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Physical Sciences

Spectroscopic and Chemical Evaluation of Clay Minerals and Their Suitability for the Manufacturing of Organic Clay, Medicinal Clay and Pillared Clay

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The levels of trace elements concentration of the clay minerals of Pakistan have been determined. Inductively Coupled Plasma (ICP) spectrometer was used for the determination of trace elements. The samples of the clay minerals were digested in the ETHOS microwave oven at $180 \pm 5^{\circ}C$. The trace elements (As, Co, Cu, Cr, Cd, Fe, Mn, Ni, Pb, Se and Zn) in the clay minerals of Pakistan fall within the global range. Except as all the trace elements fall within the technical specification of the medicinal clay. The chemical analysis also shows that (SiO_2, Al_2O_3, Fe_2O_3, CaO, MgO, Na_2O, and K_2O) these are within the technical specification of the medicinal clay. Therefore, at present they can be used for external use only. The trace elements (As, Co, Cu, Cr, Cd, Fe, Mn, Ni, Pb, Se and Zn) of clay mineral as well as the activated clay minerals were determined and it is observed that during activation Na, K and Ca ions are leached out. The presence of trace elements of transition metals which are used as catalysts in organic synthesis clearly demonstrate that the catalytic activity of clay minerals is due to these elements. If any of these elements is more than 1% in these clays minerals the clay will act as catalysts without activation. If the quantity of these trace elements is very small and leached out during activation the clay become inactive which is not explained by some workers. Pakistani clays are suitable for making pillared clays. At $550^{\circ}C$, the clay minerals are dehydroxylated and they act as Lewis acid and work as a catalyst in place of $AlCl_3$. It is observed that the clay minerals make complexes with ionic or non ionic surfactant very easily. These complexes make stable suspension in water and oil. It was observed that alkyl group gives a very good organic clay with a swelling property and stable suspension and is superior to the imported clay.

Key words: Spectroscopic evaluation, Clay mineral, Medicinal clay, Pillared clay, Catalyst, Organic clay.

Introduction

There are many medicinal, industrial and chemical uses of clay minerals, all of which depend on one or more of the unique properties of this group of minerals. These minerals have a sheet structure. They form flat, platy hexagonal crystals, white in color when pure. They naturally occur as very fine particles. Water can be absorbed between particles and can hold particles together. Particles slide over each other to form a plastic clay mass that can be absorbed between particles and can be molded and will retain shape when dry. Platy particles suspended in a liquid will link together to coat and protect a surface when dry. They can be compressed into tablets to be used as medicine (Attapulgite is sold as intestopan), melts at high temperature, thermal resistant, electrical insulator. Found in large deposits in several places and is therefore inexpensive.

*Author for correspondence

Large volumes of Na-clay minerals and Na-exchanged, Ca-Mg clay minerals and fuller's earth are directly used in foundry, oil well drilling, iron ore, feed palletizing industries also in civil engineering to impede water movement.

Large quantities of Ca-Mg clay minerals are used directly in iron foundries, in agricultural industries and for filtering and decolorizing various types of oils. A significant portion of Ca-Mg clay minerals used for decolorizing has been acid treated. Large volumes of fuller's or acid earths are commercially used for oil and grease absorbents, as carrier for insecticides, and for decolorizing of oil and fats. Significant volumes of Na-clay minerals are used for various purposes in the manufacturing of many, industrial, chemical and consumer products, such as medical, pharmaceutical, cosmetic, paint (organic clay), building-brick, sewer pipe, roofing tile, gypsum products, pottery-ceramics, radioactive waste disposal, glaze, lubricants, fertilizers, detergents, mortar, catalysts, pa-

per coating, seed coating, adhesives, water purification and other miscellaneous (Odom 1984).

Spectroscopic evaluation of clay minerals led to an enormous increase of knowledge of natural clay minerals. The chemical constitution of the clay mineral remains as important today as ever it was in its mineralogical description. It is well-known fact that a small difference in the chemical composition of clays can greatly influence the chemical and physical properties (Newman 1987), measuring this difference may be quite demanding on the analytical techniques.

In this study the clay minerals of Pakistan has been subjected to spectroscopic analysis as well as chemical analysis and their suitability for the manufacturing of the medicinal clay used in pharmaceutical and chemical industry, organic clay being used by the paint, ink, paper coating and greases. Pillared clays are used for the development of the catalysts required for the organic synthesis and industrial manufacturing. At present Pakistani clay minerals are not being used for medicinal purposes, as a catalyst in organic synthesis.

Experimental

The clay samples were digested in the ETHOS-Plus microwave system which consists of a programmable 1000-W microwave oven with fume extraction system, 12 sample holder, pressure transducer, computer console, and 12 double-wall Teflon decomposition vessels. The vessels have a capacity of 100 ml with pressure and temperature limits of 200 psi and 260°C, respectively.

Method. Clay samples (1.0 g) were accurately weighed. They were directly added to PTFE digestion vessels followed by the addition of 9 ml of 63% HNO_3 (m/m) and allowed to sit for 10 min to subdue exothermic reactions. After adding 3.0 ml of conc HCl vessels were assembled and loaded onto rotating turntable. The vessels were heated at a temperature of 180± 5°C for a period of 10 min. After digestion, vessels were allowed to cool to at least 70°C in the microwave for safety reasons and to facilitate the condensation of any volatile elements in the digest and to minimize the loss of analytes through any aerosol passing out of the vents. The turntable was then removed and placed in a fume hood to vent and open the vessels. The digests were then transferred to 100 ml volumetric flask and diluted up to mark with deionized water. The samples were then stored in a refrigerator at 4°C. The clay samples solutions after digestion were further diluted ten times before running in the ICP-OES.

The samples were analyzed by the Varion Vista-MPX CCD simultaneous ICP-OES model 1998. The ICP was first standardized by running standard solutions of 1, 10, 20, 30 and 50 ppm of the elements analyzed. The standard solutions were prepared by using metal standard solutions purchased from SCP chemical Co, Canada and are traceable to National institute of standards and technology (NIST) primary standards. Each of the elements in the samples was analyzed at multiple frequencies in the ICP for accuracy and automatic results validation. Results are given in Table 1 and 3.

Chemical analysis was carried out by the standard chemical method (Bennet and Hawley 1965). Results are given in Table 2a and 2b.

X-Rays diffraction (XRD) of the clay samples were taken by using Siemens D5000 X-Ray diffractometer. (Fig 1and 2).

The viscosity of the 30-40% clay solutions in water was measured by the RVT Brookfield viscometer. Results are given in Table 4.

Activation. Finely ground clay (about 10.0 g) was treated with conc. Slowly HCl (200 ml), which was added slowly with constant stirring, the mixture was allowed to stand overnight with occasional stirring. The clay was washed 4-5 times with distilled water till the washing becomes neutral, then dried and calcined at 500°C.

Results and Discussion

Medicinal clay minerals. There is an increasing use of Na-clay minerals in the preparation of medicine, pharmaceuticals and cosmetics. Various forms of clay have been used in medicine from time immemorial either as external or internal remedies. As an external agent kaolin is useful for its protective influence and power of absorbing moisture. As a desiccant dusting powder, it is some time applied to weeping eczemas, freely discharging ulcers, and similar conditions. A paste of kaolin in olive oil is used to protect skin around ileostomy or cecostomy opening from the digestive enzymes. Internally kaolin has been used as an adsorptive in symptomatic treatment of various forms of entrities. It is thought that kaolin provides protective coating for the irritated mucosa, and thereby decreases discomfort. It may also diminish the loss of water and electrolytes until specific therapy can be instituted.

The clay minerals used for medicinal purposes are generally water washed having high brightness in some cases must also be high in magnesium and the toxic metals such as lead must be less than 10 ppm and arsenic must be less than 2 ppm. Paul (1999) published technical specification of the medicinal clays. The trace elements limits of phybiosis by Paul (1999) are given in the Table 1 along with trace element analysis of Pakistani

Table 1
Trace elements in clay minerals of Pakistan

Trace elements in clay minerals	Pakistani clay minerals					
	Fire clay mg/kg	China clay mg/kg	Industrial clays (European commission 2001) mg/kg	Bentonite clay mg/kg	Burfab clay mg/kg	Medicinal clay mg/kg*
As	8.3	5.0	10.0	3.00	3.00	< 1
Ba	194.0	45.0	-	36.00	22.00	-
Cd	0.8	0.3	1.0	0.23	0.20	
Co	8.0	7.0	-	8.00	7.00	17
Cr	57.4	19.0	100.0	11.00	22.00	-
Cu	38.0	44.0	60.0	10.30	14.00	<29.7
Mn	99.0	210.0	-	163.00	139.00	-
Ni	27.0	15.0	75.0	10.00	25.00	-
Pb	27.0	4.6.0	75.0	2.00	26.00	13 to 22
Se	6.0	120.0		10.60	6.00	< 1
Zn	18.0	1237.0	120.0	747.00	16.50	-

(Paul 1999)

clay minerals. The elemental analysis of Pakistani clay minerals was undertaken by (ICP-OES Inductively coupled plasma, optical emission spectroscopy) the results are shown in Table 1. Literature survey showed that the trace elements present in the clay minerals of Pakistan have not been studied before.

All these elements are also present in European and American clays (Table 1) (European commission 2001). Some of these elements are essential for our body and derived from food. Some elements are needed by body in greater amount such as calcium and magnesium. The trace elements like chromium, cobalt, copper, manganese and selenium are needed in smaller quantities but they can be toxic in excess.

Arsenic is present in Pakistani clay minerals is 4-5 mg/kg. Minute traces of arsenic are found in vegetable and animal forms of life. It is a constant element of cell life and is present in eggs (Thomas 1984). Many household and garden pesticides contain various forms of arsenic. All of these are toxic if ingested or inhaled in sufficient quantity. An accumulation of arsenic in the body will cause disorders of alimentery tract, nausea, vomiting, diarrhoea, dehydration, neuritis and paralysis of wrist and ankle muscles. United States Environment Protection Agency (USEPA 2002) has set the limits of arsenic to 1 ppm or less in drinking water. It can cause skin damage or problem with circulatory system, and may have increased risk of getting cancer.

Barium compounds are usually used in paint industries, to kill pests and used to color fireworks. Poising occasionally comes from using the soluble salts in place of insoluble sulfate. USEPA (2002) has set the limits of 2 ppm or less for barium in drinking water. It causes increase in blood pressure. Discharge of drilling wastes, discharge from metal refineries and erosion of natural deposits are the main causes of the presence of barium in drinking water. Barium is quite high in Pakistani clay minerals.

Cadmium is used industrially in electroplating and in atomic reactors. Its salts are poisonous. United State Environment Protection Agency has sets the limits of cadmium in drinking water upto 0.5ppm. The cadmium determined in Pakistani clays is 0.2 to 0.9 which is within the limits of EPA. Increased amount of cadmium causes kidney damage. Corrosion of galvanized pipes, erosion of natural deposits, discharge from metal refineries, run off wastes from batteries and paints are main causes of cadmium presence in water. It is also used as a stabilizer in PVC pipes used for drinking water in Pakistan.

Cobalt can be utilized by humans only as the part of vitamin B_{12}. Average intake are 0.3mg per day. Very high doses (29.5mg per day), which were used in the treatment of certain anemias, have proved to be toxic. In Pakistani clays the quantity of cobalt is within the limits of medicinal clay standards.

Copper is associated with a number of enzymes. Deficiency has occasionally been observed in malnourished infants. Particularly, if their initial stores were depleted for example, prolonged feeding of cow's milk alone (which contain less copper than most foods) Although shell fish and liver are particularly rich in copper, the main sources in the average diet are meat, bread, and other cereal products and vegetable. USEPA (2002) has set the limits of copper in drinking water up to 1.3 ppm. Short term exposure of copper could cause

gastrointestinal distress. Long term exposure could cause liver or kidney damage. Corrosion of household plumbing systems; erosion of natural deposits are main causes of copper in drinking water. Two samples of Pakistani clays contain copper within the limits of the medicinal clay standards Table 1.

Chromium is involved in the utilization of glucose. It is fairly and widely distributed in foods those with high content include brewer's yeast, meat, wholegrain cereals, legumes and nuts. A safe level of intake believes to be more than 25 microgram per day for adults and between 0.1 and 1.0 microgram per kg per day for children and adolescents. Enviroment Protection Agency has set the limits of chromium in drinking water up to 10 ppm. It can cause allergic dermatitis (USEPA 2000). Discharge from steel and pulp mills; erosion of natural deposits are main causes of chromium in drinking water. With the exception of two samples of clay all the samples of clay have chromium within the limits of drinking water.

Manganese is present in number of enzymes and activates others. Tea is exceptionally rich in manganese and plant products, including nuts, spices and whole cereals are general much better sources of manganese than animal products. An essential element needed for normal bone metabolism. Deficiency in humans has not been demonstrated. Pakistani clays are rich in manganese.

Selenium is needed for an enzyme in the red blood cells, and the main dietary sources are meat, fish and cereal products. The selenium content of plant varies widely with level in the soil, and in some parts of the world. Animals fed on local produce develop symptoms of deficiency or excess. Neither is common in humans. In 2000 USEPA has set the limits of selenium in drinking water up to 5 ppm. It can cause hair or fin-ger nail loss; numbness in fingers or toes; circulatory problems. Discharge from petroleum refineries; erosion of natural deposits; discharge from mines are the main causes of selenium in drinking water. Pakistani clay minerals are rich in selenium.

Nickel as a nickel carbonyl is used in plating metals. It is toxic, when inhaled causing pulmonary edema. Nickel is also used as a catalyst in Ghee making industry. The quantity of nickel in Pakistani clays is less than that of in European and American industrial clays. Pakistan standard for Ghee industry is 1ppm.

Lead is a metallic element and its compounds are poisonous. Accumulation and toxicity occur if more than 0.5mg per day is absorbed. Most cases of lead poisoning occur in children eat the paint and thus develop signs of toxicity. The other sources which contribute the lead to the environment are the smoke released by the vehicle using lead containing petrol as an anti-knocking agent. Bentonite and china clay of Pakistan has the lead within the limits of the medicinal clays. All the Pakistani clays meet the standards set for the industrial clays by European commission for environment to be used for any other purposes than medicinal clays.

Zinc helps the healing of wounds, and is also associated with the activity of wide variety of enzymes. Its deficiency many contribute to growth retadation, hair loss, delayed wound healing, emotional disturbances, etc. It is still under discussion that zinc has its role in sex determination during preganancy.

About one third of the comparatively large amount is present in the bones. Zinc is present in wide ranges of foods particularly in association with protein, and meat and dairy products, which are its excellent sources. About one third of zinc in the diet is absorbed, but is reduced if large amounts of whole cereals rich in dietary fibers and phytic acid are eaten, although the amount of zinc present in the whole grain cereals is enough to of set this. Average adult intakes are 9 and 12 mg per day. High intakes from water stored in galvanized containers have caused toxicity.

National Secondary Drinking Water Regulations (NSDWR), (USEPA 2002) are non-enforceable guidelines regulating contaminants like Al, Fe, Cu, Zn, Mn, Ag that may cause cosmetic effects (such as skin or tooth discoloration) or aesthetic effects (such as taste, odor, or color) in drinking water (USEPA 2002).

Trace elements in the Pakistani clays meet the industrial standards set by the European Commission Directorate General for environment(ECDGE 2001). But there is slight variation in the standards for the medicinal clay. Chemical analyses of clay minerals of Pakistan listed in Table 2 a and b are within the limits set by the Paul (1999) for the medicinal clays. The Pakistani clay minerals are suitable for external use only such as poultices, masks, baths, creams and lotions.

The term "Medicinal clay" used in health care is relating to a clay mineral that has been extracted solely for the purpose of pharmaceutical or medical usage. It is important to differentiate ordinary clay such as the one used in the manufacture of ceramics, from medicinal clays. Although the material is the same, the methods involved in the extraction process as well as the quality standards associated with its applications are entirely different. At present in Pakistan no clay is extracted solely for medicinal purposes.

Clay catalysts. ICP spectrometric analysis of trace elements present in clay minerals and activated clay minerals are re-

Table 2 a
Chemical analysis of clays

S. no.	Element	Bentonite						Swat China clay					*Extreme composition (%)
		Clay 1	Clay 2	Clay 3 white	Clay 4	Clay 5	*Medicinal bentonite (%)	China 1	China 2	China 3	China 4	China 5	
1	Ignition loss	8.00	10.70	10.00	6.20	10.10	-	11.85	11.40	15.80	7.16	8.60	-
2	SiO_2	57.00	46.68	58.80	53.20	49.65	63.5	46.84	44.20	50.30	46.00	44.50	22.8-90
3	Al_2O_3	23.70	14.75	20.45	11.60	15.56	21.4	36.60	36.65	29.75	32.00	37.00	15.6-23
4	Fe_2O_3	7.50	3.25	0.50	10.00	3.50	3.78	0.70	0.75	0.25	0.62	1.75	5.4-6.2
5	CaO	0.10	1.40	0.70	1.40	0.70	0.66	2.10	4.25	0.70	10.22	5.02	0.4-13
6	MgO	2.26	6.08	5.87	3.54	4.16	2.03	0.67	2.06	0.50	1.00	2.98	11-12
7	Na_2O	0.47	5.40	3.00	2.40	4.80	2.70	0.50	0.54	0.27	1.65		0.1-1
8	K_2O	0.86	0.68	0.60	0.70	0.66	0.310	0.30	0.01	1.80	0.09		3.4-3.7

*Paul 1999

corded in the Table 3. These trace elements (As, Cd, Ni, Mn, Co, Cu, Pb, Fe, Se and Zn) and their compounds have been used as catalysts for the synthesis of organic compounds in the laboratory as well as on industrial scale. It is observed that the increase in any one of these elements or more in the clay minerals increases the catalytic activity of clay minerals. Ehsan *et al* (1999) carried out the analysis of red clay by XRF spectrometer. In this clay iron and titanium are present in substantial quantity(Fe 17-37%, Ti 4.75%) and even after activation the amount of these elements was less effected as compared to the other elements like Ca, Na, K. Ehsan *et al* (1999) synthesized 9, 10 dihydroanthracene by using red clay as a catalyst. We believe that unactivated clays may be treated as potential catalysts. Activated clay catalysts have been used for hydrogenation, polymerization, and hydroformylation for cracking. All of these catalysts have been used at much higher temperature than the catalytic activity we observed for our catalyst for Friedel Crafts benzylation.

The chemistry of the acid activation process and properties and chemistry of the resultant catalysts have been discussed by (Mills *et al* 1950; Thomas *et al* 1950; Hansford 1952; Miliken *et al* 1955; Ryland *et al* 1960). Thomas *et al* (1950) postulated that removal of one of pair of octahedral aluminum ion from montmorillonite, for example, removes two hydroxyl groups and leaves the other of the aluminum in four fold coordination. This tetrahedral aluminum, with its charge balancing proton, form a bronsted site and is the source of the catalytic activity. However, Mills *et al* (1950) have shown the cracking activities of acid treated bentonites from various deposits cover the range from inactive to a level of activity comparable to silica alumina. This range exists despite of almost identical physical properties after acid treatment, for both activable and non activable bentonites. A possible basis for differentiating between these two classes may be found in the observation by Milliken *et al* (1955) that acid activation of raw kaolinite and halloysite, followed by calcinations led to catalysts of inferior quality. However, if calcinations at 550°C precedes the acid leaching step the activity of the catalyst so obtained from either halloysite or kaolinite was greatly improved. Calcination of the kaolin at this temperature should have caused dehydroxyaltion with its accompanying transition to metakaolin.

The spectroscopic analysis of Pakistani clays indicates the catalytic activity is due to the presence of transition metals present in all the clays. If any of these transition elements exceeds more than 1 percent of the clay composition, the clay become active without acid activation. We believe the activity of the clay minerals is due to these transition metals.

X-Rays diffraction (XRD) pattern of the clay before and after activation indicated that both are disordered kaolinite $Al_2Si_2O_3(OH)_4$ and illite trioctahedral KO_5(Al, Fe, Mg,) 3(Si, Al)$_4O_{10}$. After acid treatment a change is observed in the proportion of transition metal (Fe, Ti) and Al. X-ray diffraction of laterite is recorded in Fig 1. The (XRD) of activated laterite is recorded in Fig 2. The peak at d = 7.1 of laterite is completely eliminated in the activated laterite. This shows that hydroxlated phase in laterite is totally de-hyroxylated and the de-hydroxylated phase is not completely amorphous (Fig 2).

The treatment with cold conc. HCl has little effect on the composition of the host layer and results in acid treated clay. The acid attack on the clay structure progresses inwards from the edge of the clay platelets leaching alkaline cations, par-

ticularly Mg, Ca, K and to some extent Fe. Acid activation causes little damage to the silicate layer and the structure in the centre of the clay platelets remain unaffected. The trace elements in all the clay minerals are affected with even cold acid treatment. This explains if the quantity of the transition trace elements in mineral clays is negligible this will become totally inactive with acid treatment. This explains why some clay minerals have no catalytic activity after activation. The Pakistani clays have all the properties to act as a catalyst itself or to act as a pillar to make a suitable catalyst for the synthesis of organic compounds.

The Pakistani clay minerals contain about 16 times more Fe and three times more Ti than the Indian clay minerals. Sabu *et al* (1993) calcined the clay then dried the catalyst after acid treatment at 110°C. It has been reported that if clay is heated upto 110°C so as to remove most of the interlamellar water until only one layer of water remains at about 5% of total water. This increases the Bronsted acidity in Friedel-Crafts reaction $AlCl_3$ is a Lewis acid that takes up an electron pair to form a co-ordinate covalent bond. Ehsan *et al* (1999) after

Table 2 b
Chemical analysis of clays

S. no.	Elements	Fire clay			Ball clay	High alumina clay	Ball clay	Clay (white)	Clay (black)	*Medicinal clay (%)
		Clay 1	Clay 2	Clay3						
1.	Ignition loss	14.20	12.20	15.30	16.00	13.40	10.40	8.20	7.20	
2.	SiO_2	44.40	47.75	46.00	31.30	33.80	56.20	61.20	58.40	56.70
3.	Al_2O_3	38.25	36.10	34.73	51.18	48.98	32.70	25.00	28.00	23.00
4.	Fe_2O_3	0.75	1.00	0.37	0.12	0.25	0.10	0.75	5.00	5.83
5.	CaO	1.40	0.32	1.40	0.70	0.70	0.15	0.27	1.40	0.45
6.	MgO	0.50	0.52	0.50	0.50	0.50	0.07	0.35	0.50	0.07
7.	Na_2O	--	0.13	0.65	--	0.03	0.09	0.15	--	0.01
8.	K_2O	--	0.26	0.86	--	0.01	0.01	0.26	--	3.6 0

*Paul 1999

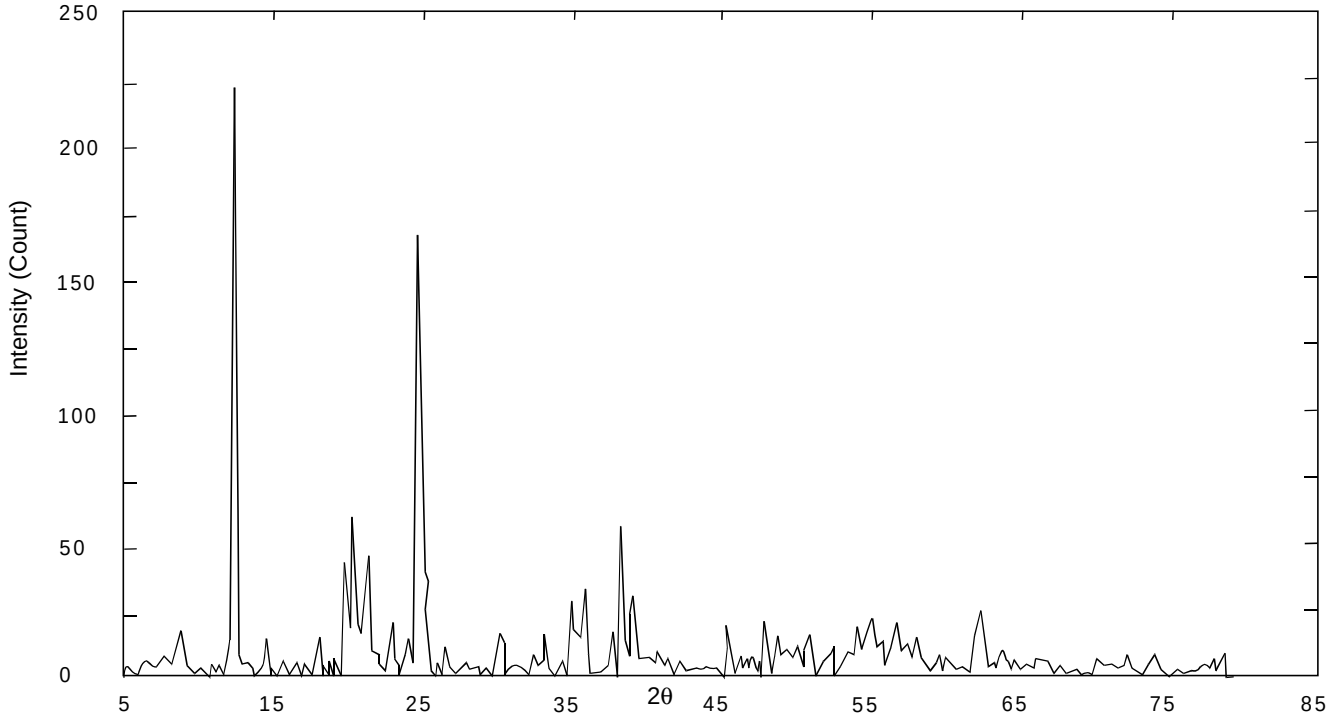

Fig 1. X-Rays diffraction of the clay samples by using Siemens D 5000 X-ray diffractometer

treating the clay with acid calcined it up to 550°C, which results in decrease in the Bronsted acidity but increase in Lewis acidity. This resulted in the formation of L 99 catalyst which is ideal for benzylation.

Commercially marketed clays are montmorrillonite and beidellite. These clays are amorphous and have good swelling property. These are marketed as cation exchanged clays or large polyatomic inorganic ions intercalated into montmorillonite clays. These catalysts are not acting at less than 100°C for Friedel Crafts benzylation.

The L99 has moderate catalytic activity as compared to $AlCl_3$. Hence the poly substitution and polymerization is not possible with L99. This property has enabled us (Ehsan *et al* 1999, 2001and 2002) to synthesize following compounds 9, 10 dihydroanthracene, biphenyl methane, benzyl naphthalene, *o* and *p* hydroxy biphenyl methane, 1-benzyl 2- napthol and 4-

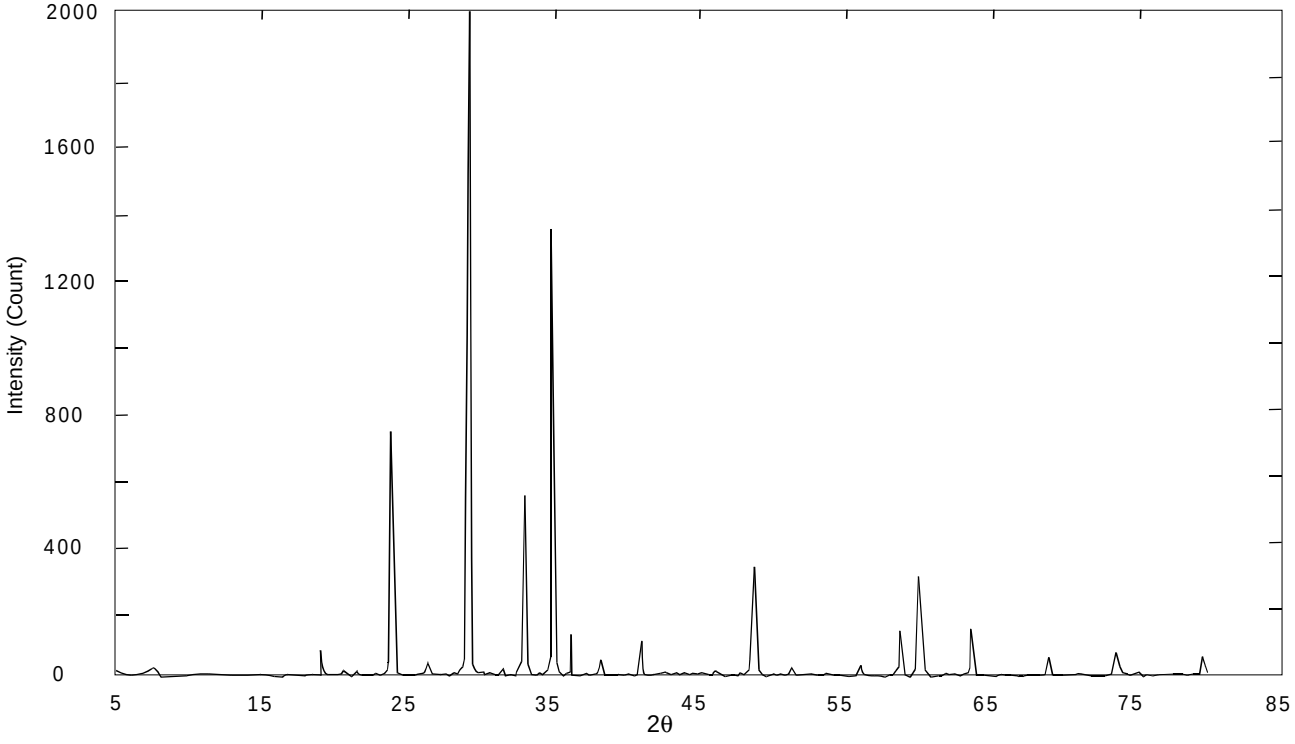

Fig 2. X-Ray diffraction of activated laterites of the clay samples by using Siemens D 5000 X-ray diffractometer

Table 3
Catalytic activity of Pakistani clay minerals

S. no	Trace elements	Bentonite		Fire clay		Laterite		China clay	
		Unactivated	Activated	Unactivated	Activated	Unactivated	Activated	Unactivated	Activated
1	As	3.0	2.4	8.3	7.0	32.0	10.0	5.0	4.00
2	Cd	0.23	0.05	0.8	0.5	9.0	7.0	0.3	0.02
3	Co	8.0	7.0	8.0	3.3	93.0	80.0	7.0	1.2
4	Cr	11.0	11.0	57.4	5.5.0	941.0	785.0	19.0	7.2
5	Cu	10.3	10.0	38.0	126.0	137.0	62.9.0	44.0	12.0
6	Fe	134.0	92.0	170.0	3627.0	-	-	11836.0	5968.0
7	Mn	163.0	162.0	99.0	17.7	308.0	93.0	210.0	48.0
8	Ni	10.0	10.0	27.0	23.0	50.0	34.0	15.0	9.3
9	Pb	2.0	2.0	27.0	12.0	61.0	38.7	4.6	6.0
10	Se	10.6	10.0	6.0	7.0	0.8.0	1.0	120.0	26.0
11	Zn	747.0	747.0	18.0	569.0	165.0	84.6	1237.0	1511.0

benzyl 1- napthol (Fig 3).

Presence of transition metals in the minerals and clay minerals, elimination of basic cations such as Na, K, Ca and Mg and the de-hydroxylation of clay mineral at 550°C is the essential requirement for the development of suitable catalyst.

Organic clay. Natural sodium clay minerals occur in commercial quantities in only few places, but Ca- Mg clay and fuller's earth deposits of considerable size occur on almost every continent. Attempts to change Ca-Mg clay minerals to so called sodium clay minerals are a common practice in the clay industry, especially where naturally occurring sodium clay deposits are rare. The ion exchange is usually performed by mixing soda ash (sodium carbonate) with crude, moist clay using various mechanical methods. A simple determination of hydration characteristics should be given priority in evaluation of clay mineral deposits as so many industrial uses are dependent on this unique property (Table 4). A num-

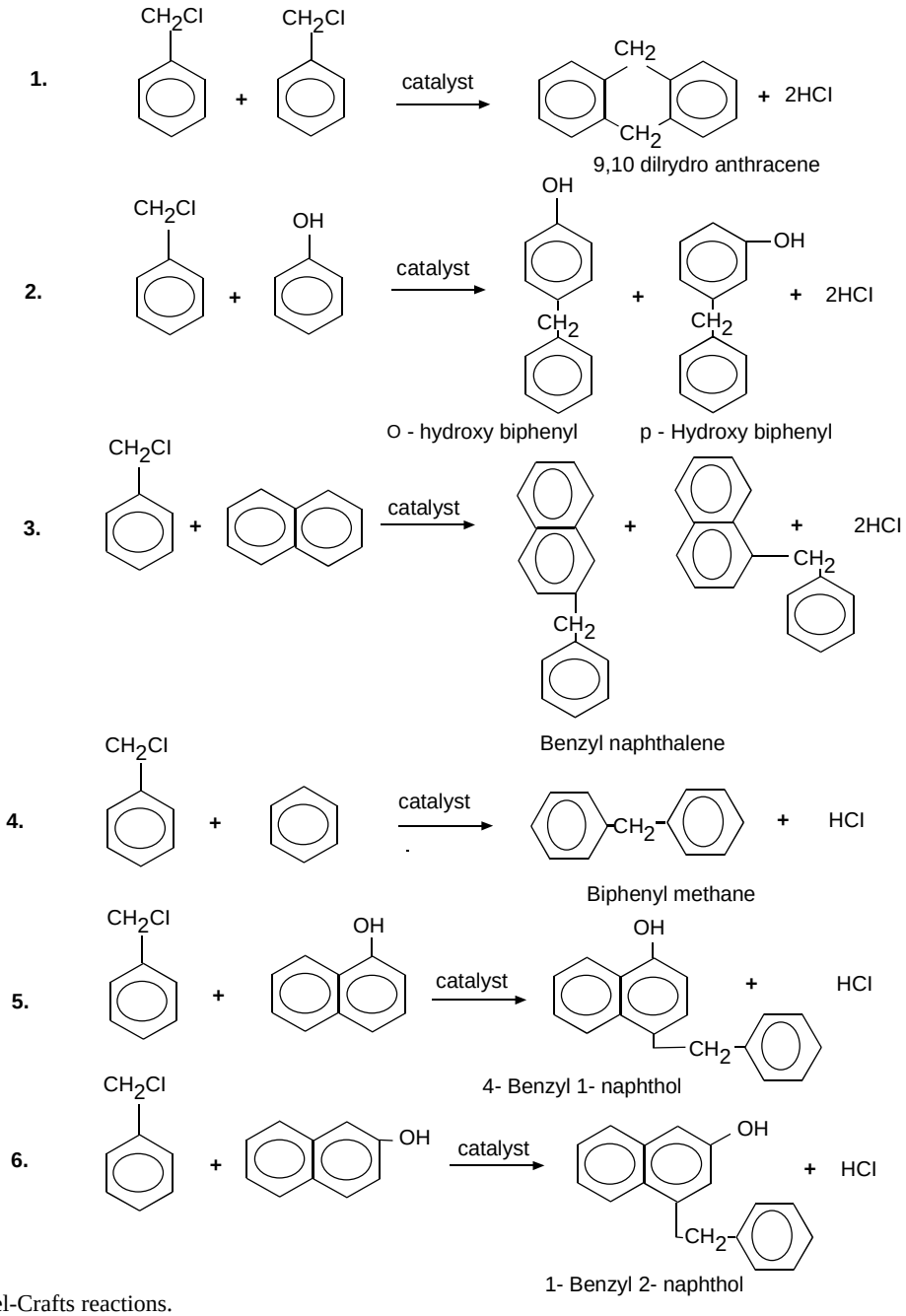

Fig 3. Friedel-Crafts reactions.

Table 4
Viscosity of clays

Sample no	Initial reading	At 30% solid leaching			At 40% solid leaching	
		After 03 h	After 24 h	After 48 h	After 24 h	After 48 h
1	15	15	90	155	200	375
2	15	15	50	65	215	390
3	15	15	120	250	285	605
4	15	15	180	270	330	425
5	15	15	30	45	110	165
6	15	15	65	110	315	450
7	15	15	20	20	370	555
8	15	15	40	40	225	400
9	15	15	15	15	150	150
10	15	15	15	65	130	235

ber of samples of clay minerals were subjected to hydration tests results are recorded in Table 4. Some of the samples swelled significantly. This swelling property showed the suitability for industrial use. The vast majority of naturally occurring clay mineral having Ca-Mg as the predominant exchangeable cations is essentially non swelling.

The property of clays to exchange inorganic cations by organic cations and also the uncharged polar compounds could enter the interlayer space without cation being released, has resulted in the development of useful number of organo-clay complexes (Odam 1984).

Organic-clad clays are widely used for stabilizing the gel properties of lubricating greases. The greases prepared with organic clad clays are said to have superior properties.

Similarly these organic clad clays are used in both oil and water based paints. Natural, Na exchanged Ca and organic clad clays are used in both oil and water based paints. The clays act as suspending and thickening agents and the gel structure is said to improve brush ability and spraying characteristics and to reduce pigment penetration into porous surfaces. They are used in printing inks to control the consistency, penetration and misting during the printing operation. These organic clad clays are not manufactured in Pakistan. At present these are being imported by the paint and grease industry.

As clay minerals make complexes with organic compounds easily. These complexes are prepared with anionic (like alkyl benzene sulfonate, alcohol ethoxy sulfates etc) and nonionic (alcohol ethoxylates, alkyl phenol ethoxylates etc) surfactants. The sedimentation test was carried out and it was observed that they are stable suspension in oil and water Table 4. Complexes with long chain alkyl ammonium ions showed the best swelling and sedimentation properties. They are better in texture and are suitable for both water and oil paints when compared with imported clays, their properties are better than the imported clays.

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References

Ehsan A, Ahsan A H, Mannan A, Iqbal M Z 1999 Development of catalyst from natural clays for friedel-crafts alkylation and new synthesis of 9, 10 dihydroanthracene. *Pak J Sci Ind Res* **42** (3), 156-160.

Ehsan A, Mannan A, Farhat J, Khan I H 2001 Catalytic activity of Pakistani clay minerals for friedel-crafts alkylation. In: *Proceedings of 7th International Symposium on Advanced Materials (ISAM 2001).* Dr A Q Khan Research Laboratories, Kahuta, Pakistan.

Ehsan A, Mannan A, Farhat J, Khan I H 2002 Catalytic activity of Pakistani clay minerals for friedel-crafts alkylation. of benzene and naphthalene. *Pak J Sci Ind Res* **45** (6) 383-385.

ECDGE 2001 European Commission Directorate General for Environment 2001 *Waste Treatment Processes*. for the Environment, European Commission B-1049 Brussels,Belgium,http://www.europa.eu.int/comm/environment/waste/waste-topics.htm.

Hansford R C 1952 Chemical concepts of catalytic cracking. *Adv Catal* **4** 1-29.

Milliken T H, Oblad A G, Mills G A 1955 Use of clays as petroleum cracking catalysts. *Clays Clay Miner* **1** 314-326.

Mills G A, Holmes J, Cornelius E B 1950 Acid activation of some bentonite clays. *J Phys Colloid Chem* **54,** 1170-1185.

Newman A C D 1987 *Chemistry of Clays and Clay Minerals.* Minerological Society Monograph No 6 John Wiley & Sons, New York, USA, pp 275-319.

Odam I E 1984 Smectite clay minerals properties and uses. *Phil Trans R Land A* **311**, 391-409.

Paul F P 1999 *Medicinal Clay: Technical Specifications.*

Phybiosis Inc, P O Box 749, Poway, California 92074, USA, www.phybiosis,com/PELOTHERAPY/Specs,-html.

Ryland L B, Tamele M W, Wilson J N 1960 Cracking catalysts. In: *Catalysis*,ed Emmettph ,pH, Reinhold Publishing Co., New York, USA, Vol **VII**, pp 1-93.

Sabu K R, Sukumar R, Lalithambika M 1993 Acidic properties and catalytic activity of natural kaolinitic clays for friedel-crafts alkylation. *Bull Chem Soc Jpn* **66** 3535-3541.

Thomas C L 1984 *Taber's Cyclopaedic Medical Dictionary*. P G Publishing Pte Led, (Singapore) by special arrangement with F A Davis Company, Philadelphia, USA.

Thomas C L, Hickey J, Stecker G 1950 Chemistry of clay cracking catalysts Ind. *Engng Chem* **42**, 866-871.

USEPA 2000 *Current Drinking Water Standards.*U S Environmental Protection Agency, July 2002, Ariel Rios Building, 1200 Pennsylvania Avenue, NW Washington, DC 20460-0003, USA, http://www.epa.gov/safewater/standards.html.

