner	

HALL – 3

SECTION V: FISHERIES, ECOLOGY, WILDLIFE, FRESHWATER BIOLOGY, MARINE BIOLOGY

SESSION IV

	Chairperson:	Prof. Dr. Q.B. Qazmi
	Co-chairperson:	Dr. Itrat Zehra
10:00 AM	Paper reading	
11:00 AM	Tea Break	

SESSION V

	Chairperson:	Prof. Dr. Aleem Ahmad Khan
	Co-chairperson:	Dr. R.R. Ghazi
11:00 AM	Paper reading	
01:00 PM	Lunch Break and Prayer Break (Zuhar)	

SESSION VI

Chairperson: Prof. Dr. M. Afzal Kazmi
Co-chairperson: Prof. Dr. N.T. Narejo

02:00 PM Paper reading
04:30 PM Tea Break and Prayer Break (Asar)
05:00 PM Executive Council Meeting
08:00 PM Dinner

THURSDAY, MARCH 20, 2008

JOINT SESSION III: (Plenary Lectures)

Chairman: Prof. Dr. M. Naseem Siddiqi
Co-chairman: Prof. Dr. Shamsuddin Shaikh

09:00 AM 1. **Dr. R.D. Bush**
Faculty of Veterinary Sciences, University of Sydney, Camden, New South Wales, Australia.
Dairy production in Pakistan: Extension of technical information to farmers.

2. **Dr. David McGill**
Charles Sturt University, Wagga Wagga, Australia.
Dairy production in Pakistan: A comparison between Australian and Pakistani system.

3. **Prof. Dr. A.R. Shakoori**
Distinguished National Professor & Director, School of Biological Sciences, University of the Punjab, Lahore.
Ethical issues in biological research.

11:00 PM Tea Break

HALL – 1

SECTION I: CELL BIOLOGY, BIOCHEMISTRY, GENETICS, MOLECULAR BIOLOGY, PHYSIOLOGY, GENETICS

SESSION VIII

	Chairperson:	Dr. Amtul Jamil
	Co-chairperson:	Dr. Naaz Abbas
11:30 AM	Paper reading	
01:30 AM	Lunch and Prayer Break	
02:30 PM	General Body Meeting	

HALL – 2

SECTION IV: PARASITOLOGY

SESSION III

	Chairperson:	Dr. Aly Khan
	Co-chairperson:	Dr. Naheed Ali
11:30 AM	Paper reading	
01:30 PM	Lunch and Prayer Break	
02:30 PM	General Body Meeting	
04:00 PM	Concluding Ceremony	
	Recitation	
	Congress Report by President ZSP	
	Award Ceremony	
	Concluding Remarks by the Chief Guest	
	Vote of Thanks	
05:00 PM	Refreshments	

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OF

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All the papers in this Proceedings were refereed by experts in respective disciplines

TWENTY EIGHTH PAKISTAN CONGRESS OF ZOOLOGY

held under auspices of

THE ZOOLOGICAL SOCIETY OF PAKISTAN

at

GOVERNMENT COLLEGE UNIVERSITY, FAISALABAD

March 18 – 20, 2008

CONTENTS

Some Abstracts

SECTION - I

CELL BIOLOGY, MOLECULAR BIOLOGY, GENETICS, PHYSIOLOGY, TOXICOLOGY

FREQUENCY OF BLOOD GROUP OCCURRENCE IN PREGNANT WOMEN: A HOSPITAL BASED STUDY

ARZOO SHABBIR, FARIYAL DEEPA, FAZLI SUBHAN, SIKANDER SULTAN AND RIFFAT SHAHEEN
Department of Zoology, Pir Mehr Ali Shah University of Arid Agriculture, Rawalpindi, Pakistan (AS), Endocrinology and Reproductive Health Department, Public Health Laboratories Division, National Institute of Health, Islamabad, Pakistan (FD, FS, SS) and Maternity and Child Health Centre, Aabpara, Islamabad, Pakistan (RS)

There is social and medical significance of blood groups, directly depicting human genetics. In humans ABO and Rh factors best describe the blood types, there are also 46 other described antigens much rare than these types. Blood transfusions from incompatible groups can cause an immunological transfusion reaction. Total 200 pregnant women (18 to 39 years old) from Islamabad and Rawalpindi Cities were checked from August 2005 to March 2006 in Maternity and Child Health (MCH) Centre Aabpara, Islamabad for their blood group by antigen and antibody agglutination test. More than 50% women were unaware of their blood group before however the percentage distribution calculated for these blood types, showed that blood group B occurs in highest frequency, B^+ (36.8%), B^- (2.2%), A^+ (24.2%), A^- (3.3%), AB^+ (9.3%), AB^- (0.5%), O^+ (21.9%) and O^- (1.6%) respectively. Rh-positive and Rh-negative subjects were 92.2% and 7.6%, respectively. Blood typing helps in transfusion services, especially during delivery.

HEMATOLOGY AND SERUM CHEMISTRY OF DESI CHICKS

SIDRA ILYAS, RASHAD HUSSAIN AND SHAMIM AKHTAR
Department of Zoology, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi
E-mail: librasidra@yahoo.com

Hematology and serum biochemistry was assessed in order to establish normal reference values for the Desi chicks (n=14). Average values for red blood cells (RBCs), white blood cells (WBCs), hemoglobin (Hb), erythrocyte sedimentation rate (ESR), and differential leucocyte count (DLC) were 2.32±0.12 10^6 cells/µL, 5.33±0.69 10^6 cells/ µL, 103.50±4.76gm/L, 0.24±.01 mm/hour, respectively. While the average concentrations of total lipid, protein, glucose, billirubin and creatinine within serum were 77.91±7.07

mg/dL, 72.94±4.58g/L, 205.91±10.60 mg/dL, 4.17±0.78 mg/dL and 1.23±0.06 mg/dL, respectively. The results of student t-test shows that there was no statistical difference between males and females in terms of above mentioned parameter except that Hb concentration was significantly ($p<0.05$) high in male than that of females (116±2.11 vs. 90.21±5.89).

STUDY ON THE DISTRIBUTION AND BIOMASS OF PHYTOPLANKTON IN SOUTHERN CASPIAN SEA

FATEMEH SADAT TAHAMI AND MAZLAN BIN ABD-GHAFFAR
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The Caspian Sea is a lake with no outlets (enclosed), which is washing shores of the five countries: Azerbaijan, Iran, Turkmenistan, Kazakhstan and Russia. The length of coastline makes 5580 km. The water of the Caspian is not fresh, but brackish. Each liter of Caspian water contains 10-13 g of salt making this water unsuitable for drinking or irrigation. The average breadth from the west to the east makes 330 km. The surface is equal to 436 000 km^2, and volume is about 77000 km^3. The maximum depth of the Caspian is 1025 m, and the average - 184 m. The Caspian Sea comprises of 3 parts Northern, Middle and Southern. The Southern Caspian has the largest volume - some 64 % of the total volume that covers area approximately 35 % of the total area of the sea. Sea – *Mnemiopsis leidyi*, which likely was introduced in the Caspian Sea in 1998-1999 from Azov-Black Sea basin through communication with the Black Sea. In the Black Sea and the Sea of Azov, the plankton, ichthyoplankton and zooplanktivorous fish stocks all underwent profound changes. And it is predicted that the impact of *M. leidyi* on the ecosystem of the southern Caspian Sea more faster and stronger than in the Black Sea. In 2001, repercussions were felt at all trophic levels, including that of the top predator, the Caspian seal. Detail study on the impact of *M. leidyi* on the phytoplankton communities is still lacking. Therefore this proposal was designed as such to investigate the occurrences of *M. leidyi* and their impact on the major primary producer in the Southern Caspian Sea In this search we want to know that after accidental introduction of *Mnemiopsis leidyi*. What is their direct impact on pelagic marine plankton communities such as species diversity, Distribution and biomass in the Southern Caspian Sea, What changes appeared in species diversity, distribution and biomass of phytoplankton and what species variety, of phytoplankton appeared and what species of phytoplankton disappeared after entering of *Mnemiopsis leidyi*? For this study we had seasonally sampling at different deeps (0, 5, 10, 20, 50 and 100) meter and in 8 line that are important on fishery in southern of Caspian sea of physicochemical and phytoplankton factors. In every line have chosen 4 station (A, B, C, D) totally (32 st.) that: A, 10m---- (0, 5, 10) m; B, 20m---- (0, 5, 10, 20) m; C, 50m---- (0, 5, 10, 20, 50) m and D, 100m---- (0, 5, 10, 20, 50,100) m. In accordance with the survey carried out in the Southern Caspian Sea, five Phyla of phytoplankton were identified of which the Crysophyta (diatome) has the maximum number and the biomass in all seasons. After the Crysophyta

phylum, the Pyrophyta phylum has the highest biomass. Three Phyla, such as Cyanophyta, Cholorophyta and Euglenophyta had very low biomass.

AN *IN-VITRO* COMPARATIVE STUDY OF GROWTH MEDIA, SERA AND FSH EFFECTS ON THE GROWTH AND MATURATION OF SYRIAN MICE PREANTRAL FOLLICLES AND THE ENCLOSED-OOCYTES

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Choosing an artificial culture system will always imply a compromise in the *in vitro* maturation (IVM) of oocytes. For culturing oocytes, the environment should be supportive of the growth of this large type of cell and should supply the essential nutrients. Fine tuning of the culture components, including the types of cell, gonadotrophin concentration and ratio, growth factors, protein source (serum supplements) and concentration, can lead to optimization of the culture system. The present study was carried out to better characterize the nature and impact of different culture media and sera on the growth and maintenance of Syrian mice follicles and the enclosed oocytes. Preantral follicles (95 ± 5 µm diameter), from 6-weeks-old Syrian mice, were cultured in NCSU23, TCM199 and L-15 culture media for 6 days. Effect of different types of sera was also evaluated and the results were recorded using inverted microscope. The follicles grown with TCM199 showed higher rates of survival (30%), GVBD (9%), oocyte maturation (4%) and follicle diameter (115 µm) as compared to those grown in L-15 and NCSU23, $P < 0.05$. An 8-days culture showed approprtaie growth of the follicles during 6-days culture as compared to days 2, 4 and 8 ($P < 0.05$). Comparative effects FCS, PGS, ESFCS and hpgMS, with different concentrations (1, 2, 5 and 10%), was evaluated for 6 days and 5% FCS showed increased follicle diameter (125 µm) and survival (41%), GVBD (20%) and oocyte maturation rates (14%) as compared to the control and other types of sera used ($P < 0.05$). while, the culture showed a significant increase in the above mentioned parameters in the presence of 100 mIU/ml FSH + 5% FCS where final diameter, survival, GVBD and oocyte maturation rates were recorded to be 197 µm, 96, 92 and 79% ($P < 0.0001$), respectively at the end of a 6-days culture period. So, this culture system has proven to be appropriate for the optimal growth of Syrian mice preantral follicles and enclosed oocytes.

A NEW APPROACH FOR THE TREATMENT OF HYPERCHOLESTEROLEMIA

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Hypercholesterolemia is a major cause of cardiovascular diseases. It is characterized by increased level of plasma LDL cholesterol which results in the form of tendom Xanthomas and atheroma leading to premature arteriosclerosis and coronary heart diseases. This problem often runs in families and is known as Familial Hypercholesterolemia (FH). The identification of specific genetic variations has provided opportunities of genetic testing and drug development. Certain drugs are being used for the treatment of hypercholesterolemia, recently a new approach has been developed, the use of biological tool, such as probiotics which include lactic acid bacteria (LAB) they have the ability to reduce serum cholesterol either by deconjugation of bile salts or by uptake of cholesterol during cell growth. Present studies were carried out to determine the prevalence of FH in Pakistani population and to develop probiotics using indigenous microbes for lowering cholesterol level in such patients. The studies have shown that FH is prevalent in our population, however genetic analysis has revealed no mutation in LDL receptor or other genes. On the other hand indigenous LAB have been isolated and characterized for bile salt tolerance. The promising ones have been checked for lowering cholesterol level. The results suggest that these have the potential to be used as probiotics. The details of these studies shall be described during presentation.

EFFECT OF SOLID STATE FERMENTATION EMPLOYING YEASTS ON SOME NUTRITIONAL PARAMETERS OF FISH FEED

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A formulated fish feed was fermented employing yeast isolates of *Saccharomyces cerevisae* (Sn1 Y and Sn-5Y) in solid state fermentation. The main focused was on the protein enrichment of feed. Solid state fermentation is a major process to increase nutritional status of feed. SSF of the feed indicated increases in protein and protease levels and decreases in total carbohydrate content and amino acid of fermented feed. Sn-l Y enhanced protein content of fermented feed up to 31.68% after 72 hours of incubation and Sn-5Y showed increase of protein content up to 32.90% after 48 hours of incubation. Protease levels were found to be increased up to 15.15% and 18.78% by Sn-lY and Sn-5Y, respectively, on 5th day of incubation. Total carbohydrate contents showed a declining trend up to 54.54% after 168 hours in feed fermented by Sn-lY and 55.39%

decline after 168 hours' of incubation By Sn-5Y. Maximum reduction in amino acid occurred unto 42.50% by Sn-lY and 16.25 by Sn-5Y after 72 hours and 48 hours of incubation, respectively. For both isolates inocula sizes were 10% and moisture content 70%. C.F.U./g of fermented feed was also determined. It is suggested that the fermentation of fish feed by the reported yeast isolates of *Saccharomyces cerevisae* may improve fish growth and economize further the development of aquaculture in this country.

ULTRASTRUCTURAL CHANGES IN THE RAT THYROID FOLLOWING ADMINISTRATION OF HEXAVALENT CHROMIUM

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Hexavalent chromium is highly toxic to animal and human bodies and enters into the environment through the industrial refuse. A group of 10 adult male Sprague Dawe1y rats was injected with an acute dose of 30 mg/kg b.w. potassium dichromate ($K_2Cr_2O_7$) intraperitoneally for 24 h. The rats received another dose of 30 mg/kg b.w. on the second day at the same time for another 24 h. The control group consisting of ten rats received 0.9% physiolpgical saline at the same quantity and for the same time period. All the nits were sacrificed after two days (total of 48 hours). The thyroid glands were collected and fixed in 5% gluteraldehyde till further processing for electron microscopy. Semithin sections of the gland were cut at 2cm and stained with toludine blue. The control and treated ultrathin sections were cut at 120-150nm. The control semithin sections showed normal follicles with abundant colloid and peripheral epithelial cells. Interfollicular spaces and connective tissue were normally formed. Follicular epithelial cells were squamous with normally placed nuclei. Basal laminae were intact. In contrast, the sections from treated rats showed increased number of irregularly shaped follicles, disruption of basal laminae and connective tissue. Collapsed and aggregated follicles were readily noticeable. Nuclei appeared deformed. In the control ultrathin sections: sections, thyroid follicles were normal and with abundant colloid. The epithelial cells were organized, having round nuclei, endoplasmic reticulum, Golgi apparatus, mitochondria, large number of lysosomes and secretory granules etc. Treated sections showed marked ultrastrUctural changes as compared to the control. Follicular architecture was abnormally irregular. There was an apparent change in the follicular size which was reduced. The follicles were more irregular shaped and flattened. The basal laminae of the follicles were disrupted; as a result follicles appeared to be collapsed and fused with one another. THe epithelial cells were shrunken. The nuclei of the cells also became reduced in size and elongated or pyknotic when compared with control sections.

The nuclear membranes were also deformed. In the cytoplasm the endoplasmic reticulum appeared disrupted, disorganized and flown away. The Golgi apparatus was also disorganized. The mitochondrial membranes were notched at their periphery. The colloid droplets also decreased. The lysosomes were -less abundant than the control. Also the collagen fibers were increased in the treated sections. The study concludes that hexavalent chromium causes disorganization of the thyroid follicles' and is potentially toxic to the cellular organelles.

ESTIMATION OF ABO GENE FREQUENCIES AND TESTS FOR GENETIC EQUILIBRIUM IN TWO CONSECUTIVE GENERATIONS FROM FAMILY DATA IN NORTHERN PAKISTAN

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A total of 412 parents and 530 offspring belonging to 206 families from Bannu, a town in the North-West Frontier Province of Pakistan, were examined for ABO blood group phenotypes during 2002. The data analyzed included a sample of 402 parents and 515 offspring belonging to 201 families only as five families were excluded because of either misdiagnosis or illegitimate children noticed during rectification of the data. The purpose was to estimate and compare the phenotype and allele frequencies, and to test the genotype equilibrium, in the two generations. The phenotypic frequencies for ABO groups in the two consecutive generations were: parents A 0.313, B 0.435, AB 0.089 and a 0.162; offspring A 0.254, B 0.412, AB 0.117 and a 0.218. A test for heterogeneity indicated a significant difference between the two generations. However, the estimates of allelic frequencies in the two generations: parents P(A) 0.234, q (B) 0.320 and r (i) 0.445; offspring P(A) 0.208, q (B) 0.315 & r *(i)* 0.476, were comparable. Tests for equilibrium using the expected ABO genotypic frequencies indicated that the sample of the offspring, but not of the parents, was at equilibrium, implying that, genotype equilibrium was attained in a single generation. Thus, the data indicated that all the conditions necessary for genetic equilibrium, including that of random mating, are met in the study population. Though culturally, marriages in Pakistan are mostly arranged, selection of spouse is practically a random process as far as the blood group genotype or phenotype is concerned. The usual approach to estimate allele frequencies is basea on the assumption of Hardy-Weinberg equilibrium. Since the assumption for the parental generation turned out to invalid, another approach was used to estimate the allele frequencies. The alternate approach involved considering all possible genotypes of parents and children in a family, and counting all the three alleles for each generation separately. The estimates of the allele frequencies based on this approach: parents P(A) 0.227, q (B) 0.364 and r (i) 0.407; offspring P(A) 0.221, q (B) 0.334 and r (i) 0.445, were comparable not only for the two generations but also with those based on the assumption of H-W equilibrium. However, the estimates of the allele frequencies obtained by alternate approach did not indicate

genetic equilibrium in either generation. The alternate approach was perhaps no better than the usual one. It may be concluded that the estimates of allele frequencies are not very sensitive to the assumption of H-W equilibrium, but even insignificant changes in allele frequencies may result in lack of equilibrium.

POTENTIAL OF BACTERIAL CELLUASES, LIPASES AND PROTEASES FOR FORMULATING BIODETERGENTS

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Biodetergents are gaining significance due to their washing efficiency coupled with environmental safety. They are being used from household level to the industrial scale. The present paper reports enzymatic profiles of four lipase and three each of cellulase and protease producing bacterial isolates. Lipase producing bacterial isolates yielded upto enzyme units 538.75 D/hr/ml in nutrient broth. All the lipase producers belonged to the genus *Bacillus*. The cellulases producers' yielded upto 40.741 µmol/ml, while protease upto 1.08 µmol/ml/min of respective enzymes. Different combinations of the cell free culture fluids from the three categories of the enzyme producers were used to assess their potential as biodetergents. It was found that the enzymatic fluids can hydrolyze and thus remove soils of lipids and proteins from experimentally blood and oil stained cloth pieces. Cellulases were found to rejuvinile the cotton fibrils. The enzymatic potential of the reported bacterial isolates demands their cultivation in un-conventional low cost media and assaying the culture fluids for the enzyme profiles.

SIGNIFICANCE OF ALPHA- FETOPROTEIN FOR TUMOUR AND FOETAL MONITORING

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Cancer is a serious disease, which often proves to be fatal if not treated properly. Early diagnosis helps in cancer death prevention by successful therapy. Tumour markers are the biochemical substances evaluated for diagnosing and monitoring of tumour. Alpha fetoprotein (AFP) is a useful marker for tumour diagnosis and for foetal monitoring (female).The aim of this study was to identify importance of AFP as a tumour marker and as foetal monitoring. Serum tumour marker AFP was studied in one hundred

and eleven patients suspected for tumour and thirty six pregnant females for foetal monitoring, received in the National Institute of Health, Islamabad (NIH) from 1998 to 2006. In case of tumour diagnosis patients were divided into two groups' *i.e*. normal and pathological on the basis of there serum AFP levels. Total of one hundred and eleven patients, seventy nine (seventy one percent) were normal and thirty two (twenty eight point eight percent) exhibited pathological levels. Z- Test was applied to analyze the data, difference in the AFP levels was highly significant between two groups ($p<0.01$) while the difference between the ages was non-significant ($p>0.05$). In case of foetal monitoring correlation was calculated between maternal serum level and foetal age. Thirty six pregnant females blood samples were analyzed and the value of co-efficient of correlation was 0.55, that showed a positive significant ($p<0.05$) correlation between maternal serum AFP levels and foetal age. Present study corroborated with significance of AFP as a tumour marker and a marker for foetal monitoring.

STATUS OF THALASSEMIA MAJOR AMONG THE CHILDREN VISITING IN THALASSEMIA CENTRE AT PAKISTAN INSTITUTE OF MEDICAL SCIENCES (PIMS), ISLAMABAD

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The present investigation was conducted to find out the status of thalassemia major among the children visiting Thalassemia Center, Pakistan Institute of Medical Sciences (PIMS), Islamabad. Furthermore, the behavior of parents towards the screening of other children to check the presence of thalassemia trait was also investigated. A total of 126 patients were counseled for different parameters based upon their age, sex, weight, native area, family background, consanguinity, family screening. The result revealed that the incidence rate was higher in Chaudry (20%) then Rajpoot (16.33%), Pathan (12.73%) and then least in other races. Incidence of thalassemia was highest in first cousin marriages (62%) then in close cousins (25%) as compared to unrelated (130/0.). Incidence was greater in males (53.7%) as compared to females (46.8%). The time of awareness of thalassemia major in the children was in very early stage as it shows symptoms or weakness, highest among the prenatal groups (66.6%) followed by 1-2 years group (14.3%), 2-3 yr group (9.5) and 9.5% for 3-5 years. 31% of the families had already screened their other siblings and 69% families had not done it. The conclusion drawn from these observations is that incidence was higher in families preferring close cousin marriages and due to unawareness of people about compatible marriages due to non-screening for the disease.

SPAWNING PATTERN AND SIZE COMPOSITION OF YELLOW FIN TUNA, *THUNNUS ALBACARES*, FROM IRANIAN GILLNET FISHERY IN OMAN SEA

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The tropical tuna, yellowfin tuna (*Thunnus albacares*) is a large, long-lived, high migratory pelagic fish with a circumuglobal distribution between 40^{o} N and 40^{o} S. Considering its world catches in second rank when compared to the total catches of tuna species, and rapidly increased harvest for last decade, especially in Indian Ocean, great concerns have been raised on the possibility of overfishing for the stock. During October 2005 to September 200, the required data for the study on spawning pattern of yellowfin tuna and its relation with length distribution have been collected from gillnet fishery at the selected landing sites located in South-east of Iran along Oman Sea. Seasonal distribution of sexual maturity stages indicated a prolonged spawning activity from March to September, peaking in April-May, which was in agreement with increased GSI values. Based on separating of mature individuals from the rest, length at first maturity (Lm50%) of yellowfin tuna obtained as 77.2 cm Fl. Combining the information on length at first maturity and length frequency distribution of landings indicates that the percentage fish captured before first maturity were around 60%. Modal length data by season suggests the appearance of young fish in autumn, which seems to be born from fish spawn in spring for the previous year. Measures such as establishing optimum mesh size could be easier to regulate and enforce and so protect the yellowfin stock from the possibility of the risk of growth-overfishing in near future.

INFORMATIVENESS OF ST14 VNTR POLYMORPHIC MARKER IN THE CARRIER DETECTION OF HEMOPHILIA A

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Hemophilia A is the most common hereditary severe disorder of blood clotting. In families affected with hemophilia, genetic analysis provides opportunities to prevent recurrence of the disease. This study establishes a diagnostic strategy for carrier-ship determination in Pakistani population using an extragenic polymorphic marker for the first time. The analysis of St14 VNTR (DXS52) was carried out by polymerase chain reaction (PCR) method, in order to determine its informativeness in terms of

heterozygosity in our population. This may be a milestone for further analysis of other polymorphic markers for carrier detection and prenatal diagnosis of hemophilia. Seventy eight blood samples (Hemophiliac = 23, Normal = 55) from 15 families were analyzed for determining informativeness of St14 VNTR in carrier detection of hemophilia A. A total of nine alleles (2400, 2100, 1750, 1690, 1630, 1570, 1390, 1300, 1220 bp) was detected in the pool of subjects. 19 out of 40 females were found to be carriers with respect to the St14 VNTR polymorphic marker. The marker was informative in 73.33% of families. The expected heterozygosity rate of the St14 VNTR was 0.86 while the observed heterozygosity was 0.7. This shows that St14 VNTR is 70% informative in our population, allowing it to be a useful marker in carrier detection, as informativeness is the direct reflection of heterozygosity of a polymorphic marker.

SEROPREVALANCE OF ANTI-HIV ANTIBODIES IN CERVICAL CANCER PATIENTS

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HIV is the causative agent of AIDS. It is a retrovirus belonging to family *Lentivirus* and consists of a core surrounded by a lipid envelope. Within the viral core are the p24 protein, p7/p9 protein, two copies of genomic RNA and the viral enzymes protease, reverse transcriptase, and integrase. The integrated form of HIV-1, provirus, is ~ 9.8 kilobases in length. HIV has several major genes coding for structural proteins that are found in all retroviruses, and several accessory genes that are unique to HIV. Envelope glycoproteins gp120, gp41 and core protein p24 are the major antigenic determinants of both HIV-1 and HIV-2. Cancer of the cervix develops due to abnormal changes in the cells of cervix, the lower part of the uterus. These changes are usually the result of HPV infection. Both HIV and HPV are sexually transmitted infections. Multiparity and poor personal hygiene also increase the risk of transmission of HPV and HIV in women. Current study investigated the prevalence of HIV in the cervical cancer patients. The prevalence of cervical cancer and HIV infection were studied among different age groups and socio-economic classes. Distribution of risk factors and signs and symptoms of HIV infection among HIV seropositive and cervical cancer patients was recorded. It was found that 3.70% of cervical cancer patients were HIV seropositive. HIV infection was more prevalent in poor socioeconomic class and in the age group 51 to 60 years. Seroprevalence rates for HIV differ widely between developing countries because of the distribution of risk factors and lack of awareness of transmission modes. Further research is required to ascertain direct correlation of HIV and HPV co-infection. It is anticipated, however, that keeping in view the similar mode of transmission of both

viruses, prospect of HPV infection will decrease with raise of awareness about precautionary measures to avoid HIV infection.

FUNCTIONAL SWITCHES OF PROTEINS INVOLVE SPECIFIC POST-TRANSLATIONAL MODIFICATIONS: PREFERRED AMINO ACID PATTERN(S) FOR DIFFERENT MODIFICATIONS

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Proteins as functional molecules play an essential role in cellular physiology. Formerly, it was hypothesized that one protein performs one function but now we know that many proteins are multifunctional. Functional switches of proteins are often regulated by dynamic protein modifications. *In vivo*, assessment of protein functions and their functional switches still remains a great challenge. *In silico* procedures may facilitate assessing and predicting the multifunctionality of proteins eventually leading to designing the directed *in vivo* studies. Extensive research is ongoing to predict the sequence of protein modification sites and analyze their dynamic nature. Post-translational modifications (PTMs) often serve as a source of dynamicity of proteins and interplay of different PTMs result in functional switches of proteins. We have analyzed the sequence patterns/motifs, in the surroundings of PTM sites, for their involvement in favoring and/or disfavoring for a specific PTM. The study demonstrated that there are some general sequence patterns/motifs preferences for a specific PTM exhibiting a general sequence requirement for a modification. Additionally, there are some specific amino acid sequence patterns/motifs required specifically for same modification depending upon the modifying amino acid and the type/isoform of enzyme involved for that PTM.

PRODUCTION AND CHARACTERIZATION OF KERATINASE ENZYME BY *BACILLUS* SP. GROWING ON CHICKEN FEATHERS

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The strain *Bacillus* sp. FBN-4 shown to be a useful mutation for biotechnological purposes such as for the degradation of chicken feathers and production of keratinase enzyme. The effect of temperature, pH, incubation time period, along with different

supplementary sources (nitrogen and carbon) as well as different concentrations of yeast extract were studied on the production of keratinase by *Bacillus* sp. FBN-4. The enzyme production occurred between 33°C and 47°C, with maximum activity and yield at temperature 37°C. Enzyme characterizations demonstrate that strain is thermostable at 60°C upto 90 min at pH 10.

ANALYSIS OF PHOSPHOELM_07 DATABASE AND MINED ASSOCIATION PATTERNS USING MAPRes

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Substantial increase in protein phosphorylation sites recognized by different experimental procedures needs to understand the effect of neighboring amino acid residues surrounding the phosphorylated sites. With the increase of massive data in molecular biology, extensive computer-assisted methods have been developed progressively utilizing throughput models to analyze and predict protein sequence patterns for phosphorylation. In this study we have analyzed PhosphoELM_07 database to study the association/correlation among phosphorylated sites and the preferred amino acids in the neighboring environment including the information of kinases involved by a method, MAPRes (Mining Association Patterns among Preferred Amino Acid Residues in the Vicinity of Modified Sites), developed in our *in silico* laboratory. The association patterns mined by MAPRes showed correlation approach and the results are in accord with more than 80% to the existing methods. These association patterns will be helpful in developing consensus sequence required for a phosphorylation site or for kinase binding and as training data for phosphorylation of Ser/Thr/Tyr in developing a high throughput prediction method for phosphorylation with reduced number of false predictions.

REGULATION OF OCT-1 FUNCTIONS BY POST-TRANSLATIONAL MODIFICATION

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Octamer DNA binding transcription factor 1 (Oct-1) contains POU (pit, oct, unc) domain composed of an *N*-terminal POU specific domain (POUs) and a *C*-terminal POU homeodomain (POUh). The POUh domain is the minimal region, which is required for sequence-specific DNA binding. It has been observed that gene expression by Oct-1 is

modulated through various types of post-translational modifications (PTMs). Regulation of Oct-1 for its role in the binding to specific DNA sequences and the activation of gene transcription are controlled by phosphorylation and dephosphorylation at particular regions. Phosphorylation of Oct-1 modifies DNA binding of POUh domain in a sequence-specific manner. It has also been observed that *O*-GlcNAc modification at the same Ser/Thr residues work analogous to phosphorylation. Utilizing computational methods for phosphorylation and *O*-GlcNAc modifications possible Yin Yang sites, sites having potential for both phosphorylation and *O*-GlcNAc modifications, in Oct-1 are predicted. Prediction results suggest that interplay of phosphorylation and *O*-GlcNAc modification regulates binding and removal of Oct-1 with the DNA that contributes to gene expression.

DEGRADATION OF CHICKEN FEATHERS BY USING *BACILLUS* STRAIN ISOLATED FROM NATURAL ENVIRONMENTS UNDER SUBMERGED FERMENTATION CULTURE

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Seven strains of *Bacillus* sp. isolated from the Poultry Feed Industry of Okara, Pakistan. Among all the *Bacillus* strain FBN-4 producing a high keratinolytic activity was rather screened and used for further optimization. This mutation when cultured on chicken feathers, the degradation of chicken feathers was greatly increased and this is further enhanced by optimizing several factors such as temperature, pH, addition of yeast extract, different concentrations of K_2HPO_4, inoculum size and incubation time period in submerged fermentation conditions. Maximum degradation was also achieved by using upflow reactor.

IDENTIFICATION OF DIFFERENT MECHANISM OF TOLERANCE AGAINST HEAVY METALS AMONG INDIGENOUS ISOLATES

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Forty three bacterial strains were isolated from potentially metal-contaminated areas, found to tolerate heavy metals, coded as CMG2K1-CMG2K43. These strains were found to tolerate heavy metals and antibiotics. In case of heavy metals 62.8% of the

strains have shown tolerance against chromate and copper, and 51.2% against nickel. Eight antibiotics were checked, 97.7% of the strains have shown tolerance against streptomycin, 44.2% against neomycin, 27.9% against ampicillin, 25.6% against tetracycline, 13.9% against chloramphenicol, 9.3% of the strains have shown tolerance against kanamycin and novobiocin, and 2.3% against rifampicin. Attempt was made to find out correlation between heavy metal tolerance and antibiotic tolerance of these strains with the assumption of multiple stress tolerance of bacteria. Statistical analysis has shown positive correlation between heavy metal tolerance and antibiotic tolerance of these strains, t-test has been conducted to test the null hypothesis of no correlation which has been rejected at t.05 level of significance. *Bacillus cereus* strain CMG2K4 tolerated high concentration of nickel. Genetic basis of heavy metal tolerance was analyzed by first screening for the known genes related to heavy metal tolerance within the genome of isolated strains. The genes screened included ncc and cnr operons for nickel tolerance which did not indicate presence of any of the related operons. Absence of such inducible operons, was confirmed by growth patterns of these strains, which showed similar growth when seeded with overnight cultures grown with and without metal, indicating a constitutive mechanism of tolerance. Most of the heavy metal tolerance mechanisms reported are plasmid borne but CMG2K4 did not harbor any plasmid confirming that the genes involved in heavy metal tolerance are located on chromosome. SDS-PAGE of crude cell extract of *Bacillus cereus* strain CMG2K4 in presence of nickel revealed ~36 kDa band which was consistent in log phase culture and stationary phase culture both in control and test, speculated to be the band of a protein involved in nickel tolerance behavior of CMG2K4. N terminal sequencing yielded 10 amino acid sequence of this ~36 kDa protein showed 100% homology with flagellin protein of several *Bacillus cereus* strains when MPsrched in Uniprot database of EBI.