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PALYNOSTRATIGRAPHIC STUDIES OF CRETACEOUS DEPOSITS OF ANAMBRA BASIN, EASTERN NIGERIA

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Upper cretaceous strata of Anambra basin were spot sampled at two locations. A palynological investigation of the forty-one samples from the outcrop section, enabled the recognition of five palynological zones based on pollen and spores. The assemblage zones which are assigned upper Campanian to Maestrichtian age are: *a). Tricolpites/Syncolporites/ Matonisporites* Assemblage zone (upper Campanian). *b). Psilamonocolpites/Zlivisporis* Assemblage zone (Campanian - Maestrichtian). *c). Retistephanocolpollenites/Monocolpollenites/Propylipollis* Assemblage zone (Maestrichtian). *d). Retidiporites/Verrucatosporites/Buttinia* Assemblage zone (Maestrichtian) and *e). Rugulatisporites/Cingulatisporites* Assemblage zone (Maestrichtian). The dominance of the palynomorph assemblage by trilete spores *(Cingulatisporites ornatus, Foveotriletes margaritae, Zlivisporis blanensis,* and *Verrucatotriletes bullatus)* and monocolpites *(Monocolpites marginatus, Longapertites* sp. and *Monocolpollenites* sp.*)* indicate a swampy environment fringed by herbaceous vegetation. While the dark grey to black, fissile, sulphur stained, pyretic, lignitic and laminated carbonaceous shale suggested a tidal flat environment of deposition. This study therefore enabled the recognition of five informal palynological zones in the upper Cretaceous sediments of Anambra basin and tidal flat environment.

Key words: Palynostratigraphic, Cretaceous deposits, Herbaceous.

Introduction

Anambra basin has received a considerable geological attention since 1903 when exploration for coal started in the basin. Over 12,000 meters of sandstones, shales, limestones and coal seams accumulated in its thickest part since Cretaceous time (Agagu and Adighije 1983). Its upper Cretaceous stratigraphic setting of interbedded sandstones and shales with occasional limestones is suitable for petroleum generation and accumulation (Agagu and Ekweozor 1982; Dankoru 1993). All Nigeria's commercial coal production to date has come within the basin with over 1.7 billion tones still in reserve (David - West 1986).

Palynological studies that have made some significant contributions to the knowledge of the biostratigraphy of the basin include (Salami 1983 and 1990; Oloto 1994). Salami (1983 and 1990) studied late Cretaceous and early Tertiary pollen and spores from Southern Nigeria sedimentary basin. He found out that the environment of deposition of some lower and upper "coal measures" (Mamu and Nsukka formations) rocks is swamp or marginal marine environment periodically inundated by marine water. Mebradu (1990) suggested three palynofacies and two palynofacies changes in the Enugu/Iva valley shales from Anambra basin while Oloto put forward the first dinoflagellate and miospore biozones for Southern Nigeria sedimentary basin.

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This study unlike the previous traditional ones of dating sediments of the basin, attempts a fine stratigraphic differentiation. This is done by concentrating on identified index palynomorphs that are useful for delineating locally defined zones and provide a means of correlation with adjoining basins. The palynostratigraphic framework established here will greatly amplify the biozonation of the flora succession of the basin in future.

*Geological setting of the basin.*This basin which is adjacent to the lower Benue trough is intracratonic (Whiteman 1982). It is an immediate precursor of the Niger delta basin and trends in a NE to SW direction. A lot of work has been undertaken to elucidate the stratigraphy (lithostratigraphy and biostratigraphy), age, palaoenvironment, paleogeography, sedimentary tectonics and origin of the deposits in the basin and the adjoining sedimentary basins. Reyment (1965) and Nwajide and Reijers (1996) delineated and described a number of lithostratigraphic subdivisions. Agagu *et al* (1985) also divided the Senonian-Maestrichtian lighostratigraphic units of the Southern Anambra basin into eight sections representing three repetitive delta-building episodes. Swamp or marginal transitional to marginal marine and tidal flat environments of deposition have been suggested (Salami 1983; Allix 1987; Akande *et al* 1992; Nwajide and Reijers 1996). Fluvio-marine sandstones and shales were said to be

deposited during the tectonically active periods while marine shales were deposited during the intervening quiescent period in response to the globally documented Albian - Maestrichtian sea level rise (Berquist 1971; Douglas *et al* 1973; Mathews *et al* 1974). The paleogeographic history of this area in terms of tectonic events which gave rise to three major depositional cycles reported by Murat (1972). These are:

(a) Abakaliki - Benue phase (Middle Albian-Coniacian).
(b) Anambra - Dahomey (Benin) phase (Campanian-Early Eocene) and
(c) Niger delta phase (Late Eocene - Pliocene).

The sediments in the basin were said to be derived mainly from Abakaliki fold belt (Petters 1978; Ojoh 1992) although some clastics were basement derived. (Hoque and Ezepue 1977).

Materials and Methods

Forty-one fresh road-cut samples were collected at two locations L1 and L2, using spot sampling method. L1 is behind Ogbete market while L2 is at 1 Kilometer along Onitsha-Enugu Express way. Grey to black shales, sand shales, siltstones and shaly sandstones were studied lithologically and processed for palynomorphs. The standard maceration technique for the preparation of palynological samples were followed in this study. This consisted essentially of:

(i) Addition of 60 ml of 50% hydrochloric acid (HCl) to 20g of each sample and washing the residue with distilled water.
(ii) Addition of concentrated hydrofluoric acid (HF) in drops until 80ml has been added. Each sample was mounted on mechanical shaker for about 8 h for the reaction to complete. The residues were washed with distilled water.
(iii) Addition of concentrated nitric acid ($HN0_3$). The residues were again washed with distilled water and finally,
(iv) 5% Potassium hydroxide (KOH) (60 ml)was added, decanted the solution and the residue thoroughly washed with distilled water three times.
(v) The unsieved residues were appropriately treated and mounted in a mixture of epoxy A (3 parts) and epoxy B (1 part) following the method of double mounting (Traverse 1988). Two slides were made from each studied sample.
(vi) Counting of grains for each sample range between 100 and 150. These were semiquantatively tallied as shown in Figs 1 and 2.

Results and Discussion

The palynostratigraphic analysis is based mainly on L2 because of its thickness (30 ml), palynomorph abundance and species diversity (28) which made biozonation possible. Five informal assemblage zones were established for the studied sections which range in age from Campanian to Maestrichtian (Figs 1 and 2). The main features of the palynozones were outlined in ascending order from the base to the top of the sequence. The assemblage zone boundaries were placed where significant changes occurred simultaneously in a number of species and age determinations rely largely on index pollen and spores found and documented in the coastal basins of West Africa and South America. Photomicrographs of selected representative palynomorphs were taken.

Zone A. Tricolpites/Syncolporites/Matonispories zone. The zone is recognized in L2. Its interval extends from 0.4m at the base to 30m at the top. The lower limit of this zone is not reached at this location. The upper limit corresponds to the simultaneous appearance of *Longapertites vaneendenburgi, Inaperturotetradites lacunosus, Monocolpites* sp. and *Proxaperitites anisosulptus.* The most common species within the zone is *Tricolpites synstriatus.* The zone is characterized by *Syncolporites marginatus* and *Psilamonocolpites medius* in addition. The zone is assigned an upper Camapnian age based on *Tricolpites synstriatus* and *Syncolporites marginatus* (Van der Hamman and Wijmstra 1964; Jardine and Magloire 1965; Jan Du Chene *et al* 1978).

Zone B. Psilamonocolpites/Longapertites/Zlivisporis zone. This zone is recognized in L2 between 30m and 8.4m. Its base is fixed at the simultaneous appearance of *Monocolpites marginatus, Anacolosidites luteoidites* and *Proxapertites anisosulptus.* The top of the zone coincides with the disappearance of *Anacolosidites luteoidites* and *Psilamonocolpites medius.* The zone is characterized among others by the presence of *Longapertites vaneendenburgi* and *Zlivisporis blanensis* which appeared and disappeared within the zone. The acme of *Psilamonocolpites medius* and *Tricolpites synstriatus* occurred here. This zone is dated Campanian - Maestrichtian based on *Zlivisporis blanensis* and *Longaper-tites veneendenburgi* (Van der Hamman 1954; Pacltova 1961).

Zone C. Retistephanocolpollenites/Monocolpollenites/Prypylipollis zone. The zone is represented in L2 and present between height 8.4m and 20.lm. The base is marked by high frequency of *Retistephanocolpollenites willamsi* and *Monocolpollenites* sp. As well as the initial appearance of *Retimonoporites pluribaculensis.* The upper boundary is defined by the disappearance of *Retistephanocolpollenites williamsi* and the simultaneous appearance of *Retidiporites*

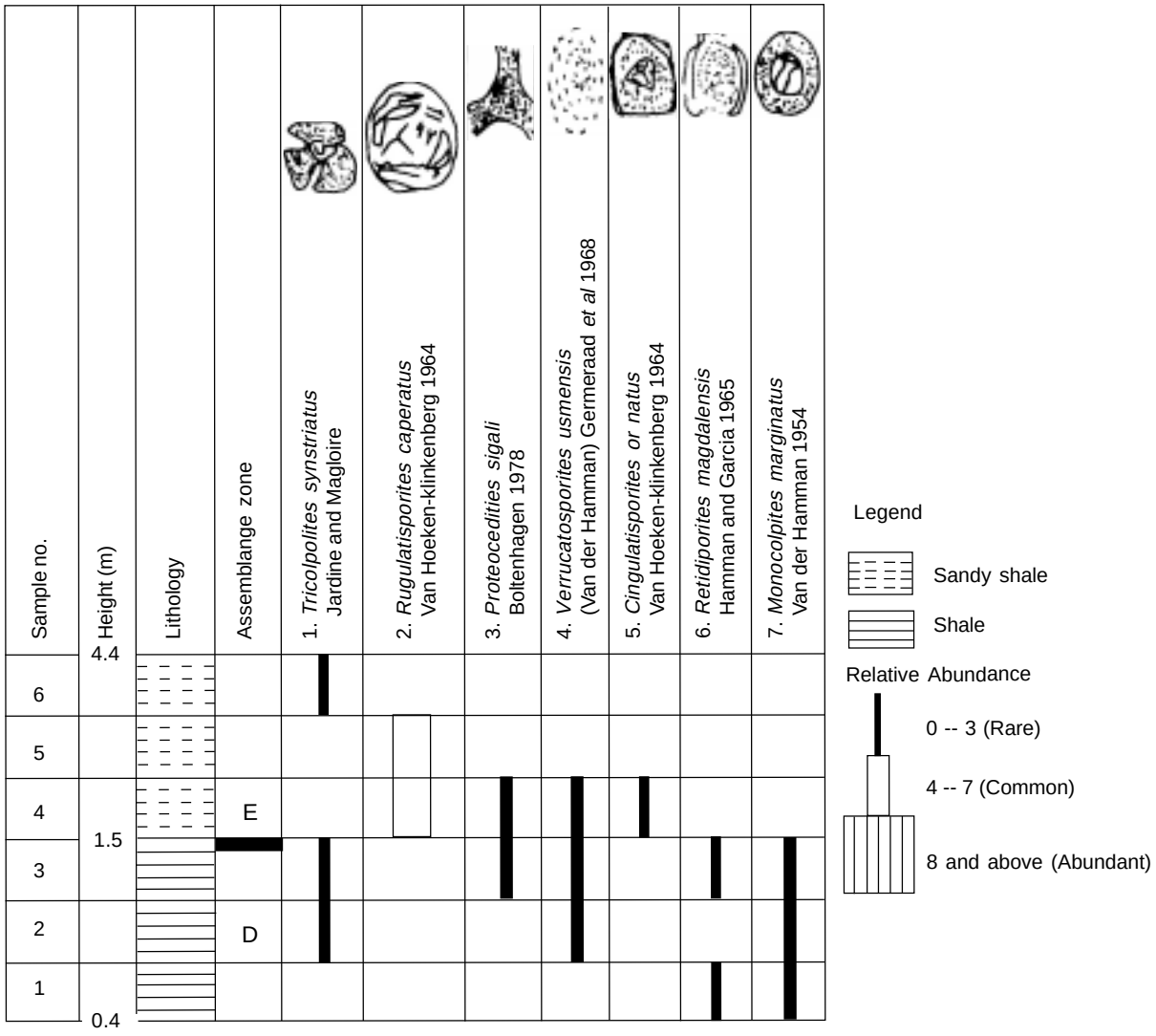


Fig 1. Stratigraphic range chart of the palynological taxa in L1.

magdalensis, Echitriporites triangulisformis and *Buttinia andreevii. Retistephanocolllenites* sp.,*Propylipollis (Proteacidites) dehaani* and *Foveotriletes margaritae* are restricted to the zone. A Campanian - Maestrichtian age is assigned to the zone based on *Propylipollis dehaani* and *Foveotriletes margaritae* (Jardine and Magloire 1965; Germeraad *et a l* 1968).

Zone D. Retidiporites verucatosporites/Buttinia zone. The zone is recognized at the interval between 20.1m and 26.1m in L2 and 0.4m and 1.5m in L1. Its base coincides with the first appearance of *Verrucatosporites usmensis, Retidiporites magdalensis, Echitriporites trianguliformis* and *Buttinia andreevii.* The upper limit is marked by the disappearance of *Longaperitites marginatus* and *Retidiporites magdalensis.* The *Monocolpites margaritae* occurred here in addition to a few poorly preserved dinoflagellates. Most species encountered in this zone also characterize the pollen sequence III of Jardine and Magloire (1965) on the co-occurrence of the foraminiferal species *Bolivina afra* Reyment. This zone is therefore, assigned to Maestrichtian age.

Zone E. Regulatisporites/Congulatisporites zone. This zone characterizes the interval between 26.1m and 29.4m in L2 and 1.5m and 4.4m in L1. Its base is fixed at the disappearance of *Longaperitites marginatus* and *Retidiporites magdalensis.* The characteristic species of the zone occur mostly in L1. These are *Longaperitites* sp., *Cingulatisporites ornatus, Proteacidites sigali* and *Rugulatisporites caperatus.* The age of this youngest zone in the study area is regarded as Maestrichtian based on *Rugulatisporites caperatus* and *Cingulatisporites ornatus* (Jardine and Magloire 1965;

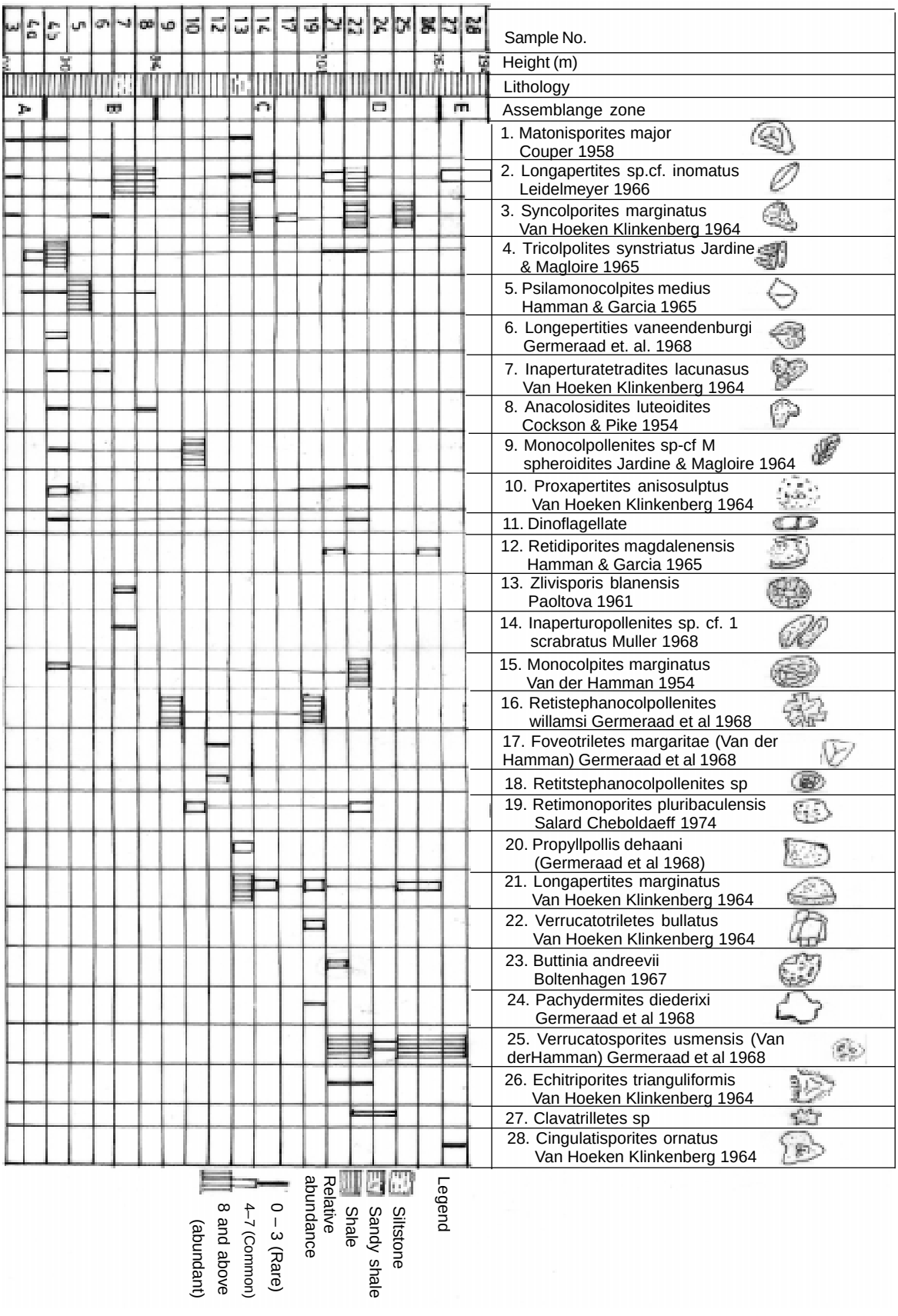
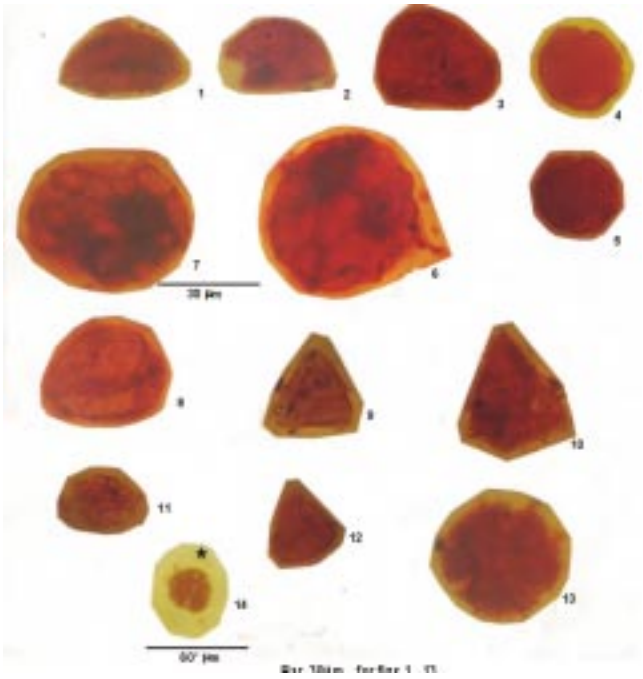


Fig 2. Stratigraphic range chart of the palynological taxa recorded in L2.



Bar 30 μm for figs 1-13
Bar 60 μm for fig 14

Plate

1, 2 *Longapertites* sp. cf *inornatus* (V.H.Kl.) Leidalmeyer 1966.
3 *Anacolosidites luteoidites* Cookson and Pike 1954.
4, 5 *Perchydermites diederixi* Germeraad *et al* 1968.
6, 7 *Zlivisporis blanensis* Palctova 1961.
8 *Inaperturopollenites* sp. cf *I. scrabratus* Muller 1968.
9,10 *Propylipollis (Proteacidites) dehaani* Germacraad *et al* 1968.
11 *Monocolpollenites* sp. cf *spheroidites* Jardine and Magloire 1965
12 *Foveotriletes margaritae* Germeraad *et al* 1968
13 *Retistephanocolpollenites williamsi* Germeraad *et al* 1968
14 *Retistephonocolpollenites* sp.

Germeraad *et al* 1968; Boltenhagen 1978; Salard-Cheboldaeff 1978).

This study delineated the five zones based on the marker species identified from the samples analysed with reference to the works of Boltenhagen (1978), Lawal and Moullade (1986), Mebradu (1990), Salami (1990) and Salard – Cheboldaeff (1990).

Paleoenvironment. With reference to the Plate 1, the brown to dark brown, fairly well preserved palynomorph assemblage indicates a swampy environment fringed by herbaceous vegetation as shown by a fairly large preponderance of trilete spores (ferns) and monocolpates (Palmae) (Salami 1990). But the dark grey to black, fissile, sulphur stained, pyritic, lignitic and laminated carbaneceous shale suggest deposition in an anoxic bottom environment in quiet water condition (Harms *et al* 1975). Hence the environment of deposition is probably tidal flat. Occasional flooding of the area of study by marine or brackish water probably occurred as indicated by the presence of more miospores in samples 4b and 22 and the incorporation of some poorly preserved dinoflagelletes at those levels (Fig. 2).

Conclusion

The knowledge of palynological zones is essential for dating formation and exploration for hydrocarbon source rocks. Outcrop samples are cheap and direct source of information on rock record. Based on the rich palynomorphs in some of the samples, the present study enabled the recognition of five informal palynological zones whose age ranges from Upper Campanian - Maestrichtian. The environment of deposition of the rock units is more of a tidal flat.

References

Agagu O K, Adighije C I 1983 Tectonic and sedimentation framework of the lower Benue Trough, Southern Nigeria. *J Afr Earth Sci* **1** 267-274.

Agagu O K, Ekweozor C M 1982 Source rock characteristics of senonian shales in the Anambra Syncline, Southern Nigeria. *J Min Geol* **19**(1),132-139.

Agagu O K, Fayose E A, Petters S W 1985 Stratigraphy and sedimentation in the senonian Anambra Basin of Eastern Nigeria. *J Min Goel* **22** (1 & 2) 25-36.

Akande S O, Hoffknecht A, Erdtmann B D 1992 Upper Cretaceous and Tertiary coals from southern Nigeria: composition, rank, depositional environments and their technological properties. *Nigerian Association of Petroleum Explorationist Bulletin* **7** (10) 26-37.

Allix P 1987 Le basin d'Anambra: Essai de characterisation de levolution tecto - sedimentaire au Cretace superior. *Bulletin des Centres de Recherches Exploration-Production Elf-Aquitaine* **11** (1) 158-159.

Berquist H R 1971 Biogeographical review of Cretaceous, foraminifera of Weatern Hemisphere: In: *Proceeding North, America Paleontologist Convention,* 1969, 1565-1609.

Boltenhagen E 1978 *Proteacidites sigali,* especе nouvelle de polln proteaceoide du senonien du Gabon. *Rev Esp Micropale* **21**(4) 13-15.

Dankoru E M 1993 Challenges of Frontier Basin Exploration in the 21st century. *Nigerian Association of Petroleum Explorationists Bulletin* **8**(2) 125-128.

David-West T 1986 Opening Address at the opening of the 22nd Annual Conference of the Nigerian Mining and Geosciences Society, Port Harcourt, 9-13.

Douglas R G, Moullade M, Nairin A E M 1973 Causes and consequences of drift in the south Atlantic. In: *Implica-*

tion of Continental Drift to the Earth Sciences,Terling DH, Runcorn Sk, New York Academic Press, USA, pp 517-537.

Germeraad J H, Hopping C A, Muller J 1968 Palynology of Tertiary sediments from tropical areas. *Rev Paleobotany, Palynolopy* **6** 189-348.

Harms J C, Southerd J B, Spearing D R, Walker R G 1975 Depositional environments as interpreted from primary sedimentary structures and stratification sequences. *Society of Economic Paleontologist and Mineralogist,* 2 Dallas, Texas USA, pp 161.

Hoque M, Ezepue M C 1977 Petrology and Paleogeography of Ajali sandstone. *J Min Geol* **14**(1) 16-22.

Jan Du Chene, R E, De Klasz T, Archibong E E 1978 Biostratigraphic study of the borehole Ojo-l SW Nigeria, with special emphasis on the Cretaceous microflora. *Rev Micropaleon,* **21**(3) 123-139.

Jardine S, Magloire L 1965 Palynologie et stratigraphie du Cretace des basisn du Senegal et de Cote d'Ivoire. *Mem Bure Rech Geol Min* **32** 187-245.

Lawal O, Moullade M 1986 Palynological biostratigraphy of Cretaceous sediments in the upper Benue basin, NE. Nigeria. *Rev Micropaleon* **29** (1) 61-83.

Mathews J L, Heezen B C, Catalano R, Coogan A, Tharp M, Natland J, Rawson M 1974 Cretaceous drowning of reefs on mid-Pacific and Japanese guyots. *Science* **184** 462-464.

Mebradu S 1990 Palynofacies of Enugu/Iva valley shales, Anambra state, Nigeria. *J Min Geol* **26** 13-20.

Murat R C 1972 Stratigraphy and paleogeography of the Cretaceous and lower Tertiary in southern Nigeria. In: *African Geology,eds* Dessauvagie TF, Whiteman AJ - University of Ibadan Press, pp 251-266.

Nwajide C S, Reijers T J A 1996 Sequence architecture in outcrops. Examples from the Anambra Basin, Nigeria. *Nigerian Associaiton of Petroleum Explorationists Bulletin* **11**(1) 23-32.

Ojoh K A 1992 The southern part of the Benue Trough (Nigeria), Cretaceous stratigraphy and geodynamic evolution of the equatorial domain of the south Atlantic. *Nigerian Association of Petroleum Explorationists Bulletin* **7** 131-152.

Oloto I N 1994 Nigerian Maastrichtian to Miocene Dinoflagellate and Miospore biozonation - A summary. *J Min Geol,* **30**(1) 61-73.

Pacltova B 1961 Nektere rostline mikrofosilieze sladkorodnich ulozenin svrchni kridy (Senon) vjihoceskych panvich. *Sborn ustr Ust Geol Od Pal* **26** 47-102.

Petters S W 1978 Stratigraphic evolution of the Benue Trough and its implications for the upper Cretaceous paleogeography of west Africa. *J Geol* **86** 311 - 322.

Reyment R A 1965 *Aspects of the Geology of Nigeria*. University of Ibadan Press, Ibadan Nigeria, pp 146.

Salami M B 1983 Some late Cretaceous and early Tertiary Pteridophytic spores from the southern Nigeria sedimentary basin. *Rev Esp Micropale* **15**(2) 257-272.

Salami M B 1990 Palynomorph taxa of the lower coal measures deposits. Campanian - Maastrichtian of Anambra Trough, southeastern Nigeria. *J Afr Earth Sci* **11**(1/2) 135-150.

Salard-Cheboldaeff M 1978 Sur la palynoflore Maestrichtienne et Tertiatre du Basin sedimentaire littoral du Cameroun. *Pollen et Spores* **20**(2) 215-260.

Salard - Chebelodaeff M 1990 Intertropical African palynostratigraphy from Cretaceous to late quaternary times. *J Afr Earth Sci* **11** (1/2) 1-24.

Traverse A 1988 *Paleopalynology.* Allen and Unwin Inc., USA, pp 600.

Van der Hammen T 1954 El desarrolo de la flora Colombiana enlos periodos geologicos. I. Maestrichtiano hasta terciario mas inferior (una investigation palinologic de la formacionde Ganduasy equivalents). *Bol Geologic* **2** (1) 49-106.

Van der Hammen T, Wijmstra T A 1964 A paynological investigation of some upper Cretaceous sediments in Nigeria. *Pollen et Spores* **6**(1) 209-231.

Whiteman A J 1982 *Nigeria: Its Petroleum Geology, Resourses and Potentials.* Graham and Trotman, London, UK **I** & **II**, pp 394 and pp 306.

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EFFECT OF MORDANTS ON COLOR SHADE AND COLOR FASTNESS OF SILK DYED WITH KIKAR AND MADDER BARKS

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The silk fabric was dyed with aqueous extract of Kikar bark *(Acacia arabica)* and Madder bark *(Rubia cordifolia)* by using various metal sulphates as mordants. The three techniques of mordanting were used for dyeing the fabric and are known as pre-mordanting, where the fabrics were first mordanted and then dyed with natural dye extract, meta-mordanting (i.e. dyeing in the presence of mordants) and post- mordanting where the dyed fabrics were treated with mordants. The fastness properties i.e. crock fastness, washing fastness and light fastness of the dyed samples were determined and comparison was made for control and samples dyed in the presence of the metal ions. The three fastness of the dyed samples were found to be good to excellent. The color of the dyed silk was investigated on computer color matching system in terms of Reflectance, K/S and CIE lab color values. The effect of different metal ions have been studied with respect to their influence on color shade and fastness properties. The mechanism of mordant interactions with the fabric has been briefly discussed.

Key words: Natural dyes, Mordants, Fastness.

Introduction

Natural dyes are generally understood to be colorants (dyes and pigments) that are obtained from animal or vegetable matter without chemical processing. They are mainly mordant dyes, although some natural vat, solvent, pigment, direct and acid types are known (Gulrajani and Gupta 1992a). In recent years concern for the environment has created an increasing interest in natural dyes. Conventional wisdom leads to the belief that natural dyes are amiable to the environment than their synthetic counterparts, although the issue is not necessarily quite so straight forward (Smith and Wagner 1995). Nevertheless, natural dyes do have tremendous commercial potential (Verma and Gupta 1994).

In recent years, the world has become increasingly aware of environmental issues. Synthetic dyestuffs in particular have come under severe criticism on the grounds of being highly polluting in their manufacturing and application. A search for safer alternatives has created a widespread renewal of interest in natural dyes. Studies conducted on the color characteristics and fastness properties of natural dyes show that the colors obtained are soft and varied and several shades have wash fastness ratings similar to those of acid dyes on wool and silk (Minagawa and Kawahara 1983; Grierson *et al* 1985; Taylor 1986; Gulrajani and Gupta 1992b).

There have been some investigations on the theoretical basis of dyeing with natural dyes (Arshad *et al* 1954; Gupta and Gulrajani 1994; Gulrajani *et al* 1999). Recently there has been growing interest in the use of natural dyes in textile applications. This is a result of the stringent environmental standards improved by many centuries in a response to the toxic and allergic reactions associated with synthetic dyes. Natural dyes exhibit better biodegradability and are generally more compatible with the environment. In spite of their inferior fastness, natural dyes are more acceptable to environmentally conscious people around the world (Deo and Desai 1999).

The present study focuses on the dyeing of silk with the Kikar and Madder bark extract which is sparingly soluble in water but is freely soluble in alcohol. For dyeing three different techniques named as pre-mordanting, meta-mordanting and post-mordanting were used. The fastness properties of silk samples dyed with natural dye with and without mordants were determined. Methods are intended for determining the resistance of the color of silk when exposed to sunlight (light fastness), washed with soap water at the given temperature (wash fastness) and rubbing off and staining other materials (crock fastness). Grey scale of society of dyers and colorist (SDC) is used as the standard rating scale (1 to 5) to determine the change in color of these fastness properties. The rating 1-2 shows poor fastness, 3-4 moderate and 4-5 good and very good fastness properties.

The changes in color properties will be discussed in terms of reflectance , K/S and CIE Lab values of the dyed substrate. The reflectance is actually the ratio of the light leaving and an

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operat versus the total light that was hitting the object and K/S is the determination of colorant strength from reflectance measurement. C1E Lab Cartesian coordinates are L*, a* and b*, where L* values represent the difference of lightness of the color, the a* value corresponds to the difference of color's position on the red-green axis and the b* value is the difference of its position on the yellow-blue axis.

Materials and Methods

A commercial sample of Kikar and Madder Bark was purchased from Akbari Mandi, Lahore. Commercially bleached silk (all 3% o.w.f) cut into rectangular pieces of 10 cm x 12 cm, weighing 2.0 and 2.5 g, respectively were used in dyeing. The fabric was washed using non-ionic detergent to remove any impurities present. Aqueous solutions containing 5 and 10g/l ferrous sulphate hepta hydrate, aluminium potassium sulphate dodecahydrate (alum) and copper sulphate pentahydrate were used as mordants. Dried barks of Kikar and Madder were crushed (10g/l) and soaked for 16 h followed by boiling for 2 h. The extract was filtered and used for dyeing silk using the same liquor ratio as for mordanting. Multifibre test fabric was used for assessment of staining.

Dyeing of silk with Kikar bark. To achieve a 1% owf shade on unmordanted silk, fabric was entered into the dye bath at 60˚C; this temperature was held for 10 min and then raised to 85˚C over 35 min at liquor ratio 30:1. After dyeing the cloth was removed and raised, soaped at the boil for 15 min, washed thoroughly and dried. Similar procedures were used for dyeing 2% and 4% (owf) shades.

The three different methods of dyeing with mordants were pre-mordanting, meta-mordanting and post-mordanting. Mordant concentration of 5 and 10g/l were used.

In the pre-mordanting method, the fabrics were first immersed in an aqueous solution of alum, copper sulphate or ferrous sulphate for 45 min at 30˚C. All of the mordanted fabrics were then dyed by the above method.

For the meta-mordanting method (i.e. dyeing in the presence of mordants) the fabrics were immersed in bath containing a mordant and the dye extract. The temperature was raised to 90˚C over 30 min and held for 1 h. The fabrics were rinsed at 60˚C, washed with water, squeezed and dried.

In post-mordanting method, dyeing was carried out in the absence of a mordant, followed by mordanting in a separate bath containing a mordant at 30˚C for 45 min. Further processing was the same as described in the meta-mordanting method.

Fastness and color measurements. Wash fastness of all dyed samples was determined by ISO CO2 method and Crock fastness was carried out according to ISO X 12 test method. Light fastness of dyed samples was determined by exposing them to sunlight according to ISO BO2. The change in color and staining on multifibre test fabric was assessed by comparing sample with Grey scale. The results have been summarized in Table 1 for Kikar bark.

Dyed samples were prepared for color measurement, which was carried out by following a standard procedure (Bryan 1987). Color values were evaluated by means of K/S and CIE Lab color-difference values by spectrophotometer with data master V 2.3 software (Data color, international, USA). Four measurements were made on each of the four samples and the variation in percentage reflectance values over a range of 400-700 nm was recorded. The reproducibility of the results was also checked and found to be satisfactory in all cases. The dyeing performance in the various processes was measured in terms of K/S value at their λ_{max} using spectrophotometer. The reflectance and K/S values were recorded for all the dyed samples by using a colorimeter and the result is recorded in Table 1. Changes in color were also measured on the basis of CIE Lab color space in terms of L^* a^* b^* (Cartesian coordinates) and the difference in values obtained for original and faded samples were measured at ΔE and the results are given in Table 3.

Dyeing of silk with madder bark. The controlled and mordanted samples of silk dyed with madder extract were prepared and their fastness properties and color measurements were determined. The results of these studies are indicated in Table 2 and 4.

Results and Discussion

In the actual dyeing process a mordant combines chemically with a soluble dye to form a very complex, aggregated, insoluble lake of high molecular weight within the textile fibre. Lake is formed when solubilized natural dye is rendered insoluble by complexing with mordanting salts and makes the fibre resistant to the external influence in washing and finishing processes. The fastness depends on the formulation of lake inside the textile fibres (Ali 1993). The silk fabric was dyed with Kikar bark and Madder bark at 2% and 4% dyeing concentrations with different mordants (alum, copper sulphate and ferrous sulphate) at 5g/l and 10g/l concentrations. The results of fastness properties of the two natural dyes are given in Table 1 and Table 2.

The metals used as mordants are well known for their ability to form co-ordination complexes and readily chelated with the dye. As the co-ordination numbers of copper and iron are 4 and 6 respectively, some co-ordination sites remained unoccupied where they interacted with the fibre. Functional groups such as amino and carboxylic acid groups on the fibre can

Table 1
Fastness properties of silk dyed with Kikar bark

Samples	Mordant concentration g/l	Crock fastness		Washing fastness						Light Fastness
		Dry	Wet	Cellulose acetate	Cotton	Nylon	Polyester	Acrylic	Wool	
Control										
Natural		3-4	3-4	2-3	2	2	3-4	3	3-4	3-4
Pre-mordanting										
Alum	5	2-3	2-3	4-5	4-5	4-5	4-5	4-5	4-5	4
Alum	10	3	3	4-5	4-5	4-5	5-5	4-5	4-5	4
Copper	5	3-4	3-4	4-5	4-5	4-5	4-5	4-5	4-5	4
Copper	10	3-4	3-4	4-5	4-5	4-5	4-5	4-5	4-5	4
Ferrous	5	3-4	3-4	4-5	5-4	5-4	5-4	5-4	5-4	4-4
Ferrous	10	4	4	4-5	5	4-5	4-5	4-5	4-5	4-5
Meta-mordanting										
Alum	5	2	2	2-3	3	3	3-4	3-4	2-3	4
Alum	10	3	3	2-3	3	3	4-5	4-5	2-3	4-5
Copper	5	2	2	4-5	2	2	4	4-5	4-5	4-5
Copper	10	2	2	4-5	2	2	4	4-5	4-5	4-5
Ferrous	5	3	3	3-4	3	2-3	4-5	4-5	4	4-5
Ferrous	10	3	3	4	4-5	4	4-5	4-5	4	4-5
Post-mordanting										
Alum	5	2-3	2-3	5	5	4-5	4-5	4-5	4-5	4
Alum	10	3	3	5	5	4-5	4-5	4-5	4-5	4
Copper	5	4	4	4-5	4-5	4-5	4-5	4-5	4-5	4
Copper	10	4	4	4-5	4-5	4-5	4-5	4-5	4-5	3-4
Ferrous	5	4	4	5	5	4-5	4-5	5	4-5	4-5
Ferrous	10	4	4	5	5	4-5	4-5	5	4-5	4

occupy these sites. These metals can form a ternary complex on one site with the fibre and on the other site with the dye. Such a strong co-ordination tendency can enhance the interaction between the fibre and the dye, resulting in high dye uptake.

*Fastness and color properties of silk dyed with Kikar bark.*The samples dyed were washed with soap water and the staining on multifibre test fabric was determined. Significant changes were noted for cellulose acetate, cotton, nylon, polyester, acrylic and wool and were compared against the original samples by Grey scale measurement. The results of wash fastness are given for each mordant in Table 1.

As shown in Table 1 the staining on the substrates mentioned above is high for control samples showing poor fastness. The staining on cotton after dyeing silk with the technique called pre-mordanting showed good wash fastness property, having high fastness value when dyed with ferrous sulphate. The samples dyed with the technique meta-mordanting showed low fastness properties as had been shown by high staining on cotton and nylon when dyed with copper sulphate as compared to alum and ferrous sulphate where the fastness is also poor. The silk dyed with these mordants by using the technique named as post-mordanting showed good fastness property for all three mordants, the Grey scale being very good for cellulose acetate, cotton and acrylic fabric for alum and ferrous sulphate.

As shown in Table 1 the crock fastness of controlled sample dyed with natural dye was found to be poor for both dry and wet crocking. The silk samples dyed under pre-mordanting technique showed poor dry and wet crock fastness for alum and copper sulphates. With ferrous sulphate dry and wet crock fastness properties were also poor. In the case of meta-mordanting the samples showed again poor dry and wet crock fastness ratings. The lowest crock fastness was obtained with copper sulphate. In the case of post-mordanting good crock fastness was found for alum and copper but for ferrous sulphate the fastness was very poor.

The results of light fastness of dyed samples are presented in Table 1. Mordanting with mordant concentrations at 5g/l and 10g/l were compared with the values found for the control samples of natural dyes. As shown in Table 1 the light fastness showed high rating of Grey scale which is good to very good.

Table 2
Fastness properties of silk dyed with Madder bark

Samples	Mordant concentration g/l	Crock fastness Dry	Wet	Washing fastness Cellulose acetate	Cotton	Nylon	Polyester	Acrylic	Wool	Light fastness
Control										
Natural		3-4	3-4	2-3	2	2	3-4	3	3-4	3-4
Pre-mordanting										
Alum	5	4	4	4	4-5	4-5	4-5	4-5	4-5	4-5
Alum	10	2-3	2-3	4-5	4-5	4-5	4-5	4-5	4-5	4
Copper	5	4	4	4-5	4-5	4-5	4-5	4-5	4-5	4-5
Copper	10	4	4	4	4-5	4	4-5	4-5	4-5	4-5
Ferrous	5	4	4	4	4-5	4	4-5	4-5	4-5	4
Ferrous	10	2-3	2-3	4	4-5	4	4-5	4-5	4-5	4
Meta-mordanting										
Alum	5	3-4	3-4	4-5	4	4-5	4-5	4-5	4-5	4-5
Alum	10	2	2	4-5	4-5	4-5	4-5	4-5	4-5	4-5
Copper	5	4	4	4-5	4-5	4-5	4-5	4-5	4-5	4-5
Copper	10	4	4	4	4-5	4	4-5	4-5	4-5	4-5
Ferrous	5	4	4	4-5	4-5	4-5	4-5	4-5	4-5	4
Ferrous	10	4	4	4	4-5	4	4-5	4-5	4-5	4-5
Post-mordanting										
Alum	5	4-5	4-5	4-5	4-5	4-5	4-5	4-5	4-5	4
Alum	10	4	4	4-5	4-5	4-5	4	4-5	4-5	4
Copper	5	4-5	4-5	4-5	4-5	4-5	4-5	4-5	4-5	4-5
Copper	10	4-5	4-5	4-5	4-5	4-5	4-5	4-5	4-5	4-5
Ferrous	5	4-5	4-5	4	4-5	4-5	4-5	4	4-5	4
Ferrous	10	4-5	4-5	4	4	4-5	4	4-5	4-5	4

Reflectance of the dyed silk with Kikar (control sample) has been found to be 9.67 and the K/S value calculated from this reflectance is 1.19.The dyeing of silk with Kikar by pre-mordanting with alum, copper and ferrous sulphate (5g/l) had shown reduction in the reflectance values for alum, copper and ferrous sulphate, respectively. By increasing the strength of mordant to 10g/l the reflectance value decreased. These values showed an increase in the strength of color of the dyed sample which is confirmed by an increase in the K/S values at 5g/l concentration of alum, copper sulphate and ferrous sulphate respectively as compared to the value of control sample.