ROLE OF COMPUTER BASED METHODS IN LIFE SCIENCES

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The use of computer assisted studies in life sciences has increased during the last two decades, and it has played a vital role in life sciences research. Completion of human genome is the most remarkable discovery that paved the way of this huge development. The efficient use of computing methods is importantly needed in research. The vital techniques used are Support Vector Machines (SVM), Hidden Markov Models (HMM), training the neural networks, data mining for developing efficient computational methods in the areas of genome sequencing, genome mapping, micro array gene expression data analysis, protein sequence and structure analysis, sequence alignment and protein modification. All the techniques have their pros and cons. Some techniques are efficient for a specific purpose while others are efficient for the others. The use of artificial intelligence is gaining popularity in bioinformatics due to its dynamic nature. It gives a

most acceptable solution as compared to other techniques. Utility of the techniques, particularly that artificial intelligence application in our work will be discussed.

FACTORS AFFECTING GESTATION LENGTH, LACTATION LENGTH AND MILK YIELD OF NILI-RAVI BUFFALOES

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Data pertaining to 426 calving records of Nili – Ravi buffaloes maintained at Livestock Research Station, National Agricultural Research Centre, Islamabad were statistically analyzed to determine the effect of age, lactation number, sex of calf and season of calving on the duration of gestation period, lactation days and milk yield. Average gestation length was 307.05±0.39 (Mean±SE) days and it ranged from 277 to 337 days, average length of lactation was found to be 273.34±2.56 (Mean±SE) days while mean milk yield was 1831.62±25.72 (Mean± SE) litres. The effect of calf sex was non – significant on gestation period, lactation length and milk yield ($P<0.05$) while that of season of calving was highly significant. Gestation period and the length of lactation prolonged during winter ($P<0.05$). Also, spring calvers produced significantly more milk (2155.5 litres) than summer calvers (1701.87 litres). The effect of age of animal and lactation number was non – significant on gestation length ($P<0.05$) but was highly significant for milk days and milk yield. The lactation length increased with the age of the animal and calving number upto 6^{th} lactation and then it declined gradually. Also, average milk yield increased progressively from 1828 litres in Ist lactation to 2125 litres in 6^{th} lactation ($P<0.05$).

LEAD CONCENTRATION IN GOAT BLOOD

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Lead is one of the environmental toxic pollutants that affects different body systems and induces deleterious health effects. Anemia, anorexia, weakness and loose feces are the important clinical symptoms in chronic lead poisoning in ruminants. Present study was designed to determine the lead concentration in goat blood of district Attock, Punjab, Pakistan whether it lies in safe range or not. Eighty blood samples were collected from the two areas (Area I and Area II) of the District Attock. Blood samples were analyzed by atomic absorption spectrophotometer to determine the concentration of lead.

The level of lead in goat blood was 4.40±0.48µg/ml and 4.31±0.58µg/ml in two different populations. Safe level of lead in blood is 0.25µg/ml, above 0.35µg/ml is toxic for ruminants and 1µg/ml is fatal. In conclusion, the lead concentration in goat blood in district Attock, Punjab exceeded the safe limits.

SURVIVAL AND REJECTION RATE OF KIDNEY TRANSPLANTATION

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The study was designed to determine the survival and rejection rate (%) of kidney transplantation. Fifty patients of kidney transplant were interviewed at Kidney Center, Rawalpindi, to get the information on sex, age, survival and rejection of kidney transplant. Overall survival rate of kidney transplant was 88%. According to sex, survival rate were 90 and 82% in males and females respectively. Survival of kidney transplant was 34, 22, 14, 10 and 8% at the age of 31-40, 21-30, 51-60 and 41-50, 10-20 years, respectively. Rejection rate of kidney transplant was 6, 2, 2 and 2% at the age of 31-40, 41-50 and 51-60, 61-70 years, respectively. In conclusion, kidney transplantation survival is higher between the ages of 31 to 40 years.

CAUSES OF END-STAGE CHRONIC RENAL FAILURE

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The study was carried out to determine the causes of end-stage chronic renal failure. Fifty patients of end stage chronic renal failure were interviewed at Kidney Center, Rawalpindi to get the information on sex, age, glomerulonephritis, hypertension, diabetes, kidney stone and idiopathic. Overall end stage chronic renal failure was 76 and 24% in male and female respectively. Glomerulonephritis, hypertension, diabetes, kidney stone, idiopathic contribute to end stage renal failure 36, 32, 22, 6 and 4%, respectively. According to sex glomerulonephritis, hypertension, diabetes, kidney stone, idiopathic contribute 30 and 6, 24 and 8, 4 and 2, 16 and 6, 2 and 2%, in male and female, respectively. End stage renal failure was 36, 26, 14, 14, 8 and 2% at the age of 31-40, 21-30, 41-50 and 51-60, 10-20, 61-70, respectively. In conclusion, glomerulonephritis is the major cause of chronic renal failure followed by hypertension and diabetes. Moreover, age between 31 and 40 years is more prone to end-stage chronic renal failure.

ANTIBIOTIC SUSCEPTIBILITY OF *ESCHERICHIA COLI* ISOLATED FROM BACTEREMIA PATIENTS

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Present study was designed to determine the susceptibility (%) of *Escherichia coli* from blood of bacterimia patients for commercially available antibiotics amikacin, amoxyl/clavul acid, ceftazidime, ciprofloxacin, pip-tazobactam, ampicillin, cefexime, ceftriaxone, gentamycin, cefoper/sulbactam, ampi/sulbactam, cefpirom, cephalexin and Imipenem. Sixty six isolates of *E. coli* were subjected to antibiotic sensitivity test using Disc Diffusion Method. The antibiotic susceptibility (%) of *E. coli* was: amikacin (89.39), amoxyl/clavul acid (25.76), ceftazidime (39.39), Ciprofloxacin (27.27), pip-tazobactam (84.85), ampicillin (4.545), cefexime (31.82), ceftriaxone (34.85), gentamycin (40.91), cefoper/sulbactam (87.88), ampi/sulbactam (6.061), cefpirom (40.91), cephalexin (15.15) and imipenem (92.42). In conclusion, *E. coli* showed maximum susceptibility to Imipenem, Amikacin, Cefoper/sulbactam and resistance to ampicillin, ampi/sulbactam and cephalexin.

ANTIBIOTIC SUSCEPTIBILITY OF *KLEBSIELLA PNEUMONIAE* ISOLATED FROM BACTEREMIA PATIENT

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Present study was designed to determine the susceptibility (%) of *Klebsiella pneumoniae* isolated from blood of bacteremia patients for commercially available antibiotics amikacin, amoxyl/clavul acid, ceftazidime, ciprofloxacin, pip-tazobactam, ampicillin, cefexime, ceftriaxone, gentamycin, cefoper/sulbactam, ampi/sulbactam, cefpirom, cephalexin and Imipenem. Forty four isolates of *Klebsiela pneumonia* were subjected to antibiotic sensitivity test using Disc Diffusion Method. The antibiotic susceptibility (%) of *Klebsiella pneumione* was: amikacin (78.05), amoxyl/clavul acid (24.39), ceftazidime (31.71), ciprofloxacin (56.1), pip-tazobactam (80.49), ampicillin (0), cefexime (21.95), ceftriaxone (24.39), gentamycin (26.83), cefoper/sulbactam (75.61), ampi/sulbactam (12.2), cefpirom (26.83), cephalexin (17.07) and imipenem (95.12). *K. pneumoniae* showed maximum susceptibility to Imipenem, pip-tazobactam Amikacin; resistance to ampicillin, ampi/sulbactam and cephalexin.

BACTERIAL DEGRADATION OF AN ORGANOPHOSPHATE INSECTICIDE

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Four species of *Pseudomonas* and one *Acinetobacter* were isolated from an insecticide contaminated locality. Inoculation of these species was profligated into the medium containing an organophosphate insecticide, malathion as sole source of carbon. They were grown both as mono and co-culture. On seventh day of inoculation, bacteria free culture fluids were obtained from these bacterial cultures by passing through syringe filters (0.2 micron meter).These cell free culture fluids as well as untreated fluids (without inoculation) were determined for degradation of the insecticide by gas chromatography and mass spectrometry (GC-MS). Malathion was not detected in the cell free culture fluids paradoxically to the untreated ones. All the species used in this study were found efficient in degrading the insecticide both as mono and co-culture.

DETECTION OF PEPTIDES IDENTICAL TO SOMATOTROPIN AND PROLACTIN SHARING THE HOMOLOGY IN THE SIGNATURE PATTERN OF THE GROWTH HORMONE AND PROLACTIN FAMILY

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The study explains the isolation and identification of two different peptides from pituitaries of water buffalo *(B.s bubalis)* and domestic cow *(Bas indicus)*. The pituitaries were collected from the freshly slaughtered animals at Baker Mandi, Lahore. The samples were immediately kept at 4°C and were latter stored at -80°C, till further use. Pituitries in each case were homogenized with Tris-HCI buffer (0.05M, pH 8.5) and centrifuged at 10,000g for 15 minutes at 4°C. A clear supernatant obtained was used as a source of protein. The pH of the supernatant was adjusted at 7.0 by adding 0.2M Tris-HCI buffer and proteins were precipitated with 30% ammonium sulfate and incubated at 4°C, overnight. The precipitates obtained after centrifugation were dissolved in Tris-HCI buffer pH 8.5 and were dialysed. The dialysate were centrifuged and the clear supernatant was subjected to SDS-PAGE. After electrophoresis with GH-Prolactin like band was identified by immunobloting. The protein bands were identified as GH-Prolactin like protein by amino acid sequence analysis. The GH-Prolactin like protein isolated from *B. indicus* showed 20% homology with prolactin and 24% with GH while the protein isolated from *B. bubalis* showed 28% homology with GH and 38% homology with

prolactin. The identified proteins also share the homology in the signature pattern of growth hormone and prolactin family. Both the identified proteins showed M.W closer to 22K.

EFFECT OF HEAVY METALS ON GROWTH AND MORPHOLOGY OF HUMAN CELLS (HeLa CELLS)

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Metal ions are ubiquitously distributed in the environment. Many of these, such as calcium, magnesium, zinc, iron, cobalt, nickel, chromium and manganese, are essential components of biological systems and therefore constitute important micronutrients. On the other hand, some metal compounds have toxic and even carcinogenic properties. Thus, besides arsenic, lead, cadmium and beryllium, also nickel, cobalt and chromium compounds are carcinogenic in humans and/or in experimental animals. In the present study, we have investigated the effects of heavy metals such as arsenic, chromium and cadmium on the growth and morphology of HeLa cell. The growth rate of Hela cells was slower in Cr^{6+}, Cd^{2+} and As^{2+} containing medium. Cell lysis was observed with the increase in the concentration of metal, and at 0.10 µg/mL concentration of Cr^{6+}, Cd^{2+} and As^{2+} the complete cell lysis was observed. The total protein profile of control as well as treated cells was checked by SDS-PAGE. Metallothionein were not observed in treated cells. With increasing concentration, heavy metals have toxic effect on human cells and cause cell lysis. Further investigation are required to find out that how heavy metal interact with human cells and causes decrease in cell growth and cell lysis.

PCR-BASED DETECTION OF CRY-II GENE FROM LOCALLY ISOLATED STRAINS OF *BACILLUS THURINGIENSIS*

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Pesticides are used in agriculture and in public health programmes as important elements in an integrated approach to vector control programme. However, they are frequently misused, and pose serious health problems to the whole population. Mosquito borne diseases such as malaria, filariasis, dengue and viral encephalitis contribute to a larger proportion of health problems of developing countries. The application of naturally occurring microorganisms with the ability to control or suppress a pest population has received modest research support over the past several decades. Among various microbial

pesticides, *Bacillus thuringiensis* is being used widely as larvicidal bacteria for mosquito control. The *B.t.* isolates were obtained from the collection centre of School of Biological Sciences and screened for the presence of *Cry-II* gene. A total of 69 already isolated strains of *Bacillus thuringiensis* were screened for the presence of *Cry-II* gene by PCR using specific primers. Seventeen *B.t.* isolates (SBS-Bt-23, SBS-Bt-29, SBS-Bt-34, SBS Bt-37, SBS-Bt-45, SBS-Bt-47, SBS-Bt-32; SBS-Bt-35, SBS-Bt-38, SBS-Bt-40, SBS-Bt-41, SBS-Bt-42, SBS-Bt-43, SBS-Bt-44, IPS-78,SBS-Bt-II, SBS-Bt-I5, and SBS-Bt-26) were found to be positive for Cry-II gene. Plasmid profile of six *B.t.* isolates positive for Cry-II gene revealed that the 23 kb plasmid was present in all *B.t.* isolates. Likewise protein profile of six *B.t.* isolates positive for Cry-II gene confirmed that the 70 kDa protein was present in all *B.t.* isolates.

ASSOCIATION BETWEEN A rs235768 VARIATION IN THE BONE MORPHOGENETIC PROTEIN-2 (BMP-2) GENE AND BONE MINERAL DENSITY IN LOCAL OSTEOPOROTIC FEMALE POPULATION

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Osteoporosis is systemic skeletal disease that results in fragility of the bone and leads to fractures. It is a polygenic condition in which many genes and environmental factors play key role. Bone morphogenetic protein 2 *(BMP-2)* has been linked to osteoporosis. The present study was conducted to determine whether polymorphism rs235768 in *BMP-2* gene has any influence on BMD (Bone Mineral Density) variation in local female osteoporotic population. BMD was used as the diagnostic tool to identify the subjects suffering from osteoporosis. Subjects were divided into three groups according to BMD *i.e.,* osteoporotic, osteopenic and normal. Average BMD (-3.133) and age of osteoporotic subjects (52.7 years) was highest among three BMD defined groups. This indicates that with the increase in age BMD becomes lower and chance of having osteoporosis increases. Most of the post menopausal females (80%) were osteoporotic. SNP rs235768 was amplified as PCR fragment and RFLP was done to determine the genotype. The results showed that 16% of the individuals had AA genotype, 34% had IT genotype and 50% had AT genotype. SNP rs235768 genotypes were tested for population based association with BMD using analysis of variance. None of the genotype of polymorphism tested reached statistical significance (p=0.05) for BMD in local osteoporotic female population. This indicates that this polymorphism does not influence BMD and thus does not contribute to osteoporotic phenotype in local female population.

STUDIES ON GROWTH RESPONSE AND METAL ACCUMULATION PATTERNS IN FISH DURING CHRONIC EXPOSURES

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Laboratory studies were conducted with nine life stages of three fish species *viz*. *Catla catla, Labeo rohita* and *Cirrhina mrigala* to determine their growth response, feed intake and feed conversion ratios under sub-lethal waterborne zinc and lead concentrations. One third of LC_{50} concentration of each metal was considered as sub-lethal level for each species of fish. This investigation also focuses on the extent of metals bioaccumulation in fish organs *viz*. gills, kidney, liver, skin, muscle and scales during chronic toxicity exposures. All the three fish species showed uniformly in their response towards feed intake, weight increment and feed conversion ratios under sub-lethal metal concentrations. Feed intake in fish increased significantly with metal exposure concentration. However, significant increase in feed intake did not product in fish weight escalation while resulted in low feed conversion ratios. Sensitivity of the fish to both zinc and lead decreased with age. Both zinc and lead accumulation patterns among three fish species varied significantly while these did not change significantly within each species of fish. Metal accumulation patterns varied significantly among fish organs. Liver and kidney exhibited significantly higher tendency to accumulate both zinc and lead. However, all the three fish species showed significantly higher tendency to accumulate zinc than lead in their body organs. Zinc accuml1lation was significantly higher in *Catla catla* than that of *Labeo rohita* and *Cirrhina mrigala* while lead accumulation appeared significantly higher in *Labeo rohita* than rest of the species. The tendency of fish to accumulate metals in their body organs increased with age. Lead bioaccumulation was predominantly found to be the highest in fish kidney, followed by that of liver and gills. In general, the accumulation of metals in fish kidney showed direct relationships with both temperature and pH of water. However, decline in water hardness resulted in significant increase of metal accumulation in fish kidney.

TERMINAL SUGARS AS CANCER RECOGNITION MARKER

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In nature more than half of eukaryotic proteins are glycosylated, and around 90% of these glycoproteins are *N*-linked. The contribution of these structures in health and disease are largely undefined. The glycosylation process produces a substantial multiplicity of chemical structures, and makes large complex molecular structures in

contrast to other cellular macromolecules such as proteins, DNA and RNA that form linear chains. Oncogenic transformation is often associated with changes in the glycosylation pattern of glycoproteins and glycolipids in cell membranes. Cancerous cells express glycoproteins with galactose (Gal) on non-reducing end of oligosaccharide chains. Thus it is becoming more important to define these oligosaccharide structures to fight the exhausting battle against cancer. Terminal Gal moieties in oligosaccharide chains of glycoproteins have several functions and may induce an immunomodulatory response or may act as a recognition marker for various lectins, like mistletoe lectin. In general the ability of a sugar to act as an epitope or a recognition marker depends on the neighboring or vicinal sugar, the anomery and the linkage. The current study is based on isolating data and searching function of specific oligosachharide structures containing terminal Gal that are specific determinants as cancer marker.

HEMATOLOGICAL AND BIOCHEMICAL RESPONSES OF BLOOD OF AN ENDANGERED SOUTH ASIAN FRESH WATER FISH, *TOR PUTITORA* AGAINST AQUATIC POLLUTION

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Fresh water quality deterioration is one of the major problem of South Asian countries. River Kabul originates in Paghman province of Afghanistan, passes through NWFP district of Pakistan and joins River Indus which flows into Arabian Sea. River Kabul is receiving city sewage and effluents from a number of industrial units without prior treatment. The drastic adverse changes in the various hematological and blood biochemical parameters of the inhabitant fish Mahaseer, *Tor putitora* compared with the fish caught from the upstream Warsak Dam indicate the level of aquatic pollution caused by the effluents. Among hematological parameters the hemoglobin concentration, RBC count, PCV and MCHC of the fish caught in the polluted part of the river showed 37.75%, 35%, 21.37% and 25.75% decrease, respectively, compared with the fish from the control site. The WBC count, however, 117.54% increase. Further down, when the river flows out of the industrial area, the fish showed these parameters respectively 12.25%, 11%, 6.98% and 19.45% lower than those in fish from control area. The WBCs showed, however, 84.53% increase. Besides that the blood protein and cholesterol levels showed, respectively, 47.24% and 51.06% decrease, blood glucose, GOT, GPT and CPK showed 28.03%, 55.98%, 124.24% and 426 fold increase in the fish caught from polluted water. In the downstream waters the blood protein, cholesterol, glucose and GPT showed 64.96%, 18.84%, 28.41% and 12.12% decrease respectively, whereas GOT and CPK activities increased 14.71% and 932 fold, respectively.

CHARACTERIZATION OF *SERRATIA MARCESCENS* FOR BIOSYNTHESIS OF A TRIPYRROLE ANTIBIOTIC

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A salt-tolerant soil bacterium, *Serratia marcescens* (BDCS-N-S 1) was isolated from the rhizoplane of rice. Differential morphological, biochemical, physiological and salt-tolerance tests were carried out for identification and characterization of bacterium. *Serratia marcescens* (BDCS-N-S 1) was found to produce cell-associated red pigment. Culture conditions for bacterial growth and pigment production were optimized by media manipulation. Shake flask cultures grown in different media at varied temperatures and incubation time showed that bacterium was tolerant to 6% NaCl and maximum pigment biosynthesis was observed at 28°C after 72 h of incubation in a medium supplemented with minerals and/or fatty acids. The red pigment was characterized as a tripyrrole antibiotic by spectrophotometric analysis. The results of this study indicate that multiple factors such as medium composition, pH, temperature and incubation time affect pigment biosynthesis.

SEQUENCE AND MUTATIONAL ANALYSIS OF HUMAN INTERFERON ß1 (IFNß1) GENE IN BLOOD SAMPLES OF ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) PATIENTS

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Acute lymphoblastic leukemia (ALL) is the most prevalent type of cancer, as well as the most common form of leukemia in children. This lymphoid malignancy, manifested by the proliferation of lymphopoietic blast cells, represents a heterogeneous group of diseases that vary with respect to morphological, cytogenetic, and immunologic features of the transformed cells. Type I interferons (IFNα, IFNß, IFNϖ) are pleiotropic cytokines that exhibit multiple biological effects on cells and tissues, including anti proliferative, antiviral, and immunomodulatory activities. Homozygous and hemizygous deletions of IFNB genes have been frequently reported in acute leukemia cell lines, primary acute leukemia cases, and gliomas. Because IFNs have an anti proliferative effect, selection against the IFNα/ß system could playa role or accompany the development of the malignant phenotype. Partial or complete loss of B-interferon (IFNB1) genes located on chromosome 9p has been demonstrated in malignant cells from patients with acute lymphoblastic leukemia. These findings may indicate that IFNs function as tumorsuppressor genes in some diseases, but the relevant gene(s) may also be located in close proximity to the IFN genes. In this study, mutational analysis of IFNB

gene was performed in ALL patients. To accomplish this work blood samples of Acute Lymphoblastic Leukemia (ALL) were collected in EDT A coated CBC vials from Children Hospital Lahore. Extraction of genomic DNA from whole blood of ALL patients was performed by using triton X-100 method. The protocol used was quite simple and fairly rapid. It does not require the use of organic solvents but rather using salt extraction to precipitate contaminating proteins. High quality of DNA can be obtained suitable for immediate PCR applications. The Polymerase Chain Reaction (PCR) was performed. Agarose gel electrophoresis was performed on the already amplified DNA samples. The quantity of gene was increased using nested PCR. Amplified Gene bands are eluted from the gel ft gene clean was performed. Single Strand Conformational Polymorphism (SSCP) and gene sequencing is being performed.

SEQUENCE AND MUTATIONAL ANALYSIS OF HUMAN INTERFERON ß1 (IFNß1) GENE IN PARAFFIN EMBEDDED TISSUES OF LARYNX/LIVER CARCINOMAS

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Liver is the largest organ inside the body. Hepatocellular carcinoma is a cancer arising from the liver. It is also known as primary liver cancer or hepatoma. Liver cancer is most common in developing countries in Africa and East Asia. In many of these countries it is the most common type of cancer. Laryngeal cancer affects the larynx, which is often called the "voice box" because it contains *vocal cords.* An estimated 2,400 cases of hypopharyngeal cancer are diagnosed each year (1,900 men and 500 women). Type I interferons (IFNα, IFNß, IFNϖ) are pleiotropic cytokines that exhibit multiple biological effects on cells and tissues, including antiproliferative, antiviral, and immunomodulatory activities. In this study, mutational analysis of IFNBI gene was performed in Paraffin embedded tissues of larynx/liver carcinoma. This work is sort of a novel work in this field. Paraffin embedded tissue samples of larynx/liver carcinoma were collected from Shaikh Zayed Hospital. DNA was isolated from the paraffin embedded larynx/liver carcinoma tissues by the manual method using tissue lysis buffer, de-waxing of the tissue, proteinase K treatment and finally preserved the DNA in the freezer. Low quality of DNA was obtained for immediate PCR applications as paraffin embedded tissues have very low level of DNA. The Polymerase Chain Reaction (PCR) was performed. The quantity of gene was increased using nested PCR. Amplified Gene bands are eluted from the gel and gene clean was performed. Single Strand Conformational Polymorphism (SSCP) and gene sequencing is being performed.

CLONING AND EXPRESSION OF *BACILLUS THURINGIENSIS Cry4B* CRYSTAL PROTEIN GENE IN *ESCHERICHIA COLI*

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The human desire to control insects has existed about as long as humans themselves have. The desire for insect control increased significantly with the realization that insects can spread human and animal diseases such as malaria and yellow fever, viral encephalitis, dengue fever, filariasis. Among bioinsecticide based strategies, special attention is given to *Bacillus thuringiensis (B. t.)* is a gram-positive, rod-shape, aerobic, and spore-forming bacterium characterized by the presence of a crystal protein within the cytoplasm of the sporulating cell. Cry4B of 130kDa is toxic to mosquito-larvae of the genus *Aedes, Anopheles,* and *Culex.* A well characterized local *B.t.* isolate named as DAB *Bt* 5 was used to clone 3.41Kb full length *cry*4B gene. The *cry4B* gene was amplified by PCR cloned in pTZ57 cloning vector and sequenced. Sequencing of cloned *cry4B* gene showed 99% homology with embIX07423.1IBlTOXD2. Sequence of full length *cry*4B gene was submitted to NCBI GenBank database and its accession number is EF468629. *cry*4B gene was cloned in pET22b expression vector and transformed in *BL21C* to take expression. The conditions for good expression of *cry*4B gene were optimized. Biotoxicity assays with recombinant Cry4B protein were done against third instar of mosquito larvae *Anopheles stephensi* showed that Cry4B of DAB *Bt* 5 was highly toxic against mosquito larvae.

CLONING OF Cry1C GENE FROM HD137 STRAIN OF *BACILLUS THURINGIENSIS*

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Gram-positive, endospore fonning, soil born bacterium *Bacillus thuringiensis,* produces a parasporal protein toxin called as insecticidal crystal protein (ICP) are selective biodegradable insecticides has alternatives to synthetic chemical insecticides. In the genes coded insecticidal proteins are located on plasmids or chromosomes. *Cry* 1 genes responsible for lepidopteron larval toxicity. Among them *cry* 1 C is the most toxic crystal protein against mosquitoes and Armyworms has been reported so far. A strain of *Bacillus thuringiensis* showed significantly high toxicity to mosquitoes and Armyworms was isolated from soil and characterized. The isolate named as MS-Bt1. The cytolytic-8 endotoxin gene crylC 3.6 Kb was amplified from genomic DNA of HD137 by PCR using primers were designed from the sequence of cry 1 C gene accession number X07518.The PCR amplified 3.6 kb product from strain was further cloned in pTZ57 cloning vector.

The cloning of 3.6Kb genes was confirmed by restriction analysis by restriction endonucleases.

MUTATIONAL ANALYSIS OF *HAP* GENE (HEMEAGGLUTANIN/PROTEASE) TOWARDS THE DEVELOPMENT OF LIVE ATTENUATED ALA AUXOTROPHIC VACCINE STRAINS VCUSM16 AND VCUSM17 FOR *VIBRIO CHOLERAE* 0139

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The hemagglutination/protease (HA/P) is involved in the proteolytic cleavage of A subunit of cholera toxin (CT) into Al and A2peptide resulting in the activation of cholera toxin. It hydrolyses crucial proteins, inactivates the filamentous phage CTXΦ particle carrying the genes for known enterotoxins and also involves in the detachment of *Vibrio cholerae* from cultured intestinal cells. PCR amplified *hap* was cloned into cloning vectors pTZ57 R/T by T/A cloning. It was followed by a deletion mutation of 703bp in *hap* using *Bstx*I restriction sites, which was followed by insertion mutation by inserting *aph*A cassette into *hap* at polished *Bstx*I restriction site. This *hap*::*aph*A was subcloned from cloning vector pTZ57 *RIT* into the suicide vector pWM91 at *Sma*I restriction site and was transferred into VCUSM2 *Vibrio cholerae* by filter matting. The resultant mutant after suicide vector's backbone excision was resistant to Kanamycin and was designated as VCUSM17. Later the *aphA* insert was excised by restricting pTZ::*hap*::*aph*A with *Psy*I followed by polishing with *T_4DNA polymerase* and self ligation with *T_4DNA ligase.* The resulting frame shifted *hap* was subcloned from pTZ57 R/T into the suicide vector p WM91 at *Sma*I restriction site and was transferred into VCUSM17 *Vibrio cholerae* by filter matting. The resultant mutant after suicide vector's backbone excision was sensitive to Kanamycin and was designated as VCUSM16. These strains were tested for colonization potential in infant mice and for toxigenic diarrhea in rabbit ileal loops. The deletion and frame shift mutations in *hap* did not result in the reduction of colonization potential necessary for GALT and MALT. A significant low fluid accumulation rate reflects non toxigenic effect of *hap* vaccine candidates. We concluded that *hap* mutation affects a lot in decreasing the effect of toxigenic diarrhea but without affecting colonization thus render least toxigenicity and with very high titers of vibriocidal, anti CT, anti HAP and anti LPS antibodies in serum of the recipient. Additionally *hem*A mutation which helps in aminolevulinic acid synthesis makes the mutants more reliable as environmentally safe aminolevulinic (ALA) auxotrophs.

ISOLATION AND IDENTIFICATION OF SPORE FORMING BACTERIA FROM DIFFERENT SOIL TYPES

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Spore-forming bacteria represent a major micro flora in natural systems. They play an important role in ecosystem development. Soil is the most common habitat of spore forming bacteria where they are found in high abundance. These microbes create symbiotic relationship with plants, some produce antibiotics against plant pest and parasite. The present study was designed to identify spore forming bacteria and to see the relation of spore forming and non spore forming bacteria to fertilized and unfertilized soil type bacteria of soil. For the isolation of bacteria, total 40 soil samples were collected from four different soil types. Out of the total (248) isolates 163 (65.7%) were spore forming and 85 (34.3%) were non spore forming bacteria. Data was analyzed by using Simpson's diversity index and two-way ANOVA. There was significant difference between spore forming and non spore forming bacteria in all soil types. Among spore forming bacteria *Bacillus subtilus* (P=0.0061) showed more significant relation to fertilize and unfertilized soil types as compared to other spore-forming bacteria. Significant change in total colony forming units was observed after the use of fertilizer (1.34×10^{12} to 3.14×10^{12}). Statistical analysis showed that great diversity of bacteria was observed in all soil types. Soil type 1 and soil type 4 have same diversity of bacteria (D=0.36) which is greater than soil type 2 (D=0.37) but less than soil type 3 (D=0.33).

PCR AMPLIFICATION OF HEPATITIS B VIRUS IN THE LIMITING DILUTION FORMAT

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Hepatitis B virus has eight genotypes based on sequence heterogeneity and at least four HBV genotypes are present in Pakistan. If infected patient has mixed infection of different HBV genotypes, PCR amplification will result the product that may be hybrid of different HBV DNA molecules. Sequence analysis of PCR product amplified from such samples is often unreliable due to ambiguities in sequencing gel/electropherogram. Research work carried out was to establish HBV DNA amplification from a single HBV molecule in a sample using limiting dilution format. For this purpose two blood samples were collected from Lahore hospitals (NA004 and NA012). Viral DNA was extracted from blood plasma and amplified a region of 1.1 Kb and 0.6 Kb by polymerase chain reaction (PCR) using two different sets of primers (NHBFl & NHBRI and B 170AS & 82833S). Two limiting DNA dilutions, 1:256< and 1:4096 were found effective to get PCR product from a single HBV DNA molecule.

IDENTIFICATION OF BACTERIAL BLIGHT RESISTANCE GENE *xa5* IN PAKISTANI RICE GERMPLASM USING GENE SPECIFIC MARKERS

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Bacterial blight (BB) caused by Xa*nthomonas oryzae* pv *oryzae* is the major disease in the irrigated rice belts. In rice season 2007, the occurrence of this disease was reported in many Basmati growing areas of Punjab. Genetic resistant to control disease is the most effective and economical. Molecular survey was conducted with the use of PCR based, gene specific marker for *xa5*, to identify the presence and absence of *xa5* in Pakistan rice germplasms. The bacterial blight resistance gene *xa5* is a recessive gene and found to show resistance against many races of this pathogen prevalent in South and Southeast Asia. The survey has been conducted on different rice lines and Basmati varieties obtained from different research institutes. During the polymorphic survey, 46 rice lines showed the amplification of 250bp fragment similar to the *xa5* positive line IRBB2 as compared to the 230bp fragment amplified from *xa5* negative IRBB7. Ten basmati and seven KSK lines obtained from Rice Research Station Kala Shah Kaku, were also surveyed. All the basmati varieties *i.e*. Basmati 198, Basmati 385, Basmati 2000, Shaheen Basmati, Kashmir Basmati, Basmati Pak. etc along with KSK lines did not show the presence of *xa5* gene. The method of identification of bacterial blight resistance genes using gene specific markers and its advantages will be discussed in detail.

MOLECULAR GENETIC AND CLINICAL FINDINGS IN PENDRED SYNDROME PATIENTS

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Pendred syndrome is characterized by sensorineural hearing loss and palpable goiter. In the present study, four Pakistani consanguineous families showing the symptoms of Pendred syndrome were studied. These families originate from different regions of the country and each of them has more than five affected individuals. Clinical history of all the family members was taken by visiting at their homes. Genetic mapping of these families was performed by genotyping microsatellite markers from the linkage intervals of corresponding genes involved. All the four families showed linkage to the PDS locus at 7q31. Further studies are being carried out to find the involvement of new mutations in each of the family.