The results also showed high values for ferrous sulphate at the concentration of 5g/l as compared to alum and copper sulphate. For meta-mordanting similar trends have been followed for the reflectance and K/S values. In this case ferrous sulphate showed high reduction in reflectance value and highest increase in K/S values at 5g/l concentration. In the case of post-mordanting the reflectance and K/S values increased at 5g/l concentration of ferrous sulphate. With the increase in the concentration of ferrous sulphate to 10g/l the values decreased for reflectance and K/S, respectively.

The color co-ordinates of dyed samples have been determined by spectrophotometer with data master V 2.3 software (Data colour, International, USA) against the control sample of natural dye and inter comparison of different techniques used for dyeing were made by measuring the difference in coordinates L*, a* and b*. The results of these studies are presented in Table 3.

As shown in Table 3 the values of L* were higher for the concentration of mordants at 5g/l as compared to 10g/l showing that color depth is maximum up to 5g/l and decreased after increasing the concentration of mordants. Similar trend was observed in all the techniques of mordanting. The variation in a* values of sample dyed with pre-mordanting and post-mordanting techniques showed negative values as compared to the a* values of samples dyed with the meta-mordanting which had positive values. The negative values of a* showed that the color moved towards the green side along the red-green axis and the positive value of a* showed that the color

Table 3
Color properties of silk dyed with Kikar bark

Sample		K/S values	Reflectance	Color coordinates CIE Lab difference		
				DL*	Da*	Db*
Control						
Natural		4.22	9.67			
Pre-mordanting						
Alum	(5 g/l)	6.34	2.72	15.91	-(4.09)	-(3.71)
Alum	(10 g/l)	5.82	2.49	9.08	-(6.05)	-(6.32)
Copper	(5 g/l)	5.12	2.20	9.54	-(9.21)	-(8.75)
Copper	(10 g/l)	4.78	2.05	8.01	-(6.50)	-(9.94)
Ferrous	(5 g/l)	7.66	3.29	8.78	-(4.45)	+11.00
Ferrous	(10 g/l)	7.50	3.22	8.46	-(2.70)	+13.55
Meta-mordanting						
Alum	(5 g/l)	12.56	5.43	13.73	+14.62	+8.53
Alum	(10 g/l)	8.54	3.67	13.82	+12.41	+14.60
Copper	(5 g/l)	14.02	6.02	9.81	+6.77	-(5.17)
Copper	(10 g/l)	9.66	3.29	11.53	+6.31	-(4.07)
Ferrous	(5 g/l)	15.27	6.56	2.26	+2.99	+6.01
Ferrous	(10 g/l)	11.36	4.88	1.75	+1.03	+8.88
Post-mordanting						
Alum	(5 g/l)	4.78	2.05	13.76	-(5.04)	+2.20
Alum	(10 g/l)	4.56	1.96	19.00	-(2.24)	+3.74
Copper	(5 g/l)	5.19	2.21	22.52	-(4.50)	-(4.15)
Copper	(10 g/l)	8.84	2.08	25.52	-(3.93)	-(3.10)
Ferrous	(5 g/l)	6.45	2.77	16.49	-(3.64)	+5.29
Ferrous	(10 g/l)	6.25	2.68	20.06	-(3.22)	+5.20

was shifted towards black axis of the CIE L a* b* space. The changes in b* values of samples dyed with pre-mordanting technique showed negative values with alum and copper indicating color shift towards the blue side along the yellow-blue axis. As compared to the ferrous sulphate which showed positive values indicating color towards the yellow side. Whereas in the case of meta-mordanting and post-mordanting, the alum and ferrous sulphate showed positive values of b* as compared to copper sulphate which showed negative b* values in the aforementioned dyeing techniques.

Fastness and color properties of silk dyed with Madder bark. Fastness to washing, crocking and light was determined as for the Kikar bark and the results presented in Table 2 showed good to very good fastness values of silk dyed with mordants as compared to the untreated or controlled sample. The post-mordanting technique of dyeing with mordants at 5g/l and 10g/l concentrations showed high rating of fastness to washing, crocking and light as compared to pre-mordanting and meta-mordanting. Dyeing of silk with the three dyeing techniques showed high rating of fastness properties with ferrous sulphate and copper sulphate as compared to alum.

The reflectance and the K/S values were determined for controlled and mordanted samples by following the same procedure as given for Kikar bark and the data obtained is presented in Table 4. The reflectance of controlled sample was decreased when the dyeing was performed with mordants by using these three techniques whereas the K/S values increased for the mordanted samples. For all the three techniques, of dyeing the samples dyed with copper sulphate at 10g/l concentration showed highest values of K/S. Increase in the K/S values of all the mordanted samples of silk showed that the strength of color was increased as compared to the controlled samples.

The color co ordinates L*, a* and b* of the controlled and mordanting samples with Madder bark are reported in Table 4. The L* values of dyed silk increased when the dyeing were performed at 10g/l concentration under pre-mordanting and post-mordanting techniques and indicated a reduction in color strength. The L* values for the mordanted samples showed an increased value at 5g/l as compared to 10g/l concentration.

The values of a* for samples dyed with pre-mordanting and post-mordanting techniques were negative indicating

Table 4
Color properties of silk dyed with Kikar bark

Sample		K/S values	Reflectance	Color coordinates CIE Lab difference		
				DL*	Da*	Db*
Control						
Natural		1.19	24.17			
Pre-mordanting						
Alum	(5 g/l)	3.02	10.23	(18.26)	-(1.07)	+7.46
Alum	(10 g/l)	4.43	9.29	(22.05)	-(0.90)	+4.73
Copper	(5 g/l)	3.94	12.65	(8.81)	-(0.56)	+13.26
Copper	(10 g/l)	7.23	6.10	(10.45)	-(2.22)	+9.12
Ferrous	(5 g/l)	3.38	11.56	10.18	-(2.02)	+8.83
Ferrous	(10 g/l)	5.87	7.32	(15.87)	-(0.89)	+6.22
Meta-mordanting						
Alum	(5 g/l)	2.13	24.09	8.01	-(3.66)	+7.84
Alum	(10 g/l)	2.83	18.28	7.11	-(2.75)	+9.82
Copper	(5 g/l)	3.68	24.01	10.44	+15.58	-(15.98)
Copper	(10 g/l)	4.85	23.02	8.68	+20.60	-(15.74)
Ferrous	(5 g/l)	2.83	10.81	4.95	+3.40	+21.66
Ferrous	(10 g/l)	1.82	8.60	4.72	+7.33	+23.76
Post-mordanting						
Alum	(5 g/l)	3.04	12.09	(5.98)	-3.71	+2.63
Alum	(10 g/l)	3.79	10.55	(7.62)	-3.59	+5.72
Copper	(5 g/l)	5.19	16.28	(18.64)	-(1.57)	-(3.43)
Copper	(10 g/l)	5.57	10.56	(24.09)	-(2.08)	-(1.34)
Ferrous	(5 g/l)	2.15	8.13	(18.77)	-3.65	+17.73
Ferrous	(10 g/l)	3.79	3.21	(21.65)	-3.08	+20.61

the shift of color on the green side along the red-green axis. On contrast the a* values for samples dyed with meta-mordanting remained positive showing the position of color on the red side of red-green axis except with alum which showed negative values. The change in the b* values of the samples dyed with pre-mordanting and post-mordanting techniques showed positive values indicating yellowish colour along the yellow-blue axis, whereas for meta-mordanting with copper sulphate at 5g/l and 10g/l negative values of b* were recorded which indicated the shift of color toward blue on yellow-blue axis. The dyeing with alum and ferrous sulphate under meta-mordanting technique, on the other hand, showed positive values of b* at the both mordants concentrations.

Conclusion

Silk fabric was dyed with Kikar and Madder bark dye extract by three different techniques named as pre-mordanting, meta-mordanting and post-mordanting using metal salts as mordant. The dyeing behaviour has been assessed by measuring K/S values and different fastness properties. In general the fastness properties obtained can be sufficiently good for practical dyeing. From the above findings that natural dyes from the two sources exist in a highly aggregated form in silk fibres, thereby exhibiting good resistance to washing, crocking and light exposures. Following these techniques this work can be expanded to other plant that yield colorants to examine the possibility of using the dye commercially as a safer substitute for synthetic dyes.

References

Ali S I 1993 Revival of natural dyes in Asia. *JSDC* **109** 13-14.

Arshad F M, Desai J N, Duff D I, Jain S A, Manencal 1954 Natural dye I. The adsorption of brazil wood and logwood coloring matters by fibres. *JSDC* **70** 392-401.

Bryan R 1987 Colorimetry and the CIE system. In: *Color Physics for Industry,* ed Roderick Mc D. The Society of Dyers and Colorists, Perkin House Bradford , West Yorkshire , England.

Deo H T, Desai B K 1999 Dyeing of cotton and jute with tea as a natural dye. *JSDC* **115** 224-232.

Grierson Su, David G D, Sinclair R S 1985 The color and fastness of natural dyes of the scottish highlands. *JSDC* **101** 220-228.

Gulrajani M L, Gupta D B 1992a *Natural Dyes and their Application to Textiles*. Department of Textile Technology, Indian Institute of Technology, New Delhi, 110 016, India.

Gulrajani M L, Gupta D B 1992b Studies on natural dyes: The color characteristics and fastness properties of rhubarb extract on wool and silk fabric. *Ind Text J* **102** 76-79.

Gulrajani M L, Gupta D, Maulik S R 1999 Studies on dyeing with natural dyes: Part I. Dyeing of annato on nylon and polyester. *Indian J Fibre Text Res* **24** 131-135.

Gupta D B, Gulrajani 1994 Kinetic and thermodynamic studies on 2-hydroxy-1,4-naphthoquinone (Lawsone). *JSDC* **110** 112-115.

Minagawa M, Kawahara A 1983 Dyeing studies with natural dyes: Dyeing of wool and silk with natural dyes. *Osaka Shiritsu Daig Sckat Kiyo* **31** 13-16.

Smith R, Wagner S 1995 Guidance for manufacture of eco-friendly text committee (India:Ciba-Geigy) pp 49.

Taylor G W 1986 Natural dyes in textile applications. *Rev Prog Col* **16** 53-61.

Verma N, Gupta N P 1994 Studies on natural dyes: Dyeing of wool fabrics with catechu and their commercial potential. *Ind Text J* **104** 82-84.

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ASSESSING THE SPECIATION PATTERN OF LEAD AND ZINC IN SURFACE WATER COLLECTED FROM ABEGEDE CREEK, IJORA AND LAGOS

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A two-stage sequential extraction procedure for the speciation of zinc and lead has been applied to surface water randomly collected from three sites in Abegede Creek, Ijora and Lagos. The determination of the labile and non-labile metals species was carried out by flame atomic absorption spectrophotometry (FAAS). The mean values of non-labile zinc and lead concentrations from the three sites, A, B and C are 0.54 ± 0.25 mg/l; 0.55 ± 0.26 mg/l; 1.13 + 0.76 mg/l, respectively for zinc and 0.13 ± 0.09; mg/l, 0.17 ± 0.07 mg/l; 0.42 ± 0.23 mg/l respectively for lead. These are higher than for the labile species in the three sites; 0.14 ± 0.07 mg/l; 0.21± 0.22 mg/l; 0.73 ± 0.82 mg/l, respectively for zinc and ND; 0.02 ± 0.04 mg/l; 0.16 ± 0.22 mg/l, respectively for lead. The statistical analysis of variance of the distribution of zinc and lead in the three sites were estimated at 95% confidence level. The values of metals obtained were compared with Nigeria's background values for some rivers and the World Health Organization limits for drinking water respectively and found to be generally higher especially for lead levels. The probable sources of zinc and lead in the Creek are from natural and point sources, although there could be non-point source contributions from urban run-offs and vehicular exhaust.

Key words: Speciation, Heavy metals, Labile, Non-labile, Flame atomic absorption spectrophotometry.

Introduction

In natural aquatic ecosystems, metals occur in low concentrations, however, the occurrence of heavy metals in excess of natural loads is of concern (Botkin and Keller 1997; Fatoki and Awofolu 2003; James and Okolo 2003). This situation has arisen as a result of increasing urbanization and industrialization, and laxity in enforcing environmental regulations especially in most developing economies (UNEP 1986; Biney *et al* 1994; Appleton *et al* 2001; Yusuf 2003).

The accumulation of metals in an aquatic environment has direct consequences on man and the ecosystem (Fergusson 1990; Holm *et al* 1995; Vincent *et al* 2001; Almeida *et al* 2001).

The bio-availability of water-bound metals is of importance from an ecotoxicological viewpoint (Deverey *et al* 1993; Botkin and Keller 1997; Calace *et al* 2000).

Legislation governing the maximum permissible levels of a polluting metal in an environmental medium such as water refers to total concentration rather than the chemical form of that metal (Ure and Davidson 1995). Most of the previous works have been limited to the determination of total concentration of the metals only. However, the determination of total concentration of the metals does not give adequate information about the bio-availability of potentially toxic metals. Fractionation (operationally defined as speciation) is now acknowledged to be a necessary tool to acquire this information (Pardor *et al* 1989; Deverey *et al* 1993; Calace *et al* 2000, He *et al* 2000, Tokalioglu *et al* 2000; Tokalioglu *et al* 2003).

The objective of this study was to determine the concentration of the labile and non-labile forms of lead and zinc in the water samples collected from the Abegede Creek, Ijora and Lagos. This is with a view to ascertain the potential bio-availability of these metals. The Creek is an important natural fresh reservoir for fishing and other recreational activities. It is adjoined upstream by some major refined petroleum oil marketing companies which discharges waste-waters into the Creek.

Materials and Methods

Water samples were collected randomly from the Abegede Creek between the months of October and November, 2001 from three different sites A, B and C indicated in Fig 1.

A – Beside Lagos State Water Corporation Headquarters, Ijora.
B – National Arts Theatre, Water Front, Iganmu.
C – A pool of water adjacent to the National Arts Theatre Iganmu Water Front off the main course of Abegede Creek.

From the samples 100 ml aliquot were acidified with 5 ml 0.01 M HNO_3 for preservation and stored in polyethylene bottles in a refrigerator before analysis (Holm *et al* 1995; APHA 1998)

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Nature of sites. An auto-mechanic workshop is located around site A, there is also a road adjoining the site, which carries a high volume of pedestrian and vehicular traffic. There is a continual discharge of effluent of petroleum products (petrol, diesel and lubricating oil) into the Creek particularly by the petroleum marketing companies. Virtually all the major petroleum marketing companies - African Petroleum, National Oil, Total and Mobil have their oil installations and storage tanks adjacent to site A. Traces of human waste (defecation) are also found in the site A. For site B, there is heavy vehicular traffic on the bridge (Eko Bridge) transversing this site. School children and others use site C as a swimming pool. There is also a road from Orile Iganmu transversing this site. This pool of water has the best aesthetic value compared to sites A and B.

Reagents. High purity chemicals and reagents (purchased from Merck and Aldrich Chemical Company), together with distilled-deionised water were used. Standards were prepared from analytical grade chemicals (Merck). Standard stock solutions were prepared from nitrate salts of Pb and Zn in 1% of HNO_3 in calibrated flasks. Diluted standard solutions were prepared from the stock standard solutions.

Resin preparation. Amberlite CG 120 cation exchange resin (100-200 mesh) sodium form was converted to the calcium form as reported earlier (Holm *et al* 1995). Sodium form resin 27.5 g was weighed and transferred into a glass column, rinsed several times with deionised-distilled water and shaken mechanically. The fine particles were decanted and the resin soaked in 1 M HNO_3 for 8 h in the glass column to remove traces of trace metals impurities. This was followed by the sequential addition of 1 M calcium acetate, 0.01 M calcium acetate and 0.01 M calcium nitrate respectively, followed by the addition of deionised/diluted water until effluent and influent pH were identical. The resin was then dried at 45˚C in a Gallenhamp Oven model 300, for 3 days.

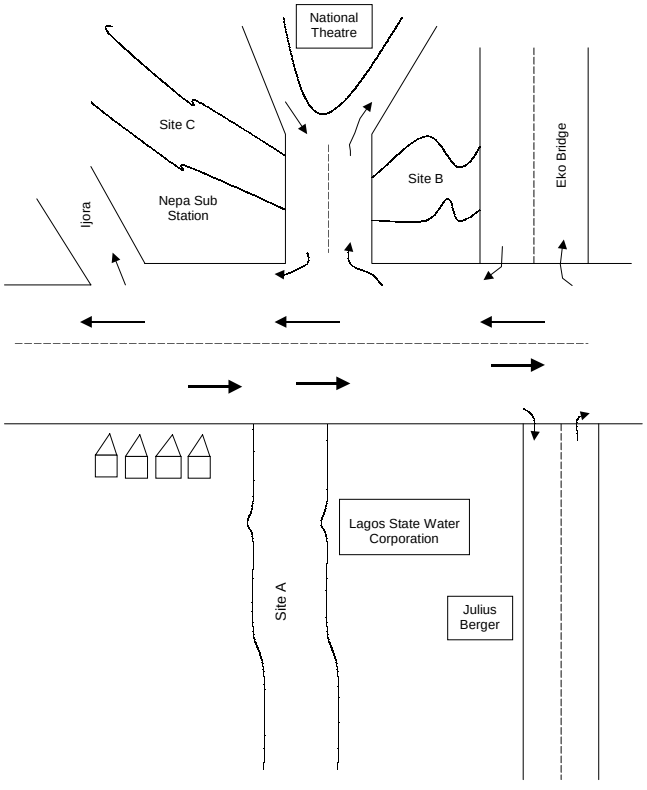


Fig 1. Sketch map of the sample sites.

Speciation procedure. A 25 ml aliquot of sample solution was added to about 400 mg of modified Amberlite resin in a bottle and shaken for 24 h in a mechanical shaker. The solution and resin were then separated by filtering under gravity. The resultant filterates were analysed for labile zinc and lead species using flame atomic absorption spectroscopy (FAAS). To the same resin was added 30 ml of 2 M HNO_3. The content was shaken vigorously for 5 min and immersed in a water bath (100˚C) with intermittent mechanical shaking for 2 h (Fig 2). The resultant filterates were then analysed for non-labile zinc and lead fractions using FAAS. The total metal was estimated by the addition of the individual labile and non-labile metal species as indicated by Calace *et al* (2000).

Instrumentation. The metals were determined with the use of a Perkin Elmer Oak Brown model 2380, Atomic Absorption Spectrophotometer. An atomizer with air/acetylene burner was used for determining zinc and lead. All instrumental settings were those recommended in manufacturer's manual. The instrument was calibrated with analytical grade metal standard stock solution in replicate. The wavelengths (Pb, 283.3 nm; Zn, 213.0 nm) used for the determinations and other working parameters are listed in Table 1.

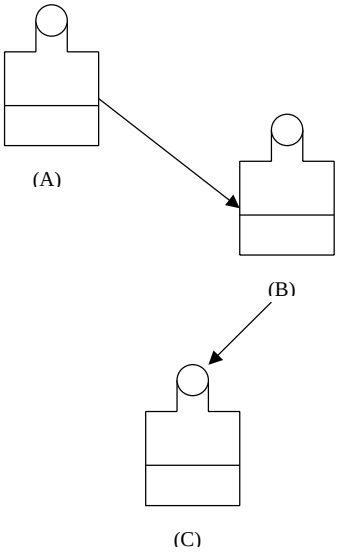
Statistical analysis. Analysis of variance was used to estimate statistically significant differences at 95% confidence level (Sokal and Rohlf 1995).

Table 1
Working parameters for the metal determination by FAAS

Element	Wavelength (nm)	Lamp current (mA)	Detection limit (ppm)	Calibration range (ppm)
Lead	283.30	1.50 - 4.00	0.05	0 - 2.00
Zinc	213.00	5.00 - 15.00	0.002	0 - 10.0

Results and Discussion

The detection limits of zinc and lead investigated and other working parameters for the FAAS are listed in Table 1. The mean concentration of the labile and non-labile zinc and lead have been determined. The results are presented in Table 2. As indicated, the mean values of the labile zinc concentration from the three sampled sites are 0.14 ± 0.07 mg/l, 0.21 ± 0.22 mg/l, and 0.73 ± 0.82 mg/l, respectively. Similarly, that of lead are ND, 0.02 ± 0.04 mg/l and 0.16 ± 0.22 mg/l respectively, for sites A, B and C. A similar trend has been observed before (Pardor *et al* 1989) in the speciation patterns of Valladolid waters. It should be noted however that some of the mean values are lower than the standard deviation. This is not uncommon for water sampled randomly over a period of time from surface and ground waters (Adeniyi and Huthman 2002; Fatoki and Awofolu 2003; James and Okolo 2003; Yusuf 2003).



(A) Stock water sample; (B) Labile (25 ml of water sample + 400 mg Amberlite resin, Stirred for 24 h); (C) Non-labile (25 ml of water sample + 2 M HNO_3 and 400 mg Amberlite resin on water bath for 2 h)

Fig 2. Experimental procedure for determination of lead and zinc in the water samples.

The non-labile zinc concentrations for the three sites are 0.54 ± 0.25 mg/l, 0.55 ± 0.26 mg/l and 1.13 ± 0.76 mg/l, respectively (Table 2 and Fig 3) Similar to labile species, non-labile levels of lead are 0.13 ± 0.09 mg/l, 0.17 ± 0.07 mg/l and 0.42 ± 0.23 mg/l, respectively for sites A, B and C (Table 2 and Fig 4). This is in agreement with earlier studies (Holm *et al* 1995). Lead has been found to occur in relatively smaller concentration than zinc in most natural water source (Pardor *et al* 1989; Garg *et al* 1992; Mathuthu *et al* 1993; Vazquez *et al* 1993; Biney *et al* 1994 ; Appleton *et al* 2001; James and Okolo 2003). This may not be unconnected with the fact that anthropogenic activities generating lead are now less widespread because of environmental pressure groups and increasing awareness of the deleterious effect of lead (Rain 1995; Botkin and Keller 1997; Tokalioglu *et al* 2000). Non-labile species are not expected to be bio-available (He *et al* 2000; Calace *et al* 2002). However, changes in certain physico-chemical conditions in the river system could result in the conversion of non-labile to labile species of metals and vice-versa (Ure and Davidson 1995; Tokalioglu *et al* 2000).

The statistical analysis of variance of the distribution of labile zinc and lead were not statistically significant at 95% confidence level. Similarly, the non-labile zinc was not significant whereas, the non-labile lead distribution was significant at 95% confidence level (Table 3).

The range of total concentrations found in the water samples are: 0.68 - 1.86 mg/l, Zn; 0.13 - 0.58 mg/l, lead. This showed that the heavy metals were present in considerable amounts in the Creek's water. Whereas, the total zinc level is lower than the World Health Organisation (WHO) (1996) limits of 0.5 mg/l for

Table 2
Levels of zinc and lead (mg/l) in fresh water samples from the Abegede Creek

Sampling sites	Metal	Labile species	Non-labile species	Total value	NBV (mg/l)	WHO (mg/l)
A	Zn	0.14±0.07	0.54±0.25	0.68±0.28	0.02 - 0.76	5.00
	Pb	ND	0.13±0.09	0.13±0.09	0.01 - 0.02	0.05
B	Zn	0.21±0.22	0.55±0.26	0.76±0.45	0.02 - 0.76	5.00
	Pb	0.02±0.04	0.17±0.07	0.19±0.06	0.01 - 0.02	0.05
C	Zn	0.73±0.82	1.13±0.76	1.86±0.97	0.02 - 0.76	5.00
	Pb	0.16±0.22	0.42±0.23	0.58±0.22	0.01 - 0.76	0.05

ND, Not detected; NBV, Nigeria's background values for some rivers; WHO, World Health Organisation limits for drinking water; Site A, Beside lagos state water corporation, Ijora; Site B, National arts theatre water front, Iganmu; Site C, Adjacent to the national arts theatre Iganmu water front off the main course of Abegede Creek.

Table 3
Statistical analysis of variance of the distribution of the labile, non- labile and total zinc and lead in the different sites

Nature of metals	$F_{cal.}$, 0.05	$F_{tab.}$, 0.05
Labile	1.23 (1.87)	3.68
Non-labile	2.44 (6.60*)	3.68
Total	3.82* (11.78*)	3.68

Values in parentheses are for lead, the rest are for zinc. *Significant difference at 95% confidence level.

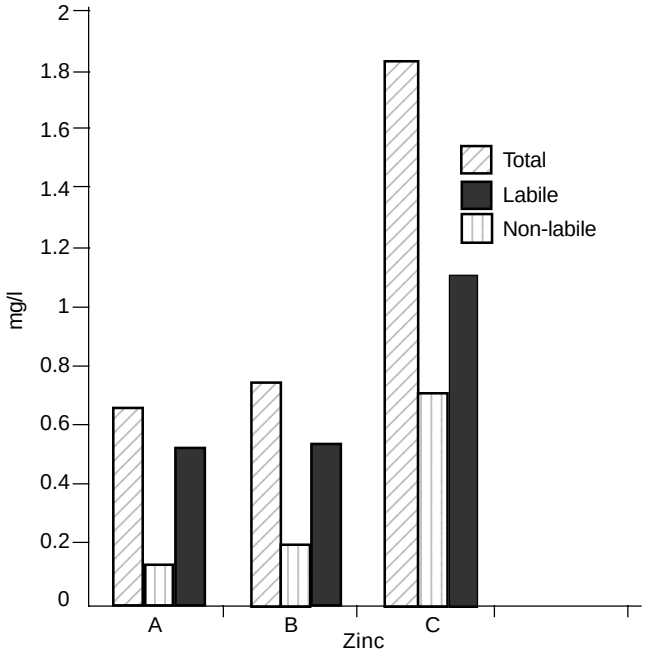


Fig 3. Concentration of zinc in three different sites A, B and C.

drinking water, it is higher than the Nigeria's background values of 0.02 -0.76 mg/l for some rivers. Zinc has low toxicity to man, but relatively high toxicity to fish (Fatoki and Awofolu 2003). In view of the fact that fishing is commonly practiced in this Creek, the levels of zinc is of great concern. Nevertheless, total lead in the water samples are generally higher than the Nigeria's background values (0.01- 0.02 mg/l) and the WHO limits for drinking water (0.5 mg/l). Undoubtedly, these relatively high values of lead are the result of acute pollution (Biney *et al* 1994; James and Okolo 2003; Yusuf 2003). This is equally of concern because of the many health problems associated with lead accumulation in the biological tissues (Needlemann *et al* 1990; Martinez-Tabche *et al* 1997; Tchernitchim *et al* 1998).

The impact of oil marketing companies in the vicinity of the Abegede Creek cannot be overlooked as heavy metals are

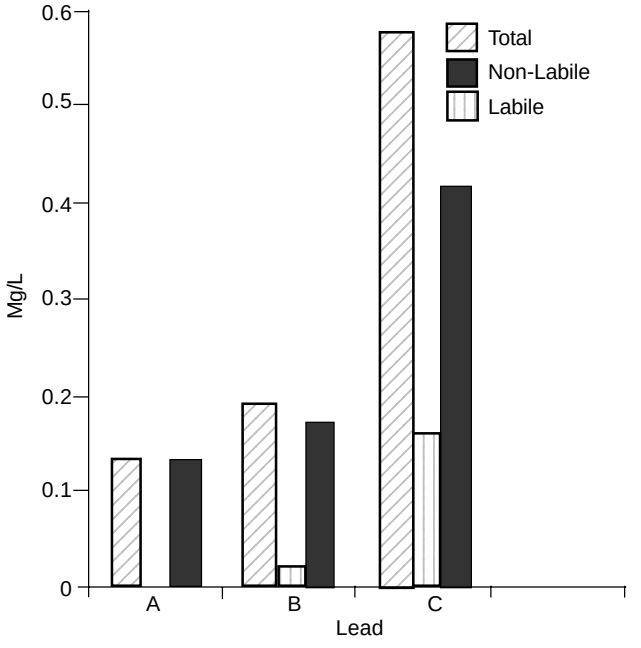


Fig 4. Concentration of lead in three different sites A, B and C.

known to be associated with petroleum products and their effluents (Onianwa 1995; Martinez-Tabche *et al* 1997; Adeniyi and Oyedeji 2001; Adeniyi and Afolabi 2002; James and Okolo 2003). With continued activities of the oil marketing companies, heavy vehicular traffic around the Creek and urban runoffs, (Arienzo *et al* 2001; Hashmi and Khani 2003) it is expected that heavy metals contamination may become wide spread in the Creek in the coming years. There is the need to evolve a sustainable environmental remediation programme to arrest the level of contamination in the Abegede Creek.

References

Adeniyi A A, Oyedeji O O 2001 Determination of total petroleum hydrocarbons (TPH), organic carbon and heavy metals in soils adjoining the national arts theatre water front and Agbegede Creek Ijora Lagos. *Fresenius Environ Bull* **10** 873-876.

Adeniyi A A, Afolabi J A 2002 Determination of total petroleum hydrocarbons and heavy metals in soils within the vicinity of facilities handling refined petroleum products in Lagos Metropolis. *Environ Int* **28** 79-82.

Adeniyi A A, Huthman A S 2002 Physico-chemical analysis of effluents from cosmetics and Toiletries industries. *ASSET Series B* **I** 77-81.

Almeida J A, Novelli E L B, Dal Pai Silva M, Alves R (Jr.) 2000 Environmental cadmium exposure and metabolic responses of Nile Tilapia. *Environ Pollut* **114** 169-175.

America Public Health Association 1998 *Standard Methods*

for the Examination of Water and Wastewater, APHA, AWWA and WPCE, 20th ed. Amer. Public Health Assoc., Byrd Progress, Springfield, New York, USA.

Appleton J D, Williams T M, Orbea H, Carrasco M 2001 Fluvial contamination associated with artisinal gold mining in the Ponce Enriquez, Portovelo-Zaruma and Nambija areas, Ecuador, water. *Air and Soil Pollut* **131** 19-39.

Arienzo M, Adamo P, Bianco M R, Violante P 2001. Impact of Landuse and Urban run-off on the contamination of the Samo River Basin in southwestern Italy. *Water Air Soil Pollut.* **131** 349-366.

Biney C, Amuzu A T, Calamari D, Kaba N, Mbome I L, Naeve H, Ochumba P B P, Osibanjo O, Radegonde V, Saad M A H 1994 Review of heavy metals in the African aquatic environment. *Ecotoxicol Environ Saf* **28** 134-159.

Botkin D B, Keller E A 1997 *Environmental Science. Earth as a Living Planet*. John Wiley, New York, USA pp 392-434.

Calace N, Petronic B B, Biebantuma E, Pietroletti M 2000 Metals speciation in sediments of polluted and non-polluted Lakes. *Toxicol Environ Chem* **76** 157-169.

Deverey E, Bitton G, Rossel D, Ramos L D, Guerrerro L M, Tarradellas J 1993 Concentration and bio-availability of heavy metals in sediments in Lake Yojoa (Honduras). *Bull Environ Contam Toxicol* **50** 253-259.

Fatoki OS, Awofolu O R 2003 Levels of Cd, Hg and Zn in some surface waters from the Eastern Cape Province, South Africa. *Water South Africa* **29** 375-380.

Fergusson J E 1990 *The Heavy Elements: Chemistry, Environmental Impact and Health Effects*. Pergamon, Oxford, USA, pp 48-67.

Garg N, Mather N, Modak D P, Singh K P, Morthy R K, Ahmed S, Chandra S V, Ray P K 1992 Trace metals trend analysis in River Ganges at Kanfor. *Environ Inter* **18** 297-305.

Hashimi D R, Khani M I Q 2003 Environmental impact assessment of air pollution in different areas of Karachi. *Pak J Sci Ind Res* **46** 399-405.

He M, Yang J, Cha Y 2000 Distribution, removal and chemical forms of heavy metals in polluted rice seed. *Toxicol Environ Chem* **76** 137-145.

Holm P E, Christensen T H, Tjell J C, McGrath S P 1995 Heavy metals in the environment. *J Environ Qual* **24**.

James M O, Okolo P O 2003 Variation of heavy metal concentrations in water and freshwater fish in Niger Delta waters - A case study of Benin River. *Pak J Sci Ind Res* **46** 439-442.

Martinez-Tabche L, Mora B R, Faz C G, Castelan T G, Ortiz M M, Gonzalez V C, Flores M O 1997 Toxic effect of sodium dodecylbenzene - sulfonate, lead and petroleum and their mixtures on the activity of acetyl - cholinesterase of *Moina macrocopa invitro*. *Environ Toxicol Water Qual* **12** 211 - 215.

Mathuthu A S, Zaranyika F M, Jonnalagadda S B 1993 Monitoring of water quality in upper Mukuvisi River in Harare, Zimbabwe. *Environ Inter* **19** 51-61.

Needlemann H L, Schell A, Bellinger D, Leviton A, Allerd E N 1990 The longterm effects of exposure to low doses of lead in childhood. An 11-Year follow-up report. *New England J Med* **322** 83-88.

Onianwa P C 1995 Petroleum hydrocarbon pollution of urban top soil in Ibadan City, Nigeria. *Environ Inter* **21** 341-343.

Pardor R, Barrado E, Arranz J M, Perez F, Vega M 1989 Level and speciation of heavy metals in water of valladolid. *Inter J Environ Anal Chem* **37** 117-123.

Rain D J 1995 Lead in the environment In: *Handbook of Ecotoxicology*, (eds.) Hoffmann D T, Rattner B A, Burton G A, Cairns J, Lewis, London, UK, pp 350-391.

Sokal R R, Rohlf F J 1995 *Biometry, The Principles and Practice of Statistics in Biological Research.* Freeman, New York, USA, pp 400 - 450.

Tchernitchim N N, Vilvagra A, Tchernitchim A N 1998 Antiestrogenic activity of lead. *Environ Toxicol Water Qual* **13** 43-53.

Tokalioglu S, Kartal S, Elci L 2000 Determination of heavy metals and their speciation in lake sediments by flame absorption spectrometry after a four-stage sequential extraction. *Anal Chim Acta* **413** 33-40.

Tokalioglu S, Kartal S, Birol G 2003 Comparison of three sequential extraction procedures for partitioning of heavy metals in car park dusts. *J Environ Monit* **5** 468 - 476.

United Nations Environment Programme (UNEP) 1986 *Environmental Capacity: An Approach to Marine Pollution Prevention*, UNEP regional Seas Reports and Studies No 80 UNEP, pp 10-14.

Ure A J, Davidson C M 1995 *Chemical Speciation in the Environment.* Blackie, Glasgow, USA, pp 67-82.

Vazqvez C F, Delgado H D, Dela Huerta C J, Aguilera L G, Sharma UK 1993 Trace and heavy metals in San Andres Lagoon, Tamaulipas, Mexico. *Environ Inter* **19** 71-77.

Vincent C D, Lawlor A J, Tipping E 2001 Accumulation of AI, Mn, Fe, Cu, Zn, Cd & Pb by the Bryophyte *Scapania undulata* in three upland waters of different pH. *Environ Pollut* **114** 93-100.

World Health Organization (WHO) 1996 *Guidelines For Drinking Water Quality.* 2nd ed., World Health Organization, Geneva, Vol. 2.

Yusuf K A 2003 Nutrients and heavy metals in Owo River and its environmental Impact on Ologe Lagoon. Ph.D thesis, University of Ibadan, Ibadan, Nigeria.

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ADSORPTION OF ACRIDINE ORANGE ON SOME METAL OXIDES

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The adsorption studies of acridine orange from aqueous solution were carried out over calcium oxide, magnesium oxide and zinc oxide. The adsorbents were subjected to a few pretreatments and the effect of pretreatment was studied on the extent and mode of adsorption. The adsorption over various pretreated surfaces exhibited three kinds of adsorption pattern, namely, S-type, L-type and H-type. The mode of adsorption is explained on the basis of the adsorption isotherm. The H-type is explained as flatwise adsorption with some ionic micellisation. The L-type has been interpreted as multilayer adsorption (flatwise) with the formation of large ionic micelles. The S-type has been explained as edge on (monodisperse) adsorption. The Freundlich adsorption isotherm are applicable within the limited range of concentration of dye. In few cases, these equations are not applicable due to the complex adsorption mechanism.

Key words: Acridine orange, Metal oxides, Aqueous solution.

Introduction

The adsorption of the cationic dye acridine orange by Nasaponite and the colloidal properties of the aqueous solution were investigated by Garfinkel-Shweky (1995) using visible spectroscopy. It was found that organic cations were adsorbed by the mechanism of cation exchange. When small amount of the dye was adsorbed, the dye penetrates into the interlayer space and most of it undergoes metachromasy due to interaction between the aromatic entity and the oxygen plane of the clay. But at greater amounts of acridine orange the metachromasy results from the aggregation of dye in the inter particle space. In excess acridine orange, the clay was gradually peptized. Grafinkel-Shweky and Yariv (1997) observed that the adsorption of cationic dye acridine orange by different monoionic laponites leads to changes in the colloids properties of this synthetic mineral in aqueous solution. The organic cation was adsorbed by the mechanism of cation exchange. Small amounts of adsorbed dye keep the clay in the peptized state with all metallic cations. Greater amount of acridine orange resulted in the neutralization of electric charge of clay, and its flocculation. In excess acridine orange the charge of clay platelets became positive and the clay was peptized. The colloidal properties were studied by the absorbance curves in which the absorbance was described as a function of the degree of saturation with constant clay concentration or with constant dye concentration. In the absorbance curves three regions can be identified. The transition between first and second or second and third region depend on the exchangeable metallic cation initially present in the clay (Granfinkel-Sheweky and Yariv 1995). It has also been found that saturated adsorption amount of the dyes on activated carbon was corelated with the electrostatic forces between charges on the carbon surface and ionic dyes. There exist the electrostatic attractive or repulsive forces between the activated carbon and ionic dyes. The adsorption forces are the sum or difference of dispersion and electrostatic forces. This conclusion was further supported by the kinetic and thermodynamic parameter that was calculated from experimental data (Minguang 1997). The adsorption characteristics of types of dyes in aqueous solutions on 9 types of amorphous oxide gel were examined. The adsorptive ability was affected by the pH of the dye solution and composition of the gel (Motoshi *et al* 1990).

The Surface Enhanced Raman Scattering (SERS) spectra of acridine in Ag solution was studied by Seong *et al* (1991). The presence of halide ions was a pre-requisite for the observation of SERS. Different SERS spectra were obtained using different pH solutions. At neutral pH, acridine was adsorbed on the Ag surface via its N lone pair electrons while in acidic medium it adsorbs as the acridinium-chloride ion pair through the Cl atom. The charge transfer effect appears to play an important role along with the electromagnetic effect. On the other hand, photoreaction readily occurs in a highly basic medium. This was concluded in terms of the competitive adsorption of Cl and O ions on Ag. Nonetheless, the acidity of acridinum ion seemed to be far greater on the surface than

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in the bulk aqueous phase(Seong *et al* 1991). Effect of pH on adsorbed amount of methyl orange was studied by Shimada, *et al* (1992) on Al_2O_3. Red colored Al_2O_3 showed adsorption of methyl orange. Langmuir type isothermal curve was obtained (Shimada *et al* 1992). Safranine T in aqueous solutions was adsorbed by different samples of silica exhibiting representative S-, L- and H-type isotherms, mostly S-type curves were observed. A typical sigmoid type adsorption behavior was found for preparative layer chromatographic silica sample. The adsorption was inhibited in an alkaline media while acidic media promotes adsorption (Mirza *et al* 1992). Adsorption isotherm of 3,6 diamino acridine on Kaoline clay showed that the dye molecules penetrate into the interlayer space of the alumino-silicate layer of the clay, while in the case of alumina the dye molecules cover only the surface. This shows that dye existed as a monolayer in the adsorbed state.The emission of dye adsorbed on clays was quenched completely even in dilute solution which shows that Al^{+3} of the alumino-silicate layer of the clay might partly be responsible for the quenching of the adsorbed dye (Ramaraj *et al* 1992).

The work with clean metal surfaces has emphasised the complexities that undoubtedly occur when metal powders chemically deposited metal films, oxides of metals and non metals are used as adsorbents.This more complicated surface phenomenon cannot therefore be restricted. In this recent study we have investigated the behavior and the extent of adsorption of acridine orange on some inorganic metal oxides. The adsorption of acridine orange at room temperature was found to be suitable for surface area measurements on a wide variety of solids, including carbon, cement, fibres, organic pigments etc. This work will provide the guideline to the chemists for the adsorption of dyes on some organic and inorganic solids.