OCCURRENCE OF YEASTS IN INDUSTRIAL EFFLUENTS AND THEIR BEHAVIOUR IN RESENCE OF CADMIUM

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Rapid growth of industries, exploding population and agricultural revolutions has affected greatly the man's physical environment. Besides drugs, antibiotics and radioactive substances, industrial wastes contain heavy metals, which are mutagenic, carcinogenic and teratogenic. A specific problem associated with heavy metals in the environment is accumulation in the food chain and persistence in the environment. Microorganisms present in industrial effluents carrying toxic chemicals show adaptation and acclimation to their environment. The present study deals with the isolation, growth and tolerance to heavy metals of yeasts, isolated from industrial wastewater. Metal uptake ability of yeasts has also been assessed with a view to using them to detoxify industrial wastes of heavy metals. Growth conditions (pH, Temp.) of isolated yeasts were ascertained to grow them in laboratory conditions. Resistance of these isolated yeasts against different heavy metal ions was checked. Yeasts showed high resistance against Cd^{2+} up to 1.3mg/ml. They were also found resistant to heavy metals *i.e.* Ni^{2+}, Zn^{2+} and Cr^{6+} up to concentration of 1.0, 1.2 and 1.1 mg/ml, respectively. The removal of cadmium from the medium after various time intervals was estimated through atomic absorption spectrophotometer. To check the effect of metals on protein synthesis total proteins of the metal treated and untreated yeasts were analyzed by SDS polyacrylamide gel electrophoresis. The detoxification ability of yeast isolates indicates that, these can be used for amelioration of cadmium polluted industrial wastewaters.

USE OF 16S rRNA GENE SEQUENCE ANALYSIS TO DETERMINE PHYLOGENETIC RELATIONSHIPS OF *BACILLUS THURINGIENSIS* GROUP MICROORGANISMS

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Bacillus thuringiensis, an extensively exploited pesticidal bacterium produces various pesticidal proteins during sporulation phase. Phenotypically and genotypically *B. thuringiensis* can be differentiated from *B. cereus* by the presence of the crystal protein and plasmid-encoded *cry* genes, but if this plasmid were lost, *B. thuringiensis* could no longer be distinguished from *B. cereus*. The sequence of the 16S rRNA gene has been widely used as a molecular clock to estimate relationships among bacteria (phylogeny), but more recently it has also become important as a means to identify an unknown bacterium to the genus or species level. The 16S rRNA gene sequences of *B. anthracis*, *B. cereus*, and *B. thuringiensis* have high levels of sequence similarity (>99%) that

support their close relationships shown by DNA hybridization. In this report, we describe the identification of *B. thuringiensis* on the bases 16S rRNA gene sequences. We have cloned and sequenced 16S ribosoamal RNA coding gene from the local isolates of *B. thuringiensis* present in our collection.

EFFECT OF A PROBIOTIC *PSEUDOMONAS PSEUDOALCALIGENES* FERMENTED FISH FEED ON BODY COMPOSITION OF *LABEO ROHITA* FINGERLINGS

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This study reports the effect of a probiotic isolate *Pseudomonas pseudoalcaligenes* on body composition of rohu *(Labeo rohita)* fingerlings. For comparison of body composition, biochemical analysis of fish muscle tissue for different contents and enzymes were performed for control as well as experimental groups. The formulated fish feed was fermented by *Pseudomonas pseudoalcaligenes* up to seven days. Fermented feed was introduced to fish (3% body weight) in two forms; with live bacteria (SSF1) and without live bacteria (SSF2). In 90 days experiment, 5 fishes in triplicates were sampled fortnightly along with morphometric measurements for biochemical analysis. In muscle tissue analysis, significant differences in protein, carbohydrates and glucose were found in control and experimental groups in last phases (60-90 days). SSF2 showed increase in DNA contents to more/less decrease in phase VI and V and no difference was seen at phase VI in control and experimental groups. When the activities of muscle tissue enzymes were investigated and it was found that alkaline phosphatase, protease and amylase showed significant difference at phase II, III in both experimental groups than that of control one. SSF1 and SSF2 showed significant increase in some contents than that of control group.

IDENTIFICATION, CLONING AND SEQUENCING OF A NOVEL COPPER-INDUCIBLE METALLOTHIONEIN GENE FROM *TETRAHYMENA TROPICALIS LAHORENSIS*

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Metallothioneins (MT) are ubiquitous, cysteine rich, multifunctional, metal binding proteins whose transcriptional activation is induced by variety of stimuli. A novel Cu-inducible metallothionein (CuMT) gene has been identified and sequenced from the locally isolated ciliate, *Tetrahymena tropicalis* lahorensis. Based on its deduced sequence the gene encodes a protein, smaller than the cadmium-inducible metallothionein,

containing 29.67% cysteine residues. These Cys residues are arranged in motifs characteristics of a typical *Tetrahymena* Cu-inducible metallothionein gene. On the basis of homology of nucleotide sequence of genomic DNA and its cDNA, CuMT has been considered as a novel gene from *Tetrahymena tropicalis*.

CLONING AND NUCLEOTIDE SEQUENCE ANALYSIS OF PRE-S1 SURFACE ANTIGEN REGION OF HEPATITIS B VIRUS

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National Centre of Excellence in Molecular Biology, University of the Punjab, Lahore.

Nucleotide sequence of the Pre-S1 region f the Surface Antigen of hepatitis B virus (HBV) from local population has been determined. Viral DNA extracted from blood plasma of two blood samples NA007 and NA013 from Service Hospital, Lahore was amplified as 1.1 Kb and 0.6 Kb fragments using two different sets of primers (NHBF1 and NHBR1; B170AS and B2833S). The amplified fragment was subcloned in pGEM-T vector. Pre-S1 region of the two samples was sequenced by non-radioactive dideoxy chain termination method. Pre-Sl region nucleotide sequence of both samples were found homologous with the Pre-S1 region of reported subtype "adr4". Sequence Alignments showed that NA007 and NA013 were 96% to 100% identical with various adr subtypes (genotype C) and had 9.8% to 23.4% nucleotide variations with other subtypes/genotypes.

THE PROBIOTIC POTENTIAL OF TWO PSEUDOMONADS AGAINST *VIBRIO ANGUILLARUM* AND *PSEUDOMONAS FLUORESCENS* IN *LABEO ROHITA* FINGERLINGS

ASMA CHAUDHARY AND JAVED IQBAL QAZI
Environmental Microbiology Laboratory, Department of Zoology, University of the Punjab, Quaid-i-Azam Campus, Lahore-54590, Pakistan.

The antibacterial properties of indigenous bacterial isolates isolated from milk and yogurt samples and their potential use as fish probiotics against fish pathogens *Vibrio anguillarum* and *Pseudomonas fluorescens* were investigated *in vivo.* The *Labeo rohita* fingerlings were exposed to both pathogens (10^5 C.F.U./ml) by bathing challenge for 5 days. No mortality was observed within 30 days. When the fishes were exposed by i-p (intraperitoneal) injection to fish pathogens, mortalities appeared in a dose dependant manner. LC_{50} for these bacterial pathogens was worked out. The pathogens injected fishes showed varying level of observable symptoms at their mortalities. These included hemorrhage, swelling of belly and anus, scale damage etc. The mortalities (10-20%) for both pathogens reduced significantly for having probiotics containing feed prior and after the injection but here more mortalities (13-26%) were recorded in groups receiving the

probiotics containing feeds prior and sterile feed after the pathogens administration. When these observations were calibrated for mortality and survival, again importance of provision of probiotics containing feed before and after the injection became evident. Highest survival rate (90%) of *P. fluorescens* administered fishes were fed with probiotics *(Pseudomonas cepacia* AsCh-A7) containing feed before and after pathogens injections as compared to group which was fed with probiotics loaded feed only before the pathogen injection. This demonstrated the importance of the probiotic loads to compete with the pathogens and thus to reduce their virulence. The bacterial probiotics obtained in this study appear to have potential to be utilized in varying conditions of the fish culture.

LAB SCALE OPTIMIZATION OF POLYHDROXYALKANOATES PRODUCTION IN MOLASSES CONTAMINATED SOIL ISOLATES

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Polyhydroxyalkanoates (PHAs) are biopolymers produced by a number of bacterial strains as their energy reserves and are considered good alternatives to petrochemical based plastics. The soil samples were collected from different molasses contaminated sites. The polyhydroxyalkanoates producing bacterial strains were isolated on PHA detection agar by using glucose as carbon source. The isolated bacterial strains were screened for PHA production by Sudan black B and Nile Blue A staining procedures. The 95% of molasses contaminated soil isolates were found to be PH A producers. The purified bacterial strains were analysed by Gram reaction and biochemical tests. All the purified strains were tested for heavy metal and antibiotic resistance. A high level of resistance was observed for penicillin (100 - 4500 µg/ml) and copper (1.5 - 17 mM/ml) in PHA producing strains. The selected PHA producing bacterial strains which have more than 65% production were optimized under different growth conditions.

COMPARATIVE EFFECTS OF SUBLETHAL DOSES OF TALSTAR ON BIOCHEMICAL COMPONENTS OF RESISTANT AND SUSCEPTIBLE ADULTS OF *RHYZOPERTHA DOMINICA*

NIGHAT S. ALI, SYED S. ALI AND ABDUL R. SHAKOORI
Toxicology and Biochemistry Laboratory, Department of Zoology (NSA, SSA), School of Biological Sciences (ARS), University of the Punjab , Quaid-i-Azam Campus, Lahore-54590, Pakistan

To evaluate the biochemical difference between malathion resistant and malathion susceptible adult populations of lesser grain borer (*Rhyzopertha dominica)* a sublethal

dose of Talstar, a pyrethroid insecticide, was administered @ 1.4 ppm for resistant and 0.22 ppm for susceptible population for a period of 48 hours. Effects on various biochemical components *i.e.,* some enzymes and metabolites were studied. Talstar produced significant increase in acid phosphatase (33 %) in resistant and decrease in alkaline phosphatase (19%) activities in susceptible population. Amylase activity increased (67 %) in susceptible while trehalase activity increased (103 %) in resistant insects. Cholinesterase activity was inhibited by 69 % in resistant insects only. Among transaminases activities (glutamate oxaloacetate transaminase, GOT and glutamate pyruvate transaminase, GPT). The GOT activity decreased (53%) in resistant adults showing derangements in the normal amino acid metabolism. Significant increase in GOT (23%) in susceptible adults is an indicative of accelerated amino acid catabolism. No change was found in GPT activity in both resistant and susceptible populations. Elevated activity (43 %) of lactate dehydrogenase (LDH) and isocitrate dehydrogenase (ICDH) in resistant insects (117 %) indicates that both catabolic pathways (glycolysis and TCA cycle) are switched on to cope with the insecticidal stress, which is obvious by significant decrease (25 %) in its glucose level. In susceptible population, LDH activity also increased (38%) with no change in ICDH activity. Decrease in trehalose contents (45 %) which may indicate its utilization for energy generation to counter the toxic effects. unchanged soluble protein with decrease (36%) in total protein contents in susceptible insects indicates that only insoluble protein may be utilized. Unchanged glycogen indicates its non-utilization. On the other hand, high total lipids contents (119 %) were observed in susceptible insects. The higher enzyme activities induced by this insecticide are explainable by elevation of RNA contents which probably indicates induction of transcription in both resistant and susceptible populations (70 % and 76 % respectively). The free amino acids (FAA) and DNA contents remained unaffected in both populations. Conclusively, Talstar under the present experimental conditions induced number of metabolic and macromolecular abnormalities in both resistant and susceptible populations of *R. dominica.* This study provides the baseline biochemical variations to adopt better control strategy for this stored grain pest.

STUDY ON THE GROSS PATHOLOGY IN BROILERS AFFECTED WITH MAREK'S DISEASE IN COMMERCIAL BROILERFARMS IN AND AROUND HYDERABAD DISTRICT, SINDH

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An Investigation was carried out to study the gross pathological changes produced by Marek's disease in different visceral organs of the broiler birds during the study period 2004-2006 in commercial broiler farms in and around Hyderabad district, *i.e.* Hyderabad, Hala, Tando Muhammad Khan, Tando Allahyar and Matyari. Five forms were selected

from each city. Clinical signs observed in infected birds included nervous derangement associated with paralysis of wings and legs, gasping, diarrhea and loss of weight in most of the birds. Lymphogenous growth varied in different visceral organs, which included in liver, spleen, kidneys, in heart, in proventiculus and others organs.

UPTAKE OF NICKEL BY CILIATES ISOLATED FROM THE INDUSTRIAL EFFLUENTS AND THEIR ROLE IN WASTEWATER CLEAN-UP OPERATIONS

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Rapid growth of industries, exploding population and agricultural revolutions has affected greatly the man's physical environment. Besides drugs, antibiotics and radioactive substances, industrial wastes contain heavy metals, which are mutagenic, carcinogenic and teratogenic. A specific problem associated with heavy metals in the environment is accumulation in the food chain and persistence in the environment. Microorganisms present in industrial effluents carrying toxic chemicals show adaptation and acclimation to their environment. The present study deals with the isolation, growth and tolerance to heavy metals of ciliate, isolated from industrial wastewater. Metal uptake ability of ciliate has also been assessed with a view to using them to detoxify industrial wastes of heavy metals. Growth conditions (pH, Temp.) of isolated yeasts were ascertained to grow them in laboratory conditions. Resistance of the isolated ciliate cells against different heavy metal ions was checked. Ciliates showed high resistance against Ni^{2+} up to 25µg/ml. They were also found resistant to heavy metals *i.e.* Cd^{2+}, Zn^{2+} and Cr^{6+} up to concentration of 10, 20 and 15 µg/ml, respectively. The detoxification ability of ciliate indicates that, these can be used for amelioration of nickel polluted industrial wastewaters.

DISTRIBUTION OF ABO AND RH BLOOD GROUPS AMONG POPULATION OF SWAT (NORTH OF PAKISTAN)

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To determine the percentage and allelic frequency of ABO an Rheuses group among population of Swat. The blood group of study population was determined by testing the blood against antisera A and antisera B (gamma biological Inc), the alletic frequencies and respective standard errors were calculated assuming that population was in Hardy- Weinberg equilibrium, as per method of Mather. The distribution of A, B, O

and RH (D) blood group was studied in random sample of 1865 individuals visiting Saidu Teaching Hospital of Swat, Pakistan, during the year 2006 and 2007. Analysis of sample of 1865 individuals suggest that blood group A, B, O and AB are n=548, (29.38%), n= 609, (32.65%), n=526, (28.2%), n=182, (9.76%) respectively. The distribution of allelic frequency of a, b, and o individual in the population understudy are computed out by using Hardy Weinberg law on the assumption that population under study is in equilibrium, yield allelic frequencies for A, B, and O, 0.2278±0.005907, 0.249064±0.006288 and 0.531±0.0149, respectively. The test for Rh factor suggests that 90.7% and 9.3% persons are positive and negative, respectively. The frequencies of D and d alleles have been calculated as 0.69722±0.0061001, 0.30278±0.02887, respectively. The chi square contribution calculated for blood group A, B, AB and O is 6.11001642, 7.6259, 4.146251761 and 0.000038743, respectively. This study reveal the percentage of different blood groups for the population of Swat that was significantly different with several of those other studies, available in Pakistan.

IDENTIFICATION OF BACTERIAL BLIGHT RESISTANCE GENE *xa5* IN PAKISTANI RICE GERMPLASM USING GENE SPECIFIC MARKERS

SHAHZAD AMIR NAVEED, MUHMMAD BABAR, IFTIKHAR ALI, MUHAMMAD ALI, TERIQ MAHMOOD ANSARI, ANJUMAN ARIF AND MUHAMMAD ARIF
National Institute for Biotechnology and Genetic Engineering (NIBGE), Faisalabad, Department of Botany. GC University, Faisalabad, Department of Environmental Science. GC University, Faisalabad and University College of Agriculture, B. Z. University, Multan

Bacterial blight (BB) caused by Xa*nthomonas oryzae* pv *oryzae* is the major disease in the irrigated rice belts. In rice season 2007, the occurrence of this disease was reported in many Basmati growing areas of Punjab. Genetic resistant to control disease is the most effective and economical. Molecular survey was conducted with the use of PCR based, gene specific marker for *xa5*, to identify the presence and absence of *xa5* in Pakistan rice germplasms. The bacterial blight resistance gene *xa5* is a recessive gene and found to show resistance against many races of this pathogen prevalent in South and Southeast Asia. The survey has been conducted on different rice lines and Basmati varieties obtained from different research institutes. During the polymorphic survey, 46 rice lines showed the amplification of 250bp fragment similar to the *xa5* positive line IRBB2 as compared to the 230bp fragment amplified from *xa5* negative IRBB7. Ten basmati and seven KSK lines obtained from Rice Research Station Kala Shah Kaku, were also surveyed. All the basmati varieties *i.e*. Basmati 198, Basmati 385, Basmati 2000, Shaheen Basmati, Kashmir Basmati, Basmati Pak. *etc*. along with KSK lines did not show the presence of *xa5* gene. The method of identification of bacterial blight resistance genes using gene specific markers and its advantages will be discussed in detail.

CHLORPYRIFOS-ROUTE OF EXPOSURE AND SUSCEPTIBILITY FOR MAMMARY LINE TUMOR DEVELOPMENT

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Susceptibility to chlorpyrifos (CPF) for mammary line tumor development, with respect to its route of exposure, was investigated in virgin female mice *(Mus musculus).* Sixty animals (3-4months old) weighing between 30-35g were randomly divided in 3groups (20each). Dams in group l and 2 received 60mg/kg CPF each as subcutaneous injection in the dorsal cervical region and oral gavages respectively. The animals in group3 were exposed to CPF fumes (25ml, 40EC) for 24hours in a 10x12x10 feet hermetically sealed room. No signs of overt toxicity were observed in any of these animals. After CPF exposure the Dams were kept for 3months under the same housing conditions. Well recognizable abdominal (mammary line) tumors appeared in 04 animals within the 60days after CPF treatment, in group 3. In group l subcutaneous dorso-cervical tumor (at the site of CPF injection) was developed only in one animal in the 2nd month after treatment. All other animals remained normal throughout this study. Our findings suggest an obvious relationship between mammary line tumors and exposure to CPF fumes for 24 h in mice.

RESPONSE OF MALATHION- RESISTANT AND MALATHION-SUSCEPTIBLE ADULTS OF *RHYZOPERTHA DOMINICA* TO THE SUBLETHAL DOSE OF DELTAMETHRIN

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Malathion- resistant adult population of lesser grain borer, *Rhyzopertha dominica* found to be Deltamethrin-susceptible and malathion-susceptible population as Deltamethrin-resistant after Deltamethrin treatment, a pyrethroid insecticide. To determine the difference of effects of Deltamethrin, on the biochemical components of the two populations, a sublethal dose was applied @ 1.50 ppm for malathion-resistant and 0.966 ppm for malathion-susceptible population for a period of 48 hours. There is highly significant increase in activities of acid phosphatase (135%), amylase (55%), cholinesterase [(ChE), 97 %], glutamate oxaloacetate transaminase [(GOT), 513 %], trehalase (348%) and concentration levels of glucose (174%) and trehalose (11%) in

malathion-susceptible as compared to those of malathion-resistant adult population. Other enzymes and metabolites as lactate dehydrogenase (LDH) and total protein contents remained unaffected in both populations. Glutamate pyruvate transaminase, GPT) activities, isocitrate dehydrogenase (ICDH), free amino acids (FAA), glycogen and RNA contents underwent prominent changes in one way or other in both populations after Deltamethrin treatment. Therefore, it can be concluded that the beetles survived after field application might develop serious molecular derangements that helps to control this stored grain pest in godowns.

SECTION - II

PESTS AND PEST CONTROL

THE EFFECT OF THE PREPARATION VIRIN HSK AGAINST *HELICOVERPA ARMIGERA* HBN

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In the last few years, pesticides based on entomopathogenic organisms have been in wide use all over the world as a result of searches for ways of control of agricultural insect pests damaging more than 50% of crops, as well as with the aim of carrying out the program of the genetic regulation of pests, owing to their safety to humans and environments. Microbiological methods, and in particular the use of viruses as agents of biological control, have been applied as alternative methods. Currently, viral insecticide preparations are considered as one of most promising and important components of the integrated system of cotton protection owing to a high specificity and ecological safety. Several viral preparations, including VIRIN HSK, were developed, after they had been tested and recommended for application against the larvae of *Helicoverpa armigera* Hbn on cotton. VIRIN HSK, which belongs to the group of viral insecticides, was developed and produced at the association Altaivitaminy Ltd. VIRIN HSK is a concentrate of suspension light-green in colot, the active agent of which is a highly virulent strain of the virus of the nuclear polyhedrosis of хлопковой совки (NPV HA), which is intended for the control of the larvae of *H. armigera* Hbn. We carried out the assessment of the biological effect of the preparation in the control of *H. armigera* on cotton plantations. The assessment of the toxic effect of VIRIN HSK was carried out in laboratory conditions. For the experiments, we selected larvae of one age, placed them into Petri dishes and left them in these dishes for 2 or 3 hrs without any food. Cotton leaves were soaked for 30 sec in the solutions of this preparation at the concentrations of 0.1, 0.4 and 1.0%. In the control, the leaves were soaked in distilled water. Then the treated were given to experimental larvae as forage. The results of the experiment documented the death of 50.3% of larvae on the third day at the application of 0.1% concentration. On days 5 and 5, the mortality reached 83.7% and 87.3%, respectively. At the concentration of 0.4%, the mortality of the *H. armigera* was 53.3; 87.5 and 92.0% on days 3, 5 and 7, respectively, while at 1% concentration, this value was 67.3; 93.7 and 99.0%, respectively. The biological effectiveness of VIRIN HSK at rate of application of the preparation at 0.1% corresponds to the criterion of the positive assessment of the preparation for the control of *H. armigera* (85.0%) on day 7. The rate of application at 0.4%, the biological effectiveness reaches the necessary value (87.3%) by day 5 post

infection, while at 1%, the biological effectiveness reaches 93.7%. Thus, data obtained suggest that the preparation VIRIN HSK is highly effective in the control of *H. armigera* and is safe for warm-blooded animals and environments. The trials of this preparation should be carried out in field conditions.

MACROCYCLOPS ALBIDUS AS A NATURAL LARVIVOROUS ORGANISM OF CULICIDAE MOSQUITOES IN UZBEKISTAN

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The results of the study of the larvivorous activity of cyclops of the genus *Macrocyclops*, which were carried out in laboratory and natural conditions, were the basis for this work. The experiments of the larvivorous activity of cyclops in laboratory conditions were carried out as described below. Each of fifty *Culex pipiens* larvae of the 1st instar and 1 adult *Macrocyclops albidus* female were placed into Petri dishes. Twenty-four hours after the experiment live and dead damaged larvae with obvious traces of the attack were counted. The experiments were carried out at the temperature of 16-25°C. We carried out 62 experiments, in which we used 3128 mosquito larvae and 62 cyclop females. The results of the experiments showed that at a drop of the temperature from 21-25°C to 16-20°C, a regular decrease in the consumption of mosquito larvae by cyclops took place, where the mortality percentage respectively ranged from 16,0-58,0% to 14,0-48,0%. The number of dead mosquito larvae was 910 individuals (29,1%); 2218 individuals were alive. Simultaneously with laboratory studies we carried out experiments on the release of cyclops *Macrocyclops albidus* into natural breeding grounds of mosquitoes. Thirty-five *Macrocyclops* individuals were released into each of two water bodies of Tashkent district (Tashkent province) at the temperature of 20-25°C in May and August. Water bodies 1 and 2 were 2 by 2 m in area and 0.5-1 m in depth. They were overgrown with macrophytes and filamentous algae. The water was grayish, odorless. The bottom was clayey. We recorded the larvae of mosquitoes *Anopheles maculipennis* (4 to 8 individuals per sq. m) and *Culex pipiens* (3 to 16 individuals per sq. m). During the control sampling, which was held once per month, we recorded a drop in the number of mosquitoes in these water bodies. Thus, in water body 1, the density of *Culex pipiens* dropped to as low as 3 individuals per sq. m – the larvae of 1st and 2nd instars in June and 1 3rd instar individual per sq. m in October. In water body 2, the number of *Culex pipiens* dropped from 10 3rd-4th instar individuals per sq. m (August to 2 3rd instar individuals per sq. m in October. *Anopheles maculipennis* dropped from 7 3rd-4th instar individuals per sq. m to 2 3rd instar individuals in October. In the control water body 3 (2 x 2x0.5 m), the larvae *Anopheles maculipennis* and *Culex pipiens* were present at 17°C in May (2 and 1 individuals per sq.m, respectively). However, an increase in the

number of mosquito larvae to 8 and 15 individuals, respectively, was recorded in June followed by a slight drop in July and August (7 and 18-10 individuals per sq. m, respectively). Thus, the results of the experiments on cyclops *Macrocyclops albidus* revealed that this species has a significant larvivorous activity and can be considered as a possible agent of the control of Culicidae populations.

THE MODERN COMPOSITION OF BLOOD-SUCKING MOSQUITOES (DIPTERA, CULICIDAE) IN UZBEKISTAN

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The first works on mosquitoes in Uzbekistan mainly described the distribution of mosquitoes of the genus *Anopheles*. Data on other species were scanty and described only from individual regions of Uzbekistan. We carried out the analysis of the literature and our own findings of the species composition of blood-sucking mosquitoes. We found out 29 mosquito species (*Diptera, Culicidae*) breeding in Uzbekistan, which belonged to 6 genera (7), *Uranotaenia* (1), *Culiseta* (3), *Coquillettidia* (1), *Aedes* (8) and *Culex* (9 species). The current fauna of blood-sucking mosquitoes in Uzbekistan is represented by 25 species of 6 genera, namely, *Anopheles* (7), *Culiseta* (2), *Uranotaenia* (1), *Coquillettidia= Mansonia* (1), *Aedes* (6) и *Culex* (8 spp.), instead of previously reported 29 species, of which: Numerous and widespread: *An*. (*Ano*.) *hyrcanus* (Pallas), 1771; *An*. (*Cel*.) *pulcherrimus* Theobald, 1902; *An*. (*Cel*.) *superpictus* Grassi, 1889; *Ae*. (*Och*.) *caspius* (Pallas), 1771; *Cx*. (B*ar*.) *modestus* Ficalbi, 1890; and *Cx*. (*Cux*.) *pipiens* Linnaeus, 1758; Not numerous, but widespread: *An*. (*Ano*.) clav*i*ger (Meigen), 1804; *U*r. (*Pfc*.) *unguiculata* Edwards, 1913; *Cs*. (*All.*) *longiareolata* (Macquart), 1838; *Cs*. (*Cus*.) *subochrea* (Edwards), 1921; *Ae*. (*Adm*.) *vexans* (Meigen), 1830; and *Ae*. (*Och*.) *detritus* (Haliday), 1833; Numerous, but not widespread: *An*. (*Ano*.) *maculipennis* Meigen, 1818; *An*. (*Ano*.) *martinius* Shingarev, 1926; and *Co*. (*Coq*.) *richiardii* (Ficalbi), 1889; Not numerous, not widespread: *Cx*. (*Bar*.) *pusillus* Macquart, 1850; *Cx*. (*Cux.) theileri* Theobald, 1903; C*x*. (*Mai.) hortensis* Ficalbi, 1889; Sole and rarely encountered: *An*. (*Ano*.) *algeriensis* Theobald, 1903; Ae. (*Fin*.) *pulchritarsis* (Rondani), 1872; *Cx*. (C*ux*.) *mimeticus* Noe, 1905; *Cx*. (*Nex*.) *territans* Walker, 1856; and *Cx*. (*Nex*.) *martinii* Medschid, 1930; Breeding grounds not revealed as yet: *Cs*. (*Cus*.) *alaskaensis* (Ludlow), 1906; *A*e. (*Och*.) *stramineus* Dubitzky, 1970; *Ae*. (*Och*.) *niphadopsis* Dyar et Knab, 1918; *Ae*. (*Och*.) *flavescens* (Muller), 1764; *Ae*. (*Och*.) *cataphylla* Dyar, 1916; *Cx*. (*Cux*.) *tritaeniorhynchus* Giles, 1901.

USAGE OF HOUSEHOLD PESTICIDES IN ISLAMABAD

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Pests are a source of nuisance in houses throughout the globe, for which household pesticides serve as a major control measure worldwide. Information about the pattern of household pesticide usage, level of exposure of public towards these chemicals and awareness level existing among people for their usage was lacking and scattered, particularly in Pakistan. Present survey was designed to answer these and many other questions which was deployed in the residential areas of Islamabad. Shops (n=129) surrounding the survey area were included under the study to assess the marketing size. Survey was conducted in 743 households out of which 650 households (87.48%) responded. A one-to-one standard questionnaire was employed to undertake this survey. Results showed greater infestation of pests (n=557; 85.69%) out of which cockroaches (n=513; 78.92%) and ants (n=407; 62.61%) were more prevalent. Most of the people were using household pesticides (n=513; 78.92%) as a pest control measure while only 8.92 per cent (n=58) reported to employ professional exterminators. 736 products were found in 650 houses with a mean of 1.21 products per household. People were found highly exposed to the pesticides. Majority of the pesticides were used weekly. Two restricted use pesticides; DDVP and Permethrin were reported to be used at household level. Boric acid was found to be an effective and safer pesticide product from environmental and hmnan heath perspective.

ESTIMATION OF LARVAL DENSITY OF *HELICOVERPA ARMIGERA* (HB.) USING SWEEP-NET TECHNIQUE IN CHICKPEA CROP

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Helicoverpa armigera (Hb.) is a major Lepidopteran pest of Chickpea, Tomato, Cotton and Tobacco crops of Pakistan. Chickpea crop is severely damaged by pod borer, *Helicoverpa armigera.* Pest damage at the podding stage is extensive, ranging from 20-96%. The caterpillars damage the crop during vegetative and podding stages. Farmers increasingly rely on synthetic insecticides to manage this pest in different crops without knowing the accurate estimates of insect numbers leading to un-reliable pest management decisions. Accurate sampling is essential for IPM in order to make optimal use of control options. It is important to check and sample larval population regularly in order to target newly hatched larvae, which are particularly susceptible to even low doses of chemical spray. Sweep-netting offers the best means for growers to assess larval densities to

provide an estimate of the large cropping areas and can be sampled very quickly. In this connection, a trial was laid out using chickpea variety, NIFA-95 planted in the NIFA field to measure the collecting efficiency of sweep-nets and to determine whether all stages of developing larvae are represented in the sample. Sweep-nets of different diameters *viz*. 30, 35 and 40 cm having 5, 10 and 15 sweeps per treatment were used to collect a representative sample of larval stages at flowering and podding stages with a view to collect different instar-wise larvae at weekly interval. Absolute counting of caterpillars per 10 chickpea plants was carried out at crop flowering, pod grain filling and podding stages for treatment comparison. Larval sampling using sweep-nets were initiated at the onset of pest incidence. The data recorded indicated that sweep-net of 30 cm dia collected mean number of 36, 45 and 51 larvae in 5, 10 and 15 sweeps respectively. In the treatment 40 cm dia , average no. of caterpillars recorded were 22 in 5 sweeps, 28 in 10 sweeps and 30 in 15 sweeps. The overall results showed that 35cm dia proved efficient in collecting maximum larvae *i.e.* 40 larvae in 5 sweeps, 53 larvae in 10 sweeps and 59 larvae in 15 sweeps.

AN ANALYSIS OF TROPHIC ASSOCIATIONS OF WEEDS IN THE SUGARCANE CROP

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Weeds are considered constraints on crop production, yet their presence in the crop fields increases phytomorphic heterogeneity for the subsistence of diverse invertebrate fauna including prey, pests and predators. Eight sugarcane associated weeds were explored for invertebrates occurring on them during an entire season of the crop in the fields of low and high input. A total of 328 specimens of invertebrates belonging to twelve orders, thirty two families and fifty three species were collected from these weed plants. The specimens belonging to Order Hymenoptera, Coleoptera, Orthoptera, and Arachnida were the dominant and economically important.

SCREENING OF DIFFERENT MARKETED INSECTICIDES AND THEIR DOSES AGAINST TERMITE, *HETEROTERMES INDICOLA*

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The present study was an investigation on different insecticides to screen out the most palatable and slow-acting toxicant for an effective control of termites under laboratory conditions at NIFA, Peshawar. Six insecticides *viz*. Perfekthion, Biomax,

Lorsban, Steward, Agenda and Dursban and control (distilled water) with 6 different doses *i.e.* 0.01 %, 0.005%, 0.0025%, 0.00125%, 0.000625% and 0.000313% were tested against termite, *Heterotermes indicola.* There were three replications for each treatment and in each replication 50 worker termites were fed on insecticide treated filter paper placed in glass Petri dishes. The results on toxicity and palatability of different concentrations of all insecticides with different doses against *H. indicola* showed the slow-acting toxicity and palatability in *H. indicola* is inversely proportional to their concentrations. As insecticide concentrations decreased from in distilled water solution the slow-acting toxicity and palatability increased. The most desirable results of 87.30% and 100% mortality were observed after 12 and 15 days respectively in Agenda at 0.000625% concentration.