Experimental

Chemically pure grade solid acridine orange from E-Merck (Germany) was used without purification. Ethyl alcohol was distilled and used whenever required. Water was doubly distilled and used for the preparation of solution as well as for washing glassware. Sodium hydroxide and conc. HCI was used for preparing 0.1 N solution for washing metal oxides.The stock solutions of dye were prepared by dissolving an appropriate amount of the dye in the solvent followed by making up the volume up to mark. For adsorption purposes, further test solutions, ranging from 1-10 ppm for acrdine orange, was prepared.The adsorption studies were carried out with acridine orange over various adsorbents namely calcium oxide, magnesium oxide and zinc oxide for investigations. In a series of adsorption experiments, calcium oxide, magnesium oxide and zinc oxide were used as such without further treatment called as "fresh" calcium oxide, magnesium oxide and zinc oxide. In third series of adsorption experiments, these adsorbents were used after washing with 0.1 N HCI solution followed by heating at 200°C in the muffle furnace for 1-2 h and subsequently cooling gradually to room temperature called as "washed". All these solids were placed in desicator ($CaC1_2$) to avoid contact of moisture with the adsorbents.

Adsorption studies. To study adsorption phenomenon the following procedure was adopted. Prepared test solution ranging in concentration from 1 ppm to 10 ppm in 100ml measuring flasks. Weighed 1g adsorbent in a series of 50 ml measuring flasks. Then to each flask added test solution from above series, made the volume up to the mark. The flasks were then tumbled for about half an hour and then kept stationary under thermostating condition away from light for a period of an hour. The amount of solute adsorbed was calculated from the difference in initial reading (without adsorbent) and final concentration of the solution measured absorptiometrically on the spectrophotometer at λ_{max} 490 nm (Mirza *et al* 1995). The absorbance of the sample should be according to Beer-Lambert law. The amount of the adsorbate removed by adsorption (x) was determined by subtrac-ting final concentration (Ce) of solution from initial concentration (Co) and amount of the adsorption per gram of the adsorbent (x/m) was calculated by dividing x with amount of adsorbent taken (usually 1g). Plotting x/m against Ce directly proved the applicability of the Freundlich adsorption isotherm.

Results and Discussion

The results obtained from the adsorption studies of acridine orange on various adsorbents such as calcium oxide, magnesium oxide and zinc oxide are discussed in the following sections.

Three major types of adsorption isotherms regarding adsorption from solution of acridine orange on different metal oxides have been classified by Giles *et al* (1964).These are discussed as H-type, L-type and S-type. The S-type (Figs 1,3 and 7) is initially convex to the solution concentration and then a plateau. The H-type (Fig 6) adsorption isotherm starts with positive value against the equilibrium concentration, usually, a sharp rise on vertical axis. In such isotherms the solution has high affinity for the adsorbent. The L-type (all other figures) adsorption isotherm is initially concave to the solution concentration and then plateau. In L-type adsorption isotherm, initially it is like H-type, then a plateau, fol-

Table 1
Mode of adsorption and monolayer capacities of acridine orange on metal oxides

Adsorbent	X/m (mono)	X/m (max)	Isotherm type	Monolayer/Multilayer	Figure
Fresh MgO	1.29	6.46	S-type	Multilayer	1
MgO washed		2.87	8.44	L-type	
in 0.1N HCl	Multilayer	2			
MgO washed		0.846	9.4	S-type	
in 0.1N Na OH	Multilayer	3			
Fresh CaO	1.13	7.45	L-type	Multilayer	4
CaO washed		0.941	8.56	L-type	
in 0.1N Na OH	Multilayer	5			
Ca O washed		1.45	6.6	H-type	
in 0.1 N HCl	Monolayer	6			
Fresh ZnO	0.4	6.63	S-type	Multilayer	7
ZnO washed		0.134	3.34	L-type	
in 0.1N NaOH	Multilayer	8			
ZnO washed		0.632	1.36	L-type	
in 0.1 N HCl	Multilayer	9			

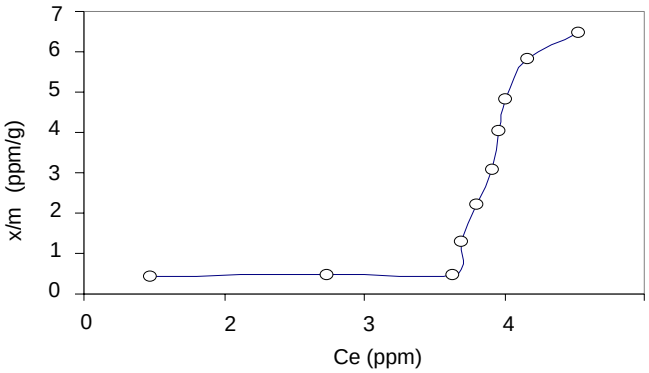

Fig 1. Adsorption of acridine orange on fresh MgO.

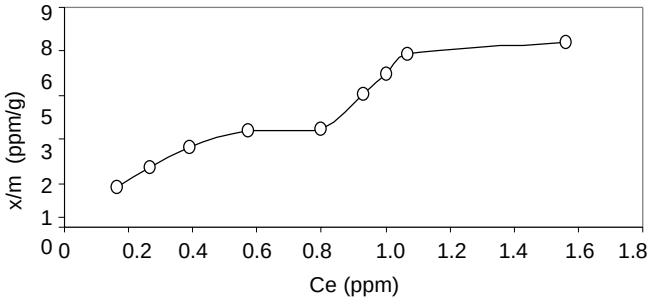

Fig 2. Adsorption of acridine orange on washed MgO in 0.1 N HCl solution.

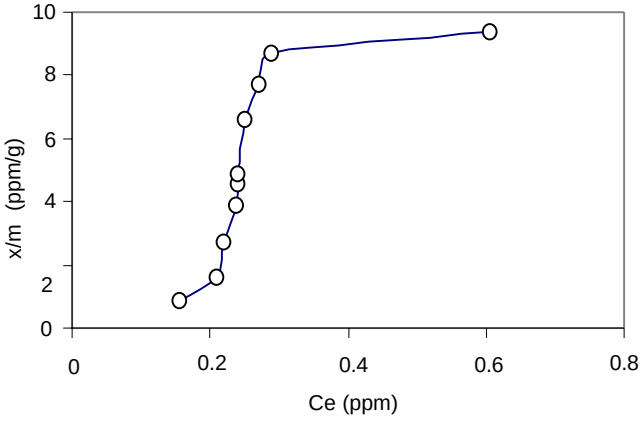

Fig 3. Adsorption of acridine orange on washed MgO in 0.1 N NaOH solution.

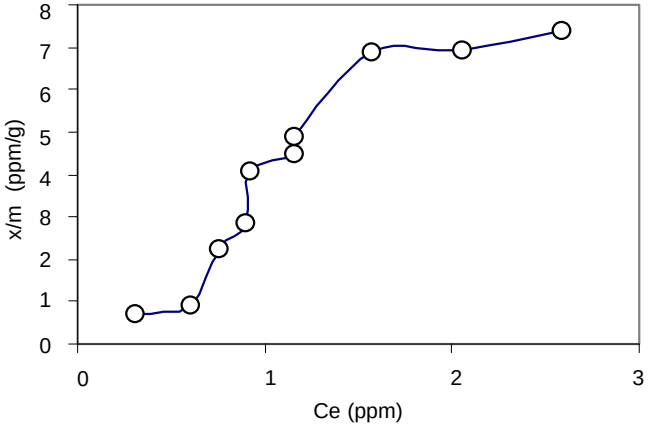

Fig 4. Adsorption of acridine orange on fresh CaO.

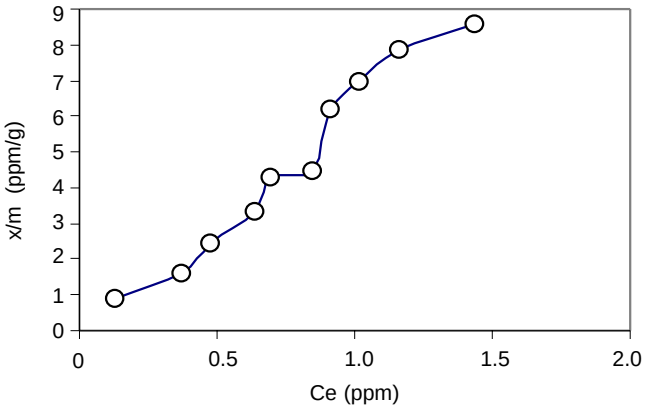

Fig 5. Adsorption of acridine orange on washed CaO in 0.1N NaOH solution.

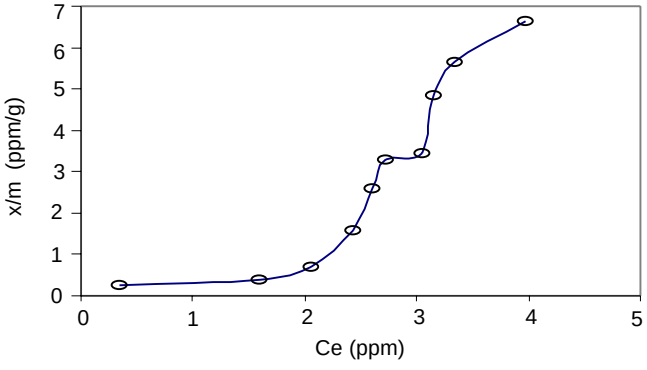

Fig 7. Adsorption of acridine orange on fresh ZnO.

lowed by another rise and possibly another plateau. The initial rise in adsorption depends upon the number of adsorption sites available. The plateau signifies the completion of monolayer i.e., saturation of surface and further adsorption takes place only on new surface developed. The length of the plateau in an isotherm indicates a high energy to be overcome before additional adsorption can occur on new sites. The start of a plateau indicates a monolayer completion. Further rise in an adsorption isotherm signifies the multilayer formation. The L-type isotherms are very commonly observed in the present studies. The isotherms are related with the mode of adsorption, orientation of adsorbed species, adsorption affinity monolayer or multilayer formation. The adsorption data like isotherm monolayer capacity, possibility of multilayer formation for acridine orange is shown in Table-1.

The monolayer adsorption capacity was determined from the plateau (Brunaur's point) on the isotherm. The results indicate that the metal oxides surface treated with sodium hydroxide possess an increase in adsorption capacity as compared to those of acid treated surfaces. This trend has been observed overall in metal oxides. This may be attributed due to acid-base interaction of the adsorbate with the adsorbent.

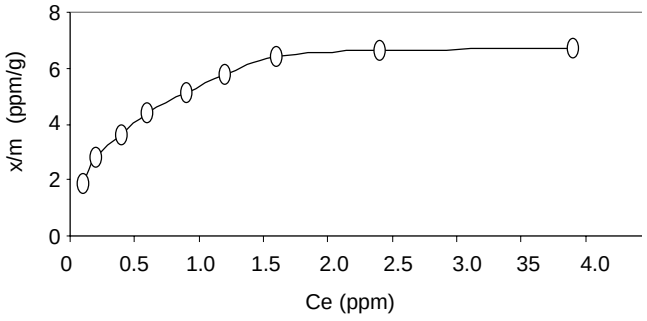

Fig 6. Adsorption of acridine orange on washed CaO in 0.1N HC1 solution.

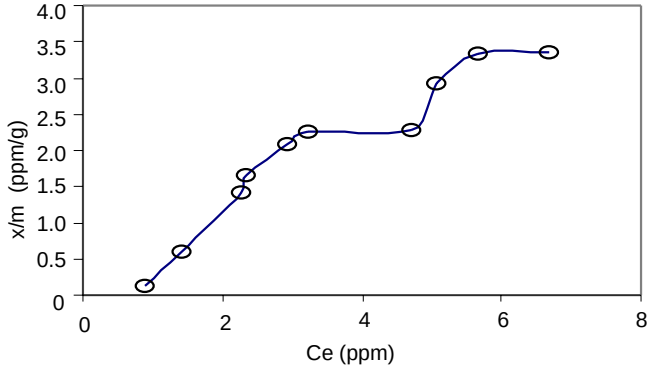

Fig 8. Adsorption of acridine organe on ZnO in 0.1 N NaOH solution.

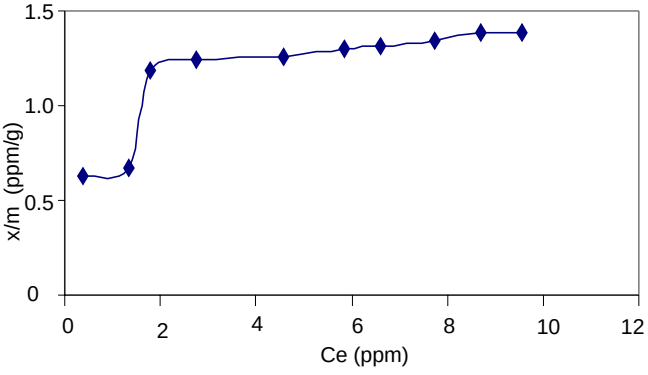

Fig 9. Adsorption of acridine orange on washed ZnO in 0.1N HC1 solution.

This is an interesting case because the basic dye shows the reverse case as was observed in our previous paper (Mirza *et al* 1988). This is due to the fact that metal oxides itself shows some interaction with the acridine. In case of fresh metal oxides , the adsorption capacity is less because there is repulsion between basic dyes and the solid oxides, whereas, in case of ZnO the situation is different because ZnO is not a basic in nature. But in case of base treated substances there is some type of interactions develop between solid metal oxides and the basic solution. In this regard there is greater interactions between the metal oxides and acridine dye. On the other hand when solid surface treated with HCl, then there

is strong force of attraction between solid surface and acid molecule appears and weak interactions between acid treated surface and dye occurs. Thus comparing to NaOH treated surface, the adsorption capacity in acid treated surface is further decreased. This anomolous behavior is due to the fact that repulsion occurs between oxide surface and dye molecules, as seen by metal oxides and acridine, both have some basic properties.Therefore as compared to acid or base treated substances adsorption is less in case of fresh metal oxide. But on the other hand if the metal oxides are treated with NaOH then metal oxides show some type of acidic behavior and more interaction with basic dyes . It is also clear that adsorption of dye on the surface of these oxides are less as compared to the acid treated dyes as well as base treated. Thus the adsorption of dyes on ZnO is less as compared to MgO and CaO due to the transition nature of the metal.

References

Grafinkel-Shweky D, Yariv S 1995 Adsorption of cationic dye acridine orange by different monomeric laponites. *Colloid Polym Sci* **273** (5) 453-463.

Grafinkel-Shweky D, Yariv S 1997 Metachromasy in claydye systems: The adsorption of acridine orange by Nasaponite. *Minerological Society* **32** (4) 653-663.

Giles C H, Nakhwa S N, Smith D 1960 A system of classification of solution adsorption isotherms and its use in diagnosis of adsorption mechanism and in measurement of specific surface areas of solids. *J Chem Soc* 3973-3993.

Minguang D J 1997 Adsorption of cationic and anionic dyes on activated carbon. *Colloid Interface Sci* **198** (1) 6-10.

Mirza M L, Wadood A, Iqbal J, Nawaz S 1995 Sorption of acridine orange on cellulose. *J Res Sci* **7**(1-2) 13-16.

Mirza M L, Wadood A, Iqbal J, Nisa Q 1992 Sorption behaviour of Safranine-T on silica. *J Nat Sci and Math* **31** (2) 185-191.

Motoshi N, Yoshio M, Hiroshi S 1990 Adsorption characteristics of amorphous mixed-oxide gels for dyes in aqueous solutions. *Hamamatsu* Japan **432** 81-87.

Seong T, Kim K, Myung S 1991 Adsorption and surface reaction of acridine in silver sol: Surface-enhanced Raman spectroscopic study. *J Phys Chem* **95**(22) 8844-8849.

Shimada H, Maru Y, Yasuoka T 1992 Development of teaching materials by using adsorption of methyl orange on alumina in aqueous solution. *KagaKuto Kyocku* **40** (3) 183-185.

Ramaraj R, Jeyanthy D R, Srinivasan C 1992 *A*dsorption characteristics and spectroscopic studies of 3, 6, diamino acridine adsorbed on clays. *J Chem Phys Biochem* **30A** (12) 1044-1046.

Mirza M L, Yasmin N, Iqbal J, Wadood A 1988 Studies on adsorption of Rhodamine-B on silica gel. *J Indian Chem Soc* ***LXV*** 28-30.

Biological Sciences

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Anatomical Characteristics of Rice Plants Influencing Resistance and Susceptibility to Yellow Stem Borer

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Plant anatomical characters presumably influencing resistance and susceptibility to yellow stem borer (YSB) infestation were studied. Rice varieties having broader and thicker sclerenchymatous hypodermis, compact parenchyma cells of ground tissue, small air spaces in the ground tissue, more vascular bundles with narrower spaces between vascular bundles, ridged stem surface containing vascular bundles and narrower pith are considered to be characters for resistance. Whereas thinner sclerenchymatous hypodermis, loose parenchyma cells of ground tissue, larger spaces between vascular bundles, wider pith and larger air cavities, might be responsible for the susceptibility to (YSB). In the present study TKM6, BR1 and Nizersail were found to be resistant to (YSB), while TN1, IR8, BR3, BR4 and BR14 were susceptible varieties.

Key words**:** Rice varieties, (YSB) yellow stem borer, Anatomical characters, Resistance.

Introduction

Stem borers are major pests of rice in Bangladesh. The predominant species is *Scirpophaga incertulas* (Walker). The other pyralid, *Chilo polychrysa* (Meyrick), *Scirpophaga innotata* (Walker) and the noctuid, *Sesamia inferens* (Walker) occur in varying proportions, depending upon the locality and the extent of cultivation of rice and other graminaceous crops. The yellow rice stem borers could be controlled considerably by modern organic insecticides, but the effectiveness of this measure depends on the proper timing of the insecticide application to coincide with the vulnerable stages of the pests. The insecticide control is temporary, and the use of insecticide is limited because they destroy natural enemies of the pest, as well as because of their toxicity to mammals and fish. Moreover, in areas where overlapping insect generations occur, repeated treatments are required to keep their numbers below levels that will cause economic damage. The problem is further complicated when frequent rains remove insecticide residues (Pathak 1967).

Thus, it is important to study control measures, which will result in cumulative reduction in the insect population. The use of relatively resistant varieties as a means of control in endemic areas, in addition to the other control methods, has great economic potential (Israel 1967). This method has been found to be particularly effective against insect pests, like yellow rice stem borer, which have high host-plant specificity and because of feeding habits and difficulty to reach with conventional methods of control. The host-plant resistant method operates at all levels of the pest population and is compatible with other methods of control.

Stem borer larvae have to eat their way into the stem, it would be expected that stems with thick culm tissues would offer resistance to larval boring (Pathak *et al* 1971). Experiments showed that varieties with a narrow stem lumen were less susceptible to borers (Seko and Kato 1950 a and b; Van and Guan 1959; Israel *et al* 1961). Stems with thick layers of lignified tissues were less infested, and distance between vascular bundles of the stem directly correlated with susceptibility. Varieties with vascular bundles arranged closer than the width of the larval head offered resistance to larval boring. Also, varieties with thick layers of sclerenchymatous tissue were generally less infested than varieties, which had thin layers. Van and Guan (1959) believed that thickness of sclerenchymatous tissue to be the major basis of resistance.

The present research work was undertaken to study the plant anatomical characters presumably influencing resistance and susceptibility to YSB infestation.

Materials and Methods

Resistant and susceptible varieties of rice plants were grown in the BAU Farm in aus and aman seasons of 1996. Twenty one rice varieties in aus (TKM6, TN1, IR8, IR29, BR1, BR2, BR3, BR6, BR7, BR8, BR9, BR12, BR14, BR15, BR16, BR20, BR21, Dular, Gomvir, Hashikalmi and Purbachi) and eleven rice varieties (TKM6, TN1, BR4, BR10, BR11, BR22, BR23, Kalizira, Nizersail, Purbachi and Tulsimala) in aman season were grown for this study. Specimens of rice stem from basal parts were collected after 100 days of transplantation and fixed in FAA (Formalin-aceto-alcohol) fixative. The stem parts were washed in running water for 5-6 h and dehy-

drated in a series of ascending grades of alcohol (i.e. 30, 50, 70, 80 and 90% upto absolute alcohol for 5-10 min each. The dehydrated specimens were kept in cedar wood oil for a period of ½ h to overnight. The cedar wood oil was removed form the tissue by placing it in xylene for ½ to 1 h. It was then transferred to melted paraffin, a commercial embedding compound of M.P 56 to 58°C and placed in an incubator at 60°C. Several changes of the paraffin (e.g. ½ xylene + ½ paraffin for 1 and ½ h liquid paraffin for 2 h pure paraffin at 60°C for overnight) were made over a period of approximately 8 h to overnight and the tissue was embedded in it and blocks were prepared.

A rotary microtome adjusted to cut section 5-10 microns thick and was used. The ribbon of section was placed in hot water bath at a temperature of 50°C and after the film was uniformly stretched the ribbon of sections was transferred to moistened slide that had been coated with a very thin film of Mayer's egg albumen fixative. Slides were usually left on the hot plate (approximately 45°C) overnight for drying. Heidenhains iron hematoxylin and safranin following the methods of Grideley (1960) and Metcalfe (1960), stained the sections in slides. The stained sections were covered by a cover slip, mounted on Canada balsam and these were examined under microscope to study the variation of anatomical cha-racteristics of resistant and susceptible varieties.

Results and Discussion

Anatomy of stem of rice varieties in transverse section is shown in Fig 1-8. The hypedermis of rice variety TKM6, is found to be composed of three layers of heavily lignified sclerenchymatous cells (Fig 1a and 1b). Vascular bundles are fairly and closely arranged in two distinct circles, the outer one is either within or attached to the hypodermis and the inner one is distributed in the middle of the ground tissue. There are five vascular bundles in the outer circle and six in the inner circle (Fig 1a).

The hypdermis of BR1 consists of three layers of sclerenchymatous cells. The parenchyma cells of ground tissue are much compact. Vascular bundles are arranged in two rows, the outer row is in the middle of the ground tissue (Fig 2a and 2b). A half ring of smaller sized compact parenchymatous tissue connects the vascular bundles of outer circle. A small air cavity is seen within the half ring. The arrangement of one vascular bundle of outer and inner circle is in the same radial line whereas there is an another vascular bundle in the inner circle appearing just below the half ring. There are two vascular bundles in the outer row and four in the inner row (Fig 2a).

The hypodermis of Nizersail consists of three layers of sclerenchymatous cell. There are two rows of vascular bundles; the outer row is located within the hypodermis and the inner is in the ground tissue (Fig 3a and 3b). The surface of the stem is not smooth, but with some ridges each containing one vascular bundle. There are four vascular bundles in the outer row and five in the inner row (Fig 3a).

The hypodermis of TN1 rice variety consists of three layer of sclerenchyma (Fig 4a and 4b). The parenchyma ground tissues are loosely arranged. There are two rows vascular bundles; the outer row embedded in the hypodermis forms a slight ridges on the epidermis and the inner row is distributed in the middle of the ground tissue. Five and four vascular bundles are observed in the outer and inner rows, respectively (Fig 4a).

The hypodermis of IR8 variety consists of two to three layers of sclerenchyma. Hair like projections is observed in the cuticle. Vascular bundles are arranged in two distinct circles, the outer circle is in the hypodermis forming slight ridges in the epidermis and the inner circle is in the middle of the ground

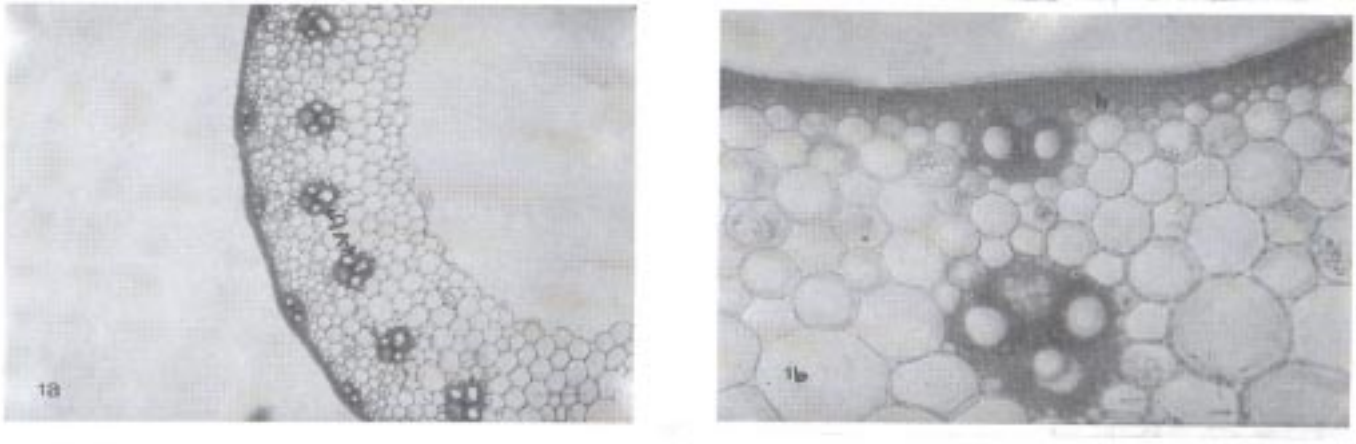


Fig 1a&b. Transverse section of the stem of TKM6 showing fairly closely arranged vascular bundles (1a) and heavily lignified hypodermis (1b) (1a, X85; 1b, X340). (h, hypodermis; vb, vascular bundle).

tissue with prominent protoxylem lacuan (Fig 5a and 5b). Three vascular bundles in the outer circle and four in the inner circle are seen (Fig 5a). Intercellular spaces of ground tissues are moderate.

Transverse sections of stem of BR3 rice are shown in Fig 6a and 6b. Three to four layers of sclerenchymatous cells are seen in the hypodermis. Epidermis forms slight ridges in which vascular bundles of outer circle are located. The vascular bundles of inner circle are not properly arranged in a row but centered in the middle of the outer circle with a prominent protoxylem lacuna. There are four vascular bundles in the outer circle and four to five in the inner circle (Fig 6a). The parenchyma cells of the ground tissue appear to be loosely arranged. Intercellular spaces are moderate.

In the transverse sections of stem of BR4 rice variety three layers of sclerenchyma are observed in the hypodermis. A row of conspicuous air cavities of unequal sizes and variable outlines, formed by the fusion of parenchymatous tissue, is

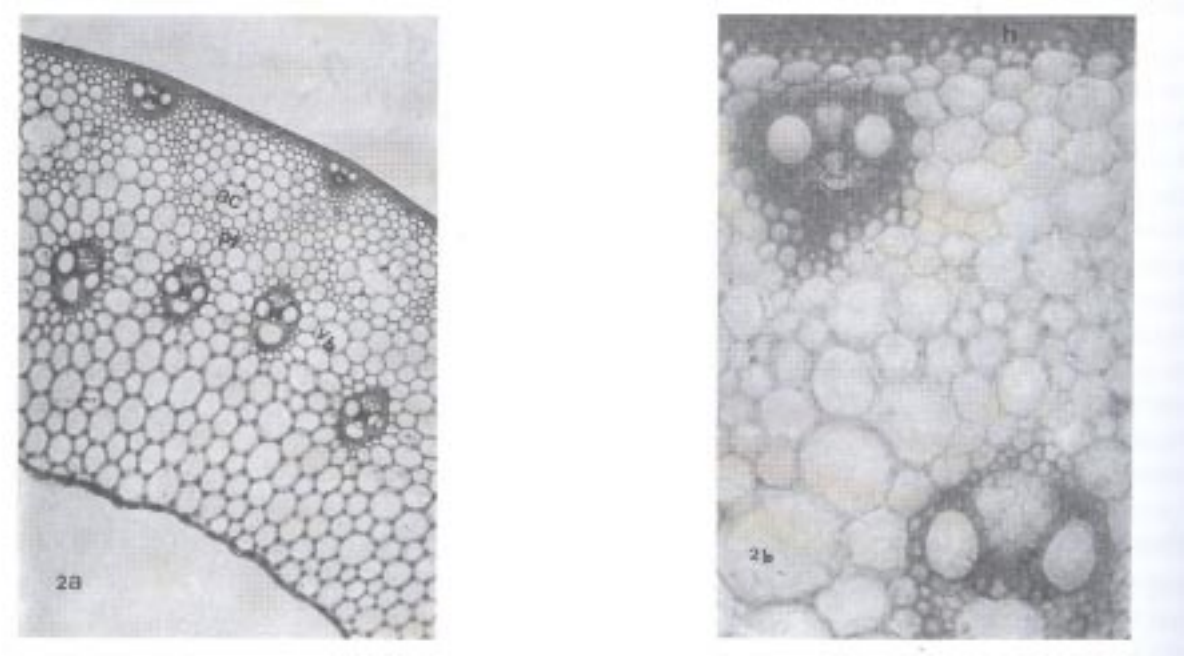


Fig 2a&b. Transverse section of the stem of BR1 showing highly lignified hypodermis (2b) and radially arranged double circled vascular bundles, the outer one connected by a half ringed compact parenchyma beneath which lie an additional vascualr bundle (X85). (ac, air cavity; h, hypodermis; pt, parenchymatous tissue; vb, vascular bundle).

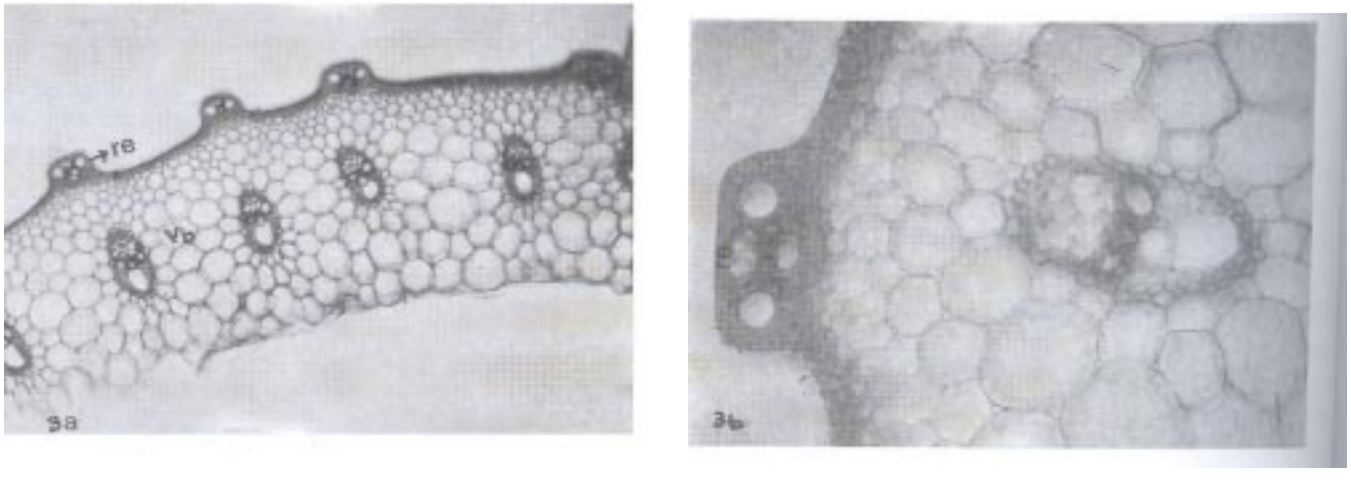
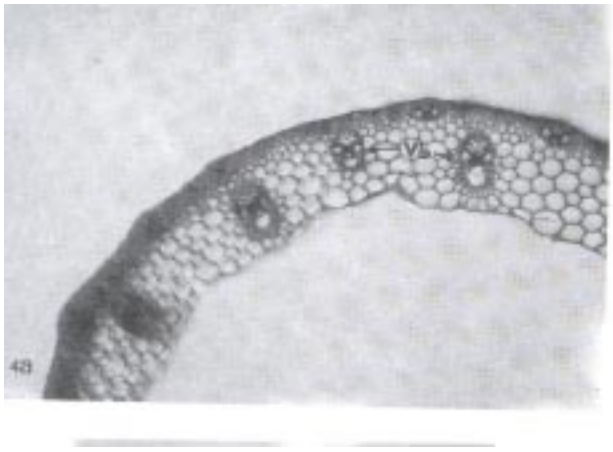


Fig 3a&b. Transverse section of the stem of Nizersail showing ridged epidermis bearing outer rows of vascular bundle, the inner rows of vascular bundle being grounded in the ground tissue (3a, X85; 3b, X340). (re, ridged epidermis; vb, vascular bundle).



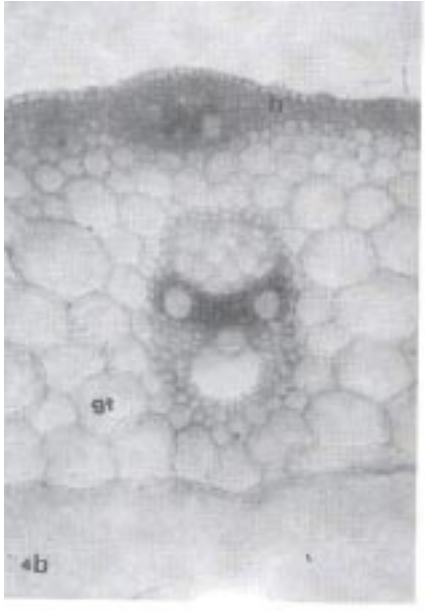
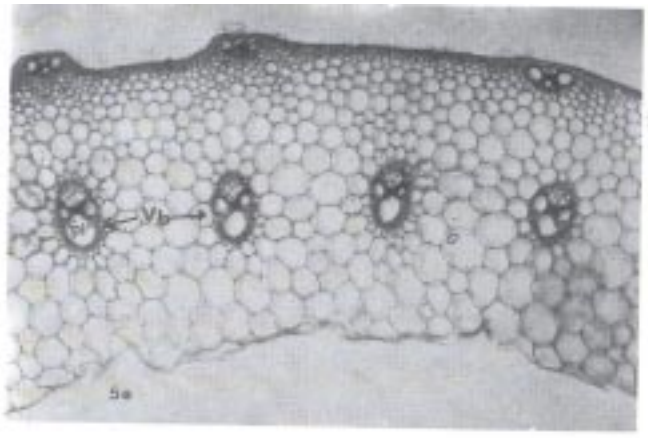


Fig 4a&b. Transverse section of the stem of TN1 showing loosely arranged ground tissues, the outer rows of vascular bundle embedded in the hypodermis and the inner rows distributed in the middle of the ground tissue (4a, 85; 4b, X340). (gt, ground tissue; h, hypodermis; vb, vascular bundle).



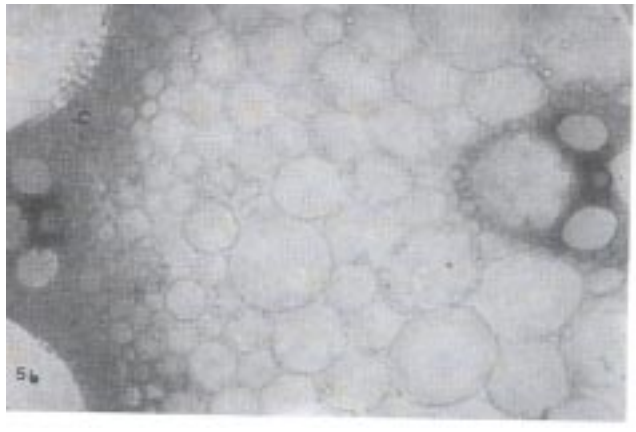


Fig 5a&b. Transverse section of the stem of IR8 showing hypodermis bearing outer rows of vascular bundle. The vascular bundle of inner rows shows prominent protoxylem lacuna (5a, X85; 5b, X340). (h, hypodermis; pl, protoxylem lacuna; vb, vascular bundle).

present in between the outer and inner circles of vascular bundles. A half ring of compact parenchyma surrounds each air cavity and this half ring connects the vascular bundles of outer circle. Vascular bundles are fairly and widely spaced and arranged in two distinct circles, the outer circle being closed to the hypodermis and the inner circle is in the ground tissue between the row of air cavities and the hollow centre of the culm (Fig 7). Only two vascular bundles in the outer circle and three in the inner circle are found (Fig 7).

In the transverse sections of stem of BR14 rice variety, three to four layers of sclerenchymatous cells are found in the hypodermis. A row of air cavities is observed in the ground tissue just in between the outer and inner circles of vascular bundles. The vascular bundles of outer circle are closed to the hypodermis, whereas the inner row is situated in between the air cavities and the hollow culm (Fig 8a and 8b). A half ring of fused parenchyma surrounds each air cavity. There are three vascular bundles in the outer circle and three in the inner circle (Fig 8a). The parenchyma cells of ground tissue are rather less compact.

The rate of infestation of YSB to different rice varieties indicates that TKM6, BR1 and Nizersail are resistant and TN1, IR8, BR3, BR4 and BR14 are susceptible to the insect pest (Shahjahan 1995). The results of the present study are in agreement with earliers (Akinsola 1973; Alam *et al* 1985; Chaudhary *et al* 1984; Heinrichs *et al* 1982) who reported that TKM6 and BR1 were resistant and TN1, IR8 and BR3 were susceptible.

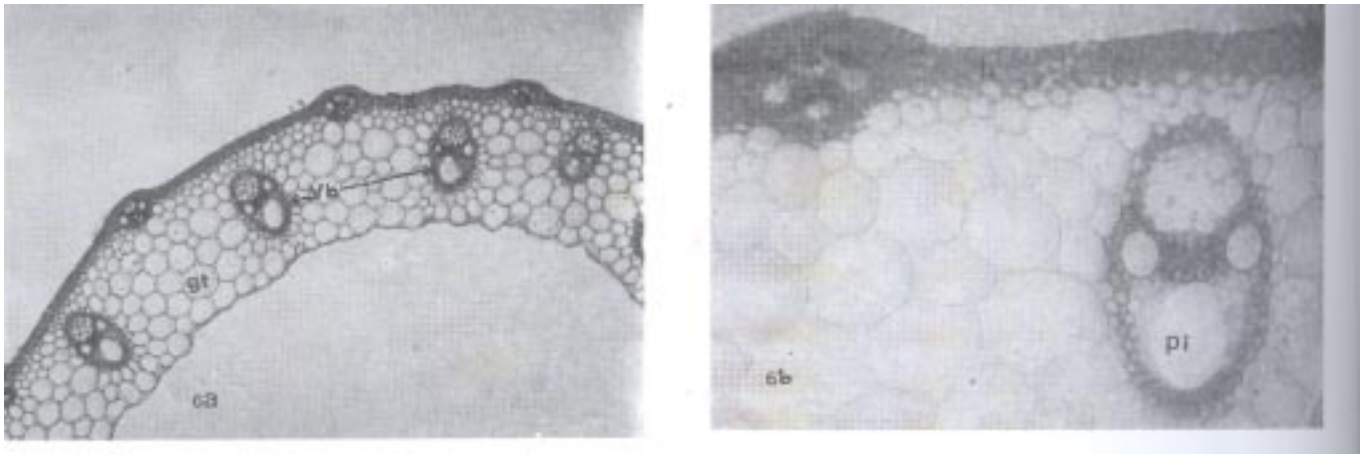


Fig 6a&b. Transverse section of the stem of BR3 showing outer circle of vascular bundles embedded in the hypodermis, the inner circle centered in the ground tissue in between the two outer bundles with a prominent protoxylem lacuna (6a, X85; 6b, X340). (h, hypodermis; gt, ground tissue; pl, protoxylem lacuan; vb, vascular bundle).

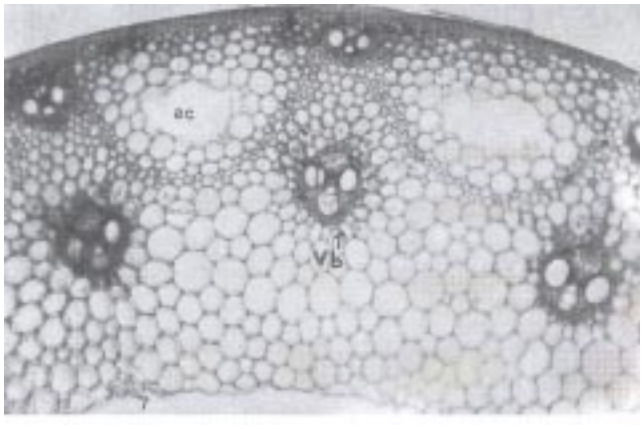


Fig 7. Transverse section of the stem of BR4 showing conspi cuous air cavities in between outer and inner circles of vascular bundle (7, X85). (ac, air cavity; pt, parenchymatous tissue; vb, vascualr bundle).