EVALUATION OF THE EFFICACY OF DIFFERENT INSECTICIDES AGAINST MANGO HOPPER *IDIOSCOPUS CLYPEALIS* (CICADELLIDAE: HOMOTERA) IN FIELD AND LABORATORY CONDITIONS

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Hopper, *Ideoscopus clypealis* (Cecadellidae: Homoptera) is the most serious pest of all verities of mango (*Mangiferae indica*) and infest mango orchards throughout Pakistan. It lays eggs on fresh tender shoots and inflorescence, both the nymphs and adults cause damage by sucking cell sap which lead drying of flowers and low yield. For the management of pest field trial and laboratory trial were conducted. Eight insecticides *i.e.* methamedophos, betacyfluthrin, deltamethrin, λ-cyhalothrin, imidaclopird, bifenthrin, acetamiprid and buprofezin were used replicated four times under RCBD in field conditions. Seven days data was recorded after every 24 hours. Statistically analyzed data revealed that after one day of pesticide application betacyfluthrin and methamedophos were found to be the most effective giving 89.13% and 87.92% respectively, followed by acetamaprid (82.61%) while bifenthrin gave the least control *i.e.* 47.37%. After seven days of pesticides application insecticidal efficacy has fallen down to a much extent, betacyfluthrin gave the best results *i.e.* 80.83% followed by λ-cyhalothrin, methamedophos and buprofezin giving mortalities 71.74%, 67.74% and 63.46% respectively while bifenthrin gave least results against mango hopper *i.e.* 33.33%. In laboratory trial ten insecticides *i.e.* profenophos, cholorpyrifos, bifenthrin, emmamectin benzoate, methamedophos, imidaclopird, λ-cyhalothrin, deltamethrin, chlorfenpyr and flufenoxuron were used replicated six times. Leaf dip bioassay method was used to assess the mortalities. Mortality data was recorded 1 hour, 2 hours, 4 hours, 8 hours, 16 hours and 24 hours after treatment. Results after one hour of treatment showed methamedophos gave the best results *i.e.* 96.67% mortality followed by imidaclopird, bifenthrin and

deltamethrin giving mortalities 70.00%, 60.00% and 53.33% respectively while emmamectin benzoate and chlorfenpyr gave least results *i.e.* 13.33% each. Cholorpyrifos and methamedophos gave 100% mortality after 4 hours while bifenthrin gave 100% mortality after 16 hours. All insecticides showed 100% mortality after 24 hours except emmamectin benzoate, deltamethrin, chlorfenpyr and flufenoxuron which gave mortality above 90%.

STUDY ON THE PEST STATUS, DISTRIBUTION AND PHALLIC COMPLEX OF *HIEROGLYPHUS PERPOLITA* (UVAROV) (HEMIACRIDINAE: ACRIDIDAE: ORTHOPERTA) WITH SPECIAL REFERENCE TO ITS ECOLOGY IN FIELD FROM DIFFERENT PROVINCES OF PAKISTAN

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The species of genus *Hieroglyphus* are very important from economic point of view they are major pest of paddy, sugarcane, wheat, and maize in Pakistan. However, they are less destructive to millets and fodder crops. For the first time observations have been made on the pest status of *H. perpolita* (Uvarov). A total of 2463 specimen were collected from the different climatic zone during the year 2005-2006. Out of this, 1895 were nymphs and 568 were adults. Incidence of *H. perpolita* reported significantly highest 40.38% and 44.20% during the month of July and August respectively, than the other months of the year. Moreover, significant variation was found in the genitalia components of male and female, compared to other species of this genus. In addition to this, close association of *H. perpolita* with *Saccharm bengalense* in the field observed *for* the first time. *H. perpolita* possesses some unique characteristics regarding their habits and habitat. *H. perpolita* are sluggish in nature and hide in the bottom of *S. bengalense.* Present study strongly recommends that if the prevailing habitat of this species is slightly disturbed they move to nearby cultivated fields and would cause extensive damage while moving from field to field over large areas.

EVALUATION OF 12 INSECTICIDES AS LURE TOXICANTS IN METHYL EUGENOL BAIT FOR MALE ANNIHILATION OF FRUIT FLIES

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Fruit flies *(Bactrocera)* are amongst the main pests of fruits and vegetables, which infest hundreds of cultivated and wild hosts throughout the world including Pakistan.

Male annihilation through lure toxicant bait traps is a better alternative of pesticide spray for the suppression and eradication of fruit flies. Males of Oriental fruit fly, *Bactrocera dorsalis* and peach fruit fly, *B. zonata* are attracted to methyl eugenol. A lure toxicant bait is widely used to detect & suppress flies population. Due to ban on existing lure toxicants, it is necessary to replace these by other easily available toxicants. For this purpose 12 insecticides *viz*. Chlorpyrifos, Thiodan, DDVP, Laser, Decis-D, Saprofan, Cypermethrin, Karate, Methyl parathion, Dimegro, Fyfenon, and Amicon were mixed @ 5% with 85% Methyl eugenol and 10% sugar. Five milliliter of each bait was used/trap on cotton wick. Traps were installed in the month of August in CR design in guava orchard at Kohat. Data were recorded on number of flies captured /week/trap for 15 weeks. The results indicated that all test insecticides caused fly mortality, none of them out classed Saprofon (standard) however, 4 insecticides, Decis-D, Cypermethrin, Karate and Fyfenon were found as effective as standard and hence can be used as good alternate toxicants in lure bait for male annihilation of two important fruit flies species *i.e. B. dorsalis* and *B. zonata.*

EFFICACY OF DIFFERENT INSECTICIDES FOR THE CONTROL OF MANGO MIDGES UNDER FIELD CONDITIONS

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Midges, *Erosomiya indica* (Cecidomyiidae: Diptera) is a serious pest of mango (*Mangiferae indica*) and has become a major pest of mango in Pakistan since last three years. It lays eggs on fresh tender leaves and inflorescence, the maggots emerged cause gall formation, destruction of flowers and holes in small fruits resulting in low yield. For the management of pest field trial was conducted. Seven insecticides *i.e*. bifenthrin, imidaclopird, lambda cyhalothrin, methamedophos, triazophos, profenophos and thiodicarb were used replicated thrice under RCBD in field conditions. Foliar method was used to check the insecticide efficacy against mango midges. Data was recorded weekly after treatment from inside of the tree. Statistically analyzed data revealed bifenthrin, lambda cyhalothrin and metahmedophos are the most effective against mango midges at field doses of 2, 4 and 50 ml per 10 liter of water respectively followed by triazophos and thiodicarb, while imidaclopird and profenophos showed no significant results.

INSECTICIDE RESISTANCE MONITORING AGAINST INSECTICIDES BEARING NOVEL MODES OF ACTION AND SYNTHETIC PYRETHROIDS IN *TRIBOLIUM CASTANEUM* (HERBST.) (COLEOPTERA: TENEBRIONIDAE)

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A survey conducted in southern region of Punjab revealed that average per acre yield for wheat crop is 1218.80 Kg. Sixty seven percent of the surveyed farmers used pesticides while 33% used different conventional methods to prevent their stored wheat grains from insect pest attack. The *Tribolium castaneum* collected from this region were tested for their relative responses to different concentrations of some synthetic pyrethrids *i.e.* cypermethrin, deltamethrin, bifenthrin, λ-cyhalothrin and β-cyfluthrin and some insecticides bearing novel modes of action *i.e.* emamectin, lufenuron, abamectin, thiamethoxam, indoxacarb and spinosad. On the basis of their LC_{50} values, the tested pyrethroids except bifenthrin were less toxic for D.G. Khan strain as compared to Multan, Rajan Pur, Vehari and Khanewal strains when tested under laboratory conditions. The LC_{50} values obtained when same strains were tested against insecticides bearing novel modes of action revealed that emamectin was the toxic of all the insecticides tested in this study followed by lufenuron, abamectin, indoxacarb and spinosad. Multan strain is 1.70, 1.55, 1.73, 0.34, 0.52 and 0.67 times resistant for abamectin, spinosad, indoxacarb, emamectin, thiamethoxam and lufenuron respectively.

RESPONSE OF DIFFERENT INSECTICIDES AT THEIR FIELD RECOMMENDED DOSES AGAINST 2ND INSTAR MANGO MEALY BUG *DROSICHA MANGIFERAE* G. (HOMOPTERA:MARGARODIDAE) UNDER LABORATORY CONDITIONS

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Mealy bug *Drosicha mangiferae* G. (Homoptera:Margarodidae) attacks on about 62 plants and fruit crops and is considered as a serious pest of Mango in Pakistan. Both nymphs and female adults suck cell sap from tender shoots, flowers and leaves, excrete honey dew and cause hindrance of photosynthetic activity by the development of fungus which lead into drying and shedding of fruits and flowers. Field recommended doses of fifteen different insecticides were used for the trial *i.e.* thiodicarb, methomyl, profenofos, chlorpyrifos, bifenthrin, emmamectin benzoate, methamedophos, chlorfenpyr, flufenoxuron, immidacloprid, acetamiprid, buprofezin, λ-cyhalothrin, deltamethrin and carbaryl. In controlled laboratory conditions leaf dip bioassay method was used under

complete randomized design (CRD) with sixteen treatments including control replicated thrice. Mortality data was taken 1 day, 2 days, 3days, 4 days, 5 days and 6 days after treatment. Statistically analyzed data revealed that 24 hours after treatment profenofos gave the best result (86.67% mortality) followed by chlorpyrifos and buprofezin with percent mortality of 80% and 73.33% respectively while emmamectin benzoate and flufenoxuron caused no morality at all. 100% mortality showed by profenofos and buprofezin 3 days after treatment while chlorpyrifos showed 100% mortality 4 days after treatment. After 5 days of treatment methamedophos and carbaryl showed 100% mortality while deltamethrin showed 100% mortality 6 days after treatment. After 6 days of treatment λ-cyhalothrin and acetamiprid gave good results with 80% and 73.33% mortality respectively while flufenoxuron and emmemectin benzoate showed least results *i.e.* 20% and 13.33% mortality, respectively.

EVALUATING THE TOXICITY OF NEEM OIL AGAINST *TRICHOGRAMMA CHILONIS*

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Laboratory tria1s were conducted to evaluate the immediate and residual toxicity of various concentrations of neem oil against *Trichogramma chilonis* adults. Three percent or more neem oil when applied fresh was as toxic to *T. chilonis* as Methamedophos or Ripcord. However, around 61 % mortality of *T. chilonis* adults was observed when exposed to freshly applied neem oil at a concentration of 1%. Studies on residual effect showed that 3 d after the application of 2% neem oil, 49% mortality of *T. chilonis* was observed. Mortality rate declined to 34% when the parasitoid adults were exposed to neem 5d after its application.

RATS IN THE DIET OF THE BARN OWL *TYTO ALBA* (SCOPOLI, 1769) AT SOME PLACES IN BALOCHISTAN

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Rats and mice are the serious vertebrate pests of Agricultural crops and vegetation in Pakistan as well as throughout the world causing tremendous losses to the field crops at different stages, grain damage in storage and structural damage in houses, shops and commercial areas. Various pesticides are being used are carrier of different type of diseases through ecto, endo-parasites and Bacteria / viruses etc. for pest control. As a

biological-control of vertebrate pests Barn owl *Tyto alba* has received highly successful results in different parts of the world. Present studies are therefore a step to examine the potential of Barn Owl *Tyto alba* for controlling rats and mice in our crop fields. During present research studies one hundred and three regurgitated (103) pellets of Barn Owl were collected from Qubba Ghaffar Khan Ghoth, Chowki Jamali and Wali Shah, Usta Muhammad, Balochistan. These were analyzed and examined, relative abundance of rats and mice were observed. It has been noted that 92.44% were rats and mice while other prey items found were shrews 7.55% and birds 1.33%. Among rats and mice *Mus* sp. was the dominant food item of the owl. This species constituted 61.77% of the Owl's diet. The relative abundance of *Millardia meltada* was 20.88%, *Rattus rattus* was 4.88%, *Suncus stoliczkanus* was 2.22%, *Nesokia indica* was 2.22%, Birds were 1.33%, *Tatera indica* was 0.88% and *Rattus meltada* was 0.44%. Studies indicate that the Barn owl *Tyto alba* can be used as a bio-control agent in our fields.

SIGNIFICANCE OF DEPLOYING SUPPLEMENTAL IRRADIATED HOSTS TO ENHANCE THE INITIAL FIELD SURVIVAL AND THE ESTABLISHMENT OF P ARASITOIDS IN THE SUGARCANE FIELDS

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Pakistan is the fourth largest among the sugarcane producing countries in the world and it is the second largest industry after textiles in the country. To manage the pests of sugarcane crop chemical and biological control strategies are adopted. But due to the devastating effects of pesticides including environmental pollution and induced pest resistance, continued interest in using biological control agents as an alternative environmentally friendly method of pest control is being applied widely. Although the value of parasitoids in augmentative biological control is well documented but their use is limited due to our inability to produce sufficient numbers at low cost. Irradiation is a technique that can be used to enhance the production of natural enemies by increasing their shelf life and parasitization potential. Provision of irradiated supplemental host may also play an important role in the initial survival and establishment of released parasitoids in the field. Here we describe the use of irradiated supplemental host to enhance the initial survival and the subsequent establishment of an egg parasitoid *Trichogramma chilonis* in the sugarcane field. Results indicated that the application of irradiated supplemental host could effectively be used to enhance the initial survival of the released *T chiloni* in the sugarcane field and also the establishment of the parasitoids was higher in the block where the supplemental irradiated host was continuously provided to the parasitoids. Temperature and relative humidity also played a significant role in the successful build up of the parasitoids as less numbers of the parasitoids were observed during May to July due to high temperature and low relative humidity in the field.

SECTION - III

ENTOMOLOGY

EFFECT OF VARIOUS TEMPERATURES ON *CHRYSOPERLA CARNEA* (STEPHENS) (NEUROPTERA: CHRYSOPIDAE) STORAGE

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Laboratory studies were conducted to determine effect of temperatures on eggs, larvae, pupae and adults of generalist predator green lacewings *Chrysoperla carnea* (Stephens). The results showed that during storage period of three weeks there was no hatching at 5°C, but it was gradually increased with the increase in temperature *viz*. 5%, 8%, 12%, 25% and 40% at 7, 9, 11, 13 and 15°C, respectively. After storage period highest survival (60%) showed at 5°C. The larvae did not survived at 5 and 7°C while highest survival 89% recorded at 13°C. The pupae during storage period did not survived while after the storage period 15°C showed highest (73%) survival. Adults highest survival (55%) was recorded at 15°C, after the completion of storage period. This phenomenon use for storage of predators which is helpful in application of biological control.

CHECK LIST OF MOTHS (LEPIDOPTERA:HETEROCERA) OF PAKISTAN

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Moths and Butterflies share common life cycle and the various ways in which this is adapted to differing climates, habitats and conditions. All adult female Lepidoptera lays eggs. In some species, mostly the largest, most specialized and longest lived, as few as 20-40 eggs are produced, more typically, medium and small species produce 100-200 or even more. The adults of most species of moth are rather short-lived, a matter of days at most, and in their adult life they are preoccupied with the essential business of mating. A minority of species lives for more than a week and some of these are adapted to particular life styles, such as hibernating or aestivating as adults. Many adult moths disperse either within the habitat or to new suitable areas elsewhere. For most adult moths, the immediate priority after emergence is to find a suitable mate; this posses a

considerable problem for a relatively small organism in a large world. The opportunities for finding a mate by chance encounter are remote and most species have very effective mechanisms for locating the opposite sex of their own species. The obvious and indeed legendary was in which this is done is for the females to emit an attractive scent and in some species this plume of perfume is effective over a distance of several kilometers, This sort of attractive chemicals, released by one individual and inducing a behavioural response in another, is called a pheromone. We have learnt to produce some of these chemicals artificially and to use them to attract the males of certain pest species to k' ling traps to control them.

PRELIMINARY STUDIES ON LONG-HORNED GRASSHOPPERS (TETTIGONIIDAE) OF SINDH

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Much work has been done on the short-horned grasshoppers of Sindh but, no attention has been paid to the long-homed grasshoppers so it was decided to start some work on identification of Tettigoniidae. A small collection of about 200 specimens were studied and species determined are: *Eueonoeephlus ineertulas* Walk. *Eueonoeephlus* sp. *eonoeephlus maeulatus* Gill, *Trigonoeorypha unieolor* Stoll and *Trigonoeorypha angastata* Uvarov. This last species is recorded for the first time from this area.

OBSERVATIONS ON BAND-WINGED GRASS HOPPERS (OEDIPODINAE) OF PAKISTAN

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Acrotylus humbertians Saussur, *A. insubricus insubricu* Scopoli, *A. insubricusinjicitu* Walker, A. *longipes longipes* Charpentier, *A. longipes subfasciatus* Bei-Bienko, *Aiolopus thalassinus thalassinus* Fabricius, *A. thalassinus tamulus* Fabricius, *A. simulatrix simulatrix* Walker, *Chloebora crassa* Saussurei, *C. grassa* Saussure, *Cophotylus splendes* Uvarov, *Gastrimargus africanus sulphureus* Bei-Bienko, *Hilethera aelopoides* Uvarov, *H. turnica* Uvarov, *Locusta migratoria* Linnaeus, *Mioscirtus wagnerirogenhoferi* Saussure, *Oedaleus abrupts* Thunberg, O. *rosescens* Uvarov, *O. senegalens is* Krauss, *Oedipoda coerulescens* Linnaeus, *O. fadtshenkoipamirica* Ramme, *O. miniata atripes* Bei-Bienko, *Scintharistanotabiles pallipes* Uvarov, *Sphingonotus akbari* Wagan & Naheed, *S. balteatus balucha* Uvarov, *S. balteatus hima layanus* Uvarov, *S. carinatus* Saussure, *S. hussaini* Wagan & Naheed, *S. longi pennis* Saussure, *S.*

maculatues fetraeus Bei-Bienko, *S. montanus* Mistchenko, *S. nebulosus persa* Saussure, *S.. obscuratus obscuratus* Walker, *S. predtetschenkyi* Mistchenko, *S. rubesecens rubesecens* Walker, *S. rubesecens afghanicus* Mistchenko, *S. savignyi* Saussure, *S. sindhensi, Trilophidia annulata* Thunberg. These band-winged grasshoppers are recorded from various provinces of Pakistan.

BIODIVERSITY OF RED COTTON BUGS: REDESCRIPTION OF *DYSDERCUS ARGILLACEUS* BERGROTH AND *OCEANICUS* BOISDUVAL (HEMIPTERA: PYRRHOCORIDAE) WITH SPECIAL REFERENCE TO THEIR GENITALIA AND THEIR RELATIONSHIPS

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The representatives of the genus *Dysdercus* Gueren-Menville are the pests of malvacian plants, mainly cotton, distributed in New and Old world. Hussey (1929) catalogued 75 species in the genus *Dysdercus*. Freeman (1947) revised the genus from the old world. He described these species *i.e. D. argillaceus* Bergroth from Kokoda, and Torres Strait, New Guinea but did not see the type which is described from Queensland, Australia and *oceanicus* Boisduval from various localities of Solomon Island of which he did not see the type which is described from New Ireland. However he considered Soloman Island specimens undoubtedly the same as the specimens originally examined by Boisduval from New Ireland. In addition Freeman (*op.cit.*) also examined specimens from Fiji and Solomon Island which were earliar described by Stål (1870) as *impictiventris* and by Walker (1872) as *albescens*. The male genitalia of all the three species according to Freeman (*op.cit.*) who examined the type of *albescens,* were identical. Stehlik (1965a and b) established the sub-generic groups of *Dysdercus*. He classified it into four distinct sub-genera *viz*., *Dysdercus* sensu stricto, *Neodysdercus* Stehlik, *Paradysdercus* Stehlik and *Megadysdercus* Breddin. Stehlik (1965a) placed *Dysdercus argillaceus* Bergroth and *D. oceanicus* Boisduval in his subgenus *Megadysdercus* Breddin. *D. argillaceus* Bergroth and *D. oceanicus* Boisduval is here described in detail with special reference to the unknown characters of genitalia and in this light their relationships within their sub generic group is also briefly discussed. Several male and female specimens collected from Solomon Island, Queensland, New Ireland, Samoan Island and Fiji Island and determined by Freeman and lodged at Natural History Museum, London (BMNH), were examined at the above museum by the courtesy of Mr. Mick Webb incharge Hemiptera section of that museum. For the inflation of the aedeagus the techniques of Ahmad (1986) and Ahmad and McPherson (1990) were followed. For the dissection of the female spermatheca, the entire abdomen was warmed on a bench lamp (after completing the external view diagram of the ovipositer) for 15 minutes. The spermatheca was dissected out in tap water after washing the specimen

thoroughly following the techniques of Ahmad *et al.* (2003). The components of male and female genitalia were preserved in glycerine in microvials pinned with the specimens.

A REVISION OF HALYINE STINK BUG GENUS *SARJU* GHAURI (HEMIPTERA, PENTATOMIDAE, HALYINI) AND THEIR CLADISTIC ANALYSIS

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Halyine stink bug genus *Sarju* was described by Ghauri (1977c) to accommodate the type species *H obscura* Westwood (1837) *Dalpada eremica* Hoberlandt and *D. pavlovskii* Kritchenko on the basis of absence of side lobes on median excavation of the ventral margin of pygophore along with five new species and three subspecies from Bengal, Iran, China and Indo-China. How ever the female of *S. farida* and *S. enigma* were not available to him which were later on described by Ahmad and Afzal (1984), enigma from northern areas of Pakistan like Manor, Kargaha, Gilgit and *S. farida* from Haripur of NWFP and Changamanga of Punjab along with redescription of males of both and the description of their new species *S. angulata* from Gilgit Pakistan. Presently the genus *Sarju* is revised with its 12 (9 species and 3 sub-species) world known species by brief distinguished features and zoogeographical distribution. The characters of each taxon are scanned from the present description and those given in the literature to date. Their apomorphic states are recognized on the bases of out group comparison within the tribe Halyini at large. A key of twelve species is given and a cladogram is constructed based on the principle of parsimony to throw light on the evolutionary relationship of the included taxa.

REDESCRIPTION OF COTTON STAINERS SPECIES *DYSDERCUS INTERMEDIUS* DISTANT (HEMIPTERA: PYRRHOCORIDAE) FROM SOUTH AND EAST AFRICA WITH SPECIAL REFERENCE TO ITS GENITALIA AND ITS BEARING ON ITS PHYLOGENETIC RELATIONSHIP

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Distant (1902) described *Dysdercus intermedius* from Tanganyika and Rhodesia. Schouteden (1912) described it as pest of cotton in Africa. Hussey (1929) catalouged it from Rhodesia. Freeman (1947)for the first time described its pygophore and paramere in

male genitalia and spermatheca in the female genitalia and on the basis of the characters of the above genitalial armature categorized it in his group1b without vertical processes of the pygophore with apex of the latter conical and spermathecal duct very long and coiled having recurved accessory gland. He called his group 1b as group of *D. intermedius* with *D. haemorhoidalis* Signoret, *D. orientalis* Shouteden and *D. pretiosus* Distant. Stehlik (1965) following Freeman (1947) named his 1b as sub genus *Neodysdercus* with *intermedius* as its type species. Qadri and Ahmad redescribed *orientalis* Schouteden and confirmed the findings of Freeman (1947) and Sthelik (1965). Presently *D. intermedius* is redescribed with reference to its characters of male and female genitalia and on this basis its phylogenetic relationship within its subgenus *Neodysdercus* is briefly discussed. The type of *D. intermedius* from Tanganyika with other specimens from Nyasaland and north and south of Rhodesia and Portuguese east Africa were examined during the visit of the first author in 2005 by the courtesy of Mr. Mick Webb Incharge Hemiptera section of the Department of Entomology, Natural History museum, London (BMNH). The male genitalia was dissected and examined following the technique of Ahmad (1986) and Ahmad and McPherson (1990) and the female genitalia following the technique of Ahmad *et al.* (2003).

REDESCRIPTION OF THE MYROCHEINE STINKBUG GENUS *PARAMECOCORIS* STÅL (HEMIPTERA: PENTATOMIDAE: PENTATOMINAE) WITH SPECIAL REFERENCE TO ITS PHYLOGENETIC RELATIONSHIPS

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The Myrocheine Stink bug genus *Paramecocoris* Stål is redescribed in detail. The genus is also compared with its closest allies and its cladistic relationships is also briefly discussed in the light of its apomorphic characters. Stål (1853) proposed *Paramecocoris* as a replacement name for *Paramecus* Fieber 1851. Fieber (1851) described the genus *Paramecus* to accommodate his species *ruficornis* which became the type species by monotypy. But *Paramecus* Fieber was a junior homonym of *Paramecus* Dejean (1829) (in the order Coleoptera) and therefore it was a preoccupied name. Stål (1853) gave it a new name as *Paramecocoris*. Stål (1861) confused his earlier named taxon as *Paramecocoris* with *Delegorguella* Spinola (1850) (about which probably he had no information) which is obvious by the fact of his list of species under his *Paramecocoris*. Stål's (1861) misidentification of *Paramecocoris* with *Delegorguella* was followed by Stål (1865 and 1876), Leithiery and Severin (1893) and Kirkaldy (1909). Actually Stål (1876) probably forgot that he himself renamed *Paramecus* Fieber because it was preoccupied in Coleoptera, considered the generic name *Paramecus* Fieber as valid and independent from that of *Paramecocoris* which Stål confused as *Delegorguella*. The

genus *Paramecocoris* Stål as senior homonym of *Paramecus* Fieber is compared with its closest allies *viz*. *Hippotiscus* Bergroth, *Praetextatus* Distant and *Valescus* Distant. The type species of *Paramecocoris* (*P. ruficornis* Fieber) from India was examined by the 2nd author during his visit to Natural History Museum in UK and Europe.

TAXONOMIC AND ECOLOGICAL STUDIES OF PHLOCID SPIDERS (ARANEAE: PHOLCIDAE) AT FAISALABAD, PAKISTAN

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Pholcids are among the dominant web building spiders in many tropical and subtropical areas and even in temperate regions of the world. They occupy a wide variety of habitats ranging from leaf litter to tree canopies and several species occur in caves and in close proximity to humans. The present work state the knowledge about Phlocids in central Punjab, Pakistan. A total of 4 genera *i.e.* Leptopholcus, Artema, Crossopriza and Phlocus and six nominal species belonging to these genera were also discussed. The population abundance and monthly fluctuations even diversity of Phlocid spiders were also discussed.

SEXUAL DIFFERENCES IN THE ATTRACTIVENESS OF FIGS TO POLLINATORS: FEMALES STAY ATTRACTIVE FOR LONGER

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There are over 750 species of fig trees *(Ficus,* Moraceae) each of which is pollinated by its own species of fig wasps (Hymenoptera, Agaonidae). About half of all *Ficus* species are functionally dioecious with separate male and female plants. Pollen and the female fig wasps that transport it are produced in figs of male plants, whereas female plants are in effect traps for the pollinators as they produce only seeds. As the short-lived female fig wasps cannot reproduce in female figs, natural selection should favour any individuals that can distinguish between the sexes of their host plant. However, fig wasps have repeatedly been shown to fail to discriminate clearly between *Ficus* sexes, probably because of intersexual mimicry, and "selection to rush" in the wasps. As others have done, we compared the relative attraction of male and female figs of the South East Asian *Ficus montana* to its pollinator *Liporrhopalum tentacularis,* but for the first time we took into account the ages of the figs. We also recorded the reproductive success of pollinators that entered figs of different ages. We also compared the reproductive success of *L.*

tentacularis females that entered figs on the first and second days of their adult lives. Age-related differences in the relative attraction of male and female figs were detected, a result that questions the validity of previous fig wasp-choice experiments that did not control for fig age. The differences in floral longevity in male and female plants of *F. montana* may be adaptive and we conclude that the host preferences shown by the pollinators reflect the reproductive strategy of the plant and are not related to the fitness of the pollinators.

A BASIC STUDY OF MULTILEVEL GEOGRAPHIC DISTRIBUTION AND PREVALENCE OF *ANOPHELES STEPHENSI* (DIPTERA: CULICIDAE) IN KARACHI, PAKISTAN

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Mosquitoes are very dangerous insect on the earth and the most medically important insects in the entire world. Different genera and species of mosquitoes found in Pakistan, many are known to be vectors (carriers) of important diseases such as Malaria, Dengue hemorrhagic fever, West Nile virus and Chikenquinea etc. Different species of *Anopheles* are responsible to transmit malaria. About 24 species and sub species of *Anopheles* mosquitoes are recorded from Pakistan, among these many are known to be vectors (carriers) of Malaria disease. *Whereas Anopheles stephensi* is one of the most important vectors of malaria in Pakistan. The present study was carried out to document a multilevel geographic distribution, density and prevalence of *Anopheles stephensi* (Diptera: Culicidae) from urban and semi urban areas of Karachi-Pakistan. The investigation was conducted on the basis of Geographical information system (GIS) in dry and wet season of (2004-2005). Adult mosquitoes were collected through netting, aspirating and by using artificial resting station from indoor and out door in and around the residential and non-residential areas included hotel, roads, school and cowsheds and one larva per container techniques were adopted. The adult and larval presence were noted from 03 districts in both dry and wet season (Density Index in dry season (D 1=3 to 6) and wet season (D 1=7 to 9) only two areas showed *Anopheles stephensi* presence in only wet season. In the residential areas, cemented houses Breteau Index (BI=30.8%); Container Index (CI= 39.08%); House Index (HI=63.6%); pupa house index (PHI= 0.2%); Larval house index (LHI=2.6%); Adult house Index (AHI=7.8%), whereas non-cemented houses (huts) showed relatively high prevalence rate of (BI=57); (CI=26.14%); (HI=77%); (PHI=51.6%); (LHI=30.6%); (AHI=29.66%), The cowshed and the animal area had rate (BI=142); (CI=62.83%); (H1=94%); (PH1=66%); (LHI=48%);(AHI=82%), Tier vehicle (48%) was the most preferred breeding place among domestic containers. *Anopheles stephensi* prevalence in the urban as well as semi urban areas has a risk of malarial infection therefore type of houses, attitude and practices can help to down the risk factors of diseases, especially elimination and checking of artificial breeding places

of mosquitoes may reduces the chance of diseases. Mosquito control has a crucial role in maintaining public health even in the absence of disease transmission, thus it is significant to understand to the professionals about the mosquito's' distribution, density, biology and their control.

BIOLOGY AND POPULATION DYNAMICS THROUGH HORIZONTAL LIFE TABLE TECHNIQUE OF COTTON MEALY BUG *PHENACOCCUS* SP. (HOMOPTERA: PSEUDOCOCCIDAE)

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Cotton mealy bugs in the recent pasts including the year 2007 produced great devastation of our cotton and vegetable crops such as little finger, brinjal, okra etc. in localized areas and caused enormous economic losses. The female lays crawlers instead of eggs and moults thrice to become wingless female in 10 to 20 days. The insects are well protected under white cottony and waxy secretion along with its antennae. The counted number of 1st and 2nd ins tars larvae of mealybug were released in different petridishes and were provided with twigs of alternate host plants, *Hebiscus esculentus.* The proportions of individuals surviving from initial to adult stages were calculated day to day. The mortality rate in one group of per thousand of individuals (qx) was calculated and survivorship curves were drawn, following Slobodkin (l962).The Age specific life budget of *Phenacoccus* sp. and the horizontal survivorship curves although showed some deviations from actual trend but apparently resembled with 3rd and 4th types of survivorship curve of Slobodkin (1962), with high mortality in initial stages and low in adults. The minimum mortality among the immature 1st and 2nd instars of *Phenacoccus* sp. was recorded during Jul. 05 (l000qx =300). The highest mortality was observed during Jan. 06 (1000qx =750). Among the 3rd instars of *Phenacoccus* sp., the minimum mortality was recorded during Jul. 05, (1000qx = 143). The unusual highest mortality in 3rd instar of *Phenacoccus* sp. was observed during Mar. 06 (1000qx = 750).

SECTION - IV

PARASITOLOGY

PCR DETECTION OF *BORELLIA BURGDORFERI* IN TICKS COLLECTED FROM WOODS OF RHODE ISLAND

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Polymerase Chain Reaction (PCR) Assay was used to test adult *Ixodes escapularis* ticks collected from woods in the adjoining areas of University of Rhode Island USA, for *Borrelia burgdorferi,* the human granulocytic ehrlichiosis (HGE) agent. Total nucleic acids were extracted from ticks with an IsoQuick nucleic acid extraction kit, which was employed according to the manufacturer's instructions, followed by phenol-chloroform extraction and isopropanol precipitation. The presence of pathogen was determined by using PCR with primer sets A2 and A4, which have previously been used to detect the pathogens.

ECOLOGICAL-MORPHOLOGICAL CHARACTERISTIC OF TREMATODES OF THE GENUS *TRICHOBILHARZIA* SKRJABIN ET ZAKHAROV, 1920, PARASTIES OF BIRDS

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The trematode genus *Trichobilharzia* comprise 42 species, the ranges of which cover significant territories of the globe. They are recorded in hydrophilous birds inhabiting tropics, subtropics and moderate zones. Life cycles of three trematodes are characterized by the change of hosts and alteration of generations – the free-living and parasitic forms. Field and experimental studies carried out in the biogeocenoses of Uzbekistan revealed the species *T. ocellata*, *T. filiformis* and *T. tatianae* in *Anseriformes*. Morpho-biological peculiarities of all stages of *T. ocellata* development were studied. Mollusks *L. auricularia*, *L. stagnalis* and *M. kainarensis* were recorded as intermediate hosts of this parasite in natural conditions of Uzbekistan. The infection of mollusks fluctuates depending on the season and biotopes from 1.1 to 6.2%. A special attention is paid to the morphological variability of the complex of traits of males, females and cercariae of these trematodes. The trematodes of the genus *Trichobilharzia* are considered by us as the combined formation of at least several groups. This is confirmed

by a presence, within the genus, of three morphological types of the structure of the excretory systems in cercariae. The first group of species is characterized by the formula of the excretory system: 2[(3)+(3)+(1)]=14. This is referred to the type species *Trichobilharzia ocellata* (La Valette, 1855). For the second group, the following formula is applied: 2[(3)+(4)+(1)]=16, which is characteristic for the species *Trichobilharzia arcuata* Islam, 1986. The formula for the third group is expressed as 2[(3)+(2)+(1)]=12, which is characterized for the species *Trichobilharzia corvi* (Yamaguti, 1941). The species of this genus are pathogenic for domestic, wild and hunted-commercial birds. *Trichobilharzia* larvae cause serious violations, which are called cercarial dermatitis, in humans. The peculiarity of the morphology, biology and ecology of trematodes of the genus *Trichobilharzia* necessitate an in-depth analysis of the structure of this genus and its status within the system of the Bilharziellidae family.

PRESENT STATUS OF CUTANEOUS LEISHMANIASIS IN SUSPECTED PAKISTANIS AND AFGHAN REFUGEES RESIDING IN NWFP, PAKISTAN

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The objective of the present study was to characterize the epidemiology of cutaneous Leishmaniasis caused by *Leishmania major* and *Leishmania tropica* in Afghan refugees and local Pakistani population in various areas of NWFP Pakistan. Parasitological and epidemiological surveys of the human population (Afghan refugee's camps and surrounding Pakistani population) were performed from the beginning of 2006 to December 2006. A total of 515 suspected people were examined of which 281 were found positive for dermal Leishmaniasis during the study period. Prevalence of Leishmaniasis was higher in local population (65.62%) than in Afghan refugees (36.41). There was an increased burden of infection among children of 0-9 years while lowest prevalence was recorded among youth of 20-29 years. Prevalence was higher among male (63.12%) as compared to female (38.06%), this may be due to male sleeping without shirts in summer giving more chances to sand flies bite. Leishmanial lesions were more common on face (61.53%) than on legs, hands and other body parts and were recorded from 1-6 per individual. Duration of lesions was from 1-9 months and dry lesions were prevalent (62.32%) over wet type (37.68%). The prevalence rate in patients, previously treated with sodium stibo-gluconate was found lower (28.98%) than untreated patient (63.92%). Isolates of *Leishmania species* were identified after culturing in NNN medium. Susceptibility to infection with *Leishmania* promastigotes was examined in 30 BALB/c mice divided in 19 groups in three different experiments. Majority of mice developed skin nodules with punch-out ulcers (lesions) by 5-8 weeks post-infection ranging from 0.1-1.2 cm in diameter.

A TEST OF NEW ANTHELMINTIC DRUGS AGAINST OF LUNG NEMATODES IN ANIMALS

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Lung nematodes are recorded in ruminants, mainly in the mixed form. Thus, one infected animal can be parasitized by 2 to 5 nematode species. The species diversity of mixed infections in Uzbekistan consists of the representatives of genera *Protostrongylus, Spiculocaulus, Muellerius, Cystocaulus* and *Dictyocaulus*. Practically, the species of these genera make up the core of the dominant nematodes in Uzbekistani biogeocenoses, while other species are recorded only sporadically. We carried out tests of a number of anthelmintics on animals experimentally infected with protostrongylides in laboratory conditions. We applied alpek 2.5%, medapek, 2.5% (water solutions) and artemizinin (oil solution), which were obtained from the Laboratory of the Institute of Plant Substances of Uzbek Academy of Sciences, and alben (granulate). The experiments on sheep showed the effect of alpek, medapek and alben, while artemizinin produced no effect on adult or larval forms. The above mentioned anthelmintics (except artemizinin) were also tested at associated infection by lung nematodes (Protostrongylidae and Dictiocaulidae) of small cattle in laboratory-industrial conditions. The studies of helminth larvae revealed no 1st instar larvae in the cattle treated with alpek and alben, while one animal remained infected after treatment with medapek, *i.e.* the effect of the former two anthelmintics reached 100%, and the latter, 90%.

DAMAGE OF PORTAL TRACT AREA AND ASSOCIATED TISSUE IN GOAT LIVER INFECTED WITH *F. GIGANTICA*

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Fasciola gigantica is a common liver parasite of herbivorus animals including sheep and goat. As the fluke migrate through the liver tissue causes damage. Bile duct hyperplasia and fatty degeneration has been reported previously. Here damage to portal tract area and associated goat liver tissue is reported. Histological sections revealed damage to portal tract area, shrinkage of tissue and fibrosis. In the liver tissue the arrangement of hepatic cords was distorted, liver cells were not identifiable, hyaline

degeneration of liver tissue with dilatation of sinusoids was obvious. In some sections, section of fluke was seen with fragmentation of liver tissue.

DESCRIPTION OF *POLYMORPHUS MOHIUDDINI* N.SP. (ACANTHOCEPHALA: POLYMORPHIDAE) FROM THE OWL *(STRIX LEPTOGRAMMICA)*

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During studies on Acanthocephala of birds, 2 male worms were collected from the small intestine of the host Owl *(Strix leptogrammica)* in Karachi, Sindh, Pakistan. Specimens were fixed in T.A.F. and stained in Mayer's carmalum. Measurements, made using an ocular micrometer and conversion table, are in micrometers unless otherwise statea. The male specimens were elongate measuring 21.14-21.44 by 0.94-0.96, Proboscis cylindrical, pyriform measuring 0.92 by 0.48 with 20-23 rows of hooks each row having 12-14 hooks measuring 0.0235 - 0.094 by 0.0076 - 0.0117. Neck measuring 0.02 by 0.48. Proboscis receptacle double walled inserted at the base of the proboscis. Lemnisci longer than the proboscis receptacle and are subequal. Reproductive system well developed comprising of two testis, four cement glands slender and elongated. Cement reservoir oval Bursa well developed. The new species is named in honour of Prof. Dr. Ahmed Mohiuddin, Department of Zoology, University of Sindh, Jamshoro.

THE EPIDEMIOLOGICAL IMPORTANCE OF THE TREMATODE *ORIENTOBILHARZIA TURKESTANICA* (SKRJABIN, 1913)

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Trematodes *Orientobilharzia turkestanica* (Skrjabin, 1913) are widespread in the biogeocenoses of zoogeographic Indo-Malay and Palaearctic provinces as components of tropic and subtropic faunas. As representatives of the order *Schistosomatida*, they became adapted to parasiting in blood vessels of about 20 mammalian species. Penetrating into non-specific hosts, the cercariae of schistosomatides cause diseases called dermatitis or cercariases. The focuses of schistosome-induced cercariases have been revealed in the territories of Tashkent and Khoresm provinces, as well as the Republic of

Karakalpakstan, where intermediate hosts of Orientobilharzia, the mollusks *Lymnaea auricularia*, are widespread. Cercariases emerge during swimming, work in water bodies, collection of mollusks, fishing in some flowing and stagnant fresh-water water bodies overgrown with plants inhabited by mollusks, intermediate hosts of these trematodes. The signs of cercariasis resemble a bite and reddening of parts of legs that were submerged into the water; a slight tingle can be felt on the skin and an intensive reddening is observed. Five to six hours later an itch is felt on the skin, which gradually increases, especially at night. By the end of the day and at night, a slight increase in body temperature is noted, as well as indisposition, general weakness and anxiety. In the following days, the itch continues and small red tubercles the size of millet grain emerge in itching places. In a week's time the itch disappears, as well as the tubercles. Therefore, orientobilharziasis in synanthropic and natural conditions focuses of the infection should be considered as a problem of the epizootological and epidemiological importance.

PLAGIORNYNCHUS KARACHIENSIS **NEW SPECIES (ACANTHOCEPHALA: PLAGIORHYNCHIDAE) FROM CROW *(CORVUS SPLENDENS* LINN.) OF KARACHI, PAKISTAN**

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Examination of six crows *(Corvus splendens* Linnaeus) from Karachi, Sindh, Pakistan revealed 2 Acanthocephalans from the small intestine of a single bird. Morphometric studies specify that the Acanthocephala are new to science and named as *Plagiorhynchus karachiensis.* The new species differ from its congeners in size of body, arrangement of hooks and egg size. This is the first record of genus *Plagiorhynchus* Luhe, 1911 from Pakistan.

TWO NEW TREMATODES FROM THE FISHES OF KARACHI COAST, PAKISTAN

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Two trematodes are describe here from the fishes of Krachi coast including, *Stephanostomum gibson* n.spi from the fish *Pomadasys olivaceum* and *Decem testis johnii* n.sp from the fish *Lutjanus johnii. Stephanostomum gibsoni* n.sp is characterized by having elongated body, narrower in the middle and wider at the ovarian region, with anterior end curved ventrally, forebody and some part of hind body is spined, rest smooth. Oral sucker, provided with two, complete, alternate, circular rows of 38-41

circum oral spines surrounding the mouth, prepharynx is small, pharynx elongated, intestinal bifurcation at the level of ventral sucker. Testes 2, located in the middle of posterior part of body, obliquely tandem, posterior is slightly bilobed, cirrus sac very long, external seminal vesicle is also bilobed, far from cirrus sac, internal seminal vesicle tubular, pars prostatica is well developed, genital opening near the dorsal anterior level of ventral sucker, ovary pretesticular, seminal vesicle pre-ovarian, large, close to external seminal vesicle, vitellaria in two lateral fields in posterior half of *Decemtestis johnii* n.sp. has a small, flattened body, forebody narrow, hindbody broader, oral sucker subterminal, acetabulum is larger than oral sucker, pre-equatorial, prepharynx absent, pharynx pear-shaped, oesophagus relatively long, cecae simple, long. Genital pore is at the base of oesopahgus, cirrus sac is long extending between anterior level of acetabulum and base of oesophagus, testes 10 in two intercecal rows, in posterior half of the body. Ovary consists of 7-8 lobes immediately anterior to testes, uterus is coiled, small, between ovary and ventral sucker extending anteriorly to the level of genital atrium. Eggs are numerous, oval in shape. Vitellaria are follicular, numerous, scattered laterally from the anterior to ventral sucker and posteriorly to the end of the body, confluent in the posterior body region. Excretory vesicle is extending to the level of anterior pair of testes.

HISTOPATHOLOGICAL STUDIES OF ALMOND ROOTS INFECTED BY ROOT-KNOT NEMATODE IN KALAT, BALOCHISTAN

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Almond *(Prunus amygdalus)* fruit is a rich and cheap source of minerals and vitamins for man and animals. Plant parasitic nematodes have been reported as an important pests of fruit plants in Pakistan. Almond roots are collected from district Kalat, Balochistan from a depth of 5-35 cm. They were cut into 12 and 1 cm long pieces with the help of a sharp blade washed free of soil for I hour fixed in F.A.A. and processed for histological technique according to Sass (1964). Sections using a rotary microtome 10-12 µm thick sections were cut and stained with Haematoxylin and eosin. Photomicrographs were taken using an automatic photographic camera mounted on a research microscope Nickon Optiphot-2 in the Department of Zoology, University of Karachi. Cell hypertrophy and syncytial formation were the most prominent responses to the nematode infection. A number of giant cell with thickened walls and cells containing yellowish masses were observed. Present study reveals the histopathological changes caused by root-knot nematode, being a root parasitic nematode it is able to penetrate into deeper layers of root. It being capable of destroying cellular layers of root and in result plant suffers with severe malabsorption of nutrients and water from the soil which leads into morbidity and mortality of the plant.

PEGOSOMUM MUNIFI N.SP. (DIGENEA: ECHINOSTOMATIDAE) FROM LIVER OF LITTLE EGRET *EGRETTA GARZETTA* (AVES: ARDEIDAE) OF HYDERABAD, SINDH, PAKISTAN

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During (1996-2004) a parasitological survey of birds from Hyderabad, Sindh, Pakistan, a new species of the genus *Pegosomum* Rátz, 1903 is described from liver of Little Egret *Egretta garzetta* (Ardeidae) and named as *Pegosomum munifi.* New species chiefly differs from its congeners in number of collar spines, extension of vitellaria, body size, egg size, genital complex, including morphology of cirrus and well developed metraterm. Accordingly, it is regarded as a new species for which the name *Pegosomum munifi* is proposed. The genus is also first report from Pakistan. The species is named in honour of Prof. Muhammad Munif Khan, Department of Zoology, University of Sindh, Jamshoro, Pakistan.

RHABDOCHONA MAGNAVESICULA NEW SPECIES (NEMATODA: RHABDOCHONIDAE) FROM THE FISH *SHIZOCYPRUS BRUCEI* REGAN, 1914 OF RIVER LONI, MUSAKHEL, BALOCHISTAN, PAKISTAN

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During an investigation on the parasitic hemlminths, a new species of nematode genus *Rhabdochona* Railliet, 1916 is described from the intestine of cyprinid fish *Shizocyprus brucei* captured in River Loni, Kingri, district Musahkel, Balochistan. The new species *R. magnavesicula* is reported from this locality of Balochistan for the first time and represent new host record for S. *brucei.* The female of new nematode species is peculiar in having large, circular, sucker-like excretory vesicle which is postequatorial in position. New species of *Rhabdochona* is proposed based mainly on findings referring to 10 prostmal teeth in both sexes, large, circular, sucker-like excretory vesicle and long, muscular vagina, directed backward in female specimens and in males two unequal and dissimilar spicules, specially the small spicule which is spindle-shaped with thread-like proximal portion and pointed distal end more than three times smaller than large spicule and 9 pairs caudal papillae including 4 preanal and 5 postanal.

A NEW RECORD OF THE GENUS *ONCHOCAMALLANUS* PETTER, 1979 (NEMATODA: CAMALLANIDAE) FROM THE FISH *RITA RITA* OF BOLAN, BALOCHISTAN, PAKISTAN

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The species of nematode genus *Onchocamallanus* Petter, 1979, *O. globoconchus* (Ali, 1960) Petter, 1979 is describe here from the intestine of the fish *Rita rita* from River Balon, Balochistan. This is the first record of *Onchocamallanus* in fishes from Balochistan and Pakistan. The newly recorded species is based on female specimens only and is closely similar to previously described species of the genus *O. globoconchus* (Ali, 1960) Petter, 1979 in morphological features such as presence of transverse thickenings on inner surface of buccal capsule with knob-like structures at the base and in having striations in double marginal wall along the entire body length and in possessing preequatorial excretory pore and postequatorial genital opening. The female of present specimens is characterized by having a large buccal capsule with transverse buccal thickenings 17 to 18 in number with 5 knob-like structures at the base, muscular esophagus is smaller than glandular esophagus almost of uniform thickness, glandular esophagus is also of same thickness simply uniting the intestine which terminate at the posterior end of the body. Genital pore is post equatorial, vulva submarginal consisting of onion ring-shaped oval lips, vagina is posterior to it, muscular and tubular, directed backward, tail relatively small terminating into a prominent bifid end, uterus extending anteriorly to the posterior level of glandular esophagus. Eggs numerous, oval to squarish in shape.

MECHANISMS THAT REDUCE NEMATODE DEVELOPMENT IN PLANTS

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Successful development of a pathogen on a compatible host involves at least three processes including penetration, development, and reproduction. Host defense mechanisms may be activated during one or more of these pest actions. The gene activated defense mechanisms of grapevines infected by *Meloidogyne arenaria* were investigated in a greenhouse at 30±3°C. Five nematode resistant grape rootstocks including 10-17 A, 10-23B, 6-19B, RS-3, RS-9 and Cabernet Sauvignon, a susceptible check, were generated from shoot tips and transferred into Deepots of 5-cm diam. x 25-em-depth. Soil was then inoculated with 500 freshly-hatched J2 of *M arenaria* pathotype

Harmony. Roots were harvested weekly, washed and stained with acid fuschin. Developmental stages within roots were then monitored and characterized as vermiform 12, swollen sausage-shaped 13 or globose adult females. Presence or absence of eggs was also noted. Response of root tissues to nematode presence was also recorded. All five resistant grape cultivars successfully reduced J2 penetration, arrested nematode development during one or more steps and eventually reduced female fecundity. Roots of resistant vines were able to elicit a hypersensitive response [HR] at their epidermis, cortex, or within vascular tissues. No HR or arrested development was evident in roots of susceptible Cabernet Sauvignon, as nematodes passed through each development stage with minimal mortality. Size of root galls and egg masses produced on roots of resistant cultivars were small compared to those on roots of the susceptible cultivar. Our findings contribute important information on several new tools available to grape growers as well as new information available for further breeding purposes.

SECTION - V

FISHERIES, ECOLOGY, WILDLIFE, FRESHWATER BIOLOGY AND MARINE BIOLOGY

A NEW RECORD OF *AQUILONASTRA IRANICA* (MORTENSEN, 1940) (ASTEROIDEA: ECHINODERMATA) FROM BOLUCHISTAN COAST, PAKISTAN

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The present paper is based on the collection obtained during the survey of Balochistan coast. Seven specimens of the Asteroid echinoderm species *Aquinolastra iranica* (Mortensen, 1940) are collected from, Gwader (Lat.25^{o} 07' 00" N Long 062^{o} 29' 00" E). It is however being reported for the first time from coastal water of Pakistan. The species is briefly described and illustrated.

HUNTING, POPULATION AND CONSERVATION OFCRANES IN SOUTHERN DISTRICTS OF NWFP, PAKISTAN

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A total of 165 camps were visited in fall 2006 and 85 camps were in 2007 spring in district Bannu, Lakki and FR Bannu. These camps were establish in Baran Dam, Kurram river, Kashu river, Kethu and Dowa in Bannu, and river Gambilla, Lunder and Chall in Lakki. During these hunting sites a total of 2080 cranes were captured at the rate of 08 per camp. Of the total captured 1580 were Demoiselle cranes and 500 were Common cranes; Of which 915 Demoiselle cranes and 290 Common cranes were captured in Bannu and 665 Demoiselle cranes and 210 Common cranes were captured in Lakki. During fall 2006 and spring 2007 a total 830 cranes were killed of which 785 were Demoiselle cranes and only 50 were Common cranes. Of the total Demoiselle cranes killed 290 were in Bannu and 210 were in Lakki while 45 Common cranes were killed in Bannu and only 05 were in district Lakki. The total hunters have 9900 cogitative cranes, of which 6600 were Demoiselle cranes and 3300 were Common cranes. The average number of Demoiselle cranes per camp was 20 pairs and 10 pairs of Common cranes per

camp. It indicates that each hunter has two pairs of Demoiselle cranes and on pair of Common crane.

CHILOTHERIUM INTERMEDIUMFROM THE DHOKPATHAN FORMATION OF THE SIWALIKS

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The material collected from the Dhok Pathan Formation of the Middle Siwaliks of Pakistan. The premolars have a constricted protocone which is the characteristic of the genus *Chilotherium.* The upper premolars are in the late stage of wear and many morphological features are not observed in this stage. However the presence of crochet and antecrochet, the constriction of protocone and bulbus hypocone allows us to identifY the genus *Chilotherium.* In the upper molars the ectoloph is flat and broad with a strong parastyle and the protocone is much less constricted off from the protoloph. In the lower dentition all the characters are observed like, V -shaped trigonid, absence of lingual and labial cingulum, the hypolophid reclines backward and the entoconid have a flat lingual margin. All the characters are observed in the studied lower dentition which clearly identify the specimens belong to genus *Chilotherium* and species *Chilotherium intermedium.*

CHILOTHERIUM INTERMEDIUMFROM THE NAGRI FORMATION OF THE SIWALIKS

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The material is described from the Nagri Village, type locality, and'probably belongs to the same animal because all the specimens collected at the one point. Eight specimens are described and assigned to species *Chilotherium intermedium.* The species is found from the Nagri Formation of the Middle Miocene comprises a late Miocene vertebrate fauna constrained to between 11.2 and 10 million years ago (ma). Characters

of the rhinocerotid material from the Nagri Fortmation here are easily recognizable as typical of Aceratheriini. The lower dentition follows the general rhinocerotid pattern with two contiguous cresents open lingually. In the studied specimens, the labial cingulum is absent in upper molars, which is the characteristic of *Chilotherium* and the studied metrical values of the specimens show the intermediate body size of the species.

A NEW FOSSIL POCKET IN THE NEOGENE OF LAVA

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The fossil pocket was found near the Lava village lies on the 14km east of the Rawalpindi - Mianawali highway. The pocket was located some 11 km south east of the village Lava, district Chakwal and the right east of the Dera Rehmataay Aali along the road opposite to Kas Badri from which we obtained fossils in a very dense form over a small area. The fossils are mostly fragmentary in nature and ratio of the postcranial fossils is more than the cranial ones. The weathering cracks, abrasion marks and byte marks are noted frequently while observing the specimens. The site is highly fossiliferous and seems to expose for the long time. Owing to the long exposure fragmentary bones and enamels present all over the site. Nearly every sandstone have some embedded vertebrate remains. The pocket is characterized by sandstone dominant and reddish shale includes it to the Chinji Formation of the Siwaliks.

ACERATHERIUM PERIMENSE FROM THE MIDDLE SIWALIKS OF THE DHOK PATHAN

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Seven specimens of *Aceratherium perimense* are described from the Tertiary Hills of the Village Dhok Pathan belonging to the Middle Siwalik continental deposits during 1983 to 2002. All the specimens are well preserved and show the features of the genus *Aceratherium.* The specimens comprise isolated upper molars; premolars and an isolated lower molar. At the entero-external corner of all the teeth there is a well developed parastyle groove or fold. This parastyle fold runs vertically for the height of the tooth along its anterior edge. This fold in the parastyle is a characteristic *Aceratherium* feature

and the specimens are identified and assigned the genus *Aceratherium* and species *A. perimense. Aceratherium perimense* is the largest species of the Siwalik rhinoceroses and is found in the Siwaliks of Pakistan. The species was well flourished during the Middle and late Miocene of the Siwaliks and known only from the skull and isolated teeth. A complete skeleton of the species is yet not found from the sediments. The species is known as "hornless Siwalik rhinoceros" found in the Middle Miocene of the continental 'deposits. The study shows that *A. perimense* is larger than the other Siwalik rhinoceroses of Pakistan.

FIRST REPORT OF THE CARIDEAN SHRIMP *LEANDRITES CELEBENSIS* (DE MAN, 1881) FROM SINDH WATERS

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A small number of the caridean shrimp *Leandrites celebensis* (De Man, 1881) has been found in the first author's collections of caridean shrimp obtained during an Higher Education Commission Pakistan funded research project (2006-2007). The shrimps were collected from Ghora Bari Creek in the Indus deltaic region. The genus *Leandrites and Leandrites celebensis* is now reported from here for the first time. It is being briefly described here, with comments on its distribution.

CATCH COMPOSITION OF FISHING TRAWLERS IN THE OMAN SEA

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One of the common fishing methods within the Iranian waters of Oman Sea is trawl. This method because of its harms affecting on demersal fishes has been banned since 1993 in the Persian Gulf and only there is an under-control fishing season within 5 months period in the Oman Sea. During this period 25-30 trawlers are issued license to do fishing within Iranian continental shelf waters farther than 10nm off coastal zone. In conformity with assess the catch composition of fish trawlers and to estimate the amount of by catch, 2 research cruises were carried out in the region with stratified random design in year 2004. The studied area is restricted to the Iranian-Pakistani waters (61° 25′E) in east, Sirik (57° 00′E) in west and covering depths of 10 up to 100 m. The total area was stratified to 7 primary strata (A to G) based on longitudinal distances and each was classified to 4 different sub layers of 10-20, 20-30, 30-50 and 50-100m depths. The

R/V Ferdows-1 equipped with a fish trawl net (cod end=80mm and headline=72m) was used for sampling from128 randomly selected stations. According to the survey design, all demersal fishes based on their importance from commercial aspect were classified into 113 species, genus or fish groups. On the other hand the total catches (after ignoring pelagic fishes) were separated as 2 main groups of commercial and non-commercial fishes. The results showed that the commercial fishes such as croaker, silver pomfret, grunt, black pomfret, snapper, grouper,… comprise 53.7% of total catch, meanwhile the amount of non-commercial fishes such as Trigillidae, Leiognathidae, Tricanthidae, Monocanthidae, Ariidae(small size) ,…was estimated 46.3% . Also by calculating catch per unit of area (CPUA) of each species or species group, the distribution pattern of them was drawn by using Arcview-GIS software and the biomass was estimated by Swept Area method. Comparing the results with previous year (2003), it is concluded that there is an ascending trend in amount of commercial fishes from 46.4% to 53.7% with 7.3% increase and it shows that management on resources has been successful with controlling the fishing effort (both seagoing days and number of vessels). The most dense stratum was found in G, namely in the east of Oman Sea and comparing 4 depth sub-layers showed that the most density of both commercial and non-commercial fishes are found in depths of 10-20m.

BIOLOGICAL STUDIES ON THE OPOSSUM SHRIMP, *INDOMYSIS ANNANDALEI*- A MENACE FOR ARTEMIA CULTURE AND A BLESSING FOR SHRIMP CULTURE

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The mysids have adapted very well to live in estuaries and are important to the economy of coastal waters, mostly as planktivores, detritivores, and rarely as carnivores are animals, as well as they preyed upon by other higher organisms. In Pakistan, mysids form one of the large groups of non penaeid shrimps; to date more than 20 species have been recorded. The occurrence of *I. annandalei* is mainly tropical and reported up to 20°N. The species has been recorded from only a few locations along Kuwait, Bahrain, Saudi Arabia, India and Pakistan. It was recorded from Pakistan in 1995 and since then is commonly observed along Sindh and Baluchistan Coasts. The present study indicates that *I. annandalei* is an extremely euryhaline, eurythermal species, and abundant in creeks and shallow waters at salinities from 4-45 ppt, whereas temperature tolerance observed was from 10 to 38°C. Present paper deals with some aspect of biology of *I. annandalei*. Unlike many other species of mysids, it is carnivore in habit. The species thrives well in Artemia ponds and feeds on Artemia and may prove to be a menace for Artemia culture. The size ranges from 1.5 to 7.2 mm in males and 1.7 to 7.9 mm in females. Females predominated almost in every sample. An overall male to female ratio was 1:2.05. Like

other mysids *I. annandalei* carries the embryos in a marsupium. The marsupium size varied between 0.1 mm-0.2 mm. The entire larval development is completed within the marsupium; five stages of development were recognized in the present samples: no of larvae in the marsupium ranged from 3 to18. Experiments were conducted to determine the maximum salinity tolerance of the species. The salinity was increased gradually @ 5ppt/ day until it reached to 130 ppt. Even at 130 ppt, they survived for 90 hours. Experiments were also conducted by increasing the salinity abruptly by adding sea salt that is giving a salinity shock of 10, 20, 30 ppt. The 30 ppt shock was found to be lethal; the species may be considered both as advantageous as well as disadvantageous to the aquaculturists. Due to it hardy nature , it could be easily cultivated in pond for use as live feed in shrimp culture, whereas, for Artemia culture, it is very detrimental.

BASELINE STUDY OF THE BIRDS OF HAMUN-E-MASHKHEL AND TAHLAB RIVER

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The study area lies in the western Balochistan in the Chagai district, and is the eastern extension of the Iranian plateau. The river system of the area is landlocked, with ephemeral rivers and streams flowing and depositing sediments into a large lake called Hamun-e-Mashkhel. The lake area fluctuates around 900sq.km. Tahlab River is one of the tributaries of the Hamun-e-Mashkhel originating from the area near Zahidan city of Iran. It receives various streams from Iran until it joins the Hamun-e-Mashkhel. The water of Hamun-e-Mashkhel and Tahlab is saline. The habitats comprise of Tahlab stream that flows for a short period after the rains in its catchments. The water level fluctuates with rainfall and dry spells in the catchments. It has some pools and marshes during non rainy season. However, during heavy downpours flash floods occur. Phytogeographically the study area falls in the Saharo-Sindian region. The climate of the area is hyper-arid with hot summers when the temperature often rises to above 51^{o}C and the winters are mild with temperature normally remaining around 5^{o}C. The precipitation is during winter. The birds were studied with Nikon binoculars having 10x 40. 6^{o}, sitting in the water or on the shore or flocks flying low and closer. However, Spotting Scope with objective diameter of 78 mm and magnification of 60 with tripod was used to study the waterfowl sitting at some distance in the water or on the shore. Shy birds like pelicans, flamingoes, and ducks were particularly observed with spotting scopes. Field Guide to Birds of Pakistan by Z B Mirza was consulted to identify the bird species in the field on the basis of field identification marks given on bird illustrations. Ground surveys are considered to be the most reliable method for counting birds. Since the area of Hamun-e-Mashkhel is large and the birds were scattered. Counting and identification of birds was done for nearly every 1,000 meters, as this range was easily covered with the spotting scope. The observations were made from the raised positions along the lake

margins to get a clear view of the wetland and the birds. Two persons were together in the count. One observed through the spotting-scope and spoke to the companion who recorded notes in the field. There was no disturbance, *i.e*., the birds were not frequently taking flight. These were well spaced out in the lake. Their number was not very large, and they were nearly stationary. The visibility was good which facilitated the identification and accurate counting. Census was done from November 1, to 11, 2007 by counting all species by individual observers or counting was done in teams. Relative abundance of bird species was calculated. The lake had abundance of Anostraca "fairy shrimp". *Daphnia* (Cladocerans) were the abundant zooplankton. The salinity of the water was so high that extensive netting revealed no fish. Non aquatic predators of zooplankton like flamingos were present in sufficient numbers. 61 species of birds were found at Hamun-e-Mashkhel and 131 bird species were present in Tahalab river habitat. Some fish feeders like Pelicans and Cormorants were also resting during the day. In the absence of fish in the water the Pelican could feed on some fairy shrimps from the lake. But since these were not seen feeding at all, it is assumed that these were there for diurnal security on this wide habitat. They must be flying at night to nearby wetlands in Iran for food. Tahalab River had abundant small Rossica Garra fish *Garra rossica.* Relative abundance of bird species at Hamun-e-Mashkhel shows that the Common Teal *Anas crecca* was highly abundant *i.e.* 30.43%. Next in abundance was Shoveler duck *Anas clypeata* 25.77%, followed by Gadwall *Anas strepera* 20.08%. Common Pochard duck was *Aythya ferina* 4.21 %. Relative Abundance of Bird Species Found in Tahlab showed that the Common Teal *Anas crecca* was again highly abundant *i.e.* 45.57%. Gadwall *Anas strepera* was on next abundance 18.42%. Spanish Sparrow *Passer hispaniolensis* and Trumpter Bulfinch *Bucanetes githagineus* roosted on Tammarisk trees which were present only in the river bed. Hamun-e-Mashkhel and Tahlab River habitats are important transit migration staging areas of migratory birds in autumn and spring. No bird was sighted at Hamun-e-Mashkhel during summer. In winter too except for some terns very low number of some water fowl were observed.

DISCOVERY OF ADULT AND JUVENILES OF *PROCLETES LEVICARINA* (BATE, 1888) (CARIDEA: PANDALIDAE) FROM OMANESE WATERS

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Marine Reference Collection & Resource Centre, University of Karachi

A small but interesting collection of deep-sea carideans of Omanese waters is available for study. Among other pandalids 1 adult female and 2 juvenile specimens of *Procletes levicarina* have been observed. The record of this species from Oman will fill the gap in the known distribution of the species. The juveniles and adults differ vastly. The two forms are dissected, illustrated and compared.

STUDY ON THE SEASONAL VARIATION OF PROTECEPHALUS FILICOLLIS (RUDOLPHI) EGGS IN *GASTEROSTEUS ACULEATUS* L.

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A study on the seasonal occurrence of *P. filicollis* eggs has shown that eggs were larger in winter and spring and smaller in summer. There was a tendency for smaller worms to contain larger eggs. Eggs with smaller diameter were observed in larger worms. The size of the hexacanth showed more consistency in the diameter and was positively correlated to the diameter of the egg. The winter eggs were found to be viable and infective to the copepod intermediate host. The structure of the egg is described and compared with previous studies on the eggs of this parasite.