Rice varieties having comparatively broad and thick sclerenchymatous hypodermis and small air spaces in the ground tissue are considered to be resistant to YSB (Van and Guan 1959; Israel *et al* 1961; Pathak 1967). Seko and Kato (1950 b,c) reported that varieties having narrower spaces between vascular bundles also exhibits resistance to YSB. Rice varieties with ridged stem surface having vascular bundles were generally reported to be less infested by YSB than those with smooth surface (IRRI 1965). In the present study it is revealed that in BR1 rice variety the hypodermis consists of compact cells and has more vascular bundles with narrower spaces in inner circle. In TKM6, the spaces between the vascular bundles are much narrower. The additional vascular bundle in the inner circle may be one of the factors of YSB resistance. There are no air spaces in the ground tissue of TKM6 and Nizersail. Thicker hypodermis and ridges containing the vascular

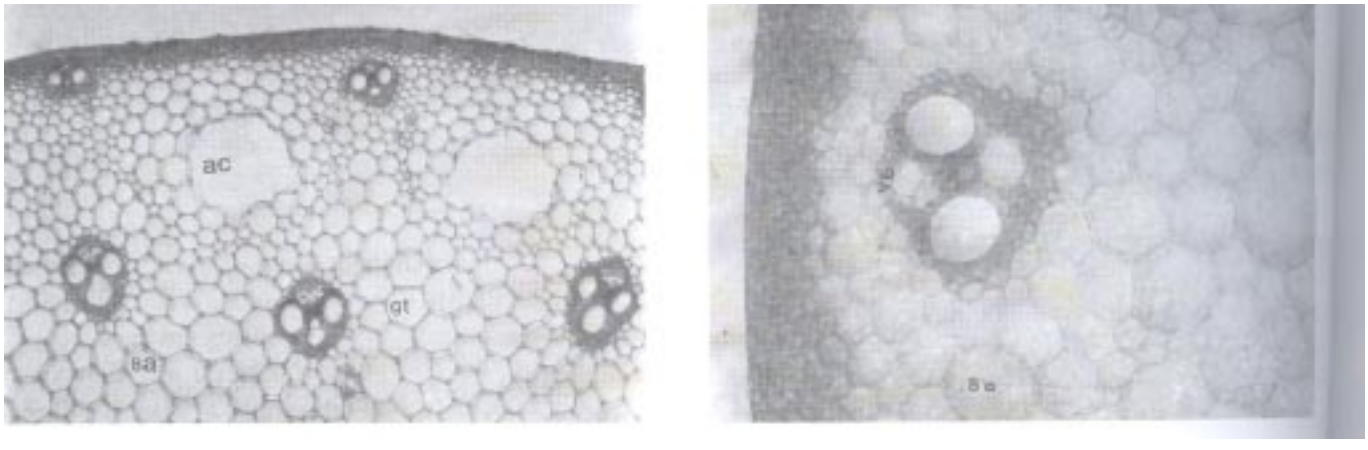


Fig 8a&b. Transverse section of the stem of BR14 showing conspicuous air cavities in the ground tissue in between outer and inner circles of vascular bundles (8a, X340). (ac, air cavity; gt, ground tissue; vb, vascular bundle).

bundles in the outer surface of epidermis might also contribute to resistance to YSB in Nizersail.

Thinner hypodermis, loose parenchyma cells of ground tissue, larger spaces between vascular bundles and wider pith might be responsible for the susceptibility of TN1, IR8, BR3, BR4 and BR14 to the YSB (Seko and Keto 1950 a and b; Israel *et al* 1961; IRRI 1964 and 1965; Israel 1967; Pathak *et al* 1971). In addition, larger air spaces of BR4 and BR14 make them more susceptible to the insect pest (Israel 1967).

References

Akinsola E A 1973 Resistance to the yellow borer, *Tryporyza incertulas* (Walker) in rice varieties. M.Sc. Thesis, Faculty of the College of Agriculture, University of the Philippines at Los Banos, Laguna, The Philippines.

Alam S, Karim A N M R, Ahmed M S 1985 Researches on rice stem borers at Bangladesh Rice Research Institute, Entomology, BRRI. *Joydevpur* pp 163.

Chaudhary R C, Khushk G S, Heinrichs E A 1984 Varietal resistance to rice stem borers in Asia. *Insect Sci Appl* **5** 447-463.

Gridley M F 1960 *Manual of Histologic and Special Staining Technique*. Mc Graw-Hill Book Company, Inc., New York, USA, pp 207.

Heinrichs E A, Medrano F G, Sunio L, Rapusas H R, Romena A, Vega C, Viajante V, Centina D, Domingo I 1982 Resistance of IR varieties to insect pests. *Int Rice Res Newsl* **7**(3) 9-10.

IRRI 1964 *Varietal Resistance to the Stem Borers*. Annual Report for 1963, International Rice Research Institute, Los Banos, The Philippines, pp 158-166.

IRRI 1965 *Entomology*. Annual Report for 1964, International Rice Research Institute, Los Banos, The Philippines, pp 157-185.

Israel P, Vademoorthy G, Rao Y S 1961 Varietal resistance to gall midge, *Pachydiplosis oryzae* Woo-Mason and other insect pests of paddy. Working paper No. 21, 4th Meeting Working Party on Rice Prod. and Protect. FAO. Int. Rice Comm., New Delhi, India, pp 5 (mimeo).

Israel P 1967 Varietal Resistance to Rice Stem Borers in India. In: *The Major Insect Pests of the Rice Plant*. John Hopkins University Press, Baltimore, Maryland, pp 391-403.

Metcalfe C R 1960 *Anatomy of the Monocotyledons*. 1. Graminae. Oxford University Press, Amen House, London, UK, EC4, pp 731.

Pathak M D 1967 Varietal resistance to rice stem borers at IRRI. In: *The Major Insect Pests of the Rice Plants*. Proc. Symp.Int. Rice Res. Inst., Sept. 1964. Johns Hopkins Press, Baltimore, Maryland, pp 405-418.

Pathak M D, Andres F, Galacgac N, Ramos R 1971 Resistance of rice varieties to striped rice borer. *IRRI Tech Bull* **11** pp 69.

Seko H, Kato I 1950a Studies on the resistance of rice plant against the attack of rice stem borer, *Chilo simplex* Butler. II. Interrelation between plant character and larval instrusion in the first generation of rice stem borer (Preliminary report). In Japanese, English summary. *Proc Crop Sci Soc Jap* **19** 204-206.

Seko H, Kato I 1950b Studies on the resistance of rice plant against the attack of rice stem borer, *Chilo simplex* Butler. III. Interrelation between plant character and the occurrence of incipient damages in the first generation of rice stem borer (Preliminary report) (In Japanese, English summary). *Proc Crop Sci Soc Jap* **19** 206-208.

Shahjahan M 1995 Studies on the resistance of yellow stem borer, *Scirpophaga incertulas* (Walker), in relation to physical and chemical factors of rice plants. PhD thesis, Bangladesh Agril. Univ. Mymensingh, Bangladesh, pp 274.

Van T K, Guan K G 1959 The resistance of *Oryza ridleyi* hook to paddy stem borer (*Chilo suppressalis* Walk.) attack. *Malayan Agric J* **42**(4) 207-210.

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A NEW SPECIES OF *TYLENCHORHYNCHUS* WITH COMMENTS ON *GEOCENAMUS RUGOSUS* (THORNE AND MALEK 1968) BREZESKI 1991 FROM SINDH

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Tylenchorhynchus fatimae sp.n was collected around the roots of coconut field, Malir and coconut plants from PCSIR campus, Karachi is described and illustrated. *T. fatimae* sp.n comes close to *T. brassicae* (Siddiqi 1961), *T. tuberosus* (Zarina and Maqbool 1994), *T. tritici* (Golden *et al* 1969) and *T. rubustoides* (Thorne and Malek 1968) but differs in stylet length, general shape of the body and DGO. *Geocenamus rugosus* (Thorne and Malek 1968) Brezeski 1991 is reported for the first time from Pakistan are listed. Measurements of *Geocenamus rugosus* are given from paratype and rest of the measurements from illustration are mentioned in Table 1.

***Key words*:** Coconut, Nematodes , *Tylenchorhynchus fatimae* sp.n.

Introduction

The genus *Tylenchorhynchus* was established by Cobb (1913) when he discoverd that *T. cylindricus* was found in soil from reclaimed coastal swamp lands in Southern California. In his excellent review of the genus *Tylenchorhynchus,* Allen established its taxonomic criteria in 1955. Golden *et al* (1987) raised the subfamily *Tylenchorhynchinae* (Eliava 1964) to family rank and provided a key to the six genera included at that time. Hooper (1978) discussed the history of the genus. In descri-bing four new *Tylenchorhynchus* species Sturhan (1966) recognized 73 valid species in the genus and indicated 10 additional forms as species inquirendae. By 1970, 96 species were described. Most of the species included under *Tylen-chorhynchus* have now been placed in new genera by diffe-rent workers. The most important characteristic used in distinguishing these genera is the number of lines, ranging from three to six, in the lateral field. *Tylenchorhynchus* now contains those species having four lines in the lateral field. Tarjan (1973) gave a valuable key and a table of diagnostic data on species. Siddiqi and Jairajpuri (1982) proposed resurrection of the subgenus *Bitylenchus* (Filpjev 1934) under the genus *Tylenchorhynchus* and Jairajpuri (1982) gave a key to 16 species of the subgenus *Bitylenchus.* By 1984, 79 species of *Tylenchorhynchus* were described.

Here one new specie of *Tylenchorhynchus* is described from Pakistan. Comments are added on *Geocenamus rugosus* which was previously described by Siddiqi (1963), who then put his genus into *Merlinius* (1970) and later he kept his genus into *Scutylenchus* as *S. rugosus* (1979). Whereas, Brezeski (1991) lateron, after a comprehensive studies placed this genus into *Geocenamus* as *G. rugosus* (1991). We have studied the specimens collected from Sindh, Pakistan and reported it.

*Author for correspondence. **Name of the specie is given in the memory of Miss Nighat Fatima, who lived for Nematology and died for Nematology.

Materials and Methods

The soil samples were collected around the roots of various Date Palm trees in PCSIR Campus and later soil samples were also taken from coconut farms, Malir. These samples were brought to the laboratory and kept at 5°C in incubator. These were processed by following Cobbs sieving and gravity method (Cobbs 1918) and later modified by Baermann's method. Nematodes were collected under stereoscopic binocular, relaxed by gentle heat and processed by slow method of glycerin according to Thorne (1961) and mounted in anhydrous glycerin and sealed with zut cement. Illustrations were made with the help of Camera Lucida attachment.

Tylenchorhynchus fatimae n.sp. (Fig 1)**

Holotype female	L=0.6 mm; a=37.2; b=5.32; c=15.2; c'=2.4; V=54.6
Paratype females	L=0.57-0.65 (0.61±1.78)mm; a=27.3-34.2 (30.75±4.8); b=5.2-6.5 (5.85±0.91); c=13.5-16.8 (15.15±2.3); c'=2.48-3.2 (2.84±0.5); V=54-56.4% (55.2±1.2); spear=14.6-14.8 (14.7±0.1)µm
Paratype male	L=0.62-0.64 (0.63±1.4)mm; a=35-38 (36.7±2.4); b=4.2-5.5 (4.85±0.56); c=14.4-15.2 (14.8±0.56); T=64.2-67

(59.1±5.1); spicules=22.2-23.4
(22.8±0.8)µm; gubuernaculum=11.5-12.2
(11.5±0.35) µm; spear=14.5-14.9
(14.7±0.2)µm

Description. Heat of the body relaxed nematode ventrally arcuate slightly. Cuticle marked by fine annulations, 1.2 µm thick near the mid body. Lip region prominent, broadly rounded, 3.2-3.6 µm in height and 6.2-6.6 µm wide; spear knobs 2.3-2.44 µm in breadth. Lateral field 4.4-4.5 µm wide in middle region of the body containing four lateral lines, outer incisures are crenate. Orifice of dorsal esophageal gland 2.4-2.6 µm behind spear base; body width at basal knobs 9.6-10.2 µm. Procorpus prominent, 34-35 µm in length and metacorpus prominent 9-10 µm with valve; Isthmus tubular, narrow, 24-33 µm long; nerve ring encircling isthmus in the middle. Excretory pore located at the distance of 88-92.5 µm from the anterior region. Hemizonids located just above a few annules anterior to distinct excretory pore. Basal oesophageal gland cylindrical 10-12 x 19-22 µm with prominent nucleus at the center; cardia conoid, 4.5-5.4 µm in length, vulva transverse, slit like and vulval opening covered by the lateral cuticular membrane, 9-11 µm long; oocytes arranged in a single row; spermathica prominent, 6-9 µm in diameter with rounded sperms. Posterior intestinal sac present. Tail subcylindrical, 43.5 µm in length bearing 35-40 annules; tail terminus smooth. Phasmids located anteriorly to the middle of the tail.

Male. Similar to female in general shape of the body. Spear 14.2 µm in length; spicules paired, cephalated with distal flanges, 21.5 µm long; gubernaculum and bursa typical of the genus with crenate margin enveloping tail.

Type locality. Collected from the soil around the roots of coconut palms, in PCSIR Campus, Karachi and also from the soil samples taken from coconut plants, Adil farms, Malir area.

Type slide. Holotype and paratype slides deposited in Nematological Collection, Food and Marine Resources Research Centre, PCSIR, Karachi.

Diagnosis. *Tylenchorhynchus fatimae* n.sp comes close to *T. brassicae* (Siddiqi 1961) in general shape of the body but differs in having smaller spear, anteriorly located vulva and presence of vulvular flap. Spicules in *T. fatimae* n.sp. 22-23.4 µm whereas in *T. brassicae* 23.6-25.3 µm. *T. fatimae* n.sp. looks apparently similar to *T. tuberosus* (Zarina and Maqbool 1994) in general body shape but differs in body length, small stylet (stylet in *T. fatimae* 14.4-14.8 µm. *T. tuberosus* 20-22 µm) *T. tuberosus* also differs from *T. fatimae* n.sp. in DGO (DGO in *T. tuberosus* 2.4-3.2 µm. *T. fatimae* 2.4-2.5 µm)

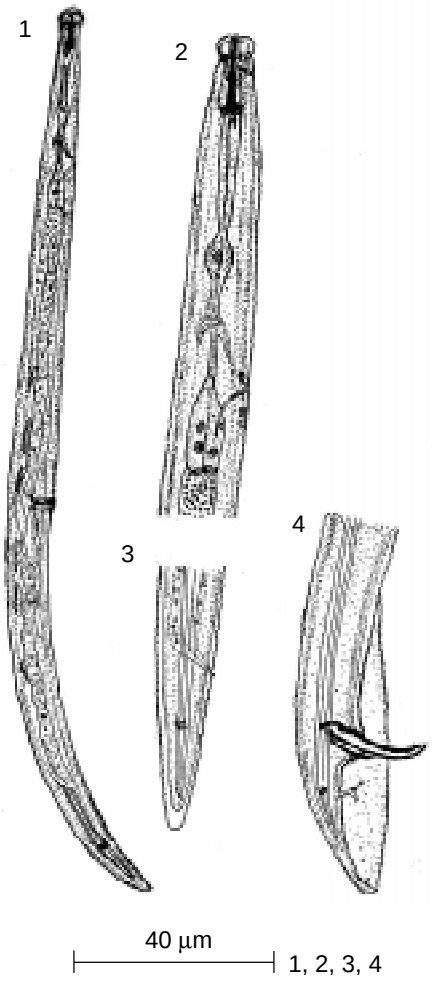

Fig 1. ***Tylenchorhynchus fatimae*** n. sp., 1=Fully body length; 2=Esophageal region; 3=Female tail and 4=Male tail.

T. fatimae is also similar to *T. tritici* (Golden *et al* 1987) in general shape of the body but differs in D.G.O length (DGO in *T. tritici* 2.1-2.5 µm. *T. fatimae* 2.4-2.5 µm). *T. fatimae* n.sp is also different from *T. tuberosus* in having well developed fasciculi (serpentine canals). Whereas small serpentine canals present in *T. tuberosus, T. fatimae* n.sp. also differs from *T. rubustoides* (Thorne and Malek 1968; Siddiqi 1986) in shape of the lip region, short stylet being 15-16 µm in *T. rubustoides* and 14.5-14.9 µm in *T. fatimae*.

Geocenamus (Thorne and Malek 1968) Brezeski 1991. *Diagnosis (amended)*. Belonolaimidae. Body length 0.5-1.1 µm. Lateral field with six distinct incisures, although additional lines may be present. Longitudinal body striations are either present or absent. Deridis may or may not be present opposite to excretory pore in four incisures of the lateral field. Labial region continues with body contour or set-off to varying degree. Labial disc rounded, hexagonal or slightly

laterally elongated. Lateral sectors of labial region may be somewhat smaller than dorsal and ventral sectors. Anterior head annuli is divided into six sectors by longitudinal ridges (not observed in *G.varianius*). Stylet thin to robust,length of known species vary from 9-132 µm, cone is seldom larger than 55-60% of total stylet length.Dorsal esophageal gland orifice 1-3 µm posterior to stylet knob. Oesophagus off-set from intestine. Female tail cylindrical to conical, c'usually 2-4, thickened terminal cuticle not pronounced. Male spicules are without distinct velum. Gubernaculum, epiptgyma and hypotgyma are present.

Type species. *Geocenamus tenuidens* (Thorne and Malek 1968; Brezeski 1991).

Syn. *Tylenchorhynchus polonicus* Sycyziel 1970.
Syn. *Merlinius polonicus* (Sycyziel 1970) Tarjan 1973.
Syn. *Geocenamus polonicus* (Sycyziel 1970) Sturhan 1981.

***Geocenamus rugosus* (Thorne & Malek 1968) Brezeski, 1991 (Fig 2)**

5 OO L=0.70-0.82 (0.76±2.6)µm; a=25.6-30.2 (27.9±3.2); b=5.3-5.6 (5.45±0.21); c=13.3-18.2 (15.75±3.46); c'=2.2-2.5 (2.35±0.21); V=53-56 (54.5±2.12); stylet=21-27 (24±4.2)µm; nerve ring=90-110 µm; lip width=13-14.7 (13.85±1.2),
Male not found.

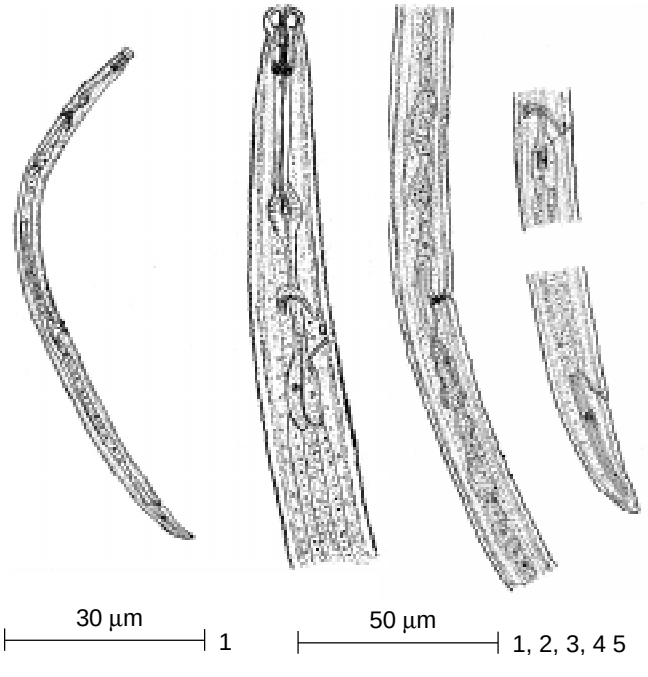

Fig 2. *Geocenamus rugosus*; 1=Fully body length; 2=Esophageal region; 3=Vulval region; 4=Esophageal gland and 5=Tail region.

Comments

Body slightly arcuate after relaxing by gentle heat, annulations of body prominent, lateral field marked with six distinct incisures, the outer most crenate. Lip width with 5-7 annules, set-off by deep constriction. Internal framework of head moderately developed, lip width (13.85 ± 1.2) µm, stylet 21-27 (24 ± 4.2) µm long, stylet knobs 4-4.5 (4.25 ± 0.35) µm diameter on anterior face. Oesophagus tapering to a narrow tube where it attaches to the median bulb. Excretory pore are 110-113 µm from anterior region or opposite to anterior of the basal bulb. Cardia somewhat discoid. Intestine is packed with large and dark granules. Female reproductive system diadelphic and amphidelphic outstretched, vulva prominent. Female tail composed of 16-24 annules of equal width. Tail slightly conoid, tapering to a smooth blunt end, phasmid somewhat variable in position. (This is the first record of this specie from Pakistan).

Remarks. *G. rugosus* (Brezeski 1991) is similar *to G. quettensis* (Maqbool *et al* 1984) in body shape but differs in stylet length (*G. rugosus* 24 ± 4.2 µm, *G. quettensis* 21 µm), in the length of excretory pore from anterior region and also differs in tail annulations (*G. rugosus* 16-24 µm, *G. quettensis* 24-30 µm). *G. rugosus* similar to *G. baluchiensis* (Maqbool *et al* 1985) in body shape but differs in c' value (*G. rugosus* 2.35 ± 0.21 µm, *G. baluchiensis* 3.0 ± 0.05 µm) and in stylet length (*G. rugosus* 24 ± 4.2 µm, *G. baluchien*sis 17 ± 0.4 µm). It also differs in length of excertory pore (*G. rugosus* 110-113 µm, *G. baluchiensis* 90-95 µm). *G. rugosus* similar to *G. niazae* (Maqbool *et al* 1983) in body shape but differs in stylet length (*G. rugosus* 21-27 µm, *G. niazae* 14-16 µm) and also in tail annuli (*G. rugosus* 16-24, 45-52 in numbers).

Other species

G. adakensis (Bernard 1985) Brezeski 1991
G. baluchiensis (Maqbool *et al* 1985) Brezeski 1991
G. bravaricus (Bavaricus 1966; Siddiqi 1970) Brezeski 1991
G. brevidens (Allen 1955; Siddiqi 1970) Brezeski 1991
G. koreanus (Choi and Geraert 1971) Brezeski 1991
G. niazae (Maqbool *et al* 1988) Brezeski 1991
G. nanus (Allen 1955; Siddiqi 1970) Brezeski 1991
G. quadarifer (Andrassy 1954) Brezeski 1991
G. quettensis (Maqbool *et al* 1984) Brezeski 1991
G. rugosus (Siddiqi 1963) Brezeski 1991
G. siddiqii (Mulk 1978) Brezeski 1991
G. sobolevi (Mukhina 1970) Brezeski 1991
G. stegus (Thorne and Malek 1968) Brezeski 1991
G. thomasi (Skwiriz 1984) Brezeski 1991
G. tetylus (Anderson and Ebsary 1982) Brezeski 1991
G. variabilis (Shahalina 1983) Brezeski 1991
G. varians (Thorne and Malek 1968) Brezeski 1991

***Geocenamus koreanus* (Choi and Geraert 1971) Brezeski 1991 (Fig 3)**

5 OO L=0.86-0.95 (0.905±0.056)mm; a=37-38.5 (37.75±1.06); b=7.2-8.6 (7.9±0.98); c=12-13.6 (12.8±1.13); c'=3.4-4 (3.7±0.42); V=68-69 (68.5±0.7); stylet=21.5-26.1 (23.8±3.25)µm
Male not found

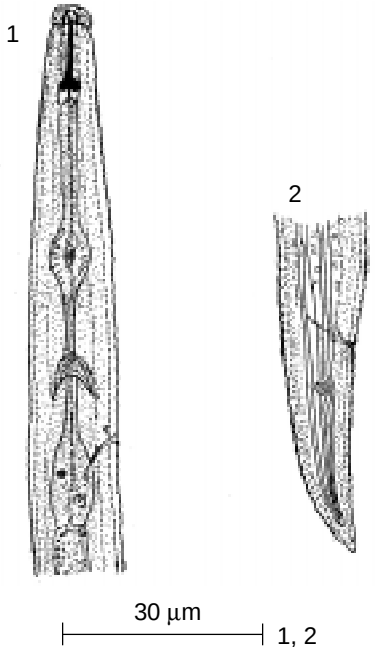

Fig 3. *Geocenamus koreanus*; 1=Anterior region, 2=Tail region.

Comments

Body cylindroid, curved ventrally after killing by gentle heat; cuticle annulated, annules in shape of small blocks. Head set-off slightly with six incisures. Cephalic frame work slightly sclerotized; amphidial aperture pore like 2.3-2.4 (2.35 ± 0.07) µm in length. Mouth aperture encircled by hexaradiate sclerotizations. Stylet 25.4 µm long, stylet knobs flattened anteriorly. Dorsal gland opening about 2.1 µm from the base of stylet knobs. Median oesophageal bulb, 12 µm in diameter. Excretory pore located opposite the end of terminal bulb;hemizonid inconspicuous. Rectum short, about half of the anal body width long. Reproductive system diadelphic amphidelphic, vulva transverse slit, symmetrical, spermatheca conspicuous but sperms not seen. Anterior end of the intestine with wide lumen. Rectum short, less than half of the anal body diameter. Tail coarsely annulated towards tip, subcylindrical; tail terminus blunt. Phasmid located in anterior third of tail. The species are reported for the first time in Pakistan. Present species coincide with original measurements.

Locality and hosts. Collected the soil around the roots of wheat, (*Tricticum vulgare*), Malakundh Agency and some unknown grasses.

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References

Allen M W 1955 A review of the nematode genus *Tylenchorhynchus*. *University of California Publications in Zoology* **61** 129-166.

Brezeski M W 1991 Taxonomy of *Geocenamus* Thorne & Malek 1968 (Nematoda: *Belonolaimidae*) E J Brill, Leiden *Nematologica* **37** 125-173.

Cobb N A 1913 New nematode genera found inhabiting fresh water and non-brackish soils. *J Wash Acad Sci* **3** 432-444.

Cobb N A 1918 Estimating the nema population of soil. *US Dept Agr Bur Plant Ind, Agr Tech Cir* **1** 1-48.

Filipjev I N 1934 The classification of the free living nematodes and their relation to parasitic nematodes. *Simithson Misc Coll* **89** 1-63.

Golden A M, Maqbool M A, Handoo Z A 1987 Description of two new species of *Tylenchorhynchus* Cobb 1913 (Nematoda: *Tylenchida*) with details on morphology and variation of *T.claytoni*. *J Nematol* **19**(1) 58-68.

Hooper D J 1978 The *Tylenchorhynchidae. The identification of Stunt Nematodes* (*Tylenchorhyniae, Merlininae* and *Trophurinae*) *Especially those in Westren Europe.* Manual prepared for workshop sponsered by the nematology group of the Association of Applied Biologists, Rothamsted Experimental station, England, pp 1-21.

Jairajpuri M S 1982 Some studies on *Tylenchorhynchinae*. The subgenus *Bitylenchus* Filipjev 1934, with description of *Tylenchorhynchus (Bitylenchus*) depress n.sp and a key to species of *Bitylenchus*,Mededelingen van de Fakultiet Landbowwetenschappen, *Rijksuniversiteit Gent* **47** 765-770.

Maqbool M A, Fatima N, Hashmi 1983 *Merlinius niazae* n.sp. (Nematoda: *Merliniinae*) and the occurrence of some members of *Merliniinae* and *Tylenchorchynchinae* in Pakistan. *Pak J Nematol* **1** 111-121.

Maqbool M A, Ghazala P, Fatima N 1984 Two new species of the family *Dolichodoridae* (Nematoda: *Tylenchida*) from Pakistan. *Pak J Nematol* **2**(2) 61-67.

Maqbool M A, Ghazala P, Fatima N, Qasim M 1985 *Pararotylenchus microstylus*. n.sp and *Scutylenchus baluchiensis* n.sp. with observation on some new records from Pakistan. *Pak J Nematol* **3**(2) 61-67.

Sycyziel A 1970 *Tylenchorhynchus polonicus* sp.n. and *Helicotylenchus pseudodigonicus* sp.n. (Nematoda, *Tylenchoidea)* from Poland. *Bull Acad Polo Sci, Class-V* **17** 685-690.

Siddiqi M R 1961 Studies on *Tylenchorhynchus* (Nematoda: *Tylenchida*) from India. *Z Parasitkde* **21** 46-64.

Siddiqi M R 1963 Four new species in the sub-family *Tylenchinae* (Nematoda) from North India. *Zeitschrift fur Parasitenkunde* **23** 397-404.

Siddiqi M R 1970 On the plant-parasitic nematode genera *Merlinius* gen.n. and *Tylenchorhynchus* Cobb and the classification of the families *Dolichodoridae* and *Belo-nolaimidae* n. rank. *Proc Helminth Soc Wash* **37** 68-77.

Siddiqi M R 1986 *Tylenchida* parasites of plant and insects. *Commonwealth Agricultural Bureaux*: *Slough*, p 645.

Sturhan D 1966 Uber Verbreitung, Pathogenitat und Taxonomie der Nematodengattung *Tylenchorhynchus. Mitteliungn aus der Biologischen Bundesanstalt fur Landund Forstwirtschaft Berlin-Dahlem* **118** 82-99.

Sturhan D 1981 Studies on *Geocenamus* species from Germany (Nematoda, *Dolichodoridae*). *Nematologica* **27** 306-314.

Tarjan A C 1973 A synopsis of the genera and species in the *Tylenchornchinae* (*Tylenchoidea*, Nematoda). *Proc Helminth Soc Wash* **40** 123-144.

Thorne G 1961 *Principles of Nematology*. McGrw-Hill Book Co., Inc., New York, USA, XIV, pp 553.

Thorne G, Malek R B 1968 Nematodes of the Northern Great Plains. Part I. *Tylenchida* (Nemata: *Secernentea*). *South Dakota State University Agricultural Experiment Station. Tech Bull* **31** pp 111.

Zarina B, Maqbool M A 1994 Description of *Tylenchorhynchus tuberosus* n.sp and observation on *Dolichorhynchus phaseoli* (Nematoda: *Dolichodoridae*) from ornamental plants of Karachi, Pakistan. *Pak J Nematol* **12**(1) 51-57.

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GENETIC VARIABILITY AND HERITIBILITY ESTIMATES OF SOME POLYGENIC TRAITS IN UPLAND COTTON

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Plant breeders are more interested in genetic variance rather than phenotypic variance because it is amenable to selection and bring further improvement in the character. Twentyeight F_2 progenies were tested in two environments so as to predict genetic variances, heritability estimates and genetic gains. Mean squares for locations were significant for all the five traits suggesting that genotypes performed differently under varying environments. Genetic variances, in most cases, however, were about equal to that of phenotypic variances consequently giving high heritability estimates and significant genetic gains. The broad sense heritability estimates were; 94.2, 92.9, 33.6, 81.9 and 86.9% and genetic gains were; 30.19, 10.55, 0.20, 0.89 and 1.76 in seed cotton yield, bolls per plant, lint %, fibre length and fibre uniformity ratio, respectively. Substantial genetic variances and high heritability estimates implied that these characters could be improved through selection from segregating populations.

Key words**:** Genetic variability, Heritability estimates, Upland cotton.

Introduction

Plant breeders can easily observe and measure phenotypic variation in plant populations, which is conditioned by the joint action of both genetic and environmental factors. However, breeders are more interested in determining the proportion of genetic variation from available total phenotypic variance, whereas environmental effect is considered unimportant and hence is neglected.

The success of any breeding venture, therefore, depends mainly on the presence of abundant genetic variability for a trait i.e. is amenable to selection. Thus, the knowledge of degree of genetic variability that is transferable to the progeny referred to as heritability is also of great importance in improving any quantitative trait.

From total genetic variability, it is again additive variance and additive gene action, which play an important role in selecting and improving multigenic traits. Though, lot of work on genetic variability and heritability estimates have already been carried out, yet the differences always existed due to either material and methodology used and environments in which the material is tested (Meredith 1984; Efe and Jancer 1998; Khadi *et al* 1998; Moser *et al* 1999; Baloch and Bhutto 2003). The main objectives of present study were to determine heritability and genetic variances and to predict genetic responses to polygenic traits.

Materials and Methods

Twenty eight F_2 progenies were developed from 28 different cross combinations so as to determine genetic variability and heritability estimates for five quantitative traits of upland cotton. Ten plants from each genotype were randomly tagged for recording on seed cotton yield per plant in g, number of bolls per plant, lint percent, fibre length measured in mm and uniformity ratio. The experiment was carried out in randomized complete block design with four replications at two locations, one at Sakrand and other at Ghotki during the crop year 2002. The combined analysis of variance was done according to Gomez and Gomez (1984) with MSTAT-C software. The row-to-row and plant-to-plant distances were 2.5 feet and 9.0 inches, respectively. All the inputs like fertilizer (150 kg/ha Nitrogen in three split doses), irrigation (eight irrigations with 15 days intervals) and insecticides (two sprays for sucking and two for bollworsm pests) were applied as recommended for our local conditions. Broad sense heritability estimates on entry mean basis were calculated as the proportions of genetic variance (σ^2g) over phenotypic variance (σ^2p) by Fehr (1987). The genetic gain was calculated according to Falconer (1989) with little modification as: $G = h^2D$

Where h^2 is broad sense heritability and D is the selection differential. Broad sense heritability is defined as the proportion of total genetic variance over the phenotypic variance as: $h^2 = \sigma^2g/\sigma^2p$, Where $D = k\sigma p$ and k is selection differential in standard units at 10% = 1.76 and σp is the square root of phenotypic variance.

Results and Discussion

Genetic variability and heritability estimates are considered as very important parameters for the improvement of any quantitative trait. The mean performance, genetic variances,

correlation coefficients, heritability estimates and genetic gains were obtained from 28 upland cotton crosses in F_2. The results depicted in (Table 1) indicated significant differences in the mean performance of genotypes for five quantitative traits, seed cotton yield per plant, number of bolls per plant, lint percent, fibre length and uniformity ratio. The seed cotton yield varied from 60.6 to 135.5 gm, bolls per plant from 21.8 to 48.5; lint % from 35.1 to 36.4; fibre length from 25.4 to 28.7 mm and uniformity ratio from 44.1 to 49.9 %.

The analysis of variance presented in (Table 2) indicates significant effect of the environment on all the five polygenic traits. The interactions between genotype x environment were also significant, further suggesting that the genotypes' performance have changed over the test environments. In other words, genotypes were not consistent in their performance over testing sites. However, the genotypes' mean squares for all the traits were greater than genotype x environment components, which also demonstrated that environment though largely affected genotype's performance, yet substantial improvement is possible in the traits studied. Correlation studies help plant breeders in two ways, i) to bring simultaneous improvement in two or more traits if they are positively correlated, ii) to improve the traits through indirect selection if the targeted trait is difficult to select. Correlation coefficients between yield and other traits were therefore workedout (Table 2) which suggested significant positive correlations with bolls per plant (r = 0.999), positive but non-significant with lint % (r = 0.450), negative and non-signifi-

Table 1

Means of 28 F_2 progenies for various polygenic traits in upland cotton evaluated in two locations

S. no.	F_2 progenies	Seed cotton yield/plant (g)	Boll per plant	Lint (%)	Fibre length (mm)	Uniformity ratio (%)
1.	VH-137 x FH-1000	127.80	46.00	35.30	26.60	46.60
2.	FH-901 x FH-1000	102.60	36.60	35.30	27.30	46.80
3.	CRIS-476 x FH-1000	116.30	41.10	35.70	27.30	46.40
4.	Cyto-51 x FH-1000	107.00	38.30	35.60	26.50	46.90
5.	CRIS-468 x FH-1000	60.60	21.80	35.60	26.60	45.60
6.	CRIS-467 x FH-945	70.70	25.50	35.10	26.90	48.00
7.	Cyto-9/91 x FH-945	91.00	32.60	36.10	27.20	47.30
8.	Cyto-51 x FH-945	76.10	27.30	35.40	26.60	46.40
9.	CIM-473 x FH-945	85.10	30.50	35.30	27.40	47.10
10.	VH-137 x FH-945	109.70	39.30	35.80	27.10	47.40
11.	FH-901 x CIM-707	91.10	32.60	36.20	27.30	44.30
12.	VH-137 x CIM-707	85.70	30.70	36.00	28.70	46.00
13.	Cyto-9/91 x CIM-707	86.90	31.10	35.50	26.70	45.80
14.	Cyto-51 x CIM-707	86.90	31.30	35.20	27.40	48.20
15.	CRIS-468 x CIM-707	69.70	25.00	35.40	26.80	46.00
16.	Cyto-9/91 x CIM-473	83.30	29.90	36.40	26.60	45.60
17.	Cyto-51 x CIM-473	110.70	39.60	35.70	25.40	46.50
18.	CRIS-468 x CIM-473	80.30	28.70	35.70	26.80	46.30
19.	CRIS-467 x CIM-473	95.60	34.20	35.70	26.40	47.10
20.	FH-901 x CIM-473	107.00	38.30	35.50	26.70	46.40
21.	Cyto-9/91 x CRIS-468	135.50	48.50	36.00	25.90	47.70
22.	FH-901 x Cyto-51	84.60	30.30	36.10	26.90	46.40
23.	Cyto-51 x CRIS-467	121.40	42.90	35.40	26.80	47.00
24.	CRIS-468 x Cyto-51	91.20	32.60	35.60	27.00	49.90
25.	CRIS-468 x Cyto-9/91	117.80	42.20	35.10	28.00	45.80
26.	VH-137 x CRIS-467	102.60	36.70	35.60	26.10	46.30
27.	CRIS-467 x Cyto-9/91	87.80	31.40	35.70	26.90	44.10
28.	CRIS-467 x CIM-707	84.60	30.30	36.10	26.60	45.30
	Population mean	**95.30**	**34.10**	**35.70**	**26.90**	**46.50**
	LSD (5%)	**5.18**	**1.88**	**0.40**	**0.62**	**0.79**

Table 2
Mean squares for five polygenic traits in studied 28 F_2 progenies of upland cotton

Source of variation	Degrees of freedom	Mean squares				
		Seed cotton yield	Bolls per plant	Lint %	Fibre length	Uniformity ratio
Location	1.0	366.18**	62.16**	208.090**	44.286**	158.458**
Rep/Loc.	6.0	29.47	3.75	0.657	0.143	0.795
Genotypes	27.0	2668.55**	335.23**	0.906**	3.075**	10.646**
Loc. X Geno.	27.0	155.39**	23.69**	0.602**	0.556	1.374**
Pooled error	162.0	27.49	3.54	0.159	0.389	0.624

Correlatios: Yield with bolls r, 0.999; Yield with lint % r, 0.450; Yield with fibre length r, -0.070; and yield with uniformity ratio r, 0.131
**Significantly different at 1%probability level

cant with fibre length (r = -0.070) and small but positive with uniformity ratio (r = 0.131). Moser *et al* (1999) also observed significant and positive correlations between lint yield and boll numbers (r = 0.98) and lint yield with lint per seed (r = 0.85).

Genetic variances (σ^2g), heritability estimates (h^2) and predicted genetic gains (G) were calculated (Table 3) so as to predict genetic advance that could be made through selection in segregating populations. The genetic variance (σ^2g = 314.145) for yield being about equal to its phenotypic variance (σ^2p = 333.568) and high heritability estimates (h^2 = 94.2 %) indicated that yield, though is influenced by the environment, yet significant improvement is possible in cotton yield. High heritability estimates of h^2 = 92.9% coupled with significant portion of genetic variance (σ^2g = 38.943) connoted that environment had rather smaller impact on the number of bolls, therefore selection in segregating generations can be very effective to improve this trait (Baloch and Bhutto 2003). Large portions of genetic variances, high heritability estimates for bolls per plant and its significant positive correlations with yield (r = 0.999), all suggested that indirect selection through bolls per plant could also make considerable improvement in yield. Khadi *et al* (1998) noted that in characters like yield and bolls per plant, phenotypic coefficient of variability was almost equal to that of genotypic coefficient of variability, thus these characters had high heritability estimates of 82.9 and 94.3%, respectively. Efe and Jancer (1998) also observed 59 and 47% broad sense heritability estimates in seed cotton yield and bolls per plant respectively from half-diallel hybrids.

Table 3
Genetic and phenotypic variances, heritability estimates and genetic gains for some polygenic traits in upland cotton

Traits	σ^2g	σ^2p	h^2	G
Yield / pl. (g)	314.145	333.568	94.2	30.19
Bolls per plant	38.943	41.904	92.9	10.55
Lint (%)	0.038	0.113	33.6	0.20
Fibre length (mm)	0.315	0.385	81.8	0.89
Fibre uniformity (%)	1.159	1.333	86.9	1.76

Lint percent in cotton was significantly affected by the environmental factors because genetic variance (σ^2g = 0.038) was smaller than its phenotypic variance (σ^2p = 0.113). Moderate heritability estimates (h^2 = 33.6%) and small but still comparable genetic variance to phenotypic variance further suggested that improvement with more efforts is also possible in lint percent. Similar to our results, Efe and Jancer (1998) also reported moderate heritability estimate of 63%, whereas, Lancon (1998) observed on an average of 72% heritability in lint % over two spacings i.e. crop spacing (normal spacing) and nursery spacing (narrow spacing). Regarding fibre length, about equal portions of genetic (σ^2g = 0.315) and phenotypic variances (σ^2p = 0.385) resulting high heritability estimates (h^2 = 81.8%), demonstrated that fibre length is significantly controlled by genetic factors, therefore, higher responses to selection from segregating populations could be obtained for this trait. Contrary to our results, Efe and Jancer (1998) reported moderate broad sense heritability estimate of 38% in fibre length. However, variable heritability estimates, ranging from 23.0 to 81.0 % were also reported by Meredith (1984) for fibre length. About equal genetic (σ^2g = 1.159) and phenotypic (σ^2p = 1.333) variances, consequently high heritability estimates (h^2= 86.9%) revealed that environment had little influence on uniformity ratio in cotton, implied that significant improvement can be expected from selection in segregating generations.