DISTRIBUTION, POPULATION STATUS AND CONSERVATION OF CHEER PHEASANT (*CATREUS WALLICHII*) IN JHELUM VALLEY, MUZAFFARABAD, AZAD KASHMIR, PAKISTAN

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Department of Zoology, The University of Azad Jammu and Kashmir Muzaffarabad

Cheer Pheasant (*Catreus wallichii*), listed as an endangered species in Red Data Book (IUCN), reported to have been extinct in all over Pakistan, but fortunately is present in some areas of Azad Jammu and Kashmir, especially in Jhelum valley where it has patchy distribution. There is little scientific data on distribution and population status of this bird in Azad Kashmir, where it is reported to be declining due to hunting and habitat degradation, since the people are unaware of its importance. There is a dire need of the time to make some strategies for conservation of this precious bird. The surveys were conducted from 1st December 2003 to 25th September 2005 in 13 main localities and 19 sub localities in District Muzaffarabad to study the distribution, population and habitat utilization of Cheer Pheasant. Each sub locality was further divided into calling sites of Cheer Pheasant. The maximum population density (17.31/Km^2) during the first year was recorded from Cheetah-I and minimum (5.33/Km^2) at Low Gali-II, however, during the second year the maximum (16.67/Km^2) population density was recorded at Shinger-II and minimum (5.33/Km^2) at Mirgran. A total of 38 calling sites were found in the study area with the total population of 249 birds. During the first year only 25 calling sites with the total population of 128 were recorded, while during the second year 13 new calling sites were explored with the increase of 121 more birds. The calling site density during the first year showed maximum value (2.88/km^2) at Cheetah-I and minimum (1.33/km^2) at Low Gali-I and Cheetah-II, while the maximum (n=3) calling sites at Cheetah-I and minimum (n=1) at Low Gali. During the second year Cheetah-I had the highest density

($2.77/km^2$) for calling sites, with maximum 3 calling sites and Low Gali-I had minimum density ($0.86/ Km^2$) with only one calling site. The phytosociological study at seven main localities showed that the *Pinus wallichiana,* among trees, *Plactranthus rugosus, Indigofera heterantha and Berberis lycium* among shrubs, while *Heteropogan contortus* and *Cynodon dactylon* among herb were common and characteristics of each habitat Correlation analysis between Cheer Pheasant population and importance values of plant species showed the negative correlation with trees ($r=-0.167$, $P>0.05$), significant correlation with shrubs ($r=0.569$, $P<0.05$) and herbs ($r=0.632$, $P<0.05$).

A SURVEY ON ICHTHYOFAUNA OF DEZ RIVER BASIN

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A research project was conducted from 2006 to 2007 to identify the ichthyfauna of Dez river basin. The study area covers the headwaters of Tireh, Gahar and Sezar rivers to the Dez river in Lorestan, Esphahan and Khoozestan provinces in the west and southwest of Iran. The main branch of Dez river has more than 520 km long. The monthly sampling were carried out in 30 stations. In this study different fish species were identified by electrofishing as the main method of sampling. Some of morphometric and meristic factors, domination and abundance of species were considered. Finally 24 species and sub-species from 15 Genera and 6 families were recognized. Most of species were endemic. The *cyprinidae* family with 15 species was more abundant. Also *Capoeta trutta* had the most abundance in the Dez river basin.

CITATIONS

RECIPIENT OF ZOOLOGIST OF THE YEAR AWARD 2008*

Prof. Dr. Muhammad Khan Lohar
Chairman, Department of Entomology, Sindh Agriculture University, Tandojam

He obtained his B.Sc. (Agri.) and later M.Sc. (Agri.) from University of Sindh, Jamshoro. He obtained Ph.D. (Entomology) from University of London, United Kingdom, and later proceeded to Okayama University, Japan for his postdoctoral work. Dr. Lohar has published 100 research papers in various national and international journals and 5 text books in Entomology. He has 10 research projects to his credit sponsored by various donor agencies. Besides that he has supervised more than 180 M.Sc., M.Phil and Ph.D. students who completed their projects in Entomology. Dr. Lohar received a Medal and Award by Matsumae International Foundation, Japan in 1986, a Gold Medal of best author by student Zone, Sindh Agriculture University, Tandojam for writing a Textbook on Applied Entomology in 1995, and Sachal Award 2003 by G.S. Multi-Media in 2004. In 2004, he also received Best University Teacher Award 2002 from Higher Education Commission, Islamabad.

Now, he is being awarded "Zoologist of the Year Award 2008" by the Zoological Society of Pakistan for his significant contribution in the field of Zoology.

*Other nominees for this award were Prof. Dr. M. Arshad Azmi, Prof. Dr. Naeem Khan, Mr. Z.B. Mirza.

RECIPIENT OF
PROF. A.R. SHAKOORI GOLD MEDAL 2008*

Dr. Farah Rauf Shakoori
Assistant Professor
Department of Zoology, Government College University, Lahore

Dr. Farah Rauf Shakoori, Assistant Professor in the Department of Zoology, Government College University, Lahore obtained her M.Sc. degree in Zoology for the University of the Punjab in 1990. Later she proceeded to USA take up reseach work for her Ph.D. degree in Molecular Biology from University of Massachutts Medical School under a collaborative arrangement with University of the Punjab, which she successfully completed in 1996.

Dr. Mrs. Shakoori has published 30 research papers on histone gene expression in heavy metal toxicity, bioinsecticides and bioremediation in most prestigious scientific journal of the world. Her research publications appeared in Nature, PNAS, JBC, Biochemistry, Journal of Cell Physiology, Journal of Cellular Biochemistry, World Journal of Microbiology and Biotechnology, Bulletin of Environmental Contamination and Toxicology, and Pakistan Journal of Zoology.

She has attended many national and international conferences. She is member of several scientific societies. She has supervised research work of several M.Sc. and M.Phil students. Presently she is supervising 3 Ph.D. students.

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*Other applicants for this award were Prof. Dr. Naeem Tariq Narejo, Mr. Khalid Abdullah, Dr. Amtul Jamil Sami.

RECIPIENT OF
PROF. DR. MIRZA AZHAR BEG GOLD MEDAL 2008*

Dr. Muhammad Mahmood-ul-Hassan
Chairman, Department of Wildlife and Ecosystem, University of Veterinary & Animal Sciences, Lahore.

He obtained his M.Sc. Degree in Zoology in 1995, and M.Phil and Ph.D. in 2004 from University of Agriculture, Faisalabad. Later he joined as lecturer in the Department of Wildlife and Ecosystem, University of Veterinary and Animal Sciences, Lahore. His field of interest is conservation biology, more specifically the behavioural and ecological responses of animals to change in their environment and the conservation implications of such changes.

*Other applicant for this award was Dr. Noor un Nisa.

RECIPIENT OF
PROF. DR. NASIMA TIRMIZI GOLD MEDAL 2008*

Prof. Dr. Syed Makhdoom Hussain
Fisheries Specialist, National Animal Plant Health Inspection Service, 28-Khalid Plaza, Block-B, Blue Area, Islamabad.

Prof. Dr. Syed Makhdoom Hussain obtained his B.Sc. degree in 1965 and Master degree in Zoology in 1967 by the University of Karachi. He did his M.Phil in 1972. He was appointed as Research Assistant in the Marine Biology Department, University of Karachi in 1973. Later when the Department was upgraded to Centre of Excellence in Marine Biology in 1983, he was promoted as Assistant Professor. He then proceeded to University of Bergen under a split programe to complete his Ph.D. work at Centre of Excellence in Marine Biology. He retired as Professor in June 2007. During his tenure in the University of Karachi, he visited Africa under the sponsorship of Pakistan Navy for conducting Oceanographic surveys. He obtained NORAD, Norwegian fellowship for about two years. He was awarded Postdoctoral fellowship by Matsumae Foundation to work at Fisheries University Kagoshima, Japan. He was awarded postdoctoral fellowship for one year by USAID programme to work at the Miami University, USA. As visiting scientist he visited London twice to work at the British Natural History Museum and Scotland University of Glasgow, U.K. He represented Pakistan on various international and national bodies, recent in March 2007 at Fisheries Observers Conference held in British Columbia, Victoria, Canada. Dr. Hussain has supervised research projects sponsored by PSF and UNESCO. He has also been a consultant of WWF and IUCN. He has supervised several M.Phil and Ph.D. students.

*Other applicant for this award was Dr. Noureen Aziz Qureshi, Mr. Abdul Hakeem Khan.

RECIPIENTS OF
GOLD MEDALS AWARDED BY THE ZOOLOGICAL SOCIETY OF PAKISTAN

1. **Muzaffar Ahmad Gold Medal 2008**
Fifteenth Muzaffar Ahmad Gold Medal 2008 was received by Ms. Ayesha Abdul Majeed for obtaining first position in the M.Sc. Zoology examination of the University of the Punjab.

Ayesha Abdul Majeed

2. **Ahmed Mohiuddin Memorial Gold Medal 2008**
Seventh Ahmed Mohiuddin Memorial Gold Medal 2008 was given to Mr. Waris Ali, who obtained first position in the M.Sc. Zoology examination of the University of Sindh, Jamshoro.

3. **The M.A.H. Qadri Memorial Gold Medal 2008**
Ninth **M.A.H. Qadri Memorial Gold Medal 2008** was given to Dr. Shamsul Islam for his Ph.D. degree in Zoology specializing in the field of Parasitology from University of Karachi.

4. **Muhammad Afzal Hussain Memorial Gold 2008**
Twelfth **Muhammad Afzal Hussain Memorial Gold 2008** was given to Ms. Amina Ismail for obtaining first position in Parasitology for her M.Sc. Zoology examination of the University of Karachi.

Amina Ismail

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ISOLATION OF PHENOL DEGRADING BACTERIA FROM INDUSTRIAL EFFLUENTS AND THEIR POTENTIAL USE IN WASTEWATER TREATMENT

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Abstract.- The present study is aimed at assessing the ability of *Bacillus megaterium* and *Micrococcus luteus* to degrade phenol into non-toxic form. Both *Bacillus megaterium* and *Micrococcus luteus* could tolerate phenol up to 300 µg/ml. *Bacillus megaterium* and *Micrococcus luteus* both showed optimum growth at pH 7 while the maximum growth of both bacteria was observed at 37ºC. Both bacterial isolates, *Bacillus megaterium* and *Micrococcus luteus*, showed high 2,3-dioxygenase activity of 505% and 523%, respectively. *Bacillus megaterium* and *Micrococcus luteus* could degrade 64% and 70% of phenol (100 µg/ml) from the medium after 12 h. The bacterial isolates can be exploited for bioremediation of phenol and phenol derivates containing wastes, since they seem to have the potential to degrade enzymatically the toxic phenol into non-toxic product form.

Keywords: Phenol degrading bacteria; *Bacillus megaterium*, *Micrococcus luteus*, bioremediation.

INTRODUCTION

Phenols and phenolic compounds are a major source of environmental pollutants. Natural phenolic compounds and their derivatives exist in nature as intermediates during biodegradation of natural polymers containing an aromatic ring such as naphthalene, anthracene, lignin, and others from aromatic amino acid precursors. Thus, the pollution related to these chemicals is associated with pulp mills, coal mines, refineries, wood preservation plants and various chemical industries. It is of utmost importance that the effluents are degraded to tolerant limits prior to being released into the environment (Van Schie and Young, 1998; Gianfreda *et al*., 2006).

Compared with physical and chemical methods, biological techniques are preferable because of economical advantages and low possibility of by products production. Screening potential microorganisms is a critical step in the

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construction of an effective remediation system. Several bacterial strains able to utilize phenol as their sole source of carbon and energy have been documented. These include species of *Arthrobacter* (Li *et al*., 2008), *Bacillus* (Kadiyala and Spain, 1998), *Burkholderia* (Bhushan *et al.*, 2000), *Ochrobactrum* (Qiu *et al*., 2007), *Pseudomonas* (Kulkarni and Chaudhari, 2007), *Rhodobacter* (Roldan *et al*., 1998), *Rhodococcus* (Shinozake *et al*., 2002) and *Strenotrophomonas* (Liu *et al*., 2007).

Phenol can be degraded through a variety of pathways. Phenol, under aerobic conditions, is usually hydroxylated to catechol and degraded via the *meta* or *ortho* pathway (Hiroyuki *et al.*, 1998). Studies conducted on these pathways dealing with the metabolism and catabolism of microorganisms adapted to ambient phenol concentration, showed that the microorganism can only resist phenol at a certain level. It is also reported that increasing phenol concentration appears to decrease overall phenol biodegradation (Van Schie and Young, 1998).

The present study deals with the isolation of phenol tolerant bacteria from a contaminated environment, their ability to degrade phenol into non-toxic product and optimization of temperature and pH for maximum phenol degradation.

MATERIALS AND METHODS

Sample collection

Wastewater samples were collected in screw capped sterilized bottles from Sheikhupura (Pakistan). Some physicochemical parameters of wastewater *viz.*, temperature, pH, dissolved oxygen and phenol were measured (APHA, 1989).

Isolation of phenol degrading bacteria

For isolation of phenol tolerating bacteria, 100 µl of the wastewater sample was spread on Luria-Bertani (LB) agar plates containing 100 µg of phenol/ml of the medium. LB agar plates were prepared by dissolving 1 g NaCl, 1 g tryptone and 0.5 g yeast extract in 100/ml distilled water, pH adjusted at 7 to 7.2 and then 1.5 g agar was added in the 250 ml flasks. The medium was autoclaved at 121ºC and 15 lbs/inch^2 pressure for 15 min. The growth of the bacterial colonies was observed after 24 hours of incubation at 37ºC. Effect of phenol on the growth of bacterial isolates was determined in Minimal Salt

Medium (MSM) which contained (mg/l): $(NH_4)_2$ SO_4, 500; $CaCl_2$, 14; $MgSO_4.7H_2O$, 120; $FeSO_4.7H_2O$, $ZnSO_4.7H_2O$ 5.0; $Na_2MoO.2H_2O$, 2.5, 5; $Na_2HPO_4.7H_2O$, 700, 0.5; KH_2PO_4, 400 (pH 7) with minor modifications and supplemented with phenol (Pesce and Wunderlin, 2004). It was again incubated at 37ºC for 24 hours. This process was repeated with successively higher concentrations of phenol until the minimum inhibitory concentration (MIC) of the bacterial isolate was obtained.

Physical and biochemical characterization

The isolates were Gram stained. For biochemical characterization the isolates were tested for catalase activity, motility, oxidase acivity, nitrate reduction, and hydrolysis of casein according to Benson (1994). Some specific tests were also performed for further characterization of the isolates up to species level such as sporulation test, acid formation test, utilization of different sugars, Voges-Proskauer test, hydrolysis of starch, and growth at 20ºC, 30ºC, 37ºC, 45ºC. The procedures of these biochemical tests were taken from Cappuccino and Sherman (2001).

Determination of optimum growth conditions

For optimum growth of the bacterial isolates, two parameters *i.e.*, temperature and pH were considered. For determination of optimum temperature, 5 ml LB broth was added in 4 sets, each of three test tubes, autoclaved and inoculated with 50 µl of freshly prepared culture of each bacterial isolate by overnight growth at 37ºC in LB broth. The four sets of tubes were incubated at 20ºC, 30ºC, 37ºC and 45ºC. After an incubation period of 12 h, their absorbance was measured at 600 nm using a LAMBDA 650 UV/Vis Spectrophotometer (PerkinElmer, USA). For determination of optimum pH, test tubes having 5 ml LB broth were prepared in 9 sets, each containing 3 test tubes and their pH was adjusted at 5.0, 6.0, 7.0, 8.0, 9.0 and 10 then autoclaved. These tubes were inoculated with 50 µl freshly prepared culture of each bacterial isolate. After an incubation period of 12 h, their absorbance was measured at 600 nm.

Effect of phenol on bacterial growth

Growth curves of bacterial isolates were determined in minimal salt medium with (100 µg of phenol/ml) and without phenol (control). For each bacterial isolate 50 ml medium was taken in one set consisting of 3 flasks, autoclaved and then inoculated with 50 µl of the freshly prepared inoculum. The

cultures were incubated at 37ºC in a shaker at 100 rpm. An aliquot of culture was taken out in an oven sterilized tube, at regular intervals of 0, 4, 8, 12, 16, 20, 24 and 28 h. Absorbance was measured at 600 nm. Growth was plotted graphically.

Crude cell extract

To prepare the crude cell free extract, the bacterial cultures were grown with phenol (100 µg/ml) in 200 ml minimal salt medium for 96 h at 37^{o}C. Cells were harvested by centrifugation at 9000 (3800 x g) for 15 min. Pellets were washed twice with 10 mM Tris HCl buffer (pH 7.2) and were suspended in 3 ml of the same buffer. Cells were disrupted by sonication for 5 min (Sonics VC 500 USA) in cold condition. The resultant homogenate was centrifuged at 8000 (3300 x g) for 30 min at 4^{o}C; the supernatant was used as a crude extract. The activity of catechol 2,3-dioxygenase (C23O) was determined by measuring catechol production in a cell suspension containing 30 µl of 30mM catechol, 300 µl of 40mM EDTA, 2.07 ml of PBS buffer (pH 7.0) and 600 µl of the crude enzyme extraction. The reaction was allowed to proceed for 30 min at 25^{o}C, and measured at 375 nm by the LAMBDA 650 UV/Vis Spectrophotometer (PerkinElmer, USA) (Liu *et al*., 2002; Hu *et al*., 2006). The crude extracts of both bacteria were also subjected to two different concentrations of phenol *i.e.*, 50 and 100 µg/ml for 12 h and crude extracts that were heated at 100^{o}C for 30 min acted as control. Phenol was assessed by spectrophotometrically (Rodenas-Torralba *et al*., 2005).

Statistical analysis

Observations were made and all the experiments run in triplicate. At least three separate flasks were usually maintained for one treatment. Each time three readings were taken, their mean, and standard error of the mean were calculated.

RESULTS AND DISCUSSION

Physicochemical characteristics of wastewater

Some physicochemical characteristics of industrial wastewater were ascertained, from where chromium tolerant bacteria were isolated. The temperature of different samples ranged between 17ºC to 36ºC, pH ranged between 5.5 and 8.6, dissolved oxygen between 0.43 ±0.03 and 1.25 ±0.01 mg/L and phenol ranging between 0.7 0±0.01 and 1.90 ±0.03 µg/ml.

Fig. 1. A site of industrial effluents (Sheikhupura), from where the wastewater samples were collected.

Isolation and identification of the bacterial isolates

A total of 12 cultures of bacteria were isolated, purified and screened for the degradation of phenol. Of all the cultures tested, 5 bacterial isolates were further screened for phenol degradation and finally 2 bacterial isolates were selected on the basis of phenol tolerance *i.e.,* 300 µg/ml. Table I shows physical and biochemical characteristics of the bacterial isolates. On the basis of these characteristics bacterial isolates have been identified as *Bacillus megaterium* and *Micrococcus luteus*. Qiu *et al.* (2009) reported that *Arthrobacter* sp. HY2 could tolerate *p*-nitrophenol up to 400 mg/l.

Optimum growth conditions

The most suitable temperature for phenol tolerating bacterial isolates was found to be 37^{o}C (Fig. 2). Both organisms also showed maximum growth at pH 7 (Fig. 3). The growth curve pattern was studied by growing the organism in the presence of phenol (100 µg/ml) and comparing with the control culture in which no phenol was added. The growth pattern of *B. megaterium* was significantly different from those of control and the lag phase delayed up to 12 h when compared with control. Similar growth pattern was also observed in *M. luteus i.e.,* 12 h delayed lag phase and the growth rate of *M. luteus* was also lower in the presence of phenol as compared to control where no phenol was added into the culture medium. The growth pattern is shown in Figure 4.

TABLE I.- MORPHOLOGICAL AND PHYSIOLOGICAL CHARACTERISTICS OF THE BACTERIAL ISOLATES.

Characteristics	*B. megaterium*	*M. luteus*
Gram stain	Positive	Positive
Motility	Positive	Positive
Sporulation	Positive	Negative
Behavior of oxygen	Aerobic	Aerobic
Casein hydrolysis	Positive	Positive
Starch hydrolysis	Positive	Positive
Reduction of nitrate to nitrite	Negative	Positive
Catalase test	Positive	Positive
Voges Proskauer test	Negative	Positive
Production of acid from		
Glucose	+	-
Mannitol	+	-
Xylose	+	-
Growth at		
20°C	+	+
30°C	+	+
37°C	+	+
45°C	+	+

(+) positive; (-) negative

Phenol degradation and crude enzyme assay

Both bacterial isolates showed very high catechol 2,3-dioxygenase activity. *B. megaterium* showed 505% activity of the enzyme while *M. luteus* showed 523% enzyme activity at a given standard assay. The cell free extracts of *B. megaterium* and *M. luteus* were exposed to two different concentrations of phenol *i.e.*, 50 and 100 μg phenol/ml and the degradation ability was assessed after 12 h of incubation at 37°C. *B. megaterium* showed phenol degradation of 68% (50 μg/ml) and 64% (100 μg/ml), respectively. Cell free extract of *M. luteus* illustrated degradation of 76% and 70% at concentrations of 50 and 100 μg phenol/ml, respectively (Table II). Phenol is widely distributed as a characteristic pollutant in effluents of many industrial processes due to its common use. Improper treatment of phenol and phenolic compounds may lead to contamination of soil and groundwater and their toxicity seriously affects living organisms even at low concentration (Kibret *et al.*, 2000). The efficient removal of these compounds is necessary and significant for environmental protection.

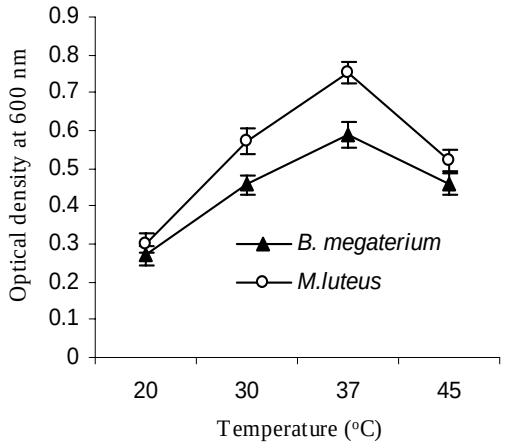

Fig. 2. Effect of temperature on the growth of bacterial isolates growing in LB medium.

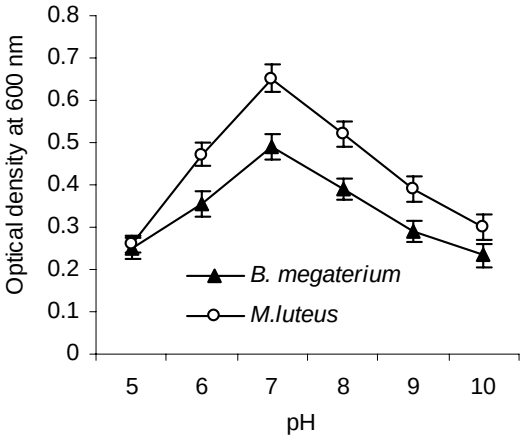

Fig. 3. Effect of pH on the growth of bacterial isolates growing in LB medium.

Phenol degradation by microorganisms may utilize many enzymes such as hydroxylase, monooxygenase and dioxygenase. The activity of catechol 2,3-dioxygenase for phenol degradation (C23O) was measured in both bacterial strains when the two bacterial isolates were grown with phenol as the sole carbon source. The results suggested that both bacterial isolates were able to degrade phenol and most of the enzymes could be induced by its degradable substrates.

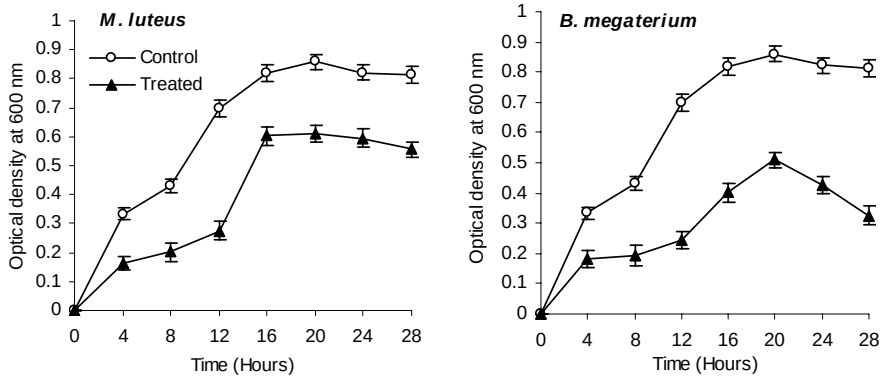

Fig. 4: Growth curves of phenol degrading *B. megaterium* and *M. luteus* in minimal salt medium containing phenol (100 µg/ml) and without phenol after incubation at 37°C.

TABLE II.- DEGRADATION OF PHENOL (%) AFTER INCUBATION WITH CRUDE CELL EXTRACTS AT DIFFERENT PHENOL CONCENTRATIONS.

Time (Hours)	at 50 (µg/ml) % degradation			at 100 (µg/ml) % degradation		
	0	**12**	**-**	**0**	**12**	**-**
Control	50	50	0	100	100	0
B. megaterium	50	16	68	100	36	64
M. luteus	50	12	76	100	30	70

CONCLUSIONS

Both bacterial isolates, *B. megaterium* and *M. luteus*, resisted phenol up to 300 mg/l and showed fairly high 2,3-dioxygenase activity of 505% and 523%, respectively. *B. megaterium* and *M. luteus* also showed excellent ability to degrade phenol to non-toxic form *i.e.,* 64% and 70% in the presence of 100 µg phenol/ml. The bacterial isolates can be exploited for bioremediation of phenol containing wastes, since it seems to have the potential to degrade the toxic phenol into non-toxic product form. Knowledge of biodegradation is important for the evaluation of the persistence of organic pollutants and the design of biodegradation facilities. Therefore, further detailed research is needed to quantify these substrates interactions in the degradation of phenol and its derivatives.

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RICE FIELDS IN RELATION TO MALARIA IN DISTRICT BANNU NWFP, PAKISTAN

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Abstract.- The relationship of malaria with rice fields was found in Bannu District (N.W.F.P) during June-November 2002. A total of 800 blood films were collected, out of which 56 were found positive, showing an average 7%. A total of 300 blood films (150 each from Ghoriwla is a rice-growing locality and Nurar where no rice can be grown because of lack of water) were collected, out of which 21 were found positive with an S.P.R. 7%. Among 21 positive slides 16 were recorded from Ghoriwala with an S.P.R. 10.66% and 5 from Nurar with an S.P.R. 3.33%. Prevalence of parasite was also analyzed in relation to age, sex and season. Males (7.18%) were found to be more infected than females (6.66%). The highest infection rate (13.33%) was found in age group of 5-10 years and lowest (4.62%) in age group of 46-50 years. The highest rate of infection was recorded in September (12%), during which rice crop were present, while the lowest infection (2%) was recorded in June. A general survey of the whole District Bannu (consisting of three sectors A, B and C) was also done from the month of July-September, during which rice crops were present. During this survey a total of 500 blood films were collected, out of which 35 were found positive with an S.P.R 7%. The highest infection (8%) was found in the rice growing area, whereas the lowest infection (5%) was noted in the non-rice growing area.

Keywords: Bannu, Ghoriwala, malaria, rice fields.

INTRODUCTION

Malaria is associated with odorous air of swamps and marshy places in the tropics. It has played an important role in the history of the world and progress of nations. It has been a major and global problem, so it is an outstanding disease in the list of WHO (Chandler and Read, 1960).

Among all the four species of malarial parasites, *Plasmodium vivax* and *P. falciparum* are most common in Indo-Pakistan Sub-Continent. The parasite is transmitted to man through female mosquito of the genus *Anopheles* (Anwar *et al.*, 1994). *P. vivax* causing benign tertian fever is the common and most widely

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distributed species, being prevalent both in tropical and temperate zones. *P. falciparum* causing malignant tertiary fever is also cosmopolitan but well adapted to tropics and subtropics. *P. malariae* causing quartan malaria has a wide distribution in warm countries, commonly found in tropical Africa, S. America, India and Malaysia and *P. ovale* causing benign tertian fever is endemic to tropical Africa (Shah and Ahmad, 1995).

In paddy fields where the water is stagnant, a very large number of eggs are laid, which develop into larvae (Katankhel, 1995). It has been noted that whilst rice irrigation schemes may provide excellent breeding sites for *An. gambiae* early in the growth cycle of the plants, this changes as the rice plants mature and form a dense canopy over the water (Ceccato *et al.,* 2005).

An. minimus larvae may also occur in rock pools, ground pools next to streams, seepage pools and rice-field terraces (Garros *et al.,* 2006). *An. maculipennis*, the historic vector of malaria in Europe and the Middle East was the first sibling species complex to be discovered among mosquitoes (Djadid *et al.,* 2007).

Mosquitoes are the most widely studied aquatic insects associated with rice fields, as this ecosystem constitutes the favored breeding sites of several species. Lacey and Lacey (1990) have given a comprehensive review of the mosquitoes in rice fields, covering aspects of their ecology, medical importance and control, and listed 137 species of mosquitoes that breed in rice fields worldwide. Abu Hassan *et al.* (1998) found 29 mosquito species biting human and bovid hosts, and 11 species breeding in rice fields in the Muda area of Malaysia. They provide details of the prevalent species breeding in different habitats within the rice ecosystem, as well as within rice fields during different stages of the rice cultivation cycle. Amerasinghe (1993) reported 26 species of mosquitoes from rice fields of the dry zone in the Eastern Province of Sri Lanka, while Bambaradeniya (2000) recorded 14 species from a rice field in the intermediate zone. Takagi *et al*. (1995, 1996) have studied the effect of rice plant canopy on the density of mosquito larvae and other insects in rice fields of Japan.

MATERIALS AND METHODS

A total of 800 blood films were collected of the people from the age between 5-50 years during the month of June up to November in 68 different localities of Bannu district. 515 blood samples were collected from males and

285 blood samples from females. For malaria control programme and surveillance, the Bannu district is divided into 3 main sectors, A, B and C. These sectors are further sub divided into 22 sub sectors. The Sector "A" includes 7 sub sectors *i.e.*, a1-a7 containing 59 index localities of which 3 sub sectors, a-1, a-2 and a-3 comprise the F.R. area having 19 localities. The sector "B" also includes 7-sub sectors *i.e.*; bl-b7 comprising 65 localities. Similarly the "C" sector includes 8-sub sectors, cl-c8 having 94 index localities; similarly each of the localities has been divided into several villages.

The blood samples collected from different sectors are as follows:

Sector	Sub-Sector	Name of localities	No. of samples collected
A	a-1	Gimbatiae and Darioba	13
	a-2	Harrsings and Markae Kila	11
	a-3	Telgi kila, and Ali Sho Baba	14
	a-4	Mohammad Khel,Dawood Shah,Haji Ghulam and Tapi Kila	17
	a-5	Baka Khel and Mamash Khel	08
	a-6	Jadid Abadi, Sokarri and Hingel Noor Baz	19
	a-7	Sikander Khel, Kachozi and Ismail Honi Khel	18
	Total	**19**	**100**
B	b-1	Domel, Spintangi and Duva Manza	15
	b-2	Sadra Van, Khan Bahadar Kila and Emerzai	21
	b-3	Kotka Moh. Khan, Azim Kila and Painda Kila	16
	b-4	Dirma Khel, Zaker Khel, Kotkaferroz and Boza Khel	20
	b-5	Jando Khel, Chack Dadan and Manduri	27
	b-6	Bazar Ahmad Khan, Shere Ahmad Kila and Fatma Khel	29
	b-7	Kala Khel, Mir Azam, Badar Khel and Mita Khel	22
	Total	**22**	**150**
C	c-1	Bada Mir Abbas Khan, Sir Mast Mera Khel, Ismai Khel and Musa Khel	32
	c-2	Barakzai and Mandew	27
	c-3	Haved, Sardi Khel and Landi dak	42
	c-4	Bharat, Hathi Khel and Tughel Khel, Kakki, Dildar Kila and Shere Oil Kila	29
	c-5	Sardar Abad, Ismail Khani and Bada Khel	31
	c-6	Landi Jalander, Sahid Zada and Mama Khel	40
	c-7	Ghoriwala Therkhewala and Degan	30
	c-8	Taji Kila and Dad Kila	19
	Total	**27**	**250**

Ghoriwala and Nurar have different local environmental conditions were surveyed for the prevalence of malaria. Ghoriwala is a rice-growing locality while in Nurar rice cannot be grown due to lack of water. A total of 300 blood samples were collected (150 from either locality) (Table I).

TABLE I.- PREVALENCE OF MALARIA IN GHORIWALA AND NURAR (BANNU) DURING JUNE-NOVEMBER, 2002.

Locality	**Total no. of samples**	***P. vivax***	***P. falciparum***	**Total no. of positive cases**	**S.P.R. (%)**
Ghoriwala	150	14	2	16	10.66
Nurar	150	05	0	5	03.33
Total	300	19	2	21	7

From the Table I it is clear that: The prevalence of infection was higher in Ghoriwala (rice growing locality) than in Nurar (non rice growing locality).