Substantial genetic variances and high heritability estimates are very important contributing parameters towards genetic gains for a trait. Results in Table 3 revealed 30.19 gm, 10.55

bolls, 0.20 %, 0.89 mm and 1.76 % genetic gains in yield, bolls per plant, lint percent, fibre length and fibre uniformity ratio, respectively. Mustafa *et al* (1995) observed that at 5% selection intensity, on an average of 10.3 and 5.9% genetic gains were obtained in seed cotton yield and bolls per plant. Genetic gains of 16.9 kg/ha per year were also reported by Moser *et al* (1999). Similar studies in upland cotton (*G. hirsutum* L.) showed genetic gains of 4.8-10.5 kg/ha per year (Bassett and Hyer 1985; Culp and Green 1992; Meredith *et al* 1997). Significantly positive correlations between yield and bolls, their higher heritability estimates consequently more genetic gains suggested that improvement in one trait could bring simultaneous improvement in another trait.

References

Baloch M J, Bhutto H U 2003 Design-11 analysis for estimating general and specific combining ability effects of cotton leaf curl virus resistant inbred parents. *Zagazig J Agric Res* **30**(3) 635-649.

Bassett D M, Hyer A H 1985 Acala cotton in California: 60 years of varietal improvement. In: *Proceedings on Beltwide Cotton Conference*, pp 76.

Culp T W, Green C C 1992 Performance of obsolete and current cultivars and Pee Dee germplasm lines of cotton. *Crop Sci* **32** 35-41.

Efe L, Jancer O 1998 Inheritance of important properties in Half-Diallel hybrids of some glandless cotton, (*Gossypium hirsutum* L.) cultivars. In: *Proceedings on World Cotton Research Conference*, September 6-12, Athens, Greece, **1** 239-243.

Falconer D S 1989 *Introduction to Quantitative Genetics.* Longman Scientific & Technical, 3rd (ed), New York, USA, pp, 187-192.

Fehr W R 1987 *Principles of Cultivar Development,* Theory and Techniques. Macmillan Publishing Company, New York, USA, Vol. **1** pp 95-102.

Gomez K A, Gomez A A 1984 *Statistical Procedures for Agricultural Research.* John Wiley & Sons Inc., 2nd (ed), New York, USA.

Khadi B M, Kulkarni V N, Narrajji S S 1998 Achieving multiple pest tolerance through manipulation of morphological features in cotton. In: *Proceedings of World Cotton Research Conference*, September 6-12, Athens, Greece, **1** 201-203.

Lancon J 1998 Evaluating F_3 lines of single crosses for yield improvements in cotton. In: *Proceedings of World Cotton Research Conference*, September 6-12, Athens, Greece, **1** 144-147.

Meredith W R J 1984 *Quantitative Genetics*, Kohel R J and Lewis C F (eds). Cottons ASA, CSSA and SSSA. Agronomy Monograph No.24, Madison, WI, USA, pp 131-150.

Meredith J, Heitholt W R, Pettigrew W T, Rayburn S T J 1997 Comparison of obsolete and modern cotton cultivars at two nitrogen levels. *Crop Sci* **37** 1453-1457.

Moser H S, Maricopa A Z, Percy R G 1999 Genetic improvement of yield, yield components and agronomic characters of Pima Cotton. In: *Proceedings of Beltwide Cotton Conferences*, National Cotton Council, Memphis, *TN* **1** 488-489.

Mustafa F, Awaad M, Awaad H 1995 Evaluation of some strains of long staple cotton hybrids at different locations in Egypt in 1993 season 1. Seed cotton yield and its contributing variables. In: *Proceedings of Beltwide Cotton Conference*, National Cotton Council, Memphis, *TN*, pp 583.

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TWO NEW *CALOGLYPHUS* BERLESE MITES (ASTIGMATA: ACARIDAE) RECORDED IN PAKISTAN

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The taxonomy of *Caloglyphus clemens* and *C. cingentis* was studied. These species were encountered from two different host materials. A key for all the known hypopodes from Pakistan, their comparison of characters, similarity matrix and phenogram have been included.

Key words**:** Acaridae, Hypopus, New mite species, *Caloglyphus,* Taxonomy.

Introduction

Mites occur widely in a variety of stored products, including foodstuffs causing considerable damage. During long-term storage of cereals like wheat, mites become more serious and damaging pest than weevils or other storage pests. Mites penetrate the seeds through the epicarp and destroy the germ as well as consume some of the endosperm. Interestingly, their damage is considered to be of great economic significance.

Genus *Caloglyphus* was erected by Berlese in 1923 and he designated *Caloglyphus berlesei* Michael (1903) as its type species. Zakhvatkin (1941) made a comprehensive review of this genus and described 4 new species and redescribed 6 species with improved descriptions. Nesbitt (1944 and 1949) and Samsinak (1966) added 1, 3 and 1 new species to this genus, respectively. Mahunka (1973, 1974 and 1978) described 2, 1 and 2 new species, respectively from his area of research. Hughes (1976) contributed a good addition of knowledge to this genus. Tseng and Hsieh (1976) redescribed 1 species with improved description. Samsinak (1980) revised the tribe *Caloglyphini*, re-established the genus *Caloglyphus* and described 1 new species. Channabasavanna *et al* (1981), Rao *et al* (1982) and Ashfaq and Chaudhri (1983) included 1, 1 and 4 new species, respectively, in this genus. Samsinak (1988) mentioned 1 new species of the tribe *Caloglyphini*. Zou and Wang (1989), Sevastyanov and Radi (1991), Sher *et al* (1991), Klimov (1996) and Eraky (1999) added 1, 3, 2, 1 and 1 new species, respectively to this genus. Klimov (2000) reviewed acarid mites of the tribe *Caloglyphini* with description of a new species. Klimov and Oconnor (2003) published phylogeny, historical ecology and systematics of some mites including full descriptions of each taxon, keys and biological informations. Multivariate analyses of variance were used to interpret morphological differences between the two species in relation to factors that influence their morphology in a laboratory and field setting. In the present study, 2 new species have been identified and described.

A large number of species in this genus are also found in Pakistan, which are necessary for undertaking the present study.

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Materials and Methods

Mites are numerous and diverse in most of the areas of Pakistan. For the purpose of present study, samples of different stored commodities were collected from various part of Pakistan, but more frequently from Punjab and N.W.F.P. provinces. Throughout the sampling, main emphasis was laid down upon the grains that were severely infested by insects to observe various species of mites. The samples were sorted into various species of genus *Caloglyphus*, using binocular and their drawings were made with the help of phase contrast microscope. The identification of both these specimens up to specific level was made by following Zakhvatkin (1941) and Hughes (1976) and compared with already reported species in the literature to help to build a background for this genus. An identification key, comparisons of characters, similarity matrix and phenogram for the already known species of this genus including the new species have been presented.

Results and Discussion

Key to Pakistan species of genus *Caloglyphus* (Hypopodes)

1. Sternum 2 (*st2*) present 2
   Sternum 2 (*st2*) absent 6

2. Apodeme 3 (*ap3*) meeting apodeme 4 (*ap4*) 5
   Apodeme 3 (*ap3*) not meeting, apodeme 4 (*ap4*) 3
3. Palposoma lateral margins parallel; paragenital seta (*pr*) bifid ..... *C. multaniensis* Ashfaq and Chaudhri (1983),
   Palposoma lateral margins not parallel; paragenital seta (*pr*) not bifid 4
4. Setae *sci* and *sce* forming straight line; apodemes 4 (*ap4*) not meeting medially ..... *C. opacatus* Ashfaq and Chaudhri (1983)
   Setae *sci* and *sce* not forming straight line; apodemes 4 (*ap4*) meeting medially ..... *C. trigonellum* Sher, Ashfaq and Parvez (1991)
5. Palposoma notched posteriorly; hysterosomal shield smooth ..... *C. merisma* Ashfaq and Chaudhri
   Palposoma not notched posteriorly; hysterosomal shield dotted ..... *C. faisalabadiensis* Sher, Asfaq and Parvez.
6. Palposoma extended beyond the body; apodemes 4 (*ap4*) meeting medially ..... *C. morosus* Ashfaq and Chaudhri
   Palposoma not extended beyond the body; apodemes 4 (*ap4*) not meeting medially 7
7. Coxal field III open; genital disc (*gdi3*) and suctorial shield with radial striations ..... *C. clemens*, n. sp.
   Coxal field III closed; genital disc (*gdi3*) and suctorial shield without radial striations ..... *C. cingentis*, n.sp.

Descriptions. Caloglyphus clemens, new species (Fig 1a, b), Hypopus.

Dorsum. Body 285 mμ long, 200 mμ wide, divided into propodosomal and hysterosomal shields. Propodosomal shield 75 mμ long, 183 mμ wide, with rostral projection antero-medially, dotted antero-laterally, remaining shield smooth; setae *vi, ve, sci, sce* and *scs*, each 1 pair, simple, measuring 12 mμ, 5 mμ, 9 mμ, 16 mμ and 28 mμ in length, respectively; *sci-sci* 28 mμ, *sce-sce* 60 mμ and *sci-sce* 20 mμ apart; setae *sci* and *sce* forming a semi-circular line. Hysterosomal shield 235 mμ long, 200 mμ wide, smooth, medially, anterior margin with broken transverse striations while lateral margins with longitudinal broken striations, turning towards ventral side. Hysterosomal shield with 11 pairs setae, 4 pairs visible pores. Setae *d1 = d2 = 6 mμ, d3 = d4* = 8 mμ; *hi* 9 mμ, *he* 10 mμ; *la* 9 mμ, *lp1 = lp2* = 13 mμ; *sae* 42 mμ, *sai* 15 mμ, long; *d1 - d1* 79 mμ, *d2 - d2* 70 mμ, *d3 - d3* 73 mμ, *d4 - d4* 80 mμ; *d1 - d2* 38 mμ, *d2 - d3* 65 mμ, *d3 - d4* 60 mμ and *la - la* 158 mμ apart. Hysterosomal shield anterior margin overlapping propodosomal shield posterior margin by 25 mμ, with transverse, broken striations Fig 1a.

Venter. Palposoma broad at base, slightly tapering anteriorly, 2 segmented, 22 mμ long (basal segment 12 mμ, distal segment 10 mμ), bifurcated anteriorly, 1 pair arista, 32 mμ long, 3 pairs small setae (Fig 1b). Apodeme 1 (*ap1*) largely Y-shaped, continuing with sternum 1 (*st1*). Sternum 1 (*st1*) free,

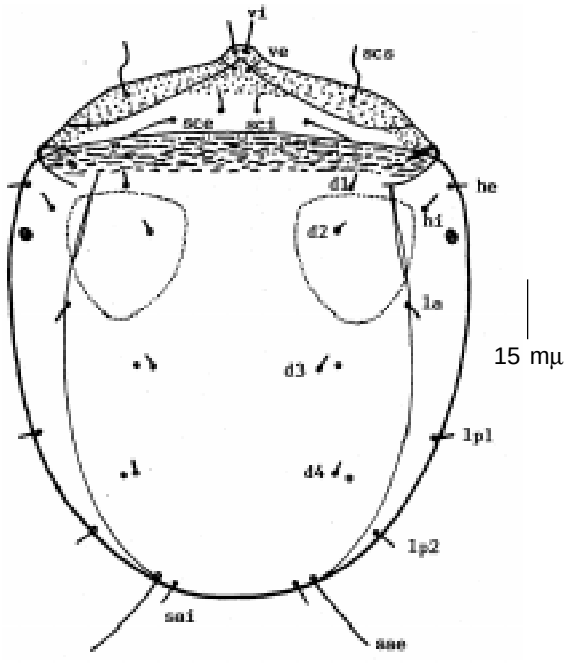

Fig 1a. Dorsal side view.

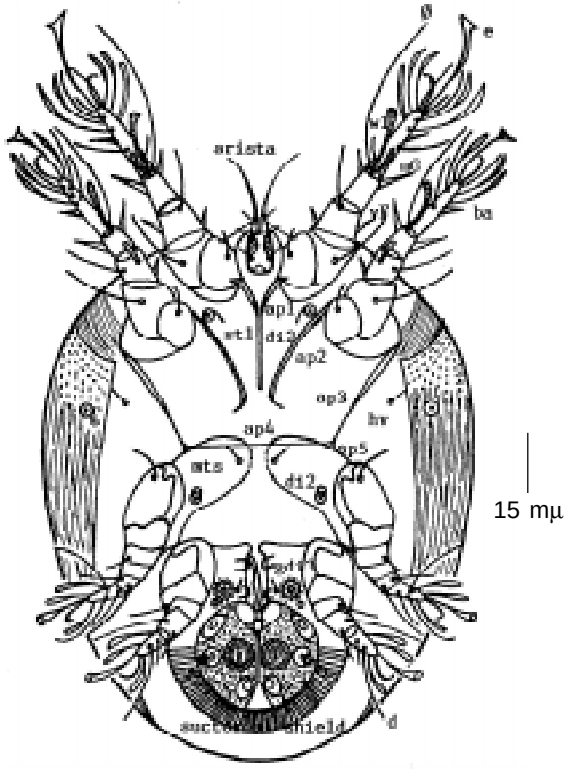

Fig 1b. Ventral side view.

46 mμ long. Apodeme 2 (*ap2*) free, curved at tip. Apodeme 3 (*ap3*) meeting apodeme 4 (*ap4*). Apodemes 4 (*ap4*) not meeting medially. Apodeme 4 (*ap4*) and apodeme 5 (*ap5*) not meeting each other but a thin membranous line continuing from the tips of both apodemes making broad, rounded tip anteriorly, not meeting with same structure from other side. Sternum 2 (*st2*) absent. Metasternal seta (*mts*) 1 small pair, each seta in encircled area of apodeme 4 (*ap4*) and apodeme 5 (*ap5*). Seta *hv* 1 pair, 15 mμ long. Coxal fields I and II, III and IV open, smooth. Ventral shield separated from genital shield. Genital shield smooth, genital slit elongated with 2 pairs genital suckers, 1 pair paragenital seta (*pr*) mesad to genital disc (*gdi3*). Coxal discs *di1* and *di2* present, conoids. Genital disc (*gdi3*) rounded with radial striations. Suctorial shield 70 mμ long, 80 mμ wide, dotted, concave anteriorly, rounded posteriorly with radial striations medio-laterally having 1 pair of suckers medially in striated area, 1 pair of anterior suckers, oval, its discs slipped out on latero-anterior side, anal suckers 1 pair, rounded with radial striations, anal suckers larger than anterior suckers, 1 pair lateral and 1 pair posterior suckers, conoids, 2 pairs vestigial suckers. Suctorial shield separated from posterior body end by 10 mμ, a distance smaller than suctorial shield length Fig 1b.

Legs. Strong and stout, I-IV measuring 108 mμ, 100 mμ, 78 mμ and 70 mμ long, respectively (trochanter base to tarsus tip). Setae and solenidia on legs I-IV segments: coxae 0-0-0-0, trochanters 1-1-1-0, femora 1-1-1-0, genua 3-3-0-1, tibiae 3-3-2-2, tarsi 14-9-7-6. Tarsi I and II 35 mμ and 30 mμ long, respectively. Seta *vF* on femora I, II and III 30 mμ, 38 mμ and 20 mμ long, respectively, absent on femur IV. Seta *e* on tarsi I-IV measuring 30 mμ, 18 mμ, 28 mμ and 25 mμ long, respectively. Seta *mG* on genua I a spine, on II simple seta; *hT* on tibiae I and II each lancet-like, 21 mμ, 11 mμ, 20 mμ and 13 mμ long, respectively. Tarsi II and I each with a solenidion *w1* 25 mμ and 22 mμ long, respectively. Tarsi III and IV short and stout. Seta σ on genua I, a simple seta 35 mμ long, on II, a solenidion 11 mμ long. Dorsal seta φ on tibiae I and II 70 mμ and 43 mμ long, respectively. Seta *ba* on tarsus I 22 mμ long. Tarsi I-IV provided with 1 spoon-shaped + 4 leaf-like; 3 leaf-like + 1 spoon-shaped; 3 leaf-like + 1 spoon-shaped; 3 leaf-like + 1 spoon-shaped setae, respectively. Seta *d* on tarsus IV 20 mμ long Fig 1b.

Type. Holotype, hypopus, collected from millet (*Panicum americanum* L.) in Charsadda on 15.10.1994 (Sarwar) and deposited in Acarology Research Laboratory, Department of Agricultural Entomology, University of Agriculture, Faisalabad.

Remarks. This new species is nearest to *C. cingentis*, another new species recorded from different host material but both the species show the following differences;

1. Palposoma with 2 pairs small setae in *C. cingentis* but with 3 pairs small setae in this species.
2. Coxal field III closed in *C. cingentis* but open in this species.
3. Genital disc (*gdi3*) without radial striations in *C. cingentis* but with radial striations in this species.
4. Tarsus I with 3 leaf-like setae in *C. cingentis* but with 4 leaf-like setae in this species.

Caloglyphus cingentis, New Species (Fig 2), Hypopus.

Dorsum. Body 255 mμ long, 180 mμ wide, divided into propodosomal and hysterosomal shields. Propodosomal shield 63 mμ long, 160 mμ wide, with rostral projection anteromedially, dotted medially, remaining shield smooth, anterolateral parts with broken striations; setae *vi*, *ve*, *sci*, *sce* and *scs*, each 1 pair, simple, measuring 14 mμ, 6 mμ, 20 mμ, 12 mμ and 22 mμ in length, respectively; *sci-sci* 32 mμ, *sce-sce* 65 mμ and *sci-sce* 8 mμ apart; setae *sci* and *sce* forming a semi-circular line. Hysterosomal shield 205 mμ long, 180 mμ wide, smooth, medially, dotted and striated anteriorly, lateral margins with broken longitudinal striations and turn towards the ventral surface. Hysterosomal shield with 11 pairs setae, with 3 pairs of visible pores. Setae *d1* 6 mμ, d2 4 mμ, *d3* 8 mμ, *d4* 4 mμ; *hi* 7 mμ, *he* 11 mμ; *la* 4 mμ, *lp1* = *lp2* = 10 mμ; *sae* 30 mμ, *sai* 12 mμ, long; *d1* - *d1* 112 mμ, *d2* - *d2* 70 mμ, *d3* - *d3* 85 mμ, *d4* - *d4* 54 mμ; *d1* - *d2* 53 mμ, *d2* - *d3* 70 mμ, *d3* - *d4* 72 mμ and *la* - *la* 184 mμ apart. Hysterosomal shield anterior margin overlapping propodosomal shield posterior margin by 15 mμ, with transverse, broken striations and dots Fig 2a.

Venter. Palposoma 2 segmented, slightly tapering anteriorly, 21 mμ long (basal segment 13 mμ, distal segment 8 mμ), bifurcated anteriorly, 1 pair long arista, 30 mμ long, 2 pairs small setae. Apodeme 1 (*ap1*) Y-shaped, sclerotized, continuing with sternum 1 (*st1*). Sternum 1 (*st1*) 45 mμ long. Apodeme 2 (*ap2*) free, curved. Apodeme 3 (*ap3*) meeting apodeme 4 (*ap4*). Apodemes 4 (*ap4*) not meeting medially. Apodeme 4 (*ap4*) and apodeme 5 (*ap5*) meeting making broad rounded tip anteriorly, not meeting with it structure from other side. Sternum 2 (*st2*) absent. Metasternal seta (*mts*) 1 pair, 7 mμ long each in encircled area of apodeme 4 (*ap4*) and apodeme 5 (*ap5*). Seta *hv* 1 pair, 8 mμ long. Coxal fields II, IV and I open, III closed all smooth. Ventral shield separated from genital shield. Genital shield smooth, genital slit elongated with 2 pairs genital suckers, 1 pair paragenital seta (*pr*) anteromedial to genital disc (*gdi3*). Coxal discs *di1* and *di2* present, conoids. Genital disc (*gdi3*) rounded, without radial striations. Suctorial shield 56 mμ long, 62 mμ wide, dotted, concave

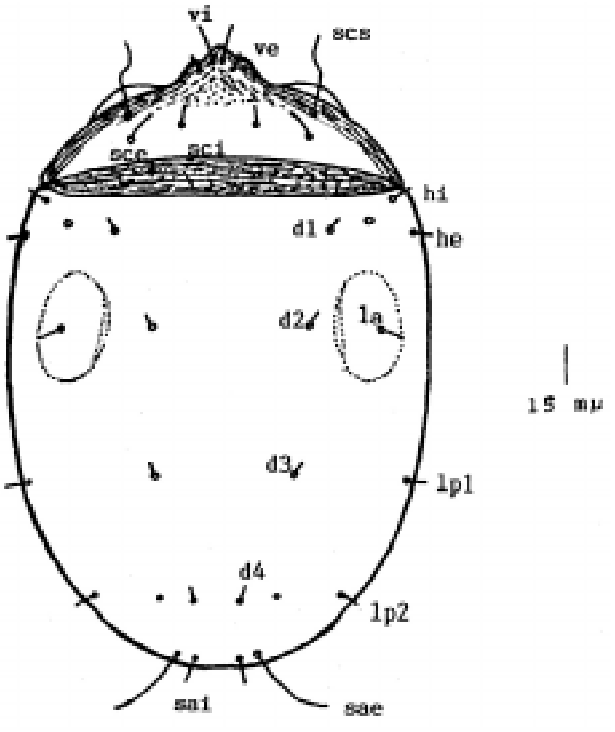

Fig 2a. Dorsal side view.

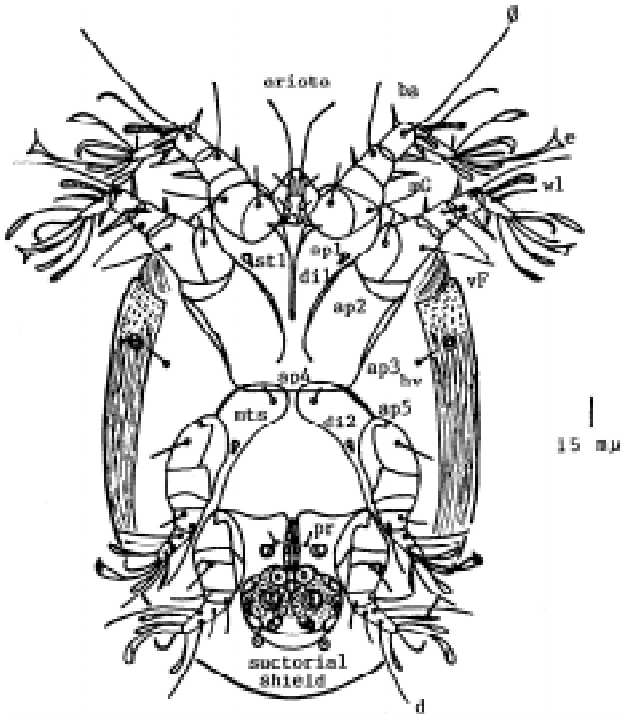

Fig 2b. Ventral side view.

anteromedially, wavy anteriorly, rounded posteriorly having 2 suckers below; anterior suckers 1 pair, anal suckers 1 pair, larger than all other suckers, 1 pair lateral and 1 pair posterior conoids, 2 pairs vestigial suckers towards periphery. Suctorial shield separated from posterior body end by 15 mμ, a distance smaller than suctorial shield length Fig 2b.

Legs. Strong and stout, I-IV measuring 103 mμ, 80 mμ, 70 mμ and 68 mμ in length, respectively (trochanter base to tarsus tip). Setae and solenidia on legs I-IV segments: coxae 0-0-0-0, trochanters 1-1-1-0, femora 1-1-0-0, genua 3-3-0-1, tibiae 3-3-2-2, tarsi 11-8-7-7. Tarsi I and II 32 mμ and 30 mμ long, respectively. Seta *vF* on femora I and II 29 mμ and 28 mμ long, respectively, absent on femora III and IV. Seta *e* on tarsi I-IV 26 mμ, 17 mμ, 15 mμ and 16 mμ in length, respectively. Seta *mG* on genu I, a spine, on genu II, a simple seta; *hT* on tibiae I and II each lancet-like, 13 mμ, 17 mμ, 16 mμ and 16 mμ long, respectively. Seta σ on genu I, a simple seta, on genu II, a solenidion 31 mμ and 9 mμ long, respectively. Tarsi II and I each with a solenidion *w1* 17 mμ and 21 mμ long, respectively. Tarsi III and IV short and stout. Dorsal seta φ on tibiae I and II 54 mμ and 40 mμ long, respectively. Seta *ba* on tarsus I 20 mμ long. Tarsi I-IV provided with 3 leaf-like + 1 spoon-shaped; 3 leaf-like + 1 spoon-shaped; 3 leaf-like + 1 spoon-shaped; 3 leaf-like + 1 spoon-shaped setae, respectively. Seta *d* on leg IV tarsus 40 mμ long Fig 2b.

Type. Holotype, hypopus, collected from Sheikhupura from rice (*Oryza sativa* L.) on 12.9.1994 (Sarwar) and deposited in Acarology Research Laboratory, Department of Agricultural Entomology, University of Agriculture, Faisalabad.

Remarks. This new species is separable from *Caloglyphus merisma* Ashfaq and Chaudhry (1983) by the presence of following characters:

1. Palposoma parallel laterally and notched posteriorly in *C. merisma* but not so in this species.
2. Sternum 2 (*st2*) present in *C. merisma* but absent in this species.
3. Apodemes 4 (*ap4*) meeting medially in *C. merisma* but not meeting in this species.
4. Suctorial shield not rounded posteriorly in *C. merisma* but rounded in this species.
5. Leg I tarsus with 5 leaf-like setae in *C. merisma* but with 3 leaf-like setae in this species.

After going through the key, this new species comes closer to *Caloglyphs clemens*, new species but can be distinguished from it due to following characters:

1. Palposoma with 3 pairs of small setae in *C. clemens* but with 2 pairs setae in this species.
2. Coxal field III opens in *C. clemens* but closed in this species.

Table 1
Comparison of characters in species of genus *Caloglyphus* Berlese

S. no.	Characters	*C. multaniensis*	*C. opacatus*	*C. merisma*	*C. morosus*	*C. faisalabadiensis*	*C. trigonellum*	*C. cingentis*	*C. clemens*
1.	Propodosomal setae (*sci*, *sce*) of equal size	-	-	+	-	-	-	-	-
2.	Propodosomal setae (*sci*, *sce*) forming a straight line	-	+	-	-	-	-	-	-
3.	Propodosomal setae (*sci*, *sce*) posterior in position	-	-	-	+	-	+	+	+
4.	Hysterosomal shield dotted	+	+	-	-	+	+	-	-
5.	Gnathosoma parallel laterally	+	-	-	-	+	-	-	-
6.	Gnathosoma notched posteriorly	-	-	+	-	-	-	-	-
7.	Gnathosoma distal fork separated from basal joint	+	+	+	+	+	+	+	+
8.	Gnathosoma with 2 pairs small setae	+	+	+	+	+	+	+	-
9.	Sternum 1 (*st1*) bifid posteriorly	+	+	-	-	+	+	-	-
10.	Sternum 2 (*st2*) absent	-	-	-	+	-	-	+	+
11.	Apodeme 3 (*ap3*) not meeting apodeme 4 (*ap4*)	+	+	-	-	-	+	-	-
12.	Apodemes 4 (*ap4*) meeting medially	-	-	+	+	-	+	-	-
13.	Coxal field III shut	+	+	+	+	+	+	+	-
14.	Ventral shield separated from genital shield	+	+	+	-	+	+	+	+
15.	Coxal discs (*di1*, *di2*) conoids	+	-	+	-	-	+	+	+
16.	Genital disc (*gdi3*) kidney-shaped	+	+	-	-	+	+	-	-
17.	Genital disc (*gdi3*) with radial striations all around	+	-	-	-	-	-	-	+
18.	Paragenital seta (*pr*) antero-medial to disc (*gdi3*)	-	+	-	+	+	+	-	-
19.	Paragenital seta (*pr*) bifid	+	-	-	-	-	-	-	-
20.	Suctorial shield rounded posteriorly	+	+	-	+	+	+	+	+
21.	Suctorial shield anal suckers equal to anterior suckers	+	-	-	-	+	+	-	-
22.	Suctorial shield with lateral and posterior conoids	+	-	+	-	+	+	+	+
23.	Seta σ on genu II a solenidion	+	-	+	-	+	+	+	+
24.	Leg I tarsus with 2 leaf-like setae	+	-	-	+	-	-	-	-
25.	Leg II tarsus with 3 leaf-like setae	+	+	-	-	-	-	+	+

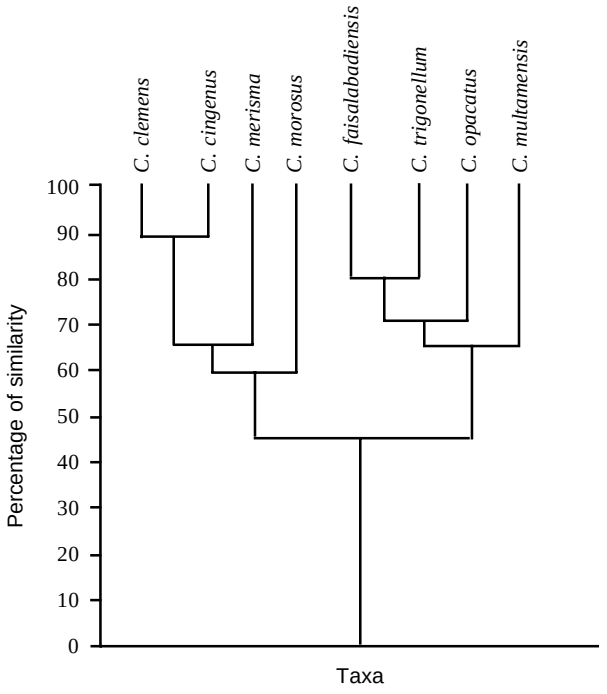

Fig 3. Phenogram of species of genus *Caloglyphus* Berlese.

3. Genital disc (*gdi3*) and suctorial shield with radial striations in *C. clemens* but not so in this species.
4. Tarsi III and I with 4 and 3 leaf-like setae, respectively in *C. clemens* but with 3 and 2 leaf-like setae, respectively in this specie.

The genus *Caloglyphus* was previously represented in Pakistan by 6 species. Now the authors have collected and described 2 new species, thus raising the total to 8 species in this genus from Pakistan. The phenogram (Fig. 3) of the species of genus *Caloglyphus* based on comparison of characters (Table 1) and similarity matrix (Table 2) indicates 2 major clusters, which show different levels of linkages with one another.

The first cluster comprises of 4 species, in this group an affinity of 88% is depicted between *clemens* and *cingentis* pair, whereas the species *merisma* and *morosus* join this pair, respectively at 66% and 60% affinity levels. As the later three species are the dwellers of arid plains, their affinity could thus be attributed to the similar ecological niche they inhabit. On the other hand, species *clemens* is a dweller of separate locality, as such their affinity could be attributed due to the sharing of common genetic characters at generic level.

The second cluster also consists of 4 species; in this cluster, the species *faisalabadiensis* and *trigonellum* constitute a pair exhibiting 80% similarity level. As these two species are the commoners of the same habitat, having the identical host materials, thus it revealed that affinity of these species could be attributed to the same ecological zones they occupy. The species *opacatus* and *multaniensis* in turn join this pair at 70% and 65.33% affinity level, respectively. Since these two species are the dwellers of similar arid ecological zones, their relationships could be the attribute of ecology. This second cluster shows a linkage of 45.75% with the first cluster.

It is noteworthy from the data that species of this genus have a wide range of distribution in Pakistan; because they have been collected from discrete, diverse ecological habitats like hills, sub-mountainous areas, arid plains and coastal areas which indicates that species have an ability to adopt diverse ecological habitats; and hence can be presumed to have a wider genetic plasticity. The linkages further show a strong genetic basis of the characters used in this study. Further, it is obvious that species collected from similar ecological habitats show a high level of affinity among them but they exhibit a relatively lower level of affinity with those collected from different ecological zones and under such conditions, the affinity could rather be the attribute of sharing of common genetic characters at generic level rather than their ecological param-

Table 2

Matrix showing percentage of similarity in species of genus *Caloglyphus* Berlese

Species	*C. multaniensis*	*C. opacatus*	*C. merisma*	*C. morosus*	*C. faisalabadiensis*	*C. trigonellum*	*C. cingentis*	*C. clemens*
C. multaniensis	XX	-	-	-	-	-	-	-
C. opacatus	60	XX	-	-	-	-	-	-
C. merisma	44	44	XX	-	-	-	-	-
C. morosus	82	56	56	XX	-	-	-	-
C. faisalabadiensis	68	72	56	52	XX	-	-	-
C. trigonellum	68	68	56	60	80	XX	-	-
C. cingentis	56	56	72	68	60	64	XX	-
C. clemens	52	44	60	56	48	52	88	XX

Letters xx are showing Zero similarity in species.

eters. The ability of these species to adapt to diverse ecological habitats and yet sharing numerous characters reflects the occurrence of stable generic characters at this level and their adaptive amplitude to varying ecological zones.

Conclusion

The present study provides a basis for the comparison of the representatives of the genus *Caloglyphus*, their characteristics are most important with reference to taxonomic point of view. More sampling is still needed in order to gain better understanding about their distribution and potential pest status. Storage habitat supports a diverse species of acaroid mites to stay there. In view of importance of storage mites, the stored commodities should be properly protected by giving the due attention to storage.

References

Ashfaq M, Chaudhri W M 1983 Four new (Hypopi) species of the genus *Caloglyphus* Berlese from Pakistan (Acarina Acaridae). *Pak Entomol* **5** (1-2) 61-78.

Berlese A 1923 Centuria sesta di Acari Nuovi. *Redia* **15** 237-262.

Channabasavanna G P, Krishna N S R, Ranganath H R 1981 A new *Caloglyphus* (Astigmata Acaridae) from poultry litter in India with taxonomic comments on the genus. *Ind J Acarol* **6** (1/2) 57-63.

Eraky S A 1999 Five new hypopial nymphs (Acari Acaridae and Histiostomatidae) described from different habitats. *Folia Entomol Hung* **60** 45-56.

Hughes A M 1976 *The Mites of Stored Food and Houses*. Tech Bull No 9 Ministry of Agriculture Food and Fisheries London, UK, pp 400.

Klimov P V 1996 A new species of acarid mite from the genus *Caloglyphus* (Acari Acaridae) from the Russian Far East. *Zool Zhur* **75** (4) 613-619.

Klimov P B 2000 A review of acarid mites of the tribe *Caloglyphini* (Acaridae Acariformes) with description of a new genus and species from Siberia and Russian Far East. *Vestnik Zoologii* Ukraine **34** (4-5) 27-35.

Klimov P B, Oconnor B M 2003 Phylogeny, historical ecology and systematics of some mushroom associated mites of the genus *Sancassania* (Acari Acaridae) with new generic synonymies. *Invertebrate Systematics* **17** 469-514.

Mahunka S 1973 Auf insekten lebende Milben (Acari Acarida Tarsonemida) aus Afrika II. *Acta Zool Hung* **19** (3-4) 289-337.

Mahunka S 1974 Auf insekten lebende Milben (Acari Acarida Tarsonemida) aus Afrika III. *Acta Zool Hung* **20** (1-2) 137-154.

Mahunka S 1978 Schizoglyphidae family new and new taxa of Acaridae and Anoetidae (Acari Acarida). *Acta Zool Hung* **24** (1-2) 107-131.

Michael A D 1903 *British Tyroglyphidae.* Ray Soc London, UK, Vol **II**, pp 183.

Nesbitt H H J 1944 Three new mites of the sub-family Rhizoglyphinae. *Canad Entomol* **76** (2) 21-27.

Nesbitt H H J 1949 Six new Mexican mites of the sub-family Rhizoglyphinae Acarina. *Pan Pacific Entomol* **25** (2) 57-70.

Rao N S K, Ranganath H R, Channabasavanna G P, Krishna N S R, Rao N S K 1982 *Caloglyphus karnatakaensis* sp nov (Acari Acaridae) from India with taxonomic comments on the genus *caloglyphus*. *Ind J Acarol* **7** (1) 37-43.

Samsinak K 1966 Die Neuerrichtung der Gattung *Cosmoglyphus* Oudmans 1932 gleichzeitig ein Beitrag zum Problem der "Copra itch". *Zool Anz* **176** (1) 27-42.

Samsinak K 1980 *Caloglyphus rodriguezi* new species with taxonomic remarks on the tribe *caloglyphini* (Acari Acaridae). *Mitt Zool Mus* Berlin **56** (2) 201-206.

Samsinak K 1988 *Sancassania ultima* a new mite of the tribe *Caloglyphini* (Acari Acaridae). *Entomol Mitt Zool Mus* Hambg **9** (133) 159-164.

Sevastyanov V D, Radi G K K K 1991 New species of the mite family Acaridae (Sarcoptiformes) from lower *Egypt*. *Entomol Rev* **8** 139-146.

Sher F, Ashfaq M, Parvez A 1991 Two new (hypopi) species of genus *Caloglyphus* Berlese (Acarina Acaridae) from Pakistan. *Pak Entomol* **13** (1-2) 27-34.

Tseng Y H, Hsieh S A 1976 A new record of acarid mite *Caloglyphus mycophagus* (Megnin) from Taiwan (Acarina Astigmata). *Taiwan Sugar Res Inst* No **74** 47-52.

Zakhvatkin A A 1941 *Fauna of USSR Arachnoidea VI (1) Tyroglyphoidea (Acari)*. Zool Inst Acad Sci USSR New ser no 28. English Translation 1959 Rateliffe A, Hughes A.M Amer Inst, Biol Sci pp 573.

Zou P, Wang X Z 1989 A new species and two new records of Acaridae associated with edible fungi from China (Acarina Acaroidea). *Acta Agric Shanghai* **5** (3) 21-24.

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SKELETAL ANOMALIES IN FISHES COLLECTED FROM KORANGI CREEK AND BACKWATER OF SANDSPIT ALONG THE COAST OF KARACHI

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Vertebral anomalies have been reported in *Liza carinata*, *Valamugil cunnesius* and *Therapon jarbua* from Korangi creek and *L. carinata* collected from backwaters of Sandspit. Detail examination of external morphology and X-rays of fishes showed kypholordosis and scoliosis in the vertebral column. It is presumed that these effects are results of pollutants in the coastal systems of Korangi creek and Sandspit backwaters where heavy pollutants and domestic sewage of the Karachi city is discharged untreated. This study suggests the need of effective management measures to save fisheries resources of the creeks and coastal waters.

***Key words*:** Skeletal anomalies, Fishes, Backwaters, Creeks, Karachi coast.

Introduction

Skeletal and body anomalies in fishes have been reported in numerous research works. Normally such anomalies in wild fish are caused by environmental degradation due to the direct discharge of untreated pollutants, nutritional deficiencies, physical shocks and infection at early developmental stages. Some of the studies on such anomalies are that of Douglas (1978); Hussain (1979); Ferguson (1989); Roberts and Bullock (1989); Schäperclaus (1992); Endo *et al* (1994); Al-Hassan and Shwafi (1997); Al-Hassan and El-Silini (1998), etc. Such abnormal fishes have also been reported from freshwater, creeks and mangrove areas along the coast of Karachi (Hussain 1979; Jafri *et al* 1998; Hussain and Khatoon 1998).

Present study reports the occurrence of abnormalities in five fishes. The detailed study of X-rays reveals scoliosis and kypholordosis resulting loss of hard parts (vertebrae, caudal skeleton and spines), which cause body deformation.

Materials and Methods

Five abnormal specimens were collected in gill nets during the regular fish samplings from Korangi creek area and backwaters of Sandspit area (Karachi coast, Northern Arabian Sea). The detail sampling technique is described in the final report of Pakistan Science Foundation Project no. 319 (PSF 2003). The described specimens are catalogued, *Valamugil cunnesius* two specimens no. 4001 and no. 4002 CEMB; *Liza carinata* two specimens no. 4003 and no. 4004 CEMB and one specimen of *Therapon jarbua* no. 6001 CEMB. Fishes were identified, measured, dissected, X-rayed and compared with normal specimens.

Abbreviations used: TL = Total length: measured from snout to the end of the caudal fin; SL = Standard length: measured from snout to last vertebrae; HL = Head length: measured from snout to the end of operculum; TW = Total weight: Number of vertebrae were counted on longitudinal axis from chondrocranium to urostyle.

Results and Discussion

Valamugil cunnesius. Specimen no.4001 CEMB, was caught by gill net mesh size, 38 mm, at Bakran creek, Station 82, Lat. $24^0 47' 03$N and long. $67^0 17' 46$E on 26th March 2001 at 11.00 A.M. Immature (sex not discriminated), TL 157 mm, SL 120 mm, TW 42.8 g. Dorsal fin IV, I, 8; pectoral fin I, 14; pelvic fin I, 5; anal fin III, 9. Scales in lateral series 34; head length 38.4% (41.6% normal specimen) in the SL; body depth 40.8% (48.0% normal specimen) in SL; eye diameter 9.6% (10.8% normal specimen) in head length; pre-dorsal distance 75.6% (83.2% normal specimen) in SL. The head and the anterior contour of the specimen develop a hump like appearance at the nape (Fig 1).

Meristic features of the abnormal fish show distinct difference from the normal specimen. The body depth is 48% of SL in normal specimen compared to 40.8% of SL in abnormal fish. Pre-dorsal distance is 83.2% in the normal fish compared to 75% of the SL in abnormal fish.