Both thick and thin blood smears were obtained on the same slide. The fingertip of the volunteer was cleaned with methylated spirit and then pricked with a sterilized lancet. A drop of blood was placed on one side of the slide and a thick smear was made with the help of the corner of another slide. Another drop was taken in the middle of the slide and the edge of the second slide was rubbed against it towards the opposite end. The slides were then left to dry, followed by fixation of thin smear with methyl alcohol and labeling. The collected slides were kept in Giemsa's Stain for 20-30 minutes. These were then cleaned with tap water, dried and screened under I00x oil immersion power of microscope for the detection of any *Plasmodium.*

All the volunteers participating in the study were healthy non-symptomatic individuals. A proforma of data report was also filled during the fieldwork in which information about the volunteer was recorded. This information included, sample number, Date, Name of volunteer, Sex, Age, Locality, Symptom of disease, Drugs taken, Rice fields Present or not, the presence or absence of stagnant water etc.

RESULTS

Out of 800 blood films 56 were found positive showing an average S.P.R. of 7%. Screening of all these blood films showed that *P.vivax* was more common

than *P. falciparum* and mixed infection of both species was not found. Whole of the work was completed in two attempts. In first attempt two different localities, Ghoriwala and Nurar with different local environmental conditions were surveyed for the prevalence of malaria. Ghoriwala is a rice-growing locality while Nurar is a non rice-growing locality. During this survey a total of 300 blood films were collected (150 from either locality), out of which 21 were found positive with an S.P.R. of 7%. From Ghoriwala, 16 were found positive indicating an S.P.R. of 10.66%. From Nurar 5 were found positive with an S.P.R. of 3.33%. (Table I, Fig. 1).

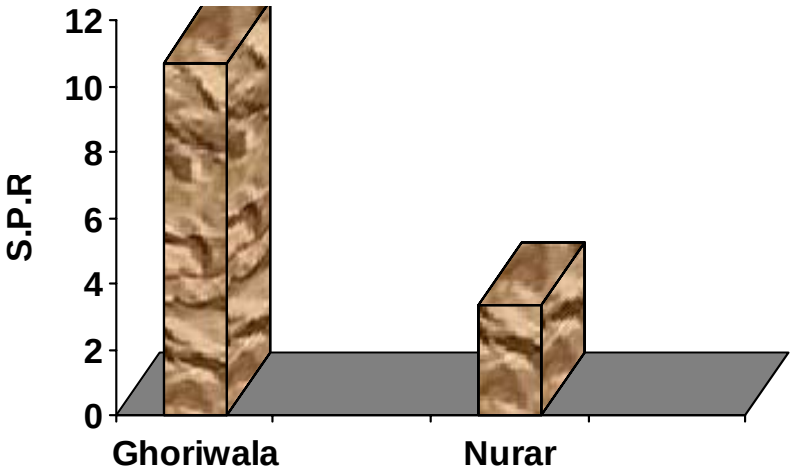

Fig. 1. Comparative prevalence of malaria in Ghoriwala and Nurar.

Age and sex wise prevalence of malaria

The age wise prevalence was found highest (13.33%) in 5-10 years age group, wile the lowest prevalence (4.62%) was found in the age group 46-50 years. Out of 800 blood slides, 515 blood films were collected from males having total 37 (7.18%) *Plasmodium* positive with 33 (6.41%) *P. vivax*, and 04 (0.78%) *P. falciparum*, whereas the remaining 285 blood samples were collected from females having total 19 (6.66%) *Plasmodium* positive with 17 (5.96%) *P. vivax* and 02 (0.70%) *P. falciparum*, showing that male infection rate was higher as compared to females (Table II).

TABLE II.- AGE AND SEXWISE PREVALENCE OF MALARIA IN BANNU DISTRICT.

S.#	Age group (Years)	Males			Females			Total		SPR %age		Grand total	
		No. examined	*P.v*	*P.f*	No. examined	*P.v*	*P.f*	*P.v*	*P.f*	% of *P.v*	% of *P.f*	Total No. examined	Grand SPR %
1	5-10	45	5	1	30	3	1	8	2	10.67	2.67	75	13.33
2	11-15	55	3	1	25	2	0	5	1	6.25	1.25	80	7.5
3	16-20	70	4	0	15	3	0	7	0	8.24	0.00	85	8.24
4	21-25	50	3	0	45	2	0	5	0	5.26	0.00	95	5.26
5	26-30	57	5	1	55	2	1	7	2	6.25	1.79	112	8.04
6	31-35	53	4	0	45	2	0	6	0	6.12	0.00	98	6.12
7	36-40	60	4	0	30	1	0	5	0	5.55	0.00	90	5.55
8	41-45	73	3	1	27	1	0	4	1	4.00	1.00	100	5.00
9	46-50	52	2	0	13	1	0	3	0	4.62	0.00	65	4.62
	Total	515	33	4	285	17	2	50	6	6.25	0.75	800	7

- Total No. of Males Examined = 515, SPR = 7.18
- Total No. of Females Examined = 285, SPR = 6.66

P.v., P. vivax; *P.f., P. falciparum.*

Prevalence of malaria in sector A, B and C

In 2nd attempt a general survey was made, during which 500 blood samples were collected throughout the Bannu district during the months of July-September. After their careful examination, 35 of the total slides were found positive showing an average S.P.R. of 7%. Blood samples out of 500 were collected from sector A, among which 5 were found positive indicating an S.P.R. of 5%. 150 of the 500 blood samples were collected from sector "B", among which 10 were found positive with an S.P.R. of 6.67%. 250 of the 500 blood sample were collected from sector "C", among which 20 were found positive with an S.P.R. of 8% (Table III, Fig. 2). These results show that there is a close relationship between malaria and rice fields. The sector wise prevalence showed that it was highest (8%) in sector "C" and the lowest prevalence (5%) was found in sector "A". In sector "B", the prevalence was 6.67%.

Out of 56 *Plasmodium* positive blood films, 50 were infected with *P. vivax* showing an overall prevalence of 6.25% and 6 with *P. falciparum* showing an overall prevalence of 0.75%.

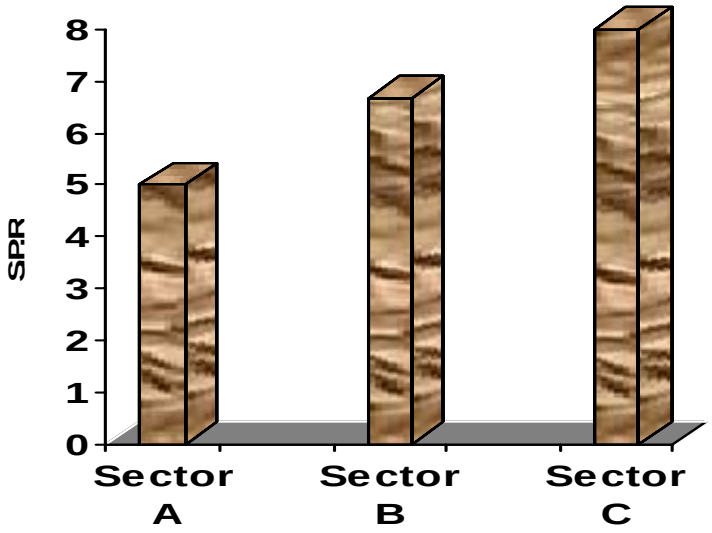

Fig. 2. Comparative prevalence of malaria in the 3 sectors of Bannu district

TABLE III.- OCCURRENCE OF MALARIA IN THE 3 SECTORS OF BANNU DISTRICT DURING JULY- SEPTEMBER-2002.

Sector	**Total no. of samples collected**	***P. vivax***	***P. falciparum***	**Total no. of samples found positive**	**S.P.R. (%)**
A	100	4	1	5	5
B	150	9	1	10	6.67
C	250	18	2	20	8
Total	500	31	4	35	7

From the table-III it is clear that: The rate of infection is higher in sector C (rice growing area) than in sector A (non rice growing area). In sector B (less rice growing area) the rate of infection is moderate.

DISCUSSION

Malaria being one of the world's significant life threatening diseases is still widely prevalent, particularly in the tropical, developing and under developed countries all over the globe. Its endemic reports today suggest wide range of morbidity and mortality among rural population as well as in those living in urban slums, where poor personal hygiene, breakdown in sanitation and socio-economic standards, tremendously contribute for the spread of the disease.

The variation in climatic conditions has profound effect on lifecycle of mosquito and development of malarial parasites. Keeping in view the environmental factors of malaria, the rice fields were studied for their relationship and effect on the prevalence of malaria in the District of Bannu. After scientific observations it was found that these rice fields have some relationship with malaria. The prevalence of infection was higher in rice growing areas than in non-rice growing areas. In rice fields huge masses of stagnant water are present. The standing water provides breeding ground for mosquitoes. The female *Anopheline* lay eggs on the surface of stagnant water. These eggs then develop into larvae. These rice fields fulfill the nutritional requirements of these larvae.

The rate of infection is low in non-rice growing areas. The scientific reason for this statement is clear. In non-rice growing areas stagnant water are scarce or absent. There is no or little breeding site for the *Anopheline* mosquitoes, which are the actual transmitters of *Plasmodium.*

The annual survey report of malaria department of Bannu district from 1975 to 2001 show the following malaria prevalence rates 1975 (7.02%), 1976 (4.38%), 1977 (0.41%), 1978 (0.09%), 1979 (0.41%), 1980 (0.94%), 1981 (1.63%), 1982 (1.74%), 1983 (1.65%), 1984 (2.05%), 1985 (1.02%), 1986 (1.08%), 1987 (1.09%), 1988 (1.16%), 1989 (5.37%), 1990 (8.64%), 1991 (7.96%), 1992 (11.24%), 1993 (13.44%), 1994 (18.97%), 1995 (23.59%), 1996 (11.04%), 1997 (5.86%), 1998 (6.67%), 1999 (6.90%), 2000 (7.80%) and 2001 (4.4%). These reports show that the prevalence was highest in 1995 (23.59%) and lowest (0.41%) in 1979. The present study showed that the incidence of malaria was 7% which is in between the annual report of malaria department of Bannu district in 1999 and 2000.

In 5-10 years age group the prevalence of malaria was highest (13.33%) and the lowest prevalence (4.62%) was found in age group 46-50 years. Illiteracy about the children health, poverty, unhygienic condition and sensitive to mosquito bites may be the few reasons responsible for high prevalence of malaria in this age group.

During the current study, the male infection rate (7.18%) was found to be high than the female infection rate (6.66%). This high prevalence may be due to the exposure of males to the environment than the females and may be due to more blood films from the males than the females.

Infection of malaria was higher (12%) in September and lower (2%) in June. This high incidence is due to the rice fields and favorable environmental conditions for mosquito breeding. The prevalence of malaria was found to be highest (8%) in sector "C" (the rice growing) area, and lowest (5%) in sector "A" (the non-rice growing area).

This high incidence may be due to the inadequate health-care facilities, unawareness about good living hygienic conditions and poor sanitary conditions. The sectors "C" is the populous and over crowded area of Bannu. The banks of River Kurram have attracted very large number of people due to fertile soils, water and other natural and artificial resources. This higher settlement of people resulted in the over crowding of the region, which is another cause of higher malaria rate in this sector.

The prevalence of malaria observed during the present survey (7%) is higher than the prevalence recorded (4.28%) in general population of Bannu district (Jan *et al.,* 2000), but lower than the prevalence rates recorded (9.84) in

selected schools of Peshawar, and also in febrile patients in Peshawar. The sex-wise prevalence of malaria observed in the District Swat (Katankhel, 1995) was very high (15.6%) as compared to the rate found in the present work. The prevalence of malaria in Afghan refugees' school going children of Mardan district (Jan and Kaleem, 1993) was slightly higher (7.91%) than that found in the present work (7%). The prevalence of malaria in refugees of Kashmir settled in Muzafferabad, Azad Kashmir (Inayat, 1998) was (7%) which is equal that detected in the present work. The parasite prevalence survey conducted by Idrees and Jan in District Dir (2001) examined the possibility that the cattle kept in house courtyard might protect occupants against malaria through Zooprophylaxis.

CONCLUSIONS

It can be concluded that along with dilapidated drainage system, less information about the disease, poor hygienic condition and domestic cattle etc., stagnant water, rice fields and age structure also play a major role in spreading of malaria disease and have close relationship with the prevalence of malaria.

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CHILOTHERIUM INTERMEDIUM FROM THE DHOK PATHAN FORMATION OF THE SIWALIKS

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Abstract.- The recovered material from the Dhok Pathan Formation of the Middle Siwalks of Pakistan are attributed to *Chilotherium intermedium.* The new remains include premolars and molars of the species. The premolars have a constricted protocone which is the characteristic of genus *Chilotherium.* In The upper molar the ectoloph is flat and broad with a strong parastyle and the protocone is much less constricted off from the protooph. In the lower dentition all the characters are observed like, V-shaped trigonid, absence of lingual and labial cingulum, the hypolophid reclines backward and the entoconid have a flat lingual margin. All the characters are observed in the studied lower dentition which clearly identify the specimens belong to genus *Chilotherium* and species *Chilotherium intermedium.*

Key words: *Chilotherium,* middle siwalks, Dhok Pathan, molars, premolars.

INTRODUCTION

Matthew (1929) revised *Rhinoceros sivalensis* var. *intermedius* described by Lydekker (1884) from the Siwalik into *C. intermedium*, and Heissig (1972) placed *C. intermedium* in the new subgenus *Subchilotherium.* Heissig (1989) raised the subgenus *Subchilotherium* to the genus rank, so the species became *S. intermedium*. The mandibular symphysis of *Subchilotherium* is narrow, and different from the widely expanded one of *Chilotherium.* According to Deng (2006), there is not any real species of *Chilotherium* in the Siwalik faunas.

Matthew (1929) first suggested that this species assigned by Lydekker to the genus *Rhinoceros* and by Pilgrim to the genus *Aceratherium,* should be

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properly classified in the genus *Chilotherium*. Matthew's opinion is followed in this present work. Although *Chilotherium intermedium* is typically of Lower Siwalik age, there are several specimens from the Middle Siwaliks in the American Museum collection that would seem to be referable to this species. The differences between these specimens and the typical *C. intermedium* do not seem to be enough to warrant their separation as a distinct form, so they are included within the species under discussion, and this species is thereby considered as ranging through the Chinji and the Middle Siwalik beds. The mandibles under consideration are quite similar to the mandible of *Chilotherium anderssoni*; an indication of the complete reduction of the incisors in this genus.

The two species supposedly representing the genus *Chilotherium* in the Siwalik deposits are very similar to each other, a fact recognized by Dr. Matthew in 1929. This [*i.e*., *Chilotherium intermedium*] is close to *C. blanfordi* Lydekker of the Bugti Hills, differs chiefly in more prominent antero-external pillar and protocone less constricted. According to him it is doubtful if the two species are really separable. *C. intermedium* is much more advanced in the evolution of the premolars, in the lesser development of the cingulum and in the development of a much larger crochet on the molars, and appears therefore to be a separate species.

The described specimens were collected from the Dhok Pathan Formation of the Middle Siwaliks. The specimens were abbreviated PUPC (Punjab University Palaeontological Collection) and housed in the Palaeontology laboratory of the Zoology Department, Punjab University, Lahore, Punjab, Pakistan. The specimens are catalogued by year and serial catalogue number for that year, *e.g*., 2007/11. Measurements were taken in millimeters using vernier calipers. Tooth cusp nomenclature follows that of Heissig (1969). Tooth length and breadth were measured at occlusal level. Paired measurements given for teeth are occlusal length and occlusal width.

SYSTEMATIC PALAEONTOLOGY

Family Rhinocerotidae Owen, 1845

Subfamily Rhinocerotinae Owen, 1845

Tribe Chilotheriini Qiu *et al*., 1987

Genus *CHILOTHERIUM* Ringstrom, 1924

Chilotherium sp.
(Figs. 1-2; Table I)

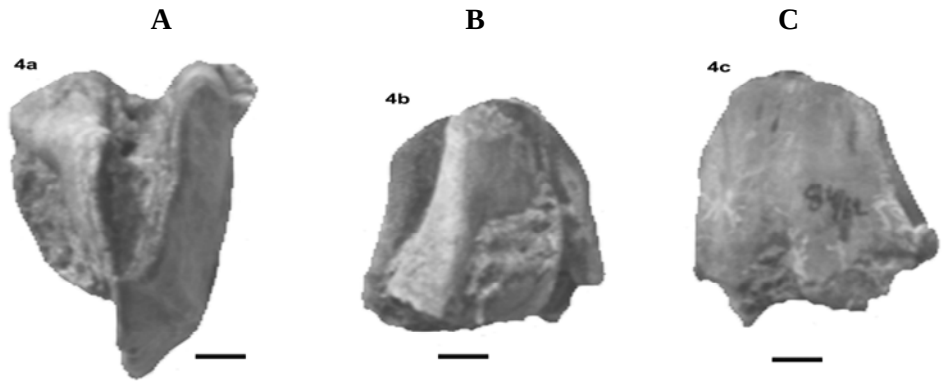

Fig. 1. *C. intermedium*, PUPC 84/62 – LM3: A, occlusal view; B, lingual view; C, buccal view. Scale bar 10 mm.

Type species

Chilotherium anderssoni Ringstrom.

Type specimen

GSI C34, a second right upper molar.

Diagnosis

A chilotherium of medium size. Upper incisor absent; cheek teeth hypsodont; parastyle fold indistinct or lacking; protocone constricted, ectoloph greatly elongated, mandibular symphysis transversely expanded. Lower incisor directed up and downwardly, slight constriction of protocone. The trigonid is angularly V-shaped. On the lower molars the lingual and labial ungula are absent, the hypolophid reclines backward and the entoconid have a flat lingual margin.

Distribution

Lower to Middle Siwaliks.

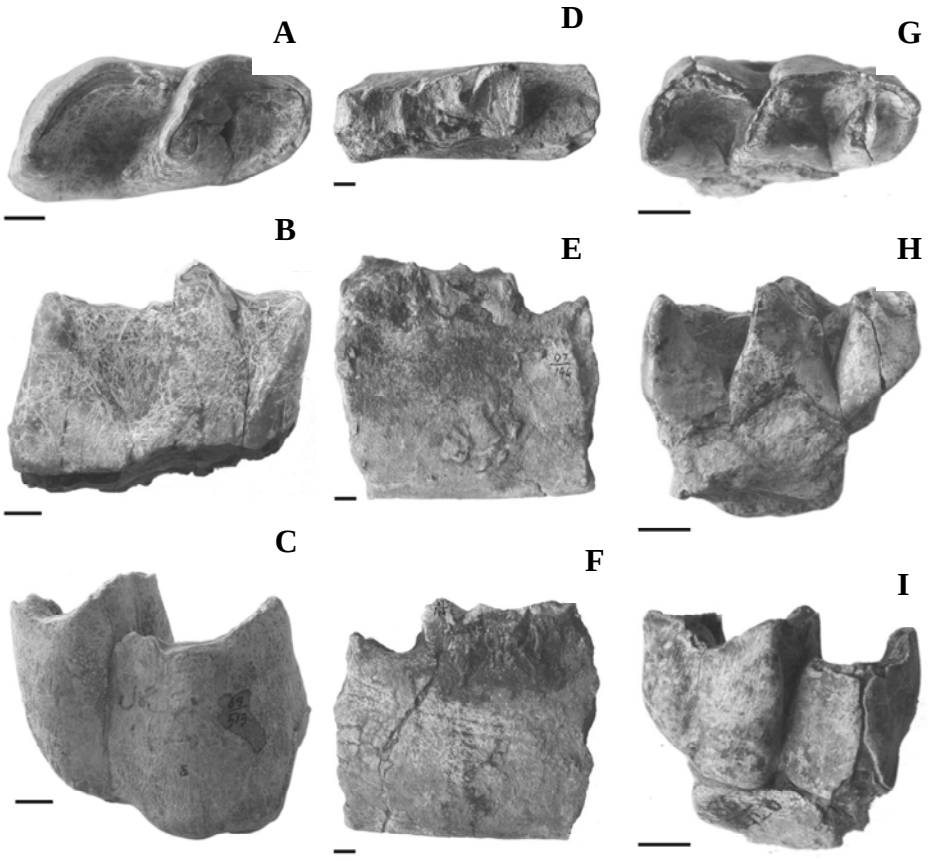

Fig. 2. *C. intermedium*, A-C, PUPC 69/513 – Lm3; D-F, PUPC 07/144 – lm2-3; G-I, PUPC 86/220 – Lm3; A, D, G, Occlusal view; B, E, H, lingual view; C, F, I, buccal view. Scale bar 10 mm.

Material

PUPC 84/62 – LM3 (left upper third molar), PUPC 87/113 – Lm3 (a left mandibular ramus with last molar), PUPC 07/144 – L m2-3 (a left mandibular ramus with molars), PUPC 07/145 – Rp4-m3 (right mandibular ramus), PUPC 86/220 – A piece of jaw bone with Lp4 (left lower fourth premolar), PUPC 86/305 – Lm3 (a left mandibular ramus with damage last molar), PUPC 69/513 – Lm3 (an isolated left lower last molar).

Locality

Dhok Pathan Formation (Middle Siwaliks).

Description

PUPC 84/62 is well preserved and triangular (Fig. 1A-C). Ectoloph and Metaloph, forming the ectometaloph structure, the characteristic feature of rhinoceros third molars are prominent. The labial wall of its ectometaloph is convex; its protocone is not constricted, and is expanded gradually towards to the base; its crochet is narrow and long, but the antecrochet is absent; its median valley is wide; its anterior cingulum is well developed and forms a shelf-like wall, but the posterior one is weak and low; its lingual cingulum is absent.

PUPC 87/113 is a mandibular fragment having a last molar complete. An anterior part of the ascending ramus is also preserved. The tooth is in early stage of wear. The tooth is two rooted; the roots are long, thick and turned posteriorly. The enamel is thick and wrinkled vertically. The occlusal outline is rectangular, longer than broader. No trace of the cement is preserved. There is neither lingual nor labial cingulum, but posteriorly the ectolophid groove is marked on the top to the base of the crown. The hypolophid is oblique but transverse in occlusal view. Trigonid is forming V-shaped structure. Anterior and posterior valleys are of different sizes. Depression is found only on the anterior side. Ectoloph have flat lingual margin. Paralophid is somehow damaged.

PUPC 07/144 (Fig. 2D-F) is preserved with the anterior part of the ascending ramus with broken dentary. Second Molar (m2) is damaged but preserved enough to show the morphological characters. Being much worn, many details have vanished due to wear. The occlusal outline is rectangular, longer than broader. Cement is moderately present. There is neither lingual nor labial cingulum. The worn paralophid is present but if it is crushed anteriorly. The hypolophid is oblique. Both the anterior and the posterior valleys are damaged. But posterior valley form U-shaped structure. The molar has a complete trigonid and the metalophid is oblique in appearance. The hypolophid, metalophid and paralophid are well developed but due to late wear too difficult to identify. The hypolophid is longer than the other ones. The metalophid and hypolophid are oblique and the paralophid is short. The lingual side of the molar is broken while the buccal or labial side is well preserved so roots can be clearly seen which are emerged inside the dentary. Labial groove is well developed. The third molar (m3) is broken, late stage of wear and has thick enamel.

PUPC 07/145 (mandibular ramus) is well preserved but the teeth are in late wear condition so all the teeth just above the root. The molars also have been worn to be close to the root. As a result, m3 becomes a quadrangle. The premolar (p4) is broken, only hypoconid and entoconid are located between them. Hypolophid is also present. The first molar is in the late stage of wear. Enamel is thick. The anterior and posterior valleys are vanished due to late wear. There is neither lingual nor labial cingulum. Lingual groove is prominent. All the lophids become united due to late wear. The second molar (m2) is preserved enough to study the basic characters. Enamel is very thick and covered the whole tooth. The second molar is birooted and possess labial groove. Anterior and posterior valleys are not clear due to late wear of the teeth. Ectolophid grooves are present but not well developed. The third molar is preserved enough to show some morphological characters. The third molar is in the late stage of wear. Trefoil shaped, talonid is present on the posterior side of the last molar and just touching the surface of the roots due to late wear. The thick enamel is partially present toward the anterior side and absent on posterior side. The last molar is longer than wide. Some posterior part of the dentary is also preserved confirms it last lower molar.

PUPC 86/220 a piece of jaw bone with Lp4 (Fig. 2G-I), is longer than wide which allows it to identify as premolar, the premolar has a posterior and anterior contact facets. The specimen is well preserved with the rooted portion of the tooth. The premolar is in the middle stage of wear and has thin enamel. Ectolophid is broken and the vertical cracks are present all over the crown, the protoconid is somewhat constricted whereas the hypoconid is prominent bulbus. Protoconid is damaged from the buccal side. The anterior valley is V-shaped and the posterior valley is U-shaped. Labial groove is prominent. Anterior and posterior facets possess depressions. Cement is markably present and dentine is not abundant. The lingual cingulum is absent, and the V-shaped labial groove is wide and shallow. The talonid is much better developed than the trigonid.

PUPC 86/305 is a left mandibular ramus with damage molar. The molar is not fully preserved; the molar is in the middle stage of wear. Thick enamel surrounds the tooth and broken from lingual side of the molar. The anterior valley is complete but the posterior valley is partially broken. The trigonid is angularly V-shaped with the narrow and short paralophid and a right angled metalophid with a slightly constricted metaconid. The root of the molar is preserved in the mandibular ramus. The paralophid is short and anterior end of it extends lingually along the anterior crescentic valley. The metalophid is

obliquely transverse with the constricted metaconid, the hypolophid and entoconid are missing in the molar. The anterior and posterior conids are uniting in this stage.

TABLE I.- *CHILOTHERIUM INTERMEDIUM*, THE COMPARATIVE MEASUREMENTS OF THE STUDIED CHEEK TEETH.

Specimens	**Position**		***Chilotherium cf. intermedium***	***C. primigenius***	***C. wimani***
PUPC 87/113	Lm3	L	45	37.5	46.9
		W	26	25	25.9
		H	34	-	-
PUPC 07/114	Lm2	L	53.7	32	46.4
		W	27	29	28.4
		H	10.7	-	-
		L	-	37.5	46.9
		W	20	25	25.9
		H	8.6	-	-
PUPC 07/145	Rp4	L	-	28.5	35.7
		W	-	24	25.8
		H	-	-	-
	Rm1	L	30.8	25.5	39.6
		W	30	25	27.7
		H	8	-	-
	Rm2	L	41	32	46.4
		W	35.3	29	28.4
		H	14.4	-	-
	Rm3	L	67	37.5	46.9
		W	37.3	25	25.9
		H	11.4	-	-
PUPC 86/220	Lp4	L	50	28.5	35.7
		W	26.7	24	25.8
		H	36.1	-	-
PUPC 86/305	Lm3	L	39.1	37.5	46.9
		W	19.2	25	25.9
		H	30.7	-	-
PUPC 69/513	Lm3	L	78	37.5	46.9
		W	39	25	25.9
		H	64	-	-
PUPC 84/63	LM3	L	33	36.5	48.7
		W	27.1	45	56.8
		H	38	-	-

PUPC 69/513 Lm3 (Fig. 2 A-C) is an isolated left lower last molar. The last molar is well preserved. It is in the middle stage of wear. Thick enamel is present. Both the anterior and the posterior valleys are located, and united. The trigonid is angularly V-shaped with the narrow and short paralophid and a right angled metalophid with a slightly constricted metaconid. There is neither lingual nor labial cingulum but posteriorly the ectolophid groove is marked to the base of the crown. The molar is anteriorly suppressed but posteriorly not suppressed which shows it is the last molar.

DISCUSSION

The material includes one upper third molar and rest of all the lower dentition collected from the Dhok Pathan Formation in the Middle Siwaliks of Pakistan. The premolars have a constricted protocone which is the characteristic of genus *Chilotherium*. The upper premolar are in late wear and many morphological features are not observed in this stage. However the presence of crochet and antecrochet, the constriction of protocone and bulbus hypocone allow us to identify the genus *Chilotherium*. In the upper molars the ectoloph is flat and broad with a strong parastyle and the protocone is much less constricted off from the protoloph (Colbert, 1935). In the lower dentition all the characters are observed like, V-shaped trigonid, absence of lingual and labial cingulum, the hypolophid reclines backward and the entoconid have a flat lingual margin. All the characters are observed in the studied lower dentition which clearly identify the specimens belong to genus *Chilotherium* and species *Chilotherium intermedium.* The specimens morphologically and meterically resemble to the species *Chilotherium intermedium* and the studied material is assigned to *Chilotherium intermedium*. Materically the measurements are overlapping by the already studied specimens (Table I).

CONCLUSION

The material described here represents a continuation of collections from the Middle Siwalik Formation of Pakistan. *Chilotherium intermedium* is an important faunal element with assemblages of bovids and proboscideans in the Dhok Pathan sediments (Khan *et al*., 2008; Khan, 2008; Akhtar, 1992). The faunal complexion indicates dense forests to grass lands suggesting mixed model fauna of the Dhok Pathan Formantion which represents hypsodont and brachydont elements. These assemblages represent different ecological environment of the Dhok Pathan Formation.

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PLAGIORHYNCHUS KARACHIENSIS, NEW SPECIES (ACANTHOCEPHALA: PLAGIORHYNCHIDAE) FROM CROW (*CORVUS SPELNDENS* LINN.) OF KARACHI, PAKISTAN

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Abstract.- Examination of six crows (*Corvus splendens* Linnaeus) from Karachi, Sindh, Pakistan revealed two Acanthocephalans from the small intestine of a single bird. Morphometric studies specify that the Acanthocephala are new to science and named as *Plagiorhynchus karachiensis*. The new species differ from its congeners in size of body, arrangement of hooks and egg size. This is the first record of genus *Plagiorhynchus* Lühe, 1911 from Pakistan.

Key words: Acanthocephala; *Plagiorhynchus karachiensis* new species, *Corvus splendens*.

INTRODUCTION

Lühe, 1911 erected the genus *Plagiorhynchus* with *P.* (*P.*) *crassiocolle* (Villot, 1875) as its type species. During study of acanthocephala from birds, two female specimens of the genus *Plagiorhynchus* Lühe, 1911 were collected by the senior author which are being described in detail.

MATERIALS AND METHODS

Crows (*Corvus splendens* Linn.) were collected from Karachi, Sindh, brought to the laboratory and immediately examined for acanthocephalan parasites. The Acanthocephala collected were fixed in F.A.A. solution under slight cover glass pressure, stand with Mayer Carmalum, dehydrated in graded series of alcohol, cleared in clove oil, and Xylol. Specimens were finally mounted permanently in Canada Balsam for detail study. Diagrams were made with the help of camera Lucida. Measurements are given in millimeter. Photomicrographs were taken using an automatic photographic camera mounted on a research microscope Nikon Optiphot-2 in the Department of Zoology, University of Karachi.

Genus *Plagiorhynchus* Lühe, 1911
Plagiorhynchus karachiensis, new species

Host:	*Corvus splendens* Linn.
Location:	Small intestine
Locality:	Karachi, Sindh, Pakistan
No. of specimens	2 from a single host recovered:

Description

Female

Body cylindrical, robust measuring 20.1-20.4 by 3.20-3.24 mm. Lacunar system well developed. Proboscis cylindrical measuring 0.68 by 0.28 with hooks in 10 to 12 rows having 6 to 12 hooks. The hooks are in straight longitudinal rows, with larger hooks in the middle of the proboscis. The larger hooks measure 0.0416-0.0418 by 0.0150-0.0152, while the smaller measure 0.0266 by 0.0098. Proboscis receptacle double walled inserted at posterior end of proboscis measuring 0.70-0.72 by 0.07-0.08. Lemnisci long and slender, subequal extending past proboscis receptacle, the left measuring 2.00 by 0.34 and the right 2.10-2.14 by 0.32-0.33.

Female genital pore terminal Eggs oval with slight polar prolongation of middle shell measuring 0.012-0.013 by 0.0036-0.0038.

Male

Not recorded.

DISCUSSION

The present specimens are larger (20.4) in length as compared to *P. crassicolle* Villot, 1875 (7); *P. lemnisalis* Belopolskaja, 1959 (8); *P. adherni* Lundstorm, 1942 (9-11); *P. paulum* Van Cleave *et* Williams, 1951 (5-3); *P. cylindraceus* (8.97-11.06); *P. formasum* (Van Cleave, 1942) Schmidt and Olsen, 1964 (9-15); *P. taiwanensis* Schmidt and Kuntz, 1966 (13-16) and *P. malagensis* (Tubangui, 1935) Schmidt and Kunz, 1966 (11.5-18.2).