Radiograph. The X-ray of the abnormal fish revealed marked rotato-scoliosis of vertebrae, with degenerative changes at the

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Fig 1a. Abnormal *Valamugil cunnesius* no: 4001 CEMB; **b.** Normal *V. cunnesius.*

Fig 3. Abnormal *Valamugil cunnesius* no: 4002 CEMB.

Fig 2. Radiograph of abnormal *Valamugil cunnesius* no: 4001 CEMB.

Fig 4. Abnormal *Liza carinata* no: 4003 CEMB.

proximal and distal vertebrae in the thoracic region. The first two vertebrae do not have neural spine; the third and fourth vertebrae have weak neural spine while the normal neural spine appears in fifth neural vertebra (Fig 2).

Valamugil cunnesius. Specimen no: 4002 CEMB, was collected by gill net mesh size 38 mm from Bakran creek at St. 112, Lat. $24^0 47' 14$ N long. $67^0 17' 44$ E on October 30th, 2001 at 10.00 A.M. TL 155 mm, SL 120 mm, TW 39 gm, immature. Dorsal fin IV, I, 8; pectoral fin I, 15; pelvic fin 1, 5; anal fin no spines, has only 6 rays. Scales in lateral line series 32; head length 25%, body depth 26.6% in SL; eye diameter 6.6% in HL, pre-dorsal distance 50%; pre-pelvic distance 16.6%; pre-anal distance 72.5% in SL. Loss of three spines and pre-sence of 6 rays in anal fin, depressed caudal fin from the dorsal side and degenerated epurals in caudal are different from normal fish (Fig 3).

Radiograph. Radiograph of specimen shows degenerated epurals and dorsal caudal rays are weak. Spines present in the anal fin in normal fish are absent.

Liza carinata. Specimen no: 4003 CEMB was collected from Shun creek at St. 94, Lat. $24^0 43' 12$N and long. $67^0 12' 05$E caught by gill net mesh size 38 mm, on 28th June 2001 at 11.30 A.M.

TL 134 mm, SL 101 mm, TW 29.9 gm, immature male dorsal fin IV, II, 7, pectoral fin I, 14, pelvic fin I, 5, anal fin III, 9, scales in the lateral line 35.

Head length 34.65% (29.56% normal specimen) in the SL; body width 33.66% (26.95% normal specimen) in the SL; eye diameter 22.8% (23.52% normal specimen) in the HL; pre-dorsal distance 52% (59.13% normal specimen) in SL.

The head of the abnormal specimen bends down at the

anterior end with hump like elevation at the origin of the dorsal fin, which is the widest part of the body while the normal specimen is straight dorsally, progressing backwards with prominent keel or crest on the head. The widest part of the normal fish is in the middle of the body.

Meristic features of abnormal fishes show slight difference from normal specimen (Fig 4).

Radiograph. X-ray of abnormal fish shows fusion of first 2^{nd} and 3^{rd} thoracic vertebrae with loss of disc space. This loss of space may be attributed to the effect of infection during early embryonic development.

Liza carinata. Specimen no: 4004 CEMB, was collected from Chari Kund channel, backwater of Sandspit, St. 70, Lat: $24^0 50' 25$N, long: $66^0 56' 52$E, on November 21^{st}, 2001 at 11.45 A.M. by gill net mesh size 38 mm.

TL 105 mm, SL 79 mm, TW 12.5 gm, HL 28 mm; BD 28 mm; eye diameter 5 mm; immature. Dorsal fin IV, I, 8; pectoral fin 14; pelvic fin I, 5, anal fin III, 8; scales in the lateral line 37; total number of vertebrae 34; eye diameter 21.4% in HL; body width 35.4%; HL 35.4% of the SL; pre-dorsal distance 79%; pre-anal distance 79.7% of the SL. The body contour of a normal fish is straight however in the abnormal fish the dorsal and anal margins have become wavy due to the abnormal rotation of the vertebrae in longitudinal axis. The head and eyes have become prominent. Anal fin is situated at the rear end and extends slightly posterior (Fig 5).

Radiograph. Vertebral column shows continuous elevation and depression giving a wavy shape to thoracic and trunk vertebrae. At places where vertebral column has rotated, the gap at the union of two vertebrae has developed indicating

Fig 5. Abnormal *Liza carinata* no: 4004 CEMB.

Fig 7. Abnormal *Therapon jarbua* no: 6001 CEMB.

Fig 6. Radiograph of abnormal *Liza carinata* no: 4004 CEMB.

Fig 8. Radiograph of the abnormal *Therapon jarbua* no: 6001 CEMB.

loss of some anterior bones of centrum and probably has weak articulation (Fig 6).

Therapon jarbua. Specimen no: 6001 CEMB, was collected by gill net mesh size 57 mm from Port Qasim at St. 105, Lat. $24^0 46' 17$N and long. $67^0 18' 57$E, on August 23rd, 2001 at 6.30 P.M., (Fig 7).

TL 165 mm, SL 123 mm, TW 77.53 g, female spent stage, dorsal fin X. I. 10; anal fin III, 8; pectoral fin rays 13; Pectoral length 27 mm, pelvic fin I, 5 rays. Scales in the lateral series 85, HL 39.83% of SL (normal fish 35.2%); body width 62.54% (normal fish 42%); Eye diameter 4% (normal fish 2.96%); distance from snout to anal fin 76.27% (normal fish 65.91%); distance from anal to caudal fin 9.32% (normal fish 16.19%) of the total length.

The abnormal specimen differs from the normal specimen in body shape. Dorsally a deep vertical depression occurs in vertebral column at the union of 1st and 2nd dorsal fin, which further extends ventrally to the end of the anal fin. The 2nd dorsal and anal fins extend further reducing the length of caudal peduncle. The shape of anal fin differs from normal specimen in having smaller base adjusting itself with the changes that have occurred in the vertebral column. The changes in the shape of vertebrae have resulted in the wide body cavity. Abnormal specimen has large liver and even large gonads. The gonads were empty without eggs suggesting spent condition.

The abnormal fish differs from normal in having bigger eyes, large body width, distance from snout to anal fin is longer, and short caudal peduncle.

Radiograph. X-rays shows marked rotato-scoliosis of vertebral bodies with degenerative changes in the thoracic and caudal vertebrae. The first three vertebrae are degenerated and fused due to which the whole vertebral column has become wavy with two swell one at the anterior and the other at the posterior end with a depression in the middle. In the normal fish all neural spines from thoracic to trunk region are curved backwards but in the abnormal fish the neural spines of thoracic and trunk vertebrae are pointed forward and backwards (Fig 8).

Body anomalies have been reported to occur in fishes collected from the western and eastern coasts of Karachi. Mostly such abnormalities are congenital in origin often occurring in laboratory reared larvae as a result of exposure to cadmium and other chemicals found in industrial and oil pollutants (Manning 1980; Woodworth and Pascoe 1982; Barahona–Fernandes 1982). Endo *et al* (1994) have reported occurrence of kypholordis by the pathogens as *Myxobolus buri* and *M. spinacurvatura* in snappers.

Reports on the presence of heavy metals and other trace elements in waters of Karachi coast are available (Beg *et al* 1992) which can be the sources for developing such skeletal deformation. High level of copper concentration in organisms is reported for functional derangement and anatomical changes (Finlayson and Verrue 1985; Reid and McDonald 1988). Korangi creek and Sandspit backwaters from where these specimens were collected are rich with pollutants. These pollutants are composed of heavy metals that affect the nursery grounds of fishes perhaps causing infection at an early larval stage and may cause high mortality, those, which survive, may develop abnormalities. Occurrences of such abnormalities are rare and show no statistical significance.

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References

Al–Hassan L A J, Shwafi N A A 1997 Asymmetry analysis in two marine teleost fishes collected from the Red Sea coast of Yemen. *Pak J Zool* **29** 23-25.

Al-Hassan L A J, El-Silini O A 1998 Additional records of fish Abnormalities from Benghazi, Libya. *Pak J Zool* **30** 266-269.

Barahona-Fernandes M H 1982 Body deformation in hatchery reared European sea bass *Dicentrarchus labrax* (L.) types, prevalence and effects on fish survival. *J Fish Biol* **21** 239-249.

Beg M A A, Mahmood S N, Yousufzai A H K 1992 Heavy metal pollution in the coastal environment of Karachi. *Pak J Mar Sci* **1** 117-126.

Douglas E R Jr 1978 Anomalous dorsal fin of white Perch. *Estuaries* **1** 64-65.

Endo M, Uehara K, Iwatsuki Y 1994 Body anomalies due to spinal curvature in two species of Snappers *Lutjanus stellatus* and *L. russelli* from the coast of Miyazaki, Southern Japan. *Japan J Ichthyol* **41** 76-79.

Ferguson H W 1989 *Systematic Pathology of Fish. A Text and Atlas of Comparative Tissue Responses in Diseases of Teleosts.* Iowa State Univ. Press, Iowa, pp 263.

Finlayson B J, Verrue K M 1985 Toxicities of butoxyethanol ester and propylene glycol butyl ether ester formulations of 2,4-dichlorophenoxy acetic acid (2,4-D) to juvenile Salmonid. *Arch Environ Contam Toxicol* **14** 153-160.

Hussain S M 1979 Record of a clupeoid fish *Nematalosa nasus* without an anal fin. *Hydrobiologia* **63** 185-188.

Hussain S M, Khatoon Z 1998 Note on *Liza subviridis* (Valen-

ciennes) without right eye. *Indian J Fish* **45** 225-226.

Jafri S I H, Narejo N T, Hussain S M 1998 Anomalous dorsal fin of a cat fish, *Rita rita. Pak J Zool* **30** 159-161.

Manning C S 1980 Vertebral anomaly in *Fundulus similis*. *Gulf Res Rep* **6** 429.

PSF 2003 Pakistan Science Foundation, *Distribution and Abundance of Juvenile Fish Stocks in Korangi Creek*. Final Report Project No.S-Ku/Bio(319), (authors: S Makhdoom Hussain, Zakia Khatoon and Samina Bano).

Reid S D, McDonald D G 1988 The effect of cadmium, copper and low pH on calcium fluxes in the rainbow trout, *Salmo gairdneri*. *Can J Fish Aquatic Sci* **45** 244-253.

Roberts R J, Bullock A M 1989 Nutritional pathology. In: *Fish Nutrition*, Halver J E (ed), Academic Press. New York, USA, 2nd ed, pp 424-475.

Schäperclaus W 1992 *Fish Diseases*. Fischkrankheiten, Akademie-Verlag, Berlin, 5th ed, pp 1398.

Woodworth J C, Pascoe D 1982 Cadmium toxicity to rainbow trout, *Salmo gairdneri* Richardson: A study of eggs and alevins. *J Fish Biol* **21** 47-57.

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STABILITY PARAMETERS OF MUNG STRAINS IN SOUTHERN PUNJAB

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Three genotypes of mung (*Vigna radiata*) i.e. BRM-188, BRM-195 and BRM-202 along with two check varieties (NM-92 and NM-98) were evaluated at five different locations (i.e. Bahawalpur, Multan, Vehari, Khanewal and Dera Ghazi Khan) in Southern Punjab for two consecutive years (2001 and 2002). The genotype BRM-195 was found to be the most stable genotype across a variety of location/environment with regression coefficient (bi) of 1.062 and dispersion (Sd^2) of 0.052 followed by BRM-188 and BRM-202. The genotype BRM-195 performed better across all the locations (3056 Kg ha^{-1}) by producing 46.2 and 60% higher yield than the check varieties i.e. NM-92 and NM-98, respectively. The genotype BRM-195 was being evaluated as a candidate variety in National Uniform Mung Yield Trials by the Coordinator Pulses, National Agricultural Research Centre, Islamabad, for its release for general cultivation in the Southern Punjab.

Key words: Mung genotypes, Stability, Locations, Grain yield.

Introduction

Pulses are the rich sources of protein and essential amino acids, particularly the lysine (cereals are low in lysine). Pulses are also highly nutritious and generally contain high percentage of protein, carbohydrate and vitamins. These are the cheapest source of protein in the indigenous diet. Protein contents range from 20.8 to 33.1%. Their seeds contain twice or thrice as high percentage of protein as cereals and 70% of the world's protein comes directly from vegetable sources (Akhtar *et al* 2003). These are the natural supplements to cereals; cereal-pulse combination thus supports the idea of **"Dal & Roti"** of Pakistani diet. It is an established fact that pulse protein is normally cheaper than the animal protein mostly obtained from meat, milk, poultry and fish.

Mungbean (green gram) (*Vigna radiata* L. Wnezck) is an important pulse crop in Asia, particularly in the Indian subcontinent and South-East Asia. This crop is generally suitable for multiple-cropping systems, enabling better use of land. Mung is rich in easily digestible protein and iron (Akhtar *et al* 2003). It grows rapidly until harvest. More clusters, more seed per pod and high seed-weight form an ideal plant type. Its seed-color is green and black.

In Pakistan, mung crop was cultivated on an area of 215.8 and 239.2 thousand acres with a production of 102 and 115.4 thousand tons, respectively, during 2001-02 (Anon 2002). Average yield was 472 and 482 Kg ha^{-1} in Punjab and Pakistan, respectively. Due to its low average yield, the plant breeders aimed at for the development of new mung cultivars that may possess improved/wider yield stability in a variety of environments. It is essential and a reported fact that the evolution of a new cultivar normally takes about 10-12 years and needs a lot of funds to develop it. For this purpose, various statistical methods have been proposed to determine the stability of a new cultivar. Most commonly used method is the joint regression analysis for yield stability (Eberhart and Russel 1966; Arain and Siddiqi 1977). According to Eberhart and Russell (1966), the regression coefficient (bi) and the average quadratic departure from the regression line (Sd^2) are the two mathematical indices for the assessment of stability parameters. Genotypes with high bi and Sd^2 react readily to changes in the environment and possess considerable variability, while cultivars with bi<1.00 and an Sd^2 near to zero react weakly to changes in growing conditions and are considered to be stable in yield (Finlay and Wilkinson 1963). According to Finlay and Wilkinson (1963), genotype with bi near to 1.00 and a high mean yield were regarded as being well adapted to all environments at growing conditions. The objective of the present studies was to assess the yield stability of newly developed mung strains (BRM-188, BRM-195 and BRM-202) in comparison with the check varieties (NM-92 and NM-98) under the local growing conditions.

Materials and Methods

Three genotypes of mungbean (*Vigna radiata*) i.e. BRM-188, BRM-195 and BRM-202 developed at the Regional Agricultural Research Institute, Bahawalpur along with two check varieties (NM-92 and NM-98) were evaluated at five different locations (i.e. Bahawalpur, Multan, Vehari, Khanewal and Dera Ghazi Khan) in the Punjab Province for two consecutive years

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Table 1
Analysis of variance of the grain yield data

Sources	df	Mean squares	F value	Probability
Replications	3	2278880.1	17.6	0.000
Locations (E)	4	12064032.8	93.2	0.000
Genotypes (G)	4	2032101.5	15.7	0.000
ExG	16	280955.1	2.2	0.010
Error	72	129492.5		

Table 2
Yield (Kg ha^{-1}) performance of mung varieties at 5 locations during the years 2001 and 2002

Varieties	BWP	Multan	Vehari	Khanewal	D.G.Khan	AV.
			Year 2001			
BRM-195	3315	3068	2950	3200	3192	3145a
BRM-188	2538	2358	2100	2669	2645	2462b
BRM-202	1482	1385	1679	1725	1319	1518d
NM-92 (check)	1895	1968	2035	1628	1754	1856c
NM-98 (check)	1628	1857	1588	1905	1547	1705c
			Year 2002			
BRM-195	3182	2713	3008	3042	2885	2966a
BRM-188	2580	2445	2710	2800	2740	2655b
BRM-202	2255	2085	2100	1972	2318	2146d
NM-92 (check)	2448	2267	2123	2295	2492	2325c
NM-98 (check)	2076	1965	2325	2100	2109	2115d

Table 3
Yield (Kg ha^{-1}) performance of mung varieties during the years 2001 and 2002 (average of 5 locations)

Genotypes	2001	2002	Av. (2 years)	% ± over checks	
				NM-92	NM-98
BRM-195	3145a	2966a	3056a	+46.2	+60.0
BRM-188	2462b	2655b	2559b	+22.4	+33.9
BRM-202	1518d	2146d	1833d	-12.4	-05.0
NM-92 (check)	1856c	2325c	2091c	-	-
NM-98 (check)	1705c	2115d	1910d	-	-

Table 4
Stability parameters

Genotypes	Regression coefficient (bi)	Dispersion (Sd^2)
BRM-188	1.054	0.049
BRM-202	1.201	0.457
BRM-195	1.062	0.052
NM-92 (check)	0.990	0.060
NM-98 (check)	0.692	0.242

2001 and 2002. The experiments were laid out according to Randomized Complete Block Design with four replications at each site during each year. Seed beds were prepared by two deep ploughings followed by plankings and use of rotavator. Six rows each of 5 meter length per genotype were sown. Row to row distance was kept as 30 cm. Normal inputs like fertilizer (1 bag DAP per acre before sowing), weedicides (1.5 litres Stomp per acre before sowing) and two irrigations were applied during the growing period. All the cultural practices were carried out during the growth period. At maturity, the central

Table 5

Consolidated results of mung national uniform yield trial-kharif-2002 [yield (Kg ha^{-1})] received from coordinator pulses, NARC, Islamabad

Code/ Source	Genotype	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10	L11	L12	L13	Av.
A / L5	98 cmg 003	1143.28	1493.25	270.36	1346.80	1085.50	776.66	507.70	554	303.72	455.62	92.67	229.90	1136.33	722.75
B / L5	98 cmg 016	1424.75	1484.50	422.91	1825.20	935.20	740.18	566.43	525	177.92	390.31	74.13	214.87	885.08	743.58
C / L5	98 cmg 018	535.15	1267.25	169.93	1863.16	1235.80	964.31	458.35	417	119.54	441.27	13.90	213.76	1311.81	693.17
D / L1	NM-1	1848.70	2204.75	411.44	2123.68	1091.07	847.90	533.41	551	266.88	416.88	90.35	136.94	709.60	864.05
E / L1	NM-2	1261.43	1527.75	353.06	2173.60	1040.97	672.41	409.36	421	235.61	394.78	76.45	252.73	1066.48	760.43
F / L10	SM-1	1310.08	1675.25	329.43	1561.56	1002.00	846.16	352.37	498	317.12	422.97	55.60	183.14	936.86	730.08
G / L4	C1/94-4-19	774.93	1805.50	575.11	1675.96	812.73	886.13	453.14	404	265.49	370.71	67.18	278.89	983.77	719.50
H / L4	C2/94-4-42	1150.23	1163.00	225.88	1883.96	779.33	740.18	472.60	432	319.70	396.71	74.13	281.12	859.72	675.27
I / L2	BRM-188	201.55	1336.75	399.63	1520.48	578.93	482.33	456.62	624	123.02	403.78	55.60	197.06	854.16	556.45
J / L2	BRM-195	378.78	2065.75	561.91	1431.56	775.07	505.61	505.61	639	93.13	391.87	39.38	90.18	698.48	628.34
K / L2	BRM-202	225.88	1415.00	212.32	1366.56	578.93	496.93	507.35	412	11.20	370.50	18.53	124.36	940.34	521.68
L / L14	Mung-1	847.90	1527.50	412.83	1517.43	935.20	834.00	454.53	593	132.05	379.29	57.92	308.95	854.50	687.32
M / L14	Mung-6	1098.10	2006.75	539.67	1789.84	912.93	617.59	518.47	407	218.93	333.31	23.17	22.67	860.06	745.58
N / L15	L1P5/5/89	427.43	1267.25	386.07	1538.68	979.73	796.47	419.09	545	401.78	443.20	121.93	157.54	954.93	647.62
O / L13	NCM-209	656.78	1510.50	555.31	1146.60	1030.03	1002.54	421.17	417	182.79	421.82	23.17	194.28	1240.23	684.78
P / L13	VC3960 A88	1078.68	1475.75	356.19	1437.80	990.87	667.20	413.53	418	311.36	449.38	74.13	212.09	955.97	680.76
Q / L13	VC3960 A89	1282.28	1369.50	747.13	1622.92	996.43	959.10	443.41	414	344.03	365.92	125.10	165.89	1134.24	766.92
R Check	NM-92	639.40	1267.25	348.20	2014.48	1024.27	948.68	377.73	417	321.09	362.13	64.87	214.32	882.65	683.23
S Check	NM-92	1120.69	1484.25	501.79	2249.52	796.03	990.38	324.91	419	398.24	356.77	143.63	228.23	944.16	765.97

Locations

L1 NIAB, Jhang Road, Faisalabad
L2 Regional Agricultural Research Institute, Bahawalpur
L3 Agricultural Research Institute, Dera Ismail Khan
L4 Arid Zone Research Institute, Bhakkar
L5 Barani Agricultural Research Institute (BARI), Chakwal
L6 KARINA, Juglote, Gilgit
L7 Pulses Research Station, Tando Jam
L8 RRI, Dokri, Sindh
L9 Agricultural Research Station, Ahmad Wala Karak
L10 Agricultural Research Station, Mingora, Swat
L11 Barani Agricultural Research Station, Jarma, Kohat
L12 Barani Agricultural Research Station, Fateh Jang
L13 NARC, Islamabad
L14 AARI, Jhang Road, Faisalabad (Trial rejected due to disease)
L15 Nuclear Institute of Agriculture (NIA), Tando Jam, Sindh

four rows of 4 meter length were harvested for grain yield determination. The data were subjected to stability analysis according to the method of Eberhart and Russell (1966).

Results and Discussion

Analysis of variance of the data for grain yield is given in Table 1, which revealed that genotypes (G), locations (environments) (E) and G x E interaction mean squares were highly significant ($P< 0.01$) for grain yield. Average grain yield (Kg ha^{-1}) of various genotypes over years and across the locations is presented in Tables 2-3. These data revealed that the genotype BRM-195 yielded the highest (3056 Kg ha^{-1}) followed by BRM-188 (2559 Kg ha^{-1}) and check NM-92 (2091 Kg ha^{-1}) at all the five locations. The genotype BRM-195 gave 46.2 and 60% higher yield than the check varieties NM-92 and NM-98, respectively. The genotype BRM-202 yielded less than both the checks. Mean yield over all locations ranged from 1833 to 3056 Kg ha^{-1}. The regression of genotype mean yield on the environmental index resulted in regression coefficient (bi) ranging from 0.692 in NM-98 to 1.054 in BRM-188 (Table 4), which means that BRM-195 (bi=1.062) was generally adapted to all environments.

The other better performing genotypes were BRM-188 and NM-92. These genotypes had regression coefficient (bi) values near to unity and had comparatively higher yield than the grand mean yield (1675 Kg ha^{-1}) over all the locations. This shows that these were less responsive to environmental changes and generally adapted to all the environments. The genotypes BRM-202 and NM-98 had the highest value of Sd^2 compared to other genotypes which means that these genotypes were less stable (Eberhart and Russell 1966). Arain and Siddiqi (1977), Sial *et al* (1999), Shindin and Lokteva (2000), Akhtar *et al* (2001) and Hussain *et al* (2002) have reported similar results.

The stable line i.e. BRM-195 is being tested in National Uniform Mung Yield Trial for its release for general cultivation in the southern Punjab. During 2002, BRM-195 yielded 2066 kg ha^{-1} in the National Trial at Bahawalpur compared with 1267 and 1484 kg ha^{-1} of the check varieties NM-92 and NM-98. BRM-195 also out yielded the check varieties NM-92 and NM-98 at Dera Ismail Khan and Mangora in NWFP and Tandojam and Dokri in the Sindh province (Table 5).

In the light of the present results, it is suggested that a new variety of any crop must not be released/approved for general cultivation until and unless it is tested across different locations of the province/country.

References

Akhtar L H, Ahmad M, Hussain M, Siddiqi S Z 2001 Stability analysis of advance stage wheat strains. *Annual Wheat Newsletter, Kansas State University,* USA **47** 129-130.

Akhtar L H, Siddiqi S Z 2003 Current status of research on pulses in Pakistan. *Sci Tech Development* **22**(1) 16-28.

Anonymous 2002 *Agricultural Statistics of Pakistan*. Ministry of Food, Agriculture and Livestock (Economic Wing), Islamabad, Government of Pakistan, pp 46-47.

Arain A G, Siddiqi K A 1977 Stability parameters of wheat mutants. *Env Exp Bot* **17** 13-18.

Eberhart S, Russell W A 1966 Stability parameters for comparing varieties. *Crop Sci* **6** 36-40.

Finlay W, Wilkinson G N R 1963 The analysis of adaptation in a plant breeding programme. *Aust J Agric Res* **14** 742-754.

Hussain A, Bakhsh A, Khan S, Mufti M U, Muhammad D 2002 Stability-analysis and genotype x environment Interaction of oat cultivars for green fodder yield and its components. *Sci Tech Development* **22**(1) 16-28.

Shindin I M, Lokteva O V 2000 Evaluation of spring wheat varieties at Primorskey for ecological plasticity. *Ann Wheat Newslett* **46** 105-106.

Sial M A, Jamali K D, Arain M A, Ahmad M 1999 Adaptability of semidwarf spring wheat in Sind Province. *Pak J Sci Ind Res* **42** (6) 342-344.

Pak. J. Sci. Ind. Res. 2004 **47**(6) 471-477

Technology

Mineral Components Important for Health from Animal Sources

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Animal samples include two different types of snakes, frog and five different types of fishes. The flesh, bones, scales and heads of the animals were separated and analysed for moisture, ash and minerals. The minerals, were not detected from any sample. Cu, Cd, Pb, Co and Cr. The moisture content was generally low. The ash was consistently highest in the bone but consistently lowest in flesh among the fish and in the scale among the snakes. The concentration of the minerals was highly varied among the body parts. The Ca/P ratios ranged from excellent to poor. The Na/K ratios were generally on the high side ranging between 0.55-1.05. The mineral safety index was generally good for Na, P, Mg and Ca, poor for Fe but very bad for Zn. It is an indication that the samples were contaminated by Fe and Zn.

Key words: Animal samples, Ca/P and Na/K ratios, Mineral safety index.

Introduction

Nigeria is predominantly an agricultural country although there is an active petrochemical industry in the Niger Delta area and industrial development is expanding, particularly in the south (Welcome 1979). Heavy urbanization along coastal rivers and lagoons, and intensive agriculture for cash crops in some other parts of the country, may give rise to local pollution problems.

Fish, an important source of animal protein of high biological value, vitamins A and D, and also contain several minerals such as Ca, Fe, Cd, Pb, etc., which may be beneficial or toxic to man depending on the exposure level (Bowen 1979; Mudambi and Rajagopal 1981). Fish is in increasing demand in Nigeria due to high population growth rate, increasing national income and increasing cost of meat and other sources of animal protein (Adeyeye 1997). The relatively high per caput consumption of fish has been attributed to greater availability of this product at relatively cheaper prices (Osajuyigbe 1981). Currently, about 40% of animal protein consumed in the country is derived from fish.

Biological magnification could lead to toxic levels of minerals in fish, even when the exposure is low. The proven toxicity of high concentrations of heavy metals in water to fishery and wild life (Cain *et al* 1980) poses the problem of an ultimate disequilibrium in the natural ecological balance. Apart from destablishing the ecosystem, the accumulation of these toxic metals in the aquatic food organisms is a potent threat to public health. The Minamata Bay epidemics remains a classic example (Goldwater 1971; Laws 1981).

Some reports have been published on the correlation between minerals in fish and their environment (Okoye 1991; Ipinmoroti and Oshodi 1993; Adeyeye 1994). The health implications of the mineral distribution in the fish samples have not been seriously emphasized. In the current report, minerals were determined in five different types of fishes, an amphibian and two types of snakes. The flesh, heads, bones and scales of all the animals were separated appropriately before each part was analysed. This separation was necessary because when fish is roasted or smoke dried (as these samples) head, flesh and bone become soft enough for easy consumption. While it is only the flesh, scales and bone that can be consumed in the snakes but not the head. Based on the mineral composition results, the following parameters were further calculated: calcium/phosphorus (Ca/P) and sodium/potassium (Na/K) ratios and mineral safety index (MSI) of the various sample parts.

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Materials and Methods

The samples of animal were purchased from the Oba market (Ado-Ekiti, Ekiti State, Nigeria) in dry form. The samples were identified and each sample was divided into head, flesh, bone and scales as appropriate. Part of every sample were homogenized in Kenwood major blender, packed in labeled plastic containers and stored in the laboratory refrigerator for pending analysis. Analyses were carried out without further processing.

Determination of moisture and ash. Between 0.5217g-0.6275g of samples were weighed for moisture content determination and dry ashing (AOAC 1990). Samples were dried at 105°C until constant weight while ashing was carried out at a temperature of 540°C in the furnace (NEY M-525) to constant weight.

Determination of minerals. Each ashed sample was transferred into a 50 ml beaker, crucible was washed with 25 ml,

20% (v/v) nitric acid into corresponding beaker and then heated the beaker to boiling to break the ash. The solution was carefully filtered and transferred into 50 ml standard volumetric flask and made up to the mark with distilled de-ionised water (AOAC 1990). The phosphorus was determined colorimetrically by Spectronic 20 (Gallankamp UK) as described by Pearson (1976). Cu, Cd, Fe, Pb, Co., Zn, Cr, Mg and Ca were determined using a Perkin Elmer model 306 Atomic Absorption Spectrophotometer, while Na and K were determined using a flame photometer (Corning, UK, Model 405). All determinations were in duplicate. Earlier, the detection limits of the metals had been determined using the methods of Varian (1975). The optimum analytical range was 0.1 - 0.5 absorbance units with a coefficient of variation of 0.87% - 2.20%. All chemicals used were of analytical grade (BDH, London). Both the moisture and ash contents were reported as g/100g while all the minerals were reported as mg/100g.

Statistical analyses. Calcium/phosphorus (Ca/P) and sodium/potassium (Na/K) ratios were calculated for all the samples (Nieman *et al* 1992) and mineral safety index (MSI) (Hathcock 1985) as appropriate. The differences between the standard mineral safety index and the mineral safety index of the samples were also calculated. Mean standard deviation and coefficients of variation percent were also calculated where appropriate (Steel and Torrie 1960).

Results and Discussion

The scientific and vernacular names as well as the body parts used in the analyses are all shown in Table 1. The heads of the snakes were not analysed because they are normally not consumed as food. The other parts analysed can be consumed although the part consumed can depend on an individual choice.

Table 2 depicts the moisture content of the various samples. Only sample E_3 had a high moisture content of 39.10g/100g, this might be due to the fact that the drying process had not been completed. All other samples had low moisture content values, this would ensure a fairly long keeping quality for the samples. This is a good advantage because frequent electricity failure is rampant here. The ash content of the samples is depicted in Table 2. The ash content of any sample is an indication of its mineral content. In all the samples, the bones (A_2, B_2, C_2, D_2, E_2, F_2, G_2, and H_2) were consistently highest in their ash levels; while scales had the lowest levels of ash in snakes (A_3 and B_3), the flesh had the lowest levels among the other aquatic samples (C_1, D_1, E_1, F_1, G_1, and H_1). The high ash values in the bones were consistent with the high ash levels in the cheliped, thoracic sterna and small thoracic appendages of the male and female samples of fresh water crab *(Sudananautes africanus africanus)* (Adeyeye 2002).

Table 1
Scientific and vernacular names of the animal samples

Animal sample	Animal part	Sample number	Vernacular name (Y)[a]	English name	Systematic name
Snake	Flesh	A_1	*Oworu*	Cobra	*Serpentes ophidia*
	Bone	A_2	"	"	"
	Scales	A_3	"	"	"
	Flesh	B_1	*Ogbara*	Wart	*Channa obscura*
	Bone	B_2	"	"	"
	Scales	B_3	"	"	"
Frog	Flesh	C_1	*Konko*	African bull frog	*Rana adspersa*
	Bone	C_2	"	"	"
	Head	C_3	"	"	"
Fish	Flesh	D_1	*Abo*	Sole	*Cynoglossus senegalensis*
	Bone	D_2	"	"	"
	Head	D_3	"	"	"
	Scales	D_4	"	"	"
	Flesh	E_1	*Kalamu*	Fresh water sardine	*Pellonula afzeliusi*
	Bone	E_2	"	"	"
	Head	E_3	"	"	"
	Flesh	F_1	*Doje*	Silver fish	*Notopterus ater*
	Bone	F_2	"	"	"
	Head	F_3	"	"	"
	Scales	F_4	"	"	"
	Flesh	G_1	*Epiya*	Tilapia	*Oreochromis niloticus*
	Bone	G_2	"	"	"
	Head	G_3	"	"	"
	Flesh	H_1	*Abori*	Mudfish	*Clarias anguillaris*
	Bone	H_2	"	"	"
	Head	H_3	"	"	"

[a]Y, Yoruba.

The mineral elements analysed for are also depicted in Table 2. The minerals were not detected in the samples are Cu, Cd, Pb, Co and Cr while Fe was not detected in D_4 and G_2, also Zn was not detected in D_4. It is gratifying that both Pb and Cd were not detected in any of the samples since both metals are not needed in the body for any biochemical process. In literature, the values of Pb in *Illisha africana* fish from fresh water ponds ranged between 0.56-2.52ppm in various body parts on dry weight basis (Adeyeye 1993), while the values in fresh, lagoon and sea water fish samples ranged from 0.000 - 0.151ppm

Table 2
Moisture, ash and mineral contents of the various samples

g/100g			Minerals (mg/100g)											
Sample	Moisture	Ash	Cu	Cd	Fe	Pb	Co	Zn	Cr	Na	K	Mg	Ca	P
A_1	7.8	11.6	ND[a]	ND	8.4	ND	ND	30.2	ND	40.2	53.0	38.3	4.7	106.3
A_2	7.4	67.0	ND	ND	6.8	ND	ND	54.5	ND	16.5	16.8	12.9	14.8	3.0
A_3	5.0	2.4	ND	ND	25.7	ND	ND	45.8	ND	158.2	290.0	115.1	158.1	405.3
B_1	10.3	9.5	ND	ND	10.3	ND	ND	54.9	ND	15.5	16.7	14.5	9.4	28.6
B_2	7.6	40.1	ND	ND	6.3	ND	ND	59.7	ND	12.6	19.2	15.3	15.8	3.2
B_3	2.2	5.2	ND	ND	37.0	ND	ND	49.1	ND	116.7	170.9	61.6	125.5	354.2
C_1	6.8	8.0	ND	ND	11.4	ND	ND	31.7	ND	17.9	22.5	16.1	17.1	18.0
C_2	5.8	57.0	ND	ND	6.2	ND	ND	31.1	ND	37.7	60.6	50.1	34.5	10.4
C_3	4.6	32.8	ND	ND	5.0	ND	ND	35.6	ND	22.4	29.4	25.2	19.7	74.1
D_1	10.7	8.7	ND	ND	5.2	ND	ND	17.4	ND	21.6	21.2	16.3	14.2	36.4
D_2	2.4	47.6	ND	ND	22.7	ND	ND	47.4	ND	220.5	323.0	242.9	193.2	33.2
D_3	6.9	23.3	ND	ND	7.7	ND	ND	15.9	ND	78.6	92.7	82.5	53.3	128.5
D_4	9.0	26.1	ND	ND	ND	ND	ND	ND	ND	784.0	950.0	821.7	558.2	1711.8
E_1	11.0	7.2	ND	ND	5.3	ND	ND	24.0	ND	14.5	20.3	16.8	10.1	25.2
E_2	6.2	57.8	ND	ND	5.7	ND	ND	38.8	ND	94.8	120.7	97.1	97.3	20.5
E_3	39.1	33.6	ND	ND	11.6	ND	ND	43.9	ND	42.1	40.1	38.7	32.7	104.9
F_1	10.3	8.5	ND	ND	4.5	ND	ND	10.8	ND	11.2	16.5	12.8	128.0	15.0
F_2	1.5	50.4	ND	ND	7.5	ND	ND	83.5	ND	196.7	191.8	150.1	136.6	28.8
F_3	2.8	25.5	ND	ND	4.6	ND	ND	39.6	ND	147.8	147.8	122.2	90.1	132.5
F_4	0.4	18.4	ND	ND	37.2	ND	ND	57.4	ND	346.3	442.1	3.9	429.4	605.0
G_1	8.9	9.7	ND	ND	7.7	ND	ND	19.2	ND	23.9	34.3	29.2	34.7	57.1
G_2	0.2	53.4	ND	ND	ND	ND	ND	36.6	ND	229.4	384.7	289.2	158.2	907.7
G_3	4.7	45.5	ND	ND	9.4	ND	ND	30.7	ND	41.5	49.4	48.5	39.5	141.4
H_1	10.1	5.0	ND	ND	8.4	ND	ND	26.9	ND	28.8	35.5	28.6	31.5	14.7
H_2	6.1	43.5	ND	ND	11.0	ND	ND	46.4	ND	94.1	116.1	110.8	102.0	24.0
H_3	12.1	16.5	ND	ND	7.9	ND	ND	26.6	ND	37.3	43.2	34.3	33.6	95.2

ND[a], not detected.

in various parts on wet weight basis (Odukoya and Ajayi 1987). The corresponding Cd values in the references were 0.10 - 0.34 ppm (Adeyeye 1993) and 0.000- 0.857ppm (Odukoya and Ajayi 1987). This means that the current samples were safer from these two metals.

It is well-known (Buss and Robertson 1976; Mertz 1981; Oshodi and Ipinmoroti 1990; Fagbemi and Oshodi 1991) that mineral elements are necessary for life. Major role of iron is in the formation of haemoglobin. Fe was highest in their group for A_3 (25.67mg/100g), B_3 (37.04mg/100g) and F_4 (37.23mg/100g) which were in all scales but ND was recorded for another scale sample (D_4). Half of the iron in meat is present as haeme iron (in haemoglobin). This is well-absorbed, about 15 -35%, a figure that can be contrasted with other forms of iron, such as that from plant foods, at 1-10% (Bender 1992). Not only is the iron of meat well absorbed but it enhances the absorption of iron from other sources for example, the addition of meat to a legume/cereal diet can double the amout of rion absorbed and so contribute significantly to the prevention of anaemia, which is so wide-spread in developing countries like Nigeria (Wheby 1974; Bender 1992). Iron facilitates the oxidation of carbohydrates, proteins and fats. Many of the samples are good sources of iron.

Zinc was widely distributed among the samples except in D_4 where ND was recorded. Zinc is present in all tissues of the body and is a component of more than 50 enzymes (Bender 1992). Meat is the richest source of zinc in the diet and supplies one third to one half of the total zinc intake of meat eaters. Zinc dietary deficiency has been found in adolescent boys in the Middle East eating a poor diet based largely on unleavened bread (Bender 1992). Families and individuals who may be using vegetable and cereal sources of protein be-

Table 3
Ratios of Na/K and Ca/P; mineral safety index of Na, Mg, P, Ca, Fe and Zn for the various samples

Ratio			Mineral Safety Index																	
			Na			Mg			P			Ca			Fe			Zn		
Sample	Na/K	Ca/P	TV[a]	CV[b]	D[c]	TV	CV	D	TV	CV	D	TV	CV	D	TV	CV	D	TV	CV	D
A_1	0.8	0.04	4.8	0.4	4.4	15	1.4	13.6	10	0.9	9.2	10	0.04	10.0	6.7	3.7	3.0	33	66.4	-33.4
A_2	1.0	5.0	4.8	0.2	4.6	15	0.5	14.5	10	0.02	10.0	10	0.12	29.9	6.7	3.0	3.7	33	119.8	-86.8
A_3	0.6	0.4	4.8	1.5	3.3	15	4.3	10.7	10	3.2	6.8	10	1.3	8.7	6.7	11.5	-4.8	33	100.8	-67.8
B_1	0.9	0.3	4.8	0.14	4.7	15	0.5	14.5	10	0.2	9.8	10	0.1	9.9	6.7	4.6	2.1	33	120.7	-87.7
B_2	0.7	4.9	4.8	0.12	4.7	15	0.6	14.4	10	0.03	10.0	10	0.13	9.9	6.7	2.6	4.1	33	131.3	-98.3
B_3	0.7	0.4	4.8	1.1	3.7	15	1.1	13.7	10	2.8	7.2	10	1.0	9.0	6.7	16.6	-9.9	33	108.1	-75.1
C_1	0.8	0.9	4.8	0.2	4.6	15	0.6	14.4	10	0.14	9.9	10	0.14	9.9	6.7	5.1	1.6	33	69.8	-36.8
C_2	0.6	3.3	4.8	0.4	4.4	15	1.9	13.1	10	0.1	9.9	10	0.3	9.7	6.7	2.8	3.9	33	68.4	-35.4
C_3	0.8	0.3	4.8	0.2	4.6	15	0.9	14.1	10	0.6	9.4	10	0.2	9.8	6.7	2.3	4.5	33	78.4	-45.4
D_1	1.0	0.4	4.8	0.2	4.6	15	0.6	14.4	10	0.3	9.7	10	0.12	29.9	6.7	2.3	4.4	33	38.4	-5.4
D_2	0.7	5.8	4.8	2.1	2.7	15	9.1	5.9	10	0.3	9.7	10	1.6	8.4	6.7	10.1	-3.4	33	104.2	-71.2
D_3	0.9	0.4	4.8	0.8	4.1	15	3.1	11.9	10	1.0	8.9	10	0.4	9.6	6.7	3.4	3.3	33	34.0	-2.0
D_4	0.8	0.3	4.8	7.5	-2.7	15	30.8	15.8	10	13.7	-3.7	10	4.7	5.4	6.7	-*	-	33	-	-
E_1	0.7	0.4	4.8	0.14	4.7	15	0.6	4.4	10	0.8	9.8	10	0.1	9.9	6.7	2.4	4.3	33	52.0	-19.0
E_2	0.8	4.7	4.8	0.9	3.9	15	3.6	1.4	10	0.2	9.8	10	0.8	9.2	6.7	2.6	4.1	33	85.3	-52.3
E_3	1.1	0.3	4.8	0.4	4.4	15	1.4	3.6	10	0.8	9.2	10	0.3	9.7	6.7	5.2	1.5	33	96.6	-63.6
F_1	0.7	81.7	4.8	0.1	4.7	10	0.5	4.5	10	0.1	9.9	10	10.2	-0.2	6.7	2.0	4.7	33	23.7	9.3
F_2	1.0	4.7	4.8	1.9	2.9	15	5.6	9.4	10	0.3	9.8	10	1.1	8.9	6.7	3.3	3.4	33	183.7	-150.7
F_3	1.0	0.7	4.8	1.4	3.4	15	4.6	10.4	10	1.1	8.9	10	0.8	9.3	6.7	2.0	4.7	33	87.0	-54.0
F_4	0.8	0.7	4.8	3.3	1.5	15	0.1	14.9	10	4.8	5.2	10	3.6	6.4	6.7	16.6	-9.9	33	126.3	-93.3
G_1	0.7	0.6	4.8	0.2	4.6	15	1.1	13.9	10	0.5	9.5	10	0.3	9.7	6.7	3.4	3.3	33	42.1	-9.1
G_2	0.6	0.2	4.8	2.2	2.6	15	10.8	4.2	10	7.3	2.7	10	1.3	8.7	6.7	-	-	33	80.5	-47.5
G_3	0.8	0.3	4.8	0.4	4.4	15	1.8	13.2	10	1.1	8.9	10	0.3	9.7	6.7	4.2	2.5	33	67.6	-34.6
H_1	0.8	2.1	4.8	0.3	4.5	15	1.1	13.9	10	0.1	9.9	10	0.3	9.7	6.7	3.7	3.0	33	59.1	-26.1
H_2	0.8	4.3	4.8	0.9	3.9	15	4.2	10.8	10	0.2	9.8	10	0.8	9.2	6.7	4.9	1.8	33	102.0	-69.0
H_3	0.9	0.4	4.8	0.4	4.4	15	1.3	13.7	10	0.8	9.3	10	0.3	9.7	6.7	3.5	3.2	33	58.6	-25.6

[a]TV, Table value; [b]CV, Calculated sample value; [c]D, Difference in value; *-, Not calculated.

cause of low incomes or as an attempt to cope with inflation may not be able to meet the zinc allowances (about 15-20mg) per day. The zinc in these sources is not available as in animal sources (NAS 1971).

The animal samples were good sources of magnesium, sodium and potassium. The sodium and potassium levels here were lower than the levels in *Cyprinus carpio* and *Clarias gariepinus* fish (Adeyeye 1996) and also lower (together with magnesium) than in three different types of land snails consumed in Nigeria (Adeyeye 1996). Magnesium is an activator of many enzyme systems and maintains the electrical potential in nerves (Shils 1973; Shils and Young 1988). Potassium is primarily an intracellular cation, mostly this cation is bound to protein and with sodium influences osmotic pressure and contributes to normal pH equilibrium (Sandstead 1967).

The calcium levels were reasonably distributed among the samples. They were particularly high in the scales followed by the bones. This meant these samples could serve as good sources of the element. Calcium, in conjunction with phosphorus, magnesium, manganese, vitamins A, C and D, chlorine and protein, are involved in bone formation but calcium is the principal contributor (Fleck 1976). Calcium plays an important role in blood clotting, in muscles contraction and in certain enzymes in metabolic processes. Calcium tends to be a kind of co-ordinator among inorganic elements, if excessive amount of potassium, magnesium or sodium are present in the body, calcium is capable of assuming a corrective role (Fleck 1976). Although rickets in children is generally attributed to the lack of vitamin D, insufficient intakes of calcium and phosphorus, as well as an imbalance of these two minerals, may

Table 4
Mean ($\bar{x}$) standard deviation (sd) and coefficient of variation percent (CV%) for the various parameters determine din the sample

	g/100g		mg/100g						Ratio			Mineral safety index					
Sample	Moisture	Ash	Zn	Fe	Na	Mg	K	Ca	P	Na/K	Ca/P	Na	Zn	Ca	Mg	Fe	P
A_1-A_3;$\bar{X}$	6.7	27.0	43.5	13.6	71.6	55.4	119.9	59.2	171.5	0.8	1.8	0.7	95.7	0.5	2.1	6.1	1.4
SD	1.5	34.9	12.3	10.5	75.9	53.2	148.4	85.8	208.9	0.2	2.8	0.7	27.1	0.7	2.0	4.7	1.7
CV%	22.4	129.3	28.3	77.1	106.0	96.0	123.7	145.0	121.8	49.0	152.8	105.8	28.3	146.9	96.2	77.0	121.9
B_1-B_3;$\bar{X}$	6.7	21.3	54.6	17.9	48.3	30.5	68.9	50.2	128.7	0.8	1.9	0.5	120.0	0.4	1.1	8.0	1.0
SD	4.1	24.2	5.3	16.7	59.3	27.0	88.3	65.3	195.7	29.0	2.6	0.6	11.6	0.5	1.0	7.5	1.6
CV%	61.6	113.7	9.7	93.6	122.9	88.7	128.1	130.0	152.1	19.7	141.6	123.9	9.7	128.6	88.6	93.6	151.5
C_1-C_3;$\bar{X}$	5.7	32.6	32.8	7.5	26.0	30.5	37.5	13.4	10.8	0.7	1.5	0.2	72.2	0.2	1.1	3.4	0.3
SD	1.1	24.5	2.5	3.4	10.4	17.6	20.3	8.7	7.0	0.1	1.6	0.1	5.4	0.1	0.7	1.5	0.3
CV%	19.4	75.2	7.5	45.2	39.9	57.8	54.1	64.7	67.0	12.3	106.0	41.7	7.5	40.0	57.9	45.2	103.7
D_1-D_4;$\bar{X}$	7.3	26.4	26.9*	11.9*	276.2	290.9	346.8	204.7	477.5	0.9	1.7	2.7	59.2	1.7	10.9	5.3	3.8
SD	3.6	16.0	17.7*	9.5*	348.7	366.4	422.3	247.9	824.1	0.1	2.7	3.3	39.0	2.1	13.7	4.2	6.6
CV%	49.6	60.7	65.9*	79.7*	126.3	126.0	121.8	121.1	172.6	16.5	156.3	126.0	65.9	121.1	121.1	79.8	172.5
E_1-E_3;$\bar{X}$	18.8	32.9	35.6	7.6	50.5	50.9	60.4	46.7	50.2	0.9	1.8	0.5	78.0	0.4	1.9	3.4	0.4
SD	17.8	25.3	10.3	3.5	40.8	41.5	53.2	45.2	47.5	0.2	2.5	0.4	23.2	0.4	1.6	1.6	0.4
CV%	94.8	77.1	29.0	46.6	80.8	81.6	88.1	96.8	94.5	20.0	139.0	81.3	29.7	97.4	82.1	46.4	95.0
F_1-F_4;$\bar{X}$	3.7	25.7	47.8	13.5	175.5	72.3	199.6	471.0	195.3	0.9	22.0	1.7	105.2	3.9	2.7	6.0	1.6
SD	4.5	17.9	30.6	15.9	138.3	74.7	178.0	526.5	278.1	0.2	39.9	1.3	67.3	4.4	2.8	7.1	2.2
CV%	120.1	69.6	64.0	118.2	78.8	103.4	89.2	111.8	142.4	19.5	181.6	78.1	64.0	111.7	103.3	118.3	142.3
G_1-G_3;$\bar{X}$	4.6	36.2	28.8	8.5[a]	98.3	122.3	156.1	77.5	368.7	0.7	0.4	0.9	63.4	0.6	4.6	3.8	3.0
SD	4.4	23.3	8.9	1.2[a]	113.9	144.9	198.1	69.9	468.7	0.12	0.2	1.1	19.5	0.6	5.4	0.5	3.7
CV%	95.2	64.4	30.8	13.8[a]	115.9	118.4	126.9	90.2	127.1	16.9	59.5	116.0	30.8	90.6	118.6	13.9	126.8
H_1-H_3;$\bar{X}$	9.4	21.6	33.3	9.1	53.4	57.9	64.9	55.7	44.6	0.8	2.2	0.5	73.2	0.5	2.2	4.1	0.4
SD	3.0	19.8	11.3	1.7	35.5	45.9	44.5	40.1	44.1	0.03	2.0	0.3	24.9	0.3	1.7	0.8	0.3
CV%	32.2	91.3	34.0	18.6	66.4	79.3	58.5	72.0	98.7	3.6	87.1	66.7	34.0	71.7	79.3	18.7	97.1

[3]D_4 not used to calculate for X, SD, CV%; [a]G_2 not used to calculate for X, SD, CV%.

result in the disease. Osteomalacia, the adult rickets may also be due to this condition. Osteoporosis (bone thinning) is said to be more common among older people, females and whites, according to Moldswer *et al* (1965), than among younger people, males and non-whites. This shows that normal level of calcium in the diet should be maintained throughout life.

Low values of phosphorus were generally observed for the bones (A_2, B_2, C_2, D_2, and E_2) but very high (907.69mg/100g) in G_2. Other samples had good yield of phosphorus. Phosphorus is always found with Ca in the body, both contributing to the supportive structure of the body. It is present in cells and in the blood as soluble phosphate ion, as well as in lipids, proteins, carbohydrates and energy transfer enzymes (NAS 1974). Phosphorus is an essential component in nucleic acids and the nucleoproteins responsible for cell division, reproduction and the transmission of hereditary traits (Hegsted 1973). Our current report in phosphorus were generally lower than the values observed for various parts of male and female fresh water crab *(Sudananautes africanus africanus)* (Adeyeye 2002).

Table 3 depicts the Na/K and Ca/P ratios as well as the mineral safety index (MSI) for Na, Mg, P, Ca, Fe and Zn. Modern diets, which are rich in animal proteins and phosphorus, may promote the loss of calcium in the urine (Shils and Young 1988). This has led to the concept of the Ca/P ratio. If the Ca/P ratio is low (low calcium, high phosphorus intake), more than the normal amount of calcium may be lost in the urine, which result to decrease the calcium level in bones. In animals, a Ca/P ratio above two (twice as much calcium as phosphorus) helps to increase the absorption of calcium in the small intestine. Such samples in our results included A_2, B_2, C_2, D_2, E_2, F_1, F_2, $H_1$and H_2. This may help to increase the calcium content of bones. Some researchers are advising that one should eat more foods that are high in calcium but low in phosphorus. Table 3 outlines the calcium to phosphorus ratio of the samples. Food is considered “good” if the ratio is above one and “poor” if the ratio is less than 0.50 (Nieman *et al* 1992). This meant 50.0% of our samples were poor in Ca/P ratio. A good consumption of vegetables will correct this anomally. This concern to the sodium and potassium ratio (Na/K). For

prevention of high blood pressure, a Na/K ratio of 0.60 is recommended (Nieman *et al* 1992). The Na/K ratio of our samples were mostly above 0.60 with 96.15% samples greater than 0.60 while only 3.846% of the samples were slightly lower (0.55, sample A3). In other words, most of the samples would promote high blood pressure disease. Foods that have low sodium, high-potassium values include mostly fruits, vegetables and low sodium cereals (Nieman *et al* 1992) which can be consumed with the animal protein samples.

The standard mineral safety index (MSI) for the minerals are Na (4.8), Mg (15), P (10), Ca (10), Fe (6.7), and Zn (33). For Na, all MSI values were low except for D_4 (with a value of 7.53) with positive values for the difference between the standard value of MSI and the calculated value of MSI. This meant that the samples might not be overloading the body rich with sodium. For MSI of Mg only D_4 was above the USRDA by 15.81 times (Hathcock 1985). For P, Ca and Fe, the odd samples out respectively were D_4 (-3.69), F_1 (-0.23), A_3 (-4.77), B_3 (-9.86), D_2 (-3.44) and F_4 (-9.94). The implication of the above is that abnormally high levels of Na, Mg and P were present in D_4; Ca in F_1 and Fe in A_3, B_3, D_2 and F_4. Samples A_3, B_3, D_2 and F_4 could cause the reduction of zinc absorption in the small intestine (O'Dell 1984) and iron poisoning particularly in children (Herbert 1987). With the exception of F_1, all the Zn MSI values were greater than 33. This meant all the samples have Zinc values far above the recommended adult intake. The minimum toxic dose is 500mg, or 33 times the RDA (Hathcock 1985). High doses of zinc can be harmful. Zinc supplements can decrease the amount of high density lipoprotein circulating in the blood, increasing risk of heart disease (Anon 1986). Excess zinc interacts with other minerals, such as copper and iron, decreasing their absorption. In animals, zinc supplements decrease the absorption of iron so much that anaemia is produced (Greger 1987). When patients are given 150 mg of zinc per day, copper deficiency results. Intakes of zinc only 3.5mg/day above the RDA decrease copper absorption (Festa 1985). In animals, copper deficiency causes scarring of the heart muscle tissue and low levels of calcium in the bone (Anon 1986). Excess zinc also decreases the functioning of the immune system. From the foregoing, most of the samples would lead to excess zinc consumption with its deleterious effects.

The sources of the high levels of iron and zinc in the samples could have been due to either from the foods they consumed or from their environments or both. Zinc is of particular interest from automotive pollution consideration and is also greatly related to vehicular emission. Iron may have its origin in corrosion of iron materials, as most vehicles on roads are aged and disjointed parts are left on the road indiscriminately (Ogunsola 1994). The inwash of the corroded vehicles into the aquatic environment could lead to high biological accumulation of iron and zinc in the samples (Montaque and Montaque 1971) hence their high concentration in the samples.

The statistical results for all the samples are shown in Table 4. All the parameters analysed for or calculated were highly varied in the various samples. The high coefficients of variation percent in the majority of the samples attested to this. However, for all the samples the Na/K ratio CV% were generally low with values ranging between 3.61 - 28.95.

From the above, it is seen that the samples were good sources of the nutritionally valuable minerals. It is also seen that each part differently concentrated the minerals to different levels. While, some of the Ca/P ratios were good and the Na/K ratios would require nutritional correction in terms of consumption of low sodium and high potassium foods with them. Efforts should be made to reduce drastically the pollution of environment by iron and zinc which could accumulate to deleterious levels.

References

Adeyeye E I 1993 Trace heavy metal distribution in *africana* fish organs and tissue I: lead and cadmium. *Ghana J Chem* **1**(8) 377-384.

Adeyeye E I 1994 Determination of trace heavy metals in *I. africana* fish and in associated water and soil sediments from some fish ponds. *Int J Environ Studies* **45** 231-238.

Adeyeye E I 1996 Waste yield, proximate and mineral composition of three different types of land snails found in Nigeria. *Int J Food Sci Nutr* **47** 111-116.

Adeyeye E I 1997 Water quality criteria and the relationship between the distribution and concentration of some mineral elements in soil sediments, ambient water and the body parts of *Clarias gariepinus* fish in a freshwater pond. *Ghana J Chem* **3** (2) 42-50.

Adeyeye E I 2002 Determination of the chemical composition of the nutritionally valuable parts of male and female common West African freshwater crab *Sudananautes africanus africanus. Int J Food Sci Nutr* **53** 189-196.

Adeyeye E I, Akinyugha N J, Fesobi M E, Tenabe V O 1996 Determination of some metals in *Clarias gariepinus*, *Cyprinus carpio* and *Oreochromis niloticus* fishes in a polyculture freshwater pond and their environments. *Aquaculture* **147** 205-241.

Anon 1986 Adverse effects of zinc megadoses. In: *Nutrition,* eds Nieman DC, Butterworth DE, Nieman CN, Wm C Brown Publishers, Dubuque, USA, pp 297-299.

AOAC 1990 *Official Methods of Analysis.* Association of

Official Analytical Chemists, Washington DC, USA, 15th ed, Sections 12.1.7; 968.08; 4.1.28.

Bender A 1992 Meat and meat products in human nutrition in developing countries. *FAO Food and Nutrition Paper* **53,** FAO, Rome, Italy, pp 46-47.

Bowen H J M 1979 Environmental chemistry of the elements. *Ghana J Chem* **3**(2), 42-50.

Buss D, Robertson J 1976 *Manual of Nutrition.* Her Majesty's Stationary Office, London, UK, 8th ed, pp 32-40.

Cain J R, Paschal D C, Hayden C M 1980 Toxicity and bioaccumulation of cadmium in the colonial green algae *(Scenedesmus obliquus). Arch Environ Contamn Toxicol* **9** 9-16.

Fagbemi T N, Oshodi A A 1991 Chemical composition and functional properties of full fat fluted pumpkin seed flour *(Telfaira occidentalis). Nig Food J* **9** 26-32.

Festa M D 1985 Effect of zinc intake on copper excretion and retention in men. *Am J Clini Nutr* **41** 285-292.

Fleck H 1976 *Introduction to Nutrition,* Macmillan, New York, USA, 3rd ed, pp 207-219.

Goldwater L J 1971 Mercury in the environment. *Sci Am* **224** (5) 15-21.

Greger J L 1987 Food, supplements and fortified foods; scientific evaluations in regard to toxicology and nutrient bioavailablity, *J Am Diete Assoc* **87** 1369-1373.

Hathcock J N 1985 Quantitative evaluation of vitamin safety. *Pharmacy Times* 104-113.

Hegsted D M 1973 Calcium and phosphorus. In: *Modern Nutrition in Health and Disease,* Ch 6, Sect A. Lea and Febiger, Philadelphia, PA, USA, In: *Introduction to Nutrition,* Fleck H 1976. Macmillan, New York, USA, 3rd ed, pp 207-213.

Herbert V 1987 Recommended dietary intakes (RDI) of iron in humans. *Am J Clini Nutr* **45** 679-686.

Ipinmoroti K O, Oshodi A A 1993 Determination of trace metals in fish, associated waters and soil sediments from fish ponds. *Disc Innov* **5** 135-138.

Laws E A 1981 *Aquatic pollution*. A Wiley-Interscience Publication, John Wiley & Sons, New York, USA, pp 301-369.

Mertz W 1981 Recommended dietary allowances up to date-trace minerals. *J Am Dietet Assoc* **64**(2) 163-167.

Moldswer M, Zimmerman S J, Collins L C 1965 Incidence of osteoporosis in elderly whites and elderly Negroes. *J Am Med Assoc* **194**(8) 859-862.

Montague K, Montague P 1971 *Mercury*. Sierra Club, San Francisco, USA, pp 1-158.

Mudambi S R, Rajagopal M V 1981 Fundamentals of food and nutrition. Wiley Eastern Limited, New Delhi, India, pp 57-63.

NAS 1971 Food and nutrition board: zinc in human nutrition. In: *Summary of Proceedings of a Workshop,* 4 – 5 December 1971. National Academy of Sciences - National Council, Washington DC, USA.

NAS 1974 Food and nutrition board: recommended dietary allowances. In: *Introduction to Nutrition*. Macmillan, National Academy of Sciences, New York, p 17.

Nieman D C, Butterworth D E, Nieman C N 1992 *Nutrition*. Wm C, Brown Publishers, Dubuque, USA, pp 237-312.

O'Dell B L 1984 Bioavailability of trace elements. *Nutr Rev* **42** 301-308.

Odukoya O O, Ajayi S O 1987 Trace heavy metals in Nigerian fishes I: Lead and cadmium. *Nig J Nutr Sci* **8**(2) 105-113.

Ogunsola O J 1994 Traffic pollution: preliminary elemental characterization of roadside dust in Lagos, Nigeria. *The Science of the Total Environment* **146/147** 175-184.

Okoye B C O 1991 Heavy metals and organisms in the Lagos lagoon. *Int J Environ Stud* **37** 285-292.

Oshodi A A, Ipinmoroti K O 1990 Determination of some nutritionally valuable minerals in *Irvingia gabonensis. Ghana J Chem* **1**(2) 138-142.

Osajuyigbe O 1981 Public investment in fisheries development in Nigeria. M.Sc thesis , Department of Agriculture Economics, University of Ife, Ile - Ife.

Pearson D 1976 *Chemical Analysis of Foods.* J A Churchill, London, UK, 7th ed, pp 7-11.

Sandstead H H 1967 Present knowledge of minerals. In: *Knowledge in Nutrition*. Chap 28, Nutrition Foundation New York, USA, In: *Introduction to Nutrition,* ed Fleck H 1976. Ma cmillan, New Yrok, USA, 3rd ed, pp 204.

Shils M E 1973 Magnesium. In: *Modern Nutrition in Health and Disease,* eds Goodhart R S, Shils M E, Chap 6, Sect B. Lea and Febiger, Philadelphia, USA. In: *Introduction to nutrition,* ed Fleck H 1976. Macmillan, New York, USA, 3rd ed, pp 214-216.

Shils M E G, Young V R 1988 *Modern Nutrition in Health and Disease,* Lea and Febiger, Philadelphia, USA. In: *Nutrition,* eds Nieman DC, Butterworth DE, Nieman CN 1992. Wm C Brown Publishers, Dubuque, USA, pp 276-282.

Steel R G D, Torrie J H 1960 *Principles and Procedures of Statistics*. Mc Graw-Hill, London, UK, pp 7-30.

Varian T 1975 *Basic Atomic Absorption Spectroscopy-A Modern Introduction*. Dominican Press, Victoria, Australia, pp 104-106.

Welcome R L 1979 *The inland fisheries of Africa*. FIFA Occ Pap 7 pp 1.

Wheby M S 1974 Systemic effects of iron deficiency. In: *Iron,* ed. Crosby WH, Medicom, New York, pp 39-45.

Review Article

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HIGH TEMPERATURE SI-B-C-N CERAMICS

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The importance of pyrolysis of preceramic organometallic compounds is increasing year by year as a key technology for the synthesis of new inorganic materials. According to this process, preceramic polymers are synthesized from monomer units. After cross-linking of such precursors, the obtained preceramic networks are transformed by pyrolysis into amorphous materials. Further increase of the temperature yields thermodynamically stable crystalline materials. In this paper, the general methods of the synthesis of ceramic materials based on the Si-B-C-N system via thermolysis of preceramic compounds have been reviewed. Bulk materials, coatings and fibers of such materials reveal quite interesting high temperature properties.

***Key words*:** Pyrolysis, Preceramic compounds, High temperature ceramics.

Introduction

In the process of the pyrolysis of preceramic compounds (Fig 1) proper monomer units are polymerized and cross-linked into organometallic preceramic networks. The networks are then subsequently pyrolized at elevated temperatures providing inorganic materials of great scientific and technical interest. Since the first proposal of this concept (Popper 1967) several research groups in Germany, USA, Japan and France have been working in this field. The achievements of these groups and the ongoing activities in the exploration of chemical synthesis routes for the production of proper preceramic networks, the controlled decomposition of the preceramics into inorganic materials as well as the characterization and technical aspects of many different materials have been reported in several articles (Laine 1986; Bill and Aldinger 1995; Birot *et al* 1995; Baldus and Jansen 1997) The idea behind the process of the pyrolysis of preceramic compounds is to build up organometallic polymeric chains of structural units of the ceramic materials. The goal is to synthesize the macromolecules at first and then to condense them at relatively low temperatures into inorganic materials.

Several aspects make this technique most attractive for the development of new materials and compounds such as:

I) With this technique one can easily produce amorphous materials with compositions not obtainable with common synthetic routes.

II) Because of the low mobility in predominantly covalent bonded materials the amorphous stage can be thermally stable to very high temperature before transforming into crystalline phases.

*Author for corresondence

III) One can control the thermal activation in the amorphous stage for material transport mechanisms. So the technique kinetically stabilizes the less stable phases and microstructures with morphologies not possible by common synthetic techniques such as melting or sintering.

IV) Taking advantage of the various fabrication capabilities of polymer process engineering, components such as fibers, coatings, infiltration and complex-shaped bulk parts can mostly be produced in an easy manner.

The purpose of this paper is to outline the general methods of the synthesis of ceramic materials via thermolysis of preceramic compounds. This outline will consist of general information taken from the literature.

Precursor synthesis. Since the type of the backbone and the functional side chains of precursor molecules substantially influence the ceramic yield, chemical composition, and microstructure of precursor-derived ceramics a variety of different compounds has been investigated as starting materials. Figure 2 shows examples of the polymers which have been synthesized from monomers for the preparation of silicon carbide and silicon nitride-based ceramics.

These polymers are characterized by a direct attachment of silicon to carbon or nitrogen. Boron-containing polycarbosilazanes (Bill *et al* 1995, Riedel *et al* 1996) carbon containing polyborosilazanes and silylated borazine derivatives (Seyferth *et al* 1991) have been shown to be excellent precursor molecules to ceramic materials in the quaternary system Si-B-C-N. In general, these molecules consist of Si-N skeletons, which are more or less cross-linked via B, B-N, B-C or borazine units, carrying different substituents bonded to the silicon centers.

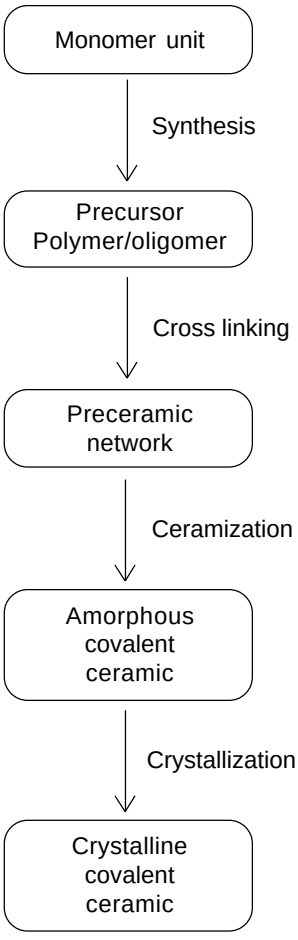

Fig 1. Material design by molecular architecture.

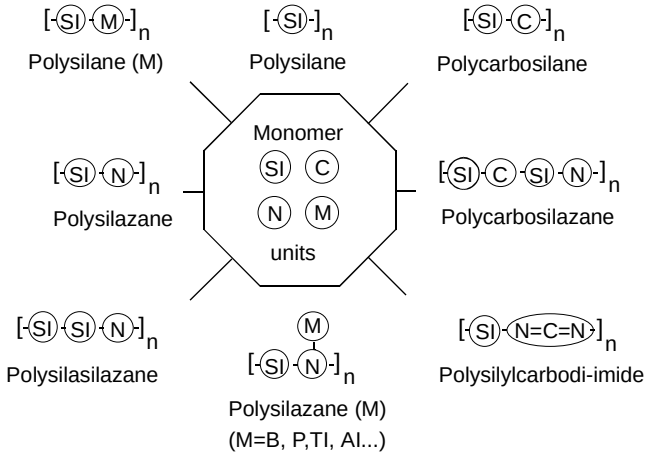

Fig 2. Formation of non-oxide organic silicon polymers from monomer units.

Recently, substantial progress has been made by the synthesis of boron-modified polysilazanes and polysilylcarbodiimides (Weinmann *et al* 1999, Bill *et al* 2000) using vinyl groups for the attachment of boron.

Thermolysis. The reactions which occur during thermolysis are still not cleared up in detail. Figure 3 describes schematically the formation of ternary Si_3N_4/SiC composites from cyclic oligosilazane (Aldinger 2002). NMR studies (Aldinger *et al* 1998, Bill *et al* 1998, Banfeld *et al* 1999) have shown that at temperatures below 500˚C cross-linking by reactions like dehydrogenation between N-H and Si-H groups occurs. Between 500˚C to 700˚C, ceramization takes place and methyl groups split off yielding an amorphous silicon carbonitride. At temperatures higher than 900˚C residual hydrogen is removed. 29Si and 13C solid state NMR studies (Aldinger *et al* 1998) were performed on a series of ternary silazanes after thermolysis of a commercial polyhydridomethylsilazane (PHMS, NCP200 of Chiosso Crop., Tokyo, Japan). The results at different temperatures revealed a short range order of the elements consisting mainly of tetrahedral SiCxNy (x + y = 4) units and sp3-hybridized carbon. However, the short range order of silicon carbonitrides is directly correlated with the molecular structure of the polymer. This has been demonstrated in the case of the thermolysis of a commercially available polyvinylsilazane (PVS, VT50, Hoechst AG, Germany)

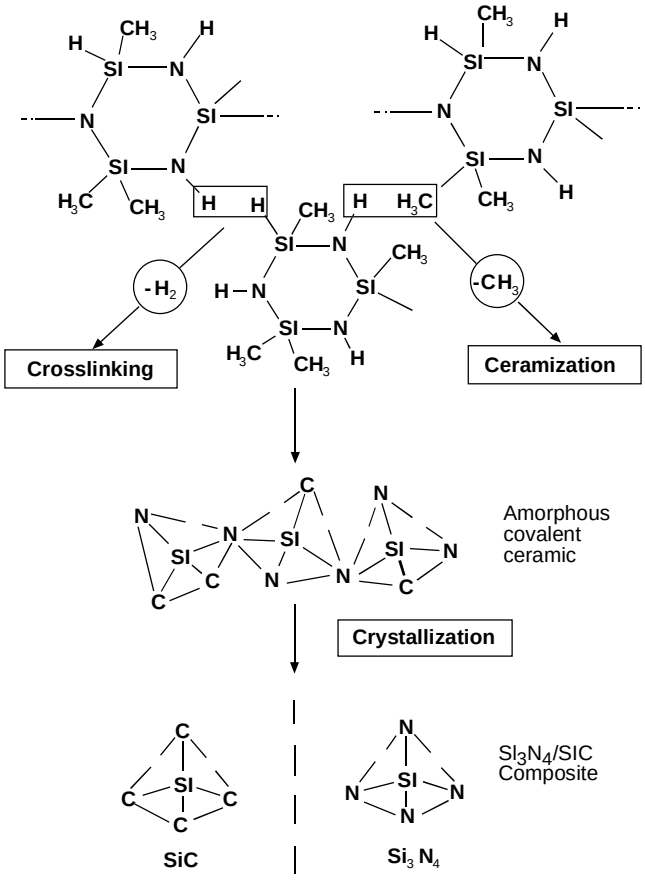

Fig 3. Schematical description of the formation of Si_3N_4/SiC composites from cyclic oligosilazane.

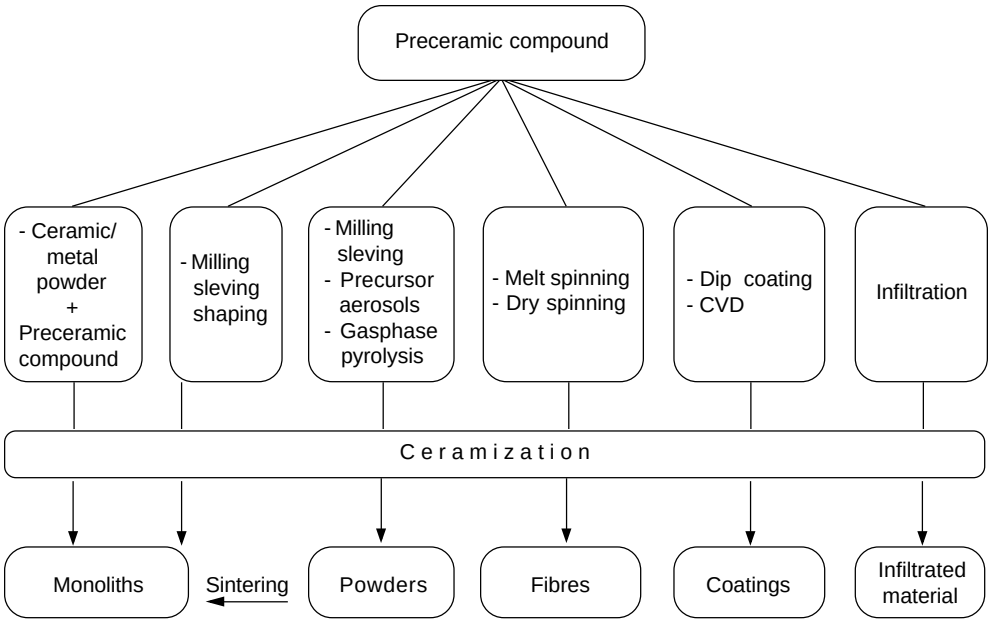

Fig 4. Preparation of ceramic materials by precursor processing.

which results in SiN4 tetrahedral units and sp 2-hybridized carbon. Against this, the effect of boron on the reactions during thermolysis is not clearly understood.

Precursor pyrolysis engineering. The production of precursor-derived ceramics takes advantage of highly developed polymer process engineering. Powders, fibers, coatings, bulk materials, infiltration and other types of preforms can be produced by techniques well known from polymer process engineering followed by a pyrolysis step (Fig 4) (Ramakrishnan *et al* 2001).

The production of dense bulks material is of special interest. This is not trivial, since the condensation reactions during pyrolysis are combined with the evolution of gases like H_2, NH_3 and CH_4. These species degas easily with the manufacture of fibers or coatings, i.e. with shapes which are thin in at least one dimension (Heimann *et al* 1995, Gadow and Kienzle 1998, Kamphowe *et al* 1998, Weinmann *et al* 1999, Bill *et al* 2000) Coatings have been produced by dip coating substrates into precursor solutes and subsequent conversion into inorganic pyrolysis.

Conclusion

It has been shown that the preparation of Si-B-C-N ceramic composites from element organic polymers is a suitable methods for the synthesis of high temperature stable materials. The polymer syntheses are realized on different reaction pathways (e.g. dehydrogenation from hydridosilazanes and borazine derivatives or by ammonolysis of suitable boron containing chlorosilanes or chlorosilylaminodichloroborane). A new class of Si-B-C-N precursors are boron containing polysilylcarbodi-imides which can be obtained from a non oxide sol-gel process of bis (trimethylsilyl) carbodi-imide and hydroborated vinylchlorosilanes. The advantage of this non oxide sol-gel process as compared to common methods (e.g. ammonolysis of chlorosilanes) significantly facilitated work-up. It has been shown that polymer solutions of boron containing polysilazanes of convenient viscosity are applicable for both, the production of oxygen resistant fibers and coatings. Moreover it has been determined that boron modified polysilylcarbodi-imides are outstanding precursors for the production of dense bulk ceramics. The obtained preceramics are then transformed into amorphous ceramics by subsequent thermolysis. Depending on the structure of the amorphous state, which is still not known in detail, the metastable amorphous ceramics crystallize at higher temperatures into the thermodynamically more stable crystalline materials.

References

Aldinger F, Weinmann M, Bill J 1998 Precursor-derived Si-B-C-N ceramics. *Pure Appl Chem* **70** (2) 439-448.

Aldinger F 2002 Thermal expansion behaviour of precursor derived amorphous Si-C-N and Si-B-C-N ceramics. *J Mater Sci Technol* **18** (2) 139-142.

Baldus H P, Jansen M 1997 Modeme Hochleistungskeramiken-amorphe anorganische Netzwerke aus molekularen Vorlaufem. *Angew Chem* **109** 338-354.

Banfeld B, Gu H, Bill J, Wakai F, Aldringer F 1999 High tem-

perature deformation of precursor-derived amorphous Si-B-C-N ceramics. *J Eur Ceram Soc* **19** 2797-2814.

Bill J, Aldinger F 1995 Precursor-derived covalent ceramics. *Adv Mater* **7** (9) 775-787.

Bill J, Kienzle A, Sasaki M, Riedel R, Aldinger F 1995 Novel routes for the synthesis of materials in the quaternary system Si-B-C-N and their characterization. In: *Advances Science and Technology 3B Ceramics*: Charting the Future, (ed) Vincenzini P Techna Srl pp 1291-1299.

Bill J, Seitz J, Thurn G, Durr J, Canel J, Janos B Z, Jalowiecki A, Sauter D, Schempp S, Lamparter H P, Mayer J, Aldinger F 1998 Structure analysis and properties of Si-C-N ceramics derived from polysilazanes. *Phys Stat Sol* **166** 269-296.

Bill J, Schuhmacher J, Muller K, Schempp S, Seitz J, Durr J, Lamparter H P, Golezewski J, Peng J, Seifert H J, Aldinger F 2000 Investigation into the structural evolution of amorphous Si-C-N ceramics from precursors. *Z Metallkd* **91** (4) 335-351.

Birot M, Pillot J P, Dunogues J 1995 Comprehensive chemistry of polycarbosilanes,polysilazanes and polycarbosilazanes as precursors of ceramics. *Chem Rev* **95** 1443-1477.

Gadow R, Kienzle A 1998 Development of ceramics via pyrolysis of metal organic polymers. *Mat wiss u Werkstofftech* **29** 371-384.

Heimann D, Bill J, Aldinger F 1995 Development of oxidation protected carbon/carbon. *Z Flugwiss Weltraumforsch* **19** 180-188.

Kamphowe T W, Weinmann M, Bill J, Aldinger F 1998 Preparation of fibre-reinforced Si-B-C-N ceramics by polymer precursor infiltration. *Sil Ind* **63** (11-12) 159-162.

Laine R M 1986 Transformation of organometallics into common and exotic materials: design and activation. In: *Proceedings of the NATO Advanced Research Workshop*, Cap D Agde France.

Popper P 1967 Brit Ceram Res Assn Special Pub location **57** 1.

Ramakrishnan P A, Wang Y T, Balzar D, An L, Haluschka C, Riedel R, Herman A 2001 Si-B-C-N ceramics: A class of high temperature dopable electronic materials. *APL* **78** (20) 3076-3078.

Riedel R, Kienzle A, Dressler W, Rumisch L, Bill J, Aldinger F, 1996 A silicoboron corbonitride ceramic stable to 2000°C. *Nature* **382** 796-798.

Seyferth D, Plenio H, Rees J W S, Buchner K 1991 *Silicon Ceramics with a Dash of Boron in Fronterrs of Organosilicon Chemistry*, The Royal Society of Chemistry, Cambridge, UK.

Weinmann M, Bill J, Aldinger F 1999 *Boron-containing polysilazanes, their manufacture, and manufacture of ceramics and coatings by thermal degradation of the polysilazanes*. Ger Offen DE 19741458.

Weinmann M, Bill J, Aldinger F 1999 *Manufacture of boron, carbon, hydrogen, nitrogen, and silicon containing polymeric precursors for manufacturing ceramics, the precursors, ceramics and coated composites obtained and their use*. Ger Offen DE 19741459.

Weinmann M, Kamphowe T, Bill J, Aldinger F 1999 *Manufacture of boron, carbon, hydrogen, nitrogen, and silicon containing polymeric precursors for manufacturing ceramics, and the precursors, ceramics and coated composites obtained*. Ger Offen DE 19741460.

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Journal Articles

In Bibliography:

Reid R W, Watson J A 1995 Reaction of lodgepole pine to attack by blue stain fungi. *Can J Bot* **45** (2 Part 1) 45-50.

Weenen H, Nkunya M H H, Bray D H, Mwasumbi L B, Kinabo L S, Kilimali V A E B 1990 Antimalarial activity of Tanzanian plants. *Plant Medic* **56**(4) 368-370.

Solhein H 1992a The early stages of fungal invasion in Norway spruce. *Can J Bot* **70** (4 Ser A) 112-119.

Solhein H 1992b Fungal succession in sapwood. *Eur J Pathol* **22** 143-158.

In Text:

(Reid and Watson 1995)

(Solhein 1992a)

(Solhein 1992a & b)

(Weenen *et al* 1990; Solhein 1992a; Reid and Watson 1995)

Chapters of Books

Demarchi DA, Marsh RD, Harcombe AP, Lea EC 1990 The environment. In: *The Birds of British Columbia*, eds Cambell R W & McNall M C E. Royal British Columbia Museum, Victoria, B C USA, Vol 2, 2nd ed, pp 366-425.

Books

Bennet O C, Myers J E 1974 *Momentum, Heat and Mass Transfer*. McGraw Hill, New York, USA, pp 540-562.

Thesis

Hu C 1989 A comparative study of peanut peroxidase isozyme. Ph.D thesis, Department of Plant Sciences, University of Western Ontario, London, Ontario, Canada.

Papers of Conferences/Symposia/Seminars

Jhonson S R, Knapp A K 1994 The role of *fire in Spartina pectinator* dominated tallgrass prairie wetlands. In: *Proceedings on 19th Tall Timbers Fire Ecology Conference*, Tallahassee, Fl, USA, November 3-6, 1993.

Technical Reports

Tiller FM, Leu WF 1984 *Solid Liquid Separation for Liquefied Coal Industries*. Final Report for RP - 1411-1. EPRI, AP-3599, Electric Power Research Institute, Palo Atto, CA, USA.

Patents

Verschuur E 1978 *Agglomerating Coal Slurry Particles*. US Patent 4126426.

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