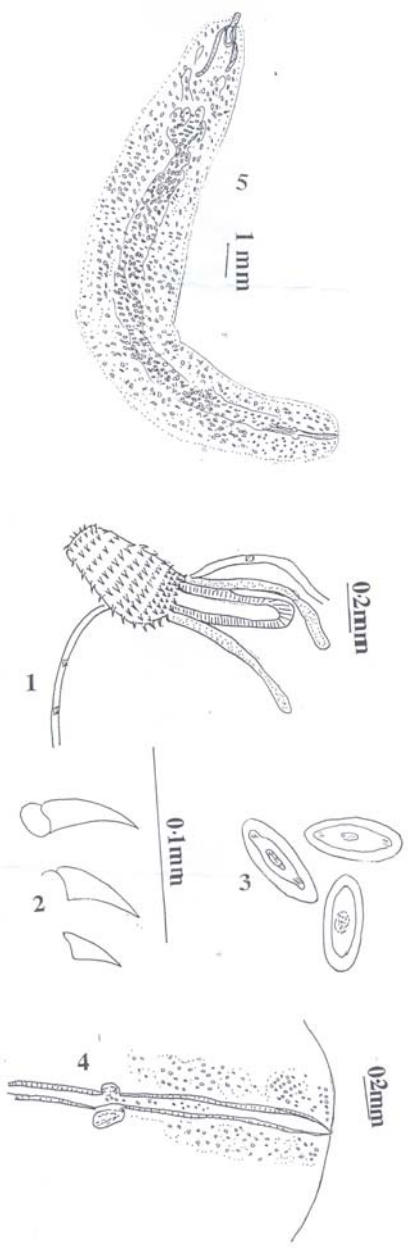

Fig. 1. *Plagiorhynchus karachiensis,* new species; 1, proboscis; 2, hooks; 3, eggs; 4, female terminalia; 5 female (entire).

The hooks in the present specimens have 10 to 12 rows having 6-12 hooks, each while in *P. crassicolle* (18 rows of 11-12 hooks); *P. charadrii* Yamaguti, 1939 (17 rows of 17-18 hooks; *P. charadriicola* (Dollfus, 1953) Golvan, 1956 (18 rows of 15-16 hooks); *P. lineare* (Westrumb, 1821) Golvan, 1956 (18 rows of 14-18 hooks); *P. menurae* (Johnston, 1912) Golvan, 1956 (26 rows of 35-40 hooks); *P. odhneri* Lundstorm, 1942 (18-19 rows of 14-16 hooks); *P. paulum* (15-16 rows of 13-16 hooks); *P. totani* (Porta, 1910) (24 rows of 18 hooks); *P. taiwanensis* (Schmidt and Kuntz, 1966) Amin *et al*., 1999 (14-17 rows of 11-16 hooks) and *P. asymmetricus* Belopolskaja, 1983 (14 rows of hooks).

The eggs in the present species are larger in length as compared to *P. charadrii* (0.0105-0.0120); *P. crassicolle* (0.011); *P. odhneri* (0.0096-0.011); *P. paulum* (0.0067-0.0082) and *P. taiwanensis* (0.0053-0.0075).

In light of these characters it is designated here as a new species and hence the name *P. karachiensis* is proposed due to locality of the host. This is the first record of the genus from Pakistan.

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DEGRADATION OF CHICKEN FEATHERS UNDER SUBMERGED FERMENTATION CULTURE BY *BACILLUS* STRAIN ISOLATED FROM NATURAL ENVIRONMENTS

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Abstract.- Seven strains of *Bacillus* sp. were isolated from the poultry feed industry of Okara, Pakistan. Among all, the *Bacillus* strain FBN-4 having comparatively high keratinolytic activity was rather screened and used for further optimization. This strain when cultured on chicken feathers, the degradation of feathers was greatly increased and it was further enhanced by optimizing several factors such as temperature, pH, and addition of yeast extract, different concentrations of K_2HPO_4, inoculum size and incubation time period in submerged fermentation conditions. Maximum degradation was also achieved by using upflow reactor.

Keywords: Submerged fermentation, feather degradation, keratinase.

INTRODUCTION

In ancient times poultry industry was not a major concern for the environment. But with the increase in human population the demand for the poultry was increased. The increase in production of the poultry industry might be accompanied with an increase in feather waste pollution. The chicken feathers recognized as solid waste is generated from poultry processing industry in abundance in the world. It is generally treated at high pressure and temperature to produce feather meal, which is used as animal feed. Feathers contain ß-keratin as major component in the form of pleated shots twisted into micro-fibrils and are resistant to biological degradation by enzymes (Illham, 2004). Keratins are hydrolyzed by keratinase, a specific protease for feather degradation. The enzyme is produced by some species of *Bacillus*, *Fervidobacterium*, saprophytic and parasitic fungi and actinomyctes (Suntronsuk *et al.,* 1999). The main objective of the present study was to develop a method for microbial degradation of indigenous keratin waste and the production of keratinases, which have wide applications in industry and to identify the microbe having the ability to degrade chicken feathers. Another important feature of this research was a step towards

sustainable development because of the industrial applications of keratinase enzyme that helps in biodegradation of chicken feathers, a kind of pollutant. Chicken feathers are also helpful in manufacturing the low cost animal feed as feather meal. In the present investigation, feather-degrading process was accelerated by inoculating the feather-degrading medium with keratinolytic *Bacillus* strain FBN-4 obtained from Poultry Feed Industry of Okara, Pakistan.

MATERIALS AND METHODS

Isolation of the bacterial strain

The soil samples were collected from the Poultry Feed Industry of Okara, Pakistan and were diluted in saline solution. The serial dilution from each sample was prepared by adding 10 g of the soil to 90 ml of saline water. The diluted samples were plated onto skimmed milk agar plates. Plates were incubated at 37^{o}C for 24 hours. Seven strains of *Bacillus* having keratinase activity were isolated and sub-cultured on nutrient agar slants for subsequent use as describe by Jayati and Rintu (2006).

Screening test

The Keratinolytic activity of the seven selected *Bacillus* strains; FBN-4, FBN-5, FBN-6, FBN-8, FBN-9, FBN-10 and FBN-11 was analyzed in seven 250 ml conical flasks separately. Each flask was containing 0.25 g chicken feathers, 0.1 g Yeast extract, 0.1 g K_2HPO_4, 0.05 g $CaCl_2$, NaCl and $MgSO_4$ each, and 50 ml of distilled water. The pH was adjusted at 7.0 with 1 N NaOH and HCl. These flasks were sterilized at 121^{o}C for 15 min and inoculated with an inoculum of each strain from 24 hours old slants. The flasks were kept on shaking in incubator by rotating at 120 rpm at 37^{o}C. After 48 hours of incubation keratinolytic activity was checked on the basis of reduction of mass of chicken feathers. Results showed that one of the strains FBN-4 was the most efficient in feather hydrolysis. This strain was examined through Gram staining and biochemical tests (citrate utilization test, gelatin hydrolysis test, indole production test, motility test and carbohydrate fermentation tests). It was confirmed as a *Bacillus* sp. This strain was selected for further uses in laboratory for both degradation of chicken feathers and production of keratinase enzyme.

Analysis of chicken feathers

Non-protein nitrogen of chicken feathers was analyzed by Kjheldhal

method described by APHA (1989). Total protein of the chicken feathers was estimated according to Lowry *et al.* (1951).

Keratinase production

Bacillus strain FBN-4 was cultured in a 250ml of conical flask in a culture medium composed of 0.5g chicken feathers, 0.5 g yeast extract, 0.1 g K_2HPO_4 and 0.05 g $CaCl_2$, NaCl and $MgSO_4$ each in 50 ml of distilled water. The pH was adjusted at 7.0. After sterilization, 5 ml of 24 hours old inoculum of *Bacillus* strain FBN-4 was inoculated and the flask was kept on shaker for 48 hours at 37^{o}C. A crude extract of enzyme was obtained after centrifugation of the culture. Keratinase production was assayed by using 2 g keratin in the medium. One unit of keratinase activity was expressed as 1 µ mol of tyrosine released per minute under the above mentioned conditions. The enzyme activity was compared with control, without *Bacillus* strain inoculum.

Keratinase assay

Keratinolytic activity was demonstrated by using a modified method of Anson (1938). The reaction mixture consisted of 2 ml keratin buffer at pH 7 containing 0.1% feathers and 1ml of enzyme solution, and was incubated for 30 min at 40^{o}C. The reaction was terminated by the addition of 3 ml of 10% trichloroacetic acid (TCA) in pH 7. After 30 min the mixture was centrifuged and 1 ml of filtrate was taken. 5 ml of 0.5 M Na_2CO_3 and 0.5 ml of Folin-Ciocalteus reagent was added into filtrate as an indicator. The amino acids liberated were measured as the absorbance at 660 nm against a reagent blank and the quantity was recorded from a standard tyrosine solution curve using a spectrophotometer.

Effect of pH, temperature and incubation time on degradation of chicken feathers

For determination of the effect of pH on degradation of chicken feathers, fermentation medium was incubated at different pH, from 6-10. Fermentation medium was containing 0.5 g chicken feathers in 250 ml conical flask with 0.5 g yeast extract, 0.1 g K_2HPO_4, and 0.05 g of each $CaCl_2$, $MgSO_4$ and NaCl and 50 ml of distilled water. The pH was adjusted at 7.0. This was kept on shaking incubator for 48 hours at 37^{o}C. After 48 hours cell biomass reduction and keratinase production was studied. The optimum temperature for the degradation of chicken feathers was determined by incubating the reaction mixture at pH 7 but at different temperatures ranging from 33^{o}C to 47^{o}C. Cell biomass reduction was determined after 48 hours of incubation.

The optimum incubation time period for the degradation of chicken feathers was demonstrated by incubating the fermentation medium at pH 7, at 37^{o}C and at different incubation time periods ranging from 24 hours to 120 hours of incubation. Cell biomass reduction of chicken feathers and keratinase production was determined after centrifugation of the medium.

Effect of inoculum size, K_2HPO_4 and yeast extract on degradation of chicken feathers

In order to determine the optimum size of inoculum for the degradation of chicken feathers, inoculum size ranging from 1 ml to 5 ml was used in fermentation medium of above mentioned composition. Effect of different concentrations of K_2HPO_4 ranging from 0.1 to 0.5 g and Yeast extract from 0.1 to 2.0 g was also determined. Reduction in cell biomass and keratinase production was determined after 48 hours of incubation time period.

Fig. 1. Upflow Reactor.

Degradation of chicken feathers in upflow reactor

For the degradation of chicken feathers in upflow reactor (Fig. 1: Air lift from Eyela Japan) of 500 ml, 10 g of feathers (2 cm. slices of 10g of chicken feathers, added in the fermentation medium as a substrate) were used with the addition of 10 g yeast extract, 2 g K_2HPO_4, 1 g of each $CaCl_2$, $MgSO_4$ and NaCl in 400 ml of distilled water. The pH of the medium was adjusted at 7.0 by adding

I N NaOH and HCl. After sterilization 100 ml of inoculum was added to 400 ml of the fermentation medium. This was kept on shaking for 48 hours at 37°C. The cell biomass and the reduction of weight in chicken feathers were determined.

RESULTS AND DISCUSSION

Soil samples were collected from the poultry feed industry of Okara, Pakistan. *Bacillus* strains were isolated from these soil samples. These strains were screened for their keratinase activity. Seven strains were chosen from the isolates to be screened for the production of keratinase enzyme. These strains included FBN-4, FBN-5, FBN-6, FBN-8, FBN-9, FBN-10 and FBN-11. For the production of keratinase enzyme, 0.25 g chicken feathers were incubated in the presence of these strains for 48 hours at 37°C. One strain, FBN-4, showed highest keratinase activity amongst all the isolates (Table I). These isolates were identified as strains of *Bacillus* as they were unicellular structure, having cylindrical spores, large colonies on agar, spreading, rough surface and off white in color, positive response for sucrose, lactose, galactose, mannitol, urease, gelatin liquefaction test and 70% good growth in NaCl broth.

TABLE I.- SCREENING OF *BACILLUS* SP. ISOLATES FOR KERATINOLYTIC ACTIVITY

Strain	pH	Weight of feathers before experiment (g/50 ml)	Weight of feathers after experiment (g/50ml)	Total reduction in weight (g/50ml)
FBN-4	7.0	0.25	0.09	0.17
FBN-5	7.0	0.25	0.18	0.13
FBN-6	7.0	0.25	0.12	0.13
FBN-8	7.0	0.25	0.15	0.09
FBN-9	7.0	0.25	0.21	0.04
FBN-10	7.0	0.25	0.15	0.099
FBN-11	7.0	0.25	0.14	0.133

Analysis of chicken feathers

Chicken feathers were first analyzed for the chemical composition and 17% of the non-protein nitrogen contents were estimated through Kjheldhal apparatus. Feughelman (2002) reported 14% nitrogen contents in feather meal in an experiment. The chemical composition of chicken feathers indicated a very moderate medium, which might not need other nutrients for culturing the degrading microorganism.

Effect of pH, temperature and incubation time on degradation of chicken feathers

The effect of the media pH was determined for strain FBN-4 by adjusting the media to different pH ranging from 6-10. The degradation of chicken feathers was high at pH 7.0. Suntronsuk (2006) also described 7.5 pH for the degradation of chicken feathers in his experiment. Effect of different temperature was also described by incubating the fermentation medium at pH 7.0 at a vide temperature range, from 33^{o}C to 47^{o}C. Degradation of chicken feathers was maximum at 37^{o}C. Incubation time period had also a remarkable effect, when fermentation medium was incubated for 24, 48, 72, 96 and 120 hours. Maximum degradation was determined after 48 hours of incubation. After 72 hours further increase in incubation time period led to decrease in keratinolytic activity. It might be due to the fact that there had been depletion of nutrients in fermentation medium (Table II).

TABLE II.- EFFECT OF PH, TEMPERATURE AND INCUBATION TIME ON DEGRADATION OF CHICKEN FEATHERS.

Process Parameters	**Weight of chicken feathers before experiment (g/50ml)**	**Weight of chicken feathers after experiment (g/50ml)**	**Total reduction in weight (g/50ml)**	**Keratinase activity (K.U./ml)**
pH (7.0)	0.5	0.18	0.32	165
Temperature (37^{o}C)	0.5	0.16	0.34	165
Incubation Time (48 hrs)	0.5	0.17	0.33	177

Effect of inoculum size, K_2HPO_4 and Yeast extract on degradation of chicken feathers

The best inoculum size was determined by the inoculation of the fermentation medium in different sizes, from 1 ml to 5 ml. The best activity was shown by 4 ml of inoculum in 50 ml of fermentation media. Genckal and Tari (2006) reported that 1 ml or 2 ml inoculation ratio didn't cause an overload of cell facing nutrients or oxygen limitations. However after 5 ml of this change, this can alter the pathways of cell growth towards enzyme synthesis.

The effect of different concentrations of K_2HPO_4 on degradation of chicken feathers was determined, from 0.1-0.5 g/50 ml of the fermentation

medium. The best degradation activity was shown by 0.5 g of K_2HPO_4. Fujiwara and Yammato (1987) reported that these ions help not only in the growth of the microbe but also act as cofactor and also help in the solute transportation to the bacterium.

The effect of different concentrations of yeast extract on degradation of chicken feathers was also demonstrated in an experiment. Concentrations were ranging from 0.1-2g/ 50 ml of the fermentation medium. The best degradation activity was determined by the addition of 2 g of yeast extract in the fermentation medium (Table III).

TABLE III.- EFFECT OF INOCULUM SIZE, K_2HPO_4 AND YEAST EXTRACT ON DEGRADATION OF CHICKEN FEATHERS.

Process Parameters	**Weight of chicken feathers before experiment (g/50ml)**	**Weight of chicken feathers after experiment (g/50ml)**	**Total reduction in weight (g/50ml)**	**Keratinase activity (K.U./ml)**
Inoculum size (4 ml/50 ml)	0.5	0.19	0.31	153
K_2HPO_4 (0.5 g/ 50 ml)	0.5	0.21	0.29	94
Yeast extract (2g /50 ml)	0.5	0.16	0.34	177

Degradation of chicken feathers in upflow reactor

The main objective of the study was the degradation of chicken feathers in upflow reactor. 10 g of chicken feathers were added in 400 ml of fermentation medium with desired nutrients. The pH of the medium was maintained before inoculating the reaction mixture. After inoculating the mixture with 100 ml of inoculum, this was incubated for 48 hours and kept on shaking with the supply of air. After 48 hours of incubation the reaction mixture was centrifuged and cell biomass was determined after the experiment. 50% of the degradation of chicken feathers was achieved (Table IV).

Taking all the results into account, FBN-4 strain was the strain of choice for further optimization study and potential candidate with keratinolytic activity for future industrial applications. Considering the biodiversity of our environment it is highly important to discover new enzymes from isolates living

under extreme conditions, which could have novel properties that could help in cleaning the environment. So a simple and cost effective method proposed for a rapid and effective bioreduction of keratin waste was explored in this work. FBN-4 was found to be a good agent for degradation of keratin waste and keratinase production. After the completion of the experimentation FBN-4 was submitted to PTCC cultural collection laboratory, FBRC, PCSIR Laboratories Complex Lahore.

TABLE IV.- DEGRADATION OF CHICKEN FEATHERS IN UPFLOW REACTOR.

No. of observations	Weight of chicken feathers before experiment (g/50ml)	Weight of chicken feathers after experiment (g/50ml)	Total reduction in weight (g/50ml)	Keratinase activity (K.U./ml)
1	10	5.6	4.4	141
2	10	4.8	5.2	130
3	10	4.3	5.7	143

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PRODUCTION AND CHARACTERIZATION OF KERATINASE ENZYME BY CULTURING *BACILLUS* STRAIN ON CHICKEN FEATHERS

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Abstract.- The purpose of the present research was to study the production and characterization of thermostable keratinase from a newly isolated *Bacillus* strain FBN-4.The study involved two steps. The first step was the production of keratinase enzyme from chicken feathers and the second was to check the stability of the enzyme. The strain of *Bacillus* sp. FBN-4 was shown to be useful for biotechnological purposes such as for the degradation of chicken feathers. The effect of temperature, pH, incubation time period, different supplementary nitrogen and carbon sources and different concentrations of yeast extract on keratinase production by *Bacillus* strain FBN-4, were optimized. The enzyme was produced between 33^{o}C and 47^{o}C, with maximum activity and yield was achieved at 37^{o}C. Enzyme characterization was also demonstrated. This enzyme was almost stable at 60^{o}C even after 90 min. of incubation at pH 10.

Key words: Keratinase, degradation of chicken feather, *Bacillus* sp.

INTRODUCTION

Keratinases are the group of enzymes that hydrolyze the keratins abundantly present in the epithelial cells of the vertebrates and represent the major constituents of skin and in appendages such as nail, hair, feathers and wool (Lin *et al.*, 1995). Worldwide poultry processing plants produce millions of tons of feathers as a waste product annually (Santos *et al.*, 1996), which consists approximately 90% of keratin. These feathers can be treated biologically for the production of keratinases by different organisms. These keratinases are biotechnologically very important and can be use in different industries. This enzyme is also used in food industry (Birch *et al.,* 1976). In the present study the production of enzyme by *Bacillus* strain FBN-4 growing on chicken feathers was analyzed and effect of different parameters on enzyme production such as substrate concentration, incubation time period, pH of the culture medium and also the presence of different supplementary nitrogen and carbon sources were recorded. Further characterization of this enzyme and the effect of various factors

on the stability of enzyme at higher temperatures and in alkaline pH were also carried out.

MATERIALS AND METHODS

Bacterial strain

Bacillus strain FBN-4 was isolated from the Poultry Feed Industry Okara, Pakistan and cultured on chicken feathers for the production of keratinase enzyme.

Keratinase production

Bacillus strain was cultured in a 250ml of conical flask containing a medium composed of 0.5g chicken feathers, 50 ml water, 0.5 g yeast extract, 0.1 g K_2HPO_4 and $CaCl_2$, NaCl and $MgSO_4$, each 0.05 g. The pH was adjusted at 7.0. A crude extract was obtained after centrifugation of the culture. Keratinase production was assayed by using 2% keratin in the medium. One unit of keratinase activity was expressed as 1 µ mol of tyrosine released per minute under the above-mentioned conditions.

Keratinase assay

Keratinolytic activity was demonstrated by using a modified method of Anson (1938). The reaction mixture consisting 2 ml keratin buffer at pH 7 containing 0.1% feathers and 1ml of enzyme solution, was incubated for 30 min at 40^{o}C. The reaction was terminated by the addition of 3 ml of 10% trichloroacetic acid (TCA) at a pH 7. After 30 min the mixture was centrifuged and 1 ml of filtrate was taken. 5 ml of 0.5 M Na_2CO_3 was added into it along with 0.5 ml of Folin-Ciocalteus as an indicator. The amino acids thus liberated were measured as the absorbance at 660 nm against a reagent blank without indicator in it. The quantity was demonstrated from a standard tyrosine solution curve using a Spectrophotometer.

Keratinase production

For the selection of a culture medium for keratinase production, 0.5 g partly ground chicken feathers in 50 ml distilled water was taken and 0.5 g yeast extract, 0.1 g K_2HPO_4 and 0.05 g of each $CaCl_2$, $MgSO_4$ and NaCl was added and pH adjusted at 7. After sterilization, 5 ml of the bacterial inoculum (*Bacillus*

strain FBN-4) was added and incubated at 37^{o}C for 48 hours. After 48 hours of incubation, keratinase activity in each flask was determined and compared with controls without the bacterium inoculum. The supernatant was collected as the crude enzyme.

Effect of pH, temperature and incubation time on keratinase production

The enzyme production showed keratinolytic activity over a pH range from 6.0 to 10.0. and from 33^{o}C to 47^{o}C. Optimum activity occurred at pH 7.0 (165 K.U/ ml) and at 37^{o}C (165 K.U/ ml). Activity was dependent on the presence of $CaCl_2$, NaCl and $MgSO_4$ in the culture medium, which played a significant role in the thermo-stability of the enzyme. The optimum temperature for the activity was measured by incubating the reaction mixture at pH 7.0 at different temperatures. The enzyme was stable upto pH 10.0 and at 47^{o}C. Incubation time also had the remarkable impact on the production of the keratinase enzyme. Incubation time period ranged from 24-120 hours. Maximum activity of keratinase was demonstrated after 48 hours of incubation.

Effect of different supplementary sources on keratinase production

The enzyme production was demonstrated with and without supplementary nitrogen and carbon sources and different concentrations of yeast extract. Nitrogen sources included Yeast extract, Beef extract, Soya bean, Trypton and Peptone. Among carbon sources Lactose, Glucose, Starch and Fructose were included. Maximum keratinase production was demonstrated in the presence of beef extract, 2% yeast extract concentration and lactose as a carbon source in the presence of 0.5% chicken feathers. Enzyme activity was demonstrated and compared with control containing only chicken feathers.

Characterization of enzyme

Stability of the enzyme was determined in the presence of a vast range of pH, different temperature ranges and incubation time periods.

Effect of pH, temperature and incubation time on the stability of keratinase enzyme

Enzyme stability was demonstrated by using different buffers in the reaction mixture. 1ml of enzyme was added in 2ml of every buffers of different pH range from 6-10. Maximum activity was determined in the presence of

glycine buffer. The optimum temperature for the stability of keratinase was measured by incubating the enzyme at maximum pH 10.0 at different temperatures, ranging from 30°C to 60°C. Optimum incubation time period for the stability and activity of keratinase enzyme was also demonstrated at pH 10.0 and at 60°C by incubating the reaction mixture at different time periods ranging from 5 to 90 min of incubation.

RESULTS AND DISCUSSION

Soil samples were collected from Okara poultry feed industry, Pakistan. *Bacillus* strains were isolated from these soil samples and were screened for their keratinase activity. Seven strains were chosen from the isolates to be screened for the production of keratinase enzyme. These strains included FBN-4, FBN-5, FBN-6, FBN-8, FBN-9, FBN-10 and FBN-11. For the production of keratinase enzyme 0.25 g chicken feathers were used in the presence of these strains and incubated for 48 hours at 37°C. One strain, FBN-4, showed highest keratinase activity amongst all the isolates. These isolates were confirmed to be the genus *Bacillus* as they possessed unicellular structure, cylindrical spores, colonies on agar were large, spreading, rough surface and off white in color, showing positive response for sucrose, lactose, galactose and mannitol, urease, gelatin liquefaction test and 70% good growth in NaCl broth.

Keratinase production

Keratinase activity of the crude enzyme was demonstrated after 24 and 48 hours of the growth period. It was observed clearly that further increase in incubation time decreased the enzyme production. It was demonstrated that in the presence of chicken feathers along with the yeast extract, $MgSO_4$, $CaCl_2$, NaCl and K_2HPO_4, the enzyme production was enhanced. *Bacillus* strain FBN-4 utilized chicken feathers in the presence of distilled water when incubated at pH 7 and at 37°C in a rotatory shaker for 48 hours. The production of enzyme was then compared with the tyrosine. The production of the keratinase enzyme by *Bacillus* strain FBN-4, was maximum in 0.5 g of the chicken feathers fermentation medium (177 K.U/ml). It was estimated, that with the increase in substrate concentration, there is a slight decrease in keratinase production. High substrate concentration may cause substrate inhibition or repressor of keratinase production (Adriano and Riffel, 2004). This was observed when Soya meal was used as a substrate during protease production by *Bacillus horikoshii* (Joo *et al.*, 2002).

Effect of pH, temperature and incubation time on enzyme production

The enzyme production showed a keratinolytic activity over a pH range from 6.0 to 10.0, optimum activity demonstrated at pH 7.0 (165 KU/ml). But with the increase in pH there was a slight decrease in keratinase production. Suntronsuk (2006) also described similar results through experiments in which he used pH 7.5 for degradation and production of keratinase. Enzyme production was also demonstrated over different temperatures ranging from 33^{o}C to 47^{o}C; optimum keratinolytic activity was observed at 37^{o}C (165 KU/ml). It was observed that further increase in temperature did not increase the enzyme production. Incubation time also had the remarkable impact on the enzyme production. Keratinolytic activity was determined in different fermentation mediums incubated at different time periods ranging from 24, 48, 72, 96 and 120 hours. Maximum keratinase production was demonstrated after 48 hours of incubation (177 K.U/ml). After 72 hours further increase in incubation time led to a decrease in keratinase activity. It might be due to the fact that there was depletion of nutrients in the fermentation medium as indicated from the experiment of Illham (2004). (Table I).

TABLE I.- EFFECT OF PH, TEMPERATURE AND INCUBATION TIME PERIOD.

Serial No.	Process Parameters		Keratinase activity (K.U/ml)
1	pH	7	165
2	Temperature	37^{o}C	165
3	Incubation Time	48 Hours	177

Effect of different supplementary sources on keratinase production

Carbon and nitrogen sources were used as supplement sources with chicken feathers in fermentation medium for keratinolytic activity of *Bacillus* strain FBN-4. The highest keratinolytic activity was shown by yeast extract (107 K.U/ml) and lactose (142 K.U/ml) at pH 7 and 37^{o}C of the fermentation medium. It was also demonstrated in another experiment that beef and yeast extract were the best supplementary sources for the enhanced production of keratinases from chicken feathers (Krishna *et al.*, 2005). Among the carbon sources, starch, glucose, sucrose and lactose proved appreciably good for the keratinase production. Lactose, starch, Soya meal and sucrose were considered good for industrial keratinase production (Sonnleitner, 1983) (Table II).

TABLE II.- EFFECT OF SUPPLEMENTARY NITROGEN AND CARBON SOURCES ON KERATINASE PRODUCTION.

Serial No.	Nitrogen sources (0.5g/50ml)	Keratinase Activity (K.U/ml)	Carbon Sources (0.5g/50ml)	Keratinase Activity (K.U/ml)
1	Yeast Extract	49	Lactose	142
2	Beef Extract	82	Glucose	116
3	Soya Bean	49	Starch	142
4	Trypton	129	Fructose	94
5	Peptone	178		

Characterization of enzyme

Partial characterization of keratinase enzyme was also carried out to check the stability and activity of the keratinase enzyme at high temperature and pH.

Effect of pH, temperature and incubation time on the stability of keratinase enzyme

Keratinase enzyme was also checked for its stability and activity carried out in various buffers of different pH values. This enzyme remained stable over a wide range of pH from 6 to 10 (Fig. 1). Maximum activity was exhibited at pH 10, which was 153 K.U/ ml, Kunamnani *et al.* (2003) reported the stability and activity of enzyme in glycine buffer at pH 10. The optimum temperature for the keratinolytic protease activity was determined by incubating the reaction mixture at different temperatures ranging from 30^{o}C to 60°C and assayed at pH 10 for 30 min. The optimum temperature for keratinolytic activity was recorded 45°C (118 K.U/ ml) (Fig. 2). Keratinase enzyme was stable up to 60^{o}C showing 82 K.U/ ml of the activity. Similar results were also described by Wellingata *et al.* (2004) about the keratinase stability at a wide range of temperatures values, from 30^{o}C to 90^{o}C. The thermo-stability of keratinase enzyme was measured by incubating the enzyme at different time intervals, 5 to 90 min. The enzyme activity was increased with the increase in incubation time period. This keratinase enzyme was stable over a broad range of incubating time period (Fig. 3). These results were similar to Hoffman (1974) who checked the stability of enzyme up to 85 min of incubation.

Taking all the results into account, *Bacillus* strain FBN-4 was decided as a strain of choice for further optimization study and potential agent with keratinolytic activity for future industrial applications. *Bacillus* sp. strain FBN-4

was a good organism for keratinase production, which ultimately brought about biodegradation of chicken feathers.

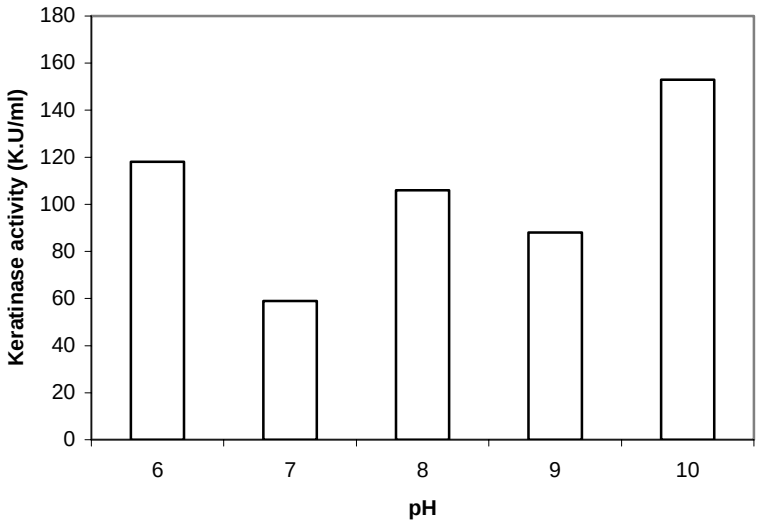

Fig. 1. Effect of different pH on stability and activity of keratinase enzyme.

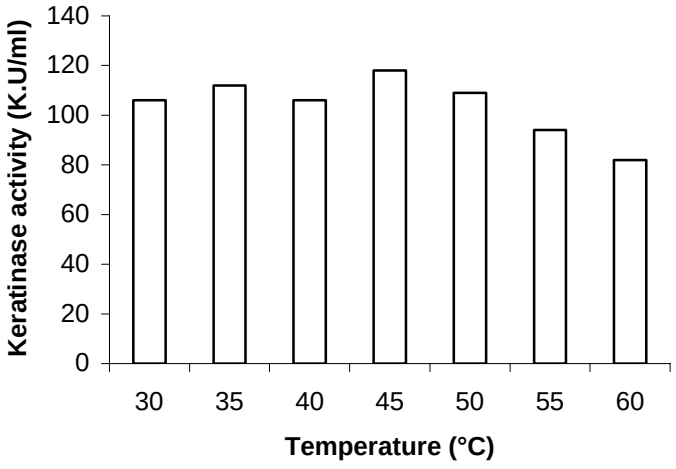

Fig. 2. Effect of Temperature on stability and activity of keratinase enzyme.

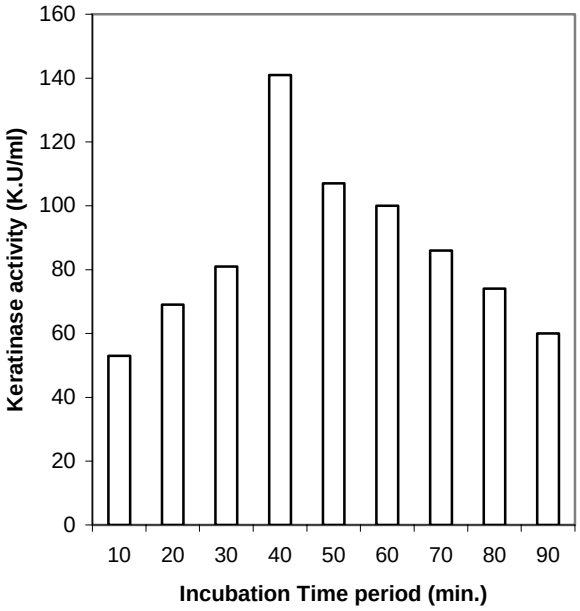

Fig. 3. Effect of Incubation Time periods on stability and activity of keratinase enzyme.

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